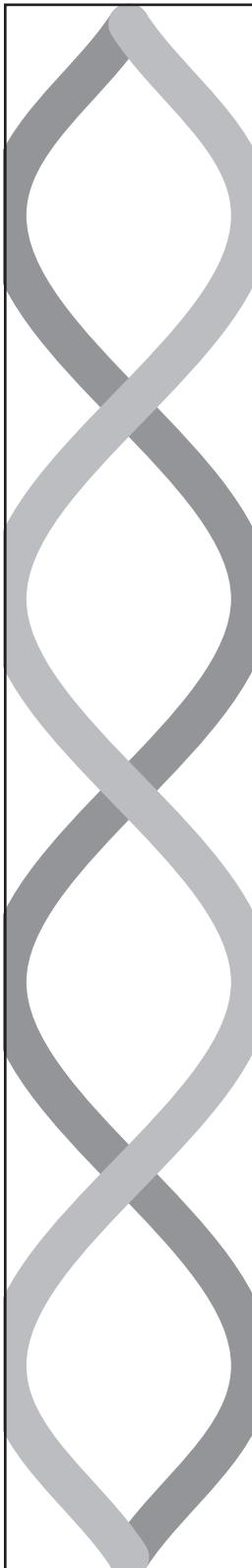




Encyclopedia of
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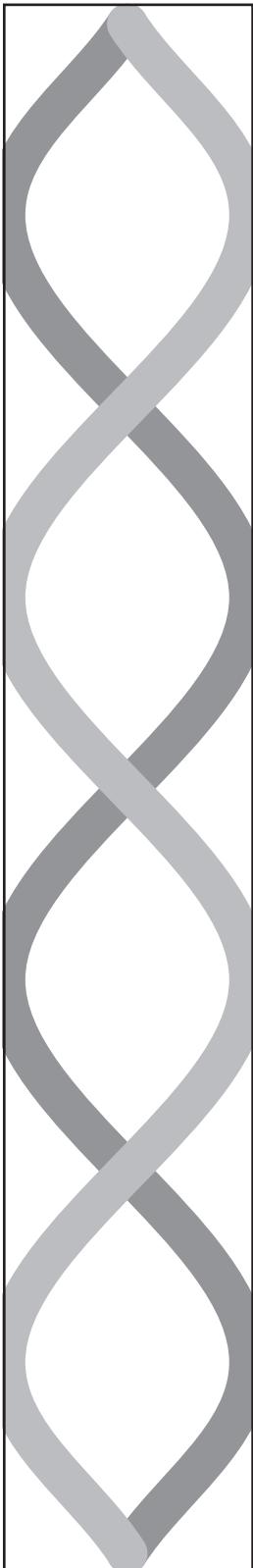
Revised Edition



Encyclopedia of Genetics

Revised Edition

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Encyclopedia of Genetics

Revised Edition

Volume 1

**Aggression –
Hybridization and Introgression**

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Publisher's Note

The award-winning *Encyclopedia of Genetics* was originally created in 1999 to provide the general reader with a thorough yet accessible overview of one of modern science's most vital and intriguing fields. This 2004 *Revised Edition* adds 64 new overview essays on current topics to the original entries, reflecting the rapid developments in an exciting and often controversial branch of science that is increasingly shaping our world.

Of the 172 original entries (168 overviews plus 4 appendices), 7 overviews ("Biotechnology," "Genetic Medicine," "Immune Deficiency Disorders," "Lethal Alleles," "Meiosis," "Methane-Producing Bacteria," and "Sheep Cloning") have been dropped because they have been superseded by other, updated or new, essays; 26 have been replaced because they were so out of date as to demand completely new coverage (two of these, "Aging" and "Mitosis and Meiosis," are from other Salem publications, deemed more up to date than the original entries); and 131 were moderately to heavily revised and updated by the editor as deemed appropriate.

All essays' bibliographies are new or are fully updated. The four appendices have been updated and two new ones, "Web Sites" and "Nobel Prizes for Discoveries in Genetics," have been added. Featured in this new edition are 25 new "sidebars," or mini-essays (500 words each). These sidebars, appearing in shaded boxes, offer coverage of particularly significant and current subtopics appended to the overview essays. A list displaying the status of the essays, designed to assist librarians in comparing the two editions, appears at the end of this note.

The result, in two volumes, is 223 overviews, 25 sidebars, and 6 appendices: a 30 percent increase based on number of overview essays, and an increase of more than 40 percent in overall word length. The set surveys this continually evolving discipline from a variety of perspectives, offering historical and technical background along with a balanced discussion

of recent discoveries and developments. Basics of biology—from the molecular and cellular levels through the organismal level, from Mendelian principles to the latest on DNA sequencing technology—constitute the core coverage. Medical topics comprise a significant number of essays, as the genetic predisposition for many illnesses and syndromes has increasingly come to light. Genetic technologies that promise a world without hunger, disease, and disability—and promise to rewrite human values—are addressed as well. The encyclopedia's scope embraces the key social and ethical questions raised by these new genetic frontiers: from cloning to stem cells to genetically modified foods and organisms.

Each essay follows a standard format, including ready-reference top matter and the following standard features:

- **Fields of study** lists one or more of a dozen subdisciplines of genetics or biology under which the topic falls.
- **Significance** provides a definition and summary of the topic's importance.
- **Key terms**, concepts central to the topic, are next identified and defined.
- **Subheads** break the main body of each essay into clearly marked subtopics.
- The **contributor's byline** lists the biologist or other area expert who wrote the essay.
- The **See also** section lists cross-references to other essays of interest.
- **Further Reading** lists sources for further study with annotations; all of these biographical sections have been fully updated and reformatted to include the latest relevant works and full citation data for easy library access.
- **Web Sites of Interest**, finally, includes annotated entries for the most authoritative free sites on the Internet, including the sponsoring organization and URL. This section,

which appears in more than half the essays, was included for topics (such as diseases and syndromes) for which authoritative government agencies, professional or academic societies, or support organizations were available, with only the occasional nod to a particularly useful personal URL. All sites were accessed by the editors in August, 2003.

A series of appendices supplements the overview essays: An updated **Time Line of Major Developments in Genetics** offers a chronological overview of the field's development. **Nobel Prizes for Discoveries in Genetics** lists Nobel Prize winners (all prizes) whose contributions altered the history of genetics. An updated **Biographical Dictionary of Important Geneticists** has more than doubled in size, now including all Nobel laureates whose discoveries in genetics garnered them the award, as well as numerous others. The updated **Glossary** provides definitions of more than 500 commonly used terms and important concepts. The updated general **Bibliography** references important works in each field of study, joining with individual articles' "Further Reading" sections to offer plentiful citations to recently published sources for additional research. The impor-

tance of the Internet to bioinformatics and to general education in genetics is reflected in the new **Web Sites** appendix.

The articles in the *Encyclopedia of Genetics, Revised Edition* are arranged alphabetically by title; an alphabetical list of contents appears at the beginning of each volume. To help readers locate topics of interest by area of study, a Category Index, a Personages Index, and a comprehensive Subject Index are included at the end of volume 2. Nearly 100 diagrams, charts, graphs, drawings, and tables elucidate complex concepts, and more than one hundred photographs illustrate the text.

We wish to thank the many biologists and other scholars who contributed both to the original edition and this revised edition; their names and academic affiliations appear in the Contributor List that follows. Special credit is due the editor of the *Revised Edition*, Dr. Bryan D. Ness of the Department of Biology, Pacific Union College. Professor Ness paid close attention to the contents of every essay, carefully updating all of the original text, elucidating complex concepts for the general reader, and making valuable contributions to the project on all levels.

Preface to the Revised Edition

In the five years since publication of the first edition of *Encyclopedia of Genetics*, the field of genetics has continued to expand, if possible, exponentially. The volume of data flowing from genetics research is so great that new methods of organizing and analyzing it are still being devised. As with any expanding field, practical applications have lagged behind predictions. The Human Genome Project, now completed, which was predicted to usher in a new era in medical genetics, has so far had only minor effects, and the many genetically modified (GM) crops that were supposed to revolutionize agriculture have caused more controversy than success. Yet, like most technologies of the past that took time to gain acceptance, the knowledge and technologies flowing from the Human Genome Project will almost certainly gain influence and acceptance over the next decades. The slow and steady application of these technologies will eventually have a world-changing impact on all aspects of life.

The current revised edition is an attempt to increase the coverage begun in the first edition and to cover as many of the new developments in genetics as possible, a daunting task considering that new discoveries seem to happen weekly. By way of perspective, consider what has occurred in the field of genetics in the past five years. The Human Genome Project, probably the most widely publicized genetic project of the past century, saw the completion of the human genome sequence two years ahead of its original schedule—appropriately, fifty years after the discovery by James Watson and Francis Crick of the double-helical structure of DNA. The field of genomics—the study of the sequence and structure of the genomes of various organisms—has now solidly entered the public consciousness and has spawned the related specialty proteomics, the study of the proteins expressed by genomes, which focuses on differences among cell types as well as differences between gene expression in health and disease. Although these studies have not yet transformed medicine, as more data accumu-

late and are analyzed, medicine will certainly become a more exact science, enabling therapy to be tailored to a person's genotype.

As spinoffs of the Human Genome Project, the genomes of many “model” organisms have also been sequenced, a process that has been accelerated by the development of technologies such as the polymerase chain reaction and automated sequencers. Only a handful of small genomes had been sequenced at the time the first edition of *Encyclopedia of Genetics* was published five years ago; today, hundreds of genomes have been sequenced, including some larger genomes such as those of *Arabidopsis thaliana* (the model mustard plant), *Drosophila melanogaster* (the fruit fly), and *Caenorhabditis elegans* (the model round worm). Many other genome sequencing projects are under way, and as more genomes are sequenced, geneticists will obtain ever clearer insights into how our genes make us who we are—not to mention how our genomes relate to those of other organisms (the focus of comparative genomics) and hence how such organisms can be manipulated genetically to our benefit.

Since the cloning of Dolly the sheep in 1996, a variety of other mammals have been cloned, including mice and, most recently, a horse. This brave new world of cloning has spawned the imaginations of filmmakers and writers as surely as space exploration did in earlier generations—fueling wild speculations about the possibility of reviving ancient life-forms, such as the dinosaurs of Michael Crichton and Stephen Spielberg’s *Jurassic Park* (1993), and, more recently, claims of human cloning that are plausible if not probable, though fraught with both technical and ethical obstacles. Shortly after Dolly was cloned, for example, it was discovered that her telomeres (the ends of her chromosomes) were shorter than normal for a juvenile sheep. Telomeres are known to shorten throughout the life spans of many organisms and have been implicated as part of the cause, or at least one of the effects, of aging. With her shortened telomeres, the big question was, how

would it affect her longevity? In February of 2003 the answer came when Dolly had to be euthanized because she was suffering significantly from a form of arthritis usually seen only in older sheep, as well as advanced lung disease. Her early decline, at six years of age, cast doubt on the hoped-for success of cloning mammals.

Of course, the cloning of Dolly fueled increased speculation about the feasibility of human cloning. Considering Dolly's premature death and other health issues, most geneticists do not consider the technology ready for human cloning. Ethically, human cloning, indeed organismal cloning of all types, is extremely controversial—with myriad implications economically, socially, politically, and ecologically (as human manipulation supplants traditional methods of natural selection as a force in evolution)—but it is even more so when the high probability of producing a debilitated human clone is considered.

Along with the budding debate over cloning is another, related issue that arose simultaneously at the beginning of the new millennium: the use of fetal stem cells in research. Some geneticists believe that stem cells—“totipotent” cells, capable of differentiating into essentially any other kind of cell—may have potential for treating a variety of neurological diseases such as Alzheimer's and Parkinson's, as well as conditions requiring new organs that might be generated by implanting stem cells with the genetic instructions to develop into ears or kidneys that could be grown on animals especially designated for this purpose. Unfortunately, the best stem cells come from developing fetuses, and the ethics of harvesting fetal stem cells from aborted fetuses is hotly debated. The much-touted potential of using adult stem cells instead is clouded by both political and social agendas and the fact that they have not yet proven to be nearly as versatile or easy to culture as fetal stem cells. In the United States, research on fetal stem cells has been limited to a small number of cell lines existing at the time that the federal government addressed the issue in 2001; it was determined to withhold funding from any research group that harvests fetal stem cells as a part of their work. Research continues on the limited stem

cell lines and on alternatives such as the less controversial adult source of stem cells.

Regardless of the eventual decisions regarding human cloning and research using fetal stem cells, the ethical questions raised by both endeavors clearly run parallel. With the endless potential uses of such technologies, where might they lead? Where does the “necessity” to solve human suffering end and the “brave new world” of self-proliferation, designer traits, worker and military subclasses, perfect progeny, and potential ecological disaster begin? What obligations do we have to fulfill the new promise of genetic science to alleviate human suffering, and what obligations do we have to limit that promise out of concern for greater detriment?

This last consideration is now a very real concern. GM crop plants were once considered the Holy Grail of agricultural genetics, seen as a solution to everything from more effectively battling pests and weeds to correcting Third World nutritional deficiencies and utilizing marginal habitats such as saline soil. Much of the early headiness surrounding the potential of high-yield and transgenic crops has dissipated and opposition has increased against the use of such plants. Objections range from fears over human health and safety to ecological and economic concerns. Most Europeans have rejected GM foods completely, and even consumers in the United States are uncomfortable using them—or at least feel that it is their right to be informed, through labeling, before making purchase decisions. If the many concerns expressed by consumers cannot be addressed, GM crops may not be embraced widely for some time, if ever.

With these advances, and many others not mentioned, the general public has become overwhelmed with the implications. Although the terms are casually thrown around in the media and strident statements are made, the general public has become increasingly uncomfortable with a technology they barely understand. As a result, opposition from many directions continues to build, epitomized by both the federal ban on stem cell research mentioned above and the Human Cloning Prohibition Act that is now making its way through Congress.

Especially with regard to GM foods, many geneticists consider the root problem to be lack of knowledge about genetics on the part of nonscientists. Although terms such as “DNA,” “cloning,” “GM food,” and “gene therapy” have entered the public vocabulary, many people gain their understanding of these technologies from the science fiction of books, film, and television rather than from the science on which they are founded. Those who are opposed to genetic technology have taken advantage of this “safety-first” attitude and have spread fear about genetic technology, coining inflammatory terms like “Franken foods” and citing the law of “unintended consequences.” Consequently, many nonscientists misunderstand the issues and mistrust the scientists doing the work, picturing them as ambitious and amoral rather than as responsible researchers. Part of the problem lies with geneticists themselves, many of whom find it difficult to communicate with nonscientists. Geneticists need to learn to communicate in a way that the general public will understand. Without a broad understanding by the general public, genetics will continue to be viewed by many in a negative light.

It is in this spirit that the current volumes were updated and expanded. The articles contained herein are written specifically with the nonscientist in mind—and specifically to explain, as simply as possible, some of the science behind the technologies and issues described above. All original topics were considered for updating, many were heavily revised, 26 were entirely replaced, and 64 are entirely new. In other cases, recent advances put a spotlight on topics—such as Bioinformatics, Biological Weapons, Smallpox, and Xenotransplants—that were too minor or obscure to have been included in the previous edition.

Other improvements in this edition include the addition of 25 “sidebars,” elucidating particularly important and timely subtopics; a new time line of Nobel laureates whose work in genetics garnered them the award; a “Biographical Dictionary of Important Geneticists” that is twice its original size; more than 100 new definitions added to the Glossary; an updated “Time Line of Major Developments in Genetics”; a heavily expanded and re-categorized

general Bibliography; and a select list of genetics Web sites aimed at students and nonspecialists. In addition, every essay carries a “Further Reading” section that has been thoroughly reviewed, updated, and annotated—to which we have added more than 400 new books and articles published since the first edition. In recognition of the influence of the Internet on student research, we for the first time include, in more than half the essays, a section headed “Web Sites of Interest” targeted at the essay’s topic. Finally, every effort has been made to make the essays user-friendly, easy to read, and clear, with the goal of improving the presentation and understandability of all the old, as well as the new, essays.

Even as this edition goes to press, it is already beginning to go out of date. Genetics is a dynamic field that deserves close attention as we begin the new millennium. Nevertheless, the basic scientific principles presented here will provide science students with insight into the topics on which they can build, and that fundamental understanding will repay students and general readers regardless of their ultimate occupations or career interests. Such an understanding behooves all of us: The potential of genetic principles to completely alter the way we live and interact with the environment is profound. We will see changes in the way doctors diagnose and treat disease, new GM crops and animals, powerful new forensics techniques, and unique ways to solve environmental and societal problems. There are potential dangers as well, and these must be carefully analyzed and vetted in the public forum. As we attempt to navigate our genetic future, knowledge will be essential if we are to take full advantage of the positive benefits, and prevent the negative consequences, of genetic technology. It is hoped that the information contained here will open a new world of understanding to the nonspecialist and encourage further exploration of the amazing world of genetics, which offers frontiers for exploration just as real as those of space or oceanic exploration—and in many ways more personal and tangible.

—Bryan D. Ness
August, 2003

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Preface to the First Edition

The science of genetics, once the purview only of serious students and professionals, has in recent decades come of age and entered the mainstream of modern life. An unparalleled explosion of new discoveries, powerful new molecular techniques, and practical applications of theories and research findings has brought genetics and its related disciplines to the forefront of public consciousness. The successful cloning of "Dolly" the sheep has sparked widespread public interest and debate and raised new questions about the ethics of this and other genetic technologies. Gene therapy has made the transition from science fiction to reality and is used to treat serious diseases, and there is increasing demand for the newest health professionals, genetic counselors, at hospitals and medical centers around the world. As we celebrate the new millennium, it is perhaps worth noting that the young science of genetics celebrates its one hundredth birthday.

Among many other events of historical importance, the year 1900 marked the rediscovery of the Austrian monk Gregor Mendel's experimental work on the inheritance of traits in the garden pea. Mendel had published his results thirty-four years earlier, but his work attracted little attention and soon faded into obscurity. By the close of the nineteenth century, however, much had happened on the scientific front. Chromosomes had been discovered, and the cellular processes of mitosis and meiosis had been observed under the microscope. The physical bases for understanding Mendel's principles of inheritance had been established, and the great significance of his pioneering work could finally be appreciated. The so-called chromosome theory of heredity was born, and the age of transmission genetics had arrived.

The first great geneticist to emerge (and some would still call him the greatest of the twentieth century) was Thomas Hunt Morgan, who established his "fly laboratory" at Columbia University and began studying the principles of transmission genetics, using the fruit fly as a model organism. All the major principles

of transmission genetics, including single and multifactorial inheritance, chromosome mapping, linkage and recombination, sex linkage, mutagenesis, and chromosomal aberrations, were first investigated by Morgan and his students.

The subdisciplines of bacterial and molecular genetics had their beginnings in the 1940's, when bacteria and their viruses became favored genetic systems for research because of their relative simplicity and the ease with which they could be grown and manipulated in the laboratory. In particular, the common intestinal bacterium *Escherichia coli* was studied intensely, and today far more is known about the biology of this single-celled organism than about any other living system. In 1952, James Watson and Francis Crick provided the molecular model for the chemical structure of DNA, the genetic material, and the next twenty years saw great progress in the understanding of the molecular nature of essential cellular processes such as DNA replication, protein synthesis, and the control of bacterial gene expression.

The 1970's witnessed the discovery of a unique class of enzymes known as restriction endonucleases, which set the stage for the development of the exciting new technology known by various names as cloning, genetic engineering, or recombinant DNA technology. Since that time, research has progressed rapidly on several fronts, with the development of genetic solutions to many practical problems in the fields of medicine, agriculture, plant and animal breeding, and environmental biology. With the help of the new technology, many of the essential questions in cell and molecular biology that were first addressed in bacteria and viruses in the 1950's and 1960's can now be effectively studied in practically any organism.

And what are the major problems remaining to be solved? No doubt there are many, some of which cannot even be articulated given the present state of scientific understanding. Two important questions, however, are drawing disproportionate shares of attention in the cur-

rent sphere of basic research. One of these is the problem variously referred to as “the second genetic code” or “protein folding.” Scientists know how a particular molecule of DNA, with a known sequence of nucleotide subunits, can cause the production of a particular unique protein composed of a known sequence of amino acid subunits. What is not understood, however, is the process by which that protein will spontaneously fold into a characteristic three-dimensional shape in which each amino acid interacts with other amino acids to produce a functional protein that has the proper pockets, ridges, holes, protuberances, and other features that it needs in order to be biologically active. If all the rules for protein folding were known, it would be possible to program a computer to create an instant three-dimensional picture of the protein resulting from any given sequence of amino acids. Such knowledge would have great applications, both for understanding the mechanisms of action of known proteins and for designing new drugs for therapeutic or industrial use.

The second “big question” at the forefront of experimental genetic inquiry relates to the control of gene expression in humans and other higher organisms. In other words, what factors come into play in turning on or turning off genes at the proper times, either during an

individual cell cycle or during the developmental cycle of an organism? How is gene expression controlled differentially—that is, how are different sets of genes turned on or off in different tissues in the same organism at the same time? Many human genetic diseases are now known or suspected to be caused by errors in gene expression—that is, too much or too little of a particular protein is made in the critical tissues at the critical developmental times—so the answers to these and related questions are sure to suggest new possibilities for gene therapy or other treatments.

The purpose of these reference volumes is twofold. First, the editors seek to highlight some of the most exciting new advances and applications of genetic research, particularly in the fields of human medical genetics and agriculture. Second, we hope to provide a solid basis for understanding the fundamental principles of genetics as they have been developed over this first one hundred years, along with an appreciation of the historical context in which the most important discoveries were made. It is our hope that such an understanding and appreciation might help to inspire a new generation of geneticists who will continue to expand the boundaries of scientific knowledge well into the next millennium.

—Jeffrey A. Knight

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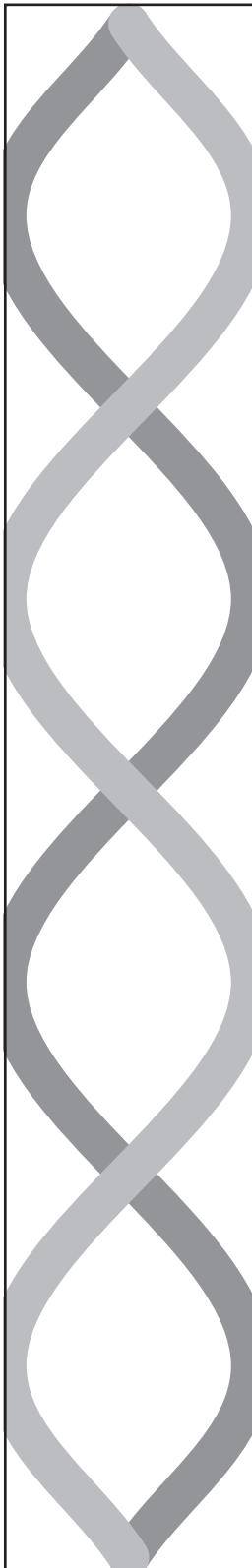
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Encyclopedia of Genetics

Revised Edition

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Aggression

Field of study: Human genetics and social issues

Significance: *Aggression refers to behavior directed toward causing harm to others. Aggressive anti-social behavior is highly heritable, and antisocial behavior (ASB) during childhood is a good predictor of ASB in adulthood and crime. Physical acts of aggression are sometimes distinguished from the more context-sensitive “covert” ASBs, including theft, truancy, and negative peer interactions.*

Key terms

ANTISOCIAL BEHAVIOR (ASB): behavior that violates rules or conventions of society and/or personal rights

IMPULSIVITY: a tendency to act quickly without planning or a clear goal in mind

IRRITABILITY: a tendency to overreact to minor stimuli; short-temperedness or volatility

LIABILITY: the risk of exhibiting a behavior; the higher one's score for a measure of liability, the greater is one's the risk of exhibiting the behavior

SEROTONIN: a neurotransmitter, 5-hydroxytryptamine (5-HT), present in blood platelets, the gastrointestinal tract, and certain regions of the brain, which plays role in initiating sleep, blood clotting, and stimulating the heartbeat, and levels of which have been correlated with aggressive behavior as well as depression and panic disorder

Aggression and Related Behaviors

Aggression or agonistic behavior in animals is usually an adaptive response to specific environmental situations during competition for resources, as in establishing dominance and a territory or in sexual competition. Rat and mice studies indicate it is partly genetic, because selective breeding produces strains that differ in levels of aggression. Human aggression can also represent a variety of natural responses to challenging situations. Measures of aggression vary, but of greatest concern are antisocial behaviors (ASBs) such as crime and delinquency and whether some individuals are more likely to engage in these behaviors than others.

The earliest evidence for a genetic contribution to these complex behaviors comes from twin and adoptee studies. Genes also increase the liability for many clinical conditions that include aggressive behaviors, such as conduct disorder (physically aggressive acts such as bullying or forced sexual activity) and antisocial personality disorder (persistent violation of social norms, including criminal behavior) and for personality traits that often accompany aggression, such as impulsivity and irritability. Differences in measuring ASBs partly account for the variability in heritability estimates, which range from 7 to 81 percent, but many studies indicate a heritability for genetic influences of 0.40-0.50, a minor influence of shared environment, and a much more significant influence of nonshared environment (environment unique to the individual).

Aggression and Human Development

Aggressive behavior develops in children through a complex interaction of many environmental and biological factors. Also increasing liability for aggression and perhaps criminality are such factors as low socioeconomic status and parental psychopathology. A consistent finding is that the measure of the activity of the central nervous system's serotonin correlates inversely with levels of lifetime aggression, tendency to physically assault, irritability, and impulsivity. Some of the implicated genes regulate serotonin synthesis, release, and reuptake as well as metabolism and receptor activation, and vary from individual to individual. Serotonergic dysfunction is also noted in alcoholism with aggression and in suicide attempters and completers. Brain injuries can also exacerbate tendencies to exhibit ASBs.

Some aggression, however, is a normal part of development. Thus, Terrie Moffitt and colleagues distinguish between “adolescent-limited aggression”—times when most adolescents are rebelling against adult authority—and “life-course persistent” ASB, which likely reflects neuropsychological deficits and specific temperaments that are often exacerbated in unsupportive family settings. Genetic factors play

a smaller role in adolescent delinquency and are consistent with aggression at this age as a developmental response to social context.

Sex Differences

A significant feature of ASB is a marked difference between the sexes. Males exhibit higher levels of physical aggression and violence at every age in all situations except in the context of partner violence (where females exceed males). More males than females are diagnosed with conduct disorder at every age. More males than females begin acts of theft and violence at every age. Males also exhibit higher rates of risk factors, such as impaired neurocognitive status, increased hyperactivity, and difficulties with peers. Females are rarely identified with the life-course persistent form of ASB; the male:female sex ratio is 10:1. Antisocial male and female adolescents tend to associate and often marry and reproduce at younger ages. The role that hormones, particularly testosterone, may play in these differences is not clear.

Social Significance

There is much controversy surrounding the efforts to identify genes associated with aggression or crime, especially now that genome sequencing is easier than ever. Many demand that the privacy of individuals be protected because the presence of specific genes does not dictate behavioral outcomes: Genes do not determine socially defined behaviors but only act on physiological systems. In addition, what constitutes acceptable or unacceptable behavior for individuals is culturally defined. Biological and environmental risk factors may increase an individual's liability to commit an act of aggression or crime, but the behavior must be interpreted within its specific context. Criminal law presumes that behavior is a function of free will, and most attempts to use genes as a mitigating factor in the courtroom have been unsuccessful. Efforts to prevent crime and violence must include consideration of all factors. Family milieu and parental competence are just as important as impaired cognitive mechanisms such as reduced serotonin activity. An imbalance in brain chemistry leading to impulsivity or aggression may be ameliorated by a

supportive home setting, by medication, or by adequate nutrition.

—Joan C. Stevenson

See also: Aging; Behavior; Biological Determinism; Criminality; DNA Fingerprinting; Forensic Genetics; Sociobiology; Steroid Hormones; XYY Syndrome.

Further Reading

Bock, Gregory R., and Jamie A. Goode. *Genetics of Criminal and Antisocial Behaviour*. New York: John Wiley & Sons, 1996. This symposium was held at the Ciba Foundation in London in 1995 and includes a representative sample of the research foci in this arena, followed by discussions.

Fishbein, Diana H., ed. *The Science, Treatment, and Prevention of Antisocial Behaviors: Application to the Criminal Justice System*. Kingston, N.J.: Civic Research Institute, 2000. An excellent set of reviews on aggression and the many associated behaviors and mental disorders.

Lesch, Klaus Peter, and Ursula Merschdorff. "Impulsivity, Aggression, and Serotonin: A Molecular Psychobiological Perspective." *Behavioral Sciences and the Law* 18, no. 5 (2000): 581-604. A wonderful review of all the interacting factors, including all the elements of the serotonin system.

Moffitt, Terrie E., Avshalom Caspi, Michael Rutter, and Phil A. Silva. *Sex Differences in Antisocial Behaviour: Conduct Disorder, Delinquency, and Violence in the Dunedin Longitudinal Study*. New York: Cambridge University Press, 2001. Sex differences are documented as children grow up.

Roush, Wade. "Conflict Marks Crime Conference." *Science* 269, no. 5232 (1995): 1808-1809. An excellent description of the pros and cons of genetic research on ASB.

Web Site of Interest

National Institutes of Health, National Institute of Mental Health. <http://www.nimh.nih.gov/publicat/violencesfact.cfm>. Provides information on child and adolescent violence and antisocial behavior, including research into the possible genetic factors of aggression.

Aging

Field of study: Human genetics and social issues

Significance: *In the light of modern science and medicine, it has become apparent that the roots of aging lie in genes; therefore, the genetic changes that take place during aging are the source of the major theories of aging currently being proposed.*

Key terms

ANTIOXIDANT: a molecule that preferentially reacts with free radicals, thus keeping them from reacting with other molecules that might cause cellular damage

FREE RADICAL: a highly reactive form of oxygen in which a single oxygen atom has a free, unpaired electron; free radicals are common by-products of chemical reactions

MITOCHONDRIAL DNA (mtDNA): the genome of the mitochondria, which contain many of the genes required for mitochondrial function

PLEIOTROPY: a form of genetic expression in which a gene has multiple effects; for example, the mutant gene responsible for cystic fibrosis causes clogging of the lungs, sterility, and excessive salt in perspiration, among other symptoms

Why Study Aging?

Biologists have long suspected that the mechanisms of aging would never be understood fully until a better understanding of genetics was obtained. As genetic information has exploded, a number of theories of aging have emerged. Each of these theories has focused on a different aspect of the genetic changes observed in aging cells and organisms. Animal models, from simple organisms such as *Tetrahymena* (a single-celled, ciliated protozoan) and *Caenorhabditis* (a nematode worm) to more complex organisms like *Drosophila* (fruit fly) and mice, have been used extensively in efforts to understand the genetics of aging. The study of mammalian cells in culture and the genetic analysis of human progeroid syndromes (that is, premature aging syndromes) such as Werner's syndrome and diseases of old age such as Alz-

heimer's disease have also improved the understanding of aging. From these data, several theories of aging have been proposed.

Genetic Changes Observed in Aging Cells

Most of the changes thus far observed represent some kind of degeneration or loss of function. Many comparisons between cells from younger and older individuals have shown that more mutations are consistently present in older cells. In fact, older cells seem to show greater genetic instability in general, leading to chromosome deletions, inversions, and other defects. As these errors accumulate, the cell cycle slows down, decreasing the ability of cells to proliferate rapidly. These genetic problems are partly a result of a gradual accumulation of mutations, but the appearance of new mutations seems to accelerate with age due to an apparent reduced effectiveness of DNA repair mechanisms.

Cells that are artificially cultured have been shown to undergo a predictable number of cell divisions before finally becoming senescent, a state where the cells simply persist and cease dividing. This phenomenon was first established by Leonard Hayflick in the early 1960's when he found that human fibroblast cells would divide up to about fifty times and no more. This phenomenon is now called the Hayflick limit. The number of divisions possible varies depending on the type of cell, the original age of the cell, and the species of organism from which the original cell was derived. It is particularly relevant that a fibroblast cell from a fetus will easily approach the fifty-division limit, whereas a fibroblast cell from an adult over age fifty may be capable of only a few divisions before reaching senescence.

The underlying genetic explanation for the Hayflick limit appears to involve regions near the ends of chromosomes called telomeres. Telomeres are composed of thousands of copies of a repetitive DNA sequence and are a required part of the ends of chromosomes due to certain limitations in the process of DNA replication. Each time a cell divides, it must replicate all of the chromosomes. The process of replication inevitably leads to loss of a portion of each telomere, so that with each new cell di-

vision the telomeres get shorter. When the telomeres get to a certain critical length, DNA replication seems to no longer be possible, and the cell enters senescence. Although the process discussed above is fairly consistent with most studies, the mechanism whereby a cell knows it has reached the limit is unknown.

A result of these genetic changes in aging humans is that illnesses of all kinds are more common, partly because the immune system seems to function more slowly and less efficiently with age. Other diseases, like cancer, are a direct result of the relentless accumulation of mutations. Cancers generally develop after a series of mutations or chromosomal rearrangements have occurred that cause the mutation of or inappropriate expression of proto-oncogenes. Proto-oncogenes are normal genes that are involved in regulating the cell cycle and often are responsible for moving the cell forward toward mitosis (cell division). Mutations in proto-oncogenes transform them into oncogenes (cancer genes), which results in uncontrolled cell division, along with the other traits displayed by cancer cells.

Progeroid Syndromes as Models of Aging

Several progeroid syndromes have been studied closely in hopes of finding clues to the underlying genetic mechanisms of aging. Although such studies are useful, they are limited in the sense that they display only some of the characteristics of aging. Also, because they are typically due to a single mutant gene, they represent a gross simplification of the aging process. Recent genetic analyses have identified the specific genetic defects for some of the progeroid syndromes, but often this has only led to more questions.

Down syndrome is the most common progeroid syndrome and is usually caused by possession of an extra copy of chromosome 21 (also called trisomy 21). Affected individuals display rapid aging for a number of traits such as atherosclerosis and cataracts, although the severity of the effects varies greatly. The most notable progeroid symptom is the development of Alzheimer's disease-like changes in the brain such as senile plaques and neurofibrillary tangles. One of the genes sometimes involved in

Alzheimer's disease is located on chromosome 21, possibly accounting for the common symptoms.

Werner's syndrome is a very rare autosomal recessive disease. The primary symptoms are severe atherosclerosis and a high incidence of cancer, including some unusual sarcomas and connective tissue cancers. Other degenerative changes include premature graying, muscle atrophy, osteoporosis, cataracts, and calcification of heart valves and soft tissues. Death, usually by atherosclerosis, often occurs by fifty or sixty years of age. The gene responsible for Werner's syndrome has been isolated and encodes a DNA helicase (called WRN DNA helicase), an enzyme that is involved in helping DNA strands to separate during the process of replication. The faulty enzyme is believed to cause the process of replication to stall at the replication fork, the place where DNA replication is actively taking place, which leads to a higher-than-normal mutation rate in the DNA, although more work is needed to be sure of its mechanism.

Hutchinson-Gilford progeria shows even more rapid and pronounced premature aging. Effects begin even in early childhood with balding, loss of subcutaneous fat, and skin wrinkling, especially noticeable in the facial features. Later, bone loss and atherosclerosis appear, and most affected individuals die before the age of twenty-five. The genetic inheritance pattern for Hutchinson-Gilford progeria is still debated, but evidence suggests it may be due to a very rare autosomal dominant gene, which may represent a defect in a DNA repair system.

Cockayne syndrome, another very rare autosomal recessive defect, displays loss of subcutaneous fat, skin photosensitivity (especially to ultraviolet, or UV, light), and neurodegeneration. Age of death can vary but seems to center around forty years of age. The specific genetic defect is known and involves the action of a few different proteins. At the molecular level, the major problems all relate to some aspect of transcription, the making of messenger RNA (mRNA) from the DNA template, which can also affect some aspects of DNA repair.

Another, somewhat less rare, autosomal recessive defect is ataxia telangiectasia. It displays

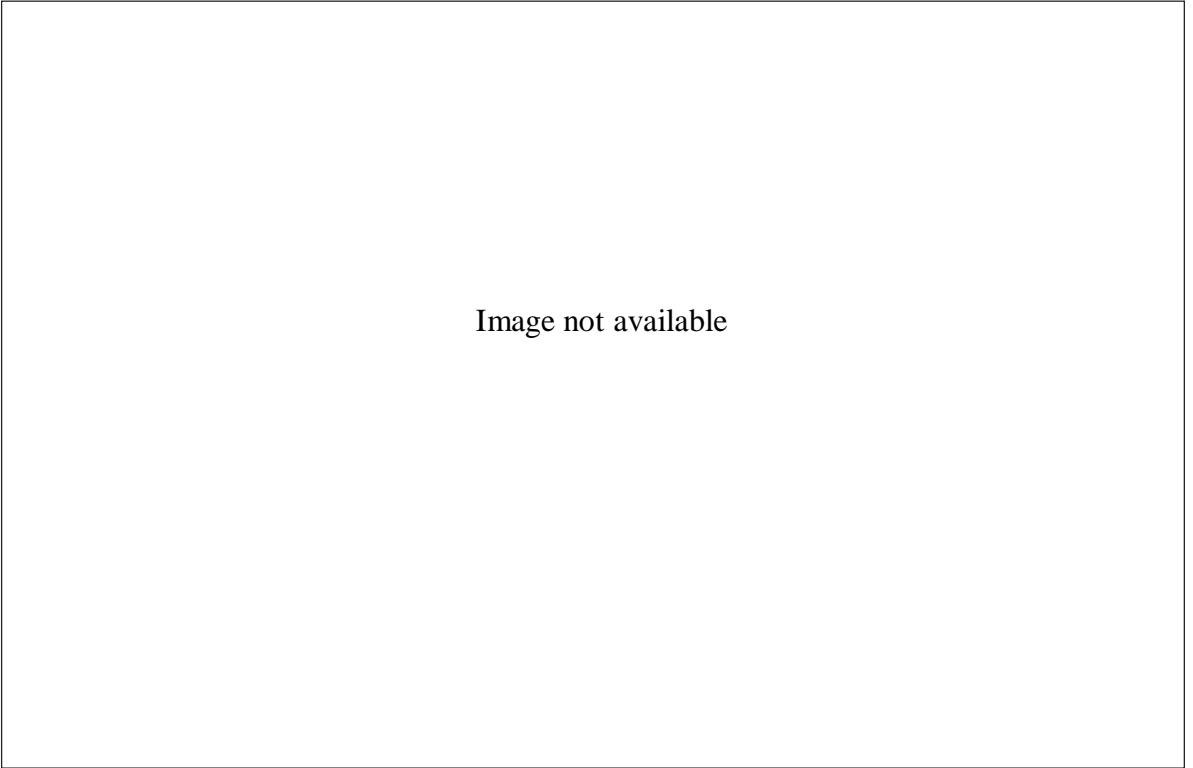


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In April, 2003, fifteen-year-old John Tacket announced the discovery of a gene that causes the disease he suffers from, progeria, a syndrome that accelerates aging. (AP/Wide World Photos)

a whole suite of premature aging symptoms, including neurodegeneration, immunodeficiency, graying, skin wrinkling, and cancers, especially leukemias and lymphomas. Death usually occurs between forty and fifty years of age. The specific defect is known to be loss of a protein kinase, an enzyme that normally adds phosphate groups to other proteins. In this case, the kinase appears to be involved in regulating the cell cycle, and its loss causes shortening of telomeres and defects in the repair of double-stranded breaks in DNA. One of the proteins it appears to normally phosphorylate is *p53*, a tumor-suppressor gene whose loss is often associated with various forms of cancer.

Although the genes involved in the various progeroid syndromes are varied, they do seem to fall into some common functional types. Most have something to do with DNA replication, transcription, or repair. Other genes are involved in control of some part of the cell cycle. Although many other genes remain to be

discovered, they will likely also be involved with DNA or the cell cycle in some way. Based on many of the common symptoms of aging, these findings are not too surprising.

Genetic Models of Aging

The increasing understanding of molecular genetics has prompted biologists to propose a number of models of aging. Each of the models is consistent with some aspect of cellular genetics, but none of the models, as yet, is consistent with all evidence. Some biologists have suggested that a combination of several models may be required to adequately explain the process of aging. In many ways, understanding of the genetic causes of aging is in its infancy, and geneticists are still unable to agree on even the probable number of genes involved in aging. Even the extent to which genes control aging at all has been debated. Early studies based on correlations between time of death of parents and offspring or on the age of death of twins

suggested that genes accounted for 40 to 70 percent of the heritability of longevity. More recent research on twins has suggested that genes may only account for 35 percent or less of the observed variability in longevity, and for twins reared apart the genetic effects appear to be even less.

Genetic theories of aging can be classified as either genome-based or mutation-based. Genome-based theories include the classic idea that longevity is programmed, as well as some evolution-based theories such as antagonistic pleiotropy, first proposed by George C. Williams, and the disposable soma theory. Mutation-based theories are based on the simple concept that genetic systems gradually fall apart from “wear and tear.” The differences among mutation-based theories generally involve the causes of the mutations and the particular genetic systems involved. Even though genome-based and mutation-based theories seem to be distinct, there is actually some overlap. For example, the antagonistic pleiotropy theory (a genome-based theory) predicts that selection will “weed out” lethal mutations whose effects are felt during the reproductive years, but that later in life lethal mutations will accumulate (a mutation-based theory) because selection has no effect after the reproductive years.

Genome-Based Theories of Aging

The oldest genome-based theory of aging, sometimes called programmed senescence, suggested that life span is genetically determined. In other words, cells (and by extrapolation, the entire organism) live for a genetically predetermined length of time. The passing of time is measured by some kind of cellular clock and when the predetermined time is reached, cells go into a self-destruct sequence that eventually causes the death of the organism. Evidence for this model comes from the discovery that animal cells, when grown in culture, are only able to divide a limited number of times, the so-called Hayflick limit discussed above, and then they senesce and eventually die. Further evidence comes from developmental studies where it has been discovered that some cells die spontaneously in a process called apoptosis. A process similar to apoptosis could be responsible

for cell death at old age. The existence of a cellular clock is consistent with the discovery that telomeres shorten as cells age.

In spite of the consistency of the experimental evidence, this model fails on theoretical grounds. Programmed senescence, like any complex biological process, would be required to have evolved by natural selection, but natural selection can only act on traits that are expressed during the reproductive years. Because senescence happens after the reproductive years, it cannot have developed by natural selection. In addition, even if natural selection could have been involved, what advantage would programmed senescence have for a species?

Because of the hurdles presented by natural selection, the preferred alternative genome-based theory is called antagonistic pleiotropy. Genes that increase the chances of survival before and during the reproductive years are detrimental in the postreproductive years. Because natural selection has no effect on genes after reproduction, these detrimental effects are not “weeded” out of the population. There is some physiological support for this in that sex hormones, which are required for reproduction earlier in life, cause negative effects later in life, such as osteoporosis in women and increased cancer risks in both sexes.

The disposable soma theory is similar but is based on a broader physiological base. It has been noted that there is a strong negative correlation among a broad range of species between metabolic rate and longevity. In general, the higher the average metabolic rate, the shorter lived the species. In addition, the need to reproduce usually results in a higher metabolic rate during the reproductive years than in later years. The price for this high early metabolic rate is that systems burn out sooner. This theory is not entirely genome-based, but also has a mutation-based component. Data on mutation rates seem to show a high correlation between high metabolic rate and high mutation rates.

One of the by-products of metabolism is the production of free oxygen radicals, single oxygen atoms with an unpaired electron. These free radicals are highly reactive and not only cause destruction of proteins and other mole-

cules, but also cause mutations in DNA. So the high metabolic rate during the reproductive years causes a high incidence of damaging DNA mutations which lead to many of the diseases of old age. After reproduction, natural selection no longer has use for the body, so it gradually falls apart as the mutations build up. Unfortunately, all attempts so far to assay the extent of the mutations produced have led to the conclusion that not enough mutations exist to be the sole cause of the changes observed in aging.

Mutation-Based Theories of Aging

The basic premise of all the mutation-based theories of aging is that the buildup of mutations eventually leads to senescence and death, the ultimate cause being cancer or the breakdown of a critical system. The major support for these kinds of theories comes from a number of recent studies that have found a larger number of genetic mutations in elderly individuals than in younger individuals, the same pattern being observed even when the same individual is assayed at different ages. The differences among the various mutation-based theories have to do with what causes the mutations and what kinds of DNA are primarily affected. As mentioned above, the disposable soma theory also relies, in part, on mutation-based theories.

The most general mutation-based theory is the somatic mutation/DNA damage theory, which relies on background radiation and other mutagens in the environment as the cause of mutations. Over time, the buildup of these mutations begins to cause failure of critical biochemical pathways and eventually causes death. This theory is consistent with experimental evidence from the irradiation of laboratory animals. Irradiation causes DNA damage, which, if not repaired, leads to mutations. The higher the dose of radiation, the more mutations result. It has also been noted that there is some correlation between the efficiency of DNA repair and life span. Further support comes from observations of individuals with more serious DNA repair deficiencies, such as those affected by xeroderma pigmentosum. Individuals with xeroderma pigmentosum have almost no ability to repair the type of DNA damage caused by exposure to UV light, and as a result they de-

velop skin cancer very easily, which typically leads to death.

The major flaw in this theory is that it predicts that senescence should be a random process, which it is not. A related theory called error catastrophe also predicts that mutations will build up over time, eventually leading to death, but it suffers from the same flaw. Elderly individuals do seem to possess greater amounts of abnormal proteins, but that does not mean that these must be the ultimate cause of death.

The free radical theory of aging is more promising and is probably one of the most familiar theories to the general public. This theory has also received much more attention from researchers. The primary culprit in this theory is free oxygen radicals, which are highly reactive and cause damage to proteins, DNA, and RNA. Free radicals are a natural by-product of many cellular reactions and most specifically of the reactions involved in respiration. In fact, the higher the metabolic rate, the more free radicals will likely be produced. Although this theory also involves a random process, it is a more consistent and predictable process, and through time it can potentially build on itself, causing accelerated DNA damage with greater age.

Significant attention has focused on mitochondrial DNA (mtDNA). Because free radicals are produced in greater abundance in respiration, which takes place primarily in the mitochondria, mtDNA should show more mutations than nuclear DNA. In addition, as DNA damage occurs, the biochemical pathways involved in respiration should become less efficient, which would theoretically lead to even greater numbers of free radicals being produced, which would, in turn, cause more damage. This kind of positive feedback cycle would eventually reach a point where the cells could not produce enough energy to meet their needs and they would senesce. Assays of mtDNA have shown a greater number of mutations in the elderly, and it is a well-known phenomenon that mitochondria are less efficient in the elderly. Muscle weakness is one of the symptoms of these changes.

The free radical theory has some appeal, in the sense that ingestion of increased amounts

of antioxidants in the diet would be expected to reduce the number of free radicals and thus potentially delay aging. Although antioxidants have been used in this way for some time, no significant increase in life span has been observed, although it does appear that cancer incidence may be reduced.

From Theory to Practice

Many of the genetic theories of aging are intriguing and even seem to be consistent with experimental evidence from many sources, but none of them adequately addresses longevity at the organismal level. Although telomeres shorten with age in individual cells, cells continue to divide into old age, and humans do not seem to die because all, or most, of their cells are no longer able to divide. Cells from older individuals do have more mutations than cells from younger individuals, but the number of mutations observed does not seem adequate to account for the large suite of problems present in old age. Mitochondria, on average, do function more poorly in older individuals and their mtDNA does display a larger number of mutations, but many mitochondria remain high functioning and appear to be adequate to sustain life.

Essentially, geneticists have opened a crack in the door to a better understanding of the causes of aging, and the theories presented here are probably correct in part, but much more research is needed to sharpen the understanding of this process. The hope of geneticists, and of society in general, is to learn how to increase longevity. Presently, it seems all that is possible is to help a larger number of people approach the practical limit of 120 years through lifestyle modification and medical intervention. Going significantly beyond 120 years is probably a genetic problem that will not be solved for some time.

—Bryan Ness

See also: Alzheimer's Disease; Autoimmune Disorders; Biochemical Mutations; Biological Clocks; Biological Determinism; Cancer; Chemical Mutagens; Developmental Genetics; Diabetes; DNA Repair; Genetic Engineering; Medical Applications; Heart Disease; Human Genetics; Human Growth Hormone; Immunogenetics;

Insurance; Mitochondrial Genes; Mutation and Mutagenesis; Oncogenes; Stem Cells; Telomeres; Tumor-Suppressor Genes.

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Web Sites of Interest

Alliance for Aging Research. <http://www.agingresearch.org>. Provides information on genetics and the aging process, including how the Human Genome Project will affect the future of health and health care.

American Geriatrics Society. <http://www.americageriatrics.org>. The national society for health care providers for older persons, posting information on genetic screening for such disorders as Alzheimer's disease.

Centagenetix. <http://www.centagenetix.com>. This group's mission is to support better understanding of the aging process and associated diseases; the site offers a scientific overview, media center, and information on related careers.

National Institute on Aging. <http://www.nia.nih.gov>. Supports research programs on the biology and genetics of aging, as well as information on aging for the public.

Albinism

Field of study: Diseases and syndromes

Significance: *Albinism—the absence of pigment such as melanin in eyes, skin, hair, scales, or feathers—is a direct result of decreased or nonexistent pigmentation of the skin, hair, and eyes. Albino*

humans are susceptible to sunburns and skin cancer, while albino animals lack the ability to adjust to environments in which nonalbino animals thrive.

Key terms

MELANISM: the opposite of albinism, a condition that leads to the overproduction of melanin

PHOTOPHOBIA: a condition, often observed in albinos, in which sunlight is painful to the eyes

PIEBALDISM: a condition involving the patchy absence of skin pigment seen in partial albinos

Occurrence and Symptoms

Tyrosine, an amino acid, is normally converted by the body to a variety of pigments called melanins, which give an organism its characteristic colors in areas such as the skin, hair, and eyes. Albinism results when the body is unable to produce melanin because of defects in the metabolism of tyrosine. Those with albinism can be divided into two subgroups: tyrosinase-negative (those who lack the enzyme tyrosinase) and tyrosinase-positive (those in whom tyrosinase is present but inactive). The most serious case is that of complete albinism or tyrosinase-negative oculocutaneous albinism, in which there is a total absence of pigment. People with this condition have white hair, colorless skin, red irises, and serious vision defects. The red irises are caused by the lack of pigmentation in the retina and subsequent light reflection from the blood present in the retina. These people also display rapid eye movements (nystagmus) and suffer from photophobia, decreased visual acuity, and, in the long run, functional blindness. People with this disorder sunburn easily, since their skin does not tan. Partial albinos have a condition known as piebaldism, characterized by the patchy absence of skin pigment in places such as the hair, the forehead, the elbows, and the knees.

Several complex diseases are associated with albinism. Waardenberg syndrome is identified by the presence of a white forelock (a lock of hair that grows on the forehead) or the absence of pigment in one or both irises, Chediak-

Higashi syndrome is characterized by a partial lack of pigmentation of the skin, and tuberous sclerosis patients have only small, localized depigmented areas. A more serious case is the Hermansky-Pudlak syndrome, a disorder that includes bleeding.

Ocular albinism is inherited and involves the lack of melanin only in the eye while the rest of the body shows normal or near-normal coloration. The condition reduces visual acuity from 20/60 to 20/400, with African Americans occasionally showing acuity as good as 20/25. Other problems include strabismus (crossed eyes or “lazy eye”), sensitivity to brightness, and nystagmus. The color of the iris may be any of the normal colors, but an optician can easily detect the condition by shining a light from the side of the eye. In ocular albinos, the light shines through the iris because of the absence of the light-absorbing pigment. Children with this condition have difficulty reading what is on a blackboard unless they are very close to it. Surgery and the application of optical aids appear to have had positive results in correcting such problems.

Albinism has long been studied in humans and captive animals. It has also been detected in wild animals, but such animals often have little chance of survival because they cannot develop normal camouflage colors, important for protection from predators. Animals in which albinism has been recorded include deer, giraffes, squirrels, frogs, parrots, robins, turtles, trout, and lobsters. Partial albinism has also been reported in wildlife. In other cases, such as the black panther of Asia, too much melanin is formed and the disorder is called melanism.

Albinism has also been observed in plants, but their life span rarely goes beyond seedline state, because without the green pigment chlorophyll, they cannot obtain energy using photosynthesis. A few species of plants, such as Indian pipes (*Monotropa*), are normally albino and obtain their energy and nutrition from decaying material in the soil.

Impact and Applications

Albinism appears in various forms and may be passed to offspring through autosomal recessive, autosomal dominant, or X-linked modes of inheritance. In the autosomal recessive case, both parents of a child with autosomal recessive albinism are carriers; that is, they each have one copy of the recessive form of the gene and are therefore not albino themselves. When both parents are carriers, there is a one-in-four chance that the child will inherit the condition. On the other hand, X-linked albinism occurs almost exclusively in males, and mothers who carry the gene will pass it on 50 percent of the time.

Albinism has not been found to affect expected life span among humans but can affect lifestyle. Treatment of the disease involves reduction of the discomfort the sun creates. Thus photophobia may be relieved by sunglasses that filter ultraviolet light, while sunburn may be re-

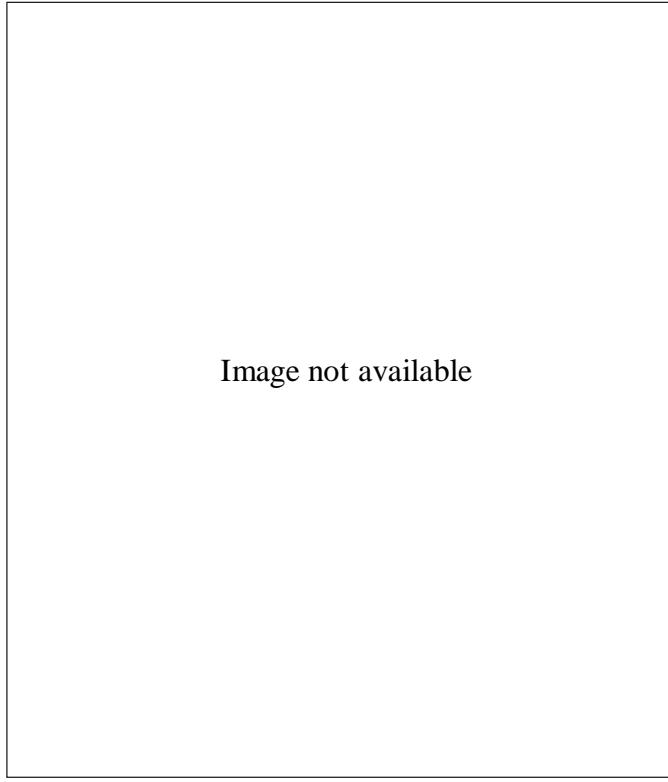


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At the Santa Lucia school in Guatemala City, an albino girl, Maria del Carmen Quel, eats a snack as she plays on a swing. Albinism is frequently associated with blindness. (AP/Wide World Photos)

duced by the use of sun protection factor (SPF) sunscreens and by covering the skin with clothing. Since albinism is basically an inherited condition, genetic counseling is of great value to individuals with a family history of albinism.

—Soraya Ghayourmanesh

See also: Biochemical Mutations; Complete Dominance; Dihybrid Inheritance; Inborn Errors of Metabolism; Monohybrid Inheritance.

Further Reading

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Web Sites of Interest

International Albinism Center, University of Minnesota. <http://www.cbc.umn.edu/iac>. Run by a team of research professionals with a variety of specialties in human albinism—clinical genetics, molecular biology, ophthalmology, dermatology, and biochemistry—who are attempting to understand the cause and effect of albinism and other forms of pigment loss. Papers, fact sheets, glossary, other resources. Links to the Albinism Database, which lists mutations associated with albinism.

National Organization for Albinism and Hypopigmentation. <http://www.albinism.org>. A volunteer organization for albinos and for those who care for people with albinism, providing resources for self-help and promoting research and education.

Alcoholism

Field of study: Diseases and syndromes

Significance: *Alcohol is one of the most widely consumed substances of abuse worldwide. Because alcohol dependence can be life-threatening, its potential genetic basis is of great interest, and a variety of genes have been found linked to its physiologic markers and to the diagnosis of alcohol dependence.*

Key terms

ALCOHOL DEPENDENCE: a medical diagnosis given when there is repeated use of alcohol over the course of at least a year, despite the presence of negative consequences, such as tolerance, withdrawal, uncontrolled use, unsuccessful efforts to quit, considerable time spent getting or using the drug, and a decrease in other important activities

CIRRHOSIS: a disease of the liver, marked by the development of scar tissue that interferes with organ functioning, that can result from chronic alcohol consumption

FETAL ALCOHOL SYNDROME: a medical condition resulting from alcohol use by a mother while pregnant, usually evidenced by facial abnormalities and mental impairments in the child and sometimes resulting in fetal death

Defining Alcoholism

“Alcoholism” is a word that is used to convey that a person is experiencing serious problems related to the use of alcohol. The technical diagnosis of alcohol dependence with physiological dependence is the diagnosis that corresponds to the notion of alcoholism. That diagnosis, formally explained in the *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR*, or *DSM* (rev. 4th ed., 2000), issued by the American Psychiatric Association, refers to physical and psychological reliance on alcohol, despite the presence of problems associated with its use.

Alcohol-related problems are typically studied and tracked by epidemiologists, physicians, psychologists, public health professionals, and basic scientists. Basic scientists tend to observe the heritability of problem acquisition and expression in lab animals, while the other professionals tend to track the problem in humans via clinical observations and research involving reports of family histories or the review of medical records.

Due to changes in how the *DSM* has developed its problem definitions over time, researchers must use care to examine how definitions of alcoholism have changed over time. Also, the social stigma associated with alcoholism may have caused over- and underrecognition of the problem in some groups. For instance, more men are recognized to have alcoholism than women. Alcoholism is also known to have its strongest genetic findings for men of alcoholic fathers. However, only about 50 percent of male cases are explained by genetics; thus, there is also a strong environmental component to this problem.

Regarding women, however, recent evidence suggests that a family history of mood disorders,

such as depression, may also be linked to how this disorder may be inherited. It may be that the heritability of depression and alcohol have something in common, that there are differences in heritability by gender, or perhaps that symptom expression by clients or recognition by professionals varies by gender as a result of other factors. Stigma, for instance, may be relevant; historically, women with alcohol problems have often been misdiagnosed with depression.

Symptom expression of alcohol problems may also differ by culture and ethnicity, because people of different cultures vary in terms of how they express physical and mental ailments. Different ethnic and racial groups may have different biological responses to therapeutic drugs and drugs such as alcohol. Some groups may even enjoy greater protection against alcoholism as a result of their genetics. Asians, for example, tend to be unable to tolerate alcohol because they generally lack an enzyme to process it out of the body. In contrast, there may be differential vulnerability to alcoholism itself, as well as differential vulnerabilities to certain types of organ damage related to alcoholism. For instance, vulnerability to cirrhosis, cardiomyopathy, pancreatitis, and Wernicke-Korsakoff’s syndrome also might be heritable and may vary by ethnicity. Latino men, for example, tend to show greater susceptibility to alcohol-related liver damage than do white men.

There is also the issue of early alcohol exposure and how such early exposure can interact with genetics to cause problems in development. Fetal alcohol syndrome, for example, can result in a child’s having mild to severe facial and dental abnormalities, mental impairments, or problems related to the skeletal and the cardiovascular systems. Problems with vision, hearing, and attention are also common. Children of alcoholic fathers also can have difficulties in learning, language, and temperament. Causes of such problems are multiple, including the contributions made by the individual’s genes as well as the environmental effects of growing up in a home that may be unstable as a result of problems in the father. In sum, parents who drink may increase the likelihood that their children will develop alcohol-

ism both through genes and through nongenetic environmental circumstances.

Alcohol Research

To date, some important physiological markers linked to alcoholism have included event-related potentials (ERPs) in electroencephalographic performance (EEGs), frontal lobe functioning, enzymes responsible for hepatic alcohol metabolism (such as alcohol dehydrogenase and aldehyde dehydrogenase), and inhibitory receptors such as gamma-aminobutyric acid (GABA) receptors. There are a variety of genes linked to such physiologic markers and to the diagnosis of alcohol dependence, including *ADH2*2*, *ADH3*1*, *ALDH2*2*, *CYP 2E1*, *GABRA6*, *GABRA1*, *COMT*, *DRD4*, *DRD2*, and *D2*.

Future Directions

The presence of alcohol in modern life may have genetic roots. Historically, it helped those who could tolerate its taste and effects to survive and be selected for when others who could not do so perished as a result of consuming contaminated water. Alcohol has a complex relationship to human life, and alcoholism will be studied for some time. Continued study of the genes associated with different patterns of alcohol problems, protective genetic effects in populations with exceptionally low rates of alcoholism, and genetically based interventions (such as matching pharmacotherapies to different populations of individuals to forestall the development of the problem) are assured. The study of genetics and alcoholism is also likely to encourage growth in the field of ethnopharmacology, the study of how different therapeutic drugs differentially affect members of specific ethnic groups.

—Nancy A. Piotrowski

See also: Aggression; Behavior; Congenital Defects; Criminality; Eugenics; Genetic Testing; Ethical and Economic Issues; Hereditary Diseases; Thalidomide and Other Teratogens.

Further Reading

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Web Site of Interest

National Institute on Alcohol Abuse and Alcoholism, ETOH. <http://etoh.niaaa.nih.gov>. ETOH is the chemical abbreviation for ethyl alcohol. This site includes reports related to alcohol dependence, including epidemiology, etiology, prevention, policy, and treatment.

Allergies

Field of study: Immunogenetics

Significance: *In economically developed countries, allergies are responsible for a large portion of illnesses and medical expenses. Many allergies have genetic components and thus tend to “run” in families; the identification of such hereditary factors can help in diagnosis and in family planning. Moreover, research into the causes of allergies may lead to a more precise understanding of how the immune system functions. This may lead ultimately to the development of better drugs to treat allergies.*

Key terms

ANTIBODY: a protein made by the body in response to an antigen; antibodies or immunoglobulins are specific for each antigen

ANTIGEN: any substance that, when injected into the body, causes antibody formation that reacts specifically to that substance; also known as an allergen or an immunogen

HYPERSensitivity: an exaggerated response of the immune system to an antigen beyond what is considered “normal”; a synonym for allergy

IMMUNE SYSTEM: the defense mechanism of the body against foreign matter (bacteria, viruses, and parasites); it is composed of different types of cells and chemical substances

The Basic Information About Allergies

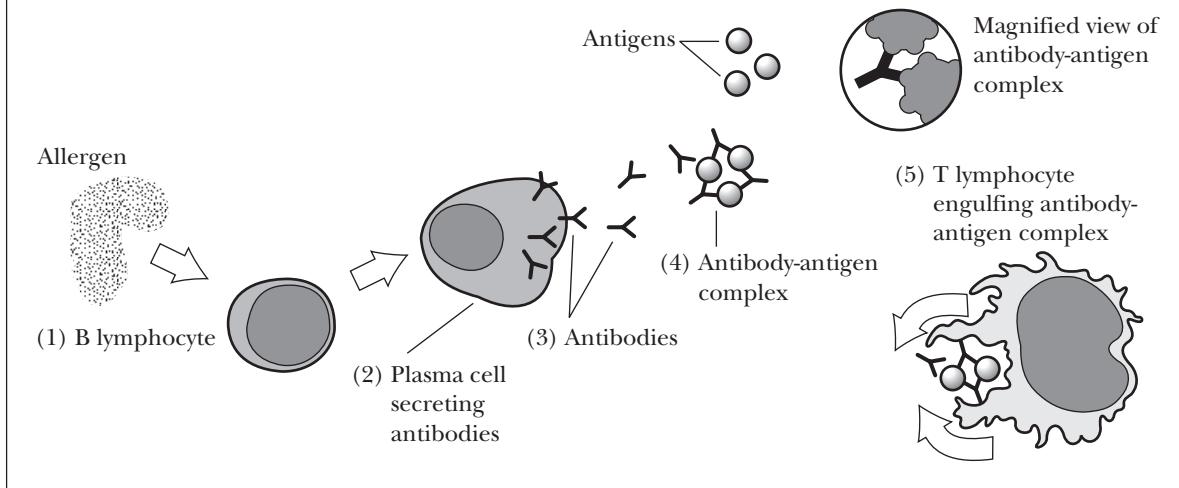
Sneezing, sniffling, and wheezing are the symptoms most often associated with allergies. Allergies, or hypersensitivities, are the human body's exaggerated response to a foreign substance such as pollen. Hypersensitivity reactions can be immediate (hay fever) or delayed (contact dermatitis—for example, a reaction to latex or poison ivy) depending upon the body's immune reaction to the antigen.

Essentially, there are three stages of an allergic reaction. The first stage causes no symptoms. It is the immune system's initial contact with the antigen. The cells of the immune system react to the antigen by producing IgE antibodies that attach to mast cells and eosinophils (two cell types of the immune system) that are circulating in the blood. When the same antigen is encountered a second time and attaches to two adjacent IgE antibodies on a mast cell, the mast cell is said to be "activated." During this second stage, the mast cell releases chemi-

cal substances (such as histamines, prostaglandins, and leukotrienes) that are responsible for many of the common allergic symptoms. The third and final stage of an allergic reaction is the prolonged immune activity caused by the chemical substances released by cells of the immune system. This prolonged or late-phase reaction can cause the immune system to continue to react and cause tissue damage.

Based on varying responses to antigens, researchers Peter Gell and Robert Coombs have classified allergies into four types: I (anaphylaxis), II (cytotoxic), III (immune complex), and IV (cell-mediated). Type I hypersensitivity—anaphylaxis, from the Greek *ana* (against) and *phylaxis* (protection), or "the opposite of protected"—can be further divided into either systemic or local response. Systemic anaphylaxis is the whole body's response to an antigen such as a bee sting. Because of the amount of chemical substances released by the cells of the immune system, the body reacts immediately by a drop in blood pressure (leading to shock), difficulty in breathing, and swelling of the airways. If not treated immediately, anaphylactic shock can be fatal. Localized anaphylactic reactions (atopy) are the most familiar of the hypersensi-

The Body's Response to Allergens



An allergic reaction is caused when foreign materials, or antigens, enter the immune system, which produces B lymphocytes (1) that cause blood plasma cells to secrete antibodies (2). The antibodies (3) link with antigens to form antibody-antigen complexes (4), which then are engulfed and destroyed by a T lymphocyte (5). (Hans & Cassidy, Inc.)

tivities. The symptoms are dependent upon the route the antigen uses to enter the body. For airborne antigens such as house dust, pollens, and animal dander, symptoms may include hay fever (itchy eyes, runny nose, sneezing, and coughing) or bronchial asthma (wheezing, coughing, and difficulty breathing). Other atopic symptoms may include hives, itchy skin, and diarrhea. Food allergies are also examples of an atopic reaction.

Type II (cytotoxic) hypersensitivity reaction involves the binding of an antigen and antibody complex to a cell that destroys the target cell. Examples of this type of hypersensitivity are incompatible blood groups (giving type B blood to a person who has type A blood), hemolytic anemia (destruction of red blood cells), and hemolytic disease of a newborn (the mother produces antibodies against the fetus based on a protein found in the blood).

Type III (immune complex) hypersensitivity reaction involves the depositing of immune complexes (an antigen bound to an antibody) on the walls of blood vessels, causing inflammation and tissue damage. Glomerulonephritis, inflammation of the blood vessels in the kidneys, is a type III hypersensitivity reaction. This disease is believed to be a reaction to a particular bacterial infection.

Contact dermatitis (for example, a reaction to poison ivy, latex, cosmetics, or jewelry) is a common example of the last category, type IV (cell-mediated) hypersensitivity. T cells (a cell type of the immune system) initially react with the antigen; upon a second exposure to the antigen, clones of the same T cell release chemical factors that cause a reaction to the antigen. This release of chemicals results in a red, itchy rash or hives. The reaction to *Mycobacterium tuberculosis* (the bacteria that causes tuberculosis) is a type IV hypersensitivity. The immune system has also been known to attack itself and cause disease. These disorders (autoimmune disorders), such as multiple sclerosis, juvenile diabetes, and systemic lupus erythematosus, are only beginning to be understood.

Impact and Applications

Treatment of allergies may include avoidance of the antigen, use of antihistamines

(drugs that block the release of histamine from mast cells) and anti-inflammatories (steroids), and desensitization (allergy shots). Efforts by scientists to learn how the immune system functions and why it overreacts to antigens will lead to the development of better, less toxic drugs to combat allergies and their symptoms.

—Mary Beth Ridenhour

See also: Autoimmune Disorders; Genetic Engineering: Risks; Immunogenetics; Synthetic Antibodies.

Further Reading

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Walsh, William. *The Food Allergy Book*. New York: J. Wiley, 2000. In this excellent guide to one prevalent form of allergy, the author presents useful background information on food allergies and a pragmatic guide to identifying and eliminating food allergens from your diet.

Web Sites of Interest

Allergic Diseases Resource Center. <http://www.worldallergy.org/allergicdiseasecenter.shtml>. Part of the World Allergy Organization, this site provides scientific and medical information on allergic diseases, research updates, and more.

National Institute of Allergy and Infectious Diseases. <http://www.niaid.nih.gov/default.htm>. An arm of the National Institutes of Health,

linking to reports and information on immunology, allergies, gene therapy, HIV/AIDS, and more.

Altruism

Field of study: Population genetics

Significance: *In a strictly Darwinian system, actions that reduce the success of individual reproduction should be selected against; however, altruism, which occurs at a cost to the altruist, is observed regularly in natural populations. This paradox may be resolved if the cost of altruism is offset by the reproductive success of relatives with which altruists share genes. Kin selection results in selection for altruistic behaviors which, if directed at relatives, preserve inclusive reproductive success, and thus Darwinian fitness.*

Key terms

ALTRUISM: behavior that benefits others at the evolutionary (reproductive) cost of the altruist

EVOLUTION: a change in the frequency of alleles resulting from the differential reproduction of individuals

HAPLODIPLOIDY: a system of sex determination in which males are haploid (developing from unfertilized eggs) and females are diploid

INCLUSIVE FITNESS: an individual's total genetic contribution to future generations, comprising both direct fitness, which results from individual reproduction, and indirect fitness, which results from the reproduction of close relatives

KIN SELECTION: an evolutionary mechanism manifest in selection for behaviors that increase the inclusive fitness of altruists

MATERNAL ALTRUISM: altruism on the part of mothers toward offspring as well as between and among members of groups comprising closely related females

NATURAL SELECTION: a process whereby environmental factors influence the survival and reproductive success of individuals; natural selection leads to genetic changes in populations over time

RECIPROCAL ALTRUISM: mutual exchange of altruistic acts typically associated with highly cohesive social groups

Reproductive Success = Survival

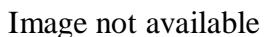
If evolutionary outcomes in a Darwinian world are described as natural economies, then individual reproduction is the currency of these economies and of natural selection. Given both naturally occurring genetic variation among individuals and a certain environmental dynamic, it follows that some individuals will be better adapted to locally changing environments than others. Such differential adaptation is expressed as a difference in the frequency with which individual genes pass into future generations. This simple scenario fulfills the genetic definition of evolution—change in allele frequencies in natural populations—by explaining environmental influences on these changes. Note that this argument emphasizes, as its central postulate, the importance of individual reproduction rather than simple survival. Survival of the fittest is therefore more properly viewed as the differential propagation of genes.

A challenge to such a scenario is the paradox of altruism. Altruism is defined as any behavior that benefits another at a cost to the altruist. Charles Darwin himself suggested that this problem was a “special difficulty . . . which at first appeared . . . insuperable, and actually fatal to [the] whole theory” of natural selection. The individual who pushes siblings from the track as he himself is killed by the rushing locomotive is an altruist; the colony sentinel that issues an alarm call to her cohort to take cover, despite the risk of drawing the attention of an approaching predator, is also acting altruistically. These behaviors make no sense in Darwin’s economy, since they appear to decrease the likelihood of individual reproduction—unless, as W. D. Hamilton suggested, Darwinian success is not limited to the success of individual bodies harboring particular genes but may be extended to include the reproductive success of relatives who share genes with the altruist. Hamilton defined inclusive fitness as the sum of an individual’s own fitness plus the influence that individual has on the fitness of rel-

atives. Kin selection is the evolutionary mechanism that selects for behaviors that increase the inclusive fitness of altruists. Even though there are potential costs to altruistic behavior, the evolutionary economy of an altruist operates in the black because actors profit (beyond associated costs) by helping others who share their genes. The bottom line is that altruists increase their inclusive fitness through the reproduction of others.

Evidence of Kin Selection

One of the best evidences for kin selection is the social structure of certain groups of insects, including the *Hymenoptera* (ants, bees, and wasps). A unique system of sex determination (haplodiploidy) in which females are diploid and males are haploid predisposes some group members to behave altruistically. In certain bees, for example, the queen is diploid and fertile. Worker bees are female, diploid, and sterile. Drones are male, developed from unfertilized eggs, and haploid. Such a situation makes for unusual patterns of genetic relationship among hive members. In diploid systems the genetic relation between parents and offspring and among offspring is symmetrical. Offspring receive half of their genetic complement from their mother and half from the father; sons and daughters are related to each parent by $\frac{1}{2}$ and sibs (siblings) are related to each other by $\frac{1}{2}$. In the haplodiploid system such genetic relationships are asymmetric. Drones are haploid and receive half of the queen’s genome. Workers are diploid and share 100 percent of their paternal genes and, on average, half of their maternal genes with their sisters. Sisters are therefore related to each other by $\frac{3}{4}$. Because sisters and their brothers share no paternal genes, and on average half of their maternal genes, they are related to drones by only $\frac{1}{4}$. In this economy it makes sense that workers should act altruistically to assist the queen in the production of sisters. What would appear to be purely altruistic acts, on the part of workers, result in greater inclusive success than if the workers had reproduced themselves. In contrast, drones contribute little to community welfare and serve only to fertilize the queen. Note that in this system there is no conscious

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The altruistic behaviors of honeybees and some other animal species may be a result of selection for behaviors that place the group, rather than the individual, at a reproductive advantage. (AP/Wide World Photos)

decision on the part of workers not to reproduce; their sterility is an inherent part of this unusual system of sex determination.

A Test of Predictions

One prediction made by the kind of kin selection described above is that, assuming the queen produces male and female offspring in equal proportion, female workers should invest three times the energy in caring for sisters that they do for brothers. Because queens are related to both male and female offspring equally, one would predict that eggs are equally divided between the sexes. Because workers are related to their sisters by $\frac{3}{4}$ and to their brothers by $\frac{1}{4}$, one would predict that they should invest three times the energy in care of eggs eventually yielding sisters that they do in the care of eggs eventually yielding brothers. Remarkably, it has been shown that certain worker ants are able to identify and then selectively care for eggs containing sisters. Kin recognition has also been studied in the house mouse, *Mus*

musculus domesticus, and in some cases individuals can distinguish full sibs from half sibs on the basis of their major histocompatibility complexes (glycoproteins important in immune system function). The specific MHC type is fairly unique for each mouse, but related individuals will have similar patterns and share some specific MHC glycoproteins. MHC glycoproteins are found in mouse urine, and individuals can distinguish these molecules by smell. Consistent with the foregoing hypothesis, the degree of female altruism toward the offspring of close relatives was predicted by the degree of relation based on MHC type and type recognition.

Maternal Altruism

Altruism may be observed in a variety of natural systems in which groups comprise individuals who share a high degree of genetic relatedness. A classic example of this sort occurs with Belding's ground squirrels. Males tend to disperse from colonies, while females remain to

create highly related maternal groups. Members of such maternal groups demonstrate altruistic behaviors such as alarm calling to warn relatives of danger. Although truly altruistic in the sense that alarm callers may incur risk of personal injury or death, they can be reasonably assured of breaking even in this economy as long as their genes live on in the bodies of those they have saved by their actions.

Reciprocal Altruism

It would seem that altruism based on Hamilton's argument of inclusive fitness would be precluded by human social organization. Scientists have predicted, however, that reciprocal altruism should exist in systems characterized by a high frequency of interaction among member individuals and life spans long enough to allow the recipients of altruistic acts to repay altruists. Note that the theoretical basis for the existence of reciprocal altruism differs from that for kin selection, and that any system in which evidence for reciprocity is found must necessarily include the development of a complex web of sophisticated social interaction. Such systems would be expected to foster traits expressing the panoply of human emotion and the development of certain moral architectures and group cohesion.

—David A. Smith

See also: Behavior; Evolutionary Biology; Homosexuality; Natural Selection; Population Genetics; Sociobiology.

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Alzheimer's Disease

Field of study: Diseases and syndromes

Significance: *Alzheimer's disease (AD) is the most common cause of irreversible dementia and accounts for approximately two-thirds of all dementia cases in the United States.*

Key terms

AMYLOID PLAQUES: plaques formed by protein fragments from amyloid precursor proteins

BETA-AMYLOID PEPTIDE: the main constituent of the neuritic plaques in the brains of Alzheimer's patients

DETERMINISTIC MUTATIONS: gene mutations associated with high risk for developing Alzheimer's

FAMILIAL ALZHEIMER'S DISEASE (FAD): inherited Alzheimer's disease

HIPPOCAMPUS: the area in the brain that encodes memory

NEUROFIBRILLARY TANGLES: abnormally twisted tau protein threads that lead to the death of brain cells

TAU PROTEIN: threads of protein in the cells of the brain that stabilize the brain's support structure

The Extent of AD

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes a gradual, irreversible, decline in memory, language, visual-spatial perceptions, and judgment which are all the result of amyloid plaques, neurofibrillary tangles, and neuronal loss. Approximately four million Americans suffer with AD, a number that is expected to increase to almost 6 million by 2020. According to the Alzheimer's Association, 14 million Americans will be diagnosed with AD by 2050 if a cure is not found. Annually, \$33 billion is lost by American businesses as a result of AD. At an annual cost of \$100 billion, AD is the third most costly disease

in the United States. Worldwide, the World Health Organization (WHO) estimates that by 2050, more than 22 million individuals worldwide will have developed AD, and some estimates are larger. AD currently accounts for between 50 and 75 percent of all dementias. Its prevalence increases from 1 percent at the age of sixty-five years to 20-35 percent by the age of eighty-five years. The average life span for AD sufferers ranges from eight to twenty years following diagnosis. As AD progresses, individuals become less and less able to perform activities of daily living because of progressive cognitive and social declines. AD will reach epidemic proportions as the human life span continues to increase. Understanding the genetic risks for developing diagnostics to identify AD and early intervention to treat AD will positively impact the quality of life of individuals who suffer with the disease.

Historical Perspectives on AD

Greeks and Romans first described symptoms of AD in their writings about dementias in old age. In 1906, a German physician, Dr. Alois

Alzheimer, described plaques and neurofibrillary tangles in the brain of a mentally disturbed woman and identified them as a component in a type of acceleration in aging. Today, these plaques and tangles in the brain are hallmarks of AD that are identified on autopsy and are the only means to definitively diagnose AD. Early-onset AD was originally referred to as presenile dementia because it occurred in individuals younger than sixty or sixty-five years of age. Late-onset AD was referred to as senile dementia because it occurred in individuals older than eighty or eight-five years of age. Until recently, AD was considered a normal consequence of aging.

Genes Associated with AD

AD is not a normal part of aging. Over the past several years, scientists have discovered genetic links to two main types of AD. In the late 1980's scientists discovered amyloid precursor protein (APP). Alpha-, beta-, and gamma-secretase enzymes hang onto APP. The beta and gamma enzymes produce a sticky protein called beta-amyloid (A-beta). A-beta builds up

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Two images of mouse-brain tissue, one (left) engineered to produce the dark protein deposits that characterize Alzheimer's disease, and the other normal. (AP/Wide World Photos)

in the fluid surrounding the neurons and is responsible for the formation of amyloid plaques in AD.

Early-onset AD is caused by mutations in *APP* that cause abnormal proteins to form: presenilin 1 and presenilin 2. *APP* mutations cause amyloid plaque to develop in the hippocampus. *APP* mutations on chromosome 21 were the first gene mutations to be described in association with early-onset AD. *APP* mutations on chromosome 14 also produce presenilin 1. *APP* mutations on chromosome 1 produce presenilin 2. Individuals with these deterministic mutations will develop early-onset AD. Early-onset AD is rare and usually affects individuals thirty to sixty years of age. Most cases of early-onset AD are inherited and are called familial AD (FAD).

Another type, late-onset AD, is the most common form of AD, occurring in individuals who are sixty-five years of age and older. Late-onset AD is caused by mutations in apolipoprotein E (ApoE). ApoE is the most common genetic risk factor for developing AD. Scientists believe that these mutations allow longer isoforms of the neurotoxic A-peptide, which lead to the death of neurons. ApoE binds to beta-amyloid. The gene that produces ApoE is in the region of chromosome 19. There are at least three forms: *e2* allele, *e3* allele, and *e4* allele. ApoE *e2* allele is rare and develops later in life. It may also protect individuals against AD. ApoE *e3* allele is the most common allele. According to researchers it appears to be neutral in AD. ApoE *e4* allele occurs in approximately 40 percent of individuals who develop late-onset AD. Individuals with ApoE *e4* allele may develop AD even if there is no family history of AD. Scientists believe that protein products from ApoE *e4* allele bind to APP and form plaques.

Chromosome 10 has also been identified as possibly containing genes that increase the risk of developing AD. Though it is still not certain whether beta-amyloid plaques cause AD or are a by-product of AD, the formation of beta-amyloid from APP is a key process in AD.

Information has been released by the National Institutes of Health (NIH) that reported that an alteration in brain-derived neurotrophic factor (BDNF) affected memory, influenced

the activation of the hippocampus, and decreased the interconnection in neurons and neuron health in humans.

Risk Factors for AD

Advancing age and heredity are the most important risk factors for the development of AD. While it is known that mutations in *APP* and presenilin 1 and presenilin 2 genes cause early-onset AD, causes of late-onset AD are not as clear-cut. Some scientists hypothesize that late-onset AD may be initiated by inefficient processing of APP or by enhanced degradation of the tau protein. Individuals with ApoE *e4* allele have a two to four times greater risk for developing AD than those without it. Still, only 30-40 percent of ApoE *e4* allele carriers develop AD. Infectious agents, environmental toxins, and metabolic errors that have not yet been identified may also be possible causes of late-onset AD.

Aside from genetic risks involved in the development of AD, other factors that increase the likelihood for developing AD include traumatic brain injury and lower socioeconomic status, being overweight, lower educational level, sedentary lifestyle, depression, elevated blood cholesterol levels, and vascular diseases such as hypertension, coronary artery disease, atrial fibrillation, and myocardial infarction. Females are also at greater risk for developing AD.

Implications and Interventions

Early diagnosis of AD is essential to ensure that proper treatment and early detection of other underlying diseases such as depression, drug interactions, vitamin deficiencies, or endocrinologic problems are ruled out. The Risk Evaluation and Education for Alzheimer's Disease (REVEAL) study investigated the impact of identifying individuals with the ApoE genotype. The ApoE genotype is the most powerful genetic risk factor for AD and may be instrumental in predicting the chance of developing AD. This study offers guidance for using genetic risk information to screen, evaluate, and educate families with relatives suffering from AD.

The National Institutes for Health Alzheimer's Disease Prevention Initiative (NIHADPI) was organized to identify factors that will assist

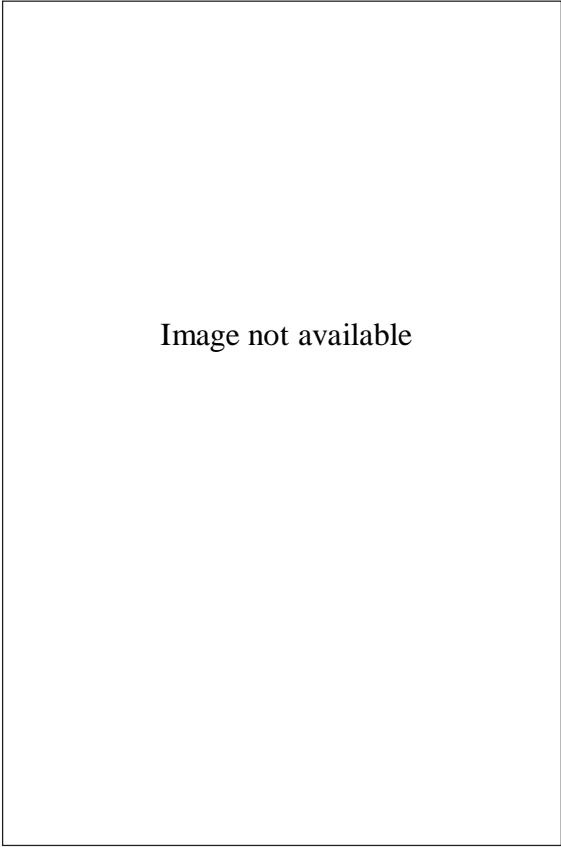


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Etsuro Uemura, a professor of veterinary science who has been working on Alzheimer's disease since the early 1970's, before the disease was named, displays tissue cultures from rat brains that he has been using in his research. (AP/Wide World Photos)

in the early diagnosis of AD and in investigating pharmacological interventions that may assist in delaying or abating the development of AD. NIHADPI investigations include biological and epidemiological research, instrument development to identify high-risk individuals, clinical trials, and alternate strategies to treat behavioral disturbances in AD patients.

Clinical trials have identified positive effects from anti-inflammatory medications, statin medications, vitamin supplements, and diet in decreasing the risk for developing AD. Pharmaceutical agents such as acetylcholinesterase inhibitors have helped to slow the progression of AD by blocking the breakdown of neurotransmitters in the brain and to lessen symptoms of mild to moderate AD. Other studies

have shown that estrogen decreases the risk for women to develop AD by 30 to 40 percent. Its antioxidant and anti-inflammatory effects enhance neuron growth and therefore memory function. However, results from controlled trials on the effects of hormone replacement therapy, conducted by the Women's Health Initiative and reported in 2003, did not yield consistent findings of any beneficial effect of estrogen on cognitive function of women with AD, and these findings combined with a clearly increased risk for some women taking hormone replacement therapy cast the benefits of estrogen for postmenopausal women in doubt. The antioxidant effects of vitamin E and selegiline hydrochloride have been promising in slowing the rate of progression of AD. Ginkgo biloba has provided moderate cognitive improvement with few ill effects. Nonsteroidal anti-inflammatory medications may reduce AD risk by 30 to 60 percent. Finally, a synthetic form of beta-amyloid protein (AN-1792) vaccine is being investigated in clinical trials.

Because the ethical, legal, and social dilemmas surrounding AD cannot be ignored, issues of confidentiality are in the forefront for all genetic testing for AD. Confidentiality in genetic testing may be compromised if such testing becomes part of a person's medical records. Once medical records mention a patient's diagnosis of AD, employers, insurance companies, and other health care organizations can theoretically obtain information that could result in discriminatory actions such as refusal to hire, insure, or provide care.

Genetic counseling allows families to be aware of their genetic predisposition for AD. Since experts still do not know what benefits are gained by knowing that one is at risk for AD, there is great controversy about whether the benefits outweigh the detrimental impact that such knowledge holds.

—Sharon Wallace Stark

See also: Aging; Alcoholism; Behavior; Biological Clocks; Cancer; Diabetes; Down Syndrome; Genetic Testing: Ethical and Economic Issues; Heart Disease; Hereditary Diseases; Hypercholesterolemia; Insurance; Prion Diseases: Kuru and Creutzfeldt-Jakob Syndrome; Proteomics; Stem Cells; Telomeres.

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Web Sites of Interest

- Alzheimer's Association. <http://www.alz.org>. This site provides a two-page genetics fact sheet and information about the Alzheimer's Disease Genetics Initiative, a study conducted by the Alzheimer's Association and the National Institute of Aging.
- Alzheimer's Disease Education and Referral Center, National Institutes of Health. <http://www.alzheimers.org>. A good general starting place for information and links to standard resources. Includes a detailed page on the genetics of AD.
- Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including Alzheimer's disease.

Amniocentesis and Chorionic Villus Sampling

Field of study: Techniques and methodologies

Significance: *Amniocentesis is a procedure for removing amniotic fluid and fetal cells from a pregnant woman. Chorionic villus sampling is a procedure to obtain fetal cells from placental tissue. These procedures, which can detect genetic disorders in the fetus, have broadened the possibilities for effective genetic counseling and for improving the child's health.*

Key terms

AMNIOTIC FLUID: the fluid in which the fetus is immersed during pregnancy

CHORIONIC VILLI: the fingerlike projections of the placenta that function in oxygen, nutrient, and waste transportation between a fetus and its mother

PRENATAL TESTING: testing that is done during pregnancy to examine the chromosomes or genes of a fetus to detect the presence or absence of a genetic disorder

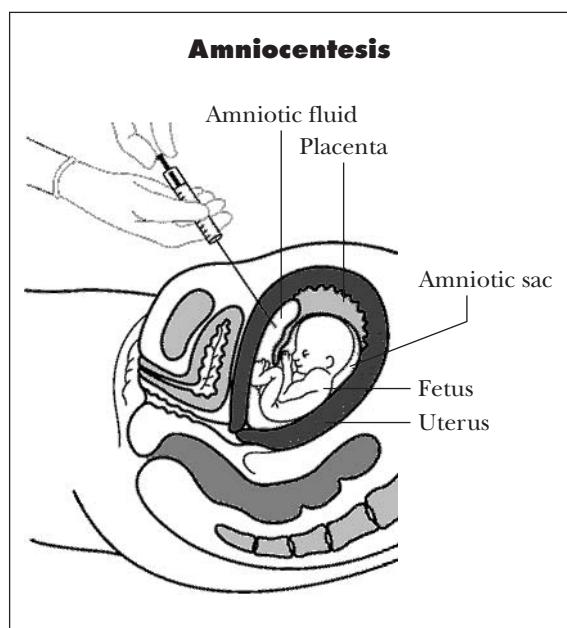
Goals of Testing

The goal of prenatal testing is to provide at-risk families with information about the chances of having a child with a specific genetic disorder or birth defect. Only a small minority of such disorders can now be detected, but the list continues to grow.

The primary techniques for prenatal testing include amniocentesis, chorionic villus sampling (CVS), ultrasonography (in which high-frequency sound waves are used to “view” the fetus and obtain information about its position and structure), fetoscopy (a procedure that uti-

lizes a fiber-optic instrument to obtain a direct image of the fetus), fetal blood sampling (in which blood cells of the fetus are obtained by inserting a needle directly into the umbilical cord), and screening for alpha fetoprotein (a fetal protein found in amniotic fluid, high levels of which may indicate the presence of neural tube defects).

Amniocentesis and chorionic villus sampling are not recommended for every pregnancy. Although the two procedures are relatively safe, they do not carry a zero risk factor and are not likely to be employed unless the risk of the procedure is lower than the risk factor for a birth defect in a specific pregnancy. The general risk for having a child with a significant birth defect is about 2 to 3 percent. Among the factors that indicate an increased risk of having a child with a birth defect are maternal age (the incidence of chromosomal defects in children increases sharply in pregnant women over age thirty-five), a previous child with a known chromosomal or genetic disorder, previous problems with spontaneous abortions or miscarriages, a history of genetic defects in the siblings or other relatives in one or both of the parents, a previous child with a neural tube defect, and marriage between closely related individuals such as first or second cousins.



Removal and analysis of fluid from the amniotic sac that surrounds a fetus during gestation can be used to rule out or confirm the presence of serious birth defects or genetic diseases. (Hans & Cassidy, Inc.)

Amniocentesis

Amniocentesis has been used safely and widely since 1967 and is used more often than other methods of prenatal testing. The procedure is usually performed on an outpatient basis between the fourteenth and eighteenth week of gestation. By this stage in the pregnancy, the volume of amniotic fluid is large enough to get an adequate sample. Also, it allows sufficient time for testing to be done in the laboratory, minimizing complications if it becomes necessary to perform a therapeutic abortion.

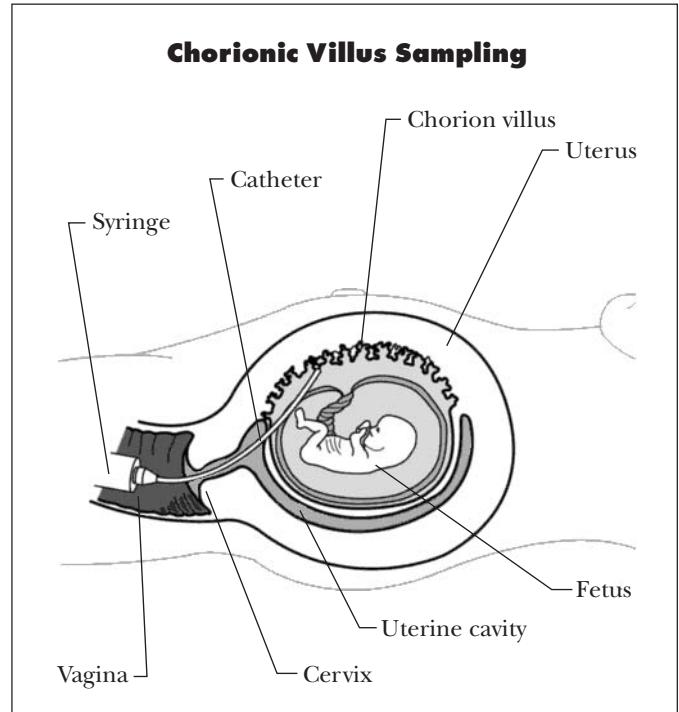
The skin of the abdomen is scrubbed, and a topical anesthetic may be applied. The exact location of the placenta and fetus is determined by ultrasound. A long, thin needle is inserted through the abdominal wall into the amniotic sac that encloses the fetus. A small amount of amniotic fluid is withdrawn. This fluid contains cells that have been sloughed off by the fetus.

The cells must be cultured in the laboratory to produce a sufficient number for testing. A variety of chromosomal analyses and biochemical tests can then be carried out. Any sort of numerical chromosomal abnormality, such as Turner syndrome and Down syndrome, can be detected. Structural abnormalities of chromosomes, such as missing or extra pieces, also can be detected. Cri du chat syndrome is one such genetic disorder. Biochemical and DNA analyses can be carried out on the cells, and some specific genetic disorders can be detected in this manner. The biochemical assays are used to detect low levels of particular enzymes involved in specific biochemical defects. Although most genetic disorders cannot be diagnosed in this manner, the list is rapidly growing. Examples of some inborn errors are galactosemia, Hurler's syndrome, and Lesch-Nyhan syndrome.

It is also possible to analyze DNA directly in cells obtained from amniocentesis. Using specific genetic probes, it is possible to identify mutant genes associated with specific genetic diseases such as sickle-cell disease, cystic fibrosis, hemophilia A, and Duchenne muscular dystrophy. This approach is not yet possible for many genes since it is necessary to know the DNA sequence of the gene involved.

Chorionic Villus Sampling

Although amniocentesis has been a successful prenatal testing procedure, it does present some disadvantages. Perhaps the major disadvantage involves the need to perform it during the sixteenth week of pregnancy, which provides a fairly narrow window in which cells can be grown in culture, tests can be carried out, and procedures can be replicated, if necessary. If procedures run past the nineteenth or twentieth week of pregnancy, the physical and psychological complications associated with a late termination of pregnancy rise considerably. The technique of chorionic villus sampling addresses some of these problems.



Chorionic villus sampling is one method of obtaining embryonic cells from a pregnant woman. Examination of these cells helps physicians determine fetal irregularities or defects, which allows time to assess the problem and make recommendations for treatment. (Hans & Cassidy, Inc.)

Like amniocentesis, chorionic villus sampling can be performed on an outpatient basis. After cleansing the vagina and cervix with an antiseptic, the physician uses ultrasound to guide the insertion of a catheter (a small, thin tube) through the cervix into the uterus. The catheter is placed in contact with the placenta, where the chorionic villi are located, and gentle suction is used to remove a small sample. Villus cells are produced by the fetus and comprise one of the outer layers of the placenta. Of major importance is the fact that this small sample of tissue contains millions of cells that can be used immediately for testing (recall that cells obtained during amniocentesis must be grown for a week or longer before testing can be done). This means that chromosomal analyses and some biochemical tests can be performed and results given to the patient before she leaves the physician's office. Chorionic villus sampling is usually performed between the ninth and twelfth weeks of pregnancy so that

complete results are likely to be reported one month earlier than for amniocentesis. A termination of pregnancy after chorionic villus sampling is expected to have fewer complications than a termination performed at a later stage in pregnancy.

Impact and Applications

Both amniocentesis and chorionic villus sampling provide significant information to couples at risk for having a child with a genetic disorder or other type of birth defect. It is estimated that approximately one-half of the women over the age of thirty-five who are pregnant utilize amniocentesis or chorionic villus sampling. Thousands of women undergo some form of prenatal genetic testing each year. Amniocentesis increases the risk of spontaneous abortion only about 0.5 percent above the overall general risk. Chorionic villus sampling probably carries an increased risk of miscarriage of 1 to 2 percent above the overall general risk. It should be kept in mind, however, that these two techniques are usually not performed unless there is some additional risk already present in a specific pregnancy.

The techniques of amniocentesis and chorionic villus sampling may assure parents at risk of having a child with a genetic disorder that their child will be born without the disorder. Results may also be such that parents must be told that their child will definitely have a certain disorder. However, even if a couple elects to continue with a pregnancy, the tests provide useful information about the nature of the disorder and about treatments that might be used after birth to prepare for raising a child with a birth defect. It is to be expected that further developments in techniques will dramatically improve the convenience and safety of prenatal testing.

—Donald J. Nash

See also: Alcoholism; Bioethics; Congenital Defects; Cystic Fibrosis; Down Syndrome; Dwarfism; Eugenics; Fragile X Syndrome; Genetic Counseling; Genetic Engineering; Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing; Ethical and Economic Issues; Hemophilia; Hereditary Diseases; Inborn Errors of Metabolism; Neural Tube De-

fects; Prader-Willi and Angelman Syndromes; Prenatal Diagnosis; RFLP Analysis; Sickle-Cell Disease; Tay-Sachs Disease; Thalidomide and Other Teratogens; Turner Syndrome.

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Web Sites of Interest

American College of Obstetricians and Gynecologists. <http://www.acog.org>. Offers a wealth of information on procedures, conditions, and ethical considerations. Searchable by keyword

Association of Women's Health, Obstetric, and Neonatal Nurses. <http://www.awhonn.org>. Offers pages for education and practice resources as well as legal policy.

March of Dimes. <http://www.marchofdimes.com>. This site is searchable by keyword and includes information on the basics of amniocentesis and chorionic villus sampling and articles on how the two procedures relate to genetics.

Key terms

DNA POLYMERASE: the enzyme that produces a complementary strand using a DNA template

PRIMER: an oligonucleotide (short strand of nucleotides typically 18-30 bases long) used as a starting point for Taq polymerase to make a complementary copy of a DNA template strand; two primers are needed that flank the DNA sequence being amplified

TAQ POLYMERASE: DNA polymerase originally isolated from the hot spring bacteria *Thermus aquaticus*, which remains stable at temperatures close to boiling (100 degrees Celsius, or 212 degrees Fahrenheit); used as the DNA polymerase in PCR reactions

THERMAL CYCLER: a machine that can rapidly heat and cool reaction tubes; used for performing PCR reactions

Gene Amplification via Polymerase Chain Reaction

Anthropologists and taxonomists have long wished that they could go back in time, collect a few samples, and return to the present so they could compare past organisms with those alive today. Until recently, the best samples available were organisms frozen long ago, mummified, or fossilized. Such samples allowed for superficial comparisons of morphology (physical characteristics), but not much else. Comparisons among extant organisms, including humans, using DNA or protein sequences have now been done for half a century, but until the 1980's ancient samples typically yielded too little material to be useful.

In 1985, R. K. Saiki and Kary B. Mullis amplified the first gene using the polymerase chain reaction (PCR). This technique allows production of billions of copies of a specific DNA sequence, even with very small amounts of template DNA. PCR caused a revolution in the molecular genetics laboratory and was soon embraced by those studying ancient humans and other organisms. So much interest was spawned by the use of PCR to study ancient DNA samples that in 1993 filmmaker Stephen Spielberg directed and produced the movie *Jurassic Park*, based on the 1990 book of the same title by Michael Crichton. Although the science in Ju-

Ancient DNA

Fields of study: Evolutionary biology; Molecular genetics

Significance: Since the development of the polymerase chain reaction (PCR), it has become possible to amplify DNA sequences from extremely small samples and from samples that are greatly degraded, which has kindled interest in studying ancient DNA samples and comparing their gene sequences with those of related modern organisms. Attempts have been made to study DNA sequences from organisms trapped in ice, from mummified individuals, and even from fossils in which some of the original tissue remains. Results have so far been mixed, and gene sequences from some of the older samples have been discredited in many cases.

Jurassic Park goes well beyond what is possible, the film brought the idea of resurrecting ancient DNA samples before the general public.

Problems of Isolation and Analysis

The two greatest concerns when attempting to isolate ancient DNA are the degree to which it has degraded and potential contamination with more recent DNA. The amount of DNA degradation is a function of the age of the sample and the conditions under which it was preserved. Samples a few thousand years old will typically yield very usable DNA, whereas samples exceeding 100,000 years in age may be so degraded, regardless of preservation conditions, that no meaningful data can be obtained. Environmental conditions at the time the organism died also play a large part. A woolly mammoth frozen shortly after death will yield much more and better-preserved DNA than will the bones of a turtle weathered for many months or years before being buried. Insects trapped in amber would seem to have very well preserved DNA, as they were likely encased while still alive, but the age of such fossils may be so great that the DNA is still degraded beyond usefulness.

Of probably greater concern than DNA degradation is contamination with modern DNA. All fossils and ancient remains have the potential to be contaminated with all manner of more recent molecules of DNA, from pollen or bacteria to mold or the skin cells of the person extracting the DNA. Even minute quantities of DNA contamination can ruin the results of PCR analysis, which is capable of amplifying a DNA sequence with only a few template molecules from which to read. For these reasons, the conditions to which the sample was exposed prior to collection must be taken into account. If the sample was encased in ice or rock, the probability of contamination is minimal, whereas if the sample was exposed to the air, contamination can almost be assured. Thus, when collecting a sample, the researcher must immediately place it into an airtight, sterile container.

Once DNA extraction begins, the specimen must be completely protected from contamination by DNA from the researcher or anything else in the environment. The work area must be

sterile and the work surfaces are commonly exposed to damaging ultraviolet (UV) rays for sixty minutes or more in order to destroy any foreign DNA. Researchers wear sterile gloves and face masks, and all laboratory equipment and containers that come in contact with the samples or solutions must be sterile and are often exposed to UV light as well. A further precaution is to analyze ancient and modern DNA samples in completely different laboratories. Finally, after the DNA has been isolated, it must still be kept from subsequent opportunities for contamination.

The first criticism typically leveled against results of ancient DNA analyses is the potential for contamination. Guidelines have been proposed, but researchers have been slow in implementing them, as they are difficult to follow. There is still considerable debate about the veracity of many of the studies so far published, and some DNA initially believed to be ancient was later found to originate in contamination from contemporary sources. In spite of some of the problems with isolating and analyzing ancient DNA, it has produced some fruitful results.

Archaeological and Anthropological Discoveries

Probably the most fruitful area of ancient DNA analysis for archaeologists has been in the study of the origins of human diseases. Traditionally, determining whether an ancient human being suffered from a particular disease relied on circumstantial evidence: stunted growth, bone scars, and other circumstances subject to interpretation. Recovery of ancient bacterial DNA from Egyptian mummies has helped establish the presence of skeletal tuberculosis as long ago as about 3000 B.C.E.

Based on historical accounts, the plague, caused by the bacterium *Yersinia pestis*, has long been considered the cause of a repeated series of epidemics over the last two millennia. Unfortunately, without any adequate medical records to confirm plague epidemic accounts, confirmation is not possible. In 1998, researchers in France unearthed the skeletons of individuals from the sixteenth and eighteenth centuries that presumably died from the plague. Using

PCR to amplify a gene from *Y. pestis* from dental pulp, they obtained proof that the plague existed at the end of the sixteenth century in France. Other diseases that have been successfully identified in this way include leprosy, schistosomiasis, malaria, and Chagas' disease.

In 2003 archaeologists discovered a mummy, accompanied by two others, that might be the famous wife of Akhenaton and co-ruler of Egypt, Nefertiti. Extraction of DNA from all three mummies, to see if they are related, could help determine whether the mummy is Nefertiti. Although DNA evidence would not remove all doubt, added to circumstantial evidence presented by some anthropologists and historians, it would strengthen their case.

Archaeologists excavating an ancient Roman and early Byzantine site in Turkey found fish remains. When DNA was isolated and analyzed, it

was discovered that the species of fish were not from the study area. Instead, they were fish that had to have come from Egypt or the Levant, hinting at potential ancient trade routes.

Dipping more deeply into the past, researchers have isolated DNA from the bones of ancient humans from Australia, Africa, Europe, and other parts of the world. The goal has been to analyze mitochondrial DNA (mtDNA) sequences and use them to help answer questions about human origins. This sort of DNA is preferred because it evolves at an appropriate rate and is more abundant in cells, so it is more likely to be present in ancient specimens. Although the results thus far have not settled some of the long-standing questions about human origins, they have shown that a remarkable similarity exists between ancient and modern DNA sequences.

Image not available

A salt crystal entrapping a 250-million-year-old bubble containing bacteria, excavated from 1,850 feet below ground near Carlsbad, New Mexico, offers scientists Russell Vreeland (left) and William Rosenzwieg the opportunity to study ancient DNA. (AP/Wide World Photos)

Ancient Dinosaur and Bacterial DNA

It had long been assumed that obtaining DNA from samples older than a few hundred thousand years would not be possible. DNA millions of years old was assumed to be so degraded that the fragments would be too small even for PCR to pull out sequences to analyze. In 1991, however, Edward Golenberg of Wayne State University isolated DNA from fossil magnolia leaves that were approximately 18 million years old. Just a year later, in 1992, researchers in California isolated DNA from a fossil bee trapped in amber and researchers at the American Museum of Natural History (New York City) isolated DNA from a termite trapped in amber. Although tantalizing, these studies have been questioned by those who suspect that the DNA that was isolated represents contamination rather than true ancient DNA. In some cases, reanalysis has shown that the supposed ancient DNA was an artifact or a contaminant. The likelihood that DNA could survive so long, even in amber, has also been questioned.

In 1994, Raúl Cano, at California Polytechnic State University, isolated 25-million-year-old DNA from bacteria in the gut of a bee trapped in amber. In 1999, Charles L. Greenblatt, at Hebrew University in Jerusalem, reported the isolation of DNA of several types of bacteria from 120-million-year-old amber. Comparisons of the DNA sequences of ribosomal RNA (rRNA) genes with modern bacteria revealed significant differences, lending support to the claim that Greenblatt and his colleagues had actually isolated ancient DNA and not contaminants.

A number of researchers have isolated DNA from dinosaur bones, ranging in age from 65 million to 80 million years old. The same doubts exist with these studies, particularly because there is no pure, undegraded DNA from ancient dinosaurs with which to compare the extracted DNA. Certainly, if the DNA is compared with bacterial or fungal DNA sequences and there is a match, then the DNA can be considered a contaminant. On the other hand, if the DNA resembles modern reptile or bird DNA, it may represent a contaminant as well but may also be dinosaur DNA, as claimed. Confidence in the results of ancient DNA anal-

yses will come only as more samples are analyzed and DNA is shown to be capable of surviving so long, and even then there will still be room for skepticism. Working with such ancient samples and avoiding all possibility of contamination is extremely difficult.

Studying rates of evolution in a population over thousands of years has also been accomplished using ancient DNA. Adélie penguins have lived in colonies in the same areas of Antarctica for many thousands of years. Excavating various colony sites, researchers have collected partially fossilized bones covering a range of ages to almost seven thousand years old. By comparing the DNA sequences of a portion of mtDNA among the different aged samples and modern samples from live penguins, they have estimated evolutionary rates of change. Because these samples were only thousands of years old, the results are much more reliable than samples from fossils.

Future Prospects in Ancient DNA Research

Some limitation in the field of ancient DNA research will never be overcome, but as more research is done, methods of extraction and analysis of ancient DNA should improve. Also, as more samples are collected and analyzed, better methods for measuring the extent of DNA degradation and for determining whether contamination is present should be developed. However, doubt about the ability of DNA to survive longer than 100,000 years, in the best of conditions, has resulted in a drop in studies of very ancient DNA. It now appears that the most fruitful use of ancient DNA is from samples on the order of a few thousands to tens of thousands of years old, rather than DNA millions of years old. Archaeologists, anthropologists, and biologists studying relatively recent extinct animals will probably be the primary researchers analyzing ancient DNA.

—Bryan Ness

See also: DNA Structure and Function; Evolutionary Biology; Genetic Code; Molecular Clock Hypothesis; Mutation and Mutagenesis; Polymerase Chain Reaction; Punctuated Equilibrium; RNA Structure and Function; RNA World.

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Animal Cloning

Field of study: Genetic engineering and biotechnology

Significance: *Animal cloning is the process of generating a genetic duplicate of an animal starting with one of its differentiated cells. Sheep, mice, cattle, goats, pigs, and a cat have been cloned. While currently an inefficient process that may pose risks to the clone, animal cloning offers the benefits of replicating valuable animals.*

Key terms

ASEXUAL REPRODUCTION: reproduction not requiring fusion of haploid gametes as a first step

CLONE: a genetic replica of a biological organism

DIFFERENTIATED CELL: a somatic cell with a specialized function

MITOCHONDRIAL GENOME: DNA found in mitochondria, coding for forty genes, involved in energy metabolism, and maternally inherited

NUCLEAR GENOME: DNA found in the nucleus, coding for 30,000 genes in higher organisms, half inherited from each parent

TELOMERE: a specialized structure at the chromosome end, which shortens in somatic cells with age

Clones and Cloning

Asexual reproduction occurs in numerous bacteria, fungi, and plants, as well as some animals, leading to genetically identical offspring or clones. In addition, humans can assist in such reproduction. For instance, cuttings from plants generate thousands of replicates. Dividing some animals, such as earthworms or flatworms, allows them to regenerate. However, most vertebrates, including all mammals, reproduce sexually, requiring fertilization of an ovum by sperm. In such species, clones occur, as in the case of identical twins, when an embryo splits into two early in development. This process can be instigated artificially using microsurgical techniques to divide a harvested early-stage embryo and reimplanting the halves into surrogate dams (mothers). While

this can be considered animal cloning, the term should be reserved for cloning from nonembryonic cells.

Cloning Procedure

Animal cloning typically refers to mammals or other higher vertebrates and involves creating a duplicate animal starting from a differentiated cell. Although such a cell only has the ability to perform its specialized function, its nucleus retains all genetic information for the organism's development. Animal cloning requires that such information be reprogrammed into an undifferentiated cell that can re-initiate the developmental process from embryo to birth and beyond.

In theory, the process is straightforward. It consists of taking a differentiated cell from an adult animal, inserting its diploid nucleus into a donor ovum whose own haploid nucleus has been removed, initiating embryonic development of this ovum, inserting the resultant embryonic mass into a receptive surrogate dam and allowing it to proceed to term. In practice, the technique is difficult and was thought to be impossible until 1997. It also appears fraught with species specificity. Various differentiated cells have been used as the starting source; mammary cells were used in the first case, while skin fibroblasts and cumulus cells are now often used. The preparation of the anucleate ovum is an important step. A limitation to clon-

Dolly the Sheep

In 1997, the world was taken aback when a group of scientists headed by embryologist Ian Wilmut at the Roslin Institute in Scotland announced the successful cloning of a sheep named Dolly. Scientists had already cloned cows and sheep, but they had used embryo cells. Dolly was the first vertebrate cloned from the cell of an adult vertebrate.

The feat was accomplished by removing cells from the udder of a six-year-old ewe and placing them in a laboratory dish filled with nutrients, where they were left to grow for five days. Then the nutrients were reduced to 5 percent of what the cells needed to continue growing, which caused the cells to enter a state resembling suspended animation, making them more receptive to becoming dedifferentiated. When the nuclei of these cells were placed in the ova of host sheep, the cytoplasm of each ovum directed the nucleus it received to enter an undifferentiated state, thus causing the cell to develop into an embryo.

Of an initial 277 adult cells introduced into sheep ova, thirteen resulted in pregnancy, and only one, Dolly, was carried to full term. Dolly was a genetic replica of the sheep from whose udder the original cells were extracted. Environmental factors would make Dolly, like any other clone, individual, but genetically she would never have the individuality that an organism produced by usual reproductive means would possess. Over the next six years, she gave birth to several, apparently healthy, offspring. In 2002, at the age of six, Dolly became lame in her left hind leg, a victim of arthritis. Although sheep commonly suf-

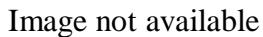
fer arthritis, a veterinarian noted that both the location and the age of onset were uncommon. Then, in February, 2003, she was euthanized after the discovery of a progressive lung disease.

Dolly's health problems have led to speculations about premature aging in clones but are complicated by her unique experiences as well. As Wilmut noted, in the early years following the announcement of her cloning, she became something of a celebrity, which led to overfeeding by visitors and in turn a period of obesity, later corrected. More significant were the discovery of her arthritis and then her lung disease—conditions not uncommon in sheep but that tend to emerge later (sheep typically live to be eleven or twelve years old).

Theories of premature aging are supported by the fact that Dolly's telomeres were shorter than normal. These cell structures function as "caps" that prevent "fraying" at the ends of DNA cells. As a cell ages, its telomeres become progressively shorter, until finally they disappear altogether and are no longer able to protect the cell, which then dies.

Was Dolly older genetically than she was chronologically? The answer to the question of whether Dolly was completely "normal" or aged prematurely as a result of being a clone must await full investigation of her autopsy results, as well as tracking of her offspring's lives and monitoring of other vertebrate clones through their life spans.

—R. Baird Shuman, updated by Christina J. Moose

Image not available

Dolly, the first animal cloned from an adult vertebrate cell, in 1997. She was euthanized in 2003 after complications from advanced arthritis and lung disease.
(AP/Wide World Photos)

ing dogs appears to be the difficulty in obtaining ova suitable for nuclear transfer. The technique for inserting the nucleus is crucial, as is the conversion to the undifferentiated embryonic state. Transfer of the embryonic cells to a receptive surrogate dam is generally a well-developed technology, although more than four viable embryos are necessary to maintain pregnancy in pigs.

Furthermore, the genetic makeup of a putative clone must be verified, to ensure that it is indeed a replica of its progenitor and not an unintended offspring of either the donor of the ovum or the surrogate dam. DNA fingerprinting via microsatellite analysis at a number of polymorphic sites is an unambiguous way to establish its genetic identity.

Identicalness

Such a clone is not absolutely identical, because of mitochondrial differences and environmental effects. While the nuclear genome must be identical to its progenitor, the mitochondrial genome of the clone will invariably be different, because it comes from the ovum used. While mitochondria make a minor contribution to the total genetic makeup, they can

influence phenotypic expression. In addition, the prenatal environment can affect some traits. Coat color and color pattern are characteristics that can be developmentally influenced; the first cloned cat was not an exact duplicate of its progenitor in coloration. Some behavioral features are also impacted during intrauterine development.

Cloned Animals

The first cloned animal was a sheep named Dolly. While she was the only live offspring generated from 277 attempts, her birth showed that animal cloning was possible. Shortly thereafter, mice and cattle were cloned. Reproducible cloning of mice is more difficult than imagined, whereas more cattle were cloned in the first five years after Dolly's birth than any other species. Goats, pigs, and a cat were subsequently also cloned.

Problems and Potential Benefits

Prominent among the problems with animal cloning is its inefficiency. Although this may not be surprising as the technology is still under development, no more than 2 percent of embryos generated lead to viable offspring. Additionally, most cloned animals are larger than normal at birth, often requiring cesarian delivery, and some have increased morbidity and mortality. Some have had smaller telomeres and shorter lives. Dolly exhibited this trait and lived for only six years (although she was euthanized, she clearly would not have lived much longer)—half of the average life span. Conversely, some cloned mice do not exhibit shortened telomeres or premature aging, even through six consecutive cloned generations. Further research will establish whether these problems are inherent to cloning, are consequences of some aspect of the current procedure or are attributable to the small numbers of cloned animals studied.

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At a news conference in July, 1998, Dr. Ryuzo Yanagimachi holds in his hands the first cloned mouse and its "parent." The cloning occurred nearly simultaneously with the cloning of Dolly the sheep. (AP/Wide World Photos)

The benefits of animal cloning would involve duplicating particularly valuable animals. Livestock with highly valued production characteristics could be targets for cloning. However, the technique is likely to be most beneficial in connection with transgenesis, to replicate animals that yield a therapeutic agent in high quantities or organs suitable for transplantation into humans. If animal cloning can be made efficient and trouble-free, its potential benefits could be fully developed.

—James L. Robinson

See also: Biopharmaceuticals; cDNA Libraries; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Knockout Genetics and Knockout Mice; Mitochondrial Genes; Parthenogenesis; Polymerase Chain Reaction; Restriction

Enzymes; Reverse Transcriptase; Shotgun Cloning; Telomeres; Transgenic Organisms; Xenotransplants.

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Pennisi, Elizabeth, and Gretchen Vogel. "Animal Cloning: Clones: A Hard Act to Follow." *Science* 288 (2000): 1722-1727. The status of animal cloning, three years after the announcement of Dolly. The problems, questions, and concerns are presented in a highly readable text.

Wilmut, Ian, Keith Campbell, and Colin Tudge. *The Second Creation: The Age of Biological Control by the Scientists That Cloned Dolly*. London: Headline, 2000. The story of the scientific

collaboration between an agricultural scientist and a cell biologist, of their perseverance and the serendipity that led to the first cloned sheep, Dolly.

Web Sites of Interest

ActionBioScience.org. <http://www.actionbioscience.org/biotech/pecorino.html>. Features the article “Animal Cloning: Old MacDonald’s Farm Is Not What It Used To Be” and several useful links to the animal cloning debate.

Roslin Institute. <http://www.roslin.ac.uk>. The site of the oldest cloning group in the world, founded in 1919, which cloned the first animal, Dolly the sheep. Includes information on genomics and animal breeding.

Anthrax

Field of study: Bacterial genetics

Significance: *Anthrax has plagued humankind for thousands of years. Naturally occurring anthrax spores have caused disease in livestock and wildlife more often than in humans, but with the rise of genetic technologies anthrax has become amenable to manipulation as an agent of bioterrorism and biowarfare.*

Key terms

PLASMIDS: extrachromosomal DNA, found most commonly in bacteria, which can be transferred between bacterial cells

POLYMERASE CHAIN REACTION (PCR): a process in which a portion of DNA is selected and repeatedly replicated

SINGLE NUCLEOTIDE POLYMORPHISM (SNP): the difference in a single nucleotide between the DNA of individual organisms

VARIABLE NUMBER TANDEM REPEAT (VNTR): the difference in the number of tandem repeats (short sequences of DNA repeated over and over) between the DNA of individual organisms

History

A disease killing cattle in 1491 B.C.E., likely to have been anthrax, is recounted in the Book

of Genesis. In Exodus 9, the Lord instructs Moses to take “handfuls of ashes of the furnace” and “sprinkle it toward the heaven in the sight of the Pharaoh.” Moses performed the deed and “it became a boil breaking forth with blains upon man and upon beast.” This may represent the first use of anthrax as a biological weapon. Greek peasants tending goats suffered from anthrax; the Greek word from which “anthrax” derives means coal, referring to the coal-black center of the skin lesion.

Anthrax became the first pathogenic bacillus to be seen microscopically when described in infected animal tissue by Aloys-Antoine Pollender in 1849. Studies by Robert Koch in 1876 resulted in the four postulates that form the basis for the study of infectious disease causation. In 1881, Louis Pasteur demonstrated the protective efficacy of a vaccine for sheep made with his attenuated vaccine strain.

The Disease

Anthrax is primarily a disease of herbivorous animals that can spread to humans through association with domesticated animals and their products. Herbivorous animals grazing in pastures with soil contaminated with anthrax endospores become infected when the spores gain entry through abrasions around the mouth and germinate in the surrounding tissues. Omnivores and carnivores can become infected by ingesting contaminated meat. Human infection is often a result of a close association with herbivores, particularly goats, sheep, or cattle (including their products of hair, wool, and hides).

The most common clinical illness in humans is skin infection (cutaneous anthrax), acquired when spores penetrate through cuts or abrasions. After an incubation period of three to five days, a papule develops, evolves into a vesicle, and ruptures, leaving an ulcer that dries to form the characteristic black scab. Inhaled spores reach the alveoli of the lung, where they are engulfed by macrophages and germinate into bacilli. Bacilli are carried to lymph nodes, where release and multiplication are followed by bloodstream invasion and the infection’s spread to other parts of the body, including the brain, where it causes meningitis. The symptoms of the illness, which begin a few days after

inhalation, resemble those of the flu and may be associated with substernal discomfort. Cough, fever, chills, and respiratory distress with raspy, labored breathing ensue. The least common type of infection is that of the gastrointestinal tract.

An effective vaccine is available for prevention, and antibiotics have been used when immediate protection is needed. Antibiotics can also successfully treat the infection. Inhalational anthrax is nearly always fatal if untreated, and even with treatment the mortality ranges from 40 to 80 percent. Mortality from treated cutaneous anthrax is less than 1 percent.

The Anthrax Bacterium

The *Bacillus anthracis* bacterium is large (1-1.2 × 3-10 microns), encapsulated, gram-

positive, and rod-shaped. It produces spores and exotoxins (toxins that are released from the cells). Spores are ellipsoidal or oval (1-2 microns) and located within the bacilli. The endospores have no reproductive significance, as only one spore is formed by each bacillus and a germinated spore yields a single bacillus. Spores form in soil and dead tissue and with no measurable metabolism may remain dormant for years. They are resistant to drying, heat, and many disinfectants.

The genetic composition of *B. anthracis* differs little from the other *Bacillus* species, and studies have demonstrated remarkable similarity within *B. anthracis* strains. The resting stage of sporulation may have contributed to the extremely similar DNA of all strains of *B. anthracis*. The circular chromosomal DNA is com-

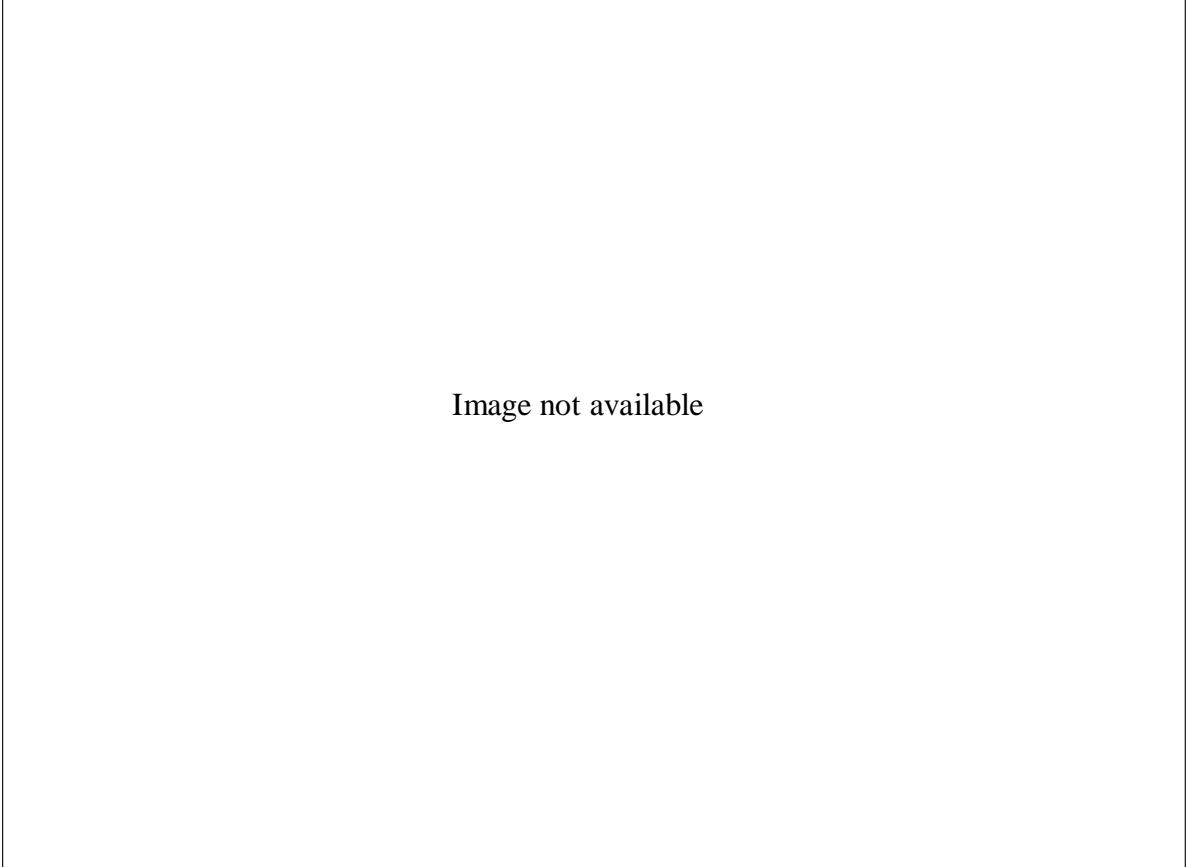


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Anthrax cells invading the spleen of a monkey, in an undated electronmicrograph from the U.S. Department of Defense Web site. The United States and the Soviet Union developed biological weapons during the Cold War, and many of these lethal organisms remain housed in facilities around the world. (AP/Wide World Photos)

posed of 5.2 million base pairs and codes for metabolic function, cell repair, and the sequential process of sporulation. Comparative genome sequencing has uncovered only four differences between the single-copy chromosomal DNA of two strains. In addition to the single-copy DNA, comprising the majority of the genome, a remaining portion consists of repetitive DNA sequences that are either dispersed or clustered into satellites. The satellite repeats occur in tandem. The number of tandem repeats varies among different strains; six chromosomal marker loci have been identified by multiple-locus variable number tandem repeat (VNTR) analysis.

In addition to its chromosome, *B. anthracis* has two large plasmids that carry genes necessary for pathogenesis. The pXO1 plasmid has 181,654 base pairs and contains the structural genes for the anthrax toxins *cya* (edema factor), *lef* (lethal factor), and *pagA* (protective antigen). The pXO2 plasmid consists of 96,231 base pairs and carries three genes required for synthesis of the capsule. These plasmids contain a much greater number of single nucleotide polymorphisms (SNPs) and VNTRs among strains than the chromosomal genome. There are a variety of reference strains, such as Pasteur (which lacks the X01 plasmid), Sterne (which lacks the X02 plasmid), and Ames (which has both plasmids and is fully virulent).

Bioterrorism

Anthrax spores can be easily packaged to act as aerosoled (airborne) agents of war, and the genome may be bioengineered to alter the virulence or effectiveness of current vaccines. Knowledge of the genetic composition of *B. anthracis* has facilitated the investigation of anthrax attacks. In 1993, the Aum Shinrikyo cult aerosoled a suspension of anthrax near Tokyo, Japan. Molecular studies of the genome from this strain revealed it to be devoid of the pXO2 plasmid (Sterne strain), explaining why only a bad odor rather than illness was the fortunate consequence. In 2001, analysis of material from letter-based attacks with anthrax in the United States demonstrated the source to be the Ames strain. Furthermore, as a result of the extensive laboratory studies associated with these at-

tacks, a sensitive and specific three-target (two-plasmid and one-chromosome) assay has been developed for rapid detection and identification of *B. anthracis*, including bioengineered strains, from both patients and the environment.

—H. Bradford Hawley

See also: Bacterial Genetics and Cell Structure; Bacterial Resistance and Super Bacteria; Biological Weapons; Plasmids; Smallpox.

Further Reading

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Read, Timothy D., et al. "Comparative Genome Sequencing for Discovery of Novel Polymorphisms in *Bacillus anthracis*." *Science* 296 (June 14, 2002): 2028-2033. Describes the complete sequencing of the anthrax genome.

Web Sites of Interest

Centers for Disease Control, Public Health Emergency Preparedness and Response. <http://www.bt.cdc.gov>. This comprehensive site offers information on how to recognize illness caused by anthrax exposure and more. Available in Spanish.

Nature. <http://www.nature.com>. The online version of the premier science journal *Nature* includes links to research articles on the genetics of anthrax, including "Focus on Anthrax."

Antibodies

Field of study: Immunogenetics

Significance: *Antibodies provide the main line of defense (immunity) in all vertebrates against infections caused by bacteria, fungi, viruses, or other foreign agents. Antibodies are used as therapeutic agents to prevent specific diseases and to identify the presence of antigens in a wide range of diagnostic procedures. Large quantities of antibodies have also been produced in plants for use in human and plant immunotherapy. Because of their importance to human and animal health, antibodies are widely studied by geneticists seeking improved methods of antibody production.*

Key terms

B CELLS: a class of white blood cells (lymphocytes) derived from bone marrow responsible for antibody-directed immunity

B MEMORY CELLS: descendants of activated B cells that are long-lived and that synthesize large amounts of antibodies in response to a subsequent exposure to the antigen, thus playing an important role in secondary immunity

HELPER T CELLS: a class of white blood cells (lymphocytes) derived from bone marrow that prompts the production of antibodies by B cells in the presence of an antigen

LYMPHOCYTES: types of white blood cells (including B cells and T cells) that provide immunity

PLASMA CELLS: descendants of activated B cells that synthesize and secrete a single antibody type in large quantities and also play an important role in primary immunity

Antibody Structure

Antibodies are made up of a class of proteins called immunoglobulins (Ig's) produced by plasma cells (descendants of activated B cells) in response to a specific foreign molecule known as an antigen. Most antigens are also proteins or proteins combined with sugars. Antibodies recognize, bind to, and inactivate antigens that have been introduced into an organism by various pathogens such as bacteria, fungi, and viruses.

The simplest form of antibody molecule is a Y-shaped structure with two identical, long polypeptides (substances made up of many amino acids joined by chemical bonds) referred to as "heavy chains" and two identical, short polypeptides referred to as "light chains." These chains are held together by chemical bonds. The lower portion of each chain has a constant region made up of similar amino acids in all antibody molecules, even among different species. The remaining upper portion of each chain, known as the "variable region," differs in its amino acid sequence from other antibodies. The three-dimensional shape of the tips of the variable region (antigen-binding site) allows for the recognition and binding of target molecules (antigens). The high-affinity binding between antibody and antigen results from a combination of hydrophobic, ionic, and van der Waals forces. Antigen-binding sites have specific points of attachment on the antigen that are referred as "epitopes" or "antigenic determinants."

Antibody Diversity

There are five classes of antibodies (IgG, IgM, IgD, IgA, and IgE), each having a distinct structure, size, and function (see the table headed "Classes, Locations, and Functions of Antibodies"). IgG is the principal immunoglobulin and constitutes up to 80 percent of all antibodies in the serum.

The human body can manufacture a limitless number of antibodies, each of which can bind to a different antigen; however, human genomes have a limited number of genes that code for antibodies. It has been proposed that random recombination of DNA segments is responsible for antibody variability. For example, one class of genes (encoding light chain) contains three regions: the L-V (leader-variable) region (in which each variable region is separated by a leader sequence), the J (joining) region, and the C (constant) region. In the embryonic B cells, each gene consists of from one hundred to three hundred L-V regions, approximately six J regions, and one C region. These segments are widely separated on the chromosome. As the B cells mature, one of the L-V regions is randomly joined to one of the J

regions and the adjacent C region by a recombination event. The remaining segments are cut from the chromosome and subsequently destroyed, resulting in a fusion gene encoding a specific light chain of an antibody. In mature B cells, this gene is then transcribed and translated into polypeptides that form a light chain of an antibody molecule. Genes for the other class of light chains as well as heavy chains are also made up of regions that undergo recombination during B-cell maturation. These random recombination events in each B cell during maturation lead to the production of billions of different antibody molecules. Each B cell has, however, been genetically programmed to produce only one of the many possible variants of the same antibody.

Production of Antibodies: Immune Response

Immunity is a state of bodily resistance brought about by the production of antibodies against an invasion by an antigen. The immune response is mediated by white blood cells known as lymphocytes that are made in the bone marrow. There are two types of lymphocytes: T cells, which are formed when lymphocytes migrate to the thymus gland, circulate in

the blood, and become associated with lymph nodes and the spleen; and B cells, which are formed in bone marrow and move directly to the circulatory and the lymph systems. B cells are genetically programmed to produce antibodies. Each B cell synthesizes and secretes only one type of antibody, which has the ability to recognize with high affinity a discrete region (epitope or antigenic determinant) of an antigen. Generally, an antigen has several different epitopes, and each B cell produces a set of different antibodies corresponding to one of the many epitopes of the same antigen. All of the antibodies in this set, referred to as “polyclonal” antibodies, react with the same antigen.

The immune system is more effective at controlling infections than the nonspecific defense response (bodily defenses against infection—such as skin, fever, inflammation, phagocytes, natural killer cells, and some other antimicrobial substances—that are not part of the immune system proper). The immune system has three characteristic responses to antigens: diverse, which effectively neutralizes or destroys various foreign invaders, whether they are microbes, chemicals, dust, or pollen; specific, which effectively differentiates between harmful and harmless antigens; and anamnestic,

Classes, Locations, and Functions of Antibodies

<i>Class</i>	<i>Location</i>	<i>Functions</i>
IgG	Blood plasma, tissue fluid, fetuses	Produces primary and secondary immune responses; protects against bacteria, viruses, and toxins; passes through the placenta and enters fetal bloodstream, thus providing protection to fetuses.
IgM	Blood plasma	Acts as a B-cell surface receptor for antigens; fights bacteria in primary immune response; powerful agglutinating agent; includes anti-A and anti-B antibodies.
IgD	Surface of B cells	Prompts B cells to make antibodies (especially in infants).
IgA	Saliva, milk, urine, tears, respiratory and digestive systems	Protects surface linings of epithelial cells, digestive, respiratory, and urinary systems.
IgE	In secretion with IgA, skin, tonsils, respiratory and digestive systems	Acts as receptor for antigens causing mast cells (often found in connective tissues surrounding blood vessels), to secrete allergy mediators; excessive production causes allergic reactions (including hay fever and asthma).

which has a memory component that remembers and responds faster to a subsequent encounter with an antigen. The primary immune response involves the first combat with antigens, while the secondary immune response includes the memory component of a first assault. As a result, humans typically get some diseases (such as chicken pox) only once; other infections (such as cold and influenza) often recur because the causative viruses mutate, thus presenting a different antigenic face to the immune system each season.

An antibody-mediated immune response involves several stages: detection of antigens, activation of helper T cells, and antibody production by B cells. White blood cells known as macrophages continuously wander through the circulatory system and the interstitial spaces between cells searching for antigen molecules. Once an antigen is encountered, the invading molecule is engulfed and ingested by a macrophage. Helper T cells become activated by coming in contact with the antigen on the macrophage. In turn, an activated helper T cell identifies and activates a B cell. The activated T cells release cytokines (a class of biochemical signal molecules) that prompt the activated B cell to divide. Immediately, the activated B cell generates two types of daughter cells: plasma cells (each of which synthesizes and releases approximately two thousand to twenty thousand antibody molecules per second into the bloodstream during its life span of four to five days) and B memory cells (which have a life span of a few months to a year). The B memory cells are the component of the immune memory system that, in response to a second exposure to the same type of antigen, produces antibodies in larger quantities and at faster rates over a longer time frame than the primary immune response. A similar cascade of events occurs when a macrophage presents an antigen directly to a B cell.

Polyclonal and Monoclonal Antibodies

Plasma cells originating from different B cells manufacture distinct antibody molecules because each B cell was presented with a specific portion of the same antigen by a helper T cell or macrophage. Thus a set of polyclonal

antibodies is released in response to an invasion by a foreign agent. Each member of this group of polyclonal antibodies will launch the assault against the foreign agent by recognizing different epitopes of the same antigen. The polyclonal nature of antibodies has been well recognized in the medical field.

In the case of multiple myeloma (a type of cancer), one B cell out of billions in the body proliferates in an uncontrolled manner. Eventually, this event compromises the total population of B cells of the body. The immune system will produce huge amounts of IgG originating from the same B cell, which recognizes only one specific epitope of an antigen; therefore, this person's immune system produces a set of antibodies referred to as "monoclonal" antibodies. Monoclonal antibodies form a population of identical antibodies that all recognize and are specific for one epitope on an antigen. Thus, someone with this condition may suffer frequent bacterial infections because of a lack of antibody diversity. Indeed, a bacterium whose antigens do not match the antibodies manufactured by the overabundant monoclonal B cells has a selective advantage.

The high-affinity binding capacity of antibodies with antigens has been employed in both therapeutic and diagnostic procedures. It is, however, unfortunate that the effectiveness of commercial preparations of polyclonal antibodies varies widely from batch to batch. In some instances of immunization, certain epitopes of a particular antigen are strong stimulators of antibody-producing cells, whereas at other times, the immune system responds more vigorously to different epitopes of the same antigen. Thus one batch of polyclonal antibodies may have a low level of antibody molecules directed against a major epitope and not be as effective as the previous batch. Consequently, it is desirable to produce a cell line that will produce monoclonal antibodies with a high affinity for a specific epitope on the antigen for commercial use. Such a cell line would provide a consistent and continual supply of identical (monoclonal) antibodies. Monoclonal antibodies can be produced by hybridoma cells, which are generated by the fusion of cancerous B cells and normal spleen cells obtained from mice

immunized with a specific antigen. After initial selection of hybridoma clones, monoclonal antibody production is maintained in culture. In addition, the hybridoma cells can be injected into mice to induce tumors that, in turn, will release large quantities of fluid containing the antibody. This fluid containing monoclonal antibodies can be collected periodically and may be used immediately or stored for future use. Various systems used to produce monoclonal antibodies include cultured lymphoid cell lines, yeast cells, *Trichoderma reesei* (ascomycetes), insect cells, *Escherichia coli*, and monkey and Chinese hamster ovary cells. Transgenic plants and plant cell cultures have been explored as potential systems for antibody expression.

Impact and Applications

The high-affinity binding capacity of antibodies may be used to inactivate antigens *in vivo* (within a living organism). The binding property of antibodies may also be employed in many therapeutic and diagnostic applications. In addition, it is a very effective tool in both immunological isolation and detection methods.

Monoclonal antibodies may outnumber all other products being explored by various biotechnology-oriented companies for the treatment and prevention of disease. For example, many strategies for the treatment of cancerous tumors as well as for the inhibition of human immunodeficiency virus (HIV) replication are based on the use of monoclonal antibodies. HIV is a retrovirus (a virus whose genetic material is ribonucleic acid, or RNA) that causes acquired immunodeficiency syndrome (AIDS). Advances in plant biotechnology have made it possible to use transgenic plants to produce monoclonal antibodies on a large scale for therapeutic or diagnostic use. Indeed, one of the most promising applications of plant-produced antibodies in immunotherapy is in passive immunization (for example, against *Streptococcus mutans*, the most common cause of tooth decay). Large doses of the antibody are required in multiple applications for passive immunotherapy to be effective. Transgenic antibody-producing plants may be one source that can supply huge quantities of antibodies in a safe

and cost-effective manner. It has been demonstrated that a hybrid IgA-IgG molecule produced by transgenic plants prevented colonization of *S. mutans* in culture, which appears to be how the antibody prevents colonization of this bacterium *in vivo*.

It has been estimated that antibodies expressed in soybeans at a level of 1 percent of total protein may cost approximately one hundred dollars per kilogram of antibody, which is relatively inexpensive in comparison with the cost of traditional antibiotics. Transgenic plants have also been used as bioreactors for the large-scale production of antibodies with no extensive purification schemes. In fact, antibodies have been expressed in transgenic tobacco roots and then accumulated in tobacco seeds. If this technology could be employed to obtain stable accumulation of antibodies in more edible plant organs such as potato tubers, it could potentially allow for long-term storage as well as a safe and easy delivery of specific antibodies for immunotherapeutic applications. In addition, plant-produced antibodies may be more desirable for human use than microbial-produced antibodies, because plant-produced antibodies undergo eukaryotic rather than the prokaryotic (bacterial) post-translational modifications. Human glycosylation (a biochemical process whereby sugars are attached onto the protein) is more closely related to that of plants than that of bacteria.

The potential use of antibody expression in plants for altering existing biochemical pathways has also been demonstrated. For example, germination mediated by phytochrome (a biochemical produced by plants) has been altered by utilizing plant-produced antibodies. In addition, antibodies expressed in plants have been successfully used to immunize host plants against pathogenic infection; for example, tobacco plants have already been immunized with antibodies against viral attack. This approach has great potential to replace the traditional methods (use of chemicals) in controlling pathogens.

—Sibdas Ghosh and Tom E. Scola

See also: Allergies; Autoimmune Disorders; Biopharmaceuticals; Blotting: Southern, Northern, and Western; Diabetes; Diphtheria;

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Antisense RNA

Field of study: Molecular genetics

Significance: *Antisense RNA and RNA interference are powerful modifiers of gene expression that act through RNA-RNA binding through complementary base pairing. This provides a flexible mechanism for specific gene regulation and has great potential for experimental studies and therapeutic action. RNA interference, a specialized form of antisense RNA, even mimics an immune system, for example, targeting RNA viruses within a cell. Processes involving antisense RNA appear in eukaryotes, eubacteria, and archaea.*

Key terms

ANTISENSE: a term referring to any strand of DNA or RNA that is complementary to a coding or regulatory sequence; for example, the strand opposite the coding strand (the sense strand) in DNA is called the antisense strand

DOWN-REGULATION: a process of gene expression in which the amount that a gene is transcribed and/or translated is reduced

GENE SILENCING: any form of genetic regulation in which the expression of a gene is completely repressed, either by preventing

transcription (pre-transcriptional gene silencing) or after a messenger RNA (mRNA) has been transcribed (post-transcriptional gene silencing)

RNA INTERFERENCE (RNAi): Sequence-specific degradation of messenger RNA (mRNA) caused by complementary double-stranded RNA

UP-REGULATION: a process of gene expression in which the amount that a gene is transcribed and/or translated is increased

Discovery

In addition to the three main types of RNA—messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA)—there are numerous other types of RNA molecules. Some have an effect, through complementary binding, on mRNA molecules. When this kind of RNA binds to an mRNA, it effectively blocks translation of the mRNA and can therefore be described as having an antisense action (that is, it blocks the expression of the message in the mRNA). Antisense RNA was first discovered in 1981 as a mechanism regulating copy number of bacterial plasmids. Other RNAs, such as small nuclear and small nucleolar RNAs (snRNA and snoRNA), act in RNA splicing and editing, with a catalytic effect guided by complementary base pairing.

Various forms of gene down-regulation were discovered throughout the 1990's, including plant post-transcriptional gene silencing (that is, preventing the mRNA from being translated), gene silencing in fungi (that is, preventing transcription of a gene), and RNA interference in the nematode *Caenorhabditis elegans*. The importance of noncoding RNA molecules, including antisense RNA, is becoming clear. They add a previously unknown level of genetic complexity, and the extent of their influence is yet to be determined fully.

Natural Function

Antisense RNA is utilized in a number of ways by bacterial plasmids. Replication of ColE1 plasmids requires an RNA preprimer, called RNA II, that interacts with the origin of replication and forms a particular secondary structure. This allows an enzyme to cut and

form the mature primer needed for DNA replication. Antisense RNA I can bind to RNA II, preventing the formation of the necessary structure. In the R1 plasmid, the CopA antisense RNA can bind and prevent the translation of the RNA transcript for replication initiation protein RepA. Thus, change in plasmid number is controlled by changing levels of antisense RNA, modifying the ability of plasmids to replicate.

Many plasmids use antisense RNA to ensure their maintenance within bacteria. The R1 plasmid transcribes Hok toxin mRNA, but interaction with antisense Sok RNA prevents its translation. Sok RNA is less stable than Hok RNA, so plasmid loss leads to Sok degradation but leaves some Hok transcripts, which are translated into a toxin that kills the cell. This is an ingenious way of selecting for plasmid propagation. Antisense regulation has also been found in some transposons and bacteriophages.

Bacteria use antisense RNA to regulate particular genes. Such RNA is often encoded in a region different from that of the target and may affect multiple genes. For example, the OxyS RNA, induced by oxidative stress, inhibits translation of fhlA mRNA (involved in formate metabolism). In conjunction with the protein Hfq, OxyS RNA binds near the ribosome-binding site in fhlA mRNA, preventing translation. MicF RNA is induced under cellular stress and binds to the mRNA of membrane pore protein ompF to prevent its translation.

One of the first examples of antisense regulatory mechanisms in eukaryotes came from the nematode *Caenorhabditis elegans*. Small antisense RNA molecules lin-4 and let-7 show imperfect base-pairing to the 3' untranslated region of their target gene mRNAs. This results in translational inhibition and is important for normal development. These small antisense RNAs are members of the microRNA (miRNA) class of molecules, which are single-stranded RNA molecules, about 21 nucleotides long, found throughout eukaryotes. They are produced by cleavage of longer molecules (about 60-100 nucleotides) which contain partial self-complementarity that produces a hairpin structure. The function of most miRNAs is unknown.

Antisense RNA has been implicated in other

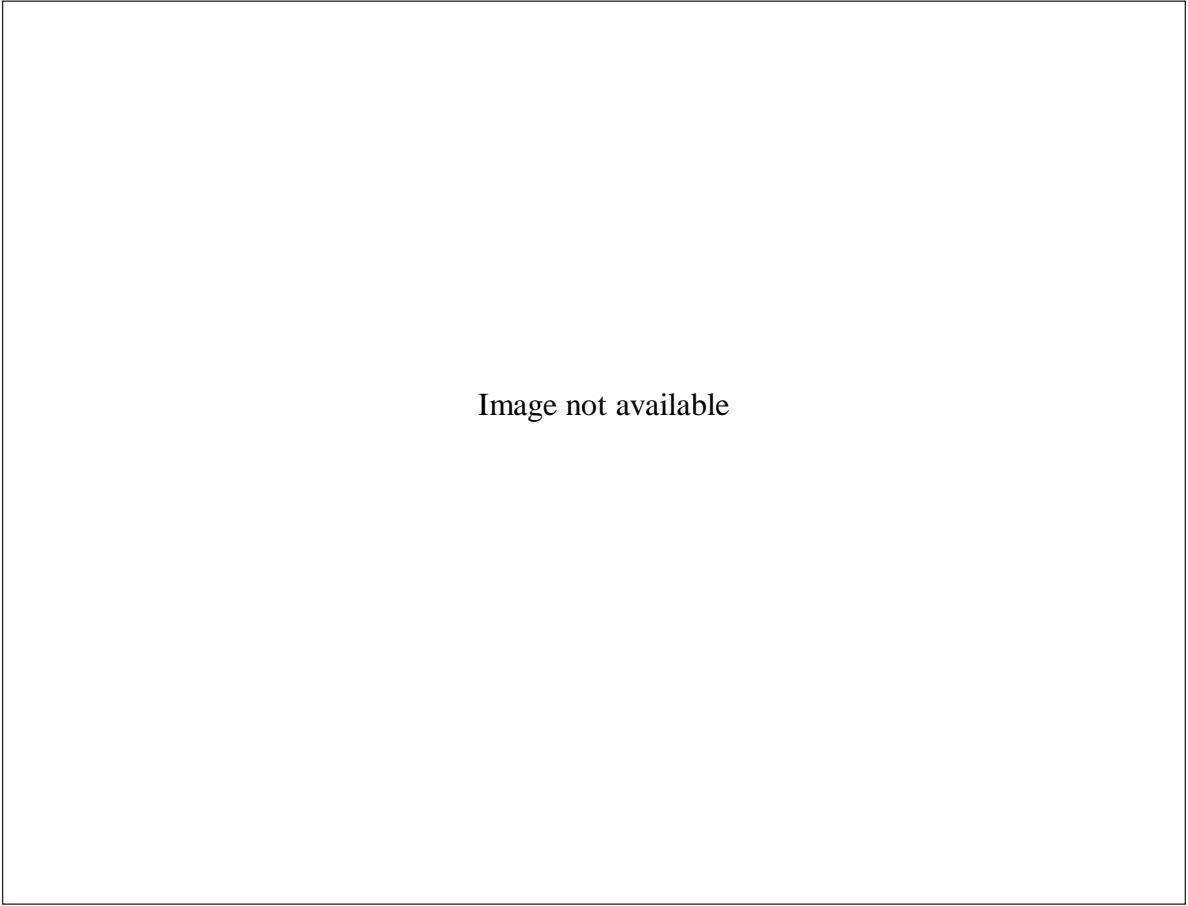
A black and white portrait photograph of Dr. Phillip A. Sharp, a man with glasses and a white lab coat.

Image not available

Dr. Phillip A. Sharp, who with Richard J. Roberts won the 1993 Nobel Prize in Physiology or Medicine. Sharp acknowledges that the discovery of antisense RNA and RNA interference has changed his cancer research. The process could theoretically offer ways of “silencing” the genes associated with cancer. (AP/Wide World Photos)

processes. Imprinted genes are often associated with antisense transcripts from the same locus, although their role is unclear. Double-stranded RNA may be capable of affecting DNA chromatin structure through methylation of homologous sequence.

RNA Interference

RNA interference (RNAi) causes sequence-specific gene silencing in response to the presence of double-stranded RNA. The process is proposed to have evolved as a mechanism of avoiding viral infection and limiting transposable element replication, since both can involve double-stranded RNA.

The process is present in a wide variety of eukaryotes (including mice and probably hu-

mans), and the steps involved are likely to be similar. RNAi begins by the recognition of long double-stranded RNA molecules by the conserved protein Dicer, which cuts these long RNAs to produce small interfering RNAs (siRNA, double-stranded RNA about 21-23 nucleotides long with overhanging 3' ends). These become part of the RNA-induced silencing complex (RISC), which uses the antisense strand of the siRNA to recognize complementary RNA sequences. These sequences are then cut and degraded. Some organisms show amplification, where siRNA stimulates production of more double-stranded RNA to be processed by Dicer. *Caenorhabditis elegans* and plants show evidence of a systemic response, whereby initial silencing in one cell spreads to other cells.

Therapeutic Applications

Many disease states are caused by abnormal gene expression and are therefore potential targets for gene therapy. One approach is to use antisense RNA or RNAi. For example, cancer cells often show overexpression of genes involved in growth and proliferation. These genes could be targeted by antisense RNA to decrease the amount of gene product produced, in hopes of preventing tumor growth. RNAi could be used to target specific mutations. The mRNA from a mutant allele could be targeted for degradation in a heterozygous patient, allowing production of the correct protein from the wild-type allele alone. Antisense techniques could be used to target viruses and prevent their replication, or could be used to correct aberrant splicing. Many of the processes described above have been successfully demonstrated in experimental systems such as cell culture or mouse models.

Many issues need to be addressed before antisense RNA therapeutics become feasible. A delivery system is needed to produce a long-lasting effect in the correct cell type. The fact that RNA interference appears to spread systemically in some organisms may facilitate its application. The safety and effectiveness of such approaches also need to be tested. Nevertheless, the approach is promising and potentially very useful.

—Peter J. Waddell and Michael J. McLachlan

See also: Gene Regulation: Eukaryotes; Human Genetics; Model Organism: *Caenorhabditis elegans*; Noncoding RNA Molecules; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World; Viral Genetics.

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Archaea

Field of study: Cellular biology

Significance: Archaea are diverse prokaryotic organisms distinct from the historically familiar bacteria. Archaea have certain molecular properties previously thought to occur only in eukaryotes. Many archaea require severe conditions for growth, and their genetic processes have adapted to these extreme conditions in ways that are not fully understood.

Key terms

CONJUGATION: the process by which one bacterial cell transfers DNA directly to another

DOMAIN: the highest-level division of life, sometimes called a superkingdom

EXTREME HALOPHILES: microorganisms that require extremely high salt concentrations for optimal growth

INSERTION SEQUENCE: a small, independently transposable genetic element

METHANOGENS: microorganisms that derive energy from the production of methane

PROKARYOTES: unicellular organisms with simple ultrastructures lacking nuclei and other intracellular organelles

SMALL SUBUNIT RIBOSOMAL RNA (ssu rRNA): the RNA molecule found in the small subunit of the ribosome; also called 16S rRNA (in prokaryotes) or 18S rRNA (in eukaryotes)

Gene Sequences Measure the Diversity of Prokaryotes

Prokaryotic microorganisms have been on earth for as many as 3.8 billion years and have diverged tremendously in genetic and metabolic terms. Unfortunately, the magnitude of this divergence has made it difficult to measure the relatedness of prokaryotes to one another. In the 1970's, Carl R. Woese addressed this problem using a method of reading short sequences of ribonucleotides from a highly conserved RNA molecule, the small subunit ribosomal RNA (ssu rRNA). Because this RNA is present in all organisms and has evolved very slowly, any two organisms have at least a few of these short nucleotide sequences in common.

The proportion of shared sequences thus provided a quantitative index of similarity by which all cellular organisms could, in principle, be compared.

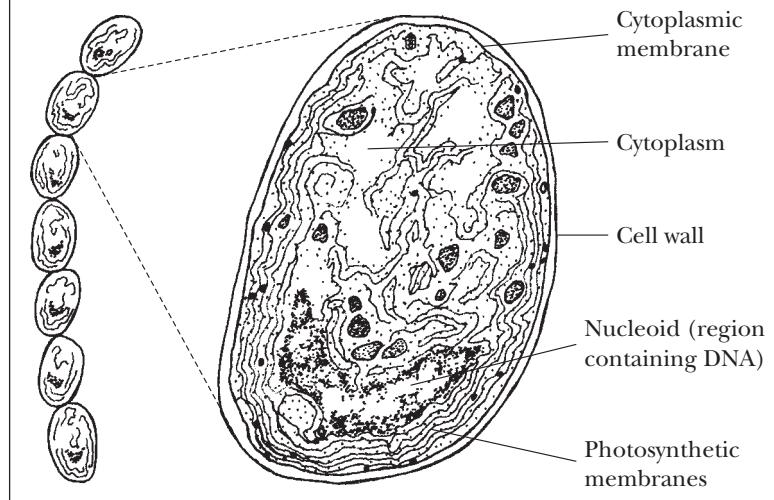
When the nucleotide sequence data were used to construct an evolutionary tree, eukaryotes (plants, animals, fungi, and protozoa) formed a cluster clearly separated from the common bacteria. Unexpectedly, however, a third cluster emerged that was equally distinct from both eukaryotes and common bacteria. This cluster consisted of prokaryotes that (1) lacked biochemical features of most bacteria (such as a cell wall composed of peptidoglycan), (2) possessed other features not found in any other organisms (such as membranes composed of isoprenoid ether lipids), and (3) occurred in unusual, typically harsh, environments.

Woese and his co-workers eventually designated the three divisions of life represented by these clusters “domains,” naming the nonbacterial prokaryotes the domain *Archaea*.

Biology of the Domain Archaea

Archaea tend to require unusual conditions for growth, which has made it challenging to determine their genetic properties. The methanogens, for example, live by converting hydrogen (H_2) and carbon dioxide (CO_2) or other simple carbon compounds into methane and are killed by even trace amounts of oxygen. The extreme halophiles, in contrast, normally live in brine lakes and utilize oxygen for growth. However, they require extremely high concentrations of salt to maintain their cellular structure. A third class of archaea, the extreme thermophiles, occur naturally in geothermal springs and grow best at temperatures ranging from 60 to 105 degrees Celsius (140–221 degrees Fahrenheit).

A Prokaryotic Cell



Archaea and bacteria are the simplest and oldest forms of life, consisting of prokaryotic cells, which differ from the cells that form higher organisms (fungi, algae, protozoa, plants, and animals), called eukaryotic cells. Based on an electron microscope image of one cell in a string forming a cyanobacterium, this depiction shows the basic features of a prokaryote. Note the lack of a defined nucleus and organelles (there are no plastids or mitochondria)—the components that house genetic information in eukaryotes. Instead, genetic material in prokaryotes is located in an unbound region called the nucleoid. (Kimberly L. Dawson Kurnizki)

Many derive energy from the oxidation or reduction of sulfur compounds. Sequencing of DNA fragments recovered from “moderate” environments, such as ocean water or soil, has revealed many additional archaeal species that presumably do not require unusual environmental conditions but have never been cultured in the laboratory.

The Genetic Machinery of Archaea

Because bacteria and eukaryotes differ greatly with respect to gene and chromosome structure and the details of gene expression, molecular biologists have examined the same properties in archaea and have found a mixture of “bacterial” and “eukaryotic” features. The organization of DNA within archaeal cells is bacterial, in the sense that archaeal chromosomes are circular DNAs of between 2 million and 4 million base pairs having single origins

of replication. As in bacteria, the genes are densely packed and often grouped into clusters of related genes transcribed from a common promoter. The promoters themselves, however, resemble the TATA box/BRE element combination of eukaryotic DNA polymerase II (Pol II) promoters, and the RNA polymerases have the complex subunit composition of eukaryotes rather than the simple composition found in bacteria. Furthermore, archaea initiate transcription by a simplified version of the process seen in eukaryotic cells: Transcription factors (TATA-binding protein and a TFIIB) first bind to regions ahead of the promoter, then recruit RNA polymerase to attach and begin transcription. Introns are rare in archaea, however, and do not interrupt protein-encoding genes. Also, the regulation of transcription in archaea seems to depend heavily on the types of repressor and activator proteins found in bacteria.

Genomes of Archaea

The availability of complete DNA sequences now enables archaeal genomes to be compared to the genomes of bacteria and eukaryotes. One pattern that emerges from these comparisons is that most of the archaeal genes responsible for the processing of information (synthesis of DNA, RNA, and proteins) resemble their eukaryotic counterparts, whereas most of the archaeal genes for metabolic functions (biosynthetic pathways, for example) resemble their bacterial counterparts. The genomes of archaea also reveal probable cases of gene acquisition from distant relatives, a process called lateral gene transfer.

A third pattern to emerge from genome comparisons is that some archaea are missing genes thought to be important or essential. For example, the genomes of at least two methanogenic archaea do not encode an enzyme that charges transfer RNA (tRNA) with cysteine. These archaea instead use a novel strategy for making cysteinyl-tRNA: Some of the seryl tRNA made by these cells is converted to cysteinyl tRNA by a specialized enzyme. Even more intriguing is the much longer list of archaea—all of which happen to be hyperthermophiles, which grow optimally at 80 degrees Celsius (176 degrees Fahrenheit) or above—that lack

genes for the DNA mismatch repair proteins found in all other organisms.

Unique Genetic Properties?

This last observation raises an important question: Has an evolutionary history distinct from all conventional genetic systems, combined with the special demands of life in unusual environments, resulted in unique genetic properties in archaea? Although basic genetic assays can be performed in only a few species, the results help identify which genetic properties of cellular organisms are truly universal and which ones may have unusual features in archaea.

The methanogen *Methanococcus voltae* transfers short pieces of chromosome from one cell to another, using particles that resemble bacterial viruses (bacteriophages). This means of gene transfer has been seen in only a few bacteria. In other methanogens, researchers have used more conventional genetic phenomena, such as antibiotic-resistance genes, plasmids, and transposable elements, to develop tools for cloning or inactivating genes. As a result, new details about the regulation of gene expression in archaea and the genetics of methane formation are now coming to light.

The extreme halophile *Halobacterium salinarum* exhibits extremely high rates of spontaneous mutation of the genes for its photosynthetic pigments and gas vacuoles. This genetic instability reflects the fact that insertion sequences transpose very frequently into these and other genes. A distantly related species, *Haloferax volcanii*, has the ability to transfer chromosomal genes by means of conjugation. Although many bacteria engage in conjugation, the mechanism used by *H. volcanii* does not resemble the typical bacterial system, since no plasmid seems to be involved, and there is no apparent distinction between donor strain and recipient strain in the transfer of DNA.

Genetic tools for the archaea from geothermal environments are less well developed, but certain selections have made it possible to study spontaneous mutation and homologous recombination in some species of *Sulfolobus*. At the normal growth temperatures of these aerobic archaea, 75-80 degrees Celsius (167-176 de-

degrees Fahrenheit), spontaneous chemical decomposition of DNA is calculated to be about one thousand times more frequent than in the organisms previously studied by geneticists. In spite of this, *Sulfolobus acidocaldarius* exhibits the same frequency of spontaneous mutation as *Escherichia coli* and significantly lower proportions of base-pair substitutions and deletions. This indicates especially effective mechanisms for avoiding or accurately repairing DNA damage, including mismatched bases, despite the fact that no mismatch repair genes have been found in *Sulfolobus* species. Also, *S. acidocaldarius*, like *H. volcanii*, has a mechanism of conjugation that does not require a plasmid or distinct donor and recipient genotypes. The transferred DNA recombines efficiently into the resident chromosome, as indicated by frequent recombination between mutations spaced only a few base pairs apart.

Finding two similar and unusual mechanisms of conjugation in two dissimilar and distantly related archaea (*H. volcanii* and *S. acidocaldarius*) raises questions regarding the possible advantages of this capability. Population genetic theory predicts that organisms that reproduce clonally (as bacteria and archaea do) would benefit from occasional exchange and recombination of genes, because this accelerates the production of beneficial combinations of alleles. Such recombination may be particularly important for archaea such as *Haloferax* and *Sulfolobus* species, whose extreme environments are like islands separated by vast areas that cannot support growth. For these organisms, frequent DNA transfer between cells of the same species may provide an efficient way to enhance genetic diversity within small, isolated populations.

—Dennis W. Grogan

See also: Antisense RNA; Bacterial Genetics and Cell Structure; Gene Regulation: Bacteria; Lateral Gene Transfer; Noncoding RNA Molecules.

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Artificial Selection

Fields of study: Evolutionary biology;
Population genetics

Significance: *Artificial selection is the process through which humans have domesticated and improved plants and animals. It continues to be the primary means whereby agriculturally important plants and animals are modified to improve their desirability. However, artificial selection is also a threat to genetic diversity of agricultural organisms as uniform and productive strains replace the many diverse, locally produced varieties that once existed around the globe.*

Key terms

GENETIC MERIT: a measure of the ability of a parent to contribute favorable characteristics to its progeny

GENETIC VARIATION: a measure of the availability of genetic differences within a population upon which artificial selection has potential to act

HERITABILITY: a proportional measure of the extent to which differences among organisms within a population for a particular character result from genetic rather than environmental causes (a measure of nature versus nurture)

Natural vs. Artificial Selection

Selection is a process through which organisms with particular genetic characteristics leave more offspring than do organisms with alternative genetic forms. The process may occur because the genetic characteristics confer upon the organism a better ability to survive and ultimately produce more offspring than individuals with other characteristics (natural selection), or it may be caused by selective breeding of individuals with characteristics valuable to humans (artificial selection). Natural and artificial selection may act in concert, as when a genetic characteristic confers a disadvantage directly to the organism. Dwarfism in cattle, for example, not only directly reduces the survival of the affected individuals but also reduces the value of the animal to the breeder. Conversely, natural selection may act in opposition to artificial selection. For example, a genetic characteristic that results in the seed being held

tightly in the head of a grass such as wheat is an advantage to the farmer, as it makes harvesting easier. That same characteristic would be a disadvantage to wild wheat because it would limit seed dispersal.

Early Applications

Artificial selection was probably conducted first by early farmers who identified forms of crop plants that had characteristics that favored cultivation. Seeds from favored plants were preferentially kept for replanting. Any characteristics that were to some degree heritable would have had the tendency to be passed on to the progeny through the selected seeds. Some favored characteristics may have been controlled by a single gene and were therefore quickly established, whereas other favored characteristics may have been controlled by a large number of genes with individually small effects, making them more difficult to estab-

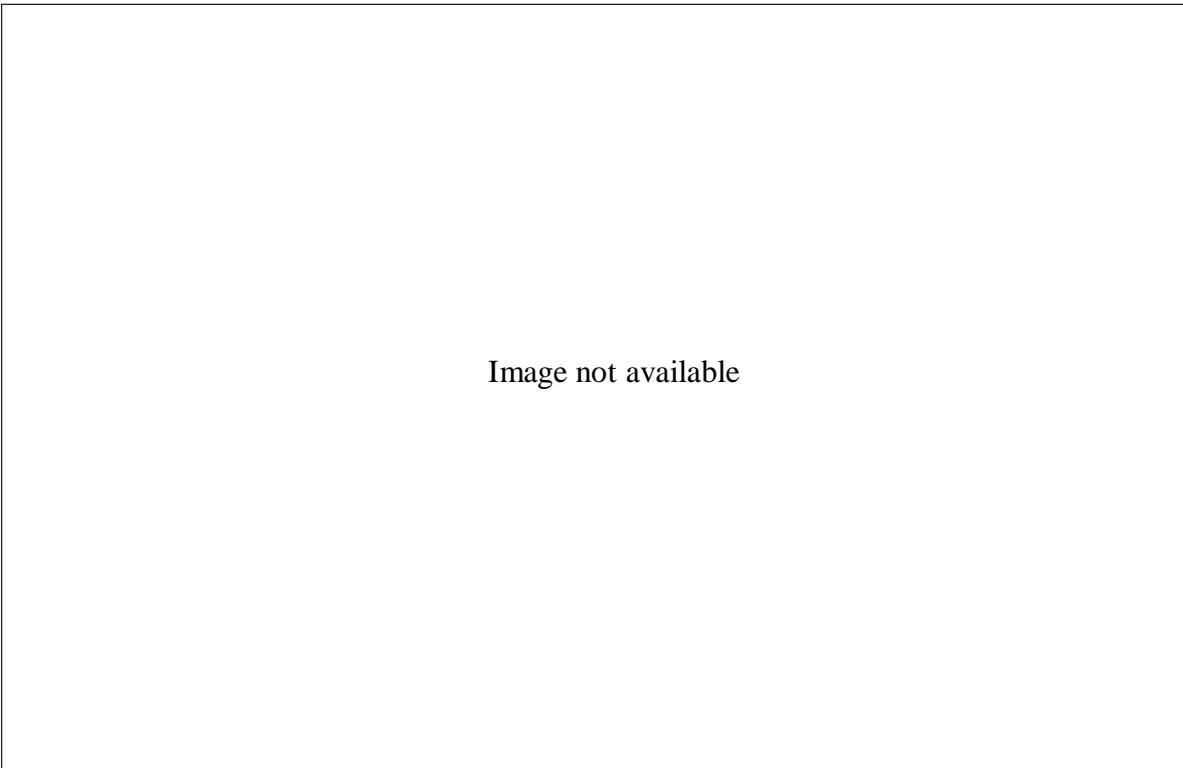


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The beefalo is created by breeding a cow and a bison, and then breeding the offspring again to a cow. Such hybridization, in both plants and animals, is a form of artificial selection that has been practiced by humans for thousands of years to meet agricultural needs. (AP/Wide World Photos)

lish. Nevertheless, seeds selected from the best plants would tend to produce offspring that were better than average, resulting in gradual improvement in the population. It would not have been necessary to have knowledge of the mechanisms of genetics to realize the favorable effects of selection. Likewise, individuals who domesticated the first animals for their own use would have made use of selection to capture desirable characteristics within their herds and flocks. The first of those characteristics was probably docile behavior, a trait known to be heritable in contemporary livestock populations.

From Pedigrees to Genome Maps

Technology to improve organisms through selective breeding preceded an understanding of its genetic basis. Recording of pedigrees and performance records began with the formal development of livestock breeds in the 1700's. Some breeders, notably Robert Bakewell, began recording pedigrees and using progeny testing to determine which sires had superior genetic merit. Understanding of the principles of genetics through the work of Gregor Mendel enhanced but did not revolutionize applications to agricultural plant and animal improvement.

Development of reliable methods for testing the efficiency of artificial selection dominated advances in the fields of plant and animal genetics during the first two-thirds of the twentieth century. Genetic merit of progeny was expected to be equal to the average genetic merit of the parents. More effective breeding programs are dependent on identifying potential parents with superior genetic merit. Computers and large-scale databases have greatly improved selection programs for crops and livestock. Genetic change on the order of 2 percent per generation became possible. However, selection to improve horticultural species and companion animals continued to rely largely on the subjective judgment of the breeder to identify superior stock. Plant and animal genome-mapping programs would facilitate the next leap forward in genetic improvement of agricultural organisms. Selection among organisms based directly on their

gene sequences promised to allow researchers to bypass the time-consuming data-recording programs upon which genetic progress of the 1990's relied.

Diversity vs. Uniformity

The ultimate limit to what can be achieved by selection is the exhaustion of genetic variants. One example of the extremes that can be accomplished by selection is evident in dog breeding: The heaviest breeds weigh nearly one hundred times as much as the lightest breeds. Experimental selection for body weight in insects and for oil content in corn has resulted in variations of similar magnitudes.

However, most modern breeding programs for agricultural crops and livestock seek to decrease variability while increasing productivity. Uniformity of the products enhances the efficiency with which they can be handled mechanically for commercial purposes. As indigenous crop and livestock varieties are replaced by high-producing varieties, the genetic variation that provides the source of potential future improvements is lost. Widespread use of uniform varieties may also increase the susceptibility to catastrophic losses or even extinction from an outbreak of disease or environmental condition. The lack of biodiversity in the wake of such species loss could threaten entire ecosystems and human beings themselves.

—William R. Lamberson

See also: Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Gene Therapy; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genomes; Genome Libraries; Hardy-Weinberg Law; High-Yield Crops; Inbreeding and Assortative Mating; Natural Selection; Polyploidy; Population Genetics; Pedigree Analysis; Punctuated Equilibrium; Quantitative Inheritance; Sociobiology; Speciation.

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Autoimmune Disorders

Fields of study: Diseases and syndromes; Immunogenetics

Significance: *Autoimmune disorders are chronic diseases that arise from a breakdown of the immune system's ability to distinguish between the body's own cells and foreign substances. Autoimmune disorders can be caused by both genetic and environmental factors and cause an individual's immune system to react against the organs or tissues of the individual's own body.*

Key terms

ANTIBODIES: molecules in blood plasma responsible for recognizing and binding to antigens

ANTIGENS: foreign substances recognized by the immune system that result in the production of antibodies and lymphocytes directed specifically against them

IMMUNE SYSTEM: the system that normally responds to foreign agents by producing antibodies and stimulating antigen-specific lymphocytes, leading to destruction of these agents

LYMPHOCYTES: sensitized cells of the immune system that recognize and destroy harmful agents via antibody and cell-mediated responses that include B lymphocytes from the bone marrow and T lymphocytes from the thymus

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC): a system of protein markers on a cell's outer membrane following infection with a virus, malignant cell, or foreign cell that signals the immune system to destroy the cell

Autoimmune Disorders and Immune System Dysfunction

Autoimmune disorders involve a large group of chronic and potentially life-threatening diseases that are initiated by an individual's own immune system attacking the organs or tissues of the individual's own body. The main function of the immune system is to defend against invading microorganisms such as bacteria, fungi, viruses, protozoa, and parasites by producing antibodies or lymphocytes that recognize and destroy the harmful agent. The ability to distinguish normal body constituents (self) from foreign substances (nonself) is crucial to appropriate immune functioning. Loss of this ability to distinguish between self and nonself can lead to serious damage to the affected organs and tissues.

Autoimmunity—the inaccurate recognition of a normal body component as foreign, followed by the mounting of an autoimmune response—results when normal autoantigens on body cells stimulate the development of autoantibodies. These autoantibodies are most often the result of a genetic defect during viral and bacterial infections or from environmental or chemical factors; they may also, according to some researchers, be a natural consequence of the aging process.

History and Classification of Autoimmune Disorders

The concept of autoimmune disorders was first proposed in 1901, but it was not until the 1950's that autoimmunity was experimentally created in animals via immunization. By the 1960's, it was recognized that autoimmunity was a direct or indirect cause of numerous human ailments. Many diseases formerly classified as collagen-vascular diseases (collagenoses) were later classified as autoimmune disorders.

Autoimmune disorders are generally categorized as organ-specific diseases and non-organ-specific (also called systemic) diseases. Organ-specific autoimmune diseases involve an attack directed against one main organ and have been documented for essentially every organ in the body. Common examples include multiple sclerosis (brain), insulin-dependent diabetes mellitus (pancreas), Graves' disease (thyroid), Addison's disease (adrenal glands), pernicious anemia (stomach), myasthenia gravis (muscles), autoimmune hemolytic anemia (blood), primary biliary cirrhosis (liver), pemphigus vulgaris (skin), and glomerulonephritis (kidneys). Non-organ-specific autoimmune diseases involve an attack by the immune system on several body areas, potentially causing diseases such as systemic lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa, scleroderma, ankylosing spondylitis, and rheumatic fever.

Some evidence suggests that other conditions (such as certain types of eye inflammation and male and female infertility) may be autoimmune related. Allergies involve hypersensitivity reactions that result in immune reactions that can lead to inflammation and tissue damage. Environmental antigens such as pollen, dust mites, food proteins, and bee venom may cause allergic reactions such as hay fever, asthma, and food intolerance in sensitive individuals via the antibody class known as immunoglobulin E (IgE). Medications such as antibiotics may also be recognized as chemical antigens, causing adverse allergic reactions.

Immunologists do not know the precise origin of most autoimmune diseases. What researchers have shown is that most autoimmune diseases occur more frequently in females than in males and that the development of autoim-

mune disorders often requires both a genetic susceptibility and additional stimuli such as exposure to a toxin. Of the numerous theories proposed for the cause of autoimmunity development, three models have received the most consideration by clinical researchers. The clonal deletion theory suggests that autoimmunity develops if autoreactive T or B cell clones are not eliminated during the fetal period or very soon after birth. The body normally does not react to its own fetal or neonatal antigens, which are recognized because the corresponding T and B cell clones are eliminated from the immune system. In the unfortunate event that "forbidden clones" of autoreactive cells remain active, antibodies are produced that are directed against its own antigens, and autoimmunity develops, frequently involving the loss of the helper T cells' ability to regulate B-cell function. A second theory suggests that some antigens that are normally nonimmunogenic (hidden antigens) somehow become immunogenic and stimulate the immune system to react against itself. A third theory suggests that autoimmunity can be initiated by an exogenous antigen, assuming that the antibodies produced to fight it cross-react with a similar determinant on the body's own cells.

Diagnosis and Treatment

Diagnosis of autoimmune disorders generally begins with the often difficult task of documenting autoantibodies and autoreactive T cells. A condition suspected to be caused by autoimmunity can also be confirmed by a number of other direct and indirect methods, such as a favorable response to immunosuppressive, corticosteroid, or anti-inflammatory drug treatment along with several other immunologic techniques.

Treatment strategies lag behind the ability to diagnose autoimmune disorders. Initial management involves the control and reduction of both pain and loss of function. Correction of deficiencies in hormones such as insulin or thyroxin caused by autoimmune damage to glands is often performed first. Replacing blood components by transfusion is also considered, but treatment effectiveness is often limited by the lack of knowledge of the precise disease mecha-

nisms. Suppression of the immune system is also often attempted, but achieving a delicate balance between controlling the autoimmune disorder and maintaining the body's ability to fight disease in general is critical.

Medication therapy commonly includes corticosteroid drugs, with more powerful immunosuppressant drugs such as cyclophosphamide, methotrexate, azathioprine, chloroquine derivatives, and small doses of antimetabolic or anticancer drugs often required. A majority of these medications can rapidly damage dividing tissues such as the bone marrow and thus must be used with caution. Plasmapheresis (removal of toxic antibodies) is often helpful in diseases such as myasthenia gravis, while other treatments involve drugs that target immune system cells such as the cyclosporines. Fish oil and antioxidant supplementation have been shown to be an effective anti-inflammatory intervention and may help suppress autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.

—Daniel G. Graetzer, updated by Bryan Ness

See also: Aging; Allergies; Antibodies; Developmental Genetics; Diabetes; Hybridomas and Monoclonal Antibodies; Immunogenetics; Organ Transplants and HLA Genes; Stem Cells; Steroid Hormones.

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Web Site of Interest

American Autoimmune Related Diseases Association. <http://www.aarda.org>. Site of the only national organization devoted specifically to autoimmune disorders and chronic illness. Includes a medical glossary, research articles, information on the science of autoimmune-related diseases, and links to related resources.

Bacterial Genetics and Cell Structure

Fields of study: Cellular biology; Bacterial genetics

Significance: *The study of bacterial structure and genetics has made tremendous contributions to the fields of genetics and medicine, leading to the development of drugs for the treatment of disease, the discovery of DNA as the master chemical of heredity, and knowledge about the regulation of gene expression in other organisms, including humans.*

Key terms

CLONING: the generation of many copies of DNA by replication in a suitable host

EUKARYOTE: an organism made up of cells having a membrane-bound nucleus that contains chromosomes

MUTATION: the process by which a DNA base-pair change or a change in a chromosome is produced; the term is also used to describe the change itself

PROKARYOTE: an organism lacking a membrane-bound nucleus

RECOMBINANT DNA: a DNA sequence that has been constructed or engineered from two or more distinct DNA sequences

Bacteria and Their Structure

The old kingdom Monera contained what has now been classified into the domains *Bacteria* and *Archaea*. Organisms in these domains are unicellular (one-celled) and prokaryotic (lacking a membrane-bound nucleus). Bacteria are among the simplest, smallest, and most ancient of organisms, found in nearly every environment on earth. While some bacteria are autotrophic (capable of making their own food), most are heterotrophic (forced to draw nutrients from their environment or from other organisms). For most of human history, the existence of bacteria was unknown. It was not until the late 1800's that bacteria were first identified. Their role in nature is that of decomposers: They break down organic molecules into their component parts. Along with fungi, they are the major recyclers in nature.

They are also capable of changing atmospheric nitrogen to a form that is usable by plants and animals.

It has long been known that some bacteria are pathogens, or causes of disease. Scientists have expended tremendous effort in describing the role bacteria play in disease and in creating agents that could kill them. Other bacteria, such as *Escherichia coli*, may be part of a mutualistic relationship with another organism, such as humans. Bacteria have been used extensively in genetics research because of their small size and because they reproduce rapidly; some bacteria produce a new generation every twenty minutes. Since they have been so thoroughly studied, a great deal is known about their structure and genetics.

Most bacteria are less than one micron (one-millionth of a meter) in length. They do not contain mitochondria (organelles that produce the energy molecule adenosine triphosphate, or ATP), chloroplasts (plant organelles in which the reactions of photosynthesis take place), lysosomes (organelles that contain digestive enzymes), or interior membrane systems such as the endoplasmic reticulum or Golgi bodies. They do, however, contain RNA, ribosomes (organelles that serve as the sites of protein synthesis), and DNA, which is organized as part of a single, circular chromosome. The circular chromosome is centrally located within the cell in a region called the nucleoid region and is capable of supercoiling. Bacteria often have additional genes carried on small circular DNA molecules called plasmids, which have been used extensively in genetic research. Some plasmids carry genes that impart antibiotic resistance to the cells that contain them.

Bacteria have three basic morphologies, or cell shapes. Bacteria that are spherical are called cocci. Some coccus bacteria form clusters (*staphylococcus*), while others may form chains (*streptococcus*). Bacteria that have a rodlike appearance are called bacilli. Spiral or helical bacteria are called spirilla (sometimes called spirochetes).

Classification of Bacteria

Bacteria fall into three basic types: those that lack cell walls, those with thin cell walls, and those with thick cell walls. Mycoplasmas lack cell walls entirely. The bacteria that cause tuberculosis, *Mycobacterium tuberculosis*, do have cell walls and, unlike *Archaea*, their cell walls are composed of peptidoglycan, a complex organic molecule made of two unusual sugars held together by short polypeptides (short chains of amino acids). In 1884, Hans Christian Gram, a Danish physician, found that certain bacterial cells absorbed a stain called crystal violet, while others did not. Those cells that absorb the stain are called gram-positive, and those that do not are called gram-negative. It has since been found that gram-positive bacteria have thick walls of peptidoglycan, while gram-negative bacteria have thin peptidoglycan walls covered by a thick outer membrane. It is this thick outer membrane that prevents crystal violet from entering the bacterial cell. Distinguishing between gram-positive and gram-negative bacteria is an important step in the treatment of disease since some antibiotics are more effective against one class than the other.

By contrast with bacteria, members of *Archaea* have cell walls that do not contain peptidoglycan. Members of *Archaea* are usually found in extreme environments, such as hot springs, extremely saline environments, and hydrothermal vents. Methanogens are the most common and are strict anaerobes, which means that they are killed by oxygen. They live in oxygen-free environments, such as sewers and swamps, and produce methane gas as a waste product of their metabolism. Halobacteria live in only those environments that have a high concentration of salt, such as salt ponds. Thermoacidophiles grow in very hot or very acidic environments.

Bacteria can be further differentiated by the presence or absence of certain surface structures. Some strains produce an outer slime layer called a “capsule.” The capsule permits the bacterium to adhere to surfaces (such as human teeth, for example, where the build-up of such bacteria causes dental plaque) and provides some protection against other microorganisms. Some strains display pili, which are fine, hairlike appendages that also allow the bacterium to adhere to surfaces. Some pilis,

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Helicobacter pylori, which causes stomach ulcers, is only one example of one of the many forms of bacteria. The inset shows a single bacterium. (AP/Wide World Photos)

such as F pili in *E. coli*, are involved in the exchange of genetic material from one bacterium to another in a process called conjugation. Some bacterial strains have one or more flagella, which allow them to be motile (capable of movement). Any bacterium may have one or more of these surface structures.

Research in molecular genetics is continuing to expand insight into bacterial classification and gene function. Many researchers have been actively sequencing the genomes of bacteria from a broad spectrum. The number of species that have been sequenced is now in the hundreds and includes many human pathogens, such as those that cause tuberculosis, bacterial pneumonia, ulcers, bacterial influenza, leprosy, and Lyme disease. The genomes of a wide range of nonpathogenic bacteria have also been sequenced. Comparisons among the genomes that have been sequenced is beginning to show extensive evidence that bacteria of different species have transferred genes back and forth many times in the past, thus making it difficult to trace their evolutionary lineages.

Bacterial Reproduction

Bacteria reproduce in nature by means of binary fission, wherein one cell divides to produce two daughter cells that are genetically identical. As bacteria reproduce, they form clustered associations of cells called colonies. All members of a colony are genetically identical to one another, unless a mutagen (any substance that can cause a mutation) has changed the DNA sequence in one of the bacteria. Changes in the DNA sequence of the chromosome often lead to changes in the physical appearance or nutritional requirements of the colony. While a bacterium is microscopic, bacterial colonies can be seen with the naked eye; changes in the colonies are relatively easy to perceive. This is one of the reasons bacteria have been favored organisms for genetic research.

For the most part, there is very little genetic variation between one bacterial generation and the next. Unlike higher organisms, bacteria do not engage in sexual reproduction, which is the major source of genetic variation within a population. In laboratory settings, however,

bacteria can be induced to engage in a unidirectional (one-way) exchange of genetic material via conjugation, first observed in 1946 by biochemists Joshua Lederberg and Edward Tatum. The unidirectional nature of the gene transfer was discovered by William Hayes in 1953. He found that one bacterial cell was a donor cell while the other was the recipient. In the 1950's, molecular biologists François Jacob and Elie Wollman used conjugation and a technique called "interrupted mating" to map genes onto the bacterial chromosome. By breaking apart the conjugation pairs at intervals and analyzing the times at which donor genes entered the recipient cells, they were able to determine a correlation between time and the distance between genes on a chromosome. The use of this technique led to a complete map of the sequence of genes contained in the chromosome. It also led to a surprise: It was use of interrupted mating with *E. coli* that first demonstrated the circularity of the bacterial chromosome. The circular structure of the chromosome was in striking contrast to eukaryotic chromosomes, which are linear.

Transformation and Transduction

The bacterium *Streptococcus pneumoniae* was used in one of the early studies that eventually led to the identification of DNA as the master chemical of heredity. Two strains of *S. pneumoniae* were used in a study conducted by microbiologist Frederick Griffith in 1928. One strain (S) produces a smooth colony that is virulent (infectious) and causes pneumonia. The other strain (R) produces a rough colony that is avirulent (noninfectious). When Griffith injected mice with living type R bacteria, the mice survived and no bacteria were recovered from their blood. When he injected mice with living type S, the mice died, and type S bacteria were recovered from their blood. However, if type S was heat-killed before the mice were injected, the mice did not die, and no bacteria were recovered from their blood. This confirmed what Griffith already knew: Only living type S *S. pneumoniae* caused lethal infections. Something interesting happened when Griffith mixed living type R with heat-killed type S, however: Mice injected with this mixture died, and

virulent type S bacteria were recovered from their blood. An unknown agent apparently transformed avirulent type R into virulent type S. Griffith called the agent the “transforming principle.” It was his belief that the transforming principle was a protein.

Sixteen years later, in 1944, bacteriologists Oswald Avery, Colin MacLeod, and Maclyn McCarty designed an experiment that showed conclusively that the transforming principle was DNA rather than protein. They showed that R bacteria could be transformed to S bacteria in a test tube. They then progressively purified their extract until only proteins and the two nucleic acids, RNA and DNA, remained. They placed some of the mixture onto agar plates (glass dishes containing a gelatin growth medium). At this point, transformation still occurred; therefore, it was clear that one of these three molecules was the transforming agent. They treated their extract with protein-degrading enzymes, which denatured (destroyed) all the proteins in the extract. Despite the denaturing of the proteins, transformation still occurred when some of the extract was plated; had protein been the transforming agent, no transformation could have occurred. Protein was eliminated as the transforming agent. The next step was to determine which of two nucleic acids was responsible for the transformation of the R strain into the S strain. They introduced RNase, an enzyme that degrades RNA, to the extract. The RNA was destroyed, yet transformation took place. RNA was thus eliminated. At this point, it was fairly obvious that DNA was the transforming agent. To conclusively confirm this, they introduced DNase to the extract. When the DNA was degraded by the enzyme, transformation did not take place, showing that DNA was the transforming agent.

Another way that genetic material can be exchanged between bacteria is by transduction. Transduction requires the presence of a bacteriophage (a virus that infects bacteria). A virus is a simple structure consisting of a protein coat called a capsid that contains either RNA or DNA. Viruses are acellular, nonliving, and extremely small. To reproduce, they must infect living cells and use the host cell's internal structures to replicate their genetic material and

manufacture viral proteins. Bacteriophages, or phages, infect bacteria by attaching themselves to a bacterium and injecting their genetic material into the cell. Sometimes, during the assembly of new viral particles, a piece of the host cell's DNA may be enclosed in the viral capsid. When the virus leaves the host cell and infects a second cell, that piece of bacterial DNA enters the second cell, thus changing its genetic makeup. Generalized transduction (the transfer of a gene from one bacterium to another) was discovered by Joshua and Esther Lederberg and Norton Zinder in 1952. Using *E. coli* and a bacteriophage called *PI*, the Lederbergs and Zinder were able to show that transduction could be used to map genes to the bacterial chromosome.

Hershey-Chase Bacteriophage Experiments

The use of bacteriophages has been instrumental in confirming DNA as the genetic material of living cells. Alfred Hershey and Martha Chase devised a series of experiments using *E. coli* and the bacteriophage *T2* that conclusively established DNA as genetic material in 1953. Bacteria are capable of manufacturing all essential macromolecules by utilizing material from their environment. Hershey and Chase grew cultures of *E. coli* in a growth medium enriched with a radioactive isotope of phosphorus, phosphorus 32. DNA contains phosphorus; as the succeeding generations of bacteria pulled phosphorus from the growth medium to manufacture DNA, each DNA strand also carried a radioactive label. *T2* phages were used to infect the cultures of *E. coli*. When the new *T2* viruses were assembled in the bacterial cells, they too carried the radioactive label phosphorus 32 on their DNA. A second culture of *E. coli* was grown in a medium enriched with radioactive sulfur 35. Proteins contain sulfur (but no phosphorus). *T2* viruses were used to infect this culture. New viruses contained the sulfur 35 label on their protein coats.

Since the *T2* phage consists of only protein and DNA, one of these two molecules had to be the genetic material. Hershey and Chase infected unlabeled *E. coli* with both types of radioactive *T2* phages. Analysis has shown that the

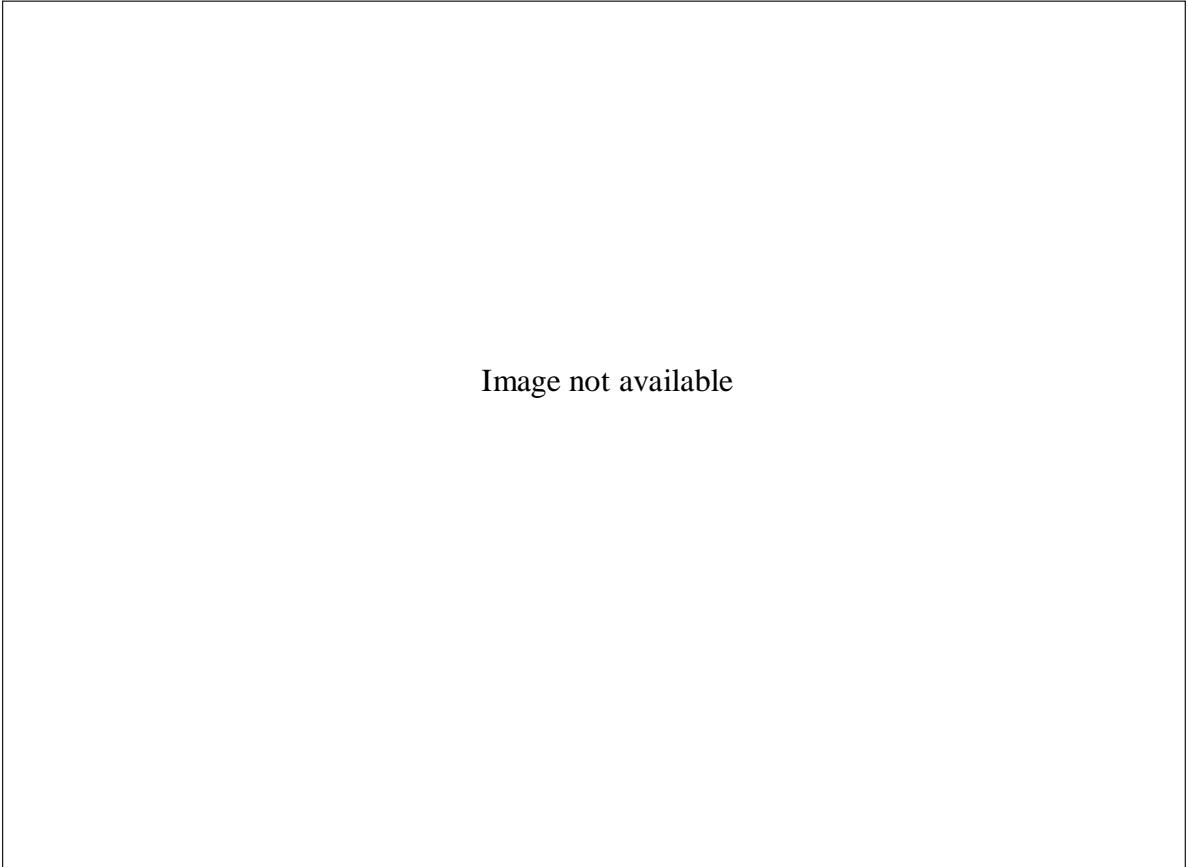


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An example of a bacterial colony formed by a pathogenic strain of *Escherichia coli*, displayed by microbiologist Jay Lewis shortly after an outbreak of food poisoning in Washington state in 1996. (AP/Wide World Photos)

phosphorus 32 label passed into the bacterial cells, while the sulfur 35 label was found only in the protein coats that did not enter the cells. Since the protein coat did not enter the bacterial cell, it could not influence protein synthesis. Therefore, protein could not be the genetic material. The Hershey-Chase experiment confirmed DNA as the genetic material.

Restriction Enzymes and Gene Expression

Using the aforementioned methods, it has been possible to construct a complete genetic map showing the order in which genes occur on the chromosome of *E. coli* and other bacteria. Certain genes are common to all bacteria. There are also several genes that are shared by bacteria and higher life-forms, including humans. Further research showed that genes can be either inserted into or deleted from bacte-

rial DNA. In nature, only bacteria contain specialized enzymes called restriction enzymes. Restriction enzymes are capable of cutting DNA at specific sites called restriction sites. The function of restriction enzymes in bacteria is to protect against invading viruses. Bacterial restriction enzymes are designed to destroy viral DNA without harming the host DNA. Hundreds of different restriction enzymes have been isolated from bacteria, and each is named for the bacterium from which it comes. The discovery and isolation of restriction enzymes led to a new field of biological endeavor: genetic engineering.

Use of these enzymes has made gene cloning possible. Cloning is important to researchers because it permits the detailed study of individual genes. Restriction enzymes have also been used in the formation of genomic libraries (a

collection of clones that contains at least one copy of every DNA sequence in the genome). Genomic libraries are valuable because they can be searched to identify a single DNA recombinant molecule that contains a particular gene or DNA sequence.

Bacterial studies have been instrumental in understanding the regulation of gene expression, or the translation of a DNA sequence first to a molecule of messenger RNA (mRNA) and then to a protein. Bacteria live in environments that change rapidly. To survive, they have evolved systems of gene regulation that can either “turn on” or “turn off” a gene in response to environmental conditions. François Jacob and Jacques Monod discovered the *lac* operon, a regulatory system that permits *E. coli* to respond rapidly to changes in the availability of lactose, a simple sugar. Other operons, such as the tryptophan operon, were soon discovered as well. An operon is a cluster of genes whose expression is regulated together and involves the interaction of regions of DNA with regulatory proteins. The discovery of operons in bacteria led to searches for them in eukaryotic cells. While none has been found, several other methods of regulating the expression of genes in eukaryotes have been described.

Impact and Applications

Diabetes mellitus is a disease caused by the inability of the pancreas to produce insulin, a protein hormone that is part of the critical system that controls the body’s metabolism of sugar. Prior to 1982, people who suffered from diabetes controlled their disease with injections of insulin that had been isolated from other animals, such as cows. In 1982, human insulin became the first human gene product to be manufactured using recombinant DNA. The technique is based on the knowledge that genes can be inserted into the bacterial chromosome; that once inserted, the gene product, or protein, will be produced; and that once produced, the protein can be purified from bacterial extracts. Human proteins are usually produced by inserting a human gene into a plasmid vector, which is then inserted into a bacterial cell. The bacterial cell is cloned until large quantities of transformed bacteria are

produced. From these populations, human proteins, such as insulin, can be recovered.

Many proteins used against disease are manufactured in this manner. Some examples of recombinant DNA pharmaceutical products that are already available or in clinical testing include atrial natriuretic factor, which is used to combat heart failure and high blood pressure; epidermal growth factor, which is used in burns and skin transplantation; factor VIII, which is used to treat hemophilia; human growth hormone, which is used to treat dwarfism; and several types of interferons and interleukins, which are proteins that have anticancer properties.

Bacterial hosts produce what are called the “first generation” of recombinant DNA products. There are limits to what can be produced in and recovered from bacterial cells. Since bacterial cells are different from eukaryotic cells in a number of ways, they cannot process or modify most eukaryotic proteins, nor can they add sugar groups or phosphate groups, additions that are often required if the protein is to be biologically active. In some cases, human proteins produced in prokaryotic cells do not fold into the proper three-dimensional shape; since shape determines function in proteins, these proteins are nonfunctional. For this reason, it may never be possible to use bacteria to manufacture all human proteins. Other organisms are used to produce what are called the second generation of recombinant DNA products.

The impact of the study of bacterial structures and genetics and the use of bacteria in biotechnology, cannot be underestimated. Bacterial research has led to the development of an entirely new branch of science, that of molecular biology. Much of what is currently known about molecular genetics, the expression of genes, and recombination comes from research involving the use of bacteria. Moreover, bacteria have had and will continue to have applications in the production of pharmaceuticals and the treatment of disease. The recombinant DNA technologies developed with bacteria are now being used with other organisms to produce medicines and vaccines.

—Kate Lapczynski, updated by Bryan Ness

See also: Archaea; Bacterial Resistance and Super Bacteria; Biopharmaceuticals; Cholera; Chromosome Walking and Jumping; Cloning; Diabetes; Diphtheria; Gene Regulation: Bacteria; Gene Regulation: *LacOperon*; Gene Regulation: Viruses; Genetic Code, Cracking of; Lateral Gene Transfer; Model Organism: *Escherichia coli*; Molecular Genetics; Plasmids; Restriction Enzymes; Transposable Elements.

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Bacterial Resistance and Super Bacteria

Field of study: Bacterial genetics

Significance: *Antibiotic-resistant bacteria have become a significant worldwide health concern. Some strains of bacteria (called super bacteria) are now resistant to most, if not all, of the available antibiotics and threaten to return health care to a preantibiotic era. Understanding how and why bacteria become resistant to antibiotics may aid treatment, the design of future drugs, and efforts to prevent other bacterial strains from becoming resistant to antibiotics.*

Key terms

ANTIMICROBIAL DRUGS: chemicals that destroy disease-causing organisms without damaging body tissues; chemicals made naturally by bacteria and fungi are also known as antibiotics

PLASMIDS: small, circular pieces of DNA that can exist separately from the bacterial chromosome; plasmids can be transferred among bacteria, and they may carry more than one R factor

RESISTANCE FACTOR (R FACTOR): a piece of DNA that carries a gene encoding for resistance to an antibiotic

TRANSPOSONS: also known as jumping genes, transposons are pieces of DNA that carry R factors and can integrate into a bacterial chromosome; they are also responsible for the spread of drug resistance in bacteria and fungi, and, like plasmids, each transposon may carry more than one R factor

History of Antibiotics

Throughout history, illnesses such as cholera, pneumonia, and sexually transmitted diseases have plagued humans. However, it was not until the early twentieth century that antibiotics were discovered. Until then, diseases such as diphtheria, cholera, and influenza were serious and sometimes deadly. With the advent of the antibiotic era, it appeared that common infectious diseases would no longer be a serious health concern. A laboratory accident led to the discovery of the first mass-produced anti-

biotic. In 1928, Scottish bacteriologist Alexander Fleming grew *Staphylococcus aureus* in petri dishes, and the plates became contaminated with a mold. Before Fleming threw out the plates, he noticed that there was no bacterial growth around the mold. The mold, *Penicillium notatum*, produced a substance that was later called penicillin, which was instrumental in saving the lives of countless soldiers during World War II. From the 1950's until the 1980's, antibiotics were dispensed with great regularity for most bacterial infections, for earaches, for colds, and as a preventive measure.

As the twentieth century progressed, however, it became apparent that the initial promise of antibiotics was mitigated by the ability of microorganisms to evolve quickly, given their relatively short life spans. Emerging infectious diseases such as multidrug-resistant tuberculosis, vancomycin-resistant enterococci, and penicillin-resistant gonorrhea became serious global health care concerns. The problem was exacerbated by the seemingly haphazard dispensing of antibiotics for viral infections (against which antibiotics are ineffective, although often prescribed as a hedge against secondary infections or simply to palliate patients).

The Rise of Bacterial Resistance

On average, bacteria can replicate every twenty minutes. Several generations of bacteria can reproduce in a twenty-four-hour period. This quick generation time leads to a rapid adaptation to changes in the environment. English naturalist Charles Darwin's *On the Origin of Species by Means of Natural Selection* (1859) first explained the theory of natural selection, the process whereby this adaptation occurs. If an organism has an advantage over other organisms (such as the ability to grow in the presence of a potentially harmful substance), that organism will survive to pass that characteristic on to its offspring while the other organisms die. The emergence of antibiotic-resistant bacteria is an excellent example of Darwin's theory of natural selection at work.

In the early twentieth century, German microbiologist Paul Ehrlich coined the term "magic bullet" in reference to chemotherapy

(the treatment of disease with chemical compounds). For a drug such as an antibiotic to be a “magic bullet,” it must have a specific target that is unique to the disease-causing agent and cannot harm the host in the process of curing the disease. In 1910, Ehrlich discovered that arsphenamine (Salvarsan), a derivative of arsenic, could be used to treat syphilis, a common sexually transmitted disease in the early twentieth century. Until that time syphilis had no known cure. The use of Salvarsan did cure some patients of syphilis, but, since it was a rat poison, it killed other patients. Generally speaking, antimicrobials have specific targets (or modes of action) within bacteria. They target the following structures or processes: synthesis of the bacterial cell wall, injury to the plasma membrane, and inhibition of synthesis of proteins, DNA, RNA, and other essential metabolites (all of these substances are building blocks for the bacteria). A good antibiotic will have a target that is unique to the bacteria so the host (the patient) will not be harmed by the drug.

Bacteria and fungi are, of course, resistant to the antibiotics they naturally produce. Other bacteria have the ability to acquire resistances to antimicrobials, and this drug resistance occurs either through a mutation in the DNA or resistance genes on plasmids or transposons. Plasmids are small, circular pieces of DNA that can exist within or independently of the bacterial chromosome. Transposons, or “jumping genes,” are pieces of DNA that can jump from one bacterial species to another and be integrated into the bacterial chromosome. The spread of plasmids and transposons that carry antibiotic resistance genes has led bacteria to become resistant to many, if not all, currently available antibiotics.

Several antimicrobial resistance mechanisms allow bacteria to become drug resistant. The first mechanism does not allow the drug to enter the bacterial cell. A decrease in the permeability of the cell wall will inhibit the antimicrobial drug from reaching its target. An alteration in a penicillin-binding protein (pbp), a protein found in the bacterial cell wall, will allow the cell to “tie up” the penicillin. Also, the pores in the cell wall can be altered so the drug cannot pass through. A second strategy is to pump the

drug out of the cell after it has entered. Such systems are found in pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These pumps are usually nonspecific and can cause bacteria to become resistant to more than one antibiotic at a time. Another method of resistance is through chemical modification of the drug. Penicillin is inactivated by breaking a chemical bond found in its ring structure. Other drugs are inactivated by the addition of other chemical groups. Finally, the target of the drug can be altered in such a manner that it is no longer affected by the drug. For example, *Mycobacterium tuberculosis*, which causes tuberculosis, became resistant to the drug rifampin by altering the three-dimensional structure of a specific protein.

Antibiotic Misuse and Drug Resistance

The misuse of antibiotics over several decades has caused many strains of bacteria to become resistant. For some bacterial infections, only one or no effective drugs are available for treatment. Many different factors of misuse, overuse, and abuse of antibiotics have led to drug-resistant diseases. Perhaps one of the most important factors in the emergence of drug-resistant bacteria is the overprescription or inappropriate use of antibiotics. Another major factor is misuse by the patient. After several days of taking an antibiotic, a patient may begin to feel better and decide not to finish all of the prescription. By not completing the full course of treatment, the patient merely kills the bacteria that are sensitive to the antibiotic, leaving the resistant bacteria to grow, multiply, pass on their resistant genes, and cause the same infection. This time, another antibiotic (if there is one available that is effective) must be used.

Another contributing factor is the ease with which the newest and best antibiotics may be obtained in many countries. In several countries in Central America, for example, one can walk into the local pharmacy and receive any antibiotic without a prescription. Another factor in the worldwide spread of drug-resistant infectious diseases is the ease of travel. Infected people can carry bacteria from one continent to another in a matter of hours and infect anyone with whom they come in contact.

The use of antibiotics is not limited to humans. They also play an important role in agriculture. Antibiotics are added to animal feed on farms to help keep herds healthy, and they are also used on fish farms for the production of fish for market. Antibiotics are used to treat domestic animals such as cats, dogs, birds, and fish and are readily available in pet stores to clear up fish aquariums. This widespread use of antibiotics allows bacteria in all environmental niches the possibility of becoming resistant to potentially useful drugs.

Emerging Resistant Infections and Super Bacteria

The misuse of antibiotics over the decades has led to more infectious diseases becoming resistant to the current arsenal of drugs. Some diseases that could be treated effectively in the 1970's and 1980's can no longer be controlled with the same drugs. Two very serious problems have emerged: vancomycin-resistant enterococci and multidrug-resistant tuberculosis. The enterococcus is naturally resistant to many types of antibiotics, and the only effective treat-

Multiple-Resistant Bacteria

They lurk in schools, nursing homes, and hospitals—perhaps even in your home. Often, you cannot see them to avoid them. Increasingly, they are a global health problem. What are these unseen purveyors of disease? Antibacterial soaps.

Antibacterial soaps contain antibacterials, a subclass of antimicrobials, which kill or inhibit the growth of bacteria and other microorganisms. Antiseptics are antimicrobial agents that are sufficiently nontoxic to be applied to human tissue. Antibiotics are chemicals that inhibit a specific pathway or enzyme in a bacterium and are critical to the treatment of a bacterial infection. When bacteria are exposed to sublethal concentrations of an antibiotic, resistance can develop through the elimination of normal bacteria, allowing the resistant ones to survive and reproduce. The question has been whether exposure to antibacterial products can promote antibiotic resistance. The answer is that the use of antibacterial products may actually *increase* the prevalence of antibiotic-resistant bacteria.

Antibiotic resistance is irreversible and unavoidable, due to the selective pressure on bacteria to become resistant. This selection is in large part a result of the widespread use of antibiotics to increase growth rates in livestock, as well as unnecessary and improper use of antibiotics to restore and maintain human health. The indiscriminate use or overuse of antibiotics has been widely blamed for the appearance of so-called super bacteria—bacteria that are unaffected by more than one antibiotic. In addition, a widely used antibacterial agent found in toothpaste, kitchen utensils and appliances, clothing, cat litter, and toys could cause resistant strains of bacteria to develop.

Triclosan is a good example of the potent antibacterial and antifungal agents that are increasingly used to produce “germ-free” consumer products. Until recently, triclosan was considered a broad-spectrum antiseptic rather than a true antibiotic. As a general biocide, triclosan was not expected to have a specific target in the bacterial cell. However, Stuart Levy and his colleagues at Tufts University School of Medicine determined that triclosan specifically interferes with an enzyme important in the synthesis of plasma membrane lipids. As triclosan kills off normal bacteria, it could make way for the growth of strains with triclosan-insensitive enzymes. More troubling, one of the front-line antibiotics commonly used to treat tuberculosis, isoniazid, targets the same enzyme, raising the possibility that the use of triclosan will lead to new drug-resistant strains of *Mycobacterium tuberculosis*.

Consumers are convinced that use of products with antimicrobial chemicals will lower their risk of infection. While this has not been demonstrated scientifically, effective handwashing has been demonstrated to prevent illness. However, the key to effective handwashing is the length of time (15-30 seconds) spent scrubbing, not the inclusion of antibacterials in the soap. Regular soap, combined with scrubbing action, physically dislodges and removes microorganisms. The constant exposure of bacteria to sublethal concentrations of triclosan promotes development of resistance; the substitution of antibacterial soap for proper handwashing techniques will eventually render triclosan ineffective. In the battle of the soaps, “plain” wins.

—Laurie F. Caslake

ment has been vancomycin. With the appearance of vancomycin-resistant enterococci, however, there are no reliable alternative treatments. The fear that vancomycin resistance will spread to other bacteria such as *staphylococci* seems well founded: A report from Japan in 1997 indicated the existence of a strain of *staphylococcus* that had become partially resistant to vancomycin. If a strain of methicillin-resistant *Staphylococcus aureus* (MRSA) also becomes resistant to vancomycin, there will be no effective treatment available against this super bacterium.

A second problem is the appearance of multidrug-resistant tuberculosis. *Mycobacterium tuberculosis* is a slow-growing bacterium that requires a relatively long course of antibiotic therapy. Tuberculosis (TB) is spread easily, and it is a deadly disease. In the United States in 1900, tuberculosis was the number-one cause of death. In the 1990's, it was still a leading cause of death worldwide. Treatment of multidrug-resistant tuberculosis requires several antibiotics taken over a period of at least six months, with a success rate of approximately 50 percent; on the other hand, susceptible strains of TB have a cure rate of nearly 100 percent.

Another contributing factor to the emergence of drug-resistant infectious diseases is the lack of basic knowledge about some bacteria. Funding for basic genetic research on tuberculosis was reduced dramatically in the mid-twentieth century when it appeared that TB would be eradicated just as smallpox had been. The appearance of multidrug-resistant tuberculosis caught scientists and physicians unprepared. Little was known about the genetics of tuberculosis or how drug resistance occurred.

Another concern about drug-resistant infections is how to control them. Hospitals are vigilant, and, in some cases, very proactive in screening for drug-resistant infections. People can be asymptomatic carriers (that is, they carry the disease-causing organism but are still healthy) of a disease such as methicillin-resistant *Staphylococcus aureus* and could infect other people without knowing it. The role of the infection-control personnel is to find the source of the infection and remove it.

Impact and Applications

There is little encouraging news about the availability of new antibiotics. The crisis of super bacteria has altered the view that few new antibiotics would be needed. Pharmaceutical companies are scrambling to discover new antimicrobial compounds and modify existing antibiotics. Policy decisions of the 1970's and 1980's requiring more and larger clinical trials for antibiotics before they are approved for use by the Food and Drug Administration have increased the price of antibiotics and the amount of time it takes to market them. It may take up to ten years from the time of "discovery" for an antibiotic to be approved for use. The scientific community has therefore had to meet the increase of drug-resistant bacterial strains with fewer and fewer new antibiotics.

The emergence of antibiotic-resistant bacteria and super bacteria is a serious global health concern that will lead to a more prudent use of available antibiotics. It has also prompted pharmaceutical companies to search for potentially new and novel antibiotics in the ocean depths, outer space, and other niches. "Rational" drug design—or RDD, drug design based on knowledge of how bacteria become drug resistant—will also be important. Exactly how scientists and physicians will be able to combat super bacteria is a question that remains to be answered. Until a more viable solution is found, prudent use of antibiotics, surveillance of drug-resistant infections, and well-orchestrated worldwide monitoring and containment of emerging diseases appear to be the answers.

—Mary Beth Ridenhour

See also: Archaea; Bacterial Genetics and Cell Structure; Chromosome Walking and Jumping; DNA Replication; Emerging Diseases; Gene Regulation: Bacteria; Gene Regulation: *Lac Operon*; Lateral Gene Transfer; Mutation and Mutagenesis; Model Organism: *Escherichia coli*; Natural Selection; Plasmids; Transposable Elements.

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Web Site of Interest

National Center for Infectious Diseases. <http://www.cdc.gov/ncidod>. Searchable on “antibiotic resistance” and other keywords to provide access to research articles.

Behavior

Field of study: Population genetics

Significance: *One of the long-standing questions pondered by biologists is, to what extent do genes control the way we behave? By the mid-1990's, researchers had identified human genes that had been linked to such behavioral characteristics as depression, homosexuality, schizophrenia, and alcoholism; however, such findings were complicated by methodological questions and by the problem of distinguishing between the effects of genetic and environmental factors.*

Key terms

EUGENICS: a process in which negative genetic traits are removed from the population and

positive genetic traits are encouraged, by controlling, in some manner, who is allowed to reproduce

GENOME: the entire set of genes required by an organism; a set of chromosomes

HERITABILITY: the probability that a specific gene or trait will be passed from parent to offspring, rendered as a number between zero and 100 percent, with zero percent being not heritable and 100 percent being completely heritable

LINKAGE: a relation of gene loci on the same chromosome; the more closely linked two loci are, the more often the specific traits controlled by these loci are expressed together

NEUROTRANSMITTER: a chemical messenger that transmits a nerve impulse between neurons

Brain Biology

As the first organ system to begin development and the last to be completed, the vertebrate nervous system—brain, spinal cord, and nerves—with the brain at the control, remains something of an enigma to biologists. It is based on neurons, special cells that generate and transmit bioelectrical impulses. The vertebrate brain consists of as many as three major areas: the brain stem, the cerebellum, and the cerebrum. A reptilian brain consists of only the brain stem, while the mammalian brain has all three, including a well-developed cerebrum (the two large hemispheres on top). The brain stem controls basic body functions such as breathing and heart rate, while the cerebrum is the ultimate control center. Consisting of billions of neurons (commonly called brain cells), the cerebrum controls functions such as memory, speech, hearing, vision, and analytical skills.

The brain is an exceedingly complex network of billions of neurons. As messages enter the brain stem from the spinal cord, groups of neurons either respond directly or transfer information to higher levels. In order to communicate with other neurons, each individual neuron generates impulses much like the impulse that carries a voice over a telephone line, and this message travels from the beginning to the

end of each neuron. At the end of one neuron and the beginning of the next in line, a small open space occurs. This space is filled with fluids, and the message is carried across to the next neuron by a chemical known as a neurotransmitter. Neurotransmitters may be of several biochemical classifications, including acetylcholines, amines, amino acids, and peptides. An individual neuron and an entire neuronal circuit may fire or not fire an impulse based on the messages carried by these neurotransmitters. For example, the signal for pain is transmitted from neuron to neuron by a peptide-based neurotransmitter known as substance P, while another peptide transmitter (endorphin) acts as a natural painkiller. Thought, memory, and behavior, then, are produced by the activity along neuronal circuits. A genetic link occurs here, since neurotransmitters are expressed either directly or indirectly based on information in genes.

By birth, the collection of approximately 21,200 genes in humans has directed the development of the nervous system. At birth, the brain consists of approximately 100 billion neurons and trillions of supporting glial cells to protect and nourish neurons. However, the intricate wiring between these neurons is yet to be determined. Studies from the 1980's and 1990's suggested that the critical networking and circuit formation between these billions of neurons that control later brain function are determined not from genes but from environmental input and experiences from birth until the brain is fully developed around age seven.

Genes and Behavior

Genes make proteins, and proteins cause biochemical responses in cells. The behavior of an animal takes place under the combined influences of its genes, expressed through the actions of proteins, and its environment. A good example is the phenomenon of mating seasons in many animals. As day length gradually increases toward spring and summer, a critical length is reached that signals the release of hormones that result in increased sexual activity, with the ultimate goal of seasonal mating. The production and activity of hormones involve genes or gene products. If the critical number

of daylight hours is not reached, the genes will not be activated, and sexual behavior will not increase.

Each neuron making up the intricate networks and circuits throughout the cerebrum (80 percent of the human brain) has protein receptors (chemoreceptors) that respond to specific signaling molecules. The production of the receptors and signaling molecules used for any type of brain activity is directly tied to genes. A slightly different gene may lead to a slightly different signaling molecule or receptor and thus a slightly different cell (neuron) response. A larger difference among genes may lead to a larger difference among signaling molecules or receptors and thus a larger variation in cell response. Since human behavior involves the response of neurons and neuron networks in the brain to specific signals, and since the response of neurons occurs because of the interaction between a signaler and a receptor built by specific genes, the genetic link seems straightforward: input, signal, response, behavior. However, when the slight variations between genes are added to the considerable variation among noncoding or regulatory sequences of DNA, the genetic connection to behavior becomes much less direct. Since a gene is under the control of one or several regulatory sequences that in turn may be under the control of various environmental inputs, the amount of genetic variation among individuals is compounded by two other critical factors: the environmental variations under which the brain develops and the daily environmental variations to which the individual is exposed. A convenient way to think of genetics and behavior is to consider that genes simply allow humans to respond to a specific stimulus by building the pathway required for a response, while behavior is defined by the degree and the manner of human response.

Eugenics

The concept of eugenics was born during the evolution and study of basic genetics in the early twentieth century. Eugenics is the categorization of a specific human behavior to an underlying genetic cause. Human characteristics such as alcoholism and laziness were thought

to be caused entirely by inherited genes. Since then, research has provided a much clearer picture of a genetic-behavior link. People inherit specific genes to build specific pathways that allow them to respond in certain ways to environmental input. With variations possible—from the gene to gene regulators to the final cellular response—it is virtually impossible to disconnect the “nature vs. nurture” tie that ultimately controls human behavior. Genes are simply the tools by which the environment shapes and reshapes human behavior. There is a direct correlation between gene and protein: Change the gene, change the protein. However, there is no direct correlation between gene and behavior: Changing the gene does not necessarily change the behavior. Behavior is a multifaceted, complex response to environmental influences that is only partially related to genetic makeup. Most studies conducted on humans based on twin and other relative data suggest that most behavioral characteristics have between a 30 and 70 percent genetic basis, leaving considerable room for environmental influence. For example, studies of twins indicate that homosexuality may be as much as 50 percent genetic, leaving 50 percent under environmental control.

Another important fact is that almost no behaviors are controlled by a single gene locus, and the more complex the behavior, the more likely that it is controlled by several to many genes. Hence, not only do environmental effects cloud the picture; each gene involved in more complex behavioral traits represents just a small part of the genetic basis for the trait. The study of the genetic basis for complex traits, therefore, involves the search for quantitative trait loci (QTLs), rather than for single genes. Searching for QTLs requires that a large number of genetic markers be identified in the human genome, and the Human Genome Project has provided numerous such markers. A QTL is identified by looking for “linkage” between a specific genetic marker and the trait being studied. Linkage occurs when a marker is close to one of the genes that control the trait. Practically speaking, this means that individuals with the behavioral trait have the marker, and those who do not have the trait lack the

marker. Thus, geneticists are not directly identifying the genes involved, but are identifying the approximate locations of the genes. Unfortunately, the more genes control a trait, the harder it is to identify QTLs. Environmental effects can also mask the existence of QTLs, causing some people to have the trait that lack a QTL and others to lack the trait but have a QTL. In spite of these difficulties, QTLs have been identified for a number of behavioral traits, such as aggression, depression, and a number of other mental disorders.

Single-Gene Behavioral Traits

Although behavioral traits controlled by a single gene have been identified, they probably require interaction with other genes in order to produce the specific characteristics of the behavior. On top of this are laid environmental effects. The most dramatic case of a single gene that controls a complex behavior was the discovery in 2002 of the gene that controls honeybee social status. This same gene is found in fruit flies and affects how actively fruit flies seek food. Bees with a more actively expressed form of the gene (called the *for* gene) were much more likely to forage than bees with a less active *for* gene. Not surprisingly, the *for* gene produces a protein that acts as a cell-signaling molecule.

In humans, only a few behavioral traits are clearly controlled by a single gene. The best examples include Huntington’s disease (a rare, autosomal dominant gene), early-onset Alzheimer’s disease (also a rare, autosomal dominant gene), and fragile X syndrome (actually involves two genes). The remaining traits, discussed below, actually represent multigene traits where one primary QTL has been identified as primarily responsible.

Several genes were identified during the late 1980’s and early to mid-1990’s with possible direct behavioral links. A gene has been identified that seems to be involved in neurotic behaviors associated with anxiety, depression, hostility, and impulsiveness. This gene produces a protein that transports a chemical called serotonin, across neuronal membranes. Serotonin is a neurotransmitter and is the chemical that is affected by the antidepressant drug Prozac and

other serotonin reuptake inhibitors (SSRIs). Scientists have also identified a gene that may be related to schizophrenia and a gene that may determine how well alcohol is cleared from the brain after overindulgence.

One of the more recent, and in some ways controversial, discoveries involved a gene for antisocial behavior (ASB). The study, started in 1972, followed the lives of 1,037 boys from birth. Children who grew up in abusive environments were more likely to display antisocial behavior later, which is not a surprise. However, about half of the boys were found to have lower levels of an enzyme called monoamine oxidase A (MAOA), which is involved in the metabolism of several neurotransmitters. The boys with the lower MAOA activity were twice as likely to have been diagnosed with conduct disorder and were three times more likely to have been convicted of a violent crime by age twenty-six. It should be noted that lower MAOA activity alone was not enough; the boys also had to be exposed to abusive upbringings. Although the link seems strong, it has not been proved, and further study is being conducted.

A better understanding of single-gene behavioral traits could open the way to better treatment and more accurate diagnoses, but it also opens the potential for discrimination. This is especially the case for traits like antisocial behavior. Those who test for low MAOA activity might be incarcerated more readily by society or be punished differently if they are considered beyond rehabilitation. To avoid such dangers, society needs to be educated better about the interplay between genes and environment. Instead of punishing offenders more severely, earlier intervention, as early as childhood, might prevent later problems. It may also be possible to stabilize MAOA activity to near-normal levels in those who have inherently low activity levels. For most behavioral traits, though, such concerns are probably unwarranted. Because most behavioral traits are controlled by many genes, all interacting with the environment, diagnosis will probably never be possible. Yet, when genetic details of the QTLs that have been discovered are learned, therapies might be developed to offset their potential harm.

Multiple-Gene Behavioral Traits

Most geneticists concede that for many behavioral traits it may never be possible to sort out the details of the underlying genetic causes. Some genes may play such a minor role that the search for some QTLs will be fruitless. Nevertheless, geneticists have been able to discover QTLs for some important behavioral traits, and the heritability of a number of traits has been determined. The better data available from the Human Genome Project has spawned a new field of study called behavioral genomics.

Some traits, such as IQ, may never be fully understood from a genetic perspective. Heritability of IQ is high, but environment also plays an important role, and numerous genes are likely involved. More success has come from focusing on specific disorders. For example, four genes have been identified so far that are involved in attention deficit hyperactivity disorder. Other QTLs have been identified in some studies but have not been found in others. This shows one of the frustrating aspects of studying the genetics of behavior. QTLs identified using one set of data will not be supported by another set of data. This may be true because such QTLs play such a small part in developing the trait that they are undetectable under certain environmental conditions. Genes and QTLs for dyslexia, schizophrenia, and homosexuality have also been discovered. The study on homosexuality has been controversial, because the QTLs discovered by one set of researchers have never been successfully identified by anyone else.

For the most complex human traits QTLs still await discovery, but twin studies have given some insights. Twin studies involve comparing the traits of identical twins that were separated from birth. The assumption is that, because they have been raised in different environments, any traits they share will be primarily due to genetics rather than environment. A study of Swedish men showed that heritability of cognitive (thinking) ability was 62 percent, while spatial ability was 32 percent. Heritability of other personality traits fell somewhere between these values. Although these kinds of studies are interesting, they may be misleading.

Consequently, a number of geneticists criticize such research, especially twin studies, which have some inherent statistical problems. Such studies can also lead to misunderstandings, especially by nonscientists, who often interpret the numbers incorrectly. Saying that cognitive ability has a 62 percent heritability does not mean that a child has a 62 percent chance of being as intelligent as his or her parents, but rather that, of the factors involved in determining a person's intelligence, genetics accounts for approximately 62 percent of the observed variation in the population.

The Future of Behavioral Genetics

Researchers are actively seeking additional and stronger links between behavior and genetics, but even when such links are found, the degree to which a particular gene is involved and the amount of variation among humans may be hard to uncover. The Human Genome Project has greatly accelerated the search for the genetic bases of behavior, but with these new data has come an even clearer realization of the complexity of the connections between genes and human behavior. If nothing else, the future should hold more precise answers to the long-standing questions about what makes human beings who they are. The current understanding makes it clear that behavior is determined neither solely by genes nor solely by the environment. Continuing studies should make the relative contributions of genes and environment more understandable.

—W. W. Gearheart, updated by Bryan Ness

See also: Aggression; Alcoholism; Altruism; Biological Clocks; Biological Determinism; Criminality; Developmental Genetics; Eugenics; Gender Identity; Genetic Engineering; Medical Applications; Genetic Engineering; Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing; Ethical and Economic Issues; Heredity and Environment; Homosexuality; Human Genetics; Inbreeding and Assortative Mating; Intelligence; Klinefelter Syndrome; Knockout Genetics and Knockout Mice; Miscegenation and Antimiscegenation Laws; Natural Selection; Sociobiology; Steroid Hormones; Twin Studies; XYY Syndrome.

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Clark, William R., and Michael Grunstein. *Are We Hardwired? The Role of Genes in Human Behavior.* New York: Oxford University Press, 2000. Explores the nexus of modern genetics and behavioral science, revealing that few elements of behavior depend upon a single gene; instead, complexes of genes, often across chromosomes, drive most of human heredity-based actions. Asserts that genes and environment are not opposing forces but work in conjunction.

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Web Sites of Interest

American Association for the Advancement of Science, Behavioral Genetics Project. <http://www.aaas.org/spp/bgenes/meetings.shtml>. Human Genome Project Information, Behavioral Genetics. http://www.ornl.gov/tech/resources/human_genome/elsi/behavior.html. This site includes information on the basics of behavioral genetics and links to related resources.

National Institute of Mental Health, Center for Genetic Studies. <http://zork.wustl.edu/nimh>. A technical site on the collecting of clinical data to help determine the possible genetic bases of certain mental disorders.

Biochemical Mutations

Fields of study: Human genetics and social issues; Molecular genetics

Significance: *The study of the biochemistry behind a particular phenotype is often necessary to understand the modes of inheritance of mutant genes. Knowledge of the biochemistry of mutant individuals is especially useful in determining treatments for genetic diseases.*

Key terms

ALLEL: a form of a gene at a specific gene locus; a locus in an individual organism typically has two alleles

BIOCHEMICAL PATHWAY: the steps in the production or breakdown of biological chemicals in cells; each step usually requires a particular enzyme

GENOTYPE: the genetic characteristics of a cell or organism, expressed as a set of symbols representing the alleles present

HETEROZYGOUS: a genotype in which a locus has two alleles that are different

HOMOZYGOUS: a genotype in which a locus has two alleles that are the same

PHENOTYPE: expressed or visible characteristics of a genotype; different genotypes often are expressed as different phenotypes but may have the same phenotype

Proteins and Simple Dominant and Recessive Alleles

In order to understand how certain genotypes are expressed as phenotypes, knowledge of the biochemistry behind gene expression is essential. It is known that the various sequences of nitrogenous bases in the DNA of genes code for the amino acid sequences of proteins. How the proteins act and interact in an organism determines that organism's phenotype.

Simple dominant and recessive alleles are the easiest to understand. For example, in the genetic disease phenylketonuria (PKU), two alleles of the PKU locus exist: p^+ , which codes for phenylalanine hydroxylase, an enzyme that converts phenylalanine (a common amino acid in proteins) to tyrosine (another common amino acid); and p , which is unable to code for the functional form of the enzyme. Individuals with two normal alleles, p^+p^+ , have the enzyme and are able to perform this conversion. However, individuals with two abnormal alleles, pp , do not have any of this enzyme and are unable to make this conversion. Since phenylalanine is not converted to tyrosine, the phenylalanine accumulates in the organism and eventually forms phenylketones, which are toxic to the nervous system and lead to mental retardation. The heterozygote, p^+p , has one normal and one abnormal allele. These individuals have phenylalanine and tyrosine levels within the normal range, since the enzyme can be used over and over again in the conversion. In other words, even when there is only one normal allele present, there is enough enzyme produced for the conversion to proceed at the maximum rate.

Many other inborn errors of metabolism follow this same pattern. In the case of albinism, for example, afflicted individuals are missing the enzyme necessary to produce the brown-black melanin pigments. Galactosemics are missing an essential enzyme for the breakdown of galactose.

Other Single-Gene Phenomena

Many other genetic phenomena can be explained by looking at the biochemistry behind them. For example, the "chinchilla coat" mutation in rabbits causes a gray appearance in the

homozygous state, $c^{ch}c^{ch}$. This occurs because the c^{ch} allele codes for a pigment enzyme that is partially defective. The partially defective enzyme works much more slowly than the normal enzyme, and the smaller amount of pigment produced leads to the gray phenotype. When this allele is heterozygous with the fully defective allele, $c^{ch}c$, there is only half as much of an enzyme that works very slowly. As one might expect, there is less pigment produced, and the phenotype is an even lighter shade of gray called light chinchilla. The enzyme concentration does affect the rate of the reaction and, ultimately, the amount of product made. This phenomenon is known as incomplete, or partial, dominance. Genes for the red pigments in such flowers as four-o'clocks and snapdragons show incomplete dominance, as do the hair, skin, and eye pigment genes of humans and the purple pigment genes of corn kernels.

Sometimes a mutation occurs that creates an enzyme with a different function instead of creating a defective enzyme. The B allele in the ABO blood-group gene codes for an enzyme that adds galactose to a short sugar chain that exists on the blood cell's surface forming the B antigen. The A allele codes for an enzyme that adds N-acetylgalactosamine to the same previously existing sugar chain, forming the A antigen. Anyone with two B alleles, I^BI^B , makes only the B antigen and is type B. Those with two A alleles, I^AI^A , make only the A antigen and are type A. Heterozygotes, I^AI^B , have the enzymes to make both antigens, and they do. Since they have both antigens on their blood cells, they are classified as type AB. This phenomenon is known as codominance and is also seen in other blood-type genes.

Biochemistry can also explain other single-gene phenomena such as the pigmentation pattern seen in Siamese cats and Himalayan rabbits. The Siamese-Himalayan allele codes for an enzyme that is so unstable that it falls apart and is completely nonfunctional at the normal body temperature of most mammals. Only at cooler temperatures can the enzyme retain its stability and function. Since mammals have lower temperatures at their extremities, it is there that the enzyme produces pigment; at more centrally located body areas, it cannot

Image not available

Many genetic phenomena can be explained by looking at the biochemistry behind them. For example, the chinchilla coat in rabbits such as this one at the Dallas Zoo is caused by a mutant allele that, in the homozygous state, codes for a pigment enzyme that is partially defective. This partially defective enzyme works much more slowly than the normal enzyme, and the smaller amount of pigment produced leads to the gray phenotype. (AP/Wide World Photos)

function. This leaves a pattern of dark pigmentation on the tail, ears, nose, feet, and scrotum, with no pigmentation at other areas.

Multiple-Gene Phenomena

Few genes act completely independently, and biochemistry can be used to explain gene interactions. One simple interaction can be seen in fruit-fly eye pigmentation. There are two separate biochemical pathways to make pigment. One produces the red pteridines, and the other produces the brown omochromes. If *b* is an allele that cannot code for an enzyme necessary to make red pigments, a *bbr^r* fly would have brown eyes. If *r* is an allele that cannot code for an enzyme necessary to make

brown pigment, a *b⁺b⁺rr* fly would have red eyes. When mated, the resulting progeny would be *b⁺br^r*. They would make both brown and red pigments and have the normal brick-colored eyes. Interbreeding these flies would produce some offspring that were *bbrr*. Since these offspring make neither brown nor red pigments, they would be white-eyed.

Another multigene phenomenon that is seen when looking at the genes of enzymes that are in the same biochemical pathway is epistasis. Consider the following pathway in dogs:

colorless → brown → black

The *a⁺* allele codes for the enzyme that converts colorless to brown, but the *a* allele cannot, and the *b⁺* allele codes for the enzyme that converts brown to black, but the *b* allele cannot. The phenotype of an organism that is *aab⁺b⁺* depends only on the *aa* genotype, since an *aa* individual produces no brown and the *b⁺b⁺* enzyme can make black only by converting brown to black. The cross *a⁺ab⁺b* × *a⁺ab⁺b* would be expected to produce the normal 9 *a⁺_b⁺_* (black) : 3 *a⁺_bb* (brown) : 3 *aab⁺_* (white) : 1 *aabb* (white) phenotypic ratio of the classic dihybrid cross, but this is more appropriately expressed as 9 black : 3 brown : 4 white ratio. (The symbol “_” is used to indicate that the second gene can be either dominant or recessive; for example, *A_* means that both *AA* and *Aa* will result in the same phenotype.) Other pathways give different epistatic ratios such as the following pathway in peas:

white → white → purple

If *A* codes for the first enzyme, *B* codes for the second enzyme, and *a* and *b* are the nonfunctional alleles, both *AAb* and *aabb* are white. Their progeny when they are crossed, *AaBb*, is

purple because it has both of the enzymes in the pathway. Interbreeding the *AaBb* progeny gives a ratio of 9 purple to 7 white.

Human pigmentation is another case in which many genes are involved. In this case, the various genes determine how much pigment is produced by nonalbino individuals. Several gene loci are involved, and the contributions of each allele of these loci is additive. In other words, the more functional alleles one has, the darker the pigmentation; the fewer one has, the lighter. Since many of the genes involved for skin, eye, and hair color are independent, ranges of color in all three areas are seen that may or may not be the same. In addition, there are genes that code for enzymes that produce chemicals that modify the expression of the pigment genes (for example, to change blue eyes to gray, convert hazel eyes to green, or change brown hair to auburn). This gives rise to the great diversity of pigmentation seen in humans today. Add to these many possible expression patterns at the biochemical level the effect of the environment, and it is clear why such great variation in phenotypic expression is possible.

—Richard W. Cheney, Jr.

See also: Chemical Mutagens; Chromosome Mutation; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Epistasis; Inborn Errors of Metabolism; Incomplete Dominance; Monohybrid Inheritance; Mutation and Mutagenesis; Oncogenes; Phenylketonuria (PKU); Tumor-Suppressor Genes.

Further Reading

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bridization. Surveys the structure, evolution, and mutational instability of the human genome and human genes, and examines mapping of the human genome, study of genetic diseases, and dissection and manipulation of genes.

Bioethics

Field of study: Bioethics; Human genetics and social issues

Significance: *Bioethics is the practice of helping society and, more specifically, families, patients, and medical teams, make tough health care decisions. This branch of philosophy focuses on helping individuals decide what is right for them while addressing the needs of families, health care providers, and society.*

Key terms

GENETIC TESTING: the use of the techniques of genetics research to determine a person's risk of developing, or status as a carrier of, a disease or other disorder

INFORMED CONSENT: the right of patients to know the risks of medical treatment and to determine what is done to their bodies

The Emergence of Bioethics

As early as the mid-1960's, advances in genetics and reproduction, life support, and transplantation technologies spurred an increased focus on ethical issues in medicine and scientific research. From the late 1960's through the mid-1970's, bioethicists were preoccupied with the moral difficulties of obtaining voluntary, informed consent from human subjects in scientific research. They concentrated on the development of ethical guidelines in research that would ensure the protection of individuals vulnerable to exploitation, including mentally or physically handicapped individuals, prisoners, and children. Beginning in the mid-1970's and continuing through the mid-1980's, bioethicists became increasingly involved in discussions of the definitions of life, death, and what it means to be human. In the mid-1980's, practitioners began to focus on cost contain-

President's Council on Bioethics

President George W. Bush established the President's Council on Bioethics by executive order on November 28, 2001. Its mission was to advise the chief executive on bioethical issues emerging from advances in biomedical science and technology. Specifically mentioned in the council's mission were embryo and stem cell research, assisted reproduction, cloning, and end-of-life issues. Other ethical and social issues identified for discussion included the protection of human research subjects and the appropriate use of biomedical technologies. The council, chaired by Leon Kass, consisted of eighteen members appointed by the president, who were eligible for reappointment. Included in that group were scientists, physicians, ethicists, social scientists, lawyers, and theologians. The council was scheduled to terminate two years after its creation unless extended.

Deeply controversial issues constituted the subject matter of the inquiries undertaken by the council. Debate among its members as well as discussions on the floors of the Senate and House of Representatives were strongly divisive, producing heated argument and disagreement. The council's members were particularly divided on the issue of human cloning, producing two recommendations for national policy. Both recommendations would ban cloning to produce children, and ten of the eighteen council members recommended a four-year moratorium on human cloning for biomedical research while the issue continued to be studied. Declining to call for an outright ban on cloning, the divided council stated that "prudent and sensible" regulation was the best

way to advance research while guarding against abuse. The minority favored regulating cloned embryos used in biomedical research, including federal licensing, oversight, and time limits on the length of time for development of cloned embryos.

President Bush stated his strong opposition to human cloning in a speech in August, 2001. The Human Cloning Prohibition Act of 2003, which banned all forms of human cloning, including cloning to create a pregnancy and cloning for medical research, passed the House of Representatives in February of 2003 by a vote of 241 to 155. It also made it a crime to "receive or import a cloned human embryo or any product derived from a cloned human embryo," punishable by \$1 million in fines and ten years' imprisonment. This part of the law essentially made it illegal to harvest embryonic stem cells for medical research.

Stem cells—undifferentiated cells that have the potential to grow into any type of tissue—are created in the first days of pregnancy. Scientists hope to direct stem cells to grow a variety of tissues for use in transplantation to treat serious illnesses such as cancer, heart disease, and diabetes. Embryos have been valued in research for their ability to produce these stem cells, but the harvesting process requires the destruction of days-old embryos (a procedure condemned by the Catholic Church, President Bush, anti-abortion activists, and women's rights organizations). Other research, however, points to similar promise using stem cells harvested from adults, so that no embryos are destroyed.

—Marcia J. Weiss

ment in health care and the allocation of scarce medical resources.

Bioethicists worry about such matters as the guarantee of privacy, especially when compulsory testing for genetic disorders is involved, and about the limits of a person's right to threaten the health of others versus the personal right to freedom of choice. For example, the dissemination of information about genetic predispositions to chronic, costly, or incapacitating conditions can result in the denial of insurance coverage, job opportunities, and admittance to educational programs. Bioethicists also debate such matters as the use of people's

reproductive materials—their eggs or sperm—to create embryos or fetuses without their explicit consent.

Beginning in 1992, the Joint Commission on Accreditation of Health Care Organizations, the U.S. agency that accredits hospitals and health care institutions, required these organizations to establish committees to formulate ethics policies and address ethical conflicts and issues. Centers for the study of biomedical ethics such as the Society for Health and Human Values and the Park Ridge Center for the Study of Health, Faith, and Ethics became important forums for public debate and research.

The overriding principle of bioethics and U.S. law is to respect each person's right to decide, free of coercion, what treatments or procedures he or she will undergo, except when the person making the decision is not competent because of youth, mental retardation, or medical deterioration. Other important rights discussed by bioethicists include a patient's right to know that medical practitioners are telling the truth and the right to know the risks of proposed medical treatment.

Impact and Applications

Advances in genetics and genetic testing have created a host of dilemmas for bioethicists, patients, and the health care establishment. For example, as the ability to forecast and understand the genetic code progresses, people will have to decide whether knowing the future, even if it cannot be altered or changed, is a good thing for them or their children.

Bioethicists help people to decide whether genetic testing can be valuable for them. Fac-

tors typically considered before a person undergoes genetic testing include the nature of the test, the timing of the test, and the options that having the test results will bring. Testing can be done prenatally to detect disorders in fetuses; it can also be done before conception to determine whether a prospective parent is a carrier of a gene for a particular disorder or disease. Tests can also provide information about whether an adult is susceptible to or even in a presymptomatic state for a genetic disorder.

Practicing bioethicists help patients to focus on whether genetic testing will help them with the nature and severity of any disorders they or their children may have, the degree of disability or discomfort they may face, the costs and rigors of treatment, and the options that might be opened or closed as a result of testing. The key for consumers of genetic testing is whether the information obtained can be provided in time and at a time when it can help to guide treatments or family planning. Some affected persons need only to make lifestyle changes or

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Leon Kass of the University of Chicago was appointed head of the President's Council on Bioethics in November, 2001. Professor Kass and a panel of scientists, doctors, lawyers, and ethicists advised the Bush administration on policy issues surrounding stem cell and other research in biology, medicine, and genetics. (AP/Wide World Photos)

take medications to help prevent or manage a disease; others learn that they or their offspring are at risk for, or even likely to develop, serious and often untreatable disorders. Knowing one's genetic fate may be more of a burden than a person wants, particularly if there is nothing that can be done to change or alter the risks the person faces. Bioethicists act as guides through the complicated and often wrenching decision process.

—Fred Buchstein

See also: Bioinformatics; Biological Determinism; Cloning: Ethical Issues; Criminality; DNA Fingerprinting; Eugenics; Eugenics: Nazi Germany; Forensic Genetics; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Human Genetics; In Vitro Fertilization and Embryo Transfer; Insurance; Miscegenation and Antimiscegenation Laws; Patents on Life-Forms; Paternity Tests; Prenatal Diagnosis; Race; Stem Cells; Sterilization Laws.

Further Reading

Bulger, Ruth Ellen, Elizabeth Heitman, and Stanley Joel Reiser, eds. *The Ethical Dimensions of the Biological and Health Sciences*. 2d ed. New York: Cambridge University Press, 2002. Designed for graduate students who will be conducting research in the medical and biological sciences. Provides essays, readings, and questions to stimulate thinking about ethical issues and implications.

Caplan, Arthur. *Due Consideration: Controversy in the Age of Medical Miracles*. New York: Wiley, 1997. A leading bioethicist analyzes the moral questions regarding scientific advancements, among them cloning, assisted suicide, genetic engineering, and treating illnesses during fetal development.

Charon, Rita, and Martha Montello, eds. *Stories Matter: The Role of Narrative in Medical Ethics*. New York: Routledge, 2002. Explores the narrative interaction of the medical field—the written and verbal communication involved in doctors' notes, patients' stories, the recommendations of ethics committees,

and insurance justifications—and the way in which this interaction profoundly affects decision making, patient health, and treatment.

Comstock, Gary L., ed. *Life Science Ethics*. Ames: Iowa State Press, 2002. Introduces ethical reasoning in the area of humankind's relationship with nature and presents twelve fictional case studies as a means to show the application of ethical reasoning.

Danis, Marion, Carolyn Clancy, and Larry R. Churchill, eds. *Ethical Dimensions of Health Policy*. New York: Oxford University Press, 2002. The three authors, from varied professions within the medical field, attempt to identify the goals of health care, examine how to connect ethical considerations with the making of health policy, and discuss specific areas of ethical controversy such as resource allocation, accountability, the needs of vulnerable populations, and the conduct of health services research.

Evans, John Hyde. *Playing God? Human Genetic Engineering and the Rationalization of Public Bioethical Debate*. Chicago: University of Chicago Press, 2002. Provides a framework for understanding the public debate, and details the various positions of the debate's players, including eugenicists, theologians, and bioethicists.

Kass, Leon R. *Life, Liberty, and the Defense of Dignity: The Challenge for Bioethics*. San Francisco: Encounter Books, 2002. Examines genetic research, cloning, and active euthanasia, and argues that biotechnology has left humanity out of its equation, often debasing human dignity rather than celebrating it.

Kristol, William, and Eric Cohen, eds. *The Future Is Now: America Confronts the New Genetics*. Lanham, Md.: Rowman & Littlefield, 2002. Brings together classic writings (George Orwell, Aldous Huxley) as well as more recent essays and congressional testimony about human cloning, genetic engineering, stem cell research, biotechnology, human nature, and American democracy.

May, Thomas. *Bioethics in a Liberal Society: The Political Framework of Bioethics Decision Making*. Baltimore: Johns Hopkins University Press, 2002. Takes the debate about biomedical

ethics into the political realm, analyzing how the political context of liberal constitutional democracy shapes the rights and obligations of both patients and health care professionals.

O'Neill, Onora. *Autonomy and Trust in Bioethics*. New York: Cambridge University Press, 2002. Examines issues surrounding reproductive and principled autonomy, trust, consent, and the media and bioethics.

Singer, Peter. *Unsanctifying Human Life: Essays on Ethics*. Edited by Helga Kuhse. Malden, Mass.: Blackwell, 2002. Singer is one of today's major bioethicists. Here he examines the role of philosophers and philosophy in such questions as the moral status of the embryo, animal rights, and how we should live.

Veatch, Robert M. *The Basics of Bioethics*. 2d ed. Upper Saddle River, N.J.: Prentice Hall, 2003. In a textbook designed for students, Veatch presents an overview of the main theories and policy questions in biomedical ethics. Includes diagrams, case studies, and definitions of key concepts.

Web Sites of Interest

American Journal of Bioethics Online. <http://www.bioethics.net>. Provides sections on cloning basics, animal cloning, stem cells, U.S. federal and state laws, the cloning debate, news, and more.

Kennedy Institute of Ethics, Georgetown University. <http://www.georgetown.edu/research/kie>. Links to many resources on bioethics as well as a "bioethics library" that in turn leads to resources on human genetics and ethics.

National Information Resource on Ethics and Human Genetics. <http://www.georgetown.edu/research/nrcbl/nirehg>. Supports links to databases, annotated bibliographies, and articles about the ethics of genetic testing and human genetics.

President's Council on Bioethics. <http://bioethics.gov>. Government arm that advises on ethical issues surrounding biomedical science and technology. Includes links to bioethics literature and other resources on ethics and human genetics.

The Hastings Center. <http://www.thehastingscenter.org>. This independent nonprofit or-

ganization specializes in bioethics, and its site contains news postings, articles on bioethics and different aspects of genetic science, and announcements of events and publications.

Biofertilizers

Field of study: Genetic engineering and biotechnology

Significance: *Biofertilizers were used in agriculture long before chemical fertilizers became prevalent during and after the Industrial Revolution. The depleted soil fertility and contamination of ecosystems caused by the extensive use of chemical fertilizers, however, has prompted the redevelopment of biofertilizers, which are designed to work according to basic principles at work in nature, taking advantage of plants and other organisms to maintain healthy soil.*

Key terms

ALGAE: minute plants that live in fresh water; they are used as biofertilizers because of their high productivity and ability to fix atmospheric nitrogen

NODULE: a symbiotic relationship between bacteria and plant roots that causes the conversion of nitrogen gas into a form readily accessible by plants

SYMBIOSIS: a mutually beneficial association between two living organisms

Overview

Biofertilizers are living microorganisms that work either alone or in association with matter and other organisms to enhance the fertility of soil. For many centuries, biofertilizers were used in organic farming in countries such as China, India, and Egypt until modernization resulted in a move toward the use of environmentally destructive chemical fertilizers. Organic agriculture integrates livestock, aquatic organisms, plants, and the scientific enhancement of natural processes to maintain ecological equilibrium, thus maximizing the production of foods and goods through complete recycling of all resources. Biofertilizers may in-

clude microorganisms, nitrogen-fixing algae, green manure, plant residues, and sewer sludge. Biofertilizers not only provide an alternative method of farming but may also provide the only way to reduce environmental contamination and soil fertility depletion caused by chemical fertilizers.

Microorganisms and Algae

Microorganisms must be applied to the soil or mixed with seeds and other ingredients before they can establish a symbiotic relationship with a plant's root system. They have been shown to stabilize manure, increase the amount of nitrogen in the soil, increase root surface area for absorption, and control the leaking of nitrogen into the groundwater. Another type of association between plant roots (particularly legumes) and nitrogen-fixing bacteria is a symbiotic relationship called a "nodule." Nodules are natural "factories" that produce ample fertilizers by converting nitrogen into ammonium, a form of nitrogen that may be used directly by plants or deposited slowly into the soil, thus enhancing its fertility. While continuous application of chemical fertilizers depletes the soil's natural fertility and destroys beneficial microorganisms, natural fertilizers produced by nodules alleviate the contamination to the ecosystem. Unfortunately, nodules are formed only between legumes and bacteria, thus limiting their use. Genetically engineered bacteria are being used to improve the efficiency of nitrogen fixation, and some researchers have attempted to transfer genes responsible for forming such symbiotic relationships from legumes to other plant species.

Algae are minute plants that are almost entirely aquatic. They grow and reproduce very rapidly during the growing season but die off during the nongrowing season. Such a "boom and bust" life cycle provides the soil with substantial amounts of nutrients through the degradation of dead algae and the deposit of the nitrogen fixed by algae during the growing season. Two types of algae, azospirillum and azotobacter, have been used as biofertilizers in rice fields for centuries in Southeast Asia. Algae are the only plants that are able to fix nitrogen by themselves. The cost of raising algae is mini-

mal, but if they are grown too densely, they may become weeds and suffocate aquatic animals.

Green Manure, Plant Residues, and Treated Sewer Sludge

Some crops, particularly legumes, are grown and harvested to be used as green manure to restore the soil's fertility. They may be used directly or mixed with microorganisms. Green manure releases nutrients slowly and provides long-term fertility for soil. After grains are harvested for food, plant residues are processed into fertilizer in one of two ways. They may be burned to extract energy, after which the ashes are applied to the soil. Alternatively, they may be fermented in a sealed underground tank to make methane to be used as "natural" gas or ethanol, which may become an increasingly important fuel source. Genetically engineered microbes convert fiber-rich crop residues such as wheat straw and corn stalks into ethanol. Solid residue left over after fermentation is used as a biofertilizer. The fermentation approach is promising because it is environmentally friendly and also creates alternative energy sources, additional income, and market opportunities for farmers.

Even though sewer sludge has been used as a fertilizer in developing countries for some time, people are more resistant to it because of the animal and human wastes present in the sludge. Proper treatments must be in place to get rid of possible heavy metals or pathogens. More research is needed on sewer sludge before it is considered for wide use as a fertilizer.

—Ming Y. Zheng

See also: Biopesticides; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetically Modified (GM) Foods; High-Yield Crops; Quantitative Inheritance; Transgenic Organisms.

Further Reading

Bethlenfalvay, G. J., and R. G. Linderman.

Mycorrhizae in Sustainable Agriculture. Madison, Wis.: American Society of Agronomy, 1992. Provides excellent discussions on the knowledge and importance of mycorrhizae to plants and the soil.

Chrispeels, M. J., and D. E. Sadava. *Plants, Genes,*

and Crop Biotechnology. Boston: Jones and Bartlett, 2003. Offers a discussion on the potential genetic modifications necessary to increase the use of microorganisms to supply nitrogen fertilizer.

Legocki, Andrezej, Hermann Bothe, and Alfred Pühler, eds. *Biological Fixation of Nitrogen for Ecology and Sustainable Agriculture*. New York: Springer, 1997. From the proceedings of the NATO Advanced Research Workshop held in Poznań, Poland, in 1996. Includes bibliographical references.

Lynch, J. M. *Soil Biotechnology: Microbiological Factors in Crop Productivity*. Boston: Blackwell Scientific, 1983. Contains some excellent information on the potential for genetically engineered microorganisms to improve crop production.

Matson, P. A., et al. "Agricultural Intensification and Ecosystem Properties." *Science* 277 (July 25, 1997): 504-509. Provides useful information on agriculture intensification and its sustainability, a strategy that can help reduce negative consequences.

Salisbury, F. B., and C. W. Ross. *Plant Physiology*. 4th ed. Belmont, Calif.: Wadsworth, 1992. Contains excellent chapters on plant nutrition and nitrogen metabolism.

Web Site of Interest

U.S. Department of Agriculture, Biotechnology: An Information Resource. <http://www.nal.usda.gov/bic>. A government site that offers dozens of links to information on applications of genetic engineering to agriculture.

Bioinformatics

Fields of study: Bioinformatics; Molecular genetics; Techniques and methodologies

Significance: *Bioinformatics is the application of information technology to the management of biological information to organize data and extract meaning. It is a hybrid discipline that combines elements of computer science, information technology, mathematics, statistics, and molecular genetics.*

Key terms

ALGORITHM: a mathematical rule or procedure for solving a specific problem; in bioinformatics, a computer program is built to implement an algorithm, but different algorithms may be used to achieve the same result—that is, to align two sequences

DATABASE: an organized collection of information within a computer system that can be used for storage and retrieval as well as for complex searches and analyses

GENBANK: a comprehensive, annotated collection of publicly available DNA sequences maintained by the National Center for Biotechnology Information and available through its Web site

GENOMICS: the use of high-throughput technology to analyze molecular events within cells at the whole genome scale (for example, all of the genes, all of the messenger RNA, or all of the proteins)

HUMAN GENOME PROJECT: a publicly funded international project to determine the complete DNA sequence of human genomic (chromosomal) DNA and to map all of the genes, which produced a "final" sequence in April, 2003

MICROARRAY: a technology to measure gene expression using nucleic acid hybridization of messenger RNA to a miniature array of DNA probes for many genes

PROTEOMICS: a collection of technologies that examine proteins within a cell in a holistic fashion, identifying or quantitating a large number of proteins within a single sample, identifying many protein-protein or protein-DNA interactions, and so on

The Need for Bioinformatics

The sequencing of cloned DNA molecules has become a routine, automated task in the modern molecular genetics laboratory, and large, publicly funded genome projects have determined the complete genomic sequences for humans, mice, fruit flies, dozens of bacteria, and many other species of interest to geneticists. All of this information is now freely available in online databases. Computational molecular biology tools allow for the design of polymerase chain reaction (PCR) primers, re-

striction enzyme cloning strategies, and even entire *in silico* experiments. This greatly accelerates the work of researchers but also changes the daily lives of many biologists so that they spend more time working with computers and less time working with test tubes and pipettors. The rapid accumulation of enormous amounts of molecular sequence data and their cryptic and subtle patterns have created a need for computerized databases and analysis tools.

Bioinformatics provides essential support services to modern molecular genetics for organizing, analyzing, and distributing data. As DNA sequencing and other molecular genetic technologies become more automated, data are generated ever more rapidly, and computing systems must be designed to store the data and make them available to scientists in a useful fashion. The use of these vast quantities of data for the discovery of new genes and genetic principles relies on the development of sophisticated new data-mining tools. The challenge of bioinformatics is in finding new approaches to deal with the volume and complexity of the data, and in providing researchers with access both to the raw data and to sophisticated and flexible analysis tools in order to advance researchers' understanding of genetics and its role in health and disease.

Database Design

The DNA sequence data collected by automated sequencing equipment can be represented as a simple sequence of letters: G, A, T, and C—which stand for the four nucleotide bases on one strand of the DNA molecule (guanine, adenine, thymine, and cytosine). These letters can easily be stored as plain text files on a computer. Similarly, protein sequences can also be stored as text files using the twenty single-letter abbreviations for the amino acids.

There is a significant advantage to storing DNA and protein sequence as plain text files, also known as flat files. Text files take up minimal amounts of hard-drive space, can be used on any type of computer and operating system, and can easily be moved across the Internet. However, a text file with a bunch of letters representing a DNA or protein sequence is essentially meaningless without some basic descriptive

information, such as the organism from which it comes, its location on the genome, the person or organization that produced the sequence, and a unique identification number (accession number) so that it can be referenced in scientific literature. This additional annotation information can also be stored as text—even in the same file with the sequence information—but there must be a consistent format, a standard.

In addition to maintaining basic flat-file structures for text data, it is useful to maintain sequence data in relational databases, which allow for much faster searching across multiple query terms and the linkage of sequence data files with other relevant information. The most sophisticated and widely used relational database system for bioinformatics is the Entrez system at the National Center for Biotechnology Information (NCBI). Entrez is a relational database that includes cross-links between all of the DNA sequences in GenBank. GenBank exchanges data with the DNA DataBank of Japan and the European Molecular Biology Laboratory on a daily basis to ensure that all three centers maintain the same set of data, and all peer-reviewed journals require the submission of sequence data to GenBank prior to publication of research articles; publicly funded sequencing projects, such as the Human Genome Project, submit new sequence data to GenBank as it is collected, so that the scientific community can have immediate access to it. Entrez also includes all of the derived protein sequences (translations from cDNAs and predicted coding sequences in genomic DNA), the scientific literature in MedLine/PubMed, three-dimensional protein structures from the Protein Data Base (PDB), and human genetic information from the Online Mendelian Inheritance in Man (OMIM) database. Relational databases are even more important for more complex types of genomic data, such as gene expression microarrays and genetic variation and genotyping data sets.

Key Algorithms

Some of the key algorithms used in bioinformatics include sequence alignment (dynamic programming), sequence similarity

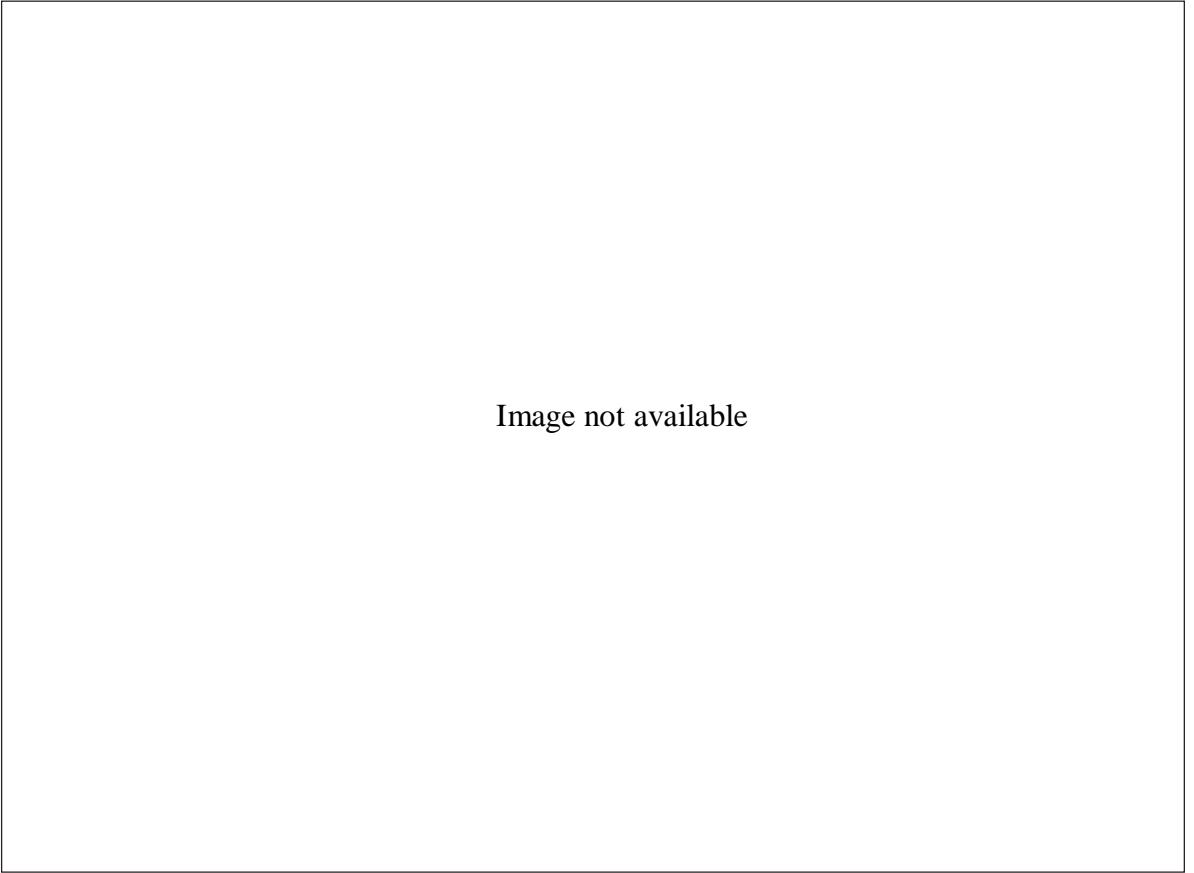


Image not available

Steven Brenner, of the University of California at Berkeley, next to a computer running bioinformatics software in November, 2001. He advocates distributing information freely as “open source code,” claiming that this is the best way to debug bioinformatics software and advance research. (AP/Wide World Photos)

(word matching from hash tables), assembly of overlapping fragments, clustering (hierarchical, self-organizing maps, principal components, and the like), pattern recognition, and protein three-dimensional structure prediction. Bioinformatics is both eclectic and pragmatic: Algorithms are adopted from many different disciplines, including linguistics, statistics, artificial intelligence and machine learning, remote sensing, and information theory. There is no consistent set of theoretical rules at the core of bioinformatics; it is simply a collection of whatever algorithms and data structures have been found to work for the current data-management problems being faced by biologists. As new types of data become important in the work of molecular geneticists, new algorithms for bioinformatics will be invented or adopted.

New Types of Data

In addition to DNA and protein sequences, bioinformatics is being called upon to organize many other types of biological information that are being collected in ever greater amounts. Gene expression microarrays collect information on the amounts of mRNA produced from tens of thousands of different genes in a single tissue sample. Proteomics technologies are automating the process of mass spectroscopy, which allows investigators to identify and measure thousands of proteins in a single cell extract sample. Genes and proteins can also be organized into gene families based on sequence similarity, homology across organisms (comparative genomics), and function in metabolic or regulatory pathways. Many new technologies are being developed to measure genetic

variation: genetic tests either for alleles of well-studied genes or for anonymous single nucleotide polymorphisms (SNPs) identified from genome sequence data. As these genotyping technologies are improved, it is becoming possible to collect data in an automated fashion for many genetic loci from a single DNA sample, or to test a single genetic locus on many thousands of DNA samples in parallel. These new data types require new database designs and the inclusion of new types of algorithms (from statistics, population genetics, and other disciplines) in bioinformatics data-management solutions.

Integration

The biggest challenge facing bioinformatics is the integration of various types of data in a form that allows scientists to extract meaningful insights into biology from the masses of information in molecular genetic databases. Genome browsers are one example of this challenge. It is extremely difficult to provide a display that allows someone to view all of the relevant information about a gene or a chromosomal region, including the identity of encoded proteins, protein structure and functional information, involvement in metabolic and regulatory pathways, developmental and tissue-specific gene expression, evolutionary relationships to proteins in other organisms, DNA motifs bound by regulatory proteins, genetic synteny with other species (that is, having genes with loci on the same chromosome), phenotypes of mutations, and known alleles and SNPs and their frequency in various populations.

Another, much more modest, goal would be simply to alert a person viewing a DNA or protein sequence in one database of the existence of additional information about that entity in other databases. At the present time, such cross-database links are inconsistent and unreliable. The NCBI cross-references its own databases—from DNA to proteins to three-dimensional structures to PubMed articles to genomes. Most special subject databases, such as those that focus on a particular species or on a particular type of molecule, link DNA and protein sequences back to the corresponding “reference”

entries in GenBank; however, these links are not reciprocal. Someone looking at a GenBank cDNA sequence in the Entrez browser would have no way of knowing that a corresponding protein entry is present in a database dedicated to *Drosophila* genetics or to G-protein coupled receptor mutants. It is never possible for scientists to be certain that they have collected all of the relevant information about a molecule of interest from all online databases.

—Stuart M. Brown

See also: cDNA Libraries; DNA Fingerprinting; DNA Sequencing Technology; Forensic Genetics; Genetic Testing: Ethical and Economic Issues; Genetics, Historical Development of; Genomic Libraries; Genomics; Human Genome Project; Icelandic Genetic Database; Linkage Maps; Proteomics.

Further Reading

- Baxevanis, Andreas D., and B. F. Francis Ouellette. *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*. 2d ed. Hoboken, N.J.: John Wiley & Sons, 2003. This book provides a sound foundation of basic concepts of bioinformatics, with practical discussions and comparisons of both computational tools and databases relevant to biological research. The standard text for most graduate-level bioinformatics courses.
- Claverie, Jean-Michel, and Cedric Notredame. *Bioinformatics for Dummies*. Hoboken, N.J.: John Wiley & Sons, 2003. A practical introduction to bioinformatics: computer technologies that biochemical and pharmaceutical researchers use to analyze genetic and biological data. This reference addresses common biological questions, problems, and projects while providing a UNIX/Linux overview and tips on tweaking bioinformatic applications using Perl.
- Krawetz, Stephen A., and David D. Womble. *Introduction to Bioinformatics: A Theoretical and Practical Approach*. Totowa, N.J.: Humana Press, 2003. Aimed at undergraduates, graduate students, and researchers. Four sections: “Biochemistry: Cell and Molecular Biology,” “Molecular Genetics,” “Unix Operating System,” and “Computer Applications.”

Mount, David W. *Bioinformatics: Sequence and Genome Analysis*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2001. A textbook written for the biologist who wants to acquire a thorough understanding of popular bioinformatics programs and molecular databases. It does not teach programming but does explain the theory behind each of the algorithms.

Nucleic Acids Research 31, no. 1 (2003). This widely respected journal produces a special issue in January of each year devoted entirely to online bioinformatics databases. The articles represent the definitive statement by the directors of each of the major public databases of molecular biology data regarding the types of information and analysis tools in their databases and plans for development in the immediate future.

Web Sites of Interest

Bioinformatics Organization. <http://www.bioinformatics.org>. Provides a helpful tutorial on bioinformatics.

European Bioinformatics Institute. <http://www.ebi.ac.uk>. Maintains databases concerning nucleic acids, protein sequences, and macromolecular structures, as well as postings of news and events and descriptions of ongoing scientific projects.

Biological Clocks

Field of study: Human genetics and social issues

Significance: *Biological clocks control those periodic behaviors of living systems that are a part of their normal function. The rhythms may be of a daily, monthly, yearly, or even longer periodicity. In some cases, the clocks may be "programmed" to regulate processes that may occur at some point in the lifetime of the individual, such as those processes related to aging. Altered or disturbed rhythms may result in disease.*

Key terms

ALZHEIMER'S DISEASE: a disorder characterized by brain lesions leading to loss of memory,

personality changes, and deterioration of higher mental functions

CIRCADIAN RHYTHM: a cycle of behavior, approximately twenty-four hours long, that is expressed independent of environmental changes

FREE-RUNNING CYCLE: the rhythmic activity of an individual that operates in a constant environment

HUNTINGTON'S DISEASE: an autosomal dominant genetic disorder characterized by loss of mental and motor functions in which symptoms typically do not appear until after age thirty

SUPRACHIASMATIC NUCLEUS (SCN): a cluster of several thousand nerve cells that contains a central clock mechanism that is active in the maintenance of circadian rhythms

Types of Cycles

Biological clocks control a number of physiological functions, including sexual behavior and reproduction, hormonal levels, periods of activity and rest, body temperature, and other activities. In humans, phenomena such as jet lag and shift-work disorders are thought to result from disturbances to the innate biological clock.

The most widely studied cycles are circadian rhythms. These rhythms have been observed in a variety of animals, plants, and microorganisms and are involved in regulating both complex and simple behaviors. Typically, circadian rhythms are innate, self-sustaining, and have a cyclicity of nearly, but not quite, twenty-four hours. Normal temperature ranges do not alter them, but bursts of light or temperature can change the rhythms to periods of more or less than twenty-four hours. Circadian rhythms are apparent in the activities of many species, including humans, flying squirrels, and rattlesnakes. They are also seen to control feeding behavior in honeybees, song calling in crickets, and hatching of lizard eggs.

What is known about the nature of the biological clock? The suprachiasmatic nucleus (SCN) consists of a few thousand neurons or specialized nerve cells that are found at the base of the hypothalamus, the part of the brain that controls the nervous and endocrine systems. The

SCN appears to play a major role in the regulation of circadian rhythms in mammals and affects cycles of sleep, activity, and reproduction. The seasonal rhythm in the SCN appears to be related to the development of seasonal depression and bulimia nervosa. Light therapy is effective in these disorders. Blind people, whose biological clocks may lack the entraining effects of light, often show free-running rhythms.

Genetic control of circadian rhythms is indicated by the findings of single-gene mutations that alter or abolish circadian rhythms in several organisms, including the fruit fly (*Drosophila*) and the mouse. A mutation in *Drosophila* affects the normal twenty-four-hour activity pattern so that there is no activity pattern at all. Other mutations produce shortened (nineteen-hour) or lengthened (twenty-nine-hour) cycles. The molecular genetics of each of these mutations is known.

A semidominant autosomal mutation, CLOCK, in the mouse produces a circadian rhythm one hour longer than normal. Mice that are homozygous (have two copies) for the CLOCK mutation develop twenty-seven- to twenty-eight-hour rhythms when initially placed in darkness and lose circadian rhythmicity completely after being in darkness for two weeks. No anatomical defects have been seen in association with the CLOCK mutation.

Biological Clocks and Aging

Genes present in the fertilized egg direct and organize life processes from conception until death. There are genes whose first effects may not be evident until middle age or later. Huntington's disease (also known as Huntington's chorea) is such a disorder. An individual who inherits this autosomal dominant gene is "programmed" around midlife to develop in-

Image not available

A squirrel hibernates in the hands of University of Minnesota biochemist Matt Andrews. Because the ground squirrel possesses the ability to put its body into this form of stasis, it is nearly immune to strokes. The genetics of such biological clocks may one day lead to better treatments for strokes in humans. (AP/Wide World Photos)

voluntary muscle movement and signs of mental deterioration. Progressive deterioration of body functions leads to death, usually within fifteen years. It is possible to test individuals early in life before symptoms appear, but such tests, when no treatment for the disease is available, are controversial.

Alzheimer's disease (AD) is another disorder in which genes seem to program processes to occur after middle age. AD is a progressive, degenerative disease that results in a loss of intellectual function. Symptoms worsen until a person is no longer able to care for himself or herself, and death occurs on an average of eight years after the onset of symptoms. AD may appear as early as forty years of age, although most people are sixty-five or older when they are diagnosed. Age and a family history of AD are clear risk factors. Gene mutations associated with AD have been found on human chromosomes 1, 14, 19, and 21. Although these genes, especially the apolipoprotein *e4* gene, increase the likelihood of a person getting AD, the complex nature of the disorder is underscored when it is seen that the mutations account for less than half of the cases of AD and that some individuals with the mutation never get AD.

Impact and Applications

Evidence has accumulated that human activities are regulated by biological clocks. It has also become evident that many disorders and diseases, and even processes that are associated with aging, may be affected by abnormal clocks. As understanding of how genes control biological clocks develops, possibilities for improved therapy and prevention should emerge. It may even become possible to slow some of the harmful processes associated with normal aging.

—Donald J. Nash

See also: Aging; Alzheimer's Disease; Biological Determinism; Cancer; Developmental Genetics; Huntington's Disease; Telomeres.

Further Reading

Finch, Caleb Ellicott. *Longevity, Senescence, and the Genome*. Reprint. Chicago: University of Chicago Press, 1994. Provides a comparative

review of research on organisms from algae to primates, expanding traditional gerontological and geriatric issues to intersect with behavioral, developmental, evolutionary, and molecular biology. Illustrated.

Hamer, Dean, and Peter Copeland. *Living with Our Genes: Why They Matter More than You Think*. New York: Doubleday, 1998. Links DNA and behavior and contains a good chapter on biological clocks and aging.

Medina, John J. *The Clock of Ages: Why We Age, How We Age—Winding Back the Clock*. New York: Cambridge University Press, 1996. A book written especially for the general reader. Covers aging on a system-by-system basis and includes a large section on the genetics of aging.

Nelson, James Lindemann, and Hilde Lindemann Nelson. *Alzheimer's: Answers to Hard Questions for Families*. New York: Main Street Books, 1996. Reviews Alzheimer's disease for the lay reader, guides caregivers through the difficult moral and ethical problems associated with the disease, and discusses support services.

Zallen, Doris Teichler. *Does It Run in the Family? A Consumer's Guide to DNA Testing for Genetic Disorders*. New Brunswick, N.J.: Rutgers University Press, 1997. Focuses on the practical aspects of obtaining genetic information, clearly explaining how genetic disorders are passed along in families. Provides useful information on genetic disorders, including Huntington's disease and Alzheimer's disease.

Web Sites of Interest

National Institute of Mental Health, How Biological Clocks Work. <http://www.nimh.nih.gov/publicat/bioclock.cfm>. An introductory site that focuses on the molecular basis of the biological clock. Includes references for further study.

National Science Foundation, Center for Biological Timing. <http://www.cbt.virginia.edu/cbtdocs>. This site covers the science of biological clocks and provides a biological timing tutorial.

Biological Determinism

Field of study: Human genetics and social issues

Significance: *Biological determinists argue that there is a direct causal relationship between the biological properties of human beings and their behavior. From this perspective, social and economic differences between human groups can be seen as a reflection of inherited and immutable genetic differences. This contention has been used by groups in power to claim that stratification in human society is based on innate biological differences. In particular, biological determinism has been used to assert that certain ethnic groups are biologically defective and thus intellectually, socially, and morally inferior to others.*

Key terms

DETERMINISM: the doctrine that everything, including one's choice of action, is determined by a sequence of causes rather than by free will

INTELLIGENCE QUOTIENT (IQ): performance on a standardized test, often assumed to be indicative of an individual's level of intelligence

REDUCTIONISM: the explanation of a complex system or phenomenon as merely the sum of its parts

REIFICATION: the oversimplification of an abstract concept such that it is treated as a concrete entity

The Use of Inheritance to Promote Social Order

The principle of biological determinism lies at the interface between biology and society. A philosophical extension of the use of determinism in other sciences, such as physics, biological determinists view human beings as a reflection of their biological makeup and hence simple extensions of the genes that code for these biological processes. Long before scientists had any knowledge of genetics and the mechanisms of inheritance, human societies considered certain groups to be innately superior by virtue of their family or bloodlines (nobility) while others were viewed as innately inferior (peasantry). Such views served to preserve the social order. According to evolutionary biologist Stephen Jay Gould, Plato himself circulated a myth that certain citizens were "framed differently" by God, with the ranking of groups in society based on their inborn worth.

As science began to take a more prominent role in society, scientists began to look for evidence that would justify the social order. Since mental ability is often considered to be the most distinctive feature of the human species, the quantification of intelligence was one of the main tactics used to demonstrate the inferiority of certain groups. In the mid-1800's, measurements of the size, shape, and anatomy of the skull, brain, and other body features were compiled by physician Samuel George Morton and surgeon Paul Broca, among others. These measurements were used to depict races as separate species, to rank them by their mental and moral worth, and to document the subordinate status of various groups, including women. In the first decades of the twentieth century, such measurements were replaced by the intelligence quotient (IQ) test. Although its inventor, Alfred Binet, never intended it to be used in this way, psychologists such as Lewis M. Terman and Robert M. Yerkes promoted IQ as a single number that captured the complex, multifaceted, inborn intelligence of a person. IQ was soon used to restrict immigration, determine occupation, and limit access to higher education. Arthur Jensen, in 1979, and Richard Herrnstein and Charles Murray, in 1994, reasserted the claim that IQ is an inherited trait that differs among races and classes.

Problems with the Principle of Biological Determinism

Geneticists and sociobiologists (who study the biological basis of social behavior) have uncovered a variety of animal behaviors that are influenced by biology. However, the genetic makeup of an organism ("nature") is expressed only within the specific context of its environment ("nurture"). Thus genes that are correlated with behavior usually code for predispositions rather than inevitabilities. For such traits, the variation that occurs within a group is usually greater than the differences that occur be-

tween groups. In addition, the correlation between two entities (such as genes and behavior) does not necessarily imply a causal relationship (for example, the incidences of ice cream consumption and drowning are correlated only because both increase during the summer). Complex, multifaceted behaviors such as intelligence and violence are often reified, or treated as discrete concrete entities (as IQ and impulse control, respectively), in order to make claims about their genetic basis. Combined with the cultural and social bias of scientific researchers, reification has led to many misleading claims regarding the biological basis of social structure.

Biological and cultural evolution are governed by different mechanisms. Biological evolution occurs only between parents and offspring (vertically), while cultural evolution occurs through communication without regard to relationship (horizontally) and thus can occur quickly and without underlying genetic change. Moreover, the socially fit (those who are inclined to reproduce wealth) are not necessarily biologically fit (inclined to reproduce themselves). The reductionist attempt to gain an understanding of human culture through its biological components does not work well in a system (society) shaped by properties that emerge only when the parts (humans) are put together. Cultures cannot be understood as biological behaviors any more than biological behaviors can be understood as atomic interactions.

Impact and Applications

Throughout history, biological determinism has been used to justify or reinforce racism, genocide, and oppression, often in the name of achieving the genetic improvement of the human species (for example, the “racial health” of Nazi Germany). Gould has noted that claims of biological determinism tend to be revived during periods when it is politically expedient to do so. In times of economic hardship, many find it useful to adopt an “us against them” attitude to find a group to blame for social and economic woes or to free themselves from the responsibility of caring for the “biologically inferior” underprivileged. As advances in molecular genetics lead to the identification of addi-

tional genes that influence behavior, society must guard against using this information as justification for the mistreatment or elimination of groups that are perceived as “inferior” or “undesirable” by the majority.

—Lee Anne Martínez

See also: Aggression; Aging; Alcoholism; Altruism; Behavior; Bioethics; Biological Clocks; Cloning; Ethical Issues; Criminality; Developmental Genetics; Eugenics; Eugenics: Nazi Germany; Gender Identity; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing: Ethical and Economic Issues; Heredity and Environment; Human Genetics; Intelligence; Miscegenation and Antimiscegenation Laws; Natural Selection; Race; Sociobiology; Twin Studies; XYY Syndrome.

Further Reading

Begley, Sharon. “Gray Matters.” *Newsweek*, March 27, 1995. Discusses the differences between the brains of males and females.

Gould, Stephen Jay. *The Mismeasure of Man*. New York: Norton, 1996. Refutes Richard Herrnstein and Charles Murray’s argument and presents an engaging historical overview of how pseudoscience has been used to support racism and bigotry.

Herrnstein, Richard, and Charles Murray. *The Bell Curve: Intelligence and Class Structure in American Life*. New York: Simon and Schuster, 1994. Asserts that IQ plays a statistically important role in the shaping of society by examining such sociological issues as school dropout rates, unemployment, work-related injury, births out of wedlock, and crime.

Moore, David S. *Dependent Gene: The Fallacy of Nature vs. Nurture*. New York: W. H. Freeman, 2001. Few books examine the ways the genes and the environment interact to produce everything from eye color to behavioral tendencies. This book lays to rest the popular myth that some traits are purely genetic and others purely a function of environment; rather, all traits are the result of complex, dependent interactions of both—interactions that occur at all stages of biological and psychological development. An informed argument against simplistic determinism.

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Biological Weapons

Fields of study: Genetic engineering and biotechnology; Human genetics

Significance: *Just as twentieth century discoveries in chemistry and physics led to such devastating weapons as poison gases and nuclear bombs, so humanity in the twenty-first century faces the prospect that the biotechnological revolution will lead to the development and use of extremely deadly biological weapons.*

Key terms

ANTHRAX: an acute bacterial disease that affects animals and humans and that is especially deadly in its pulmonary form

BIOLOGICAL WEAPON (BW): the military or terrorist use of such organisms as bacteria and viruses to cause disease and death in people, animals, or plants

BIOTERRORIST: an individual or group that coercively threatens or uses biological weapons, often for ideological reasons

ETHNIC WEAPONS: genetic weapons that target certain racial groups

GENETIC ENGINEERING: the use of recombinant DNA to alter the genetic material in an organism

IMMUNE SYSTEM: the biological defense mechanism that protects the body from disease-causing microorganisms

RECOMBINANT DNA: DNA prepared by transplanting and splicing genes from one species into the cells of another species

SMALLPOX: an acute, highly infectious, often fatal disease characterized by fever followed by the eruption of pustules

Early History

Biological warfare antedates by several centuries the discovery of the gene. Just as the history of genetics did not begin with Gregor Mendel, whose pea-plant experiments eventually helped found modern genetics, the history of biological warfare began long before the Japanese dropped germ-filled bombs on several Chinese cities during World War II. For example, the Assyrians, six centuries before the common era, knew enough about rye ergot, a fungus disease, to poison their enemies' wells. The ancient Greeks also used disease as a military weapon, and the Romans catapulted diseased animals into enemy camps. A famous medieval use of biological weapons occurred during the Tatar siege of Kaffa, a fortified Black Sea port, then held by Christian Genoans. When Tatars started dying of the bubonic plague, the survivors catapulted cadavers into the walled city. Many Genoans consequently died of the plague, and the remnant who sailed back to Italy contributed to the spread of the Black Death into Europe.

Once smallpox was recognized as a highly contagious disease, military men made use of it in war. For example, the conquistador Francisco Pizarro presented South American natives with smallpox-contaminated clothing, and, in an early case of ethnic cleansing, the British and Americans used deliberately induced smallpox epidemics to eliminate native tribes from desirable land.

As scientists in the nineteenth and twentieth centuries learned more about the nature and modes of reproduction of such diseases as anthrax and smallpox, germ warfare began to become part of such discussions as the First International Peace Conference in The Hague (1899). The worldwide revulsion against the chemical weapons used in World War I, along with a concern that biological weapons would be more horrendous, led to the Geneva Proto-

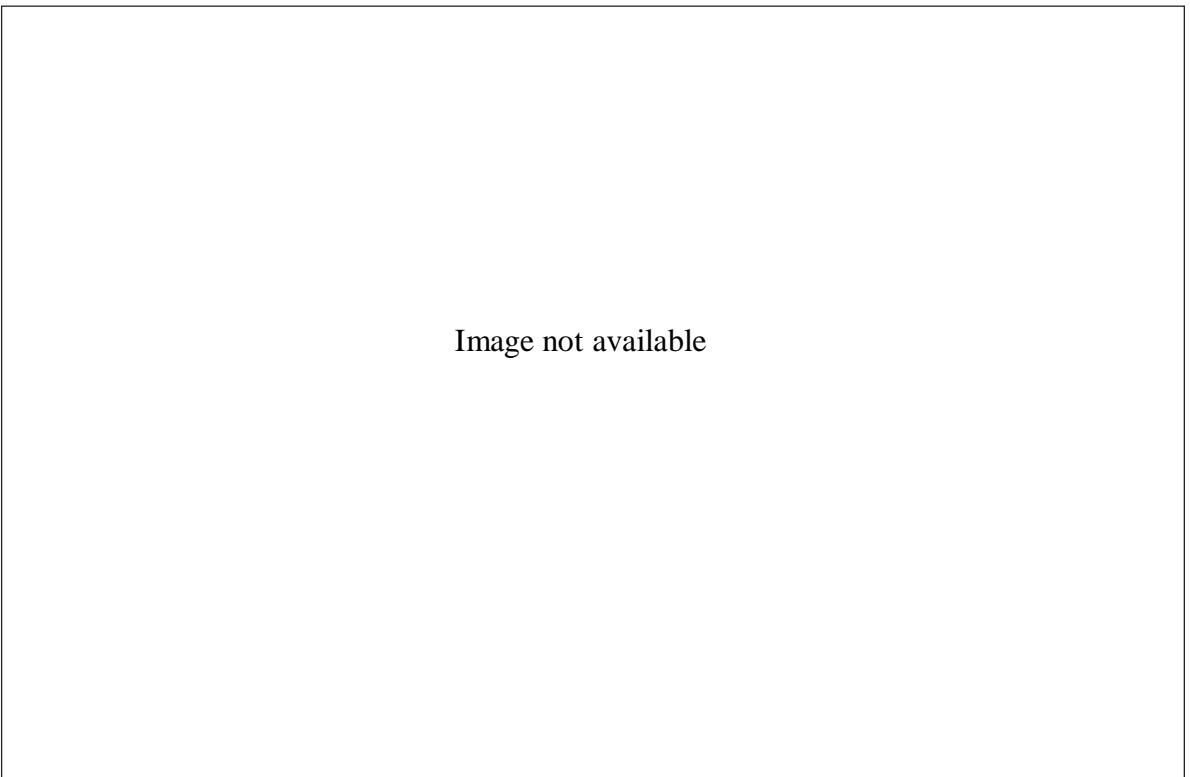


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Anthrax colonies grow on culture in a petri dish in Mexico City, where in 2001 two germ banks housed dozens of these cultures virtually unguarded. (AP/Wide World Photos)

col (1925), which prohibited the first use of germ weapons, but not their development.

From Germ Warfare to Genetic Weapons

With the accelerating knowledge about the genetics of various disease-causing microorganisms, several countries became concerned with the threat to their security posed by the weaponizing of these pathogens. Although several states signed the Geneva Protocol in the late 1920's, others signed only after assurances of their right to retaliate. The United States, which did not ratify the treaty until 1975, did extensive research on germ weapons during the 1950's and 1960's. American scientists were able to make dry infectious agents that could be packed into shells and bombs, and estimates were made that ten airplanes with such bombs could kill or seriously disable tens of millions of people. Unknown to Congress and the American people, tests using apparently harmless microbes were performed on such large commu-

nities as San Francisco. When news of these secret tests was made public, many questioned their morality. Extensive criticism of the research and development of these weapons, together with the realization that these weapons posed a threat to the attackers as well as the attacked, led President Richard Nixon to end the American biological weapons program formally in 1969.

Abhorrence of biological weapons extended to the world community, and in 1972 the Biological and Toxin Weapons Convention (BTWC)—a treaty that prohibited the development, production, and stockpiling of bacteriological weapons—was signed in Washington, D.C., London, and Moscow and was put into force in 1975. Although it was eventually signed by most members of the United Nations, the nations that signed the pact failed to reach agreement on an inspection system that would control the proliferation of these weapons. A pivotal irony of the BTWC is that while most of the world was

renouncing germ warfare, biologists were learning how to manipulate DNA, the molecule that carries genetic information, in powerful new ways. This knowledge made possible the creation of “superbugs,” infectious agents for which there are no cures.

Some scientists warned the public and international agencies about these new germ weapons. Other investigators discovered that American researchers were creating infectious agents that would confuse diagnosticians and defeat vaccines. Similarly, Soviet researchers on an island in the Aral Sea, described as the world’s largest BW test site, were producing germ weapons that could be loaded on missiles. When Boris Yeltsin became president of Russia, he discovered that the secret police and military officials had misinformed him about BW programs, in which deadly accidents had occurred. Also troubling was the spread of biological agents to such countries as Iraq. American and French companies legally shipped anthrax

and botulinum bacteria to Iraq, whose scientists later acknowledged that they had used these microbes to make tons of biological weapons during the 1980’s.

With the demise of the Soviet Union and increasing violence in the Balkans and Middle East, politicians became fearful that experts who had dedicated their careers to making biological weapons would now sell their knowledge to rogue nations or terrorist groups. Indeed, deadly pathogens were part of world trade, since the line separating legitimate and illegitimate research, defensive and offensive BWs, was fuzzy. In the 1980’s members of a religious cult spread salmonella, a disease-causing bacterium, in an Oregon town, causing more than seven hundred people to become very ill. The same company that sold salmonella to this religious cult also sold pathogens to the University of Baghdad. Bioterrorism had become both a reality and a threat.

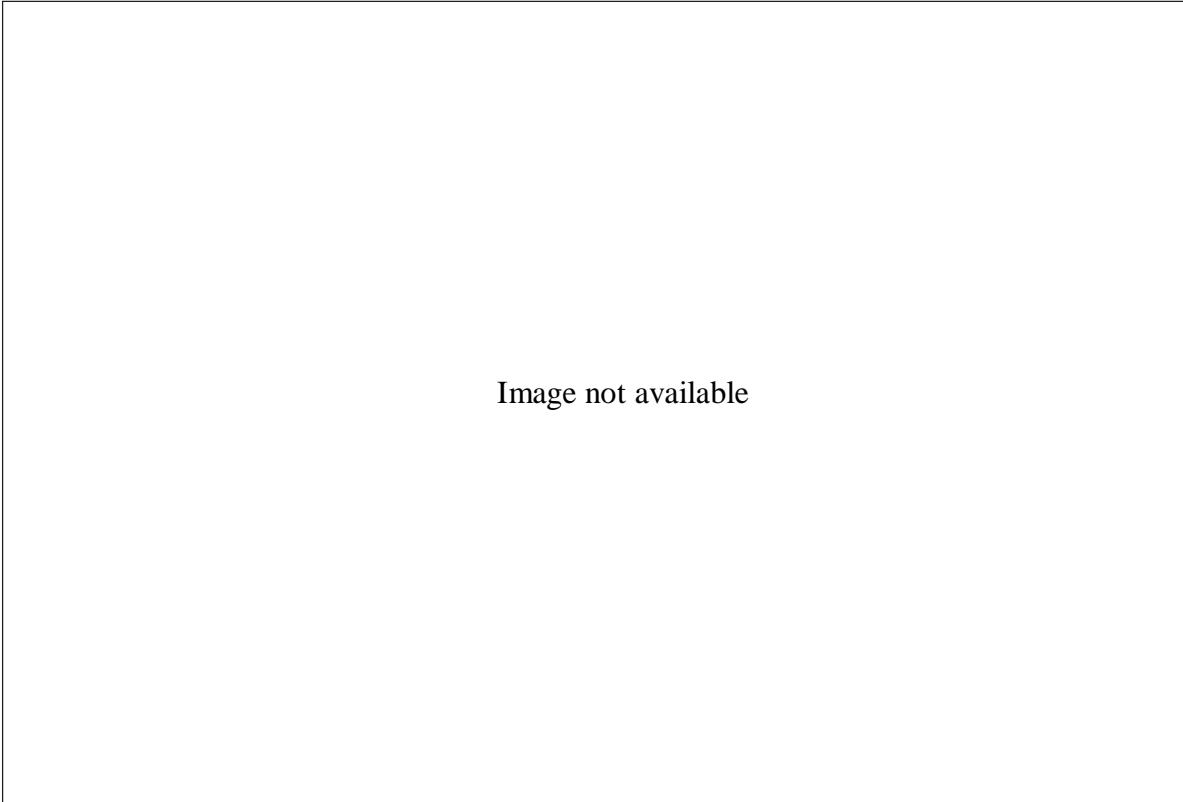


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In 1996, U.N. inspectors supervise the destruction of growth media for biological weapons in Iraq. (AP/Wide World Photos)

The Future of Genetic Weapons

Some scientists and politicians believe that a nation's best defense against bioterrorism is advanced genetic knowledge, so that vaccines can be tailored to respond to traditional and new BWs. For example, the Human Genome Project, which succeeded in mapping the human genetic material, has the potential for revealing both the vulnerabilities and defenses of the immune system. (The human genome sequence contains 3.2 billion bases and approximately 34,000 genes. These data freely are available on the Internet in a variety of forms, including text files and graphical "genome browsers.") On the other hand, such knowledge could prove dangerous if the genetic vulnerabilities of certain ethnic groups could be targeted by bioengineered microbes. Some scientists find these speculations about genocidal BWs unevidenced and unsubstantial. Genetic similarities between different ethnic groups are more significant than their differences. Other scientists point out that dramatic genetic differences between ethnic groups are a reality. For example, milk is a poison for certain Southeast Asian populations. Other genetic differences could therefore be exploited to create BWs to attack group-specific gene clusters. Believers in ethnic BWs point to existing techniques for selectively killing certain cells and for inactivating certain DNA sequences. These techniques, developed with the hope of curing genetic diseases, could also be used to cause harm. Knowledge of the structure of the human genome will increasingly lead to knowledge of its function, and this knowledge will make it possible to manipulate, in both benign and malign ways, these information-laden molecules. Modern biotechnology thus presents humanity with both a great promise, to better health and life in peace, and a great peril, to multiply sickness and death in war. The hope of many scientists, politicians, and ordinary people is that humanity will choose the path of promise.

—Robert J. Paradowski

See also: Anthrax; Bioethics; Biopesticides; Biopharmaceuticals; Emerging Diseases; Eugenics; Eugenics: Nazi Germany; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Smallpox.

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Web Site of Interest

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Biopesticides

Field of study: Genetic engineering and biotechnology

Significance: As an alternative to chemical pesticides, agricultural scientists have begun using ecologically safer methods such as biopesticides to protect plants from insects.

Key terms

Agrobacterium tumefaciens: a species of bacteria that is able to transfer genetic information into plant cells

Bacillus thuringiensis: a species of bacteria that produces a toxin deadly to caterpillars, moths, beetles, and certain flies

BACULOVIRUS: a strain of virus that is capable of causing disease in a variety of insects

TRANSFORMATION: the process of transferring a foreign gene into an organism

TRANSGENIC ORGANISM: an organism synthesizing a foreign protein; the gene of which was obtained from a different species of organism

Bacillus thuringiensis

Hungry insects are the bane of gardeners. This problem is worsened for farmers, whose livelihoods depend on keeping fields free of destructive insects. Although effective, chemical pesticides have a variety of drawbacks. The increasing popularity of organically grown produce that is untreated by chemicals suggests that consumers are wary that human-made pesticides may hold hidden dangers. In response to consumers' worries over chemical-pesticide

safety, agricultural biologists have turned to nature to solve pest problems. Biopesticides are insecticides taken from nature. They are designed not in a laboratory but through evolution, making them very specific and effective. A biopesticide may be sprayed directly on crops or may be genetically engineered to be produced by a crop itself.

Since the 1950's, the bacterial pesticide *Bacillus thuringiensis* (*Bt*) has been used on crops susceptible to destruction by insect larvae. Upon sporulation, *Bt* produces a crystallized protein that is toxic to many forms of larvae. The protein is synthesized by the bacteria as an

Image not available

Although the biopesticide Bt has been genetically integrated into crops to make them resistant to insects and other pests, there is evidence that new pests are emerging and old pests, such as the bollworm—against which the Bt crops were engineered to protect—are evolving their own resistance to the Bt toxin. Here a farmer in India displays the bollworm damage to his Bt cotton. (AP/Wide World Photos)

Biopesticides and Nontarget Species

Researchers have long had a concern as to the effect of chemical insecticides on nontarget species. Target species frequently display resistance to chemical controls due to large effective population sizes and prior histories of exposure to chemical agents which favors the increase in resistance alleles in a population. The exact opposite is typically true for non-target species that occupy the treatment area. When biopesticides such as the Cry1ab endotoxins, derived from the soil bacterium *Bacillus thuringiensis* (*Bt*), were first proposed as control agents, many scientists believed that the collateral effects on non-target species would be significantly limited.

Initially these toxins were sprayed on crops, thus potentially increasing the exposure of nontarget species. Even with the development of transgenic crops such as corn, it was possible for *Bt* to move from the treatment area to the feeding grounds of nontarget species through pollen dispersal. One of the first documented accounts of *Bt*-induced mortality in a nontarget species was provided in 1999 by researchers at Cornell University. They demonstrated that the pollen from *Bt*-treated corn increased mortality among monarch butterflies (*Danaus plexippus*) when applied to the surfaces of milkweed plants, the butterfly's primary food source. In this study, monarchs exposed to *Bt* had a slower rate of growth and increased mortality. It was suggested that field monarchs could also be exposed to corn pollen containing *Bt* endotoxins. Given the popularity of the monarch and the noticeable decline in North American populations during the 1990's, it appeared that the future of biopesticides was dim.

Since then, additional studies indicate a less significant effect of *Bt* toxins on nontarget species. The dispersal of *Bt* pollen is not believed to occur more than a few meters from the edges of the treatment area, and even at these distances the levels have been shown to be sublethal. Research involving monarchs and swallowtail butterflies (*Papilio* species) has indicated that lethality is not elevated at low-level *Bt* exposure, although there is evidence of reduced growth rates. Furthermore, only a fraction of the nontarget organism's population would be exposed at a given time, and frequently the larval periods of the target and nontarget organisms do not overlap. This evidence suggests that biopesticides are not producing the observed decrease in nontarget populations.

It is likely that there may be a limited effect of biopesticides on nontarget species, and most researchers agree that additional research needs to be conducted. The genetics of *Bt* resistance have been determined for a number of insects, although for others the exact mechanism has remained elusive. However, the greatest threat to the nontarget organisms rests with habitat destruction. A decrease in the population size due to reduced resources may serve to weaken the population and enhance the sublethal effects of biopesticide production. The physiological effect and population genetics of *Bt* susceptibility in nontarget species will need to be examined in some detail to prove to the public the value of biopesticides.

—Michael Windelspecht

inactive proenzyme. After it is digested, enzymes in the insect's gut cleave the protein into an active, toxic fragment. The active toxin binds to receptors in the insect's midgut cells and blocks those cells from functioning. Only caterpillars (tobacco hornworms and cotton bollworms), beetles, and certain flies have the gut biochemistry to activate the toxin. The toxin does not kill insects that are not susceptible, nor does it harm vertebrates in any way.

The drawbacks of *Bt* are its expense and its short-lived effect. In the early 1990's, scientists overcame these two drawbacks through applied genetic engineering. They produced transgenic

cotton plants that generated their own *Bt* toxin. The toxin gene was first isolated from *Bt* cells and ligated (enzymatically attached) into a Ti plasmid. A Ti plasmid is a circular string of double-stranded DNA that originates in the *Agrobacterium tumefaciens* bacteria. The *A. tumefaciens* has the ability to take a portion of that Ti plasmid, called the T-DNA, and transfer it and whatever foreign gene is attached to it into a plant cell. Cotton plants were exposed to the *A. tumefaciens* carrying the toxin gene and were transformed. The transgenic plants synthesized the *Bt* toxin and became resistant to many forms of larvae.

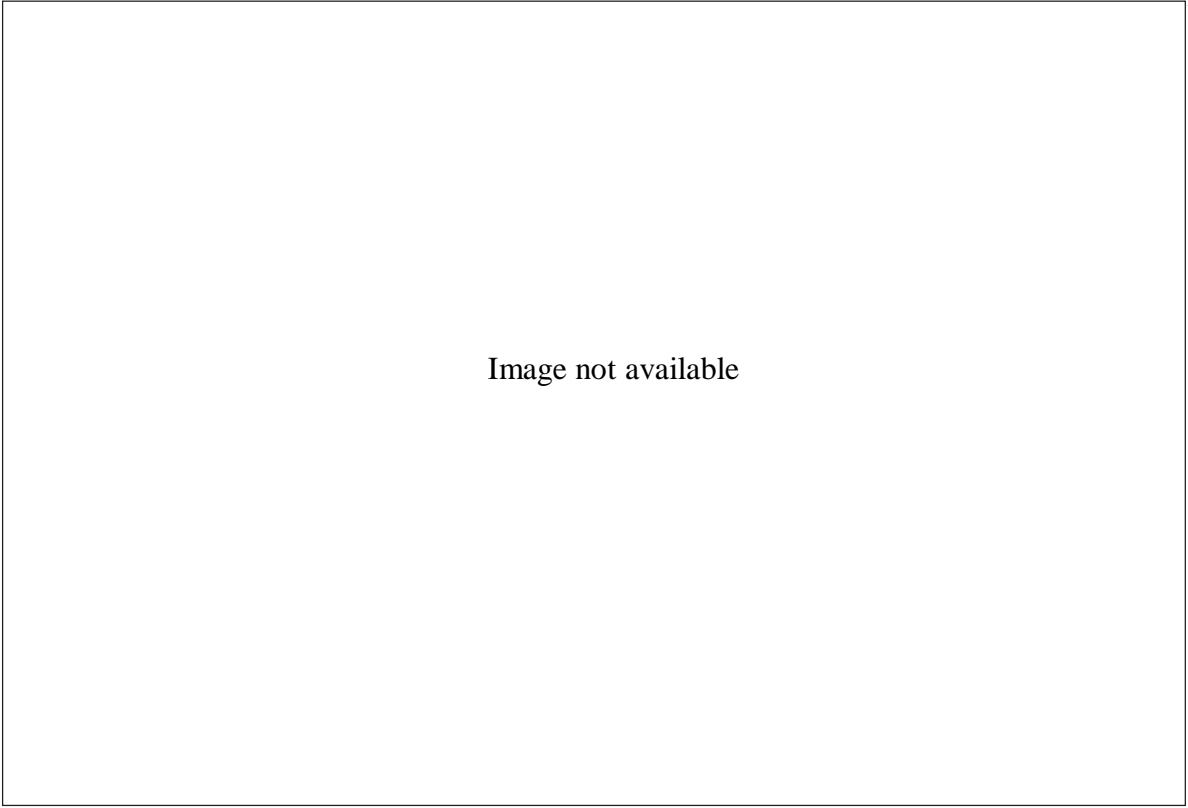


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At the University of Florida's Institute of Food and Agricultural Sciences, Dov Borovsky has developed a "diet pill" for mosquitoes that causes them to starve to death. It may help eradicate mosquitoes and the diseases they transmit. (AP/Wide World Photos)

Many crystal toxins have been isolated from various strains of *Bt*. These toxins make up a large collection of proteins active against pests from nematodes to aphids. Researchers are in the process of reengineering the toxin genes to improve upon their characteristics and to design better methods of transporting genes from one *Bt* strain to another.

Other Biopesticides

Several species of fungi have been found to be toxic to insects, including *Verticillium lecanii* and *Metarhizium anisopliae*. Scientists have just begun to study these fungi, which are not yet commercially available to farmers.

In the mid-1990's, a viral biopesticide called baculovirus became widely popular. Baculoviruses are sprayed onto high-density pest populations just like chemical pesticides. Baculoviruses have several advantages over conventional pesticides. The most important advan-

tage is their strong specificity against moths, sawflies, and beetles but not against beneficial insects. Also, viruses, unlike bacteria, tend to persist in the environment for a longer period. Finally, baculoviruses are ideal for use in developing countries because they can be produced cheaply and in great quantity with no health risks to workers. One limitation of baculovirus is that it must be administered in a precise temporal and spatial framework to be effective. Knowledge of insect behavior after hatching, the insect population's distribution within the crop canopy, and the volume of foliage ingested by each larva is essential. For example, moths usually do the most damage at the late larval stage. To minimize crop damage from moths, one must spray baculovirus as early as possible before the insects reach that late stage.

One final biopesticide approach has been to make transgenic plants that manufacture proteins isolated from insect-resistant plant spe-

cies. Tomatoes naturally make an enzyme inhibitor that deters insects by keeping their digestive enzymes (trypsin and chymotrypsin) from functioning. These inhibitors were isolated by Clarence Ryan at the University of Washington. Ryan transformed tobacco plants with two different forms of inhibitor (inhibitors I and II from tomato). The tomato proteins were effectively produced in tobacco and made the transgenic plants resistant to tobacco hornworm larvae.

Biopesticide Resistance

As with chemical pesticides, over time insect populations grow resistant to biopesticides. *Bt*-resistant moths can now be found around the world. Resistance arises when pesticides are too effective and destroy more than 90 percent of a pest population. The few insects left are often very resistant to the pesticide, breed among themselves, and create large, resistant populations.

Entomologists have suggested strategies for avoiding pesticide-resistant insect populations. One strategy suggests mixing biopesticide-producing and nonproducing plants in the same field, thereby giving the pesticide-susceptible part of the insect population places of refuge. These refuges would allow resistant and nonresistant insects to interbreed, making the overall species less resistant. Other strategies include synthesizing multiple types of *Bt* toxin in a single plant to increase the toxicity range and reduce resistance, making other biological toxins besides *Bt* in a single plant, and reducing the overall exposure time of insects to the biopesticides.

—James J. Campanella

See also: Biofertilizers; Genetic Engineering: Agricultural Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Population Genetics.

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Web Site of Interest

U.S. Environmental Protection Agency. <http://www.epa.gov/pesticides>. Government site with a link to information on biopesticides, including the Federal Insecticide, Fungicide, and Rodenticide Act, enacted to monitor the harmful effects of toxic pesticides on humans and the environment and ensure industry compliance.

Biopharmaceuticals

Fields of study: Diseases and syndromes; Genetic engineering and biotechnology

Significance: *Biopharmaceuticals encompass a class of drugs that are designed by combining genetics with biotechnology. Biopharmaceutical products are typically derived from proteins, such as enzymes or antibodies, and are genetically engineered in an attempt to treat a specific disease. They differ from traditional pharmaceuticals, which are usually simpler compounds that are produced by chemical synthesis.*

Key terms

CLINICAL TRIAL: an experimental research study used to determine the safety and effectiveness of a medical treatment or drug

HUMANIZED ANTIBODY: a human antibody that has been engineered to contain a portion of a nonhuman variable region with known therapeutic activity

PHARMACOGENOMICS: the field of science that examines how variations in genes alter the metabolism and effectiveness of drugs

History of Biopharmaceuticals

Drugs have been used by humans for thousands of years. The Sumerians are the first people known to have compiled medical information in a written form that outlined symptoms and treatments for different diseases more than three thousand years ago. Most ancient cultures used medicines derived from plants and animals. These drugs are different from modern biopharmaceuticals in many ways, but the most significant difference is that there was no engineering used to shape the drugs for a

particular disease. Since there was no real understanding of the underlying problem, a rational approach to drug selection and design was difficult if not impossible. One philosophy of medicine that developed to address this problem was called the doctrine of similitudes, in which treatments were based on similarities of structure with disease manifestation. For example, the leaves of St John's wort look similar to damaged skin, so it was thought that extracts from this plant would be beneficial for treating cuts and burns.

It was not until the twentieth century that the underlying genetic basis for disease was discovered. The discovery that DNA is the genetic material and provides the instructions to make proteins was revolutionary. In the mid-1900's it was demonstrated that sickle-cell disease was caused by a single nucleotide change from an A (adenine) to a T (thymine) in the hemoglobin beta-chain gene. This small change alters the shape of a red blood cell from a biconcave disc to a sharply pointed crescent. Even though this finding showed it was possible to identify genetic mutations, there was still no way to manipulate or make changes to the genetic information itself.

The advent of recombinant DNA technology in the 1970's provided the first chance to engineer, or manipulate, genes. Restriction enzymes became an important tool of this technology. Restriction enzymes were first found in bacteria, where they function to protect the cell from foreign DNA by cutting it up. Restriction enzymes cut DNA at specific sequences, which are usually palindromes of the letters that signify the four nucleotides that make up DNA: guanine (G), adenine (A), thymine (T), and cytosine (C). Most restriction enzymes cut the DNA in such a way that an overhang, called a sticky end, is created. Since DNA readily recombines with complementary strands, these sticky ends can be used to splice different pieces of DNA together. The resulting sequence is called recombinant DNA.

With the ability to engineer DNA now possible, scientists looked again to bacteria to provide a way to convert that DNA into protein. Bacteria are ideal for protein production because they reproduce quickly, are easy to ma-

nipulate, and can be grown in large quantities. Many bacteria contain circular pieces of DNA apart from their genome, called plasmids. These plasmids can be readily transferred between bacteria and are also inherited by the daughter cells when a bacterium divides. With the use of restriction enzymes, plasmids can be taken from bacteria and engineered to contain a foreign gene. The resulting recombinant plasmid can be put back in bacteria, transforming them into protein factories that work nonstop transcribing and translating the recombinant gene. The first biopharmaceutical produced in bacteria was recombinant human insulin, which was marketed in 1982.

The future for biopharmaceuticals looks bright. In 1991, there were only fourteen biopharmaceuticals approved for use by the U.S. Food and Drug Administration (FDA). By 2001, nearly three hundred had been approved for use, with an additional fifty in phase III clinical trials, and by 2003 more than 330 major companies in the United States were working to produce and develop biopharmaceuticals.

Design of Biopharmaceuticals

A popular method for identifying disease-related genes is called genomics. This method uses gene chip analysis to screen thousands of genes in a single experiment. This approach is dramatically faster and more efficient than traditional methods and can be used for any disease, even those that are not hereditary.

Once the genomic information is obtained, it is used to build a broad understanding of how a disease gene functions and what role it plays in the cell. This information is gathered through the use of experimental models, genetic analysis, biochemical analysis, and structural analysis. Experimental models can range from cell culture to transgenic mice and provide physiological information about the disease. Genetic analysis provides information about where and when the gene is expressed. At the molecular level, biochemical analysis provides information about protein-protein interactions, post-translational modifications, and enzymatic activity. Structural analysis yields extremely detailed information about the physical arrangement of the atoms that make up the

protein. All of these approaches can provide clues as to what may be important targets for treatment of the disease.

A better understanding of the disease at the genetic and molecular levels facilitates an attempt at designing a biopharmaceutical to treat the disease. Once a disease is understood, it becomes possible to target a key pathway or protein involved in the disease. The resultant drug and the way that it is used clinically will vary from disease to disease. For example, type I diabetes is caused by a deficiency in the hormone insulin. Without insulin, the body is not able to regulate the level of glucose in the blood. Lack of insulin is easily corrected by a simple injection of recombinant human insulin, the first biopharmaceutical. Another example of a biopharmaceutical that is currently in use is the enzyme tissue plasminogen activator (tPA). Most heart attacks are caused by a blood clot blocking the flow of blood through a coronary artery. Formation and removal of blood clots is a highly regulated and well-understood process. The enzyme tPA is known to be one of the key players in blood clot removal. This knowledge led to the development of recombinant tPA, which can be provided by injection or infusion to heart attack patients. Once in the bloodstream, the tPA breaks up the coronary artery clots and restores blood flow to the heart, preventing any further muscle damage.

Clinical Trials

Before a biopharmaceutical can be used to treat disease, it must undergo a clinical trial to test its safety and effectiveness. There are four phases to a clinical trial. Phase I trials involve studies on a small number of patients (fewer than one hundred) to determine drug safety and dosage. Phase II trials involve more patients (up to five hundred) to determine effectiveness and additional safety information, such as potential side effects. Phase III trials are the most extensive and involve large numbers of people (between one thousand and three thousand) to establish risk-benefit information and comparisons with other currently used treatments. Phase IV trials determine the drug's optimal use in a clinical setting. In 2003, the en-

tire process of drug design—from discovery to clinical trials—cost approximately \$802 million and took an average of twelve years. Many years of research and millions of dollars are wasted, because only one in five thousand potential drugs actually makes it to market.

Biopharmaceuticals Today

Two examples of approved biopharmaceuticals are Aralast and Campath. Aralast is marketed by Baxter and was approved for use by the FDA in 2003. Aralast is the trade name for the recombinant human protein known as alpha-1 proteinase inhibitor (A1PI). A1PI deficiency results in the destruction of lung tissue, which can lead to emphysema. Aralast is given to patients intravenously each week and helps protect them against future lung damage. The second drug, Campath, is marketed by Millennium Pharmaceuticals and was approved by the FDA in 2001. Campath is the trade name for a humanized antibody against the CD52 antigen found on lymphocytes. The antibody is used to treat chronic lymphocytic leukemia and works by destroying lymphocytes through agglutination and complement activation.

The current trend in pharmaceutical research is the production of designer drugs through the new field of pharmacogenomics. Instead of giving a patient a drug that works for the average person with the average form of a disease, a patient will be given a drug that is specifically matched to his or her genetic profile and to his or her particular form of the disease. Currently 100,000 people die each year because of adverse drug reactions. With the use of pharmacogenomics it will be possible to determine a patient's genetic profile prior to treatment and avoid adverse drug reactions. Research in pharmacogenomics will also increase the pool of drugs available to treat disease. Currently, many drugs never make it to market because they work for only a small subset of patients. Pharmacogenomic research will allow these specific patients to be identified and enable previously abandoned drugs to be used to treat disease.

—Matthew J. F. Waterman

See also: Cloning; Cloning: Ethical Issues; Cloning Vectors; Gene Therapy; Gene Therapy;

Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; Molecular Genetics; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Web Site of Interest

- American Chemical Society, The Pharmaceutical Century. <http://pubs.acs.org/journals/pharmcent>. Posts articles about the science of biopharmaceuticals, including the role played by genetics and the Human Genome Project in the development of new drugs.

BloTTing: Southern, Northern, and Western

Field of study: Techniques and methodologies

Significance: *BloTTing is a technique that allows identification of a specific nucleic acid or amino acid sequence even when it is mixed in with all of the other material from a cell. This allows the rapid identification of the changes associated with mutant alleles.*

Key terms

BLOTTING: the transfer of nucleic acids or proteins separated by gel electrophoresis onto a filter paper, which allows access by molecules that will interact with only one specific sequence

HYBRIDIZATION: incubation of a target sequence with an identifying probe, which allows the formation of annealed hybrids

NORTHERN BLOT: a blot designed to detect messenger RNA

PROBE: a nucleic acid sequence or antibody that can attach to a specific DNA or RNA sequence or protein; the probes are often labeled with radioactive compounds or enzymes so their position can be determined

SOUTHERN BLOT: a blot designed to detect specific DNA fragments

WESTERN BLOT: a blot that uses antibodies to detect specific proteins

Limitations of Gel Electrophoresis

Using gel electrophoresis to separate proteins and nucleic acids has been an invaluable tool in analyzing living systems. Changes in these molecules—such as a mobility shift in a mutant protein or the change in the size of a plasmid that has received a DNA insert—can be easily detected using this technique. However, the ability to differentiate between types of molecules is quite limited. An extract of red blood cell proteins run through an acrylamide gel might show one major band for hemoglobin which can be discerned from the many other proteins in the cell. However, the hundreds of different proteins that might be produced in a liver extract will produce a tight ladder of bands that are impossible to tell apart.

The situation can be even worse with DNA. A restriction enzyme digest of a plasmid or simple virus might yield fewer than six pieces of DNA that could be easily separated on an agarose gel. If one were to digest the total genomic DNA of even a simple organism, such as *Escherichia coli*, with a typical restriction enzyme such as *Eco*RI, the result would be a thousand bands of numerous sizes (4×10^6 base pairs of DNA, since *Eco*RI recognizes a six-base-pair site, which should occur, on average, every 4^6 or 4,096 bp). After separation on a gel, the result would be a smear with no individual bands visible. Working with an even more complex genome, such as the human genome, would result in millions of bands. The only way to study a specific protein or nucleic acid sequence using gel electrophoresis, therefore, would be to find

a way to label it specifically so that it could be differentiated from the general background.

Basic Blotting Techniques

In 1975, Ed Southern developed a method that allowed the detection of specific DNA sequences after they had been separated by agarose gel electrophoresis. What makes a piece of DNA unique is the sequence of the nucleotides. This is most efficiently detected by the hybridization of the antiparallel strand. This can only occur if the two strands are separated into single strands. Therefore, the first step is to soak the agarose gel in a strong base, such as 1 molar sodium hydroxide, and high salt, which stabilizes the single-stranded form. The base is then neutralized with a strong buffer, such as tris-hydrochloride, again in high salt. The DNA can now be analyzed by its ability to hybridize to a radioactive piece of single-stranded DNA. Since this radioactive DNA can “explore” the different sequences to find the one matching sequence, it is also known as a probe (an instrument or device that can be used to explore and send back information).

Although the agarose is porous, it would be very slow and inefficient to try to perfuse the gel with radioactive probe and then remove the pieces that did not hybridize. Southern realized that he needed to move the DNA to a thin material to be able to probe it efficiently. The material chosen was nitrocellulose, consisting of a variant of paper (cellulose) with reactive nitro groups attached. The treated gel is placed on a sponge soaked with a high-salt solution. The nitrocellulose sheet is placed onto the gel and then a stack of dry paper towels is laid on top. The salt solution is drawn through the gel to the dry towels and carries the DNA from the gel up into the paper. The positively charged nitro groups on the nitrocellulose stick to the negatively charged DNA, thereby holding the DNA in a pattern matching the band locations in the gel. The nitrocellulose is removed from the gel and baked at 80 degrees Celsius (176 degrees Fahrenheit) or treated with ultraviolet light, both of which covalently cross-link the DNA to the paper, locking it in its position. The filter is soaked in a solution that promotes reassociation of single-stranded DNA, and ra-

dioactive, single-stranded DNA is added. Since the added DNA could stick nonspecifically to the nitrocellulose, the paper is pretreated with unrelated DNA, such as sheared salmon DNA, which will bind the available nitro groups but not react with the probe.

A large molar excess of probe must be used to drive the hybridization reaction (reforming the “hybrid” of two matching antiparallel strands together), which means that it is necessary to make sure that enough probe is available in the solution to randomly run into the correct sequence on the paper and reanneal to it. The hybridization is done at an elevated temperature—often 50–65 degrees Celsius (122–149 degrees Fahrenheit), so that only strands that match exactly will stay together and those with short, random matches will come apart. After overnight hybridization, the paper is washed multiple times with a detergent-salt solution, which removes the DNA that did not hybridize. The paper is placed against a piece of X-ray film, and the radioactive emissions from the probe darken the film next to them. When the film is developed, a pattern of bands appears that corresponds to the position in the original gel of the DNA piece for which the researcher was probing.

Expanded Techniques to Study RNA and Proteins

The basic method of blotting has been expanded to include the study of RNA and proteins. James Alwine developed a very similar method to transfer messenger RNA (mRNA) that had been separated on an agarose gel. Since the mRNA started as single-stranded, there was no need to treat the gel with denaturant. However, to block the formation of internal double-stranded regions, which could alter the migration during electrophoresis, the gel contained an organic solvent. Other than that, the two methods are very similar. Although the DNA transfer system was named the Southern blot in honor of Ed Southern, Alwine decided to defer the credit and called his system the Northern blot to indicate that it was related but in a different direction.

Similarly, when W. N. Burnette developed a system for transferring and detecting specific

proteins, he named the system Western blotting. This system of naming has been expanded: A technique for detecting viral DNA in tree leaves was named the Midwestern blot and a variant of the Northern blot developed in Israel was named the Middle Eastern blot.

Since proteins are generally smaller than DNA fragments, they are usually separated on polyacrylamide gels, which have a much smaller pore size than agarose gels. It is therefore necessary to use electrical current to pull the proteins out of the gel. The nitrocellulose is pressed onto the gel with a porous plastic pad. The gel is then placed in a buffer tank and electrodes are placed on either side. When a voltage is applied, the current that flows through the gel carries the proteins onto the nitrocellulose. The reactive side chains of the nitrocellulose also bind proteins very effectively, so they are all retained on the paper. The specific probe used to detect a protein is an antibody that either can be radioactively labeled or can have an enzymatic side chain attached, which will produce light or a colored dye when the appropriate chemicals are added. Since the antibody is a protein, it could also stick nonspecifically to the paper, so the blot is pretreated with a general protein such as serum albumin before the antibody is added.

Blotting in Genetic Analysis

The ability to detect individual molecules in a large background has been very important for genetic analyses. For instance, restriction fragment length polymorphism (RFLP) analysis is a method that uses the change in the size of a DNA fragment in the genome, generated by restriction enzyme digestion as a genetic marker. The isolation of many disease genes, including the one causing Huntington’s disease, depended on RFLP mapping to localize the gene. It would not be possible to detect the changes in a single DNA fragment out of the millions generated by digesting the human genome without having the Southern blot to pick out the correct piece. Many other mutations that change a specific region of DNA—such as deletions, inversions, and duplications—are often detected by changes in a Southern blot pattern. The sensitivity of hybridization can be

tuned to a level where probes that differ by only a single nucleotide will not attach efficiently. This allows the rapid identification of the positions of point mutations. When polymerase chain reaction (PCR) is used to amplify DNA from a crime scene or to detect human immunodeficiency virus (HIV) in the bloodstream, the presence of DNA pieces on a gel is not sufficient proof that the correct DNA has been found. The DNA must be blotted and probed with the expected sequence to confirm that it is the correct piece.

Northern blot analysis allows scientists to see how mRNA is altered in different mutants. Northern blots can indicate if a mutant allele is no longer transcribed or if the level of mRNA produced has been dramatically decreased or increased. Deletions or insertions will also show up as shortened or lengthened messages. Alternative splicing can be seen as multiple bands on a Northern blot which hybridize to the same probe. Point mutations that do not detectably alter the mRNA can still dramatically alter the protein product. Changes of a single amino acid can alter the electrophoretic mobility and the difference in apparent molecular weight can often only be detected by Western blot. These changes can also alter protein stability, which can be detected as decreased protein levels showing up on the Western. The ability to detect changes at the DNA, RNA, and protein level through blotting techniques has greatly increased the ability of scientists to study genetic alterations.

Future Directions

Blotting techniques are the most generally efficient methods for detecting specific proteins or nucleic acids. Most improvements in the past years have been aimed at speeding up the transfer process using vacuums or pressure or the hybridization process by changing the conditions. The next step will be developing silicon chips that can interact with specific nucleic acid or amino acid sequences and produce an electrical output when they "hybridize" with the correct sequence. This will diminish the time required to confirm a sequence from several hours to minutes.

—J. Aaron Cassill

See also: Antibodies; DNA Sequencing Technology; Gel Electrophoresis; Genetic Testing; Huntington's Disease; Immunogenetics; Model Organisms; Polymerase Chain Reaction; Repetitive DNA; RFLP Analysis.

Further Reading

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- Southern, E. M. "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis." *Journal of Molecular Biology* 98, no. 3 (1975): 503-517. The original description of Southern blotting and of blotting in general. This is one of the most often cited articles in biology research.

Breast Cancer

Field of study: Diseases and syndromes

Significance: While the majority of breast cancers are caused by acquired mutations, about 5 percent of all breast cancers are caused by inherited mutations that greatly increase the chances of developing the disease. Germ-line mutations in the BRCA1 and BRCA2 genes are associated with most of these inherited breast cancers.

Key terms

BRCA1 AND BRCA2 GENES: the genes associated with most inherited breast cancers

CELL CYCLE: the sequence of events of a dividing cell

EXON: the coding sequence (part of a messenger RNA, or mRNA) that specifies the amino acid sequence of the protein produced during translation

GERM-LINE MUTATION: a heritable change in the genes of an individual's reproductive cells, often linked to hereditary diseases

p53 GENE: a tumor-suppressor gene, the first gene identified in an inherited breast cancer

TUMOR-SUPPRESSOR GENE: a gene that produces a protein product that limits cell division and therefore acts to inhibit the uncontrolled cell growth of cancers

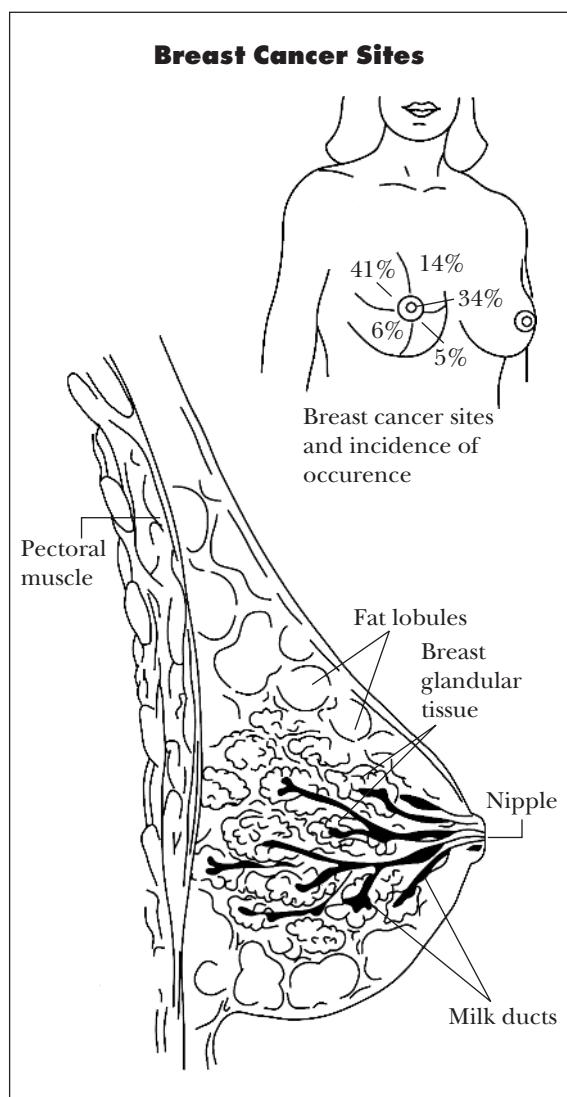
Genes Associated with Breast Cancer

Approximately one in eight women develops breast cancer over the course of her lifetime. In the United States there are approximately 180,000 new cases of breast cancer yearly. By 2002, more than forty different genes had been found to be altered in breast cancers. Those breast cancers that are not familial (inherited)

are termed "sporadic." It is estimated that about 5 to 10 percent of all breast cancers are familial. Approximately 80 to 85 percent of these can be attributed to mutations in the *BRCA1* or *BRCA2* gene.

The first gene identified in an inherited breast cancer was *p53*, which is mutated in Li-Fraumeni syndrome. It is a tumor-suppressor gene that encodes a protein transcription factor that stops the cell cycle until DNA repair has occurred; a defective *p53* gene no longer stops cell division, and unrepaired DNA can be replicated, resulting in accumulated mutations in the cell. About 1 percent of women who develop breast cancer before the age of thirty have germ-line mutations in *p53*. Families with this syndrome have extremely high rates of brain tumors and other cancers in both children and adults.

Some gene mutations may predispose an individual to develop breast cancer. For example, there is an increased incidence of breast cancer associated with the ataxia telangiectasia (*AT*) gene and the *HRAS1* gene. A mutated form of the *AT* gene is found in the rare recessive hereditary disorder ataxia telangiectasia, which has a very wide range of symptoms, including cerebellar degeneration, immunodeficiency, balance disorder, high risk of blood cancers, extreme sensitivity to ionizing radiation, and an increased risk of breast cancer. Individuals with one mutated copy of the *AT* gene have an increased risk of cancer. The *AT* gene was identified as a phosphatidylinositol-3 kinase (an enzyme that adds a phosphate group to a lipid molecule) that transmits growth signals and other signals from the cell membrane to the cell interior. The *AT* gene was found to be similar in sequence to other genes that are known to have a role in blocking the cell cycle in cells whose DNA is damaged by ultraviolet light or X rays. It is possible that the mutated *AT* gene does not stop the cell from dividing, and the damaged DNA may lead to cancers. It is disturbing to note that individuals with a mutated *AT* gene may be more sensitive to ionizing radiation. It must be determined if these individuals should then avoid low X-ray doses, such as those received from a mammogram used to detect the early stages of breast cancer.



(Hans & Cassidy, Inc.)

Discoveries of Breast Cancer Genes

Prior to the discoveries of *BRCA1* and *BRCA2*, there were many hints that susceptibility to at least some breast cancers was inherited. The time line below shows some of the discoveries leading up to the discoveries of *BRCA1* and *BRCA2* as well as later discoveries about breast cancer genes.

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| <p>1966 Henry Lynch began the first studies on inherited cancers.</p> <p>1970 The first cancer-causing gene (oncogene) was reported in chickens by Peter Vogt.</p> <p>1976 J. Michael Bishop and Harold Varmus reported the discovery of oncogenes in the DNA of normal chromosomes.</p> <p>1978 M. H. Bronstein et al. see a link between Cowden disease, an inherited tumorogenic syndrome, and breast cancer.</p> <p>1979 Arnold Levine and David Baltimore discover <i>p53</i>, a gene mutated in approximately half of all known cancers, including breast cancer.</p> <p>1985 The mutant <i>p53</i> gene is cloned by Arnold Levine.</p> <p>1987 Michael Swift et al. report a hereditary link between ataxia telangiectasia mutated (<i>ATM</i>) and many cancers, including breast cancer.</p> <p>1988 Dennis Slamon reports that the <i>HER-2/neu</i> growth factor gene is overexpressed in 30 percent of the most aggressive breast cancers.</p> <p>1990 Mary Claire King and coworkers report the discovery of <i>BRCA1</i> in Ashkenazi Jewish women and locate it on chromosome 17.</p> <p>1990 David Malkin et al. report a link between the <i>p53</i> gene product and breast cancer.</p> <p>1991 Elizabeth Claus et al. do a statistical analysis of familial breast cancer and predict a dominant breast cancer gene will be found.</p> <p>1993 Theodore Krontiris et al. report an association between <i>HRAS1</i> (Harvey rat sarcoma oncogene 1) and breast cancer.</p> | <p>1994 Yoshio Miki et al. announce the cloning of <i>BRCA1</i> on chromosome 17.</p> <p>1995 Richard Wooster et al. announce the discovery and cloning of <i>BRCA2</i> on chromosome 13.</p> <p>1996 Prasanna Athma et al. report that heterozygotes for the recessive allele <i>ATM</i> are more susceptible to breast cancer.</p> <p>1997 Danny Liaw et al. find that germ-line mutations in the <i>PTEN</i> gene lead to Cowden disease and associated breast cancer.</p> <p>1998 Dennis Slamon tests Herceptin, a monoclonal antibody that targets the product of <i>HER-2/neu</i>, against aggressive breast cancers.</p> <p>1999 François Ugolini et al. implicate <i>FGFR1</i> (fibroblast growth factor receptor gene 1) in some breast cancers.</p> <p>2000 Tommi Kainu et al. propose a <i>BRCA3</i> gene to explain non-<i>BRCA1</i>/<i>BRCA2</i> hereditary breast cancers in several families.</p> <p>2001 Paul Yaswen reports that multiple copies of the gene <i>ZNF217</i> are seen in 40 percent of breast cancers.</p> <p>2001 Minna Allinen et al. find a mutation in <i>CHEK2</i> gene that leads to hereditary breast cancers. This is proposed as <i>BRCA3</i>.</p> <p>2002 Alan D'Andrea et al. report that the same inherited mutations in the six genes that cause Fanconi anemia also increase the susceptibility to breast cancer.</p> |
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—Richard W. Cheney, Jr.

Possible Functions of Breast Cancer Genes

The *BRCA1* gene is on chromosome 17 and encodes a protein that is 1,863 amino acids long. Germ-line mutations of *BRCA1* are associated with 50 percent of hereditary breast cancers and with an increased risk of ovarian can-

cer. The *BRCA2* gene is on chromosome 13q12-13 and encodes a protein of 3,418 amino acids. Germ-line mutations of *BRCA2* are thought to account for approximately 35 percent of families with multiple-case, early-onset female breast cancer. Mutations of *BRCA2* are also associated

with an increased risk of male breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer.

Although *BRCA1* was cloned in 1994 and *BRCA2* in 1995, the function of these genes has been difficult to identify. Part of the difficulty has been that the proteins coded by these genes do not resemble any proteins of known function. In 1997, David Livingston and coworkers of the Dana-Farber Cancer Institute found that the *BRCA1* gene product associates with repair protein RAD51. A few months later, Allan Bradley of Baylor College of Medicine and Paul Hasty of Lexicon Genetics reported that the *BRCA2* protein binds to the RAD51 repair protein. This work suggests that both genes may be in the same DNA-repair pathway. Bradley and Hasty also showed that embryonic mouse cells with inactivated mouse *BRCA2* genes are unable to survive radiation damage, again suggesting that the *BRCA* genes are DNA-repair genes. Initially, it was thought that the breast cancer genes were typical tumor-suppressor genes that

normally function to control cell growth. The 1997 work suggests that the breast cancer gene mutations act indirectly to disrupt DNA repair and allow cells to accumulate mutations, including mutations that allow cancer development. In 2002 the detailed structure of the *BRCA2* protein was determined. It has structural motifs that show it to be capable of binding to DNA. Although the specific role of the *BRCA2* protein is uncertain, it is now clear that it does play a role in repairing double-stranded breaks in DNA. The understanding of the function of *BRCA1* and *BRCA2* is incomplete, but what is known will encourage additional studies.

Social Implications of Genetic Screening

With the cloning of the *BRCA1* and *BRCA2* genes, it became possible to test them for mutations. Such testing has been controversial, raising a number of social and psychological issues. There is a concern that the technical ability to test for genetic conditions is ahead of the ability to predict outcomes or risks, prescribe the

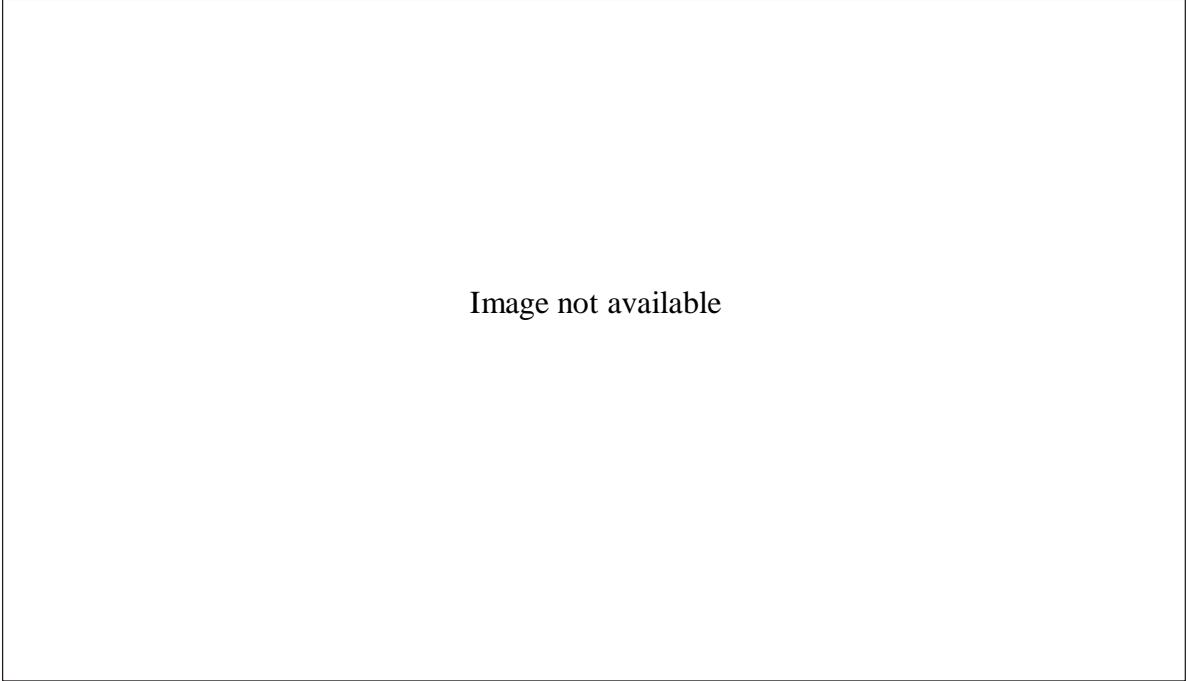


Image not available

Myriad Genetic Laboratories president Gregory Critchfield in 2002, posing in front of walls lined with the DNA data on BRCA1 and BRCA2 genes from just one woman. A mutation on either of these genes increases the risk of developing breast cancer significantly, but the ability to identify the mutation in individuals may also dramatically increase the chances for early detection and survival. (AP/Wide World Photos)

most effective treatment, or counsel individuals. Part of the dilemma about testing is the uncertainty about the meaning of the test results. If a test confirms the presence of a mutation in a breast cancer gene in a woman with a family history of breast cancer, there is a high risk, but not a certainty, that the woman will develop breast cancer. Even if a test is negative, it does not mean the woman is not at risk for breast cancer, because the large majority of breast cancers are not inherited. If a test is positive, it is not clear what the best course for the woman would be. Increased monitoring with mammography and even removal of both breasts as a preventive measure should reduce the chances of developing cancer but do not guarantee a cancer-free life. Even if a woman does not yet have cancer, she may feel the additional psychological stress of knowing she has a high risk of developing cancer.

There is also concern that test results may be misused by employers or insurers. A number of states have passed laws that prevent health insurance companies from using genetic test results to discriminate against patients. In 1996, the National Cancer Institute established the Cancer Genetics Network as a means for individuals with a family history of cancer to enroll in research studies and learn of their genetic status while receiving counseling.

—Susan J. Karcher, updated by Bryan Ness

See also: Aging; Cancer; Cell Cycle, The; DNA Repair; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Human Genome Project; Model Organism: *Mus musculus*; Mutation and Mutagenesis; Oncogenes; Tumor-Suppressor Genes.

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Yang, Haijuan, et al. "BRCA2 Function in DNA Binding and Recombination from a BRCA2-DSS1-ssDNA Structure." *Science* 297 (September 13, 2002): 1837-1848. This study presents evidence that the failure of *BRCA2* in DNA repair through homologous recombination may account for unsuppressed tumor growth.

Web Sites of Interest

American Cancer Society, All About Breast Cancer. <http://www.cancer.org>. Searchable information on breast cancer, including an overview, a detailed guide, and practical resources.

National Cancer Institute, National Institutes of Health. <http://www.nci.nih.gov/breast>. Provides information on the genetics of breast cancer and useful links.

National Institutes of Health, National Library of Medicine. Genetics Home Reference. <http://www.nlm.nih.gov>. This site includes information on breast cancer genetics.

particularly common in cases associated with acquired immunodeficiency syndrome (AIDS). Burkitt's lymphoma grows very rapidly, with a doubling time of approximately twenty-four hours, and thus prompt diagnosis is essential. A healthy child may become critically ill in about four to six weeks. These children often exhibit a head or neck mass or a large abdominal mass with fluid (ascites) accumulation. Other symptoms include vomiting, pain, anemia, and increased bleeding.

Burkitt's Lymphoma

Field of study: Diseases and syndromes

Significance: *Burkitt's lymphoma, a cancer of B lymphocytes, is the most common tumor among children and young adults in Central Africa and New Guinea. It is one of the most aggressive malignancies known. However, early clinical and laboratory diagnosis usually leads to effective treatment and survival.*

Key terms

B LYMPHOCYTE: an antibody-producing lymphocyte

ONCOGENE: a mutated or improperly expressed gene that can cause cancer; the normal form of an oncogene, called a proto-oncogene, is involved in regulating the cell cycle

RECIPROCAL TRANSLOCATION: a chromosomal abnormality in which there is an exchange of chromosome segments between nonhomologous chromosomes

SARCOMA: a cancer arising from cells of mesodermal origin

The Discovery of Burkitt's Lymphoma

Burkitt's lymphoma was first described by Denis Burkitt, a surgeon working in Uganda in the 1950's, as a sarcoma of the jaw in African children. Males are affected more commonly than females. The mean age for affected children in Africa is seven years, whereas the mean age in the United States is eleven years. Tumor infiltration usually occurs in abdominal sites such as bowels, kidneys, ovaries, or other organs. Rare cases occur as acute leukemia with circulating Burkitt tumor cells. The acute lymphocytic leukemia (ALL) presentation is

Diagnosis

The diagnosis of Burkitt's lymphoma is usually made by a needle biopsy from a suspected disease site such as the bone marrow, ascites, or a lymph node. Microscopic analysis is used to determine if the disease is present and, if so, its stage of development. Early clinical and laboratory diagnosis spares the child any life-threatening complications from the rapid tumor growth. Other common diagnostic tests may include a complete blood count (CBC), a platelet count, a bone marrow aspiration, a biopsy, and a lumbar puncture. Further tests may include specialized radiographic exams such as a computer-assisted tomography (CAT) scan to look for hidden tumor masses. The National Cancer Institute (NCI) stages Burkitt's lymphoma according to the amount of the disease present. The less disease, the better the outlook for improvement after treatment. Patients who remain free of disease for more than one year from the time of diagnosis are considered to be cured.

Culprit or Consort?

As with many other cancers, the exact cause of Burkitt's lymphoma is not known. In patients from equatorial Africa, however, there is a close correlation with the Epstein-Barr virus (EBV). More than 97 percent of lymphomas from equatorial Africa carry the EBV genome. By contrast, only 15 to 20 percent of sporadic cases of Burkitt's lymphoma in Europe and North America are positive for EBV. EBV has a single, linear, double-stranded DNA genome and was the first herpesvirus to be completely sequenced. EBV infection is not limited to areas where Burkitt's lymphoma is found. It in-

fects people worldwide without producing symptoms. EBV is also the causative agent of infectious mononucleosis, a common disease in which B cells are infected.

At least two EBV subtypes have been identified in human populations: EBV-1 is detected more commonly in Western societies, whereas EBV-1 and EBV-2 subtypes seem to be equally distributed in Africa. Although EBV is identified as a possible causative agent of African Burkitt's lymphoma, it appears that non-African Burkitt's lymphoma EBV may be just one factor in a multistep process of development. Burkitt's lymphoma is a monoclonal proliferation of B lymphocytes. The lymphocytes have membrane receptors for EBV. African children who develop Burkitt's lymphoma are thought to be unable to mount an appropriate immune response to primary EBV infection, possibly because of coexistent malaria, which is immunosuppressive. As time passes, excessive B-cell proliferation occurs. The precise role of EBV in the development of Burkitt's lymphoma remains unclear, but much research in this area continues to be done.

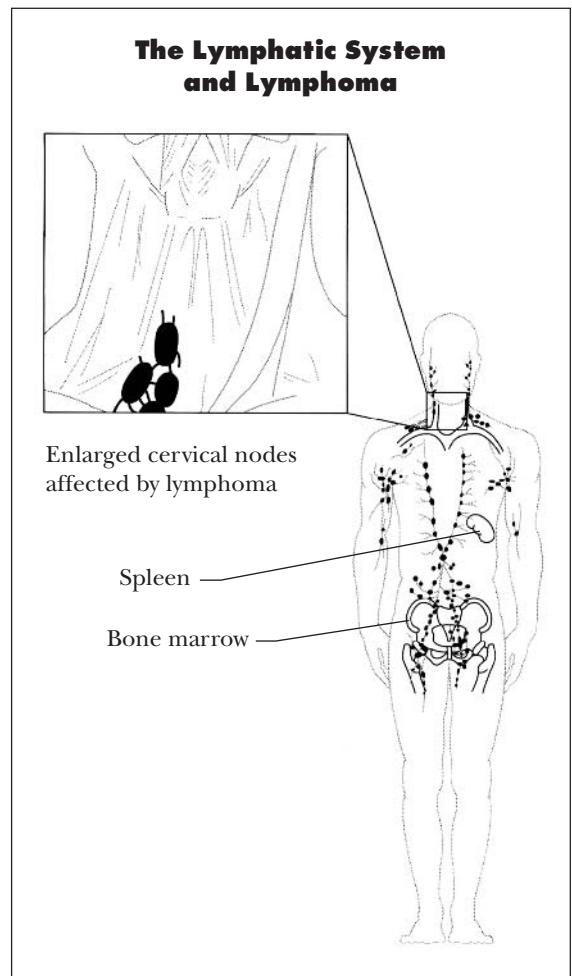
Good Genes and Bad Genes

The cells of Burkitt's lymphoma are characterized by a specific chromosomal defect known as a balanced reciprocal translocation. Observation of fresh Burkitt's lymphomas and cultured cells has revealed an additional DNA fragment at the end of the long arm of chromosome 14, while the end of one chromosome, 8, was consistently absent. Researchers suggested that the missing part of chromosome 8 was translocated to chromosome 14. This 8/14 translocation has also been observed in sporadic Burkitt's tumors from America, Japan, and Europe and has been observed in Burkitt's tumors with or without EBV markers.

The part of chromosome 8 involved in the translocation is known as the *c-myc* proto-oncogene. Proto-oncogenes normally help control the cell cycle by regulating the number of cell divisions. They are especially active when high rates of cell division are needed, as in embryonic development, wound healing, or regeneration. The proto-oncogene is transformed into an oncogene when the chromosomes break

and reunite, resulting in a reciprocal translocation. The rearrangement of genes in this kind of translocation causes the *c-myc* gene to become an oncogene by forming an abnormal fusion protein that triggers the onset of cancer. More than sixty human proto-oncogenes have now been localized to a specific chromosome or chromosome region. The new location of the *c-myc* gene results in deregulation and subsequent overexpression. A normal-acting proto-oncogene is transformed into an abnormally active oncogene.

Ninety percent of Burkitt's tumors are associated with a reciprocal translocation involving



Anatomy of the lymphatic system, showing major lymph nodes. Enlarged lymph nodes may occur for a wide variety of reasons, including but not limited to lymphoma (cancer). (Hans & Cassidy, Inc.)

chromosomes 8 and 14. As additional tumors have been examined, two other related translocations involving chromosome 8 have been observed. The variant translocations involved chromosomes 2 or 22. However, no unified theory exists to explain the role of chromosome abnormalities in the activation of oncogenes. The Epstein-Barr virus has been implicated in Burkitt's lymphoma and is known to be a B-cell mitogen (a substance that stimulates cell division). As a mitogen, it stimulates inactive cells to transform into actively dividing cells. Perhaps EBV plays a role in the origin of 8/14 translocation abnormalities simply by increasing the number of B cells undergoing DNA replication. This could increase the chances for developing a chromosome abnormality with the potential to become cancerous.

—Phillip A. Farber

See also: Cancer; Oncogenes; Tumor-Suppressor Genes.

Further Reading

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Web Sites of Interest

American Cancer Society. <http://www.cancer.org>. Comprehensive and searchable site covering all aspects of cancer.

Lymphoma Research Foundation, Getting the Facts on Non-Hodgkin's Lymphoma. <http://www.lymphoma.org>. Site has searchable information on Burkitt's lymphoma (non-Hodgkin's lymphoma), including online guides and information on obtaining guides by mail.

The Leukemia and Lymphoma Society. <http://www.leukemia-lymphoma.org>. Searchable site that includes a free, sixty-four-page downloadable guide on all types of lymphomas, including Burkitt's.

Cancer

Field of study: Diseases and syndromes

Significance: *At its root cancer is a genetic disease.*

It is characterized by unrestrained growth and reproduction of cells, loss of contact inhibition, and, eventually, metastasis (the wandering of cancer cells from a primary tumor to other parts of the body). All of these changes represent underlying mutations or inappropriate expression of genes involved in the control of the cell cycle and related processes.

Key terms

CARCINOGEN: a substance or other environmental factor that produces or encourages cancer

ONCOGENES: genes that cause cancer but that, in their normal form, called proto-oncogenes, are important in controlling the cell cycle and related processes

TUMOR: a mass formed by the uncontrolled growth of cells, which may be malignant (considered cancerous) or benign (nonmalignant)

TUMOR-SUPPRESSOR GENES: genes involved in regulating the cell cycle and preventing cell division until an appropriate time; when mutated, these genes can cause cancer

The Problem of Cancer

Cancer is characterized by abnormal cell growth that leads to the invasion and destruction of healthy tissue by cells that meet certain criteria. Normal cells in the human body are continuously growing but are under normal cell control mechanisms. Cancer cells begin as normal cells that, due to genetic mutations, start to grow uncontrollably, escaping from the normal rules regulating cell growth and behavior. Contact inhibition, in which cells contacting other cells prevent unrestrained growth, is lost in cancer cells. Normal cells also remain in one location, or at least in the same tissue, but malignant tumors, in their later stages, metastasize, allowing their cells to wander freely in the body, leading to the development of tumors in other organs. A final common feature is that cancer cells lose their normal cell shape.

The area where cancer begins to form is called the primary site. Most types of cancer begin in one place (the breast, lung, or bowel, for example) from which the cells invade neighboring areas and form secondary tumors. To make matters more complicated, some types of cancer, such as leukemia, lymphoma, and myeloma, begin in several places at the same time, usually in the bone marrow or lymph nodes. Primary tumors begin with one abnormal cell. This cell, as is true of all cells, is extremely small, no more than 0.002 or 0.003 millimeter across (about one-twentieth the width of a human hair). Therefore early cancer is very difficult to locate. Even if there are more than 100,000 cancer cells in a tumor, it is barely visible except under a microscope.

Cancer cells divide and reproduce about every two to six weeks. If they divide on the average of once per month, a single cell will multiply into approximately four thousand cells by the end of a year. After twenty months, there will be one million cells, which would form a tumor about the size of a pinhead and would still be undetectable. A tumor can be discovered only when a lump of approximately one billion cells is present. This would be about the size of a small grape. It would take about two and one-half years for a single cancer cell to reach this size. Within seven months, the one billion cells would grow to more than 100 billion cells, and the tumor would weigh about four ounces. By the fortieth month of growth, the lump of cancer cells would weigh about two pounds. By the time a tumor has reached this size, death often occurs. Death normally occurs about three and one-half years after the first cancer cell begins to grow. It takes about forty-two cell doublings to reach the lethal stage. The problem is that, in most cases, tumors are detectable only after thirty doublings. By this time, cancer cells may have invaded many other areas of the body beyond the primary site.

How Cancer Cells Grow and Invade

Cancer cells are able to break down the barriers that normally keep cells from invading other groups of cells. With the aid of a microscope, cancer cells can be observed breaking

through the boundary between cells, called the basement membrane. Cancer cells can make substances that break down the intercellular matrix, the “glue” that holds cells together. The intercellular matrix is a complex mixture of substances, including collagen, a strong, fibrous protein that gives strength to tissues. Cancer cells produce collagenase, an enzyme that breaks down collagen. Cancer cells also produce hyaluronidase, which further breaks down the intercellular matrix. This causes cancer cells to lose their normal shape and allows them to push through normal boundaries and establish themselves in surrounding tissues. Cancer cells have jagged edges, are irregular in shape, and have hard-to-detect borders, making them relatively easy to identify microscopically. Normal cells, on the other hand, have a regular, smooth edge and shape.

There are many steps involved in the process of metastasizing, not all of which are understood by researchers. First is the entry into a blood vessel or lymph channel. Lymph channels, or lymphatics, comprise a network of vessels that carry lymph from the tissues to the bloodstream. Lymph is a colorless liquid that drains from spaces between cells. It consists mainly of water, salts, and proteins and eventually enters the bloodstream near the heart. The function of lymph is to filter out bacteria and other foreign particles that might enter the blood and cause infections. A mass of lymph vessels is called a lymph node. In the human body, lymph nodes are found in the neck, under the arms, and in several other places. Every body tissue has a network of lymph and blood vessels running through it.

Once a malignant tumor develops and metastasizes, the cells often travel through the body using the lymphatic system, a network of vessels that filter pathogens and transport lymph, a fluid similar to blood plasma. Cancer cells may gain entry into a nearby lymph vessel by breaking down defensive enzymes. Once in the lymph system, they can travel to nodes (gland-like masses of cells that produce white blood cells) and eventually into the bloodstream. Whatever route they take, groups of cancer cells can break away from the primary site of the tumor and float along whatever vessel they

have invaded, forming numerous secondary tumors along the way. Because cancer cells are not considered foreign substances, such as bacteria or viruses, they are able to evade the body’s immune system. Because of their overall resemblance to normal cells, cancer cells fool the body into thinking they are normal and therefore not dangerous.

Cancer cells eventually enter narrow blood vessels called capillaries and stay there for a brief period before they enter tissues such as lungs, bones, skin, and muscle. The secondary tumors then capture their own territory. As a tumor establishes itself, its cells often secrete signal proteins that stimulate new blood vessels to form (a process called angiogenesis) to increase blood supply to the growing tumor. The body thus not only fails to destroy developing tumors, but unwittingly helps establish them as well.

The Genetics of Cancer

Cancer has been known since antiquity, but it was not until the twentieth century that the underlying causes of cancer began to be explored. In 1910, Peyton Rous discovered a type of cancer in chickens called a sarcoma (a cancer of connective tissue) that could be passed on to other chickens. He demonstrated this by removing tumors from affected chickens, grinding the tumors up, filtering the grindate, and then injecting the filtrate into healthy chickens. Injected chickens invariably developed sarcoma tumors, suggesting that something smaller than the tumor cells was being passed on and was stimulating cancer development in otherwise normal cells. It is now known that the filtrate contained a cancer-causing virus, now called the Rous sarcoma virus. Similar types of viruses were discovered to be responsible for cancers in a variety of animals, but none was discovered in humans initially.

As the genetic material of some of the tumor viruses was later analyzed, all of them were discovered to contain genes called oncogenes, because they promoted oncogenesis (tumor development). Even more surprising was the discovery that humans have genes in their genome that are homologous (having a high degree of similarity) to viral oncogenes. The human genes did not seem to cause cancer under

normal circumstances and were called proto-oncogenes. In cancer cells, some of these proto-oncogenes were discovered to have mutations or, in some cases, were simply overexpressed. In recognition of their abnormal state, these genes were called cellular oncogenes, to distinguish them from viral oncogenes. It is now known that proto-oncogenes are important in controlling the cell cycle by stimulating cell division only at the appropriate time. When they are transformed into oncogenes, uncontrolled cell growth and division occurs, two of the hallmarks of cancer.

A second type of cancer-causing gene, called a tumor-suppressor gene, was discovered to be the cause of retinoblastoma, a cancer of the retina, most often occurring in children. Tumor-suppressor genes have an effect opposite to that of proto-oncogenes; they suppress cell division and thus prevent unrestrained cell proliferation. If both alleles of a tumor-suppressor gene have a mutation that makes them non-functional, then cell division can occur un-

checked. Retinoblastoma occurs in children when they inherit one faulty copy from a parent. If the other copy experiences a mutation, which frequently occurs, then retinoblastoma develops.

How Cancer Develops

The development of cancer is typically more complicated than implied above. It generally requires mutations in more than a single proto-oncogene or tumor-suppressor gene. Any factors that increase mutation rates or decrease a cell's ability to repair mutations will increase the likelihood that cancer will develop. Inheritance of already mutated genes can also greatly increase a person's chance of developing cancer, which accounts for the above-normal occurrence of cancer in some families.

One of the best-studied cases of oncogenesis involves colorectal cancer, which takes years to develop from a small cluster of abnormal cells into life-threatening cancer. It involves the loss or mutation of three tumor-suppressor genes

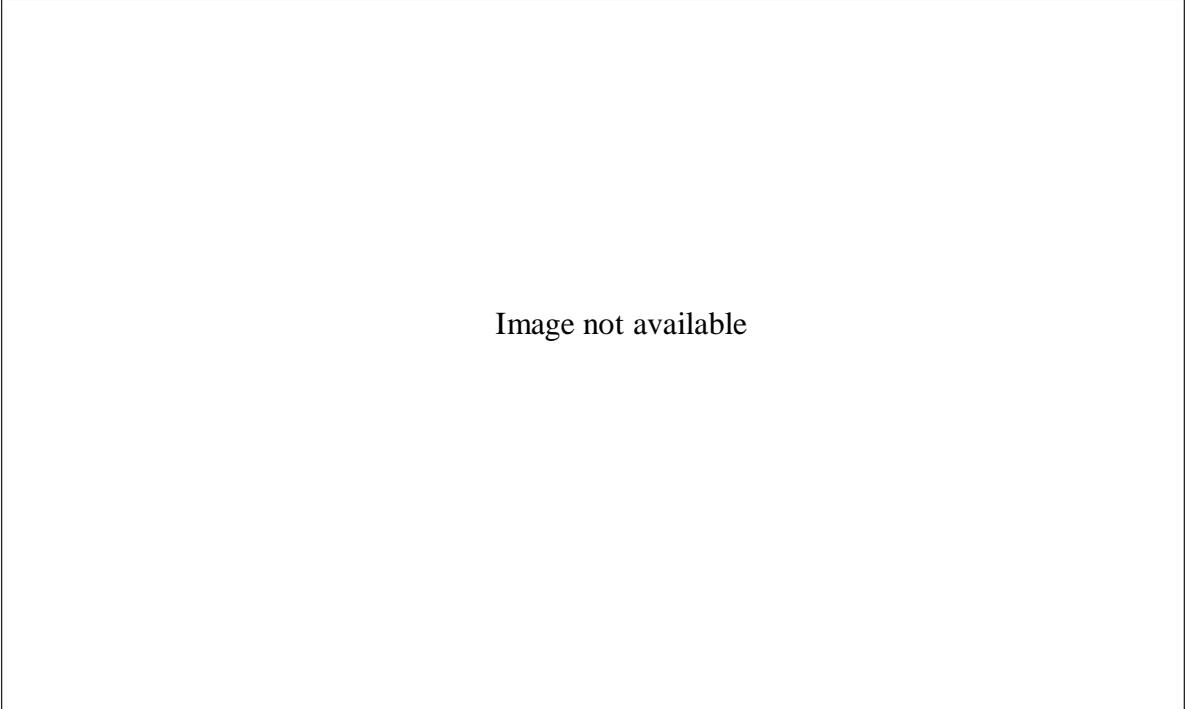


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Leland H. Hartwell, co-winner of the 2001 Nobel Prize in Physiology or Medicine with R. Timothy Hunt and Paul M. Nurse, at the Fred Hutchinson Cancer Research Center in Seattle, Washington, shortly after the Nobel Foundation's announcement. The three men won for their work on cell division and its implications for cancer research. (AP/Wide World Photos)

and one proto-oncogene. Often colorectal cancer runs in families, because the loss of the first gene, the *APC* tumor-suppressor gene, is often inherited, resulting in an increased chance of developing colorectal cancer. Loss of this gene causes increased cell growth and some other genetic changes. In the next step, the *ras* oncogene is mutated, causing even more cell growth. Two more tumor-suppressor genes are lost, *DCC* and *p53*, at which point a tumor called a carcinoma has developed. Additional gene loss, which occurs much more easily in tumor cells, leads to metastasis, and the cancer then spreads to other organs and tissues.

Inheritance of a gene loss or mutation does not mean a person will get cancer; it simply means they have a higher chance of developing cancer. Although development of cancer is ultimately genetically based, environmental factors also play a part. In the case of colorectal cancer, a diet low in roughage is often considered to increase colorectal cancer rates. Exposure to carcinogens, chemicals, or other factors, such as radiation, can also increase the likelihood of cancer. Exposure can occur in the diet, as a result of skin exposure, or inhalation. For example, smoking cigarettes is known to increase the occurrence of lung cancer, as well as a variety of other cancers. Excess exposure to damaging UV rays in sunlight or other sources is known to significantly increase the occurrence of skin cancer. Carcinogens promote cancer because they cause damage to DNA, and if the damage happens to occur to a tumor-suppressor gene or oncogene, then cancer may occur.

Inheritance of some mutations is particularly potent in increasing the chances of developing cancer. One example is the genetic disease xeroderma pigmentosa. Individuals with this disease develop skin cancer in response to even relatively brief exposure to UV radiation and must therefore avoid exposure to sunlight. A small percentage of breast cancers are also highly heritable and are now known to involve mutations in the *BRCA1* and *BRCA2* genes. In these types of highly heritable cancers, it appears that the mutations cause some kind of deficiency in the cellular DNA repair systems. As a result of a decreased ability to repair mutations, it is just a matter of time before mutations oc-

cur in proto-oncogenes or tumor-suppressor genes, so that the only way to prevent cancer is to control exposure to as many environmental carcinogens as possible and to aggressively screen for tumors.

Cancer Treatment

Cancers vary in their severity and rate of growth, which means that proper treatment depends on correctly diagnosing the type of cancer. For example, some forms of prostate cancer grow extremely slowly, and metastasis is rare until very late stages in the disease, sometimes many years after initial diagnosis. Treatment may comprise simply monitoring the tumor, avoiding carcinogenic exposure as much as possible, and possibly changing lifestyle. On the other hand, some types of skin cancer progress so rapidly that aggressive treatment may be required, unless it is caught very early. Although survival rates for many types of cancer have risen, treatment for most cancers is still only partially successful, and the later a tumor is detected, the greater chance that it will be untreatable.

New therapies are constantly being developed, but most cancers are still treated using surgery (removal of tumors), chemotherapy, and radiation therapy, either singly or, more often, in combination. More important than the specific treatment used is detecting tumors in their earliest stages, before they have extensively invaded surrounding tissues or metastasized. Survival rates are high for most cancers when treated very early.

The very nature of cancer makes treatment difficult. Because the cells involved are difficult for the immune system to recognize as dangerous, the body is typically inefficient at destroying them. Many of the treatments, other than surgical removal, rely on the fact that cancer cells divide much faster and more frequently than normal cells. Therefore, any agent that can cause higher mortality in rapidly dividing cells has potential as a cancer treatment. Chemotherapeutic agents are essentially toxic chemicals that are most toxic to dividing cells. Thus, they kill cancer cells much more readily than most other body cells, but any other body cells undergoing cell division are susceptible,

so chemotherapy also kills some normal cells. Cancer patients often feel very ill during chemotherapy because of this.

Radiation therapy works similarly, being more damaging to dividing cells. An added advantage of radiation therapy, if the tumor has not yet metastasized, is that it can be focused more intensely in the vicinity of the tumor, preventing damage to other tissues. If the tumor has metastasized, then more widespread exposure to radiation may be used, with the obvious drawback that many other normal cells will also be damaged. Radiation therapy is often used to treat leukemia. Radiation is used to kill the patient's bone marrow, and then new bone marrow is transplanted from a compatible donor. The new bone marrow can then restore normal function to production of blood cells.

Genetics has played a part in improving chemotherapy. It has long been known that some people will respond better than others to certain chemotherapeutic drugs. It is now known that some of these differences are genetic, and the underlying genetic differences have been uncovered in some cases. Therefore, as part of cancer treatment for some kinds of cancer, a person may be tested genetically to make more intelligent choices about which drugs to use. As more genetic data become available, it is anticipated that more effective and personalized treatments will be developed.

Innovations and Future Treatments

Although the immune system cannot normally identify cancer cells accurately, there has been some success in immunological approaches. A recent new treatment involves Herceptin, a specially designed monoclonal antibody that attacks cells that overproduce a protein called HER-2. It has shown some promise in treating breast cancer and is being studied as an agent for treating other forms of cancer. Unfortunately, some of the possible side effects include damage to the heart and lungs and serious allergic reactions. Careful monitoring is required to prevent life-threatening damage. Research is also progressing on development of vaccines against cancer, but so far this approach is still in its early experimental stages.

Angiogenesis inhibitors are also at the experimental stage. As noted above, many cancer cells secrete agents that encourage angiogenesis (the formation of blood vessels) in the region of the tumor to increase blood supply to the rapidly dividing cells. It is hoped that angiogenesis inhibitors might counteract this activity and essentially "starve" the cancer cells. Shrinkage of tumors in laboratory animals has been observed using this approach.

Photodynamic therapy also shows promise. It is based on the observation that certain chemicals, when ingested by single-celled organisms, release damaging oxygen radicals when exposed to light, thus killing the organisms. It has been observed that cancer cells retain these chemicals longer than normal cells. Treatment involves administering the chemical by injection, then waiting for a specified period for it to be retained by cancer cells and flushed out of normal cells. Then the tissue in which the cancer cells are located is exposed to laser light. This method works on any tissues that can be exposed to laser light, which includes any part of the body accessible to endoscopy.

The ultimate treatment for cancer would be replacement or repair of the mutated genes responsible. Currently such treatment is not possible. There are many hurdles to overcome, including designing safe methods for inserting corrected gene copies. There is danger that improper gene therapy methods could actually make things worse, causing additional tumors or other diseases. A much better understanding of the genetics of cancer and future improvements in gene therapy techniques hold promise of someday being able to cure or prevent most kinds of cancer.

—Leslie V. Tischauser, updated by Bryan Ness

See also: Aging; Breast Cancer; Burkitt's Lymphoma; Cell Culture: Animal Cells; Cell Cycle, The; Cell Division; Chemical Mutagens; Chromosome Mutation; Developmental Genetics; DNA Repair; Gene Therapy; Genetic Engineering: Medical Applications; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Homeotic Genes; Human Genome Project; Huntington's Disease; Hybridomas and Monoclonal Antibodies; Hypercholesterolemia; Insurance; Model Organism: *Caenorhab-*

ditis elegans; Model Organism: *Mus musculus*; Mutation and Mutagenesis; Nondisjunction and Aneuploidy; Oncogenes; Proteomics; Reverse Transcriptase; RNA Transcription and mRNA Processing; Signal Transduction; Stem Cells; Steroid Hormones; Telomeres; Tumor-Suppressor Genes.

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Web Sites of Interest

American Cancer Society. <http://www.cancer.org>. Comprehensive and searchable site covering all aspects of cancer.

National Cancer Institute. <http://www.cancer.gov>. Site links to comprehensive information on genetics and cancer, including a cancer-basics tutorial.

cDNA Libraries

Fields of study: Bioinformatics; Techniques and methodologies

Significance: A cDNA library is a set of cloned DNA copies of the RNAs found in a specific cell type at a specific time. This library can be used to construct probes for mapping these genes, to study the changing expression of genes over time (during development, for example), or to clone genes into organisms for further study or production of proteins.

Key terms

COMPLEMENTARY DNA (cDNA): also known as copy DNA, a form of DNA synthesized by reverse transcribing RNAs (usually messenger RNAs) into DNA

DNA LIBRARY: a collection of DNA fragments cloned from a single source, such as a genome, chromosome, or set of mRNAs

IN SITU HYBRIDIZATION: a technique that uses a molecular probe to determine the chromosomal location of a gene

INTRONS: noncoding segments of DNA within a gene that are removed from mRNA copies of the gene before polypeptide translation

REVERSE TRANSCRIPTASE: an enzyme, isolated from retroviruses, that synthesizes a DNA strand from an RNA template

Gene Cloning and DNA Libraries

In order to study and map genes, researchers need to take potentially very large sections of DNA (such as a chromosome or whole genome), break them into smaller, manageable fragments, and clone these fragments to construct a DNA library. A genomic or chromosome library may contain many thousands of cloned fragments, many of which will represent stretches of noncoding DNA between genes. If the researcher is interested in studying the protein-coding regions, or genes, of the DNA,

it is better to start with the messenger RNAs (mRNAs) of the cell, which represent the genes being actively transcribed in the cell at that time. By constructing and cloning complementary DNA (cDNA) copies of these mRNAs, researchers can create a library that contains copies of only the active genes.

cDNA Library Construction

DNA copies of mRNAs are synthesized using the enzyme reverse transcriptase. This enzyme was independently discovered by Howard Temin and David Baltimore in 1970 in retroviruses, which “reverse transcribe” their RNA genomes into DNA after infecting their host cells. In the late 1970’s, researchers began using the enzyme to make DNA copies of mRNAs, and later to construct cDNA libraries.

To create a cDNA library from a sample of cells, mRNAs from the cells are isolated and purified. Reverse transcriptase is used to synthesize a complementary DNA strand using each mRNA strand as a template, resulting in a collection of double-stranded RNA-DNA hybrids. To obtain double-stranded cDNAs suitable for cloning, the enzyme RNase H is used to digest the RNA strand, and DNA polymerase I is used to synthesize the second DNA strand using the first as a template. If desired, “sticky ends” can be added to the cDNAs for cloning into a vector. The set of recombinant vectors are inserted into bacterial cells in the process of transformation, resulting in a cloned cDNA library. The library is maintained as a collection of bacterial colonies, each colony containing a different cloned DNA fragment.

Applications

A cDNA library represents the coding sequences of genes that were actively expressed in the original cell sample at the time the sample was taken. In effect, it can represent a snapshot of active genes in the cells at that time. Comparing the cDNAs of different tissues from the same organism can reveal the differences in gene expression of these tissues. Also, comparing cDNAs of cells in the same tissue over time can show how gene expression changes in the same cells. This approach has been especially fruitful in developmental genetic re-

search, because the developmental pattern of an organism can be correlated with the activity of specific genes.

Cloned cDNAs can also be used to find the chromosomal location of an expressed gene. One strand of the cDNA clone is labeled with a fluorescent tag and used as a molecular probe. In the technique of *in situ* hybridization, the probe will base pair, or hybridize, to the complementary sequence in a preparation of partially denatured chromosomes, and the chromosomal location of the original gene will be visible because of the fluorescent label. Such a probe can also be used to screen a chromosome or genomic library for the cloned fragment containing the target gene. Using the entire cDNA library to probe a genome will generate a cDNA map that suggests the most biologically and medically important parts of the genome, aiding researchers in the search for disease genes.

Genes of eukaryotes (nonbacterial organisms) usually contain introns, noncoding segments that are transcribed but removed from mRNAs before translation, but bacterial genes do not. Often, a eukaryotic gene put into a bacterial cell will not produce a functional polypeptide because the cell does not have the biochemical machinery for removing introns. If the goal of the research is to have a bacterium make the protein product of a gene, it may be necessary to clone a cDNA version of the gene, which lacks introns, using a special expression vector that allows the cell to transcribe the inserted gene and translate it to the proper polypeptide.

Advantages and Disadvantages

Because cDNA libraries contain only DNA of expressed genes, they are much smaller and more easily managed and studied than chromosome or genomic libraries that have all coding and noncoding regions. The cDNA versions of genes have only the protein-coding sequence, without introns, so that cloning them in bacteria allows expression of the protein products of the genes. In contrast to other DNA libraries, cDNA libraries can be used to study variable patterns of gene expression among cell types or over time. In eukaryotes,

cDNA copies of genes are not identical to the original sequences of the genes and also lack the promoter region necessary for proper transcription of the gene. However, using cDNA as a molecular probe can lead to the identification of the original gene.

—Stephen T. Kilpatrick

See also: Bioinformatics; DNA Fingerprinting; DNA Sequencing Technology; Forensic Genetics; Genetic Testing; Ethical and Economic Issues; Genetics, Historical Development of; Genomic Libraries; Genomics; Human Genome Project; Icelandic Genetic Database; Linkage Maps; Proteomics; Reverse Transcriptase.

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MICROPROPAGATION: removal of small pieces of plant tissue for growth in culture

PRIMARY CELLS: explants removed from an animal

TRANSFORMATION: any physical change to a cell, but generally the change of a normal cell into a cancer cell

Early History

Methodology for maintaining tissues *in vitro* (in laboratory vessels) began in 1907 with Ross Harrison at Yale College. Harrison placed tissue extracts from frog embryos on microscope slides in physiological fluids such as clotted frog lymph. The material was sealed with paraffin and observed; specimens could be maintained for several weeks. In 1912, Alexis Carrel began the maintenance of cardiac tissues from a warm-blooded organism, a chicken, in a similar manner. The term “tissue culture” was originally applied to the cells maintained in the laboratory in this manner, reflecting the origin of the technique. More appropriate to modern techniques, the proper terminology is “cell culture,” since it is actually individual cells which are grown, developing as explants from tissue. Nevertheless, the terms tend to be used interchangeably for convenience.

Types of Cell Culture

The most common form of mammalian cell culture is that of the primary explant. Cells are removed from the organism, preferably at the embryonic stage, treated with an enzyme such as trypsin, which serves to disperse the cells, and placed in a laboratory growth vessel. Most vessels used today are composed of polystyrene or similar forms of plastic.

Most forms of cells are anchorage-dependent, meaning they will attach and spread over a flat surface. Given sufficient time, such cells will cover the surface in a layer one cell thick, known as a monolayer.

A few forms of cells, mainly hematopoietic (blood-forming) or transformed (cancer) cells, are anchorage-independent and will grow in suspension as long as proper nutrients are supplied.

Similar procedures are used in preparation of nonmammalian cell lines such as those from

Cell Culture: Animal Cells

Fields of study: Cellular biology; Techniques and methodologies

Significance: *The ability to grow and maintain cells or tissues in laboratory vessels has provided researchers with a means to study cell genetics and has contributed to the understanding of what differentiates “normal” cells from cancer cells. The technology involved in growing viruses in cell culture has proved vital both to understanding virus replication and for development of viral vaccines.*

Key terms

CELL LINES: cells maintained for an indeterminate time in culture

HELA CELLS: the first human tumor cells shown to form a continuous cell line

poikilotherms (cold-blooded organisms such as fish) or insects. Insect lines have become particularly important as techniques were developed for cloning genes in insect pathogens known as baculoviruses. Such cells can often be maintained at room temperature in suspension.

Development of Cell Lines

A characteristic of primary cells is that of a finite life span; normal cells will replicate approximately fifty times, exhibit symptoms of “aging,” and die. When primary cells are removed from a culture and cultured separately, they become known as a cell strain.

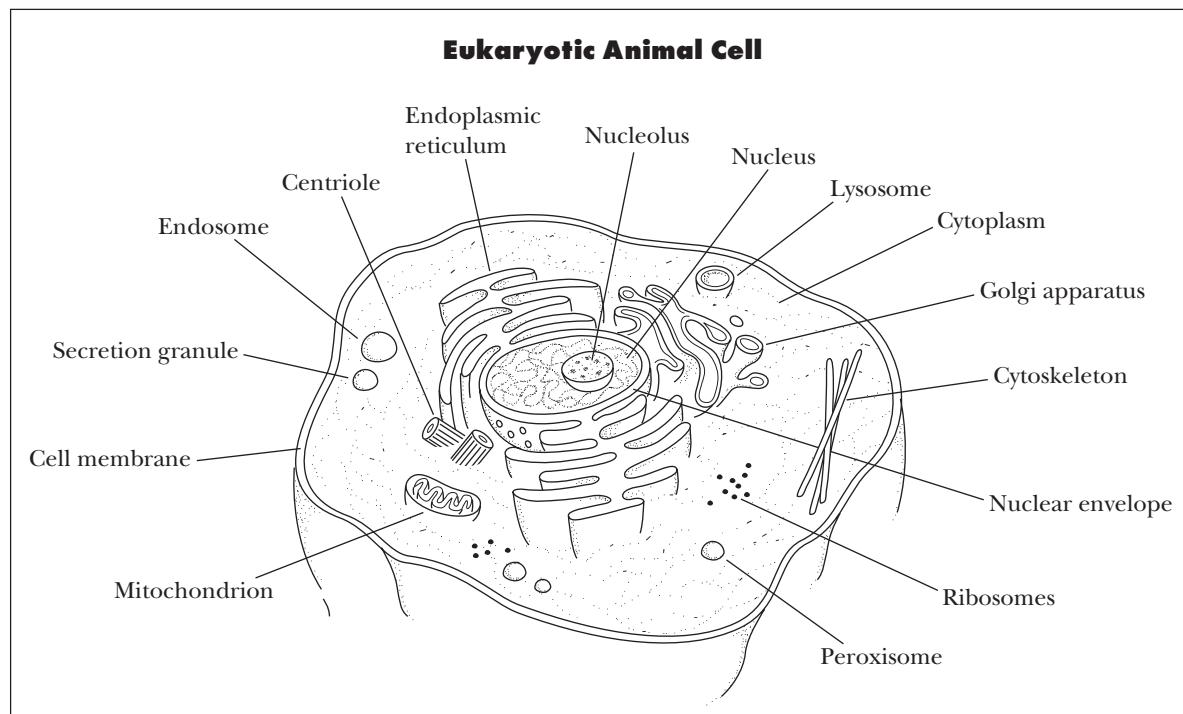
A few rare cells may enter “crisis” and begin to exhibit characteristics of abnormal cells such as anchorage-independence or unusual chromosome numbers. If these cells survive, they represent what is called a “cell line.” Cell lines express characteristics of cancer cells and are often immortal.

During the first half century of work in cell culture, only nonhuman cells were grown in culture. In 1952, George Gey, a physician at

Johns Hopkins Hospital, demonstrated that human cells could also be grown continuously in culture. Using cervical carcinoma explants from a woman named Henrietta Lacks, Gey prepared a continuous line from these cells. Known as HeLa cells, these cultures became standard in most laboratories studying the growth of animal viruses. Ironically, growth of HeLa cells was so convenient and routine that the cells frequently contaminated other cultures found in the same laboratories.

Nutrient Requirements

Particular cells may have more stringent requirements for growth than other types of cells; in addition, primary cells have greater requirements than cell lines. However, certain generalities apply to the growth requirements for all cells. All cells must be maintained in a physiological salt solution. Required vitamins and amino acids are included in the mixture. Antibiotics such as penicillin and streptomycin are routinely added to suppress the growth of unwanted microorganisms. Nevertheless, sterility



All animal cells are eukaryotic cells, which differ from more primitive prokaryotic cells in having a nucleus that houses the primary genetic material. This drawing depicts the basic features of a eukaryotic animal cell. (Electronic Illustrators Group)

is of utmost importance since some organisms are unaffected by these antibiotics. Depending upon the type of cell, the particular pH, or acid content, of the culture may be variable. Most mammalian cells grow best at a pH of 7-7.2. For this reason, cells are generally grown in special incubators which utilize a relatively high CO₂ atmosphere.

Replication of animal cells requires the presence of certain growth factors to be present in the medium. Historically, the source of such factors has been serum, usually obtained from fetal bovines. Genetic engineering techniques have resulted in production of commercially available growth factors, eliminating the requirement for expensive serum for growth of some forms of cells in culture.

Genetics of Cells in Culture

Study of cultured animal cells has resulted in significant advancement in understanding many areas of cell regulation. For example, the role played by cell receptors in response to the presence of extracellular ligands such as hormones and other metabolites was clarified by studying the response of cells to such stimulation. Intracellular events, including the role of enzymes in cell activities, was clarified and remains a primary area of research.

The ability to transform mammalian cells using isolated DNA has allowed for significant applications in genome analysis. Such genetic manipulation has led to a greater understanding in the role specific genes play in cell regulation. In particular, use of cultured cells was instrumental in clarifying the role played by specific gene products in intracellular trafficking, the movement of molecules to specific sites within the cell. Similar techniques continue to be used to further understand the regulatory process.

Mammalian Cells and Oncogenesis

During the 1960's, Leonard Hayflick at the Wistar Institute in Philadelphia, Pennsylvania, observed that primary cells in culture exhibit a finite life span; normal cells generally divide no more than approximately fifty times (a phenomenon now called the Hayflick limit). Any cells that survive generally take on the characteristics of cancer cells.

During the same period, Howard Temin at the University of Wisconsin, while studying the growth of RNA tumor viruses in cultured cells, reported the apparent requirement for DNA production by these viruses in transforming normal cells into cancer cells. Temin's and Hayflick's investigations contributed significantly to the question of how cancer cells differ from normal cells, and the understanding of genes involved in such changes. Eventually, this led to the discovery of oncogenes.

The term "oncogene" is somewhat misleading. Its definition was originally based on the fact that mutations in such genes may contribute to transformation of cells from normal to cancerous. The study of these genes in cultured cells clarified their role: Most oncogene products can be classified as growth factors, which stimulate cell growth; receptors, which respond to such stimulation; or intracellular molecules, which transfer such signals to the cell DNA. In other words, the normal function of the oncogene is to regulate replication of normal cells; only when these proteins are inappropriately expressed do they result in transformation of the cell.

Application of Cell Culture to Virology

The use of mammalian cells for the study of viruses represented among the earliest, and arguably among the most important, applications of the technique of cell culture. Prior to the 1940's, study of most animal viruses, including those that cause disease in humans, was confined to *in vivo* studies in animals. For example, the study of poliovirus required inoculation of the virus directly into the brains of suitable monkeys.

In 1949, John Enders and his co-workers demonstrated the growth of poliovirus in human embryonic cells, eliminating the requirement for monkeys. Their work played a critical role in the later development of poliovirus vaccines by Jonas Salk and Albert Sabin. The ability to grow viruses in cells maintained in the laboratory opened the field to nearly all virologists and biochemists, rather than restricting such studies to those with access to animal facilities.

—Richard Adler

See also: Cancer; Cell Culture: Plant Cells; Cell Cycle, The; Cell Division; Gene Regulation: Eukaryotes; Gene Regulation: Viruses; Mitosis and Meiosis; Oncogenes; Stem Cells; Totipotency; Tumor-Suppressor Genes; Viral Genetics.

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Cell Culture: Plant Cells

Fields of study: Cellular biology; Techniques and methodologies

Significance: *Plant cell culture is the establishment and subsequent growth of various plant cells, tissues, or organs in vitro, using an artificial nutritional medium usually supplemented by various plant growth regulators. It has become a tool that plant geneticists use for purposes ranging from the basic study of plant development to the genetic improvement of economically important agricultural plant species.*

Key terms

CALLUS: a group of undifferentiated plant cells growing in a clump

MORPHOGENESIS: the induction and formation of organized plant parts or organs

PLANT GROWTH REGULATORS: hormonelike substances that profoundly affect plant growth and development

SOMATIC EMBRYOS: asexual embryoid structures derived from somatic cells

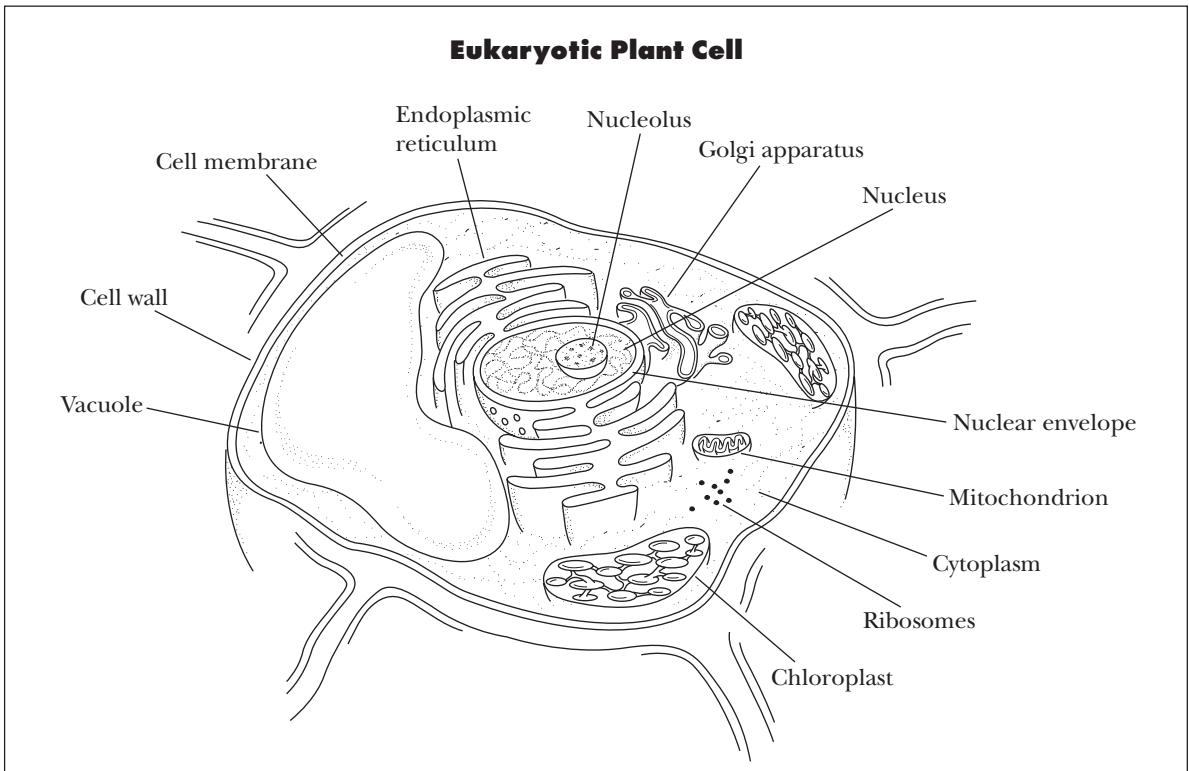
TOTIPOTENCY: the ability of a plant cell or part to regenerate into a whole plant

Culturing Plant Cells

Plant cell cultures are typically initiated by taking explants—such as root, stem, leaf, or flower tissue—from an intact plant. These explants are surface-sterilized and then placed in vitro on a formulated, artificial growth medium containing various inorganic salts, a carbon source (such as sucrose), vitamins, and various plant growth regulators, depending on the desired outcome. There are many commercially available media formulations; the two most common include MS (murashige and skoog) and WPM (woody plant media). Alternatively, customized formulations may be necessary for culturing certain plant species. One of the most important uses of plant tissue culture has been for the mass propagation of economically important agricultural and horticultural crops. Since the 1980's, however, plant cell culture has become an important tool allowing for direct genetic manipulations of several important agricultural crops, including corn, soybeans, potatoes, cotton, and canola, to name only a few.

Appearance in Culture

The underlying basis for the prevalent and continued use of plant cell culture is the remarkable totipotent ability of plant cells and tissues. They are able to dedifferentiate in culture, essentially becoming a nondifferentiated clump of meristematic, loosely connected cells termed callus. Callus tissue can be systematically subcultured and then, depending on exposure to various plant growth regulators incorporated in the growth media, induced to undergo morphogenesis. Morphogenesis refers to the redifferentiation of callus tissue to form specific plant organs, such as roots, shoots, or subsequent whole plants. Many plant species can also be manipulated in culture to form somatic embryos, which are asexual embryoid



All plant cells, like animal cells, are eukaryotic cells. However, plant cells contain chloroplasts, the “factories” that produce chlorophyll during photosynthesis. This drawing depicts the basic features of a eukaryotic plant cell. (Electronic Illustrators Group)

structures that can then develop into plantlets. The totipotency of plant cells thus allows for a single cell, such as a plant protoplast, to be able to regenerate into a complete, whole plant. An analogous comparison of the totipotency of plant cells would be that of stem cells in animals. Genetic manipulation of individual plant cells coupled with their totipotency makes plant cell culture a powerful tool for the plant geneticist.

Role of Plant Growth Regulators

Hormones or plant growth regulators (PGRs) are naturally occurring or synthetic compounds that, in small concentrations, have tremendous regulatory influence on the physiological and morphological growth and development of plants. There are several established classes of PGRs, including auxins, cytokinins, gibberellins, abscisic acid (ABA), and ethylene. Additionally, several other compounds, such as polyamines, oligosaccharides, and sterols,

exert hormonelike activity in plant cell cultures. While each class has a demonstrative and unique effect on overall whole plant growth and development, auxins and cytokinins continue to be the most widely used in manipulating plant growth in vitro. Auxins (such as IAA, NAA, and 2,4-D) and cytokinins (such as zeatin, kinetin, and BAP) are frequently used in combination in plant tissue culture. Generally, a high auxin-to-cytokinin ratio results in the induction of root tissue from callus, while a high cytokinin-to-auxin ratio results in the induction of shoot formation. For many plant species, an intermediate ratio of auxin to cytokinin results in continued callus formation.

There are also specific uses of certain PGRs in plant cell culture. For example, 2,4-D is typically used to induce somatic embryogenesis in cultures but then must be removed for subsequent embryoid development. Gibberellins, such as GA₄ and GA₇, can be inhibitory to morphogenesis. Some PGRs may even elicit oppo-

site morphogenic effects in two different plant species. Nevertheless, the use of PGRs remains essential in plant cell culture to direct morphological development.

Applications and Potential

Plant cell culture as a tool has greatly enhanced the ability of the plant geneticist in the area of crop improvement. Haploid cell cultures initiated from pollen can result in homozygous whole plants, which are very useful as pure lines in breeding programs. In such plants, recessive mutations are easily identified.

The enzymatic removal of the plant cell wall yields naked plant protoplasts, which are more amenable to genetic manipulation. Protoplasts of different species can be chemically or electrically fused to give somatic hybrids that may not be obtained through traditional sexual crossing due to various types of sexual incompatibility. As they divide and regenerate cell walls, these somatic hybrids can then be selected for desired agriculture characteristics, such as insect or disease resistance.

The isolation of plant protoplasts from leaves results in millions of individual cells. As they divide, grow, and differentiate into whole plants, some may contain spontaneous mutations or other changes which can be selected for. Screening for such characteristics, such as salt tolerance or disease resistance, can be done *in vitro*, thereby saving time and space.

A relatively recent use of plant cell culture in crop improvement involves directed genetic transformation. Genes from other species, including bacteria, animals, and other plants, have been introduced into cell cultures, resulting in genetically modified (GM) plants. The most common technique used to transfer desired genes uses the bacterium *Agrobacterium tumefaciens*. Other techniques include electroporation, microinjection, and particle bombardment with "gene guns." As genetic engineering of plants proceeds and is refined, plant cell culture will continue to play a vital role as a tool in this effort.

—Thomas J. Montagno

See also: Cancer; Cell Culture: Animal Cells; Cell Cycle, The; Cell Division; Gene Regulation: Eukaryotes; Genetic Engineering: Ag-

ricultural Applications; Genetically Modified (GM) Foods; High-Yield Crops; Mitosis and Meiosis; Oncogenes; Shotgun Cloning; Stem Cells; Totipotency.

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The Cell Cycle

Field of study: Cellular biology

Significance: During the phases of the cell cycle, cells divide (mitosis and cytokinesis), grow (G_1), replicate their DNA (S), and prepare for another cell division (G_2). Protein signals regulate progress through these phases of the cell cycle. Mutations that alter signal structure, time of synthesis, or how it is received can cause cancer.

Key terms

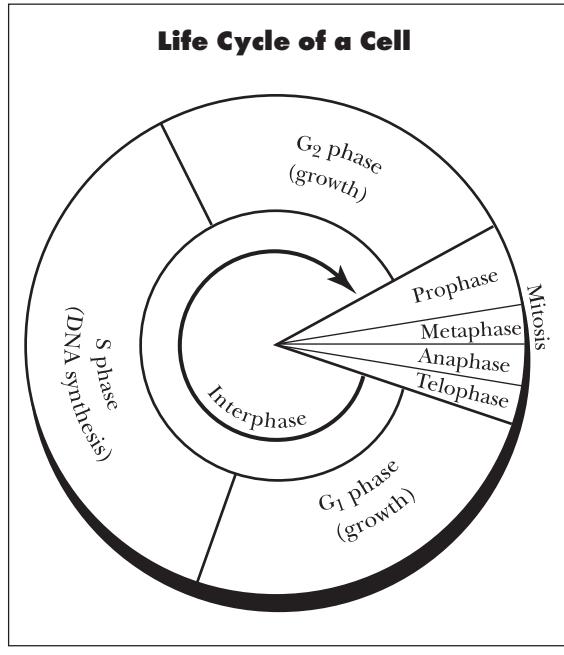
CHECKPOINT: the time in the cell cycle when molecular signals control entry to the next phase

CYCLINS: proteins whose levels rise and fall during the cell cycle

KINASE: an enzyme that catalyzes phosphate addition to molecules

ONCOGENE: a gene whose products stimulate inappropriate cell division, causing cancer

TUMOR SUPPRESSOR: a gene whose product normally prevents or slows cell division; when mutated, these genes can lead to uncontrolled cell division



(Electronic Illustrators Group)

Defining Cell Cycle Phases

The eukaryotic cell cycle is defined by five phases. Two of these, mitosis and cytokinesis, do not last long. Mitosis itself has five phases:

- (1) prophase, when duplicated attached chromatids with replicated DNA condense and become visible as chromosomes, each composed of two sister chromatids
- (2) metaphase, when the chromosomes attach to spindle fibers and move to the middle of the cell
- (3) anaphase, when sister chromatids separate
- (4) telophase, when the separated sister chromatids, now chromosomes, move to opposite poles of the cell, during which cytokinesis often starts
- (5) interphase, a time between successive mitoses when cells approximately double in size

An early experiment showed that DNA replicates long before mitosis. After a short exposure (pulse) of cells to radioactive thymidine to allow synthesis of radioactive DNA, the “hot” nucleotide was removed and cells were allowed to grow for different chase times in a medium containing nonradioactive nucleotides. (The

term “chase” refers to the second part of what is called a radioactive pulse-chase experiment.) Autoradiography showed that after the pulse period, 40 percent of cells were labeled, but only interphase nuclei were radioactive. This established that DNA is not actually synthesized during mitosis. Labeled mitotic chromosomes were seen only in cells chased for 4 to 14 hours. After longer chase times, label was again confined to interphase nuclei. From this kind of study, the cell cycle could be divided into five major phases (times listed are typical of a cell in an adult organism):

- (1) mitosis (M), one hour
- (2) cytokinesis (C), thirty minutes
- (3) gap 1 (G_1), a time of cell growth, which lasts the generation time minus the times of the other phases
- (4) synthesis (S), nine hours of DNA synthesis
- (5) gap 2 (G_2), four to five hours of preparation for the next mitosis

Identification of Cell Cycle Switches

Cells reproduce at different rates. Embryonic cells divide hourly or more often, while neurons stop dividing altogether shortly after birth. Cells divide, or stop dividing, in response to chemical signals. When mitotic cells are fused with G_1 , S, or G_2 cells, fused cells at first contain the chromosomes of the mitotic cell alongside the intact nucleus of the other cell. After a few minutes, the intact nucleus disintegrates and its chromosomes also condense, suggesting that some chemical signal is causing the intact nucleus to respond as if it were undergoing mitosis. This suggests that cells in mitosis contain a substance that induces nondividing cells to become “mitotic.”

The first chemical signal controlling the cell cycle was discovered in studies of amphibian oogenesis. Oocyte maturation begins with the first meiotic division, when the germinal vesicle (the oocyte nucleus) breaks down and chromosomes and spindle fibers first appear at one pole of the oocyte. In a key experiment, cytoplasm taken from oocytes during germinal vesicle breakdown was injected into immature oocytes. Condensed chromosomes quickly appeared in the injected oocytes. A protein called

MPF (maturation promoting factor) was purified from the older oocytes. MPF was later found in developing frog embryos, where its levels fluctuated, peaking just before the embryonic cells began mitosis. Thus, MPF also controls mitosis as well as meiosis and is often called mitosis-promoting factor. MPF consists of cyclin and cyclin-dependent protein kinase (cdk). Cyclin-bound cdk catalyzes phosphorylation of other cellular proteins. Levels of cdk were shown to be constant in the cell, while cyclin levels rose and peaked late in G₂, explaining why MPF activity is highest during mitosis and why mitotic cells induce nuclear breakdown and chromosome condensation when fused to nonmitotic cells.

To study cell cycle regulation further, researchers turned to yeast, a model single-cell eukaryote easily subject to genetic manipulation. Mutagenized yeast was screened for temperature-sensitive mutations that reproduced at lower temperatures but were blocked at one or another point in the cell cycle when grown at higher temperatures. One such temperature-sensitive mutant was arrested in G₂ at the higher temperature. These cells had a defective cell-division-cycle-2 (*cdc2*) gene encoding a yeast version of the frog cdk in MPF. Cellular cdc2 levels are stable, but its kinase activity depends on a yeast cyclin whose levels peak at the end of G₂. The active yeast MPF triggers passage through the G₂ checkpoint, committing the cell to mitosis. Other mutants were found encoding separate G₁ cyclin and G₁ cdk proteins that together form an active kinase that triggers passage through a G₁ checkpoint into the S phase of the cell cycle. Among higher eukaryotes, different combinations of cyclins and cdk's act at still other checkpoints in the cell cycle.

How MPF and G₁ cdk's Work

The proteins phosphorylated by yeast MPF and G₁ cyclin-cdk catalysis function in pathways that promote mitosis and cytokinesis, on one hand, and DNA replication, on the other. How are cdk's activated, what proteins do they phosphorylate, and what do these phosphorylated proteins do?

Yeast MPF made by joining late G₂ cyclin and cdk is not active until it is itself phosphorylated. MPF first receives two phosphates. Then the addition of a third phosphate causes the first two to come off in a peculiar MPF activation pathway. In fact, MPF remains unphosphorylated and inactive in cells experimentally prevented from replicating. In normal cells, blocking premature activation of MPF could prevent condensing chromosomes from damaging DNA that is still replicating. When properly activated, MPF phosphorylates (a) proteins that break down the nuclear membrane, (b) histones and other chromatin proteins thought to start chromosome condensation, and (c) microtubule-associated proteins associated with mitotic spindle formation.

G₁ cyclin and cdk production occur when cells reach a suitable size during G₁ and when they are stimulated by a growth factors. For example, EGF (epidermal growth factor) stimulates embryonic cell growth by binding to cell membrane receptors. EGF-receptor binding converts the intracellular domain of the receptor into an active protein kinase that catalyzes self-phosphorylation. The auto-phosphorylated receptor activates a G-protein encoded by the *ras* gene, which binds GTP. Then, ras-GTP activates the first in a series of protein kinases, setting off an intracellular kinase cascade. Sequential phosphorylations finally stimulate synthesis of G₁ cyclin and G₁ cdk. Active cyclin-bound G₁ cdk then phosphorylates the Rb protein, causing it to detach from protein EF2, which becomes an active transcription factor that stimulates synthesis of proteins needed for replication in the S phase.

To summarize, MPF is activated by a phosphorylation pathway in which the kinase itself becomes phosphorylated, while G₁ cdk is made in response to growth factors like EGF that initiate phosphorylation cascades, resulting in the eventual synthesis of cyclin and cdk. MPF phosphorylates other proteins, permitting transition across the G₂ checkpoint, while the G₁ cdk allows progress through the G₁ checkpoint.

The Cell Cycle and Cancer

With the discovery of the first MPF, scientists had already begun to suspect that mutations in

genes encoding proteins involved in cell cycling might cause the uncontrollable cell divisions associated with cancer. Many cancers are associated with oncogenes (called proto-oncogenes when they function and are expressed correctly) encoding proteins involved in cell cycle control. Some oncogenes are carried by viruses, but most arise by mutation of their normal counterparts, resulting in inappropriate activity of the protein encoded by the gene. Representative human oncogenes include *neu* (a growth-factor-receptor oncogene associated with breast and ovarian cancers), *trk* (a receptor oncogene associated with colon cancer), *ras* (a G-protein oncogene), *L-myc* (a transcription-factor oncogene causing small-cell lung cancer), *cdk-4* (a cyclin-dependent kinase oncogene causing a muscle sarcoma), and *CYCD1* (a cyclin oncogene associated with lymphoma). Each of these oncogenes produces proteins that promote unrestricted passage through the cell cycle. In contrast, retinoblastoma is a rare eye cancer in which the *Rb* oncogene product is not made, so that *EF2* transcription factor is always active and genes involved in replication are continuously on. Because *Rb* restrains unwanted cell divisions, it is called a tumor-suppressor gene. Unfortunately, the *Rb* oncogene is also associated with more common human adult lung, breast, and bladder cancers. Another tumor-suppressor gene, *p53*, is also implicated in several human cancers; a defective *p53* gene allows cells with damaged DNA to replicate, increasing the chances of cancer development.

In the brief history of cell cycle studies, the discovery of an oncogene identifies the cause of a cancer while the newfound actor in a phosphorylation cascade is a candidate for an oncogene. The study of the cell cycle is an excellent example of the synergy between basic and applied science: The fundamental drive to know how cells grow and divide has merged with a fervent desire to conquer a group of human diseases increasingly prevalent in our aging population.

—Gerald K. Bergstrom

See also: Cancer; Cell Division; Chemical Mutagens; Chromosome Mutation; Cytokinesis; DNA Repair; Gene Regulation; Eukaryotes;

Mitosis and Meiosis; Mutation and Mutagenesis; Oncogenes; Stem Cells; Telomeres; Totipotency; Tumor-Suppressor Genes.

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Cell Division

Field of study: Cellular biology

Significance: *Eukaryotic cell division (mitosis and cytokinesis) are a short part of the cell cycle. In the longer time between successive cell divisions cells grow and replicate their DNA. Molecular signals tell cells when to enter each stage of the cycle.*

Key terms

ASEXUAL REPRODUCTION: a form of reproduction wherein an organism's cell DNA doubles and is distributed equally to progeny cells

BINARY FISSION: cell division in prokaryotes in which the plasma membrane and cell wall grow inward and divide the cell in two

CHROMATID: one-half of a replicated chromosome

CHROMATIN: the material that makes up chromosomes; a complex of fibers composed of DNA, histone proteins, and nonhistone proteins

CHROMOSOME: a self-replicating structure, consisting of DNA and protein, that contains part of the nuclear genome of a eukaryote; also used to describe the DNA molecules comprising the prokaryotic genome

CYCLINS: proteins whose levels rise and fall during the cell cycle

CYCLIN-DEPENDENT PROTEIN KINASES (CDK's): proteins that regulate progress through the eukaryotic cell cycle

CYTOKINESIS: movements of and in a cell resulting in the division of one eukaryotic cell into two

DNA REPLICATION: synthesis of new DNA strands complementary to parental DNA

GENOME: the species-specific, total DNA content of a single cell

MEIOSIS: a type of cell division that leads to production of gametes (sperm and egg) during sexual reproduction

MITOSIS: nuclear division, a process of allotting a complete set of chromosomes to two daughter nuclei

PHASES OF MITOSIS AND MEIOSIS: periods—including prophase, metaphase, anaphase, telophase—characterized by specific chromosomal events during cell division

PHASES OF THE CELL CYCLE: mitosis, cytokinesis, G₁ (gap 1), S (DNA synthesis), and G₂ (gap 2)

PHOSPHORYLATION: a chemical reaction in which a phosphate is added to a molecule, common in the control of cell activity, including the regulation of passage through different stages of the cell cycle

Asexual vs. Sexual Reproduction

A cell's genetic blueprint is encoded in genes written in the four-letter alphabet of DNA, which stands for the four nucleotides that make up the strands of DNA: guanine (G), adenine (A), thymine (T), and cytosine (C).

Reproduction of this blueprint is an essential property of life. Prokaryotes (cells without nuclei) contain a single chromosome in the form of a circular double helix. They replicate their DNA and reproduce asexually by binary fission. Eukaryotic cells, with two or more pairs of linear, homologous chromosomes in a nucleus, replicate their DNA and reproduce asexually by mitosis. In sexual reproduction in higher organisms, special cells called germ cells are set aside to form gametes by meiosis. During meiosis, the germ cells duplicate their chromosomes and separate the homologs into gametes. After mitosis, new cells have a copy of all of the chromosomes originally present in the parent cell; after meiosis, gametes (sperm or egg) contain only one of each homologous chromosome originally present in the parent cell. Though their chromosomal outcomes are quite different, the cellular events of mitosis and meiosis share many similar features, discussed below mostly in the context of mitosis. The focus here is on when cells replicate their DNA, when they physically divide, and how they partition duplicate sets of genetic information into progeny cells.

Binary Fission vs. Meiosis, Mitosis, and Cytokinesis

During binary fission, which occurs in prokaryotic cells (cells that have no nucleus—primary bacteria), these small cells grow larger, become pinched in the middle, and eventually produce two new cells. A specific base sequence in the circular bacterial DNA molecule attaches to the cell membrane. When this sequence replicates during DNA synthesis it also attaches to the cell membrane, but on the opposite side of the cell. As the bacterial cell grows and divides, the two DNA attachment points become separated into the progeny cells, ensuring that each gets a copy of the original circular DNA molecule. DNA replication and cell division in prokaryotes are therefore simultaneous processes.

Mitosis (and meiosis) and cytokinesis, by contrast, are processes well separated in time from DNA replication. When first observed in the microscope in the 1880's, mitosis seemed to be a busy time in the life of a cell. During

prophase (the initial phase of mitosis), nuclei seem to disintegrate in a matter of minutes at the same time that chromosomes take shape from nondescript nuclear substance. Spindle fibers form at opposite poles and grow toward the center of the cell. After about thirty minutes, cells are in metaphase. The spindle fibers extend across the cell, attaching to fully formed chromosomes lined up at the metaphase plate in the middle of the cell. Each chromosome is actually composed of two attached strands, or chromatids.

During anaphase the chromatids of each chromosome pull apart and move toward opposite poles of the cell. Telophase is characterized by the re-formation of nuclei around the chromosomes and the de-condensation of the chromosomes back to the shapeless substance now called chromatin.

Cytokinesis, meaning “cell movement,” begins during telophase, lasts about thirty minutes, and is the actual division of the parent cell into two cells, each of which gets one of the newly forming nuclei. The processes of mitosis and cytokinesis, which together typically last about 1.5 hours, ensures that duplicated pairs of chromosomes are partitioned correctly into progeny cells.

Meiosis actually consists of two cell divisions, each progressing through prophase, metaphase, anaphase, and telophase. In the first division, homologous chromosomes with their chromatids are separated into progeny cells; in the second, chromatids are pulled apart into the cells that will become gametes. The result is to produce haploid eggs or sperm, rather than the diploid progeny with paired homologous chromosomes that result from mitosis.

The Cell Cycle

Early histologists studying mitosis noted that it often took cells about twenty hours to double, implying a long period between successive cell divisions. This period was called interphase, meaning simply “between” the mitotic phases. An interphase also separates the first meiotic division from a prior mitosis, though there is not always an interphase between the first and second meiotic divisions. One might have suspected that cells were not just biding their time

between mitoses, but it was only in the middle of the twentieth century that the cell cycle was fully characterized, showing interphase to be a long and very productive time in the life of a cell.

In an elegant experiment, cultured cells were exposed to radioactive thymidine, a DNA precursor. After a few minutes, radioactive DNA was detected in the nuclei of some cells. However, no cells actually in mitosis were radioactive. This meant that DNA is not synthesized during mitosis. Radioactive condensed mitotic chromosomes were detected only four to five hours after cells had been exposed to the radioactive DNA precursor, suggesting that replication had ended four to five hours before the beginning of mitosis. Studies like this eventually revealed the five major intervals of the cell cycle: mitosis, cytokinesis, gap 1 (the G₁ phase, a time of cell growth), DNA synthesis (the S phase of DNA synthesis), and gap 2 (the G₂ phase, during which a cell continues growing and prepares for the next mitosis).

The overall length of the cell cycle differs for different cell types. Human neurons stopped dividing shortly after birth, never to be replaced. Many other differentiated cells do not divide but are replaced periodically by stem cells that have the capacity to continue to divide and differentiate. Clearly, human genes must issue instructions telling cells when and when not to reproduce.

Controlling the Cell Cycle

Sometimes cells receive faulty instructions (for example, from environmental carcinogens) or respond inappropriately to otherwise normal commands from other cells. Cancer is a group of diseases in which normal regulation of the cell cycle has been lost and cells divide out of control. In research published in the 1970's, cells synchronized in mitosis were mixed with others synchronized in other phases of the cell cycle in the presence of polyethylene glycol (the main ingredient in automobile antifreeze). The antifreeze caused cells to fuse. Right after mixing, chromosomes and a mitotic spindle could be seen alongside an intact nucleus in the fused cells. Later, the intact nucleus broke down and chromosomes condensed. The conclusion from studies like this is that mitosing

cells contain a substance that causes nuclear breakdown and chromosome condensation in nonmitosing cells. Similar results were seen when cells in meiosis were fused with non-meiotic cells. When purified, the substances from meiotic and mitotic cells could be injected into nonmitosing cells, where they caused nuclear breakdown and the appearance of chromosomes from chromatin. The substance was called maturation (or mitosis) promoting factor (MPF). MPF contains one polypeptide called cyclin and another called cyclin-dependent protein kinase (cdk). The kinase enzyme catalyzes transfer of a phosphate to other proteins; it is active only when bound to cyclin—hence the name. The kinase is always present in cells, while cyclin concentrations peak at mitosis and then fall. This explains why MPF activity is highest during mitosis and why mitotic cells fused to G₁ cells, for example, can cause the G₁ cell nucleus to disappear and chromosomes to emerge from chromatin.

Since the initial discovery of MPF, studies of eukaryotic cells, from yeast cells to human cells, have revealed many different cyclin-dependent kinases and other regulatory proteins that exert control at different checkpoints on the cell cycle, determining whether or not cells progress from one stage to another. Scientists remain ignorant of the exact causes of most cancers, but because of the compelling need to know, researchers are beginning to understand the normal controls on cellular reproduction.

A final word on the cyclin-dependent protein kinase: This enzyme is one of a large number of kinases that participate in regulating cell chemistry and behavior in response to many extracellular signals (such as hormones). The phosphorylation of cellular proteins has emerged as a major theme in the regulation of many cellular activities, including cell division.

—Gerald K. Bergstrom

See also: Cell Culture: Animal Cells; Cell Culture: Plant Cells; Cell Cycle, The; Cytokinesis; Gene Regulation: Eukaryotes; Mitosis and Meiosis; Polyploidy; Totipotency.

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Central Dogma of Molecular Biology

Field of study:

Molecular genetics

Significance: *The central dogma states precisely how DNA is processed to produce proteins. Originally thought to be a unidirectional process proceeding from DNA to RNA and then to protein, it is now known to include reverse transcription and the enzymatic activity of certain RNA molecules. The central dogma lies at the core of molecular genetics, and understanding it, and particularly reverse transcription, is key to comprehending both the way viruses cause disease and methods that have revolutionized biology.*

Key terms

CODON: three nucleotides in DNA or RNA that correspond with a particular amino acid or stop signal

COLINEARITY: the exact correspondence between DNA or RNA codons and a protein amino acid sequence

COMPLEMENTARY BASES: the nucleic acid bases in different strands of nucleic acid in RNA and DNA that pair together through hydrogen bonds: guanine-cytosine and adenine-thymine (in DNA and RNA) and adenine-uracil (in RNA)

EXON: the part of the coding sequence of mRNA that specifies the amino acid sequence of a protein

HYDROGEN BOND: a weak chemical bond that forms between atoms of hydrogen and atoms of other elements, including oxygen and nitrogen

INTRON: a noncoding intervening sequence present in many eukaryotic genes that is transcribed but removed before translation

RETROVIRUS: a virus that carries reverse transcriptase that converts its RNA genome into a DNA copy that integrates into the host chromosome

REVERSE TRANSCRIPTION: the conversion of RNA into DNA catalyzed by the enzyme reverse transcriptase

RIBOZYME: catalytic RNA

SUBUNIT: a polypeptide chain of a protein

Original Central Dogma

Nobel Prize winner Francis Crick, who was co-discoverer with James Watson of the double helical structure of DNA, coined the term “central dogma” in 1958 to describe the fact that the processing of genetic information contained in DNA proceeded unidirectionally by its conversion first to an RNA copy, called messenger RNA (mRNA), in a molecular process called transcription. Then the genetic information contained in the sequence of bases in the mRNA was read in the ribosome, and the appropriate amino acids carried by transfer RNAs (tRNAs) were assembled into protein according to the genetic code in a process called translation. The basis of these reactions stemmed from the properties of DNA, particularly its double helical structure. The fact that the two strands of DNA were held together by hydrogen bonds between specific nucleic acid bases (guanine-cytosine, adenine-thymine) on the two strands clearly suggested how the molecule could be duplicated. Watson and Crick postulated that if they split the double-stranded structure at the

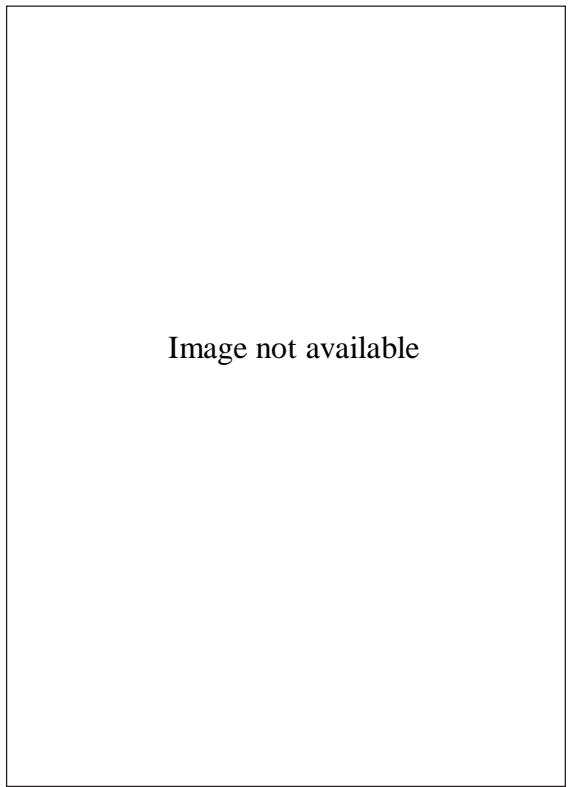


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Francis Crick in 2003, who with James Watson won the 1962 Nobel Prize in Physiology or Medicine for their discovery of the double helix structure of DNA. Crick articulated the “central dogma” of molecular biology and coined the term. (AP/Wide World Photos)

hydrogen bonds, attaching new complementary bases, and reforming the hydrogen bonds, precise copies identical to the original DNA would result. In an analogous manner, RNA was produced by using one DNA strand as a template and adding the correct complementary bases according to what came to be called Watson Crick base pairing. Thus the original dogma stated that transfer of genetic information proceeded unidirectionally, that is, only from DNA to RNA to protein. The only exception was the duplication of DNA in a process called replication.

Modified Central Dogma

Several discoveries made it necessary to change the central dogma. The first and most heretical information came from the study of retroviruses, including the human immunode-

ficiency virus (HIV). Howard Temin reported that viruses of this group contained an enzyme called reverse transcriptase, which was capable of converting RNA to DNA and thus challenging the whole basis of molecular reactions and the central dogma. Temin and David Baltimore were subsequently awarded Nobel Prizes for their work describing this new enzyme. They were able to show that it synthesizes a DNA strand complementary to the RNA template, and then the DNA-RNA hybrid is converted to a DNA-DNA molecule, which inserts into the host chromosome. Only then can transcription and translation take place.

The second significant change was finding that RNA can act as a template for its own synthesis. This situation occurs in RNA bacteriophages such as MS2 and QB. These phages are very simple, with genomes specifying only three proteins, a coat and attachment proteins and an RNA replicase subunit. This subunit combines with three host proteins to form the mature RNA replicase that catalyzes the replication of the single-stranded RNA. Thus translation to form the protein subunit of RNA replicase occurs using the RNA genome as mRNA upon viral infection without transcription taking place. Only then is the RNA template successfully replicated.

The third natural modification of the original dogma also concerned the properties of RNA. Thomas Cech in 1982 discovered that introns could be spliced out of eukaryotic genes without proteins catalyzing the process. For the discovery and characterization of catalytic RNA, Cech and Sidney Altman were awarded Nobel Prizes for their work in 1989. Their experiments demonstrated that RNA introns, also called ribozymes, had enzymatic activity that could produce a functional mRNA. This process occurred by excising the introns and combining the exons, thus restoring colinearity of DNA and amino acid sequence. RNA processing thus demonstrates another needed modification of the central dogma: The colinearity of gene and protein in prokaryotes predicts that gene expression results directly from the sequence of bases in its DNA. In the case of eukaryotic genes with multiple introns, however, colinearity does not result until the RNA pro-

cessing has taken place. Therefore, the correspondence of the codons in the original DNA sequence containing the introns does not correspond to the order of amino acids in the protein product.

Numerous examples also exist of DNA rearrangements occurring before final gene expression takes place. Examples include the formation of antibodies, the expression of different mating types in yeast, and the expression of different surface antigens in parasites, such as the trypanosome protozoan parasite, which causes sleeping sickness. All of these gene products are produced as a result of gene rearrangements, and the original DNA sequences are not colinear with the amino acid sequences in the protein.

Importance and Applications

The theoretical importance of the central dogma is unquestioned. For example, one modern-day scourge, the human immunodeficiency virus (HIV), replicates its genetic material by reverse transcription (central dogma modification), and one of the drugs shown to contain this virus, azidothymidine (AZT), targets the reverse transcriptase enzyme. Perhaps even more important is the use of the reverse transcription polymerase chain reaction (RT-PCR), one application of the polymerase chain reaction originally devised in 1983 by Kary B. Mullis, formerly of Cetus Corporation. RT-PCR employs reverse transcriptase to form a double-stranded molecule from RNA, resulting in a revolutionary technique that can generate usable amounts of DNA from extremely small quantities of DNA or from poor-quality DNA. Also of practical importance is the laboratory modification of hammerhead ribozymes (central dogma modification), found naturally in plant pathogens, for clinical uses, such as to target RNA viruses infecting patients, including HIV and papillomavirus.

—Steven A. Kuhl

See also: DNA Structure and Function; Gene Regulation: Viruses; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Protein Synthesis; Reverse Transcriptase; RNA Structure and Function; RNA World.

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Chemical Mutagens

Field of study: Molecular genetics

Significance: *Mutagens are naturally occurring or human-made chemicals that can directly or indirectly create mutations or changes in the information carried by the DNA. Mutations may cause birth defects or lead to the development of cancer.*

Key terms

DEAMINATION: the removal of an amino group from an organic molecule

TAUTOMERIZATION: a spontaneous internal rearrangement of atoms in a complex biological molecule which often causes the molecule to change its shape or its chemical properties

The Discovery of Chemical Mutagens

The first report of mutagenic action of a chemical occurred in 1946, when Charlotte Auерbach showed that nitrogen mustard (a component of the poisonous "mustard" gas widely used in World War I) could cause mutations in fruit flies (*Drosophila melanogaster*). Since that time, it has been discovered that many other chemicals are also able to induce mutations in a variety of organisms. This led to the birth of genetic toxicology during the last half of the twentieth century, dedicated to identifying potentially mutagenic chemicals in food, water, air, and consumer products. Continued research has identified two modes by which mutagens cause mutations in DNA: (1) by interacting directly with DNA, and (2) indirectly, by tricking the cell into mutating its own DNA.

Chemical Mutagens with Direct Action on DNA

Base analogs are chemicals that structurally resemble the organic bases purine and pyrimidine and may be incorporated into DNA in place of the normal bases during DNA replication. An example is bromouracil, an artificially created compound extensively used in research. It resembles the normal base thymine and differs only by having a bromine atom instead of a methyl (CH_3) group. Bromouracil is incorporated into DNA by DNA polymerase, which pairs it with an adenine base just as it would thymine. However, bromouracil is more unstable than thymine and is more likely to change its structure slightly in a process called tautomerization. After the tautomerization process, the new form of bromouracil pairs better with guanine rather than adenine. If this happens to a DNA molecule being replicated, DNA polymerase will insert guanine opposite bromouracil, thus changing an adenine-thymine pair to guanine-cytosine by way of the two intermediates involving bromouracil. This type of mutation is referred to as a transition, in which a purine is replaced by another purine and a pyrimidine is replaced by another pyrimidine.

Another class of chemical mutagens are those that alter the structure and the pairing properties of bases by reacting chemically with them. An example is nitrous acid, which is

formed by digestion of nitrite preservatives found in some foods. Nitrous acid removes an amino (NH_3) group from the bases cytosine and adenine. When cytosine is deaminated, it becomes the base uracil, which is not a normal component of DNA but is found in RNA. It is able to pair with adenine. Therefore, the action of nitrous acid on DNA will convert what was a cytosine-guanine base pair to uracil-guanine, which, if replicated, will give rise to a thymine-adenine pair. This is also a transition type of mutation.

Alkylating agents are a large class of chemical mutagens that act by causing an alkyl group (which may be methyl, ethyl, or a larger hydrocarbon group) to be added to the bases of DNA. Some types of alkylation cause the base to become unstable, resulting in a single-strand break in the DNA; this type of event can cause a mutation if the DNA is replicated with no base present or can lead to more serious breaks in the DNA strand. Other alkylation products will change the pairing specificity of the base and create mutations when the DNA is replicated.

Intercalating agents such as acridine orange, proflavin, and ethidium bromide (which are used in labs as dyes and mutagens) have a unique mode of action. These are flat, multiple-ring molecules that interact with bases of DNA and insert themselves between them. This insertion causes a “stretching” of the DNA duplex, and the DNA polymerase is “fooled” into inserting an extra base opposite an intercalated molecule. The result is that intercalating agents cause frame-shift mutations in which the “sense” of the DNA message is lost, just as if an extra letter were inserted into the phrase “the fat cat ate the hat” to make it “the ffa tca tat eth eha t.” This occurs because genes are read in groups of three bases during the process of translation. This type of mutation always results in production of a nonfunctional protein.

Chemical Mutagens with Indirect Action

Aromatic amines are large molecules that bind to bases in DNA and cause them to be unrecognizable to DNA polymerase or RNA polymerase. An example is N-2-acetyl-2-amino-fluorine (AAF), which was originally used as an insecticide. This compound and other aromatic

amines are relatively inactive on DNA until they react with certain cellular enzymes, after which they react readily with guanine. Mutagens of this type and all others with indirect action work by triggering cells to induce mutagenic DNA repair pathways, which results in a loss of accuracy in DNA replication.

One of the oldest known environmental carcinogens is the chemical benzo(α)pyrene, a hydrocarbon found in coal tar, cigarette smoke, and automobile exhaust. An English surgeon, Percivall Pott, observed that chimney sweeps had a high incidence of cancer of the scrotum. The reason for this was later found to be their exposure to benzo(α)pyrene in the coal tar and soot of the chimneys. Like the aromatic amines, benzo(α)pyrene is activated by cellular enzymes and causes mutations indirectly.

Another important class of chemical mutagens with indirect action are agents causing cross-links between the strands of DNA. Such cross-links prevent DNA from being separated into individual strands as is needed during DNA replication and transcription. Examples of cross-linking agents are psoralens (compounds found in some vegetables and used in treatments of skin conditions such as psoriasis) and cis-platinum (a chemotherapeutic agent used to fight cancer).

Another important class of chemical mutagens are those that result in the formation of active species of oxygen (oxidizing agents). Some of these are actually created in the body by oxidative respiration (endogenous mutagens), while others are the result of the action of chemicals such as peroxides and radiation. Reactive oxygen species cause a wide variety of damage to the bases and the backbone of DNA and may have both direct and indirect effects.

Detection of Chemical Mutagens

The Ames test, developed by biochemistry professor Bruce Ames and his colleagues, is one of the most widely used screening methods for chemical mutagens. It employs particular strains of the bacterium *Salmonella typhimurium* that require the amino acid histidine because of mutations in one of the genes controlling histidine production. The bacteria are exposed

to the potential mutagen and then spread on an agar medium lacking histidine. The strains can grow only if they develop a mutation restoring function to the mutated gene required for histidine synthesis. The degree of growth indicates the strength of the mutagen; mutagens of different types are detected by using bacterial strains with different mutations. Mutagens requiring metabolic activation are detected by adding extracts of rat liver cells (capable of mutagen activation) to the tested substance prior to exposure of the bacteria. The Ames test and others like it involving microorganisms are rapid, safe, and relatively inexpensive ways to detect mutagenic chemicals, but it is not always clear how the results of the Ames test should be interpreted when determining the degree of mutagenicity predicted in humans.

Impact and Applications

Mutations can have serious consequences for cells of all types. If they occur in gametes, they can cause genetic diseases or birth defects. If they occur in somatic (body) cells of multicellular organisms, they may alter a growth-controlling gene in such a way that the mutated cell begins to grow out of control and forms a cancer. DNA is subject to a variety of types of damage by interaction with a wide array of chemical agents, some of which are ubiquitous in the environment, while others are the result of human intervention. Methods of detection of chemicals with mutagenic ability have made it possible to reduce the exposure of humans to some of these mutagenic and potentially carcinogenic chemicals.

—Beth A. Montelone

See also: Biochemical Mutations; Cancer; DNA Repair; DNA Replication; Mutation and Mutagenesis; Oncogenes; Repetitive DNA; Tumor-Suppressor Genes.

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Chloroplast Genes

Field of study: Molecular genetics

Significance: *Plants are unique among higher organisms in that they meet their energy needs through photosynthesis. The specific location for photosynthesis in plant cells is the chloroplast, which also contains a single, circular chromosome composed of DNA. Chloroplast DNA (cpDNA) contains many of the genes necessary for proper chloroplast functioning. A better understanding of the genes in cpDNA has improved the understanding of photosynthesis, and analysis of the DNA sequence of these genes has also been useful in studying the evolutionary history of plants.*

Key terms

CHLOROPLAST: the cell structure in plants responsible for photosynthesis

GENOME: all of the DNA in the nucleus or in one of the organelles such as a chloroplast

OPEN READING FRAMES: DNA sequences that contain all the components found in active genes, but whose functions have not yet been identified

PHOTOSYNTHESIS: the process in which sun-light is used to take carbon dioxide from the air and convert it into sugar

The Discovery of Chloroplast Genes

The work of nineteenth century Austrian botanist Gregor Mendel showed that the inheritance of genetic traits follows a predictable pattern and that the traits of offspring are determined by the traits of the parents. For example, if the pollen from a tall pea plant is used to pollinate the flowers of a short pea plant, all the offspring are tall. If one of these tall offspring is allowed to self-pollinate, it produces a mixture of tall and short offspring, three-quarters of them tall and one-quarter of them short. Similar patterns are observed for large numbers of traits from pea plants to oak trees. Because of the widespread application of Mendel's work, the study of genetic traits by controlled mating is often referred to as Mendelian genetics.

In 1909, German botanist Carl Erich Correns discovered a trait in four-o'clock plants (*Mirabilis jalapa*) that appeared to be inconsistent with Mendelian inheritance patterns. He discovered four-o'clock plants that had a mixture of leaf colors on the same plant: Some were all green, many were partly green and partly white (variegated), and some were all white. If he took pollen from a flower on a branch with all-green leaves and used it to pollinate a flower on a branch with all-white leaves, all the resulting seeds developed into plants with white leaves. Likewise, if he took pollen from a flower on a branch with all-white leaves and used it to pollinate a flower on a branch with all-green leaves, all the resulting seeds developed into plants with green leaves. Repeated pollen transfers in any combina-

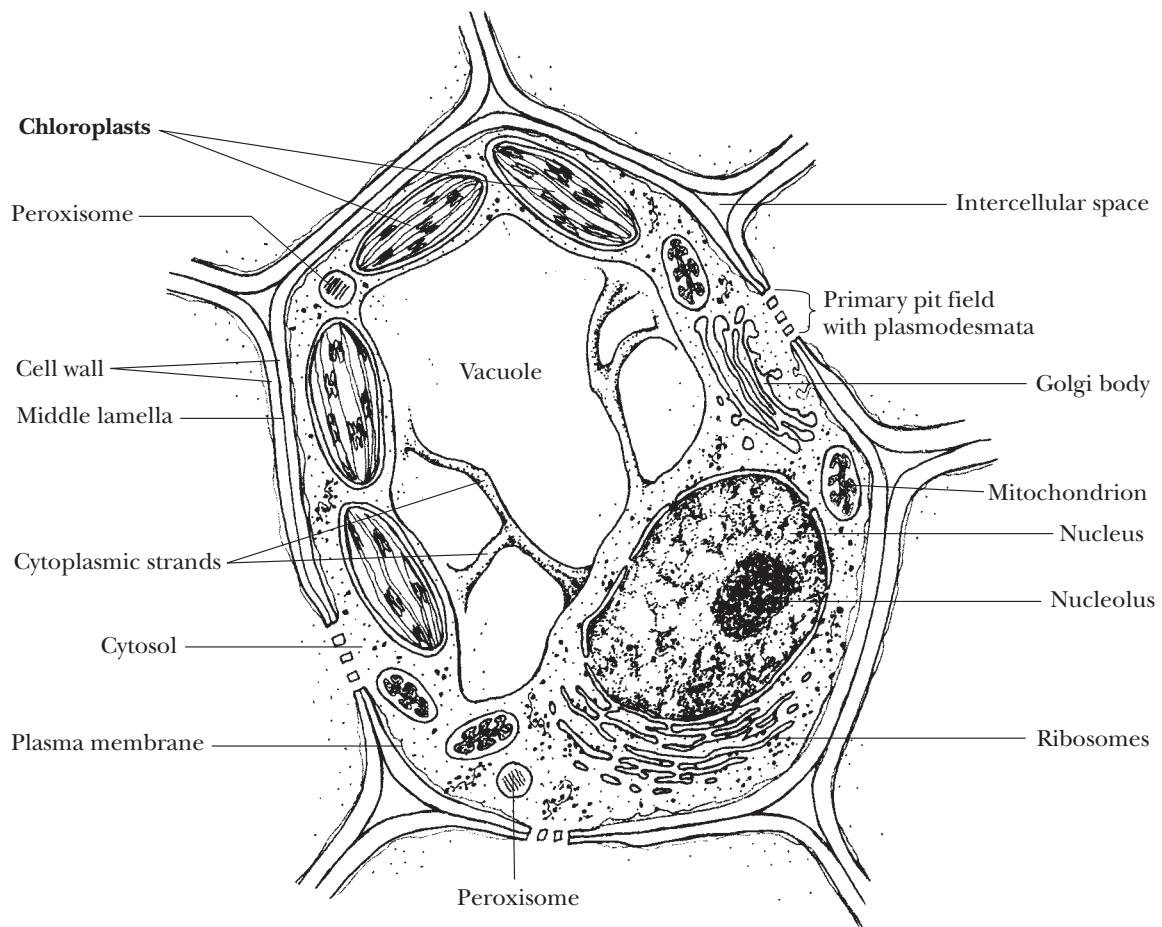
tion always resulted in offspring whose leaves resembled those on the branch containing the flower that received the pollen—that is, the maternal parent. These results could not be explained by Mendelian genetics.

Since Correns's discovery, many other such traits have been discovered. It is now known that the reason these traits do not follow Mendelian inheritance patterns is that their genes are not on the chromosomes in the nucleus of the cell where most genes are located. Instead, the gene for the four-o'clock leaf color trait is located on the single, circular chromosome found in chloroplasts. Because chloroplasts are specialized for photosynthesis, many of the genes on the single chromosome produce proteins or RNA that either directly or indirectly affect synthesis of chlorophyll, the pigment primarily responsible for trapping energy from light. Because chlorophyll is green and because mutations in many chloroplast genes cause chloroplasts to be unable to make chlorophyll, most mutations result in partially or completely white or yellow leaves.



Carl Erich Correns, whose experiments with four-o'clock plants led to the discovery of chloroplast genes. (National Library of Medicine)

Chloroplasts and Other Parts of a Plant Cell



(Kimberly L. Dawson Kurnizki)

Identity of Chloroplast Genes

Advances in molecular genetics have allowed scientists to take a much closer look at the chloroplast genome. The size of the genome has been determined for a number of plants and algae and ranges from 85 to 292 kilobase pairs (kb, or one thousand base pairs), with most being between 120 kb and 160 kb. The complete DNA sequence for many different chloroplast genomes of plants and algae have been determined. Although a simple sequence does not necessarily identify the role of each gene, it has allowed the identity of a number of genes to be determined, and it has al-

lowed scientists to estimate the total number of genes. In terms of genome size, chloroplast genomes are relatively small and contain a little more than one hundred genes.

Roughly half of the chloroplast genes produce either RNA molecules or polypeptides that are important for protein synthesis. Some of the RNA genes occur twice in the chloroplast genomes of almost all land plants and some groups of algae. The products of these genes represent all the ingredients needed for chloroplasts to carry out transcription and translation of their own genes. Half of the remaining genes produce polypeptides directly required

for the biochemical reactions of photosynthesis. What is unusual about these genes is that their products represent only a portion of the polypeptides required for photosynthesis. For example, the very important enzyme ATPase—the enzyme that uses proton gradient energy to produce the important energy molecule adenosine triphosphate (ATP)—comprises nine different polypeptides. Six of these polypeptides are products of chloroplast genes, but the other three are products of nuclear genes that must be transported into the chloroplast to join with the other six polypeptides to make active ATPase. Another notable example is the enzyme ribulose biphosphate carboxylase (RuBP carboxylase, or Rubisco), which is composed of two polypeptides. The larger polypeptide, called *rbcL*, is a product of a chloroplast gene, whereas the smaller polypeptide is the product of a nuclear gene.

The last thirty or so genes remain unidentified. Their presence is inferred because they have DNA sequences that contain all the components found in active genes. These kinds of genes are often called open reading frames (ORFs) until the functions of their polypeptide products are identified.

Impact and Applications

The discovery that chloroplasts have their own DNA and the further elucidation of their genes have had some impact on horticulture and agriculture. Several unusual, variegated leaf patterns and certain mysterious genetic diseases of plants are now better understood. The discovery of some of the genes that code for polypeptides required for photosynthesis has helped increase understanding of the biochemistry of photosynthesis. The discovery that certain key chloroplast proteins such as ATPase and Rubisco carboxylase are composed of a combination of polypeptides coded by chloroplast and nuclear genes also raises some as yet unanswered questions. For example, why would an important plant structure like the chloroplast have only part of the genes it needs to function? Moreover, if chloroplasts, as evolutionary theory suggests, were once free-living bacteria-like cells, which must have had all the genes needed for photosynthesis, why and how

did they transfer some of their genes into the nuclei of the cells in which they are now found?

Of greater importance has been the discovery that the DNA sequences of many chloroplast genes are highly conserved—that is, they have changed very little during their evolutionary history. This fact has led to the use of chloroplast gene DNA sequences for reconstructing the evolutionary history of various groups of plants. Traditionally, plant systematists (scientists who study the classification and evolutionary history of plants) have used structural traits of plants such as leaf shape and flower anatomy to try to trace the evolutionary history of plants. Unfortunately, there are a limited number of structural traits, and many of them are uninformative or even misleading when used in evolutionary studies. These limitations are overcome when gene DNA sequences are used.

A DNA sequence several hundred base pairs in length provides the equivalent of several hundred traits, many more than the limited number of structural traits available (typically much fewer than one hundred). One of the most widely used sequences is the *rbcL* gene. It is one of the most conserved genes in the chloroplast genome, which in evolutionary terms means that even distantly related plants will have a similar base sequence. Therefore, *rbcL* can be used to retrace the evolutionary history of groups of plants that are very divergent from one another. The *rbcL* gene, along with a few other very conservative chloroplast genes, has already been used in attempts to answer some basic questions about the origins and evolution of some of the major flowering plant groups. Less conservative genes and ORFs show too much evolutionary change to be used at higher classification levels but are extremely useful in answering questions about the origins of closely related species, genera, or even families. As analytical techniques are improved, chloroplast genes show promise of providing even better insights into plant evolution.

—Bryan Ness

See also: Cell Culture: Plant Cells; DNA Isolation; Extrachromosomal Inheritance; Genomics; Hybridization and Introgression; Model Organism: *Chlamydomonas reinhardtii*.

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ing epidemics that resulted in countless deaths. By the early twentieth century, cholera had been confined mostly to Asia. In 1961, however, a cholera pandemic beginning in Indonesia spread to Africa, the Mediterranean nations, and North America. In the poorer nations of the world, cholera is still widespread and occurs where sanitation is inadequate. In industrialized nations, where sanitation is generally good, only a few cases occur each year. These usually result from the return of afflicted travelers from regions where cholera is endemic. Because cholera has a 50 to 60 percent fatality rate when its symptoms are not treated quickly, occasional cases cannot be ignored; both the consequences to afflicted people and the potential for the outbreak of epidemics are great.

Cholera is an infection of the small intestine caused by the comma-shaped bacterium *Vibrio cholerae*. Infection is almost always caused by consumption of food or water contaminated with the bacterium. It is followed in one to five days by watery diarrhea that may be accompanied by vomiting. The diarrhea and vomiting may cause the loss of as much as a pint of body water per hour. This fluid loss depletes the blood water and other tissues so severely that if left unchecked it can cause death within a day. Treatment of cholera combines oral or intravenous rehydration of afflicted individuals with saline-nutrient solutions and chemotherapy with antibiotics, especially tetracycline. The two-pronged therapy replaces lost body water and destroys all *V. cholerae* in infected individuals. Antibiotic prophylaxis, which destroys the bacteria, leads to the cessation of production of cholera toxin, the substance that causes diarrhea, vomiting, and death.

Cholera

Fields of study: Bacterial genetics; Diseases and Syndromes;

Significance: *Cholera is an extremely dangerous intestinal disease that has the potential to kill millions of people. Understanding of its genetic basis simplifies treatment and may lead to its eradication.*

Key terms

ENDEMIC: prevalent and recurring in a particular geographic region, specific to a particular region

PANDEMIC: an epidemic that occurs over a large area

PROPHYLAXIS: prevention or cure of a disease

Cholera, Its Symptoms, and Its Cure

Cholera arose centuries ago in India and was disseminated throughout Asia and Europe by trade and pilgrimage. It was devastating, caus-

Genetics and Cholera

The disease occurs when cholera toxin binds to intestinal cells and stimulates the passage of water from the blood into the intestine. This water depletion and resultant cardiovascular collapse are major causes of cholera mortality. Study of the genetics and the biochemistry of cholera has shown that the toxin is a protein composed of portions called A and B subunits, each produced by a separate gene. When a bacterium secretes a molecule of cholera toxin, it

binds to a cell of the intestinal lining (an intestinal mucosa cell) via B subunits. Then the A subunits cause the mucosal cell to stimulate the secretion of water and salts from the blood to produce diarrhea. Lesser amounts of the watery mix are vomited and exacerbate dehydration.

The use of bacterial genetics to compare virulent *V. cholerae* and strains that did not cause the disease helped in the discovery of the nature of the cholera toxin and enabled production of vaccines against the protein. These vaccines are useful to those individuals who visit areas where cholera is endemic, ensuring that they do not become infected with it during

these travels. Unfortunately, the vaccines are only effective for about six months.

The basis for the operation of cholera toxin is production of a hormone substance called cyclic adenosine monophosphate (cAMP). The presence of excess cAMP in intestinal mucosa cells causes movement of water and other tissue components into the intestine and then out of the body. The accumulation of cAMP is caused by the ability of the cholera toxin to modify an enzyme protein, adenyl cyclase, to make it produce excess cAMP via modification of a control substance called a G-protein. This modification, called adenine ribosylation, is a mechanism similar to that causing diphtheria, another dan-

Cholera in Marine Plankton

Outbreaks of cholera can occur in nonendemic areas when an infected person travels to another country or when infected water is carried in the ballast of ships to another country. These two processes alone, however, could not explain all of the outbreaks of cholera observed worldwide. In the late 1960's, *Vibrio cholerae* was found in the ocean associated with marine plankton. This association, along with climate change, helps to explain the spread of cholera.

Plankton are the small organisms suspended in the ocean's upper layers. Plankton can be divided into two groups, phytoplankton (small plants) and zooplankton (small animals). *Vibrio cholerae* is found associated with the surface and gut of copepods, which are members of the zooplankton group. These small crustaceans act as a reservoir for the cholera bacteria, allowing them to survive in the ocean for long periods of time. Then, a change in weather that causes the ocean temperature to rise could also cause currents that stir up nutrients from lower layers of the ocean to the upper layers. Numbers of phytoplankton, which live in the upper layers of ocean waters, increase in these periods as a result of the warmer temperatures and greater availability of nutrients. Zooplankton numbers increase as well, because of the increase in main food source, the phytoplankton. Consequently, the number of cholera bacteria increase to a level that can cause the disease. Thus, climate change can result in an outbreak of cholera in a region where cholera is endemic, or, if currents move the plankton to other coastal areas, in a new, nonendemic region. This scenario is believed

to explain the 1991 cholera epidemic in Peru, when the oceanic oscillation known as El Niño caused a warming of ocean temperature.

Because of the association of *V. cholerae* with plankton, scientists believe they may be able to track or identify future epidemics by the use of satellite imagery. Increases in phytoplankton turn the ocean color from blue to green. Thus, changes in green areas in the ocean on satellite pictures show where the phytoplankton and, by association, zooplankton and cholera bacteria, are relocating or increasing in number.

The association of cholera with zooplankton has also helped reveal a new way to prevent the disease. People get cholera by ingesting several thousand cholera bacteria at one time. A single copepod can harbor ten thousand bacteria; therefore, the ingestion of one infected copepod can cause disease in a person. Researchers have found a simple and inexpensive way to reduce this risk from copepods dramatically. Filtering water through four layers of fabric used to make saris, which are commonly worn in regions plagued by cholera, removes 99 percent of copepods from water containing high levels of plankton.

Now that the entire genetic sequence of *V. cholerae* has been determined, scientists are armed with additional genetic data to elucidate the relationship of the bacterium with copepods, which may help them find more ways of controlling the spread of the disease.

—Vicki J. Isola

gerous disease that can be fatal, although in diphtheria other tissues and processes are affected.

Impact and Applications

Cholera has, for centuries, been a serious threat to humans throughout the world. During the twentieth century, its consequences to industrialized nations diminished significantly with the advent of sound sanitation practices that almost entirely prevented the entry of *V. cholerae* into the food and water supply. In poorer nations with less adequate sanitation, the disease flourishes and is still a severe threat.

It must be remembered that handling cholera occurs at three levels. The isolation and identification of cholera toxin, as well as development of current short-term cholera vaccines, were highly dependent on genetic methodology. Vaccine protects most travelers from the disease. However, wherever the disease afflicts individuals, its treatment depends solely upon rehydration and use of antibiotics. Finally, modern cholera prevention focuses solely on adequate sanitation. It is thus essential to produce a long-lasting vaccine for treatment of cholera to enable prolonged immunization at least at the ten-year level of tetanus shots. Efforts aimed at this goal are ongoing and utilize molecular genetics to define more clearly why long-term vaccination has so far been unsuccessful. Particularly useful will be fine genetic sequence analysis and the use of gene amplification followed by DNA fingerprinting.

—Sanford S. Singer

See also: Anthrax; Archaea; Bacterial Genetics and Cell Structure; Bacterial Resistance and Super Bacteria; Diphtheria; Emerging Diseases; Gene Regulation: Bacteria; Gene Regulation: *Lac Operon*; Transgenic Organisms.

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Web Site of Interest

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Chromatin Packaging

Field of study: Molecular genetics

Significance: *The huge quantity of DNA present in each cell must be organized and highly condensed in order to fit into the discrete units of genetic material known as chromosomes. Gene expression can be regulated by the nature and extent of this DNA packaging in the chromosome, and errors in the packaging process can lead to genetic disease.*

Key terms

CHROMATIN: the material that makes up chromosomes; a complex of fibers composed of DNA, histone proteins, and nonhistone proteins

HISTONE PROTEINS: small, basic proteins that are complexed with DNA in chromosomes and that are essential for chromosomal structure and chromatin packaging

NONHISTONE PROTEINS: a heterogeneous group of acidic or neutral proteins found in chromatin that may be involved with chromosome structure, chromatin packaging, or the control of gene expression

NUCLEOSOME: the basic structural unit of chromosomes, consisting of 146 base pairs of DNA wrapped around a core of eight histone proteins

Chromosomes and Chromatin

Scientists have known for many years that an organism's hereditary information is encrypted in molecules of DNA that are themselves organized into discrete hereditary units called genes and that these genes are organized into larger subcellular structures called chromosomes. James Watson and Francis Crick elucidated the basic chemical structure of the DNA molecule in 1953, and much has been learned since that time concerning its replication and expression. At the molecular level, DNA is composed of two parallel chains of building blocks called nucleotides, and these chains are coiled around a central axis to form the well-known "double helix." Each nucleotide on each chain attracts and pairs with a complementary nucleotide on the opposite chain, so a DNA molecule can be described as

consisting of a certain number of these nucleotide base pairs. The entire human genome consists of more than six billion base pairs of DNA, which, if completely unraveled, would extend for more than 2 meters (6.5 feet). It is a remarkable feat of engineering that in each human cell this much DNA is condensed, compacted, and tightly packaged into chromosomes within a nucleus that is less than 10^{-5} meters in diameter. What is even more astounding is the frequency and fidelity with which this DNA must be condensed and relaxed, packaged and unpackaged, for replication and expression in each individual cell at the appropriate time and place during both development and adult life. The essential processes of DNA replication or gene expression (transcription) cannot occur unless the DNA is in a more open or relaxed configuration.

Chemical analysis of mammalian chromosomes reveals that they consist of DNA and two distinct classes of proteins, known as histone and nonhistone proteins. This nucleoprotein complex is called chromatin, and each chromosome consists of one linear, unbroken, double-stranded DNA molecule that is surrounded in predictable ways by these histone and nonhistone proteins. The histones are relatively small, basic proteins (having a net positive charge), and their function is to bind directly to the negatively charged DNA molecule in the chromosome. Five major varieties of histone proteins are found in chromosomes, and these are known as H1, H2A, H2B, H3, and H4. Chromatin contains about equal amounts of histones and DNA, and the amount and proportion of histone proteins are constant from cell to cell in all higher organisms. In fact, the histones as a class are among the most highly conserved of all known proteins. For example, for histone H3, which is a protein consisting of 135 amino acid "building blocks," there is only a single amino acid difference in the protein found in sea urchins as compared with the one found in cattle. This is compelling evidence that histones play the same essential role in chromatin packaging in all higher organisms and that evolution has been quite intolerant of even minor sequence variations between vastly different species.

Nonhistones as a class of proteins are much more heterogeneous than the histones. They are usually acidic (carrying a net negative charge), so they will most readily attract and bind with the positively charged histones rather than the negatively charged DNA. Each cell has many different kinds of nonhistone proteins, some of which play a structural role in chromosome organization and some of which are more directly involved with the regulation of gene expression. Weight for weight, there is often as much nonhistone protein present in chromatin as histone protein and DNA combined.

Nucleosomes and Solenoids

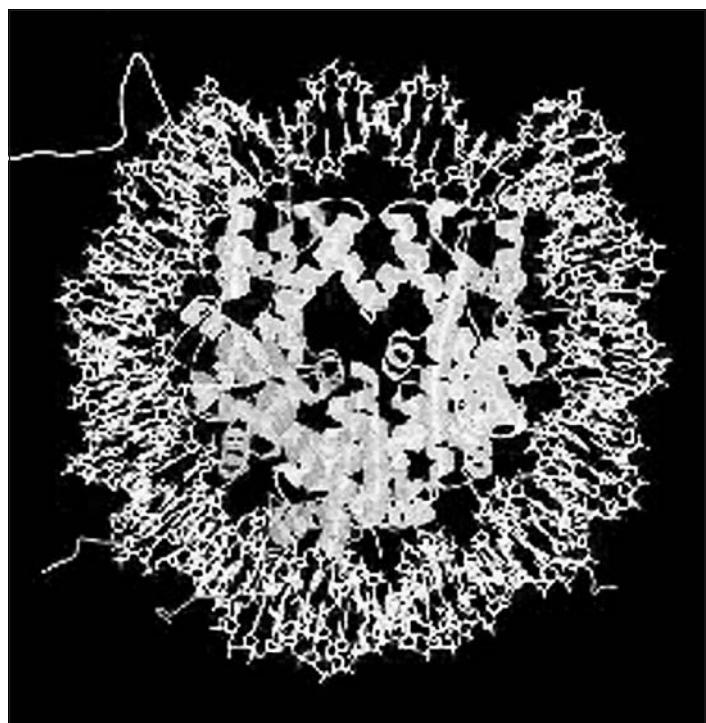
The fundamental structural subunit of chromatin is an association of DNA and histone proteins called a “nucleosome.” First discovered in the 1970’s by Ada and Donald Olins and Chris Woodcock, each nucleosome consists of a core of eight histone proteins: two each of the histones H2A, H2B, H3, and H4. Around this histone octamer is wound 146 base pairs of DNA in one and three-quarter turns (approximately eighty base pairs per turn). The overall shape of each nucleosome is similar to that of a lemon or a football. Each nucleosome is separated from its adjacent neighbor by about 55 base pairs of “linker DNA,” so that in its most unraveled state they appear under the electron microscope to be like tiny beads on a string. Portions of each core histone protein protrude outside of the wound DNA and interact with the DNA that links adjacent nucleosomes.

The next level of chromatin packaging involves a coiling and stacking of nucleosomes into a ribbon-like arrangement, which is twisted to form a chromatin fiber about 30 nanometers (nm) in diameter commonly called a “solenoid.” Formation of solenoid fibers requires the interaction of histone H1, which binds to the linker DNA between

nucleosomes. Each turn of the chromatin fiber contains about 1,200 base pairs (six nucleosomes), and the DNA has now been compacted by about a factor of fifty. The coiled solenoid fiber is organized into large domains of 40,000 to 100,000 base pairs, and these domains are separated by attached nonhistone proteins that serve to both organize and to control their packaging and unpackaging.

Long DNA Loops and the Chromosome Scaffold

Physical studies using the techniques of X-ray crystallography and neutron diffraction have suggested that solenoid fibers may be further organized into giant supercoiled loops. The extent of this additional looping, coiling, and stacking of solenoid fibers varies, depending on the cell cycle. The most relaxed and extended chromosomes are found at interphase, the period of time between cell divisions. Inter-



This image, captured through neutron crystallography, shows the molecular structure of the fundamental repeating unit of the chromosome, the nucleosome core complex: 146 base pairs of DNA wrapped around a core of eight histone proteins. (U.S. Department of Energy Genomes to Life Program, <http://doegenomestolife.org>)

phase chromosomes typically have a diameter of about 300 nm. Chromosomes that are getting ready to divide (metaphase chromosomes) have the most highly condensed chromatin, and these structures may have a diameter of up to 700 nm. One major study on the structure of metaphase chromosomes has shown that a skeleton of nonhistone proteins in the shape of the metaphase chromosome remains even after all of the histone proteins and the DNA have been removed by enzymatic digestion. If the DNA is not digested, it remains in long loops (10 to 90 kilobase pairs) anchored to this nonhistone protein scaffolding.

In the purest preparations of metaphase chromosomes, only two scaffold proteins are found. One of these forms the latticework of the scaffold, while the other has been identified as topoisomerase II, an enzyme that is critical in DNA replication. This enzyme cleaves double-stranded DNA and then rapidly reseals the cut after some of the supercoiling has been relaxed, thus relieving torsional stress and preventing tangles in the DNA. Apparently this same enzyme activity is necessary for the coiling and looping of solenoid fibers along the chromosome scaffold that occurs during the transition between interphase and metaphase chromosome structure. In the most highly condensed metaphase chromosomes, the DNA has been further compacted by an additional factor of one hundred.

Impact and Applications

Studies of chromatin packaging continue to reveal the details of the precise chromosomal architecture that results from the progressive coiling of the single DNA molecule into increasingly compact structures. Evidence suggests that the regulation of this coiling and packaging within the chromosome has a significant effect on the properties of the genes themselves. In fact, errors in DNA packaging can lead to inappropriate gene expression and developmental abnormalities. In humans, the blood disease thalassemia, several neuromuscular diseases, and even male sex determination can all be explained by the altered assembly of chromosomal structures.

Chromatin domains, composed of coiled so-

lenoid fibers, may contain several genes, or the boundary of a domain can lie within a gene. These domains have the capacity to influence gene expression, and this property is mediated by specific DNA sequences known as locus control regions (LCRs). An LCR is like a powerful enhancer that activates transcription, thereby turning on gene expression. The existence of such sequences was first recognized from a study of patients with beta-thalassemia and a related condition known as hereditary persistence of fetal hemoglobin. In these disorders, there is an error in the expression of a cluster of genes, known as the beta-globin genes, that prevents the appearance of adult type hemoglobin. The beta-globin genes are linearly arrayed over a 50-kilobase-pair chromatin domain, and the LCR is found upstream from this cluster. Affected patients were found to have normal beta-globin genes, but there was a deletion of the upstream LCR that led to failure to activate the genes appropriately. Further investigation led to the conclusion that the variation in expression of these genes observed in different patients was caused by differences in the assembly of the genes into higher-order chromatin structures. In some cases, gene expression was repressed, while in others it was facilitated. Under normal circumstances, a nonhistone protein complex was found to bind to the LCR, causing the chromatin domain to unravel and making the DNA more accessible to transcription factors, thus enhancing gene expression.

DNA sequencing studies have demonstrated a common feature in several genes whose altered expression leads to severe human genetic disease. For example, the gene that causes myotonic dystrophy has a large number of repeating nucleotide triplets in the DNA region immediately adjacent to the protein-encoding segment. Physical studies have shown that this results in the formation of unusually stable nucleosomes, since these repeated sequences create the strongest naturally occurring sites for association with the core histones. It has been suggested that these highly stable nucleosomes are unusually resistant to the unwinding and denaturation of the DNA that must occur in order for gene expression to begin. RNA polymerase is the enzyme that makes an RNA

transcript of the gene, and its movement through the protein-coding portion of the gene is inhibited if the DNA is unable to dissociate from the nucleosomes. Thus, although the necessary protein product would be normal and functional if it could be made, it is a problem with chromatin unpackaging that leads to reduced gene expression that ultimately leads to clinical symptoms of the disease. Both mild and severe forms of myotonic dystrophy are known, and an increase in the clinical severity correlates exactly with an increased number of nucleotide triplet repeats in the gene. Similar triplet repeats have been found in the genes responsible for Kennedy's disease, Huntington's disease (Huntington's chorea), spinocerebellar ataxia type I, fragile X syndrome, and dentatorubral-pallidoluysian atrophy.

Fascinating and unexpected recent research results have suggested that a central event in the determination of gender in mammals depends on local folding of DNA within the chromosome. Molecular biologists Peter Goodfellow and Robin Lovell-Badge successfully cloned a human gene from the Ychromosome that determines maleness. This *SRY* gene (named from the sex-determining region of the Ychromosome) encodes a protein that selectively recognizes a specific DNA sequence and helps assemble a chromatin complex that activates other male-specific genes. More specifically, binding of the *SRY* protein causes the DNA to bend at a specific angle and causes conformation that facilitates the assembly of a protein complex to initiate the cascade of gene activation leading to male development. If the bend is too tight or too wide, gene expression will not occur, and the embryo will develop as a female.

The unifying lesson to be learned from these examples of DNA packaging and disease is that DNA sequencing studies and the construction of human genetic maps will not by themselves provide all the answers to questions concerning human variation and genetic disease. An understanding of human genetics at the molecular level depends not only on the primary DNA sequence but also on the three-dimensional organization of that DNA within the chromosome. Compelling genetic and biochemical ev-

idence has left no doubt that the packaging process is an essential component of regulated gene expression.

—Jeffrey Knight

See also: Cell Division; Central Dogma of Molecular Biology; Chromosome Structure; Developmental Genetics; Fragile X Syndrome; Gene Regulation: Eukaryotes; Huntington's Disease; Mitosis and Meiosis; Molecular Genetics.

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in the early years of the twentieth century, geneticists who worked with it were the first to discover chromosome mutations. Calvin Bridges proposed deletions in 1917, duplications in 1919, and translocations in 1923 as explanations of phenomena he had observed in genetic experiments. Alfred Sturtevant proposed inversions in 1926 to explain experimental genetic data. Their proposals were directly confirmed as chromosome mutations when methods for microscopic examination of chromosomes were developed in the 1920's and 1930's.

Chromosome Mutation

Fields of study: Cellular biology; Molecular genetics

Significance: *Unlike gene mutations, which alter individual genes, chromosome mutations delete, duplicate, or rearrange chromosome segments. Chromosome mutations may create gene mutations if they delete genes or if the breakpoints of rearranged segments disrupt gene structure or alter gene expression. Even when they do not create gene mutations, chromosome mutations may reduce fertility and are an important cause of inherited infertility in humans. They also play important roles in the evolution of species.*

Key terms

DELETION: a missing chromosome segment

DUPLICATION: a chromosome segment repeated in the same or in a different chromosome

FISSION: separation of a single chromosome into two chromosomes

FUSION: joining of two chromosomes to become a single chromosome

INVERSION: a chromosome segment with reversed orientation when compared to the original chromosome structure

TRANSLOCATION: a chromosome segment transferred from one chromosome to a nonhomologous chromosome

Discovery

As the fruit fly *Drosophila melanogaster* became a premier organism for genetic research

Deletions

A deletion results when a chromosomal segment is lost. A deletion creates an imbalance in the genetic material because a relatively large segment of it is missing. Most deletions are lethal, even when heterozygous. Some small deletions persist in the heterozygous state but are usually lethal when homozygous. These small deletions are usually characterized by deleted portions of only one or two genes and behave genetically as recessive alleles when paired with a typical recessive allele of the affected gene.

Duplications

A duplication arises when a chromosomal segment is duplicated and inserted either into the same chromosome, as its parent segment, or into another chromosome. Duplications are present in most genomes. Genome projects (including the Human Genome Project) have revealed large duplicated segments containing multiple genes dispersed throughout the chromosomes in most species. Some duplications are repeated in tandem in the same chromosome and are subject to unequal crossing over, a process in which duplicated segments mispair with one another and a crossover takes place within the mispaired segment. Unequal crossing over increases the number of tandem duplications in one chromosome and decreases that number in the other.

Inversions

Two breaks within the same chromosome may liberate a chromosome segment. If the segment is reinserted into the same chromosome, but in reverse orientation, an inversion

results. Also, rare crossing over between duplicated segments in the same chromosome may produce an inversion. If a breakpoint of the inversion lies within a gene, it disrupts the gene, causing a gene mutation. Additionally, an inversion may place a gene in another location in the chromosome, removing the gene from its regulatory elements and altering its expression, a phenomenon known as the position effect.

When one chromosome carries an inversion and its homologous partner does not, the individual carrying these two chromosomes is said to be an inversion heterozygote. The two homologous chromosomes in an inversion heterozygote cannot pair properly in meiosis; one of them must form a loop in the inverted region. A crossover within the inversion loop results in chromosomes that carry large deletions and duplications. Because of the imbalance of chromosomal material created by the deletions and duplications, progeny resulting from such crossovers usually do not survive. In genetic experiments, crossing over appears to be suppressed within an inversion, whereas, in reality, crossing over does take place within the inversion but crossover-type progeny fail to survive. For this reason, inversion heterozygotes may suffer a reduction in fertility that is proportional to the size of the inversion. An individual who is homozygous for an inversion, however, suffers no loss of fertility, because the chromosomes pair normally.

Translocations

A break in a chromosome may liberate a chromosome fragment, which if reattached to a different chromosome is called a translocation. Most translocations are reciprocal: Two chromosome breaks, each in a different chromosome, liberate two fragments, and each fragment reattaches to the site where the other fragment was originally attached; in other words, the two fragments exchange places. If the breakpoint of a translocation is within a gene, a gene mutation may result. Also, a gene at or near the breakpoint may undergo a change in its expression because of position effect.

Translocations alter chromosome pairing in meiosis. During meiosis in a reciprocal trans-

location heterozygote, the two chromosomes with translocated segments pair with two other chromosomes without translocations. The pairing of these four chromosomes forms an X-shaped structure called a quadrivalent, so named because it contains four chromosomes paired with one another, instead of the usual two. Depending on the orientation of the quadrivalent during meiosis, some gametes may receive a balanced complement of chromosomes and others an unbalanced complement with large duplications and deletions. Typically, about half of all gametes in a reciprocal translocation heterozygote carry an unbalanced chromosome complement, a situation that significantly reduces the individual's fertility. However, translocation homozygotes suffer no loss of fertility, because the chromosomes pair normally with no quadrivalent.

Fusions

Very rarely, two chromosomes may fuse with one another to form a single chromosome. Chromosomes with centromeres at or very near the ends of the chromosomes may undergo breakage at the centromeres and fuse with each other in the centromeric region, resulting in a single chromosome with the long arms of the original chromosomes on either side of the fused centromere. Such a chromosome fusion is called a Robertsonian translocation. In other cases, two chromosomes may fuse with one another producing a dicentric chromosome (a chromosome with two centromeres). For the fused chromosome to persist, one of the centromeres ceases to function, leaving the other centromere as a single, functional centromere for the fused chromosome.

Fissions

A chromosome break produces two fragments, which may function as individual chromosomes if each has telomeres on both ends and a functional centromere. Typically, chromosome breakage produces one fragment with a telomere on one end and a centromere, and another fragment with a telomere on one end and no centromere. For both fragments to function as chromosomes, one must acquire a telomere and the other a centromere and a

telomere. These events are highly unlikely, so fissions are rarer than fusions. However, complex translocations with other chromosomes may rarely produce functional chromosomes from a fission event, and cases of functional chromosomes arising from fissions have been documented.

Impact on Human Genetics and Medicine

Chromosome mutations are responsible for several human genetic disorders. For example, about 20 percent of hemophilia A cases result from a gene mutation caused by an inversion with a breakpoint in the *F8C* gene, which encodes blood clotting factor VIII. Cri du chat syndrome, a severe disorder characterized by severe mental retardation and distinctive physical features, is usually caused by deletion of a small chromosomal region near the end of chromosome 5. A few cases of this syndrome are associated with deletions that result from a translocation with a breakpoint near the end of chromosome 5 or crossovers within a small inversion in that chromosome region. Robertsonian translocations that fuse the long arm of chromosome 21 with the long arm of another chromosome (usually chromosome 14) are responsible for some inherited cases of Down syndrome. A reciprocal translocation between chromosomes 9 and 22, called the Philadelphia chromosome, causes increased susceptibility to certain types of cancer by altering the expression of a gene located at the breakpoint of the translocation. Other translocations are likewise associated with certain cancers. Chromosome mutations may also cause infertility in humans. Reciprocal translocations are especially notorious, although certain inversions are also associated with infertility.

Implications for Evolution

Heterozygous carriers of inversions, translocations, fusions, and fissions often suffer losses of fertility, but homozogotes do not. Thus, natural selection may disfavor heterozygotes while favoring homozygotes either for the original chromosome structure or for the mutation. Accumulation of different chromosome mutations in isolated populations of a species may eventually differentiate the chromosomes to

such a degree that the isolated populations diverge into separate species. Their members can no longer produce fertile offspring when hybridized with members of another population because the chromosomes cannot properly pair with one another. Indeed, accumulated chromosome mutations are often evident when geneticists compare the chromosomes of closely related species. For example, the chromosomes of different *Drosophila* species are differentiated mostly by translocations and fusions. Comparison of human, chimpanzee, gorilla, and orangutan chromosomes reveals numerous inversions that distinguish the chromosomes of these species. One of the most striking cases of chromosome evolution is the origin of human chromosome 2. This chromosome matches two separate chromosomes in the great apes and apparently arose from a fusion of these two chromosomes after the divergence of the human and chimpanzee lineages. The presence in human chromosome 2 of DNA sequences corresponding to a nonfunctional centromere and telomere at sites corresponding to these structures in the great ape chromosomes is strong evidence of a chromosome fusion during evolution of the human lineage.

—Daniel J. Fairbanks

See also: Cell Cycle, The; Cell Division; Central Dogma of Molecular Biology; Chemical Mutagens; Chromosome Structure; Chromosome Theory of Heredity; Congenital Disorders; Cystic Fibrosis; Down Syndrome; Epistasis; Evolutionary Biology; Hemophilia; Hereditary Diseases; Huntington's Disease; Inborn Errors of Metabolism; Infertility; Mitosis and Meiosis; Molecular Genetics; Mutation and Mutagenesis; Punctuated Equilibrium.

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Chromosome Structure

Fields of study: Cellular biology; Classical transmission genetics

Significance: *The separation of the alleles in the production of the reproductive cells is a central feature of the model of inheritance. The realization that the genes are located on chromosomes and that chromosomes occur as pairs that separate during meiosis provides the physical explanation for the basic model of inheritance. When chromosome structure is modified, changes in information transmission produce abnormal developmental conditions, most of which contribute to early miscarriages and spontaneous abortions.*

Key terms

HISTONES: a class of proteins associated with DNA

HOMOLOGOUS CHROMOSOMES: chromosomes that have identical physical structure and contain the same genes; humans have twenty-two pairs of homologous chromosomes and a pair of sex chromosomes that are only partially homologous

KARYOTYPING: an analysis or physical description of all the chromosomes found in an organism's cells; often includes either a drawing or photograph of the chromosomes

SPINDLE FIBERS: minute fibers composed of the protein tubulin that are involved in distributing the chromosomes during cell division

Discovery of Chromosomes' Role in Inheritance

The development of the microscope made it possible to study what became recognized as the central unit of living organisms, the cell. One of the most obvious structures within the cell is the nucleus. As study continued, dyes were used to stain cell structures to make them more visible. It became possible to see colored

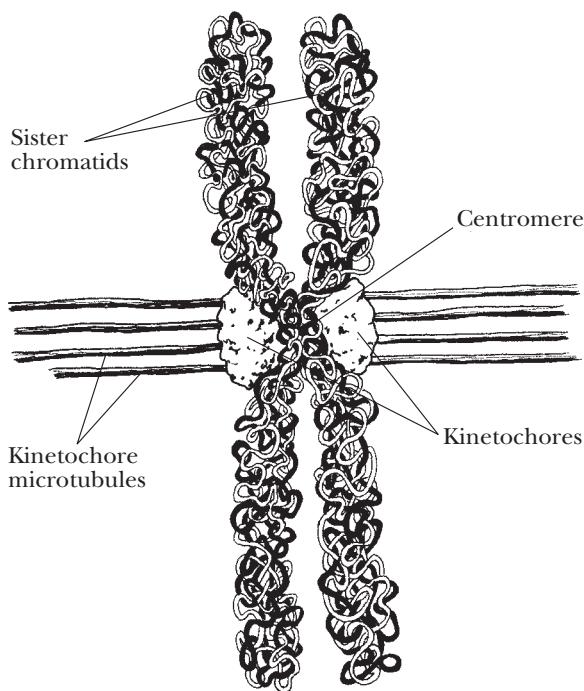
structures called chromosomes ("color bodies") within the nucleus that became visible when they condensed as the cell prepared to divide.

The association of the condensed, visible state of chromosomes with cell division caused investigators to speculate that the chromosomes played a role in the transmission of information. Chromosome counts made before and after cell division showed that the chromosome number remained constant from generation to generation. When it was observed that the nuclei of two cells (the egg and the sperm) fused during sexual reproduction, the association between information transport and chromosome composition was further strengthened. German biologist August Weismann, noting that the chromosome number remained constant from generation to generation despite the fusing of cells, predicted that there must be a cell division that reduced the chromosome number in the egg and sperm cells. The reductional division, meiosis, was described in 1900.

Following the rediscovery of Gregor Mendel's rules of inheritance in 1900, the work of Theodor Boveri and Walter Sutton led to the 1903 proposal that the character-determining factors (genes) proposed by Mendel were located on the chromosomes and that the factor segregation that was a central part of the model occurred because the like chromosomes of each pair separated during the reductional division that occurs in meiosis. This hypothesis, the "chromosome theory of heredity," was confirmed in 1916 by the observations of the unusual behavior of chromosomes and the determining factors located on them by Calvin Bridges.

Chromosome Structure and Relation to Inheritance

With the discovery of the nucleic acids came speculation about the roles of DNA and the associated proteins. During the early 1900's, it was generally accepted that DNA formed a structural support system to hold critical information-carrying proteins on the chromosomes. The identification of the structure of DNA in 1953 by American biologist James Watson and English physicist Francis Crick and the recogni-

External Structure of a Chromosome

(Kimberly L. Dawson Kurnizki)

tion that DNA, not the proteins, contained the genetic information led to study of chromosome structure and the relationships of the DNA and protein components.

It is now recognized that each chromosome contains one DNA molecule. Each plant and animal species has a specific number of chromosomes. Humans have twenty-three kinds of chromosomes, present as twenty-three pairs. Each chromosome can be recognized by its overall length and the position of constrictions, called centromeres, that are visible only when the cell is reproducing. At all other stages of the cell's life, the chromosome material is diffuse and is seen only as a general color within the nucleus. When the cell prepares for division, the fibrous DNA molecule tightly coils and condenses into the visible structures. Since there must be information for the two cells that result from the process of division, the chromosomes are present in a duplicated condition when they first become visible.

A major feature of the visible, copied chromosomes is the centromere. This constriction may be located anywhere along the chromosome, so its position is useful for identifying chromosomes. In karyotyping, the standard system used to identify human chromosomes, the numbering begins with the longest chromosome with the constriction nearest the center (chromosome 1) and are referred to as having a metacentric centromere placement. Chromosomes with nearly the same length but with the centromere constriction removed from the center position have higher numbers (chromosomes 2 and 3) and are referred to as acrocentric. Shorter chromosomes with a centromere near the middle are next, and the numbering proceeds based on the distance the centromere is removed from the central position. Short chromosomes with a centromere near one end have the highest numbers and are referred to as telocentric.

Most of the chromosomes have a centromere that is not centrally located, which results in arms of unequal length.

The short arm is referred to as "petite" and is designated the *p* arm. The long arm is designated the *q* arm. This nomenclature is useful for referring to features of the chromosome. For example, when a portion of the long arm of chromosome 15 has been lost, the arm is shorter than normal. The loss, a deletion, is designated $15q^-$ (chromosome 15, long arm, deletion). Prader-Willi syndrome, in which an infant has poor sucking ability and poor growth, and later becomes a compulsive eater, results from this deletion. Cri du chat ("cry of the cat") syndrome results from $5p^-$, a deletion of a portion of the short arm of chromosome 5. The cry of these individuals is like that of a cat, and they are severely mentally retarded and have numerous physical defects.

Some chromosomes have additional constrictions referred to as secondary constrictions. The primary centromere constrictions are located where the spindle fibers attach to the chromosomes to move them to the appropriate poles during cell division. The second-

ary constrictions are sites of specific gene activity. Both of these regions contain DNA base sequence information that is specific to their functions.

Histones

The DNA of the chromosomes is wound around special proteins called histones. This results in an orderly structure that condenses the DNA mass so that the bulky DNA does not require as much storage space. The wrapped DNA units then fold into additional levels of compaction, by means of a process called condensation. The exact processes involved in these higher levels of folding are not fully understood, but the overall condensation reduces the bulk of the DNA nearly one thousandfold. If the DNA is removed from a condensed chromosome, the proteins remain and have nearly the same shape as the chromosome, indicating that it is the proteins that form the chromosome shape. The presence of these proteins and the fact that the DNA is wrapped around them raises many questions about how the DNA is copied in preparation for cell division and how the DNA information is read for gene activity. These are areas of active research.

The histone proteins form a structure called a “nucleosome” (“nuclear body”). There are four kinds of histones, and two of each kind join together to form a cylinder-shaped nucleosome structure. The fibrous DNA molecule wraps around each nucleosome approximately two and one-half times with a sequence of unwound DNA between each nucleosome along the entire length of the DNA molecule. The structure, called chromatin, looks like a string of beads when isolated sections are viewed with an electron microscope. When the chromatin is digested with enzymes that break the DNA backbone in the unwound regions, repeated lengths of chromatin are recovered, showing that the nucleosome wrapping is very regular. These nucleosome regions join together to form the additional folding as the chromosome condenses when the cell prepares for division.

In addition to the histone proteins, nonhistone proteins attach to the chromatin. With an electron microscope, chromatin loops can be seen extending from a protein matrix. There is

evidence that these loops represent replication units along the chromosome, but how the DNA molecule is freed from the histone proteins to be replicated is a major unsolved puzzle.

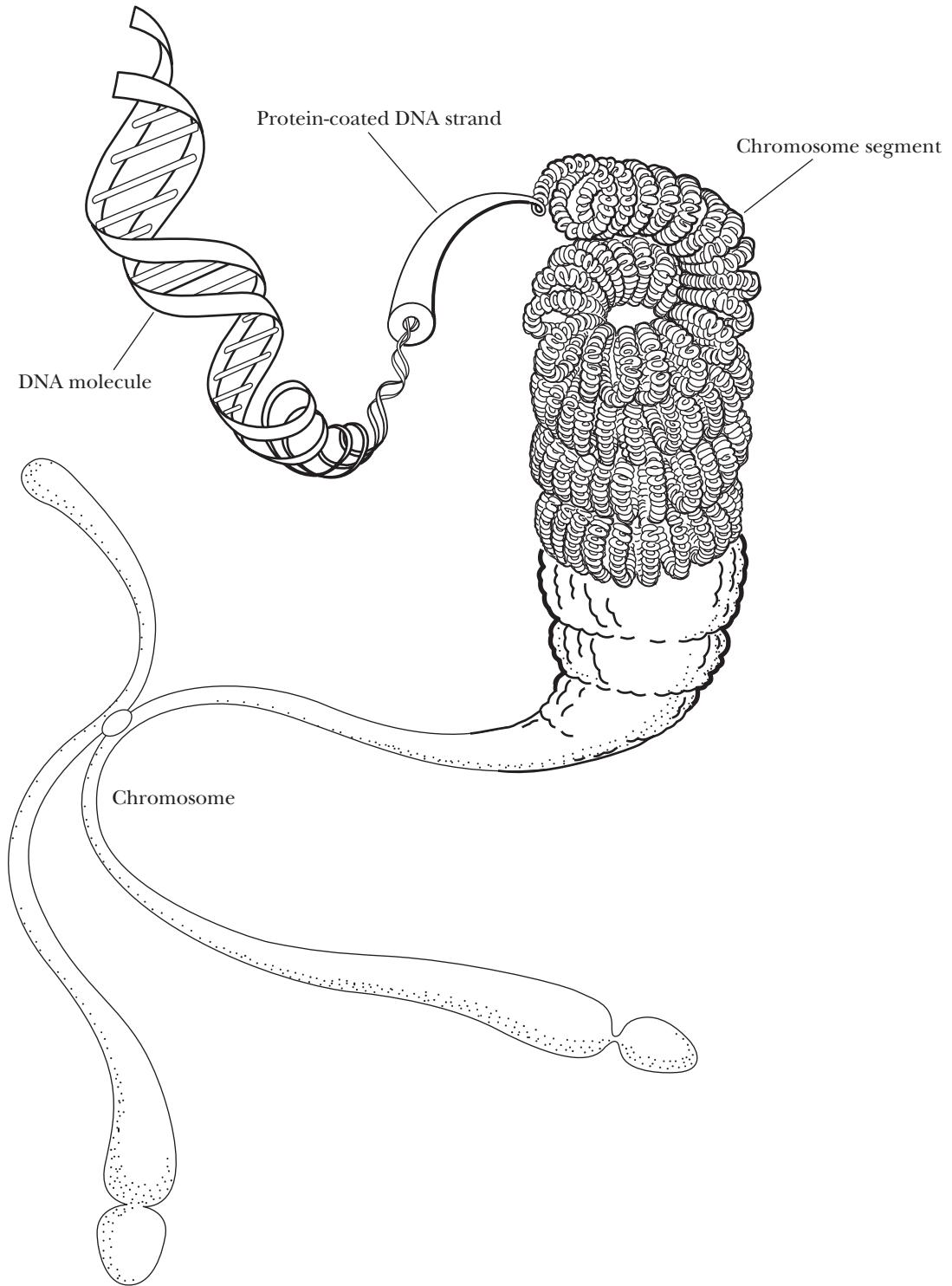
The condensation of the chromatin is not uniform over the entire chromosome. In the regions immediately adjacent to the centromere, the chromatin is tightly condensed and remains that way throughout the visible cycle. All of the available evidence indicates that this chromatin does not contain actively expressed genes. It also replicates later than the remaining DNA. This more highly condensed chromatin is called heterochromatin (“the other chromatin”). The remaining chromatin is referred to as euchromatin (“true chromatin”) because it contains actively expressed genes and it replicates as a unit.

Giemsia Stain and Chromosome Painting

When chromosomes are treated with a dye called Giemsa stain, regular banding patterns appear. The bands vary in width, but their positions on the individual chromosomes are consistent. This makes the bands useful in identifying specific chromosome regions. When a chromosome has a structural modification, such as an inversion—which results when two breaks occur and the region is reversed when the fragments are rejoined—the change in the banding pattern makes it possible to recognize where it has occurred. When a loss of a chromosomal region produces a deficiency disorder, changes in the banding patterns of a chromosome can identify the missing region. Karyotype analysis is a useful tool in genetic counseling because disorders caused by chromosome structure modifications can be identified. Associations between disorders and missing chromosome regions are useful in identifying which functions are associated with specific regions. Other stains produce different banding patterns and, when used in combination with the Giemsa banding patterns, allow diagnosis of structure modifications that can be quite complex.

It is also possible to use fluorescent dyes, in a process called chromosome painting, to identify the DNA of individual chromosomes, which allows the recognition of small regions

Internal Structure of a Chromosome



that have been exchanged between chromosomes that are too small to be recognized otherwise. Color differences within chromosomes or at their tips clearly show which chromosomes have exchanged DNA, how much DNA each has been exchanged, and where on the chromosomes the exchanges have taken place. Many cancer cells, for example, have multiple chromosome modifications, with DNA from two or three chromosomes associated in one highly modified chromosome structure.

Chromosome Disorders

At the ends of the chromosomes are structures called telomeres, which are composed of specific repetitive DNA sequences that help protect the ends of chromosomes from damage and prevent DNA molecules from sticking together. Research that began in the early 1990's led to the discovery that the telomere regions of the chromosomes are shortened at each DNA replication. When the telomeres have been reduced to some critical point, the cell is no longer able to divide and often dies not long after, a phenomenon called apoptosis. Other observations indicate that the telomere is returned to its normal length in tumor cells, suggesting that this might contribute to the long life of tumor cells, possibly making them immortal. The relationship of cell age to telomere length and the mechanisms that lead to telomere shortening are not understood clearly, but this is an area of active research because it has implications for aging and cancer treatment.

The DNA of each chromosome carries a unique part of the information code in the sequence of the bases. The specific sequences are in linear order along the chromosome and form linked sequences of genes called linkage groups. When the like chromosomes pair and separate during meiosis, one copy of each chromosome is transmitted to the offspring. During meiosis, there may be an exchange of material between the paired chromosomes, but this does not change the information content because the information is basically the same for both chromosomes in any region. There may be differences in the coding sequences, but functionally the same informational content is

transmitted. Extreme changes in chromosome structure that result in the moving of information to another chromosome may have consequences for how specific information is expressed; a change in position might result in different regulation or in changes in how the information is transmitted during meiosis.

Each chromosome has a specific arrangement of genes. Although homologous chromosomes exchange DNA during meiosis, as long as this process occurs normally, the gene arrangement on the chromosomes remains unchanged. Position affects result when genes are moved to different regions of the same chromosome or to another chromosome. A normal allele may show a mutant phenotype expression in a new position in the chromosome set. The best-known case occurs when a gene is placed adjacent to a heterochromatic region. The relocated DNA is condensed like the heterochromatic-region DNA and normally active genes now remain inactive. Ninety percent of patients with the disorder chronic myelogenous leukemia have an exchange of material called a translocation, between chromosomes 9 and 22. Chromosome 22 is shorter than normal and is called the Philadelphia chromosome, after the city in which it was discovered. The placing of a specific gene from chromosome 9 within the broken region adjacent to a gene on chromosome 22 causes the uncontrolled expression of both of the genes and uncontrolled cell reproduction, the hallmark of leukemia.

The separation of like chromosomes during meiosis occurs because the two chromosome arms are attached to a specific centromere. When the centromere is moved to one of the poles, the arms are pulled along, ensuring movement of all of the material of the paired chromosomes to the opposite poles and inclusion in the newly formed cells. Translocations occur when chromosomes are broken and material is placed in the wrong position by the repair system, causing a chromosome region to become attached to a different centromere. This leads to an inability to properly separate the regions of the arm, which can result in duplication of some of the chromosomal regions (when two copies of the same arm move to one

cell) or deficiencies (when none of the material from a chromosome arm moves into a cell). This is a common outcome with translocation heterozygotes (individuals with both normal chromosomes and translocated chromosomes in the same cells). Pairing of like chromosome regions occurs, but rather than two chromosomes paired along their entire lengths, the arms of the two translocated chromosomes are paired with the arms of their normal pairing partners. The separation of the chromosomes produces duplications of material from one chromosome arm or a deficiency of that material 50 percent of the time. If these cells are involved in fertilization, the offspring will show duplication or deficiency disorders.

—D. B. Benner, updated by Bryan Ness

See also: Chromatin Packaging; Cell Cycle, The; Cell Division; Central Dogma of Molecular Biology; Chromosome Mutation; Chromosome Theory of Heredity; Chromosome Walking and Jumping; Classical Transmission Genetics; Dihybrid Inheritance; DNA Replication; DNA Structure and Function; Epistasis; Extrachromosomal Inheritance; Incomplete Dominance; Mendelian Genetics; Mitosis and Meiosis; Molecular Genetics; Monohybrid Inheritance; Multiple Alleles; Mutation and Mutagenesis; Non-disjunction and Aneuploidy; Parthenogenesis; Penetrance; Polygenic Inheritance; RNA Structure and Function; Transposable Elements.

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Chromosome Theory of Heredity

Field of study: Classical transmission genetics; History of genetics

Significance: *The chromosome theory of heredity originated with American geneticist Walter Sutton, who first suggested that genes were located on chromosomes. This theory guided much of genetic research in the early twentieth century, including development of the earliest genetic maps based on linkage. In 1931, several experiments confirmed the chromosome theory by demonstrating that certain rearrangements of the heritable traits (or genes) were always accompanied by corresponding rearrangements of the microscopically observable chromosomes.*

Key terms

CROSSING OVER: the breakage of chromosomes followed by the interchange of the resulting fragments; also, the recombination of genes that results from the chromosomal rearrangement

GENETIC MAPPING: the locating of gene positions along chromosomes

INDEPENDENT ASSORTMENT: the inheritance of genes independently of one another when they are located on separate chromosomes

LINKAGE: the frequent inheritance of two or more genes together as a unit if they are located close together on the same chromosome

LINKAGE MAPPING: a form of genetic mapping that uses recombination frequencies to estimate the relative distances between linked genes

PHYSICAL MAPPING: a form of genetic mapping that associates a gene with a microscopically observable chromosome location

Mendel's Law of Independent Assortment

In a series of experiments first reported in 1865, Austrian botanist Gregor Mendel established the first principles of genetics. Mendel showed that the units of heredity were inherited as particles that maintained their identity across the generations; these units of heredity are now known as genes. These genes exist as pairs in all the body's cells except for the egg and sperm cells. When Mendel studied two traits at a time (dihybrid inheritance), he discovered that different genes were inherited independently of one another, a principle that came to be called the law of independent assortment. For example, if an individual inherits genes *A* and *B* from one parent and genes *a* and *b* from the other parent, in subsequent generations the combinations *AB*, *Ab*, *ab*, and *ab* would all occur with equal frequency. Gene *A* would go together with *B* just as often as with *b*, and gene *B* would go with *A* just as often as with *a*. Mendel's results were ignored for many years after he published his findings, but his principles were rediscovered in 1900 by Erich Tschermak von Seysenegg in Vienna, Austria, Carl Erich Correns in Tübingen, Germany, and Hugo de Vries in Amsterdam, Holland. Organized research in genetics soon began in various countries in Europe and also in the United States.

Sutton's Hypothesis

Mendel's findings had left certain important questions unanswered: Why do the genes exist

in pairs? Why do different genes assort independently? Where are the genes located? Answers to these questions were first suggested in 1903 by a young American scientist, Walter Sutton, who had read about the rediscovery of Mendel's work. By this time, it was already well known that all animal and plant cells contain a central portion called the nucleus and a surrounding portion called the cytoplasm. Division of the cytoplasm is a very simple affair: The cytoplasm simply squeezes in two. The nucleus, however, undergoes mitosis, a complex rearrangement of the rod-shaped bodies called chromosomes, which exist in pairs. Sex cells (eggs or sperm) are "haploid," with one chromosome from each pair. All other body cells, called somatic cells, have a "diploid" chromosome number in which all chromosomes are paired. During mitosis, each chromosome becomes duplicated; then the two strands (or chromatids) split apart and separate. One result of mitosis is that the chromosome number of each cell is always preserved. Sutton also noticed that eggs in most species are many times larger than sperm because of a great difference in the amount of cytoplasm. The nuclei of egg and sperm are approximately equal in size, and these nuclei fuse during fertilization, a process in which two haploid sets of chromosomes combine to make a complete diploid set. From these facts, Sutton concluded that the genes are probably in the nucleus, not the cytoplasm, because the nucleus divides carefully and exactly while the cytoplasm divides inexactly. Also, if genes were in the cytoplasm, one would expect the mother's contribution to be much greater than the father's, contrary to the repeated observation that the parental contributions to heredity are usually equal.

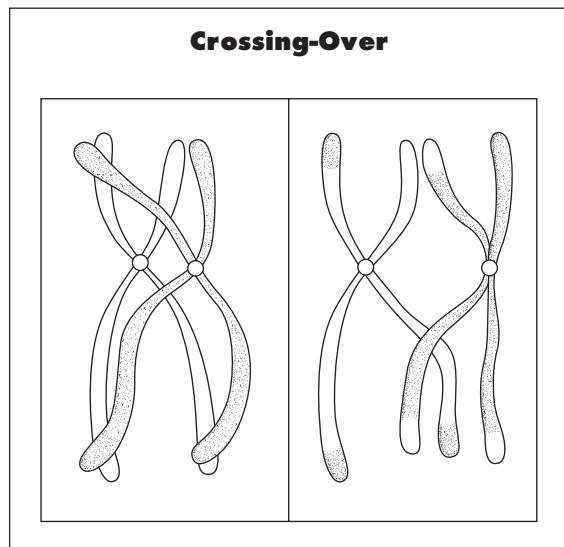
Of all the parts of diploid cells, only the chromosomes were known to exist in pairs. If genes were located on the chromosomes, it would explain why they existed in pairs (except singly in eggs and sperm cells). In fact, the known behavior of chromosomes exactly paralleled the postulated behavior of Mendel's genes. Sutton's hypothesis that genes were located on chromosomes came to be called the chromosome theory of heredity. According to Sutton's hypothesis, Mendel's genes assorted indepen-

dently because they were located on different chromosomes. However, there were only a limited number of chromosomes (eight in fruit flies, fourteen in garden peas, and forty-six in humans), while there were hundreds or thousands of genes. Sutton therefore predicted that Mendel's law of independent assortment would only apply to genes located on different chromosomes. Genes located on the same chromosome would be inherited together as a unit, a phenomenon now known as linkage.

In 1903, Sutton outlined his chromosomal theory of heredity in a paper entitled "The Chromosomes in Heredity." Many aspects of this theory were independently proposed by Theodor Boveri, a German researcher who had worked with sea urchin embryos at the Naples Marine Station in Italy.

Linkage and Crossing Over

Sutton had predicted the existence of linked genes before other investigators had adequately described the phenomenon. The subsequent discovery of linked genes lent strong support to Sutton's hypothesis. English geneticists William Bateson and Reginald C. Punnett described crosses involving linked genes in both poultry and garden peas, while American geneticist Thomas Hunt Morgan made similar discoveries in the fruit fly (*Drosophila melanogaster*). Instead of assorting independently, linked genes most often remain in the same combinations in which they were transmitted from prior generations: If two genes on the same chromosome both come from one parent, they tend to stay together through several generations and to be inherited as a unit. On occasion, these combinations of linked genes do break apart, and these rearrangements were attributed to "crossing over" of the chromosomes, a phenomenon in which chromosomes were thought to break apart and then recombine. Some microscopists thought they had observed X-shaped arrangements of the chromosomes that looked like the result of crossing over, but many other scientists were skeptical about this claim because there was no proof of breakage and recombination of the chromosomes in these X-shaped arrangements.



In the crossing-over process, chromosomes meet (left) and recombine (right). (Electronic Illustrators Group)

Genetic Mapping

Sutton had been a student of Thomas Hunt Morgan at Columbia University in New York City. When Morgan began his experiments with fruit flies around 1909, he quickly became convinced that Sutton's chromosome theory would lead to a fruitful line of research. Morgan and his students soon discovered many new mutations in fruit flies, representing many new genes. Some of these mutations were linked to one another, and the linked genes fell into four linkage groups corresponding to the four chromosome pairs of fruit flies. In fruit flies as well as other species, the number of linkage groups always corresponds to the number of chromosome pairs.

One of Morgan's students, Alfred H. Sturtevant, reasoned that the frequency of recombination of linked genes should be small for genes located close together and higher for genes located far apart. In fact, the frequency of crossing over between linked genes could serve as a rough measure of the distance between them along the chromosome. Sturtevant assumed that the frequency of recombination would be roughly proportional to the distance along the chromosome; recombination between closely linked genes would be a rare event, while recombination between genes fur-

ther apart would be more common. Sturtevant first used this technique in 1913 to determine the relative positions of six genes on one of the chromosomes of *Drosophila*. For example, the genes for white eyes and vermillion eyes recombined about 30 percent of the time, and the genes for vermillion eyes and miniature wings recombined about 3 percent of the time. Recombination between white eyes and miniature wings took place 34 percent of the time, close to the sum of the two previously mentioned frequencies (30 percent plus 3 percent). Therefore, the order of arrangement of the genes was:

$$\text{white} \leftarrow 30 \text{ units} \rightarrow \text{vermillion} \leftarrow 3 \text{ units} \rightarrow \text{miniature}$$

Since the distances were approximately additive (the smaller distances added up to the larger distances), Sturtevant concluded that the genes were arranged along each chromosome in a straight line like beads on a string. In all, Sturtevant was able to determine such a linear arrangement among six genes in his initial study (an outgrowth of his doctoral thesis) and many more genes subsequently. Calvin Bridges, another one of Morgan's students, worked closely with Sturtevant. Over the next several years, Sturtevant and Bridges conducted numerous genetic crosses involving linked genes. They used recombination frequencies to determine the arrangement of genes along chromosomes and the approximate distances between these genes, thus producing increasingly detailed genetic maps of several *Drosophila* species.

The use of Sturtevant's technique of making linkage maps was widely copied. As each new gene was discovered, geneticists were able to find another gene to which it was linked, and the new gene was then fitted into a genetic map based on its linkage distance to other genes. In this way, geneticists began to make linkage maps of genes along the chromosomes of many different species. There are now more than one thousand genes in *Drosophila* whose locations have been mapped using linkage mapping. Extensive linkage maps have also been developed for mice (*Mus musculus*), humans (*Homo sapiens*), corn or maize (*Zea mays*), and bread mold

(*Neurospora crassa*). In bacteria such as *Escherichia coli*, other methods of genetic mapping were developed based on the order in which genes were transferred during bacterial conjugation. These mapping techniques reveal that the genes in bacteria are arranged in a circle or, more precisely, in a closed loop resembling a necklace. This loop can break at any of several locations, after which the genes are transferred from one individual to another in the order of their location along the chromosome. The order can be determined by interrupting the process and testing to see which genes had been transferred before the interruption.

Confirmation of the Chromosome Theory

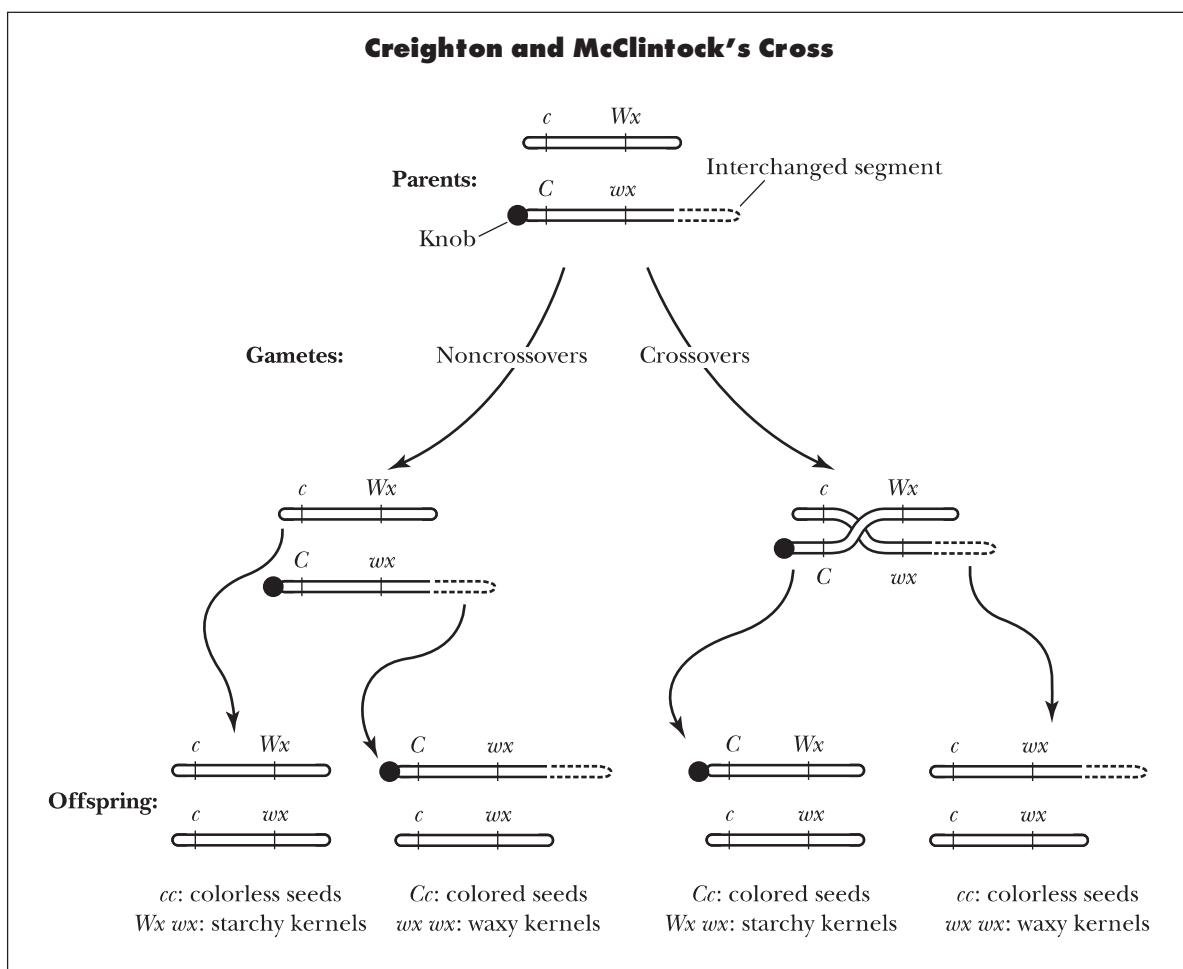
The first confirmation of the chromosome theory was published in 1916 by Bridges, who studied the results of a type of abnormal cell division. When egg or sperm cells are produced by meiosis, only one chromosome of each chromosome pair is normally included in each of the resultant cells. In a very small proportion of cases, one pair of chromosomes fails to separate (or "disjoin"), so that one of the resultant cells has an extra chromosome while the other cell is missing that chromosome. This abnormal type of meiosis is called nondisjunction. In fruit flies, as in humans and many other species, females normally have two X chromosomes (XX) and males have two unequal chromosomes (XY). Bridges discovered some female fruit flies that had the unusual chromosome formula XXY; he suspected that these unusual females had originated from nondisjunction, in which two X chromosomes had failed to separate during meiosis. Bridges studied one cross using a white-eyed XXY female mated to a normal, red-eyed male. (The gene for white eyes was known to be sex-linked; it was carried on the X chromosome.) Bridges was able to predict both the genetic and chromosomal anomalies that would occur as a result of this cross. Among the unusual predictions that were verified experimentally was the existence of a chromosome configuration (XYY) that had never been observed before. Using the assumption that the gene for white eyes was carried on the X chromosome in this and other crosses, Bridges was able to make unusual pre-

dictions of both genetic and chromosomal results. These studies greatly strengthened the case for the chromosomal theory.

In 1931, Harriet Creighton and Barbara McClintock were able to confirm the chromosomal theory of inheritance much more directly. Creighton and McClintock used corn plants whose chromosomes had structural abnormalities on either end, enabling them to recognize the chromosomes under the microscope. One chromosome, for example, had a knob at one end and an attached portion of another chromosome at the other end, as shown in the figure headed “Creighton and McClintock’s Cross.” Creighton and McClintock then crossed plants differing in two genes located along this chromosome. One gene controlled

the color of the seed coat while the other produced either a starchy or waxy kernel. The parental gene combinations (*C* with *wx* on the abnormal chromosome and *c* with *Wx* on the other chromosome) were always preserved in noncrossovers. However, a crossover between the two genes produced two new gene combinations: *C* with *Wx* and *c* with *wx*.

In this cross, Creighton and McClintock observed that the chromosomal appearance in the offspring could always be predicted from the phenotypic appearance: Seeds with colorless seed coats and starchy kernels had normal chromosomes, seeds with colored seed coats and waxy kernels had chromosomes with the knob at one end and the extra interchanged chromosome segment at the other end, seeds



with colorless seed coats and waxy kernels had the interchanged segment but no knob, and seeds with colored coats and starchy kernels had the knob but not the interchanged segment. In other words, whenever the two genes showed rearrangement of the parental combinations, a corresponding switch of the chromosomes could be observed under the microscope. The interchange of chromosome segments was always accompanied by the recombination of genes, or, in the words of the original paper,

cytological crossing-over . . . is accompanied by the expected types of genetic crossing-over. . . . Chromosomes . . . have been shown to exchange parts at the same time they exchange genes assigned to these regions.

In short, genetic recombination (the rearranging of genes) was always accompanied by crossing-over (the rearranging of chromosomes). This historic finding established firm evidence for the chromosomal theory of heredity. Later that same year, Curt Stern published a paper describing a very similar experiment using fruit flies.

Physical Mapping and Further Confirmation

Other evidence that helped confirm the chromosome theory came from the study of rare chromosome abnormalities. In 1933, Thomas S. Painter called attention to the large salivary gland chromosomes of *Drosophila*. Examination of these large chromosomes made structural abnormalities in the chromosomes easier to identify. When small segments of a chromosome were missing, a gene was often found to be missing also. These abnormalities, called chromosomal deletions, allowed the first physical maps of genes to be drawn. In all cases, the physical maps were found to be consistent with the earlier genetic maps (or linkage maps) based on the frequency of crossing over.

When Bridges turned his attention to the “bar eyes” trait in fruit flies, he discovered that the gene for this trait was actually another kind of chromosome abnormality called a “duplication.” Again, a chromosome abnormality that could be seen under the microscope could be

related to a genetic map based on linkage. Larger chromosome abnormalities included “inversions,” in which a segment of a chromosome was turned end-to-end, and “translocations,” in which a piece of one chromosome became attached to another. There were also abnormalities in which entire chromosomes were missing or extra chromosomes were present. Each of these chromosomal abnormalities was accompanied by corresponding changes in the genetic maps based on the frequency of recombination between linked genes. In cases in which the location of a chromosomal abnormality could be identified microscopically, this permitted an anchoring of the genetic map to a physical location along the chromosome. The correspondence between genetic maps and chromosomal abnormalities provided important additional evidence in support of the chromosomal theory. Other forms of physical mapping were developed decades later in mammals and bacteria. The increasingly precise mapping of gene locations led the way to the development of modern molecular genetics, including techniques for isolating and sequencing individual genes.

The discovery of restriction endonuclease enzymes during the 1970’s allowed geneticists to cut DNA molecules into small fragments. In 1980, a team headed by David Botstein measured the sizes of these “restriction fragments” and found many cases in which the length of the fragment varied from person to person because of changes in the DNA sequence. This type of variation is generally called a “polymorphism.” In this case, it was a polymorphism in the length of the restriction fragments (known as a restriction fragment length polymorphism, or RFLP). The use of the RFLP technique has allowed rapid discovery of the location of many human genes. The Human Genome Project (an effort by scientists worldwide to determine the location and sequence of every human gene) would never have been proposed had it not been for the existence of this mapping technique.

—Eli C. Minkoff

See also: Cell Division; Chromosome Mutation; Chromosome Structure; Classical Transmission Genetics; Genetic Code; Genetic Code,

Cracking of; Linkage Maps; Mendelian Genetics; Mitosis and Meiosis; Model Organism: *Drosophila melanogaster*; Restriction Enzymes; RFLP Analysis; Transposable Elements.

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Chromosome Walking and Jumping

Fields of study: Genetic engineering and biotechnology; Techniques and methodologies

Significance: *Chromosome walking and jumping* are mapping methods used to find defective genes that cause hereditary diseases. Walking is a much slower process, whereas jumping has cut down the time it takes to locate defective genes sometimes by years. These techniques assist in curing diseases, seeking preventive measures, and detecting genetic carriers.

Key terms

GENOMIC LIBRARY: a group of cloned DNA fragments representative of an organism's genome

KILOBASE PAIRS (kb): a measurement of 1,000 base pairs in DNA

MARKER: a unique DNA sequence with a known location with respect to other markers or genes

Gene Hunting

Several geneticists autonomously recognized the possibilities of chromosome walking and jumping to locate genes. Hans Lehrach suggested such techniques at the European Molecular Biology Laboratory, and Sherman Weissman posed similar methods at Yale University. Weissman's student Francis S. Collins

elaborated his mentor's chromosome-jumping concepts. Interested in identifying disease-causing genes, Collins sought to examine sizable areas of genetic material for unknown genes believed to be responsible for triggering erratic biochemical behavior. His novel exploratory method enabled researchers to span chromosomes expeditiously and bypass repetitive or insignificant genetic information.

Investigators have adopted the chromosome-jumping procedure as a reliable, efficient molecular biology tool. Gene searching became less time-consuming and resulted in the identification of defective genes that code for abnormal proteins and cause such diseases as cystic fibrosis. Understanding the nature of such mutations makes the development of treatments and cures more likely and can lead to the ability to detect the presence of the mutated gene in carriers.

Procedure

Geneticists initiate chromosome walking and jumping by collecting genetic samples from people who have a specific disease and from their close relatives. For walking, researchers select a cloned DNA fragment from a genomic library that contains the marker closest to the gene being sought. A small part of the cloned DNA fragment that is on the end nearest the gene being sought is subcloned. The subcloned fragment is then used to screen the genomic library for a clone with a fragment closer to the gene. Then a small part of this new cloned fragment is subcloned to be used to screen for the next closer fragment. This series of steps is repeated as many times as needed, until a fragment is found that appears to contain a gene. This fragment is carefully analyzed, and if it does contain the gene of interest, the process is halted; if not, chromosome walking is continued. Chromosome walking is slow, and repetitive DNA sequences or regions that do not appear in the library can halt the process.

Geneticists choose chromosome jumping to maneuver to genes more quickly and to bypass troublesome regions of DNA. Using chromosome jumping, researchers can travel the same distance they can using chromosome walking—and farther—in one step requiring less

time, because chromosome jumping uses much larger fragments. Chromosome jumping is achieved by selecting a large DNA segment from the area where geneticists believe the desired gene is located and joining the ends to form a circle. This moves DNA sequences together that naturally would occur at distances of several kilobases. Researchers cut out and clone these junctions to form libraries. They use probes from the DNA sample to seek clones with matching start and end sequences and jump along the chromosome. After each jump, bidirectional walking is often done in the new region. A combination of chromosome jumping and walking can be done until the gene is found.

Gene Discovery

Chromosome walking to find the gene for cystic fibrosis, *CF*, would have required eighteen years, while chromosome jumping reduced that time to four years. Collaborating with Lap-Chee Tsui and researchers at Toronto's Hospital for Sick Children, Collins examined DNA from patients suffering from cystic fibrosis. Tsui realized that the *CF* gene was located on chromosome 7. That chromosome consists of 150 million DNA base pairs. Using markers Tsui made from chromosome 7 library fragments, researchers, applying chromosome jumping, scanned the genetic material to target where they should use chromosome walking to find the *CF* gene.

They discovered the *CF* gene in 1989. Analysis revealed that mutation is a deletion of DNA base pairs. This gene codes the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Tsui determined that the shape of CFTR and how it functions are affected by the mutated gene's coding. The abnormal CFTR is unable to create a release channel to remove chloride and sodium from cells. Mucus builds up, adhering to lungs and organs, and bacteria proliferate. Cystic fibrosis is the most frequent fatal hereditary disease in Caucasians. Geneticists estimate that one in twenty-five white Americans has a recessive *CF* gene, and one in two thousand white babies are born with cystic fibrosis. Internationally, researchers associated with Tsui's Toronto-based consortium continue

to study DNA fragments for additional *CF* gene mutations and have detected at least one thousand distinct mutations.

Applications

Chromosome walking and jumping have been utilized to find other disease-causing genes. Collins and his team identified the tumor-producing neurofibromatosis gene in 1990. Three years later, they located the gene for Huntington's disease (Huntington's chorea), an extreme neurological disorder. This method also detected the location on the X chromosome of the choroideremia gene, which causes gradual blindness, mostly in males, as the retina and choroid coat degenerate. Investigating Duchenne muscular dystrophy, Louis Kunkel at the Harvard Medical School used chromosome walking to detect the absence of a gene on the X chromosome that codes the dystrophin protein for muscles. Not all genes found by these methods are linked to diseases. Andrew Sinclair and his team in London applied chromosome walking to seek the gene that signals development of testes in many embryonic mammals.

Although these techniques are useful, they raise ethical concerns. As soon as genes with disease-causing mutations are identified, people can undergo testing to determine whether they carry the mutations. This information can affect reproductive choices, particularly if both partners have a recessive allele for a potentially lethal disease. Fetal material can be genetically analyzed, resulting in complex decisions to continue or terminate a pregnancy if the fetus has the mutation.

Once the mapping of the human genome was completed in 2003, however, geneticists arrived at a time when they no longer needed to depend on chromosome walking and jumping as tools to seek human genes. However, investigators continue to use walking and jumping to locate genes of other organisms, particularly such agricultural plants as rice and wheat.

—Elizabeth D. Schafer

See also: Cystic Fibrosis; Genetic Screening; Genetic Testing; Genomic Libraries; Linkage Maps.

Further Reading

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Classical Transmission Genetics

Field of study: Classical transmission genetics; History of genetics

Significance: *In sexual reproduction, parents produce specialized cells (eggs and sperm) that fuse to produce a new individual. Each of these cells contains one copy of each of the required units of information, or genes, which provide the blueprint necessary for the offspring to develop into individual, functioning organisms. Transmission genetics refers to the passing of the information needed for the proper function of an organism from parents to their offspring as a result of reproduction.*

Key terms

CHROMOSOMES: structures in haploid cells (eggs and sperm) that carry genetic information from each parent

CROSS: the mating of parents to produce offspring during sexual reproduction

GENE: a sequence of base pairs that specifies a product (either RNA or protein); the average gene in bacteria is one thousand base pairs long

LINKAGE: a relation of gene loci on the same chromosome; the more closely linked two loci are, the more often the specific traits controlled by these loci are expressed together

MEIOSIS: the process of nuclear division during sexual reproduction that produces cells that contain half the number of chromosomes as the original cell

SEXUAL REPRODUCTION: reproduction that requires fusion of haploid gametes, each of which contains one copy of the respective parent's genes, as a first step

Discovery of Transmission Genetics

The desire to improve plant and animal production is as old as agriculture. For centuries, humans have been using selective breeding programs that have resulted in the production of thousands of varieties of plants and breeds of animals. The Greek philosopher-scientist Hippocrates suggested that small bits of the body of the parent were passed to the offspring during reproduction. These small bits of arms, heads, stomachs, and livers were thought to develop into a new individual. Following the development of the microscope, it became possible to see the cells, the small building blocks of living organisms. Study of the cell during the 1800's showed that sexual reproduction was the result of the fusion of specialized cells from two parents (eggs and sperm). It was also observed that these cells contained chromosomes ("color bodies" visible when the cells reproduced) and that the number and kind of these chromosomes was the same in both the parents and the offspring. This suggested that the chromosomes carried the genetic information and that each parent transmitted the same number and kinds of chromosomes. For example, humans have twenty-three kinds of chromosomes. The offspring receives one of each kind from each parent and so has twenty-three chromosome pairs. Since the parents and the offspring have

the same number and kinds of chromosomes, and since each parent transmits one complete set of the chromosomes, it was thought that there must be a process of cell division that reduces the parent number from two sets of chromosomes to one set in the production of the egg or sperm cells. The parents would each have twenty-three pairs (forty-six) chromosomes, but their reproductive cells would each contain only one of each chromosome (twenty-three).

In the 1860's, the Austrian botanist Gregor Mendel repeated studies of inheritance in the garden pea and, using the results, developed a model of genetic transmission. The significance of Mendel's work was not recognized during his lifetime, but it was rediscovered in 1900. In that same year, the predicted reductional cell division during reproduction was fully described, and the science of genetics was born.

A Study of Variation

In many respects, genetics is the study of variation. It is recognized that a particular feature of an animal or plant is inherited because there is variation in the expression of that feature, and variation in expression follows a recognizable inheritance pattern. For example, it is known that blood types are inherited, both because there is variation (blood types A, B, and O) and because examination of family histories reveals patterns that show transmission of blood-type information from parents to children.

Variation in character expression may have one of two sources: environmental conditions or inherited factors. If a plant is grown on poor soil, it might be short. The same plant grown on good soil might be tall. A plant that is short because of an inherited factor cannot grow tall even if it is placed on richer soil. From this example, it can be seen that there may be two different ways to determine whether a specific character expression is environmentally or genetically determined: testing for environmental influences and testing for inherited factors. Many conditions are not so easily resolved as this example; there may be many complex environmental factors involved in producing a con-

dition, and it would be impossible to test them all. Knowledge of inheritance patterns can, however, help in determining whether inherited factors play a role in a condition. Cancer-associated genes have been located using family studies that show patterns consistent with a genetic contribution to the disease. There are certainly environmental factors that influence cancer production, but those factors are not as easily recognized.

The patterns of transmission genetics were discovered because the experimenters focused their attention on single, easily recognized characteristics. Mendel carefully selected seven simple characteristics of the pea plant, such as height of the plant, color of the flower, and color of the seeds. The second reason for success was the use of carefully controlled crosses. The original parents were selected from varieties that did not show variations in the characteristic of interest. For example, plants from a pure tall variety were crossed with plants from a pure short variety. Control of the information passed by the parents allowed the experimenter to follow the variation of expression from parents to offspring through a number of generations.

Transmission Patterns

The classic genetic transmission pattern is the passing of information for each characteristic from each parent to each offspring. The offspring receives two copies of each gene. (The term “gene” is used to refer to a character-determining factor; Mendel’s original terminology was “factor.”) Each parent also had two copies of each gene, so in the production of the specialized reproductive cells, the number must be reduced. Consider the following example. A tall pea plant has two copies of the information for height, and both copies are for tall height (tall/tall). This plant is crossed with a plant with two genes for short height (short/short). The information content of each plant is reduced to one copy: The tall plant transmits one tall gene, and the short plant transmits one short gene. The offspring receive both genes and have the information content tall/short.

The situation becomes more complex and more interesting when one or both of the par-

ents in a cross have two different versions of the gene for the same characteristic. If, for example, one parent has the height genes tall/short and the other has the genes short/short, the cells produced by the tall/short parent will be of two kinds: $\frac{1}{2}$ carry the tall gene and $\frac{1}{2}$ carry the short gene. The other parent has only one kind of gene for height (short), so all of its reproductive cells will contain that gene. The offspring will be of two kinds: $\frac{1}{2}$ will have both genes (tall/short), and $\frac{1}{2}$ will have only one kind of gene (short/short). Had it been known that the one parent had one copy for each version of the gene, it could have been predicted that the offspring would have been of two kinds and that each would have an equal chance of appearing. Had it not been known that one of the parents had the two versions of the gene, the appearance of two kinds of offspring would have revealed the presence of both genes. The patterns are repeatable and are therefore useful in predicting what might happen or revealing what did happen in a particular cross. For example, blood-type patterns or DNA variation patterns can be used to identify the children that belong to parents in kidnapping cases or in cases in which children are mixed up in a hospital.

In a second example, the pattern is more complex, because both parents carry both versions of the gene: a tall/short to tall/short cross. Each parent will produce $\frac{1}{2}$ tall-gene-carrying cells and $\frac{1}{2}$ short-gene-carrying cells. Any cell from one parent may randomly join with any cell from the other parent, which leads to the following patterns: $\frac{1}{2}$ tall $\times \frac{1}{2}$ tall = $\frac{1}{4}$ tall/tall; $\frac{1}{2}$ tall $\times \frac{1}{2}$ short = $\frac{1}{4}$ tall/short; $\frac{1}{2}$ short $\times \frac{1}{2}$ tall = $\frac{1}{4}$ short/tall; $\frac{1}{2}$ short $\times \frac{1}{2}$ short = $\frac{1}{4}$ short/short. Tall/short and short/tall are the same, yielding totals of $\frac{1}{4}$ tall/tall; $\frac{1}{2}$ tall/short; and $\frac{1}{4}$ short/short, or a 1:2:1 ratio.

This was the ratio that Mendel recognized and used to develop his model of transmission genetics. Mendel used pure parents (selected to breed true for the one characteristic), so he knew when he had a generation in which all of the individuals had one of each gene.

As in the previous example, if it had been known that each of the parents had one of each

gene, the ratio could have been predicted; conversely, by using the observed ratio, the information content of the parents could be deduced. Using a blood-type example, if one parent has blood-type genes AO and the other parent has the genes BO, the possible combinations observed in their offspring would be AB, AO, BO, and OO, each with the same probability of occurrence ($\frac{1}{2}$ A gene-bearing and $\frac{1}{2}$ O gene-bearing cells in one parent $\times \frac{1}{2}$ B gene-bearing and $\frac{1}{2}$ O gene-bearing cells in the other parent).

Reductional Division

Transmission genetics allows researchers to make predictions about specific crosses and explains the occurrence of characteristic expressions in the offspring. In genetic counseling, probabilities of the appearance of a genetic disease can be made when there is an affected child in the family or a family history of the condition. This is possible because, for most inherited characteristics, the pattern is established by the reduction of chromosome numbers that occurs when the reproductive cells are produced and by the random union of reproductive cells from the two parents. The recognition that the genes are located on the chromosomes and the description of the reductional division in which the like chromosomes separate, carrying the two copies of each gene into different cells during the reductional division of meiosis, provide the basis of the regularity of the transmission pattern. It is this regularity that allows the application of mathematical treatments to genetics. Two genes are present for each character in each individual, but only one is passed to each offspring by each parent; therefore, the 50 percent (or $\frac{1}{2}$) probability becomes the basis for making predictions about the outcome of a cross for any single character.

The classical pattern of transmission genetics occurs because specialized reproductive cells, eggs and sperm, are produced by a special cell reproduction process (meiosis) in which the chromosome number is reduced from two complete sets to one set in each of the cells that result from the process. This reduction results because each member of a pair of chromosomes recognizes its partner, and the chromo-

somes come together. This joining (pairing) appears to specify that each chromosome in the pair will become attached to a "motor" unit from an opposite side of the cell that will move the chromosomes to opposite sides of the cell during cell division. The result is two new cells, each with only one of the chromosomes of the original pair. This process is repeated for each pair of chromosomes in the set.

Independent Genes

Humans have practiced selective breeding of plants and animals for centuries, but it was only during the nineteenth and twentieth centuries that the patterns of transmission of inherited characters were understood. This change occurred because the experimenters focused on a single characteristic and could understand the pattern for that characteristic. Previous attempts had been unsuccessful because the observers attempted to explain a large number of character patterns at the same time. Mendel expanded his model of transmission to show how observations become more complex as the number of characteristics examined is expanded. Consider a plant with three chromosomes and one simple character gene located on each chromosome. In the first parent, chromosome 1 contains the gene for tall expression, chromosome 2 contains the gene for expression of yellow seed color, and chromosome 3 contains the gene for purple flower color. In the other parent, chromosome 1 contains a gene for short height, chromosome 2 contains a gene for green seed color, and chromosome 3 contains a gene for white flower color. Each parent will transmit these genes to their offspring, who will have the genes tall/short, yellow/green, and purple/white. In the production of reproductive cells, the reductional division of meiosis will pass on one of the character expression genes for each of the three characters. (It is important to remember that the products of the reductional cell division have one of each chromosome. If this did not occur, information would be lost, and the offspring would not develop normally.) The characteristics are located on different chromosomes, and during the division process, these chromosome pairs act independently.

This means that the genes that came from any one parent (for example, the tall height, yellow seed, and purple flower expression genes from the one parent) do not have to go together during the division process. Since chromosome pairs act independently, different segregation patterns occur in different cells. The results from one meiosis may be a cell with the tall, green, and purple genes and one with the short, yellow, and white genes. In the same plant, another meiosis might produce a cell with the short, yellow, and purple genes, and the second cell would have the tall, green, and white genes.

Since these genes are independent, height does not influence seed color or flower color, nor does flower color influence seed color or height. The determining gene for each characteristic is located on a different chromosome, so the basic transmission model can be applied to each gene independently, and then the independent patterns can be combined. The tall/short height genes will segregate so that $\frac{1}{2}$ of the cells will contain the tall gene and $\frac{1}{2}$ will contain the short gene. Likewise, the yellow/green seed color genes will separate so that $\frac{1}{2}$ of the cells will contain the yellow gene and $\frac{1}{2}$ will contain a green gene. Finally, $\frac{1}{2}$ of the cells will contain a purple flower gene and $\frac{1}{2}$ will contain a white gene. These independent probabilities can be combined because the probability of any combination is the product of the independent probabilities. For example, the combination tall, purple, white will occur with a probability of $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}$. This means that one should expect eight different combinations of these characters. The possible number of combinations for n chromosome pairs is 2^n . For humans, this means that any individual may produce 2^{23} different chromosome combinations. This is the same idea as tossing three coins simultaneously. Each coin may land with a head or a tail up, but how each coin lands is independent of how the other coins land. Knowledge of transmission patterns based on chromosome separation during meiosis allows researchers to explain the basic pattern for a single genetic character, but it also allows researchers to explain the great variation that is observed among individuals within a population in

which genes for thousands of different characters are being transmitted.

Continuous Variation

The principles of transmission genetics were established by studying characters with discrete expressions—plants were tall or dwarf, seeds were yellow or green. In 1903, Danish geneticist Wilhelm Johannsen observed that characteristics that showed continuous variation, such as weight of plant seeds, fell into recognizable groups that formed a normal distribution. These patterns could also be explained by applying the principles of transmission genetics.

Assume a plant has two genes that influence its height and that these genes are on two different chromosomes (for example, 1 and 3). Each gene has two versions. A tall gene stimulates growth (increases the height), but a short gene makes no contribution to growth. A plant with the composition tall-1/tall-1, tall-3/tall-3 would have a maximum height because four genes would be adding to the plant's height. A short-1/short-1, short-3/short-3 plant would have minimum height because there would be no contribution to its height by these genes. Plants could have two contributing genes (tall-1/short-1, tall-3/short-3) or three contributing genes (tall-1/short-1, tall-3/tall-3). The number of offspring with each pattern would be determined by the composition of the parents and would be the result of gene segregation and transmission patterns. Many genes contributing to a single character expression apply to many interesting human characteristics, such as height, intelligence, amount of skin pigmentation, hair color, and eye color.

Linkage Groups

Mendel's model of the transmission of genes was supported by the observations of chromosome pair separation during the reductional division, but early in the twentieth century, it was recognized that some genes did not separate independently. Work in American geneticist Thomas Hunt Morgan's laboratory, especially by an undergraduate student, Alfred Sturtevant, showed that each chromosome contained determining genes for more than

one characteristic and established that genes located close together on the same chromosome stayed together during the separation of the paired chromosomes during meiosis. If a pea plant had a chromosome with the tall height gene and, immediately adjacent to it, a gene for high sugar production, and if the other version of this chromosome had a gene for short height and a gene that limited the sugar production, the most likely products from meiosis would be two kinds of cells: one with the genes for tall height and high sugar production and one with the genes for short height and limited sugar production. These genes are said to be "linked," or closely associated on the same chromosome, because they go together as the chromosomes in the pair separate. It is generally accepted that humans contain approximately 21,000 genes, but there are only twenty-three kinds of chromosomes. This means that each chromosome contains many different genes. Each chromosome is considered a linkage group, and one of the goals of genetic study is to locate the gene responsible for each known characteristic to its proper chromosome.

A common problem in medical genetics is locating the gene for a specific genetic disease. Family studies may show that the disease is transmitted in a pattern consistent with the gene being on one of the chromosomes, but there is no way of knowing its location. Variations in DNA structure are also inherited in the classic pattern, and these DNA pattern modifications can be determined using modern molecular procedures. DNA variation patterns are analyzed for linkage to the disease condition. If a specific DNA pattern always occurs in individuals with the disease condition, it indicates that the DNA variation is on the same chromosome and close to the gene of interest because it is transmitted along with the disease-producing gene. This information locates the chromosome position of the gene, allowing further work to be done to study its structure. With the completion of the Human Genome Project, it is predicted that tracking down the genes responsible for genetic defects will be a much faster process than before. Many more genetic

markers have now been identified, which, in theory, should greatly enhance the techniques used to locate a faulty gene.

—D. B. Benner

See also: Cell Division; Chromosome Mutation; Chromosome Structure; Chromosome Theory of Heredity; Dihybrid Inheritance; Epistasis; Extrachromosomal Inheritance; Genetic Code; Genetic Code: Cracking of; Hybridization and Introgression; Incomplete Dominance; Lamarckianism; Linkage Maps; Mendelian Genetics; Mitochondrial Genes; Mitosis and Meiosis; Monohybrid Inheritance; Multiple Alleles; Nondisjunction and Aneuploidy; Parthenogenesis; Penetrance; Polygenic Inheritance.

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Cloning

Field of study: Genetic engineering and biotechnology

Significance: *Cloning includes both gene cloning and organismal cloning. Gene cloning, an important technique for understanding how cells work, has produced a multitude of useful products, including human medicines. Organismal cloning includes reproductive cloning and therapeutic cloning. Ethical and safety concerns have led to a consensus that human cloning should be banned, but therapeutic cloning is more controversial, since it could lead to treatments for many human diseases.*

Key terms

CLONING VECTOR: a plasmid or virus into which foreign DNA can be inserted to amplify the number of copies of the foreign DNA in the host cell or organism

DNA: deoxyribonucleic acid, a long-chain macromolecule, made of units called nucleotides and structured as a double helix joined by weak hydrogen bonds, that forms genetic material for most organisms

DNA HYBRIDIZATION: formation of a double-stranded nucleic acid molecule from single-stranded nucleic acid molecules that have complementary base sequences

LIGASE: an enzyme that joins recombinant DNA molecules together

PLASMID: a DNA molecule that replicates independently of chromosomes

RECOMBINANT DNA TECHNOLOGY: methods used to splice a DNA fragment from one organism into DNA from another organism and then clone the new recombinant DNA molecule

REPRODUCTIVE CLONING: cloning to produce individual organisms

RESTRICTION ENZYME: a protein (an enzyme) that recognizes a specific nucleotide sequence in a piece of DNA and causes a sequence-specific cleavage of the DNA

STEM CELLS: cells that are able to divide indefinitely in culture and to give rise to specialized cells

THERAPEUTIC CLONING: cloning to produce a treatment for a disease

Types of Cloning

There are three different definitions of a clone. One is a group of genetically identical cells descended from a single common ancestor. This type of clone is often made by plant cell tissue culture in which a whole line of cells is made from a single cell ancestor. A second type of clone is a gene clone, or recombinant DNA clone, in which copies of a DNA sequence are made by genetic engineering. A third type of clone is an organism that is descended asexually from a single ancestor. A much-celebrated example of an organismal clone is the sheep Dolly (1997-2003), produced by placing the nucleus of a cell from an ewe's udder, with its genetic material (DNA), into an unfertilized egg from which the nucleus had been removed.

DNA Cloning

DNA is cloned to obtain specific pieces of DNA that are free from other DNA fragments. Clones of specific pieces of DNA are important for basic research. Once a piece of DNA is cloned, it can be sequenced (to determine the order of the four repeating nucleotides) to learn the details of genes within that DNA. Where does the gene begin and end? What type of control regions does the gene have? Cloned DNAs can be used as hybridization probes, where sequences that are complementary to the cloned DNA can be detected. Such DNA hybridization is useful to detect similarities between genes from different organisms, to detect the presence of specific disease genes, and to determine in what tissues that gene is expressed. The gene is expressed when a messenger RNA (mRNA) is made from the gene and the mRNA is translated into a protein product. A DNA clone is also used to produce the protein product for which that gene codes. When a clone is expressed, the protein made by that gene can be studied or an antibody against that protein can be made. An antibody is used to show in which tissues of an organism that protein is found. Also, a DNA clone may be expressed because the gene codes for a useful product. This is a way to obtain large amounts of the specific protein.

Products of Recombinant DNA Technology

Recombinant DNA technology has produced clones put to use for a wide variety of human purposes. For example, rennin and chymosin are used in cheese making. One of the most important applications, however, is in medicine. Numerous recombinant DNA products are useful in treating human diseases, including the production of human insulin (Humalin) for diabetics. Other human pharmaceuticals produced by gene cloning include clotting factor VIII to treat hemophilia A, clotting factor IX to treat hemophilia B, human growth hormone, erythropoietin to treat certain anemias, interferon to treat certain cancers and hepatitis, tissue plasminogen activator to dissolve blood clots after a heart attack or stroke, prolactin to treat genetic emphysemas, thrombate III to correct a genetic antithrombin III deficiency, and parathyroid hormone.

The advantages of the cloned products are their high purity, greater consistency from batch to batch, and the steady supply they offer.

How to Clone DNA

DNA is cloned by first isolating it from its organism. Vector DNA must also be isolated from bacteria. (A vector is a plasmid or virus into which DNA is inserted.) Both the DNA to be cloned and the vector DNA are cut with a restriction enzyme that makes sequence-specific cuts in the DNAs. The ends of DNA molecules cut with restriction enzymes are then joined together with an enzyme called ligase. In this way the DNA to be cloned is inserted into the vector. These recombinant DNA molecules (vector plus random pieces of the DNA to be cloned) are then introduced into a host, such as bacteria or yeast, where the vector can replicate. The recombinant molecules are analyzed to find the ones that contain the cloned DNA of interest.

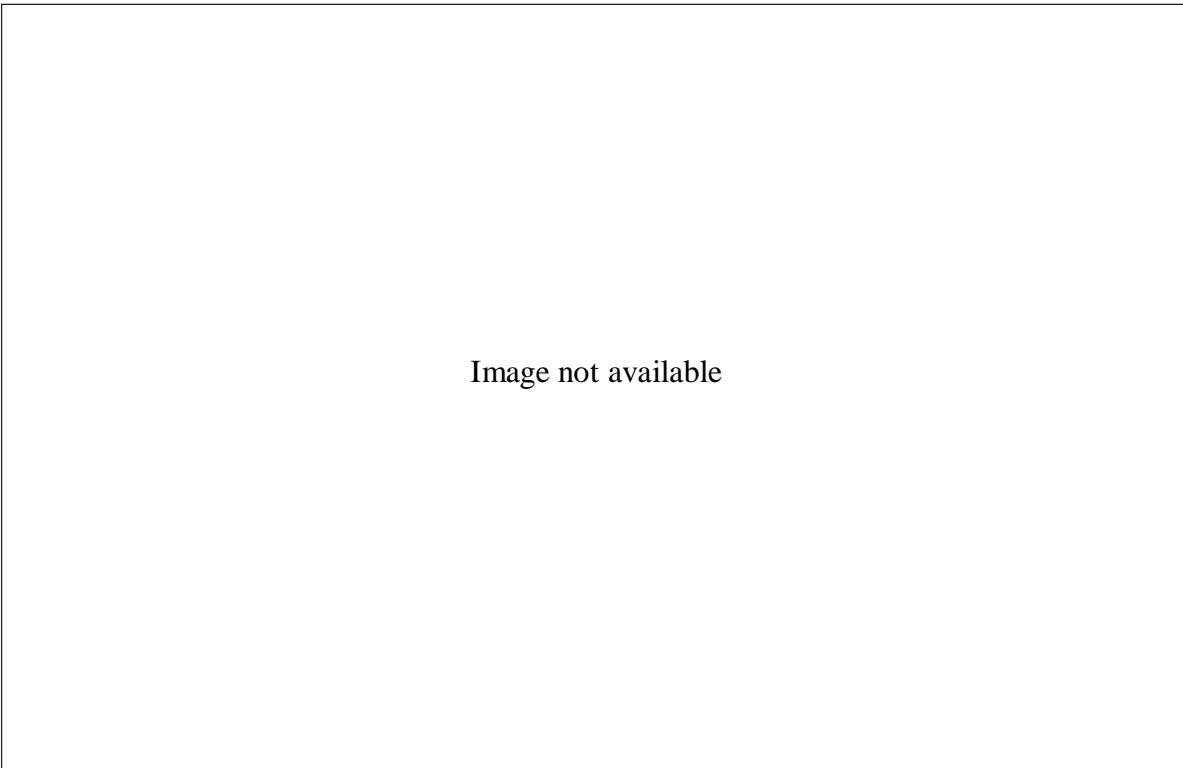


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These five cows on an Iowa farm in April, 2000, were cloned by Robert Lanza and colleagues of Advanced Cell Technologies in Worcester, Massachusetts. The cows' cells—unlike those of the first cloned vertebrate, Dolly the sheep—appeared to have a prolonged youth. (AP/Wide World Photos)

Regulation of DNA Cloning

In the 1970's the tools to permit cloning of specific pieces of DNA were developed. There was great concern among scientists about the potential hazards of some combinations of DNA from different sources. Concerns included creating new bacterial plasmids with new drug resistances and putting DNA from cancer-causing viruses into plasmids. In February, 1975, scientists met at a conference center in Asilomar, California, to discuss the need to regulate recombinant DNA research. The result of this conference was the formation of the Recombinant DNA Molecule Program Advisory Committee at the National Institutes of Health, and guidelines for recombinant DNA work were established.

Genetically Modified Organisms

Numerous cloned genes have been introduced into different organisms to produce genetically modified organisms (GMOs). Genes for resistance to herbicides and insects have been introduced into soybean, corn, cotton, and canola, and these genetically engineered plants are in cultivation in fields in the United States and other countries. Fish and fruit and nut trees that mature more rapidly have been created by genetic engineering. Edible vaccines have been made—for example, a vaccine for hepatitis B in bananas. A tomato called the Flavr Savr is genetically engineered to delay softening. Plants that aid in bioremediation by taking up heavy metals such as cadmium and lead are possible.

Concerns about genetically modified organisms include safety issues—for example, concerns that foreign genes introduced into food plants may contain allergens and that the antibiotic resistance markers used in creating the GMOs might be transferred to other organisms. There are concerns about the environmental impact of GMOs; for example, if these foreign genes are transferred to other plants by unintended crossing of a GMO with a weed plant, weeds may become difficult or impossible to eradicate and jeopardize crop growth. There is a concern about the use of genetically modified organisms as food. There is a concern about loss of biodiversity if only one, genetically mod-

ified, variety of a crop plant is cultivated. There are also ethical concerns surrounding whether certain GMOs might be made available only in rich countries, and there are concerns about careful labeling of GMOs so that consumers will be aware when they are using products from GMOs. All of these questions remain in flux as the marketing of GMOs proceeds.

Organismal Cloning

A goal of organismal cloning is to develop ways of efficiently altering animals genetically in order to reproduce certain animals that are economically valuable. Animals have been altered by the introduction of specific genes, such as human proteins that will create drug-producing animals. Some genes have been inactivated in organisms to create animal models of human diseases. For example, “knockout mice” are used as models for diabetes research. Another goal is to conduct research that might lead to the development of human organs for transplant produced from single cells. Similarly, animals might be genetically engineered to make their organs better suited for transplantation to humans. Finally, the cloning of a human might be a solution to human infertility.

Are Organismal Clones Normal?

There is, however, a concern about the health of cloned animals. First of all, when inserting a new nucleus into an egg from which the nucleus has been removed, and then implanting such eggs into surrogate mothers, only very few of the eggs develop properly. There are suggestions of other abnormalities in cloned animals that might be due to the cloning process. The first vertebrate to be successfully cloned, Dolly (1997-2003), developed first arthritis and then a lung disease when six years old; although neither condition was unusual in sheep, both appeared years earlier than normal, and Dolly was euthanized. Was she genetically older than her chronological age?

Stem Cells

Stem cells are unspecialized cells that are able to divide continuously and with the proper conditions be induced to give rise to specialized cell types. In the developing embryo they

give rise to the hundreds of types of specialized cells that comprise the adult body. Embryonic stem cells can be isolated from three- to five-day-old embryos. Some tissues in the adult, such as bone marrow, brain, and muscle, contain adult stem cells that can give rise to cell types of the tissue in which they reside.

A goal of research on stem cells is to learn how stem cells become specialized cells. Human stem cells could be used to generate tissues or organs for transplantation and to generate specific cells to replace those damaged as a result of spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, rheumatoid arthritis, and other conditions.

Regulation of Organismal Cloning

Until the cloning of the sheep Dolly in 1997, it was thought that adult specialized cells could not be made to revert to nonspecialized cells that can give rise to any type of cell. However, Dolly was created from a specialized adult cell from a ewe's udder. After the publicity about Dolly, U.S. president Bill Clinton asked the National Bioethics Advisory Commission to form recommendations about the ethical, religious, and legal implications of human cloning. In June, 1997, that commission concluded that attempts to clone humans are "morally unacceptable" for safety and ethical reasons. There was a moratorium on using federal funds for human cloning. In January, 1998, the U.S. Food and Drug Administration (FDA) declared that it had the authority to regulate human cloning and that any human cloning must have FDA approval.

While there is general agreement in the United States and in many other countries that reproductive human cloning should be banned because of ethical and safety concerns, there is ongoing debate about whether or not to allow therapeutic cloning to treat human disease or research cloning to study how stem cells develop. The Human Cloning Prohibition Act of 2001 to ban both reproductive and therapeutic cloning passed in the U.S. House of Representatives, but the Senate did not support the ban. The ban was again considered by the lawmakers in 2002. In the meantime, individual states such as California and New Jersey have passed

bills that approve of embryonic stem cell research. Such research might lead to treatments for diseases such as Parkinson's, diabetes, and Alzheimer's. The research is controversial because embryos must be destroyed to obtain the stem cells, and some groups believe that constitutes taking a human life. The embryos used are generally extra embryos left over from in vitro fertilizations. In December, 2002, and January, 2003, a company called Clonaid announced the births of several babies they claimed were the result of human cloning but then failed to produce any scientific evidence that the babies were clones. February, 2003, the U.S. Congress considered a ban on both reproductive and therapeutic cloning. In late February, the House passed the Human Prohibition Cloning Act of 2003, banning the cloning of human beings but allowing limited research on some existing stem cell lines.

The tension between scientific possibility, public policy, and societal values continues in the arena of cloning. Through therapeutic cloning there is great potential for the treatment of human diseases, but the ethical concerns about such procedures must be carefully considered as well.

—Susan J. Karcher

See also: Animal Cloning; Biopharmaceuticals; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; Knockout Genetics and Knockout Mice; Plasmids; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Stem Cells; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Web Site of Interest

Human Genome Project Information, Cloning Fact Sheet. <http://www.doe-genomes.org>. Site links to information on cloning types—including DNA, reproductive, and therapeutic cloning—cloning organs for transplants, cloning risks, and more.

Cloning: Ethical Issues

Fields of study: Bioethics; Genetic engineering and biotechnology; Human genetics

Significance: Although cloning of plants has been performed for hundreds of years and cloning from embryonic mammalian cells became commonplace in the early 1990's, the cloning of the sheep Dolly from adult cells in 1996 raised concerns that cloning might be used in a dangerous or unethical manner.

Key terms

BIOETHICS: the study of human actions and goals in a framework of moral standards relating to use and abuse of biological systems

CLONE: an identical genetic twin of any organism or DNA sequence; clones can occur naturally or experimentally

CLONING: the process of producing a genetic twin in the laboratory by experimental means

Bioethics and Cloning

Bioethics was founded as a discipline by ethicist Van Rensselaer Potter (1911-2001) in the early 1970's as the formal study and application of ethics to biology and biotechnology. The discipline was initially created as an ethical values system to help guide scientists and others in making decisions that could affect the environment. The world has become even more complex since Potter's original vision of a planet challenged by ecological catastrophe. Humans have developed the ability to take genes from one organism and transfer them to another, creating something entirely new to nature, with unknown consequences. Moreover, humans have the ability to make endless genetic copies of these organisms by cloning. Bioethics now includes asking hard ethical questions about biotechnology, and, as Potter suggests, "promot[ing] the evolution of a better world for future generations."

Cloning involves making a genetic twin of an organism or of a DNA sequence. The focus of this article is on the cloning of whole organisms. The process of cloning has actually been performed with plants for centuries.

Cuttings can be removed from many species and induced to make roots. These cuttings are then grown into full-size, genetically identical copies of the parent plant. The emergence of crops that cannot be propagated in the standard fashion, such as seedless navel oranges, has led to whole groves of cloned siblings. Few would suggest that such cloning is inherently wrong or unethical. Animal cloning has been quietly occurring since the early 1990's. Eggs fertilized *in vitro* are allowed to develop to the eight-cell stage, at which point the cells are separated. Each individual cell then develops into an embryo that is implanted in a female. Thus, a single zygote can be used to make eight identical individuals. This type of cloning has been used routinely in animal husbandry to propagate desirable genetic traits.

In 1996, a team of scientists in Scotland headed by Ian Wilmut cloned a mammal—a sheep named Dolly—from adult cells for the first time. While bioethicists had seen no wrong in cloning orange trees and embryonic mammals, they were troubled by the cloning of a sheep. It is important to realize that the cloning of Dolly was not the key bioethical issue. Rather, the issue that worried the ethicists was the implication of the clone's existence: that scientists were only a small step away from cloning a human. If bioethics is concerned with protecting the evolution of future generations of humans, did the cloning of Dolly represent a potential threat? Could the natural progress of humans toward an unknown evolutionary future be sidetracked or derailed by the intervention and effects of cloning? What would be the social ramifications of human cloning? Would it have the potential to change humanity as it is now known forever? Was cloning simply wrong? Christian bioethicists, for example, were troubled by the implications of humans being able to manipulate themselves in this way, many considering it morally wrong.

Many scientists, including Wilmut, were quick to point out that they would never support human cloning but did not believe that cloning itself was unethical. Most ethicists agreed that cloning animals could help human society in many ways. Genetically engineered animals had the potential to be used to create

vast quantities of protein-based therapeutic drugs. Commercial animals that are top producers, such as cows with high milk yields, could also be cloned. Human replacement organs could be grown in precisely controlled environments.

However, cloning, if misapplied, has frightening possibilities in the minds of many. Although only science fiction now, it is possible to envision a future world of human clones designed to fill certain roles, as genetically programmed soldiers, workers, or even an elite society of "perfect" cloned individuals. Others have envisioned the possibility of cloning an extra copy of themselves as donors of perfectly matched organs during old age. Even the possibility that individuals might be cloned without their knowledge or permission has been anticipated.

Human Cloning

Something about human cloning chafes at the human conscience. Bioethicist Karen Rothenberg, in statements delivered to the U.S. Senate's Public Health and Safety Subcommittee of the Labor and Human Resources Committee in the 1990's, suggested why society is made uneasy by the potential implications of human cloning. She broke her argument down into three *I*s. The first *I* is "interdependence." Cloning makes humans uneasy because it requires only one parent. People are humbled because it takes two humans to produce a baby. If part of the definition of humanity is the interdependence upon one another to reproduce, then a cloned human begs the question of just what is human. Rothenberg's second *I* is "indeterminateness." Cloning removes all randomness from human reproduction. With cloning, people predetermine whether they want to reproduce any physical or mental type available. They can control all possible genetic variables in cloning with a predicted outcome. However, does the same genetic variability that decides one's hereditary fate at conception also define some part of humanity? The last *I* is "individuality." It is disconcerting for people to imagine ten or one hundred copies of themselves walking around. Twins and triplets are common now, but what would such a vast change mean

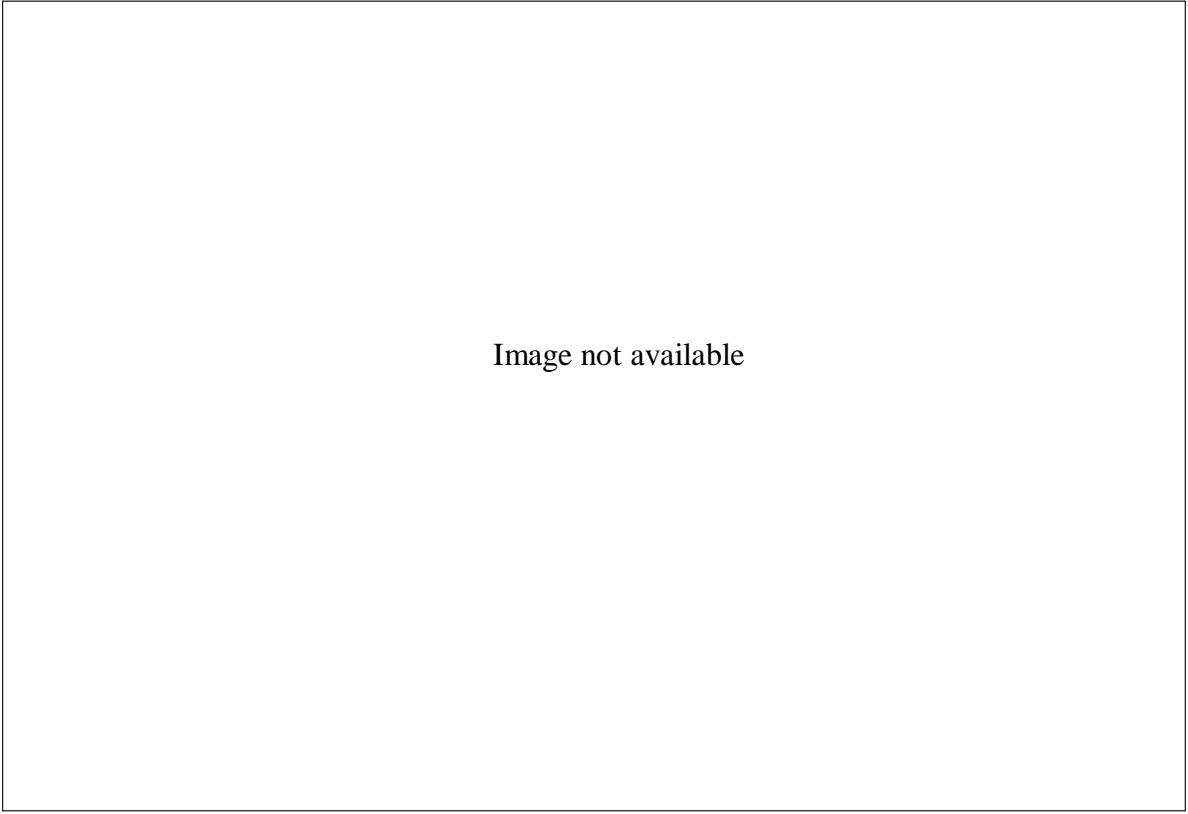


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Chief executive of Clonaid Brigitte Boisselier (left) and the founder of the Raelian movement, Claude Vorilhon, announced in January, 2003, the birth of a human clone, as well as imminent births of other cloned children. Physical evidence and independent confirmation of the cloning were never offered and the announcement was concluded to be a hoax, but in the wake of media attention the issue of human cloning became the focus of renewed public debate. (AP/Wide World Photos)

to individuality and the concept of the human soul? In closing, Rothenberg asked whether “the potential benefits of any scientific innovation [are] outweighed by its potential injury to our very concept of what it means to be human.”

Andrew Scott of the Urban Institute takes a different view. He believes that bioethics does not apply to cloning but only to what happens after cloning. Cloning does not present a moral dilemma to Scott, assuming that the process does not purposely create “abnormalities.” Scott states that “the clone [would] simply be another, autonomous human being . . . carrying the same genes as the donor, and [living] life in a normal, functional way.” He suggests that as long as clones are not programmed to be “human drones” and are not used in an unethical way, cloning should not be a bioethical

worry. Many nonscientists miss the point that a clone is simply a genetically identical copy, not a copy in every aspect. If someone were to have cloned Albert Einstein, the cloned Einstein would not be identical behaviorally or in other ways to the original. What made Einstein who he was involved not merely his genes but also his many life experiences, which are impossible to duplicate in a clone. The same would be true of a cloned child brought to life by grieving parents who have lost their original child in an accident. The clone would be like a twin, not the same child.

Perhaps the right questions are not being asked. Better questions may be: Can humans be trusted not to abuse the technology of cloning? Can those in positions of power be trusted not to use cloning to their advantage and the endangerment of humanity? Probably the most

basic question is, What compelling reason is there to clone a human in the first place? Carl B. Feldbaum, the president of the Biotechnology Industry Organization, believes that people should be wary of anyone who asks them to allow human cloning and states:

In the future, society may determine that there are sound reasons to clone certain animals to improve the food supply, produce biopharmaceuticals, provide organs for transplantation and aid in research. I can think of no ethical reason to apply this technique to human beings, if in fact it can be applied.

The ethical issues are even more complicated than they first appear. Is the actual process of cloning, as performed by Wilmut, ethical if applied to humans? Wilmut's cloning process produced many failures before Dolly was conceived; only she survived of her 277 cloned sisters. Her early death at the age of six was also potentially precipitated by the cloning process. Bioethicists question whether manipulating human embryos to produce clones with only a 0.4 percent success rate is moral; to someone who believes that human life begins at conception, the cloning procedure as performed by Wilmut would almost certainly be unacceptable.

Of course, these questions remain irrelevant in most of the world. As of 2003, many developed nations had banned human cloning, including the United States. There is also some question as to whether the technology has progressed enough to make human cloning possible. Some believe the technology has reached this point and that cloning has been attempted secretly, at least somewhere in the world. A few even speculate that somewhere it might have already succeeded. Geneticists in several laboratories have carried out human cloning through the very early stages of embryogenesis, but there is no official case where it has been accomplished to the point of a healthy child being born.

Cloning offers a new and perhaps frightening view of life and the biological universe and brings with it a renewed respect for life. If almost any cell in the body can be used as the basis to clone an entirely new organism, this

makes each cell the potential equivalent of a fertilized egg. While respect for life is renewed from this insight, life is simultaneously cheapened. If each cell contains all the genetic information needed to create a new individual, then what is a single cell worth among millions of copies? The answer may be "very little." When one million or one hundred million potential copies exist, then one copy is worth almost nothing. Therefore, the two contrary feelings of reverence and irreverence linger side by side. The question one must ask is, Which will win out in the end?

—James J. Campanella, updated by Bryan Ness

See also: Animal Cloning; Bioethics; Biological Weapons; Cloning; Cloning Vectors; Eugenics; Eugenics: Nazi Germany; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetics in Television and Films; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Stem Cells; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Web Sites of Interest

ActionBioScience.org. <http://www.actionbioscience.org/biotech/mcgee.html>. A site that includes a “primer” on the ethics of cloning and useful links.

The President’s Council on Bioethics. <http://bioethics.gov>. Government council that advises on ethical issues surrounding biomedical science and technology, including cloning. Includes links to bioethics literature.

Cloning Vectors

Field of study: Genetic engineering and biotechnology

Significance: *Cloning vectors are one of the key tools required for propagating (cloning) foreign DNA sequences in cells. Cloning vectors are vehicles for the replication of DNA sequences that cannot otherwise replicate. Expression vectors are cloning vectors that provide not only the means for replication but also the regulatory signals for protein synthesis.*

Key terms

BACTERIOPHAGE: a virus that infects bacterial cells, often simply called a phage

FOREIGN DNA: DNA taken from a source other than the host cell that is joined to the DNA of the cloning vector; also known as insert DNA

PLASMID: a small, circular DNA molecule that replicates independently of the host cell chromosome

RECOMBINANT DNA MOLECULE: a molecule of DNA created by joining DNA molecules from different sources, most often vector DNA joined to insert DNA

RESTRICTION ENZYME: an enzyme capable of cutting DNA at specific base pair sequences, produced by a variety of bacteria as a protection against bacteriophage infection

The Basic Properties of a Cloning Vector

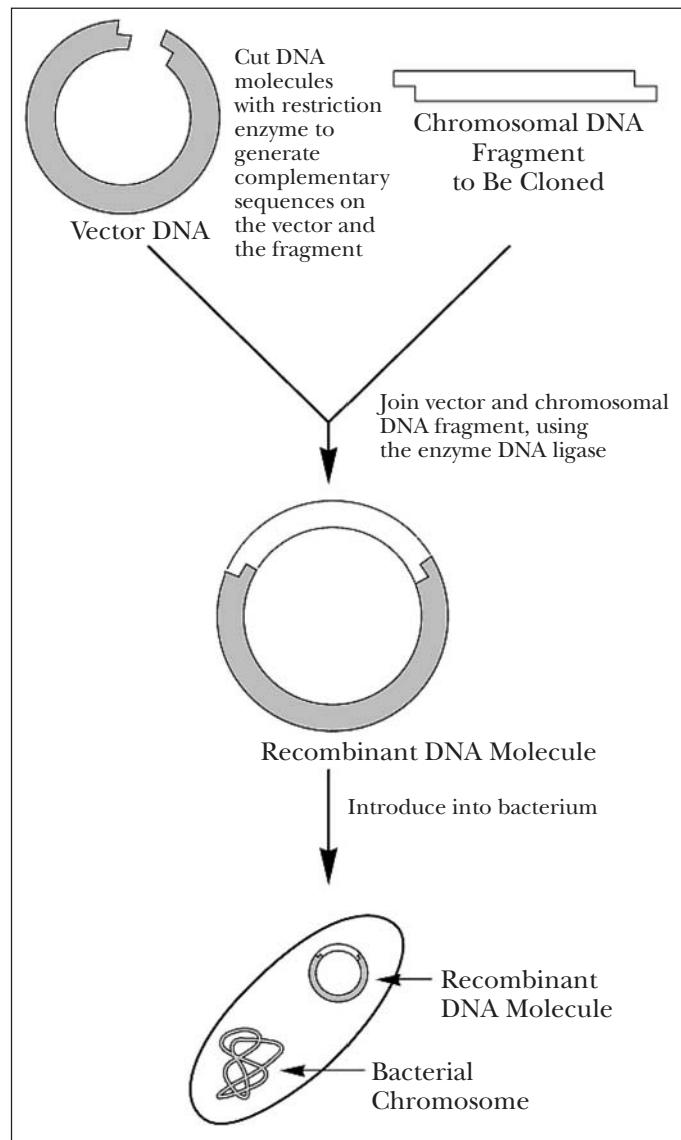
Cloning vectors were developed in the early 1970’s from naturally occurring DNA molecules found in some cells of the bacteria *Escherichia coli* (*E. coli*). These replicating molecules, called plasmids, were first used by the American scientists Stanley Cohen and Herbert Boyer as vehicles, or vectors, to replicate other pieces of DNA (insert DNA) that were joined to them. Thus the first two essential features of cloning vectors are their ability to replicate in an appropriate host cell and their ability to join to foreign DNA sequences to make recombinant molecules. Plasmid replication requires host-cell-specified enzymes, such as DNA polymerases that act at a plasmid sequence called the “origin of replication.” Insert DNA is joined (ligated) to plasmid DNA through the use of two

kinds of enzymes: restriction enzymes and DNA ligases. The plasmid DNA sequence must have unique sites for restriction enzymes to cut. Cutting the double-stranded circular DNA at more than one site would cut the plasmid into pieces and would separate important functional parts from one another. However, when a restriction enzyme cuts the circular plasmid at one unique site, it converts it to a linear molecule. Linear, insert DNA molecules, produced by cutting DNA with the same restriction enzyme as was used to cut the plasmid vector, can be joined to cut plasmid molecules using the enzyme DNA ligase. This catalyzes the covalent joining of the insert DNA and plasmid DNA ends to create a circular, recombinant plasmid molecule. Most cloning vectors have been designed to have many unique restriction enzyme cutting sites all in one stretch of the vector sequence. This part of the vector is referred to as the multiple cloning site.

In addition to an origin of replication and a multiple cloning site, most vectors have a third element: a selective marker. In order for the vector to replicate, it must be present inside an appropriate host cell. Introducing the vector into cells is often a very inefficient process. Therefore, it is very useful to be able to select, from a large population of host cells, those rare cells that have taken up a vector. This is the role of the selectable marker. The selectable marker is usually a gene that encodes resistance to an antibiotic to which the host is normally sensitive. For example, if a plasmid vector has a gene that encodes resistance to the antibiotic ampicillin, only those *E. coli* cells that harbor a plasmid will be able to grow on media containing ampicillin.

Many vectors have an additional selective marker that is rendered inactive when a plasmid is recombinant. A commonly used marker gene of this

kind is the *lacZ* gene, which encodes the enzyme beta-galactosidase. This enzyme breaks the disaccharide lactose into two monosaccharides. The pUC plasmid vector has a copy of the *lacZ* gene which has been carefully engineered to contain a multiple cloning site within it,



Segments of DNA from any organism can be cloned by inserting the DNA segment into a plasmid—a small, self-replicating circular molecule of DNA separate from chromosomal DNA. The plasmid can then act as a “cloning vector” when it is introduced into bacterial cells, which replicate the plasmid and its foreign DNA. This diagram from the Department of Energy’s Human Genome Program site illustrates the process. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis/>)

while maintaining the functionality of the expressed enzyme. When a DNA fragment is inserted into the multiple cloning site, the *lacZ* gene is no longer capable of making functional beta-galactosidase. This loss of function can be detected by putting X-gal into the growth media. X-gal has a structure similar to lactose but cannot be broken down by beta-galactosidase. Rather, beta-galactosidase modifies X-gal and produces a blue color. Thus, colonies of the bacterium *E. coli* containing recombinant plasmids will be normal colored, whereas those that have normal, nonrecombinant plasmids will be blue. Typical selection media then contain ampicillin and X-gal. The ampicillin only allows *E. coli* which contain a plasmid to grow, and the X-gal identifies which colonies have recombinant plasmids.

There are a number of procedures for introducing the plasmid vector into the host cell. Transformation is a procedure in which the host cells are chemically treated so that they will allow small DNA molecules to pass through the cell membrane. Electroporation is a procedure that uses an electric field to create pores in the host cell membrane to let small DNA molecules pass through.

Viruses and Cloning Vectors

In addition to plasmid cloning vectors, some bacteriophages (or phages) have been modified to serve as cloning vectors. Bacteriophages, like other viruses, are infectious agents that are made of a genome, either DNA or RNA, that is surrounded by a protective protein coat. Phage vectors are used similarly to the way plasmid vectors are used. The vector and insert DNAs are cut by restriction enzymes so that they subsequently can be joined by DNA ligase. The newly formed recombinant DNA molecules must enter an appropriate host cell to replicate. In order to introduce the phage DNA into cells, a whole phage particle must be built. This is referred to as “packaging” the DNA. The protein elements of the phage are mixed with the recombinant phage DNA and packaging enzymes to create an infectious phage particle. Appropriate host cells are then infected with it. The infected cells then make many copies of each recombinant molecule, along with the

proteins needed to make a completed phage particle. In many cases, the final step of viral infection is the lysis of the host cell. This releases the mature phage particles to infect nearby host cells. Phage vectors have two advantages relative to plasmid vectors: First, viral delivery of recombinant DNA to host cells is much more efficient than the transformation or electroporation procedures used to introduce plasmid DNA into host cells, and second, phage vectors can be used to clone larger fragments of insert DNA.

Viruses that infect cells other than bacteria have been modified to serve as cloning vectors. This permits cloning experiments using many different kinds of host cells, including human cells. Viral vectors, just like the natural viruses from which they are derived, have specific host and tissue ranges. A particular viral vector will be limited for use in specific species and cell types. The fundamental practice of all virally based cloning vectors involves the covalent joining of the insert DNA to the viral DNA to make a recombinant DNA molecule, introduction of the recombinant DNA into the appropriate host cell, and then propagation of the vector through the natural mechanism of viral replication. There are two fundamentally different ways that viruses propagate in cells. Many viruses, such as the phages already described, enter the host cell and subvert the cell’s biosynthetic machinery to its own reproduction, which ultimately leads to lysis and thereby kills the host cell as the progeny viruses are released. The second viral life strategy is to enter the host cell and integrate the viral DNA into the host cell chromosome so that the virus replicates along with the host DNA. Such integrating viruses can be stably maintained in the host cell for long periods. The retroviruses, of which the human immunodeficiency virus (HIV) is an example, are a group of integrating viruses that are potentially useful vectors for certain gene therapy applications. Using cloning vectors and host cells other than bacteria allows scientists to produce some proteins that bacteria cannot properly make, permits experiments to determine the function of cloned genes, and is important for the development of gene therapy.

The Ti Plasmid of *Agrobacterium*

Two species of naturally occurring plant pathogenic bacteria, *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, infect many plant species and have been harnessed through biotechnology to effect permanent genetic transformation of plants. Virulent (disease-causing) *Agrobacterium* species can infect plants and transfer a small portion of their own bacterial DNA, called T-DNA (transferred DNA), into the plant. The T-DNA is actually a small fragment of a large (approximately 200-kilobase-pair) plasmid called the Ti (tumor-inducing) plasmid in *A. tumefaciens* and the Ri (root-inducing) plasmid in *A. rhizogenes*.

The T-DNA fragment of the Ti plasmid is defined on both ends by 24-base-pair direct repeat sequences called the left-hand and right-hand border sequences. The T-DNA fragment is released from the plasmid by the action of endonucleases, which cut the DNA at specific points within the right-hand and left-hand border sequences. The endonucleases are two of the *Vir* (virulence) genes encoded on the Ti plasmid adjacent to the T-DNA. Several other *Vir* genes are produced when *Agrobacterium* cells are introduced into plant tissue, usually through a wound. Following infection of a plant, *Agrobacterium* cells sense the presence of phenolic wound compounds and the acidic environment within wounded plant tissues. These conditions trigger a series of several *Vir* genes to produce *Vir* proteins that direct excision of the T-DNA and facilitate transport and incorporation of the T-DNA segment into the plant's genome. Once the T-DNA is incorporated into the plant genome, expression of the T-DNA-encoded genes causes the plant to produce unusual quantities

of plant hormones and other compounds that cause the plant cells to grow abnormally near the infection site, producing characteristic tumors.

Purposeful genetic transformation of plants requires a tool that can be used to insert new genes into a plant. This tool, regardless of its derivation, is called a vector. To date, the most common means for stable genetic transformation of plants involves the use of vectors derived from bacteria of the genus *Agrobacterium*. Biotechnologists have harnessed *Agrobacterium* to insert new genes of interest into plants by modifying the T-DNA segment of the bacterial DNA using standard recombinant methods. By deleting the genes on the T-DNA that cause tumors and then inserting desirable genes in their place, a wide variety of vectors can be produced to transfer desirable genes into plants. The genes transferred by way of *Agrobacterium* vectors become a permanent part of the plant's genome. DNA from plants, animals, bacteria, and viruses can be introduced into plants in this way.

One major drawback of *Agrobacterium* transformation is that insertion of T-DNA into the plant genome is essentially random. The genes on the T-DNA segment may not be efficiently transcribed at their location or the insertion of T-DNA may knock out an important plant gene by inserting in the middle of it. Therefore, a plant genetically transformed using an *Agrobacterium* vector is not necessarily guaranteed to perform as desired. A final drawback is that the vector works only with dicots, while many of the world's most important crops are monocots, such as wheat, rice, corn, and many other grain crops.

—Robert A. Sinnott

Expression Vectors

Expression vectors are cloning vectors designed to express the gene contained in the recombinant vector. In order to accomplish this, they must also provide the appropriate regulatory signals for the transcription and translation of the foreign gene. Regulatory sequences, which direct the cellular transcription machinery, are very different in bacteria and higher organisms. Thus, unless the vector provides the appropriate host regulatory sequences, foreign genes will not normally be expressed.

Expression vectors make it possible to pro-

duce proteins encoded by eukaryotic genes (that is, genes from higher organisms) in bacterial cells. Furthermore, producing proteins in this way often results in higher production rates than in the cells from which the gene was obtained. This technology not only is of immense benefit to scientists who study proteins but also is used by industry (particularly the pharmaceutical industry) to make valuable proteins. Proteins such as human insulin, growth hormone, and clotting factors that are difficult and extremely expensive to isolate from their natural sources are readily available because

they can be produced much more cheaply in bacteria. An added benefit of expression vectors is that actual human proteins are produced by bacteria and therefore do not provoke allergic reactions as frequently as insulin that is isolated from other species.

Artificial Chromosomes

In 1987, a new type of cloning vector was developed by David Burke, Maynard Olson, and their colleagues. These new vectors, artificial chromosomes, filled the need created by the Human Genome Project (HGP) to clone very large insert DNAs (hundreds of thousands to millions of base pairs in length). One of the goals of the HGP—to map and ultimately sequence all the chromosomes of humans, as well as a number of other “model” organisms’ genomic sequences—required a vector capable of propagating much larger DNA fragments than plasmid or phage vectors could propagate. The first artificial chromosome vector was developed in the yeast *Saccharomyces cerevisiae*. All the critical DNA sequence elements of a yeast chromosome were identified and isolated, and these were put together to create a yeast artificial chromosome (YAC). The elements of a YAC vector are an origin of replication, a centromere, telomeres, and a selectable marker suitable for yeast cells. A yeast origin of replication (similar to the origin of replication of bacterial plasmids) is a short DNA sequence that the host’s replicative enzymes, such as DNA polymerase, recognize as a site to initiate DNA replication. In addition to replicating, the new copies of a chromosome must be faithfully partitioned into daughter cells during mitosis. The centromere sequence mediates the partitioning of the chromosomes during cell division because it serves as the site of attachment for the spindle fibers in mitosis. Telomeres are the DNA sequences at the ends of chromosomes. They are required to prevent degradation of the chromosome and for accurate replication of DNA at the ends of chromosomes.

YACs are used much as plasmid vectors are. Very large insert DNAs are joined to the YAC vector, and the recombinant molecules are introduced into host yeast cells in which the artificial chromosome is replicated just as the host’s

natural chromosomes are. YAC cloning technology allows very large chromosomes to be subdivided into a manageable number of pieces that can be organized (mapped) and studied. YACs also provide the opportunity to study DNA sequences that interact over very long distances. Since the development of YACs, artificial chromosome vectors for a number of different host cells have been created.

Impact and Applications

Cloning vectors are one of the key tools of recombinant DNA technology. Cloning vectors make it possible to isolate particular DNA sequences from an organism and make many identical copies of this one sequence in order to study the structure and function of that sequence apart from all other DNA sequences. Until the development of the polymerase chain reaction (PCR), cloning vectors and their host cells were the only means to collect many copies of one particular DNA sequence. For long DNA sequences (those over approximately ten thousand base pairs), cloning vectors are still the only means to do this.

Gene therapy is a new approach to treating and perhaps curing genetic disease. Many common diseases are the result of defective genes. Gene therapy aims to replace or supplement the defective gene with a normal, therapeutic gene. One of the difficulties faced in gene therapy is the delivery of the therapeutic gene to the appropriate cells. Viruses have evolved to enter cells, sometimes only a very specific subset of cells, and deliver their DNA or RNA genome into the cell for expression. Thus viruses make attractive vectors for gene therapy. An ideal vector for gene therapy would replace viral genes associated with pathogenesis with therapeutic genes; the viral vector would then target the therapeutic genes to just the right cells. One of the concerns related to the use of viral vectors for gene therapy is the random nature of the viral insertion into the target cell’s chromosomes. Insertion of the vector DNA into or near certain genes associated with increased risk of cancer could theoretically alter their normal expression and induce tumor formation.

Plasmid DNA vectors encoding immuno-

genic proteins from pathogenic organisms are being tested for use as vaccines. DNA immunization offers several potential advantages over traditional vaccine strategies in terms of safety, stability, and effectiveness. Genes from disease-causing organisms are cloned into plasmid expression vectors that provide the regulatory signals for efficient protein production in humans. The plasmid DNA is inoculated intramuscularly or intradermally, and the muscle or skin cells take up some of the plasmid DNA and express the immunogenic proteins. The immune system then generates a protective immune response. There are two traditional vaccination strategies: One uses live, attenuated pathogenic organisms, and the other uses killed organisms. The disadvantage of the former is that, in rare cases, the live vaccine can cause disease. The disadvantage of the latter strategy is that the killed organism does not enter the patient's cells and make proteins like the normal pathogen. Therefore, one part of the immune response, the cell-mediated response, is usually not activated, and the protection is not as good. In DNA immunization, the plasmids enter the patient's cells, and the immunogenic proteins produced there result in a complete immune response. At the same time, there is no chance that DNA immunization will cause disease, because the plasmid vector does not carry all of the disease-causing organism's genes.

—Craig S. Laufer, updated by Bryan Ness

See also: Animal Cloning; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; DNA Replication; DNA Sequencing Technology; Gene Therapy; Genetic Engineering; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genomic Libraries; Knockout Genetics and Knockout Mice; Plasmids; Polymerase Chain Reaction; Protein Synthesis; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Stem Cells; Synthetic Genes; Telomeres; Transgenic Organisms; Xenotransplants.

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Color Blindness

Field of study: Diseases and syndromes

Significance: *Color blindness is a condition in people whose eyes lack one or more of the three color receptors present in most human eyes. It is an important condition to understand because so many people experience it to some degree. It is also a window into the inner workings of the eye and a marvelous example of the workings of Mendelian genetics.*

Key terms

CONES: the light-sensitive structures in the retina that function as color receptors and are the basis for color vision

DEUTERANOPES: people who lack the second kind of color receptor, the M cone, and thus are more sensitive to green

DICHROMATS: people whose eyes have only two of the three cones

PROTANOPES: people who lack the first kind of color receptor, the L cone, and thus are more sensitive to red

TRICHRONMATS: people whose eyes have all three color receptors, or cones

TRITANOPES: people who lack the third kind of color receptor, the S cone, and thus are more sensitive to blue

Color Vision

Light-sensitive structures in the retina called cones are the basis for color vision. A person with normal vision can distinguish seven pure hues (colors) in the rainbow: violet, blue, cyan, green, yellow, orange, and red. People with normal vision are trichromats, meaning that they have three types of cones: L, M, and S, named for particular sensitivities to light of long, medium, and short wavelengths. The human vision system detects color by comparing the relative rates at which the L, M, and S cones react to light. For example, yellow light causes the M and L cones to signal at about the same rate, and the person “sees” yellow. Strangely, the right amounts of green and red stimulate these cones in the same fashion, and the person will again see the color yellow even though there is no yellow light present. Since people have only three types of color receptors, it takes the proper mix of intensities of only three primary colors to cause a person to “see” all the colors of the rainbow. A tiny droplet of water on the screen of a color television or computer monitor will act like a magnifying lens and reveal that the myriad colors that are displayed are formed from tiny dots of only blue, green, and red.

Dichromats

People are referred to as “color blind” if they are dichromats, that is, if they have only two of

the three types of cones. Approximately 1.2 percent of males and 0.02 percent of females are protanopes (lack L cones); 1.5 percent of males and 0.01 percent of females are deuteranopes (lack M cones); but only 0.001 percent of males and females are tritanopes (lack S cones).

Dichromats can match all of the colors they see in the rainbow by mixing only two primary colors of light, but they see fewer (and different) hues in the rainbow than a person with normal vision. Protanopes and deuteranopes cannot distinguish between red and green. More exactly, protanopes tend to confuse reds, grays, and bluish blue-greens, while deuteranopes tend to confuse purples, grays, and greenish blue-greens.

S Pigment Genes

Tritanopes cannot distinguish between blue (especially greenish shades) and yellow. The genetic code for the S pigment lies on chromosome 7. The fact that the S pigment gene lies on an autosome explains why yellow-blue color blindness is manifested equally in males and females. The inheritance pattern is that of an autosomal dominant trait: Only one arm of the two arms of chromosome 7 has the defective allele in the affected parent, and since there is a 50 percent chance a child will receive the defective arm, 50 percent of the children will inherit the defect. In fact, the trait is often incompletely expressed, so that the majority of affected individuals retain some reduced S-cone function.

L and M Pigment Genes

Anomalous trichromats are more common than dichromats. They need three primary colors to match the hues of the rainbow, but they match them with different intensities than normal trichromats do because the peak sensitivities of their cones occur at wavelengths slightly different from normal. Their color confusion is similar to that of the dichromats, but less severe. About 1 percent of males and 0.03 percent of females have anomalous L cones, while 4.5 percent of males and 0.4 percent of females have anomalous M cones. The fact that far more males than females have some degree of red-green color blindness implies that the ge-

netic information for the pigments in L and M cones lies on the X chromosome.

The gene structures for M-cone and L-cone pigments are 96 percent the same, so it is likely that one began as a mutation of the other. Small mutations in either gene can slightly shift the color of peak absorption in the cones and produce an anomalous trichromat. Generally these mutations make M and L cones more alike. The similarity between the genes and the fact that they are adjacent to each other on the X chromosome can lead to a variety of copying errors during meiosis. People with normal color vision have one L-cone gene and one to three M-cone genes. The complete omission of either type of gene will result in severe red-green color blindness: protanopia or deutanopia. Hybrid genes that are a combination of L-cone and M-cone genes lead to less severe types of red-green color blindness, especially if there is also a normal copy of the gene present.

Red-green color blindness follows an X-gene recessive inheritance pattern. Suppose that Grandfather has a defective X gene (and is therefore color blind) and Grandmother is normal. Their male children are normal because they inherited their X genes from their mother, but their female children will be carriers because they had to inherit one X gene from their father. If the daughters married normal men, 50 percent of the grandsons got the defective gene from their mothers and were color blind, and 50 percent of the grandsons were normal. Likewise, 50 percent of the granddaughters were normal and 50 percent inherited the defective gene from their mothers and became carriers.

—Charles W. Rogers

See also: Classical Transmission Genetics; Congenital Defects; Dihybrid Inheritance; Hereditary Diseases; Monohybrid Inheritance.

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Web Sites of Interest

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Howard Hughes Medical Institute, Seeing, Hearing, and Smelling the World. <http://www.hhmi.org/senses>. Site that includes the articles "Color Blindness: More Prevalent Among Males" and "How Do We See Colors?"

Complementation Testing

Field of study: Techniques and methodologies

Significance: *Complementation testing is used to determine whether or not two mutations occur within the same gene.*

Key terms

ALLEL: a form of a gene; each gene (locus) in most organisms occurs as two copies called alleles

CISTRON: a unit of DNA that is equivalent to a gene; it encodes a single polypeptide

INBORN ERRORS OF METABOLISM: conditions that result from defective activity of an enzyme or enzymes involved in the synthesis, conversion, or breakdown of important molecules within cells

LOCUS (*pl. LOCI*): the location of a gene, often used as a more precise way to refer to a gene; each locus occurs as two copies called alleles

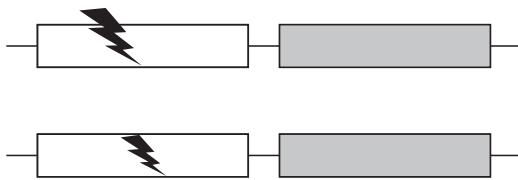
Finding Mutations

Most traits are the result of products from

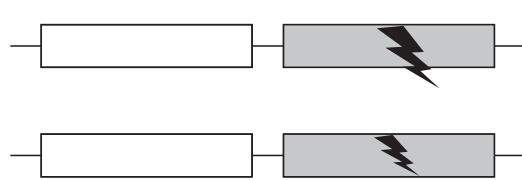
several genes. Mutations at any one of these genes may produce the same mutant phenotype. If the same mutant phenotype is observed in two different strains of an organism, there is no way, using simple observation, to determine whether this shared mutant phenotype represents a mutation in the same or different genes, or loci. One way of solving this problem is through complementation testing. If the mutations are alleles of the same locus, then a cross between mutant individuals from the two strains will only produce offspring with the mutant phenotype. In genetic terms, they fail to com-

Complementation

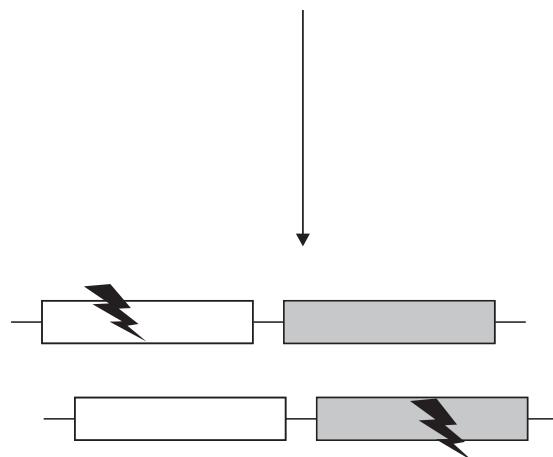
MALE



FEMALE



Two genes (boxes) reside on one chromosome. Each parent has two copies of every chromosome. The male parent is homozygous for a mutation in gene a (white box); the female is homozygous for a mutation in gene b (gray box).



The offspring has one non-mutated copy of each gene and as a consequence has a wildtype phenotype. The mutations therefore are said to **complement** each other.

plement each other and are therefore members of the same complementation group. If from the same cross, all the offspring are normal; the two mutations are at the same locus and they are said to complement each other. Researchers often want to define multiple alleles of a single gene in order to understand the gene's function better.

Often a researcher is interested in the genetic control of a particular biological process, such as the biochemistry of eye color in fruit flies. As a first step, researchers often screen large numbers of individuals to find abnormal phenotypes involving the process in which they are interested. For instance, researchers studying eye color in fruit flies may screen hundreds of thousands of fruit flies for abnormal eye colors. Complementation testing is then used to organize the mutations into complementation groups.

Complementation Testing and Inborn Errors of Metabolism

Human genetic diseases that affect the function of cellular enzymes are known as inborn errors of metabolism and were defined by Sir Archibald Garrod long before DNA was determined to be the hereditary material. Garrod studied families with alkaptonuria, a disease that causes urine to turn dark upon exposure to air. He determined that this biochemical defect was inherited in a simple Mendelian fashion.

George Beadle and Edward Tatum studied mutant strains of *Neurospora* and expanded on Garrod's work. They used radiation to generate random mutations that resulted in strains of *Neurospora* that could not grow without specific nutritional supplements (essentially creating yeast with inborn errors of metabolism). Some of the mutant strains required the addition of a specific amino acid to the media. Each mutant strain had its own specific requirements for growth, and each strain was shown to have a single defective step in a metabolic pathway. When strains that had different defects were grown together, they were able to correct each other's metabolic defect. This correction was termed metabolic complementation. Using complementation tests, Beadle and Tatum were able to establish the number of

genes required for a particular pathway. These studies formed the basis for the "one gene-one enzyme hypothesis": Each gene encodes a single enzyme required for a single step in a metabolic pathway. This hypothesis has since been renamed the "one gene-one polypeptide hypothesis" because some enzymes consist of multiple polypeptides, each of which is encoded by a single gene.

The Biochemical Basis for Complementation Testing

Complementation testing is useful for locating and identifying the genes affected by recessive or loss-of-function alleles. A researcher crosses two organisms that are each homozygous for a recessive mutation. If these two alleles affect the same gene, they will not complement each other, because the first-generation (F_1) offspring will inherit one mutant copy of the gene from one parent and a second mutant copy of the gene from the other parent, thus having no normal copies of the gene. If the mutations are alleles of two different genes, genes *A* and *B*, the F_1 offspring will receive a normal copy of *A* and a mutant copy of *B* from one parent and a mutant copy of *A* and a normal copy of *B* from the other, thus having one normal copy of each of the two genes and having a wild-type (normal) phenotype. The mutations are said to complement each other.

If a scientist is interested in a particular gene, obtaining as many alleles of that gene as possible will lead to a better understanding of how the gene works and what parts of the gene are essential for function. One way to identify new alleles of a gene is through an F_1 noncomplementation screen. In this type of screen, the researcher treats the model organism with radiation or chemicals to increase the rate of mutation. Any individuals from the screen that segregate the desired phenotype (white eyes, for example) in a Mendelian fashion are crossed with individuals carrying a known mutation in the gene of interest. If the progeny of this cross have white eyes (the mutant phenotype), then the two mutations have failed to complement each other and are most likely alleles of the same gene. Such noncomplementation screens have been used to identify genes involved in a

wide variety of processes ranging from embryo development in fruit flies to spermatogenesis in *Caenorhabditis elegans*.

—Michele Arduengo and Bryan Ness

See also: Biochemical Mutations; Chemical Mutagens; Chromosome Mutation; Inborn Errors of Metabolism; Linkage Maps; Mutation and Mutagenesis; Model Organism: *Caenorhabditis elegans*; Model Organism: *Neurospora crassa*.

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Complete Dominance

Field of study: Classical transmission genetics

Significance: *Complete dominance represents one of the classic Mendelian forms of inheritance. In an individual that is heterozygous for a trait, the allele that displays complete dominance will determine the phenotype of the individual. Knowing whether the pattern of expression of a trait is dominant or recessive helps in making predictions concerning the inheritance of a particular genetic condition or disorder in a family's history.*

Key terms

ALLELES: different forms of a gene at a specific locus; for each genetic trait there are typically two alleles in most organisms, including humans

GENOTYPE: a description of the alleles at a gene locus

HETEROZYGOTE: an individual with two different alleles at a gene locus

HOMOZYGOTE: an individual with two like alleles at a gene locus

INCOMPLETE DOMINANCE: the expression of a trait that results when one allele can only partly dominate or mask the other

LOCUS (*pl. LOCI*): a gene, located at a specific location on a chromosome, which in humans and many other organisms occurs in the same location on homologous chromosomes

PHENOTYPE: the observed expression of a gene locus in an individual

The Discovery and Definition of Dominance

Early theories of inheritance were based on the idea that fluids carrying materials for the production of a new individual were transmitted to offspring from the parents. It was assumed that substances in these fluids from the two parents mixed and that the children would therefore show a blend of the parents' characteristics. For instance, individuals with dark hair mated to individuals with very light hair were expected to produce offspring with medium-colored hair. The carefully controlled breeding studies carried out in the 1700's and 1800's did not produce the expected blended phenotypes, but no other explanation was suggested until Gregor Mendel proposed his model of inheritance. In the 1860's, Mendel repeated studies using the garden pea and obtained the same results seen by other investigators, but he counted the numbers of each type produced from each mating and developed his theory based on those observations.

One of the first observations Mendel dealt with was the appearance of only one of the parental traits in the first generation of offspring (the first filial, or F_1 , generation). For example, a cross of tall plants and dwarf plants resulted in offspring that were all tall. Mendel proposed that the character expression (in this case height) was controlled by a determining "factor," later called the "gene." He then proposed that there were different forms of this controlling factor corresponding to the different expressions of the characteristic and termed these "alleles." In the case of plant height, one allele produced tall individuals and the other produced dwarf individuals. He further proposed that in the cross of a tall (D) plant and a dwarf (d) plant, each parent contributed one factor for height, so the offspring were Dd . (Uppercase letters denote dominant alleles, while low-

ercase letters denote recessive alleles.) These plants contained a factor for both the tall expression and the dwarf expression, but the plants were all tall, so “tall” was designated the dominant phenotype for the height trait.

Mendel recognized from his studies that the determining factors occurred in pairs—each sexually reproducing individual contains two alleles for each inherited characteristic. When he made his crosses, he carefully selected pure breeding parents that would have two copies of the same allele. In Mendel’s terminology, the parents would be homozygous: A pure tall parent would be designated *DD*, while a dwarf parent would be designated *dd*. His model also proposed that each parent would contribute one factor for each characteristic to each offspring, so the offspring of such a mating should be *Dd* (heterozygous). The tall appearance of the heterozygote defines the character expression (the phenotype) as dominant. Dominance of expression for any characteristic cannot be guessed but must be determined by observation. When variation is observed in the phenotype, heterozygous individuals must be examined to determine which expression is observed. For phenotypes that are not visible, such as blood types or enzyme activity variations, a test of some kind must be used to determine which phenotype expression is present in any individual.

Mendel’s model and the appearance of the dominant phenotype also explains the classic 3:1 ratio observed in the second (F_2) generation. The crossing of two heterozygous individuals ($Dd \times Dd$) produces a progeny that is $\frac{1}{4} DD$, $\frac{1}{2} Dd$, and $\frac{1}{4} dd$. Because there is a dominant phenotype expression, the $\frac{1}{4} DD$ and the $\frac{1}{2} Dd$ progeny all have the same phenotype, so $\frac{3}{4}$ of the individuals are tall. It was this numerical relation that Mendel used to establish his model of inheritance.

The Functional Basis of Dominance

The development of knowledge about the molecular activity of genes through the 1950’s and 1960’s provided information on the nature of the synthesis of proteins using the genetic code passed on in the DNA molecules. This knowledge has allowed researchers to explain

variations in phenotype expression and to explain why a dominant allele behaves the way it does at the functional level. An enzyme’s function is determined by its structure, and that structure is coded for in the genetic information. The simplest situation is one in which the gene product is an enzyme that acts on a specific chemical reaction that results in a specific chemical product, the phenotype. If that enzyme is not present or if its structure is modified so that it cannot properly perform its function, then the chemical action will not be carried out. The result will be an absence of the normal product and a phenotype expression that varies from the normal expression. For example, melanin is a brown pigment produced by most animals. It is the product of a number of chemical reactions, but one enzyme early in the process is known to be defective in albino animals. Lacking normal enzyme activity, these animals cannot produce melanin, so there is no color in the skin, eyes, or hair. When an animal has the genetic composition *cc* (*c* designates colorless, or albino), it has two alleles that are the same, and neither can produce a copy of the normal enzyme. Animals with the genetic composition *CC* (*C* designates colored, or normal) have two copies of the allele that produces normal enzymes and are therefore pigmented. When homozygous normal (*CC*) and albino (*cc*) animals are crossed, heterozygous (*Cc*) animals are produced. The *c* allele codes for production of an inactive enzyme, while the *C* allele codes for production of the normal, active enzyme. The presence of the normal enzyme promotes pigment production, and the animal displays the pigmented phenotype. The presence of pigment in the heterozygote leads to the designation that the pigmented phenotype is dominant to albinism or, conversely, that albinism is a recessive phenotype because it is seen only in the homozygous (*cc*) state.

The same absence or presence of an active copy of an enzyme explains why blood types A and B are both dominant to blood type O. When an *A* allele or a *B* allele is present, an active enzyme promotes the production of a substance that is identified in a blood test; the blood type A expression or the blood type B expression is seen. When neither of these alleles

is present, the individual is homozygous *OO*. There is no detectable product present, and the blood test is negative; therefore, the individual has blood type O. When the *A* allele and the *B* allele are both present in a heterozygous individual, each produces an active enzyme, so both the *A* and the *B* product are detected in blood tests; such an individual has blood type AB. The two phenotypes are both expressed in the heterozygote, a mode of gene expression called codominance.

When there are a number of alleles present for the expression of a characteristic, a dominance relation among the phenotype expressions can be established. In some animal coats, very light colors result from enzymes produced by a specific allele that is capable of producing melanin but at a much less efficient rate than the normal version of the enzyme. In the rabbit, chinchilla (*c^{ch}*) is such an allele. In the *Cc^{ch}* heterozygote, the normal allele (*C*) produces a normal, rapidly acting enzyme, and the animal has normal levels of melanin. The normal pigment phenotype expression is observed because the animals are dark in color, so this expression is dominant to the chinchilla phenotype expression. In the heterozygote *c^{ch}c*, the slow-acting enzyme produced by the *c^{ch}* allele is present and produces pigment, in a reduced amount, so the chinchilla phenotype expression is observed and is dominant to the albino phenotype expression. The result is a dominance hierarchy in which the normal pigment phenotype is dominant to both the chinchilla and the albino phenotypes, and the chinchilla expression is dominant to the albino expression.

It is important to note that the dominant phenotype is the result of the protein produced by each allele. In the previous examples, both the albino allele and the chinchilla allele produce a product—a version of the encoded enzyme—but the normal allele produces a version of the enzyme that produces more pigment. The relative ability of the enzymes to carry out the function determines the observed phenotype expression and therefore the dominance association. The *C* allele does not inhibit the activity of either of the other two alleles or their enzyme products, and the allele does not,

therefore, show dominance; rather, its enzyme expression does.

Dominant Mutant Alleles

Dominance of a normal phenotype is fairly easy to explain at the level of the functioning protein because the action of the normal product is seen, but dominance of mutant phenotypes is more difficult. Polydactyly, the presence of extra fingers on one hand or extra toes on one foot, is a dominant phenotype. The mechanism that leads to this expression and numerous other developmental abnormalities is not yet understood. One insight comes from the genetic expression of enzymes that are composed of two identical polypeptide subunits. In this situation, the gene locus codes for one polypeptide, but it takes two polypeptide molecules joined together to form a functional enzyme molecule. In order to function normally, both of the polypeptide subunits must be normal. A heterozygote can have one allele coding for a normal polypeptide and the other allele coding for a mutant, nonfunctional polypeptide. These polypeptides will join together at random to form the enzyme. The possible combinations will be defective-defective, which results in a nonfunctional enzyme; defective-normal, which also results in a nonfunctional enzyme; and normal-normal, which is a normal, functional enzyme. The majority of the enzyme molecules will be nonfunctional, and their presence will interfere with the action of the few normal units. The normal function will be, at best, greatly reduced, and the overall phenotype will be abnormal. One form of hereditary blindness is dominant because the presence of abnormal proteins interferes with the transport of both protein types across a membrane to their proper location in the cells that react to light. The abnormal phenotype appears in the heterozygote, so the abnormal phenotype is dominant. A number of human disease conditions, including some forms of cancer, display a dominant mode of inheritance.

Sometimes a trait that appears to be dominant is actually more complex. The Manx trait in cats, which results in a very short, stubby tail, occurs only in heterozygous individuals. On the surface, this would appear to be a simple

case of dominance, where the Manx allele, *T*, is dominant to the normal tail allele, *t*. Recall that when two heterozygotes are mated, the expected phenotype ratio in the offspring is 3:1, dominant:recessive. If two Manx cats are mated, the phenotype ratio in the offspring is 2:1, Manx:normal, because kittens that are homozygous for the Manx allele (*TT*) die very early in development and are reabsorbed by the mother cat. Therefore, the Manx allele does not display complete dominance, but rather incomplete dominance. The Manx allele is lethal in the homozygous state and causes a short, stubby tail in the heterozygous state. This occurs because the Manx allele causes a developmental defect that affects spinal development. If one normal allele is present, the spine develops enough for the cat to survive, although it will display the Manx trait. In mutant homozygotes (*TT*) the spine is unable to develop, which proves lethal to the developing fetus.

Impact and Applications

One of the aims of human genetic research is to find cures for inherited conditions. When a condition shows the recessive phenotype expression, treatment may be effective. The individual lacks a normal gene product, so supplying that product can have a beneficial effect. This is the reason for the successful treatment of diabetes using insulin. There are many technical issues to be considered in such treatments, but current successes give hope for the treatment of other recessive genetic conditions.

Dominant disorders, on the other hand, will be much more difficult to treat. An affected heterozygous individual has a normal allele that produces normal gene product. The nature of the interactions between the products results in the defective phenotype. Supplying more normal product may not improve the situation. A great deal more knowledge about the nature of the underlying mechanisms will be needed to make treatment effective.

—D. B. Benner, updated by Bryan Ness

See also: Biochemical Mutations; Dihybrid Inheritance; Epistasis; Incomplete Dominance; Mendelian Genetics; Monohybrid Inheritance; Multiple Alleles.

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Congenital Defects

Field of study: Diseases and syndromes

Significance: *Congenital defects are malformations caused by abnormalities in embryonic or fetal development that may interfere with normal life functions or cause a less severe health problem. The defect may be morphological or biochemical in nature. Understanding the causes of birth defects has led to improved means of detection and treatment.*

Key terms

SENSITIVE PERIOD: a critical time during development when organs are most susceptible to teratogens

TERATOGEN: any agent that is capable of causing an increase in the incidence of birth defects

TERATOLOGY: the science or study of birth defects

Normal Development

In order to understand the causes of birth defects, it is necessary to have some understanding of the stages of normal development. If the time and sequence of development of each organ are not correct, an abnormality may result. It has been useful to divide human pregnancy into three major periods: the preembryonic stage, the embryonic stage, and the fetal stage.

The preembryonic stage is the first two weeks after fertilization. During this stage, the fertilized egg undergoes cell division, passes down the Fallopian tube, and implants in the uterine wall, making a physical connection with the mother. It is of interest to note that perhaps as many as one-half of the fertilized eggs fail to implant, while the half, which do implant, do not survive the second week. The second stage, the embryonic stage, runs from the

beginning of the third week through the end of the eighth week. There is tremendous growth and specialization of cells during this period, as all of the body's organs are formed. The embryonic stage is the time during which most birth defects are initiated.

The fetal stage runs from the beginning of the ninth week to birth. Most organs continue their rapid growth and development during this final period of gestation leading up to birth. By the end of the eighth week, the embryo, although it has features of a human being, is only about 1 inch (2.54 centimeters) long. Its growth is amazing during this period, reaching 12 inches (30 centimeters) by the end of the fifth month and somewhere around 20 inches (50 centimeters) by birth. It is evident from the description of normal development that the changes the embryo and fetus undergo are very rapid and complicated. It is not

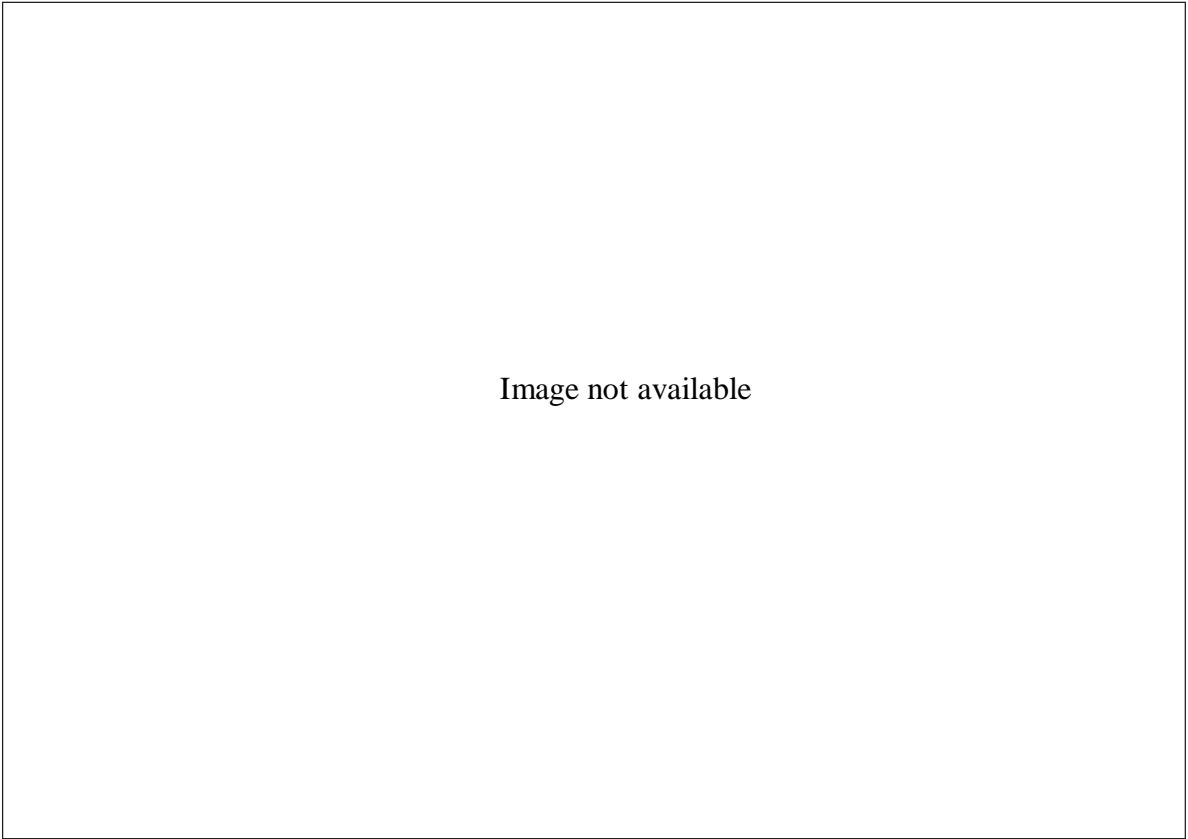
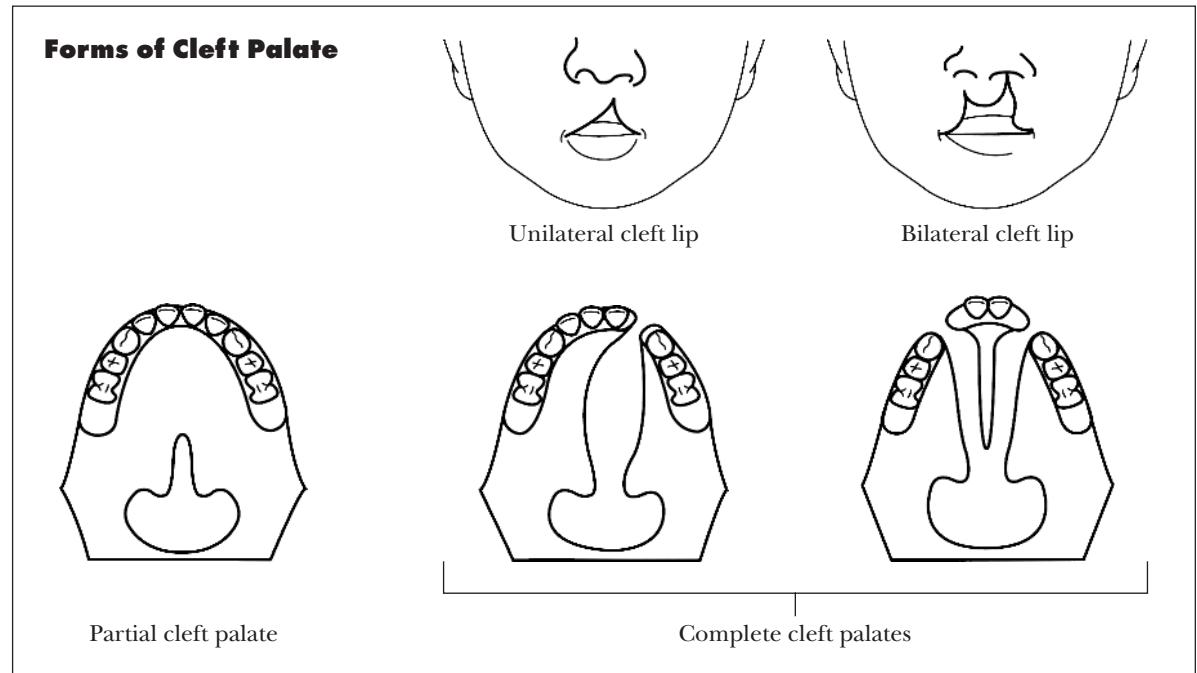


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Writer Firdaus Kanga of Bombay was born with the disease osteogenesis imperfecta. A film based on his autobiographical novel Try to Grow was produced in the mid-1990's. (AP/Wide World Photos)



(Hans & Cassidy, Inc.)

unexpected that mistakes can happen, leading to congenital disorders.

Causes of Birth Defects

Throughout history, examples of birth defects have been described by all cultures and ethnic groups. Although the incidence of specific malformations may vary from group to group, the overall incidence of birth defects is probably similar in all people on earth. It is estimated that three out of every hundred newborns have some sort of major or minor disorder. An additional 2 to 3 percent have malformations that fully develop sometime after birth. When it is also realized that perhaps another 5 percent of all fertilized eggs have severe enough malformations to lead to an early, spontaneous abortion, the overall impact of birth defects is considerable.

Humans have long sought an explanation for why some couples have babies afflicted with serious birth defects. Such children were long regarded as "omens" or warnings of a bad event to come. The word "teratology" (Greek for "monster causing") was coined by scientists to reflect the connection of "monster" births with

warnings. Frequently, ancient people sacrificed such babies. It was thought that such pregnancies resulted from women mating with animals or evil spirits. Maternal impression has long been invoked as an explanation for birth defects, and from early Greek times until even relatively recent times, stories and superstitions abounded.

Of the birth defects in which a specific cause has been identified, it has been found that some are caused by genetic abnormalities, including gene mutations and chromosomal changes, while others are caused by exposure of the pregnant woman and her embryo or fetus to some sort of environmental toxin such as radiation, viruses, drugs, or chemicals.

Examples of Birth Defects

Many birth defects are caused by changes in the number or structure of chromosomes. The best-known chromosomal disorder is Down syndrome, which results from individuals having an extra chromosome 21, giving them forty-seven chromosomes rather than the normal forty-six. A person with Down syndrome characteristically has a flattened face, square-shaped

ears, epicanthal folds of the eye, a short neck, poor muscle tone, slow development, and subnormal intelligence. Cystic fibrosis is an example of a defect caused by a single gene. Affected people inherit a recessive gene from each parent. The disorder is physiological in nature and results in a lack of digestive juices and the production of thick and sticky mucus that tends to clog the lungs, pancreas, and liver. Respiratory infections are common, and death typically occurs by the age of thirty. Cleft lip, or cleft palate, is multifactorial in inheritance (some cases are caused by chromosomal abnormalities or by single-gene mutations). Multifactorial traits are caused by many pairs of genes, each having a small effect, and are usually influenced by factors in the environment. The result is that such traits do not follow precise, predictable patterns in a family.

Genetic factors account for the great majority (perhaps 85 to 90 percent) of the birth defects in which there is a known cause. The remaining cases of known cause are attributed to maternal illness; congenital infections; exposure to chemicals, drugs, and medicines; and physical factors such as X rays, carbon dioxide, and low temperature. The "government warning" on liquor bottles informs pregnant women that if they drink alcohol during a sensitive period of prenatal development, they run the risk of having children with fetal alcohol syndrome. There is a wide variation in the effects of alcohol on a developing fetus. Alcohol exposure can lead to an increased frequency of spontaneous abortion, and it depresses growth rates, both before and after birth. Facial features of a child exposed to alcohol may include eye folds, a short nose, small mid-face, a thin upper lip, a flat face, and a small head. These characteristics are likely to be associated with mental retardation. Frequently, however, otherwise normal children have learning disorders and only a mild growth deficiency. Variation in the symptoms of prenatal alcohol exposure has made it difficult to estimate the true incidence of the fetal alcohol syndrome. Estimates for the United States range from 1 to 3 per 1,000 newborns.

In 50 to 60 percent of babies born with a major birth disorder, no specific cause can be

identified. Because of this rather large gap in knowledge, nonscientific explanations about the causes of birth defects flourish. What is known is that most congenital defects, whether caused by a genetic factor or an environmental factor, are initiated during the embryonic period. It is also known that some disorders, such as learning disorders, frequently result from damage to the fetus during the last three months of pregnancy. Knowledge about what can be done by parents to avoid toxic exposure and activity that could cause birth defects is critical.

—Donald J. Nash

See also: Albinism; Color Blindness; Consanguinity and Genetic Disease; Cystic Fibrosis; Developmental Genetics; Down Syndrome; Dwarfism; Fragile X Syndrome; Hemophilia; Hereditary Diseases; Hermaphrodites; Huntington's Disease; Inborn Errors of Metabolism; Klinefelter Syndrome; Metafemales; Mitochondrial Diseases; Neural Tube Defects; Phenylketonuria (PKU); Prader-Willi and Angelman Syndromes; Prion Diseases; Kuru and Creutzfeldt-Jakob Syndrome; Pseudohermaphrodites; Sickle-Cell Disease; Tay-Sachs Disease; Testicular Feminization Syndrome; Thalidomide and Other Teratogens; Turner Syndrome; XYY Syndrome.

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Written for the general public, a guidebook to clinical and research information on hereditary conditions and birth defects. Includes a general essay on the basics of genetic science and its medical applications.

More than 600 cross-referenced entries are alphabetically arranged and cover genetic anomalies, diagnostic procedures, causes of mutations, and high risk groups.

Web Sites of Interest

March of Dimes Birth Defects Foundation. <http://www.marchofdimes.com>. Includes fact sheets and links to resources on birth defects.

Medline Plus. <http://www.nlm.nih.gov/medlineplus>. Medline, sponsored by the National Institutes of Health, is one of the first stops for any medical question, and it offers information and references on most genetic diseases, birth defects, and disorders.

National Birth Defects Network. <http://www.nbdpn.org>. National support group with information, resources, links.

National Institutes of Health, National Library of Medicine. <http://www.nlm.nih.gov/medlineplus/birthdefects.html>. Government site that includes dozens of links to resources on birth defects, with information on genetics, treatments, statistics, and more.

Consanguinity and Genetic Disease

Fields of study: Diseases and syndromes; Population genetics

Significance: *The late onset of sexual maturity and the random mating habits of most humans make studying rare mutations in human populations especially difficult. Small, isolated communities in which mates are chosen only from within the population lead to consanguineous populations that can serve as natural laboratories for the study of human genetics, especially in the area of human disease.*

Key terms

ALLELES: genetic variants of a particular gene

CONSANGUINEOUS: literally, "of the same blood," or sharing a common genetic ancestry; members of the same family are consanguineous to varying degrees

ISOLATE: a community in which mates are chosen from within the local population rather than from outside populations

The Importance of Isolates

When studying the genetics of the fruit fly or any other organism commonly used in the laboratory, a researcher can choose the genotypes of the flies that will be mated and can observe the next few generations in a reasonable amount of time. Experimenters can also choose to mate offspring flies with their siblings or with their parents. As one might expect, this is not possible when studying the inheritance of human characteristics. Thus, progress in human genetics most often relies on the observation of the phenotypes of progeny that already exist and matings that have already occurred. Many genetic diseases only appear when a person is homozygous for two recessive alleles; thus a person must inherit the same recessive allele from both parents. Since most recessive alleles are rare in the general population, the chance that both parents carry the same recessive allele is small. This makes the study of these diseases very difficult. The chance that both parents carry the same recessive allele is increased whenever mating occurs between individuals who share some of the same genetic background. These consanguineous matings produce measurably higher numbers of offspring with genetic diseases, especially when the degree of consanguinity is at the level of second cousin or closer.

In small religious communities in which marriage outside the religion is forbidden, and in small, geographically isolated populations in which migration into the population from the outside is at or near zero, marriages often occur between two people who share some common ancestry; therefore, the level of consanguinity can be quite high. These communities thus serve as natural laboratories in which to study genetic diseases. Geographically isolated mountain and island communities are found in many areas of the world, including the Caucasus Mountains of Eurasia, the Appalachian Mountains of North America, and many areas in the South Pacific. Culturally isolated communities are also of worldwide distribution.

Among the Druse, a small Islamic sect, first-cousin marriages approach 50 percent of all marriages. The Amish, Hutterites, and Dunkers in the United States are each descended from small groups of original settlers who immigrated in the eighteenth and nineteenth centuries and rarely mated with people from outside their religions.

The Amish

There are many reasons the Amish serve as a good example of an isolate. The original immigration of Amish to America consisted of approximately two hundred settlers. In subsequent generations, the available mates came from the descendants of the original settlers. With mate choice this limited, it is inevitable that some of the marriages will be consanguineous. Consanguinity increases as further marriages take place between the offspring of consanguineous marriages. Current estimates are that the average degree of consanguinity of Amish marriages in Lancaster County, Pennsylvania, is at the level of marriages between second cousins.

Other factors that make the Amish good subjects for genetic research are their high fertility and their high level of marital fidelity. Thus, if both parents happen to be heterozygous for a particular genetic disease, the chance that at least one of the offspring will show the disease is high. In families of two children, there is a 44 percent chance that at least one child will show the trait. This increases to 70 percent of the families with four children and to more than 91 percent of the families with eight children, a common number among the Amish. Because of the high marital fidelity, researchers do not have to worry about illegitimacy when making these estimates.

Many genetic diseases that are nearly nonexistent in the general population are found among the Amish. The allele for a type of dwarfism known as the Ellis-van Creveld syndrome is found in less than 0.1 percent of the general population; among the Lancaster Amish, however, the allele exists in approximately 7 percent of the population. Other genetic diseases at higher levels among the Amish include cystic fibrosis, limb-girdle muscular

dystrophy, pyruvate kinase-deficient hemolytic anemia, and several inherited psychological disorders. Having more families and individuals with these diseases to study helps geneticists and physicians discover ways to treat the problems and even prevent them from occurring.

—Richard W. Cheney, Jr.

See also: Cystic Fibrosis; Dwarfism; Genetic Load; Hardy-Weinberg Law; Hereditary Diseases; Heredity and Environment; Inbreeding and Assortative Mating; Lateral Gene Transfer; Mendelian Genetics; Natural Selection; Polyploidy; Population Genetics; Punctuated Equilibrium; Quantitative Inheritance; Sociobiology; Tay-Sachs Disease.

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Key terms

METABOLIC PATHWAY: a biochemical process that converts certain chemicals in the body to other, often more useful, chemicals with the help of proteins called enzymes

NEUROTRANSMITTER: a chemical that carries messages between nerve cells

Biochemical Abnormalities

Early attempts to identify the root of the tendency for criminal behavior fell under the auspices of biological determinism, which sought to explain and justify human society as a reflection of inborn human traits. For example, Italian physician Cesare Lombroso reported in *L'uomo delinquente* (1876; criminal man) that certain "inferior" groups, by virtue of their apish appearance, were evolutionary throwbacks with criminal tendencies. Since that time, more sophisticated scientific methods have been employed to seek the "root causes" of criminality. Among the most prominent findings are those that indicate that certain biochemical imbalances, particularly in neurotransmitters, may lead to a range of "abnormal" behaviors. For example, levels of the neurotransmitter serotonin have been found to be low in many people who have attempted suicide and in people with poor impulse control, such as children who torture animals and impulsive arsonists. However, the environment itself may lower or raise serotonin levels, calling into question the importance of genetic influence. The psychological effects of serotonin are also far-reaching, with antidepressant drugs such as Prozac functioning by increasing the amount of time serotonin remains in the system after its release (latency).

Abnormalities in dopamine levels (the primary neurotransmitter in the brain's "pleasure center") have also been implicated in aggressive, antisocial behavior. In 1995, researchers reported that increased latency of dopamine might be associated with a tendency toward violence among alcoholics. A genetic abnormality on the X chromosome that causes a defect in the enzyme monoamine oxidase A was reported by researchers in 1993. This enzyme is responsible for degrading certain neurotransmitters, including dopamine and epinephrine. This defect was linked to a heritable history of low in-

Criminality

Field of study: Human genetics and social issues

Significance: *The pursuit of genetic links to criminality is a controversial field of study that has produced several intriguing examples of the apparent contribution of genetic defects to criminal behavior. However, most of these defects involve major alterations in metabolic pathways that, in turn, affect numerous characteristics. Experts disagree on the validity and significance of these data. These research efforts have also come under strong criticism by opponents who fear that such discoveries will be used to charge that certain ethnic or racial groups are genetically predisposed to deviant behaviors such as criminality.*

telligence quotient (IQ) and violent acts in one Dutch family. Males who possess an extra Y chromosome (XYY syndrome males) also demonstrate a variety of behavioral difficulties and are overrepresented in prisons and mental institutions. However, no link to criminal behavior has been established.

In all cases, such genetic abnormalities affect numerous characteristics (often including mental capabilities) and manifest themselves as any number of unassociated antisocial behaviors ranging from exhibitionism to arson. Since criminality simply refers to a violation of the law and since there are numerous types of crimes and motivations for them (such as anger, revenge, and financial gain), it is difficult to make claims of definitive, nonenvironmental links between biochemical disorders and criminal behavior. Poorly defined, multifaceted social descriptors (for example, violence, aggression, and intelligence) are usually used to represent such behaviors and, as such, cannot be considered true "characters." As child psychiatrist Michael Rutter has said, to claim that there is a gene for crime is "like saying there's a gene for Roman Catholicism."

Impact and Applications

Genetic links to criminality entered the public spotlight in the early 1990's as part of the U.S. government's Violence Initiative, championed by Secretary of Health and Human Services Louis Sullivan. The uproar began in 1992 when Frederick Goodwin, then director of the Alcohol, Drug Abuse, and Mental Health Administration, made comments comparing urban youth to aggressive jungle primates. The public feared that research on genetic links to criminality would be used to justify the disproportionate numbers of African Americans and Hispanics in the penal system. Psychiatrist Peter Breggin also warned that unproved genetic links would be used as an excuse to screen minority children and give them sedating drugs to intervene in their impending aggression and criminality. After all, forced sterilization laws had been enacted in thirty U.S. states in the 1920's to prevent reproduction by the "feebleminded" and "moral degenerate." In 1993, public protest led to the temporary cancella-

tion of Genetic Factors in Crime, a federally funded conference organized by philosopher David Wasserman. A similar symposium, Genetics of Criminal and Antisocial Behaviour, was held in London in 1995. However, the public remains highly suspicious of the motivation for such research.

In an era in which genes have been implicated in everything from manic depression to the propensity to change jobs, the belief that genes are responsible for criminal behavior is very enticing. However, such a belief may have severe ramifications. To the extent that society accepts the view that crime is the result of pathological and biologically deviant behavior, it is possible to ignore the necessity to change social conditions such as poverty and oppression that are also linked to criminal behavior. Moreover, this view may promote the claim by criminals themselves that their "genes" made them do it. While biochemical diagnosis and treatment with drugs may be simpler and therefore more appealing than social intervention, it is reminiscent of the days when frontal lobotomy (surgery of the brain) was the preferred method of biological intervention for aggressive mental patients. In the future, pharmacological solutions to social problems may be viewed as similarly inhumane.

—Lee Anne Martínez

See also: Aggression; Alcoholism; Altruism; Behavior; Biological Determinism; Developmental Genetics; DNA Fingerprinting; Eugenics; Eugenics: Nazi Germany; Forensic Genetics; Hardy-Weinberg Law; Heredity and Environment; RFLP Analysis; Sociobiology; Sterilization Laws; XYY Syndrome.

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Cystic Fibrosis

Field of study: Diseases and syndromes

Significance: *Cystic fibrosis, although a rare disease, is the most common lethal inherited disease among Caucasians in the United States and the United Kingdom. Advances in genetic screening and treatment may someday result in the prevention or elimination of this disease.*

Key terms

EPITHELIAL CELLS: cells responsible for transporting salt and water

RECESSIVE GENE: a gene that in diploid organisms gets expressed only when it represents both copies at a gene locus

Causes and Symptoms

Cystic fibrosis is caused by an abnormal recessive gene that must be inherited from both parents. If both parents carry the gene, their child has a 25 percent chance of inheriting the abnormal gene from both parents and thus having the disease. The child has a 50 percent chance of having one normal and one abnormal gene, thus becoming a carrier of the disease.

Cystic fibrosis is chronic and has no known cure. Generally, symptoms are apparent shortly after birth and become progressively more serious. Abnormally thick mucus blocks the ducts

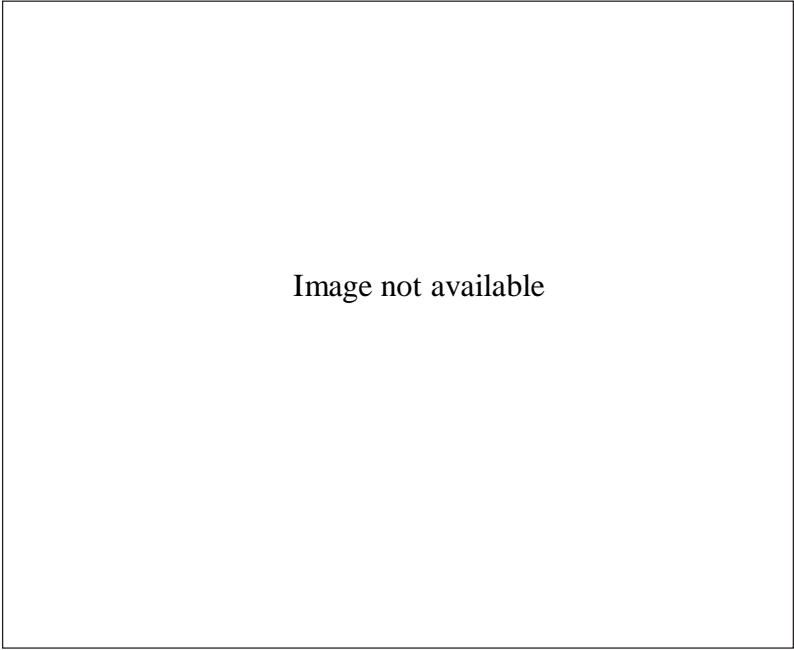


Image not available

At the National Heart, Lung, and Blood Institute in Bethesda, Maryland, Dr. Ronald Crystal works on a nasal spray of proteins that have been genetically engineered to break down mucus in the lungs of individuals with cystic fibrosis. (AP/Wide World Photos)

in sweat glands and glands in the lungs and pancreas. Diagnosis usually involves a simple test that measures excessive sodium and chloride (salt) in the person's sweat. Ten percent of newborns with cystic fibrosis cannot excrete undigested material in their intestines and so develop a blocked intestine. Symptoms of this condition include a swollen abdomen, vomiting, constipation, and dehydration. The child may become severely undernourished because the digestive system is not functioning properly. In turn, the malnutrition causes poor weight gain, impaired blood clotting, slow bone growth, and poor overall growth. Digestive problems usually increase as the child ages.

Although cystic fibrosis can damage other organs, the most serious complications from the disease involve the lungs. Respiratory problems develop as the affected person ages. The lungs may appear normal in early life but will later malfunction. Normal lungs fight off infection by secreting mucin, the primary component of mucus. The mucin helps trap germs and foreign particles in the lungs. The mucus is swept toward the throat by hairlike projec-

tions known as cilia and is then expelled from the respiratory tract. Lungs also produce a natural antibiotic called defensin, which destroys germs.

For people with cystic fibrosis, the defense mechanisms are crippled. The lungs produce mucin that is too thick and sticky to be flushed away by the cilia. The mucus stays in the lungs and provides an ideal breeding ground for bacteria. The person suffers from repeated respiratory infections such as bronchitis and pneumonia. In addition, cystic fibrosis prevents the body from properly absorbing salt into the epithelial cells. The lungs cannot absorb the salt, causing a buildup of salt outside the

cells. This buildup disables the natural antibiotic defensin, which in turn leads to bacterial infections that cause the increased production of mucus. The damage from infections and the buildup of mucus in the airways eventually makes breathing impossible.

Treatment and Outlook

Approximately thirty thousand Americans have cystic fibrosis, and one in twenty-five Caucasians are carriers of the disease. In the 1950's, an infant with cystic fibrosis seldom survived past the first year or two. With aggressive medical treatment and therapy to relieve the severe symptoms, the average life span of a person with cystic fibrosis had increased to twenty-nine years by the early 1990's, and a person with the disorder may live as long as forty years.

Treatment depends on the organs involved. People with cystic fibrosis follow special diets and take dietary supplements so that they have proper nutrition and receive the necessary digestive enzymes, salts, and vitamins that cannot pass through the blocked ducts. The disease makes the digestion and absorption of fats and

proteins difficult because certain enzymes are depleted as a result of blocked ducts in the pancreas.

The affected person also undergoes daily backslapping designed to break up the mucus in the lungs. Antibiotics can reduce infections of the lungs. In the 1990's, therapy involving the inhalation of a special enzyme began. This enzyme helps break down the thick mucus so that it is easier to cough out. In late 1997, a potent antibiotic was recommended to the Food and Drug Administration. This antibiotic, called Tobi (tobramycin for inhalation), was the first inhaled antibiotic and appeared to increase the lung function of some cystic fibrosis patients.

Research continues in an effort to determine the nature of the genetic defect that causes cystic fibrosis so that the normal function of the defective gene can be replaced by specially designed therapies. Recent and ongoing attempts at gene therapy have not yet been successful. A corrected form of the gene has been engineered and recombinant adenoviruses have been used to try to deliver the corrected gene to epithelial cells in the lungs. The recombinant viruses can easily be inhaled by atomizing a solution containing the viruses. Unfortunately, patients in clinical trials have often developed immune responses to the adenoviruses and the transfer efficiency of the adenoviruses was too low to cause much lasting im-

Gene Therapy for Cystic Fibrosis

Once scientists discovered the cystic fibrosis gene, *CF*, and its protein product, cystic fibrosis transmembrane regulator (CFTR), attempts at gene therapy were quickly initiated. Since most of the life-threatening complications of this disease are seen in the respiratory system, that system became the main target for gene replacement therapy.

Early attempts at gene therapy involved the attachment of a functional *CF* gene to a virus that acts as a vector and the subsequent introduction of this virus to the respiratory epithelium in an aerosol. Several problems arose. Although a cystic fibrosis patient's immune system does not function properly, especially in the respiratory system, the immune system is active enough to prevent many of the viruses from entering the target cells. Those that did penetrate and inserted the normal *CF* gene induced only a transient benefit. This most likely occurred because of the high turnover rate of surface epithelial cells. The epithelial cells could incorporate the gene that codes for normal CFTR, but cells that had not been repaired would soon replace the repaired cells. Continued aerosol applications were also not helpful, because the body began producing antibodies to the viral vector, which further reduced the virus's ability to enter cells and introduce an active *CF* gene. Another problem was the inflammation caused by the virus itself.

To surmount some of these difficulties, other approaches have been tried. A team of Australian researchers has looked at preconditioning the respira-

tory epithelium with a detergent-like substance found in normal lungs as a way of increasing virus uptake by the epithelium. This system has had success in mice and has led to longer-term improvements of lung function. These researchers speculate that long-term improvement occurs when some epithelial stem cells have had defective DNA replaced by the DNA for functional CFTR. In Cleveland, researchers have tried to insert the *CF* gene directly into cells without a viral vector. They have accomplished this by compacting the DNA into a particle small enough to enter the cell.

Another novel gene therapy has been labeled SMaRT by its proponents. This therapy takes advantage of the need to remove introns (noncoding intervening sequences) from pre-messenger RNA (pre-mRNA) in eukaryotes and then to splice the exons (coding sequences) together to form functional mRNA. In this procedure, multiple copies of a "minigene" that contain a good copy of the exon that normally contains the defect in the *CF* gene are introduced to the epithelial cells. When the pre-mRNA is processed, there are so many more copies of the corrected exon that it is usually spliced into the CFTR mRNA. This technique has the advantage of not disrupting the cells' normal regulation of the CFTR protein. However, the viruses involved in the transfer of the minigenes face the same barriers that all viral vectors face in cystic fibrosis gene therapy.

—Richard W. Cheney, Jr.

provement. Researchers still hope to improve gene therapy methods and eventually find ways either to cure cystic fibrosis fully or at least to reduce some of the more serious symptoms.

Genetic testing for cystic fibrosis can locate the defect responsible for the disease in about 75 percent of afflicted people. Since cystic fibrosis can cause more than one hundred other genetic mutations, however, a simple test to detect the variations may be very difficult to develop. In addition, the symptoms of the disease can vary from severe to extremely mild. Research focuses on the development of inexpensive and accurate diagnosis as well as sound genetic counseling in order to reduce the occurrence of the disease. In early 1997, the National Institutes of Health (NIH) recommended that all couples planning to have children should be offered the option of testing for the cystic fibrosis gene mutations.

—Virginia L. Salmon, updated by Bryan Ness

See also: Amniocentesis and Chorionic Villus Sampling; Biochemical Mutations; Chromosome Mutation; Chromosome Walking and Jumping; Congenital Defects; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering; Genetic Screening; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Human Genetics; Inborn Errors of Metabolism; Multiple Alleles.

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Web Sites of Interest

Cystic Fibrosis Foundation. <http://www.cff.org>. This national organization's site includes information on the genetics of cystic fibrosis.

Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including cystic fibrosis.

Cytokinesis

Field of study: Cellular biology

Significance: Cytokinesis is a process, usually occurring concurrent with mitosis, in which the cytoplasm and organelles are divided into two new cells. In eukaryotes, mitosis and meiosis involve division of the nucleus, while cytokinesis is the division of the cytoplasm.

Key terms

- BINARY FISSION:** cell division in prokaryotes in which the plasma membrane and cell wall grow inward and divide the cell in two
- CELL CYCLE:** a regular and repeated sequence of events during the life of a cell; it ends when a cell completes dividing
- DAUGHTER CELLS:** cells that result from cell division
- INTERPHASE:** the phase that precedes mitosis in the cell cycle, a period of intense cellular activities that include DNA replication
- MEIOSIS:** a type of cell division that leads to production of gametes (sperm and egg) during sexual reproduction
- MITOSIS:** nuclear division, a process of allotting a complete set of chromosomes to two daughter nuclei

Events Leading to Cytokinesis

Cytokinesis is the division or partitioning of the cytoplasm during the equal division of genetic material into the daughter cells. Before a cell can divide, its genetic material, DNA, has to be duplicated through DNA replication. The identical copies of DNA are then separated into one of the two daughter cells through a multistep process, which varies among prokaryotes, plants, and animals. With a single chromosome and no nucleus, prokaryotes (such as bacteria) utilize a simple method of cell division called binary fission (meaning “splitting in two”). The single circular DNA molecule is replicated rapidly and split into two. Each of the two circular DNAs then migrates to the opposite pole of bacterial cell. Eventually, one bacterial cell splits into two through binary fission. On average, a bacterial cell can go through the whole process of cell division within twenty minutes.

In eukaryotes, cell division is a more complex process given the presence of a nucleus and multiple DNA molecules (chromosomes). Each chromosome needs to be replicated in preparation for the division. The replication process is completed during the interphase. Once replicated, the copies of each chromosome, called sister chromatids, are connected together in a region called the centromere. The chromosomes then go through a process

of shortening, condensing, and packing with proteins that make them visible using a light microscope. Chromosomes then migrate and line up at the equator of the parent cell. Then the sister chromatids are separated and pulled to opposite poles. These multiple steps include interphase (cell growth and DNA replication), prophase (disintegration of nuclear envelope, formation of spindle fibers, condensation of chromosomes), metaphase (lining up of chromosomes at equator plate), anaphase (split of two sister chromatids), and telophase (completion of migration of chromatids to opposite poles). Although animal and plant cells share many common features in DNA replication and mitosis, some noticeable differences in interphase and cytokinesis exist. Even within the animal kingdom, cytokinesis may vary with the type of cell division. Particularly during oogenesis (the process of forming egg), both meiosis I and meiosis II engage in unequal partitioning of cytoplasm that is distinct from normal mitosis of animal and plant cells. In some cases, a cell will complete mitosis without cytokinesis, resulting in a multinucleate cell.

Cytokinesis in Animals

In animal cells, cytokinesis normally begins during anaphase or telophase and is completed following the completion of chromosome segregation. First, microfilaments attached to the plasma membrane and form a ring around the equator of the cell. This ring then contracts and constricts the cell’s equator, forming a cleavage furrow, much like pulling the drawstring around the waist of a pair of sweatpants. Eventually the “waist” is pinched through and contracts down to nothing, partitioning the cytoplasm equally into two daughter cells. Partitioning the cytoplasm includes distributing cellular organelles so each daughter cell has what is needed for cellular processes.

Cytokinesis in Plants

Cytokinesis in plant cells is different from that in animal cells. The presence of a tough cell wall (made up of cellulose and other materials) makes it nearly impossible to divide plant cells in the same manner as animals cells. Instead, it begins with formation of a cell plate. In

early telophase, an initially barrel-shaped system of microtubules called a phragmoplast forms between the two daughter nuclei. The cell plate is then initiated as a disk suspended in the phragmoplast.

The cell plate is formed by fusion of secretory vesicles derived from the Golgi apparatus. Apparently, the carbohydrate-filled vesicles are directed to the division plane by the phragmoplast microtubules, possibly with the help of motor proteins. The vesicles contain matrix molecules, hemicelluloses, and/or pectins. As the vesicles fuse, their membranes contribute to the formation of the plasma membrane on either side of the cell plate. When enough vesicles have fused, the edges of the cell plate merge with the original plasma membrane around the circumference of the cell, completing the separation of the two daughter cells. In between the two plasma membranes is the middle lamella. Each of the two daughter cells then deposits a primary wall next to the middle lamella and a new layer of primary wall around the entire protoplast. This new wall is continuous with the wall at the cell plate. The original wall of the parent cell stretches and ruptures as the daughter cells grow and expand.

Cytokinesis in Sexual Reproduction

In animal oogenesis, the formation of ova, or eggs, occurs in the ovaries. Although the daughter cells resulting from the two meiotic divisions receive equal amounts of genetic material, they do not receive equal amounts of cytoplasm. Instead, during each division, almost all the cytoplasm is concentrated in one of the two daughter cells. In meiosis I, unequal partitioning of cytoplasm during cytokinesis produces the first polar body almost void of cytoplasm, and the secondary oocyte with almost all cytoplasm from the mother cell. During meiosis II, cytokinesis again partitions almost all cytoplasm to one of the two daughter cells, which

will eventually grow and differentiate into a mature ovum, or egg. Another daughter cell, the secondary polar body, receives almost no cytoplasm. This concentration of cytoplasm is necessary for the success of sexual reproduction because a major function of the mature ovum is to nourish the developing embryo following fertilization.

—Ming Y. Zheng

See also: Cell Cycle, The; Cell Division; Mitosis and Meiosis; Polyploidy; Totipotency.

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Developmental Genetics

Field of study: Developmental genetics

Significance: *The discovery of the genes responsible for the conversion of a single egg cell into a fully formed organism has greatly increased our understanding of development. Common developmental mechanisms exist for diverse organisms and experimental manipulation of particular genes could potentially lead to treatments or cures for cancers and developmental abnormalities in humans.*

Key terms

DIFFERENTIATION: the process by which a cell changes its phenotype, or outward appearance, and becomes different from its parent cell, usually by altering its gene expression

EPIGENESIS: the formation of differentiated cell types and specialized organs from a single, homogeneous fertilized egg cell without any preexisting structural elements

GENE EXPRESSION: the combined biochemical processes, called transcription and translation, that convert the linearly encoded information in the bases of DNA into the three-dimensional structures of proteins

INDUCTION: an easily observed event in which a cell or group of cells signals an adjacent cell to pursue a different developmental pathway and so become differentiated from its neighboring cells

MORPHOGEN: a chemical compound or protein made by cells in an egg that creates a concentration gradient affecting the developmental fate of surrounding cells by altering their gene expression or their ability to respond to other morphogens

Early Hypotheses of Development in Diverse Organisms

From the earliest times, people noted that a particular organism produced offspring very much like itself in structure and function, and the fully formed adult consisted of numerous cell types and other highly specialized organs and structures, yet it came from one simple egg cell. How could such simplicity, observed in the egg cell, give rise to such complexity in the adult and always reproduce the same structures?

In the seventeenth century, the “preformationism” hypothesis was advanced to answer these questions by asserting that a miniature organism existed in the sperm or eggs. After fertilization, this miniature creature simply grew into the fully formed adult. Some microscopists of the time claimed to see a “homunculus,” or little man, inside each sperm cell. That the preformationism hypothesis was ill-conceived became apparent when others noted that developmental abnormalities could not be explained satisfactorily, and it became clear that another, more explanatory hypothesis was needed to account for these inconsistencies.

In 1767, Kaspar Friedrich Wolff published his “epigenesis” hypothesis, in which he stated that the complex structures of chickens developed from initially homogeneous, structureless areas of the embryo. Many questions remained before this new hypothesis could be validated, and it became clear that the chick embryo was not the best experimental system for answering them. Other investigators focused their efforts on the sea squirt, a simpler organism with fewer differentiated tissues.

Work with the sea squirt, a tiny sessile marine animal often seen stuck to submerged rocks, led to the notion that development followed a mosaic pattern. The key property of mosaic development was that any cell of the early embryo, once removed from its surroundings, grew only into the structure for which it was destined or determined. Thus the early embryo consisted of a mosaic of cell types, each determined to become a particular body part. The determinants for each embryonic cell were found in the cell’s cytoplasm, the membrane-bound fluid surrounding the nucleus. Other scientists, most notably Hans Driesch in 1892 and Theodor Boveri (working with sea urchin embryos) in 1907, noted that a two-cell-stage embryo could be teased apart into separate cells, each of which grew into a fully formed sea urchin. These results appeared to disagree with the mosaic developmental mechanism. Working from an earlier theory, the “germ-plasm” theory of August Weismann (1883), Driesch and Boveri proposed a new mechanism called regulative development.

The key property of regulative development was that any cell separated from its embryo could regulate its own development into a complete organism. In contrast to mosaic development, the determinants for regulative development were found in the nuclei of embryonic cells, and Boveri hypothesized that gradients of these determinants, or morphogens, controlled the expression of certain genes. Chromosomes were assumed to play a major role in controlling development; however, how they accomplished this was not known, and Weismann mistakenly implied that genes were lost from differentiated cells as more and more specific structures formed.

In spite of the inconsistencies among the several hypotheses, a grand synthesis was soon formed. Working with roundworm, mollusc, sea urchin, and frog embryos, investigators realized that both mosaic and regulative mechanisms operate during development, with some organisms favoring one mechanism over the other. The most important conclusion coming from these early experiments suggested that certain genes on the chromosomes interacted with both the cytoplasmic and nuclear morphogenetic determinants to control the proliferation and differentiation of embryonic cells. What exactly were these morphogens, where did they originate, and how did they form gradients in the embryo? How did they interact with genes?

The Morphology of Development

Before the “how and why” mechanistic questions of morphogens could be answered, more answers to the “what happens when” questions were needed. Using new, powerful microscopes in conjunction with cell-specific stains, many biologists were able to precisely map the movements of cells during embryogenesis and to create “fate maps” of such cell migrations. Fate maps were constructed for sea squirt, roundworm, mollusc, sea urchin, and frog embryos, which showed that specific, undifferentiated cells in the early embryo gave rise to complex body structures in the adult.

In addition, biologists observed an entire stepwise progression of intervening cell types and structures that could be grouped into various stages and that were more or less consistent

from one organism to another. Soon after fertilization, during the very start of embryogenesis, specific zones with defining, yet structureless, characteristics were observed. These zones consisted of gradients of different biochemical compounds, some of which were morphogens, and they seemed to function by an induction process. Some of these morphogen gradients existed in the egg before fertilization; thus it became evident that the egg was not an entirely amorphous, homogeneous cell but one with some amount of preformation. This preformation took the form of specific morphogen gradients.

After these early embryonic events and more cell divisions, in which loosely structured patterns of morphogen gradients were established to form the embryo’s polar axes, the cells aggregated into a structure called a “blastula,” a hollow sphere of cells. The next stage involved the migration of cells from the surface of the blastula to its interior, a process called gastrulation. This stage is important because it forms three tissue types: the ectoderm (for skin and nerves), the mesoderm (for muscle and heart), and the endoderm (for other internal organs). Continued morphogenesis generates a “neurula,” an embryo with a developing nervous system and backbone. During axis formation and cell migrations, the embryonic cells are continually dividing to form more cells that are undergoing differentiation into specialized tissue types such as skin or muscle. Eventually, processes referred to as “organogenesis” transform a highly differentiated embryo into one with distinct body structures that will grow into a fully formed adult.

Experimental Systems for Studying Developmental Genes

In order to understand the details of development, biologists normally study organisms with the simplest developmental program, ones with the fewest differentiated cell types that will still allow them to answer fundamental questions about the underlying processes. Sea squirts and roundworms have been valuable, but they exhibit a predominantly mosaic form of development and are not the best systems for studying morphogen-dependent induction.

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Bahri Karacay, a postdoctoral fellow at the University of Iowa, studies the development of the nervous system in a mouse embryo as part of a project that seeks to treat brain tumors in humans. (AP/Wide World Photos)

Frog embryogenesis, with both mosaic and regulatory processes, was well described and contributed greatly to answering the “what and when” questions of sequential events, but no effective genetic system existed for examining the role of genes in differentiation necessary for answering the “why and how” questions.

The issue was finally resolved by focusing once again on the morphogens. These mediators of cellular differentiation were found only in trace amounts in developing embryos and thus were difficult if not impossible to isolate in pure form for experimental investigation. An alternative to direct isolation of morphogens was to isolate the genes that make the morphogens. The organism deemed most suitable for such an approach was the fruit fly *Drosophila melanogaster*; even though its development was more complex than that of the roundworm. Fruit flies could be easily grown in large numbers in the laboratory, and many mutants could be generated quickly; most important, an effec-

tive genetic system already existed in *Drosophila*, making it easier to create and analyze mutants. The person who best used the fruit fly system and greatly contributed to the understanding of developmental genetics was Christiane Nüsslein-Volhard, who shared a 1995 Nobel Prize in Physiology or Medicine with Edward B. Lewis and Eric Wieschaus.

The Genes of Development

The first important developmental genes discovered in *Drosophila* were the latest acting in morphogenesis, which led to the isolation of the gene for one of the morphogens controlling the anterior-posterior axis of the embryo, the *bicoid* gene. The study of mutants, such as those with legs in place of antennae, allowed the discovery of many other developmental genes, referred to generally as “homeotic” genes.

The *bicoid* gene’s discovery validated the gradient hypothesis originally proposed by Boveri

because its gene product functioned as a “typical” morphogen. It was a protein that existed in the highest concentration at the egg’s anterior pole and diffused to lower concentrations toward the posterior pole, thus forming a gradient. Through the use of more fruit fly mutants, geneticists showed that the BICOID protein stimulated the gene expression of another early gene, called *hunchback*, which in turn affected the expression of other genes: *Krüppel* and *knirps*. The BICOID protein controls the *hunchback* gene by binding to the gene’s control region.

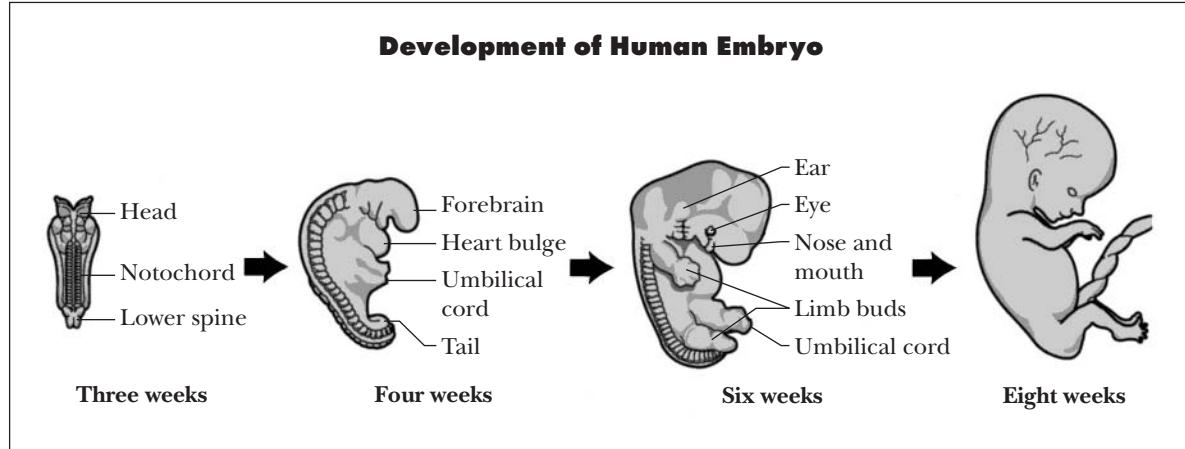
Since these initial discoveries, a plethora of new developmental genes have been discovered. It is now clear that some fifty genes are involved in development of a fruit fly larva from an egg, with yet more genes responsible for development of the larva into an adult fly. These genes are grouped into three major categories: maternal effect genes, segmentation genes, and homeotic genes. Maternal effect genes include the *bicoid* gene. These genes, located in special “nurse” cells of the mother, make proteins that contribute to the initial morphogen gradients along the egg’s axes before fertilization. Segmentation genes comprise three subgroups: gap, pair-rule, and segment polarity genes. Each of these types of segmentation genes determines a different aspect of the segments that make up a developing fruit fly. The *hunchback*, *Krüppel*, and *knirps* genes are all gap genes. Homeotic genes ultimately determine

the segment identity of previously differentiated cell groups.

Pattern Formation

Through the use of highly specific stains to track the morphogens in normal and mutant embryos, a fascinating picture of the interactions among developmental genes has emerged. Even before fertilization, shallow, poorly defined gradients are established by genes of the mother, such as the *bicoid* gene and related genes. These morphogen gradients establish the anterior-to-posterior and dorsal-to-ventral axes. After fertilization, these morphogens bind to the control regions of gap genes, whose protein products direct the formation of broadly defined zones which will later develop into several specific segments. The gap proteins then bind to the control regions of pair-rule genes, whose protein products direct further refinements in the segmentation process. The last group of segmentation genes, the segment polarity genes, direct the completion of the segmentation patterns observable in the embryo and adult fly, including definition of the anterior-posterior orientation of each segment. Homeotic genes then define the specific functions of the segments, including what appendages will develop from each one. Mutations in any of these developmental genes cause distinct and easily observed changes in the developing segment patterns. Genes such as *hunchback*, *giant*, *gooseberry*, and

Development of Human Embryo



***Caenorhabditis* Studies Tracing Cell Fates**

Caenorhabditis elegans, a free-living soil nematode (a type of worm) 1 millimeter in length, has proved invaluable as a model organism for studying development. In addition to its small size, it has a rapid life cycle, going from egg to sexual maturity in three and a half days and living only two to three weeks. The presence of rudimentary physiological systems, including digestive, nervous, muscular, and reproductive systems, enables comparative studies between *Caenorhabditis* and “higher” organisms, such as mice and humans. Because the animal is transparent, the formation of every cell in the 959-celled adult can be observed microscopically and manipulated to illuminate its developmental program.

In 1963 Sydney Brenner set out to learn everything there was to know about *Caenorhabditis elegans*. In a 1974 publication he demonstrated how specific mutations could be induced in the *C. elegans* genome through chemical mutagenesis and showed how these mutations could be linked to specific genes and specific effects on organ development. Proving the utility of the organism as a genetic model encouraged a cadre of researchers to pursue research with *C. elegans*.

One of Brenner’s students, John Sulston, developed techniques to track and study cell divisions in the nematode, from fertilized egg through adult. Microscopic examination of individual cell nuclei of the animal as it developed, along with electron microscopy of serial sections of the animal, enabled scientists to trace each of the adult worm’s 959 cells back to a single fertilized egg. This “lineage map” was

then used to track the fates of cells in animals that had been experimentally manipulated. Using a fine laser beam, scientists could kill a single cell at some point in development of the animal, then determine what changes, if any, awaited the remaining cells. These studies proved that the *C. elegans* cell lineage is invariant; that is, every worm underwent exactly the same sequence of cell divisions and differentiation.

Studies on cell fate and lineage mapping also led to the discovery that specific cells in the lineage always die through programmed cell death. Robert Horvitz, another of Brenner’s students, discovered two “death genes” in *C. elegans* as well as genes that protect against cell death and direct the elimination of the dead cell. He also identified the first counterparts of the death gene in humans.

The characterization of the invariant cell lineage of *C. elegans* and the genetic linkages have been of great value to understanding basic principles of development, including signaling pathways in multicellular organisms and pathways controlling cell death. This knowledge has been invaluable to medicine, where it has helped researchers to understand mechanisms by which bacteria and viruses invade cells and has provided insights into the cellular mechanisms involved in neurodegenerative diseases, autoimmune disorders, and cancer. For their pioneering work in the “genetic regulation of organ development and programmed cell death” Brenner, Sulston, and Horvitz were awarded the 2002 Nobel Prize in Physiology or Medicine.

—Karen E. Kalumuck

hedgehog were all named with reference to the specific phenotypic changes that result from improper control of segmentation.

Homeotic genes are often called the “master” genes because they control large numbers of other genes required to make a whole wing or leg. Several clusters of homeotic genes have been discovered in *Drosophila*. Mutations in a certain group of genes of the *bithorax* complex result in adult fruit flies with two sets of wings. Similarly, mutations in some of the genes in the *antennapedia* complex can result in adult fruit flies with legs, rather than antennae, on the head.

A general principle applying to develop-

mental processes in all organisms has emerged from the elegant work with *Drosophila* mutants: Finer and finer patterns of differentiated cells are progressively formed in the embryo along its major axes by morphogens acting on genes in a cascading manner, in which one gene set controls the next in the sequence until a highly complex pattern of differentiated cells results. Each cell within its own patterned zone then responds to the homeotic gene products and contributes to the formation of distinct, identifiable body parts.

Another important corollary principle was substantiated by the genetic analysis of development in *Drosophila* and other organisms.

In direct contrast to Weismann's implication about gene loss during differentiation, convincing evidence showed that genes were not systematically lost as egg cells divided and acquired distinguishing features. Even though a muscle cell was highly differentiated from a skin cell or a blood cell, each cell type retained the same numbers of chromosomes and genes as the original, undifferentiated, but fertilized egg cell. What changed in each cell was the pattern of gene expression, so that different proteins were made by specific genes while other genes were turned off. The morphogens, working in complex combinatorial patterns during the course of development, determined which genes would stay "on" and which would be turned "off."

Impact and Applications

The discovery and identification of the developmental genes in *Drosophila* and other lower organisms led to the discovery of similarly functioning genes in higher organisms, including humans. The base-pair sequences of many of the developmental genes, especially shorter subregions coding for sections of the morphogen that bind to the control regions of target genes, are conserved, or remain the same, across diverse organisms. This conservation of gene sequences has allowed researchers to find similar genes in humans. For example, some forty homeobox genes have been found in mice and humans, even though only eight were initially discovered in *Drosophila*. Some of the late-acting human homeobox genes are responsible for such developmental abnormalities as fused fingers and extra digits on the hands and feet. One of the most interesting abnormalities is craniosynostosis, a premature fusion of an infant's skull bones that can cause mental retardation. In 1993, developmental biologist Robert Maxson and his research group at the University of Southern California's Norris Cancer Center were the first to demonstrate that a mutation in a human homeobox gene *MSX2* was directly responsible for craniosynostosis and other bone/limb abnormalities requiring corrective surgeries. Maxson made extensive use of "knockout" mice, genetically engineered mice lacking particular genes, to

test his human gene isolates. He and his research group made great progress in understanding the role of the *MSX2* gene as inducer of surrounding cells in the developing embryo. When this induction process fails because of defective *MSX2* genes, the fate of cells destined to participate in skull and bone formation and fusion changes, and craniosynostosis occurs.

A clear indication of the powerful cloning methods developed in the late 1980's was the discovery and isolation in 1990 of an important mouse developmental gene called *brachyury* ("short tails"). The gene's existence in mutant mice had been inferred from classical genetic studies sixty years prior to its isolation. In 1997, Craig Basson, Quan Yi Li, and a team of co-workers isolated a similar gene from humans and named it T-box *brachyury* (*TBX5*). Discovered first in mice, the "T-box" is one of those highly conserved subregions of a gene, and it allowed Basson and Li to find the human gene. When mutated or defective in humans, *TBX5* causes a variety of heart and upper limb malformations referred to as Holt-Oram syndrome. *TBX5* codes for an important morphogen affecting the differentiation of embryonic cells into mesoderm, beginning in the gastrulation phase of embryonic development. These differentiated mesodermal cells are destined to form the heart and upper limbs.

One of the important realizations emerging from the explosive research into developmental genetics in the 1990's was the connection between genes that function normally in the developing embryo but abnormally in an adult, causing cancer. Cancer cells often display properties of embryonic cells, suggesting that cancer cells are reverting to a state of uncontrolled division. Some evidence indicates that mutated developmental genes participate in causing cancer. Taken together, the collected data from many isolated human developmental genes, along with powerful reproductive and cloning technologies, promise to lead to cures and preventions for a variety of human developmental abnormalities and cancers.

—Chet S. Fornari, updated by Bryan Ness

See also: Aging; Animal cloning; Cell Cycle, The; Cell Division; Congenital Defects; Cytoki-

nesis; DNA Structure and Function; Evolutionary Biology; Genetic Engineering; Hereditary Diseases; Homeotic Genes; In Vitro Fertilization and Embryo Transfer; Model Organism: *Caenorhabditis elegans*; Model Organism: *Drosophila melanogaster*; Model Organism: *Mus musculus*; RNA Structure and Function; Stem Cells; Telomeres; Totipotency; X Chromosome Inactivation.

Further Reading

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- Nüsslein-Volhard, Christiane. "Gradients That Organize Embryo Development." *Scientific American* (August, 1996). The Nobel laureate reports on findings from the *Drosophila* studies.
- Nüsslein-Volhard, Christiane, and J. Kratzschmar, eds. *Off Fish, Fly, Worm, and Man: Lessons from Developmental Biology for Human Gene Function and Disease*. New York: Springer,

2000. Designed for researchers, a consideration of the next phase of biology following the sequencing of several large genomes (accomplished at the turn of the millennium): determining the functions of genes and the interplay between them and their protein products.

Web Sites of Interest

- Institute for Developmental Genetics. <http://www.gsf.de/idg>. Based in Germany, maintains data on mouse and zebra fish genetics "to unravel the molecular genetic networks controlling neuronal pattern formation, neuronal specification and differentiation during development."
- Society for Developmental Biology. <http://sdb.bio.purdue.edu>. Professional society for biologists and geneticists interested in problems of development and growth of organisms. Site links to developmental biology sites and features the "Developmental Biology Cinema," which links to video sequences of developing organisms.
- Virtual Library of Developmental Biology, Society for Developmental Biology. <http://sdb.bio.purdue.edu>. Primarily a collection of annotated links to laboratories by subject or organism, useful for its lists of departments, programs, and organizations.

Diabetes

Field of study: Diseases and syndromes

Significance: *Diabetes mellitus is a syndrome in which the body cannot metabolized glucose appropriately. The subsequent elevated levels cause significant damage to the eyes, heart, kidneys, and other organs. Diabetes is a significant public health problem with more than 17 million persons affected in the United States alone, of whom more than 90 percent have adult-onset (Type II) diabetes.*

Key terms

AUTOIMMUNE RESPONSE: an immune response of an organism against its own cells

LOCUS (*pl. LOCI*): the physical location of a gene, which in most organisms occurs as two copies called alleles, one copy on each of the chromosomes in a homologous pair

Types of Diabetes

Diabetes mellitus actually comprises a number of different diseases, broadly categorized into Type I and Type II diabetes. In both forms, the body's ability to process sugars is impaired, with consequences that can lead to death if untreated. Genetics plays a role in both types of diabetes, although both are thought to be the result of the interaction between genetics and the environment.

Glucose is a simple sugar that is required by all cells for normal functioning. Most of the body's glucose initially comes from carbohydrates broken down during digestion. Normally, the glucose level in a person's blood rises when carbohydrates are ingested. When the blood glucose reaches a certain level, it triggers the pancreas to release insulin, which causes the glucose level in the blood to drop by increasing its uptake in muscle, fat, the liver, and the gut. One theory is that as the glucose level drops, the person becomes hungry. Eating begins a new cycle of blood sugar elevation and insulin response.

Patients with either type of diabetes have difficulty metabolizing glucose, with a subsequent rise in fasting and postprandial blood sugar levels. In Type I diabetes, also called juvenile-onset or insulin-dependent diabetes, this is due to destruction of the insulin-secreting cells in the pancreas. In Type II, also called adult-onset, maturity-onset, or non-insulin-dependent diabetes, cells become resistant to the effects of insulin even though the pancreas is still producing some insulin. Both types lead to increased risk of heart and vascular disease, kidney problems, blindness, neurological problems, and other serious medical consequences.

Type I Diabetes

Type I diabetes mellitus is a chronic autoimmune disease that results from a combination of genetic and environmental factors. Certain persons are born with a genetic susceptibility to the disease. Before the disease can develop,

however, some kind of trigger in the environment must be present. The environmental trigger, possibly a viral infection like measles or mumps, triggers an autoimmune response; that is, the person's own immune system aids in the destruction of the beta cells, those responsible for the secretion of insulin, in the pancreas. Typically, autoantibodies appear a few years ahead of the actual disease. The first recognizable symptom is a condition called pre-diabetes in which the usual insulin release in response to elevated blood sugar levels in the blood is diminished. At a certain point, most commonly between the ages of ten and fourteen, the person develops full-blown diabetes, with excessive thirst and urination, as well as weight loss despite adequate or increased caloric intake. If untreated, the person can become comatose or even die. Regular treatment with insulin (by injection or by an insulin pump) is required for the rest of the person's life. There is also evidence that weight reduction and exercise can alleviate symptoms to some extent.

The earliest evidence for a genetic basis for Type I diabetes was the observation by epidemiologists that it often occurs in families. In white Americans, the risk of diabetes is 0.12 percent overall, but in children of persons with diabetes the risk is much greater, 1-15 percent. Worldwide, the incidence of Type I diabetes is highest in Scandinavia, Northern Europe, and areas of the United States where Scandinavian immigrants settled. The lowest incidence of this type of diabetes is in China and parts of South America.

The genetic basis for developing Type I diabetes appears not so much to involve mutant genes per se, but rather a bad combination of particular alleles. Most of the genes implicated so far are found in the major histocompatibility complex, known as the HLA (human leukocyte antigen) complex. Certain combinations of alleles at these loci seem to confer a much higher susceptibility than normal. Some of the autoantigens themselves have also been identified, insulin being one of them, which should be no surprise. In addition, a rare type of autoimmune diabetes, resembling Type I, occurs as part of a syndrome called autoimmune polyendocrinopathy-candidiasis-ectodermal

dystrophy (APECED), which is caused by mutation in *Aire*, an autoimmune regulator gene. Although the function of *Aire* is not known, expression of the gene has been detected in the thymus, pancreas, and adrenal cortex, and developmental studies suggest that mutations in *Aire* might cause the thymus (which is integral to proper immune system function) to develop incorrectly.

Type II Diabetes

Diabetes mellitus Type II is by far the more common type of diabetes. For example, about 90 percent of diabetics in the United States have Type II. It is a disease that occurs primarily in older adults, although the incidence in younger people is increasing as the incidence of obesity increases and as more children lead sedentary lives. The children at greatest risk in the United States are those from ethnic minority backgrounds.

Type II diabetes appears to be a group of diseases, rather than a single disease, in which there are two defects: (1) beta-cell dysfunction, leading to somewhat decreased production of insulin (although elevated levels of insulin also occur), and (2) tissue resistance to insulin. As with Type I, it appears that people who develop Type II are born with a genetic susceptibility but the development of actual disease is dependent upon an environmental trigger. Some possible triggers include aging, sedentary lifestyle, and abdominal obesity. Obesity plays a particularly significant role in the development of Type II diabetes. Among North Americans, Europeans, and Africans with Type II diabetes, between 60 and 70 percent are obese.

As with Type I, epidemiologic evidence suggests a strong genetic component to Type II diabetes. In identical twins over forty years of age, for example, the likelihood is about 70 percent that the second twin will develop Type II diabetes once the first twin has developed the disease. Diabetes Type II is also found in nearly 100 percent of obese Pima Indians and some Pacific Islanders.

Mutant alleles for a number of genes have been implicated in susceptibility and development of Type II diabetes. The first genes to be implicated were the insulin gene, genes encod-

ing important components of the insulin secretion pathways, and other genes involved in glucose homeostasis. Mutations are very diverse and can include mutation not only in the genes themselves but also in transcription factors and control sequences. As more genes and their mutant alleles are discovered, better treatment options should become available, possibly even some that are tailored to specific types of mutations.

Treatments for Type II diabetes available at the beginning of the twenty-first century include such lifestyle changes as increased activity and weight loss, as well as oral drugs that increase tissue sensitivity to circulating insulin, stimulate increased insulin secretion, or alter insulin action. Some patients may ultimately have to add insulin to their treatment regimen. Once the genetic factors have been completely elucidated for both types of diabetes, treatments to modify the genes may become a reality.

—Rebecca Lovell Scott and Bryan Ness

See also: Autoimmune Disorders; Bacterial Genetics and Cell Structure; Biopharmaceuticals; Cloning; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Heart Disease; Hereditary Diseases.

Further Reading

American Diabetes Association. *American Diabetes Association Complete Guide to Diabetes: The Ultimate Home Reference from the Diabetes Experts*. New York: McGraw-Hill, 2002. Written for the consumer, this book includes everything a person with diabetes or a caregiver needs to know, including information on symptoms, complications, exercise and nutrition, blood sugar control, sexual issues, drug therapies, insulin regimes, and daycare.

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Becker, Gretchen. *The First Year: Type 2 Diabetes, An Essential Guide for the Newly Diagnosed*. New York: Marlowe, 2001. The author suffers from Type II diabetes and provides firsthand

advice for coping with the disease, beginning with the day of diagnosis, day by day through the first week, and beyond.

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Milchovich, Sue K., and Barbara Dunn-Long. *Diabetes Mellitus: A Practical Handbook*. 8th ed. Boulder, Colo.: Bull, 2003. A basic reference book that contains comprehensive information on living with diabetes.

Notkins, Abner Louis. "Immunologic and Genetic Factors in Type I Diabetes." *The Journal of Biological Chemistry* 277, no. 46 (2002): 43,545-43,548. An overview of the major lines of evidence used to consider Type I diabetes primarily an autoimmune disease. Also provides specifics about the gene defects involved in Type I diabetes.

Web Sites of Interest

American Diabetes Association. <http://www.diabetes.org>. Site includes information on genetics and diabetes.

National Institute of Diabetes & Digestive & Kidney Diseases. <http://www.niddk.nih.gov>. This arm of the National Institutes of Health offers resources and links to research on lactose intolerance.

Dihybrid Inheritance

Field of study: Classical transmission genetics

Significance: *The simultaneous analysis of two different hereditary traits may produce more information than the analysis of each trait separately. In addition, many important hereditary traits are controlled by more than one gene. Traits controlled by two genes serve as an introduction to the more complex topic of traits controlled by many genes.*

Key terms

ALLELES: different forms of the same gene; any gene may exist in several forms having very similar but not identical DNA sequences

DIHYBRID: an organism that is heterozygous for both of two different genes

HETEROZYGOUS: a condition in which the two copies of a gene in an individual (one inherited from each parent) are different alleles

HOMOZYGOUS: a condition in which the two copies of a gene in an individual are the same allele; synonymous with "purebred"

Mendel's Discovery of Dihybrid Inheritance

Austrian botanist Johann Gregor Mendel was the first person to describe both monohybrid and dihybrid inheritance. When he crossed purebred round-seed garden peas with purebred wrinkled-seed plants, they produced only monohybrid round seeds. He planted the monohybrid round seeds and allowed them to fertilize themselves; they subsequently produced $\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled seeds. He concluded correctly that the monohybrid generation was heterozygous for an allele that produces round seeds and another allele that produces wrinkled seeds. Since the monohybrid seeds were round, the round allele must be dominant to the wrinkled allele. He was able to explain the 3:1 ratio in the second generation by assuming that each parent contributes only one copy of a gene to its progeny. If *W* represents the round allele and *w* the wrinkled allele, then the original true-breeding parents are *WW* and *ww*. When eggs and pollen are produced, they each contain only one copy of the

gene. Therefore the monohybrid seeds are heterozygous Ww . Since these two alleles will separate during meiosis when pollen and eggs are produced, $\frac{1}{2}$ of the eggs and pollen will be W and $\frac{1}{2}$ will be w . Mendel called this “segregation.” When the eggs and pollen combine randomly during fertilization, $\frac{1}{4}$ will produce WW seeds, $\frac{1}{2}$ will produce Ww seeds, and $\frac{1}{4}$ will produce ww seeds. Since W is dominant to w , both the WW and Ww seeds will be round, producing $\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled seeds. When Mendel crossed a purebred yellow-seed plant with a purebred green-seed plant, he observed an entirely analogous result in which the yellow allele (G) was dominant to the green allele (g).

Once Mendel was certain about the nature of monohybrid inheritance, he began to experiment with two traits at a time. He crossed purebred round, yellow pea plants with purebred wrinkled, green plants. As expected, the dihybrid seeds that were produced were all round and yellow, the dominant form of each trait. He planted the dihybrid seeds and allowed them to fertilize themselves. They produced $\frac{9}{16}$ round, yellow seeds; $\frac{3}{16}$ round, green seeds; $\frac{3}{16}$ wrinkled, yellow seeds; and $\frac{1}{16}$ wrinkled, green seeds. Mendel was able to explain this dihybrid ratio by assuming that in the dihybrid flowers,

the segregation of W and w was independent of the segregation of G and g . Mendel called this “independent assortment.” Thus, of the $\frac{3}{4}$ of the seeds that are round, $\frac{3}{4}$ should be yellow and $\frac{1}{4}$ should be green, so that $\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$ should be round and yellow, and $\frac{3}{4} \times \frac{1}{4} = \frac{3}{16}$ should be round and green. Of the $\frac{1}{4}$ of the seeds that are wrinkled, $\frac{1}{4}$ should be yellow and $\frac{1}{4}$ green, so that $\frac{1}{4} \times \frac{3}{4} = \frac{3}{16}$ should be wrinkled and yellow, and $\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$ should be wrinkled and green. This relationship can be seen in the table headed “Dihybrid Inheritance and Sex Linkage.”

Sex Chromosomes

Humans and many other species have sex chromosomes. In humans, normal females have two X chromosomes and normal males have one X and one Y chromosome. Therefore, sex-linked traits, which are controlled by genes on the X or Y chromosome, are inherited in a different pattern than the genes that have already been described. Since there are few genes on the Y chromosome, most sex-linked traits are controlled by genes on the X chromosome.

Every daughter gets an X chromosome from each parent, and every son gets an X from his mother and a Y from his father. Human red-

Dihybrid Inheritance and Sex Linkage

		Pollen			
		$W;G$	$W;g$	$w;G$	$w;g$
Eggs	$W;G$	$WW;GG$ round, yellow	$WW;Gg$ round, yellow	$Ww;GG$ round, yellow	$Ww;Gg$ round, yellow
	$W;g$	$WW;Gg$ round, yellow	$WW;gg$ round, green	$Ww;Gg$ round, yellow	$Ww;gg$ round, green
	$w;G$	$Ww;GG$ round, yellow	$Ww;Gg$ round, yellow	$ww;GG$ wrinkled, yellow	$ww;Gg$ wrinkled, yellow
	$w;g$	$Ww;Gg$ round, yellow	$Ww;gg$ round, green	$ww;Gg$ wrinkled, yellow	$ww;gg$ wrinkled, green

Note: Semicolons indicate that the two genes are on different chromosomes.

green color blindness is controlled by the recessive allele (*r*) of an X-linked gene. A red-green color-blind woman (*rr*) and a normal man (*RY*) will have normal daughters (all heterozygous *Rr*) and red-green color-blind sons (*r Y*). Conversely, a homozygous normal woman (*RR*) and a red-green color-blind man (*rY*) will have only normal children, since their sons will get a normal X from the mother (*RY*) and the daughters will all be heterozygous (*Rr*). A heterozygous woman (*Rr*) and a red-green color-blind man (*rY*) will have red-green color-blind sons (*rY*) and daughters (*rr*), and normal sons (*RY*) and daughters (*Rr*) in equal numbers.

A dihybrid woman who is heterozygous for red-green color blindness and albinism (a recessive trait that is not sex linked) can make four kinds of eggs with equal probability: *R;A*, *R;a*, *r;A*, and *r;a*. A normal, monohybrid man who is heterozygous for albinism can make four kinds of sperm with equal probability: *R;A*, *R;a*, *Y;A*, and *Y;a*. By looking at the table headed "Mixed Sex-Linked and Autosomal Traits," it is easy to predict the probability of each possible kind of child from this mating.

The probabilities are $\frac{1}{16}$ normal female, $\frac{1}{16}$ albino female, $\frac{1}{16}$ normal male, $\frac{1}{16}$ red-green

color-blind male, $\frac{1}{16}$ albino male, and $\frac{1}{16}$ albino, red-green color-blind male. Note that the probability of normal coloring is $\frac{1}{4}$ and the probability of albinism is $\frac{1}{4}$ in both sexes. There is no change in the inheritance pattern for the gene that is not sex linked.

Other Examples of Dihybrid Inheritance

A hereditary trait may be controlled by more than one gene. To one degree or another, almost every hereditary trait is controlled by many different genes, but often one or two genes have a major effect compared with all the others, so they are called single-gene or two-gene traits. Dihybrid inheritance can produce traits in various ratios, depending on what the gene products do. A number of examples will be presented, but they do not exhaust all of the possibilities.

The comb of a chicken is the fleshy protuberance that lies on top of the head. There are four forms of the comb, each controlled by a different combination of the two genes that control this trait. The first gene exists in two forms (*R* and *r*), as does the second (*P* and *p*). In each case, the form represented by the uppercase letter is dominant to the other form. Since there are two copies of each gene (with

Mixed Sex-Linked and Autosomal Traits

	Sperm			
	<i>A;R</i>	<i>a;R</i>	<i>A;Y</i>	<i>a;Y</i>
<i>A;R</i>	<i>A A;R R</i> normal female	<i>A a;R R</i> normal female	<i>A A;R Y</i> normal male	<i>A a;R Y</i> normal male
<i>A;r</i>	<i>A A;R r</i> normal female	<i>A a;R r</i> normal female	<i>A A;r Y</i> red-green color-blind male	<i>A a;r Y</i> red-green color-blind male
<i>a;R</i>	<i>A a;R R</i> normal female	<i>a a;R R</i> albino female	<i>A a;R Y</i> normal male	<i>a a;R Y</i> albino male
<i>a;r</i>	<i>A a;R r</i> normal female	<i>a a;r r</i> albino female	<i>A a;r Y</i> red-green color-blind male	<i>a a;r Y</i> albino, red-green color-blind male

Note: Semicolons indicate that the two genes are on different chromosomes.

		Pollen			
		<i>A;B</i>	<i>A;b</i>	<i>a;B</i>	<i>a;b</i>
Eggs	<i>A;B</i>	<i>A A;B B</i> red	<i>A A;B b</i> medium red	<i>A a;B B</i> medium red	<i>A a;B b</i> light red
	<i>A;b</i>	<i>A A;B b</i> medium red	<i>A A;B b</i> light red	<i>A a;B b</i> light red	<i>A a;B b</i> very light red
	<i>a;B</i>	<i>A a;B B</i> medium red	<i>A a;B b</i> light red	<i>a a;B B</i> light red	<i>a a;B b</i> very light red
	<i>a;b</i>	<i>A a;B b</i> light red	<i>A a;B b</i> very light red	<i>a a;B b</i> very light red	<i>a a;B b</i> white

Note: Semicolons indicate that the two genes are on different chromosomes. Dihybrid ratios may change if both genes are on the same chromosome.

the exception of genes on sex chromosomes), the first gene can be present in three possible combinations: *RR*, *Rr*, and *rr*. Since *R* is dominant, the first two combinations produce the same trait, so the symbols *R*_ and *P*_ can be used to represent either of the two combinations. Chickens with *R*_;*P*_ genes have what is called a walnut comb, which looks very much like the meat of a walnut. The gene combinations *R*_;*pp*, *rr*;*P*_, and *rr*;*pp* produce combs that are called rose, pea, and single, respectively. If two chickens that both have the gene combination *Rr*;*Pp* mate, they will produce progeny that are $\frac{1}{16}$ walnut, $\frac{3}{16}$ rose, $\frac{3}{16}$ pea, and $\frac{1}{16}$ single (see Table 1 for an explanation of these numbers).

White clover synthesizes small amounts of cyanide, which gives clover a bitter taste. There are some varieties that produce very little cyanide (sweet clover). When purebred bitter clover is crossed with some varieties of purebred sweet clover, the progeny are all bitter. However, when the hybrid progeny is allowed to fertilize itself, the next generation is $\frac{1}{16}$ bitter and $\frac{15}{16}$ sweet. This is easy to explain if it is assumed that bitter/sweet is a dihybrid trait. The bitter parent would have the gene combination *AA*;*BB* and the sweet parent *aa*;*bb*, where *A* and

B are dominant to *a* and *b*, respectively. The bitter dihybrid would have the gene combination *Aa*;*Bb*. When it fertilizes itself, it would produce $\frac{1}{16}$ *A*_;*B*_, which would be bitter, and $\frac{3}{16}$ *A*_;*bb*, $\frac{3}{16}$ *aa*;*B*_, and $\frac{1}{16}$ *aa*;*bb*, all of which would be sweet. Clearly, both the *A* allele and the *B* allele are needed in order to synthesize cyanide. If either is missing, the clover will be sweet.

Absence of Dominance

In all of the previous examples, there was one dominant allele and one recessive allele. Not all genes have dominant and recessive alleles. If a purebred snapdragon with red flowers (*RR*) is crossed with a purebred snapdragon with white flowers (*rr*), all the monohybrid progeny plants will have pink flowers (*Rr*). The color depends on the number of *R* alleles present: two *R*s will produce a red flower, one *R* will produce a pink flower, and no *R*s will produce a white flower. This is an example of partial dominance or additive inheritance.

Consider a purebred red wheat kernel (*AA*;*BB*) and a purebred white wheat kernel (*aa*;*bb*) (see the table headed “Partial Dominance”). If the two kernels are planted and the resulting plants are crossed with each other,

the progeny dihybrid kernels will be light red ($Aa;Bb$). If the dihybrid plants grown from the dihybrid kernels are allowed to self-fertilize, they will produce $\frac{1}{16}$ red ($AA;BB$), $\frac{1}{16}$ medium red ($AA;Bb$ and $Aa;BB$), $\frac{1}{16}$ light red ($AA;bb$, $Aa;Bb$, and $aa;BB$), $\frac{1}{16}$ very light red ($Aa;bb$ and $aa;Bb$), and $\frac{1}{16}$ white ($aa;bb$). The amount of red pigment depends on the number of alleles (A and B) that control pigment production. Although it may appear that this is very different than the example in the first table, they are in fact very similar.

All of the inheritance patterns that have been discussed are examples of “independent assortment,” in which the segregation of the alleles of one gene is independent of the segregation of the alleles of the other gene. That is exactly what would be expected from meiosis if the two genes are not on the same chromosome. If two genes are on the same chromosome and sufficiently close together, they will not assort independently and the progeny ratios will not be like any of those described. In that case, the genes are referred to as “linked” genes.

—James L. Farmer

See also: Chromosome Theory of Heredity; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Epistasis; Incomplete Dominance; Linkage Maps; Mendelian Genetics; Monohybrid Inheritance; Multiple Alleles.

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Diphtheria

Fields of study: Bacterial genetics; Diseases and syndromes

Significance: *Diphtheria* is an acute bacterial disease known best for damaging the respiratory system. Afflicted individuals die from this as well as from damage to the heart, nerves, and kidney. Genetic research has led to better understanding of diphtheria’s cause, action, and treatment.

Key terms

ANAPHYLAXIS: a severe, sometimes fatal allergic reaction

ANTIBODIES: proteins that help identify and destroy foreign pathogens and other molecules in the body

ANTITOXIN: a vaccine containing antibodies against a specific toxin

CUTANEOUS: related to or affecting skin

Diphtheria Symptoms and Cure

The acute bacterial disease diphtheria is caused by rod-shaped *Corynebacterium diphtheriae* (*C. diphtheriae*), discovered in 1883 by Edwin Klebs and Friedrich Löffler. Diphtheria involves the respiratory tract, nerves, and heart in ways that can be lethal. After 1950, the disease became uncommon in industrialized nations because of immunization by vaccination with antitoxin originally isolated from horses by Emil Adolf von Behring in the 1880’s. In such nations, diphtheria is contracted by contact with travelers coming from developing nations, where it is much more common, who may be asymptomatic carriers or have active diphtheria.

C. diphtheriae usually enters the body through mucous membranes of the mouth or nose, though it can also enter via breaks in the skin

(cutaneous route). After infection and a two-to-five-day incubation period, diphtheria's first symptoms are localized inflammation that kills cells in the respiratory tract or skin. Respiratory diphtheria initially appears as a sore throat in which a dirty gray membrane (diphtheria pseudomembrane) forms and spreads through the respiratory system. The pseudomembrane (made mostly of dead cells, bacteria, and white blood cells) causes a husky voice and is accompanied by swollen lymph glands. In severe cases, diphtheria kills by heart failure or throat paralysis as little as one day after the initial symptoms appear. Fortunately, such lethality occurs mostly in unimmunized individuals. Cutaneous *C. diphtheriae* infections most often produce only skin lesions, though they can cause death if the bacteria spreads widely through the blood and damages the heart, nerves, and kidneys. Damage depends upon the bacterial entry site, individual immunization status, and the amount of toxin made.

Although most people in industrialized nations are immunized, the consequences of diphtheria can be so severe that therapy by diphtheria antitoxin should begin as soon as symptoms suggest the disease. Cure of diphtheria requires, in addition to the antitoxin, destruction of all *C. diphtheriae* in afflicted individuals. Immunization is the first line of defense, so it is crucial to ensure that the suspected diphtheria sufferer is not sensitive to antitoxin because incautious antitoxin administration may cause lethal anaphylaxis in sensitive people. Individual sensitivity is identified by scratch tests with diluted antitoxin. In sensitive people, desensitization is achieved through the sequential administration of increasing doses of antitoxin in an intensive care unit until effective doses are safely reached.

Diphtheria is so dangerous that all patient contacts are tested for *C. diphtheriae*. Afflicted individuals are given penicillin, erythromycin, and/or antitoxin, depending on the presence, absence, and severity of diphtheria symptoms. Though adequate universal immunization is a sure diphtheria control, booster shots—like those for tetanus prophylaxis—should be given every ten years in addition to childhood shots. Because of the extremely infectious and fatal

nature of diphtheria, all people positive for *C. diphtheriae* must be kept in bed, isolated, and treated until symptoms and bacteria are absent after antibiotic therapy stops. This may require four to six weeks.

Genetics and Diphtheria

Diphtheria symptoms are caused by diphtheria toxin, a protein so lethal that 6 micrograms will kill a 150-pound human. Most often, the toxin first localizes in respiratory mucosa cells or cutaneous sites, where it causes diphtheria pseudomembrane or skin lesions by interacting with the protein translocase. Translocase is essential to synthesis of proteins needed for body cell growth, survival, and reproduction. Diphtheria toxin and translocase interact through a process called adenine ribosylation, similar to that in cholera. Diphtherial adenine ribosylation inactivates translocase, preventing its action and killing affected cells. Dead respiratory cells form diphtheria pseudomembrane, which closes off the throat. In skin, toxin-killed cells cause skin lesions. Destruction of nerve, heart, and kidney cells leads to damage in those tissues.

The diphtheria pseudomembrane may cut off breathing. In such cases, suffocation is prevented by a tracheotomy (a surgical incision in the neck that creates an airway). Major causes of quick diphtheria fatality are damage to nerves and the heart. The toxin is a protein made by genes that are present only in certain strains, and *C. diphtheriae* strains that do not produce the toxin are harmless. In addition, genetic studies have identified interaction of the toxin with respiratory mucosa cell translocase as well as similar action in many other tissues. Use of bacterial genetics has also enabled more scientific production of diphtheria antitoxin. The antitoxin is useful to visitors of regions where the disease is common. Its universal use has led to a worldwide decrease in diphtheria fatalities to fewer than five deaths per million people. The immunization is effective for ten years.

Impact and Applications

Diphtheria has long been a serious, worldwide threat. During the twentieth century, its

danger greatly diminished in industrialized nations with the advent of antitoxin and the wide use of antibiotics to kill *C. diphtheriae*. In poorer nations, diphtheria still flourishes and is a severe threat, partly because of less advanced medical practices and the public's fear of immunization.

Prevention of diphtheria relies mostly on immunization via antitoxin. The isolation and identification of diphtheria toxin and the development of antitoxin have depended on genetic methods that now protect most people from the disease. Wherever it afflicts people, diphtheria treatment also requires the use of antibiotics. Hence, advanced diphtheria prevention and treatment will be best effected by using genetic, immunologic, and biochemical methods to produce vaccines effective for more than ten years and to produce more potent antibiotics. Efforts toward these ends will most likely utilize molecular genetics to clearly define why diphtheria is intractable to lifelong vaccination. Especially valuable will be DNA sequence analysis, when a genome sequence becomes available.

—Sanford S. Singer

See also: Cholera; Emerging Diseases; Hereditary Diseases; Smallpox.

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Web Site of Interest

Diphtheria Hub. <http://www.healthubs.com/diphtheria>. Provides dozens of links to information on diphtheria, including overviews, diagnosis, and treatment.

DNA Fingerprinting

Field of study: Human genetics and social issues

Significance: *DNA fingerprinting includes a variety of techniques in which individuals are uniquely identified through examination of specific DNA sequences that are expected to vary widely among individuals. Uses for these technologies include not only practical applications in forensic analysis and paternity tests but also basic research in paternity, breeding systems, and ecological genetics for many nonhuman species.*

Key terms

MICROSATELLITE: a type of VNTR in which the repeated motif is 1 to 6 base pairs; synonyms include simple sequence repeat (SSR) and short tandem repeat (STR)

MINISATELLITE: a type of VNTR in which the repeated motif is 12 to 500 base pairs in length

POLYMERASE CHAIN REACTION (PCR): a laboratory procedure for making millions of identical copies of a short DNA sequence

VARIABLE NUMBER TANDEM REPEAT (VNTR): a type of DNA sequence in which a short sequence is repeated over and over; chromosomes from different individuals frequently have different numbers of the basic repeat, and if many of these variants are known, the sequence is termed a hypervariable

Genetic Differences Among Individuals

All individuals, with the exception of twins and other clones, are genetically unique. Theoretically it is therefore possible to use these genetic differences, in the form of DNA sequences, to identify individuals or link samples of blood, hair, and other features to a single individual. In practice, individuals of the same species typically share the vast majority of their DNA sequences; in humans, for example, well over 99 percent of all of the DNA is identical. For individual identification, this poses a problem: Most of the sequences that might be examined are identical (or nearly so) among randomly selected individuals. The solution to this problem is to focus only on the small regions of the DNA that are known to vary widely among individuals. These regions, termed hypervariable, are typically based on repeat sequences in the DNA.

Imagine a simple DNA base sequence, such as AAC (adenine-adenine-cytosine), which is repeated at a particular place (or locus) on a human chromosome. One chromosome may have eleven of these AAC repeats, while another might have twelve or thirteen, and so on. If one could count the number of repeats on each chromosome, it would be possible to specify a diploid genotype for this chromosomal locus: An individual might have one chromosome with twelve repeats, and the other with fifteen. If there are many different chromosomal variants in the population, most individuals will have different genotypes. This is the conceptual basis for most DNA fingerprinting.

DNA fingerprint data allow researchers or investigators to exclude certain individuals: If, for instance, a blood sample does not match an individual, that individual is excluded from further consideration. However, if a sample and

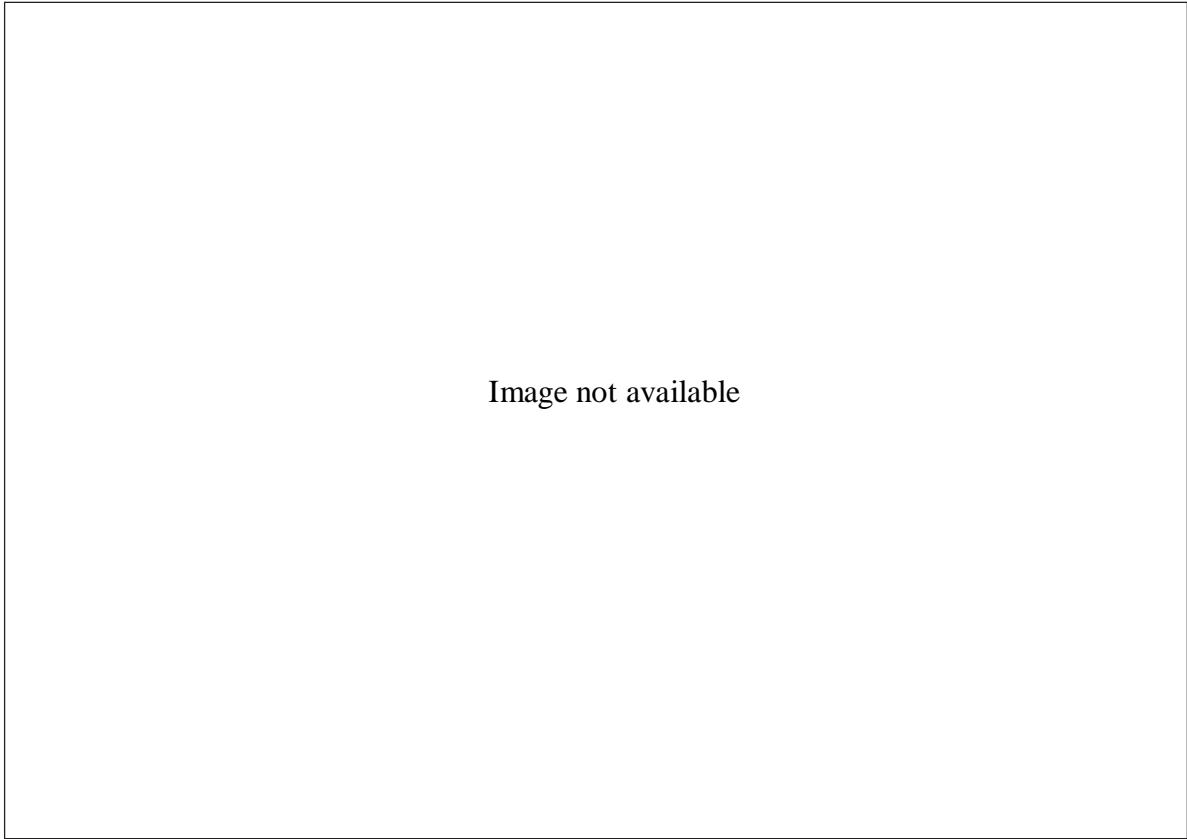


Image not available

A criminalist at the Phoenix Police Department prepares samples of DNA taken from a crime scene for comparison to the DNA fingerprints of suspects. (AP/Wide World Photos)

an individual match, this is not proof that the sample came from that individual; other individuals might have the same genotype. If a second locus is examined, it becomes less likely that two individuals will share the same genotype. In practice, investigators use enough independent loci that it is extremely unlikely that two individuals will have the same genotypes over all of the loci, making it possible to identify individuals within a degree of probability expressed as a percentage, and very high percentages are possible.

The First DNA Fingerprints

Alec Jeffreys, at the University of Leicester in England, produced the first DNA fingerprints in the mid-1980's. His method examined a twelve-base sequence that was repeated one right after another, at many different loci in the human genome. Once collected from an individual, the DNA was cut using restriction enzymes to create DNA fragments that contained the repeat sequences. If the twelve-base sequence was represented by more repeats, the fragment containing it was that much longer. Jeffreys used agarose gel electrophoresis to separate his fragments by size, and he then used a specialized staining technique to view only the fragments containing the twelve-base repeat. For two samples from the same individual, each fragment, appearing as a band on the gel, should match. This method was used successfully in a highly publicized rape and murder case in England, both to exonerate one suspect and to incriminate the perpetrator.

While very successful, this method had certain drawbacks. First, a relatively large quantity of DNA was required for each sample, and results were most reliable when each sample compared was run on the same gel. This meant that small samples, such as individual hairs or tiny blood stains, could not be used, and also that it was difficult to store DNA fingerprints for use in future investigations.

Variable Number Tandem Repeat Loci

The type of sequence Jeffreys exploited is now included in the category of variable number tandem repeats (VNTRs). This type of DNA sequence is characterized, as the name implies,

by a DNA sequence which is repeated, one copy right after another, at a particular locus on a chromosome. Chromosomes vary in the number of repeats present.

VNTRs are often subcategorized based on the length of the repeated sequence. Minisatellites, like the Jeffreys repeat, include repeat units ranging from about twelve to several hundred bases in length. The total length of the tandemly repeated sequences may be several hundred to several thousand bases. Many different examples have since been discovered, and they occur in virtually all eukaryotes. In fact, the Jeffreys repeat first discovered in humans was found to occur in a wide variety of other species.

Shorter repeat sequences, typically 1 to 6 bases in length, were subsequently termed microsatellites. In humans, AC (adenine-cytosine) and AT (adenine-thymine) repeats are most common; an estimate for the number of AC repeat loci derived from the Human Genome Project suggests between eighty thousand and ninety thousand different AC repeat loci spread across the genome. Every eukaryote studied to date has had large numbers of microsatellite loci, but they are much less common in prokaryotes.

The Polymerase Chain Reaction

The development of the polymerase chain reaction (PCR) in the mid-1980's, and its widespread use and optimization in DNA labs a few years later offered an alternative approach to DNA fingerprinting. The PCR technique makes millions of copies of short segments of DNA, with the chromosomal location of the fragments produced under the precise control of the investigator. PCR is extremely powerful and can be used with extremely small amounts of DNA. Because the fragments amplified are small, PCR can also be used on partially degraded samples. The size and chromosomal location of the fragments produced depends on the DNA primers used in the reaction. These are short, single-stranded DNA molecules that are complementary to sequences that flank the region to be amplified.

With this approach, an investigator must find and determine the DNA sequence of a re-

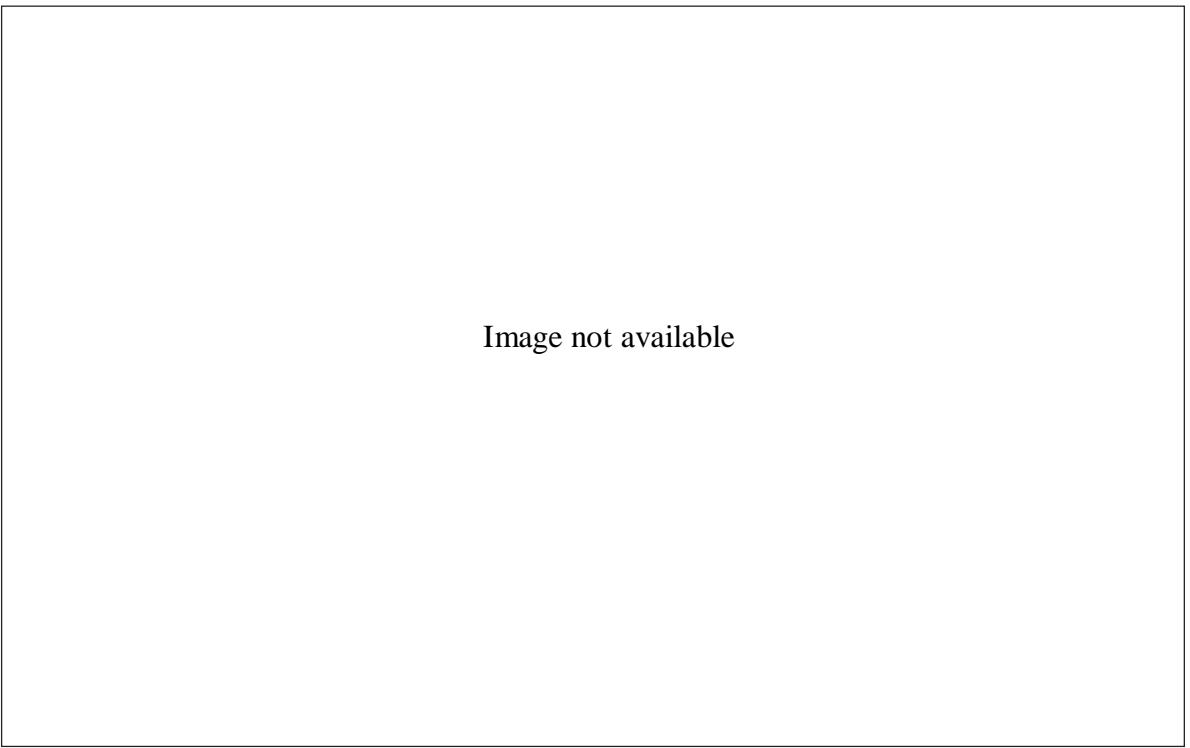


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Co-founder of the San Diego DNA laboratory Annette Peer testifies about DNA evidence at the David Westerfield murder trial on June 20, 2002. (AP/Wide World Photos)

gion containing a VNTR. Primers are designed to amplify the VTNR region, together with some flanking DNA sequences on both ends. The fragments produced in the reaction are then separated by length using gel electrophoresis so that differences in length, attributable to different numbers of the repeat, become apparent. For a dinucleotide repeat like AC, fragments representing different numbers of repeats, and hence different alleles, differ by a multiple of two. For instance, a researcher might survey a number of individuals and find fragments of 120, 122, 124, 128, and 130 base pairs in length.

Current Approaches

Most current approaches to DNA fingerprinting use data collected simultaneously from a number of different VNTR loci, most commonly microsatellites. Preferably, the loci are PCR amplified using primers with fluorescent dyes attached, so that fragments from different loci are uniquely tagged with different

colors. The fragments are then loaded in polyacrylamide DNA gels of the type used for DNA sequencing and separated by size. The fluorescent colors and sizes of the fragments are determined automatically, using the same automated machines typically used for DNA sequencing.

DNA fingerprint data generated in this way are easily stored and saved for future comparisons. Since each allelic variant is represented by a specific DNA fragment length, and because these are measured very precisely, the initial constraint of running samples for comparison on the same gel is avoided.

Human Forensic and Paternity Testing

Although several different systems have been developed and used, a widely employed current standard comprises the Federal Bureau of Investigation's Combined DNA Index System (CODIS), with thirteen core loci. These thirteen are tetranucleotide (TCTA) microsatellite repeat loci, located on autosomes. Each

locus has many known alleles, in some cases more than forty; the genetic variation is well characterized, and databases of variation within a variety of ethnic groups are available.

In addition to its role in criminal cases, this technique has seen widespread use to establish or exclude paternity, in immigration law to prove relatedness, and to identify the remains of casualties resulting from military combat and large disasters.

Other Uses for VNTR Genotyping

Soon after VNTRs were discovered in humans and used for DNA fingerprinting, researchers demonstrated that the same or similar types of sequences were found in all animals, plants, and other eukaryotes. The method pioneered by Jeffreys was, only a few years later, used for studies of paternity in wild bird populations. Since then, microsatellite analysis has come to dominate studies of relatedness, paternity, breeding systems, and other questions of individual identification in wild species of all kinds, including plants, insects, fungi, and vertebrates. Researchers now know, for example, that among the majority of birds which appear monogamous, between 10 and 15 percent of all progeny are fathered by males other than the recognized mate.

—Paul R. Cabe

See also: Criminality; Forensic Genetics; Genetic Testing; Genetics, Historical Development of; Human Genetics; Paternity Tests; Repetitive DNA.

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Iowa State University Extension and Office of Biotechnology, DNA Fingerprinting in Agricultural Genetics Programs. http://www.biotech.iastate.edu/biotech_info_series. Site links to a comprehensive and illustrative article on the role of DNA fingerprinting in agriculture.

DNA Isolation

Fields of study: Genetic engineering and biotechnology; Molecular genetics

Significance: Before it can be manipulated and studied, DNA must be isolated from other substances such as complex carbohydrates, proteins, and RNA. The isolation process is central to biotechnology and genetic engineering.

Key terms

CHLOROFORM/ISOAMYL ALCOHOL (CIA): a mixture of two chemicals used in DNA isolation to rid the extract of the contaminating compound phenol

LYSIS: the breaking open of a cell

OSMOTIC SHOCK: the lysing of cells by moving them from a liquid environment with a high solute concentration to an environment with a very low solute concentration

PHENOL: a simple chemical used in DNA extraction to precipitate proteins and aid in their removal

DNA Discovery and Extraction

Deoxyribonucleic acid (DNA) was discovered in 1869 by the Swiss physician Friedrich Miescher, who studied white blood cells in pus obtained from a surgical clinic. Miescher found that when bandages that had been removed from the postoperative wounds of injured soldiers were washed in a saline solution, the cells on the bandages swelled into a gelatinous mass that consisted largely of DNA. Miescher had isolated a denatured form of DNA—that is, DNA not in the normal double-stranded conformation. After a series of experiments, Miescher concluded that the substance he had isolated originated in the nuclei of the blood cells; he first called the substance nuclein and later nucleic acid.

The first problem when extracting DNA is lysing, or breaking open, the cell. Bacteria, yeast, and plant cells usually have a thick cell wall protecting their plasma membrane, which makes lysis more difficult. Bacteria, such as *Escherichia coli*, are the easiest of these cells to open by a process called alkaline lysis, in which cells are treated with a solution of sodium hydroxide and detergent that degrades both the cell wall and the cell membrane. Yeast cells are often broken open with enzymes such as lysozyme that degrade cell walls or by using a “French press,” a piston in an enclosed chamber that forces cells open under high pressure. Plant tissue is usually mechanically broken into a fine cell suspension before extraction by grinding frozen tissue in a mortar and pestle. Once the suspension of cells is obtained, the tissue may be treated with a variety of enzymes to

break down cell walls or with strong detergents, such as sodium lauryl sarcosine, that disrupt and dissolve both cell walls and cell membranes. Animal cells, such as white blood cells, do not have cell walls and can generally be opened by osmotic shock, the lysing of cells by moving them from a liquid environment with a high solute concentration to an environment with a very low solute concentration.

Isolation and Purification

Although lysis methods differ according to cell type, the process of DNA isolation and purification is more standardized. The isolation process may be imagined as a series of steps designed to remove either naturally occurring biological contaminants from the DNA or contaminants added by the scientist during the extraction process. The biological contaminants already present in cells are proteins and ribonucleic acid (RNA); additionally, plant cells have high levels of complex carbohydrates. Contaminants intentionally added by scientists may include salts and various chemicals.

After cells are lysed, a high-speed centrifugation is performed to form large-scale, insoluble cellular debris, such as membranes and organelles, into a pellet. The liquid extract remaining still contains dissolved proteins, RNA, and DNA. If salts are not already present in the extract, they are added; salt must be present later for the DNA to precipitate efficiently. Proteins must be removed from the extract since some not only degrade DNA but also inhibit enzymatic reactions with DNA. Proteins are precipitated by mixing the extract with a chemical called phenol. When phenol and the extract are mixed in a test tube, they separate into two parts like oil and water. If these fluids are centrifuged, precipitated proteins will actually collect between the two liquids at a spot called the interphase. The liquid layer containing the dissolved DNA is then drawn up and away from the precipitated protein.

The protein-free solution still contains DNA, RNA, salts, and traces of phenol dissolved into the extract. To remove the contaminating phenol, the extract is mixed with a chloroform/isoamyl alcohol solution (CIA). Again like oil and water, the DNA extract and CIA separate

Differential Isolation of Organelle DNA

Discussions of DNA isolation usually concern isolation of DNA from the nucleus. While the nucleus is the location of most of the genetic information in the cell, DNA molecules also exist in other organelles, such as mitochondria and chloroplasts. Chromosomes of these organelles, referred to as nonnuclear or cytoplasmic DNA, contain a small subset of genes, mostly encoding proteins needed by these organelles.

Most standard DNA isolation techniques isolate both nuclear and nonnuclear DNA together. For a person working with nuclear DNA, this is usually not a concern because the amount of nuclear DNA is much greater than the amount of nonnuclear DNA. In working with nonnuclear DNA however, the presence of nuclear DNA can often cause problems. Some techniques used to examine nonnuclear DNA, such as the polymerase chain reaction (PCR), are not affected by the presence of nuclear DNA, but for other techniques, pure nonnuclear DNA is required.

Isolation strategies for nonnuclear DNA usually involve two steps. The first step is the isolation of intact mitochondria or chloroplasts from the cells, followed by the lysing of the mitochondria or chloroplasts to release the DNA so it can be purified. The process is the same for isolation of both mitochondrial and chloroplast DNA. Isolation of intact mitochondria (for example) requires that the membranes of the cells be lysed in a way that does not rupture the mitochondria. To achieve this goal, gentle mechanical, chemical, or enzymatic methods (de-

pending on the nature of the cell membrane and whether there is a cell wall) are employed to break open the cells and release the cytoplasmic contents. The lysis of the cells is usually done in an osmotically stabilized buffer. The solutes in this buffer match the concentration of the solutes inside the mitochondria, which prevents the mitochondria from bursting when the cells are lysed.

Once the cells are lysed, the lysate is centrifuged at low speed (usually between one thousand and three thousand times the force of gravity) to remove nuclei, membrane fragments, and other debris. The resulting supernatant contains the mitochondria in suspension. To concentrate the mitochondria, the supernatant is centrifuged at high speed (twelve thousand times the force of gravity). The pellet formed by this centrifugation will contain mitochondria and can be suspended in a small volume of liquid to create a concentrated suspension of mitochondria. This suspension may be treated with the enzyme DNase, which will degrade any nuclear DNA that remains without crossing the intact mitochondrial membrane. The enzyme will then be deactivated, and the mitochondria will be lysed. Lysis of the mitochondria is achieved by adding a strong detergent to the suspension of the mitochondria. Once the mitochondria have been lysed, the free mitochondrial DNA can be purified just as nuclear DNA would be, using phenol extraction and ethanol precipitation.

—*Douglas H. Brown*

into two layers. If the two layers are mixed vigorously and separated by centrifugation, the phenol will move from the DNA extract into the CIA layer. At this point the extract—removed to a new test tube—contains RNA, DNA, and salt.

The extract is next mixed with 100 percent ethanol, inducing the DNA to precipitate out in long strands. The DNA strands may be isolated by either spooling the sticky DNA around a glass rod or by centrifugation. If spooled, the DNA is placed in a new test tube; if centrifuged, the liquid is decanted from the pellet of DNA. The precipitated DNA, with salt and RNA present, is still not pure. It is washed for a final time with 70 percent ethanol, which does not dis-

solve the DNA but forces salts present to go into solution. The DNA is then reisolated by spooling or centrifugation and dried to remove all traces of ethanol. At this point, only DNA and RNA are left; this mixture can be dissolved in a low-salt buffer containing the enzyme RNase, which degrades any RNA present, leaving pure DNA.

Technological advances have allowed deproteinization by the use of “spin columns” without the employment of toxic phenol. The raw DNA extract is placed on top of a column containing a chemical matrix that binds proteins but not DNA; the column is then centrifuged in a test tube. The raw extract passes through the chemical matrix and exits protein-free into the

collection tube. These newer methods not only increase safety and reduce the production of toxic waste; they are also much faster.

—James J. Campanella

See also: Ancient DNA; DNA Replication; DNA Sequencing Technology; DNA Structure and Function; RFLP Analysis; RNA Isolation; RNA Structure and Function.

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DNA Repair

Field of study: Molecular genetics

Significance: *To protect the integrity of their genetic material, cells are able to correct damage to DNA. Many of these mechanisms are found in organisms ranging from bacteria to humans, indicating that they evolved early in the history of life. Disruption of DNA repair mechanisms in humans has been associated with the development of cancers.*

Key terms

BASE: the component of a nucleotide that gives it its identity and special properties

NUCLEOTIDE: the basic unit of DNA, consisting of a five-carbon sugar, a nitrogen-containing base, and a phosphate group

DNA Structure and DNA Damage

All living things are continually exposed to agents such as radiation or chemicals that can damage their genetic material. In addition, damage to DNA can occur spontaneously, or as a by-product of other cellular processes. Because DNA is the blueprint for directing the functions of the cell; it must be accurately maintained. The integrity of DNA also assures that all daughter cells receive the same genetic information. DNA damage can include a change in the meaning of a gene (a mutation), a break in a DNA molecule, or the abnormal joining of two DNA molecules. To a bacterial cell, DNA damage may mean death. To a multicellular organism, damaged DNA in some of its cells may mean loss of function of organs or tissues or it may lead to cancer.

A brief overview of the structure of DNA will clarify how it can be damaged. DNA is assembled from nucleotides, of which there are four types, each defined by the base it contains. If the DNA double helix is pictured as a twisted ladder, the outside supports (sometimes referred to as the “backbone” of the DNA) are alternating units of sugar and phosphate, and the “rungs” of the ladder are bases. There are four types of bases found in DNA: the double-ring purines, adenine and guanine, and the single-ring pyrimidines, cytosine and thymine. The structure of each base allows it to pair with only one other base: adenine pairs with thymine, and cytosine pairs with guanine. Base pairing holds the two strands of the double helix together and is essential for the synthesis of new DNA molecules (DNA replication) and for the transfer of information from DNA to RNA in the process of transcription. DNA replication is carried out by an enzyme called DNA polymerase, which reads the information (the sequence of bases) on a single strand of DNA, brings the appropriate nucleotide to pair with the existing strand one nucleotide at a time, and joins it to the end of a growing chain. In addition to copying entire long strands of DNA every time a cell divides, DNA polymerases are also responsible for repairing short, damaged regions of DNA. Transcription occurs through a process similar to DNA replication, except that an RNA polymerase copies only a portion of one of the strands of DNA (a gene), making an RNA copy. The RNA can then direct the production of a particular protein, which is the ultimate product of the most genes.

One of the most frequent forms of DNA damage is loss of a base. Purines are particularly unstable, and many are lost each day in human cells. If a base is absent, the DNA cannot be copied correctly during DNA replication. Another common type of DNA damage is a pyrimidine dimer, an abnormal linkage between two cytosines, two thymines, or a cytosine and a thymine next to each other in a DNA strand. These are caused by the action of ultraviolet light on DNA. A pyrimidine dimer creates a distortion in the double helix that interferes with the processes of DNA replication and transcription. Another form of DNA damage is a break

in the backbone of one or both strands of the double helix. Breaks can block DNA replication, create problems during cell division, or cause rearrangements of the chromosomes. DNA replication itself can cause problems by inserting an incorrect base or an additional or too few bases in a new strand. While DNA replication errors are not DNA damage as such, they can also lead to mutations and are subject to repair.

DNA Repair Systems

DNA repair systems are found in most organisms. Even some viruses, such as bacteriophages (which infect bacteria) and herpesviruses (which infect animals), are capable of repairing some damage to their genetic material. The DNA repair systems of single-celled organisms, including bacteria and yeasts, have been extensively studied for many years. In the 1980's and 1990's, techniques including the use of recombinant DNA methods revealed that DNA repair systems of multicellular organisms such as humans, animals, and plants are quite similar to those of microorganisms.

Scientists generally classify DNA repair systems into three categories on the basis of complexity, mechanism, and the fate of the damaged DNA. “Damage reversal” systems are the simplest: They usually require only a single enzyme to directly act on the damage and restore it to normal, usually in a single step. “Damage removal” systems are somewhat more complicated: These involve cutting out and replacing a damaged or inappropriate base or section of nucleotides and require several proteins to act together in a series of steps. “Damage tolerance” systems are those that respond to and act on damaged DNA but do not actually repair the original damage. Instead, they are ways for cells to cope with DNA damage in order to continue growth and division.

Damage Reversal Systems

Photoreactivation is one of the simplest and perhaps oldest known repair systems: It consists of a single enzyme that can split pyrimidine dimers in the presence of light. An enzyme called photolyase catalyzes this reaction; it is found in many bacteria, lower eukaryotes, in-

sects, and plants but seems to be absent in mammals (including humans). A similar gene is present in mammals but may code for a protein that functions in another type of repair.

X rays and some chemicals such as peroxides can cause breaks in the backbone of DNA. Simple breaks in one strand are rapidly repaired by the enzyme DNA ligase. Mutant strains of microorganisms with reduced DNA ligase activity tend to have high levels of recombination since DNA ends are very “sticky” and readily join with any other fragment of DNA. While recombination is important in generating genetic diversity during sexual reproduction, it can also be dangerous if DNA molecules are joined inappropriately. The result can be aberrant chromosomes that do not function properly.

Damage Removal Systems

Damage removal systems are accurate and efficient but require the action of several enzymes and are more energetically “expensive” to the cell. There are three types of damage removal systems that work in the same general way but act on different forms of DNA damage. In “base excision” repair, an enzyme called a DNA glycosylase recognizes a specific damaged or inappropriate base and cuts the base-sugar linkage to remove the base. Next, the backbone is cut by another protein that removes the baseless sugar; then a new nucleotide is inserted to replace the damaged one by a DNA polymerase enzyme. Finally, the break in the backbone is sealed by DNA ligase. There are a number of specific glycosylases for particular types of DNA damage caused by radiation and chemicals.

The “nucleotide excision” repair system works on DNA damage that is “bulky” and that creates a block to DNA replication and transcription, such as ultraviolet-induced pyrimidine dimers and some kinds of DNA damage created by chemicals. It probably does not recognize a specific abnormal structure but sees a distortion in the double helix. Several proteins joined in a complex scan the DNA for helix distortions. When one is found, the complex binds to the damage and creates two cuts in the DNA strand containing the damaged bases on either side of the damage. The short segment

with the damaged bases (around twenty-seven nucleotides in humans) is removed from the double helix, leaving a short gap that can be filled by DNA polymerase using the intact nucleotides in the other DNA strand as a guide. In the last step, DNA ligase rejoins the strand. Mutants that are defective in nucleotide excision repair have been isolated in many organisms and are extremely sensitive to mutation induction by ultraviolet light and similar-acting chemical mutagens. Humans with the hereditary disease xeroderma pigmentosum are sun-light-sensitive and have a very high risk of skin cancers on sun-exposed areas of their bodies. These individuals have defective copies of genes that code for proteins involved in nucleotide excision repair. A comparison of the genes defective in xeroderma pigmentosum patients and those involved in nucleotide excision repair in simpler organisms reveals a great deal of similarity, indicating that this repair system evolved early in the history of life.

“Mismatch” repair occurs during DNA replication as a last “spell check” on its accuracy. By comparing mutation rates in *Escherichia coli* bacteria that either have or lack mismatch repair systems, scientists have estimated that this process adds between one hundred and one thousand times more accuracy to the replication process. It is carried out by a group of proteins that can scan DNA and look for incorrectly paired bases (or unpaired bases). The incorrect nucleotide is removed as part of a short stretch, and then the DNA polymerase gets a second try to insert the correct sequence. In 1993, Richard Fishel, Bert Vogelstein, and their colleagues isolated the first genes for human mismatch repair proteins and showed that they are very similar to those of the bacterium *Escherichia coli* and the simple eukaryote baker’s yeast. Further studies in the 1990’s revealed that mismatch repair genes are defective in people with hereditary forms of colon cancer.

Damage Tolerance Systems

Not all DNA damage is or can be removed immediately; some of it may persist for a while. If a DNA replication complex encounters DNA damage such as a pyrimidine dimer, it will normally act as a block to further replication of

that DNA molecule. In eukaryotes, however, DNA replication initiates at multiple sites and may be able to resume downstream of a damage site, leaving a “gap” of single-stranded, unreplicated DNA in one of the two daughter molecules. The daughter-strand gap is potentially just as dangerous as the original damage site, if not more so. The reason for this is that if the cell divides with a gap in a DNA molecule, there will be no way accurately to repair that gap or the damage in one of its two daughter cells. To avoid this problem, cells have developed a way to repair daughter-strand gaps by recombination with an intact molecule of identical or similar sequence. The “recombinational” repair process, which requires a number of proteins, yields two intact daughter molecules, one of which still contains the original DNA damage. In addition to dealing with daughter-strand gaps, recombinational repair systems can also repair single- and double-strand breaks caused by the action of X rays and certain chemicals on DNA. Many of the proteins required for recombinational repair are also involved in the genetic recombination that occurs in meiosis, the sexual division process of higher cells. In 1997, it was shown that the products of the breast cancer susceptibility genes *BRCA1* and *BRCA2* participate in both recombinational repair and meiotic recombination.

An alternative choice for a DNA polymerase blocked at a DNA damage site is to change its specificity so that it can insert any nucleotide opposite the normally nonreadable damage and continue DNA replication. This type of “damage bypass” is very likely to cause a mutation, but if the cell cannot replicate its DNA, it will not be able to divide. In *Escherichia coli* bacteria, there is a set of genes that are turned on when the bacteria have received a large amount of DNA damage. Some of these gene products alter the DNA polymerase and allow damage bypass. This system has been termed the “SOS response” to indicate that it is a system of last resort. Other organisms, including humans, seem to have similar damage bypass mechanisms that allow a cell to continue growth despite DNA damage at the price of mutations. For this reason, damage bypass systems are

sometimes referred to as “error-prone” or mutagenic repair systems.

Impact and Applications

DNA repair systems are an important component of the metabolism of cells. Studies in microorganisms have shown that as little as one unrepaired site of DNA damage per cell can be lethal or lead to permanent changes in the genetic material. The integrity of DNA is normally maintained by an elaborate series of interrelated checks and surveillance systems. The greatly increased risk of cancer suffered by humans with hereditary defects in DNA repair shows how important these systems are in avoiding genetic changes. As the relationship between mutations in DNA repair genes and cancer susceptibility becomes clearer, this information may be used in directing the course of cancer therapy and possibly in providing gene therapy to individuals with cancer.

—Beth A. Montelone

See also: Aging; Biochemical Mutations; Breast Cancer; Cancer; Chemical Mutagens; DNA Structure and Function; Human Genetics; Immunogenetics; Model Organism: *Escherichia coli*; Mutation and Mutagenesis; Oncogenes; Protein Structure; Protein Synthesis; RNA Structure and Function; RNA Transcription and mRNA Processing; Telomeres; Tumor-Suppressor Genes.

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DNA Replication

Field of study: Genetic engineering and biotechnology

Significance: Cells and organisms pass hereditary information from generation to generation. To assure that offspring contain the same genetic information as their parents, the genetic material must be accurately reproduced. DNA replication is the molecular basis of heredity and is one of the most fundamental processes of all living cells.

Key terms

REPLICATION: the process by which one DNA molecule is converted to two DNA molecules identical to the first

TRANSCRIPTION: the process of forming an RNA according to instructions contained in DNA

TRANSLATION: the process of forming proteins according to instructions contained in an RNA molecule

X-RAY DIFFRACTION: a method for determining the structure of molecules which infers structure by the way crystals of molecules scatter X rays as they pass through

DNA Structure and Function

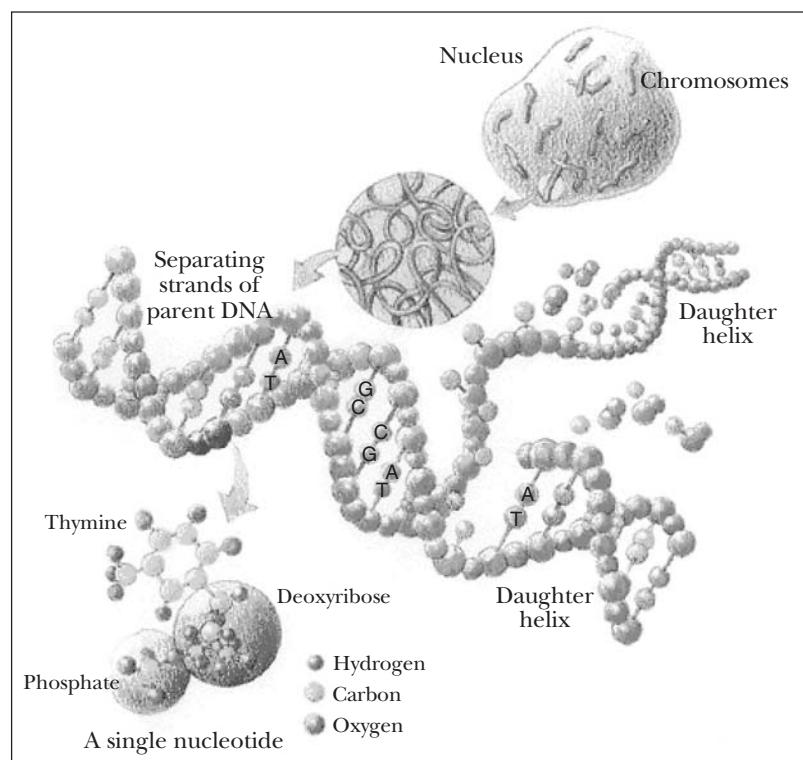
The importance of chromosomes in heredity has been known since early in the twentieth century. Chromosomes consist of both DNA and protein, and in the early twentieth century there was considerable controversy concerning which component was the hereditary molecule. Early evidence favored the proteins. In 1944, however, a series of classic experiments by Oswald Avery, Maclyn McCarty, and Colin MacLeod lent strong support to the proponents favoring DNA as the genetic material. They showed that a genetic transforming agent of bacteria was DNA and not protein. In experiments reported in 1952, Alfred Hershey and Martha Chase provided evidence that DNA was the genetic material of bacteriophages (viruses that infect bacteria). Combined with additional circumstantial evidence from many sources, DNA became favored as the hereditary molecule, and a heated race began to determine its molecular structure.

In 1953, James Watson and Francis Crick published a model for the atomic structure of DNA. Their model was based on known chemical properties of DNA and X-ray diffraction data obtained from Rosalind Franklin and Maurice Wilkins. The structure itself made it clear that DNA was indeed the molecule of heredity and provided evidence for how it might be copied. The molecule resembles a ladder. The “rails” are composed of repeating units of sugar and phosphate, forming a backbone for the molecule. Each “rung” consists of a pair of nitrogenous bases, one attached to each of the two rails and held together in the middle through weak bonds called hydrogen bonds. Since there are thousands to hundreds of millions of units on a DNA molecule, the hydrogen bonds between each pair of bases add up to a strong force that holds the two strands together. DNA, then, consists of two strands, each consisting of a repeating sugar-phosphate backbone and nitrogenous bases with the two strands held together by base-pair interactions. The two strands are oriented in opposite directions. The ends of a linear DNA molecule can be distinguished by which part of the backbone sugar is exposed and are referred to as the 5' (five prime) end and the 3' end, named for a particular carbon atom on the ribose sugar. If one DNA strand is oriented 5' to 3', its complementary partner is oriented 3' to 5'. This organization has important implications for the mechanism of DNA replication.

There are four different bases: adenine (A), guanine (G), cytidine (C), and thymine (T). They can be arranged in any order on a DNA strand, allowing the

enormous diversity necessary to encode the blueprint of every organism. A key feature of the double-stranded DNA molecule is that bases have strict pairing restrictions: A can only pair with T; G can only pair with C. Thus if a particular base is known on one strand, the corresponding base is automatically known on the other. Each strand can serve as a template, or mold, dictating the precise sequence of bases on the other. This feature is fundamental to the process of DNA replication.

The genome (the complete DNA content of an organism) stores all the genetic information that determines the features of that organism. The features are expressed when the DNA is transcribed to a messenger molecule, mRNA, which is used to construct a protein. The proteins encoded by the organism's genes in its DNA carry out all of the activities of the cell.



This illustration from the Human Genome Program of the Department of Energy shows the basic context of DNA replication from the cellular nucleus, which contains the chromosomes, to the separation of DNA strands and their replication at the molecular and atomic levels into daughter helices. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

The Cell Cycle

In eukaryotic organisms (most organisms other than bacteria), cells progress through a series of four stages between cell divisions. The stages begin with a period of growth (G_1 phase), followed by replication of the DNA (S phase). A second period of growth (G_2 phase) is followed by division of the cell (M phase). The two cells resulting from the cell division each go through their own cell cycle or may enter a dormant stage (G_0 phase). The passage from one stage to the next is tightly regulated and directed by internal and external signals to the cell.

The transition from G_1 into S phase marks the beginning of DNA replication. In order to enter S phase, the cell must pass through a checkpoint or restriction point in which the cell determines the quality of its DNA: If there is any damage to the DNA, entry into S phase will be delayed. This prevents the potentially lethal process of beginning replication of a DNA molecule that has damage that would prevent completion of replication. If conditions are determined to be acceptable, a “molecular switch” is thrown, triggering the initiation of DNA replication. What is the nature of this molecular switch? There are many different proteins that participate in the process of DNA replication, and they can have their activity turned off and on by other proteins. Addition or removal of a chemical group called a phosphate is a common mechanism of chemical switching. This reaction is catalyzed by a class of enzymes called kinases. Certain key proteins are phosphorylated at the boundary of the G_1 and S phases of the cell cycle by kinases, switching on DNA replication.

Origins and Initiation

If the human genome were replicated from one end to the other, it would take several years to complete the process. The DNA molecule is simply too large to be copied end to end. Instead, replication is initiated at many different sites called origins of replication, and DNA synthesis proceeds from each site in both directions until regions of copied DNA merge. The region of DNA copied from a particular origin is called a replicon. Using hundreds to tens of thousands of initiation sites and replicons, the

genome can be copied in a matter of hours. The structure of replication origins has been difficult to identify in all but a few organisms, most notably yeast. Origins consist of several hundred base pairs of DNA comprising sequences that attract and bind a set of proteins called the origin recognition complex (ORC). The exact mechanism by which the origin is activated is still under investigation, but a favored model is supported by all of the available evidence.

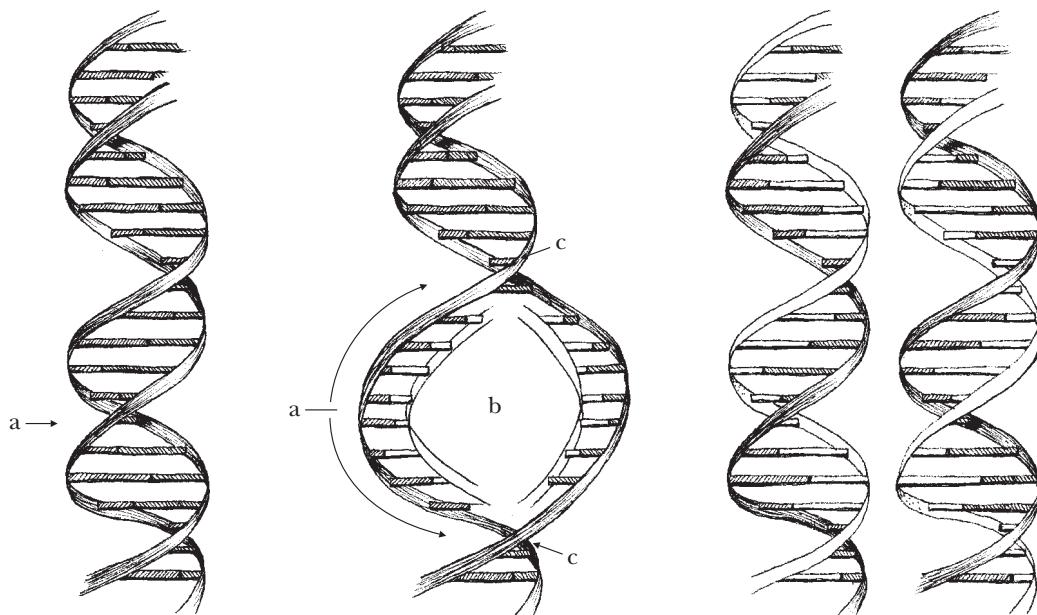
The ORC proteins are believed to be bound to the origin DNA throughout the cell cycle but become activated at the G_1/S boundary through the action of kinases. Kinases add phosphate groups to one or more of the six ORC proteins, activating them to initiate DNA replication. Different replicons are initiated at different times throughout S phase. It is unclear how the proposed regulatory system distinguishes between replicons that have been replicated in a particular S phase and those that have not, since each must be used once and only once during each cell division cycle.

A number of different enzymatic activities are required for the initiation process. The two strands of DNA must be unwound or separated, exposing each of the parent strands so they can be used as templates for the synthesis of new, complementary strands. This unwinding is mediated by an enzyme called a helicase. Once unwound, the single strands are stabilized by the binding of proteins called single-strand binding proteins (SSBs). The resulting structure resembles a “bubble” or “eye” in the DNA strand. This structure is recognized by the DNA replication machinery that is recruited to the site, and DNA replication begins. As replication proceeds, the DNA continues to unwind through the action of helicase. The site at which unwinding and DNA synthesis are occurring is at either end of the expanding eye or bubble, called a replication fork.

DNA Synthesis

The DNA synthesis machinery is not able to synthesize a strand of DNA from scratch; rather, a short stretch of RNA is used to begin the new strands. The synthesis of the RNA is catalyzed by an enzyme called primase. This

Stages in DNA Replication



At left, a double-stranded DNA molecule, with its sides formed by sugar-phosphate molecules and its “rungs” formed by base pairs. Replication begins at point (a), with the separation of a base pair as a result of the action of a special initiator protein (b). The molecule splits, or “unzips,” in opposite directions (c) as each parental strand is used as a template for the daughter strand, which is formed when bases form hydrogen bonds with their appropriate “mate” bases to form new ladder “rungs.” Finally (right), one parental strand and its newly synthesized daughter strand form a new double helix, while the other parental strand and its daughter strand form the second double helix. (Kimberly L. Dawson Kurnizki)

short piece of RNA, or primer, is extended using DNA nucleotides by the enzymes of DNA synthesis, called DNA polymerases. The RNA primer is later removed and replaced by DNA. Nucleotide monomers align with the exposed template DNA strand one at a time and are joined by the DNA polymerase. The joining of nucleotides into a growing DNA chain requires energy. This energy is supplied by the nucleotide monomers themselves. A high-energy phosphate bond in the nucleotide is split, and the breakage of this high-energy bond provides the energy to drive the polymerase reaction.

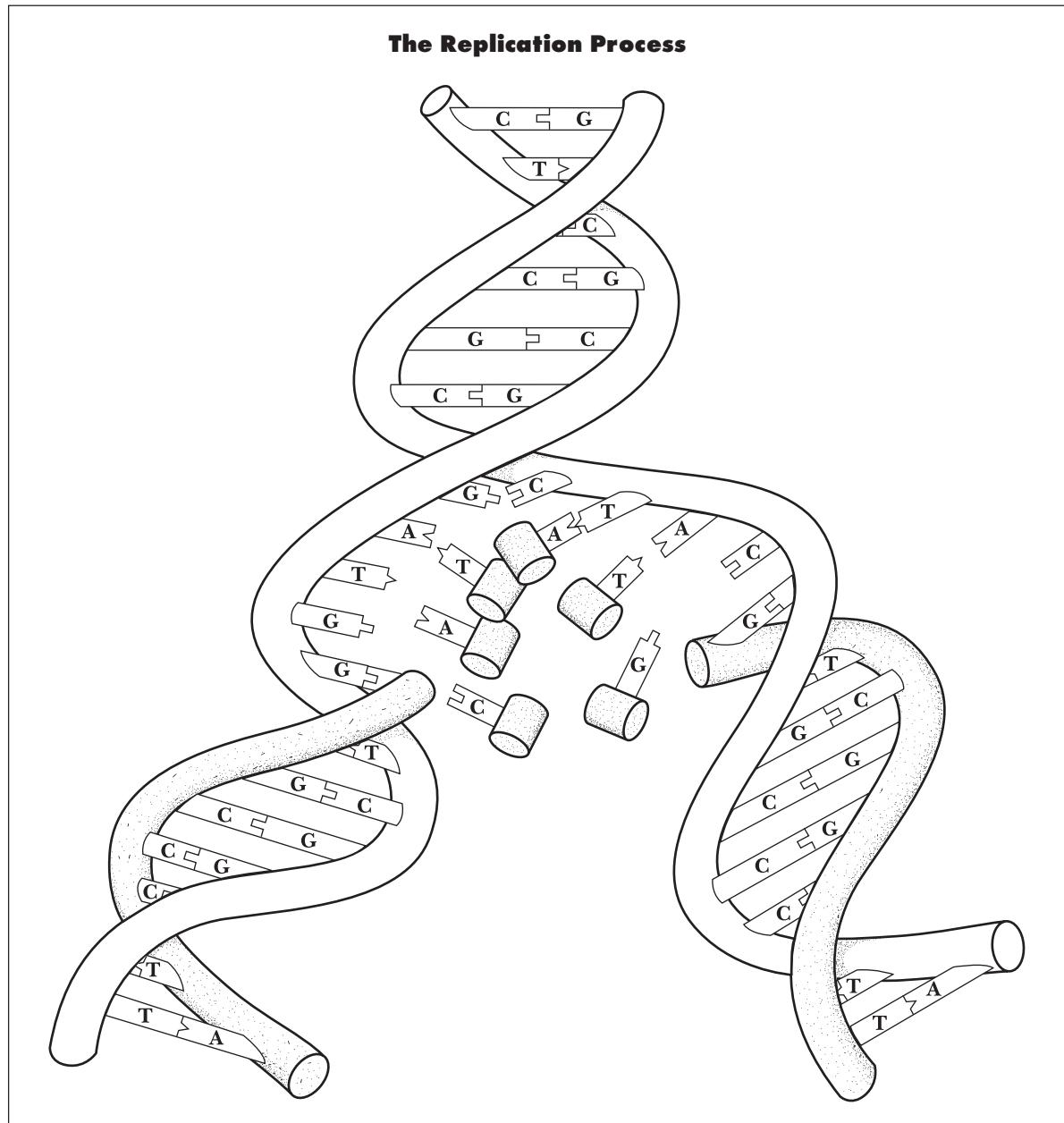
The two strands of DNA are not synthesized in the same way. The two strands are oriented opposite one another, but DNA synthesis only occurs in one direction: 5' to 3'. Therefore, one strand, called the leading strand, is synthesized continuously in the same direction that the replication fork is moving, while the lagging

strand is synthesized away from the direction of fork movement. Since the lagging-strand DNA synthesis and fork movement are in opposite directions, this strand of DNA must be made in short pieces that are later joined. Lagging-strand synthesis is therefore said to be discontinuous. These short intermediates are called Okazaki fragments, named for their discoverer, Reiji Okazaki. Overall, DNA replication is said to be semidiscontinuous.

The DNA synthesis machine operating at the replication fork is a complex assembly of proteins. Many different activities are necessary to carry out the process of DNA replication efficiently. Several proteins are necessary to recognize the unwound origin and assemble the rest of the complex. Primase must function to begin both new strands and is then required periodically throughout synthesis of the lagging strand. A doughnut-shaped clamp called

PCNA functions as a “processivity factor” to keep the entire complex attached to the DNA until the job is completed. Helicase is continuously required to unwind the template DNA and move the fork along the parent molecule. As the DNA is unwound, strain is created on

the DNA ahead of the replication fork. This strain is alleviated through the action of topoisomerase enzymes. Single-strand binding proteins are needed to stabilize the regions of unwound DNA that exist before the DNA is actually copied. Finally, an enzyme called ligase is



A detailed schematic of DNA replication, in which a double-standard parent helix splits apart and reassembles into two identical daughter helices. The amino acid base pairs are reproduced exactly, because cytosine (C) pairs only with guanine (G), and adenine (A) pairs only with thymine (T). (Electronic Illustrators Group)

necessary to join the regions replicated from different origins and to attach all of the Okazaki fragments of the lagging strand. All of these proteins are part of a well-orchestrated, efficient machine ideally suited to its task of copying the genetic material.

DNA polymerases are not perfect. At a relatively low frequency, they can add an incorrect nucleotide to a growing chain, one that does not match the template strand as dictated by the base-pairing rules. However, because the DNA molecules are so extremely large, novel mechanisms for proofreading have evolved to ensure that the genetic material is copied accurately. DNA polymerases can detect the misincorporation of a nucleotide and use an additional enzymatic activity to correct the mistake. Specifically, the polymerase can “back up” and cut out the last nucleotide added, then try again. With this and other mechanisms to correct errors, the observed error rate for DNA synthesis is a remarkable one error in every billion nucleotides added.

Impact and Applications

DNA replication is a fundamental cellular process: Proper cell growth cannot occur without it. It must be carefully regulated and tightly controlled. Despite its basic importance, the details of the mechanisms that regulate DNA replication are poorly understood. Even with all of the checks and balances that have evolved to ensure a properly replicated genome, occasional mistakes do occur. Attempting to replicate a genome damaged by chemical or other means may simply lead to death of a single cell. Far more ominous are genetic errors that lead to loss of regulating mechanisms. Without regulation, cell growth and division can proceed without normal limits, resulting in cancer. Much of the focus for the study of cell growth and regulation is to set a foundation for the understanding of how cancer cells develop. This knowledge may lead to new techniques for selective inhibition or destruction of cancer cells.

Manipulation of DNA replication and cell cycle control are the newest tools for progress in genetic engineering. In early 1997, the first successful cloning of an adult mammal, Dolly the sheep, raised important new issues about

the biology and ethics of manipulating mammalian genomes. The technology now exists to clone human beings, although such experiments are not likely to be carried out. More relevant is the potential impact on agriculture. It is now possible to select for animals that have the most desirable traits, such as lower fat content or disease resistance, and create herds of genetically identical animals. Of direct relevance to humans is the potential impact on the understanding of fertility and possible new treatments for infertility.

A new class of genetic diseases was discovered in the 1980's called triplet repeat diseases. Regions of DNA consist of copies of three nucleotides (such as CGG) that are repeated up to fifty times. Through unknown mechanisms related to DNA replication, the number of repeats may increase from generation to generation, at some point reaching a threshold level at which disease symptoms appear. Diseases found to conform to this pattern include fragile X syndrome, Huntington's disease (Huntington's chorea), and Duchenne muscular dystrophy.

The process of aging is closely related to DNA replication. Unlike bacteria, eukaryotic organisms have linear chromosomes. This poses problems for the cell, both in maintaining intact chromosomes (ends are unstable) and in replicating the DNA. The replication machinery cannot copy the extreme ends of a linear DNA molecule, so organisms have evolved alternate mechanisms. The ends of linear chromosomes consist of telomeres (short, repeated DNA sequences that are bound and stabilized by specific proteins), which are replicated by a separate mechanism using an enzyme called telomerase. Telomerase is inactivated in mature cells, and there may be a slow, progressive loss of the telomeres that ultimately leads to the loss of important genes, resulting in symptoms of aging. Cancer cells appear to have reactivated their telomerase, so potential anticancer therapies are being developed based on this information.

—Michael R. Lentz

See also: Animal Cloning; Cancer; Cell Cycle, The; Cell Division; Cloning; DNA Sequencing Technology; DNA Structure and Function;

Genetic Code; Genetic Engineering; Molecular Genetics; Mutation and Mutagenesis; Protein Structure; Protein Synthesis; Restriction Enzymes; RNA Structure and Function; RNA Transcription and mRNA Processing; Telomeres.

Further Reading

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DePamphilis, Melvin L., ed. *Concepts in Eukaryotic DNA Replication*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999. A broad account of the basic principles of DNA replication and related functions such as DNA repair and protein phosphorylation. One chapter surveys the most recent advances in the field.

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Kornberg, Arthur. *For the Love of Enzymes: The Odyssey of a Biochemist*. Reprint. Cambridge, Mass.: Harvard University Press, 1991. Kornberg discovered the enzymes that replicate DNA and was awarded the Nobel Prize for his work. This autobiography is a rich history of the process of science and discovery.

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DNA Sequencing Technology

Field of study: Genetic engineering and biotechnology

Significance: *The genetic code is contained in the ordered, linear arrangement of the four DNA nucleotides: adenine, cytosine, guanine, and thymine. Determining this sequence is called DNA sequencing. Advances in DNA sequencing technology have increased speed and accuracy of sequencing by several orders of magnitude.*

Key terms

AUTOMATED FLUORESCENT SEQUENCING: a modification of dideoxy termination sequencing which uses fluorescent markers to identify the terminal nucleotides, allowing the automation of sequencing in which robots can carry out large scale projects

BASE PAIR (bp): often used as a measure of the size of a DNA fragment or the distance along a DNA molecule between markers; both the singular and plural are abbreviated bp

MAXAM-GILBERT SEQUENCING: A method of base-specific chemical degradation to determine DNA sequence

PRIMER: A short piece of single-stranded DNA that can hybridize to denatured DNA and provide a start point for extension by a DNA polymerase

SANGER SEQUENCING: Also known as dideoxy termination sequencing, a method using nucleotides that are missing the 3' hydroxyl group in order to terminate the polymerization of new DNA at a specific nucleotide

The Need for Sequencing

DNA was first discovered in the 1869 as a viscous material in pus, and its basic chemical composition was well established by the 1930's. By 1950, the critical role of DNA as the hereditary material was clearly determined. In the 1950's, the classic papers by James Watson and Francis Crick and Matthew Meselson and Frank Stahl gave scientists a clear picture of the structure and mode of replication of DNA. Crick demonstrated that the genetic code con-

sisted of triplet codons in 1961. However, there was no system to read the sequence and uncover the actual words that spelled out the code of life.

The discovery of rapid sequencing methods in the 1970's created a flood of new discoveries in biology. The coding region and control elements could be identified and compared. The sequence changes in different alleles of the same gene could be identified, homologous genes could be identified in divergent species, and evolutionary changes could be studied. Today, an entire genome can be sequenced, meaning the identification of every nucleotide in the correct order along every chromosome, in a matter of months. This ability to sequence the genomes of entire organisms has created a whole new field called genomics, the study and comparison of whole genomes of different organisms. Sequencing is now at the core of many of the new discoveries in biology.

Principles of DNA Sequencing

Molecular biologists cannot observe DNA molecules directly, even through a microscope, so they must devise controlled chemical reactions whose outcomes are indicative of what occurs at the submicroscopic level. In DNA sequencing, the key is to use a chemical method that allows analysis of the base sequence one base at a time. Such a method needs to produce a collection of DNA fragments whose lengths can be used to detect the identity of the base located at the end of each different-sized fragment. For example, if fragments of the short DNA sequence ACGTCCGATCG can be predictably produced, then the size of each fragment could be used to determine the locations of each base. If the fragment is cut to the right of each of the thymine bases, two fragments of 4 and 9 base pairs (bp) will be produced. Doing the same for the other three nucleotides could identify their positions. Reading from smallest to largest fragment and seeing which reaction generates each piece provides the DNA sequence. Although this is a very simple example, the principles apply to all current sequencing methods. Electrophoresis in denaturing polyacrylamide gels (to keep the DNA single-stranded) is used to separate fragments that are



Frederick Sanger developed one of the first methods for sequencing DNA and published the first genome sequence. (© The Nobel Foundation)

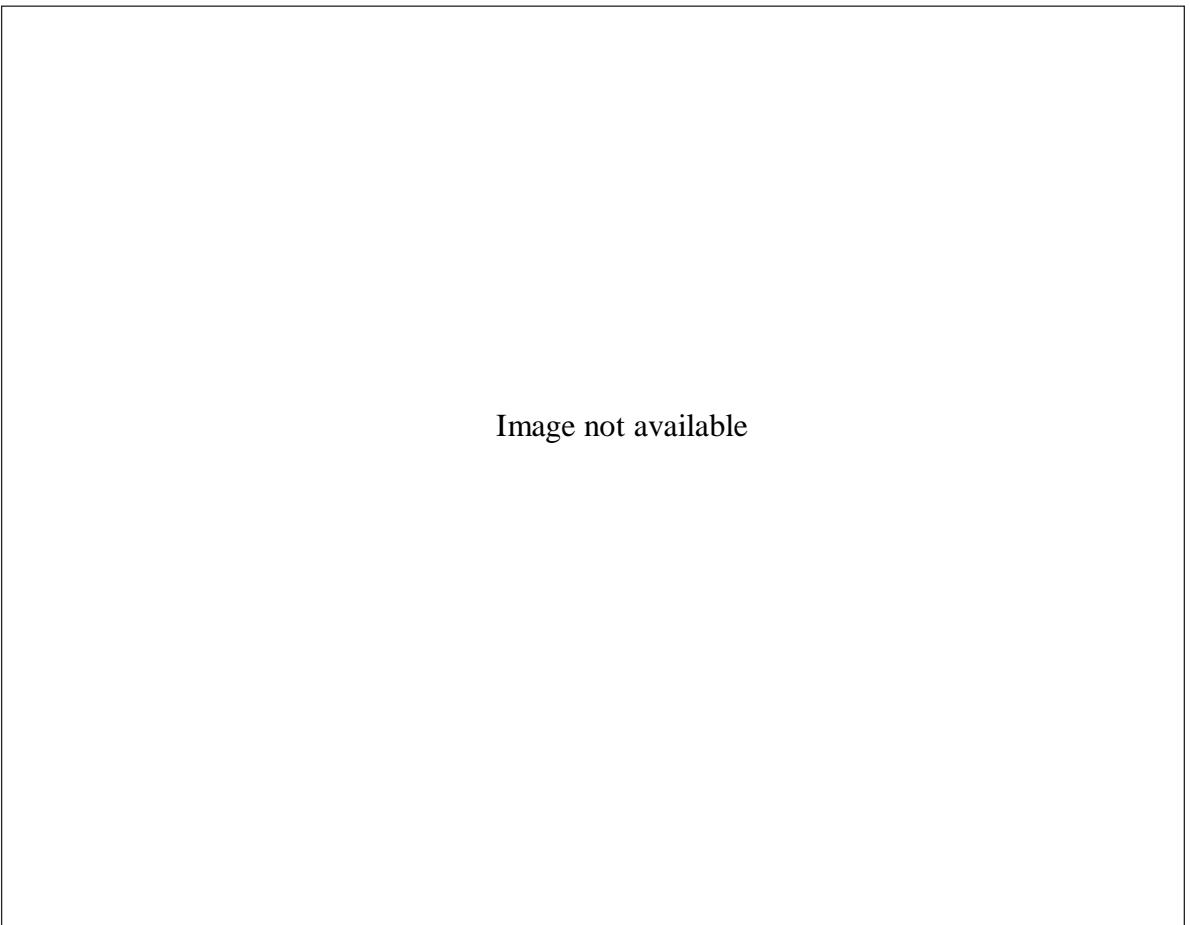


Image not available

A DNA sequencing program at work, displaying the bar-code-like DNA sequence on a computer screen. (AP/Wide World Photos)

hundreds of base pairs in length but differ by only a single nucleotide. The DNA is labeled with either radioactive or fluorescent markers so that the bands can be detected.

Maxam-Gilbert Sequencing

Organic chemists working with DNA have identified many chemicals that react with specific bases and cleave the backbone of the strand at that location. To sequence DNA with this method, the DNA fragment to be sequenced is isolated and the 5' end of only one of the strands is labeled by the placement in the terminal phosphate of the radioactive atom phosphorus 32. This creates the endpoint. In separate tubes, the DNA is reacted with chemicals that will cleave at one of the four nucleotides. Since only one strand is broken, it is nec-

essary to denature the DNA to separate the strands before separating them by size so that the fragments will correspond in length to the distance from the terminal phosphate label to the cleavage point. The method requires dangerous chemicals and does not easily lend itself to automation, so it is rarely used today.

Sanger Sequencing

This method requires that the sequence of a short stretch of DNA adjacent to the region to be sequenced is known so that a short synthetic oligonucleotide can be made which can hybridize onto the region to act as a primer for DNA synthesis in the direction of the DNA to be sequenced. This is usually not a problem, since the DNA to be sequenced is often cloned into a plasmid vector whose sequence is known.

The DNA is denatured and the primer is allowed to anneal. A DNA polymerase is added and extends the DNA for a short distance in the presence of radioactive nucleotides, which labels the new DNA. The reaction is then divided into four equal parts and added into four separate reaction tubes. Each tube contains all four DNA nucleotides, but a small percentage of one nucleotide is missing the 3' hydroxyl group. Without the hydroxyl group, no more bases can be added and the reaction terminates. Since the dideoxy nucleotide constitutes only a small percentage of the available nucleotides, the reactions will terminate at random places along the DNA strand. Since the terminated fragment is attached to the larger template strand, the DNA must be denatured before gel electrophoresis so that the size will correspond accurately to the position of the terminated base.

Automated Sequencing

Automated sequencing is a variant of Sanger sequencing. Each of the four dideoxy bases has a different fluorescent dye attached. When the elongation is blocked, the fragment will also be labeled with a specific color indicating which nucleotide is in the terminal position. As a result, four separate reactions (one for each nucleotide) are not needed. Also, the polymerase chain reaction (PCR), which requires much smaller amounts of DNA, is often used in automated sequencers. The reaction products are electrophoresed through a narrow tube of polyacrylamide with a laser and fluorescence detector at the bottom. As the different-sized fragments reach the bottom, the detector will register the colors as they pass. The data are logged on a computer, which outputs the DNA sequence. This system has been automated with robot arms moving the samples into reaction tubes and loading them into the tube gels and computers compiling and comparing the sequence data. An automated sequencer may cost more than \$250,000 but can generate 10,000 bp of new sequence data per day.

Future Directions

Scientists have developed silicon chips that can bind to DNA and change their electrical

properties when they bind. Currently, the technology allows interaction only with specific sequences. However, the goal is to develop new systems that will generate electrical outputs indicative of the sequence of the DNA with which the chip interacts. This would allow the generation of new data at phenomenal rates, would allow field scientists to sequence the genomes of new organisms instantly, and would allow police investigators to generate sequence evidence right at the crime scene. While such developments would be very exciting from a scientific perspective, they would also open many new questions about personal privacy as prospective employers (and mates) could easily scan a person's genome for undesirable sequences.

—J. Aaron Cassill

See also: Cloning; Cloning Vectors; DNA Replication; Genetic Code; Genetic Engineering; Genome Libraries; Genome Size; Genomics; Human Genome Project; Knockout Genetics and Knockout Mice; Model Organism: *Escherichia coli*; Molecular Clock Hypothesis; Polymerase Chain Reaction; Population Genetics; Pseudogenes; Repetitive DNA; Restriction Enzymes; Reverse Transcriptase; RFLP Analysis; Shotgun Cloning; Synthetic Genes; Transposable Elements.

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DNA Structure and Function

Field of study: Molecular genetics

Significance: Structurally, DNA is a relatively simple molecule; functionally, however, it has wide-ranging roles in the cell. It functions primarily as a stable repository of genetic information in the cell and as a source of genetic information for the production of proteins. Greater knowledge of the characteristics of DNA has led to advances in the fields of genetic engineering, gene therapy, and molecular biology in general.

Key terms

DOUBLE HELIX: the molecular shape of DNA molecules, which resembles a ladder that twists, or spirals

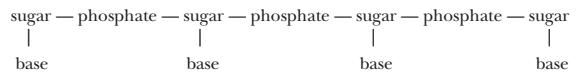
GENE EXPRESSION: the processes (transcription and translation) by which the genetic information in DNA is converted into protein

TRANSCRIPTION: the process by which genetic information in DNA is converted into messenger RNA (mRNA)

TRANSLATION: the process by which the genetic information in an mRNA molecule is converted into protein

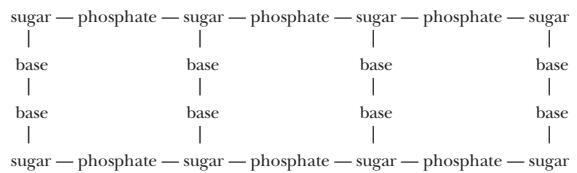
Chemical and Physical Structure of DNA

Deoxyribonucleic acid (DNA) is the genetic material found in all cells. Chemically, it is classified as a nucleic acid, a relatively simple molecule composed of nucleotides. A nucleotide consists of a sugar (deoxyribose), a phosphate group, and one of the nitrogenous bases: adenine (A), cytosine (C), guanine (G), or thymine (T). In fact, nucleotides differ only in the particular nitrogenous base that they contain. Ribonucleic acid (RNA) is the other type of nucleic acid found in the cell; however, it contains ribose as its sugar instead of deoxyribose and has the nitrogenous base uracil (U) instead of thymine. Nucleotides can be assembled into long chains of nucleic acid via connections between the sugar on one nucleotide and the phosphate group on the next, thereby creating a sugar-phosphate “backbone” in the molecule. The nitrogenous base on each nucleotide is positioned such that it is perpendicular to the backbone, as shown in the following diagram:



Any one of the four DNA nucleotides (A, C, G, or T) can be used at any position in the molecule; it is therefore the specific sequence of nucleotides in a DNA molecule that makes it unique and able to carry genetic information. The genetic information is the sequence itself.

In the cell, DNA exists as a double-stranded molecule; this means that it consists of two chains of nucleotides side by side. The double-stranded form of DNA can most easily be visualized as a ladder, with the sugar-phosphate backbones being the sides of the ladder and the nitrogenous bases being the rungs of the ladder, as shown in the following diagram:



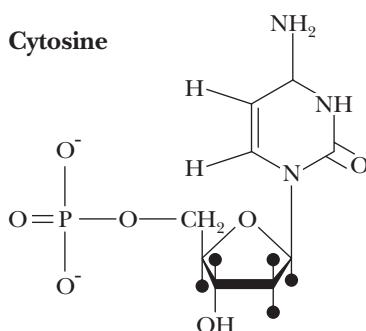
This ladder is then twisted into a spiral shape. Any spiral-shaped molecule is called a “helix,” and since each strand of DNA is wound into a spiral, the complete DNA molecule is often called a “double helix.” This molecule is extremely flexible and can be compacted to a great degree, thus allowing the cell to contain large amounts of genetic material.

The Discovery of DNA as the Genetic Material

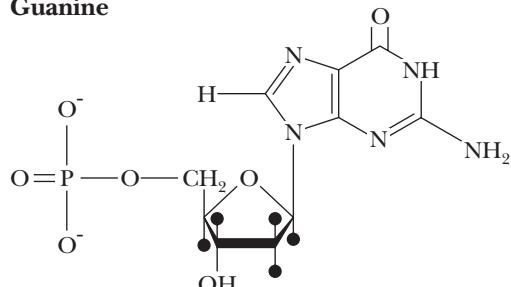
Nucleic acids were discovered in 1869 by the physician Friedrich Miescher. He isolated these molecules, which he called nuclein, from the nuclei of white blood cells. This was the first association of nucleic acids with the nucleus of the cell. In the 1920’s, experiments performed by other scientists showed that DNA could be located on the chromosomes within the nucleus. This was strong evidence for the role of DNA in heredity, since at that time there was already a link between the activities of chromosomes during cell division and the inheritance of particular traits, largely because of the work

The Four Nucleotides That Compose DNA

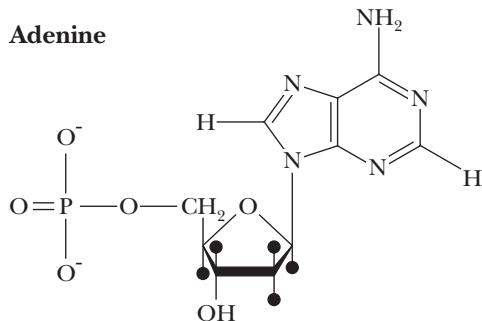
Cytosine



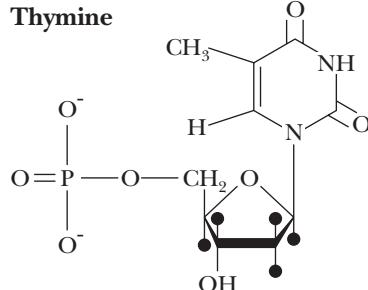
Guanine



Adenine



Thymine



of the geneticist Thomas Hunt Morgan about ten years earlier.

However, it was not immediately apparent, based on this evidence alone, that DNA was the genetic material. In addition to DNA, proteins are present in the nucleus of the cell and are an integral part of chromosomes as well. Proteins are also much more complex molecules than nucleic acids, having a greater number of building blocks; there are twenty amino acids that can be used to build proteins, as opposed to only four nucleotides for DNA. Moreover, proteins tend to be much more complex than DNA in terms of their three-dimensional structure as well. Therefore, it was not at all clear in the minds of many scientists of the time that DNA had to be the genetic material, since proteins could not specifically be ruled out.

In 1928, the microbiologist Frederick Griffith supplied some of the first evidence that eventually led to the identification of DNA as the genetic material. Griffith's research involved the bacterium *Streptococcus pneumoniae*, a

common cause of lung infections. He was working primarily with two different strains of this bacterium: a strain that was highly virulent (able to cause disease) and a strain that was nonvirulent (not able to cause disease). Griffith noticed that if he heat-killed the virulent strain and then mixed its cellular debris with the living, nonvirulent strain, the nonvirulent strain would be "transformed" into a virulent strain. He did not know what part of the heat-killed virulent cells was responsible for the transformation, so he simply called it the "transforming factor" to denote its activity in his experiment. Unfortunately, Griffith never took the next step necessary to reveal the molecular identity of this transforming factor.

That critical step was taken by another microbiologist, Oswald Avery, and his colleagues in 1944. Avery essentially repeated Griffith's experiments with two important differences: Avery partially purified the heat-killed virulent strain preparation and selectively treated this preparation with a variety of enzymes to see if

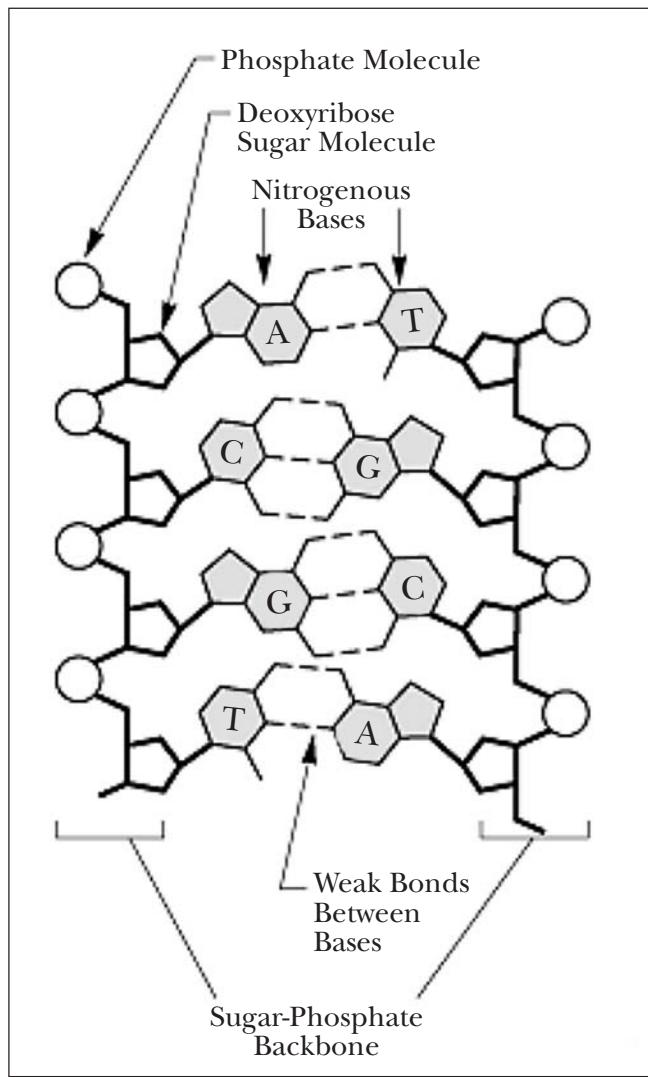
the transforming factor could be eliminated, thereby eliminating the transformation itself. Avery showed that transformation was prevented only when the preparation was treated with deoxyribonuclease, an enzyme that specifically attacks and destroys DNA. Other enzymes that specifically destroy RNA or proteins could not prevent transformation from occurring. This was extremely strong evidence that the genetic material was DNA.

Experiments performed in 1952 by molecular biologists Alfred Hershey and Martha Chase using the bacterial virus *T2* finally demonstrated conclusively that DNA was indeed the genetic material. Hershey and Chase studied how *T2* infects bacterial cells to determine what part of the virus, DNA or protein, was responsible for causing the infection, thinking that whatever molecule directed the infection would have to be the genetic material of the virus. They found that DNA did directly participate in infection of the cells by entering them, while the protein molecules of the viruses stayed outside the cells. Most strikingly, they found that the original DNA of the “parent” viruses showed up in the “offspring” viruses produced by the infection, directly demonstrating inheritance of DNA from one generation to another. This was an important element of the argument for DNA as the genetic material.

The Watson-Crick Double-Helix Model of DNA

With DNA conclusively identified as the genetic material, the next step was to determine the structure of the molecule. This was finally accomplished when the double-helix model of DNA was proposed by molecular biologists James Watson and Francis Crick in 1953. This model has a number of well-defined and experimentally determined characteristics. For example, the diameter of the molecule, from one sugar-phosphate backbone to the other, is 20 angstroms. (There are 10 million angstroms in one

millimeter, which is one-thousandth of a meter.) There are 3.4 angstroms from one nucleotide to the next, and the entire double helix makes one turn for every ten nucleotides, a distance of about 34 angstroms. These measurements were determined by the physicists Maurice Wilkins and Rosalind Franklin around 1951 using a process called X-ray diffraction, in which crystals of DNA are bombarded with X rays; the resulting patterns captured on film



A schematic showing the major components of a DNA molecule, including the four bases that compose DNA—adenine (A) and thymine (T), cytosine (C) and guanine (G)—and how they form the “rungs” of the DNA “ladder” by forming hydrogen bonds. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

gave Wilkins and Franklin, and later Watson and Crick, important clues about the physical structure of DNA.

Another important aspect of Watson and Crick's double-helix model is the interaction between the nitrogenous bases in the interior of the molecule. Important information about the nature of this interaction was provided by molecular biologist Erwin Chargaff in 1950. Chargaff studied the amounts of each nitrogenous base present in double-stranded DNA from organisms as diverse as bacteria and humans. He found that no matter what the source of the DNA, the amount of adenine it contains is always roughly equal to the amount of thymine; there are also equal amounts of guanine and cytosine in DNA. This information led Watson and Crick to propose an interaction, or "base pairing," between these sets of bases such that A always base pairs with T (and vice versa) and G always base pairs with C. Another name for this phenomenon is "complementary base pairing": A is said to be the "complement" of T, and so on.

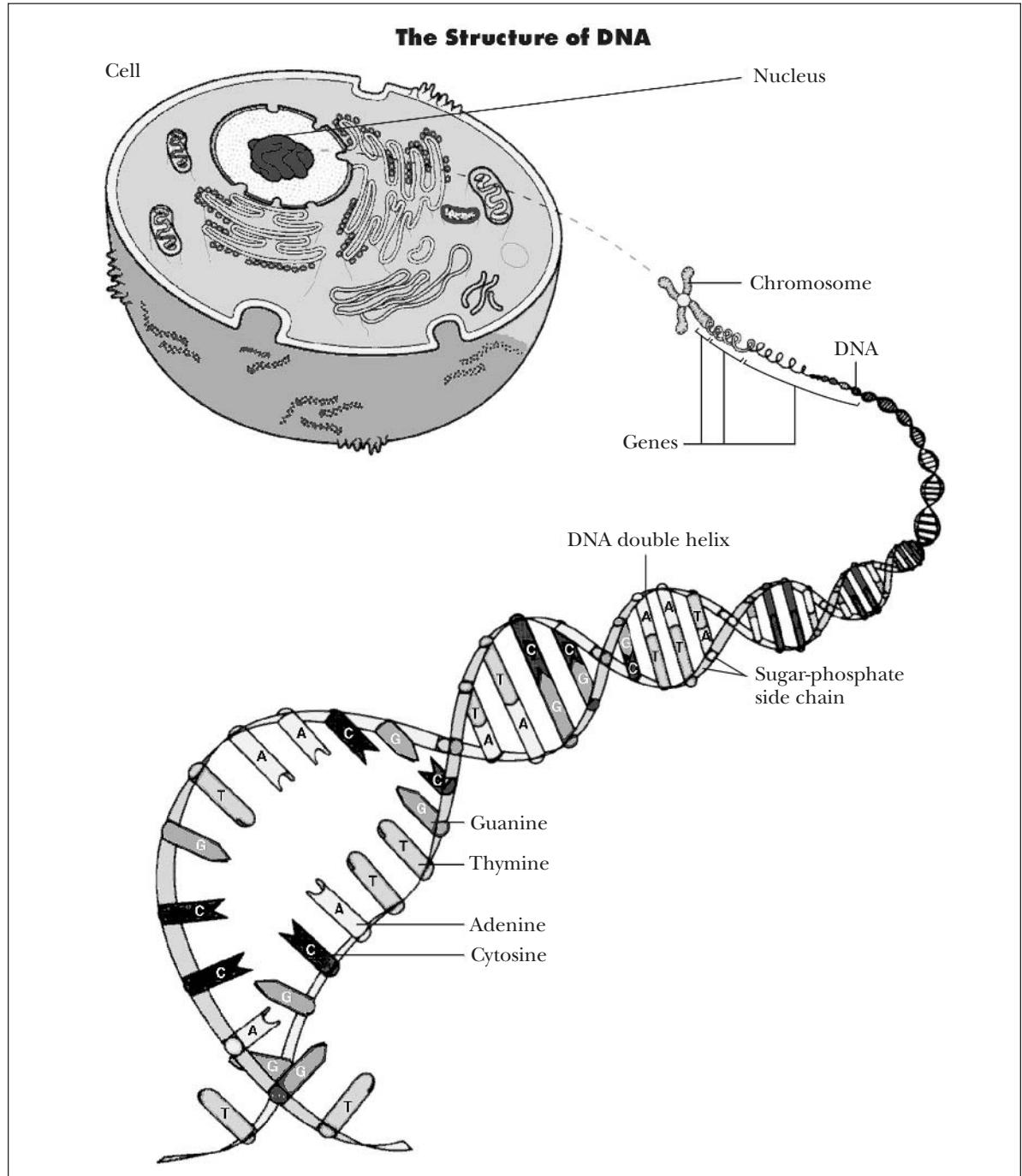
The force that holds complementary bases, and therefore the two strands of DNA, together is a weak chemical interaction called a "hydrogen bond," which is created whenever a hydrogen atom in one molecule has an affinity for nitrogen or oxygen atoms in another molecule. The affinity of the atoms for each other draws the molecules together in the hydrogen bond. A-T pairs have two hydrogen bonds between them because of the chemical structure of the bases, whereas G-C pairs are connected by three hydrogen bonds, making them slightly stronger and more stable than A-T pairs. The entire DNA double helix, although it is founded upon the hydrogen bond, one of the weakest bonds in nature, is nonetheless an extraordinarily stable structure because of the combined force of the millions of hydrogen bonds holding most DNA molecules together. However, these hydrogen bonds can be broken under certain conditions in the cell. This usually occurs as part of the process of the replication of the double helix, in which the two strands of DNA must come apart in order to be duplicated. In the cell, the hydrogen bonds are broken with the help of enzymes. Under artificial condi-

tions in the laboratory, hydrogen bonds in the double helix can easily be broken just by heating a solution of DNA to high temperatures (close to the boiling point).

Other Features of the Watson-Crick Model

Watson and Crick were careful to point out that their double-helix model of DNA was the first model to immediately suggest a mechanism by which the molecule could be replicated. They knew that this replication, which must occur before the cell can divide, would be a necessary characteristic of the genetic material of the cell and that an adequate model of DNA must help explain how this duplication could occur. Watson and Crick realized that the mechanism of complementary base pairing that was an integral part of their model was a potential answer to this problem. If the double helix is separated into its component single-strand molecules, each strand will be able to direct the replacement of the opposite, or complementary, strand by base pairing properly with only the correct nucleotides. For example, if a single-strand DNA molecule has the sequence TTAGTCA, the opposite complementary strand will always be AATCAGT; it is as if the correct double-stranded structure is "built in" to each single strand. Additionally, as each of the single strands in a double-strand DNA molecule goes through this addition of complementary nucleotides, two new DNA double helices are produced where there was only one before. Further, these new DNA molecules are completely identical to each other, barring any mistakes that might have been made in the replication process.

A strand of DNA also has a certain direction built into it; the DNA double helix is often called "antiparallel" in reference to this aspect of its structure. "Antiparallel" means that although the two strands of the DNA molecule are essentially side by side, they are oriented in different directions relative to the position of the deoxyribose molecules on the backbone of the molecule. To help keep track of the orientation of the DNA molecule, scientists often refer to a 5' to 3' direction. This designation comes from numbering the carbon atoms on the deoxyribose molecule (from 1' to 5') and takes



note of the fact that the deoxyribose molecules on the DNA strand are all oriented in the same direction in a head-to-tail fashion. If it were possible to stand on a DNA molecule and walk down one of the sugar-phosphate backbones,

one would encounter a 5' carbon atom on a sugar, then the 3' carbon, and so on all the way down the backbone. If one were walking on the other strand, the 3' carbon atom would always be encountered before the 5' carbon. The con-

cept of an antiparallel double helix has important implications for the ways that DNA is produced and used in the cell. Generally, the cellular enzymes that are involved in processes concerning DNA are restricted to recognizing just one direction. For example, DNA polymerase, the enzyme that is responsible for making DNA in the cell, can only make DNA in a 5' to 3' direction, never the reverse.

Watson and Crick postulated a right-handed helix as part of their double-helix model; this means that the strands of DNA turn to the right, or in a counterclockwise fashion. This is now regarded as the “biological” (B) form of DNA because it is the form present inside the nucleus of the cell and in solutions of DNA. However, it is not the only possible form of DNA. In 1979, an additional form of DNA was discovered by molecular biologist Alexander Rich that exhibited a zigzag, left-handed double helix; he called this form of DNA Z-DNA. Stretches of alternating G and C nucleotides most commonly give rise to this conformation of DNA, and scientists think that this alternative form of the double helix is important for certain processes in the cell in which various molecules bind to the double helix and affect its function.

The Function of DNA in the Cell

DNA plays two major roles in the cell. The first is to serve as a storehouse of the cell’s genetic information. Normally, cells have only one complete copy of their DNA molecules, and this copy is, accordingly, highly protected. DNA is a chemically stable molecule; it resists damage or destruction under normal conditions, and, if it is damaged, the cell has a variety of mechanisms to ensure the molecule is rapidly repaired. Furthermore, when the DNA in the cell is duplicated in a process called DNA replication, this duplication occurs in a regulated and precise fashion so that a perfect copy of DNA is produced. Once the genetic material of the cell has been completely duplicated, the cell is ready to divide in two in a process called mitosis. After cell division, each new cell of the pair will have a perfect copy of the genetic material; thus these cells will be genetically identical to each other. DNA thus provides a

mechanism by which genetic information can be transferred easily from one generation of cells (or organisms) to another.

The second role of DNA is to serve as a blueprint for the ultimate production of proteins in the cell. This process occurs in two steps. The first step is the conversion of the genetic information in a small portion of the DNA molecule, called a gene, into messenger RNA (mRNA). This process is called transcription, and here the primary role of the DNA molecule is to serve as a template for synthesis of the mRNA molecule. The second step, translation, does not involve DNA directly; rather, the mRNA produced during transcription is in turn used as genetic information to produce a molecule of protein. However, it is important to note that genetic information originally present in the DNA molecule indirectly guides the synthesis and final amino acid sequence of the finished protein. Both of these steps, transcription and translation, are often called gene expression. A single DNA molecule in the form of a chromosome may contain thousands of different genes, each providing the information necessary to produce a particular protein. Each one of these proteins will then fulfill a particular function inside or outside the cell.

Impact and Applications

Knowledge of the physical and chemical structure of DNA and its function in the cell has undoubtedly had far-reaching effects on the science of biology. However, one of the biggest effects has been the creation of a new scientific discipline: molecular biology. With the advent of Watson and Crick’s double-helix model of DNA, it became clear to many scientists that, perhaps for the first time, many of the important molecules in the cell could be studied in detail and that the structure and function of these molecules could also be elucidated. Within fifteen years of Watson and Crick’s discovery, a number of basic genetic processes in the cell had been either partially or completely detailed, including DNA replication, transcription, and translation. Certainly the seeds of this revolution in biology were being planted in the decades before Watson and Crick’s 1953 model, but it was the double helix that al-

lowed scientists to investigate the important issues of genetics on the cellular and molecular levels.

An increased understanding of the role DNA plays in the cell has also provided scientists with tools and techniques for changing some of the genetic characteristics of cells. This is demonstrated by the rapidly expanding field of genetic engineering, in which scientists can precisely manipulate DNA and cells on the molecular level to achieve a desired result. Additionally, more complete knowledge of how the cell uses DNA has opened windows of understanding into abnormal cellular processes such as cancer, which is fundamentally a defect involving the cell's genetic information or the expression of that information.

Through the tools of molecular genetics, many scientists hope to be able to correct almost any genetic defect that a cell or an organism might have, including cancer or inherited genetic defects. The area of molecular biology that is concerned with using DNA as a way to correct cellular defects is called gene therapy. This is commonly done by inserting a normal copy of a gene into cells that have a defective copy of the same gene in the hope that the normal copy will take over and eliminate the effects of the defective gene. It is hoped that this sort of technology will eventually be used to overcome even complex problems such as Alzheimer's disease and acquired immunodeficiency syndrome (AIDS).

One of the most unusual and potentially rewarding applications of DNA structure was introduced by computer scientist Leonard Adleman in 1994. Adleman devised a way to use short pieces of single-stranded DNA in solution as a rudimentary "computer" to solve a relatively complicated mathematical problem. By devising a code in which each piece of DNA stood for a specific variable in his problem and then allowing these single-stranded DNA pieces to base pair with each other randomly in solution, Adleman obtained an answer to his problem in a short amount of time. Soon thereafter, other computer scientists and molecular biologists began to experiment with other applications of this fledgling technology, which represents an exciting synthesis of two formerly

separate disciplines. It may be that this research will prove to be the seed of another biological revolution with DNA at its center.

—Randall K. Harris

See also: Ancient DNA; Antisense RNA; Chromosome Structure; DNA Isolation; DNA Repair; DNA Replication; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Noncoding RNA Molecules; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; Repetitive DNA; RNA Isolation; RNA Structure and Function; RNA Transcription and mRNA Processing.

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Web Sites of Interest

Deakin University, Australia. http://agrippina.bcs.deakin.edu.au/bcs_courses/forensic/chemical%20detective/dna_type.htm. A richly illustrated guide to the molecular structure and function of DNA.

Left-Handed DNA Hall of Fame. <http://www.lecb.ncifcrf.gov/~toms/lefthanded.DNA.html>. Molecular information theorist Tom Schneider created this site to document media and book illustrations in which DNA is shown incorrectly twisting to the left.

University of Massachusetts. DNA Structure. <http://molvis.sdsu.edu/dna/index.htm>. An interactive, animated, downloadable tutorial on the molecular composition and structure of DNA for high school students and college freshmen. Available in Spanish, German, and Portuguese.

Key terms

ANEUPLOID: a cell or individual with one or a few missing or extra chromosomes

MEIOSIS: a process of nuclear division that produces cells containing half the number of chromosomes as the original cell

NONDISJUNCTION: the failure of homologous chromosomes to separate correctly during cell division

TRISOMY: the condition of having an extra chromosome in a set present, such as having three copies of chromosome 21, as in Down syndrome

Discovery and Cause

Down syndrome is one of the most common chromosomal defects in human beings. According to some studies, it occurs in one in seven hundred live births; other studies place the number at one in nine hundred. Further, it occurs in about one in every two hundred conceptions. This syndrome (a pattern of characteristic abnormalities) was first described in 1866 by the English physician John Langdon Down. While in charge of an institution housing the profoundly mentally retarded, he noticed that almost one in ten of his patients had a flat face and slanted eyes causing Down to use the term "mongolism" to describe the syndrome; this term, however, is misleading. Males and females of every race and ethnicity can and do have this syndrome. To eliminate the unintentionally racist implications of the term "mongolism," Lionel Penrose and his colleagues changed the name to Down syndrome.

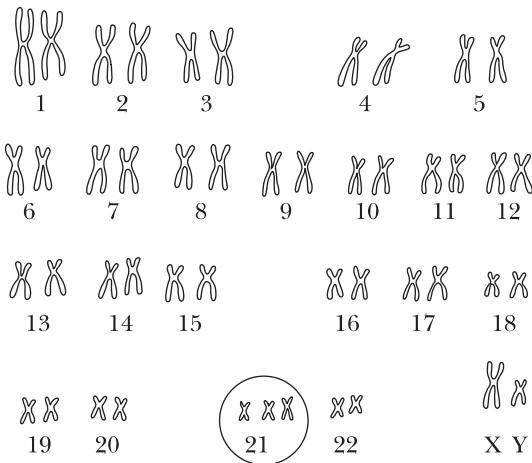
Although Down syndrome was observed and reported in the 1860's, it was almost one hundred years before the cause was discovered. In 1959, the French physician Jérôme Lejeune and his associates realized that the presence of an extra chromosome 21 was the apparent cause. This fact places Down syndrome in the broader category of aneuploid conditions. All human cells have forty-six chromosomes or strands made up of the chemical called deoxyribonucleic acid (DNA). The sections or subdivisions along these forty-six strands, called genes, are responsible for producing all the proteins that determine our specific human characteristics. An aneuploid is a cell with forty-

Down Syndrome

Field of study: Diseases and syndromes

Significance: *Down syndrome is one of the most thoroughly studied genetic diseases. The discovery that the syndrome is usually caused by the presence of an extra chromosome was a landmark in the understanding of the causes of genetic defects.*

The Cause of Down Syndrome



Down syndrome, or trisomy 21, is caused by the presence of an abnormal third chromosome in pair 21. (Electronic Illustrators Group)

five or forty-seven or more chromosomes, with the missing or extra strands of DNA leaving the individual with too few or too many genes. This aneuploid condition then results in significant alterations in one's traits and a great number of potential abnormalities.

In a normal individual, the forty-six strands are actually twenty-three pairs of chromosomes that are referred to as homologous because each pair is the same size and contains the same genes. In most cases of Down syndrome, there are three copies of chromosome 21. An aneuploid with three of a particular chromosome is called trisomic; thus Down syndrome is often called trisomy 21. The extra chromosome is gained because either the egg or sperm that came together at fertilization contained an extra one. This error in gamete (egg or sperm) production is called nondisjunction and occurs during the process of meiosis. When meiosis proceeds normally, the homologous chromosome pairs are separated from each other, forming gametes with twenty-three chromosomes, one from each pair. If nondisjunction occurs, a pair fails to separate, producing a gamete type with twenty-two chromosomes and a second gamete type with twenty-four chromo-

somes. If the pair that has failed to separate is chromosome 21, then the potential exists for twenty-three chromosomes in a normal gamete to combine with a gamete containing twenty-four, creating a trisomic individual with forty-seven chromosomes.

Symptoms of Down Syndrome

The slanted appearance of the eyes first reported by Down is caused by a prominent fold of skin called an epicanthic fold (a fold in the upper eyelid near the corner of the eye). This fold of skin is accompanied by excess skin on the back of the neck and abnormal creases in the skin of the palm. In addition, the skull is wide, with a flat back and a flat face. The hair on the skull is sparse and straight. The rather benign physical abnormalities are minor compared to the defects in internal organ systems. Almost 40 percent of Down syndrome patients suffer from serious heart defects. They are very prone to cancer of the white blood cells (acute leukemia), the formation of cataracts, and serious recurring respiratory infections. Short of stature with poorly formed joints, they often have poor reflexes, weak muscle tone, and an unstable gait. The furrowed, protruding tongue that often holds the mouth partially open is an external sign of the serious internal digestive blockages frequently present. These blockages must often be surgically repaired before the individual's first birthday. Many suffer from major kidney defects that are often irreparable. Furthermore, a suppressed immune system can easily lead to death from an infectious disease such as influenza or pneumonia.

With all these potential physical problems, it is not surprising that nearly 50 percent of Down syndrome patients die before the age of one. For those who live, there are enormous physical, behavioral, and mental challenges. The mental retardation that always accompanies Down syndrome ranges from quite mild to profound. This mental retardation makes all learning difficult and speech acquisition in particular very slow. Yet most Down syndrome individuals have warm, loving personalities and enjoy art and music very much.

Modern Understanding of Down Syndrome

Although this syndrome was recognized by Down in 1866, true understanding of it dates from the work that Lejeune began in 1953. The seemingly innocuous characteristic of abnormal palm prints and fingerprints fostered an important insight for him. Since those prints are laid down very early in the child's prenatal development, they suggest a profoundly altered embryological course of events. His intuition told him that not one or two altered genes but rather a whole chromosome's genes must be at fault. In 1957, he discovered, by the culturing of cells from Down syndrome children in dishes in the laboratory, that those cells contained forty-seven chromosomes. This work eventually resulted in his 1959 publication,

Image not available

Down syndrome is characterized by impaired mental ability and a complex of physical traits that may include short stature, stubby fingers and toes, protruding tongue, a single transverse palm crease, slanting of the eyes, small nose and ears, abnormal finger orientation, congenital heart defects, and other traits that vary from individual to individual. Despite some or all of these traits, many Down syndrome children, supported by parents and organizations such as the Special Olympics and Boy Scouts, engage fully in life. (AP/Wide World Photos)

which was soon followed by the discovery that the extra chromosome present was a third copy of chromosome 21.

The modern development of more sophisticated methods of identifying individual parts of chromosomes has shed much light on the possible mechanisms by which the symptoms are caused. Some affected individuals do not have a whole extra chromosome 21; rather, they possess a third copy of some part of that chromosome. A very tiny strand of DNA, chromosome 21 contains only about fifteen hundred genes. Of these fifteen hundred, only a few hundred are consistently present in those who suffer from Down syndrome, namely the genes in the bottom one-third of the chromosome. Among those genes are several that could very likely cause certain symptoms associated with Down syndrome. A leukemia-causing gene and a gene for a protein in the lens of the eye that could trigger cataract formation have both been identified. A gene for the production of the chemicals called purines has been located. The overabundance of purines produced when three copies of this gene are present has been linked to the mental retardation usually seen. Even the fact that Down syndrome individuals have a greatly reduced life expectancy is validated by the presence of an extra gene for the enzyme superoxide dismutase, which seems involved in the normal aging process. Like Alzheimer's disease patients, Down syndrome patients who live past forty years of age have gummy tangles of protein strands called amyloid fibers in their brains. Since one form of inherited Alzheimer's is caused by a gene on chromosome 21, scientists continue to search for links between the impaired mental functioning characteristic of both diseases.

Other modern research has shed light on the long-recognized relationship between the age of the mother and an increased risk of having a Down syndrome child. Using more and more elaborate methods of chromosome banding, geneticists can determine whether the extra

Familial Down Syndrome

Down syndrome always involves either an extra portion of or a complete extra copy of chromosome 21. There are three mechanisms by which this can occur. Between 92 and 95 percent of cases result from non-disjunction during meiosis, in which two copies of chromosome 21 migrate to the same pole and end up in the same daughter cell. This most often happens in women, and if an egg with two copies of chromosome 21 is fertilized, the zygote will have three copies and all cells throughout the developing fetus will have an extra chromosome 21.

The second mechanism, mosaic trisomy 21, involves an error in cell division shortly after conception. This error produces two populations or lines of cells, some with 46 chromosomes and some with 47—the ones that have the additional chromosome 21. This mechanism occurs in 2 to 4 percent of Down syndrome live births. Covert mosaicism in parents used to be suspected as causing familial Down syndrome but is no longer indicated.

Between 1 and 4 percent of children with Down syndrome have translocation trisomy 21, which occurs when extra genetic material from chromosome 21 has been translocated to another chromosome. A family history of Down syndrome is an indication that this may be the cause of the defect. The occurrence of more than one case of Down syndrome in a family is relatively rare, but when it does occur, translocation trisomy is often suspected. Carrier parents usually do not display any genetic abnormalities. Not until there is miscarriage of a fetus with Down syndrome or birth of a child with Down syn-

drome do couples discover that one of them is a translocation carrier. Carriers can produce (1) non-carrier, chromosomally normal, children (which usually happens); (2) carrier children, just like the carrier parent, who are translocation heterozygotes; or (3) children with Down syndrome. Carrier mothers produce children with Down syndrome about 12 percent of the time. Carrier fathers produce children with Down syndrome about 3 percent of the time. Why greater risk exists for mothers is not clear.

Though maternal age is the most frequent predisposing factor for Down syndrome, it is uncorrelated with familial Down syndrome; translocation trisomy 21 occurs with equal frequency in younger and older women. Rarely, a carrier parent will have a translocation between both twenty-first chromosomes, a translocation carrier homozygote. This parent has a 100 percent chance for producing children with Down syndrome.

Ever since the genes on chromosome 21 were fully mapped, pedigree research (family recurrence studies) and epidemiological research (studies of chance occurrence among populations) have supported that these chromosomal abnormalities and uneven distributions of genetic material are inherited, and most often through mothers. Though cryptic parental mosaicism is no longer suspected and there is promising research investigating mitochondrial DNA in the form of cytoplasmic inheritance, the specific genetic mechanism of familial Down syndrome remains elusive.

—Paul Moglia

chromosome 21 came from the mother or the father. In 94 percent of children, the egg brings the extra chromosome. Since the first steps of meiosis to produce her future eggs occur before the mother's own birth, the older the mother, the longer these egg cells have been exposed to potentially harmful chemicals or radiation. On the other hand, paternal age is not a factor because all the steps of meiosis in males occur in cells produced in the few weeks before conception. The continued study of the age factor as well as new insights from genomics are leading to a greater understanding for all those affected by Down syndrome.

—Grace D. Matzen

See also: Aging; Amniocentesis and Chorionic Villus Sampling; Congenital Defects; Fragile X Syndrome; Genetic Counseling; Genetic Testing; Genetics, Historical Development of; Intelligence; Mutation and Mutagenesis; Non-disjunction and Aneuploidy; Prenatal Diagnosis; Proteomics.

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mental retardation, its causes, and prevention strategies. Topics include parenting issues, educational implications, health care needs, employment and economic matters, and legal issues. Glossary.

Web Sites of Interest

Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including Down syndrome.

National Down Syndrome Society. <http://www.ndss.org>. A comprehensive site that includes information on research into the genetics of the syndrome and links to related resources.

Dwarfism

Field of study: Diseases and syndromes

Significance: *Dwarfism in humans is a term used to describe adults who are less than 50 inches (127 centimeters) in height. Dwarfism can be caused by genetic factors, endocrine malfunction, acquired conditions, or growth hormone deficiency. Individuals with dwarfism usually have normal intelligence and have an average life span. Dwarfism may result in multiple medical problems that can lead to death. Dwarfs who have normal body proportions are referred to as midgets. Disproportioned human dwarfs are referred to as dwarfs. Both scientific terms are socially unacceptable; human dwarfs prefer to be referred to as little people.*

Key terms

ALLEL: one of the different forms of a particular gene (locus)

AUTOSOMAL DOMINANT ALLELE: an allele of a gene (locus) on one of the nonsex chromosomes that is always expressed, even if the other allele is normal

AUTOSOMAL RECESSIVE ALLELE: an allele of a gene which will be expressed only if there are two identical copies

Types and Symptoms of Dwarfism

Dwarfism, of which there are several hundred forms, occurs in approximately one in every ten thousand births. Most dwarfs are born to parents of average height. The most common type of dwarfism, achondroplasia, is an autosomal dominant trait, but in 80 percent of cases it appears in children born to normal parents as a result of mutations in the sperm or egg.

Dwarfisms in which body proportions are normal usually result from metabolic or hormonal disorders in infancy or childhood. Chromosomal abnormalities, pituitary gland disorders, problems with absorption, malnutrition, kidney disease, and extreme emotional distress can also interfere with normal growth. When body parts are disproportional, the dwarfism is usually due to a genetic defect.

Skeletal dysplasias are the most common causes of dwarfism and are the major cause of disproportionate types of dwarfism. More than five hundred skeletal dysplasias have been identified. Chondrodystrophic dwarfism occurs when cartilage cells do not grow and divide as they should and cause defective cartilage cells. Most chondrodystrophic dwarfs have abnormal body proportions. The defective cells occur only in the spine or only in the arms and legs.

Short-limb dwarfism includes individuals with achondroplasia, diastrophic dysplasia, and Hunter-Thompson chondrodysplasia. Achondroplasia is the most common skeletal dysplasia and affects more than 50 percent of all dwarfs. Achondroplasia is caused by an autosomal dominant allele and is identified by a disproportionate short stature consisting of long trunk and short upper arms and legs. Eighty percent of all cases of achondroplasia result from a mutation on chromosome 4 in a gene that codes for a fibroblast growth factor receptor. Achondroplasia is seen in both males and females, occurs in all races, and affects approximately one in every twenty thousand births. If one parent has achondroplasia and the other does not, a child born to them would have a 50 percent chance of inheriting achondroplasia. On the other hand, if both parents have achondroplasia, their offspring have a 50 percent

chance of inheriting achondroplasia, a 25 percent chance of being normal, and a 25 percent chance of inheriting the abnormal allele from each parent and suffering often fatal skeletal abnormalities. Children who do not inherit the defective gene will never have achondroplasia, and cannot pass it on to their offspring, unless a mutation occurs in the sperm or egg of the parents. Geneticists have observed that fathers who are 40 years of age or older are more likely to have children with achondroplasia due to mutations in their sperm.

Diastrophic dysplasia is a relatively common form of short-limb dwarfism that occurs in approximately one in 100,000 births and is identified by the presence of short arms and calves, clubfeet, and short, broad fingers with a thumb that has a hitchhiker type appearance. Infant mortality can be high as a result of respiratory complications, but if they survive infancy, short-limbed dwarfs have a normal life span. Orthopedic dislocations of joints are common. Scoliosis is seen especially in the early teens, and progressive cervical kyphosis and partial dislocation of the cervical spine eventually cause compression of the spinal cord. Diastrophic dysplasia is an inherited autosomal recessive condition linked to chromosome 5. Parents have a 25 percent chance that each additional child will get diastrophic dysplasia.

Short-trunk dwarfism includes individuals with spondyloepiphyseal dysplasia, which results from abnormal growth in the spine and long bones that leads to a shortened trunk. In spondyloepiphyseal dysplasia tarda, the lack of growth may not be recognized until five to ten years of age. Those affected have progressive joint and back pain and eventually develop osteoarthritis. Spondyloepiphyseal dysplasia congenita is caused by autosomal dominant gene mutations and is evidenced by a short neck and trunk, and barrel chest at birth. It is not uncommon for cleft palate, hearing loss, myopia, and retinal detachment to be present. Morquio syndrome, which was first described in 1929, is classified as a mucopolysaccharidosis (MPS) disease caused by the body's inability to produce enzymes that help to breakdown and recycle dead cells. Consequently, wastes are stored in the body's cells.



Image not available

Fred (left) and Toby Gill, in a photograph taken September 6, 1996. The twins were born with a form of short-limb dwarfism known as diastrophic dysplasia, which occurs in approximately one in 100,000 births and is identified by the presence of short arms and calves, clubfeet, and short, broad fingers with a thumb that has a hitchhiker-type appearance. (AP/Wide World Photos)

Hunter-Thompson chondrodysplasia is a form of dwarfism caused by a mutation in growth factor genes. Affected individuals have shortened and misshapened bones in the lower arms, the legs, and the joints of the hands and feet. Fingers are shortened and toes are ball-shaped.

Growth hormone, a protein that is produced by the pituitary ("master") gland, is vital for normal growth. Hypopituitarism results in a deficiency of growth hormone and afflicts between ten thousand and fifteen thousand children in the United States.

Turner syndrome affects one in every two thousand female infants and is characterized by the absence of or damage to one of the X

chromosomes in most of the cells in the body. Short stature and the failure to develop sexually are hallmarks of Turner syndrome. Learning difficulties, skeletal abnormalities, heart and kidney problems, infertility, and thyroid dysfunction may also occur. Turner syndrome can be treated with human growth hormones and by replacing sex hormones.

Treatments for Dwarfism

Some forms of dwarfism can be treated through surgical and medical interventions such as bone-lengthening procedures, reconstructive surgery, and growth and sex hormone replacement. The Human Genome Project continues to investigate genetic links to dwarfism. Prenatal counseling and screening for traits of dwarfism, along with genetic counseling and support groups, are avenues to pursue for family and individual physical, psychological, and social well-being and to make informed choices. Family and public education regarding dwarfism and growth problems offers a means to bring greater awareness of dwarfism to communities.

—Sharon Wallace Stark

See also: Congenital Defects; Consanguinity and Genetic Disease; Hereditary Diseases; Human Growth Hormone; Pedigree Analysis.

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Web Sites of Interest

Centralized Dwarfism Resources. <http://www.dwarfism.org>. Offers information on types of dwarfism and links to other informative sites.

National Center for Biotechnology Information. Online Mendelian Inheritance in Man. <http://www.ncbi.nlm.nih.gov/Omim>. A catalog on genes and genetic disorders, including dwarfism, searchable by keyword.

Emerging Diseases

Fields of study: Diseases and syndromes; Viral genetics

Significance: *Pandemics caused by emerging diseases have the potential to sweep the globe and kill millions of persons. These diseases typically arise by evolutionary processes—natural selection or artificial selection via the misuse of antibiotics—by swapping of plasmids or other genetic elements, or else they represent pathogens normally found in animals that make the jump into humans.*

Key term

DRUG RESISTANCE: a phenomenon in which pathogens no longer respond to drug therapies that used to control them; resistance can arise by recombination, by mutation or by several methods of gene transfer, or by misuse of existing drugs

EMERGING DISEASE: a disease whose incidence in humans or other target organisms has increased

INTERSPECIFIC TRANSMISSION: when a pathogen infecting one host infects another host of a different species

A Global Scare

Early in 2003, a new virus appeared. The illness it caused, severe acute respiratory syndrome (SARS), began with flu-like symptoms—fever, chills, headache followed by a dry cough—but appeared to be quite deadly. By mid-April of the year, more than 3,500 persons around the globe had been diagnosed with SARS. Of those, more than 150 had died. As the illness spread from its apparent source in Guangdong Province, China, individuals infected with or exposed to it were quarantined. International travel was restricted, damping an already sluggish global economy. Also by mid-April of that year, the World Health Organization announced that an international team of researchers had determined that a coronavirus—a type of virus related to those that cause the common cold—was the cause of the syndrome. However, it was of a type never before seen. SARS is a prime example of the potential threat posed by emerging infectious diseases.

Epidemiological Background

Emerging diseases are new illnesses or old ailments whose incidence in humans or other target organisms, such as economically important plants or animals, have increased. The key to an emergent or reemergent disease is that the change in its status is sudden and unexpected. For example, acquired immunodeficiency syndrome (AIDS)—caused by the human immunodeficiency virus (HIV)—is an example of a new disease that emerged from an isolated, mysterious wasting syndrome to a pandemic that has killed millions. Tuberculosis, caused by *Mycobacterium tuberculosis*, is an example of an old, more or less controlled condition that has reemerged in an age when many people have compromised immune systems and when antibiotic misuse has promoted the development of drug-resistant strains.

In many cases, emerging diseases are not the result of genetic changes. They instead gain a foothold in a new species by transmission from another host. The probability of interspecific transmission rises as contacts increase between species that formerly had few opportunities to interact. For example, the Ebola virus may have made the leap into humans as humans encroached upon the forests of central Africa and came into greater contact with the natural hosts of the disease. HIV may have made a similar leap. Influenza viruses infect humans, swine, horses, poultry, and waterfowl. New strains of the virus often emerge in regions, such as southern China, where an agricultural mode of life prevails and humans live in close proximity to farm animals. The close proximity of several susceptible hosts makes it easy for mutant strains to develop in one species and spread to others.

The Mutants

In 1997, a new strain of influenza emerged in Hong Kong. The strain (H5N1) leapt from chickens to humans and was armed with a mutation that allowed it to disable a part of the immune system. H5N1 killed only six people—but that small number was one-third of the persons infected, a very large proportion. Normally, the flu virus would be contained within

the lungs or intestines of the chicken, but this particular strain spread to other tissues and had the potential to kill victims quickly. Subsequent research traced the change to one gene, *PB2*. The mutation in *PB2* gave H5N1 the ability to infect people. Because of the dangers posed by the 1997 strain, Hong Kong authorities ordered the destruction of more than a million fowl. The mass killing was repeated during another outbreak in 2001 and 2002, during which 4 million fowl were destroyed.

A mutation can affect as little as a single base pair in a strand of DNA or RNA, or it can involve much longer segments. The classes of mutations include substitutions, deletions, and insertions. Substitutions occur when a nitrogenous base or a sequence of bases in a nucleic acid is replaced by another sequence of identical length. Substitutions can be silent—that is, they can have no effect because the changed sequence calls for the same amino acid as the original. However, substitutions can cause missense mutations, in which a different amino acid is called for, or nonsense mutations, which signals the end of a polypeptide chain. Deletions occur when a portion of a gene is cut out and lost. Insertions occur when more nucleotides are inserted into the sequence of an existing gene.

Reshuffling the Genome

Some of the world's most lethal pandemics arise through recombination in the genome of the pathogen. Influenza is a prime example. The flu viruses are notorious for their ability to change—one reason flu vaccinations are given annually. The 1918 Spanish flu pandemic, caused by a strain that actually originated in North America, spread quickly, infecting about half of the human population at the time and killed tens of millions before running its course.

Genetic analysis of the Spanish flu virus revealed changes in the gene that codes for a protein, hemagglutinin, that helps the virus attach to host cells. Parts of the Spanish flu hemagglutinin gene resembled that of strains that typically infected

humans. Other parts resembled those of strains that normally infected swine. Such swapping of similar genetic sequences is called recombination. The new combinations may have arisen as the swine strain infected humans, or as the human strain infected swine—or they may have arisen through many infections back and forth. While no one is sure how the combination of human and swine characteristics proved so deadly—or how it arose—no one can dispute its lethal effectiveness.

Image not available

In the spring and early summer of 2003, a new pandemic, severe acute respiratory syndrome or SARS, emerged from China's Guangdong Province and was quickly spread across the globe by world travelers. After it was recognized as a new and highly infectious coronavirus, the World Health Organization issued guidelines for its containment, which seemed to occur as the summer drew to a close. Here a woman stands in front of a poster in Taipei, Taiwan, that urges anti-SARS practices on the part of the public. (AP/Wide World Photos)

Mix and Match and Mix

One of the types of recombination seen in influenza viruses is a called reassortment. It occurs in viruses, like influenza viruses, with segmented genomes—their genetic material does not come in one piece, like a chromosome does, but in separate units. If two or more strains of a virus infect a host, they can exchange those units via reassortment and give rise to a new strain with characteristics of the parental strains. The rotaviruses are another group of viruses that recombine their genomes via reassortment. A human rotavirus is one of the most common causes of gastroenteritis in small children around the world and kills hundreds of thousands of children each year.

Viruses are capable of classical recombination, in which homologous segments of DNA or RNA break away and reattach to related strands of genetic material. Another type of recombination requires DNA or double-stranded RNA. It is called copy-choice recombination and occurs when the polymerase enzyme used to make a copy of a nucleic acid strand switches strands in the middle of the process.

Bacterial Gene Swapping

Bacteria swap via plasmids—tiny rings of DNA that can be transferred directly from bacterial cell to bacterial cell via conjugation. Plasmids can also be transferred indirectly as some cells release them and others take them up. Plasmids can also be transferred via bacteriophages—viruses that infect bacteria. Viruses also stash their own genetic material inside the bacterial genome.

Such gene swapping is the primary mechanism by which bacteria acquire new characteristics such as antibiotic resistance—which is now a serious threat in some circumstances. The resistance itself may arise through a mutation but spreads among other microbes via gene exchange. Jim Henson, the renowned puppeteer who created the Muppets, died in 1990 at a young and typically healthy age from an infection of drug-resistant *Streptococcus*, a bacterium that normally gives humans only a sore throat and that normally responds quickly to antibiotic treatment.

New Threats

Influenza is an ancient scourge of humankind, but because of its ability to change, remains a significant pandemic threat. HIV and severe acute respiratory syndrome (SARS) are much more recent emergent viral diseases. Others include Ebola, described in 1976; hepatitis C, identified in 1989; Sin nombre virus, isolated in 1993; the H5N1 strain of the flu virus; and West Nile virus, which emerged in 2002 in the United States.

Several bacterial diseases have likewise emerged in recent decades. They include Legionnaires' disease, caused by *Legionella pneumophila*; *Escherichia coli* strain O157, a virulent form of a bacterium that normally lives in the human gut; and Lyme disease, caused by *Borrelia burgdorferi*. Antibiotic resistance itself is on the rise and poses an increasing danger to humans. Bacteria for which resistance has been documented include *Mycobacterium tuberculosis*, *Neisseria gonorrhoea*, *Staphylococcus aureus*, and *Streptococcus*.

Monitoring

Public health agencies have recognized the importance of surveillance in any attempt to contain emerging or reemerging diseases. Disease monitoring has been going on for some time: The U.S. Congress in 1878 authorized the U.S. Marine Hospital Service to collect data on four diseases—cholera, smallpox, plague, and yellow fever—from U.S. consuls around the world. The Centers for Disease Control and Prevention now administer disease surveillance activities within the United States. The World Health Organization (WHO) in 2001 established a Department of Communicable Disease Surveillance and Response in order to improve monitoring activities globally. Reports of new diseases, such as SARS, are forwarded to the WHO headquarters in Geneva, Switzerland, which coordinates a global response.

—David M. Lawrence

See also: Bacterial Resistance and Super Bacteria; Biological Weapons; Diphtheria; Mutation and Mutagenesis; Smallpox; Swine Flu; Viral Genetics; Viroids and Virusoids.

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- Kolata, Gina. *Flu: The Story of the Great Influenza Pandemic of 1918 and the Search for the Virus That Caused It*. New York: Simon and Schuster, 2001. The discovery of pieces of this flu virus in Arctic remains, as well as government laboratories, prompted this history of the pandemic. Describes the science surrounding the possible recurrence of one of the deadliest plagues to attack humankind.
- Levy, Stuart B. *The Antibiotic Paradox: How Miracle Drugs Are Destroying the Miracle*. New York: Plenum Press, 1992. Levy presents a frightening and authoritative indictment of how misuse of antibiotics is leading to the emergence of drug-resistant microbes against which humanity has no defense. This classic and accessible account is still timely.
- McNeill, William H. *Plagues and Peoples*. New York: Anchor Books, 1998. An updated edition of a classic work on the effect of disease outbreaks on humans throughout history.

Web Sites of Interest

National Center for Infectious Diseases,
Emerging Infectious Diseases Resources.

<http://www.cdc.gov/ncidod>. This searchable government site includes information on emerging diseases such as Severe Acute Respiratory Syndrome (SARS) and others.

The DNA Files, Genetics of Infectious Disease. <http://www.dnafiles.org/resources>. Site includes articles on emerging diseases, a link to the site of the CDC's 2002 international conference on emerging diseases, and a useful bibliography for further study.

World Health Organization. <http://www.who.int/en>. This U.N. agency monitors disease outbreaks and emerging diseases worldwide, working with government health agencies to coordinate scientific and clinical efforts.

Epistasis

Field of study: Classical transmission genetics

Significance: *Epistasis is the interaction of genes such that the alleles at one locus can modify or mask the expression of alleles at another locus. Dihybrid crosses involving epistasis produce progeny ratios that are non-Mendelian, that is, different from the kinds of ratios discovered by Gregor Mendel.*

Key terms

ALLEL: an alternate form of a gene at a particular locus; a single locus can possess two alleles

DIHYBRID CROSS: a cross between parents that involve two specified genes, or loci

F₁: first filial generation, or the progeny resulting from the first cross in a series

F₂: second filial generation, or the progeny resulting from cross of the F₁ generation

LOCUS (pl. LOCI): a more precise word for gene; in diploid organisms, each locus has two alleles

Definition and History

The term “epistasis” is of Greek and Latin origin, meaning “to stand upon” or “stoppage.” The term was originally used by geneticist William Bateson at the beginning of the twentieth

century to define genes that mask the expression of other genes. The gene at the initial location (locus) is termed the epistatic gene. The genes at the other loci are “hypostatic” to the initial gene. In its strictest sense, it describes a nonreciprocal interaction between two or more genes, such that one gene modifies, suppresses, or otherwise influences the expression of another gene affecting the same phenotypic (physical) character or process. By this definition, simple additive effects of genes affecting a single phenotypic character or process would not be considered an epistatic interaction. Similarly, interactions between alternative forms (alleles) of a single gene are governed by dominance effects and are not epistatic. Epistatic effects are interlocus interactions. Therefore, in terms of the total genetic contribution to phenotype, three factors are involved: dominance effects, additive effects, and epistatic effects. The analysis of epistatic effects can suggest ways in which the action of genes can control a phenotype and thus supply a more complete un-

derstanding of the influence of genotype on phenotype.

A gene can influence the expression of other genes in many different ways. One result of multiple genes is that more phenotypic classes can result than can be explained by the action of a single pair of alleles. The initial evidence for this phenomenon came out of the work of Bateson and British geneticist Reginald C. Punnett during their investigations on the inheritance of comb shape in domesticated chickens. The leghorn breed has a “single” comb, brahmas have “pea” combs, and wyandottes have “rose” combs. Crosses between brahmas and wyandottes have “walnut” combs. Inter-crosses among walnut types show four different types of F_2 (second-generation) progeny, in the ratio 9 walnut: 3 rose: 3 pea: 1 single. This ratio of phenotypes is consistent with the classical F_2 ratio for dihybrid inheritance. The corresponding ratio of genotypes, therefore, would be 9 $A_B : 3 A_bb : 3 aaB_ : 1 aabb$, respectively. (The underscore is used to indicate that the

A Punnett Square Showing Flower Pigmentation

		White CCpp	\times		White ccPP
	F_1		\downarrow		
		Purple CcPp			
		\downarrow			
	F_2	CP	CP	Cp	cP
		CCPP purple	CCPp purple	CcPP purple	CcPp purple
		CCPp purple	CCpp white	CcPp purple	Ccpp white
		CcPP purple	CcPp purple	ccPP white	ccPp white
		CcPp purple	Ccpp white	ccPp white	ccpp white

When white-flowered sweet pea plants were crossed, the first-generation progeny (F_1) all had purple flowers. When these plants were self-fertilized, the second-generation progeny (F_2) revealed a ratio of nine purple to seven white. This result can be explained by the presence of two genes for flower pigmentation, P (dominant) and p (recessive) and C or c . Both dominant forms, P and C , must be present in order to produce purple flowers.

second gene can be either dominant or recessive; for example, $A_$ means that both AA and Aa will result in the same phenotype.) In this example, one can recognize that two independently assorting genes can affect a single trait. If two gene pairs are acting epistatically, however, the expected 9:3:3:1 ratio of phenotypes is altered in some fashion. Thus, although the preceding example involves interactions between two loci, it is not considered a case of epistasis, because the phenotype ratio is a classic Mendelian ratio for a dihybrid cross. Five basic examples of two-gene epistatic interactions can be described: complementary, modifying, inhibiting, masking, and duplicate gene action.

Complementary Gene Action

For complementary gene action, a dominant allele of two genes is required to produce a single effect. An example of this form of epistasis again comes from the observations of Bateson and Punnett of flower color in crosses between two white-flowered varieties of sweet peas. In their investigation, crosses between these two varieties produced an unexpected result: All of the F_1 (first-generation) progeny had purple flowers. When the F_1 individuals were allowed to self-fertilize and produce the F_2 generation, a phenotypic ratio of nine purple-flowered to seven white-flowered individuals resulted. Their hypothesis for this ratio was that a homozygous recessive genotype for either gene (or both) resulted in the lack of flower pigmentation. A simple model to explain the biochemical basis for this type of flower pigmentation is a two-step process, each step controlled by a separate gene and each gene having a recessive allele that eliminates pigment formation. Given this explanation, each parent must have had complementary genotypes ($AA\ bb$ and $aa\ BB$), and thus both had white flowers. Crosses between these two parents would produce double heterozygotes ($Aa\ Bb$) with purple flowers. In the F_2 generation, $\frac{9}{16}$ would have the genotype $A_ B_$ and would have purple flowers. The remaining $\frac{7}{16}$ would be homozygous recessive for at least one of the two genes and, therefore, would have white flowers. In summary, the phenotypic ratio of the F_2 generation would be 9:7.

Modifying Gene Action

The term “modifying gene action” is used to describe a situation whereby one gene produces an effect only in the presence of a dominant allele of a second gene at another locus. An example of this type of epistasis is aleurone color in corn. The aleurone is the outer cell layer of the endosperm (food storage tissue) of the grain. In this system, a dominant gene ($P_$) produces a purple aleurone layer only in the presence of a gene for red aleurone ($R_$) but expresses no effect in the absence of the second gene in its dominant form. Thus, the corresponding F_2 phenotypic ratio is 9 purple:3 red:4 colorless. The individuals without aleurone pigmentation would, therefore, be of the genotype $P_ rr$ ($\frac{3}{16}$) or $pp rr$ ($\frac{1}{16}$). Again, a two-step biochemical pathway for pigmentation can be used to explain this ratio; however, in this example, the product of the second gene (R) acts first in the biochemical pathway and allows for the production of red pigmentation and any further modifications to that pigmentation. Thus, the phenotypic ratio of the F_2 generation would be 9:3:4.

Inhibiting Gene Action

Inhibiting action occurs when one gene acts as an inhibitor of the expression of another gene. In this example, the first gene allows the phenotypic expression of a gene, while the other gene inhibits it. Using a previous example (the gene R for red aleurone color in corn seeds), the dominant form of the first gene R does not produce its effect in the presence of the dominant form of the inhibitor gene I . In other words, the genotype $R_ i_$ results in a phenotype of red aleurone ($\frac{3}{16}$), while all other genotypes result in the colorless phenotype ($\frac{13}{16}$). Thus gene R is inhibited in its expression by the expression of gene I . The F_2 phenotypic ratio would be 13:3. This ratio, unlike the previous two examples, includes only two phenotypic classes and highlights a complicating factor in determining whether one or two genes may be influencing a given trait. A 13:3 ratio is close to a 3:1 ratio (the ratio expected for the F_2 generation of a monohybrid cross). Thus it emphasizes the need to look at an F_2 population of sufficient size to discount the possibility of a single

gene phenomenon over an inhibiting epistatic gene interaction.

Masking Gene Action

Masking gene action, a form of modifying gene action, results when one gene is the primary determinant of the phenotype of the offspring. An example of this phenomenon is fruit color in summer squash. In this example, the F_2 ratio is 12:3:1, indicating that the first gene in its dominant form results in the first phenotype (white fruit); thus this gene is the primary determinant of the phenotype. If the first gene is in its recessive form and the second gene is in its dominant form, the fruit will be yellow. The fruit will be green at maturity only when both genes are in their recessive form ($\frac{1}{16}$ of the F_2 population).

Duplicate Gene Interaction

Duplicate gene interaction occurs when two different genes have the same final result in terms of their observable influence on phenotype. This situation is different from additive gene action in that either gene may substitute for the other in the expression of the final phenotype of the individual. It may be argued that duplicate gene action is not a form of epistasis, since there may be no interaction between genes (if the two genes code for the same protein product), but this situation may be an example of gene interaction when two genes code for similar protein products involved in the same biochemical pathway and their combined interaction determines the final phenotype of the individual. An example of this type of epistasis is illustrated by seed capsule shape in the herb shepherd's purse. In this example, either gene in its dominant form will contribute to the final phenotype of the individual (triangular shape). If both genes are in their recessive form, the seed capsule has an ovoid shape. Thus, the phenotypic ratio of the F_2 generation is 15:1.

Impact and Applications

Nonallelic gene interactions have considerable influence on the overall functioning of an individual. In other words, the genome (the entire genetic makeup of an organism) deter-

mines the final fitness of an individual, not only as a sum total of individual genes (additive effects) or by the interaction between different forms of a gene (dominance effects) but also by the interaction between different genes (intra-genomic or epistatic effects). This situation is something akin to a chorus: Great choruses not only have singularly fine voices, but they also perform magnificently as finely tuned and coordinated units. Knowledge of what contributes to a superior genome would, therefore, lead to a fuller understanding of the inheritance of quantitative characters and more directed approaches to genetic improvement. For example, most economically important characteristics of agricultural species (such as yield, pest and disease resistance, and stress tolerance) are quantitatively inherited, the net result of many genes and their interactions. Thus an understanding of the combining ability of genes and their influence on the final appearance of domesticated breeds and crop varieties should lead to more efficient genetic improvement schemes. In addition, it is thought that many important human diseases are inherited as a complex interplay among many genes. Similarly, an understanding of genomic functioning should lead to improved screening or therapies.

—Henry R. Owen

See also: Chromosome Structure; Chromosome Theory of Heredity; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Extrachromosomal Inheritance; Hybridization and Introgression; Incomplete Dominance; Lamarckianism; Mendelian Genetics; Monohybrid Inheritance; Multiple Alleles; Nondisjunction and Aneuploidy; Parthenogenesis; Penetrance; Polygenic Inheritance; Quantitative Inheritance.

Further Reading

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Eugenics

Field of study: Human genetics and social issues

Significance: *The eugenics movement sought to speed up the process of natural selection through the use of selective breeding and led to the enactment of numerous laws requiring the sterilization of “genetically inferior” individuals and limiting the immigration of supposedly defective groups. Such flawed policies were based on an inadequate understanding of the complexity of human genetics, an underestimation of the role of the environment in gene expression, and the desire of certain groups to claim genetic superiority and the right to control the reproduction of others.*

Key terms

BIOMETRY: the measurement of biological and psychological variables

NEGATIVE EUGENICS: improving human stocks through the restriction of reproduction

POSITIVE EUGENICS: improving human stocks by encouraging the “naturally superior” to breed extensively with other superior humans

The Founding of the Eugenics Movement

With the publication of Charles Darwin's *On the Origin of Species by Means of Natural Selection* (1859), the concept of evolution began to revolutionize the way people thought about the human condition. Herbert Spencer and other proponents of what came to be known as social Darwinism adhered to the belief that social class structure arose through natural selection,

seeing class stratification in industrial societies, including the existence of a permanently poor underclass, as a reflection of the underlying, innate differences between classes.

During this era there was also a rush to legitimize all sciences by using careful measurement and quantification. There was a blind belief that attaching numbers to a study would ensure its objectivity.

Francis Galton, an aristocratic inventor, statistician, and cousin of Darwin, became one of the primary promoters of such quantification. Obsessed with mathematical analysis, Galton measured everything from physiology and reaction times to boredom, the efficacy of prayer, and the beauty of women. He was particularly interested in the differences between human races. Galton eventually founded the field of biometry by applying statistics to biological problems.

A hereditarian, Galton assumed that talent in humans was subject to the laws of heredity. Although Galton did not coin the term “eugenics” until 1883, he published the first discussion of his ideas in 1865, in which he recognized the apparent evolutionary paradox that those of talent often have few, if any, children and that civilization itself diminishes the effects of natural selection on human populations. Fearing that medicine and social aid would lead to the propagation of weak individuals, Galton advocated increased breeding by “better elements” in the population (positive eugenics), while at the same time discouraging breeding of the “poorer elements” (negative eugenics).

Like most in his time, Galton believed in “blending inheritance,” whereby hereditary material would mix together like different colors of paint. Trying to reconcile how superior traits would avoid being swamped by such blending, he came up with the statistical concept of the correlation coefficient, and in the process connected Darwinian evolution to the “probability revolution.” His work focused on the bell-shaped curve or “normal distribution” demonstrated by many traits and the possibility of shifting the mean by selection pressure at either extreme. His statistical framework deepened the theory of natural selection. Unfortunately, the mathematical predictability he

studied has often been misinterpreted as inevitability. In 1907, Galton founded the Eugenics Education Society of London. He also carefully cataloged eminent families in his *Hereditary Genius* (1869), wherein the Victorian world was assumed to be the ultimate level that society could attain and the cultural transmission of status, knowledge, and social connections were discounted.

Early Eugenics in Britain

Statistician and social theorist Karl Pearson was Galton's disciple and first Galton Professor of Eugenics at the Galton Laboratory at the University of London. His *Grammar of Science* (1892) outlined his belief that eugenic management of society could prevent genetic deterioration and ensure the existence of intelligent rulers, in part by transferring resources from inferior races back into the society. According to philosopher David J. Depew and biochemist Bruce H. Weber, even attorney Thomas Henry Huxley, champion of Darwinism, balked at this "pruning" of the human garden by the administrators of eugenics. For the most part, though, British eugenicists focused on improving the superior rather than eliminating the inferior.

Another of Galton's followers, comparative anatomist Walter Frank Weldon, like Galton before him, set out to measure all manner of things, showing that the distribution of many human traits formed a bell-shaped curve. In a study on crabs, he showed that natural selection can cause the mean of such a curve to shift, adding fuel to the eugenicists' conviction that they could better the human race through artificial selection.

Population geneticist Ronald A. Fisher was Pearson's successor as the Galton Professor of Eugenics. Fisher cofounded the Cambridge Eugenics Society and became close to Charles Darwin's sons, Leonard and Horace Darwin. In a speech made to the Eugenics Education Society, Fisher called eugenicists the "agents of a new phase of evolution" and the "new natural nobility," with the view that humans were becoming responsible for their own evolution. The second half of his book *The Genetical Theory of Natural Selection* (1930) deals expressly with

eugenics and the power of "good-making traits" to shape society. Like Galton, he believed that those in the higher social strata should be provided with financial subsidies to counteract the "resultant sterility" caused when upper class individuals opt to have fewer children for their own social advantage.

British embryologist William Bateson, who coined the terms "genes" and "genetics," championed the Mendelian genetics that finally unseated the popularity of Galton's ideas in England. In a debate that lasted thirty years, those that believed in Austrian monk Gregor Mendel's particulate inheritance argued against the selection touted by the biometrists, and vice versa. Bateson, who had a deep distrust of eugenics, successfully replicated Mendel's experiments. Not recognizing that the two arguments were not mutually exclusive, Pearson and Weldon rejected genetics, thus setting up the standoff between the two camps.

Fisher, on the other hand, tried to model the trajectory of genes in a population as if they were gas molecules governed by the laws of thermodynamics, with the aim of converting natural selection into a universal law. He used such "genetic atomism" to propose that continuous variation, natural selection, and Mendelian genetics could all coexist. Fisher also mathematically derived Galton's bell-shaped curves based on Mendelian principles. Unfortunately, by emulating physics, Fisher underestimated the degree to which environment dictates which traits are adaptive.

Early Eugenics in the United States

While Mendelians and statisticians were debating in Britain, in the United States, Harvard embryologist Charles Davenport and others embarked on a mission of meshing early genetics with the eugenics movement. In his effort, Davenport created the Laboratory for Experimental Evolution at Cold Springs Harbor, New York. The laboratory was closely linked to his Eugenics Record Office (ERO), which he established in 1910. Davenport raised much of the money for these facilities by appealing to wealthy American families who feared unrestricted immigration and race degeneration. Though their wealth depended on the availabil-

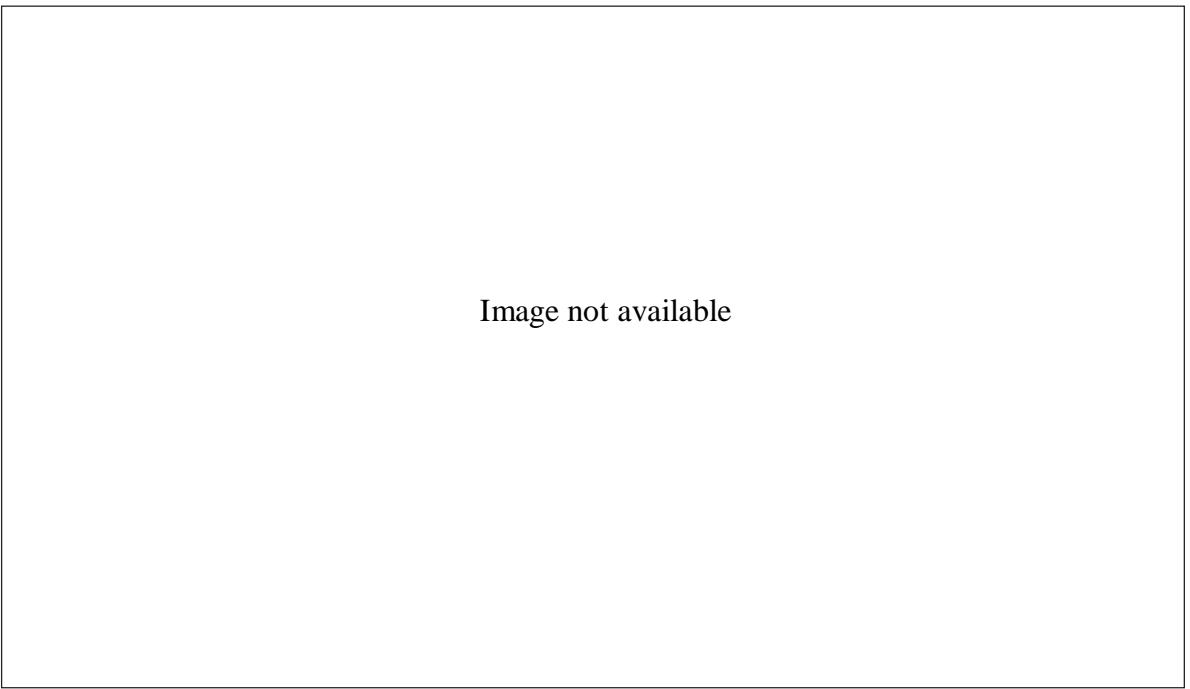


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In the first half of the twentieth century, thousands of people in the United States, many of them teenagers thought to be weak or abnormal, were sterilized to prevent their genes from passing on to the next generation. Here Sarah Jane Wiley revisits the Virginia Colony of the Epileptic and Feebleminded in Lynchburg, Virginia, where she and her brother were both sterilized in 1959. (AP/Wide World Photos)

ity of cheap labor guaranteed by immigration, these American aristocrats feared the cultural impact of a flood of “inferior immigrants.”

Unlike the British, U.S. eugenicists thought of selection as a purifying force and thus focused on how to stop the defective from reproducing. Davenport wrongly felt that Mendelian genetics supported eugenics by reinforcing the effects of inheritance over the environment. He launched a hunt to identify human defects and link specific genes (as yet poorly understood entities) to specific traits. His primary tool was the family pedigree chart. Unfortunately, these charts were usually based on highly subjective data, such as questionnaires given to schoolchildren to determine the comparative social traits of various races.

The Eugenics Research Association was founded in 1913 to report the latest findings. In 1918, the Galton Society began meeting regularly at the American Museum of Natural History in New York, and in 1923 the American Eugenics Society was formed. These efforts paid

off. By the late 1920’s and early 1930’s, eugenics was a topic in high school biology texts and college courses across the United States.

Among eugenics supporters was psychologist Lewis M. Terman, developer of the Stanford-Binet intelligence quotient (IQ) test, and Harvard psychologist Robert M. Yerkes, developer of the Army IQ test, who both believed that IQ test performance (and hence intelligence) was hereditary. The administration of such tests to immigrants by eugenicist Henry Goddard represented a supposedly “objective and quantitative tool” for screening immigrants for entry into the United States. Biologist Garland Allen reports that Goddard, in fact, determined that more than 80 percent of the Jewish, Hungarian, Polish, Italian, and Russian immigrants were mentally defective.

Fear that immigrants would take jobs away from hardworking Americans, supported by testimony from ERO’s superintendent, Harry Laughlin, and the findings of Goddard’s IQ tests, resulted in the Johnson Act of 1924, which

severely restricted immigration. In the end, legal sterilization and immigration restrictions became more widespread in the United States than in any country other than Nazi Germany. By 1940, more than thirty states in the United States had enacted compulsory sterilization laws. Most were not repealed until after the 1960's.

Eugenics and the Progressive Era

During the Progressive Era, the eugenics movement became a common ground for such diverse groups as biologists, sociologists, psychologists, militarists, pacifists, socialists, communists, liberals, and conservatives. The progressive ideology, exemplified by Theodore Roosevelt's Progressive Party, sought the scientific management of all parts of society. Eugenics attracted the same crowd as preventative medicine, since both were seen as methods of harnessing science to reduce suffering and misfortune. For example, cereal entrepreneur John Harvey Kellogg founded the Race Betterment Foundation, mixing eugenics with hygiene, diet, and exercise. During this period, intellectuals of all stripes were attracted by the promise of "the improvement of the human race by better breeding." The genetics research of this time focused on improving agriculture, and eugenics was seen as the logical counterpart to plant and animal husbandry.

Davenport did not hesitate to play on their sympathies by making wild claims about the inheritance of "nomadism," "shiftlessness," "love of the sea," and other "traits" as if they were single Mendelian characteristics. Alcoholism, pauperism, prostitution, rebelliousness, criminality, feeble-mindedness, chess expertise, and industrial sabotage were all claimed to be determined by one or two pairs of Mendelian genes. In particular, the progressives were lured by the idea of sterilizing the "weak minded," especially after the publication of articles about families in Appalachia and New Jersey that supposedly documented genetic lines cursed by a preponderance of habitual criminal behavior and mental weakness.

Having the allure of a "social vaccination," the enthusiasm to sterilize the "defective" spread rapidly among intellectuals, without

regard to political or ideological lines. Sweden's Social Democrats forcibly sterilized some sixty thousand Swedes under a program that lasted from 1935 to 1976 organized by the state-financed Institute for Racial Biology. Grounds for sterilization included not only "feeble-mindedness" but also "gypsy features," criminality, and "poor racial quality." The low class or mentally slow were institutionalized in the Institutes for Misled and Morally Neglected Children and released only if they would agree to be sterilized. Involuntary sterilization policies were also adopted in countries ranging from Switzerland and Austria to Belgium and Canada, not to be repealed until the 1970's.

Hermann Müller, a eugenicist who emigrated to the Soviet Union (and later returned to the United States), attacked Davenport's style of eugenics at the International Eugenics Congress in 1932. Müller, a geneticist who won the 1946 Nobel Prize for Physiology or Medicine for his discovery of the mutagenic power of X rays, instead favored the style of eugenics envisioned by English novelist Aldous Huxley's *Brave New World* (1932), with state nurseries, artificial insemination, and the use of other scientific techniques to produce a genetically engineered socialist society.

According to journalist Jonathan Freedland, the British left, including a large number of socialist intellectuals such as playwright George Bernard Shaw and philosopher Bertrand Russell, was convinced that it knew what was best for society. Concerned with the preservation of their higher intellectual capacities, they joined the fashionable and elitist Eugenics Society in the 1930's, where they advocated the control of reproduction, particularly favoring the idea of impregnating working-class women with sperm of men with high IQs.

The American Movement Spreads to Nazi Germany

The eugenics movement eventually led to grave consequences in Nazi Germany. Negative eugenics reached its peak there, with forced sterilization, euthanasia or "mercy killing," experimentation, and ultimately genocide being used in the name of "racial hygiene." Eugenicists in the United States and Germany formed

close and direct alliances, especially after the Nazis came to power in 1933. The ERO's Laughlin gave permission for his article "Eugenical Sterilization" to be reprinted in German in 1928. It soon became the basis of Nazi sterilization policy. Davenport even arranged for a group of German eugenicists to participate in the three hundredth anniversary of Harvard's founding in 1936.

Inspired by the U.S. eugenics movement and spurred by economic hardship that followed World War I, the Nazi Physician's League took a stand that those suffering from incurable disease caused useless waste of medications and, along with the crippled, the feeble-minded, the elderly, and the chronic poor, posed an economic drain on society. Hereditary defects were considered to be the cause of such maladies, and these people were dubbed "lives not worth living." In 1933, the German Law on Preventing Hereditarily Diseased Progeny made involuntary sterilization of such people, including the blind, deaf, epileptic, and poor, legal. The Nazis set up "eugenics courts" to decide cases of involuntary sterilization. Frederick Osborn, secretary of the American Eugenics Society, wrote a 1937 report summarizing the German sterilization programs, indicative of the fascination American eugenicists had for the Nazi agenda and the Nazi's ability to move this experiment to a scale never possible in the United States.

The Demise of Eugenics

With the Great Depression in 1929, the U.S. eugenics movement lost much of its momentum. Geneticist and evolutionary biologist Sewall Wright, although himself a member of the American Eugenics Society, found fault with the genetics and the ideology of the movement: "Positive eugenics seems to require . . . the setting up of an ideal of society to aim at, and this is just what people do not agree on." He also wrote several articles in the 1930's challenging the assumptions of Fisher's genetic atomism model. In a speech to the Eugenics Society in New York in 1932, Müller pointed out the economic disincentive for middle and upper classes to reproduce, epitomized by the failure of many eugenicists to have children. Galton

himself died childless. This inverse relationship between fertility and social status, coupled with the apparent predatory nature of the upper class, seemed to doom eugenics to failure.

Evolutionary biologist Stephen Jay Gould claimed that the demise of the eugenics movement in the United States was more a matter of Adolf Hitler's use of eugenic arguments for sterilization and racial purification than it was of advances in genetic knowledge. Once the Holocaust and other Nazi atrocities became known, eugenicists distanced themselves from the movement. Depew and Weber have written that Catholic conservatives opposed to human intervention in reproduction and progressives, who began to abandon eugenics in favor of behaviorism (nurture rather than nature), were political forces that began to close down the eugenics movement, while Allen points out that the movement had outlived its political usefulness. Russian geneticist Theodosius Dobzhansky had by this time recognized the prime importance of context in genetics and consequently rejected the premise of eugenics, helping to push it into the realm of phony genetics.

Implications

The term "euphenics" is used to describe human genetic research that is aimed at improving the human condition, replacing the tainted term eugenics. Euphenics deals primarily with medical or genetic intervention that is designed to reduce the impact of defective genotypes on individuals (such as gene therapy for those with cystic fibrosis). However, in this age of increasing information about human genetics, it is necessary to keep in mind the important role played by environment and the malleability of human traits.

Allen argues that the eugenics movement may reappear (although probably under a different name) if economic problems again make it attractive to eliminate "unproductive" people. His hope is that a better understanding of genetics, combined with the lessons of Nazi Germany, will deter humans from ever again going down that path that journalist Jonathan Freedland calls "the foulest idea of the 20th century."

—Lee Anne Martínez

See also: Artificial Selection; Bioethics; Bioinformatics; Biological Determinism; Cloning: Ethical Issues; Eugenics: Nazi Germany; Evolutionary Biology; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing: Ethical and Economic Issues; Heredity and Environment; Human Genetics; Insurance; Intelligence; Miscegenation and Antimiscegenation Laws; Natural Selection; Patents on Life-Forms; Paternity Tests; Race; Sociobiology; Stem Cells; Sterilization Laws.

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National Reference Center for Bioethics Literature. <http://www.georgetown.edu/research/nrcbl/scopenotes/sn28.html>. An introduction to eugenics and a comprehensive annotated bibliography of sources for further study.

Eugenics: Nazi Germany

Field of study: History of genetics; Human genetics and social issues;

Significance: Fueled by economic hardship and racial prejudice, the largest-scale application of eugenics occurred in Nazi Germany, where numerous atrocities, including genocide, were committed in the name of the genetic improvement of the human species. The German example raised worldwide awareness of the dangers of eugenics and did much to discredit eugenic theory.

Key terms

ARYAN: a "race" believed by Nazis to have established the civilizations of Europe and India

EUTHANASIA: the killing of suffering people, sometimes referred to as “mercy” killing

NORDIC: the northernmost of the Aryan groups of Europe, believed by the Nazis to be the highest and purest racial group

Origins of Nazi Eugenic Thought

Nazi eugenic theory and practice grew out of two traditions: the eugenics movement, founded by British scientist Francis Galton, and racial theories of human nature. Most historians trace the origin of modern racial theories to French diplomat and writer Joseph-Arthur de Gobineau, who maintained that all great civilizations had been products of the Aryan, or Indo-Germanic, race. Through the late nineteenth and early twentieth centuries, German thinkers applied Galton’s ideas to the problem of German national progress. The progress of the nation, argued scientists and social thinkers, could be best promoted by improving the German people through government-directed control of human reproduction. This type of eugenic thinking became known as “racial hygiene”; in 1904, eugenicists and biologists formed the Racial Hygiene Society in Berlin.

The Aryan mythology of Gobineau also grew in popularity. In 1899, an English admirer of Germany, Houston Stewart Chamberlain, published a widely read book entitled *The Foundations of the Nineteenth Century*. Chamberlain, heavily influenced by Gobineau, maintained that Europe’s accomplishments had been the work of ethnic Germans, members of a healthy and imaginative race. Opposed to the Germans were the Jews, who were, according to Chamberlain, impure products of crossbreeding among the peoples of the Middle East.

Basics of Nazi Eugenics

The Law to Prevent Hereditarily Sick Offspring, requiring sterilization of people with hereditary diseases and disabilities, was drafted and decreed in Germany in 1933. Before the Nazis came to power, many segments of German society had supported sterilization as a way to improve future generations, and Adolf Hitler’s emergence as a national leader provided the pressure to ensure the passage of the

law. Between 1934 and 1945, an estimated 360,000 people (about 1 percent of the German population) who were believed to have hereditary ailments were sterilized. Despite this law, the Nazis did not see eugenics primarily as a matter of discouraging the reproduction of unhealthy individuals and encouraging the reproduction of healthy individuals. Following the theories of Chamberlain, Adolf Hitler and his followers saw race, not individual health or abilities, as the distinguishing characteristic of human beings.

The Schutzstaffel (SS) organization was a key part of Nazi eugenic activities. In January, 1929, Heinrich Himmler was put in charge of the SS, a police force aimed at establishing order among the street fighters who formed a large part of the early Nazi Party. In addition to disciplining rowdy Nazis, the SS quickly emerged as a racial elite, the spearhead of an intended German eugenic movement. Himmler recruited physicians and biologists to help ensure that only those of the purest Nordic heritage could serve in his organization. In 1931, the agriculturalist R. Walther Darre helped Himmler draw up a marriage code for SS men, and Himmler appointed Darre head of an SS Racial Office. Himmler hoped to create the seeds of a German super race by directing the marriages and reproduction of the “racially pure” members of the SS.

Since the Nazis saw Germans as a “master race,” a race of inherently superior people, they attempted to improve the human stock by encouraging the birth of as many Germans as possible and by encouraging those seen as racially pure to reproduce. The Nazis declared that women should devote themselves to bearing and caring for children. Hitler’s mother’s birthday was declared the Day of the German Mother. On this day, public ceremonies awarded medals to women with large numbers of children. The SS set up and maintained an organization of maternity homes for unmarried mothers of acceptable racial background and orphanages for their children; these institutions were known as the Lebensborn (“fountain of life”). There is some evidence that young women with desired racial characteristics who were not pregnant were brought to the



On Wehrmacht Day, 1935, in Nazi Germany (from left): German chancellor Adolf Hitler, head of the air force Hermann Göring, army commander Werner von Fritsch, minister of war Eduard von Blomberg, commander of the navy Erich Raeder, and other Nazi officials. During the late 1930's and early 1940's, the Nazi government conducted the extreme and brutal form of eugenics that culminated in the Holocaust and the murder of millions of innocent Jews and other "undesirables." (Library of Congress)

Lebensborn to have children by the SS men to create “superior” Nordic children.

Impact

In addition to encouraging the reproduction of those seen as racially pure, the Nazis sought to eliminate the unhealthy and the racially undesirable. In August, 1939, a committee of physicians and government officials, operating under Hitler’s authority, issued a secret decree under which all doctors and midwives would have to register births of malformed or handicapped children. By October of that year, Hitler had issued orders for the “mercy killing” of these children and all those with incurable diseases. This euthanasia movement expanded from sick and handicapped children to those believed to belong to “sick” races. The T4 euthanasia organization, designed for efficient and secret killing, experimented with lethal injections and killing by injection and became a pilot program for the

mass murder of the Jews during the Holocaust.

German racial hygienists had long advocated controlling marriages of non-Jewish Germans with Jews in order to avoid “contaminating” the German race. In July, 1941, Nazi leader Hermann Göring appointed SS officer Reinhard Heydrich to carry out the “final solution” of the perceived Jewish problem. At the Wannsee Conference in January, 1942, Hitler and his close associates agreed on a program of extermination. According to conservative estimates, between four million and five million European Jews died in Nazi extermination camps. When the murderous activities of the Nazis were revealed to the world after the war, eugenics theory and practice fell into disrepute.

—Carl L. Bankston III

See also: Bioethics; Bioinformatics; Biological Determinism; Eugenics; Evolutionary Biology; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering; Social and Ethical Issues; Genetic Screening;

Genetic Testing: Ethical and Economic Issues; Heredity and Environment; Human Genetics; Insurance; Intelligence; Miscegenation and Antimiscegenation Laws; Patents on Life-Forms; Paternity Tests; Race; Sociobiology; Stem Cells; Sterilization Laws.

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Web Site of Interest

National Information Resource on Ethics and Human Genetics. <http://www.georgetown.edu/research/nrcbl/nirehg/quickbibs.htm>. Searchable “quick bib” on resources about eugenics and Nazi Germany.

Evolutionary Biology

Fields of study: Evolutionary biology; Population genetics

Significance: While the existence of evolutionary change is firmly established, many questions remain about its causes in particular groups of organisms. The science of evolutionary biology focuses on reconstructing the actual history of life and on understanding how evolutionary mechanisms operate in nature.

Key terms

ADAPTATION: a genetically based characteristic that increases the ability of an organism to survive and reproduce under prevailing environmental conditions

EVOLUTION: the process of change in the genetic structure of a population over time; descent with modification

FITNESS: the relative reproductive contribution of one individual to the next generation as compared to that of others in the population

GENETIC DRIFT: chance fluctuations in allele frequencies within a population, resulting from random variation in the number and genotypes of offspring produced by different individuals

GENOTYPE: the genetic makeup of an individual or group

NATURAL SELECTION: the phenomenon of differing survival and reproduction rates among various genotypes; the frequency of the favored genotypes increases in succeeding generations

PHYLOGENY: the history of descent of a group of species from a common ancestor

An Evolutionary Context

Life is self-perpetuating, with each generation connected to previous ones by the thread of DNA passed from ancestors to descendants. Life on earth thus has a single history much like the genealogy of a family, the shape and characteristics of which have been determined by internal and external forces. The effort to uncover that history and describe the forces that shape it constitutes the field of evolutionary biology.

As an example of the need for this perspective, consider three vertebrates of different species, two aquatic (a whale and a fish) and one terrestrial (a deer). The two aquatic species share a torpedolike shape and oarlike appendages. These two species differ, however, in that one lays eggs and obtains oxygen from the water using gills, while the other produces live young and must breathe air at the surface. The terrestrial species has a different, less streamlined, shape and appendages for walking, but it too breathes air using lungs and produces live young. All three species are the same in having a bony skeleton typical of vertebrates. In order to understand why the various organisms display the features they do, it is necessary to consider what forces or historical constraints influence their genotypes and subsequent phenotypes.

It is logical to hypothesize that a streamlined shape is beneficial to swimming creatures, as is the structure of their appendages. This statement is itself an evolutionary hypothesis; it implies that streamlined individuals will be more successful than less streamlined ones and so will become prevalent. It may initially be difficult to reconcile the differences between the two aquatic forms swimming side-by-side with the similarities between one of them and the terrestrial species walking around on dry land. However, if it is understood that the whale is more closely related to the terrestrial deer than it is to the fish, much of the confusion disappears. Using this comparative approach, it is unnecessary, and scientifically unjustified, to construct an elaborate scenario whereby breathing air at the surface is more advantageous to a whale than gills would be; the simpler explanation is that the whale breathes air because it (like the deer) is a mammal, and both species inherited this trait from a common ancestor sometime in the past.

Organisms are thus a mixture of two kinds of traits. Ecological traits are those the particular form of which reflects long-term adaptation to the species' habitat. Two species living in the same habitat might then be expected to be similar in such features and different from species in other habitats. Evolutionary characteristics, on the other hand, indicate common ancestry rather than common ecology. Here, similarity

between two species indicates that they are related to each other, just as familial similarity can be used to identify siblings in a crowd of people. In reality, all traits are somewhere along a continuum between these two extremes, but this distinction highlights the importance of understanding the evolutionary history of organisms and traits. The value of an evolutionary perspective comes from its comparative and historical basis, which allows biologists to place their snapshot-in-time observations within the broader context of the continuous history of life.

Early Evolutionary Thought

Underlying evolutionary theory is Mendelian genetics, which provides a mechanism whereby advantageous traits can be passed on to offspring. Both Mendelian genetics and the theory of evolution are at first glance (and in retrospect) remarkably simple. The theory of evolution, however, is paradoxical in that it leads to extremely complex predictions and thus is often misunderstood, misinterpreted, and misapplied.

It is important to distinguish between the phenomenon of evolution and the various processes or mechanisms that may lead to evolution. The idea that species might be mutable, or subject to change over generations, dates back to at least the mid-eighteenth century, when the French naturalist Georges-Louis Leclerc, comte de Buffon, the Swiss naturalist Charles Bonnet, and even the Swedish botanist Carolus Linnaeus suggested that species (or at least "varieties") might be modified over time by intrinsic biological or extrinsic environmental factors. Other biologists after that time also promoted the idea that populations and species could evolve. Nevertheless, with the publication of *On the Origin of Species by Means of Natural Selection* in 1859, Charles Darwin became the first to propose that all species had descended from a common ancestor and that there was a single "tree of life." These claims regarding the history of evolution, however, are distinct from the problem of how, or through what mechanisms, evolution occurs.

In the first decade of the nineteenth century, Jean-Baptiste Lamarck promoted the the-



Charles Darwin is credited as the father of the theory of natural selection, on which modern evolutionary biology is based. (Library of Congress)

ory of inheritance of acquired characteristics to explain how species could adapt over time to their environments. His famous giraffe example illustrates the Lamarckian view: Individual giraffes acquire longer necks as a result of reaching for leaves high on trees, then pass that modified characteristic to their offspring. According to Lamarck's theories, as a result of such adaptation, the species—and, in fact, each individual member of the species—is modified over time. While completely in line with early nineteenth century views of inheritance, this view of the mechanism of evolution has since been shown to be incorrect.

Darwinian Evolution: Natural Selection

In the mid-nineteenth century, Darwin and Alfred Russel Wallace independently developed the theory of evolution via natural selection, a theory that is consistent with the genetics of inheritance as first described by Gregor Mendel. Both Darwin's and Wallace's arguments center on four observations of nature and a logical conclusion derived from those observations

(presented here in standard genetics terminology, although Darwin and Wallace used different terms).

First, variation exists in the phenotypes of different individuals in a population. Second, some portion of that variation is heritable, or capable of being passed from parents to offspring. Third, more individuals are produced in a population than will survive. Fourth, some individuals are, because of their particular phenotype, better able to survive and reproduce than others. From this, Darwin and Wallace deduced that those individuals whose phenotypes conferred on them greater fitness for survival would produce more offspring (genetically and phenotypically similar to themselves) than would less fit individuals; therefore, the frequency of individuals with the favored genotype would increase in the next generation, though each individual would be unchanged throughout its lifetime. This process would continue as long as new genetic variants continued to arise and selection favored some over others. The theory of natural selection provided a workable and independently testable natural mechanism by which evolution of complex and sometimes very different adaptations could occur within and among species.

Evolutionary Biology After Darwin

Despite their theoretical insight, Darwin and Wallace had no knowledge of the genetic basis of inheritance. Mendel published his work describing the particulate theory of inheritance in 1866 (he had reported the results before the Natural Sciences Society earlier, in February and March of 1865), but Darwin and Wallace appear to have been unaware throughout their lives that this vexing problem had been solved. In fact, Mendel's work went unnoticed by the entire scientific community for nearly fifty years; it was rediscovered, and its significance appreciated, in the first decade of the twentieth century. Over the next three decades, theoreticians integrated Darwin's theory of natural selection with the principles of Mendelian genetics. Simultaneously, Ernst Mayr, G. Ledyard Stebbins, George Gaylord Simpson, and Julian Huxley demonstrated that evolution of species and the patterns in the fossil record could be

readily explained using Darwinian principles. This effort culminated in the 1930's and 1940's in the "modern synthesis," a fusion of thought that resulted in the development of the field of population genetics, a discipline in which biologists seek to describe and predict, quantitatively, evolutionary changes in populations and higher groups of organisms.

Since the modern synthesis (also called the neo-Darwinian synthesis), biologists have concentrated their efforts on applying the theories of population genetics to understanding the evolutionary dynamics of particular groups of organisms. More recently, techniques of phylogenetic systematics have been developed to provide a means of reconstructing phylogenetic relationships among species. This effort has emphasized the need for a comparative and evolutionary approach to biology, which is essential to correct interpretation of data.

In the 1960's, Motoo Kimura proposed the neutral theory of evolution, which challenged the "selectionist" view that patterns of genetic and phenotypic variation in most traits are determined by natural selection. The "neutralist" view maintains that much genetic variation, especially that seen in the numerous alleles of enzyme-coding genes, has little effect on fitness and therefore must be controlled by mechanisms other than selection. The last remaining frontier in the quest for a unified model of evolution is the integration of evolutionary theory with the understanding of the processes of development.

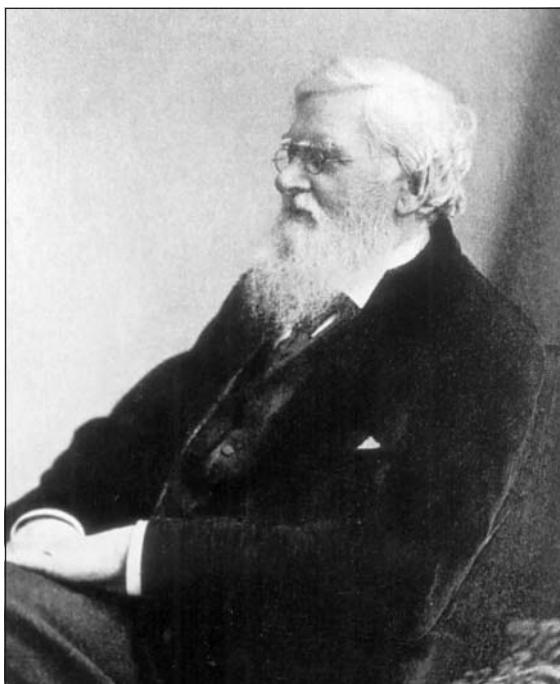
Evolutionary Mechanisms

Natural selection as described by Darwin and Wallace leads to the evolution of adaptations. However, many traits (perhaps the majority) are not adaptations; that is, differences in the particular form of those traits from one member of the species to the next do not lead to differences in fitness among those individuals. Such traits cannot evolve through natural selection, yet they can and do evolve. Thus, there must be additional mechanisms that lead to changes in the genetic structure of biological systems over time.

Evolutionary mechanisms are usually envisioned to act on individual organisms within

a population. For example, natural selection may eliminate some individuals while others survive and produce a large number of offspring similar to themselves. As a result, evolution occurs within those populations. A key tenet of Darwinian evolution (which distinguishes it from Lamarckian evolution) is that populations evolve, but the individual organisms that constitute that population do not. While evolution of populations is certainly the most familiar scenario, this is not the only level at which evolution occurs.

Richard Dawkins energized the scientific discussion of evolution with his book *The Selfish Gene*, first published in 1976. Dawkins argued that natural selection could operate on any type of "replicator," or unit of biological organization that displayed a faithful but imperfect mechanism of copying itself and that had differing rates of survival and reproduction among the variant copies. Under this definition, it is possible to view individual genes or strands of DNA as focal points for evolutionary mechanisms such as selection. Dawkins used this



Alfred Russel Wallace is now considered the co-author of modern evolutionary theory along with Darwin. (National Library of Medicine)

framework to consider how the existence of DNA selected to maximize its chances of replication (or “selfish DNA”) would influence the evolution of social behavior, communication, and even multicellularity.

Recognizing that biological systems are arranged in a hierarchical fashion from genes to genomes (or cells) to individuals through populations, species, and communities, Elisabeth Vrba and Niles Eldredge in 1984 proposed that evolutionary changes could occur in any collection of entities (such as populations) as a result of mechanisms acting on the entities that make up that collection (individuals). Because each level in the biological hierarchy (at least above that of genes) has as its building blocks the elements of the preceding one, evolution may occur within any of them. Vrba and Eldredge further argued that evolution could be viewed as resulting from two general kinds of mechanisms: those that introduce genetic variation and those that sort whatever variation is available. At each level, there are processes that introduce and sort variation, though they may have different names depending on the level being discussed.

Natural selection is a sorting process. Other mechanisms that sort genetic variation include sexual selection, whereby certain variants are favored based on their ability to enhance reproductive success (though not necessarily survival), and genetic drift, which is especially important in small populations. While these forces are potentially strong engines for driving changes in genetic structure, their action—and therefore the direction and magnitude of evolutionary changes that they can cause—is constrained by the types of variation available and the extent to which that variation is genetically controlled.

Processes such as mutation, recombination, development, migration, and hybridization introduce variation at one or more levels in the biological hierarchy. Of these, mutation is ultimately the most important, as changes in DNA sequences constitute the raw material for evolution at all levels. Without mutation, there would be no variation and thus no evolution. Nevertheless, mutation alone is a relatively weak evolutionary force, only really significant in driv-

ing evolutionary changes when coupled with processes of selection or genetic drift that can quickly change allele frequencies. Recombination, development, migration, and hybridization introduce new patterns of genetic variation (initially derived from mutation of individual genes) at the genome, multicellular-organism, population, and species levels, respectively.

The Reality of Evolution

It is impossible to prove that descent with modification from a common ancestor is responsible for the diversity of life on earth. In fact, this dilemma of absolute proof exists for all scientific theories; as a result, science proceeds by constructing and testing potential explanations, gradually accepting those best supported by new observations until they are either clearly disproved or replaced by another theory even more consistent with the data.

Darwin’s concept of a single tree of life is supported by vast amounts of scientific evidence. In fact, the theory of evolution is among the most thoroughly tested and best-supported theories in all of science. The view that evolution has and continues to occur is not debated by biologists; there is simply too much evidence to support its existence, across every biological discipline.

On a small scale, it is possible to demonstrate evolutionary changes experimentally or through observation. Spontaneous mutations that introduce genetic variation are well documented; the origination and spread of drug-resistant forms of viruses and other pathogens is clear evidence of this potential. Agricultural breeding programs and other types of artificial selection illustrate that the genetic structure of lineages containing heritable variation can be changed over time. For example, work by John Doebley begun in the late 1980’s suggested that the evolution of corn from its wild ancestor teosinte may have involved changes in as few as five major genes and that this transition likely occurred as a result of domestication processes established in Mexico between seven thousand and ten thousand years ago. The effects of natural selection can likewise be observed in operation: Peter Grant and his colleagues demonstrated that during drought periods, when seed

is limited, deep-billed individuals of the Galápagos Island finch *Geospiza fortis* increase in proportion to the general population of the species, as only the deep-billed birds can crack the large seeds remaining after the supply of smaller seeds is exhausted. These and similar examples demonstrate that the evolutionary mechanisms put forward by Darwin and others do occur and lead to microevolution, or evolutionary change within single species.

Attempts to account for larger-scale macroevolutionary patterns, such as speciation and the origin of major groups of organisms, rely on indirect tests using morphological and genetic comparisons among different species, observed geographic distributions of species, and the fossil record. Such comparative studies rely on the concept of homology, the presence of corresponding and similarly constructed features among species.

At the most basic level, organization of the genetic code is remarkably similar across species; only minor variations exist among organisms as diverse as archaea (bacteria found in extreme environments such as hot springs, salt lakes, and habitats lacking in oxygen), bacteria, and eukaryotes (organisms whose cells contain a true nucleus, including plants, animals, fungi, and their unicellular counterparts). This genetic homology extends as well to the presence of shared and similarly functioning gene sequences across biological taxa, such as homeotic genes, common among all eukaryotes. Morphological homologies are also widespread; the limbs of mammals, birds, amphibians, and reptiles, for example, are all built out of the same arrangement of bones (although the particular shapes of these bones can vary greatly among groups). The conclusion that emerges from this weight of independent evidence is that structural homologies reflect an underlying evolutionary homology, or descent from a common ancestor.

Punctuated Equilibrium

Although the order of appearance of organisms in the fossil record is consistent with evolutionary theory in general, it has been troubling in two major ways: (1) there are no unequivocal transition fossils for the major evolutionary

splits, such as reptiles to birds or nonflowering plants to flowering plants; and (2) paleontologists have long emphasized that gradualism—that is, evolution by gradual changes, eventually producing major changes—is not supported by the fossil record. The fossil record more often shows a pattern of almost no change over millions of years, followed by much shorter periods of rapid change. Stephen Jay Gould and Niles Eldredge, both paleontologists, proposed a new theory called punctuated equilibrium to explain this discrepancy.

Gould and Eldredge's theory accepts the fact that the fossil record shows long periods of stasis (no change) followed by periods of rapid change and consider this the primary mode for evolution. Instead of the strict neo-Darwinian view of gradual changes leading to large changes over time, Gould and Eldredge suggest that large changes are the result of a series of larger steps over a much shorter period of time. Some of the discoveries in developmental biology—of genes that, when mutated, can cause fruit flies (*Drosophila melanogaster*) to grow legs on their heads instead of antennae, or that can cause every other body segment to be missing—have helped provide some plausible mechanisms for rapid change. If genes like those in the fruit fly, which are master control genes, undergo mutation, the result could be large changes in a very short time. When first proposed, the punctuated equilibrium theory was not readily accepted, but it has gained more acceptance over time.

The Practice of Evolutionary Biology

Contemporary evolutionary biology builds upon the theoretical foundations established by Darwin, Wallace, the framers of the modern synthesis, and now Gould and Eldredge. While the reality of evolution is no longer in doubt, considerable debate remains about the importance of the various mechanisms in the history of particular groups of organisms. Much effort continues to be directed at reconstructing the particular historical path that life on earth has taken and that has led to the enormous diversity of species. Likewise, scientists seek a fuller understanding of how new species arise, as the process of splitting lineages represents a water-

shed event separating microevolution and macroevolution.

Unlike many other fields of biology, evolutionary biology is not amenable to tests of simple cause-and-effect hypotheses. Much of what evolutionary biologists are interested in understanding occurred in the past and over vast periods of time. In addition, the evolutionary outcomes observed in nature depend on such a large number of environmental, biological, and random factors that re-creating and studying the circumstances that could have led to a particular outcome is virtually impossible. Finally, organisms are complex creatures exposed to conflicting evolutionary pressures, such as the need to attract mates while simultaneously attempting to remain hidden from predators; such compromise-type situations are hard to simulate under experimental conditions.

Many evolutionary studies rely on making predictions about the patterns one would expect to observe in nature if evolution in one form or another were to have occurred, and such studies often involve synthesis of data derived from fieldwork, theoretical modeling, and laboratory analysis. While such indirect tests of evolutionary hypotheses are not based on the sort of controlled data that are used in direct experiments, if employed appropriately the indirect tests can be equally valid and powerful. Their strength comes from the ability to formulate predictions based on one species or type of data that may then be supported or refuted by examining additional species or data from another area of biology. In this way, evolutionary biologists are able to use the history of life on earth as a natural experiment, and, like forensic scientists, to piece together clues to solve the greatest biological mystery of all.

—Doug McElroy, updated by Bryan Ness

See also: Ancient DNA; Artificial Selection; Classical Transmission Genetics; Genetic Code; Genetic Code, Cracking of; Genetics, Historical Development of; Hardy-Weinberg Law; Human Genetics; Lamarckianism; Mendelian Genetics; Molecular Clock Hypothesis; Mutation and Mutagenesis; Natural Selection; Population Genetics; Punctuated Equilibrium; Repetitive DNA; RNA World; Sociobiology; Speciation; Transposable Elements.

Further Reading

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Harvard University, Department of Organismic and Evolutionary Biology. <http://www.oeb.harvard.edu>. Site has a timeline of significant figures in the history of evolutionary biology and a survey of its foundations.

Society for the Study of Evolution. <http://www.evolutionandsociety.org>. This site offers an illustrative guide, "Evolution, Science, and Society and the National Research Agenda," which includes discussion of the foundations, social effects, and future of evolutionary biology.

ents, but the site of non-nuclear DNA, the cytoplasm, is almost always contributed by the female parent. The understanding of this extrachromosomal inheritance is crucial, since many important traits in plants and animals—as well as mutations implicated in disease and aging—display this type of transmission.

Key terms

GENOME: hereditary material in the nucleus or organelle of a cell

PLASMAGENE: a self-replicating gene in a cytoplasmic organelle

PLASMON: the entire complement of genetic factors in the cytoplasm of a cell (plasmagenes or cytogenes); a plastid plasmon is referred to as a "plastome"

PLASTID: organelles, including chloroplasts, located in the cytoplasm of plant cells which form the site for metabolic processes such as photosynthesis

MITOCHONDRIA: small structures enclosed by double membranes found in the cytoplasm of all higher cells, which produce chemical power for the cells and harbor their own DNA

Discovery of Extrachromosomal Inheritance

Carl Correns, one of the three geneticists who rediscovered Austrian botanist Gregor Mendel's laws of inheritance in 1900, and Erwin Baur first described, independently, extrachromosomal inheritance of plastid color in 1909. However, they did not know then that they were observing the transmission patterns of organelle genes. Correns studied the inheritance of plastid color in the albomaculata strain of four-o'clock plants (*Mirabilis jalapa*), whereas Baur investigated garden geraniums (*Pelargonium zonate*). Correns observed that seedlings resembled the maternal parent regardless of the color of the male parent (uniparental-maternal inheritance). Seeds obtained from plants with three types of branches—with green leaves, white leaves, and variegated (a mixture of green and white) leaves—provided interesting results. Seeds from green-leaved branches produced only green-leaved seedlings, and seeds from white-leaved branches produced only white-

Extrachromosomal Inheritance

Field of study: Cellular biology

Significance: *Extrachromosomal inheritance refers to the transmission of traits that are controlled by genes located in non-nuclear organelles such as chloroplasts and mitochondria. Nuclear or chromosomal traits are determined equally by both par-*

leaved seedlings. However, seeds from branches with variegated leaves resulted in varying ratios of green-leaved, white-leaved, and variegated-leaved offspring. The explanation is that plastids in egg cells of the green-leaved branches and white-leaved branches were only of one type (homoplasmic or homoplastic)—that is, normal chloroplasts in the green-leaved cells and white plastids (leukoplasts) in the white-leaved cells. The cells of the variegated branches, on the other hand, contained both chloroplasts and leukoplasts (heteroplasmic or heteroplastic) in varying proportions. Some descendants of the heteroplastic cells received only chloroplasts, some received only leukoplasts, and some received a mixture of the two types of plastids in varying proportions in the next generation, hence variegation.

Baur observed similar progeny from reciprocal crosses between normal green and white *Pelargonium* plants. Progeny in both cases were of three types: green, white, and variegated, in varying ratios. This indicated that cytoplasm was inherited from the male as well as the female parent; however, the transmission of plastids was cytoplasmic. Male transmission of plastids has also been observed in oenothera, snapdragons, beans (*Phaseolus*), potatoes, and rye. Rye is the only member of the grass family that exhibits both maternal and paternal inheritance of plastids.

The investigations on plastid inheritance also clearly established that in plants exhibiting uniparental-maternal inheritance, a variegated maternal parent always produces green, white, and variegated progeny in varying proportions because of its heteroplastic nature. Crosses between green and white plants always yield green or white progeny, depending upon the maternal parent, when the parental plants are homoplasmic for plastids.

Extrachromosomal Inheritance vs. Nuclear Inheritance

Extrachromosomal inheritance has been found in many plants, including barley, maize, and rice. Traits are inherited through chloroplasts, mitochondria, or plasmids (small, self-replicating structures). Inheritance of traits that are controlled by organelle genomes

(plasmons) can be called nonnuclear or cytoplasmic. The cytoplasm contains, among other organelles, mitochondria in all higher organisms and mitochondria and chloroplasts in plants. Because cytoplasm is almost always totally contributed by the female parent, this type of transmission may also be called maternal or uniparental inheritance.

Most chromosomally inherited traits obey Mendel's law of segregation, which states that a pair of alleles or different forms of a gene separate from each other during meiosis (the process that halves the chromosome number in gamete formation). They also follow the law of independent assortment, in which two alleles of a gene assort and combine independently with two alleles of another gene. Such traits may be called Mendelian traits. Extrachromosomal inheritance is one of the exceptions to Mendelian inheritance. Thus, it can be called non-Mendelian inheritance. (Mendel only studied and reported on traits controlled by nuclear genes.) Mendelian heredity is characterized by regular ratios in segregating generations for qualitative trait differences and identical results from reciprocal crosses. On the contrary, non-Mendelian inheritance is characterized by a lack of regular segregation ratio and non-identical results from reciprocal crosses.

The mitochondria are the sites of aerobic respiration (the breaking down of organic substances to release energy in the presence of oxygen) in both plants and animals. They are, like plastids, self-replicating entities and exhibit genetic continuity. The mitochondrial genes do not exhibit the Mendelian segregation pattern either. Mitochondrial genetics began around 1950 with the discovery of "petite" mutations in baker's yeast (*Saccharomyces cerevisiae*). Researchers observed that one or two out of every one thousand colonies grown on culture medium were smaller than normal colonies. The petite colonies bred true (produced only petite colonies). The petite mutants were respiration deficient under aerobic conditions. The slow growth of the petite colonies was related to the loss of a number of respiratory (cytochrome) enzymes that occur in mitochondria. These mitochondrial mutants, termed "vegetative petites," can be induced with acriflavine and re-

lated dyes. Another type of mutation, called a “suppressive petite,” was found to be caused by defective, rapidly replicating mitochondrial DNA (mtDNA). Petite mutants that are strictly under nuclear gene control have also been reported and are called segregational petite mutants. Most respiratory enzymes are under both nuclear and mitochondrial control, which is indicative of collaboration between the two genetic systems.

In the fungus *Neurospora*, mitochondrial inheritance has been demonstrated for mutants referred to as “poky” (a slow-growth characteristic). The mutation resulted from an impaired mitochondrial function related to cytochromes involved in electron transport. The mating between poky female and normal male yields only poky progeny, but when the cross is reversed, the progeny are all normal, confirming maternal inheritance for this mutation.

According to a 1970 study, cytoplasmic male sterility is found in about eighty plant species. The molecular basis of cytoplasmic male sterility in maize through electrophoretic separation of restriction-endonuclease-created fragments of DNA was traced to mitochondrial DNA. Cytoplasmic male sterility can be overcome by nuclear genes. The plasmids that reside in mitochondria are also important extrachromosomal DNA molecules that are especially important in antibiotic resistance. Plasmids have been found to be extremely useful in genetic engineering.

Mutator Genes

Plastome mutations can be induced by nuclear genes. A gene that increases the mutation rate of another gene is called a “mutator.” One such gene is the recessive, nuclear *iojap* (*ij*) mutation in maize. In the homozygous (*ij ij*) condition, it induces a plastid mutation. The name “*iojap*” has been derived from “Iowa” (the maize strain in which the mutation is found) and “japonica” (a type of striped variety that the mutation resembles). Once the plastid gene mutation caused by the *ij* gene has been initiated, the inheritance is non-Mendelian, and it no longer depends on the nuclear *ij* gene. As long as the *iojap* plants are used as female parents, the inheritance of the trait is sim-

ilar to that for plastids in the *albomaculata* variety of four o’clock plants.

The *chm* mutator gene causes plastid mutations in the plant *Arabidopsis*, and mutator “*striata*” in barley causes mutations in both plastids and mitochondria. Cases of mutator-induced mutations in the plastome have also been reported in rice and catnip.

Chloroplast and Mitochondrial DNA

Plastids contain DNA, have their own DNA polymerase (the enzyme responsible for DNA replication), and undergo mutation. The chloroplast DNA (cpDNA) is a circular, self-replicating system that carries genetic information that is transcribed (from DNA to RNA) and translated (from RNA to protein) in the plastid. It replicates in a semiconservative manner—that is, an original strand of DNA is conserved and serves as the template for a new strand in a manner similar to replication.

The soluble enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) is involved in photosynthetic carbon dioxide fixation. In land plants and green algae, its large subunit is a cpDNA product, while its small subunit is controlled by a nuclear gene family. Thus, the Rubisco protein is, as are chloroplast ribosomes, a product of the cooperation between the nuclear and chloroplast genes. In all other algae, both the large and small subunits of Rubisco are encoded in cpDNA.

Mitochondrial DNA (mtDNA) molecules are also circular and self-replicating. Human, yeast, and higher plant mtDNAs are the major systems that have been studied. The human mtDNA has a total of 16,569 base pairs. The yeast mtDNA is five times larger than that (84 kilobases), and maize mtDNA is much larger than the yeast mtDNA. Every base pair of human mtDNA may be involved in coding for a mitochondrial messenger RNA (mRNA) for a protein, a mitochondrial ribosomal RNA (rRNA), or a mitochondrial transfer RNA (tRNA). It is compact, showing no intervening, noncoding base sequences between genes. It has only one major promoter (a DNA region to which an RNA polymerase binds and initiates transcription) on each strand. Most codons—triplets of nucleotides (bases) in messenger

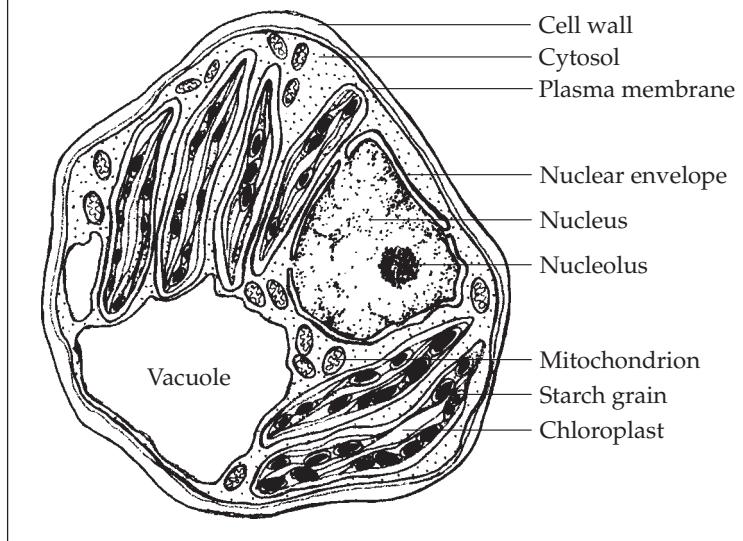
RNA carrying specific instructions from DNA—have the same meaning as in the universal genetic code, except the following differences: UGA represents a “stop” signal (universal), but represents tryptophan in yeast and human mtDNA; AUA represents isoleucine (universal), but methionine in human mtDNA; CUA represents leucine (universal), but threonine in yeast mtDNA; and CGG represents arginine (universal), but tryptophan in plant mtDNA.

The mtDNA carries the genetic code (plasmagene names in parentheses) for proteins, such as cytochrome oxidase subunits I (coxl), II (cox2), and III (cox3); cytochrome B (cytb); and ATPase subunits 6 (atp6), 8 (atp8), and 9 (atp9). It also contains the genetic codes for several ribosomal RNAs, such as mtrRNA 16s and 12s in the mouse; mtrRNA 9s, 15s, and 21s in yeast; and mtrRNA 5s, 18s, and 26s in maize. In addition, twenty-two transfer RNAs in mice, twenty-four in yeast, and three in maize are encoded in mtDNA.

Chlamydomonas reinhardtii

Chlamydomonas reinhardtii is a unicellular green alga in which chloroplast and mitochondrial genes show uniparental transmission. In 1954, Ruth Sager discovered the chloroplast genetic system. Resistance to high levels of streptomycin (a trait controlled by chloroplast genes) has been shown to be transmitted uniparentally by the mt^+ mating type parent. The mt^- mating type transmits the mitochondrial genes uniparentally. Mutants in chloroplasts have been identified for antibiotic and herbicide resistance. Genetic recombination is common in *C. reinhardtii*, which occurs in zygotes (the fused gametes of opposite sexes) when biparental cytogenes are in a heterozygous (union of unlike genes) state. This is an ideal system among plants for recombination

Parts of a Eukaryotic Plant Cell



This depiction of a eukaryotic plant cell shows the organelles where extrachromosomal DNA is found: mitochondria, and in plants chloroplasts and other plastids. (Kimberly L. Dawson Kurnizki)

studies, since there is only one large plastid per cell. In higher plants, study of genetic recombination is difficult because of a large number of plastids in cells and a lack of genetic markers.

Mutations in mitochondria of *C. reinhardtii* can be induced with acriflavine or ethidium bromide dyes. Point mutations for myxothiazol resistance mapping in the *cytb* gene have been isolated. The mitochondrial genome of this species of algae has been completely sequenced. It encodes five of more than twenty-five subunits of the reduced nicotinamide-adenine dinucleotide (NADH) dehydrogenase of complex I (*nad1*, *nad2*, *nad4*, *nad5*, and *nad6*), the COX I subunit of cytochrome oxidase (*cox1*), and the apocytochrome b (*cob*) subunit of complex III. All of these proteins have a respiratory function.

Origin of Plastid and Mitochondrial DNA

According to the endosymbiont theory, plastids and mitochondria in eukaryotes are the descendants of prokaryotic organisms that invaded primitive eukaryotes. Subsequently, they developed a symbiotic relationship and became

dependent upon each other. There is much support for this theory. Researchers in 1972 showed homology (genetic similarity) between ribosomal RNA from cyanobacteria and DNA from the chloroplasts of *Euglena gracilis*. This provided support for chloroplasts as the descendants of cyanobacteria. Mitochondria are believed to have come from primitive bacteria and plastids from blue-green algae. Molecular evidence strongly supports the endosymbiotic origin of mitochondria from alpha purple bacteria.

In 1981, Lynn Margulis summarized evidence for this theory. There are many similarities between prokaryotes and organelles: Both have circular DNA and the same size ribosomes, both lack histones and a nuclear membrane, and both show similar response to antibiotics that inhibit protein synthesis. Both also show a primitive mode of translation that begins with formulated methionine. The discovery of promiscuous DNA (DNA segments that have been transferred between organelles or from a mitochondrial genome to the nuclear genome) in eukaryotic cells also lends support to this theory.

Impact and Applications

Genetic investigations have helped tremendously in constructing a genetic map of maize cpDNA. Important features of the map, including two large, inverted, repeat segments containing several rRNA and tRNA genes, are now known. Detection and quantification of mutant mtDNA are essential for the diagnosis of diseases and for providing insights into the molecular basis of pathogenesis, etiology, and ultimately the treatment of diseases. This should help enhance the knowledge of mitochondrial biogenesis. Mitochondrial dysfunction, resulting partly from mutations in mtDNA, may play a central role in organismal aging.

A number of human diseases associated with defects in mitochondrial function have been identified. Large-scale deletions and tRNA point mutations (base changes) in mtDNA are associated with clinical mitochondrial encephalomyopathies. Heteroplasmy (the coexistence of more than two types of mtDNA) has provided experimental systems in which the transmis-

sion of mtDNA in animals can be studied. Numerous deleterious point mutations of mtDNA are associated with various types of human disorders involving deficiencies in the mitochondrial oxidative phosphorylation (respiration) apparatus. Leigh disease is caused by a point mutation in mtDNA. Deletions of mtDNA have been associated with diseases such as isolated ocular myopathy, chronic progressive external ophthalmoplegia, Kearns-Sayre syndrome, and Pearson's syndrome.

The influence of the mitochondrial genome and mitochondrial function on nuclear gene expression is poorly understood, but progress is being made toward understanding why a few genes are still sequestered in the mitochondria and toward developing new tools to manipulate mitochondrial genes.

—Manjit S. Kang

See also: Ancient DNA; Chloroplast Genes; Genetic Code; Human Genetics; Mitochondrial Diseases; Mitochondrial Genes; Model Organism: *Chlamydomonas reinhardtii*; RNA World.

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Forensic Genetics

Field of study: Human genetics and social issues

Significance: *Forensic genetics uses DNA or the inherited traits derived from DNA to identify individuals involved in criminal cases. Blood tests and DNA testing are used to determine the source of evidence, such as blood stains or semen, left at a crime scene.*

Key terms

ALLELES: alternative versions of genes at a genetic locus that determine an individual's traits

DNA FINGERPRINTING: a DNA test used by forensic scientists to aid in the identification of criminals or to resolve paternity disputes

FORENSIC SCIENCE: the application of scientific knowledge to analyze evidence used in civil

and criminal law, especially in court proceedings

Forensic Science and DNA Analysis

Forensic scientists use genetics for two major legal applications: identifying the source of a sample of blood, semen, or other tissue, and establishing the biological relationship between two people in paternity or maternity suits. Forensic scientists are frequently called upon to testify as expert witnesses in criminal trials. One of the most useful sources of inherited traits for forensic science purposes is blood. Such traits include blood type, proteins found in the plasma, and enzymes found in blood cells. The genes in people that determine such inherited traits have many different forms (alleles), and the specific combination of alleles for many of the inherited blood traits can be

Image not available

A serologist at the Massachusetts State Police Crime Lab displays forms used to collect and identify blood samples for the state's DNA database of people convicted of certain crimes. In 1998, a group of prisoners brought a suit against the state to overturn a law requiring blood samples from anyone convicted of any of thirty-three different crimes. (AP/Wide World Photos)

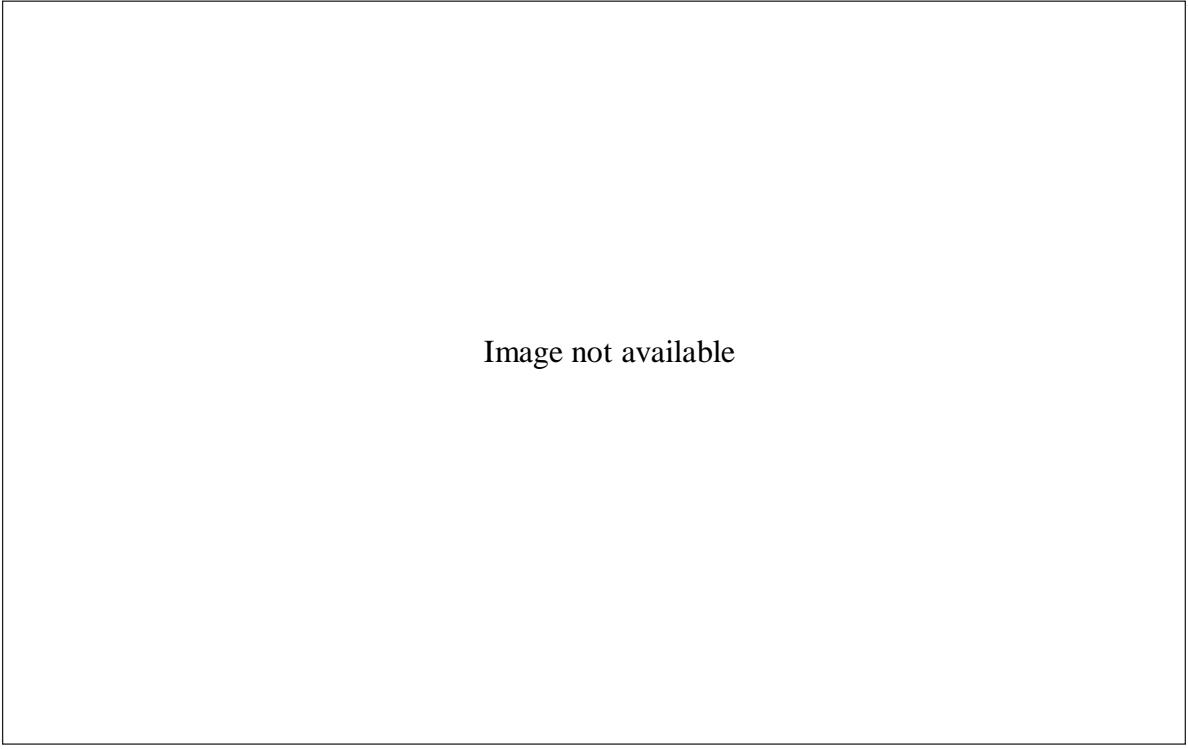


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O.J. Simpson and attorneys discuss strategy for cross-examining a forensic scientist during Simpson's 1995 murder trial. Despite DNA evidence that blood found near Simpson's home and in his car matched that of the murder victims, Simpson was acquitted by a jury upon testimony that the evidence might have been contaminated. (AP/Wide World Photos)

used to identify an individual. The number of useful blood group systems is small, however, which means that a number of individuals might have blood groups identical to those of the subject being tested.

The ultimate source of genetic information for identification of individuals is the DNA found in the chromosomes. Using a class of enzymes known as restriction enzymes, technicians can cut strands of DNA into segments, forming bands similar to a supermarket bar code that vary with individuals' family lines. The pattern, termed a DNA "fingerprint" or profile, is inherited much as are the alleles for blood traits. DNA fingerprinting can be used to establish biological relationships (including paternity) with great reliability, because a child cannot have a variation that is not present in one of the parents. Since DNA is stable and can be reliably tested in dried blood or semen even years after a crime has been committed, DNA fingerprinting has revolutionized the solution

of criminal cases in which biological materials are the primary evidence. The likelihood of false matches ranges from one per million to one per billion. These numbers, however, do not include the possibility of mishandling of evidence, laboratory errors, or planting of evidence.

Criminal Cases Involving DNA Evidence

On November 6, 1987, serial rapist Tommy Lee Andrews became the first American ever convicted in a case involving DNA evidence. Samples of semen left at the crime scene by the rapist and blood taken from Andrews were sent to a New York laboratory for testing. Using the techniques of DNA fingerprinting, the laboratory isolated DNA from each sample, compared the patterns, and found a DNA match between the semen and the blood. Andrews was sentenced to twenty-two years in prison for rape, aggravated battery, and burglary.

The 1990-1991 *United States v. Yee* homicide

trial in Cincinnati, Ohio, was the first major case that challenged the soundness of DNA testing methods. DNA analysis by the Federal Bureau of Investigation (FBI) showed a match between blood from the victim's van and from Steven Yee's car. The defense claimed that the matching DNA data were ambiguous or inconsistent, citing what they claimed to be errors, omissions, lack of controls, and faulty analysis. However, after a fifteen-week hearing, the judge accepted the DNA testing as valid.

In 1994, former football star O. J. Simpson was arrested and charged with the murders of his ex-wife, Nicole Brown, and her friend, Ronald Goldman. Blood with DNA that matched Simpson's was found at Brown's home, and blood spots in Simpson's car contained DNA matching Brown's, Goldman's, and Simpson's. Furthermore, blood at Simpson's home contained DNA that matched Brown's and Goldman's. For the most part, the defense admitted the accuracy of the DNA tests and did not scientifically challenge the results of the DNA fingerprinting. Instead, they argued that the biological evidence had been contaminated by shoddy laboratory work and by planting of evidence; the jury found Simpson not guilty of the charges against him.

Impact and Applications

DNA evidence is used in thousands of criminal investigations and tens of thousands of paternity tests annually in the United States. In addition, in numerous cases forensic DNA testing has been used to free previously convicted and incarcerated individuals, some of whom have been in prison for more than a decade. Most states now have data banks containing DNA profiles of people already convicted of sexual or related offenses; when law enforcement officials investigate a crime, they are now able to test DNA collected at the scene to see if it matches that of anyone in the data bank with a history of a similar offense.

—Alvin K. Benson

See also: Biological Determinism; Criminality; DNA Fingerprinting; Eugenics; Eugenics: Nazi Germany; Human Genetics; Insurance; Paternity Tests; Sociobiology; Sterilization Laws.

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Web Sites of Interest

Department of Justice, Federal Bureau of Investigation, Handbook of Forensic Services, DNA Examinations. <http://www.fbi.gov/hq/lab/handbook/examsdna.htm>. The FBI's step-by-step guide to the collecting, securing, and submitting of DNA evidence from crime scenes.

Earl's Forensic Page. <http://members.aol.com/EarlNMeyer/DNA.html>. Summarizes how DNA fingerprinting works and its use in crime investigations and in determining paternity.

Fragile X Syndrome

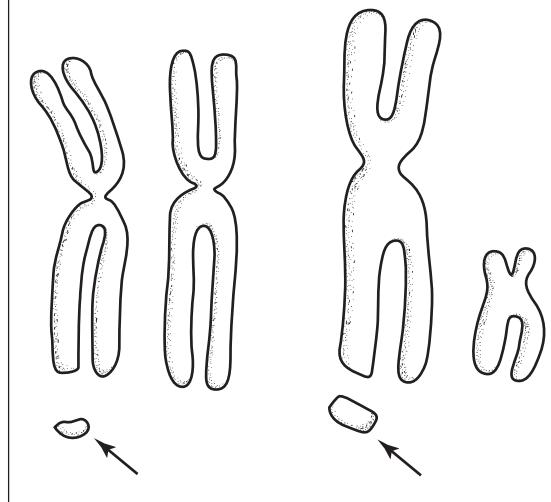
Field of study: Diseases and syndromes

Significance: *There are more than fifty mental retardation disorders associated with the X chromosome, but their frequencies are rare. Fragile X syndrome is the most common inherited form of mental retardation, affecting an estimated one in fifteen hundred males and one in twenty-five hundred females.*

Key terms

SEX CHROMOSOMES: the chromosomes, X and Y, that determine sex; the presence of two X chromosomes codes for females and an X chromosome paired with a Y chromosome codes for males; these chromosomes are received from an individual's parents, each of whom contributes one sex chromosome to their offspring

The Cause of Fragile X Syndrome



Fragile X syndrome in a female chromosome pair (left) and in a male pair (right). Note the apparently detached tips of the X chromosomes, the defect that gives the syndrome its name. (Electronic Illustrators Group)

SEX-LINKED TRAIT: Any characteristic controlled by genes on the X or Y chromosome

History of Fragile X Syndrome

In 1969, geneticists studied a family of four mentally retarded brothers who had X chromosomes whose tips appeared to be detached from the rest of the chromosome. It is now recognized that this fragile site occurs in the vicinity of the *FMRI* gene. Males affected with fragile X syndrome have moderate to severe mental retardation and show distinctive facial features, including a long and narrow face, large and protruding ears, and a prominent jaw. Additional features include velvet-like skin, hyperextensible finger joints, and double-jointed thumbs. These features are generally not observed until maturity. Prior to puberty, the only symptoms a child may have are delayed developmental milestones, such as sitting, walking, and talking. Fragile X children may also display an abnormal temperament marked by tantrums, hyperactivity, or autism. A striking feature of most adult fragile X males is an enlarged testicular volume (macroorchidism). This en-

largement is not a result of testosterone levels, which are normal. Fragile X men are fertile, and offspring have been documented, but those with significant mental retardation rarely reproduce.

The intelligence quotient (IQ) of the majority of affected males is in the moderate to severely retarded range. Only a few affected males have IQs above seventy-five. Fragile X males frequently show delayed speech development and language difficulties. Repetitive speech patterns may also be present.

Mode of Inheritance

In males, any abnormal gene on the X chromosome is expressed because males have only one X chromosome. In females, two copies of the fragile X chromosome must be present for them to be affected. This is the classic pattern for X-linked, or sex-linked, traits (traits whose genes are located on the X chromosome.)

The pattern of inheritance for fragile X is unusual. Fragile X syndrome increases in severity through successive generations. This is explained by a worsening of the defect in the *FMR1* gene as it is passed from mothers to sons. Since males contribute the Y chromosome to their sons, fathers do not pass the fragile X gene to their sons. They will, however, contribute their X chromosome to their daughters. Because these daughters also receive an X chromosome from their mothers, they generally appear normal or only mildly affected. It is only when these daughters have a son that the condition is expressed.

An explanation for this increasing severity through generations was discovered by analyzing the DNA sequence of the *FMR1* gene. The molecules composing DNA are adenine (A), thymine (T), cytosine (C), and guanine (G) and are referred to collectively as "bases." In fragile X syndrome, a sequence in which the

Image not available

Seventeen-year-old Jake Porter (wearing Mohawks jersey 45) suffers from fragile X syndrome. Here he is being honored by teammates during halftime at Motor City Bowl in Detroit on December 26, 2002, for scoring a touchdown during an earlier game in McDermott, Ohio. (AP/Wide World Photos)

three bases CGG are repeated over and over was found. The repetitive sequence is found in normal copies of the *FMR1* gene, but in individuals with fragile X syndrome there are many times more copies of the CGG triplet. The longer repetitive sequence in the *FMR1* gene prevents it from being expressed. Individuals not having the fragile X syndrome have a working *FMR1* gene.

—Linda R. Adkison, updated by Bryan Ness

See also: Behavior; Chromatin Packaging; Classical Transmission Genetics; Congenital Defects; DNA Replication; Down Syndrome; Intelligence; Repetitive DNA.

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Web Sites of Interest

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FRAXA Research Foundation. <http://www.fraxa.org>. The foundation supports research aimed at treatment, and this site offers information on managing the syndrome.

National Fragile X Foundation. Xtraordinary Accomplishments. <http://www.nfxf.org>. General information about the disorder, with advice for caregivers on testing, medical treatment, education, and life planning.

Gel Electrophoresis

Field of study: Techniques and methodologies

Significance: *Gel electrophoresis is a laboratory technique involving the movement of charged molecules in a buffer solution when an electric field is applied to the solution. The technique allows scientists to separate DNA, RNA, and proteins according to their size. The method is the most widely used way to determine the molecular weight of these molecules and can be used to determine the approximate size of most DNA molecules and proteins.*

Key terms

DENATURING: a method of disrupting the normal three-dimensional structure of a protein or nucleic acid so that it stretches out more or less linearly

GEL: a support matrix formed by interconnecting long polymers into a porous, solid material that retards the movement of molecules entrapped in it

STAINING DYE: a chemical with a high affinity for DNA, RNA, or proteins that causes a visible color to develop that allows the detection of these molecules in the gel

Basic Theory of Electrophoresis

Biologists often need to determine the approximate size of DNA fragments, RNA, or proteins. All of these molecules are much too small to visualize using conventional methods. The size of a piece of DNA capable of carrying all the information needed for a single gene may be only 2 microns long and 20 angstroms wide, while the protein encoded by this gene might form into a globular ball only 2.5 to 10 nanometers in diameter. Therefore, some indirect method of “seeing” the length of these molecules must be used. The easiest and by far most common way to do this is by gel electrophoresis. Electrophoresis is based on the theory that if molecules can be induced to move in the same direction through a tangled web of material, smaller molecules will move farther through the matrix than larger molecules. Thus, the distance a molecule moves will be related to its size, and knowing the basic chemical nature of

the molecule will allow an approximation of its relative molecular weight.

As an analogy, imagine a family with two children picnicking by a thick, brushy forest. Their small dog runs into the brush, and the whole family runs in after it. The dog, being the smallest, penetrates into the center of the forest. The six-year-old can duck through many of the branches and manages to get two-thirds of the way in; the twelve-year-old makes it halfway; the mother gets tangled up and must stop after only a short distance; the father, too large to fit in anywhere, cannot enter at all. This is what happens to molecules moving through a gel: Some travel through unimpeded, others are separated into easily visualized size groups, and others cannot even enter the matrix.

The Electrophoresis Setup

The gel is typically composed of a buffer solution containing agarose or acrylamide, two polymers that easily form a gel-like material at room temperature. At first the buffer/polymer solution is liquid and is poured into a casting chamber composed of a special tray or of two plates of glass with a narrow space between them. A piece of plastic with alternating indentations like an oversized comb is pushed into one end of the gel while it is still liquid. When the gel has solidified, the “comb” is removed, leaving small depressions in the matrix (wells) into which the DNA, RNA, or protein sample is applied. The gel is then attached to an apparatus that exposes the ends of the gel to a buffer, each chamber of which is attached to an electric power supply. The buffer allows an even application of the electric field.

Since the molecules of interest are so small, matrices with small pore size must be created. It is important to find a matrix that will properly separate the molecules being studied. The key is to find a material that creates pores large enough to let DNA or proteins enter but small enough to impede larger molecules. By using different concentrations of agarose or acrylamide, anything from very short pieces of DNA that differ only by a single nucleotide to whole chromosomes can be separated.

Agarose is composed of long, linear chains of multiple monosaccharides (sugars). At high temperatures, 95 degrees Celsius (203 degrees Fahrenheit), the agarose will “melt” in a buffer solution. As the gel cools to around 50 degrees Celsius (122 degrees Fahrenheit), the long chains begin to wrap around each other and solidify into a gel. The concentration of agarose determines the pore size, since a larger concentration will create more of a tangle. Agarose is usually used with large DNA or RNA molecules.

Acrylamide is a short molecule made up of a core of two carbons connected through a double bond with a short side-chain with a carboxyl and amino group. When the reactive chemicals ammonium persulfate and TEMED are added, the carbon ends fuse together to create long chains of polyacrylamide. If this were the only reaction, the end result would be much like agarose. However, a small number (usually 5 percent or less) of the acrylamides are the related molecule called bis-acrylamide, a two-headed version of the acrylamide molecule. This allows the formation of interconnecting branch points every twenty to fifty acrylamide residues on the chain, which creates a pattern more like a net than the tangled strands of agarose. This results in a narrower pore size than agarose, which allows the separation of much smaller fragments. Acrylamide is used to separate proteins and small DNA fragments and for sequencing gels in which DNA fragments differing in size by only a single nucleotide must be clearly separated.

Why Nucleic Acids and Proteins Move in a Gel

DNA and RNA will migrate in an electric field since every base has a net negative charge. This means that DNA molecules are negatively charged and will migrate toward the positive pole if placed in an electric field. In fact, since each base contributes the same charge, the amount of negative charge is directly proportional to the length of the DNA. This means that the electromotive force on any piece of DNA or RNA is directly proportional to its length (and therefore its mass) and that the rate of movement of DNA or RNA molecules of the same length should be the same.

The charge on different amino acids varies considerably, and the proportions of the various amino acids vary widely from protein to protein. Therefore, the charge on a protein has nothing to do with its length. To correct for this, proteins are mixed with the detergent sodium dodecyl sulfate, or SDS (the same material that gives most shampoos their suds), before being loaded onto the gel. The detergent coats the protein evenly. This has two important effects. The first is that the protein becomes denatured, and the polypeptide chain will largely exist as a long strand (rather than being compactly bunched, as it normally is). This is important because a tightly balled protein would more easily pass through the polyacrylamide matrix than a linear molecule, and proteins with the same molecular weight might appear to be different sizes. More important, each SDS molecule has a slight negative charge, so the even coating of the protein results in a negative charge that is directly proportional to the size of the protein.

Once the molecules have been subjected to the electric field long enough to separate them in the gel, they must be visualized. This is done by soaking the gel in a solution that contains a dye that stains the molecules. For DNA and RNA, this dye is usually ethidium bromide, a molecule that has an affinity for nucleic acids and slips between the strands or intercalates into the helix. The dye, when exposed to ultraviolet light, glows orange, revealing the location of the nucleic acid in the gel. For proteins, the dye Coomassie blue is usually used, a stain that readily binds to proteins of most types.

—J. Aaron Cassill, updated by Bryan Ness

See also: Blotting; Southern, Northern, and Western; DNA Fingerprinting; Genetic Testing; Proteomics; RFLP Analysis; Shotgun Cloning.

Further Reading

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Gender Identity

Field of study: Human genetics and social issues

Significance: Researchers have long sought an understanding of the basis of human gender identity. Discoveries in the field of human genetics have opened the way to examine how genes affect sexual behavior and sexual identity.

Key terms

HERMAPHRODITE: an individual who has both male and female sex organs

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP): a technique involving the cutting of DNA with restriction endonucleases (restriction enzymes) that allows researchers to compare genetic sequences from various sources

SEX DETERMINATION: the chromosomal sex of an individual; normal human females have two X chromosomes; normal human males have one X and one Y chromosome

SEXUAL ORIENTATION: the actual sexual behavior exhibited by an individual

Boy or Girl?

The question of what is “male” and what is “female” can have a variety of answers, depending on whether one is thinking of chromosomal (genetic) sex, gonadal sex, phenotypic sex, or self-identified gender. Chromosomal sex is determined at the time of conception. The fertilized human egg has a total of forty-six chromosomes, including one pair of sex chromosomes. If the fertilized egg has a pair of X chromosomes, its chromosomal, or genetic, sex is female. If it has one X chromosome and one Y chromosome, its genetic sex is male. Toward the end of the second month of prenatal development, processes are initiated that lead to the development of the gonadal sex of the individual; the embryo develops testes if male, ovaries if female. Although the chromosomal sex may be XX, the sexual phenotype will not always be female; likewise, if the chromosomal sex is XY, the sexual phenotype does not always turn out to be male. Naturally occurring chromosomal variations or single-gene mutations

may interfere with normal development and differentiation, leading to sexual phenotypes that do not correspond to the chromosomal sex.

One such case is that of hermaphrodites, individuals who possess both ovaries and testes. They usually carry both male and female tissue. Some of their cells may be of the female chromosomal sex (XX), and some may be of the male chromosomal sex (XY). Such individuals are called sex chromosome mosaics, and their resulting phenotype may be related to the number and location of cells that are XX and those that are XY. Another example is testicular feminization syndrome, in which a single gene affects sexual differentiation. Individuals with this syndrome have the chromosomal sex of a normal male but have a female phenotype. XY males with this gene, located on the X chromosome, exhibit initial development of the testes and normal production of male hormones. However, the mutant gene prevents the hormones from binding to receptor cells; as a result, female characteristics develop.

Gender Identity Disorder

Gender identity disorder, or transsexualism, is defined by researchers as a persistent feeling of discomfort or inappropriateness concerning one's anatomic sex. The disorder typically begins in childhood and is manifested in adolescence or adulthood as cross-dressing. About one in eleven thousand men and one in thirty thousand women are estimated to display transsexual behavior. Hormonal and surgical sex reassignment are two forms of available treatment for those wanting to take on the physical characteristics of their self-identified gender. Little is known about the causes of gender identity disorder. In some cases, research shows a strong correlation between children who exhibit cross-gender behavior and adult homosexual orientation. Adults with gender identity disorder and adult homosexuals often recall feelings of alienation beginning as early as preschool.

Although some clinical aspects are shared, however, gender identity disorder is different from homosexuality. One definition for homosexuality proposed by Paul Gebhard is "the

physical contact between two individuals of the same gender which both recognize as being sexual in nature and which ordinarily results in sexual arousal." Other researchers have underscored the difficulty in defining and measuring sexual orientation. Whatever measure is used, homosexuality is far more common than transsexualism.

Impact and Applications

Biological and genetic links to gender identity have been sought for more than a century. Studies on twins indicate a strong genetic component to sexual orientation. There appears to be a greater chance for an identical twin of a gay person to be gay than for a fraternal twin. Heritability averages about 50 percent in the combined twin studies. The fact that heritability is 50 percent rather than 100 percent, however, may indicate that other biological and environmental factors play a role. One study using restriction fragment length polymorphisms (RFLPs) to locate a gene on the X chromosome associated with male homosexual behavior showed a trend of maternal inheritance. However, not all homosexual brothers had the gene, and some heterosexual brothers shared the gene, indicating that other factors, whether genetic or nongenetic, influence sexual orientation.

Although some genetic factors have been found to influence sexual orientation, most researchers believe that no single gene causes homosexuality. It is also apparent that gender identity and homosexuality are influenced by complexes of factors dictated by biology, environment, and culture. Geneticists and social scientists alike continue to design studies to define how the many factors are interrelated.

—Donald J. Nash

See also: Behavior; Biological Clocks; Hermaphrodites; Homosexuality; Human Genetics; Metafemales; Pseudohermaphrodites; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

Further Reading

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Web Sites of Interest

About Gender. <http://www.gender.org.uk>. A site that looks at the nature versus nurture debate in research on gender roles, identity, and variance, with special emphasis on genetics.

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization

which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes or links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

Gene Families

Field of study: Molecular genetics

Significance: Gene families contain multiple copies of structurally and functionally related genes, derived from duplications of an original gene. Some gene families represent multiple identical copies of an important gene, while others contain different versions of a gene with related functions. Evolution of gene families can lead some genes to take on completely new functions, allowing greater complexity of the genome and perhaps the organism.

Key terms

CONCERTED EVOLUTION: a process in which the members of a gene family evolve together

PSEUDOGENES: nonfunctional segments of DNA that resemble functional genes

REPETITIVE DNA: a DNA sequence that is repeated two or more times in a DNA molecule or genome

Evolutionary Origin of Gene Families

Gene families are a class of low or moderately repetitive DNA, consisting of structurally and functionally related genes resulting from gene duplication events. Usually, members of gene families are clustered together on a chro-

mosome, but members of a family can be located on more than one chromosome. Several mechanisms can generate tandem copies of genes: chromosome duplication, unequal crossing over, and replication slippage. Duplication of chromosomal segments is often a result of crossing over in inversion heterozygotes and creates tandem repeated segments. Unequal crossing over occurs when homologous segments do not line up correctly during meiosis and one of the crossover products has a duplicated segment. Replication slippage occurs when the DNA polymerase “slips” during DNA replication and copies part of the template strand again. Once there are two copies of a gene in tandem, the latter two mechanisms are more likely to generate additional copies.

A member of a gene family may be functional or functionless. If the gene was not copied completely or further mutations render it nonfunctional, it is called a pseudogene. Further sequence changes in a functional copy may result in a gene with an altered function, such as producing a similar but different form of a protein that can serve some biochemical need or a protein that has a much different function than the original.

Identical Gene Families

Identical gene families contain functional member genes that produce proteins that are identical or very nearly so. These gene families usually contain genes for protein products that need to be found in abundance in the cell because of a crucial function. Multiple copies of the genes allow greater transcription and protein production.

For example, in eukaryotes, ribosomal RNA (rRNA) genes are repeated in tandem several hundred times. In contrast, there are only seven copies of rRNA genes in the prokaryote *Escherichia coli*, and they are dispersed throughout its single chromosome. The rRNA products of these genes make up part of the structure of the ribosome, the organelle responsible for the important process of protein synthesis.

The genes for eukaryotic histone proteins, which are important in maintaining the structure of DNA in chromosomes and in regulating

the rate of transcription of many genes, are another example of clustered repeats of the same set of genes. In this case, there are five histone genes, separated by short, unrelated noncoding sequences, repeated several hundred times. The repeats are found in tandem in many invertebrate animal genomes but are dispersed in mammalian genomes.

Nonidentical Gene Families

The human beta-globin gene family is an example of a nonidentical gene family, which has functional member genes that serve different, but usually related, functions. In this case, the different protein products are alternate forms of the same type of protein, perhaps expressed at different times in the organism’s development. There are five functional genes and one pseudogene clustered together on chromosome 11. One gene is expressed in the human embryo stage, two in the fetus, and two in the adult. The related alpha-globin gene family, with three genes and four pseudogenes, is a cluster on chromosome 16.

Evolutionary Role of Gene Families

Gene families serve as an example of how genes may be accidentally duplicated by several possible processes, and then by mutation and further duplication the various copies can diverge in function. It is known that long-term genomic evolution (with the exceptions of symbiotic and parasitic genomes) usually involves increases in the number of genes. Although there are a number of mechanisms for this, including polyploidization, it is believed that the formation of gene families can be a first step toward the evolution of “new” genes. Mutations in different members of the gene family cause them to diverge independently, and some may evolve to produce completely different proteins. The presence of gene copies still coding for the original protein allows redundant copies to evolve freely without detrimental changes to cellular physiology.

Although gene family members can evolve to be more different, they may also undergo concerted evolution, in which the various copies evolve together. Unequal crossing over not only changes the number of copies of members

of a gene family but also does so by actual duplication, so that some copies are identical. Repeated events of this type can result in all of the genes in the family being identical. In fact, natural selection will sometimes favor this process if it is to the organism's advantage to have multiple identical copies, as with the rRNA and histone identical gene families.

—Stephen T. Kilpatrick

See also: DNA Replication; Evolutionary Biology; Genomics; Multiple Alleles; Mutations and Mutagenesis; Pseudogenes; Repetitive DNA.

Further Reading

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Gene Regulation: Bacteria

Fields of study: Bacterial genetics; Cellular biology; Molecular genetics

Significance: *Gene regulation is the process by which the synthesis of gene products is controlled. The study of gene regulation in bacteria has led to an understanding of how cells respond to their external and internal environments.*

Key terms

ALLELLE: an alternative form of a gene; for example, *lacI⁺*, *lacI⁻*, and *lacI^S* are alleles of the *lacI* gene

CONTROLLING SITE: a sequence of base pairs to which regulatory proteins bind to affect the expression of neighboring genes

GENE: a sequence of base pairs that specifies a product (either RNA or protein); the average gene in bacteria is one thousand base pairs long

OPERON: one or more genes plus one or more controlling sites that regulate the expression of the genes

TRANSCRIPTION: the use of DNA as the template in the synthesis of RNA

TRANSLATION: the use of an RNA molecule as the guide in the synthesis of a protein

The Discovery of Gene Regulation

In 1961, a landmark paper by French researchers François Jacob and Jacques Monod outlined what was known about genes involved in the breakdown of sugars, the synthesis of amino acids, and the reproduction of a bacterial virus called lambda phage (λ phage). Jacob and Monod described in detail the induction of enzymes that break down the sugar lactose. These enzymes were induced by adding the sugar or, in some cases, structurally related molecules to the media. If these inducer molecules were removed, the enzymes altering lactose were no longer synthesized. Bacteria without the *lacI* gene (*lacI⁻*) produced the enzymes for metabolizing lactose whether or not the inducer was present.

Although bacteria normally have only one copy of each gene locus, they can be given extra copies of selected genes by transforming them

with a plasmid containing the genes of interest. Thus, bacteria that are heterozygous at a locus can be produced. When Jacob and Monod produced bacteria heterozygous for the *lacI* gene (*lacI⁻*/*lacI⁺*), they functioned like normal bacteria (*lacI⁺*), indicating that the *lacI⁺* allele was dominant to the *lacI⁻* allele. Certain alleles of the operator site, *lacO^C*, result in the synthesis of lactose-altering enzymes whether or not the inducer was present and even when *lacI⁺* was present. These observations suggested that the *lacI⁺* gene specified a repressor that might bind to *lacO⁺* and block transcription of the genes involved in lactose metabolism. Jacob and Monod concluded that inducers interfered with the repressor's ability to bind to *lacO⁺*. This allowed transcription and translation of the lactose operon. In their model, the repressor protein is unable to bind to the altered operator site, *lacO^C*. This explained how certain mutations in the operator caused the enzymes for lactose metabolism to be continuously expressed.

Seeing a similarity between the expression of the genes for lactose metabolism, the genes for amino acid synthesis, and the genes for lambda phage proliferation, Jacob and Monod proposed that all genes might be under the control of operator sites that are bound by repressor proteins. An operon consists of the genes that the operator controls. Although the vast majority of operons have operators and are regulated by a repressor, there are some operons without operator sites that are not controlled by a repressor. Generally, these operons are regulated by an inefficient promoter or by transposition of the promoter site, whereas some are inhibited by attenuation, a more complex interaction occurring during transcription and translation. The only controlling site absolutely necessary for gene expression is the promoter site, where RNA polymerase binds.

Lactose Operon: Negatively Controlled Genes

The lactose operon (*lacZYA*) consists of three controlling sites (*lacCRP*, *lacP_{ZYA}*, and *lacO*) and three structural genes (*lacZ*, *lacY*, and *lacA*). The lactose operon is controlled by a neighboring operon, the lactose regulatory operon, consisting of a single controlling site

(*lacP_I*) and a single structural gene (*lacI*). The order of the controlling sites and structural genes in the bacterial chromosome is *lacP_I*, *lacI*, *lacCRP*, *lacP_{ZYA}*, *lacO*, *lacZ*, *lacY*, *lacA*. Transcription of the regulatory operon proceeds to the right from the promoter site, *lacP_I*. Similarly, transcription of the L-arabinose operon occurs rightward from *lacP_{ZYA}*. A cyclic-adenosine monophosphate receptor (CRP) bound by cyclic-adenosine monophosphate (cAMP), referred to as a CRP-cAMP complex, attaches to the *lacCRP* site.

The *lacI* gene specifies the protein subunit of the lactose repressor, a tetrameric protein that binds to the operator site, *lacO*, and blocks transcription of the operon. The *lacZ* gene codes for beta-galactosidase, the enzyme that cleaves lactose into galactose plus glucose. This enzyme also converts lactose into the effector molecule allolactose, which actually binds to the repressor inactivating it. The *lacY* gene specifies the enzyme, known as the "lactose permease," that transports lactose across the plasma membrane and concentrates it within the cell. The *lacA* gene codes for an enzyme called transacetylase, which adds acetyl groups to lactose.

In the absence of lactose, the repressor occasionally diffuses from the operator, allowing RNA polymerase to attach to *lacP_{ZYA}* and make a single RNA transcript. This results in extremely low levels of enzymes called the "basal" level. With the addition of lactose, a small amount of allolactose binding to the repressor induces a conformational change in the repressor so that it no longer binds to *lacO*. The levels of permease and beta-galactosidase quickly increase, and within an hour the enzyme levels may be one thousand times greater than they were before lactose was added.

Normally, cells do not produce levels of lactose messenger RNA (mRNA) or enzymes that are more than one thousand times greater than basal level because the lactose operon is regulated by catabolite repression. As cells synthesize cellular material at a high rate, lactose entrance and cAMP synthesis are inhibited, whereas cAMP secretion into the environment is increased. This causes most of the CRP-cAMP complex to become CRP. CRP is unable to bind

to *lacCRP* and promote transcription from *lacP_{BAD}*.

If lactose is removed from the fully induced operon, repressor quickly binds again to *lacO* and blocks transcription. Within a few hours, lactose mRNA and proteins return to their basal levels. Since the lactose operon is induced and negatively regulated by a repressor protein, the operon is classified as an inducible, negatively controlled operon.

Arabinose Operon: Positively Controlled Genes

The L-arabinose operon (*araBAD*) has been extensively characterized since the early 1960's by American researchers Ellis Englesberg, Nancy Lee, and Robert Schleif. This operon is under the control of a linked regulatory operon consisting of (*araC*, *araO2*) and (*araP_C*, *araO1*). The parentheses indicate that the regions overlap: *araO2* is an operator site in the middle of *araC*, whereas *araP_C* and *araO1* represent a promoter site and an operator site respectively, which overlap. The order of the controlling sites and genes for the regulatory operon and the L-arabinose operon is as follows: (*araC*, *araO2*) (*araP_C*, *araO1*), *araCRP*, *araI1*, *araI2*, *araP_{BAD}*, *araB*, *araA*, *araD*. RNA polymerase binding to *araP_C* transcribes *araC* leftward, whereas RNA polymerase binding to *araP_{BAD}* transcribes *araBAD* rightward.

The *araA* gene specifies an isomerase that converts L-arabinose to L-ribulose, the *araB* gene codes for a kinase that changes L-ribulose to L-ribulose-5-phosphate, and the *araD* gene contains the information for an epimerase that turns L-ribulose-5-phosphate into D-xylulose-5-phosphate. Further metabolism of D-xylulose-5-phosphate is carried out by enzymes specified by genes in other operons.

The *araC* product is in equilibrium between two conformations, one having repressor activity and the other having activator activity. The conformation that functions as an activator is stabilized by the binding of L-arabinose or by certain mutations (*araC^C*). In the absence of L-arabinose, almost all the *araC* product is in the repressor conformation; however, in the presence of L-arabinose, nearly all the *araC* product is in the activator conformation.

In the absence of L-arabinose, bacteria will synthesize only basal levels of the lactose regulatory protein and the enzymes involved in the breakdown of L-arabinose. The repressor binding to *araO2* prevents *araC* transcription beginning at *araP_C* from being completed, whereas repressor binding to *araI1* prevents *araBAD* transcription beginning at *araP_{BAD}*.

The addition of L-arabinose causes repressor to be converted into activator. Activator binds to *araI1* and *araI2* and stimulates *araBAD* transcription. Activator is absolutely required for the metabolism of L-arabinose since bacterial cells with a defective or missing L-arabinose regulatory protein, *araC⁻*, only produce basal levels of the L-arabinose enzymes. This is in contrast to what happens to the lactose enzymes when there is a missing lactose regulatory protein, *lacI⁻*. Because of the absolute requirement for an activator, the L-arabinose operon is considered an example of a positively controlled, inducible operon.

Transcription of the *araBAD* operon is also dependent upon the cyclic-adenosine monophosphate receptor protein (CRP), which exists in two conformations. When excessive adenosine triphosphate (ATP) and cellular constituents are being synthesized from L-arabinose, cAMP levels drop very low in the cell. This results in CRP-cAMP acquiring the CRP conformation and dissociating from *araCRP*. When this occurs, the *araBAD* operon is no longer transcribed. The L-arabinose operon is controlled by catabolite repression very much like the lactose operon.

Tryptophan Operon: Genes Controlled by Attenuation

The tryptophan operon (*trpLEDCBA*) consists of the controlling sites and the genes that are involved in the synthesis of the amino acid tryptophan. The order of the controlling sites and genes in the tryptophan operon is as follows: (*trpP*, *trpO*), *trpL*, *trpE*, *trpD*, *trpC*, *trpB*, *trpA*. RNA polymerase binds to *trpP* and initiates transcription at the beginning of *trpL*.

An inactive protein is specified by an unlinked regulatory gene (*trpR*). The regulatory protein is in equilibrium between its inactive and its repressor conformation, which is sta-

lized by tryptophan. Thus, if there is a high concentration of tryptophan, the repressor binds to *trpO* and shuts off the tryptophan operon. This operon is an example of an operon that is repressible and negatively regulated.

The tryptophan operon is also controlled by a process called attenuation, which involves the mRNA transcribed from the leader region, *trpL*. The significance of leader region mRNA is that it hydrogen-bonds with itself to form a number of hairpinlike structures. Hairpin-III interacts with the RNA polymerase, causing it to fall off the DNA. Any one of several hairpins can form, depending upon the level of tryptophan in the environment and the cell. When there is no tryptophan in the environment, the operon is fully expressed so that tryptophan is synthesized. This is accomplished by translation of the leader region right behind the RNA polymerase up to a couple of tryptophan codons, where the ribosomes stall. The stalled ribosomes cover the beginning of the leader mRNA in such a way that only hairpin-II forms. This hairpin does not interfere with transcription of the rest of the operon and so the entire operon is transcribed.

When there is too much tryptophan, the operon is turned off to prevent further synthesis of tryptophan. This is accomplished by translation of the leader region up to the end of the leader peptide. Ribosomes synthesizing the leader peptide cover the leader mRNA in such a way that only hairpin-III forms. This hairpin causes attenuation of transcription.

In some cases, the lack of amino acids other than tryptophan can result in attenuation of the tryptophan operon. In fact, cells starved for the first four amino acids (N-formylmethionine, lysine, alanine, and isoleucine) of the leader peptide result in attenuation. When these amino acids are missing, hairpins-I and III both form, resulting in attenuation because of hairpin-III.

Flagellin Operons: Operons Controlled by Transposition

Some pathogenic bacteria change their flagella to avoid being recognized and destroyed by the host's immune system. This change in flagella occurs by switching to the synthesis of another flagellar protein. The phenomenon is

known as phase variation. The genes for flagellin are in different operons. The first operon consists of a promoter site, an operator site, and the structural gene for the first flagellin (*flgP_{H1}*, *flgO1*, *flgH1*). The first operon is under the negative control of a repressor specified by the second operon. The second operon also specifies the second flagellin and a transposase that causes part of the second operon to reverse itself. This portion of the operon that "flips" is called a "transposon." The promoter sites for the transposase gene (*flgT2*), flagellin gene (*flgH2*), and repressor gene (*flgR2*) are located on either side of the transposase gene in sequences called inverted repeats. Transcription from both promoters in the second operon occurs from left to right: *flgP_{T2}*, *flgT2*, *flgP_{H2R2}*, *flgH2*, *flgR2*.

When the second operon is active, the repressor binds to *flgO1*, blocking the synthesis of the first flagellin (*flgH1*). Consequently, all bacterial flagella will be made of the second flagellin (*flgH2*). Occasionally, the transposase will catalyze a recombination event between the inverted repeats, which leads to the transposon being reversed. When this occurs, neither *flgH2* nor *flgR2* is transcribed. Consequently, the first operon is no longer repressed by *flgR2*, and *flgH1* is synthesized. All the new flagella will consist of *flgH1* rather than *flgH2*.

Impact and Applications

Many of the genetic procedures developed to study gene regulation in bacteria have contributed to the development of genetic engineering and the production of biosynthetic consumer goods. One of the first products to be manufactured in bacteria was human insulin. The genes for the two insulin subunits were spliced to the lactose operon in different populations of bacteria. When induced, each population produced one of the subunits. The cells were cracked open, and the subunits were purified and mixed together to produce functional human insulin. Many other products have been made in bacteria, yeast, and even plants and animals.

Considerable progress has been made toward introducing genes into plants and animals to change them permanently. In most

cases, this is difficult to do because the controlling sites and gene regulation are much more complicated in higher organisms than in bacteria. Nevertheless, many different species of plants have been altered to make them resistant to desiccation, herbicides, insects, and various plant pathogens. Although curing genetic defects by introducing good genes into animals and humans has not been very successful, animals have been transformed so that they produce a number of medically important proteins in their milk. Goats have been genetically engineered to release tissue plasminogen activator, a valuable enzyme used in the treatment of heart attack and stroke victims, into their milk. Similarly, sheep have been engineered to secrete human alpha-1 antitrypsin, useful in treating emphysema. Cattle that produce more than ten times the milk that sheep or goats produce may potentially function as factories for the synthesis of all types of valuable proteins specified by artfully regulated genes.

—Jaime S. Colomé

See also: Bacterial Genetics and Cell Structure; Central Dogma of Molecular Biology; Gene Regulation: Eukaryotes; Gene Regulation: *Lac Operon*; Gene Regulation: Viruses; Model Organism: *Escherichia coli*; Molecular Genetics; Transposable Elements.

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Gene Regulation: Eukaryotes

Fields of study: Cellular biology; Molecular genetics

Significance: *Gene regulation refers to the processes whereby the information encoded within a DNA sequence is expressed at the required level. For eukaryotes, this primarily pertains to the selective expression of particular proteins during development or in specific tissues.*

Key terms

ANTISENSE TECHNOLOGY: use of antisense oligonucleotides that base pair with mRNA to prevent translation

BASAL TRANSCRIPTION FACTOR: protein that is required for initiation of transcription at all promoters

CHROMATIN REMODELING: any event that changes the nuclease sensitivity (DNA accessibility) of chromatin

CORE PROMOTERS: DNA elements that direct initiation of transcription by the basal RNA polymerase machinery

ENHANCER: a DNA element that serves to enhance transcriptional activity above basal levels

INSULATOR: a DNA element that, when placed between an enhancer and a promoter, prevents activation of that particular gene

RNA INTERFERENCE (RNAi): small, interfering RNAs that cause gene silencing

TRANSCRIPTION FACTOR: a protein that is involved in initiation of transcription but is not part of the RNA polymerase

Nuclear RNA Polymerases

Three nuclear RNA polymerases share the responsibility of transcribing eukaryotic genes.

RNA polymerase I transcribes genes encoding ribosomal RNA (rRNA), RNA polymerase II transcribes protein-coding genes and some small nuclear RNA genes, and RNA polymerase III transcribes genes encoding transfer RNA (tRNA), the 5S rRNA, and some small nuclear RNA. DNA elements known as promoters serve to recruit the RNA polymerases to their transcriptional start sites. The RNA polymerases do not bind their promoters directly. Instead, transcription factors bind to the promoter, and the RNA polymerases are recruited by binding their cognate transcription factors. Promoters for RNA polymerases I and III have limited variability and are recognized by a finite set of transcription factors. In contrast, promoters for RNA polymerase II show significant diversity, and the number of transcription factors involved in recruiting the polymerase is huge. For each polymerase, the core promoter elements are recognized by a set of basal transcription factors which are required for initiation of transcription at all promoters. RNA polymerase, together with its basal transcription factors, constitutes the basal transcription apparatus. Activated or repressed transcription is measured with respect to basal levels.

Transcription can be divided into three phases: Initiation, elongation, and termination. Most regulation occurs at the level of initiation. For RNA polymerases I and III, regulation is generally global and involves a repression of transcription. For RNA polymerase II, regulation is gene-specific, allowing specific regulation of each of thousands of protein-coding genes. RNA polymerase II promoters function only at very low efficiency with the basal transcription factors, and activation is the common mode of regulation. This overview will focus on regulation of protein-coding genes.

Basal Transcription by RNA Polymerase II

RNA polymerase II promoters are modular. The core promoter, which directs transcription by the basal transcription apparatus, typically extends about 35 base pairs upstream or downstream of the transcriptional start site. Core promoters can vary considerably from gene to gene, and there are no universal core promoter elements. Common core promoter elements

include the TATA-box, an AT-rich sequence that is located about 25 base pairs upstream of the transcriptional start, and the region immediately surrounding the start site, known as the initiator. The downstream promoter element, DPE, is typically found about 30 base pairs downstream of the transcriptional start, and it is mainly found in genes that do not have a TATA-box. The strength of a given promoter, as defined by the level of basal transcription, depends on which combination of promoter elements is present and on their respective sequences.

The core promoter elements are recognized by basal transcription factors that for RNA polymerase II are named TFII X , where X is a letter that identifies the individual factor. For example, the TATA-box is bound by the TATA-binding protein, which is a subunit of the transcription factor known as TFIID. A subset of TATA-boxes feature a sequence immediately upstream that serves as a recognition site for TFIIIB. TFIIIB, in turn, recruits the polymerase. Ultimately, the core promoter is also the target for the factors that regulate transcriptional activity.

Activated Transcription

Transcription is the key step at which gene expression is controlled. Transcriptional initiation is regulated by enhancers, which are DNA elements that function to increase levels of transcription above basal levels. Enhancers may be located on either side of the gene, up to several thousand base pairs from the transcriptional start. Enhancers are recognized by transcriptional activators that mediate an increase in transcriptional activity. Transcriptional activators show great variability in terms of cell type and gene specificity, thus allowing unique regulation of individual genes. Activator proteins are modular, containing both a DNA-binding domain and an activation domain. Some activators function by directly interacting with components of the transcription apparatus to stimulate transcription.

Cellular DNA is not naked but packaged into highly organized and compacted nucleoprotein structures known as chromatin. Packaging of DNA into chromatin can occlude protein-

binding sites—for example, interfering with binding of basal transcription factors. Accordingly, activation of transcription may be accomplished by relieving the repression caused by chromatin formation. Indeed, many activators function by recruiting protein complexes whose function is to remodel chromatin to increase DNA accessibility. Decompaction of chromatin at promoters is not always sufficient, as RNA polymerase II may need to transcribe thousands of base pairs. Efficient transcription, therefore, may also depend on specific elongation factors that travel with the RNA polymerase to destabilize chromatin structure.

Since enhancers may activate genes that are located at some distance, mechanisms exist to specify that a certain gene not be the target of a given enhancer. A DNA insulator serves this function: When placed between the enhancer and the promoter, it prevents activation of that particular gene. Unlike enhancers, insulators must therefore be located at a specific position to work. Recruitment of DNA-binding proteins to these insulator elements prevents activation of a specific gene.

Post-transcriptional Control

The pre-messenger RNA (pre-mRNA) transcript is subject to several types of post-transcriptional processing: Intervening sequences (introns) are removed by splicing, a “cap” structure is added to the 5' end, and a polyadenosine (poly-A) tail is added to the 3' end, following cleavage of the transcript. Although historically referred to as post-transcriptional events, this processing occurs during, not after, transcription; the largest RNA polymerase II subunit has a carboxy-terminal domain which serves to recruit proteins involved in mRNA splicing, polyadenylation, and capping, thus securing a tight association between these processes.

Capping and polyadenylation affect both stability of the mRNA and the efficiency of translation. Since most intracellular RNA degradation is in the form of nuclease-mediated degradation from either end, protecting the ends by cap-binding proteins and polyA-binding proteins, respectively, prevents degradation. Short-lived mRNAs often contain elements within the

region downstream of the stop codon that explicitly recruit nuclease complexes that degrade the RNA. In general, genes that encode “house-keeping” proteins produce mRNAs with long half-lives, whereas genes whose expression must be rapidly controlled tend to generate mRNAs with short half-lives.

Additional protein diversity may be generated by alternative splicing, a process whereby different combinations of coding sequences, or exons, are incorporated into the final spliced mRNA product. In this fashion, multiple versions of a protein may be made from a single gene.

Finally, the mRNA sequence affects the efficiency with which it is translated. For instance, folding of the mRNA region upstream of the start codon can interfere with binding of the ribosome, and the sequence adjacent to the start codon affects the efficiency of translation initiation. Nucleotide sequences in the untranslated regions of mRNA are also recognized by specific proteins that may anchor the mRNA to specific cellular structures to ensure their translation and accumulation at the appropriate locations.

Changing Gene Expression

Several techniques exist for modification of gene expression. The principle behind antisense technology is the base pairing of a complementary oligonucleotide to a target mRNA that results in the prevention of translation. As natural oligonucleotides (RNA and DNA) are rapidly degraded in the cell, more stable, artificial oligonucleotides with modified backbones are typically used. This strategy has great potential to reduce expression of genes involved in disease states.

RNA interference, or RNAi, also results in sequence-specific gene silencing. The exposure to double-stranded RNA that matches the sequence of coding regions results in loss of the corresponding mRNA. The double-stranded RNA triggers the assembly of a nuclease complex that targets the homologous mRNA for degradation. In plants, for example, RNAi has been suggested to play an important role in resistance to pathogens. RNAi has also evolved into a powerful tool for probing gene activity

and for developing gene-silencing therapeutics.

Hundreds of different cell types exist that are specialized to perform unique roles. Since each of these cells contains the same tens of thousands of genes, their specialization requires tightly controlled gene regulation. As summarized above, gene regulation occurs at multiple levels, with DNA sequences from the promoter region to the untranslated mRNA sequences dictating the rates of transcription, pre-mRNA processing, and translation. Although the exact mechanisms are only beginning to be elucidated, knowing the DNA sequence therefore has the potential to reveal which exact modes of regulation are in effect. Understanding normal gene regulation will in turn lead to an understanding of how misregulation may lead to disease.

—Anne Grove

See also: Antisense RNA; Bacterial Genetics and Cell Structure; Central Dogma of Molecular Biology; Gene Regulation: Bacteria; Gene Regulation: *Lac* Operon; Gene Regulation: Viruses; Model Organism: *Escherichia coli*; Molecular Genetics; Transposable Elements.

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Gene Regulation: *Lac* Operon

Fields of study: Bacterial genetics; Cellular biology; Molecular biology

Significance: *Studies of the regulation of the lactose (lac) operon in Escherichia coli have led to an understanding of how the expression of a gene is turned on and off through the binding of regulator proteins to the DNA. This has served as the groundwork for understanding not only how bacterial genes work but also how genes of higher organisms are regulated.*

Key terms

ACTIVATOR: a protein that binds to DNA to enhance a gene's conversion into a product that can function within the cell

OPERATOR: a sequence of DNA adjacent to (and usually overlapping) the promoter of an operon; binding of a repressor to this DNA prevents transcription of the genes that are controlled by the operator

OPERON: a group of genes that all work together to carry out a single function for a cell

PROMOTER: a sequence of DNA to which the gene expression enzyme (RNA polymerase) attaches to begin transcription of the genes of an operon

REPRESSOR: a protein that prevents a gene from being made into a functional product when it binds to the operator

Inducible Genes and Repressible Genes

In order for genes or genetic information stored in DNA to be used, the information must first be transcribed into messenger RNA (mRNA); mRNA is synthesized by an enzyme, RNA polymerase, which uses the DNA as a template for making a single strand of RNA that can be translated into proteins. The proteins are the functional gene products that act as enzymes or structural elements for the cell. The process by which DNA is transcribed and then translated is referred to as expression of the genes.

Some genes are always expressed in bacterial cells; that is, they are continually being transcribed into mRNA, which is translated into functional proteins (gene products) of the cell.

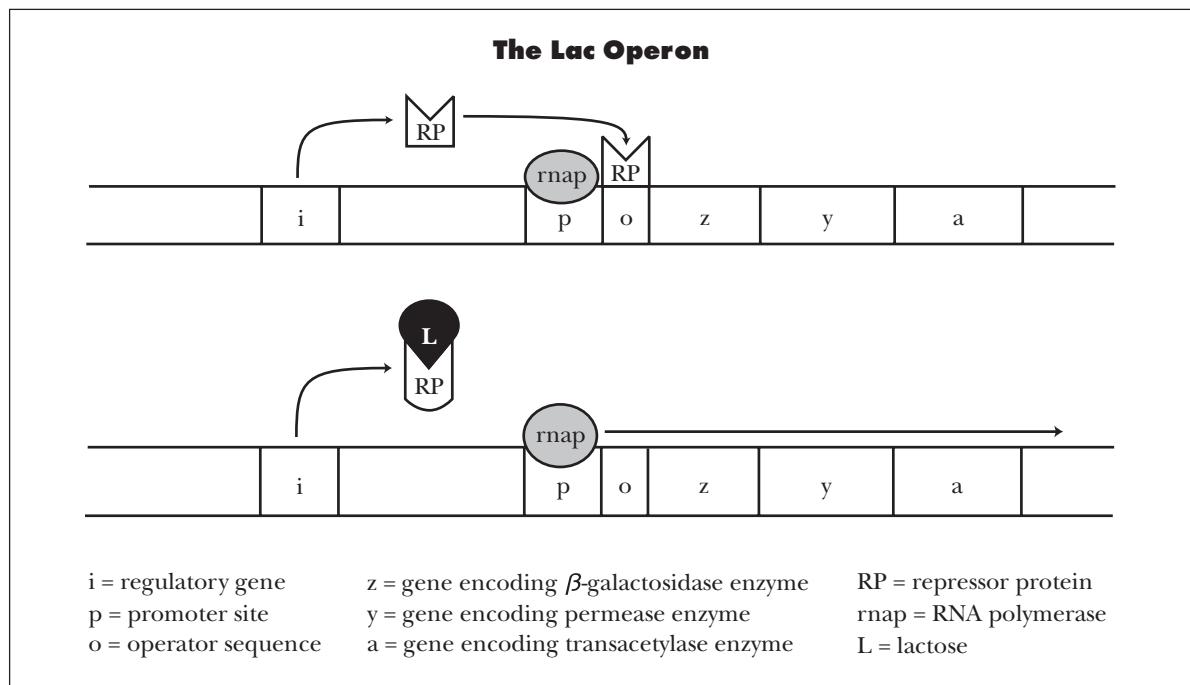
The genes involved in using glucose as an energy source are included in this group. Other genes are inducible (expressed only under certain circumstances). The genes for using lactose as an energy source are included in this group. beta-galactosidase, the enzyme that converts lactose into glucose and galactose so that the sugar can be easily metabolized by a cell, is only made in cells when lactose is present. Synthesizing proteins uses a large amount of energy. In order for the cell to conserve energy, it produces proteins only when they are needed. There is no need to make beta-galactosidase if there is no lactose in the cell, so it is synthesized only when lactose is present.

As early as the 1940's François Jacob, Jacques Monod, and their associates were studying the mechanisms by which beta-galactosidase was induced in *Escherichia coli*. They discovered that when there is no lactose in a cell, a repressor protein binds to the DNA of the operon at the operator site. Under these conditions, transcription of genes in the operon cannot occur since the RNA polymerase is physically pre-

vented from binding to the promoter when the repressor is in place. This occurs because the promoter and operator sequences are overlapping. The lactose (*lac*) operon is, therefore, under negative control. When lactose is present, an altered form of the lactose attaches to the repressor in such a way that the repressor can no longer bind to the operator. With the operator sequence vacant, it is possible for the RNA polymerase to begin transcription of the operon genes at the promoter. Lactose (or its metabolite) serves as an inducer for transcription. Only if it is present are the lactose operon genes expressed. The lactose operon is, therefore, an inducible operon under negative control. In 1965, Jacob and Monod were awarded the Nobel Prize in Physiology or Medicine in recognition of their discoveries concerning the genetic control of enzyme synthesis.

Lac Operon Expression in the Presence of Glucose

When a culture of *E. coli* is given equal amounts of glucose and lactose for growth and



1. In the absence of lactose, the repressor protein binds to the operator, blocking the movement of RNA polymerase. The genes are turned off.
2. When lactose is present, it preferentially binds the repressor protein, freeing up the operator and allowing RNA polymerase to move through the operon. The genes are turned on.

is compared with cultures given either glucose alone or lactose alone, the cells given two sugars do not grow twice as fast, but rather show two distinct growth cycles. Beta-galactosidase is not synthesized initially; therefore, lactose is not used until all the glucose has been metabolized. Laboratory observations show that the presence of lactose is necessary but not a sufficient condition for the lactose (*lac*) operon to be expressed. An activator protein must bind at the promoter in order to unravel the DNA double helix so that the RNA polymerase can bind more efficiently. The activator protein binds only when there is little or no glucose in the cell. If glucose is available, it is preferred over other sugars because it is most easily metabolized to make energy in the form of adenosine triphosphate (ATP). ATP is made through a series of reactions from an intermediate molecule, cyclic adenosine monophosphate (cAMP). The cAMP concentration decreases when ATP is being made but builds up when no ATP synthesis occurs. When the glucose has been used, the concentration of cAMP rises. The cAMP binds to the activator protein to enable it to bind at the operon's promoter. With the activator bound, transcription of the genes, including the beta-galactosidase, occurs.

The activation of a DNA-binding protein by cAMP is a global control mechanism. The lactose operon is only one of many that are controlled in this way. Global control allows bacteria to prevent or turn on transcription of a group of genes in response to a single signal. It ensures that the bacteria always utilize the most efficient energy source if more than one is available. This type of global control only occurs, however, when the operon is also under the control of another DNA-binding protein (the *lac* repressor in the case of the *lac* operon), which makes the operon inducible or repressible or both. Control of transcription through the binding of an activator protein is an example of positive control, since binding of the activator turns on gene expression.

Impact and Applications

Jacob and Monod developed the concept of an operon as a functional unit of gene expres-

sion in bacteria. What they learned from studying the *lac* operon has led to a more general understanding of gene transcription and the use of mRNA as an information-bearing intermediate in the process of gene expression. The operon concept has proven to be a universal mechanism by which bacteria organize their genes. Although genes of higher cells (eukaryotes) are not usually organized in operons and although negative control of expression is rare in them, similar positive control mechanisms occur in both bacterial and eukaryotic cells. Studies of the *lac* operon have made possible the understanding of how DNA-binding proteins can attach to a promoter to enhance transcription.

—Linda E. Fisher

See also: Bacterial Genetics and Cell Structure; Gene Regulation: Bacteria; Gene Regulation: Eukaryotes; Gene Regulation: Viruses; Model Organism: *Escherichia coli*.

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Gene Regulation: Viruses

Fields of study: Cellular biology; Molecular biology; Viral genetics

Significance: *Gene regulation in viruses typically resembles that of the hosts they infect. Because viruses are not alive and are incapable of self-replication, gene regulation at the time of initial infection depends on its host's control systems. Once infection is established, regulation is generally mediated by gene products of the virus's own DNA or RNA.*

Key terms

BACTERIOPHAGE: general term for a virus that infects bacteria

LAMBDA (λ) PHAGE: a virus that infects bacteria and then makes multiple copies of itself by taking over the infected bacterium's cellular machinery

LYSOGENY: a process whereby a virus integrates into a host chromosome as a result of non-lytic, nonproductive, infection

OPERATOR: a sequence of DNA adjacent to (and usually overlapping) the promoter site, where a regulatory protein can bind and either increase or decrease the ability of RNA polymerase to bind to the promoter

PROMOTER: a sequence of DNA to which the gene expression enzyme (RNA polymerase) attaches to begin transcription of the genes of an operon

moter, or they increase or decrease the strength of RNA polymerase binding. These secondary control sequences, therefore, act as switches for turning on or off their associated genes. Some may also act like a dimmer switch, increasing or decreasing the rate at which a gene is expressed.

Viruses are incapable of self-replication and must rely on the host cells they infect. In order to replicate successfully, a virus must be compatible with the host's cell biochemistry and gene-regulation systems. When a virus first enters a host cell, its genes are regulated by the host. Thus, viral promoters and other control elements must be compatible with those of its host. The control elements associated with promoters in prokaryotes are called operators. An operator represents a site where a regulatory protein (a product of yet another gene) can bind and either increase or decrease the ability of RNA polymerase to bind to the promoter of its associated gene or group of genes.

Eukaryotic systems (cells of plants and animals) are more complex and involve a number of proteins called transcription factors, which bind to or near the promoter and assist RNA polymerase binding. There are also enhancer proteins, which bind to other control sequences somewhere upstream from the gene they influence. Because of this greater complexity, viruses that infect eukaryotic cells are also more genetically complex than are viruses infecting prokaryotes.

General Aspects of Regulation

Regardless of the type of organism, DNA is the genetic material that allows species to survive and pass their traits to the next generation. Genes are encoded, along with control sequences that the cell uses to control expression of their associated genes. Although details of these control sequences vary between prokaryotes and eukaryotes, they still function in similar ways. One element common to all genes is a promoter, a sequence that acts as the binding site for RNA polymerase, the enzyme that transcribes the gene into RNA so it can be translated into a protein product. Other control sequences, if present, simply help control whether or not RNA polymerase can bind to the pro-

Viral Genomes

All cells, including bacteria, are subject to infection by parasitic elements such as viruses. Viruses which specifically infect bacteria are known as bacteriophages, from the Greek *phagos*, "to eat." The genetic information in viruses may consist of either RNA or DNA. All forms of viruses contain one or the other, but never both. Regardless of the type of genetic material, gene regulation does have certain features in common.

The size of the viral genome determines the number of potential genes that can be encoded. Among the smallest of the animal viruses are the hepadnaviruses, including hepatitis B virus, the DNA of which consists of some

3 kilobase pairs (3 kbp, or 3,000 base pairs), enough to encode approximately seven proteins. The largest known viruses are the poxviruses, consisting of 200-300 kbp, enough to encode several hundred proteins. Lambda is approximately average in size, with a DNA genome of 48 kbp, enough to encode approximately fifty genes.

Lambda as a Model System: The Lytic Cycle

Following infection of the bacterial host, most bacteriophages replicate, releasing progeny as the cell falls apart, or lyses. Lambda phage is unusual in that, while it can complete a lytic cycle, it is also capable of a nonproductive in-

fection: following infection, the viral genome integrates into the host chromosome, becoming a prophage in a process known as lysogeny. Such phages are known as temperate viruses.

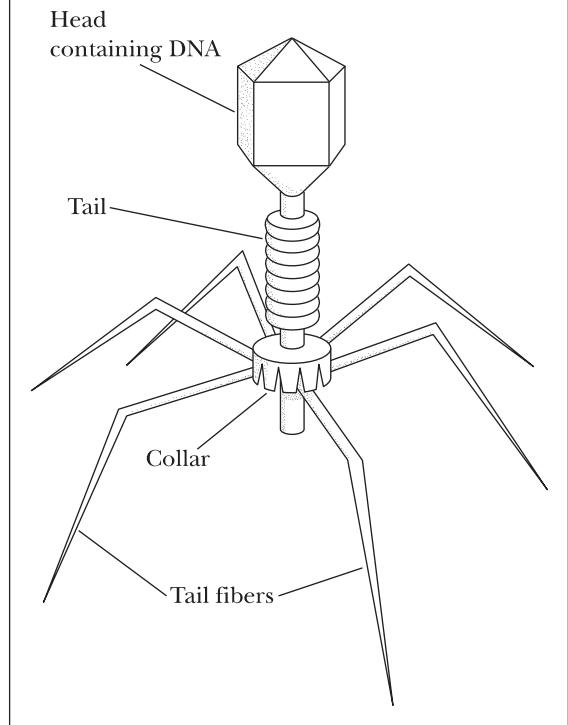
Most viruses, including lambda, exhibit a temporal control of regulation: Gene expression is sequential. Three classes of proteins are produced, classified based on when after infection they are expressed. “Immediate early” genes are expressed immediately after infection, generally using host machinery and enzymes. “Early” genes are expressed at a later time and generally require proteins expressed from early genes. “Late” genes are expressed following genome replication of the virus. The various temporal classes of gene products may also be referred to as lambda, beta, and gamma proteins.

The lytic cycle of lambda represents a prototype of temporal control. Lambda immediate early gene expression begins following infection of the host cell, *Escherichia coli*. Host cell enzymes catalyze the process. Transcription of lambda DNA begins at a site called a promoter, a region recognized by the host RNA polymerase, which catalyzes transcription. Lambda DNA is circular after entering the cell, and two promoters are recognized: One regulates transcription in a leftward direction (P_L), while the other regulates transcription from the opposite strand in a rightward transcription (P_R).

Among the immediate early genes expressed is one encoding the N protein, expression of which is under the control of P_L . Generally, transcription occurs through a set of genes and is terminated at a specific point. The N protein is an example of an antiterminator, a protein that allows “read-through” of the stop signal for transcription and expression of additional genes. A second protein is encoded by the *cro* gene, the product of which plays a vital role in determining whether the infection is lytic or becomes lysogenic. *Cro* gene expression is controlled through P_R , as are several “early class” genes which regulate viral DNA replication (*O* and *P* genes), repressor synthesis (*cII*), and early gene expression (*Q* gene).

Both the *cro* and *Q* proteins are involved in regulating “late” genes, those expressed following DNA replication. Like the N protein, the *Q*

Bacteriophage Structure



Bacteriophages, or “phages,” are viruses that attach themselves to bacteria and inject their genetic material into the cell. Sometimes, during the assembly of new viral particles, a piece of the host cell’s DNA may be enclosed in the viral capsid. When the virus leaves the host cell and infects a second cell, that piece of bacterial DNA enters the second cell, thus changing its genetic makeup. (Electronic Illustrators Group)

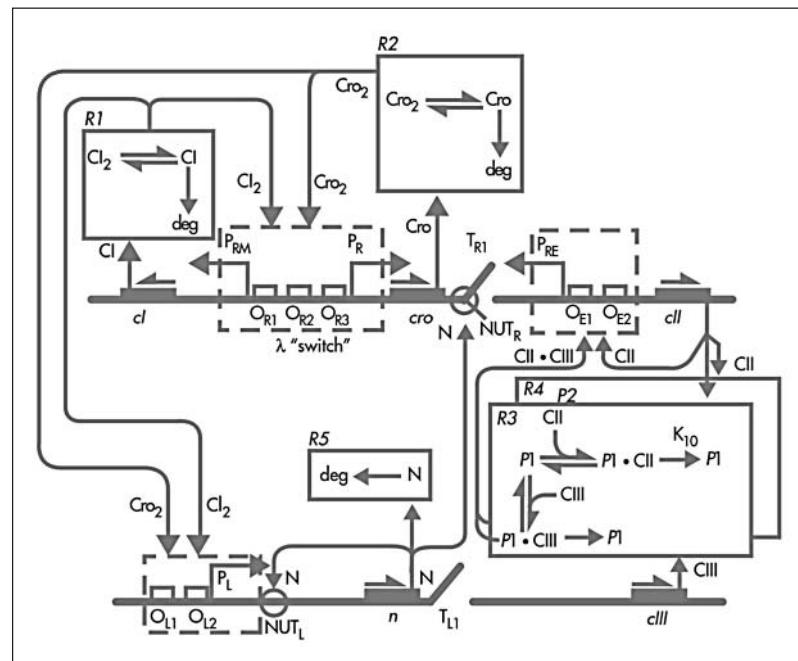
protein is an antiterminator. Late gene products include those that become the structural proteins of the viral capsid. Other late proteins cause cell lysis, releasing progeny phage particles from the cell. The entire process is completed in approximately thirty minutes.

Lambda: The Lysogenic Cycle

Lambda is among those bacterial viruses that can also carry out lysogeny, a nonlytic infection in which the virus integrates within the host chromosome. Lysogeny is dependent on the interaction between two gene products: the repressor, a product of the *cI* gene, and the cro protein.

The cII protein, an early gene product, activates the expression of *cI*, the gene that encodes the repressor. At this point in the cycle, it becomes a “race” (literally) between the activity of the repressor and the cro protein. Each has affinity for the operator regions (O_L and O_R) which control access to the respective promoters, P_L and P_R . If the repressor binds the operator regions before the cro protein, access to these sites by RNA polymerase is blocked, and the virus enters a lysogenic state. If the cro product binds first, repressor action is blocked, and the virus continues in a lytic cycle.

Lambda can remain in lysogeny for an indefinite length of time. Because it is integrated with the host’s genome, every time the host reproduces, lambda is also reproduced. Lambda typically remains in the lysogenic phase, unless its host gets into difficulty. For example, if the host is “heat shocked,” it produces heat shock proteins that inadvertently destroy the lambda repressor protein. Without the repressor protein to block expression of the early genes, lambda enters the lytic phase. This switch to the



The pathway kinetics model of gene regulation in the bacterial virus lambda shows the “decision circuit” that determines the phage’s life cycle: either lytic, in which the virus replicates and destroys its host cell, or lysogenic, in which the viral DNA is incorporated into the host cell’s genome and lies dormant. The model, adapted from A. Arkin et al. (Genetics 149, 1633-48, 1998), was generated using a supercomputer and is consistent with experimental observations. (U.S. Department of Energy Genomes to Life Program, <http://doegenomestolife.org>)

lytic phase allows lambda to reproduce and leave its host before it is potentially destroyed with the host.

Regulation in Other Viral Systems

While lambda is unusual among the complex bacteriophages in carrying out both lytic and lysogenic cycles, regulation among other viruses, including those which infect animals, has certain features in common. Most viruses exhibit a form of temporal control. Regulation in T_{even} bacteriophage infection (T2, T4, or T6) is accomplished by altering the specificity of the RNA polymerase β, resulting in the recognition of alternate promoters at different times after infection. Bacteriophage T7 accomplishes the same task by encoding an entirely new polymerase among its own genes.

The complexity of animal viruses varies significantly; the greater the coding capacity, the more variability in regulation. Some animal vi-

ruses, such as the influenza viruses, encode different proteins on unique segments of genetic material, in this case RNA. DNA viruses such as the human herpesviruses (HHV) or poxviruses utilize the same form of temporal control as described above. In place of antiterminators, products of each time frame regulate subsequent gene expression. In some cases, unique polymerase enzymes encoded by the virus carry out transcription of these genes.

Despite their apparent complexity, viruses make useful models in understanding gene expression in general. Control elements resembling operators and promoters are universal among living cells. In addition, an understanding of regulation unique to certain classes of viruses, such as expression of new enzymes, provides a potential target for novel treatments.

—Richard Adler

See also: Bacterial Genetics and Cell Structure; Gene Regulation: Bacteria; Gene Regulation: Eukaryotes; Gene Regulation: *Lac Operon*; Genomic Libraries; Viral Genetics; Viroids and Virusoids.

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Gene Therapy

Fields of study: Genetic engineering and biotechnology; Human genetics and social issues

Significance: *Gene therapy is the result of a compilation of recombinant DNA technologies, which allows the replacement or supplementation of defective or undesirable genes within a person's genome with functional copies of that gene and/or complementary genes. The primary goal of gene therapy is to reverse the effects of a genetic disease.*

Key terms

EXPRESSION CASSETTE: a synthetic genetic construct that contains the target gene and other DNA elements, which allow the gene to be moved about easily and properly expressed in cells

ONCORETROVIRUS: an RNA-containing viruses that may cause cancerous mutations

VECTOR: a tool for packaging and transferring a gene into a cell

A Brief Background

Gene therapy can be defined quite simply as the use of recombinant DNA technologies to effect a treatment or cure for an inherited (genetic) disease. The term "gene therapy" evokes mixed emotions in scientists and the population at large. In the 1990's, the first positive results using gene therapy to cure genetic diseases in humans began to appear in the medical literature. The topic of gene therapy is alive with scientific, legal, and ethical controversy. By any measure, gene therapy is a very active area of research with tremendous potential to help human beings control previously incurable diseases. However, before the full potential of gene therapy is realized, new scientific technologies will need to be developed, legal and ethical considerations will need to be addressed, and potential risks, many of which are still unforeseen, will need to be minimized to achieve an acceptable risk-benefit ratio.

In many ways, gene therapy is a logical extension of the human desire to improve our surroundings by manipulating evolution, which is a genetically controlled process. People first

started altering the process of natural selection many thousands of years ago, when farmers began selectively breeding certain forms of plants and animals found desirable in a process called artificial selection. Artificial selection has been refined over many thousands of years of successful use. In the twentieth century, with the discovery of DNA as the molecule of inheritance and rapid evolution of laboratory methods to isolate and manipulate DNA, it became possible to change the genetic composition of living organisms. The lengthy processes of traditional breeding could theoretically be bypassed, and a major barrier of traditional breeding, generally limited to breeding only within members of the same phylogenetic “family,” broke down.

In the broadest sense, gene therapy offers the potential of replacing defective genes within the human genome, be it one or many genes, with new genetic “patches” that can counteract the effect of the defective genes. Additionally, new, beneficial genes that impart desirable characteristics—such as enhanced life span, cancer resistance, or resistance to other diseases—can theoretically be inserted into the human genome even in the absence of defective genes. Finally, what makes gene therapy especially exciting, and simultaneously alarming, is the fact that genes from any living organism, including all animals, bacteria, plants, and even viruses, could potentially be used for gene therapy in humans. No evolutionary boundaries apply in gene therapy.

The Theory of Gene Therapy

The primary goal of gene therapy is to correct a genetic disease by replacing defective genes with functional or supplemental genes that will alleviate the disorder. The driving forces behind gene therapy are recombinant DNA technologies. Recombinant technologies allow the extraction, manipulation, and reinsertion of cellular DNA within and between living organisms. The development of recombinant DNA technologies began in the 1970’s, rapidly evolved through the 1980’s and the 1990’s, and is currently at a level where gene therapy, at least for some conditions, is technically feasible. There have been tremendous ad-

vancements in routinely available materials and equipment—including fast, efficient, and affordable laboratory equipment, an explosive proliferation of available biochemicals, and streamlined laboratory procedures. Many key laboratory procedures, which once were very expensive and time-consuming, are now available in reasonably priced and easy-to-use kits, which has greatly increased the speed of biomedical research. The Human Genome Project, completed in April, 2003, offers an abundance of information about the sequence and location of genes within the human genome and will be a tremendous boost to future gene therapy research.

The simplest and most logical targets for gene therapy are hereditary single-gene defects. In these cases, a single faulty gene causes a genetic disease. There are many examples of these single-gene disorders, including certain types of hemophilia, muscular dystrophy, cystic fibrosis, and an immune disorder known as severe combined immunodeficiency disorder (SCID). Theoretically, getting a “good copy” of the defective gene into people with these disorders might cure these types of diseases. In reality, however, controlling factors—such as gene insertion, gene expression, gene targeting, and immune response—pose tremendous technical challenges that researchers are currently working to overcome. Gene therapy may also target complex diseases, such as cancer, cardiovascular disease, neurological diseases, and infectious diseases. It is possible that even these complex diseases may one day be treated routinely with gene therapies.

Key Technologies

Although gene therapy and cloning may be employed together in certain scientific and medical research projects, gene therapy is very different from cloning. In the process of cloning, the entire genome of an organism is duplicated to produce a genetically identical organism. In gene therapy, only portions of a genome, usually only one or a few genes, are manipulated at a time, with the goal of correcting a specific genetic disorder. Many of the same legal and ethical questions do apply to both cloning and gene therapy, and both tech-

nologies do result in the production of a genetically modified organism (GMO).

Genetic diseases have been studied for many centuries. In many single-gene-defect diseases, the faulty gene has been identified, located, and sequenced. In many cases, the structure and function of the gene product is known in great detail. Through routine molecular biology techniques, functional copies of the gene, suitable for gene therapy, can be isolated from normal human tissues in the laboratory. This functional gene itself may be altered or put together with other genes to create an “expression cassette.” An expression cassette is a synthetic genetic construct that contains the target gene and other DNA elements, which allow the gene to be moved about easily and properly expressed in cells.

Once the functional gene is isolated and placed into an expression cassette, the gene is still not ready for use in gene therapy. Because of physical barriers within the human body and the efficiency of the immune system in defending the body from pathogens (disease causing organisms), delivery and expression of foreign gene constructs in the human body are not easy to accomplish. To deliver therapeutic genes into the body, scientists most often harness the power of viruses, since they are very adept at getting around the physical and immune defenses of the body. For safety purposes, most potentially harmful viral genes that might trigger disease or elicit a severe immune response are removed to produce what is called a “disarmed” viral vector.

Viral Vectors

Currently, several classes of virus are used to produce viral vectors for human gene therapy trials. These include oncoretroviruses, such as the Moloney murine leukemia virus (MLV), a virus that causes leukemia in mice; lentiviruses (retroviruses), such as human immunodeficiency virus (HIV), the virus that causes AIDS in humans; adenoviruses, which are extremely infectious viruses that cause cold or flulike symptoms in humans; and herpesviruses, the family of viruses that cause cold sores, genital herpes, and chickenpox in humans. All of these viruses have different applications in human

gene therapy, depending on the specific cells or tissues in the body that are being targeted. For example, herpesvirus vectors have been used for gene therapy research in cells of the nervous system, while oncoretroviruses and lentiviruses have been used for transforming cells of the circulatory system and stem cells.

In addition to being able to transfer “good” genes into the body, vectors must be genetically stable, able to be propagated in cell culture, and able to be purified to a high concentration. After the vector is built, propagated, and purified, the job is still not complete. A growing number of techniques are used to deliver the vector to the correct cells and tissues in the body. In most cases, the cells, tissues, or organs to receive the gene are specifically targeted for delivery. Targeting can be accomplished either by exposing certain cells to the vector outside the body (*ex vivo*), such as in a culture tube, or by introducing the vector in a targeted way inside the body (*in vivo*), such as introducing the vector into an organ through a specific blood vessel. Both *in vitro* and *in vivo* targeted delivery methods have been used for human gene therapy trials. Targeted delivery appears to be a critical aspect of human gene therapy, in order to increase efficacy and reduce potential risks.

The use of potentially dangerous viruses to transfer genes into the human body is one of the major concerns that surround gene therapy. Even with proper precautions in the design and building of the vector, research and human trials are conducted according to strict biohazard containment procedures in an attempt to prevent the unintentional spread of the gene therapy vector to laboratory and medical personnel.

Clinical Trials

In 1990, the first clinical trial of human gene therapy was conducted in children who were afflicted with severe combined immunodeficiency disorder (SCID). A single defective gene for an enzyme, adenosine deaminase (ADA), had been linked with this fatal disorder, which prevents the immune system from maturing and functioning properly, and so it appeared to be an attractive target for human gene therapy. It was thought that introducing a functional

copy of the ADA gene into some cells of the immune system would result in a cure for this genetic disease. Since the ADA gene had already been cloned in 1984 and an early gene therapy vector, derived from a mouse retrovirus, was available, the stage was set for the first test of human gene therapy. Hematopoietic stem cells (very young blood cells) were isolated from young patients with SCID. In laboratory flasks, the stem cells from each patient were exposed to the viral vector containing the good copy of the ADA gene. The goal was to cause genetic transformation of some of the stem cells. The stem cells were then transferred back into the young patients. The good news was that the functional ADA gene

did appear in some blood cells of the participants. The bad news was that this genetic modification did not correct the young patients' SCID. As it turned out, ADA production alone was not enough to reverse the SCID disease condition. Overall, this early and heroic attempt at gene therapy—despite the fact that it was not successful in curing the targeted disorder—resulted in useful data and led to tremendous advances in future attempts and eventually to success almost a decade later.

In September, 1999, the first human death attributable to a human gene therapy clinical trial was reported. An eighteen-year-old participant in a human gene therapy trial for hereditary ornithine transcarbamylase (OTC) deficiency died of multiorgan failure caused by a severe immunological reaction to the disarmed adenovirus vector used in the trial. It appears that this patient's immune system may have been sensitized by a previous infection with a wild-type adenovirus and, when exposed to the adenovirus vector, even though it was a disarmed vector, his immune system overreacted, resulting in severe complications and eventually death. This tragic death not only un-

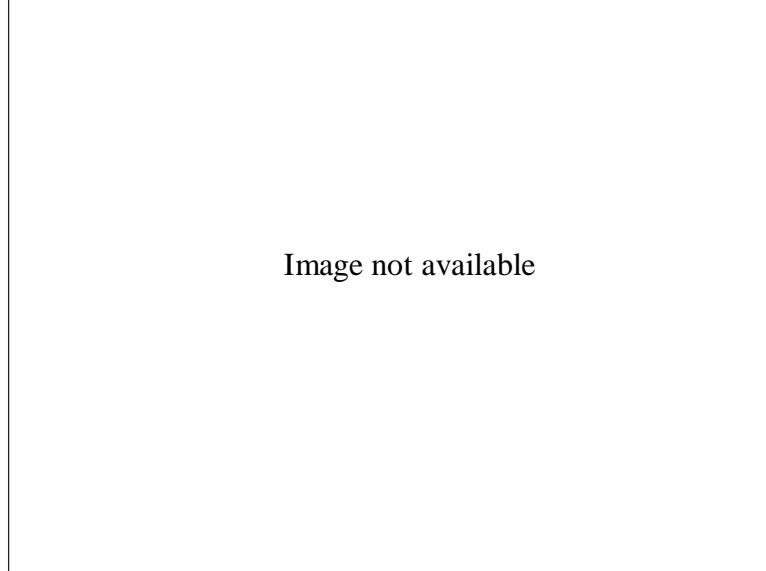


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Patient Donovan Decker, who has suffered from muscular dystrophy much of his life, prepares to be injected by Dr. Jerry Mendell with genes to help correct the condition. Donovan was the first person to receive gene therapy for muscular dystrophy, in September, 1999. (AP/Wide World Photos)

derscored the unforeseen risks associated with human gene therapy trials but also alerted researchers to the need to assess the immune status of gene therapy candidates, especially regarding prior exposure to pathogenic viruses.

In April, 2000, the first successful report of human gene therapy to correct a human genetic disease was published in the journal *Science*. The report contained the details surrounding children afflicted with lethal X-linked severe combined immunodeficiency syndrome (SCID-X). These children were successfully treated at the Necker Hospital for Sick Children in Paris using gene therapy. Eventually, nine of the eleven patients included in this clinical study were cured of their genetic disorder. The techniques used were evolved from the earlier, unsuccessful SCID clinical trials using the ADA gene. In this case, the gene that was introduced into the hematopoietic stem cells was a cytokine receptor gene rather than the ADA gene, and the results were greatly improved.

However, as might be expected in pioneering medical research, unforeseen adverse events soon marred what had been celebrated

as an unqualified success. In September, 2002, a three-year-old participant in the SCID-X human gene therapy trials began exhibiting a leukemia-type lymphoproliferative disorder (an inappropriate proliferation of white blood cells). Subsequently another child from the same SCID-X trial showed signs of the same disorder. It was determined that this disorder was most probably due to the nature of the retrovirus vector used. The vector had apparently inserted the therapeutic gene construct into the genome of at least one of the stem cells at a place where it inadvertently activated an oncogene (a cancer-causing gene). These cells with an activated oncogene went on to multiply in the children and cause their leukemia-type disease. Subsequently, another child in the SCID-X trial tested positive for the same oncogene activation, although he did not exhibit the leukemia-type disorder. The combination of successes and unforeseen adverse events underscored the fact that human gene therapy is currently operating in uncharted areas and undoubtedly involves very complex biological factors, some of which are probably still unknown.

Future Prospects: Benefits and Risks

Future prospects are really limited only by imagination and the constraints of currently available technology. The Human Genome Project, which maps all the gene sequences as well as their location in the human genome, will revolutionize the development of human gene therapy. Using these data, scientists will discover targets for gene therapy that can be examined in their native context within the human genome, which should result in the production of much better expression cassettes and allow targeted insertion of therapeutic genes, which in turn should greatly decrease undesirable side effects such as those seen in the SCID-X trial. Gene therapy may within a few decades provide physicians with tools to treat or prevent all sorts of genetic diseases, both simple and complex.

The same technologies developed to correct defective genes may also give scientists the power to insert "desirable genes," possibly from other types of living organisms, to increase life span, impart cancer resistance, provide protec-

tion from environmental toxins, and function as permanent vaccines against infectious disease.

This notion of desirable genes raises the prospect of creating "designer humans"—humans with beneficial or targeted genetic traits, even aesthetic genetic modifications—and all the attendant legal, political, and ethical ramifications.

—Robert A. Sinnott

See also: Bioethics; Bioinformatics; Cloning Vectors; Cystic Fibrosis; DNA Structure and Function; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering: Historical Development; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Human Genetics; Human Genome Project; Inborn Errors of Metabolism; Insurance; Knockout Genetics and Knockout Mice; RNA World; Stem Cells; Transgenic Organisms; Tumor-Suppressor Genes.

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Web Sites of Interest

American Cancer Society, Gene Therapy: Questions and Answers. <http://www.cancer.org>. Site has searchable information on gene therapy. Topics covered include "What Is Gene Therapy?" and "How Does Gene Therapy Work?"

Genethon. Gene Therapies Research and Applications Center. http://www.genethon.fr/php/index_us.php. Supported by the French Muscular Dystrophy Association, Genethon sponsors research in genetic and cellular therapies for rare diseases. This site offers a section accompanied by computer graphics on the theory of gene therapy.

Gene Therapy: Ethical and Economic Issues

Fields of study: Bioethics; Genetic engineering and biotechnology; Human genetics and social issues

Significance: *Gene therapy has the potential to cure many diseases once viewed as untreatable, such as cystic fibrosis. At the same time, gene therapy presents ethical dilemmas ranging from who decides who will benefit from new therapies to questions of ethics and social policy, such as whether humans should attempt to manipulate natural evolutionary processes. Although there are strong economic incentives for developing new therapies, ethical concerns must be addressed.*

Key terms

GERM CELLS: reproductive cells such as eggs and sperm

GERM-LINE THERAPY: alteration of germ cells resulting in a permanent genetic change in the organism and succeeding generations

INSULIN: a pancreatic hormone that is essential to metabolize carbohydrates, used in the control of diabetes mellitus

RECOMBINANT DNA: genetically engineered DNA prepared by cutting up DNA molecules and splicing together specific DNA fragments, often from more than one species of organism

SOMATIC CELL THERAPY: treatment of specific tissue with therapeutic genes

Gene Therapy

Advances in molecular biology and genetics near the end of the last century have presented tantalizing possibilities for new treatment for medical conditions once viewed as incurable. Gene therapy for the treatment of human genetic diseases can take two forms: somatic cell therapy and germ-line therapy. Somatic cell therapy is less controversial, because it only modifies nonreproductive cells, and therefore the changes cannot be passed on to a person's children. Still, caution is needed, as with any new technology, to be sure the benefits outweigh the risks. Germ-line therapy is more permanent in that the changes include modification of reproductive cells, and thus the changes can be passed on to a person's children. This has led to much greater controversy, because all the same cautions apply to this approach as to somatic cell therapy, with the added problem that any defects introduced by the technology could become permanent features of the human population. Because of this, germ-line gene therapy is currently banned in the United States and in much of the rest of the world.

Somatic Cell Therapy

Somatic cell therapy could provide some clear benefits. For example, it could potentially free insulin-dependent diabetics from reliance on external sources of insulin by restoring the ability of the patient's own body to manufacture it. Scientists have already succeeded in genetically engineering bacteria to grow recombinant insulin, eliminating the need to harvest it from animal pancreatic tissue obtained from slaughterhouses. The next step would seem to be the use of somatic cell therapy to treat individual diabetics.

The primary ethical concerns about treating a disease like diabetes using somatic cell therapy primarily relate to cost and technological

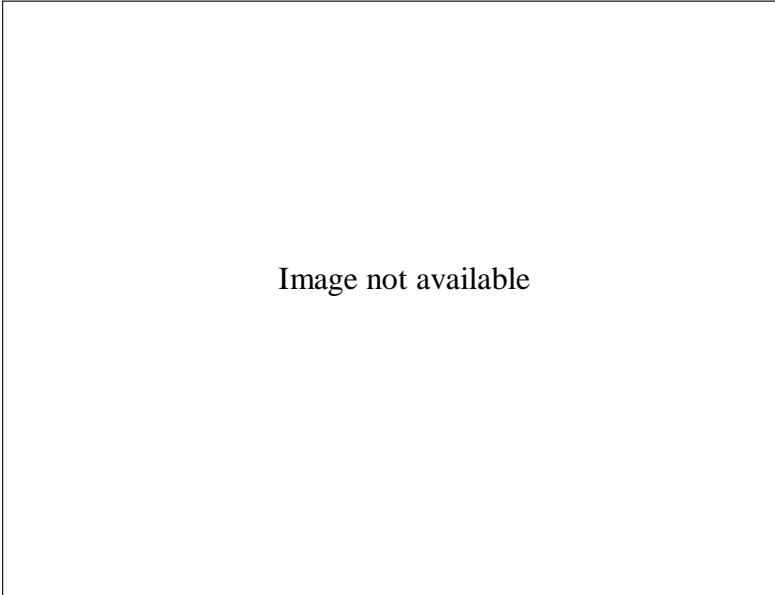


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Three-year-old Wilco Conradi at the zoo in Amsterdam on August 18, 2002. After living isolated in a plastic-enclosed space for most of his life, he received gene therapy for the fatal "bubble boy" syndrome, severe combined immunodeficiency disorder (SCID). The results of this treatment appeared promising until it was noted that several children so treated were developing a leukemia-type disorder, likely caused when an oncogene was activated by the vector used to insert the therapeutic gene. Although for children afflicted with SCID, the alternative to no therapy was much worse, such mixed results nevertheless raise ethical concerns. (AP/Wide World Photos)

proficiency. Currently, the potential costs of gene therapy put it out of reach for most people. Is it ethical to develop a technological solution to a problem that will be available to only a few? Of course, this same concern could be directed at many other expensive medical procedures, such as organ transplants, which are often out of reach for most people yet are now well entrenched in medical practice.

A more serious ethical concern, at present, is whether the technology is safe enough to use on humans. Clinical trials of some somatic cell therapies have been halted due to unforeseen complications, including deaths and the development of cancer in some cases. These events have led some ethicists to question whether gene therapy trials should be considered at all. Is it fair to expect individuals who are managing their diabetes with conventional methods to accept the unknown risks inherent in such a complex and poorly understood technology? Do we know so little at this point that we cannot

even adequately assess potential risks? Caution has been advised by many, in light of these concerns.

Assuming that the technological hurdles can be overcome, somatic cell therapy to cure diabetes mellitus appears to offer a fairly clear-cut case for treatment. What about less threatening conditions, such as the insufficient production of growth hormone? A shortage of human growth hormone can result in dwarfism. The use of somatic cell therapy to correct the condition clearly would be beneficial, but growth hormone deficiencies vary, and even otherwise normal children can be shorter than average. In a society in which height is associated with success, wealthy parents have been known to pressure doctors to prescribe human growth hormone to their

children who are only slightly smaller than average and not truly suffering from a pituitary gland disorder. If somatic cell gene therapy became widely available for human growth, how many parents would succumb to the temptation to give their children a boost in height? The same potential for abuse is present for any number of perceived defects that might be cured by gene therapy, with only those who are rich being able to afford the technology. When the defect is not life threatening, or even particularly debilitating, do parents have the right to decide that their children receive these treatments?

Germ-Line Therapy

Germ-line gene therapy faces all the same ethical objections as somatic cell therapy, and introduces what some consider more serious ethical concerns. Germ-line therapy changes the characteristics an organism passes on to its offspring. Humans suffer from a variety of in-

herited diseases, including hemophilia, Huntington's disease (Huntington's chorea), and cystic fibrosis, and physicians have long recognized that certain conditions, such as coronary artery disease and diabetes, seem to run in some families. It is tempting to consider the possibility of eliminating these medical conditions through germ-line therapies: Not only would the person suffering from the disease be cured, but his or her descendants would never have to worry about passing the condition on

to their offspring. Eventually, at least in theory, the genes that cause the disease could be eliminated from the general population.

Tempting though it is to see this as a good thing, ethicists believe that such an approach could be extremely susceptible to abuse. They view discussions of human germ-line therapy as an attempt to resurrect the failed agenda of the eugenics movement of the 1920's and 1930's. If scientists are allowed to manipulate human heredity to eliminate certain characteristics, what

FDA Limits Gene Therapy Trials

Because of an adverse reaction—a leukemia-like disorder reported in two patients who had undergone successful SCID-X gene therapy—human gene therapy trials are proceeding with caution. The available data suggest that the retrovirus vector used for the SCID-X gene therapy trials, derived from a cancer-causing mouse virus, may be largely responsible. Retrovirus vectors have the ability to insert genes permanently into the human genome, which is desirable to obtain long-term results in gene therapy. A problem occurs, however, if a retrovirus inserts the therapeutic gene near, or in, certain genes called oncogenes or tumor-suppressor genes: Cancerous mutations can develop in the transformed cells. When cells with cancerous mutations replicate over time, cancers can develop. That appears to have happened in at least two of the patients who participated in the SCID-X trials. In response, an advisory committee that monitors data from gene-therapy trials for the U.S. Food and Drug Administration (FDA) recommended that gene therapy for SCID-X be moved to a second-line treatment, meaning that it should be used only in the absence of other medical treatment options, such as a bone marrow transplant from a matched donor.

These results from the SCID-X trials reinvigorated the ethical and legal questions surrounding gene therapy. Moral questions originally arose when scientists became able to alter the human genome and were complicated with the rise of research into embryonic stem cells (cells obtained from human embryos). Embryonic stem cells are an attractive target for researchers in the area of gene therapy because these very young, undifferentiated cells are the progenitors of all the other cells in the human body. Performing gene therapy on embryonic stem

cells and then manipulating these cells to develop into specific tissues or organs would allow the quintessential degree of targeted gene therapy. While there is currently no comprehensive ban on the use of embryonic stem cells in gene therapy research, only certain exempted cell lines can be used in federally funded research projects.

The economics of gene therapy may also affect its actual impact on human health care. The technologies involved in gene therapy are currently very expensive and probably will remain so for the foreseeable future. Most gene therapy trials are considered experimental procedures and are therefore not covered by health insurance. These and other real economic conditions, particularly in countries with no national health care policy, may make gene therapy affordable to some and not to others. In this way, gene therapy may increase the disparity in health care services available to people of different socio-economic groups.

Finally, since the terrorist attacks on the United States of September 11, 2001, any discussion of gene therapy must include the possibility that some of the technologies developed to correct genetic diseases could also be used by people with no moral or legal restraints to cause tremendous human suffering. By using infectious viral vectors developed for gene therapy and incorporating expression cassettes containing harmful or lethal genes, terrorists and others could develop biological weapons with relative ease compared to "traditional" threats such as nuclear weapons. The deliberate spread of these malicious constructs, especially in densely populated areas, could have catastrophic results.

—Robert A. Sinnott

is to prevent those same scientists from manipulating the human genome to enhance other characteristics? Would parents be able to request custom-tailored offspring, children who would be tall with predetermined hair color and eyes? Questions concerning class divisions and racial biases have also been raised. Would therapies be equally available to all people who requested them, or would such technology lead to a future in which the wealthy custom-tailor their offspring while the poor must rely on conventional biology? Would those poor people whose parents had been unable to afford germ-line therapy then find themselves denied access to medical care or employment based on their "inferior" or "unhealthy" genetic profiles?

In addition, many ethicists and scientists raise cautionary notes about putting too much faith in new genetic engineering technologies too soon. Most scientists concede that not enough is known about the interdependency of various genes and the roles they play in overall health and human evolution to begin a program to eliminate so-called bad genes. Genes that in one combination may result in a disabling or life-threatening illness may in another have beneficial effects that are not yet known. Germ-line therapy could eliminate one problem while opening the door to a new and possibly worse condition. Thus, while the economic benefits of genetic engineering and gene therapies can be quite tempting, ethicists remind us that many questions remain unanswered. Some areas of genetic research, particularly germ-line therapy, may simply be best left unexplored until a clearer understanding of both the potential social and biological cost emerges.

—Nancy Farm Männikkö, updated by Bryan Ness

See also: Bioethics; Bioinformatics; Cloning Vectors; Cystic Fibrosis; DNA Structure and Function; Gene Therapy; Genetic Counseling; Genetic Engineering: Historical Development; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Human Genetics; Human Genome Project; Inborn Errors of

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Web Sites of Interest

American Medical Association. <http://www.ama-assn.org/ama/pub/printcat/2827.html>. The AMA's page on gene therapy, with links to news stories.

Genethon. Gene Therapies Research and Applications Center. http://www.genethon.fr/php/index_us.php. Supported by the French Muscular Dystrophy Association, Genethon sponsors research in genetic and cellular therapies for rare diseases. This site offers a section accompanied by computer graphics on the theory of gene therapy.

National Information Resource on Ethics and Human Genetics. <http://www.georgetown.edu/research/nrcbl/nirehg>. Site supports links to databases, annotated bibliographies, and articles about the ethics of gene therapy and human genetics in general.

Genetic Code

Field of study: Molecular genetics

Significance: *The molecules of life are made directly or indirectly from instructions contained in DNA. The instructions are interpreted according to the genetic code, which describes the relationship used in the synthesis of proteins from nucleic acid information.*

Key terms

CODON: a three-nucleotide unit of nucleic acids (DNA and RNA) that determines the amino acid sequence of the protein encoded by a gene

NUCLEOTIDES: long nucleic acid molecules that form DNA and RNA, linked end to end; the sequences of these nucleotides in the DNA chain provides the genetic information

READING FRAME: the phasing of reading codons, determined by which base the first codon begins with; certain mutations can also change the reading frame

RNA: ribonucleic acid, a molecule similar to DNA but single-stranded and with a ribose rather than a deoxyribose sugar; RNA molecules are formed using DNA as a template and then use their complementary genetic information to conduct cellular processes or form proteins

TRANSFER RNA (tRNA): molecules that carry amino acids to messenger RNA (mRNA) codons, allowing amino acid polymerization into proteins

TRANSLATION: the process of forming proteins according to instructions contained in an mRNA molecule

Elements of the Genetic Code

Every time a cell divides, each daughter cell receives a full set of instructions that allows it to grow and divide. The instructions are contained within DNA. These long nucleic acid molecules are made of nucleotides linked end to end. Four kinds of nucleotides are commonly found in the DNA of all organisms. These are designated A, G, T, and C for the variable component of the nucleotide (adenine, guanine, thymine, and cytosine, respectively).

The Genetic Code					
second position →	T	C	A	G	third position ↓
first position ↓					
T	Phenylalanine Phenylalanine Leucine Leucine	Serine Serine Serine Serine	Tyrosine Tyrosine END CHAIN END CHAIN	Cysteine Cysteine END CHAIN Tryptophan	T C A G
	Leucine Leucine Leucine Leucine	Proline Proline Proline Proline	Histidine Histidine Glutamine Glutamine	Arginine Arginine Arginine Arginine	T C A G
A	Isoleucine Isoleucine Isoleucine Methionine	Threonine Threonine Threonine Threonine	Asparagine Asparagine Lysine Lysine	Serine Serine Arginine Arginine	T C A G
	Valine Valine Valine Valine	Alanine Alanine Alanine Alanine	Aspartic Acid Aspartic Acid Glutamic Acid Glutamic Acid	Glycine Glycine Glycine Glycine	T C A G

The amino acid specified by any codon can be found by looking for the wide row designated by the first base letter of the codon shown on the left, then the column designated by the second base letter along the top, and finally the narrow row marked on the right, in the appropriate wide row, by the third letter of the codon. Many amino acids are represented by more than one codon. The codons TAA, TAG, and TGA do not specify an amino acid but instead signal where a protein chain ends.

The sequence of the nucleotides in the DNA chain provides the information necessary for manufacturing all the proteins required for survival, but information must be decoded.

DNA contains a variety of codes. For example, there are codes for identifying where to start and where to stop transcribing an RNA molecule. RNA molecules are nearly identical in structure to the single strands of DNA molecules. In RNA, the nucleotide uracil (U) is used in place of T and each nucleotide of RNA contains a ribose sugar rather than a deoxyribose sugar. RNA molecules are made using DNA as a template by a process called transcription. The resulting RNA molecule contains the same information as the DNA from which it was made, but in a complementary form. Some RNAs function directly in the structure and activity of cells, but most are used to produce proteins with the help of ribosomes. This latter type is known as messenger RNA (mRNA). The ribo-

some machinery scans the RNA nucleotide sequence to find signals to start the synthesis of polypeptides, the molecules of which proteins are made. When the start signals are found, the machinery reads the code in the RNA to convert it into a sequence of amino acids in the polypeptide, a process called translation. Translation stops at termination signals. The term “genetic code” is sometimes reserved for the rules for converting a sequence of nucleotides into a sequence of amino acids.

The Protein Genetic Code: General Characteristics

Experiments in the laboratories of Har Gobind Khorana, Heinrich Matthaei, Marshall Nirenberg, and others led to the deciphering of the protein genetic code. They knew that the code was more complicated than a simple one-to-one correspondence between nucleotides and amino acids, since there were about twenty

different amino acids in proteins and only four nucleotides in RNA. They found that three adjacent nucleotides code for each amino acid. Since each of the three nucleotide positions can be occupied by any one of four different nucleotides, sixty-four different sets are possible. Each set of three nucleotides is called a codon. Each codon leads to the insertion of one kind of amino acid in the growing polypeptide chain.

Two of the twenty amino acids (tryptophan and methionine) have only a single codon. Nine amino acids are each represented by a pair of codons, differing only at the third position. Because of this difference, the third position in the codons for these amino acids is often called the wobble position. For six amino acids, any one of the four nucleotides occupies the wobble position. The three codons for isoleucine can be considered as belonging to this class, with the exception that AUG is reserved for methionine. Three amino acids (leucine,

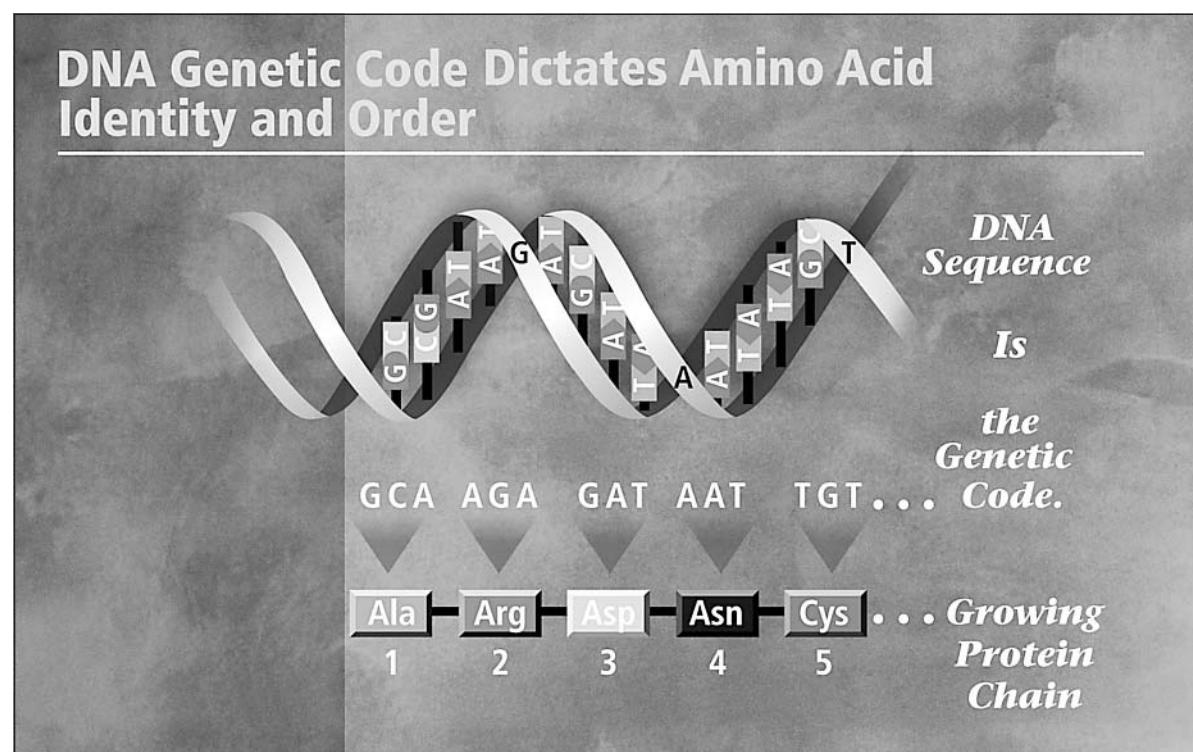
arginine, and serine) are unusual in that each can be specified by any one of six codons.

Punctuation

The protein genetic code is often said to be “commaless.” The bond connecting two codons cannot be distinguished from bonds connecting nucleotides within codons. There are no spaces or commas to identify which three nucleotides constitute a codon. As a result, the choice of which three nucleotides are to be read as the first codon during translation is very important. For example, if “EMA” is chosen as the first set of meaningful letters in the following string of letters, the result is gibberish:

TH EMA NHI TTH EBA TAN DTH EBA TBI
THI M.

On the other hand, if “THE” is chosen as the first set of three letters, the message becomes clear:



This figure from the Genome Image Gallery of the Department of Energy makes clear the concept of the reading frame and how the genetic code translates into amino acids and hence proteins. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

THE MAN HIT THE BAT AND THE BAT BIT HIM.

The commaless nature of the code means that one sequence of nucleotides can be read three different ways, starting at the first, second, or third letter. Still, the genetic code does have “punctuation.” The beginning of each coding sequence has a start codon, which is always the AUG. Each coding sequence also has a stop codon, which acts like a period at the end of a sentence, denoting the end of the coding sequence.

These ways of reading are called reading frames. A frame is said to be open if there are no stop codons for a reasonable distance. In most mRNAs, only one reading frame is open for any appreciable length. However, in some mRNAs, more than one reading frame is open. Some mRNAs can produce two, rarely three, different polypeptide sequences.

The Near Universality of the Code

The universal genetic code was discovered primarily through experiments with extracts from the bacterium *Escherichia coli* and from rabbit cells. Further work suggested that the code was the same in other organisms. It came to be known as the universal genetic code. The code was deciphered before scientists knew how to determine the sequence of nucleotides in DNA efficiently. After nucleotide sequences began to be determined, scientists could, using the universal genetic code, predict the sequence of amino acids. Comparison with the actual amino acid sequence revealed excellent overall agreement.

Nevertheless, the universal genetic code assignments of codons to amino acids had apparent exceptions. Some turned out to be caused by programmed changes in the mRNA information. In selected codons of some mRNA, a C is changed to a U. In others, an A is changed so that it acts like a G. Editing of mRNA does not change the code used by the ribosomal machinery, but it does mean that the use of DNA sequences to predict protein sequences has pitfalls.

Some exceptions to the universal genetic code are true variations in the code. For exam-

ple, the UGA universal stop codon codes for tryptophan in some bacteria and in fungal, insect, and vertebrate mitochondrial DNA (mtDNA). Ciliated protozoans use UAA and UAG, reserved as stop codons in all other organisms, for the insertion of glutamine residues. Methionine, which has only one codon in the universal genetic code (AUG), is also encoded by AUA in vertebrate and insect mtDNA and in some, but not all, fungal mitochondria. Vertebrate mtDNA also uses the universal arginine codons AGA and AGG as stop codons. AGA and AGG are serine rather than arginine codons in insect mtDNA.

Interpreting the Code

How is the code interpreted? The mRNA codons organize small RNA molecules called transfer RNA (tRNA). There is at least one tRNA for each of the twenty amino acids. They are *L*-shaped molecules. At one end tRNAs have a set of three nucleotides (the anticodon) that can pair with the three nucleotides of the mRNA codon. They do not pair with codons for other amino acids. At the other end tRNAs have a site for the attachment of an amino acid.

Special enzymes called aminoacyl tRNA synthetases (RS enzymes) attach the correct amino acids to the correct tRNAs. There is one RS enzyme for each of the twenty amino acids. Interpretation is possible because each RS enzyme can bind only one kind of amino acid and only to tRNA that pairs with the codons for that amino acid. The key to this specificity is a special code in each tRNA located near where the amino acid gets attached. This code is sometimes referred to as the “second genetic code.” After binding the correct amino acid and tRNA, the RS enzyme attaches the two molecules with a covalent bond. These charged tRNAs, called aminoacyl-tRNAs, are ready to participate in protein synthesis directed by the codons of the mRNA. Information is stored in RNA in forms other than the triplet code. A special tRNA for methionine exists to initiate all peptide chains. It responds to AUG. However, proteins also have methionines in the main part of the polypeptide chain. Those methionines are carried by a different tRNA that also responds to AUG.

The “Second” Genetic Code

The fidelity of translating codons of messenger RNA (mRNA) into amino acids of the protein product requires that each transfer RNA (tRNA) be attached to the proper amino acid. Twenty distinct aminoacyl tRNA synthetases (RS enzymes) are found in cells; each is specific for a particular amino acid which it attaches to an appropriate tRNA. Because some amino acids (such as isoleucine and valine) are similar in structure, some RS enzymes have an editing feature, which allows them to cleave a mistakenly attached amino acid. The site at which the attachment reaction occurs is distinct from the editing site. The end result is that fewer than one in ten thousand amino acids is attached to the wrong tRNA.

Each RS enzyme must also recognize an appropriate tRNA. One might imagine that the anticodon found in the tRNA would be the recognition site. However, only in a few cases is it the major or sole determinant. Because the anticodon is at one end of the L-shaped tRNA and the amino acid is attached at the other end, this is perhaps not surprising. While tRNA molecules have the same general shape, they typically consist of seventy-six nucleotides, which provide numerous opportunities to distinguish themselves from one another.

The “second” genetic code is sometimes used to refer to the sequence of the tRNA that ensures that the correct one is recognized by its corresponding RS enzyme. Surprisingly, different elements are used by the various RS enzymes. In some cases, elements

near the amino acid attachment site are important. This is the case for alanine tRNA, where the primary recognition is a G₃-U₇₀ base pair. Incorporating this element into a cysteine tRNA will cause it to accept alanine despite the fact that the anticodon remains that for cysteine. In other cases, structures in the middle of the tRNA molecule are important, such as the variable loop or the D-loop. Usually multiple elements contribute to the recognition and ensure that the correct tRNA is recognized by its respective RS enzyme.

A mutation in the anticodon of a tRNA will usually not restrict its being attached to its designated amino acid. Such a mutation is referred to as a suppressor mutation if it overrides another mutation that leads to a chain termination mutation. For example, a point mutation in the CAG glutamine codon in a gene can convert it to a UAG chain termination codon. This would usually be deleterious because the resultant protein would be shorter than normal. However, if the normal GUA anticodon on tyrosine tRNA is mutated to CUA, it would pair with the UAG in the messenger RNA (mRNA) during protein synthesis; it would suppress the chain termination mutation by inserting tyrosine for the original glutamine in the protein, which may retain its function. This mutated tRNA would, however, insert a tyrosine for the normal UAG chain termination for other genes.

—James L. Robinson

The ribosome and associated factors must distinguish an initiating AUG from one for an internal methionine.

Distinction occurs differently in eukaryotes and bacteria. In bacteria, AUG serves as a start codon only if it is near a sequence that can pair with a section of the RNA in the ribosome. Two things are required of eukaryotic start (AUG) codons: First, they must be in a proper context of surrounding nucleotides; second, they must be the first AUG from the mRNA beginning that is in such a context. Context is also important for the incorporation of the unusual amino acid selenocysteine into several proteins. In a limited number of genes, a special UGA stop codon is used as a codon for selenocysteine. Se-

quences additional to UGA are needed for selenocysteine incorporation. Surrounding nucleotide residues also allow certain termination codons to be bypassed. For example, the mRNA from tobacco mosaic virus encodes two polypeptides, both starting at the same place; however, one is longer than the other. The extension is caused by the reading of a UAG stop codon by tRNA charged with tyrosine.

The production of two proteins with identical beginnings but different ends can also occur by frame shifting. In this mechanism, signals in the mRNA direct the ribosome machinery to advance or backtrack one nucleotide in its reading of the mRNA codons. Frame shifting occurs at a specific sequence in the RNA. Often the

code for a frame shift includes a string of seven or more identical nucleotides and a complex RNA structure (a “pseudoknot”).

Further codes are embedded in DNA. The linear sequence of amino acids, derived from DNA, has a code for folding in three-dimensional space, a code for its delivery to the proper location, a code for its modification by the addition of other chemical groups, and a code for its degradation. The production of mRNA requires nucleotide codes for beginning RNA synthesis, for stopping its synthesis, and for stitching together codon-containing regions (exons) should these be separated by noncoding regions (introns). RNA also contains signals that can tag them for rapid degradation. DNA has a code recognized by protein complexes for the initiation of DNA replication and signals recognized by enzymes that catalyze DNA rearrangements.

Impact and Applications

A major consequence of the near universality of the genetic code is that biotechnologists can move genes from one species into another and they are still expressed correctly. Since the code is the same in both organisms, the same protein is produced. This has resulted in the large-scale production of specific proteins in bacteria, yeast, plants, and domestic animals. These proteins are of immense pharmaceutical, industrial, and research value.

Scientists developed rapid methods for sequencing nucleotides in DNA in the 1970's. Since the genetic code was known, it suddenly became easier to predict the amino acid sequence of a protein from the nucleotide sequence of its gene than it did to determine the amino acid sequence of the protein by chemical methods. The instant knowledge of the amino acid sequence of a particular protein greatly simplified predictions regarding protein function. This has resulted in the molecular understanding of many inherited human diseases and the potential development of rational therapies based on this new knowledge.

—Ulrich Melcher, updated by Bryan Ness

See also: Central Dogma of Molecular Biology; Chromosome Structure; Chromosome

Theory of Heredity; DNA Replication; DNA Structure and Function; Evolutionary Biology; Genetic Code, Cracking of; Genetics, Historical Development of; Mendelian Genetics; Molecular Genetics; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World.

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- Clark, David, and Lonnie Russell. *Molecular Biology: Made Simple and Fun*. 2d ed. Vienna, Ill.: Cache River Press, 2000. A detailed and accessible account of molecular biology.
- Judson, Horace Freeland. *The Eighth Day of Creation*. Rev. ed. Cold Harbor Spring, N.Y.: Cold Spring Harbor Laboratory Press, 1997. A noted and fascinating history of molecular biology that details the deciphering of the genetic code.
- Kay, Lily E. *Who Wrote the Book of Life? A History of the Genetic Code*. Stanford, Calif.: Stanford University Press, 2000. Brings myriad sources together to describe research on the genetic code between 1953 and 1970, the rise of communication technosciences, the intersection of molecular biology with cryptanalysis and linguistics, and the social history of postwar Europe and the United States.
- Trainor, Lynn E. H. *The Triplet Genetic Code: The Key to Molecular Biology*. River Edge, N.J.: World Scientific, 2001. Intended for nonspecialists and professionals, surveys the fundamentals of the genetic code and how it has come to revolutionize thinking about living systems as a whole, especially regarding the connection between structure and function.

Web Site of Interest

Oak Ridge National Laboratory, The Genetic Code. http://www.ornl.gov/techresources/human_genome/graphics/slides/images1.html. Site has link to an image of the genetic code, with discussion.

Genetic Code, Cracking of

Field of study: History of genetics;
Molecular genetics

Significance: *The deciphering of the genetic code was a significant accomplishment for molecular biologists. The identification of the “words” used in the code explained how the information carried in DNA can be interpreted, via an RNA intermediate, to direct the specific sequence of amino acids found in proteins.*

Key terms

ANTICODON: a sequence of three nucleotide bases on the transfer RNA (tRNA) that recognizes a codon

CODON: a sequence of three nucleotide bases on the messenger RNA (mRNA) that specifies a particular amino acid

The Nature of the Puzzle

Soon after DNA was discovered to be the genetic material, scientists began to examine the relationship between DNA and the proteins that are specified by the DNA. DNA is composed of four deoxyribonucleotides containing the bases adenine (A), thymine (T), guanine (G), and cytosine (C). Proteins are composed of twenty different building blocks known as amino acids. The dilemma that confronted scientists was to explain the mechanism by which the four bases in DNA could be responsible for the specific arrangement of the twenty amino acids during the synthesis of proteins.

The solution to the problem arose as a result of both theoretical considerations and laboratory evidence. Experiments done in the laboratories of Charles Yanofsky and Sydney Brenner provided evidence that the order, or sequence, of the bases in DNA was important in determining the sequence of amino acids in proteins. Francis Crick proposed that the bases formed triplet “code words.” He reasoned that if a single base specified a single amino acid, it would only be possible to have a protein made up of four amino acids. If two bases at a time specified amino acids, it would only be possible to code for sixteen amino acids. If the four bases were used three at a time, Crick proposed, it

would be possible to produce sixty-four combinations, more than enough to specify the twenty amino acids. Crick also proposed that since there would be more than twenty possible triplets, some of the amino acids might have more than one code word. The eventual assignment of multiple code words for individual amino acids was termed “degeneracy.” The triplet code words came to be known as codons.

Identifying the Molecules Involved

Since DNA is found in the nuclei of most cells, there was much speculation as to how the codons of DNA could direct the synthesis of proteins, a process that was known to take place in another cellular compartment, the cytosol. A class of molecules related to DNA known as ribonucleic acids (RNAs) were shown to be involved in this process. These molecules consist of ribonucleotides containing the bases A, C, and G (as in DNA) but uracil (U) rather than thymine (T). One type of RNA, ribosomal RNA (rRNA), was found to be contained in structures known as ribosomes, the sites where protein synthesis occurs. Messenger RNA (mRNA) was shown to be another important intermediate. It is synthesized in the nucleus from a DNA template in a process known as transcription, and it carries an imprint of the information contained in DNA. For every A found in DNA, the mRNA carries the base U. For every T in DNA, the mRNA carries an A. The Gs in DNA become Cs in mRNA, and the Cs in DNA become Gs in mRNA. The information in mRNA is found in a form that is complementary to the nucleotide sequence in DNA. The mRNA is transported to the ribosomes and takes the place of DNA in directing the synthesis of a protein.

Deciphering the Code

The actual assignment of codons to specific amino acids resulted from a series of elegant experiments that began with the work of Marshall Nirenberg and Heinrich Matthaei in 1961. They obtained a synthetic mRNA consisting of polyuridylic acid, or poly (U), made up of a string of Us. They added poly (U) to a cell-free system that contained ribosomes and all other ingredients necessary to make proteins *in vitro*. When the twenty amino acids were

added to the system, the protein that was produced contained a string of a single amino acid, phenylalanine. Since the only base in the synthetic mRNA was U, Nirenberg and Matthaei had discovered the code for phenylalanine: UUU. Because UUU in mRNA is complementary to AAA in DNA, the actual DNA bases that direct the synthesis of phenylalanine are AAA. By convention, the term “codon” is used to designate the mRNA bases that code for specific amino acids. Therefore UUU, the first code word to be discovered, was the codon for phenylalanine.

Using cell-free systems, other codons were soon discovered by employing other synthetic mRNAs. AAA was shown to code for lysine, and CCC was shown to code for proline. Scientists working in the laboratory of Severo Ochoa began to synthesize artificial mRNAs using more than one base. These artificial messengers produced proteins with various proportions of amino acids. Using this technique, it was shown that a synthetic codon with twice as many Us as Gs specified valine. It was not clear, however, if the codon was UUG, UGU, or GUU. Har Gobind Khorana and his colleagues began to synthesize artificial mRNA with predictable nucleotide sequences, and the use of this type of mRNA contributed to the assignment of additional codons to specific amino acids.

In 1964, Philip Leder and Nirenberg developed a cell-free protein-synthesizing system in which they could add triplet codons of known sequence. Using this new system, as well as Khorana’s synthetic messengers, scientists could assign GUU to valine and eventually were able to assign all but three of the possible codons to specific amino acids. These three codons, UAA, UAG, and UGA, were referred to as “nonsense” codons because they did not code for any of the twenty amino acids. The nonsense codons were later found to be a type of genetic punctuation mark; they act as stop signals to specify the end of a protein.

There is no direct interaction between the mRNA codon and the amino acid for which it codes. Yet another type of RNA molecule was found to act as a bridge or, in Crick’s terminology, an “adaptor” between the mRNA codon and the amino acid. This type of RNA is a small

Image not available

The assignment of codons to specific amino acids resulted from a series of elegant experiments that began with the work of Marshall Nirenberg (above) and Heinrich Matthaei in 1961. (Jim Willier-Stokes Imaging)

molecule known as transfer RNA (tRNA). Specific enzymes connect the amino acids to their corresponding tRNA; the tRNA then carries the amino acid to the appropriate protein assembly location specified by the codon. The tRNA molecules contain recognition triplets known as anticodons, which are complementary to the codons on the mRNA. Thus, the tRNA that carries phenylalanine and recognizes UUU contains an AAA anticodon.

By 1966, all the codons had been discovered. Since some codons had been identified as “stop” codons, scientists had begun searching for one or more possible “start” codons. Since all proteins were shown to begin with the amino acid methionine or a modified form of methionine (which is later removed), the methionine codon, AUG, was identified as the start codon for most proteins. It is interesting that AUG also codes for methionine when this amino acid occurs at other sites within the protein.

The cracking of the genetic code gave sci-

tists a valuable genetic tool. Once the amino acid sequence was known for a protein, or for even a small portion of a protein, knowledge of the genetic code allowed scientists to search for the gene that codes for the protein or, in some cases, to design and construct the gene itself. It also became possible to predict the sequence of amino acids in a protein if the sequence of nucleotide bases in a gene were known. Knowledge of the genetic code became invaluable in understanding the genetic basis of mutation and in attempts to correct these mutations by gene therapy. The discovery of the genetic code was therefore key to the development of genetics in the late twentieth century, perhaps outshined only by the discovery of DNA's double-helical structure in 1953 and the completion of the Human Genome Project in 2003.

—Barbara Brennessel

See also: Central Dogma of Molecular Biology; Chromosome Structure; Chromosome Theory of Heredity; DNA Replication; DNA Structure and Function; Evolutionary Biology; Genetic Code; Genetics, Historical Development of; Human Genome Project; Mendelian Genetics; Molecular Genetics; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World.

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Edey, Maitland A., and Donald C. Johnson. *Blueprints: Solving the Mystery of Evolution*. Reprint. New York: Viking, 1990. Focuses on evolution from the molecular genetic perspective and emphasizes the process of sci-

entific discovery; three chapters are devoted to the genetic code.

Judson, Horace Freeland. *The Eighth Day of Creation*. Rev. ed. Cold Harbor Spring, N.Y.: Cold Spring Harbor Laboratory Press, 1997. A noted and fascinating history of molecular biology that details the deciphering of the genetic code.

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Trainor, Lynn E. H. *The Triplet Genetic Code: The Key to Molecular Biology*. River Edge, N.J.: World Scientific, 2001. Intended for nonspecialists as well as professionals, surveys the fundamentals of the genetic code and how it has come to revolutionize thinking about living systems as a whole.

Web Site of Interest

Cracking the Code of Life. <http://www.pbs.org/wgbh/nova/genome>. The companion Web site to the 2001 PBS broadcast of the same name. Discusses heredity, gene manipulation, DNA sequencing, a "journey into DNA," and more.

Genetic Counseling

Field of study: Human genetics and social issues

Significance: *Genetic counseling involves helping individuals or families cope with genetic syndromes or diseases that exist, or could potentially occur, in a family setting. Genetic counselors pro-*

vide information regarding the occurrence or risk of occurrence of genetic disorders, discuss available options for dealing with those risks, and help families determine their best course of action.

Key terms

GENETIC SCREENING: the process of investigating a specific population of people to detect the presence of genetic defects

NONDIRECTIVE COUNSELING: a practice that values patient autonomy and encourages patients to reach a decision that is right for them based upon their personal beliefs and values

PEDIGREE ANALYSIS: analysis of a family's history by listing characteristics such as age, sex, and state of health of family members, used to determine the characteristics of a genetic disease and the risk of passing it on to offspring

PRENATAL DIAGNOSIS: the process of detecting a variety of birth defects and inherited disorders before a baby is born by various imaging technologies, genetic tests and biochemical assays

The Establishment of Genetic Counseling

Historically, people have long understood that some physical characteristics are hereditary and that particular defects are often common among relatives. This concept was widely accepted by expectant parents and influenced the thinking of many scientists who experimented with heredity in plants and animals. Many efforts were made to understand, predict, and control the outcome of reproduction in humans and other organisms. Gregor Mendel's experiments with garden peas in the mid-1800's led to the understanding of the relationship between traits in parents and their offspring. During the early twentieth century, Walter Sutton proposed that newly discovered hereditary factors were physically located on complex structures within the cells of living organisms. This led to the chromosome theory of inheritance, which explains mechanically how genetic information is transmitted from parent to offspring in a regular, orderly manner. In 1953, James Watson and Francis Crick (along with Maurice Wilkins and Rosalind Franklin)

discovered the double-helix structure of DNA, the molecule that carries the genetic information in the cells of most living organisms. Three years later, human cells were found to contain forty-six chromosomes each.

These discoveries, along with other developments in genetics, periodically generated efforts (often misguided) to control the existence of "inferior" genes, a concept known as eugenics. Charles F. Dight, a physician influenced by the eugenics movement, left his estate in 1927 "To Promote Biological Race Betterment—betterment in Human Brain Structure and Mental Endowment and therefor[e] in Behavior." In 1941 the Dight Institute for Human Genetics began to shift their emphasis from eugenics to genetic studies of individual families. In 1947, Sheldon Reed began working at the Dight Institute as a genetic consultant to individual families. Reed believed that his profession should put the clients' needs before all other considerations and that it should be separated from the concept of eugenics. He rejected the older names for his work, such as "genetic hygiene," and substituted "genetic counseling" to describe the type of social work contributing to the benefit of the family. As a result, the field of genetic counseling was born and separated itself from the direct concern of its effect upon the state or politics. In fact, Reed predicted that genetic counseling would have been rejected had it been presented as a form of eugenics.

Genetic counseling developed as a preventive tool and became more diagnostic in nature as it moved from academic centers to the major medical centers. In 1951, there were ten genetic counseling centers in the United States employing academically affiliated geneticists. Melissa Richter and Joan Marks were instrumental in the development of the first graduate program in genetic counseling at Sarah Lawrence College in New York in 1969. By the early 1970's, there were nearly nine hundred genetic counseling centers worldwide. By 2002 there were approximately two thousand genetic counselors in the United States not only working with individual families concerning genetic conditions but also involved in teaching, research, screening programs, public health, and

the coordination of support groups. In 1990, the Human Genome Project began as a fifteen-year effort coordinated by the U.S. Department of Energy and the National Institutes of Health to map and sequence the entire human genome, prepare a model of the mouse genome, expand medical technologies, and study the ethical, legal, and social implications of genetic research.

The Training of the Genetic Counselor

Most genetic counseling students have undergraduate degrees in genetics, nursing, psychology, biology, social work, or public health. Training programs for genetic counselors are typically two-year masters-level programs and include field training in medical genetics and counseling in addition to a variety of courses focusing on genetics, psychosocial theory, and counseling techniques. During the two-year program, students obtain an in-depth background in human genetics and counseling through coursework and field training at genetic centers. Coursework incorporates information on specific aspects of diseases, including the prognoses, consequences, treatments, risks of occurrence, and prevention as they relate to individuals or families. Field training at genetic centers enables students to develop research, analytical, and communication skills necessary to meet the needs of individuals at risk for a genetic disease.

Many genetic counselors work with M.D. or Ph.D. geneticists and may also be a part of a health-care team that may include pediatricians, cardiologists, psychologists, endocrinologists, cytologists, nurses, and social workers. Other genetic counselors are in private practice or are engaged in research activities related to the field of medical genetics and genetic counseling. Genetic counseling most commonly takes place in medical centers, where specialists work together in clinical genetics units and have access to diagnostic facilities, including genetic laboratories and equipment for prenatal screening.

The Role of the Genetic Counselor

Prior to the 1960's, most genetic counselors were individuals with genetic training who con-

sulted with patients or physicians about specific risks of occurrence of genetic diseases. It was not until 1959, when French geneticist Jérôme Lejeune discovered that children with Down syndrome have an extra chromosome 21, that human genetics was finally brought to the attention of ordinary physicians. Rapid growth in knowledge of inheritance patterns, improvements in the ability to detect chromosomal abnormalities, and the advent of screening programs for certain diseases in high-risk populations all contributed to the increased interest in genetic counseling. Development of the technique of amniocentesis, which detects both chromosomal and biochemical defects in fetal cells, led to the increased specialization of genetic counseling. By the 1970's, training of genetic counselors focused on addressing patients' psychosocial as well as medical needs. Genetic counseling thus became a voluntary social service intended exclusively for the benefit of the particular family involved.

Genetic counselors provide information and support to families who have members with genetic disorders, individuals who themselves are affected with a genetic condition, and families who may be at risk for a variety of inherited genetic conditions, including Huntington's disease (Huntington's chorea), cystic fibrosis, and Tay-Sachs disease. The counselor obtains the family medical history and medical records in order to interpret information about the inherited genetic abnormality. Genetic counselors analyze inheritance patterns, review risks of recurrence, and offer available options for the genetic condition. Other functions of genetic counselors include discussing genetic risks with blood-related couples considering marriage, contacting parents during the crisis following fetal or neonatal death, preparing a community for a genetic population screening program, and informing couples about genetically related causes of their infertility. A pregnant patient is most commonly referred to a genetic counselor by an obstetrician because of her advanced age (thirty-five years or older).

In addition to obtaining accurate diagnosis of the genetic abnormality, genetic counselors strive to explain the genetic information as

clearly as possible, making sure that the individual or family understands the information fully and accurately. The genetic counselor must evaluate the reliability of the diagnosis and the risk of occurrence of the genetic disease. Because the reliability of various tests will affect a patient's decision about genetic testing and abortion, the counselor must give the patient a realistic understanding of the meaning and inherent ambiguity of test results. Most genetic counselors practice the principle of nondirectiveness and value patient autonomy. They present information on the benefits, limitations, and risks of diagnostic procedures without recommending a course of action, encouraging patients to reach their own decisions based on their personal beliefs and values. This attitude reflects the historical shift of genetic counseling away from eugenics toward a focus on the individual family. The code of ethics of the National Society of Genetic Counselors states that its members strive to "respect their clients' beliefs, cultural traditions, inclinations, circumstances, and feelings as well as provide the means for their clients to make informed independent decisions, free of coercion, by providing or illuminating the necessary facts and clarifying the alternatives and anticipated consequences."

Diagnosis of Genetic Abnormalities

In the latter half of the twentieth century, discoveries in genetics and developments in reproductive technology contributed to the advancements in prenatal diagnosis and genetic counseling. Prenatal diagnostic procedures eventually became an established part of obstetrical practice with the development of amniocentesis in the 1960's, followed by ultrasound, chorionic villus sampling (CVS), and fetal blood sampling. Amniocentesis, CVS, and fetal blood sampling are ways to obtain fetal cells for analysis and detection of various types of diseases. Amniocentesis, a cytogenetic analysis of the cells within the fluid surrounding the fetus, is performed between the fifteenth and twentieth weeks of gestation and detects possible chromosomal abnormalities such as Down syndrome and trisomy 18. The information obtained from CVS is similar to that obtained from amniocentesis, except the testing can be

performed earlier in the pregnancy (during the tenth to twelfth weeks of gestation). Fetal blood sampling can be performed safely only after eighteen weeks of pregnancy. An ultrasound, offered to all pregnant women, uses high-frequency sound waves to create a visual image of the fetus and detects anatomical defects such as spina bifida, cleft lip, or certain heart malformations. Pedigree analysis may also be used for diagnostic purposes and to determine the risk of passing a genetic abnormality on to future generations. A pedigree of the family history is constructed, listing the sex, age, and state of health of the patient's close relatives; from that, recurrent miscarriages, stillbirths, and infant deaths are explored.

Prenatal diagnostic techniques are used to identify many structural birth defects, chromosomal abnormalities, and more than five hundred specific disorders. Genetic counselors who believe that their client is at risk for passing on a particular disease may suggest several genetic tests, depending on the risk the patient may face. Screening of populations with high frequencies of certain hereditary conditions, such as Tay-Sachs disease among Ashkenazi Jews, is encouraged so that high-risk couples can be identified and their pregnancies monitored for affected fetuses. Pregnant women may also be advised to undergo testing if an abnormality has been found by the doctor, the mother will be thirty-five years of age or older at the time of delivery, the couple has a family history of a particular genetic abnormality, the mother has a history of stillbirths or miscarriages, or the mother is a carrier of metabolic disorders (for example, hemophilia) that can be passed from mothers to their sons.

The Human Genome Project is expected to have a dramatic impact on presymptomatic diagnosis of individuals carrying specific diseases, multigene defects involved in common diseases such as heart disease and diabetes, and individual susceptibility to environmental factors that interact with genes to produce diseases. The isolation and sequencing of genes associated with genetic abnormalities such as cystic fibrosis, kidney disease, Alzheimer's disease, and Huntington's disease (Huntington's chorea) allow for individuals to be tested for

those specific conditions. Many genetic tests have been developed so that the detection of genetic conditions can be made earlier and with more precision.

Ethical Aspects of Genetic Counseling

With advancements in human genetics and reproductive technology, fundamental moral and ethical questions may arise during difficult decision-making processes involving genetic abnormalities for which families may be unprepared. Diagnosis of a particular genetic disease may allow individuals or families to make future plans and financial arrangements. However, improvements in the capability to diagnose numerous hereditary diseases often exceed the ability to treat such diseases. The awareness that an unborn child is genetically predisposed toward a disease with no known cure may lead to traumatic anxiety and depression. The psychological aspects of genetic counseling and genetic centers must therefore continue to be explored in genetic centers throughout the world.

Questions about who should have access to the data containing patients' genetic makeup must also be considered as the ability to screen for genetic diseases increases. Violating patients' privacy could have devastating consequences, such as genetic discrimination in job hiring and availability of health coverage. Employers and insurance companies have already denied individuals such opportunities based on information found through genetic testing. Disclosure of genetic information not only contributes to acts of discrimination but also may result in physical and psychological harm to individuals.

With data derived from the Human Genome Project increasing rapidly, problems arising from the application of new genetic knowledge in clinical practice must be addressed. The norm of nondirective counseling will be challenged, raising questions of who provides and who receives information and how it is given. Many believe that genetic counseling is beneficial to those faced with genetic abnormalities, while others fear that genetic counseling is a form of negative eugenics, an attempt to "improve" humanity as a whole by discouraging

the birth of children with genetic defects. Since most genetic conditions can be neither treated nor modified in pregnancy, abortion is often the preventive measure used. Thus, ethical issues concerning the respect for autonomy of the unborn child must also be considered.

—Jamalynne Stuck and Doug McElroy

See also: Amniocentesis and Chorionic Villus Sampling; Bioethics; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Human Genetics; In Vitro Fertilization and Embryo Transfer; Insurance; Linkage Maps; Pedigree Analysis; Prenatal Diagnosis.

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Web Sites of Interest

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Human Genome Project Information, Genetic Counseling. <http://www.doegenomes.org>. Site links to genetic counseling information and related resources.

National Society of Genetic Counselors. <http://www.nsge.org>. Offers a search engine for locating genetic counselors in the United States and a newsroom with press releases and fact sheets about the counseling services.

nostic, and therapeutic applications that promise to help prevent and treat a wide range of genetic diseases.

Key terms

BIOTECHNOLOGY: the application of recombinant DNA technology to the development of specific products and procedures

CLONING: the process by which large amounts of a single gene or genome (the entire genetic content of a cell) are reproduced

COMPLEMENTARY BASE PAIRING: hydrogen bond formation that only occurs between adenine and thymine or cytosine and guanine

DNA SEQUENCE ANALYSIS: chemical methods that permit the determination of the order of nucleotide bases in a DNA molecule

GENOMIC LIBRARY: a collection of clones that includes the entire genome of a single species as fragments ligated to vector DNA

PROBE HYBRIDIZATION: a method that permits the identification of a unique sequence of DNA bases using a single-stranded DNA segment complementary to the unique sequence and carrying a molecular tag allowing identification

TRANSGENIC ORGANISM: a species in which the genome has been modified by the insertion of genes obtained from another species

VECTOR: a segment of DNA, usually derived from viruses, bacteria, or yeast, that contains regulatory sequences that permit the amplification of single genes or genetic segments

Restriction Enzymes

Many of the methods used in genetic engineering represent adaptations of naturally occurring genetic processes. One of the earliest and most significant discoveries was the identification of a family of DNA enzymes called restriction endonucleases, more commonly called restriction enzymes. Restriction enzymes are DNA-modifying enzymes produced by microorganisms as a protection against viral infection; their uniqueness and utility in recombinant DNA technology reside in their ability to cleave DNA at precise recognition sites based on DNA sequence specificity. Several hundred restriction enzymes have been isolated, and many recognize unique DNA segments and ini-

Genetic Engineering

Field of study: Genetic engineering and biotechnology

Significance: *The development of the tools of recombinant DNA technology used in genetic engineering has generated unprecedented inquiry into the nature of the living system and has revolutionized the study of genetics. The implications of this research are far-reaching, ranging from a better understanding of basic biological principles and molecular mechanisms to pharmacological, diag-*

tiate DNA cleavage only at these sites. The site-specific cleavages generated by restriction enzymes can be used to produce a unique set of DNA segments that can be used to “map” individual genes and distinguish them from all other genes. This type of genetic analysis, based on differences in the sizes of DNA segments from different genes or different individuals when cleaved with restriction enzymes, is referred to as restriction fragment length polymorphism (RFLP) analysis.

If genes or DNA segments from different sources or species are cleaved with the same restriction enzyme, the DNA segments produced, though genetically unrelated, can be mixed together to produce recombinant DNA. This occurs because most restriction enzymes produce complementary, linear, single-stranded DNA ends that can join together. An additional enzyme called DNA ligase is used to seal the link between the DNA molecules with covalent bonds. This procedure, developed in the 1970’s, is at the core of recombinant DNA technology and can be used to analyze the structure and function of the genome at the molecular level.

Another key development has been the use of vectors to amplify DNA fragments. Vectors are specially designed DNA molecules derived from viruses, bacteria, or other microorganisms, such as yeast, that contain regulatory sequences permitting the amplification or expression of a DNA fragment or gene. Vectors are available for numerous applications.

Vectors

Plasmids are small, circular DNAs that have been isolated from many species of bacteria. These naturally occurring molecules often encode antibiotic resistance genes that can be transferred from one bacterial cell to another in a process called transformation. In the laboratory, plasmids can be used as vectors in the amplification of genes inserted by restriction enzyme treatment of both vector and insert DNA, followed by DNA ligation to produce recombinant plasmids. The recombinant DNA is then inserted into host bacterial cells by transformation, a routine process in which bacterial cells are made “competent,” that is, able to take up DNA from their surroundings. Once inside

the host cell, the recombinant plasmid will be replicated by the host cell, along with the host’s own genome. Bacterial cells reproduce rapidly and generate large colonies of cells, each cell containing a copy of the recombinant plasmid. By this process the fragment of DNA in the recombinant vector is “cloned.”

The cloned DNA can then be isolated from the bacterial cells and used for other applications or studies. Plasmids are useful for cloning small genes or DNA fragments; larger fragments can be cloned using viral vectors such as the bacterial virus (bacteriophage) lambda (phage λ). This virus can infect bacterial cells and reproduce to high copy number. If nonessential viral genes are removed, recombinant viruses containing genes of interest can be produced. Synthetic recombinant vectors incorporating bacterial and viral components, called cosmids, have also been developed. In addition, synthetic minichromosomes called yeast artificial chromosomes (YACs), which incorporate large segments of chromosomal DNA and which are capable of replication in bacterial or eukaryotic systems, have been developed.

DNA Sequence Analysis

A further key discovery in genetic engineering has been the development of chemical methods of DNA sequence analysis. These methods permit a determination of the linear sequence of nucleotide bases in DNA. DNA sequence analysis permits a direct determination of gene structure with respect to regulatory and protein-coding regions and can be used to predict the structure and function of proteins encoded by specific genes.

There are many important applications of the basic principles of genetic engineering. Notable examples include the Human Genome Project, the identification and characterization of human disease genes, the production of large amounts of proteins for therapeutic or industrial purposes, the creation of genetically engineered plants that are disease-resistant and show higher productivity, the creation of genetically engineered microorganisms that can help clean up pollution, and the treatment of genetic disorders using gene therapy.

Gene Cloning

The ability to clone DNA fragments has directly facilitated DNA sequence analysis. In addition to allowing the better understanding of specific genes, cloning was an integral tool in the Human Genome Project, an international effort to elucidate the structure of the entire human genome. The Human Genome Project offers the promise of greatly increasing the understanding of the genes responsible for inherited single-gene disorders as well as the involvement of specific genes in multifactorial disorders such as coronary heart disease.

The underlying genetic defects for a number of disease-causing genes have been identified, including sickle-cell disease (which results from a single nucleotide base substitution in one of the globin genes), Duchenne muscular dystrophy (caused by deletions in the muscle protein gene for dystrophin), and cystic fibrosis (caused by a variety of mutations in the gene

for the chloride channel conductance protein). The identification of these disease genes has permitted the design of diagnostic tests and in some cases therapeutic strategies, including attempts to replace defective genes.

The analysis of gene function has been made possible by a process called site-directed mutagenesis, in which specific mutations can be introduced into cloned genes. These mutant genes can then be inserted into expression vectors, where the faulty protein can be produced and studied. Alternatively, the mutant genes can be introduced into animals, such as mice, to explore the effects of specific mutations on development and cell function.

Transgenic Organisms

One of the earliest successes in producing transgenic organisms was when *Escherichia coli* bacteria were engineered to produce human insulin for the treatment of diabetes. The tech-

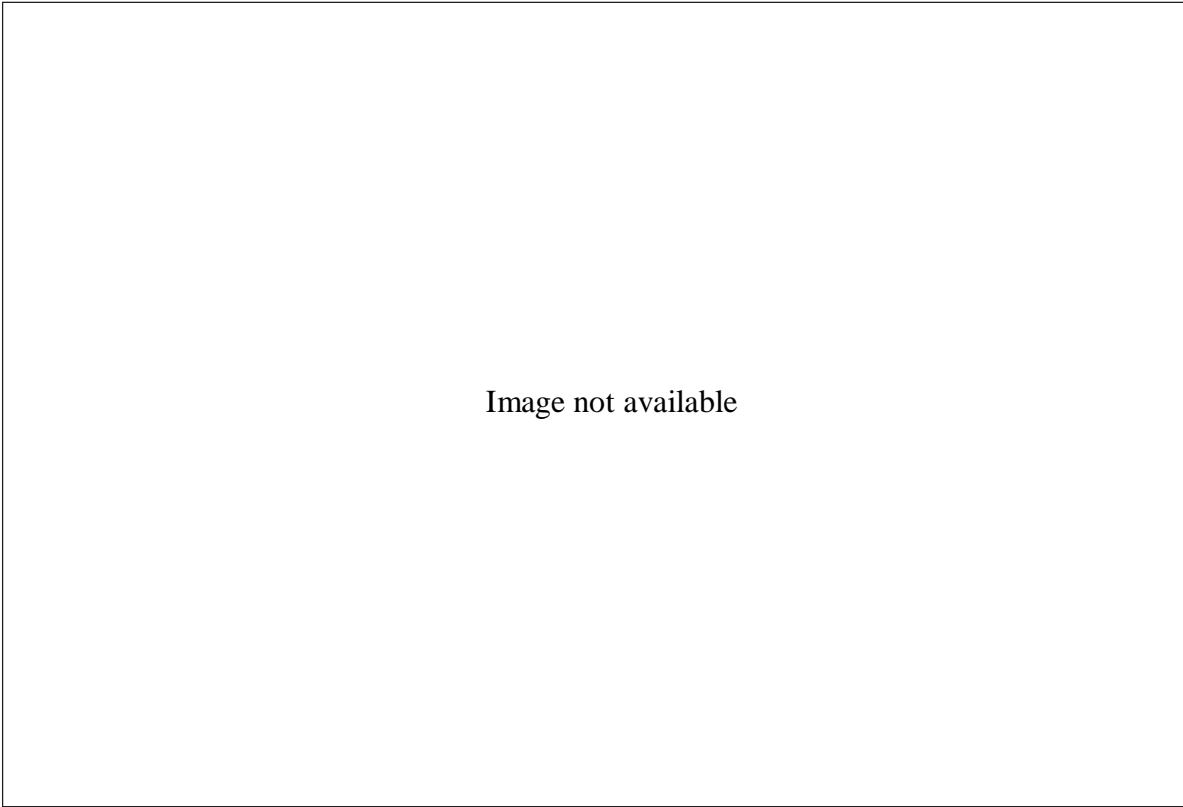


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Among less well known genetic engineering projects is the work of Oregon State University professor Steve Strauss and his colleagues, who are genetically modifying poplar trees to grow larger leaves in order to find genes that affect growth. (AP/Wide World Photos)

nology involved the cloning of the human insulin gene and its insertion into bacterial expression vectors. Subsequently, many gene products have been produced by genetically engineered microorganisms, including clotting factors (used in the treatment of hemophilia), growth factors such as epidermal growth factor (used to accelerate wound healing) and colony-stimulating factors (used to stimulate blood cell formation in the bone marrow), and interferons (used in the treatment of immune system disorders and certain types of cancer). The advantages of using genetically engineered products are enormous: Therapeutic proteins or hormones can be produced in much larger amounts than could be obtained from tissue isolation, and the genetically engineered products are free of viruses and other contaminants.

Introduction of foreign genes into the fertilized eggs of host animals is called germ-line transformation and involves the insertion of individual genes into fertilized eggs. After the eggs are implanted in foster mothers, the resulting transgenic offspring will have the mutated gene in all their cells and will be able to pass the gene on to their future offspring.

Many of the methods for introducing foreign genes into host cells take advantage of the naturally occurring processes facilitated by viruses. Genetically engineered retroviruses, for example, can be used to insert a foreign gene into a recipient cell following viral infection. Foreign genes may also be incorporated into lipid membranes to form liposomes, which then can bind to the target cell and insert the gene. Chemical methods of gene transfer include the use of calcium phosphate or dextran sulfate to generate pores in the recipient cell membrane through which the foreign DNA enters the cell. Microinjection involves the use of microscopic needles to insert foreign DNA directly into the nucleus of the target cell and is often used to insert genetic material into fertilized eggs. Electroporation involves the use of an electric current to open pores in the cell membrane, permitting DNA uptake by the recipient cell. Finally, particle bombardment represents a method of gene transfer in which metal pellets coated with DNA are transferred into target cells under high pressure using

“gene guns.” This method is particularly useful for inserting genes into plant cells that are resistant to DNA uptake because of thick cell walls.

Genetically engineered transgenic species have many biological uses. Transgenic animals have been used to analyze the functions of specific genes in development and to generate animal models of human diseases. For example, a transgenic mouse strain incorporating a human breast cancer gene has been developed to explore the mechanisms by which this disease occurs. In addition, transgenic mice have been used to analyze the normal functions of specific genes by producing “knockout” mice, whose genomes contain mutated, nonfunctional copies of the genes of interest. This technology, developed by Mario Capecchi, uses homologous recombination, in which only complementary nucleotide base pairs carry out the genetic exchange within the host chromosome. Thus, the effects of the inserted gene, or transgene, on development and physiology can be examined. Knockout mice lacking a functional adenosine deaminase (*ADA*) gene, for example, show disease characteristics comparable to those of humans with severe combined immunodeficiency disorder (SCID). These mice have been very useful for determining the efficacy of novel treatments, including the replacement of the faulty gene by gene therapy.

Transgenic animals have also been developed to produce therapeutic gene products in large quantities. For example, transgenic sheep have been developed that secrete the human protein alpha-1 antitrypsin (AAT) in their milk. AAT is used to treat an inherited form of emphysema. The process involves the microinjection of fertilized sheep eggs with the human *AAT* gene linked to regulatory sequences that allow the gene to be actively expressed in the mammary tissue. Although the process of generating transgenic animals is inefficient, individual transgenic animals can produce tremendous amounts of gene products that can be readily purified from the milk. Additional transgenic livestock have been engineered to produce tissue plasminogen activator (used in the treatment of blood clots), hemoglobin (used as

a blood substitute), erythropoietin (used to stimulate red blood cell formation in kidney dialysis patients), human growth hormone (used to treat pituitary dwarfism), and factor VIII (used to treat hemophilia).

Transgenic plants have also been produced, using the Ti (tumor-inducing) plasmid. This plasmid is found naturally in the bacterium *Agrobacterium tumefaciens*. The Ti plasmid has been used to transfer a toxin gene from the bacterium *Bacillus thuringiensis* that kills insect pests, thereby avoiding the use of pesticides.

Genetically Engineered Viruses

An additional medical application involves the use of genetically engineered viruses in the treatment of genetic diseases. Retroviruses are the most important group of viruses used for these purposes, since the life cycle of the virus involves the incorporation of the viral genome into host chromosomes. Removal of most of the virus's own structural genes removes its ability to cause disease, while the regulatory genes are retained and ligated to the therapeutic gene. The recombinant retrovirus then becomes harmless; however, it can still enter a cell and become integrated into the host cell genome, where it can direct the expression of the therapeutic gene. The first successful clinical application was the use of genetically engineered retroviruses in the treatment of severe combined immunodeficiency disorder (SCID). Viruses with a functional copy of the ADA gene were able to reverse SCID. However, in 2002 researchers in France and the United States discovered that this treatment appears to lead to a greatly increased risk of developing leukemia, and clinical trials were suspended.

Similar methods have been used to develop recombinant vaccines. For example, a recombinant vaccinia virus has been produced by the insertion of genes from other viruses. During the process of infection, the recombinant vaccinia virus produces proteins from the foreign genes, which act as antigens which lead to immunity following vaccination. This strategy is particularly useful in the development of vaccines against viruses that are highly pathogenic such as the human immunodeficiency virus (HIV), in which it is not possible to use a whole

killed or attenuated (weakened) live viral vaccine because of the risk of developing the disease from the vaccination. Genetically engineered viruses may also be useful in the treatment of diseases such as cancer since they could be designed to target specific cells with abnormal cell surface receptors. Recombinant adenoviruses containing a single gene mutation have been engineered that are capable of lethal infection in cancer cells but not in normal tissues of the body.

Impact and Applications

The methods of recombinant DNA technology have revolutionized our understanding of the molecular basis of life and have led to a variety of useful applications. Some of the most important discoveries have involved an increased understanding of the molecular basis of disease processes, which has led to new methods of diagnosis and treatment. Genetically engineered animals can be used to produce unlimited amounts of therapeutic gene products and can also serve as genetic models to enhance understanding of the physiological basis of disease. Plants can be genetically engineered for increased productivity and disease resistance. Genetically engineered viruses have been developed as vaccines against infectious disease. The methods of recombinant DNA technology were originally developed from natural products and processes that occur within the living system. The ultimate goals of this research must involve applications that preserve the integrity and continuity of the living system.

—Sarah Crawford Martinelli,
updated by Bryan Ness

See also: Animal Cloning; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain

Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Web Sites of Interest

- Centers for Disease Control, Office of Genomics and Disease Prevention. <http://www.cdc.gov/genomics/default.htm>. Offers information on the genetic discoveries and prevention of diseases in humans. Includes links to related resources.
- U.S. Department of Agriculture, Biotechnology: An Information Resource. <http://www.nal.usda.gov/bic>. A government site that offers dozens of links to information on genetic engineering (biotechnology). glossary, and an annotated bibliography.

Genetic Engineering: Agricultural Applications

Field of study: Genetic engineering and biotechnology

Significance: *Genetic engineering is the deliberate manipulation of an organism's DNA by introducing beneficial or eliminating specific genes in the cell. For agricultural applications, the technology enables scientists to isolate, modify, and insert genes into the same or different crop, clone an adult plant from a single cell of a parent plant, and create genetically modified (GM) foods.*

Key terms

CLONING: regeneration of full-grown adult group of organisms from some form of asexual reproduction—for example, from protoplasts

EXOGENOUS GENE: a gene produced or originating from outside an organism

GENOME: the collection of all the DNA in an organism

PLASMID: a small, circular DNA molecule that occurs naturally in some bacteria and yeasts

PROTOPLASTS: plant cells whose cell walls have been removed by enzymatic digestion

RECOMBINANT DNA: a molecule of DNA formed by the joining of DNA segments from different sources

TRANSGENIC CROP PLANT: a crop plant that contains a gene or genes that have been artificially inserted into its genome

VECTOR: a carrier organism, or a DNA molecule used to transmit genes in a transformation procedure

Producing Transgenic Crop Plants

To produce a transgenic crop, a desirable gene from another organism, of the same or a different species, must first be spliced into a vector such as a virus or a plasmid. In some cases additional modification of the gene may be attempted in the laboratory. The most common vector used for producing transgenic plants is the "Ti" plasmid, or tumor-inducing plasmid, found in the cells of the bacterium called *Agrobacterium tumefaciens*. *A. tumefaciens* infection causes galls or tumorlike growths to

develop on the tips of the plants. Botanists use the infection process to introduce exogenous genes of interest into host plant cells in order to generate entire crop plants that express the novel gene.

Unfortunately, it was discovered that *A. tumefaciens* could infect only dicotyledons such as potatoes, apples, pears, roses, tobacco, and soybeans. Monocotyledons like rice, wheat, corn, barley, and oats could not be infected with the bacterium. Three primary methods are used to overcome this problem: particle bombardment, microinjection, and electroporation. Particle bombardment is a process in which microscopic DNA-coated pellets are shot through the cell wall using a gene gun. Microinjection involves the direct injection of DNA material into a host cell using a finely drawn micropipette needle. In electroporation, the recipient plant cell walls are removed with hydrolyzing enzymes to make protoplasts, and a few pulses of electricity are used to produce membrane holes through which some DNA can randomly enter.

Reducing Damage from Pests, Predators, and Disease

Geneticists have identified many of the genes for resistance to insect predation and damage caused by viral, bacterial, and fungal diseases in agricultural plants. For instance, seeds of common beans produce a protein that blocks the digestion of starch by two insect pests, cowpea weevil and Azuki bean weevil. The gene for this protein has now been transferred to the garden pea to protect stored pea seeds from pest infestation.

Bacillus thuringiensis (Bt), a common soil bacterium, produces an endotoxin called the *Bt* toxin. The *Bt* toxin, considered an environmentally safe insecticide, is toxic to a number of caterpillars, including the tobacco hornworm and gypsy moth. An indirect approach to pest management completely bypasses the problem of plant transformation. This involves inserting the *Bt* gene into the genome of a bacterium that colonizes the leaf, synthesizes, and secretes the pesticide on the leaf surface. Transgenic corn and cotton have also been modified with the *Bt* gene, enabling the plants to manu-

facture their own pesticide, which is nontoxic to humans.

Glyphosate, the most widely used nonselective herbicide, and other broad-spectrum herbicides are often toxic to crop plants, as well as the weeds they are intended to kill. A major thrust is to identify and transfer herbicide resistance genes into crop plants. Cotton plants, for example, have been genetically engineered to be resistant to certain herbicides.

Improving Crop Yield and Food Quality

Genetic engineering is now being used to modify crops, to improve the quality of food taste, fatty acid profile, protein content, sugar composition, and resistance to spoilage. New, useful or attractive horticultural varieties are also produced, by transforming plants with new or altered genes. For example, plants have been engineered that have additional genes for enzymes that produce anthocyanins, which has resulted in flowers with unusual colors and patterns.

Cereals are the staple food and major source of protein for a large percentage of the earth's population, and contain 10 percent protein in the dry weight. Grains unfortunately lack one or more essential amino acids and therefore provide incomplete nutrition. There are efforts to engineer missing amino acids into cereal protein and to insert genes for higher yields. The development of a high-yielding dwarf rice plant so dramatically helped the nutritional status of millions of people in Southeast Asia that it has been called the "miracle rice."

Researchers based at the Swiss Federal Institute of technology in Zurich have genetically engineered a more nutritious type of rice by inserting three genes into rice that make the plant produce beta-carotene or pro-vitamin A. The color that is imparted to the rice by the vitamin gives it the name "golden rice." Mammals, including humans use beta-carotene, from their food to produce vitamin A, which is necessary for good eye-

sight. It is estimated in 2003 that about 124 million children in the world lack vitamin A, which puts them at risk of permanent blindness and other serious diseases. Golden rice could help alleviate the serious problem of vitamin A deficiency. Iron deficiency is the world's worst nutrition disorder, causing anemia that affects 2 billion people worldwide. The scientists have also managed to insert genes into rice to make it iron-rich.

For improved quality of fruit after harvest, genetic engineers are inserting genes to slow the rate of senescence (aging) and thus slow spoilage of harvested crops, especially fruits. For example, scientists at Calgene (Davis, California) have inserted a gene into tomato plants that blocks the synthesis of the enzyme polygalacturonase, which causes tomato softening, thereby delaying aging (hence rotting) of the fruit.

Improved tolerance to environmental stress for agricultural plants is also being explored by biotechnology, especially for drought, saline conditions, chilling temperatures, high light intensities, and extreme heat. Some plants have genes that enable them to adapt naturally to harsh environments, and genetic engineers are

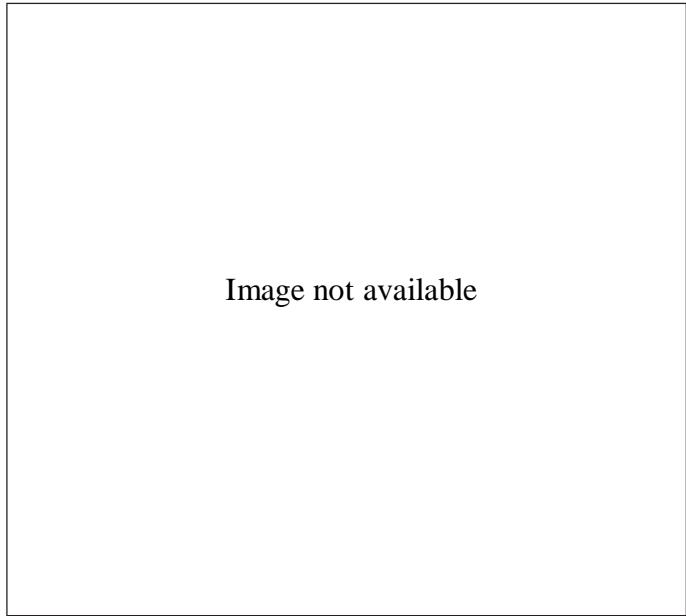
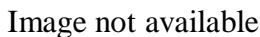


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Tobacco engineered to have no nicotine became economically important to this Amish farmer during the drought of 2002. (AP/Wide World Photos)

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A demonstration in Seattle against Starbucks' usage of genetically engineered ingredients, dubbed "frankenfoods" by protesters. Although many in the developed nations of Europe and North America are concerned over unintended consequences, genetic engineering in agriculture has made it possible to breed varieties of desirable crop plants with a wider range of tolerance for climatic and soil conditions, as well as pests. Such crops offer hope that poorer nations will be able to feed their growing populations. (AP/Wide World Photos)

using these genes to produce similar effects in crop plants.

Biotechnology has produced a marked increase in crop productivity worldwide. In 1999, about 50 percent of the soybean, 33 percent of the corn, and 35 percent of the cotton crops in the United States and 62 percent of the canola crop in Canada were planted with genetically modified seed. In 1996, genetically engineered corn and soybeans were first grown commercially on 1.7 million hectares (4.2 million acres). The land planted in these crops had swelled to 39.9 million hectares (98.8 million acres) by 2003.

Impact and Implications

The various applications of genetic engineering to agriculture have made it possible to alter genes and modify crops for the benefit of humankind, in addition to industrial and medical applications. This should be a subject of in-

terest to everyone because it impacts every aspect of our daily living, and calls for ideas to be tapped from all sectors of our communities. It is a modern innovative trend that has become a major thrust in agriculture by production of genetically modified (GM) foods that are more nutritious and better preserved, but raises concerns as well because of potential dangers of microbial infections and chemical hazards.

Many nonscientists, and some scientists, are leery of GM foods, feeling that too little is understood about the environmental effects of growing GM plants and the potential health dangers of eating GM foods. Resistance to GM foods is widespread in Europe and parts of Asia, and a number of environmental groups strongly oppose all GM crops. Some have gone so far as to call them "frankenfoods." So far they appear to be safe and successful, holding out great promise to solve the problem of world hunger. They make it possible to breed variet-

ies of desirable crop plants with a wider range of tolerance for different climatic and soil conditions, offering hope for the promotion of global agriculture to feed poorer nations. Genetic engineering must be seen as an indispensable component of modern scientific advancement and social development for every nation, if handled wisely without exposing living organisms to harmful microorganisms and toxic chemicals in the process.

—Samuel V. A. Kisseadoo

See also: Animal Cloning; Biofertilizers; Biological Weapons; Biopesticides; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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National Academic of Sciences. Transgenic Plants and World Agriculture. <http://www.nap.edu/html/transgenic>. An online, downloadable pamphlet, published in July of 2000 by a consortium of leading research societies around the world, assesses the need to modify crops genetically in order to feed the increasing world population and then discusses examples of the technology, safety, effect on the environment, funding sources, and intellectual property issues.

Genetic Engineering: Historical Development

Field of study: Genetic engineering and biotechnology; History of genetics

Significance: *Genetic engineering, or biotechnology, is the use of biology, genetics, and biochemistry to manipulate genes and genetic materials in a highly controlled fashion. It has led to major advancements in the understanding of the molecular*

organization, function, and manipulation of genes. The methods have been used to identify causes and solutions to many different human genetic diseases and have led to the development of many new medicines, vaccines, plants, foods, animals, and environmental cleanup techniques.

Key terms

CLONE: a group of genetically identical cells

PLASMIDS: small rings of DNA found naturally in bacteria and some other organisms

RECOMBINANT DNA: a DNA molecule made up of two or more sequences derived from different sources

Foundations of Genetic Engineering

Microbial genetics, which emerged in the mid-1940's, was based upon the principles of heredity that were originally discovered by Gregor Mendel in the middle of the nineteenth century and the resulting elucidation of the principles of inheritance and genetic mapping during the first forty years of the twentieth century. Between the mid-1940's and the early 1950's, the role of DNA as genetic material became firmly established, and great advances occurred in understanding the mechanisms of gene transfer between bacteria. A broad base of knowledge accumulated from which later developments in genetic engineering would emerge. The discovery of the structure of DNA by James Watson and Francis Crick in 1953 provided the stimulus for the development of genetics at the molecular level, and, for the next few years, a period of intense activity and excitement evolved as the main features of the gene and its expression were determined. This work culminated with the establishment of the complete genetic code in 1966, which set the stage for advancements in genetic engineering.

Initially, the term "genetic engineering" included any of a wide range of techniques for the manipulation or artificial modification of organisms through the processes of heredity and reproduction, including artificial selection, control of sex type through sperm selection, extrauterine development of an embryo, and development of whole organisms from cultured cells. However, during the early 1970's, the term came to be used to denote the nar-

rower field of molecular genetics, involving the manipulation, modification, synthesis, and artificial replication of DNA in order to modify the characteristics of an individual organism or a population of organisms.

The Development of Genetic Engineering

Molecular genetics originated during the late 1960's and early 1970's in experiments with bacteria, viruses, and free-floating rings of DNA found in bacteria known as plasmids. In 1967, the enzyme DNA ligase was isolated. This enzyme can join two strands of DNA together, acting like a molecular glue. It is the prerequisite for the construction of recombinant DNA molecules, which are DNA molecules that are made up of sequences not normally joined together in nature.

The next major step in the development of genetic engineering came in 1970, when researchers discovered that bacteria make special enzymes called restriction endonucleases, more commonly known as restriction enzymes. Restriction enzymes recognize particular sequences of nucleotides arranged in a specific order and cut the DNA only at those specific sites, like a pair of molecular scissors. Whenever a particular restriction enzyme or set of restriction enzymes is used on DNA from the same source, the DNA is cut into the same number of pieces of the same length and composition. With a molecular tool kit that included isolated enzymes of molecular glue (ligase) and molecular scissors (restriction enzymes), it became possible to remove a piece of DNA from one organism's chromosome and insert it into another organism's chromosome in order to produce new combinations of genes (recombinant DNA) that may not exist in nature. For example, a bacterial gene could be inserted into a plant, or a human gene could be inserted into a bacterium.

The first recombinant DNA molecules were generated by Paul Berg at Stanford University in 1971, and the methodology was extended in 1973 when DNA fragments were joined to *Escherichia coli* (*E. coli*) plasmids. These recombinant molecules could replicate when introduced into *E. coli* cells, and a colony of identical cells, or clones, could be grown on agar plates.

This development marked the beginning of the technology that has come to be known as gene cloning, and the discoveries of 1972 and 1973 triggered what became known as "the new genetics." The use of the new technology spread very quickly, and a sense of urgency and excitement prevailed. However, because of rising concerns about the morality of manipulating the genetic material of living organisms, as well as the fear that potentially harmful organisms might accidentally be produced, U.S. biologists

called for a moratorium on recombinant DNA experiments in 1974, and the National Institutes of Health (NIH) issued safety guidelines in 1976 to control laboratory procedures for gene manipulation.

In 1977, the pioneer genetic engineering company Genentech produced the human brain hormone somatostatin, and, in 1978, Genentech produced human insulin in *E. coli* by the plasmid method of recombinant DNA. Human insulin was the first genetically engi-

The Asilomar Conference

Rising concerns related to safety and ethical issues surrounding experiments involving recombinant DNA technology led the National Institutes of Health (NIH) and the National Institute of Medicine (NIM) to appoint the Recombinant DNA Advisory Committee (RAC) to study the matter in 1973. RAC consisted of twelve experts from the areas of molecular biology, genetics, virology, and microbiology. Not only was there adverse public opinion in reaction to recombinant DNA (recombinant DNA) experiments, but many specialists in the field of genetic engineering were beginning to doubt their own ability to make important decisions that could impact society.

In February, 1975, the Asilomar Conference was convened under the direction of the NIH in Pacific Grove, California, to address the relevant issues. A total of 140 prominent international researchers and academicians, including Dr. Phillip Sharp, Nobel Laureate Professor at the Massachusetts Institute of Technology's Center for Cancer Research, met to discuss their opinions about recombinant DNA experiments.

Some of the issues debated at the Asilomar Conference included whether or not genetically altered microorganisms that posed a health hazard to humans and other living things might escape from lab facilities, how different genetically tailored recombinant DNA organisms should be classified, and what guidelines should be established to regulate recombinant DNA technology. The scientists concluded that only "safe" bacteria and plasmids that could not escape from the laboratory should be developed. They called for a moratorium on recombinant DNA experiments and demanded that the federal government establish guidelines regulating these experi-

ments. Appropriate safeguards on both physical and biological contaminant procedures would have to be in effect before recombinant DNA experiments continued. Within a year, the NIH had developed guidelines based upon the recommendations made at the Asilomar Conference.

Many positive outcomes resulted from the Asilomar Conference. Scientists demonstrated to the public their genuine concern for the development of safe scientific technology. It marked the first time in history that scientists themselves halted scientific research until the potential hazards could be properly assessed. It also became clear that for future meetings on recombinant DNA technology it would be wise to include scientists with training in infectious diseases, epidemiology, and public health, as well as people from other disciplines, in order to establish a more complete picture of the potential problems and solutions. As a result, a variety of scientists and nonscientists became part of national and local review boards on biotechnology.

Conferences that followed focused on "worst case scenarios" of recombinant DNA experiments. For the first time, debate of scientific issues spread beyond the scientific community to include the general public. Broad social, ethical, environmental, and ecological issues became part of conference agendas and discussions. The RAC membership was changed to include experts in epidemiology, infectious diseases, botany, tissue culture, and plant pathology, as well as nonscientists. NIH guidelines for federally funded research involving recombinant DNA molecules were published on June 23, 1976. As recombinant DNA research continued to progress, appropriate modifications to the NIH guidelines were made.

—Alvin K. Benson

neered product to be approved for human use. By 1979, small quantities of human somatostatin, insulin, and interferon were being produced from bacteria by using recombinant DNA methods. Because such research was proven to be safe, the NIH gradually relaxed the guidelines on gene splicing between 1978 and 1982. The 1978 Nobel Prize in Physiology or Medicine was shared by Hamilton O. Smith, the discoverer of restriction enzymes, and Daniel Nathans and Werner Arber, the first people to use these enzymes to analyze the genetic material of a virus.

By the early 1980's, genetic engineering techniques could be used to produce some biomolecules on a large scale. In December, 1980, the first genetically engineered product was used in medical practice when a diabetic patient was injected with human insulin generated in bacteria; in 1982, the Food and Drug Administration (FDA) approved the general use of insulin produced from bacteria by recombinant DNA procedures for the treatment of people with diabetes. During the same time period, genetically engineered interferon was tested against more than ten different cancers. Methods for adding genes to higher organisms were also developed in the early 1980's, and genetic researchers succeeded in inserting a human growth hormone gene into mice, which resulted in the mice growing to twice their normal size. By 1982, geneticists had proven that genes can be transferred between plant species to improve nutritional quality, growth, and resistance to disease.

In 1985, experimental guidelines were approved by the NIH for treating hereditary defects in humans by using transplanted genes; the more efficient polymerase chain reaction (PCR) cloning procedure for genes, which produces two double helixes *in vitro* that are identical in composition to the original DNA sample, was also developed. The following year, the first patent for a plant produced by genetic engineering, a variety of corn with increased nutritional value, was granted by the U.S. Patent and Trademark Office. In 1987, a committee of the National Academy of Sciences concluded that no serious environmental hazards were posed by transferring genes between species of

organisms, and this action was followed in 1988 by the U.S. Patent and Trademark Office issuing its first patent for a genetically engineered higher animal, a mouse that was developed for use in cancer research.

Impact and Applications

The application of genetic engineering to gene therapy (the science of replacing defective genes with sound genes to prevent disease) took off in 1990. On September 14 of that year, genetically engineered cells were infused into a four-year-old girl to treat her adenosine deaminase (ADA) deficiency, an inherited, life-threatening immune deficiency called severe combined immunodeficiency disorder (SCID). In January, 1991, gene therapy was used to treat skin cancer in two patients. In 1992, small plants were genetically engineered to produce small amounts of a biodegradable plastic, and other plants were manufactured to produce antibodies for use in medicines.

By the end of 1995, mutant genes responsible for common diseases, including forms of schizophrenia, Alzheimer's disease, breast cancer, and prostate cancer, were mapped, and experimental treatments were developed for either replacing the defective genes with working copies or adding genes that allow the cells to fight the disease. In February, 1997, a lamb named Dolly was cloned from the DNA of an adult sheep's mammary gland cell; it was the first time scientists successfully cloned a fully developed mammal. By the end of 1997, approximately fifty genetically engineered products were being sold commercially, including human insulin, human growth hormone, alpha interferon, hepatitis B vaccine, and tissue plasminogen activators for treating heart attacks. In 1998, strong emphasis was placed on research involving gene therapy solutions for specific defects that cause cancer, as well as on a genetically engineered hormone that can help people with damaged hearts grow their own bypass vessels to carry blood around blockages.

In spite of the many successes and optimism that prevailed for many years, there have also been some serious setbacks. In 1999 a healthy eighteen-year-old participating in a gene therapy clinical trial at the University of Pennsylva-

nia died unexpectedly, casting doubt on the safety of some types of gene therapy. In another set of clinical trials in France in 2002, involving the treatment of children with SCID, two of the children developed leukemia, raising doubts about the safety of yet another gene therapy protocol. As a result of these events, gene therapy trials of many types were put on hold and extensive discussions and investigations ensued. Still, scientists are hopeful that these kinds of obstacles can be overcome, leading to much greater availability of cures for genetic diseases.

—Alvin K. Benson

See also: Animal Cloning; Biofertilizers; Biological Weapons; Biopesticides; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Genetic Engineering: Industrial Applications

Field of study: Genetic engineering and biotechnology

Significance: *Industrial applications of genetic engineering include the production of new and better fuels, medicines, products to clean up existing pollution, and tools for recovering natural resources. Associated processes may maximize the use and production of renewable resources and biodegradable materials, while minimizing the generation of pollutants during product manufacture and use.*

Key terms

BIOMASS: any material formed either directly or indirectly by photosynthesis, including plants, trees, crops, garbage, crop residue, and animal waste

BIOREMEDIATION: biologic treatment methods to clean up contaminated water and soils

CLONING VECTOR: a DNA molecule that maintains and replicates a foreign piece of DNA in a cell type of choice, typically the bacterium *Escherichia coli*

GENETIC TRANSFORMATION: the transfer of extracellular DNA among and between species

NANOTECHNOLOGY: development and use of devices that have a size of only a few billionths of a meter

PLASMIDS: small rings of DNA found naturally in bacteria and some other organisms, used as cloning vectors

RECOMBINANT DNA: a DNA molecule made up of sequences that are not normally joined together

Foundations in Medical Advancements

Since the 1970's, numerous industrial processes have involved applications of genetic engineering and biotechnology, ranging from the production of new medicines and foods to the manufacture of new materials for cleaning up the environment and enhancing natural resource recovery. With these applications, a primary focus has been the development of industrial processes that reduce or eliminate the production of waste products and consume low amounts of energy and nonrenewable resources. The chemical, plastic, paper, textile, food, farming, and pharmaceutical industries are positively impacted by biotechnology.

The dawn of the age of genetic engineering was 1971, when Herbert Boyer and Stanley Cohen successfully spliced a toad gene between two recombined ends of bacterial DNA. After further experimentation and resulting successes, Boyer and Robert Swanson in 1976 formed Genentech, a company devoted to the development and promotion of biotechnology and genetic engineering applications. In 1978, Boyer discovered a synthetic version of the human insulin gene and inserted it into *Escherichia coli* (*E. coli*) bacteria. The *E. coli* served as cloning vectors to maintain and replicate large amounts of human insulin. This application of recombinant DNA technology to produce human insulin for diabetics was a foundation for the future of industrial applications of genetic engineering and biotechnology. The Eli Lilly company began manufacturing large quantities of human insulin by vector cloning in 1982. Growth hormones for children and antibodies for cancer patients were soon being similarly cloned in bacteria. The pharmaceutical industry was revolutionized.

Later applications of genetic engineering to

the medical industry include the production of new vaccines for use in fighting a variety of diseases. One approach is to use genetically altered viruses to insert manufactured vaccines directly into the cells of diseased animals and humans. In other cases, antigens that invoke immunity to certain diseases are being produced from genetically modified viruses, bacteria, fungi, and other disease-causing microorganisms.

Cleaning up Waste

Genetic engineering methods are being employed in myriad applications to help clean up waste and pollution worldwide. The idea had its beginning in 1972, when Ananda Chakrabarty, a researcher at General Electric (who would later join the college of medicine at the University of Illinois at Chicago), applied for a patent on a genetically modified bacterium that could partially degrade crude oil. Other scientists quickly recognized that toxic wastes might be cleaned up by pollution-eating microorganisms. After a financial downturn for a number of years, a resurgence in bioremediation technology occurred in the late 1980's and early 1990's, when genetically engineered bacteria were produced that could accelerate the breakdown of oil, as well as a diversity of unnatural and synthetic compounds, such as plastics, chlorinated insecticides, herbicides, and fungicides. In 1987 and 1988, bacterial plasmid transfer was used to degrade a variety of hydrocarbons found in crude oil. In the 1990's, naturally occurring and genetically altered bacteria were employed to degrade crude oil spills, such as the major spill that occurred in Alaska's Prince William Sound after the *Exxon Valdez* accident.

Some genetically altered bacteria have been designed to concentrate or transform toxic metals into less toxic or nontoxic forms. In 1998, a gene from *E. coli* was successfully transferred into the bacterium *D. radiodurans*, allowing this microbe to resist high levels of radioactivity and convert toxic mercury II into less toxic elemental mercury. Other altered microbial genes have been added to this bacterium, allowing it to metabolize the toxic organic chemical toluene, a carcinogenic constituent of gasoline. Genetically altered plants have

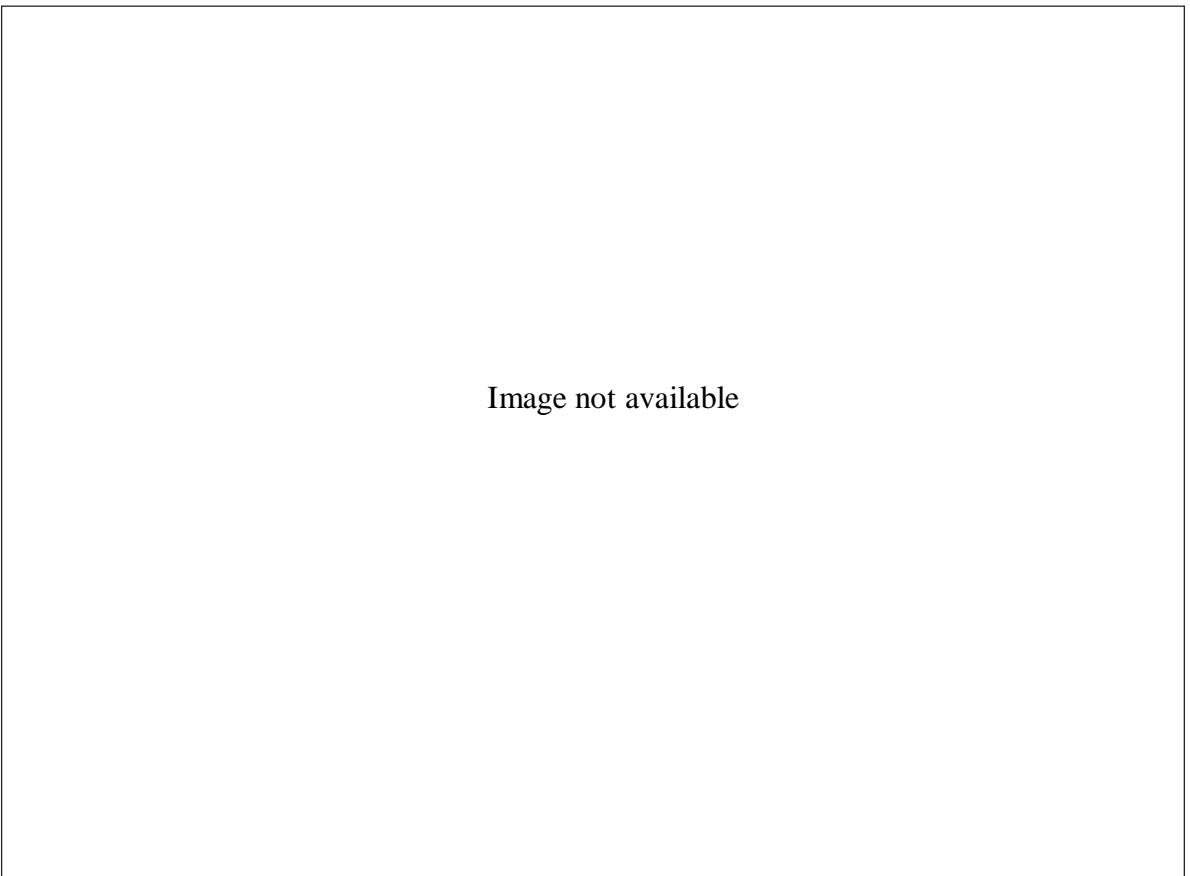


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A genetically engineering enzyme developed from a hybrid poplar tree, shown here by researchers Arun Goyal (left) and Neil Nelson, could reduce the cost of manufacturing paper by replacing chlorine used for pulp bleaching, and might also become a component of animal feed and a means of decomposing harmful toxic pollution. (AP/Wide World Photos)

been produced that absorb toxic metals, including lead, arsenic, and mercury, from polluted soils and water. At Michigan State University, naturally occurring bacteria have been combined with genetically modified bacteria to degrade polychlorinated biphenyls (PCBs). A genetically altered fungus that helps clean up toxic substances discharged when paper is manufactured also produces methane as a by-product, which can be used as a fuel.

Biomass and Materials Science

Genetically altered microorganisms can transform animal and plant wastes into materials usable by humans. Bioengineered bacteria and fungi are being developed to convert biomass wastes, such as sewage solid wastes (paper, garbage), agricultural wastes (seeds, hulls, corn

cobs), food industry by-products (cartilage, bones, whey), and products of biomass, such as sugars, starch, and cellulose, into useful products like ethanol, hydrogen gas, and methane.

Commercial amounts of methane are being generated from animal manure at cattle, poultry, and swine feed lots, sewage treatment plants, and landfills. Biofuels will be cleaner and generate less waste than fossil fuels. In a different application involving fuel technology, genetically modified microbes are being used to reduce the pollution associated with fossil fuels by eating the sulfur content from these fuels.

In applications involving the generation of new materials, a gene generated in genetically modified cotton can produce a polyester-like substance that has the texture of cotton, is even

warmer, and is biodegradable. Other genetically engineered biopolymers are being produced to replace synthetic fibers and fabrics. Polyhydroxybutyrate, a feedstock used in producing biodegradable plastics, is being manufactured from genetically modified plants and microbes. Natural protein polymers, very similar to spider silk and the adhesives generated by barnacles, are being produced from the fermentation of genetically engineered microbes. Sugars produced by genetically altered field corn are being converted into a biodegradable polyester-type material for use in manufacturing packaging materials, clothing, and bedding products. Genetically tailored yeasts can produce a variety of plastics. Such biotechnological advancements will help eliminate the prevalent use of petroleum-based chemicals that has been necessary in the creation of plastics and polyesters.

The fields of biotechnology and nanotechnology are being merged in some materials science applications. Genetic codes discovered in microorganisms can be used as codes for nanostructures, such as task-specific silicon chips and microtransistors. Nanotech production of bioactive ceramics may provide new ways to purify water, since bacteria and viruses stick to these ceramic fibers. Recombinant DNA technology combined with nanotechnology provides the promise for the production of a variety of commercially useful polymers. Carbon nanotubes possessing great tensile strength may be used as computer switches and hydrogen energy storage devices for vehicles. When these nanotubes are coated with reaction specific biocatalysts, many other specialized applications are apparent. In the future, DNA fragments themselves may be used as electronic switching devices.

Natural Resource Recovery

Bioengineered microbes are being developed to extract and purify metals from mined ores and from seawater. The microbes obtain energy by oxidizing metals, which then come out of solution. Chemolithotrophic bacteria, such as *Bacillus cereus*, are energized when they oxidize nickel, cobalt, and gold. They may be used to filter out and concentrate precious

metals from seawater. Iron and sulfur-oxidizing bacteria can also concentrate and release precious metals from seawater. Genetically modified thermophilic bacteria are being produced to extract precious metals from sands. Some genetically altered microorganisms can withstand extreme environments of high salinity, acidity, heavy metals, temperature, and/or pressure, like those that exist around hydrothermal vents where precious minerals are present near the bottom of the ocean.

Genetically engineered strains of the bacteria *Pseudomonas* and *Bacillus* are being produced that can extract oil from untapped reservoirs and store it rather than digest it. These bacteria can be extracted and processed to recover the oil. Other strains are being developed to absorb oil from the vast supplies of oil shale in North America. The process would involve drilling into the oil shale and breaking it into pieces with chemical explosives. A solution of the bioengineered microbes would then be injected through a well into the rock fragments, where they would grow and absorb the oil. The solution would be pumped back to the surface through another well and the bacteria processed to remove the oil. Since this process would eliminate the need for large, open-pit oil shale mines, as well as the need to store oil shale at the surface, the negative environmental impact of oil recovery from shale would be greatly reduced.

—Alvin K. Benson

See also: Animal Cloning; Biofertilizers; Biological Weapons; Biopesticides; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Genetic Engineering: Medical Applications

Fields of study: Genetic engineering and biotechnology; Human genetics and social issues

Significance: *Genetic engineering has produced a wide range of medical applications, including recombinant DNA drugs, transgenic animals that produce pharmaceutically useful proteins, methods for the diagnosis of disease, and gene therapy to introduce a functional gene to replace a defective one.*

Key terms

CLONE: in recombinant DNA technology, a piece of DNA into which a gene of interest has been inserted to obtain large amounts of that gene

GENE TARGETING: the process of introducing a gene that replaces a resident gene in the genome

GENE THERAPY: any procedure to alleviate or treat the symptoms of a disease or condition by genetically altering the cells of the patient

GERM-LINE GENE THERAPY: a genetic change in gametes or fertilized ova so all cells in the organism will have the change and the change will be passed on to offspring

KNOCKOUT: the inactivation of a specific gene within a cell (or whole organism, as in the case of knockout mice), to determine the effects of loss of function of that gene

SOMATIC GENE THERAPY: a genetic change in a specific somatic tissue of an organism, which will not be passed on to offspring

STEM CELL: a an undifferentiated cell that retains the ability to give rise to other, more specialized cells

TRANSGENIC ANIMAL: an animal in which introduced foreign DNA is stably incorporated into the germ line

Multiple Applications: Drug Production

Genetic engineering, the manipulation of DNA to obtain a large amount of a specific gene, has produced numerous medical applications. As a result of the completion in 2003 of the Human Genome Project—the determination of the DNA sequences of all the chromosomes in humans—genetic engineering will continue at an accelerated pace and result in even more important medical applications.

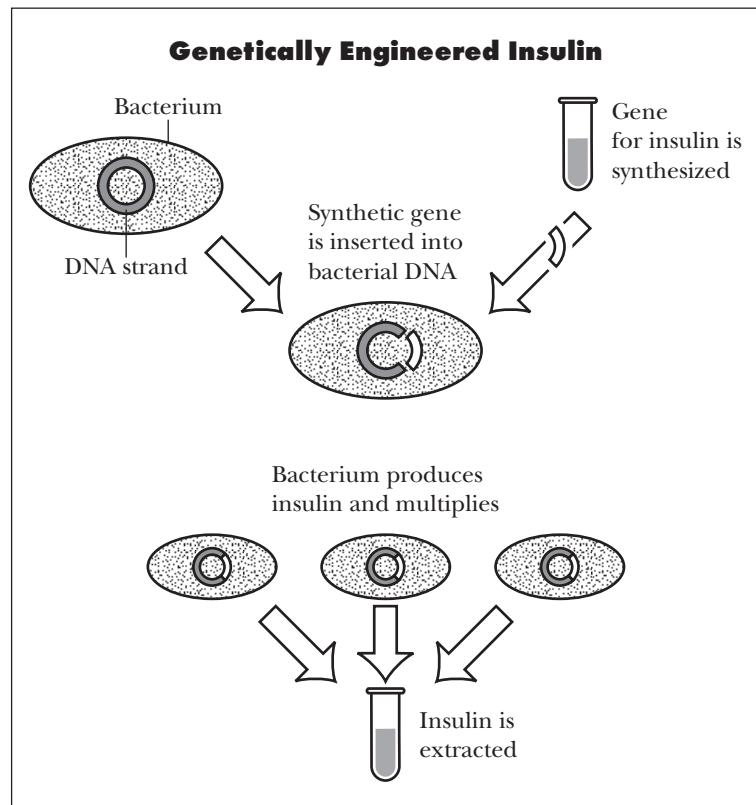
Recombinant DNA technology can be used to mass-produce protein-based drugs. The gene for the protein of interest is cloned and expressed in bacteria. For example, insulin needed for people with Type I diabetes mellitus was isolated from the pancreases of cattle or pigs in slaughterhouses, an expensive and far from ideal process. There are some small chemical differences between human and cow and pig insulin. About 5 percent of those receiving cow insulin have an allergic reaction to it and therefore need insulin from other animals or human cadavers. In 1982, the human gene for insulin was isolated, and a transgenic form called Humulin was successfully produced using *Escherichia coli* bacteria grown in a con-

trolled environment by pharmaceutical companies.

Many other protein-based drugs are produced in bacteria using recombinant DNA technology. Among these are human growth hormone, to treat those deficient in the hormone; factor VIII, to promote blood clotting in hemophiliacs; tissue plasminogen activator, to dissolve blood clots in heart attack and stroke victims; renin inhibitor, to lower blood pressure; fertility hormones, to treat infertility; epidermal growth factor, to increase the rate of healing in burn victims; interleukin-2, to treat kidney cancer; and interferons, to treat certain leukemias and hepatitis.

Transgenic Pharming

Sometimes a protein from a higher organism that is expressed in bacteria does not function properly because bacteria cannot perform certain protein modifications. In such cases, the protein can be produced in a higher organism. In transgenic pharming, a gene that codes for a pharmaceutically useful protein is introduced into an animal such as a cow, pig, or sheep. For example, a transcriptional promoter from a sheep gene that is expressed in sheep's milk is spliced to the gene of interest, such as for alpha-1-antitrypsin, ATT, a glycoprotein (a protein modified with sugar groups) in blood serum that helps the microscopic air sacs of the lungs function properly. People who lack ATT are at risk for developing emphysema. This sheep promoter and ATT gene are injected into the nuclei of fertilized sheep ova that are implanted in surrogate mother sheep. The offspring are examined, and if the procedure is successful, a few of the female lambs will produce the ATT protein in their milk. Once a transgenic animal is created that expresses the ATT gene, transgenic animals expressing the



Genetic engineering is being used to synthesize large quantities of drugs and hormones such as insulin for therapeutic use. (Hans & Cassidy, Inc.)

gene can be bred to each other to produce a whole flock of sheep making ATT—an easier way to obtain ATT than isolating it from donated human blood.

Vaccines

Recombinant DNA methods can be used to produce DNA vaccines that are safer than vaccines made from live viruses. Edible vaccines have also been created by introducing plant genes that will cause a specific immune response. For example, a vaccine for hepatitis has been made in bananas. The idea is that by eating the fruit, individuals will be vaccinated.

Diagnosis

Recombinant DNA methods are used in the diagnosis, as well as treatment, of diseases. Oligonucleotide DNA sequences specific for, and which will only bind to, a particular mutation are used to show if that particular mutation is

present. Also, DNA microarrays are important for gene expression profiling, to aid in cancer diagnosis. For example, oligonucleotides representing portions of many different human genes can be fixed to special "chips" in an array. Messenger RNAs from a cancer patient are bound to the array to show which genes are expressed in that cancer. A certain subtype of cancers express a certain group of genes. This knowledge can be used to design specific treatment regimens for each subtype of cancer.

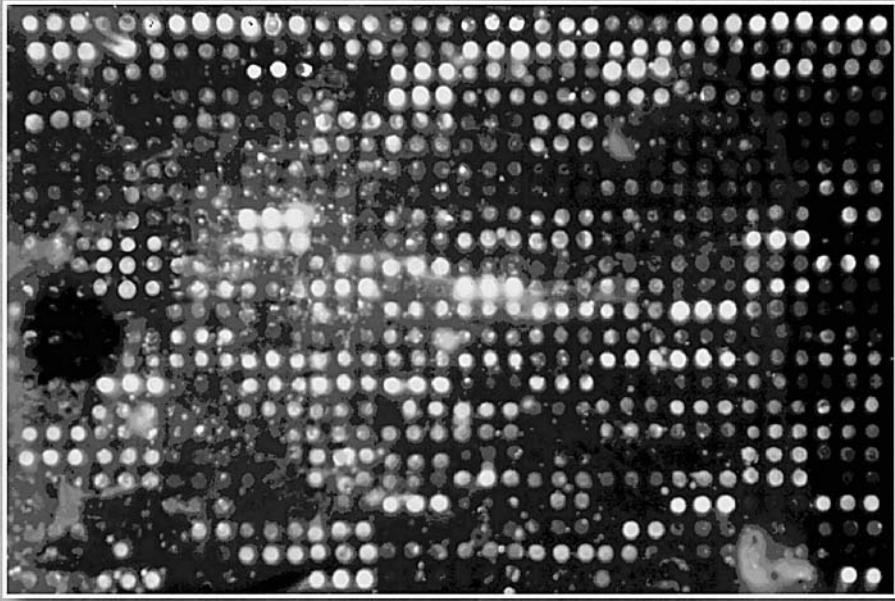
Mice and other animals are used as models for human diseases. Through recombinant DNA technology, a specific gene is "knocked out" (inactivated) to study the effect of the loss of that gene. Mice models are particularly useful in the study of diseases such as diabetes, Parkinson's disease, and severe combined immunodeficiency disorder (SCID).

Gene Therapy

In gene therapy, a cloned functional copy of a gene is introduced into a person to compensate for the person's defective copy. Due to ethical concerns, germ-line gene therapy is not being conducted. Many geneticists and bioethicists oppose germ-line therapy because any negative consequences of the therapy would be passed on to future generations. Therefore, germ-line therapy must wait until scientists, policymakers, and legislators are more confident of consistently positive outcomes. In general, there is support for somatic gene therapy, where the somatic tissue of an individual is modified to produce the correct gene product.

Gene therapy has been attempted for a number of diseases, including SCID and hemophilia. Gene therapy trials have been under close scrutiny, however, during clinical trials

Gene Chips Reveal Susceptibilities



Y-GG 00-0479

DNA microarrays such as the one above can show which genes are expressed in a cancer, knowledge that can be used to design specific treatment regimens for each subtype of cancer. (Mitch Doktycz, Life Sciences Division, Oak Ridge National Laboratory; U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

for gene therapy, one young man died in 1999 and two cases of leukemia in children were detected. These trials used inactivated viruses as vectors, which may have played a role in the death and leukemia cases. Efforts are therefore focusing on the development of DNA delivery systems that do not use viruses.

Future Prospects

In the future, stem cells may be used to generate tissues to replace defective tissues. Catalytic RNAs (ribozymes) may be used to repair genetically defective messenger RNAs. RNA-mediated interference may be used to partially inactivate, rather than knock out, genes to determine the genes' functions in the cell. With the completion of the DNA sequence of the human genome, more genes will inevitably be identified and their functions determined, leading to many more applications to medical diagnosis and therapy.

—Susan J. Karcher

See also: Animal Cloning; Biofertilizers; Biological Weapons; Biopesticides; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Lewis, Ricki. *Human Genetics: Concepts and Applications*. 5th ed. Boston: McGraw-Hill, 2003. A well-written introductory text. Includes chapters on genetically modified organisms, gene therapy, and the Human Genome Project. Illustrations, color photos, problems, glossary, index. Lists links to Web sites.

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Web Sites of Interest

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Centers for Disease Control, Office of Genomics and Disease Prevention. <http://www.cdc.gov/genomics/default.htm>. Offers information on the genetic discoveries and prevention of diseases in humans. Includes links to related resources.

Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes.

National Center for Biotechnology Information. Online Mendelian Inheritance in Man. <http://www.ncbi.nlm.nih.gov/Omim>. A catalog of human genes and genetic disorders for scientists, offering maps of genes and diseases, statistical summaries, and links to similar sites devoted to medical literature and biotechnology.

Genetic Engineering: Risks

Fields of study: Bioethics; Genetic engineering and biotechnology

Significance: *The application of biotechnology, specifically genetic engineering, creates real and foreseeable risks to humans and to the environment. Furthermore, like any new technology, it may cause unforeseen problems. Predicting the occurrence and severity of both anticipated and unexpected problems resulting from biotechnology is a subject of much debate in the scientific community.*

Key terms

FITNESS: the probability of a particular genotype surviving to maturity and reproducing

GENOME: the genetic content of a single set of chromosomes

GENOTYPE: the genetic makeup of an individual, referring to some or all of its specific genetic traits

SELECTION: a natural or artificial process that removes genotypes of lower fitness from the population and results in the inheritance of traits from surviving individuals

TRANSGENIC ORGANISM: an organism that has had its genome deliberately modified using genetic engineering techniques and that is usually capable of transmitting those changes to offspring

The Nature of Biotechnological Risks

Most of the potential risks of biotechnology center on the use of transgenic organisms. Potential hazards can result from the specific protein products of newly inserted or modified genes; interactions between existing, altered, and new protein products; the movement of transgenes into unintended organisms; or

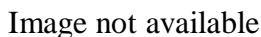
changes in the behavior, ecology, or fitness of transgenic organisms. It is not the process of removing, recombining, or inserting DNA that usually causes problems. Genetically modifying an organism using laboratory techniques creates a plant, animal, or microbe that has DNA and RNA that is fundamentally the same as that found in nature.

Risks to Human Health and Safety

The problem most likely to result from ingesting genetically modified (GM) foods is unexpected allergenicity. Certain foods such as milk or Brazil nuts contain allergenic proteins that, if placed into other foods using recombinant DNA technology, could cause the same allergic reactions as the food from which the allergenic protein originally came. Scientists and policymakers will, no doubt, guard against or severely restrict the movement of known allergens into the food supply. New or unknown allergens, however, could necessitate extensive testing of each GM food product prior to general public consumption. Safety testing will be especially important for proteins that have no history of human consumption.

Unknown, nonfunctional genes that produce compounds harmful or toxic to humans and animals could become functional as a result of the random insertion of transgenes into an organism. Unlike traditional breeding methods, recombinant DNA technology provides scientists with the ability to introduce specific genes without extra genetic material. These methods, however, usually cannot control where the gene is inserted within the target genome. As a result, transgenes are randomly placed among all the genes that an organism possesses, and sometimes "insertional mutagenesis" occurs. This is the disruption of a previously functional gene by the newly inserted gene. This same process may also activate previously inactive genes residing in the target genome. Early testing of transgenic organisms would easily reveal those with acute toxicity problems; however, testing for problems caused by the long-term intake of new proteins is difficult.

Many human and animal disease organisms are becoming resistant to antibiotics. Some

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Greenpeace has been active in protesting genetically engineered organisms, especially for use as food. Near Live Oak, California, one protester warns passersby of a rice "pharm" crop that has been engineered to produce human proteins for drug production. Environmentalists fear the effects such experimentation might have on the food supply and wild-type species.

(AP/Wide World Photos)

scientists worry that biotechnology may accelerate that process. Recombinant DNA technologies usually require the use of antibiotic resistance genes as "reporter" genes in order to identify cells that have been genetically modified. Consequently, most transgenic plants contain antibiotic resistance genes that are actively expressed. Although unlikely, it is possible that resistance genes could be transferred from plants to bacteria or that the existence of plants carrying active antibiotic resistance genes could encourage the selection of antibiotic-resistant bacteria. As long as scientists continue using nat-

urally occurring antibiotic resistance genes that are already commonly found in native bacterial populations, there is little reason to believe that plants with these genes will affect the rate of bacteria becoming resistant to antibiotics.

Another possible problem associated with antibiotic resistance genes is the reduction or loss of antibiotic activity in individuals who are taking antibiotic medication while eating foods containing antibiotic resistance proteins. Would the antibiotic be rendered useless if transgenic foods were consumed? Scientists have found that this is not the case for the most commonly used resistance gene, NPTII (neomycin phosphotransferase II), which inactivates and provides resistance to kanamycin and neomycin. Studies have shown this protein to be completely safe to humans, to be broken down in the human gut, and to be present in the current food supply. Each person consumes, on average, more than one million kanamycin-resistant bacteria daily through the ingestion of fresh fruit and vegetables. These results are probably similar for other naturally occurring resistance genes of bacterial origin.

Risks to the Environment

If environmentally advantageous genes are added to transgenic crops, then those crops, or crop-weed hybrids, may become weeds, or their weediness may increase. For example, tolerance to high-salt environments is a useful and highly desirable trait for many food crops. The addition of transgenes for salt tolerance may allow crop-weed hybrids to displace naturally occurring salt-tolerant species in high-salt environments. Most crop plants are poor competitors in natural ecosystems and probably would not become weeds even with the addition of one or a few genes conferring some competitive advantage. Hybrids between crops and related weed species, however, can show increased weediness, and certain transgenes may also contribute to increased weediness.

Biotechnology may accelerate the development of difficult-to-control pests. Crops and domesticated animals are usually protected from important diseases and insect pests by specific host resistance genes. Genetic resistance is the most efficient, effective, and environmen-

tally friendly means for controlling and preventing agricultural losses caused by pests. Such genes are bred into plants and animals by mating desirable genotypes to those that carry genes for resistance. This method is limited to those species that can interbreed. Biotechnology provides breeders with methods for moving resistance genes across species barriers, which was not possible prior to the 1980's. Bacteria and viruses, however, have been moving bits of DNA in a horizontal fashion (that is, across species and kingdom barriers) since the beginning of life. The widespread use of an effective, specific host resistance gene in domes-

ticated species historically has led to adaptation in the pest population eventually making the resistance gene ineffective. Recombinant methods will likely accelerate the loss of resistance genes as compared with traditional methods because one resistance gene can be expressed simultaneously in many species, is often continuously expressed at high levels within the host, and will more likely be used over large areas because of the immediate economic benefits such a gene will bring to a grower or producer.

Hybrid plants carrying genes that increase fitness (through, for example, disease resistance or drought tolerance) may decrease the

Gene Flow from Crop Plants to Wild Relatives

Crop plants commonly exchange genes with related wild plants that are growing nearby, in a process known as gene flow. Pollen seems to be the most effective agent for gene flow, introducing genes of the parent plant to the recipient plant through fertilization of egg cells. Concern has arisen that genes engineered into crop plants, called transgenes, might spread to their nondomesticated relatives. As bioengineered varieties continue to be developed and as farmers grow the resulting transgenic plants on a commercial scale, the chances of transgenes escaping both to other crop plants and to nondomesticated, wild relatives will increase.

Agriculturally useful traits engineered into crop plants include resistance to herbicides, insects, and pathogens, and tolerance of harsh environmental conditions such as cold, drought, and high salinity. These traits not only give the crops a survival edge under appropriate conditions but also might do the same for nearby wild relatives that acquire the transgenes. As a result, farmers face the possibility that wild plants invigorated by transgenes coding for herbicide resistance could turn into "superweeds," requiring more expensive or more environmentally harmful herbicides.

Further, if transgenes permit a crop to be grown closer to locally rare, wild relatives because it can tolerate an environmental stress that it could not tolerate before, the previously isolated species might hybridize. If hybridization occurs repeatedly, the risk of extinction for the wild population increases.

Another fear is that the spread of transgenes could diminish the genetic diversity of agronomi-

cally important native plants. For example, in Mexico, which is located in the evolutionary cradle of corn, concerns about the spread of transgenes to ancient, native corn varieties, which conventional corn breeders value as genetic reservoirs, led the Mexican government to outlaw the planting of bioengineered corn in 1998.

In addition, wild plants that acquire transgenes for insecticidal properties could harm insects that the crop bioengineers had not targeted. For example, moth and butterfly species, whose larvae depend for food primarily on these wild plants, might be vulnerable if acquired transgenes endow their food plants with insect-killing abilities.

The potential for transgene flow from crops to wild relatives varies with the crop and the geographic location. Most cultivated plants spontaneously mate with one or more wild cousins somewhere in their agricultural distributions. In the United States, some of the major genetically engineered crops, including corn and soybeans, generally have no nearby, wild relatives. About twenty other U.S. crops (some already having transgenic varieties), however, are grown near nondomesticated kin. These crops include rice, sorghum, canola, strawberries, and turf grasses. The hazards from transgene flow to wild relatives, though, could prove lower than the risks of crop-to-crop gene flow, because of the prospect that transgenes for production of pharmaceuticals or other industrial chemicals could make their way into food crops.

—Jane F. Hill

native genetic diversity of a wild population through competitive or selection advantage. As new genes or genes from unrelated species are developed and put into domesticated species, engineered genes may move, by sexual out-crossing, into related wild populations. Gene flow from nontransgenic species into wild species has been taking place ever since crops were first domesticated, and there is little evidence that such gene flow has decreased genetic diversity. In most situations, transgene flow will likewise have little or no detrimental effects on the genetic diversity of wild populations; however, frequent migration of transgenes for greatly increased fitness could have a significant impact on rare native genes in the world's centers of diversity. A center of diversity harbors most of the natural genetic resources for a given crop and is a region in which wild relatives of a crop exist in nature. These centers are vital resources for plant breeders seeking to improve crop plants. The impact of new transgenes on such centers should be fully investigated before transgenic crops are grown near their own center of diversity.

Impact and Applications

The risks associated with genetically modified organisms have been both overstated and understated. Proponents of biotechnology have downplayed likely problems while opponents have exaggerated the risks of the unknown. As with any new technology, there will be unforeseen problems; however, as long as transgenic organisms are scientifically and objectively evaluated on a case-by-case basis prior to release or use, society should be able to avoid the obvious or most likely problems associated with biotechnology and benefit from its application.

—Paul C. St. Amanda

See also: Animal Cloning; Biofertilizers; Biological Weapons; Biopesticides; Biopharmaceuticals; Cloning; Cloning: Ethical Issues; Cloning Vectors; DNA Replication; DNA Sequencing Technology; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering;

Medical Applications; Genetic Engineering; Social and Ethical Issues; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Polymerase Chain Reaction; Restriction Enzymes; Reverse Transcriptase; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Web Sites of Interest

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The Edmonds Institute. <http://www.edmonds-institute.org>. A site that offers "A Brief History of Biotechnology Risk Debates and Policies in the United States" and "Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms."

Genetic Engineering: Social and Ethical Issues

Fields of study: Bioethics; Genetic engineering and biotechnology; Human genetics and social issues

Significance: *New technologies for manipulating the genetic makeup of living organisms raise serious questions about the social desirability of controlling genes and the moral right of humans to redesign living beings.*

Key terms

BIODIVERSITY: the presence of a wide variety of forms of life in an environment

BIOTECHNOLOGY: the technological manipulation of living organisms; genetic engineering is the most common form of biotechnology

RECOMBINANT DNA: a new combination of genes spliced together on a single piece of DNA; recombinant DNA is the basis of genetic engineering technology

TRANSGENIC ORGANISM: a organism into which the DNA of another species has been inserted

Genetic Engineering as a Social and Ethical Problem

English author Mary Shelley's 1818 horror novel *Frankenstein*, about a scientist who succeeds in bringing a creature to life, expressed anxiety about the possibility of human control over the basic mysteries of existence. The novel's continuing popularity and the many films and other works based on it attest to deep-seated feelings that unrestrained science may violate essential principles of nature and religion and that human powers may grow to exceed human wisdom. With the rise of genetic engineering in the 1970's, many serious philosophers and social critics feared that the Frankenstein story was moving from the realm of science fiction into reality.

The basic blueprint of all living beings was found in 1953, when Francis Crick and James Watson discovered the structure of DNA. A little less than two decades later, in 1970, it became possible to conceive of redesigning this

blueprint when Hamilton Smith and Daniel Nathans of The Johns Hopkins University discovered a class of "restriction" enzymes that could be used as scissors to cut DNA strands at specific locations. In 1973, two researchers in California, Stanley Cohen and Herbert Boyer, spliced recombinant DNA strands into bacteria that reproduced copies of the foreign DNA. This meant that it would be possible to combine genetic characteristics of different organisms. In 1976, Genentech in San Francisco, California, became the first corporation formed to develop genetic engineering techniques for commercial purposes.

By the 1990's, genetic engineering was being used on plants, animals, and humans. The Flavr Savr tomato, the first genetically modified (GM) food to be approved by the U.S. government, was developed when biotechnologists inserted a gene that delayed rotting in tomatoes. Transgenic animals (containing genes from humans and other animals) became commonplace in laboratories by the middle of the 1990's. The year 1990 saw the first successful use of genetic engineering on humans, when doctors used gene therapy to treat two girls suffering from an immunodeficiency disease. The long-felt discomfort over scientific manipulation of life, the suddenness of the development of the new technology, and the application of the technology to humans all combined to make many people worry about the social and ethical implications of genetic engineering. The most serious concerns were over genetic manipulation of humans, but some critics also pointed out possible problems with the genetic engineering of plants and animals.

Engineering of Plants and Animals

According to a Harris Poll survey conducted for the U.S. Office of Technology in the fall of 1968, a majority of Americans were not opposed to using recombinant DNA techniques to produce hybrid agricultural plants. Some social critics, such as Jeremy Rifkin, have argued that such ready acceptance of the genetic engineering of plants is shortsighted. These critics question the wisdom of intervening in the ecological balance of nature. More specifically, they maintain that manipulating the genetic

structure of plants tends to lead to a reduction in the diversity of plant life, making plants less resistant to disease. It could also lead to the spread of diseases from one plant species to another, as genes of one species are implanted in another. Furthermore, new and unnatural varieties of food plants could have unforeseen health risks for human beings.

Since genetic engineering is a highly technical procedure, those who control technology have great power over the food supply. Thus, both corporate power over consumers and the power of more technologically advanced nations over less technologically advanced nations could be increased as GM foods fill the marketplace. In addition, plants that are genetically engineered to produce more often re-

quire more fertilizer and greater amounts of irrigated water than ordinary plants. The technology would therefore serve the interests of corporate agribusiness at the expense of small-scale, low-income farmers.

Many of the concerns about the genetic engineering of animals are similar to those about the engineering of plants. Loss of biodiversity, vulnerability to disease, and business control over livestock are all frequently mentioned objections to the genetic manipulation of animals. Moral issues tend to become more important, though, when opponents of genetic engineering discuss its use with animals. Many religious beliefs hold that the order of the world, including its division into different types of creatures, is divinely ordained. From the perspective of such beliefs, the relatively common experimental practice of injecting human growth genes into mice could be seen as the sacrilegious creation of monsters. Opponents of the genetic alteration of animals argue, further, that animals will suffer. They point out that selective breeding, a slow process, has led to about two hundred diseases of genetic origin in purebred dogs. Genetic engineering brings about change much faster than breeding, increasing the probability of genetic diseases.

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In Battle Creek, Michigan, a demonstration outside the headquarters of Kellogg highlights the company's use of genetically altered crops without labeling. Those in favor of labeling the use of genetically altered ingredients maintain that the public has a right to know about the use of such ingredients so they can make informed purchase decisions. (AP/Wide World Photos)

Engineering of Humans

Some of the greatest ethical and social problems with genetic engineering involve its use on humans. Gene therapy seeks to cure inherited diseases by altering the defective genes that cause them. Those who favor gene therapy maintain that it can be a powerful tool to overcome human misery. Those who oppose this type of medical procedure usually focus on three major ethical issues. First, critics maintain that this technology raises the problem of ownership of human life. In the early 1990's, the National Institutes of Health (NIH) began filing for patents on human genes, meaning that the blueprints for human life could actually be owned. Because all human DNA comes from human tissue, the question arises of whether participants in genetic experiments own their own DNA or it belongs to the researchers who have extracted it.

The second problem involves eugenic impli-

cations. Eugenics is the practice of trying to produce "better" humans. If scientists can alter genes to produce humans with more desirable health characteristics, then scientists can also alter genes to produce humans with more desirable characteristics of personality or physical appearance. In this way, genetic engineering poses the risk of becoming an extreme and highly technological form of discrimination. The third problem is related to both of the first two: the reduction of humans to objects. When human life becomes something that can be owned and redesigned at will, some ethicists claim, it will cease to be seen as a sacred mystery and will become simply another piece of biological machinery. As objects, people will gradually lose the philosophical justification for their political and moral rights.

Impact and Applications

Concerns about the social and ethical implications of genetic engineering have led to a number of attempts to limit or control the technology. The environmental group Greenpeace has campaigned against GM agricultural products and called for the clear labeling of all foods produced by genetic manipulation. In September, 1997, Greenpeace filed a legal petition against the U.S. Environmental Protection Agency (EPA), objecting to the EPA's approval of GM plants.

Activist Jeremy Rifkin became one of the most outspoken opponents of all forms of genetic engineering. Rifkin and his associates called on the U.S. NIH to stop government-funded transgenic animal research. A number of organizations, such as the Boston-based Council for Responsible Genetics (CRG), lobbied to increase the legal regulation of genetic engineering. In 1990, in response to pressure from critics of genetic engineering, the Federal Republic of Germany enacted a gene law to govern the use of biotechnology. In the United States, the federal government and many state governments considered laws regarding genetic manipulation. A 1995 Oregon law, for example, granted ownership of human tissue and genetic information taken from human tissue to the person from whom the tissue was taken.

—Carl L. Bankston III

See also: Animal Cloning; Biological Weapons; Biopharmaceuticals; Cloning: Ethical Issues; Eugenics; Cloning Vectors; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetically Modified (GM) Foods; High-Yield Crops; Knockout Genetics and Knockout Mice; Organ Transplants and HLA Genes; Paternity Tests; Patents on Life-Forms; Shotgun Cloning; Synthetic Genes; Transgenic Organisms; Xenotransplants.

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Evans, John Hyde. *Playing God? Human Genetic Engineering and the Rationalization of Public Bioethical Debate*. Chicago: University of Chicago Press, 2002. Chapters include "Framework for Understanding the Thinning of a Public Debate," "The Eugenicists and the Challenge from the Theologians," "Gene Therapy, Advisory Commissions, and the Birth of the Bioethics Profession," and "The President's Commission: The 'Neutral' Triumph of Formal Rationality."

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Reiss, Michael J., and Roger Straughan, eds. *Improving Nature? The Science and Ethics of Genetic Engineering*. New York: Cambridge University Press, 2001. Elucidates the ethical issues surrounding genetic engineering for the nonbiologist. Chapters examine genetic engineering in microorganisms, plants, animals, and humans.

Rifkin, Jeremy. *The Biotech Century: Harnessing the Gene and Remaking the World*. New York: Putnam, 1998. One of the best-known critics of biotechnology warns that procedures such as cloning and genetic engineering could be disastrous for the gene pool and for the natural environment.

Yount, Lisa, ed. *The Ethics of Genetic Engineering*. San Diego: Greenhaven Press, 2002. Essays written by scientists, science writers, ethicists, and consumer advocates present the growing controversy over genetically modi-

fying plants and animals, altering human genes, and cloning humans.

Veatch, Robert M. *The Basics of Bioethics*. 2d ed. Upper Saddle River, N.J.: Prentice Hall, 2003. In a textbook designed for students, Veatch presents an overview of the main theories and policy questions in biomedical ethics. Includes diagrams, case studies, and definitions of key concepts.

Web Sites of Interest

American Medical Association. <http://ama-assn.org>. The AMA has posted its guidelines on the ethics of genetic engineering.

Council for Responsible Genetics. <http://www.gene-watch.org>. An organization that encourages debate on issues concerning genetic technologies.

National Information Resource on Ethics and Human Genetics. <http://www.georgetown.edu/research/nrcbl/nirehg>. Site supports links to databases, annotated bibliographies, and articles about the ethics of genetic engineering and human genetics.

Genetic Load

Field of study: Population genetics

Significance: *Genetic load is a measure of the number of recessive deleterious (lethal or sublethal) alleles in a population. These alleles are maintained in populations at equilibrium frequencies by mutation (which introduces new alleles into the gene pool) and selection (which eliminates unfavorable alleles from the gene pool). Genetic load is one of the causes of inbreeding depression, the reduced viability of offspring from closely related individuals. For this reason, genetic load is a primary concern in the fields of agriculture, animal husbandry, conservation biology, and human health.*

Key terms

DELETERIOUS ALLELES: alternative forms of a gene that, when expressed in the homozygous condition in diploid organisms, may be lethal or sublethal—in the latter case typically resulting in an aberrant phenotype with low fitness

INBREEDING DEPRESSION: reduced fitness of an individual or population arising as the result of decreased heterozygosity across loci

Genetic Load in Diploid Populations

Genetic diversity is a measure of the total number of alleles within a population and it is mutation, the ultimate source of all genetic variation, that gives rise to new alleles. Favorable mutations are rare and are greatly outnumbered by mutations that are selectively neutral or deleterious (that is, lethal or sublethal). In diploid organisms most mutant (deleterious) alleles are hidden from view because they are masked by a second, normal, or wild-type, allele; that is they are typically (but not always) recessive. On the other hand, in haploid organisms lethal and deleterious genes are immediately exposed to differential selection.

Genetic load is defined as an estimate of the number of deleterious alleles in a population. Total genetic load is therefore the sum of two major components, the lethal load (L) and the detrimental, but nonlethal load (D). Empirical and theoretical studies suggest detrimental alleles rather than lethals constitute the greater majority of the genetic load in natural populations. When expressed in the homozygous condition, the primary effect of deleterious alleles within the gene pool on individuals is straightforward: death or disability accompanied by lower fitness. However, the impact of lethal and sublethal alleles on the mean fitness of populations, as opposed to individuals, is dependent upon many factors, such as their frequency within the gene pool, the number of individuals in the population, and whether or not those individuals are randomly mating.

How and why are recessive alleles maintained within a population at all? Why are they not eliminated by natural selection? First, recessive deleterious alleles must obtain a sufficient frequency before homozygous individuals occur

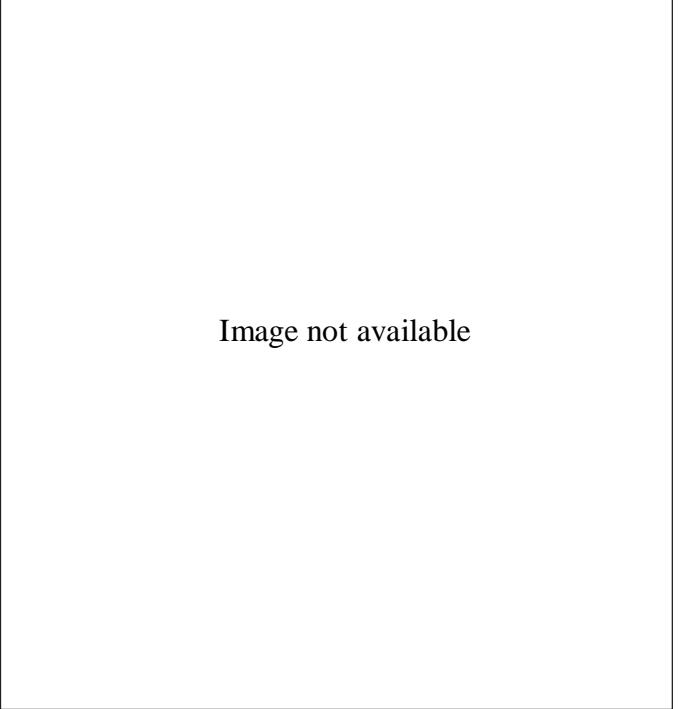


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The April 25, 1986, accident at the Chernobyl nuclear power plant in the Ukraine released 5 percent of the radioactive reactor core into the atmosphere, contaminating large areas of Belarus, Ukraine, and Russia and quite possibly increasing genetic loads in affected populations. (AP/Wide World Photos)

in sufficient number to be detected. Second, in some situations recessive alleles that are deleterious or lethal in the homozygous state are advantageous in heterozygotes. Third, new deleterious alleles are constantly introduced into the population by mutation or are reintroduced by back mutation. Finally, the rate at which deleterious genes are purged from the population critically depends upon the “cost of selection” against them, and selection coefficients may vary considerably depending upon the allele and intra- or extracellular environments. In large randomly mating diploid populations, genetic load theoretically reaches an “equilibrium value” maintained by a balance between the mutation rate and the strength of selection. Finally, it should be borne in mind that non-lethal alleles that are not advantageous under present circumstances nevertheless constitute a pool of alleles that may be advantageous in a different (or changing) environment or in a

different genetic background. In other words, some neutral and nonlethal mutations may have unpredictable “remote consequences.”

Population Size, Inbreeding, and Genetic Load

As it is used among population geneticists, genetic load is most appropriately defined as the proportionate decrease in the average fitness of a population relative to that of the optimal genotype. The “proportionate decrease in the average fitness” is, of course, due to the presence of lethal and deleterious nonlethal alleles that are maintained in equilibrium by mutation and selection. Genetic load within populations may be substantially increased under certain circumstances. Small populations, species whose mating system involves complete or partial inbreeding, and populations with increased mutation rates all are expected to accumulate load at values exceeding that of large outbreeding populations. Small populations face multiple genetic hazards, including inbreeding depression.

Inbreeding decreases heterozygosity across loci and, relative to randomly mating populations, the fitness of inbred individuals is typically depressed. Inbreeding causes rare recessive alleles to occur more frequently in the homozygous condition, increasing the frequency of aberrant phenotypes that are observed. Complete or partial inbreeding (or, in plants, self-fertilization) leads to the accumulation of deleterious mutations that increase genetic load. Paradoxically, continued inbreeding results in lower equilibrium frequencies of deleterious alleles because they are expressed with greater frequency in the homozygous state. Thus, inbreeding populations may eliminate, or “purge,” some proportion of their genetic load via selection against deleterious recessive alleles. Nevertheless it is true that, compared to large genetically diverse populations, small inbred populations with reduced genetic diversity are more likely to go extinct. For these reasons, population sizes, inbreeding, and genetic load are among the primary concerns of conservation biologists working to ensure the survival of rare or endangered species.

As previously mentioned, increased muta-

tion rates may also increase genetic load. For example, the rate of nucleotide substitution in mammalian mitochondrial DNA (mtDNA) is nearly ten times that of nuclear DNA. The ten-fold mutation rate difference is postulated to be due to highly toxic, mutagenic reactive oxygen species produced by the mitochondrial electron transport chain and/or relatively inefficient DNA repair mechanisms. Thus, mitochondrial genomes accumulate fixed nucleotide changes rapidly via “Müller’s ratchet.” Mutation rates and genetic load may also be increased by exposure to harmful environments. For example, an accident on April 25, 1986, at the Chernobyl nuclear power plant in the Ukraine released 5 percent of the radioactive reactor core into the atmosphere, contaminating large areas of Belarus, Ukraine, and Russia. Radiation exposure of this kind and toxic chemicals (such as heavy metals) in watersheds pose significant human health risks that, over time, may be associated with increased genetic loads in affected populations.

—J. Craig Bailey

See also: Consanguinity and Genetic Disease; Hardy-Weinberg Law; Heredity and Environment; Inbreeding and Assortative Mating; Lateral Gene Transfer; Natural Selection; Pedigree Analysis; Polyploidy; Population Genetics; Punctuated Equilibrium; Quantitative Inheritance; Sociobiology; Speciation.

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Wallace, Bruce. *Genetic Load: Its Biological and Conceptual Aspects*. Englewood Cliffs, N.J.: Prentice-Hall, 1970. This 116-page treatise provides an introduction to the concept of genetic load in individuals and populations and discusses how genetic load is calculated. It also provides a discussion of how the interplay among mutation rates, selection, and inbreeding influences the dynamics of genetic load within populations.

Genetic Screening

Field of study: Human genetics and social issues

Significance: *Genetic screening is a preventive health measure that involves the mandatory or voluntary testing of certain individuals for the purpose of detecting genetic disorders or identifying defective genes that can be transmitted to offspring. The primary goals of genetic screening include the prevention and/or treatment of genetic disorders and the option to make informed and rational decisions about conception and birth. It has raised concerns about confidentiality, discrimination, and the right to privacy.*

Key terms

ALLEL: a form of a gene at a locus; each locus in an individual's chromosomes has two alleles, which may be the same or different

CARRIER: a healthy individual who has one normal allele and one defective allele at the same gene locus

GENETIC DISORDER: a disorder caused by a change in a gene or chromosome

INBORN ERROR OF METABOLISM: an inherited disease caused by a mutation in a gene that codes for an enzyme important in a metabolic pathway

LOCUS (*pl. LOCI*): the actual location of a gene on a chromosome

Neonatal Screening

The most widespread use of genetic screening is the testing of newborn babies, called neonatal screening. Every year, millions of newborn babies are tested for inborn errors of metabolism.

The purpose of this kind of screening is to provide immediate treatment after birth if a defect is detected, so that the newborn has a chance of having a normal life. A classic example of neonatal screening in the United States and many countries is the mandatory mass screening of newborn babies for phenylketonuria (PKU), a disorder that causes irreversible brain damage when not treated. Individuals with PKU lack the enzyme phenylalanine hydroxylase, which converts the essential amino acid phenylalanine into the amino acid tyrosine. PKU results in accumulation of phenylalanine in the body, which causes the brain damage.

Newborn babies are screened for PKU using the Guthrie test, named after its inventor, Robert Guthrie. The Guthrie test detects high levels of phenylalanine in the blood of newborns. Blood samples are taken from the heels of newborn babies in the hospital nursery, placed on

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Many states mandate that newborns be screened for phenylketonuria (PKU), hypothyroidism, and, in some cases, other inborn errors of metabolism such as galactosemia. (AP/Wide World Photos)

filter papers as dried spots, and sent off to appropriate laboratories for analysis. Newborns with positive results can be effectively treated with a diet low in phenylalanine (low-protein foods). The level of phenylalanine in the blood is regularly monitored. If treatment is not initiated within the first two months of life, irreversible brain damage will occur.

In the United States, only one other neonatal test is performed routinely in all states: for hypothyroidism. There is no federal mandate for which tests must be provided to everyone, so each state decides independently. Many states also screen for galactosemia, an inherited disorder characterized by seizures, mental retardation, vomiting, and liver disease, caused by the accumulation of galactose in the blood. Many states also screen for maple syrup urine disease, homocystinuria, and biotinidase deficiency. A majority of states also screen for sickle-cell disease, an inherited blood disorder characterized by anemia, pain in the abdomen and joints, and damage to organs.

Carrier Screening

Carrier screening is the voluntary testing of healthy individuals of reproductive age who may be carriers of heritable, defective genes, the purpose being to inform couples of their risk of having a child with a genetic disorder. In the United States, screening has been limited to some ethnic groups known to have a high incidence of a specific genetic disorder. In the 1970's, for example, Tay-Sachs screening of Ashkenazi Jews of reproductive age was successfully implemented. Tay-Sachs disease is an inherited, progressive disease in infants characterized by a startle response to noise, blindness, seizures, paralysis, and death in infancy caused by the absence of an enzyme called hexosaminidase A. Tay-Sachs screening measures the level of hexosaminidase A in the blood. People with Tay-Sachs disease have no detectable level of the enzyme, while carriers have a below-normal level of the enzyme.

In the early 1970's, mandatory, large-scale screening of African American couples and some schoolchildren was implemented in an effort to identify carriers of the gene for sickle-cell disease. Blood samples taken from individ-

uals were tested for the presence of distorted or sickled-shaped red blood cells caused by the production of abnormal hemoglobin, the molecule that transports oxygen in the body. The laws mandating screening were later repealed amid charges of racial discrimination.

After successful identification of the cystic fibrosis gene in 1989, the scientific and medical community began debating the costs and benefits of screening millions of carriers of the gene in the United States. Cystic fibrosis is a common inherited disorder characterized by accumulation of mucus in the lungs and pancreas; it affects Caucasian children and young adults. The proportion of carriers in the population varies by ethnicity. Northern Europeans have the highest proportion of carriers (1 in 22), while African Americans have a much lower proportion (1 in 65). Some companies have begun voluntary screening for the cystic fibrosis gene in couples with a family history of the disorder.

Impact and Applications

The benefits of genetic screening include early intervention and treatment, detection of new mutations by researchers, and the education of people about genetic disorders so that they are able to make informed and responsible decisions about reproductive issues. However, screening for genetic defects has raised ethical and social issues over confidentiality, discrimination, and the right to privacy.

One example is the sickle-cell screening program of the early 1970's. Screening results were not kept in strictest confidence; consequently, many healthy African Americans who were carriers of the sickle-cell gene were stigmatized and discriminated against in terms of employment and insurance coverage. There were also charges of racial discrimination because carriers were advised against bearing children.

Although the sickle-cell disease screening programs were unsuccessful, there were some successes in other screening programs. For example, the number of newborns with Tay-Sachs disease has dropped dramatically as a result of carrier screening programs. The screening programs succeeded because of the tremendous effort put forth to educate Ashkenazi Jewish communities about the disorder and the conse-

quent acceptance of the programs by these communities.

Genetic screening may be even more useful in the future, when genetic disorders that are currently incurable might be treated by gene therapy (the replacement of a defective or missing gene with a normal, functional copy of the gene). The first clinical trials of gene therapy began in 1990 for severe combined immunodeficiency disorder (SCID), a lethal genetic disorder in which a person has no functional immunity. Much attention has been focused on gene therapy as a significant treatment option for patients with disorders such as cystic fibrosis. So far, gene therapy has garnered mixed results, and much progress will be needed before it becomes possible, much less routinely feasible. However, although it may well be decades or more before that occurs, future generations may well enjoy the results of today's research.

—Oluwatoyin O. Akinwunmi

See also: Amniocentesis and Chorionic Villus Sampling; Bioethics; Cystic Fibrosis; Down Syndrome; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Human Genetics; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Insurance; Linkage Maps; Phenylketonuria (PKU); Prenatal Diagnosis; Sickle-Cell Disease; Tay-Sachs Disease.

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Roberts, Leslie. "To Test or Not to Test?" *Science* 247 (January, 1990). Although a bit dated, outlines well the basic issues surrounding cystic fibrosis screening.

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Web Sites of Interest

American Medical Association. <http://ama-assn.org>. The AMA's guidelines on the ethics of genetic screening.

Centers for Disease Control, Genomics and Disease Prevention. <http://www.cdc.gov/genomics/info/reports/program/population.htm>. A journal article on genetic screening, entitled "Population Screening in the Age of Genomic Medicine."

Genetic Testing

Field of study: Human genetics and social issues

Significance: *Genetic testing comprises any procedure used to detect the presence of a genetic disorder or a defective gene in a fetus, newborn, or adult. The results of genetic tests can be useful in family planning, treatment decisions and medical research. Genetic testing has significant implications with respect to reproductive choices, privacy, insurance coverage, and employment.*

Key terms

GENETIC DISORDER: a disorder caused by a mutation in a gene or chromosome

GENETIC MARKER: a distinctive DNA sequence that shows variation in the population and can therefore potentially be used for identification of individuals and for discovery of disease genes

Prenatal Diagnosis

Prenatal diagnosis is the testing of a developing fetus in the womb, or uterus, for the presence of a genetic disorder. The purpose of this type of genetic testing is to inform a pregnant woman of the chances of having a baby with a genetic disorder. Prenatal diagnosis is limited to high-risk individuals and is usually recommended only if a woman is thirty-five years of age or older, if she has had two or more spontaneous abortions, or if there is a family history of a genetic disorder. Hundreds of genetic disorders can be tested in a fetus. One of the most common genetic disorders screened for is Down syndrome, or trisomy 21, a form of mental retardation caused by having an extra copy of chromosome 21. The incidence of Down syndrome increases sharply in children born to women over the age of forty.

The technique most commonly used for prenatal diagnosis is amniocentesis. It is performed between the sixteenth and eighteenth week of pregnancy. Amniocentesis involves the insertion of a hypodermic needle through the abdomen into the uterus of a pregnant woman. The insertion of the needle is guided by ultrasound, a technique that uses high-frequency sound waves to locate a developing fetus or internal organs and presents a visual image on a video monitor. A small amount of amniotic fluid, which surrounds and protects the fetus, is withdrawn. The amniotic fluid contains fetal secretions and cells sloughed off the fetus that are analyzed for genetic abnormalities. Chromosomal disorders such as Down syndrome, Edwards' syndrome (trisomy 18), and Patau syndrome (trisomy 13) can be detected by examining the chromosome number of the fetal cells. Certain biochemical disorders such as Tay-Sachs disease, a progressive disorder characterized by a startle response to sound, blindness, paralysis, and death in infancy, can be determined by testing for the presence or absence of a specific enzyme activity in the amniotic fluid. Amniocentesis can also determine the sex of a fetus and detect common birth defects such as spina bifida (an open or exposed spinal cord) and anencephaly (partial or complete absence of the brain) by measuring levels of alpha fetoprotein in the amniotic fluid. The limitations of amniocentesis include inability to detect most genetic disorders, possible fetal injury or death, infection, and bleeding.

Chorionic villus sampling (CVS) is another technique used for prenatal diagnosis. It is performed earlier than amniocentesis (between the eighth and twelfth week of pregnancy). Under the guidance of ultrasound, a catheter is inserted into the uterus via the cervix to obtain a sample of the chorionic villi. The chorionic villi are part of the fetal portion of the placenta, the organ that nourishes the fetus. The chorionic villi can be analyzed for chromosomal and biochemical disorders but not for congenital birth defects such as spina bifida and anencephaly. The limitations of this technique are inaccurate diagnosis and a slightly higher chance of fetal loss than in amniocentesis.

Neonatal Testing

The most widespread genetic testing is the mandatory testing of every newborn infant for the inborn error of metabolism (a biochemical disorder caused by mutations in the genes that code for the synthesis of enzymes) phenylketonuria (PKU), a disorder in which the enzyme for converting phenylalanine to tyrosine is nonfunctional. The purpose of this type of testing is to initiate early treatment of infants. Without treatment, PKU leads to brain damage and mental retardation. A blood sample is taken by heel prick from a newborn in the hospital nursery, placed on filter papers as dried spots, and subsequently tested, using the Guthrie test, for abnormally high levels of phenylalanine. In infants who test positive for PKU, a diet low in phenylalanine is initiated within the first two months of life. Newborns can be tested for many other disorders such as sickle-cell disease and galactosemia (accumulation of galactose in the blood), but the cost benefit ratio is only acceptable in the more common genetic diseases, and most tests are only per-

formed if there is a family history of the genetic disease or some other reason to suspect its presence.

Carrier Testing

A healthy couple contemplating having children can be tested voluntarily to determine if they carry a defective gene for a disorder that runs in the family. This type of testing is known as carrier testing because it is designed for carriers (individuals who have a normal gene paired with a defective form of the same gene but have no symptoms of a genetic disorder). Carriers of the genes responsible for Tay-Sachs disease, cystic fibrosis (accumulation of mucus in the lungs and pancreas), Duchenne muscular dystrophy (wasting away of muscles), and hemophilia (uncontrolled bleeding caused by lack of blood clotting factor) can be detected by DNA analysis.

When the gene responsible for a specific genetic disorder is unknown, the location of the gene on a chromosome can be detected indirectly by linkage analysis. Linkage analysis is a

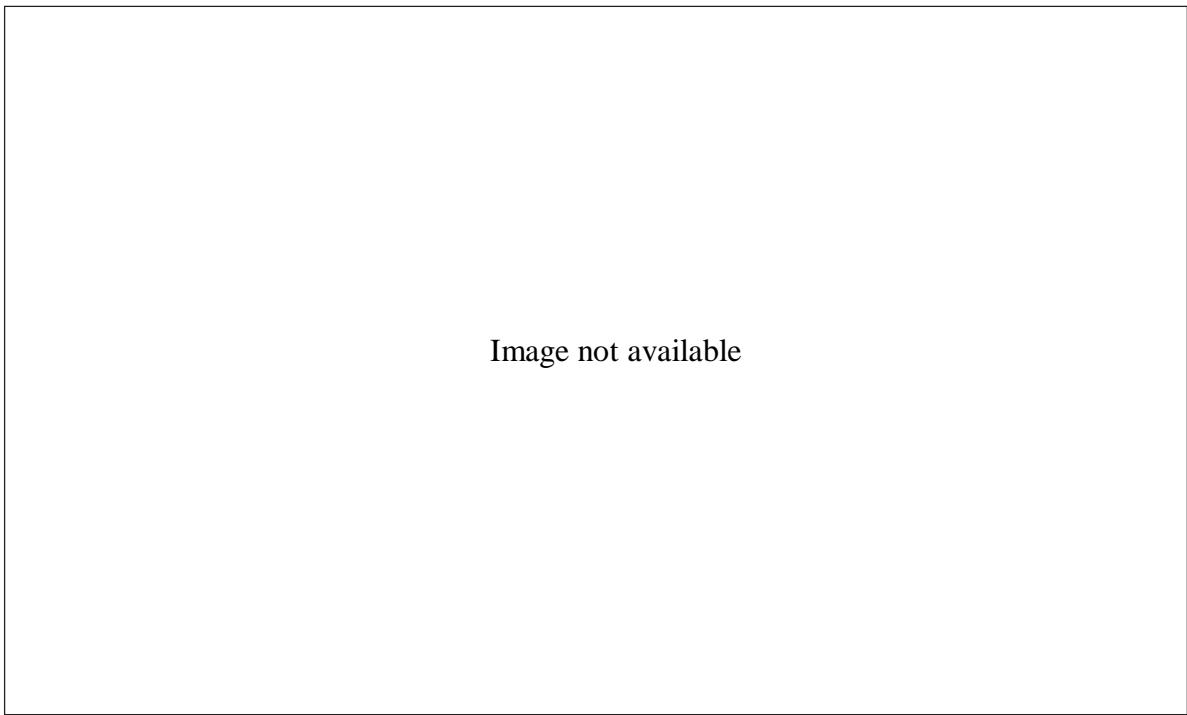


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DNA samples from patients are removed by an eight-needle apparatus and deposited into a tray for genetic testing at Myriad Genetics in Salt Lake City. (AP/Wide World Photos)

technique in which geneticists look for consistent patterns in large families where the mutated gene and a genetic marker always appear together in affected individuals and those known to be carriers. If a genetic marker lies close to the defective gene, it is possible to locate the defective gene by looking for the genetic marker. The genetic markers used commonly for linkage analysis are restriction fragment length polymorphisms (RFLPs). When human DNA is isolated from a blood sample and digested at specific sites with special enzymes called restriction endonucleases, RFLPs are produced. RFLPs are found scattered randomly in human DNA and are of different lengths in different people, except in identical twins. They are caused by mutations or the presence of varying numbers of repeated copies of a DNA sequence and are inherited. RFLPs are separated by gel electrophoresis, a technique in which DNA fragments of varying lengths are separated in an electric field according to their sizes. The separated DNA fragments are blotted onto a nylon membrane, a process known as Southern blotting. The membrane is probed and then visualized on X-ray film. The characteristic pattern of DNA bands visible on the film is similar in appearance to the bar codes on grocery items.

An early successful example of linkage analysis involved the search for the gene that causes Huntington's disease, an always fatal neurological disease that typically shows onset after 35 or 40 years of age. In 1983, James Gusella, Nancy Wexler, and Michael Conneally reported a correlation between one specific RFLP they named *G8* and Huntington's disease (Huntington's chorea). After studying numerous RFLPs of generations of an extended Venezuelan family with a history of Huntington's disease, they discovered that *G8* was present in members afflicted with the genetic disorder and was absent in unaffected members.

High-risk individuals or families can be tested voluntarily for the presence of a mutated gene that may indicate a predisposition to a late-onset genetic disorder such as Alzheimer's disease or to other conditions such as hereditary breast, ovarian, and colon cancers. This type of testing is called predictive testing. Un-

like tests for many of the inborn errors of metabolism, predictive testing can only give a rough idea of how likely an individual may be to develop a particular genetic disease. It is not always clear how such information should be used, but at least in some cases lifestyle or therapeutic changes can be instituted to lessen the likelihood of developing the disease.

Impact and Applications

Genetic testing has had a significant impact on families and society at large. It provides objective information to families about genetic disorders or birth defects and provides an analysis of the risks for genetic disorders through genetic counseling. Consequently, many prospective parents are able to make informed and responsible decisions about conception and birth. Some choose not to bear children, some terminate pregnancy after prenatal diagnosis, and some take a genetic gamble and hope for a normal child. Genetic testing can have a profound psychological impact on an individual or family. A positive genetic test could cause a person to experience depression, while a negative test result may eliminate anxiety and distress. Questions have been raised in the scientific and medical community about the reliability and high costs of tests. There is concern about whether genetic tests are stringent enough to ensure that errors are not made. DNA-based diagnosis can lead to errors if DNA samples are contaminated. Such errors can be devastating to families. People at risk for late-onset disorders such as Huntington's disease can be tested to determine if they are predisposed to developing the disease. There is, however, controversy over whether it is ethical to test for diseases for which there are no known cures or preventive therapies. The question of testing also creates a dilemma in many families. Unlike other medical tests, predictive testing involves the participation of many members of a family. Some members of a family may wish to know their genetic status, while others may not.

While there has been great enthusiasm over genetic testing, there are also social, legal, and ethical issues such as discrimination, confidentiality, reproductive choice, and abuse of genetic information. Insurance companies and

employers may require prospective customers and employees to submit to genetic testing or may inquire about a person's genetic status. Individuals may be denied life and health insurance coverage because of their genetic status, or a prospective customer may be forced to pay exorbitant insurance premiums. The potential for discrimination with respect to employment and promotions also exists. For example, as a result of the sickle-cell screening programs of the early 1970's, many African Americans with sickle-cell disease were denied employment and insurance coverage and some were denied entry into the U.S. Air Force. The Americans with Disabilities Act, signed into federal law in 1990, contained provisions safeguarding employees from genetic discrimination by employers. By 1994, companies with fifteen or more employees had to comply with the law, which prohibits employment discrimination because of genetic status and also prohibits genetic testing by employers.

As genetic testing becomes standard practice, the potential for misuse of genetic tests and genetic information will become greater. Prospective parents may potentially use prenatal diagnosis as a means to ensure the birth of a "perfect" child. Restriction fragment length polymorphism analysis, used in genetic testing, has applications in DNA fingerprinting or DNA typing. DNA fingerprinting is a powerful tool for identification of individuals used to generate patterns of DNA fragments unique to each individual based on differences in the sizes of repeated DNA regions in humans. It is used to establish identity or nonidentity in immigration cases and paternity and maternity disputes; it is also used to exonerate the innocent accused of violent crimes and to link a suspect's DNA to body fluids or hair left at a crime scene. Several states in the United States have been collecting blood samples from a variety of sources, including newborn infants during neonatal testing and individuals convicted of violent crimes, and have been storing genetic information derived from them in DNA databases for future reference. Such information could be misused by unauthorized people.

—Oluwatoyin O. Akinwunmi,
updated by Bryan Ness

See also: Amniocentesis and Chorionic Villus Sampling; Bioethics; Breast Cancer; Cystic Fibrosis; DNA Fingerprinting; Down Syndrome; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Screening; Genetic Testing: Ethical and Economic Issues; Hemophilia; Hereditary Diseases; Human Genetics; Huntington's Disease; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Insurance; Linkage Maps; Paternity Tests; Phenylketonuria (PKU); Prenatal Diagnosis; RFLP Analysis; Sickle-Cell Disease; Tay-Sachs Disease.

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1999. Gives lay readers insight into basic consumer health information about a range of medical tests. Topics covered include general screening tests, medical imaging tests, genetic testing, newborn screenings, and sexually transmitted disease tests, as well as Medicare, Medicaid, and other information on paying for medical tests.

Web Site of Interest

National Institutes of Health, National Library of Medicine. <http://www.nlm.nih.gov>. This site links to comprehensive information on genetic testing, research, and more. Also available in Spanish.

Genetic Testing: Ethical and Economic Issues

Field of study: Bioethics; Human genetics and social issues

Significance: *Using a suite of molecular, biochemical, and medical techniques, it is now possible to identify carriers of a number of genetic diseases and to diagnose some genetic diseases even before they display physical symptoms. In addition, numerous genes that predispose people to particular diseases such as cancer, alcoholism, and heart disease have been identified. These technologies raise important ethical questions about who should be tested, how the results of tests should be used, who should have access to the test results, and what constitutes normality.*

Key terms

DOMINANT TRAIT: a genetically determined trait that is expressed when a person receives the gene for that trait from either or both parents

RECESSIVE TRAIT: a genetically determined trait that is expressed only if a person receives the gene for the trait from both parents

The Dilemmas of Genetic Testing

Historically, it was impossible to determine whether a person was a carrier of a genetic disease or whether a fetus was affected by a genetic disease. Now both of these things and much

more can be determined through genetic testing. Although there are obvious advantages to acquiring this kind of information, there are also potential ethical problems. For example, if two married people are both found to be carriers of cystic fibrosis, each child born to them will have a 25 percent chance of having cystic fibrosis. Using this information, they could choose not to have any children, or, under an oppressive government desiring to improve the genetics of the population, they could be forcibly sterilized. Alternatively, they could choose to have each child tested prenatally and abort any child that tests positive for cystic fibrosis. Ethical dilemmas similar to these are destined to become increasingly common as scientists develop tests for more genetic diseases.

Another dilemma arises in the case of diseases such as Huntington's disease (Huntington's chorea), which is caused by a single dominant gene and is always lethal but which does not generally cause physical symptoms until middle age or later. A parent with such a disease has a 50 percent chance of passing it on to each child. Now that people can be tested, it is possible for a child to know whether he or she has inherited the deadly gene. If a person tests positive for the disease, he or she can then choose to remain childless or opt for prenatal testing to guard against the possibility of bringing a child into the world under a death sentence.

Tests for deadly, untreatable genetic diseases in offspring have an even darker side. If the test is negative, the person may be greatly relieved; if it is positive, however, doctors can offer no hope. Is it right to let someone know that they will die sometime around middle age or shortly thereafter if there is nothing the medical community can do to help them? The psychological trauma associated with such disclosures can sometimes be severe enough to result in suicide. Additionally, who should receive information about the test, especially if it shows positive for the disease? If the information is kept confidential, a person with the disease could buy large amounts of life insurance, to the financial advantage of beneficiaries, at the same price as an unaffected person. On the other hand, if

health and life insurance companies were allowed to know the results of such tests, they might use the information to refuse insurance coverage of any kind. Finally, none of the genetic tests is 100 percent accurate. There will be occasional false positives and false negatives. With so much at stake, how can doctors and genetic counselors help patients understand the uncertainties?

How Should Genetic Testing Information Be Used?

Scientists are now able to test for more than just specific, prominent genetic defects. Genetic tests are now available for determining potential risks for such things as cancer, alcoholism, Alzheimer's disease, and obesity. A positive result for the alcoholism gene does not mean that a person is doomed to be an alcoholic but rather that they have a genetic tendency toward behavior patterns that lead to alcoholism or other addictions. Knowing this, a person can then seek counseling, as needed, to prevent alcoholism and make lifestyle decisions to help prevent alcohol abuse.

Unfortunately, a positive test for genes that predispose people to diseases such as cancer may be more ominous. It is believed that people showing a predisposition can largely prevent the eventual development of cancer with aggressive early screening (for example, breast exams and colonoscopies) and lifestyle changes. Some pre-emptive strategies, however, have come under fire. For example, some women at risk for breast cancer have chosen prophylactic mastectomies. In some cases, however, cancer still develops after a mastectomy, and some studies have shown lumpectomy and other less radical treatments to be as effective as mastectomy.

Another concern centers on who should have access to the test results. Should employers be allowed to require genetic testing as a screening tool for hiring decisions? Should insurance companies have access to the records when making policy decisions? These are especially disturbing questions considering the fact that a test for one of the breast cancer genes, for example, only predicts a significantly higher probability of developing breast cancer than is typical for the general population. Making such

testing information available to employers and insurance companies would open the door to discrimination based on the probability that a prospective employee or client will become a future financial burden. A number of states have banned insurance companies from using genetic testing data for this very reason.

Impact and Applications

The long track record and accuracy of some tests, such as the tests for cystic fibrosis and Tay-Sachs disease, has led to the suggestion that they could be used to screen the general population. Although this would seem to provide positive benefits to the population at large, there is a concern about the cost of testing on such a broad scale. Would the costs of testing outweigh the benefits? What other medical needs might not receive funding if such a program were started? The medical community will have to consider the options carefully before more widespread testing takes place.

As more genetic tests become available, it will eventually be possible to develop a fairly comprehensive genetic profile for each person. Such profiles could be stored on CD-ROMs or other storage devices and be used by individuals, in consultation with their personal physicians, to make lifestyle decisions that would counteract the effects of some of the defects in their genetic profiles. The information could also be used to determine a couple's genetic compatibility before they get married. When a woman becomes pregnant, a prenatal genetic profile of the fetus could be produced; if it does not match certain minimum standards, it could be aborted. The same genetic profile could be used to shape the child's life and help determine the child's profession. Although such comprehensive testing is now prohibitively expensive, the costs should drop as the tests are perfected and made more widely available.

Access to genetic profiles by employers, insurance companies, advertisers, and law enforcement agencies could result in considerable economic savings to society, allowing many decisions to be made with greater accuracy, but at what other costs? How should the information be used? How should access be limited? How much privacy should individuals have with

regard to their own genetic profiles? As genetic testing becomes more widespread, these questions will need to be answered. Ultimately, the relationship between the good of society and the rights of the individual will need to be redefined.

—Bryan Ness

See also: Amniocentesis and Chorionic Villus Sampling; Bioethics; Breast Cancer; Cystic Fibrosis; DNA Fingerprinting; Down Syndrome; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Screening; Genetic Testing; Hemophilia; Hereditary Diseases; Human Genetics; Huntington's Disease; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Insurance; Linkage Maps; Paternity Tests; Phenylketonuria (PKU); Prenatal Diagnosis; RFLP Analysis; Sickle-Cell Disease; Tay-Sachs Disease.

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Web Sites of Interest

American Medical Association. <http://ama-assn.org>. The AMA's guidelines on the ethics of genetic testing and screening of adults and children and of multiplex testing.

National Information Resource on Ethics and Human Genetics. <http://www.georgetown.edu/research/nrcbl/nirehg>. Site supports links to databases, annotated bibliographies, and articles about the ethics of genetic testing and human genetics.

Genetically Modified (GM) Foods

Field of study: Genetic engineering and biotechnology

Significance: *Genetically modified foods are produced through the application of recombinant DNA technology to crop breeding, whereby genes from the same or different species are transferred and expressed in crops that do not naturally harbor those genes. While GM crops offer great potential for food production in agriculture, their release has spurred various concerns among the general public.*

Key terms

Bacillus thuringiensis (Bt) toxin: a toxic compound naturally synthesized by bacterium *Bacillus thuringiensis*, which kills insects

GENETIC ENGINEERING: the manipulation of genetic material for practical purposes; also referred to as recombinant DNA technology, gene splicing, or biotechnology

GENETICALLY MODIFIED ORGANISMS (GMOs): genetically modified organisms, created through the use of genetic engineering or biotechnology

HERBICIDE RESISTANCE: a trait acquired by crop plants through recombinant DNA technology that enables plants to resist chemicals designed to control weeds

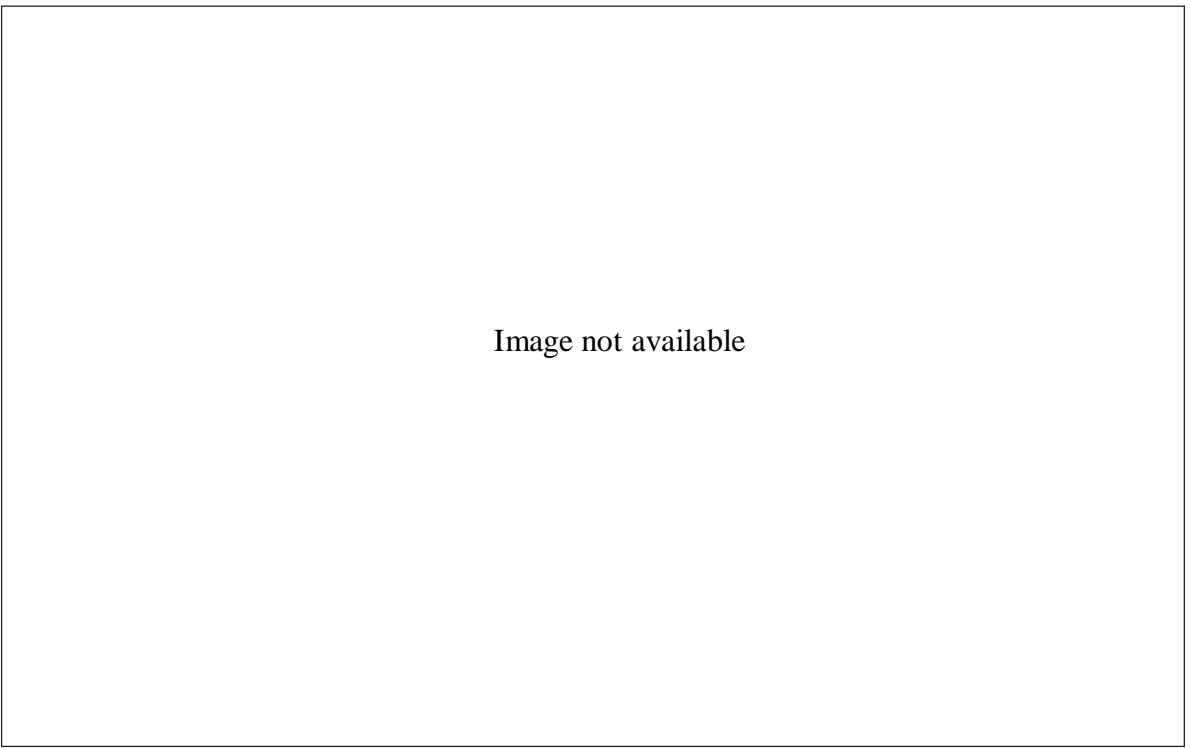


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In 1991, chief executive officer of CalGene Roger Salquist examines genetically modified tomatoes that are able to ripen on the vine before shipping, instead of having to be picked green. (AP/Wide World Photos)

The Technology

Genetically modified (GM) foods are food products derived from genetically modified organisms (GMOs). GMOs may have genes deleted, added, or replaced for a particular trait; they constitute one of the most important means by which crop plants will be improved in the future. The advantage of using genetic engineering is quite obvious: It allows individual genes to be inserted into organisms in a way that is both precise and simple. Using molecular tools available, DNA molecules from entirely different species can now be spliced together to form a recombinant DNA molecule.

The recombinant DNA molecule can then be introduced into a cell or tissue through genetic transformation. When a particular gene that codes for a trait is successfully introduced to an organism and expressed, that organism is defined as a transgenic or GM organism.

Most of the GM crops in production thus far have modified crop protection characteristics, mainly improving protection against insects

and competition (herbicide resistance). Some have improved nutritional quality and longer shelf life. Yet others under development will lift yield caps previously not possible to overcome by conventional means. Because of the direct access to and recombination of genetic material from any source, the normal reproductive barrier among different species can now be circumvented. All these modifications offer great potential for creating transgenic animals and plants useful to humankind, but GMOs also pose the possibility of misuse and unintended outcomes.

Conceivable Benefits of GM Foods

The potential benefits of using genetic engineering to develop new cultivars are evident. Crop yields can be increased by introducing genes that increase the crop's resistance to various pathogens or herbicides and enhance its tolerance to various stresses. The increased food supply is vital to support a growing population with shrinking land. One well-known example

is the introduction of *Bt* gene from the bacterium *Bacillus thuringiensis* to several crops, including corn, cotton, and soybeans. When the *Bt* gene is transferred to plants, the plant cells produce a protein toxic to some insects and hence become resistant to these insects. The grains of *Bt* maize were also found to contain low mycotoxin, thus exhibiting better food safety than non-GM corns. Another example is the successful insertion of a gene resistant to the herbicide glyphosate, reducing production costs and increasing grain purity.

Food quality can be improved in other ways. Soybeans and canola with reduced saturated fats (healthier oil) have been developed. Alterations in the starch content of potatoes and the nutritional quality of protein in maize kernels are being developed. More precise gene transfer is also being used to produce desirable products that the plant does not normally make. The potential products include pharmaceutical proteins (for example, vaccines), vitamins, and plastic compounds. "Golden rice" has been engineered to produce significantly

higher vitamin A precursors. This GM rice plays an important role in alleviating vision loss and blindness caused by vitamin A deficiency among those who consume rice as their main staple food. Attempts are being made to increase nitrogen availability, a limiting factor in crop production, by transferring genes responsible for nitrogen fixation into crops such as wheat and maize. In addition, the reduction in the use of fertilizers, insecticides, and herbicides for GM crops not only saves billions of dollars in costs but also alleviates the damage to wild organisms and ecosystems.

Concerns About GM Foods

Like any other technological innovation, genetic engineering in crop breeding and production does not come without risk or controversy. Some of the common questions raised by consumers include concerns over what plant and animal organisms they are now putting into their bodies, whether these are safe, whether they have been tested, why they are not labeled as GM foods, and whether GM foods might not

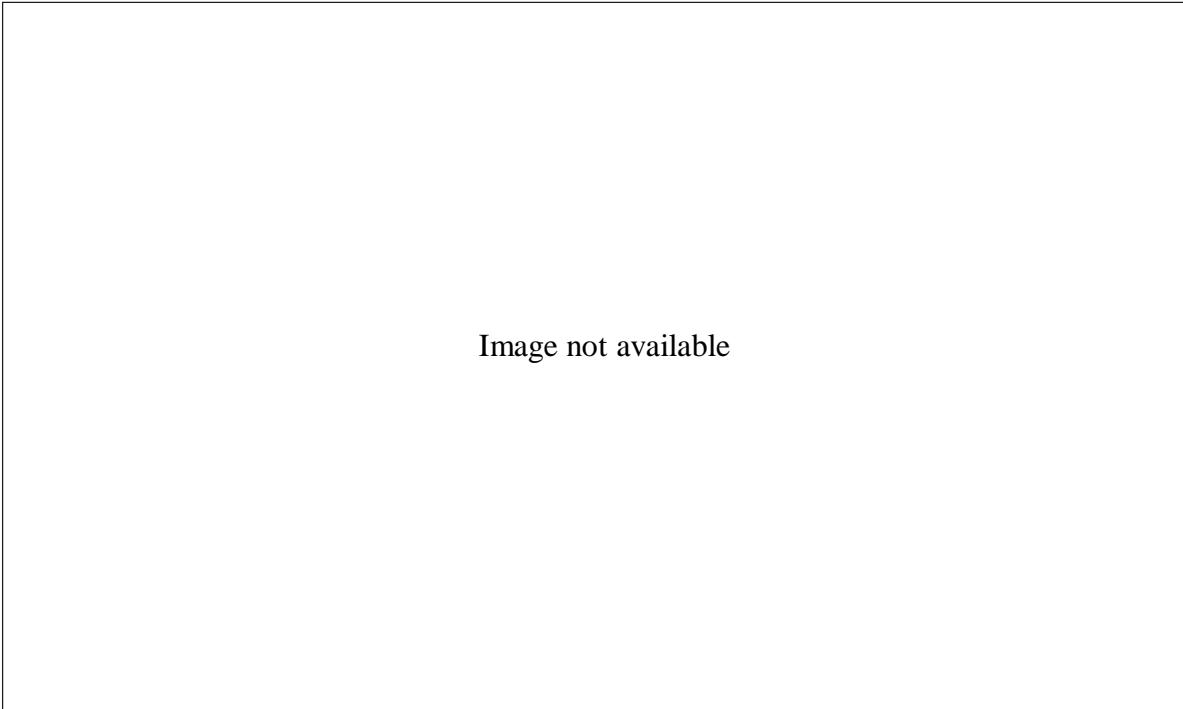


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Demonstrators in front of the San Diego Convention Center in 2001, dressed as "killer tomatoes," protest the annual conference of the Biotechnology Industry Organization. (AP/Wide World Photos)

contain toxins or allergens not present in their natural counterparts. Although most of these questions are understandable, the public uproar concerning the GM crops and other foods, particularly in Great Britain and Europe, are, from a scientific standpoint, an overreaction. Most of the general public does not understand much about the genetic engineering technology, and scientists need to increase their efforts to educate the public.

Second, most people are not aware of the strict regulations imposed on GM research and active safeguards by most governments. In the United States, research and chemical analyses by many scientists working with the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), or independently have concluded that biotechnology is a safe means of producing foods. Thousands of tests over fifteen years in the United States, along with the consumption of GM foods in the United States for four years, have revealed no evidence of harmful effects related to GM foods. Most food safety problems arise from handling (for example, microbial contamination), for GM and non-GM foods alike.

A third reason for the societal concern is rooted in negative media opinion, opposition by activists, and mistrust of the industry. Most current complaints about GM foods can be categorized into three major areas: the possible detrimental health effects, the potential environmental threats such as “superweeds,” and the social, economic, and ethical implications of genetic engineering. Some activists have taken extreme measures, such as destroying field plots and even firebombing a research laboratory. Although the majority of the public do not agree with the extreme measures taken by some activists, some continue to push for mandatory labeling of all foods whose components have derived from GMOs. Activist groups and media also continue to create myths and release misinformation regarding GM foods: GMOs have no benefit to the consumer, they may harm the environment, they are unsafe to eat, the only beneficiary of GM foods is big corporations, GM crops do not benefit small farmers, or they will drive organic farmers out of business.

Broader Issues in Biotechnology

Although some concerns are genuine—particularly ecological concerns regarding gene flow from GM plants to wild relatives—one should not ignore the fact that safety is a relative concept. Agriculture and animal husbandry have inherent dangers, as do the consumption of their products, regardless of GM or non-GM foods. In response to the demands of activist groups, the European Union (EU) and its member states adopted strict regulations over the import and release of GMOs. GM crops and foods are being subjected to more safety checks and tighter regulation than their non-GM counterparts. Through extensive studies and analyses, both the USDA and the EU have found no perceptible difference between conventional and GM foods. Of course, one cannot ensure consumers of absolute, zero risk with regard to any drug or food product, regardless of how they are produced. The demand for zero risk is more of an emotional reaction than realistically possible. Mandatory labeling on all GM foods is both impractical and technically difficult and would drive food prices to much higher levels than consumers are willing to pay. Farmers and the food industry would have to sort every GMO and store and process them separately. Realizing the complexity, federal agencies like the FDA and USDA have recommended a voluntary labeling system by which the organic and non-GM food products can be marked for consumers who are willing to pay the premium.

Where Do We Go from Here?

Development of new crops is vital for the future of the world. Since conventional breeding cannot keep up with the population explosion, biotechnology may be the best tool available to produce a greater diversity and high quality of safe food on less land, while conserving soil, water, and genetic diversity. To ensure the safety and success of GM crops, scientists and regulators will need to have open and honest communications with the public, building trust through better education and more effective regulatory oversights. In the meantime, the media will also need to convey more credible, balanced information to the public.

As Nobel laureate Norman Borlaug, father of the Green Revolution, stated, “I now say that the world has the technology that is either available or well advanced in the research pipeline to feed a population of 10 billion people. The more pertinent question today is: Will farmers and ranchers be permitted to use this new technology?”

—Ming Y. Zheng

See also: Biofertilizers; Biopesticides; Cell Culture: Plant Cells; Cloning; Cloning: Ethical Issues; Cloning Vectors; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; High-Yield Crops; Hybridization and Introgression; Lateral Gene Transfer; Transgenic Organisms.

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Potrykus, Ingo. “Golden Rice and Beyond.” *Plant Physiology* 125 (2001): 1157-1161. The originator of the wonder rice presents scientific, ethical, intellectual, and social challenges of developing and using the GMOs. Illuminating and insightful.

Web Sites of Interest

AgBioWorld.org. <http://www.agbioworld.org>. This site advocates the use of biotechnology and GM foods.

Agriculture Network Information Center. <http://www.agnic.org>. Searchable by keyword or

subject category, this site offers “quality” information on topics including transgenic crops.

Transgenic Crops. <http://www.colostate.edu/programs/lifesciences/transgeniccrops>. This richly illustrated site provides information on genetically modified (GM) foods, including news updates, the history of plant breeding, the making of transgenic plants, government regulations, and risks and concerns. This site is also available in Spanish.

Genetics, Historical Development of

Fields of study: Evolutionary biology; Genetic engineering and biotechnology; History of genetics

Significance: *Genetics is a relatively new branch of biology that explores the mechanisms of heredity. It impacts all branches of biology as well as agriculture, pharmacology, and medicine. Advances in genetics may one day eliminate a wide variety of diseases and disorders and change the way that life is defined.*

Key terms

CHROMOSOME THEORY OF HEREDITY: the theory put forth by Walter Sutton that genes are carried on cellular structures called chromosomes

MENDELIAN GENETICS: genetic theory that arose from experiments conducted by Gregor Mendel in the 1860's, from which he deduced the principles of dominant traits, recessive traits, segregation, and independent assortment

MODEL ORGANISMS: organisms, from unicellular to mammals, that are suitable for genetic research because they are small and easy to keep alive in a laboratory, reproduce a great number of offspring, and can produce many generations in a relatively short period of time

ONE GENE-ONE ENZYME HYPOTHESIS: the notion that a region of DNA that carries the information for a gene product codes for a

particular enzyme, later refined to the “one gene-one protein” hypothesis and then to “one gene-one polypeptide” principle

Charles Darwin

The prevailing public attitude of the mid-nineteenth century was that all species were the result of a special creation and were immutable; that is, they remained unchanged over time. The work of Charles Darwin challenged that attitude. As a young man, Darwin served as a naturalist on the HMS *Beagle*, a British ship that mapped the coastline of South America from 1831 to 1836. Darwin’s observations of life-forms and their adaptations, especially those he encountered on the Galápagos Islands, led him to postulate that living species shared common ancestors with extinct species and that the pressures of nature—the availability of food and water, the ratio of predators to prey, and competition—exerted a strong influence over which species were best able to exploit a given habitat. Those best able to take advantage of an environment would survive, reproduce, and, by reproducing, pass their traits on to the next generation. He called this response to the pressures of nature “natural selection”: Nature selected which species would be capable of surviving in any given environment and, by so doing, directed the development of species over time.

When Darwin returned to England, he shared his ideas with other eminent scientists but had no intention of publishing his notebooks, since he knew that his ideas would bring him into direct conflict with the society in which he lived. However, in 1858, he received a letter from a young naturalist named Alfred Russel Wallace. Wallace had done the same type of collecting in Malaysia that Darwin had done in South America, had observed the same phenomena, and had drawn the same conclusions. Wallace’s letter forced Darwin to publish his findings, and in 1859, a joint paper by both men on the topic of evolution was presented at the London meeting of the Linnean Society. In 1859, Darwin reluctantly published *On the Origin of Species by Means of Natural Selection*. The response was immediate and largely negative. While the book became a best-seller, Darwin

found himself under attack from religious leaders and other prominent scientists. In his subsequent works, he further delineated his proposals on the emergence of species, including man, but was never able to answer the pivotal question that dogged him until his death in 1882: If species are in fact mutable (capable of change over long periods of time), by what mechanism is this change possible?

Gregor Mendel

Ironically, it was only six years later that this question was answered, and nobody noticed. Today, Gregor Mendel is considered the “father” of genetics, but, in 1865, he was an Augustinian monk in a monastery in Brunn, Austria (now Brno, Czech Republic). From 1856 to 1863, he conducted a series of experiments using the sweet pea (*Pisum sativum*), in which he cultivated more than twenty-eight thousand plants and analyzed seven different physical traits. These traits included the height of the plant, the color of the seed pods and flowers, and the physical appearance of the seeds. He cross-pollinated tall plants with short plants, expecting the next generation of plants to be of medium height. Instead, all the plants produced from this cross, which he called the F₁ (first filial) generation, were tall. When he crossed plants of the F₁ generation, the next generation of plants (F₂) were both tall and short at a 3:1 ratio; that is, 75 percent of the F₂ generation of plants were tall, while 25 percent were short. This ratio held true whether he looked at one trait or multiple traits at the same time. He coined two phrases still used in genetics to describe this phenomenon: He called the trait that appeared in the F₁ generation “dominant” and the trait that vanished in the F₁ generation “recessive.” While he knew absolutely nothing about chromosomes or genes, he postulated that each visible physical trait, or phenotype, was the result of two “factors” and that each parent contributed one factor for a given trait to its offspring. His research led him to formulate several statements that are now called the Mendelian principles of genetics.

Mendel’s first principle is called the principle of segregation. While all body cells contain two copies of a factor (what are now called

genes), gametes contain only one copy. The factors are segregated into gametes by meiosis, a specialized type of cell division that produces gametes. The principle of independent assortment states that this segregation is a random event. One factor will segregate into a gamete independently of other factors contained within the dividing cell. (It is now known that there are exceptions to this rule: Two genes carried on the same chromosome will not assort independently.)

To make sense of the data he collected from twenty-eight thousand plants, Mendel kept detailed numerical records and subjected his numbers to statistical analysis. In 1865, he presented his work before the Natural Sciences Society. He received polite but indifferent applause. Until Mendel, scientists rarely quantified their findings; as a result, the scientists either did not understand Mendel's math or were bored by it. In either case, the scientists completely overlooked the significance of his findings. Mendel published his work in 1866. Unlike Darwin's work, it was not a best-seller. Darwin himself died unaware of Mendel's work, in spite of the fact that he had an unopened copy of Mendel's paper in his possession. Mendel died in 1884, two years after Darwin, with no way of knowing the eventual impact his work was to have on the scientific community. That impact began in 1900, when three botanists, working in different countries with different plants, discovered the same principles as had Mendel. Hugo De Vries, Carl Correns, and Erich Tschermark von Seysenegg rediscovered Mendel's paper, and all three cited it in their work. Sixteen years after his death, Mendel's research was given the respect it deserved, and the science of genetics was born.

Pivotal Research in Genetics

In 1877, Walter Flemming identified structures in the nuclei of cells that he called chromosomes; he later described the material of which chromosomes are composed as "chromatin." In 1900, William Bateson introduced the term "genetics" to the scientific vocabulary. Wilhelm Johannsen expanded the terminology the following year with the introduction of the terms "gene," "genotype," and "pheno-

type." In fact, 1901 was an exciting year in the history of genetics: The ABO blood group was discovered by Karl Landsteiner; the role of the X chromosome in determining gender was described by Clarence McClung; Reginald Punnett and William Bateson discovered genetic linkage; and De Vries introduced the term "mutation" to describe spontaneous changes in the genetic material. Walter Sutton suggested a relationship between genes and chromosomes in 1903. Five years later, Archibald Garrod, studying a strange clinical condition in some of his patients, determined that their disorder, called alkaptonuria, was caused by an enzyme deficiency. He introduced the concept of "inborn errors of metabolism" as a cause of certain diseases. That same year, two researchers named Godfrey Hardy and Wilhelm Weinberg published their extrapolations on the principles of population genetics.

From 1910 to 1920, Thomas Hunt Morgan, with his graduate students Alfred Sturtevant, Calvin Bridges, and Hermann Müller, conducted a series of experiments with the fruit fly *Drosophila melanogaster* that confirmed Mendel's principles of heredity and also confirmed the link between genes and chromosomes. The mapping of genes to the fruit fly chromosomes was complete by 1920. The use of research organisms such as the fruit fly became standard practice. For an organism to be suitable for this type of research, it must be small and easy to keep alive in a laboratory and must produce a great number of offspring. For this reason, bacteria (such as *Escherichia coli*), viruses (particularly those that infect bacteria, called bacteriophages), certain fungi (such as *Neurospora*), and the fruit fly have been used extensively in genetic research.

During the 1920's, Müller found that the rate at which mutations occur is increased by exposure to X-ray radiation. Frederick Griffith described "transformation," a process by which genetic alterations occur in pneumococci bacteria. In the 1940's, Oswald Avery, Maclyn McCarty, and Colin MacLeod conducted a series of experiments that showed that the transforming agent Griffith had not been able to identify was, in fact, DNA. George Beadle and Edward Tatum proposed the concept of "one

gene, one enzyme"; that is, a gene or a region of DNA that carries the information for a gene product codes for a particular enzyme. This concept was further refined to the "one gene, one protein" hypothesis and then to "one gene, one polypeptide." (A polypeptide is a string of amino acids, which is the primary structure of all proteins.)

During the 1940's, it was thought that proteins were the genetic material. Chromosomes are made of chromatin; chromatin is 65 percent protein, 30 percent DNA, and 5 percent RNA. It was a logical conclusion that if the chromosomes were the carriers of genetic material, that material would make up the bulk of the chromosome structure. By the 1950's, however, it was fairly clear that DNA was the genetic material. Alfred Hershey and Martha Chase were able to prove in 1952 that DNA is the heredi-

tary material in bacteriophages. From that point, the race was on to discover the structure of DNA.

For DNA or any other substance to be able to carry genetic information, it must be a stable molecule capable of self-replication. It was known that along with a five-carbon sugar and a phosphate group, DNA contains four different nitrogenous bases (adenine, thymine, cytosine, and guanine). Erwin Chargaff described the ratios of the four nitrogenous bases in what is now called Chargaff's rule: adenine in equal concentrations to thymine, and cytosine in equal concentrations to guanine. What was not known was the manner in which these constituents bonded to each other and the three-dimensional shape of the molecule. Groups of scientists all over the world were working on the DNA puzzle. A group in Cambridge, En-

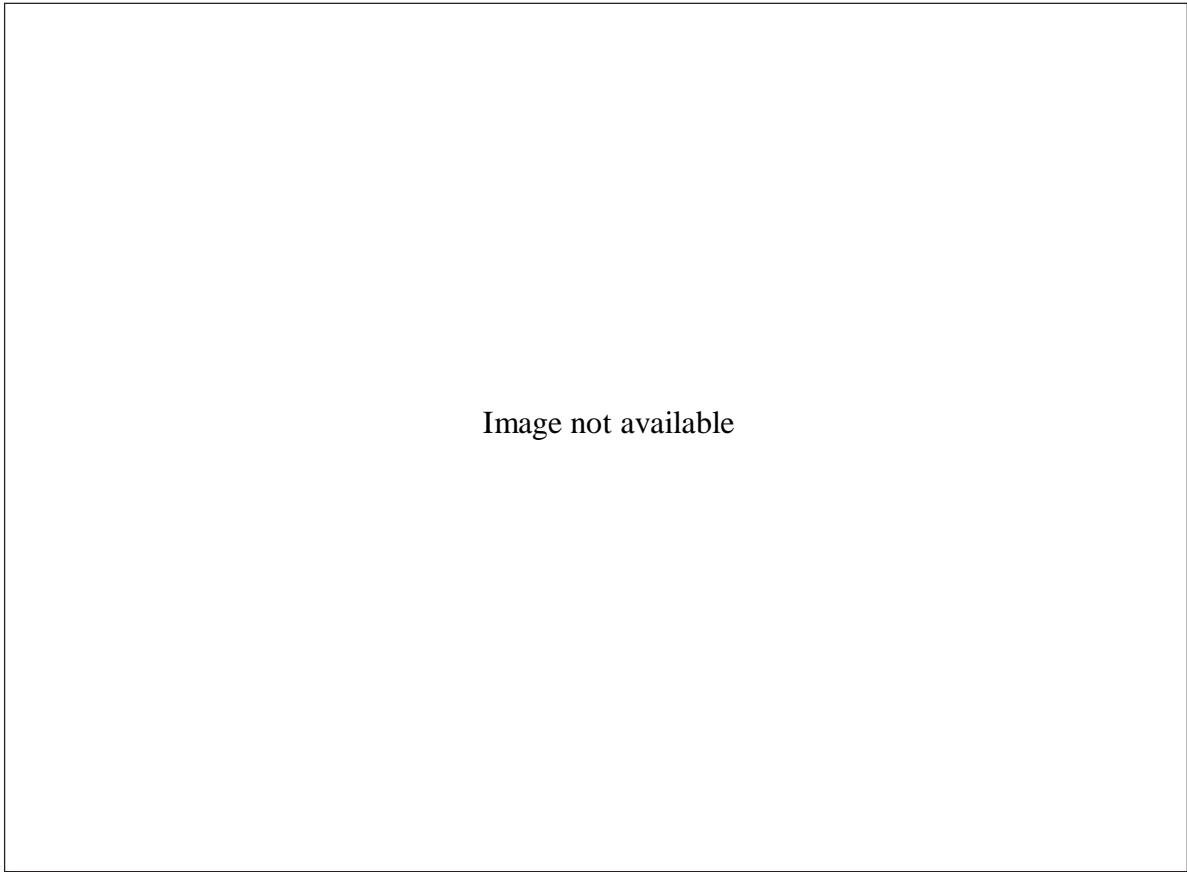


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James Watson (left) and Francis Crick pose with a model of the double-helical structure of DNA. They won the 1962 Nobel Prize in Physiology or Medicine, along with Maurice Wilkins. (Hulton Archive)

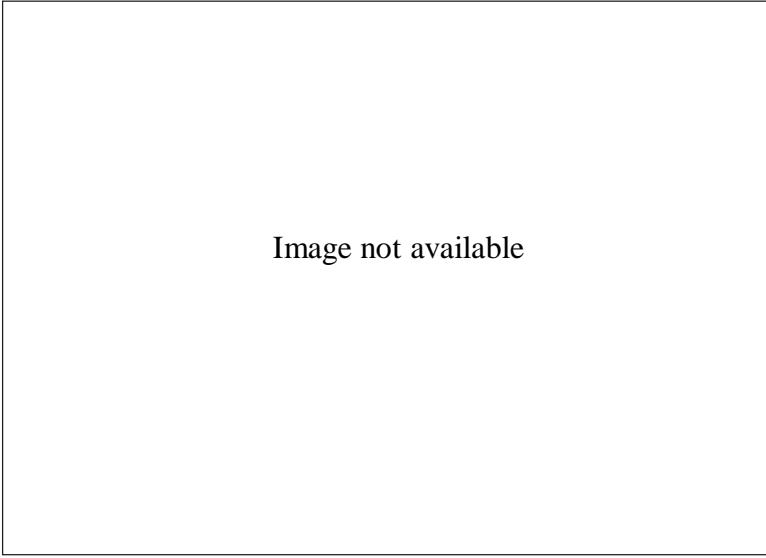


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Maurice Wilkins poses with a model of a DNA molecule at a London celebration of the fiftieth anniversary of the discovery of the double helix. Wilkins, with Rosalind Franklin, was able to elucidate the molecule's physical structure using X-ray crystallography. (AP/Wide World Photos)

gland, was the first to solve it. James Watson and Francis Crick, supported by the work of Maurice Wilkins and Rosalind Franklin, described the structure of DNA in a landmark paper in *Nature* in 1953. They described the molecule as a double helix, a kind of spiral ladder in which alternating sugars and phosphate groups make up the backbone and paired nitrogenous bases make up the rungs. Arthur Kornberg created the first synthetic DNA in 1956. The structure of the molecule suggested ways in which it could self-replicate. In 1958, Matthew Meselson and Franklin Stahl proved that DNA replication is semiconservative; that is, each new DNA molecule consists of one template strand and one newly synthesized strand.

The Information Explosion

Throughout the 1950's and 1960's, genetic information grew exponentially. This period saw the description of the role of the Y chromosome in sex determination; the description of birth defects caused by chromosomal aberrations such as trisomy 21 (Down syndrome), trisomy 18 (Edwards' syndrome), and trisomy 13 (Patau syndrome); the description of operon and gene regulation by François Jacob and

Jacques Monod in 1961; and the deciphering of the genetic code by Har Gobind Khorana, Marshall Nirenberg, and Severo Ochoa in 1966.

The discovery of restriction endonucleases (enzymes capable of splicing DNA at certain sites) led to an entirely new field within genetics called biotechnology. Mutations, such as the sickle-cell mutation, could be identified using restriction endonucleases. Use of these enzymes and DNA banding techniques led to the development of DNA fingerprinting. In 1979, human insulin and human growth hormone were synthesized in *Escherichia coli*. In 1981, the first cloning experiments were successful when

the nucleus from one mouse cell was transplanted into an enucleated mouse cell. By 1990, cancer-causing genes called oncogenes had been identified, and the first attempts at human gene therapy had taken place. In 1997, researchers in England successfully cloned a living sheep. As the result of a series of conferences between 1985 and 1987, an international collaboration to map the entire human genome began in 1990. A comprehensive, high-density genetic map was published in 1994, and in 2003 the human genome was completed.

Impact and Applications

The impact of genetics is immeasurable. In less than one hundred years, humans went from complete ignorance about the existence of genes to the development of gene therapies for certain diseases. Genes have been manipulated in certain organisms for the production of drugs, pesticides, and fungicides. Genetic analysis has identified the causes of many hereditary disorders, and genetic counseling has aided innumerable couples in making difficult decisions about their reproductive lives. DNA analysis has led to clearer understanding of the manner in which all species are

linked. Techniques such as DNA fingerprinting have had a tremendous impact on law enforcement.

Advances in genetics have also given rise to a wide range of ethical questions with which humans will be struggling for some time to come. Termination of pregnancies, in vitro fertilization, and cloning are just some of the technologies that carry with them serious philosophical and ethical problems. There are fears that biotechnology will make it possible for humans to "play God" and that the use of biotechnology to manipulate human genes may have unforeseen consequences for humankind. For all the hope that biotechnology offers, it carries with it possible societal changes that are unpredictable and potentially limitless. Humans may be able to direct their own evolution; no other species has ever had that capability. How genetic technology is used and the motives behind its use will be some of the critical issues of the future.

—Kate Lapczynski

See also: Central Dogma of Molecular Biology; Chromosome Theory of Heredity; Classical Transmission Genetics; DNA Structure and Function; Evolutionary Biology; Genetic Code, Cracking of; Genetic Engineering: Historical Development; Genetics in Television and Films; Genomics; Human Genome Project; Lamarckianism; Mendelian Genetics; Sociobiology; Biographical Dictionary of Important Geneticists (appendix); Nobel Prizes for Significant Discoveries in Genetics (appendix); Time Line of Major Developments in Genetics (appendix).

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_____. *In Mendel's Footnotes: An Introduction to the Science and Technologies of Genes and Genetics from the Nineteenth Century to the Twenty-Second*. London: Jonathan Cape, 2000. Investigates the world of biotechnologies, including cloning, genomics, and genetic engineering. Bibliography, index.

Watson, James. *The Double Helix*. 1968. Reprint. New York: Simon and Schuster, 2001. Discusses the race to solve the structure of the DNA molecule.

Web Sites of Interest

Dolan DNA Learning Center, DNA from the Beginning. <http://www.dnabtb.org>. Sponsored by the Cold Spring Harbor Laboratory, an animated site aimed at those looking for a general introduction to DNA, genes, genetics, and heredity, and their scientific histories. Organized by key concepts.

Electronic Scholarly Publishing Project, Classic Genetics: Foundations. <http://www.esp.org>. A collection of classic papers marking the development of genetics. Includes a time line.

popularized genetics and how it can be applied to transform, extend, and enrich lives. With the exception of documentaries such as *The DNA Revolution* (1998), few films and programs featuring genetics are realistic and accurate.

Science-fiction films and television programs usually depict genetics as a wondrous endeavor that can abruptly go awry. Genetics is often appropriated to provoke rather than to resolve dilemmas. In film and television, genetic engineering is usually equated with power—power that genetically superior characters occasionally abuse. Plots frequently contrast extremes, such as good and evil scientists pitted against each other or combating corrupt administrators and greedy entrepreneurs. Many depictions of genetics perpetuate stereotypes such as mad scientists isolated in laboratories and unaccountable to humankind for their research and creations. A host of biotechnological monsters and mutants populate films.

Genetics in Television and Films

Field of study: History of Genetics; Human genetics and social issues

Significance: *Popular culture expresses attitudes regarding genetics. Films and television programs present biotechnology in extremes of either promoting genetics as a valuable investigative and reproductive tool or demeaning it as a dangerous science which is hazardous to people and environments. Most genetic depictions in these media are more entertaining than accurate.*

Key terms

EUGENICS: the selective application of genetics to produce superior offspring

GENETIC DETERMINISM: how genes might influence behavioral characteristics

Science Fiction

In the 1950's, science-fiction films and television programming gradually incorporated references to genetics. The expansion of biotechnology research in the 1970's inspired fictional plots that focused on genetics to amuse audiences more than educate them. Box-office successes such as *Jurassic Park* (1993) and hit television series, including *The X-Files* (1993-2002),

DNA and Identity

CSI: Crime Scene Investigation, a television series that first aired in 2000, is representative of crime-based television shows that became popular in the late 1990's, in part because of public fascination with the O. J. Simpson murder trial and other high-profile cases in which DNA evidence was showcased in the media. Both episodic drama programming and true-crime shows such as *Cold Case Files* rely on sets that are filled with genetic tools. Scenes depict characters collecting DNA samples from crime scenes and evaluating the tissues in laboratories to identify victims, prove criminals' guilt, or exonerate the falsely accused.

Soap-opera writers often appropriate genetics as a plot device. Characters test DNA to confirm paternity, establish identity, or prove a person's presence at a crime scene. These daytime serials usually restrict access to DNA knowledge to medical and police personnel. Some characters manipulate DNA evidence by switching samples or tampering with laboratory records. In 2002, *Days of Our Lives* introduced a story line involving the genetically engineered Gemini Twins, who displayed previously undocumented DNA patterns.

Cloning Characters

Clones are often depicted as evil creatures that prey on humans. The feature film *The Boys from Brazil* (1978) reveals the potential horrific results if Nazi sympathizers successfully cloned Adolf Hitler. Clones are sometimes shown to be dutiful, almost robotic, helpers. In *Star Wars Episode II: Attack of the Clones* (2002), thousands of clonetroopers are created as soldiers during the clone wars. In these movies, cloning concepts are more futuristic than realistic.

Jurassic Park and its two sequels captured worldwide attention for cloning. Those movies are based on the concept that scientists cloned dinosaurs from DNA preserved in amber. Scientists criticize this movie's premise of cloning a dinosaur from fragments of ancient genetic material as improbable, stating that locating an egg and host animal capable of transforming the DNA into a dinosaur would be difficult if not impossible. More important, DNA of the age required for dinosaurs (more than 65 million years old), even if recovered, would almost certainly be far too degraded to make cloning possible.

The Brazilian soap opera *O clone* (2001–2002; the clone) chronicles Dr. Albieri secretly creating the clone Leo. Albieri is concerned for Leo's health, referring to cloned sheep Dolly's premature aging, and addresses ethical issues related to cloning. Leo suffers identity problems, questions his potential life span, and represents unwanted public attention.

Designer Plots

Hollywood is intrigued with the idea of human genetic modification. Films and programs explore the possibilities of manipulating genes to give characters unnatural advantages. Often these genetic changes create designer bodies in an almost eugenic effort to attain physical perfection and perceived superiority. These presentations usually simultaneously address determinism and how genes might control both positive and negative behaviors unrelated to appearance.

In *Gattaca* (1997), genetically altered characters have power in a futuristic soci-

ety over normal characters who have not benefited from biotechnology and are relegated to an underclass because of their imperfections. Vincent, a frustrated janitor who aspires to become an astronaut, uses DNA to adopt the appearance of the elite. His genetic transformation enables him to achieve his professional ambition. Vincent's emotional traits are shown to be superior to the physical beauty of the genetically engineered people.

Beginning in 2002, MTV aired *Clone High*, a controversial cartoon featuring clones of significant historical leaders. These characters are presented as angst-ridden teenagers whom the scripts hint represent genetic determinism. For example, Joan of Arc is an atheist, suggesting that she might have been genetically prone to that behavior if she had not been influenced by cultural factors.

Reactions

Genetic-based movie and television programming impacts audiences by influencing

Image not available

The film Jurassic Park (1993) posited that dinosaurs could be cloned from ancient DNA—*theoretically plausible, but practically impossible due to the extreme degradation of DNA the age of dinosaurs.* (AP/Wide World Photos)

how people perceive and accept or reject biotechnology. Although these media expand awareness of genetics, they usually are not reliable educational resources and perpetuate misunderstandings. Advertisements for the fictional *O clone*, designed as news broadcasts, were so realistic that many viewers thought an actual person had been cloned.

Errors detract from programs being credible cinema. Movies and series offer simplified depictions of complex scientific processes, suggesting they require minimal time and effort and consistently produce positive results. Viewers develop unrealistic expectations that genetics can quickly solve mysteries because of the immediacy of DNA testing in brief episodes.

Dr. J. Craig Venter criticizes popular culture's concentration on genetic determinism because such emphasis and negative cinematic portrayals might cause people to reject biotechnology instead of recognizing its merits. Experts worry about cinema geneticists acting irresponsibly and unprofessionally. In an effort to improve depictions, some scientists have served as genetics advisers for film and television productions.

—Elizabeth D. Schafer

See also: Ancient DNA; Biological Determinism; Chromosome Theory of Heredity; Classical Transmission Genetics; Cloning; Cloning: Ethical Issues; Criminality; DNA Fingerprinting; Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Forensic Genetics; Genetic Code, Cracking of; Genetic Engineering; Historical Development; Genetic Engineering: Social and Ethical Issues; Human Genetics; Human Genome Project; Lamarckianism; Mendelian Genetics; Patents on Life-Forms; Paternity Tests; Race; Sociobiology.

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Web Site of Interest

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Genome Size

Field of study: Molecular genetics

Significance: *Genome size, the total amount of genetic material within a cell of an organism, varies 200,000-fold among species. Since the 1950's it has been clear that there is no obvious link between an organism's complexity and the size of its genome, although numerous hypotheses to explain this paradox exist.*

Key terms

C-VALUE: the characteristic genome size for a species

CHROMOSOME: a self-replicating structure, consisting of DNA and protein, that contains part of the nuclear genome of a eukaryote; also used to describe the DNA molecules comprising the prokaryotic genome

GENOME: the entire genetic complement of an organism

JUNK DNA: a disparaging (and now known to be inaccurate) characterization of the non-coding DNA content of a genome

REASSOCIATION KINETICS: a technique that uses hybridization of denatured DNA to reveal DNA classes differing in repetition frequency

REPETITIVE DNA: a DNA sequence that is repeated two or more times in a DNA molecule or genome

Genome Sizes in Prokaryotes vs.

Eukaryotes

Wide variation in genome size exists among species, from 580,000 bases in the bacterium *Mycoplasma genitalium* to 670 billion bases in the protist *Amoeba dubia*. In general, prokaryotic genomes are smaller than the genomes of eukaryotes, although a few prokaryotes have genomes that are larger than those of some eukaryotes. The largest known prokaryotic genome (10 million bases in the cyanobacterium *Nostoc punctiforme*) is several times larger than the genomes of parasitic eukaryotic microsporidia, with genome sizes of approximately 3 million bases. Within the prokaryotes, the archaea have a relatively small range of genome sizes, with the majority of species in the 1- to 3-million-base range, while bacterial species have been found with genomes differing by twentyfold.

Contrary to expectations, there is no obvious correlation between genome size and organismal complexity in eukaryotes. For example, the genome of a human is tenfold smaller than the genome of a lily, twenty-fivefold smaller than the genome of a newt, and two-hundred-fold smaller than the genome of an amoeba. The characteristic genome size of a species is called the C-value; the lack of relationship between genome size, number of genes, and organismal complexity has been termed the “C-value paradox.”

Reasons for Size Differences

The majority of DNA in most eukaryotes is noncoding. Previously known as “junk” DNA, this DNA (comprising up to 98.5 percent of some genomes) does not contain the coding sequences for proteins. The complexity of DNA can be characterized using a technique called reassociation kinetics. DNA is sheared into pieces of a few hundred bases, heated to denature into single strands, then allowed to renature during cooling. The rate of renaturation is related to the sequence complexity: DNA sequences present in numerous copies

will renature more rapidly than unique DNA sequences. Unique DNA sequences usually represent protein-coding regions, whereas repetitive DNA generally does not encode traits. In many genomes, three types of DNA can be identified by reassociation kinetics: highly repetitive DNA, middle repetitive DNA, and unique DNA. Prokaryotes have little or no repetitive DNA. Among eukaryotes, the amounts of the three types of DNA varies. The share of the genome dedicated to genes is relatively constant, whereas the amount of repetitive DNA, 10-70 percent of the total, varies widely even within families of organisms. The existence of noncoding DNA appears to account for the lack of correlation between genome size and complexity because complexity may be more directly related to number of genes, a number which does appear to have more correlation to organismal complexity.

The variation in the amount of repetitive DNA, even within families, may be related to the spontaneous rate of DNA loss. Small genomes may be small because they throw away junk DNA very efficiently, whereas large genomes may be less able to weed out unnecessary DNA. Studies on several invertebrates support this hypothesis: Species within a family with large genomes have substantially lower spontaneous DNA losses.

Genome size does have a positive correlation with cell size and a negative correlation with cell division rate in a number of taxa. Because of these correlations, genome size is associated with developmental rate in numerous species. This correlation is not exact, however. For some organisms (particularly plants) with relatively simple developmental complexity, developmental rate is constrained by external factors such as seasonal changes, while for others (amphibians with time-limited morphogenesis) developmental complexity overwhelms the effects of developmental rate.

Differences in Chromosome Number

The genomes of eukaryotes are organized into sets of two or more linear DNA molecules, each contained in a chromosome. The number of chromosomes varies from 2 in females of the ant species *Myrmecia pilosula* to 46 in humans to

94 in goldfish. These numbers represent the diploid number of chromosomes. A genome that contains three or more full copies of the haploid chromosome number is polyploid. As a general rule polyploids can be tolerated in plants but are rarely found in animals. One reason is that the sex balance is important in animals and variation from the diploid number results in sterility. Chromosome number appears to be unrelated to genome size or to most other biological features of the organism.

For most of the prokaryotes studied, the prokaryotic genome is contained in a single, circular DNA molecular, with the possible addition of small, circular, extrachromosomal DNA molecules called plasmids. However, some prokaryotes have multiple chromosomes, some of which are linear; and some prokaryotes have several very large plasmids, nearly the size of the bacterial chromosome.

—Lisa M. Sardinia

See also: Ancient DNA; Evolutionary Biology; Gene Families; Genomics; Human Genetics; Molecular Clock Hypothesis; Noncoding RNA Molecules; Plasmids; Pseudogenes; Repetitive DNA; Transposable Elements.

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of the clones together contain most or all of the genome. To find a specific gene, scientists can screen the library using labeled probes of various kinds.

Key terms

GENOME: all the genetic material carried by a cell

LAMBDA (λ) PHAGE: a virus that infects bacteria and then makes multiple copies of itself by taking over the infected bacterium's cellular machinery

LIGATION: the joining together of two pieces of DNA using the enzyme ligase

What Is a Genomic Library?

Scientists often need to search through all the genetic information present in an organism to find a specific gene. It is thus convenient to have collections of genetic sequences stored so that such information is readily available. These collections are known as genomic libraries.

The library metaphor is useful in explaining both the structure and function of these information-storage centers. If one were interested in finding a specific literary phrase, one could go to a conventional library and search through the collected works. In such a library, the information is made up of letters organized in a linear fashion to form words, sentences, and chapters. It would not be useful to store this information as individual words or letters or as words collected in a random, jumbled fashion, as the information's meaning could not then be determined. The more books a library has, the closer it can come to having the complete literary collection, although no collection can guarantee that it has every piece of written word. The same is true of a genomic library. The stored pieces of genetic information cannot be individual bits but must be ordered sequences that are long enough to define a gene. The longer the string of information, the easier it is to make sense of the gene they make up, or "encode." The more pieces of genetic information a library has, the more likely it is to contain all the information present in a cell. Even a large collection of sequences, however, cannot guarantee that it contains every piece of genetic information.

Genomic Libraries

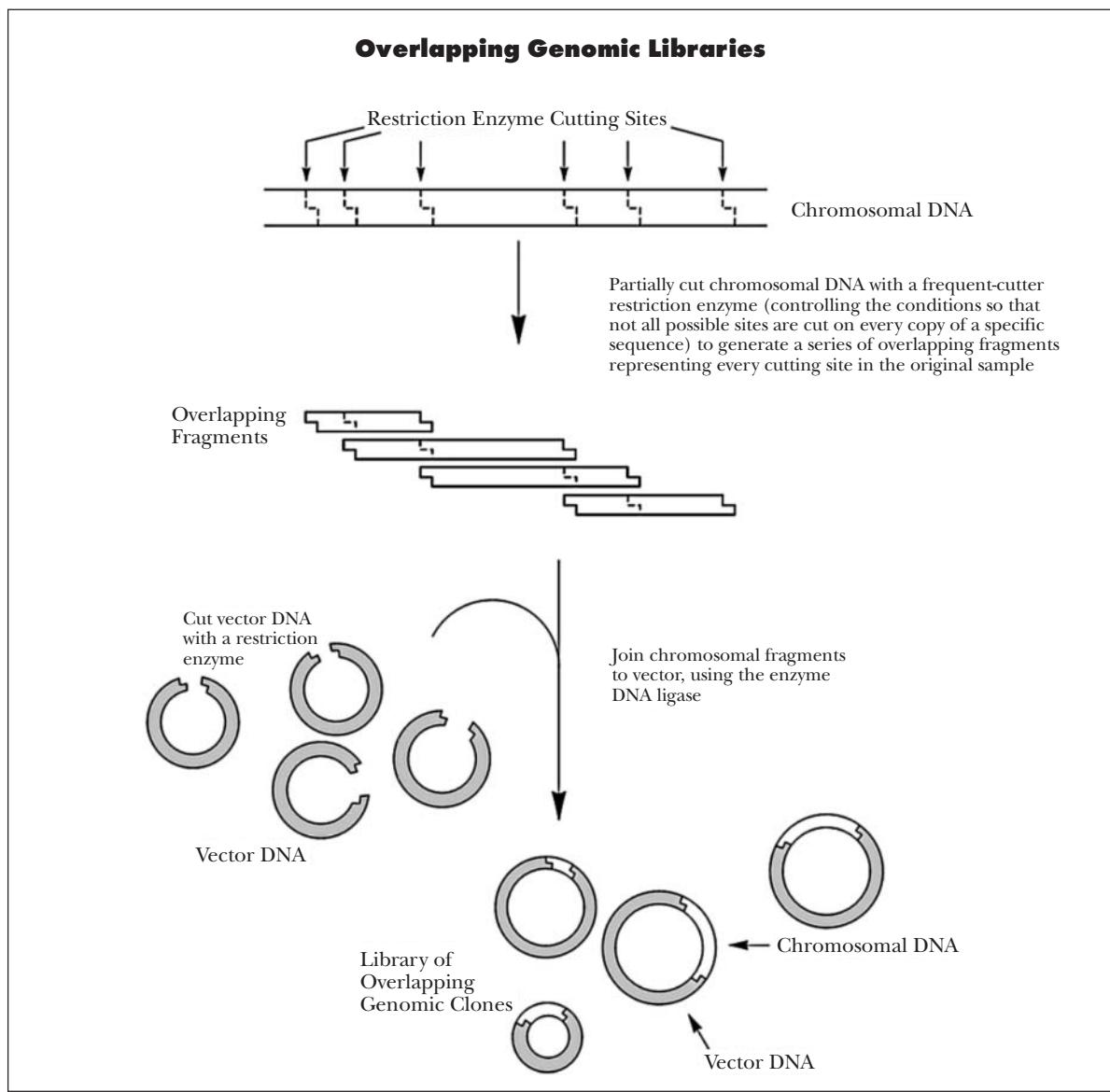
Fields of Study: Bioinformatics; Techniques and methodologies

Significance: *A genomic library is a collection of clones of DNA sequences, each containing a relatively short piece of the genome of an organism. All*

How Is a Genomic Library Created?

In order for a genomic library to be practical, some method must be developed to put an entire genome into discrete units, each of which contains sufficiently large amounts of information to be useful but which are also easily replicated and studied. The method must also generate fragments that overlap one another for short stretches. The information exists in

the form of chromosomes composed of millions of units known as base pairs. If the information were fragmented in a regular fashion—for example, if it were cut every ten thousand base pairs—there would be no way to identify each fragment's immediate neighbors. It would be like owning a huge, multivolume novel without any numbering system: It would be almost impossible to determine with which book to



Genomic libraries are collections of clones of chromosomal DNA segments. These must be constructed in such a way that the order of the segments can be determined. To accomplish this, regions of each cloned segment overlap with other segments. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

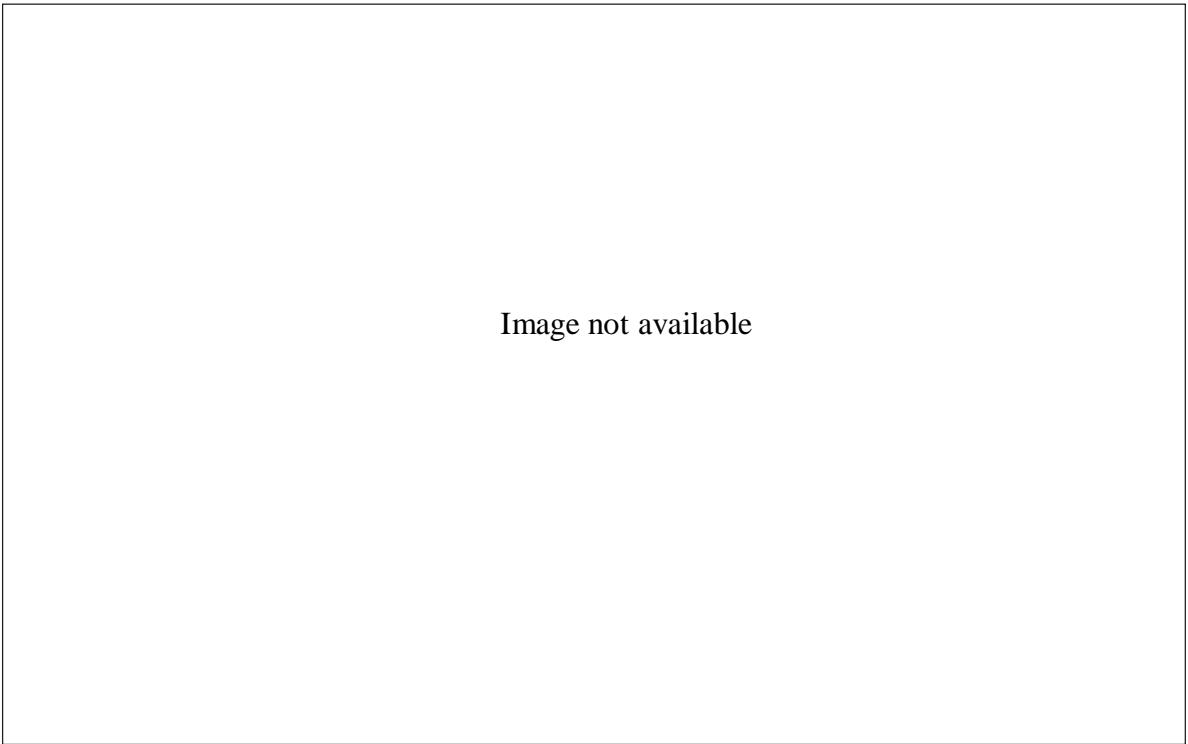


Image not available

DNA samples are stored in the "Big Bertha" freezer at the Armed Forces Institute of Pathology in Gaithersburg, Maryland. (AP/Wide World Photos)

start and which to proceed to next. Similarly, without some way of tracking the order of the genetic information, it would be impossible to assemble the sequence of each subfragment into the big continuum of the entire chromosome. The fragments are thus cut so that their ends overlap. With even a few hundred base pairs of overlap, the shared sequences at the end of the fragments can be used to determine the relative position of the different fragments. The different pieces can then be connected into one long unit, or sequence.

There are two common ways to fragment DNA, the basic unit of genetic information, to generate a library. The first is to disrupt the long strands of DNA by forcing them rapidly through a narrow hypodermic needle, creating forces that tear, or shear, the strands into short fragments. The advantage of this method is that the fragment ends are completely random. The disadvantage is that the sheared ends must be modified for easy joining, or ligation. The other method is to use restriction endonu-

cleases, enzymes that recognize specific short stretches of DNA and cleave the DNA at specific positions. To create a library, scientists employ restriction enzymes that recognize four-base-pair sequences for cutting. Normally, the result of cleavage with such an enzyme would be fragments with an average size of 256 base pairs. If the amount of enzyme in the reaction is limited, however, only a limited number of sites will be cut, and much longer fragments can be generated. The ends created by this cleavage are usable for direct ligation into vectors, but the distribution of cleavage sites is not as random as that produced by shearing.

In a conventional library, information is imprinted on paper pages that can be easily replicated by a printing press and easily bound into a complete unit such as a book. Genetic information is stored in the form of DNA. How can the pieces of a genome be stored in such a way that they can be easily replicated and maintained in identical units? The answer is to take the DNA fragments and attach, or ligate, them

into lambda phage DNA. When the phage infects bacteria, it makes copies of itself. If the genomic fragment is inserted into the phage DNA, then it will be replicated also, making multiple exact copies (or clones) of itself.

To make an actual library, DNA is isolated from an organism and fragmented as described. Each fragment is then randomly ligated into a lambda phage. The pool of lambda phage containing the inserts is then spread onto an agar plate coated with a “lawn” or confluent layer of bacteria. Wherever a phage lands, it begins to infect and kill bacteria, leaving a clear spot, or “plaque,” in the lawn. Each plaque contains millions of phages with millions of identical copies of one fragment from the original genome. If enough plaques are generated on the plate, each one containing some random piece of the genome, then the entire genome may be represented in the summation of the DNA present in all the plaques. Since the fragment generation is random, however, the completeness of the genomic library can only be estimated. It takes 800,000 plaques containing an average genomic fragment of 17,000 base pairs to give a 99 percent probability that the total will contain a specific human gene. While this may sound like a large number, it takes only fifteen teacup-sized agar plates to produce this many plaques. A genetic library pool of phage can be stored in a refrigerator and plated out onto agar petri dishes whenever needed.

How Can a Specific Gene Be Pulled out of a Library?

Once the entire genome is spread out as a collection of plaques, it is necessary to isolate the one plaque containing the specific sequences desired from the large collection. To accomplish this, a dry filter paper is laid onto the agar dish covered with plaques. As the moisture from the plate wicks into the paper, it carries with it some of the phage. An ink-dipped needle is pushed through the filter at several spots on the edge, marking the same spot on the filter and the agar. These will serve as common reference points. The filter is treated with a strong base that releases the DNA from the phage and denatures it into single-stranded form. The base is neutralized, and the filter is

incubated in a salt buffer containing radioactive single-stranded DNA. The radioactive DNA, or “probe,” is a short stretch of sequence from the gene to be isolated. If the full gene is present on the filter, the probe will hybridize with it and become attached to the filter. The filter is washed, removing all the radioactivity except where the probe has hybridized. The filters are exposed to film, and a dark spot develops over the location of the positive plaque. The ink spots on the filter can then be used to align the spot on the filter with the positive plaque on the plate. The plaque can be purified, and the genomic DNA can then be isolated for further study.

It may turn out that the entire gene is not contained in the fragment isolated from one phage. Since the library was designed so that the ends of one fragment overlap with the adjacent fragment, the ends can be used as a probe to isolate neighboring fragments that contain the rest of the gene. This process of increasing the amount of the genome isolated is called genomic walking.

—J. Aaron Cassill

See also: Bioinformatics; cDNA Libraries; DNA Fingerprinting; DNA Sequencing Technology; Forensic Genetics; Genetic Testing; Ethical and Economic Issues; Genetics, Historical Development of; Genomics; Human Genome Project; Icelandic Genetic Database; Linkage Maps; Proteomics; Restriction Enzymes; Reverse Transcriptase.

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categories: regions, maps, and variations of the human genome.

National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov>. A central repository for biological information, including links to genome projects and genomic science. Maintains GenBank, a comprehensive, annotated collection of publicly available DNA sequences.

Genomics

Field of study: Molecular genetics

Significance: *Genomics involves studying the entire complement of genes that an organism possesses. A genomic approach to biology uses modern molecular and computational techniques in conjunction with large-scale experimental approaches to sequence, identify, map, and determine the function of genes. It is also concerned with the structure and evolution of the genome as a whole.*

Key terms

BACTERIAL ARTIFICIAL CHROMOSOMES (BACs): cloning vectors that hold inserts of 100-200 kilobase pairs of foreign DNA

EXPRESSED SEQUENCE TAGS (EST) LIBRARY: a survey of expressed sequence tags, which are partial sequences from messenger RNA (mRNA)

Definition

A genome comprises all of the DNA that occurs in each cell of an organism. For prokaryotes, which are always single-celled, it comprises all of the DNA within the bacterial cell that is specific to that species. Other DNA molecules may also reside in a bacterial cell, such as plasmids (small extra pieces of circular DNA) and bacteriophage DNA (bacterial virus DNA). These extra pieces are not considered a part of the genome. In eukaryotes, the genome typically includes just the DNA in the nucleus, which is composed of linear chromosomes. All eukaryotic cells also have DNA in their mitochondria, the organelle that carries out a portion of cellular respiration. It is a circular

molecule and is sometimes referred to as the mitochondrial genome or simply mitochondrial DNA (mtDNA). Plants and some single-celled organisms have, in addition to mito-

chondria, another type of organelle called a chloroplast, which also has a circular DNA molecule. This DNA is called the chloroplast genome, or simply chloroplast DNA (cpDNA).

Sequenced Organisms

Many genomes—from vertebrate mitochondria at about 16,000 base pairs (bp) to mammals at more than 3 billion bp—have been completed, and although there still is no one repository for all these data, the National Center for Biotechnology Information maintains GenBank, which keeps track of many. Prokaryotic genomes (both *Eubacteria*, or simply *Bacteria*, and *Archaea*) are now relatively minor projects on the order of 0.6–8 megabase pairs (Mbp), and the number completed is now in the hundreds, because large sequencing centers are capable of completing thirty or more per month.

Compared to the prokaryotes, eukaryotic genomes generally involve much more work. Vertebrate genomes that have either been completed or mostly sequenced and are awaiting assembly include *Homo sapiens* (humans) at about 3.3 billion bp, *Mus musculus* (the mouse) at about 3 billion bp, *Rattus norvegicus* (the rat) at about 2.8 billion bp, *Danio rerio* (the zebra fish) at about 1.7 bp, *Fugu rubripes* (the pufferfish) at about 3.6 million bp, and *Tetraodon nigroviridis* (another form of pufferfish) at 3.8 million bp. Projects for which more than 90 percent of sequencing may be complete by 2010 (if not sooner) include *Pan paniscus* (the chimpanzee) at about 3.3 billion bp, *Macaca mulatta* (the rhesus monkey), *Papio cynocephalus* (the yellow baboon), *Bos taurus* (the cow), *Sus scrofa* (the pig), *Canis familiaris* (the dog), *Felis catus* (the cat), *Equus caballus* (the horse), *Oryctolagus cuniculus* (the rabbit), *Gallus gallus* (the chicken), *Xenopus tropicalis* (a frog), and *Xenopus laevis* (another species of frog). These include most of the well-known experimental vertebrates as well as others of commercial importance. As in the Human Genome Project, annotation, closing gaps, and checking assemblies may require additional years.

Beyond the next few years, there is strong advocacy for genomic sequences of less well-known experimental animals, including *Peromyscus* (the deer mouse) and *Tupaia* (the tree shrew), as well as representatives of distinct evolutionary lineages such as elephants. Sequencing the genomes of such animals is important, since the best animals for comparative genomics are not necessarily experimentally or commercially im-

portant. For example, the small size of the *Fugu* genome or the intermediate size of the marsupial genome makes these valuable because of their uniqueness, while at the same time they possess copies of different variants of many of the same genes. Such comparisons may provide insights into gene function and interactions among genes and their products.

Nonvertebrate animal genomes have been sequenced for *Ciona intestinalis* (the sea-squirt), *Anopheles gambiae* (the malaria mosquito), *Drosophila melanogaster* (the fruit or vinegar fly), and *Caenorhabditis briggsae* and *C. elegans* (nematode worms). Projects soon to be completed include *Apis mellifera ligustica* (the honeybee), *Culex* and *Aedes* (mosquitoes), *Glossina morsitans* (the tsetse fly), and *Brugia malayi* (the nematode that causes elephantiasis). For comparative reasons a cnidarian and a mollusk would be valuable.

Fungi projects include *Aspergillus* species, *Candida albicans* (which causes thrush infections), *Cryptococcus neoformans*, *Neurospora crassa* (orange bread mold), *Phanerochaete chrysosporium* (white wood rot), *Saccharomyces cerevisiae* (baker's and brewer's yeast), *Schizosaccharomyces pombe* (fission yeast), and *Pneumocystis carinii* (which causes pneumonia). Many more are soon to start.

Plants often have very large genomes because of duplication events (tetraploidy). *Arabidopsis thaliana* (thale cress) at about 115 Mbp and *Oryza sativa* (rice) at about 430 Mbp have been completed, and large-scale EST sequencing projects are under way for wheat, potato, cotton, tomato, barley and corn, which all have much larger genomes.

A wide variety of parasites are also being sequenced: *Cryptosporidium parvum*, which causes diarrhea; *Plasmodium falciparum*, which causes malaria; *Toxoplasma gondii*, a microsporidian; *Encephalitozoon cuniculi*, kinetoplastids; *Leishmania major*, which causes leishmaniasis; *Trypanosoma brucei*, which causes sleeping sickness; *Trypanosoma cruzi*, which causes Chagas' disease; *Thalassiosira pseudonana*, a diatom; *Dictyostelium discoideum*, a slime mold; and *Entamoeba histolytica*, which causes amebic dysentery.

—Peter J. Waddell and Michael J. McLachlan

Because the genome includes all of the genes that are expressed in an organism, knowing its nucleotide sequence is considered the first step in a complete understanding of the genetics of an organism. It must be emphasized that much more work follows this first step, because just knowing the nucleotide sequence of all the genes does not necessarily identify their function or how they interact with other genes. One important side benefit of having the complete genome sequence is that it can greatly speed the discovery of mutant genes. The human genome sequence, completed by the Human Genome Project in 2003, has already enabled medical geneticists to find a number of genes for genetic defects.

Sequencing Whole Genomes

A number of complementary strategies are involved in sequencing a genome. One approach is the shotgun sequencing of mapped clones. Large sections of DNA are cloned into vectors such as bacterial artificial chromosomes (BACs). A physical map of each BAC is made using techniques such as restriction mapping or the assignment of previously known sequence elements. The BAC maps are compared to identify overlapping clones, forming a map of long contiguous regions of the genome. BACs are selected from this map and the inserts are randomly fragmented into short pieces, 1-2 kilobase pairs (kb), and subcloned into vectors. Subclones are selected at random and sequenced. Many subclones are sequenced (often enough to provide sevenfold coverage of the clone) and then assembled to yield the contiguous sequence of the original BAC insert. The sequences from overlapping BACs are then assembled. In the finishing stage, additional bridging sequences are obtained to close gaps where there were no overlapping clones.

Whole genome shotgun sequencing involves randomly fragmenting the whole genome and sequencing clones without an initial map. Small clones (up to around 2 kb) are sequenced and assembled into contiguous regions with the help of sequences from larger (10-50 kb) clones that form a scaffold. The sequence is then linked to a physical map of the organism's chromosomes. This method works effectively

on bacterial genomes because of their small size and lack of repetitive DNA. However, the amount of repetitive sequence in eukaryotes can lead to difficulties for sequence assembly and gap filling. Therefore, a mapped, clone-based approach may be needed to finish such sequences. A genomic sequencing project may use a combination of the mapped clone method and whole genome shotgun sequencing to produce a completed genomic sequence.

An important adjunct to the genomic sequence is an extensive catalog of expressed sequence tags (ESTs), or full-length mRNAs from many different tissue types. This is achieved by reverse transcribing mRNA to complementary DNA (cDNA) and then sequencing. If a genome is impractically large to sequence at present (due to large amounts of noncoding DNA), this stage alone can yield much useful information.

Annotation

The annotation process involves gathering and presenting information about the location of genes, regulatory elements, structural elements, repetitive DNA, and other factors. It is important to integrate any previously known information regarding the genome, such as location of ESTs, at this stage. A powerful approach to identifying genes is to map ESTs and mRNAs to the genome. This will identify many of the protein-coding genes and can reveal the intron-exon structure plus possible alternative splicing of the gene. It will not identify most functional RNA genes, and how to do so effectively is an open question. Indeed, how many functional RNA genes there may be in eukaryotic genomes is unclear. For example, in humans approximately thirty-five thousand protein-coding genes have been identified, but there is evidence of many more transcribed sequences, and exactly what these are is unknown.

Some genes can be identified in the genomic sequence by the comparative approach—that is, by showing significant sequence similarity (for example, via BLAST algorithm) with annotated genes from other organisms. Such an approach becomes more powerful as the genomes of more organisms are published.

Computational methods can also be used to predict regions of the sequence that may represent genes. These rely on identifying patterns in the genomic sequence that resemble known properties of protein-coding genes, such as the presence of an open reading frame or sequence elements associated with promoters, intron-exon boundaries, and the 3' tail.

Functional Genomics

The availability of information identifying the majority of genes in an organism allows new kinds of experiments to be devised, and on a larger scale than ever before. Functional genomics, for example, aims to assign a functional role to each gene and identify the tissue type and developmental stage at which it is expressed. Identifying all genes in a genome makes it possible to determine the effect of altering the expression of each gene, through the use of knockouts, gene silencing, or transgenic experiments. Technologies such as microarray analysis allow mRNA expression levels to be measured for tens of thousands of genes simultaneously, while proteomic methods such as mass spectroscopy are beginning to allow high-throughput measurements of proteins. In these areas genomics overlaps with transcriptomics, proteomics, and specialties such as glycomics.

Structural Genomics

Structural genomics touches upon proteomics in the need to consider structural changes when there are post-translational changes or binding with other molecules. Structural genomics aims to define the three-dimensional folding of all protein products that an organism produces. The structure of a protein can provide insights into its function and mode of action. Identifying all the genes in a genome allows the amino acid sequence of each protein to be inferred from the DNA, and comparisons between them allow proteins (or characteristic sections of a protein, called folds or domains) to be identified and classified into families.

Structural prediction typically proceeds via each gene being cloned and then expressed. The protein product is then purified, and its

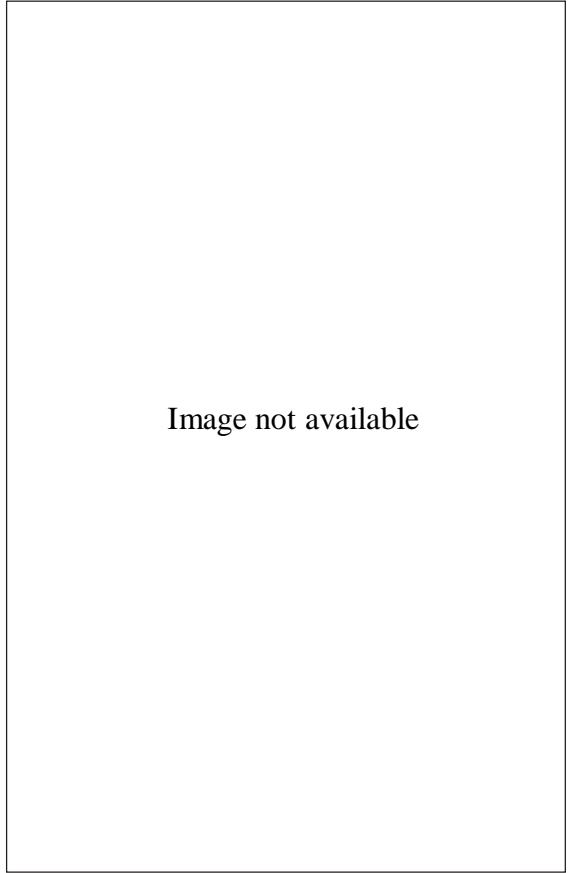


Image not available

A researcher examines DNA samples at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois, Urbana. (AP/Wide World Photos)

structures are experimentally determined using methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. Computational methods of structural prediction, either *ab initio* (from the beginning) or alternatively by computational prediction, aided by the known structure of a related protein, are generally inferior to direct experimental approaches, but these fields are rapidly advancing and are the key to the future.

Comparative Genomics

Comparative genomics expands knowledge through the comparison of different organisms' genomes. This is essential to the annotation of genomic sequences. For example, both otherwise unknown genes and particularly regulatory elements in humans and mice were first

revealed by identifying conserved regions of their genomic sequences. This can identify genes homologous to those in other species or identify a new member of a gene family. Comparing genomes can give insights into evolutionary questions about a particular gene or the organisms themselves. Important information can also be discovered about the regulation of different genes, the effects of different gene expression patterns between different species, and how the genome of each species came to be the way it is. Comparative genomics essentially rests upon phylogenetic methodology to describe the pattern and process of molecular evolution (phylogenomics).

—Peter J. Waddell and Michael J. McLachlan

See also: Bioinformatics; cDNA Libraries; Chromosome Walking and Jumping; DNA Sequencing Technology; Gene Families; Genetic Engineering; Genome Size; Genomic Libraries; Human Genome Project; Molecular Clock Hypothesis; Protein Structure; Protein Synthesis; Proteomics; Reverse Transcription; RNA World.

Further Reading

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ect. The whole journal issue contains many other papers considering the structure, function, and evolution of the human genome.

Venter, J. C., et al. "The Sequence of the Human Genome." *Science* 291, no. 5507 (2001): 1304-1351. Report on the Celera Genomics human genome project.

Web Sites of Interest

Department of Energy Joint Genome Institute. <http://www.jgi.doe.gov>. A collaboration between the Department of Energy's Lawrence Berkeley, Lawrence Livermore, and Los Alamos National Laboratories. Includes an introduction to genomics, a research time line that starts with Darwin's work in 1859, and links.

Human Genome Sequencing Center. <http://www.hgsc.bcm.tmc.edu>. Baylor College of Medicine. Posts an ongoing "counter" of human genome sequencing completed worldwide.

National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov>. A central repository for biological information, including links to genome projects and genomic science. Maintains GenBank, a comprehensive, annotated collection of publicly available DNA sequences.

Hardy-Weinberg Law

Field of study: Population genetics

Significance: *The Hardy-Weinberg law is the foundation for theories about evolution in local populations, often called microevolution. First formulated in 1908, it continues to be the basis of practical methods for investigations in fields from plant breeding and anthropology to law and public health.*

Key terms

ALLEL FREQUENCY: the proportion of all the genes at one chromosome location (locus) within a breeding population

GENE FLOW: movement of alleles from one population to another by the movement of individuals or gametes

GENE POOL: the total set of all the genes in all individuals in an interbreeding population

GENETIC DRIFT: random changes in allele frequencies caused by chance events

Introduction

The Hardy-Weinberg law can be phrased in many ways, but its essence is that the genetic makeup of a population, which meets certain assumptions, will not change over time. More important, it allows quantitative predictions about the distribution of genes and genotypes within and among generations. It may seem strange that theories about fundamental mechanisms of evolution are based on a definition of conditions under which evolution will not occur. It is the nature of science that scientists must make predictions about the phenomena being studied. Without something with which to compare the results of experiments or observations, science is impossible. Sir Isaac Newton's law of inertia plays a similar role in physics, stating that an object's motion will not change unless it is affected by an outside force.

After the rediscovery of Mendelian genetics in 1900, some scientists initially thought dominant alleles would become more common than recessive alleles, an error repeated in each generation of students. In 1908, Godfrey Hardy published his paper "Mendelian Proportions in a Mixed Population" in the journal *Science* to

counteract that belief, pointing out that by themselves, sexual reproduction and Mendelian inheritance have no effect on an allele's commonness. Implicit in Hardy's paper was the idea that populations could be viewed as conglomerations of independent alleles, what has come to be called a "gene pool." Alleles randomly combine in pairs to make up the next generation. This simplification is similar to Newton's view of objects as simple points with mass.

Hardy, an English mathematician, wrote only one paper in biology. Several months earlier, Wilhelm Weinberg, a German physician, independently and in more detail had proposed the law that now bears both their names. In a series of papers, he made other contributions, including demonstrating Mendelian heredity in human families and developing methods for distinguishing environmental from genetic variation. Weinberg can justifiably be regarded as the father of human genetics, but his work, like Mendel's, was neglected for many years. The fact that his law was known as Hardy's law until the 1940's is an indictment of scientific parochialism.

The Hardy-Weinberg Paradigm

The Hardy-Weinberg "law" is actually a paradigm, a theoretical framework for studying nature. Hardy and Weinberg envisioned populations as collections of gametes (eggs and sperm) that each contain one copy of each gene. Most populations consist of diploid organisms that have two copies of each gene. Each generation of individuals can be regarded as a random sample of pairs of gametes from the previous generation's gamete pool. The proportion of gametes that contain a particular allele is the "frequency" of that allele.

Imagine a population of one hundred individuals having a gene with two alleles, *A* and *a*. There are three genotypes (combinations of alleles) in the population: *AA* and *aa* (homozygotes), and *Aa* (heterozygotes). If the population has the numbers of each genotype listed in the table "Genome Frequencies," then the genotype frequencies can be computed as shown.

The individuals of each genotype can be

Genome Frequencies

Genotype	Number	Genotype frequency
AA	36	36/100 = 0.36
AB	48	48/100 = 0.48
BB	16	16/100 = 0.16
Total	100	1.00

viewed as contributing one of each of their alleles to the gene pool, which has the composition shown in the table headed “Gene Pool Composition.”

Gene Pool Composition

Genotype	A gametes	B gametes	Genotype contributions
AA	36 + 36 = 72		72
AB		48	96
BB		16 + 16 = 32	32
Total	120	80	200
Allele Frequency	120/200 = 0.6	80/200 = 0.4	200/200 = 1.0

This population can be described by the genotype ratio $AA:Aa:aa = 0.36:0.38:0.16$ and the allele frequencies $A:a = 0.6:0.4$. Note that allele frequencies must total 1.0, as must genotype frequencies.

The Hardy-Weinberg Law and Evolution

Allele and genotype frequencies would be of little use if they only described populations. By making a Punnett square of the gametes in the population and using allele frequencies, the table showing predicted genotype frequencies in the next generation will be obtained.

The predicted frequencies of homozygotes are 0.36 and 0.16; the frequency of Aa is 0.48

(adding the frequencies of Aa and aA). These are the same as the previous generation.

Hardy pointed out that if the frequency of $A = p$ and the frequency of $a = q$, then $p + q = 1$. Random mating can be modeled by the equation $(p + q) \times (p + q) = 1$, or more compactly $(p + q)^2 = 1$. This can be expanded to provide the genotype frequencies: $p^2 + 2pq + q^2 = 1$. In other words, the ratio of $AA:Aa:aa = p^2:2pq:q^2$. Substituting 0.6 for p and 0.4 for q produces the figures shown in the preceding table, but more compactly and easily. The Hardy-Weinberg concept may also be extended to genes with more than two alleles. Therefore, three predictions

may be made for a Hardy-Weinberg population: Frequencies of alleles p and q sum to 1.0 and will not change; the frequencies of genotypes AA , Aa , and aa will be $p^2:2pq:q^2$ respectively, will sum to 1.0, and will not change (that is, they are in equilibrium); and if the genotype frequencies are not initially at equilibrium ratios, they will eventually reach equilibrium.

There are within-generation and between-generation predictions. Within any one generation, the ratios of the genotypes are predictable if allele frequencies are known; if the frequency of a genotype is known, allele frequencies can be estimated. Between generations, allele and genotype frequencies will not change, as long as the following assumptions are met: (1) there are no mutations, (2) there is no gene flow with other populations, (3) mating is totally random, (4) the population is of infinite size, and (5) there is no natural selection. Violations of these assumptions define the five major evolutionary forces: mutation,

Predicted Genotype Frequencies

Sperm	Eggs	
	A (frequency = 0.6)	B (frequency = 0.4)
A (frequency = 0.6)	AA (frequency = $0.6 \times 0.6 = \mathbf{0.36}$)	BA (frequency = $0.6 \times 0.4 = \mathbf{0.24}$)
B (frequency = 0.4)	AB (frequency = $0.6 \times 0.4 = \mathbf{0.24}$)	BB (frequency = $0.4 \times 0.4 = \mathbf{0.16}$)

gene flow, nonrandom mating, genetic drift, and natural selection, respectively.

Despite its seeming limitations, the Hardy-Weinberg law has been crucially useful in three major ways. First, its predictions of allele and genotype frequencies in the absence of evolution provide what statisticians call the “null hypothesis,” which is essential for statistically rigorous hypothesis tests. If measured frequencies do not match predictions, then evolution is occurring. This redefines evolution from a vague “change in species over time” to a more useful, quantitative “change in allele or genotype frequencies.” However, it is a definition that cannot be used in the domain of “macroevolution” and paleontology above the level of biological species. Similarly, Newton’s definition of a moving object does not apply in quantum physics. Second, Hardy-Weinberg provides a conceptual framework for investigation. If evolution is happening, a checklist of potential causes of evolution can be examined in turn. Finally, the Hardy-Weinberg paradigm provides the foundation for mathematical models of each evolutionary force. These models help biologists determine whether a specific evolutionary force could produce observed changes.

Using the Hardy-Weinberg Law

Sickle-cell disease is a severe disease of children characterized by reduced red blood cell number, bouts of pain, fever, gradual failure of major organs, and early death. In 1910, physicians noticed the disease and associated it with distortion (“sickling”) of red blood cells. They realized that victims of the disease were almost entirely of African descent. Studies showed that the blood of about 8 percent of adult American blacks exhibited sickling, although few actually had the disease. By the 1940’s, they knew sickling was even more common in some populations in Africa, India, Greece, and Italy.

In 1949, James Neel proved the disease was caused by a recessive gene: Children homozygous for the sickle allele developed the disease and died. Heterozygotes showed the sickle trait but did not develop the disease. Using the Hardy-Weinberg law, Neel computed the allele frequency among American blacks as follows: Letting p = the frequency of the sickle allele,

$2pq$ is the frequency of heterozygotes (8 percent of adult African Americans). Since $p + q = 1$, $q = 1 - p$ and $2p(1 - p) = 0.08$. From this he computed $p = 0.042$ (about 4 percent). From the medical literature, Neel knew the frequency of the sickle trait in several African populations and computed the sickle allele frequency to be as high as 0.10 (since then the frequency has been found to be as high as 0.20). These are extraordinarily high frequencies for a lethal recessive allele and begged the question: Why was it so common?

The Hardy-Weinberg assumptions provided a list of possibilities, including nonrandom mating (mathematical models based on Hardy-Weinberg showed nonrandom mating distorts genotype frequencies but cannot change allele frequencies), mutation (for the loss of sickle alleles via death of homozygotes to be balanced by new mutations, scientists estimated the mutation rate from normal to sickle allele would have to be about three thousand times higher than any known human mutation rate, which seemed unlikely), and gene flow (models showed gene flow reduces differences between local populations caused by other evolutionary forces; gene flow from African populations caused by slavery explained the appearance of the sickle allele in North America but not high frequencies in Africa).

Another possibility was genetic drift. Models had shown deleterious alleles could rise to high frequencies in very small populations (smaller than one thousand). It was possible the sickle allele “drifted” to a high frequency in a human population reduced to small numbers by some catastrophe (population “bottleneck”) or started by a small number of founders (the “founder effect”). If so, the population had since grown far above the size at which drift is significant. Moreover, drift was random; if there had been several small populations, some would have drifted high and some low. It was unlikely that drift would maintain high frequencies of a deleterious allele in so many large populations in different locations. Therefore, the remaining possibility, natural selection, was the most reasonable possibility: The heterozygotes must have some selective advantage over the normal homozygotes.

A few years later, A. C. Allison was doing field work in Africa and noted that the incidence of the sickle-cell trait was high in areas where malaria was prevalent. A search of the literature showed this was also true in Italy and Greece. In 1954, Allison published his hypothesis: In heterozygotes, sickle-cell alleles significantly improved resistance to malaria. It has been repeatedly confirmed. Scientists have found alleles for several other blood disorders that also provide resistance to malaria in heterozygotes.

Impact and Applications

The Hardy-Weinberg law has provided scientists with a more precise definition of evolution: change in allele or genotype frequencies. It allows them to measure evolution, provides a conceptual framework for investigation, and continues to serve as the foundation for the theory of microevolution. Beyond population genetics and evolution, the Hardy-Weinberg paradigm is used in such fields as law (analysis of DNA “fingerprints”), anthropology (human migration), plant and animal breeding (maintaining endangered species), medicine (genetic counseling), and public health (implementing screening programs). In these and other disciplines, the Hardy-Weinberg law and its derivatives continue to be useful.

The Hardy-Weinberg law also has implications for social issues. In the early twentieth century, growing knowledge of genetics fueled a eugenics movement that sought to improve society genetically. Eugenicists in the 1910's and 1920's promoted laws to restrict immigration and promote sterilization of “mental defectives,” criminals, and other “bad stock.” The Hardy-Weinberg law is often credited with the decline of eugenics. The ratio $2pq/q^2 = 1$ makes it clear that if a recessive trait is rare (as most deleterious alleles are), most copies of a recessive allele are hidden in apparently normal heterozygotes. Selecting against affected individuals will be inefficient at best. However, a host of respected scientists championed eugenics into the 1920's and 1930's, long after the implications of Hardy-Weinberg were understood. It was really the reaction to the horrors of Nazi leader Adolf Hitler's eugenics program that made eugenics socially unacceptable. More-

over, it is premature to celebrate the end of the disturbing questions raised by eugenics. Progress in molecular biology makes it possible to detect deleterious alleles in heterozygotes, making eugenics more practical. Questions of whether genes play a major role in criminality and mental illness are still undecided. Debate about such medical and social issues may be informed by knowledge of the Hardy-Weinberg law, but decisions about what to do lie outside the domain of science.

—Frank E. Price

See also: Consanguinity and Genetic Disease; Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Genetic Load; Genome Size; Heredity and Environment; Inbreeding and Assortative Mating; Natural Selection; Polyploidy; Population Genetics; Punctuated Equilibrium; Quantitative Inheritance; Sickle-Cell Disease; Sociobiology; Speciation.

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Heart Disease

Field of study: Diseases and syndromes

Significance: Individual susceptibility to cardiovascular (heart) disease involves the interaction of complex genetic traits, as well as factors loosely defined as “environmental.” Only in rare circumstances do specific mutations result in disease; more often, quantitative differences in gene products reflect a minimum threshold necessary for overt disease.

Key terms

ANGINA PECTORIS: chest pain that can be indicative of heart disease

ANGIOTENSIN-CLEAVING ENZYME (ACE): a protein indirectly involved in regulation of blood pressure

APOLIPOPROTEIN A1 (APO A1): a molecule that binds cholesterol in the bloodstream, facilitating its removal by the liver

ATHEROSCLEROSIS: narrowing of coronary arteries that results from plaque formation in the vessel

CHOLESTEROL: a steroid derivative that is a normal constituent of cell membranes, but which plays a role in plaque formation in arteries

INTEGRIN B₃ (ITGB₃): a glycoprotein found in platelet membranes that plays a role in adherence to capillary surfaces

LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLR): a protein on surface of liver cells that removes cholesterol and other lipids from the bloodstream

QUANTITATIVE TRAIT LOCUS (QTL) (*pl. QUANTITATIVE TRAIT LOCI*): a group of genes that interact in defining physical or biochemical characteristics such as development of disease

Forms and Genetic Basis

Coronary heart disease (CHD) is arguably the leading cause of death among older adults. While CHD may take a variety of forms, most commonly it is associated with a narrowing of the coronary arteries that supply oxygen and other nutrients to heart tissue. Eventually, the artery may be completely blocked. While cholesterol or other lipids certainly play a role in the process, other factors or systems are also involved. Most of these involve genetically encoded proteins or protein-utilizing systems.

Since heart disease often runs in families, there is clearly a genetic element in its development. Generally, development of disease in populations can be described as a continuum, with the quantitative level and rate of CHD development varying among individuals; even accounting for the role of the environment, some individuals are more susceptible than others. The genes that are involved can be mapped, in some cases to specific sites on chromosomes, and until the genes are actually identified they are referred to as quantitative trait loci (QTLs).

At least 250 genes or QTLs have now been linked in some manner with CHD development. This is not to say that environment plays

only a limited role. The form or quantity of the gene product may define susceptibility to disease; often, however, the environment may play a major role in determining the significance played by that product.

Cholesterol

Cholesterol was the first component in blood in which the concentration could be correlated with risk for heart disease. While other molecules found in the bloodstream are now known to also play undefined roles in CHD, elevated cholesterol concentration remains one of the more important risk factors.

The concentration of cholesterol is responsive to a variety of processes under genetic control. These take the form of either cholesterol “packaging” or removal. Once in the bloodstream, cholesterol becomes linked with any of a variety of proteins or other molecules. Low-density lipoprotein (LDL) is often referred to as “bad” cholesterol, while high-density lipoprotein (HDL) is called the “good” cholesterol. The apo A1 protein, product of the *APOA1* gene, binds with cholesterol in forming HDLs. At least certain forms of CHD are associated with variants of the gene product apo A1, which form lower concentrations of HDL. Likewise, the *APOA1* gene can express variants that increase the concentration of LDLs, with a concordant increase in risk of CHD.

The concentration of “packaged” cholesterol is also reflected by its rate of removal from the bloodstream. In part, this is a function played by the liver. Liver cells have LDL receptors, the function of which is to bind and remove LDLs. At least 350 genetic variants have been described for the *LDLR* gene, resulting in significant quantitative differences among persons in their efficiency of LDL removal. Persons with those receptor variants that function inefficiently often are at increased risk of heart disease. For example, hypercholesterolemia, a condition in which cholesterol may be three to four times the normal level, is often the result of reduced numbers of LDL receptors. Persons with this condition are at extremely high risk for development of atherosclerosis and may suffer heart attacks even as young adults.

Defects in the Blood-Clotting System

While the buildup of plaque in coronary arteries is a major factor in heart disease, a heart attack is often triggered by clot formation at the site of narrowing. The uneven nature or structure of the plaque may itself be sufficient for clot formation. Nevertheless, the discovery that persons with variants of certain clot-associated or inflammatory factors may show increased risk of clot formation suggests genetic factors may also play a role.

Clot formation begins when blood platelets attach to the surface at the site of an injury. Among the molecules found in the cell membrane of platelets is a glycoprotein encoded by the *ITGB3* gene. The normal function of this molecule is to enable the platelet to attach to the surface of the blood capillary. However, a large proportion of middle-aged adults suffer-

ing from CHD have been shown to express an unusual variant of this gene, resulting in an increased capacity of platelets to initiate clot formation.

A second gene in the blood-clotting category encodes the protein thrombospondin (TSP), one of a family of proteins that regulate adhesion of cells to capillary surfaces. Epidemiological studies of persons with coronary artery disease have shown that certain variations of this gene are found in a large proportion of patients, while other variants seem associated with a decreased risk of disease.

Quantitative Trait Loci

Quantitative traits are those expressed at varying levels in the population. To date, most QTL studies have involved genetic crosses of rats or mice; mice in particular are simple to inbreed and share some genetic similarities with humans. Other genes have been found as a result of the Human Genome Project.

Approximately thirty QTLs have been defined in humans. Individual genes that make up QTLs are themselves heterogeneous, existing as multiple variants or alleles. Clearly, CHD is a complex disorder involving the interaction of many gene products of numerous alleles, and the specific role played by such loci in most forms of CHD remains to be explained. The association of one type of QTL linked to a risk factor for CHD, that of left ventricular hypertrophy (LVH), provides a prototype for understanding the interaction of gene products in development of disease.

LVH represents a condition in which the mass of the lower portion of the heart increases, raising the level of blood pressure and increasing the strain on the heart. Since LVH is a significant risk factor in devel-

Image not available

At the Nebraska Heart Institute in August, 2002, Dr. Vish Bhoopalam discusses gene therapy used to stimulate growth of new capillaries near the heart. (AP/Wide World Photos)

opment of coronary disease, an understanding of the process is important in its prevention.

Using crosses among inbred strains of rats, researchers have found that genetic markers for LVH can be mapped to certain QTLs. The quantitative expression of a protein, atrial natriuretic factor (ANF), appeared to correlate with the extent of LVH in rats: The higher the concentration of ANF, the lower was the ventricular mass. A specific gene, natriuretic peptide precursor A (*Nppa*), was found in the region, and it encoded the ANF precursor protein.

The relationship between QTLs and CHD remains more elusive. Human populations obviously do not lend themselves to similar forms of crosses, so identification of candidate QTLs has generally been limited to studies between twins, or at least siblings. These studies have demonstrated that both genetics and the environment play roles of indeterminate importance in CHD. If one sibling develops disease, the other is at significantly higher risk for the same. At the same time, certain behavioral risk factors such as smoking or obesity also increase the incidence of cardiovascular disease.

Most candidate QTLs have been limited to an association with hypertension; mutations in certain such genes have been observed in hypertensive cases responsive to reduced levels of salt intake. The actual function of the gene products has not been determined. Since there exists a clear relationship between elevated blood pressure and development of heart disease, these would qualify as candidate loci.

Blood Pressure

The regulation of blood pressure by the body involves a number of complementary systems. One of these mechanisms involves the molecule angiotensin II. Produced within the liver, angiotensin II constricts small arteries, increasing blood pressure, and regulates salt uptake by the kidney.

Angiotensin II is first synthesized as an inactive precursor molecule, angiotensinogen; subsequent cleavage is required for activation. Regulation of angiotensin concentration is in part a function of two different gene products. The product of the *ACE* gene, an angiotensin-

cleaving enzyme, converts the inactive precursor to the active form. Inactivation of angiotensin II is carried out by the product of the *ACE2* gene, angiotensin-converting enzyme 2.

It is known that high blood pressure in some individuals may be aggravated by diets high in salt. Animals that exhibit similar characteristics are often found to express decreased concentrations of the *ACE2* enzyme, resulting in greater salt retention as well as higher blood pressure. Whether a similar situation exists in humans is unclear, though there is evidence that reduced levels of *ACE2* production may play an analogous role in humans.

Nature vs. Nurture

For most populations, the risk of CHD represents a continuum, with some individuals at low risk and others at increasingly elevated risk. Excluding those situations in which specific genetic variation is directly the cause of CHD, as in the situation of hypercholesterolemia, in most persons the genetic pattern merely relates to the ability of environmental factors to trigger disease. For example, while elevated blood pressure is a significant factor in long-term cardiovascular injury, environmental factors such as obesity, diet, level of stress, and level of exercise may themselves determine the extent of hypertension.

The single most important environmental factor associated with CHD that can be controlled is that of tobacco use. While genetic variation may play a role in susceptibility to the effects of tobacco smoke, there is no question that a cause-and-effect relationship between the extent of smoking and increased risk for disease exists.

As the role played by specific genes in development of CHD becomes more apparent, not only is there the potential for improved treatments, but methods both for screening and prevention become possible. Persons at greater risk may be identified on the basis of possessing certain genetic variants, and methods of treatment may be determined as a result of knowing a specific cause.

—Richard Adler

See also: Congenital Defects; Diabetes; Genetic Testing; Hereditary Diseases; Heredity

and Environment; Human Genetics; Human Genome Project; Hypercholesterolemia; Organ Transplants and HLA Genes; Prenatal Diagnosis.

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Web Sites of Interest

American Heart Association. <http://www.americanheart.org>. The AHA's Web site offers education regarding the different forms of heart disease, symptoms, and treatments. A page devoted to congenital disorders as well as a search on "gene" and similar words offer information and articles on specific hereditary defects and conditions.

National Institutes of Health, National Library of Medicine. <http://www.nlm.nih.gov>. This site offers comprehensive information on heart disease, including genetics and research.

Hemophilia

Field of study: Diseases and syndromes

Significance: *Hemophilia is a sex-linked inherited genetic disorder in which the blood does not clot adequately. Although incidents of hemophilia are relatively rare, the study of this disease has yielded important information about genetic transmission and the factors involved in blood clotting.*

Key terms

- HEMOPHILIA A:** a blood disease with a deficiency of clotting factor VIII
- HEMOPHILIA B:** a blood disease with a deficiency of clotting factor IX
- HEMOSTASIS:** the process by which blood flow is stopped at an injury site

Causes and Symptoms

When an injury occurs that involves blood loss, the body responds by a process known as hemostasis. Hemostasis involves several steps that result in the blood clotting and stopping the bleeding. With hemophilia, an essential substance is absent. For blood to clot, a series of chemical reactions must occur in a "domino effect." The reaction starts with a protein called the Hageman factor or factor XII, which cues factor XI, which in turn cues factor X and so on until factor I is activated. Each factor is expressed by a different gene. If one of the genes is defective, the blood will not clot properly. Hemophilia A is the most common type, affect-

Alleles and Hemophilia

		Father's Sperm Cells	
		X	Y
Mother's Egg Cells	X	XX Normal Girl	XY Normal Boy
	X _h	XX _h Normal Girl (carrier)	X _h Y Hemophiliac Boy

The daughters produced by the union depicted in this table will be physically normal, but half will be carriers of hemophilia. Half the sons produced by the union will be hemophiliacs.

ing over 80 percent of all hemophiliacs and resulting when clotting factor VIII is deficient. Hemophilia B (also known as Christmas disease) affects about 15 percent of hemophiliacs and results when clotting factor IX is deficient.

Hemophilia affects males almost exclusively because it is an X-linked (often called sex-linked) recessive trait. Although it is possible for women to have hemophilia, it is extremely rare, because women must have two copies of the defective gene to be affected. A female has two X chromosomes, and a male has an X and Y chromosome. Even though the trait is recessive, because men have a single X chromosome, recessive X-linked genes are expressed as if they were dominant. Thus, hemophilia in males is inherited, along with their X chromosome, from the mother. The daughter of a hemophiliac father will carry the disease because she inherits one X chromosome (with the abnormal gene) from the father and one from the mother. Any son born to a carrier has a 50 percent chance of having hemophilia, since she will either pass on the X chromosome with the normal gene or the one with the abnormal gene. In order for a female to have hemophilia, she would have to inherit the abnormal gene on the X chromosomes from both her mother and her father.

Hemophilia can be mild, moderate, or severe, depending on the extent of the clotting factor deficiency. Mild hemophilia may not

be evident until adulthood when prolonged bleeding is observed after surgery or a major injury. The symptoms of moderate or severe hemophilia often appear early in life. These symptoms may include easy bruising, difficulty in stopping minor bleeding, bleeding into the joints, and internal bleeding without any obvious cause (spontaneous bleeding). When bleeding occurs in the joints, the person experiences severe pain, swelling, and possible deformity in the affected joint. The weight-bearing joints such as ankles and knees are usually affected. Internal bleeding requires immediate hospitalization and could result in death if severe. People who experience prolonged or abnormal bleeding are often tested for hemophilia. Testing the specific blood-clotting factors can determine the type and severity of hemophilia. Although a family history of hemophilia may help in the diagnosis, approximately 20 percent of hemophiliacs have no such history of the disease.

Impact and Applications

Hemophilia is not curable, although advances in the treatment of the disease are prolonging life and preventing crippling deformities. Symptoms of hemophilia can be reduced by replacing the deficient clotting factor. People with hemophilia A may receive antihemophilic factors to raise their blood-clotting factor above normal levels so that the blood clots appropriately. People with hemophilia B may receive clotting factor IX during bleeding episodes in order to increase the clotting factor levels. The clotting factors may be taken from plasma (the fluid part of blood), although it takes a great deal of plasma to produce a small amount of the clotting factors. Risks include infection by the hepatitis virus or human immunodeficiency virus (HIV), although advanced screening procedures have greatly reduced such risks. In 1993 the FDA approved a new recombinant form of factor VIII and in 1997 the FDA approved a new recombinant form of factor IX for treating individuals with hemophilia A and B, respectively. The advantage of recombinant factors is that they are automatically free of plasma-derived viruses, thus reducing one of the primary risks endured by previous hemo-

philiacs. Patients with mild hemophilia may be treated with a synthetic hormone known as desmopressin acetate (DDAVP).

Treatment with the plasma clotting factors has increased longevity and quality of life. In addition, many patients are able to treat bleeding episodes as outpatients with home infusions or self-infusions of the clotting factors. However, problems do exist with the treatment of hemophilia. Various illnesses, such as HIV, liver disease, or cardiovascular disease, have resulted from contamination of the clotting factors. Several techniques are used to reduce the risk of contamination, and most difficulties were largely eliminated by the mid-1990's. Bleeding into the joints is often controlled by

the use of elastic bandages and ice. Exercise is recommended to help strengthen and protect the joint. Painkillers are used to reduce the chronic pain associated with joint swelling and inflammation, although hemophiliacs cannot use products containing aspirin or antihistamines because they prolong bleeding. Patients and their families have also benefited from genetic education, counseling, and testing. Hemophilia centers can provide information on how the disease is transmitted, potential genetic risks, and whether a person is a carrier. This knowledge provides options for family planning as well as support in coping with the disease.

—Virginia L. Salmon, updated by Bryan Ness

Recombinant Factor VIII

Prior to the development of recombinant factor VIII, patients with hemophilia were treated with coagulation factors prepared from the blood of thousands of different donors. While these coagulation factor concentrates were highly effective in treating acute bleeding episodes, they also proved to be the source of infection with hepatitis and human immunodeficiency (HIV) viruses. Many patients with hemophilia became seriously ill and died from a treatment that was designed to save their lives.

Once the risk of viral infection from these pooled donations was recognized in the early 1980's, biomedical manufacturers introduced measures to inactivate the viruses during the process of preparing the concentrates. The next, even more important, step in improving hemophilia treatment was the development of recombinant factors VIII and IX using DNA technology. Early studies demonstrated that the recombinant factors were as effective as the pooled blood concentrates and had few adverse effects.

The first recombinant factor VIII concentrate was introduced in 1987. Large-scale multinational studies of the safety and effectiveness of recombinant factor VIII began in human subjects in 1989. All of these studies are classified as "prospective" or "cohort" studies where patients are enrolled, treated, and followed through many years. Since prospective studies are considered the most methodologically sound, they yield scientific information that is highly respected.

The results are encouraging. Previously untreated patients with hemophilia who have had severe bleeding episodes have responded well to recombinant products. The majority of the bleeds (71-91 percent) in most studies resolved with a single dose. Patients rarely have side effects, and those they experience are mild. About one-third of patients developed inhibitors to recombinant factor VIII, but several of these inhibitors disappeared over time. No one has found evidence of the transmission of infectious agents in the recombinant factor concentrates. Newer studies show that treatment at home by the patients themselves, preventive treatment prior to necessary surgery, and treatment in previously treated patients are effective and safe, with minimal adverse effects.

In the United States, recombinant factor VIII was licensed for use in 1992. These products are now used in United States, Canada, Europe, Japan, and elsewhere. Recombinant factors are considered in most areas the treatment of choice for the treatment of patients with severe hemophilia. Unfortunately, these products are not readily available and are extremely costly, meaning that physicians must select which patients are most appropriate for using recombinant factor VIII. In general, patients who have not been treated before and who are not infected with hepatitis or HIV viruses are the candidates most likely to receive these products until the supplies are greater and the costs lower.

—Rebecca Lovell Scott

See also: Amniocentesis and Chorionic Villus Sampling; Bacterial Genetics and Cell Structure; Chromosome Mutation; Cloning; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Engineering; Genetic Engineering: Medical Applications; Genetic Testing; Hereditary Diseases.

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- Potts, D. M., and W. T. W. Potts. *Queen Victoria's Gene: Haemophilia and the Royal Family*. Stroud: Sutton, 1999. Explores the source of hemophilia in the royal families of Europe and the effect it had on history. Illustrations, plates, genealogical tables, map.
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Web Sites of Interest

- Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including hemophilia.
- National Hemophelia Foundation. <http://www.hemophelia.org>. Site that includes information on research and links to related organizations.

Hereditary Diseases

Field of study: Diseases and syndromes

Significance: Scientists are discovering the genetic bases of an ever-increasing number of diseases affecting children and adults. The Human Genome Project was begun in 1990 with the goal of determining and mapping all human genes by the year 2005, a task which was largely completed by April, 2003. As knowledge about the genetics underlying different diseases is gained, opportunities should increase for the diagnosis, prevention, and treatment of these diseases.

Key terms

CHROMOSOMAL DEFECTS: defects involving changes in the number or structure of chromosomes

CONGENITAL DEFECTS: birth defects, which may be caused by genetic factors, environmental factors, or interactions between genes and environmental agents

HEMIZYGOUS: characterized by being present only in a single copy, as in the case of genes on the single X chromosome in males

MENDELIAN DEFECTS: also called single-gene defects; traits controlled by a single gene pair

MITOCHONDRIAL DISORDERS: disorders caused by mutations in mitochondrial genes

MODE OF INHERITANCE: the pattern by which a trait is passed from one generation to the next

MULTIFACTORIAL DISORDERS: disorders determined by one or more genes and environmental factors

Causes and Impact of Hereditary Diseases

Twentieth century medicine was hugely successful in conquering infectious diseases. Elimination, control, and treatment of diseases such as smallpox, measles, diphtheria, and plague have greatly decreased infant and adult mortality. Improved prenatal and postnatal care have also decreased childhood mortality. Shortly after the rediscovery of Mendelism in the early 1900's, reports of genetic determination of human traits began to appear in medical and biological literature. For the first half of the twentieth century, most of these reports were regarded as interesting scientific reports of isolated clinical diseases that were incidental to the practice of medicine. The field of medical genetics is considered to have begun in 1956 with the first description of the correct number of chromosomes in humans (forty-six). Between 1900 and 1956, findings were accumulating in cytogenetics, Mendelian genetics, biochemical genetics, and other fields that began to draw medicine and genetics together.

The causes of hereditary diseases fall into four major categories:

- (1) single-gene defects or Mendelian disorders (such as cystic fibrosis, Huntington's disease [Huntington's chorea], color blindness, and phenylketonuria)
- (2) chromosomal defects involving changes in the number or alterations in the structure of chromosomes (such as Down syndrome, Klinefelter syndrome, and Turner syndrome)
- (3) multifactorial disorders, caused by a combination of genetic and environmental factors (such as congenital hip dislocation, cleft palate, and cardiovascular disease)
- (4) mitochondrial disorders caused by mutations in mitochondrial genes (such as Leber hereditary optic neuropathy)

These four categories are relatively clear-cut. It is likely that genetic factors also play a less well-defined role in all human diseases, including susceptibility to many common diseases and degenerative disorders. Genetic factors may affect a person's health from the time before birth to the time of death.

Congenital defects are birth defects and may be caused by genetic factors, environmental factors (such as trauma, radiation, alcohol, infection, and drugs), or the interaction of genes and environmental agents. Alan Emery and David Rimoin noted that the proportion of childhood deaths attributed to nongenetic causes was estimated to be 83.5 percent in London in 1914 but had declined to 50 percent in Edinburgh by 1976, whereas childhood deaths attributed to genetic causes went from 16.5 percent in 1914 to 50 percent in 1976. These changes reflect society's increased ability to treat environmental causes of disease, resulting in a larger proportion of the remaining diseases being caused by genetic defects. Rimoin, J. Michael Connor, and Reed Pyeritz estimate that single-gene disorders have a lifetime frequency of 20 in 1000, chromosomal disorders have a frequency of 3.8 in 1000, and multifactorial disorders have a frequency of 646 in 1000. It is evident that hereditary diseases are and will be of major concern for some time.

Single-Gene Defects

Single-gene defects result from a change or mutation in a single gene and are referred to as Mendelian disorders or inborn errors of metabolism. In 1865, Gregor Mendel described the first examples of monohybrid inheritance. In a trait governed by a single locus with two alleles, individuals inherit one allele from each parent. If the alleles are identical, the individual is said to be homozygous. If the alleles are different, the individual is said to be heterozygous. Single-gene defects are typically recessive. A single copy of a dominant allele will be expressed the same in homozygous and heterozygous individuals. A recessive allele, on the other hand, is expressed in homozygous individuals (often called homozygotes). In heterozygotes, the dominant allele "hides" or masks the expression of the recessive allele. This helps explain why recessive single-gene defects predominate. Dominant single-gene defects are always expressed when present and never remain "hidden." As a result, natural selection quickly removes these defects from the population.

Genes can be found either on sex chromosomes or non-sex chromosomes (called auto-

Some Genetic Disorders

<i>Disorder</i>	<i>Genetic Characteristics</i>	<i>Disorder</i>	<i>Genetic Characteristics</i>
Achondroplasia	Autosomal dominant disorder	Hemochromatosis	Autosomal recessive disorder
Albinism	Autosomal recessive disorder	Hemophilia	X-linked recessive disorder
Alzheimer's disease, familial early onset	Mutations in <i>PS1</i> , <i>PS2</i>	Huntington's disease	Autosomal dominant disorder
Alzheimer's disease, late onset	Mutations in <i>APOE</i>	Hypercholesterolemia	Autosomal dominant disorder
Angelman syndrome	Deletion in chromosome 15	Klinefelter syndrome	Males that are XXY autosomal dominant disorder
Beta-thalassemia	Mutations in or impaired expression of the gene for beta-globin	Kuru	Prion disease
Breast cancer	Mutations in <i>BRCA1</i> , <i>BRCA2</i> , <i>p53</i> cause predisposition	Lactose intolerance	Autosomal recessive disorder
Burkitt's lymphoma	Reciprocal translocation involving chromosomes 8 and 14 (or occasionally 22 or 2)	Marfan syndrome	Autosomal dominant disorder
Cancer	Mutations in proto-oncogenes and tumor-suppressor genes or in the control regions of these genes cause predisposition	Metafemale (multiple X syndrome)	Females with more than two X chromosomes
Color blindness (common form)	Sex-linked recessive disorder	Neurofibromatosis (NF)	Types 1 and 2 both autosomal dominant disorders
Creutzfeldt-Jakob syndrome	Prion disease	Phenylketonuria (PKU)	Autosomal recessive disorder
Cystic fibrosis	Autosomal recessive disorder	Polycystic kidney disease	Autosomal dominant disorder
Diabetes, Type I	Mutations in the gene for insulin	Prader-Willi syndrome	Deletion in chromosome 15
Diabetes, Type II	Mutations in the gene for insulin	Pseudohermaphroditism	Autosomal recessive disorder
Down syndrome	Trisomy 21	Sickle-cell disease	Autosomal incompletely dominant disorder (sometimes considered autosomal recessive)
Down syndrome, familial	Translocation of part of chromosome 21	Tay-Sachs disease	Autosomal recessive disorder
Dwarfism (achondroplasia)	Autosomal dominant disorder	Testicular feminization syndrome	Form of pseudohermaphroditism, and also an autosomal recessive disorder
Fragile X syndrome	X-linked showing imprinting	Turner syndrome	Monosomy
		XYY syndrome	Males with an extra Y chromosome

—Bryan Ness

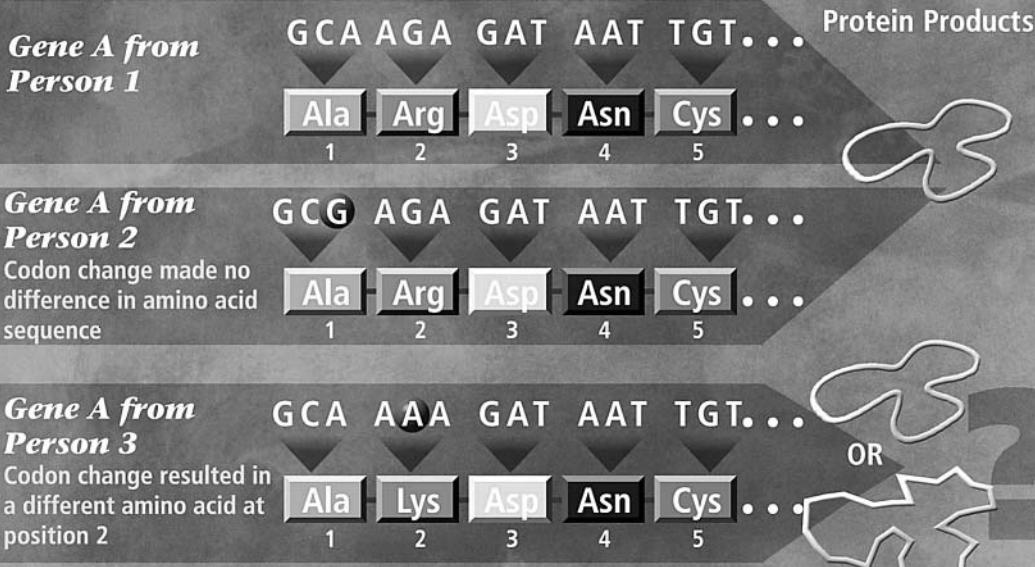
somes). One pair of chromosomes (two chromosomes of the 46 in humans) have been designated sex chromosomes because the combination of these two chromosomes determines the sex of the individual. Human males have an unlike pair of sex chromosomes, one called the X chromosome and a smaller one called the Y chromosome. Females have two X chromosomes. Genes on the X or Y chromosomes are considered sex-linked. However, since Y chromosomes contain few genes, "sex-linked" usually refers to genes on the X chromosome; when greater precision is required, genes on the X chromosome are referred to as "X-linked." Inheritance patterns for X-linked traits are different than for autosomal traits. Because males only have one X chromosome, any allele, whether normally recessive or dominant, will be expressed. Therefore, recessive X-linked traits are typically much more common in men than in women, who must have two recessive al-

leles to express a recessive trait. Additionally, a male inherits X-linked alleles from his mother, because he only gets a Y chromosome from his father.

Chromosomal Disorders

Chromosomal disorders are a major cause of birth defects, some types of cancer, infertility, mental retardation, and other abnormalities. They are also the leading cause of spontaneous abortions. Deviations from the normal number of forty-six chromosomes, or structural changes, usually result in abnormalities. Variations in the number of chromosomes may involve just one or a few chromosomes, a condition called aneuploidy, or complete sets of chromosomes, called polyploidy. Polyploidy among live newborns is very rare, and the few polyploid babies who are born usually die within a few days of birth as a result of severe malformations. The vast majority of embryos and fetuses

DNA Sequence Variation in a Gene Can Change the Protein Produced by the Genetic Code



Whereas some variations in an individual's genetic code will not affect the protein produced, others will, possibly resulting in disease or sensitivity to environmental triggers for the disease. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

with polyploidy are spontaneously aborted.

Aneuploidy typically involves the loss of one chromosome from a homologous pair, called monosomy, or possession of an extra chromosome, called trisomy. Monosomy involving a pair of autosomes usually leads to death during development. Individuals have survived to birth with forty-five chromosomes, but they suffered from multiple, severe defects. Most embryos and fetuses that have autosomal trisomies abort early in pregnancy. Invariably, trisomics that are born have severe physical and mental abnormalities. The most common trisomy involves chromosome 21 (Down syndrome), with much rarer cases involving chromosome 13 (Patau syndrome) or chromosome 18 (Edwards' syndrome). Infants with trisomy 13 or 18 have major deformities and invariably die at a very young age. Down syndrome is the most common (about one in seven hundred births) and is the best known of the chromosomal disorders. Individuals with Down syndrome are short and have slanting eyes, a nose with a low bridge, and stubby hands and feet; about one-third suffer severe mental retardation. The risk of giving birth to a child with Down syndrome increases dramatically for women over thirty-five years of age.

Variations in the number of sex chromosomes are not as lethal as those involving autosomes. Turner syndrome is the only monosomy that survives in any number, although 98 percent of them are spontaneously aborted. Patients have forty-five chromosomes consisting of twenty-two pairs of autosomes and only one X chromosome. They are short in stature, sterile, and have underdeveloped female characteristics but normal or near-normal intelligence. Other diseases caused by variations in the number of sex chromosomes include Klinefelter syndrome, caused by having forty-seven chromosomes, including two X and one Y chromosome (affected individuals are male with small testes and are likely to have some female secondary sex characteristics such as enlarged breasts and sparse body hair) and multiple X syndrome, or metafemale (affected individuals are females whose characteristics are variable; some are sterile or have menstrual irregularities or both).

Variations in the structure of chromosomes include added pieces (duplications), missing pieces (deletions), and transfer of a segment to a member of a different pair (translocation). Most deletions are likely to have severe effects on developing embryos, causing spontaneous abortion. Only those with small deletions are likely to survive and will have severe abnormalities. The cri du chat ("cry of the cat") syndrome produces an infant whose cry sounds like a cat's meow. There is also a form of Down syndrome, called familial Down syndrome, that is caused by a type of reciprocal translocation between two chromosomes.

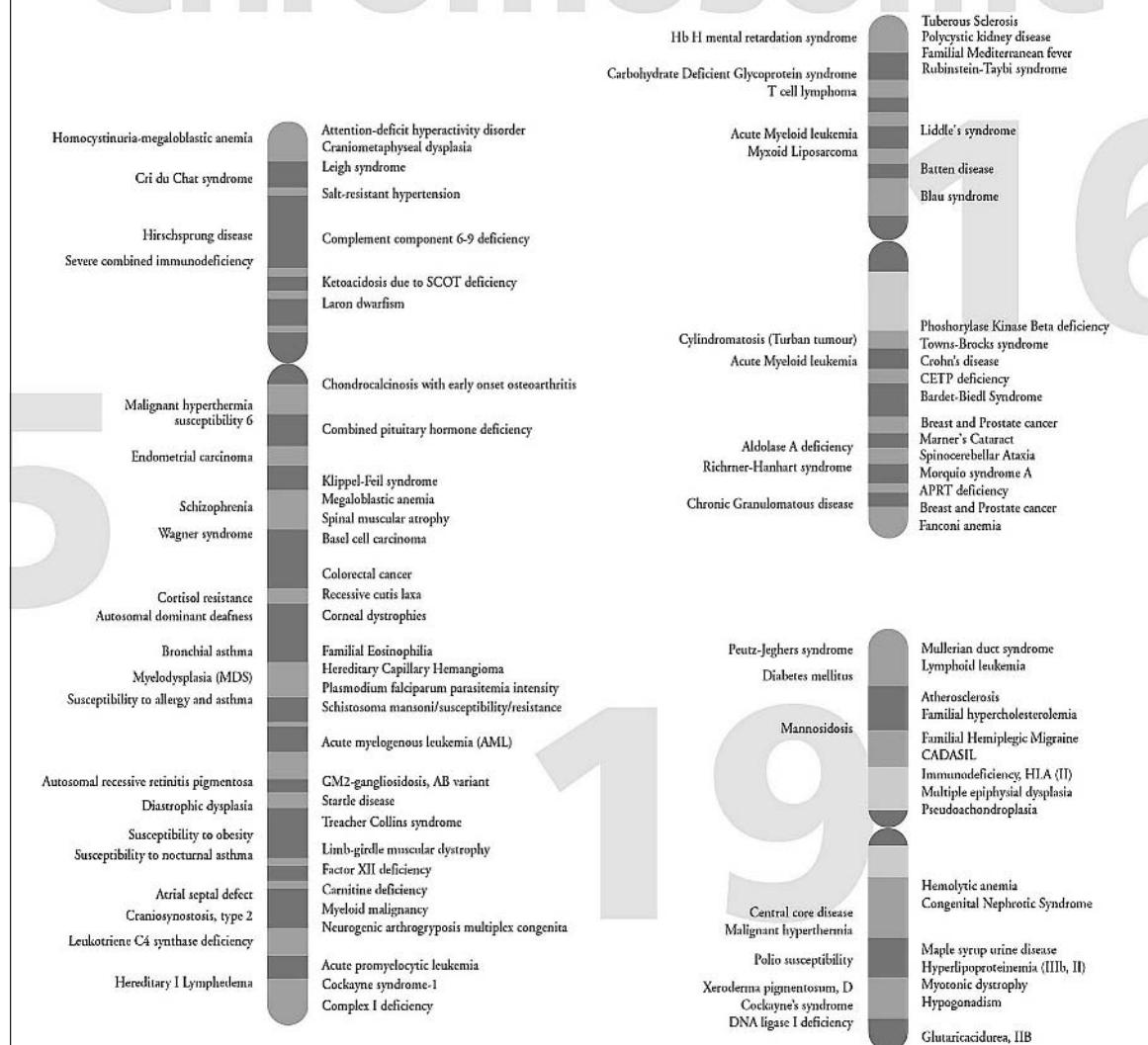
Multifactorial Traits

Multifactorial traits (sometimes referred to as complex traits) result from an interaction of one or more genes with one or more environmental factors. Sometimes the term "polygenic" is used for traits that are determined by multiple genes with small effects. Multifactorial traits do not follow any simple pattern of inheritance and do not show distinct Mendelian ratios. Such diseases show an increased recurrence risk within families. "Recurrence risk" refers to the likelihood of the trait showing up multiple times in a family; in general, the more closely related someone is to an affected person, the higher the risk. Recurrence risk is often complicated by factors such as the degree of expression of the trait (penetrance), the sex of the affected individual, and the number of affected relatives. For example, pyloric stenosis, a disorder involving an overgrowth of muscle between the stomach and small intestine, is the most common cause of surgery among newborns. It has an incidence of about 0.2 percent in the general population. Males are five times more likely to be affected than females. For an affected male, there is a 5 percent chance his first child will be affected, whereas for a female, there is a 16 percent chance her first child will be affected.

It is necessary to develop separate risks of recurrence for each multifactorial disorder. Multifactorial disorders are thought to account for 50 percent of all congenital defects. In addition, they play a significant role in many adult disorders, including hypertension and other

Sequencing Targets and Associated Diseases

Chromosome



This poster from the Joint Genome Institute shows the location of genes associated with diseases in three human chromosomes. (U.S. Department of Energy's Joint Genome Institute, Walnut Creek, California, <http://www.jgi.doe.gov>)

cardiovascular diseases, rheumatoid arthritis, psychosis, dyslexia, epilepsy, and mental retardation. In total, multifactorial disorders account for more genetic diseases than do single-gene and chromosome disorders combined.

Impact and Applications

In 2003, the Human Genome Project achieved its goal of mapping the entire human genome. The complete specifications of the genetic material on each of the twenty-two autosomes and the X and Y chromosomes will improve the understanding of the biological and molecular bases of hereditary diseases. Once the location of a gene is known, it is possible to make a better prediction of how that gene is transmitted within a family and of the probability that an individual will inherit a specific genetic disease.

For many hereditary diseases, the protein produced by the gene and its relation to the symptoms of the disease are not known. Locating a gene facilitates this knowledge. It becomes possible to develop new diagnostic tests and therapies. The number of hereditary disorders that can be tested prenatally and in newborns will increase dramatically. In the case of those single genes that do not produce clinical symptoms until later in life, many more of these disorders will be diagnosed before symptoms appear, opening the way for better treatments and even prevention. Possibilities will exist to develop the means of using gene therapy to repair or replace the disease-causing gene. The identification and mapping of single genes and those identified as having major effects on multifactorial disorders will greatly affect hereditary disease treatment and genetic counseling techniques. It is evident that knowledge of genes, both those that cause disease and those that govern normal functions, will begin to raise many questions about legal, ethical, and moral issues.

—Donald J. Nash, updated by Bryan Ness

See also: Albinism; Alcoholism; Alzheimer's Disease; Autoimmune Disorders; Breast Cancer; Burkitt's Lymphoma; Cancer; Color Blindness; Congenital Defects; Consanguinity and Genetic Disease; Cystic Fibrosis; Diabetes; Down Syndrome; Dwarfism; Emerging Diseases; Frag-

ile X Syndrome; Gender Identity; Heart Disease; Hemophilia; Hermaphrodites; Homosexuality; Human Genetics; Human Genome Project; Huntington's Disease; Hypercholesterolemia; Inborn Errors of Metabolism; Infertility; Klinefelter Syndrome; Lactose Intolerance; Metafemales; Mitochondrial Diseases; Monohybrid Inheritance; Neural Tube Defects; Phenylketonuria (PKU); Prader-Willi and Angelman Syndromes; Prion Diseases; Kuru and Creutzfeldt-Jakob Syndrome; Pseudohermaphrodites; Sickle-Cell Disease; Smallpox; Swine Flu; Tay-Sachs Disease; Testicular Feminization Syndrome; Thalidomide and Other Teratogens; Turner Syndrome; XY Syndrome.

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- Wynbrandt, James, and Mark D. Ludman. *The Encyclopedia of Genetic Disorders and Birth Defects*. 2d ed. New York: Facts On File, 2000. Six hundred entries cover the spectrum of clinical and research information on hereditary conditions and birth defects in a non-technical manner. Illustrated.

Web Sites of Interest

Centers for Disease Control, Office of Genomics and Disease Prevention. <http://www.cdc.gov/genomics/default.htm>. Offers information on the genetic discoveries and prevention of diseases in humans. Includes links to related resources.

Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes.

Genetic Alliance. <http://www.geneticalliance.org>. An international advocacy group of those with genetic conditions. This site provides links to information on the diseases, public policy, and support organizations for a broad array of hereditary diseases.

Medline Plus. <http://www.nlm.nih.gov/medlineplus>. Medline, sponsored by the National Institutes of Health, is one of the first stops for any medical question, and it offers information and references on most genetic diseases, birth defects, and disorders.

Heredity and Environment

Field of study: Human genetics

Significance: "Heredity and environment" is the modern incarnation of the age-old debate on the effects of nature versus nurture. Research in the field has implications ranging from the improvement of

crop plants to the understanding of the heritability of behavioral traits in humans.

Key terms

GENOTYPE: the genes that are responsible for physical or biochemical traits in organisms
HERITABILITY: a measure of the genetic variation for a quantitative trait in a population
PHENOTYPE: the physical and biochemical traits of a plant or animal

PHENOTYPIC PLASTICITY: the ability of a genotype to produce different phenotypes when exposed to different environments

QUANTITATIVE TRAIT LOCUS (QTL) MAPPING: a molecular biology technique used to identify genes controlling quantitative traits in natural populations

REACTION NORM: the graphic illustration of the relationship between environment and phenotype for a given genotype

Nature vs. Nurture and the Origin of Genetics

Is human behavior controlled by genes or by environmental influences? The “nature vs. nurture” controversy has raged throughout human history, eventually leading to the modern antithesis between hereditarianism and environmentalism in biological research. These two schools of thought have shaped a dispute that is at once a difficult scientific problem and a thorny ethical dilemma. Many disciplines, chiefly genetics but also the cognitive sciences, have contributed to the scientific aspect of the discussion. At the same time, racist and sexist overtones have muddled the inquiry and inextricably linked it to the implementation of social policies. Nevertheless, the relative degree of influence of genes and environments in determining the characteristics of living organisms is a legitimate and important scientific question, apart from any social or ethical consideration.

At the beginning of the twentieth century, scientists rediscovered the laws of heredity first formulated by Gregor Mendel in 1865. Mendel understood a fundamental concept that underlies all genetic analyses: Each discrete trait in a living organism, such as the color of peas, is influenced by minute particles inside the body

that behave according to simple and predictable patterns. Mendel did not use the term “gene” to refer to these particles (he called them “factors”), and his pioneering work remained largely unknown to the scientific community for the remainder of the nineteenth century. Immediately following the rediscovery of Mendel’s laws in 1900, the Danish biologist Wilhelm Johannsen proposed the fundamental distinction between “phenotype” and “genotype.” The phenotype is the ensemble of all physical and biochemical traits of a plant or animal. The composite of all the genes of an individual is its genotype. To some extent, the genotype determines the phenotype.

Reaction Norm: Environments and Genes Come Together

It was immediately clear to Johannsen that the appearance of a trait is the combined result of both the genotype and the environment, but to understand how these two factors interact took the better part of the twentieth century and is still a preeminent field of research in ecological genetics. One of the first important discoveries was that genotypes do not always produce the same phenotype but that this varies with the particular environment to which a genotype is exposed. For instance, if genetically identical fruit flies are raised at two temperatures, there will be clear distinctions in several aspects of their appearance, such as the size and shape of their wings, even though the genes present in these animals are indistinguishable.

This phenomenon can be visualized in a graph by plotting the observed phenotype on the *y*-axis versus the environment in which that phenotype is produced on the *x*-axis. A curve describing the relationship between environment and phenotype for each genotype is called a reaction norm. If the genotype is insensitive to environmental conditions, its reaction norm will be flat (parallel to the environmental axis); most genotypes, however, respond to alterations in the environment by producing distinct phenotypes. When the latter case occurs, that genotype is said to exhibit phenotypic plasticity. One can think of plasticity as the degree of responsiveness of a given genotype to changes in

its environment: The more responsive the genotype is, the more plasticity it displays.

The first biologist to fully appreciate the importance of reaction norms and phenotypic plasticity was the Russian Ivan Schmalhausen, who wrote a book on the topic in 1947. Schmalhausen understood that natural selection acts on the shape of reaction norms: By molding the genotype's response to the environment, selection can improve the ability of that genotype to survive under the range of environmental conditions it is likely to encounter in nature. For example, some butterflies are characterized by the existence of two seasonal forms. One form exists during the winter, when the animal's activity is low and the main objective is to avoid predators. Accordingly, the coloration of the body is dull to blend in with the surroundings. During the summer, however, the butterflies are very active, and camouflage would not be an effective strategy against predation. Therefore, the summer generation develops brightly colored "eyespots" on its wings. The function of these spots is to attract predators' attention away from vital organs, thereby affording the insect a better chance of survival. Developmental geneticist Paul Brakefield demonstrated, in a series of works published in the 1990's, that the genotype of these butterflies codes for proteins that sense the season by using environmental cues such as photoperiod and temperature. Depending on the perceived environment, the genotype directs the butterfly developmental system to produce or not produce the eyespots.

Quantitative Genetics of Heredity and Environment

An important aspect of modern science is the description of natural phenomena in mathematical form. This allows predictions on future occurrences of such phenomena. In the 1920's, Ronald Fisher developed the field of quantitative genetics, a major component of which is a powerful statistical technique known as analysis of variance. This allows a researcher to gather data on the reaction norms of several genotypes and then mathematically partition the observed phenotypic variation (V_p) into its three fundamental constituents:

$$V_p = V_g + V_e + V_{ge}$$

where V_g is the percentage of variation caused by genes, V_e is the percentage attributable to environmental effects, and V_{ge} is a term accounting for the fact that different genotypes may respond differently to the same set of environmental circumstances. The power of this approach is in its simplicity: The relative balance among the three factors directly yields an answer to any question related to the nature-nurture conundrum. If V_g is much higher than the other two components, genes play a primary role in determining the phenotype ("nature"). If V_e prevails, the environment is the major actor ("nurture"). However, when V_{ge} is more significant, this suggests that genes and environments interact in a complex fashion so that any attempt to separate the two is meaningless. Anthony Bradshaw pointed out in 1965 that large values of V_{ge} are indeed observable in most natural populations of plants and animals.

The quantity V_g is particularly important for the debate because when it is divided by V_p , it yields the fundamental variable known as heritability. Contrary to intuition, heritability does not measure the degree of genetic control over a given trait but only the relative amount of phenotypic "variation" in that trait that is attributable to genes. In 1974, Richard Lewontin pointed out that V_g (and therefore heritability) can change dramatically from one population to another, as well as from one environment to another, because V_g depends on the frequencies of the genes that are turned on (active) in the individuals of a population. Since different sets of individuals may have different sets of genes turned on, every population can have its own value of V_g for the same trait. Along similar lines, some genes are turned on or off in response to environmental changes; therefore, V_g for the same population can change depending on the environment in which that population is living. Accordingly, estimates of heritability cannot be compared between different populations or species and are only valid in one particular set of environmental conditions.

Molecular Genetics

The modern era of the study of nature-nurture interactions relies on the developments in molecular genetics that characterized the whole of biology throughout the second half of the twentieth century. In 1993, Carl Schlichting and Massimo Pigliucci proposed that specific genetic elements known as plasticity genes supervise the reaction of organisms to their surroundings. A plasticity gene normally encodes a protein that functions as a receptor of environmental signals; the receptor gauges the state of a relevant environmental variable such as temperature and sends a signal that initiates a cascade of effects eventually leading to the production of the appropriate phenotype. For example, many trees shed their leaves at the onset of winter in order to save energy and water that would be wasted by maintaining structures that are not used during the winter months. The plants need a reliable cue that winter is indeed coming to best time the shedding process. Deciduous trees use photoperiod as an indicator of seasonality. A special set of receptors known as phytochromes sense day length, and they initiate the shedding whenever day length becomes short enough to signal the onset of winter. Phytochromes are, by definition, plasticity genes.

Research on plasticity genes is a very active field in both evolutionary and molecular genetics. Johanna Schmitt's group has demonstrated that the functionality of photoreceptors in plants has a direct effect on the fitness of the organism, thereby implying that natural selection can alter the characteristics of plasticity genes. Harry Smith and collaborators have contributed to the elucidation of the action of photoreceptors, uncovering an array of other genes that relate the receptor's signals to different tissues and cells so that the whole organism can appropriately respond to the change in environmental conditions. Similar research is ongoing on an array of other types of receptors that respond to nutrient availability, water supply, temperature, and a host of other environmental conditions.

From an evolutionary point of view, it is important not only to uncover which genes control a given type of plasticity but also to find

out if and to what extent these genes are variable in natural populations. According to neo-Darwinian evolutionary theory, natural selection is effective only if populations harbor different versions of the same genes, thereby providing an ample set of possibilities from which the most fit combinations are passed to the next generation. Thomas Mitchell-Olds pioneered a combination of statistical and molecular techniques known as quantitative trait loci (QTL) mapping, which allows researchers to pinpoint the location in the genome of those genes that are both responsible for phenotypic plasticity and variable in natural populations. These genes are the most likely targets of natural selection for the future evolution of the species.

Complex Traits: Behavior and Intelligence

The most important consequence of nature-nurture interactions is their application to the human condition. Humans are compelled to investigate questions related to the degree of genetic or environmental determination of complex traits such as behavior and intelligence. Unfortunately, such a quest is a potentially explosive mixture of science, philosophy, and politics, with the latter often perverting the practice of the first. For example, the original intention of intelligence quotient (IQ) testing in schools, introduced by Alfred Binet at the end of the nineteenth century, was simply to identify pupils in need of special attention in time for remedial curricula to help them. Soon, however, IQ tests became a widespread tool to support the supposed "scientific demonstration" of the innate inferiority of some races, social classes, or a particular gender (with the authors of such studies usually falling into the "superior" race, social class, or gender). During the 1970's, ethologist Edward Wilson freely extrapolated from behavioral studies on ant colonies to reach conclusions about human nature; he proposed that genes directly control many aspects of animal and human behavior, thereby establishing the new and controversial discipline of sociobiology.

The reaction against this trend of manipulating science to advance a political agenda has, in some cases, overshot the mark. Some well-

intentioned biologists have gone so far as to imply either that there are no genetic differences among human beings or that they are at least irrelevant. This goes against everything that is known about variation in natural populations of any organism. There is no reason to think that humans are exceptions: Since humans can measure genetically based differences in behavior and problem-solving ability in other species and relate these differences to fitness, the argument that such differences are somehow unimportant in humans is based on social goodwill rather than scientific evidence.

The problem with both positions is that they do not fully account for the fact that nature-nurture is not a dichotomy but a complex interaction. In reality, genes do not control behavior; their only function is to produce a protein, whose only function is to interact with other proteins at the cellular level. Such interactions do eventually result in what is observed as a phenotype—perhaps a phenotype that has a significant impact on a particular behavior—but this occurs only in a most indirect fashion and through plenty of environmental influences. On the other hand, plants, animals, and even humans are not infinitely pliable by environmental occurrences. Some behaviors are indeed innate, and others are the complex outcome of a genotype-environment feedback that occurs throughout the life span of an organism. In short, nature-nurture is not a matter of “either/or” but a question of how the two relate and influence each other.

As for humans, it is very likely that the precise extent of the biological basis of behavior and intelligence will never be determined because of insurmountable experimental difficulties. While it is technically feasible, it certainly is morally unacceptable to clone humans and study their characteristics under controlled conditions, the only route successfully pursued to experimentally disentangle nature and nurture in plants and animals. Studies of human twins help little, since even those separated at birth are usually raised in similar societal conditions, with the result that the effects of heredity and environment are hopelessly confounded from a statistical standpoint. Regardless of the failure of science to answer these

questions fully, the more compelling argument that has been made so far is that the actual answer should not matter to society, in that every human being is entitled to the same rights and privileges of any other one, regardless of real and sometimes profound differences in genetic makeup. Even the best science is simply the wrong tool to answer ethical questions.

—Massimo Pigliucci

See also: Aggression; Alcoholism; Altruism; Artificial Selection; Behavior; Biological Clocks; Biological Determinism; Criminality; Developmental Genetics; Eugenics; Gender Identity; Genetic Engineering; Medical Applications; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Heredity and Environment; Homosexuality; Human Genetics; Inbreeding and Assortative Mating; Intelligence; Miscegenation and Antimiscegenation Laws; Natural Selection; Sociobiology; Twin Studies; XYY Syndrome.

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Key terms

GENOTYPE: an organism's complete set of genes
GONAD: an organ that produces reproductive cells and sex hormones; termed ovaries in females and testes in males

KARYOTYPE: a description of the chromosomes of an individual's cells, including the number of chromosomes and a physical description of them (normal female is 46,XX and normal male is 46,XY)

PHENOTYPE: the physical and biochemical characteristics of an individual based on the interaction of genotype and environment

Early Human Sexual Development

Up to the ninth week of gestation, the external genitalia (external sexual organs) are identical in appearance in both male and female human embryos. There is a phallus that will become a penis in males and a clitoris in females and labioscrotal swelling that will become a scrotum in males and labial folds in females. A person's development into a male or female is governed by his or her sex chromosome constitution (the X and Ychromosomes). An individual who has two X chromosomes normally develops into a female, and one who has one X and one Ychromosome normally develops into a male. It is the Ychromosome that determines the development of a male. The Ychromosome causes the primitive gonads (the gonads that have not developed into either an ovary or a testis) to develop into testes and to produce testosterone (the male sex hormone). It is testosterone that acts on the early external genitalia and causes the development of a penis and scrotum. If testosterone is not present, regardless of the chromosome constitution of the embryo, normal female external genitalia will develop.

Hermaphrodites

Hermaphrodites are individuals who have both male and female gonads. At birth, hermaphrodites can have various combinations of external genitalia, ranging from completely female to completely male genitalia. Most hermaphrodites have external genitalia that are ambiguous (genitalia somewhere between normal male and normal female) and often consist

Hermaphrodites

Field of study: Diseases and syndromes

Significance: *Hermaphrodites are people born with both male and female sexual parts. Early identification and thorough medical evaluation of these individuals can help them lead relatively normal lives.*

of what appears to be an enlarged clitoris or a small penis, hypospadias (urine coming from the base of the penis instead of the tip), and a vaginal opening. The extent to which the genitalia are masculinized depends on how much testosterone was produced by the testicular portion of the gonads during development. The gonadal structures of a hermaphrodite can range from a testis on one side and an ovary on the other side, to testes and ovaries on each side, to an ovotestis (a single gonad with both testicular and ovarian tissue) on one or both sides.

Hermaphroditism has different causes. The chromosomal or genotypic sex of a hermaphrodite can be 46,XX (58 percent have this karyotype), 46,XY (12 percent), or 46,XX/46,XY (14 percent), while the rest have different types of mosaicism, such as 46,XX/47,XXY or 45,X/46,XY. Individuals with a 46,XX/46,XY karyotype are known as chimeras. Chimerism usually occurs through the merger of two different cell lines (genotypes), such as when two separate fertilized eggs fuse together to produce one embryo. This can result in a single embryo with some cells being 46,XX and some being 46,XY. Mosaicism means having at least two different cell lines present in the same individual, but the different cell lines are caused by losing or gaining a chromosome from some cells early in development. An example would be an embryo that starts out with all cells having a 47,XXY chromosome constitution and then loses a single Y chromosome from one of its cells, which then produces a line of 46,XX-containing cells. This individual would have a karyotype written as 46,XX/47,XXY. In a chimera or mosaic individual, the proportion of developing gonadal cells with Y chromosomes determines the appearance of the external genitalia. More cells with a Y chromosome mean that more testicular cells are formed and more testosterone is produced.

The cause of hermaphroditism in the majority of affected individuals (approximately 70 percent) is unknown, although it has been postulated that those hermaphrodites with normal male or female karyotypes may have hidden chromosome mosaicism in just the gonadal tissue.

Impact and Applications

Hermaphrodites with ambiguous genitalia are normally recognized at birth. It is essential that these individuals have a thorough medical evaluation, since other causes of ambiguous genitalia besides hermaphroditism can be life-threatening if not recognized and treated promptly. Once hermaphroditism is diagnosed in a child, the decision must be made whether to raise the child as a boy or a girl. This decision is made by the child's parents working with specialists in genetics, endocrinology, psychology, and urology. Typically, the karyotype and appearance of the external genitalia of the child are the major factors in deciding the sex of rearing. Previously, most hermaphrodites with male karyotypes who had either an absent or an extremely small penis were reared as females. The marked abnormality or absence of the penis was thought to prevent these individuals from having fulfilling lives as males. This practice has been challenged by adults who are 46,XY but who were raised as females. Some of these individuals believe that their conversion to a female gender was the wrong choice, and they prefer to think of themselves as male. Hermaphrodites with a female karyotype and normal or near-normal female external genitalia are typically reared as females.

The debate over what criteria should be used to decide sex of rearing of a child is ongoing. An increasingly important part of this debate is the concept of gender identity, which describes what makes people male or female in their own minds rather than according to what sex their genitalia are. This is an especially important issue for those individuals with chimerism or mosaicism who have both a male and female karyotype. Currently, the decision to raise these individuals as boys or girls is made primarily on the basis of the degree to which their external genitalia are masculinized or feminized.

Those hermaphrodites who have normal female or male genitalia at birth are at risk for developing abnormal masculinization in the phenotypic females or abnormal feminization in the phenotypic males at puberty if both testicular and ovarian tissue remains present. Thus it is usually necessary to remove the gonad that is

not specific for the desired sex of the individual. An additional reason to remove the abnormal gonad is that the cells of the gonad(s) that have a 46,XY karyotype are at an increased risk of becoming cancerous.

—Patricia G. Wheeler

See also: Gender Identity; Homosexuality; Metafemales; Pseudohermaphrodites; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

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Web Sites of Interest

Androgen Insensitivity Syndrome Support Group. <http://www.medhelp.org/www/ais>. A comprehensive educational and informational site with links to related resources.

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which

works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

National Organization for Rare Disorders (NORD). <http://www.rarediseases.org>. Offers information and articles about rare genetic conditions and diseases, including true hermaphroditism, in several searchable databases.

High-Yield Crops

Field of study: Genetic engineering and biotechnology

Significance: *The health and well-being of the world's large population is primarily dependent on the ability of the agricultural industry to produce high-yield food and fiber crops. Advances in the production of high-yield crops will have to continue at a rapid rate to keep pace with the needs of an ever-increasing population.*

Key terms

CULTIVAR: a subspecies or variety of plant developed through controlled breeding techniques

GREEN REVOLUTION: the introduction of scientifically bred or selected varieties of grain (such as rice, wheat, and maize), which, with high enough inputs of fertilizer and water, greatly increased crop yields

MONOCULTURE: the agricultural practice of continually growing the same cultivar on large tracts of land

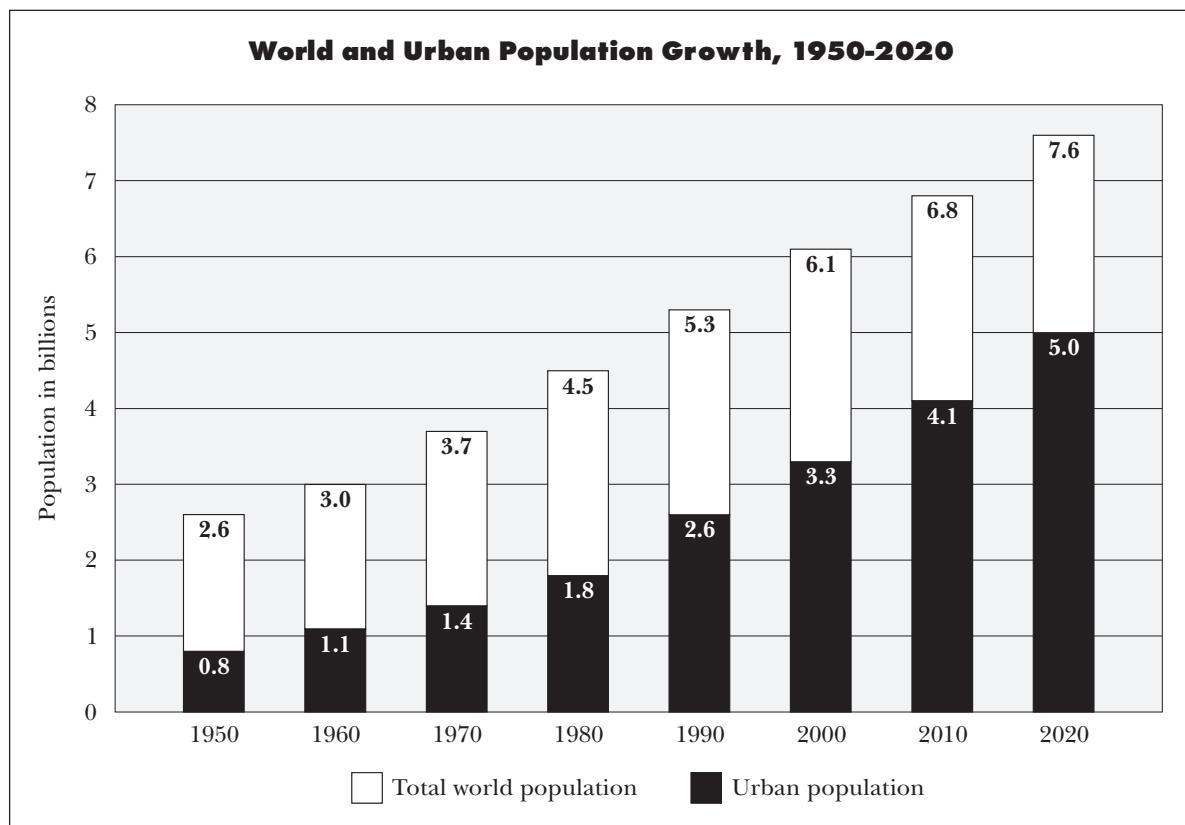
The Historical Development of High-Yield Crops

No one knows for certain when the first crops were cultivated, but by six thousand years ago, humans had discovered that seeds from certain plants could be collected, planted, and later gathered for food. As human populations continued to grow, it was necessary to select and produce higher-yielding crops. The Green Revolution of the twentieth century helped to make this possible. Agricultural scientists developed new, higher-yielding varieties, particularly grains that supply most of the world's calories. In addition to greatly increased yields, the new crop varieties also led to an increased reliance on monoculture, the practice of growing only one crop over a vast number of acres. Current production of high-yield crops is extremely

mechanized and highly reliant on agricultural chemicals such as fertilizers and pesticides. It also requires less human power, and encourages extensive monocropping.

Methods of Developing High-Yield Crops

The major high-yield crops are wheat, corn, soybeans, rice, potatoes, and cotton. Each of these crops originated from a low-yield native plant. The two major ways to improve yield in agricultural plants is to produce a larger number of harvestable parts (such as fruits or leaves) per plant or to produce plants with larger harvestable parts. For example, to increase yield in corn, the grower must either produce more ears of corn per plant or produce larger ears on each plant. Numerous agricultural practices are required to produce higher yields, but one



The exponential and ongoing rise in the globe's human population makes the need for high-yield, dependable food crops ever more compelling.

Source: Data are from U.S. Bureau of the Census International Data Base and John Clarke, "Population and the Environment: Complex Interrelationships," in *Population and the Environment* (Oxford, England: Oxford University Press, 1995), edited by Bryan Cartledge.

of the most important is the selection and breeding of genetically superior cultivars.

Throughout most of history, any improvement in yield was primarily based on the propagation of genetically favorable mutants. When a grower observed a plant with a potentially desirable gene mutation that produced a change that improved some yield characteristic such as more or bigger fruit, the grower would collect seeds or take cuttings (if the plant could be propagated vegetatively) and propagate them. This selection process is still one of the major means of improving yields. Sometimes a high-yield cultivar is developed which has other undesirable traits, such as poor flavor or undesirable appearance. Another closely related cultivar may have good flavor or desirable appearance, but low yield. Traditional breeding techniques can be used to form hybrids between two such cultivars, in hopes that all the desirable traits will be combined in a new hybrid cultivar.

Genetic Modification

The advent of recombinant DNA technology has brought greater precision into the process of producing high-yield cultivars and has made it possible to transfer genetic characteristics between any two plants, regardless of how closely related. The first step generally involves the insertion of a gene or genes that might increase yield into a piece of circular DNA called a plasmid. The plasmid is then inserted into a bacteria, and the bacteria is then used as a vector to transfer the gene into the DNA of another plant. This technology has resulted in genetically modified crops such as "golden rice" (fortified with vitamin A), herbicide-resistant soybeans, and new strains such as triticale, which promise to ameliorate world hunger at the same time that they threaten to reduce biodiversity and alter other plants through genetic drift.

Impact and Applications

As the human population grows, pressure on the world's food supply will increase. Consequently, researchers are continually seeking better ways to increase food production. In order to accomplish this goal, advances in the

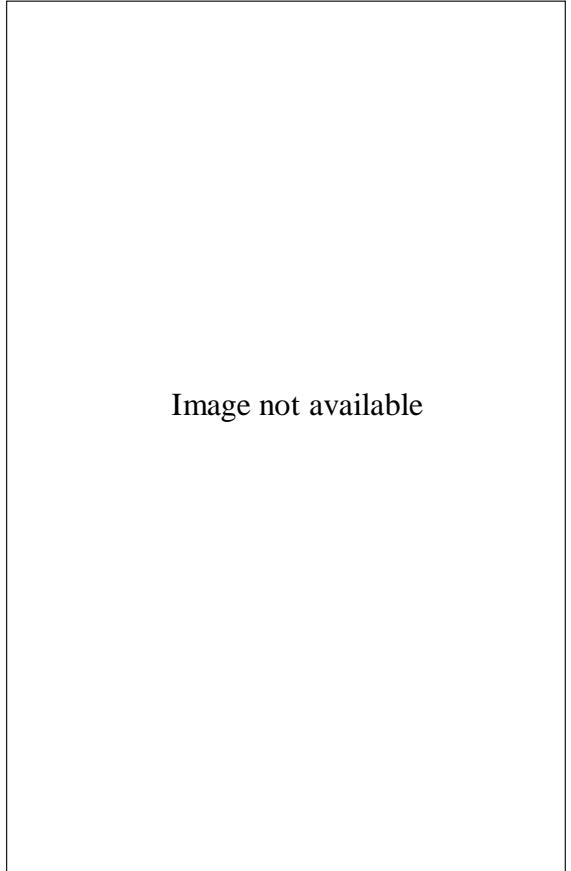


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Grain crops such as rice, wheat, and (above) corn, grown here for research by DeKalb Genetics Corporation, are among those that have been genetically modified to increase yield and nutritional value. (AP/Wide World Photos)

production of high-yield crops will have to continue at a rapid rate to keep pace. New technologies will have to be developed, and many of these new technologies will center on advances in genetic engineering. It is hoped that such advances will lead to the development of new high-yield crop varieties that require less water, fertilizer, and chemical pesticides.

—D. R. Gossett, updated by Bryan Ness

See also: Biofertilizers; Biopesticides; Cell Culture; Plant Cells; Cloning; Cloning: Ethical Issues; Cloning Vectors; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Geneti-

cally Modified (GM) Foods; Hybridization and Introgression; Lateral Gene Transfer; Transgenic Organisms.

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agriculture student, which serves as one of the most valuable sources available on the practical aspects of the production of high-yield crops.

Web Site of Interest

Food and Agriculture Organization of the United Nations. Biotechnology in Food and Agriculture. <http://www.fao.org>. Addresses the role of biotechnology in worldwide food production.

Homeotic Genes

Field of study: Developmental genetics

Significance: *Embryonic development and organogenesis proceed by way of a complex series of cascaded gene activities, which culminate in the activation of the homeotic genes to specify the final identities of body parts and shapes. The discovery of homeotic genes has provided the key to understanding these patterns of development in multicellular organisms. Knowledge of homeotic genes not only is helping scientists understand the variety and evolution of body shapes (morphology) but also is providing new insights into genetic diseases and cancer.*

Key terms

PROMOTER: the control switch in genes where transcription factors bind to activate or repress the conversion of DNA information into proteins

TRANSCRIPTION FACTOR: a protein with specialized structures that binds specifically to the promoters in genes and controls the gene's activity

The Discovery of Homeotic Genes

One of the most powerful tools in genetic research is the application of mutagenic agents (such as X rays) that cause base changes in the DNA of genes to create mutant organisms. These mutants display altered appearances, or phenotypes, giving the geneticist clues about how the normal genes function. Few geneticists have used this powerful research tool as well as the recipient of the 1995 Nobel Prize in

Physiology or Medicine recipient Christiane Nüsslein-Volhard (who shared the award with Edward B. Lewis and Eric Wieschaus). She and her colleagues, analyzing thousands of mutant *Drosophila melanogaster* fruit flies, discovered many of the genes that functioned early in embryogenesis.

Among the many mutant *Drosophila* flies studied by these and other investigators, two were particularly striking. One mutant had two sets of fully normal wings; the second set of wings, just behind the first set, displaced the normal halteres (flight balancers). The other mutant had a pair of legs protruding from its head in place of its antennae. These mutants were termed “homeotic” because major body parts were displaced to other regions. Using such mutants, Lewis was able to identify a clustered set of three genes responsible for the extra wings and map or locate them on the third chromosome of *Drosophila*. He called this gene cluster the bithorax complex (*BX-C*). The second mutation was called *antennapedia*, and its complex, with five genes, was called *ANT-C*. If all the *BX-C* genes were removed, the fly larvae had normal head structures, partially normal middle or thoracic structures (where wings and halteres are located), but very abnormal abdominal structures that appeared to be nothing more than the last thoracic structure repeated several times. From these genetic studies, it was concluded that the *BX-C* genes controlled the development of parts of the thorax and all of the abdomen and that the *ANT-C* genes controlled the rest of the thorax and most of the head.

The *BX-C* and *ANT-C* genes were called homeotic selector genes: “selector” because they acted as major switch points to select or activate whole groups of other genes for one developmental pathway or another (for example, formation of legs, antennae, or wings from small groups of larval cells in special compartments called *imaginal disks*). Although geneticists knew that these homeotic selector genes were arranged tandemly in two clusters on the third *Drosophila* chromosome, they did not know the molecular details of these genes or understand how these few genes functioned to cause such massive disruptions in the *Drosophila* body parts.

The Molecular Properties of Homeotic Genes

With so many mutant embryos and adult flies available, and with precise knowledge about the locations of the homeotic genes on the third chromosome, the stage was set for an intensive molecular analysis of the genes in each complex. In 1983, William Bender’s laboratory used new, powerful molecular methods to isolate and thoroughly characterize the molecular details of *Drosophila* homeotic genes. He showed that the three bithorax genes constituted only 10 percent of the whole *BX-C* cluster. What was the function of the other 90 percent if it did not contain genes? Then William McGinnis’ and J. Weiner’s laboratories made another startling discovery: The base sequences (the order of the nucleotides in the DNA) of the homeotic genes they examined contained nearly the same sequence in the terminal 180 bases. This conserved 180-base sequence was termed the “homeobox.” What was the function of this odd but commonly found DNA sequence? What kind of protein did this homeobox-containing gene make?

Soon it was discovered that homeotic genes and homeoboxes were not confined to *Drosophila*: All animals had them, both vertebrates, such as mice and humans, and invertebrates, such as worms and even sea sponges. The homeobox sequence was not only conserved within homeotic and other developmental genes, but it was also conserved throughout the entire animal kingdom. All animals seemed to possess versions of an ancestral homeobox gene that had duplicated and diverged over evolutionary time.

New discoveries about homeobox genes flowed out of laboratories all over the world in the late 1980’s and early 1990’s; it was discovered that the order of the homeobox genes in the gene clusters from all animals was roughly the same as the order of the eight genes found in the original *BX-C* and *ANT-C* homeotic clusters of *Drosophila*. In more complex animals such as mice and humans, the two *Drosophila*-type clusters were duplicated on four chromosomes instead of just one. Mice have thirty-two homeotic genes, plus a few extra not found in *Drosophila*. Frank Ruddle hypothesized that

the more anatomically complex the animal, the more homeotic genes it will have in its chromosomes. Experimental evidence from several laboratories has supported Ruddle's hypothesis.

The questions posed earlier about the functions of extra DNA in the homeotic clusters and the role of the homeobox in gene function were finally answered. It seems that all homeotic genes code for transcription factors, or proteins that control the activity or expression of other genes. The homeobox portion codes for a section of protein that binds to base sequences in the promoters of other genes, thus stimulating those genes to express their proteins. The earlier idea of homeotic genes as selector genes makes sense; the protein products of homeotic genes bind to the promoter control regions of many other genes and activate them to make complex structures such as legs and wings. The homeotic genes themselves are under the control of other genes making transcription factors that bind to the extra DNA in the homeotic clusters. The bound transcription factors control the differential expression of homeotic genes in many different cellular environments throughout the developing embryo, all along its anterior to posterior axis. Embryonic development and organogenesis proceed by way of a complex series of cascaded gene activities, which culminate in the activation of the homeotic genes to specify the final identities of body parts and shapes.

Impact and Applications

In a 1997 episode of the television series *The X-Files*, a mad scientist transforms his brother into a monster with two heads. Federal Bureau of Investigation (FBI) agent Dana Scully patiently explains to her partner Fox Mulder that the scientist altered his brother's homeobox genes, causing the mutant phenotype. Science fiction indeed—but with the successful cloning of Dolly the sheep in 1997, the prospect of manipulating homeobox genes in embryos is no longer far-fetched.

The first concern of scientists is to elucidate more molecular details about the actual processes by which discrete genes transform an undifferentiated egg cell into a body with per-

fectly formed, bilateral limbs. Sometimes mutations in homeobox genes cause malformed limbs, extra digits on the hands or feet, or fingers fused together, conditions known as syndactyly; often limb and hand deformities are accompanied by genital abnormalities. Several reports in 1997 provided experimental evidence for mutated homeobox genes in certain leukemias and cancerous tumors. Beginning in 1996, the number of reports describing correlations between mutated homeobox genes and specific cancers and other developmental abnormalities increased dramatically. Although no specific gene-based therapies have been proposed for treating such diseases, the merger between the accumulated molecular knowledge of homeotic genes and the practical gene manipulation technologies spawned by animal cloning will likely lead to new treatments for limb deformities and certain cancers.

—Chet S. Fornari

See also: Developmental Genetics; Evolutionary Biology; Model Organism: *Drosophila melanogaster*.

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Homosexuality

Field of study: Human genetics and social issues

Significance: *The debate over whether individuals choose to whom they are attracted or their orientation is determined primarily by genetic or social factors is ongoing. Interest persists in part because individuals' sexual orientation appears to extend beyond sexuality to influence gender and in part because individuals erroneously believe that social acceptance and treatment of homosexuals may differ depending upon whether gay and lesbian individuals are free agents or are responding to biological imperatives.*

Key terms

CONCORDANCE: the presence of a trait in both members of a pair of twins

HERITABILITY: the proportion of phenotypic variation that is due to genes rather than the environment

SEX-LINKED TRAITS: Characteristics that are encoded by genes on the X or Y chromosome

Biological vs. Environmental Factors

Sexual orientation is a fundamental aspect of human sexuality that usually results in females mating with males (heterosexuality). Sexual orientation may be closely linked to sexual experience, but many factors (social, religious, or logistic) can decrease the correlation. As a result, the frequency of homosexuality (a sexual orientation or attraction to persons of the same sex) varies from approximately 2 to 10 percent of the population, depending on how homosexuality is defined and measured. In general, there appears to be a continuum, from exclusive heterosexuality (90-92 percent) to exclusive homosexuality (1-4 percent) with many people falling somewhere between. Like most complex behaviors, homosexuality is probably influenced by both biological and environmental factors. The exact mechanism may differ for individuals who appear to exhibit similar behavioral patterns.

Genetic Influences

The genetic basis of homosexuality has been

assessed using twin studies and pedigree analysis. Lesbians are approximately three times as likely as heterosexual women to have lesbian sisters and generally have more lesbian relatives as well, which suggests that genes as well as environmental factors influence homosexuality in women. Similarly, among men, concordance in sexual orientation among monozygotic (MZ) twins is greater than that for dizygotic (DZ) twins or nontwin brothers. Since MZ twins share 100 percent of their genes but are not always either both straight or both gay, sexual orientation cannot be 100 percent due to genes.

Heritability of homosexuality has been estimated at 30-75 percent for men and at 25-76 percent for women. The different rates of heritability and frequency, with lesbians typically representing a smaller proportion of the population than gay men, suggests that men's and women's sexuality may have different origins. The X-linked locus associated with homosexuality in some men (*Xq28*, according to Hamer et al., 1993) does not appear to be associated with lesbianism (Hu et al., 1995). Further, research suggests that men's orientation is bimodal in distribution relative to the Kinsey scale of sexual orientation, whereas women's orientation is distributed more continuously and is more likely than men's to change through adulthood.

Neurohormonal Influences

Adult homosexuals do not differ from their heterosexual counterparts in terms of circulating levels of sex hormones. Instead, the neuroendocrine theory predicts that prenatal exposure to high levels of androgens masculinizes brain structures and influences sexual orientation. Consistent with this, women with congenital adrenal hyperplasia (CAH) who experience atypically high levels of androgens prenatally appear to be somewhat more likely to engage in same-sex sexual fantasies and behavior compared to heterosexual women, whereas XY women with complete androgen insensitivity syndrome (cAIS) do not exhibit increased expression of lesbianism. Exposure to the synthetic estrogen DES, which is also thought to have a demasculinizing effect on the brain, also appears to influence women's sexuality mod-

estly and to induce higher levels of homosexuality.

Stress hormones generally reduce the production of sex hormones. The level and timing of stress experienced by women during pregnancy may therefore also affect the amount of sex hormones experienced prenatally and hence the sexual differentiation and organizational phase of early brain development. Studies suggest that some women who experience stress during pregnancy may be more likely to have homosexual children, but the data are still preliminary.

Given that most homosexuals do not have one of the aforementioned hormonal conditions and most individuals who do have them are heterosexual, the neuroendocrine theory alone does not appear to account for the origin of homosexuality.

Neuroanatomical Influences

Although stereotypes exist, there is no overall lesbian or gay physique. There is some evidence that gay men's brains may differ from heterosexual men's in some structures where sexual dimorphism also occurs (for example, interstitial nuclei of the anterior hypothalamus 3, suprachiasmatic nucleus in the anterior hypothalamus and the anterior commissure), presumably due to the organizational effects of sex hormones. Structure size varies considerably both within and between sexes; however, all three structures appear to differ significantly in size for gay versus heterosexual men. It is not yet clear whether these differences cause homosexual activity or are caused by it.

Evolutionary Perspective

Evolutionary biologists have suggested that homosexuality may persist because there is little cost associated with the behavior. In situations in which homosexuality is not exclusive (that is, most individuals engage in heterosexual as well as homosexual liaisons) homosexuals would experience little or no decline in reproductive success. This could occur when marriage is compulsory, where there are strict gender roles and religious requirements, or when homosexual behavior is situational or opportunistic. Similarly, in situations in which in-

dividuals are exclusively homosexual and experience no direct individual fitness (that is, no offspring are produced), homosexuals can reduce the reproductive cost by increasing their inclusive fitness via contributions to relatives' offspring. Consistent with the latter hypothesis, there is some evidence that gay men exhibit increased levels of empathy, an accepted indicator of altruism.

Homosexuality is one of the three most common expressions of human sexual orientation and has been observed throughout human history and across religions and cultures. Like other complex behavioral traits, sexual orientation appears to be influenced by both biological and environmental factors. There is some evidence that situational or opportunistic homosexuality may differ from obligatory homosexuality and that the mechanisms influencing sexual orientation may be different in gay men and lesbians.

—Cathy Schaeff

See also: Behavior; Biological Clocks; Gender Identity; Heredity and Environment; Hermaphrodites; Human Genetics; Metafemales; Pseudohermaphrodites; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

Further Reading

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Web Sites of Interest

Parents, Families, and Friends of Lesbians and Gays. <http://www.pflag.org>. Site includes a section on frequently asked questions and information about local chapters, news, and public advocacy.

Sexuality Information and Education Council of the United States. <http://www.siecus.org>. A vast resource on all aspects of sex and sexuality. Includes links for teenagers, public policy issues, school health, a searchable bibliography database, and more.

Human Genetics

Fields of study: Human genetics and social issues

Significance: *Human genetics is concerned with the study of the human genome. The study of human genetics includes identifying and mapping genes; determining their function, mode of transmission, and inheritance; and detecting mutated or nonfunctioning genes. Important aspects of modern human genetics include gene testing or genetic screening, gene therapy, and genetic counseling.*

Key terms

BIOINFORMATICS: The science of compiling and managing genetic and other biology data using computers, requisite in human genome research

DYSMORPHOLOGY: Abnormal physical development resulting from genetic disorder

FORENSIC GENETICS: the application of genetics, particularly DNA technology, to the analysis of evidence used in civil cases, criminal cases, and paternity testing

GENE THERAPY: the use of a viral or other vector to incorporate new DNA into a person's cells with the objective of alleviating or treating the symptoms of a disease or condition

GENE TRANSFER: Using a viral or other vector to incorporate new DNA into a person's cells. Gene transfer is used in gene therapy

GENETIC SCREENING: the use of the techniques of genetics research to determine a person's risk of developing, or his or her status as a carrier of, a disease or other disorder

GENETIC TESTING: the process of investigating a specific individual or population of people to detect the presence of genetic defects

GENOMICS: the branch of genetics dealing with the study of the genetic sequences of organisms, including the human being

PHARMACOGENOMICS: The branch of human medical genetics that evaluates how an individual's genetic makeup influences his or her response to drugs

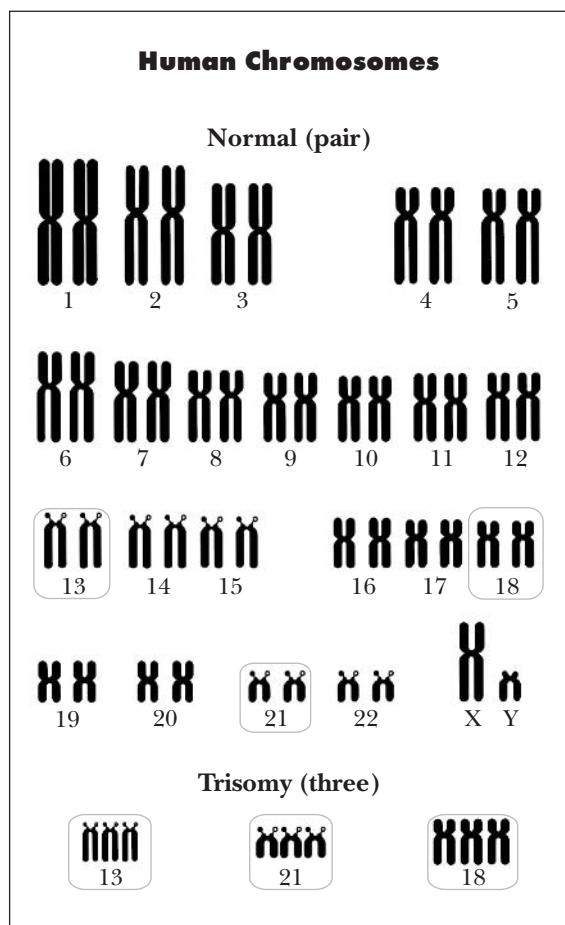
PROTEOMICS: the study of how proteins are expressed in different types of cells, tissues, and organs

TOXICOGENOMICS: evaluating ways in which genomes respond to chemical and other pollutants in the environment

Human Genome Project

Human genetics is the discipline concerned with identifying and studying the genes carried by humans, the control and expression of traits caused by these genes, their transmission from generation to generation, and their expression in offspring. Modern human genetics properly begins with the elucidation of the structure of DNA in 1953 by James D. Watson and Francis H. Crick. This discovery led to very rapid advances in acquisition of genetic information and ultimately spawned the Human Genome Project (HGP), which was initiated in 1986 by the DOE (Department of Energy). In 1990 the DOE combined efforts with the National Institutes of Health (NIH) and private collaborators,

including the Wellcome Trust of the United Kingdom, along with private companies based in Japan, France, Germany, and China. The ultimate goal of HGP was to determine the precise genetic makeup of humans as well as explore human genetic variation and human gene function. The first high-quality draft of the human genetic sequence was completed in April of 2003, thereby providing a suitable salute to the fiftieth anniversary of the discovery of DNA, which opened the modern era of human genetics.



Genetic diseases are caused by defects in the number of chromosomes, in their structure, or in the genes on the chromosome (mutation). Shown here is the human complement of chromosomes (23 pairs) and three errors of chromosome number (trisomies) that lead to the genetic disorders Patau's syndrome (trisomy 13), Edward's syndrome (trisomy 18), and the more common Down syndrome (trisomy 21). (Hans & Cassidy, Inc.)

Almost all modern human genetics is directly related to the enormous mass of genetic data obtained and made available by the HGP. Some of the many themes now being explored include medical genetics, genetic bioinformatics, proteomics, toxicogenomics, the inheritance and prevention of gene-related cancers and other diseases, and policy and ethical issues related to genetic concerns of humans.

The human genome consists of genes located in chromosomes, along with a much smaller gene content, found in mitochondria, that is called mitochondrial DNA or mtDNA. About 99.7 percent of the human genome is located in the chromosomes, and another 0.3 percent consists of the mtDNA genome, which encodes for a number of enzymes involved in cellular respiration. The mtDNA is inherited almost entirely through the female line, so its genetic transmission and expression differ from that of classical Mendelian genetics. Studies of human mtDNA have revealed a number of medical pathologies associated with this unique mode of inheritance transmission. Studies have also proven useful in determining significant trends in the evolutionary development of *Homo sapiens* and elucidating relationships with the near-species *Homo neanderthalensis* (the now extinct Neanderthals).

The HGP effort decoded the genetic arrangement—the gene sequence of roughly 3 billion nucleotide base pairs of between 25,000 and 45,000 genes that collectively form the human genome. Many, but not all, of these have been sequenced and their locations on chromosomes mapped. Structurally, base-sequencing studies reveal that human genes showed great variations in size, ranging from several thousand base pairs to some genes comprising nearly half a million base pairs. The genetic functions have been determined for about half of the human genes that have been identified and sequenced. HGP provided so much information that a new field called bioinformatics was developed to handle the enormous amounts of genetic sequencing data for the human genome.

Bioinformatics

The purpose of bioinformatics is to help organize, store, and analyze genetic biological in-

formation in a rapid and precise manner, dictated by the need to be able to access genetic information quickly. In the United States the online database that provides access to these gene sequences is called GenBank, which is under the purview of the National Center for Biotechnology Information and has been made available on the Internet. In addition to human genome sequence records, GenBank provides genome information about plants, bacteria, and other animals.

Proteomics

Bioinformatics provides the basis for all modern studies of human genetics, including analysis of genes and gene sequences, determining gene functions, and detecting faulty genes. The study of genes and their functions is called proteomics, which involves the comparative study of protein expression. That is, exactly what is the metabolic and morphological relationship between the protein encoded within the genome and how that protein works. Geneticists are now classifying proteins into families, superfamilies, and folds according to their configuration, enzymatic activity, and sequence. Ultimately proteomics will complete the picture of the genetic structure and functioning of all human genes.

Toxicogenomics

Another newly developing field that relies on bioinformatics is the study of toxicogenomics, which is concerned with how human genes respond to toxins. Currently, this field is specifically concerned with evaluating how environmental factors negatively interact with messenger RNA (mRNA) translation, resulting in disease or dysfunction.

Medical Genetics

Almost all of modern human medical genetics rests on the identification of human gene sequences that were provided by the HGP and made accessible through bioinformatics. Human medical genetics begins with recognition of defective genes that are either nonfunctioning or malfunctioning and that cause diseases or tissue malformation. Once defective genes have been identified and cataloged, patients

A Punnett Square Showing Alleles for Blood Type

		Father's Sperm Cells	
		B	O
Mother's Egg Cells	A	AB (AB blood)	AO (A blood)
	O	BO (B blood)	OO (O blood)

A heterozygous AO mother and a heterozygous BO father can produce children with any of the four blood types.

can be screened with gene testing procedures to determine if they carry such genes. Following detection of a defective gene, several options may be explored and implemented, including genetic counseling, gene therapy, and pharmacogenetics.

At least four thousand diseases of humans are known to have a genetic basis and can be passed from generation to generation. In addition to many kinds of human cancers, all of which have a genetic basis, human genetic disorders include diabetes, heart disease, and cystic fibrosis. Other diseases and disorders that have been directly linked to human genetic anomalies include predispositions for colon cancer, Alzheimer's disease, and breast cancer.

Gene Testing

In a gene-testing protocol, a sample of blood or body fluids is examined to detect a genetic anomaly such as the transposition of part of a chromosome or an altered sequence of the bases that comprise a specific gene, either of which can lead to a genetically based disorder or disease. Currently more than six hundred tests are available to detect malfunctioning or nonfunctioning genes. Most gene tests have focused on various types of human cancers, but other tests are being developed to detect genetic deficiencies that cause or exacerbate infectious and vascular diseases.

The emphasis on the relationship between genetics and cancer lies in the fact that all hu-

man cancers are genetically triggered by genes or have a genetic basis. Some cancers are inherited as mutations, but most result from random genetic mutations that occur in specific cells, often precipitated by viral infections or environmental factors not yet well understood.

At least four types of genetic problems have been identified in human cancers. The normal function of oncogenes, for example, is to signal the start of cell division. However, when mutations occur or oncogenes are overexpressed, the cells keep on dividing, leading to rapid growth of cell masses. The genetic inheritance of certain kinds of breast cancers and ovarian cancers results from the nonfunctioning tumor-suppressor genes that normally stop cell division. When genetically altered tumor-suppressor genes are unable to stop cell division, cancer results. Conversely, the genes that cause inheritance of colon cancer result from the failure of DNA repair genes to correct mutations properly. The accumulation of mutations in these “proofreading” genes makes them inefficient or less efficient, and cells continue to replicate, producing a tumor mass.

If a gene screening reveals a genetic problem several options may be available, including gene therapy and genetic counseling. If the detected genetic anomaly results in disease, then pharmacogenomics holds promise of patient-specific drug treatment.

Gene Therapy

The science of gene therapy uses recombinant DNA technology to cure diseases or disorders that have a genetic basis. Still in its experimental stages, gene therapy may include procedures to replace a defective gene, repair a defective gene, or introduce healthy genes to supplement, complement, or augment the function of nonfunctional or malfunctioning genes. Several hundred protocols are being used in gene therapy trials, and many more are under development. Current trials focus on two major types of gene therapy, somatic gene therapy and germ-line gene therapy.

Somatic gene therapy focuses on altering a defective gene or genes in human body cells in an attempt to prevent or lessen the debilitating

impact of a disease or other genetic disorder. Some examples of somatic cell gene therapy protocols now being tested include ones for adenosine deaminase (ADA) deficiency, cystic fibrosis, lung cancer, brain tumors, ovarian cancer, and AIDS.

In somatic gene therapy a sample of the patient's cells may be removed and treated, and then reintegrated into body tissue carrying the corrected gene. An alternative somatic cell therapy is called gene replacement, which typically involves insertion of a normally functioning gene. Some experimental delivery methods for gene insertion include use of retroviral vectors and adenovirus vectors. These viral vectors are used because they are readily able to insert their genomes into host cells. Hence, adding the needed (or corrective) gene segment to the viral genome guarantees delivery into the cell's nuclear interior. Nonviral delivery vectors that are being investigated for gene replacement include liposome fat bodies, human artificial chromosomes, and naked DNA (free DNA, or DNA that is not enclosed in a viral particle or any other “package”).

Another type of somatic gene therapy involves blocking gene activity, whereby potentially harmful genes such as those that cause Marfan syndrome and Huntington's disease are disabled or destroyed. Two types of gene-blocking therapies now being investigated include the use of antisense molecules that target and bind to the messenger RNA (mRNA) produced by the gene, thereby preventing its translation, and the use of specially developed ribozymes that can target and cleave gene sequences that contain the unwanted mutation.

Germ-line therapy is concerned with altering the genetics of male and female reproductive cells, the gametes, as well as other body cells. Because germ-line therapy will alter the individual's genes as well as those of his or her offspring, both concepts and protocols are still very controversial. Some aspects of germ-line therapy now being explored include human cloning and genetic enhancement.

The next steps in human genetic therapy involve determining the underlying mechanisms by which genes are transcribed, translated, and expressed, which is called proteomics.

Clinical Genetics

Clinical genetics is that branch of medical genetics involved in the direct clinical care of people afflicted with diseases caused by genetic disorders. Clinical genetics involves diagnosis, counseling, management, and support. Genetic counseling is a part of clinical genetics directly concerned with medical management, risk determination and options, and decisions regarding reproduction of afflicted individuals. Support services are an integral feature of all genetic counseling themes.

Clinical genetics begins with an accurate diagnosis that recognizes a specific, underlying genetic cause of a physical or biochemical defect following guidelines outlined by the NIH Counseling Development Conference. Clinical practice includes several hundred genetic tests that are able to detect mutations such as those associated with breast and colon cancers, muscular dystrophies, cystic fibrosis, sickle-cell disease, and Huntington's disease.

Genetic counseling follows clinical diagnosis and focuses initially on explaining the risk factors and human problems associated with the genetic disorder. Both the afflicted individual and family members are involved in all counseling procedures. Important components include a frank discussion of risks, of options such as preventive operations, and of options involved in reproduction. All reproductive options are described along with their potential consequences, but genetic counseling is a support service rather than a directive mode. That is, it does not include recommendations. Instead, its ultimate mission is to help both the afflicted individuals and their families recognize and cope with the immediate and future implications of the genetic disorder.

Pharmacogenomics

That branch of human medical genetics dealing with the correlation of specific drugs to fit specific diseases in individuals is called pharmacogenomics. This field recognizes that different individuals may metabolically respond differentially to therapeutic medicines based on their genetic makeup. It is anticipated that testing human genome data will greatly speed the development of new drugs that not only tar-

get specific diseases but also will be tailored to the specific genetics of patients.

Policy and Ethical Concerns and Issues in Human Genetics

The "new genetics" of humans has raised a number of critical concerns that are currently being addressed on a number of levels. Some of these concerns are related to the ownership of genetic information obtained by the Human Genome Project, privacy issues, and use of genetic information in risk assessment and decision making.

Privacy issues have focused on psychological impact, possible discrimination, and stigmatization associated with identifying personal genetic disorders. For example, policy guarantees must be established to protect the privacy of persons with genetic disorders to prevent overt or covert societal discrimination against the affected individual. Another question arising from this is exactly who has the right to the genetic information of persons.

Use of information obtained by the Human Genome Project has provided entrepreneurial opportunities that will undoubtedly prove economically profitable. That is, the limits of commercialization of products, patents, copyrights, trade secrets, and trade agreements have to be determined. If patents of DNA sequences are permitted, will they limit accessibility and free scientific interchange among and between peoples of the world? This question becomes critical when it is recognized that the human genome is properly the property of all humans.

Noncoding "Junk" DNA

Like that of other organisms, the human genome consists of long segments of DNA that contain noncoding sequences called introns (intervening sequences). These vary from a few hundred to several thousand base pairs in length and often consist of repetitive DNA elements with no known function; that is, they do not code for proteins. Because they appear functionless but take up valuable chromosomal space, these noncoding sequences have been considered useless and have been termed junk DNA or selfish DNA. Recent studies, however, lend strong support to the possibility that the

seemingly useless repetitive DNA may actually play a number of important genetic roles, from providing a substrate on which new genes can evolve to maintaining chromosome structure and participating in some sort of genetic control. Consequently, it is now out of fashion among geneticists to refer to these parts of the genome as junk DNA, but rather as DNA of unknown function.

Forensic Genetics

Law enforcement agencies are increasingly relying on a branch of human genetics called forensic genetics. The aims of forensic genetics typically are to determine the identity or non-identity of suspects in crimes, based on an analysis of DNA found in hair, blood, and other body substances retrieved from the scene of the crime in comparison with that of suspects. Popularly called DNA fingerprinting, forensic genetics relies on the fact that the DNA of every human carries unique tandem repeats of 20 or more kilobase pairs that can be compared and identified using radioactive probes. Thus, comparisons can establish identity or nonidentity to a very high level of probability. DNA fingerprinting is also used in recognizing genetic parentage of children, identifying victims—sometimes from fragments of bodies—and identifying relationships of missing children.

Phylogeny and Evolution

Another rapidly developing field in human genetics is the use of human gene sequences in both nuclear and mitochondrial DNA (mtDNA) to explore questions of human origins, evolution, phylogeny, bioarchaeology, and past human migration patterns.

Much of the analytical work has involved mtDNA to study relationships. Because it is inherited strictly through the egg line or female component, mtDNA is somewhat more useful, but comparisons of DNA sequences along the Y chromosome of human populations have also yielded valuable information regarding human origins and evolution.

One of the more interesting of these studies involves comparing mtDNA over a broad spectrum of global human populations. Comparisons of DNA sequencing of these populations

has revealed differences in DNA sequences of about 0.33 percent, which is considerably less than seen in other primate species. These minor differences strongly suggest that all members of the human species, *Homo sapiens*, are far more closely related to one another than are members of many other vertebrate species.

A separate study compared human gene sequences among different human populations across the globe. This study revealed that the highest variations in DNA sequences are found among the modern human populations of Africa. Since populations that exhibit the highest genome variations are thought to be the oldest populations (because chance mutations have a longer time to accumulate in older populations as opposed to younger populations), these results strongly suggest that modern humans originated in Africa and subsequently dispersed into other regions of the world. This “out of Africa” theory has received compelling support from the DNA evidence, and the theory also explains why all other human populations are so remarkably similar. Since all other global human populations show minimal DNA sequence differences, it is hypothesized that a small group of humans emigrated from Africa to spread across and eventually colonize the other continents. Tests of gene sequences along Y chromosomes show similar patterns, leading to the proposal that all humans today came from a mitochondrial Eve and a Y chromosome Adam who lived between 160,000 and 200,000 years ago.

DNA-based phylogeny studies are also shedding light on the relationship between the Neanderthals (*Homo neanderthalensis*), a species that disappeared between 30,000 and 60,000 years ago, and the modern Cro-Magnon humans (*Homo sapiens*) that replaced them. Comparisons of mtDNA between the two *Homo* species indicate that Neanderthals began diverging from modern humans half a million years ago and were significantly different in genomic content to be placed in a separate species. These findings also support the suggestion that Neanderthals were ecologically replaced by modern humans rather than genetically amalgamated into present human populations, as was once proposed. Although such arguments

are not universally accepted, many more geneticists, paleoanthropologists, and forensic scientists are now using comparative analysis of DNA sequences among and between human populations to study questions of human evolutionary history.

—Dwight G. Smith

See also: Aggression; Aging; Bioethics; Bioinformatics; Biological Determinism; Criminality; DNA Fingerprinting; Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Forensic Genetics; Gender Identity; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Human Genome Project; Human Growth Hormone; In Vitro Fertilization and Embryo Transfer; Insurance; Intelligence; Miscegenation and Antimiscegenation Laws; Patents on Life-Forms; Paternity Tests; Prenatal Diagnosis; Race; Sterilization Laws.

Further Reading

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Web Sites of Interest

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- Association of Professors of Human or Medical Genetics (APHMG). <http://www.faseb.org/genetics/aphmg/aphmg1.htm>. This site of the North American group of academicians in medical and graduate schools features information on core curricula and workshops.
- Johns Hopkins University. The Genome Database. <http://gdbwww.gdb.org>. The official

central storage center for gene mapping data compiled in the Human Genome Initiative, an international effort to decode and analyze human DNA. Intended for scientists, the site presents information in three categories: regions, maps, and variations of the human genome.

National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov>. Maintains GenBank, a comprehensive, annotated collection of publicly available DNA sequences.

Sanger Centre, Wellcome Trust. <http://www.sanger.ac.uk>. One of the premier genome research centers, focusing on large-scale sequencing projects and analysis. Offers many data resources, software, databases, and information on career opportunities.

Human Genome Project

Fields of study: History of genetics; Human genetics; Techniques and methodologies

Significance: *The Human Genome Project will have a profound effect in the twenty-first century, providing the means to identify disease-causing mutations (including those involved in cancer), to design new drugs, to provide human gene therapy, to learn how genes control development, and to understand the origins and evolution of the human race.*

Key terms

GENOME: the entire complement of genetic material (DNA) in a cell

GENOMICS: that branch of genetics dealing with the study of genetic sequences

PROTEOMICS: that branch of genetics dealing with the expression, function, and structure of proteins

SINGLE NUCLEOTIDE POLYMORPHISM (SNP): differences at the individual nucleotide level among individuals

Perspective

April 25, 2003, was the fiftieth anniversary of the publication of the double helix model of DNA by James Watson and Francis Crick, based

on the experimental data of Rosalind Franklin and others. It was fitting then, that fifty years later, in April of 2003, the complete sequence of the human genome was published, marking probably one of the greatest achievements not only in genetics but in all of science. During the years ahead, thousands of scientists will mine these data for information about the human body, how its genes shape development and behavior, and the role mutations play in diseases.

Origins of the Human Genome Project

The Human Genome Project (HGP) began as a result of the catastrophic events of World War II: the dropping of atomic bombs on the Japanese cities of Nagasaki and Hiroshima. There were many survivors who had been exposed to high levels of radiation, known to cause mutations. Such survivors were stigmatized by society and were considered poor marriage prospects, because of potential genetic damage. The U.S. Atomic Energy Commission of the U.S. Department of Energy (DOE) established the Atomic Bomb Casualty Commission in 1947 to assess mutations in such survivors. However, there were no suitable methods to measure these mutations, and it would be many years before suitable techniques would be developed. Knowing the sequence of the human genome would be the greatest tool for identifying human mutations.

Advances in Molecular Biology

As in all areas of science, progress in molecular biology was limited by available technology. Many advances in molecular biology made feasible the undertaking of the HGP. Starting in the 1970's, techniques were developed to isolate and clone individual genes. By 1977, Walter Gilbert and Frederick Sanger had independently developed methods for sequencing DNA, and in 1977 Sanger's group published the sequence of the first genome, the small bacterial virus Phi X174. In 1985, Kary Mullis and colleagues developed the method of polymerase chain reaction (PCR), in which extremely small amounts of DNA could be amplified billions of times, providing significant amounts of specific DNA for analysis. Finally, in 1986, Leroy Hood and Applied Biosystems developed

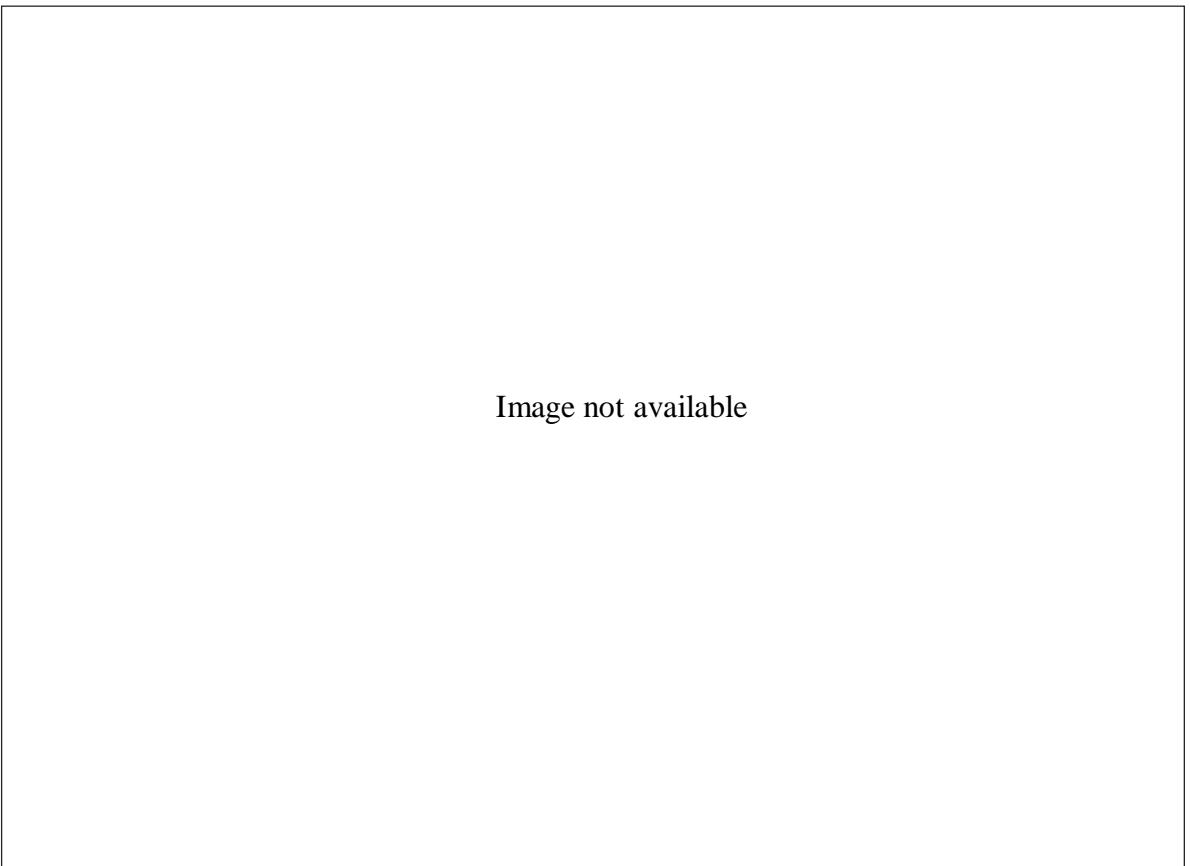


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Craig Venter of Celera Genomics (at the microphone) and Francis Collins, Director of the National Institutes of Health (right), announce the initial sequencing of the human genome on June 26, 2000, with President Bill Clinton in attendance. (AP/Wide World Photos)

an automated DNA sequencer that could sequence DNA hundreds of times faster than was previously possible. Additional advances in computer technology now made it possible to sequence the human genome.

The “Holy Grail” of Molecular Biology

In 1985 a conference of leading scientists was held at the University of California, Santa Cruz, to discuss the feasibility of sequencing the entire human genome. Biologists were looking for the equivalent of a Manhattan Project for biology. The Manhattan Project was the concerted effort of physicists to develop atomic weapons during World War II and resulted in huge increases of government funding for physics research. Walter Gilbert called the HGP the Holy Grail of molecular biology. With impetus

from the DOE and the National Research Council, the Human Genome Project was launched in 1990 with James Watson as head. The goal was completion in 2005 at a cost of \$1.00 per base pair. In 1992, Watson resigned over a controversy surrounding the patenting of human sequences. Francis Collins took over as head of the HGP at the National Institutes of Health (NIH). The sequencing of genetic model organisms, in addition to the human genome, was one of the goals of the HGP. This included genomes of the bacterium *Escherichia coli*, yeast, the fruit fly *Drosophila melanogaster*, the round worm *Caenorhabditis elegans*, and other organisms. Moreover, 10 percent of the funding was to be directed toward studies of the social, ethical, and legal implications of learning the human genome.

Competition Between the Public and Private Sectors

Craig Venter, a former National Institutes of Health researcher, left the NIH and formed a private company, The Institute for Genome Research (TIGR). TIGR, using a different approach (known as the shotgun method) was able to sequence the 1.8 million-base-pair genome of the first free-living organism, the bacterium *Haemophilus influenzae*, in less than a year. In 1998 Venter along with Perkin-Elmer

Corporation formed the biotech company Celera Genomics to sequence the human genome privately. Celera had more than three hundred of the world's fastest automated sequencers and a supercomputer to analyze data. Meanwhile, public funds supported scientists in the United States, the United Kingdom, Japan, Canada, Sweden, and fourteen other countries working on HGP sequencing. The public sector was now in competition with Celera. To assure free access, each day new sequence data from the public projects were made available on the Internet.

The Human Genome Project Is Completed

In 2001 the first draft of the human genome was published in the February 15 issue of *Nature* and the February 16 issue of *Science*. There are many short, repeated sequences of DNA in the genome, and certain regions that were difficult to sequence needed to be sequenced again for accuracy. Thus in April, 2003, the final sequence of the human genome was achieved. It is remarkable that a government-funded project was completed two and a half years ahead of schedule and under budget. April 25, 2003, was designated National DNA Day.

Findings from the Human Genome Project

Perhaps the most surprising finding from the HGP is the relatively low number of human genes. Scientists had predicted the human genome would contain about 100,000 genes, yet the actual number of protein-coding sequences is approximately 21,000, representing only about 1 percent of the entire genome. In comparison, yeast has about 6,000 genes, the fruit fly about 13,000, and the *Caenorhabditis*



Although all human beings share the same DNA, slight variations in DNA sequences, including single nucleotide polymorphisms (SNPs), occur commonly across individuals. One individual, for example, might have the base A (adenine) where another has the base C (cytosine); several different combinations of these bases can often code for the same amino acid and hence protein, so the differences often have little or no effect. However, these SNPs can account for variations in our reactions to pathogens, drugs, and other environmental conditions. Knowing these variations may help researchers identify the genes associated with complex conditions such as cancer, diabetes, and cardiovascular diseases. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>.)

about 18,000. It was surprising that a complex human had less than twice the number of genes as the lowly roundworm. The human genome also contains 740 genes that encode stable RNAs. The genome of the mouse, another model genetic organism, will provide interesting comparisons to the human genome.

Whose Genome Is It?

Although more than 99.99 percent of the DNA sequences of all humans are identical, there are many differences. An important question is, Whose genome is it? Craig Venter has acknowledged that Celera has been sequencing mostly his DNA. However, the final sequence database is an “average” or “consensus” genome. Every human carries many and perhaps even hundreds of mutations. Even before the HGP was completed, databases listing single nucleotide polymorphisms were being established. These databases list the types of genetic variations that occur at individual nucleotides in the genome. For example, a cancer gene database lists the types of mutations that have been identified in specific cancer-causing genes and the frequency of such mutations. Mutations in genes such as *BRCA1* and *BRCA2* are responsible for breast and ovarian cancers, while mutations in the tumor-suppressor gene *p53* have been found in the majority of human tumors.

The Future: Genomics and Proteomics

The Human Genome Project has given rise to two new fields of study. Genomics is the study of genomes. To do so requires databases and search engines to seek out information from these sequences. Today there are hundreds of such databases already established. Scientists can search for complete gene sequences if they know only a short segment of a gene. They can look for related sequences within the same genome or among different species. From such information one can study the evolution of particular genes.

The next step is to define the human proteome, giving rise to the field of proteomics. Proteomics seeks to determine the expression patterns of genes, the functions of the proteins produced, and the structure of specific pro-

teins derived from their DNA sequence. If a particular protein is involved in a disease process, specific drugs to interfere with it may be designed. Humanity is just beginning to reap the benefits from the Human Genome Project.

—Ralph R. Meyer

See also: Behavior; Bioinformatics; Chromosome Theory of Heredity; Genetic Code, Cracking of; Genetic Engineering; Genomic Libraries; Genomics; Hereditary Diseases; Human Genetics; Icelandic Genetic Database; Polymerase Chain Reaction; Proteomics; Race.

Further Reading

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International Human Genome Sequencing Consortium. “Initial Sequencing and Analysis of the Human Genome.” *Nature* 409, no. 6822 (2001): 860-921. The publication of the first draft of the Human Genome Project. The whole journal issue contains many other papers considering the structure, function, and evolution of the human genome.

_____. “The Human Genome.” *Science* 291 (2001): 1145-1434. This special issue provides data from the first draft of the human genome sequence.

Sulston, John, and Georgina Ferry. *The Common Thread: A Story of Science, Politics, Ethics, and the Human Genome*. Washington, D.C.: Joseph Henry Press, 2002. A chronicle of the race for the HGP from the perspective of a British Nobel Laureate Sir John Sulston, head of Sanger Centre, the British research unit involved in the HGP. Describes the effort to ensure public access to the genome data.

Wolfsberg, Tyra G., Kris A. Wetterstrand, Mark S. Guyer, Francis S. Collins, and Andreas D.

Baxevanis. "A User's Guide to the Human Genome." *Nature Genetics Supplement* 32 (2002): 1-79. This supplement nicely illustrates how one can search the human genome database. It is set up as a series of questions with step-by-step color Web page illustrations of such searches. The supplement also lists major Web resources and databases.

Web Sites of Interest

Department of Energy. Office of Science. <http://doegenomes.org>. Along with the National Human Genome Research Institute, conducted the Human Genome Project. Site includes discussion of the ethical, legal, and social issues surrounding the project, a genome glossary, and "Genetics 101."

National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/gene> map99. Starting with a general introduction to the human genome and the process of gene mapping, this site provides charts of the known genes on each chromosome, articles about the Genome Project and gene-related medical research, and links to other genome sites and databases.

National Human Genome Research Institute. <http://www.genome.gov>. One of the major gateways to the Human Genome Project, with a brief but thorough introduction to the project, fact sheets, multimedia education kits for teachers and students, a glossary, and links.

New York University/Bell Atlantic/Center for Advanced Technology. The Student Genome Project. <http://www.cat.nyu.edu/sgp/parent.html>. Uses interactive multimedia and three-dimensional technology to present tutorials and games related to the human genome and genetics for middle and high school students.

The Institute for Genomic Research (TIGR). <http://www.tigr.org>. The organization founded by Craig Venter, focusing on structural, functional, and comparative analysis of genomes and gene products. Provides databases, gene indices, and educational resources.

Human Growth Hormone

Field of study: Human genetics and social issues; Molecular genetics

Significance: *Human growth hormone (HGH) determines a person's height, and abnormalities in the amount of HGH in a person's body may cause conditions such as dwarfism, gigantism, and acromegaly. Genetic research has led to the means to manufacture enough HGH to correct such problems and expand the understanding of HGH action and endocrinology.*

Key terms

ENDOCRINE GLAND: a gland that secretes hormones into the circulatory system

HYPOPHYSECTOMY: surgical removal of the pituitary gland

PITUITARY GLAND: an endocrine gland located at the base of the brain; also called the hypophysis

TRANSGENIC PROTEIN: a protein produced by an organism using a gene that was derived from another organism

Growth Hormones and Disease

Symptoms

The pituitary (hypophysis) is an acorn-sized gland located at the base of the brain that makes important hormones and disseminates stored hypothalamic hormones. The hypothalamus controls the activity of the pituitary gland by sending signals along a network of blood vessels and nerves that connects them. The main portion of the pituitary gland, the adenohypophysis, makes six trophic hormones that control many body processes by causing other endocrine glands to produce hormones. The neurohypophysis, the remainder of the pituitary, stores two hypothalamic hormones for dissemination.

Dwarfism is caused by the inability to produce growth hormone. When humans lack only human growth hormone (HGH), resultant dwarfs have normal to superior intelligence. However, if the pituitary gland is surgically removed (hypophysectomy), the absence of other pituitary hormones causes additional mental and gender problems. The symptoms

of dwarfism are inability to grow at a normal rate or attain adult size. Many dwarfs are two to three feet tall. In contrast, some giants have reached heights of more than eight feet. The advent of gigantism often begins with babies born with pituitary tumors that cause the production of too much HGH, resulting in continued excess growth. People who begin oversecreting HGH as adults (also caused by tumors) do not grow taller. However, the bones in their feet, hands, skull, and brow ridges overgrow, causing disfigurement and pain, a condition known as acromegaly.

Dwarfism that is uncomplicated by the absence of other pituitary hormones is treated with growth hormone injections. Humans undergoing such therapy can be treated with growth hormones from humans or primates. Growth hormone from all species is a protein made of approximately two hundred amino acids strung into a chain of complex shape. However, differences in amino acids and chain arrangement in different species cause shape differences; therefore, growth hormone used for treatment must be extracted from a related species. Treatment for acromegaly and gigantism involves the removal of the tumor. In cases where it is necessary to remove the entire pituitary gland, other hormones must be given in addition to HGH. Their replacement is relatively simple. Such hormones usually come from animals. Until recently, the sole source of HGH was pituitaries donated to science. This provided the ability to treat fewer than one thousand individuals per year. Molecular genetics has solved that problem by devising the means to manufacture large amounts of transgenic HGH.

Growth Hormone Operation and Genetics

In the mid-1940's, growth hormone was isolated and used to explain why pituitary extracts increase growth. One process associated with HGH action involves cartilage cells at the ends of long bones (such as those in arms and legs). HGH injection causes these epiphyseal plate cells (EPCs) to rapidly reproduce and stack up. The EPCs then die and leave a layer of protein, which becomes bone. From this it

has been concluded that growth hormone acts to cause all body bones to grow until adult size is reached. It is unclear why animals and humans from one family exhibit adult size variation. The differences are thought to be genetic and related to production and cooperation of HGH, other hormones, and growth factors.

Genetic research has produced transgenic HGH in bacteria through the use of genetic engineering technology. The gene that codes for HGH is spliced into a special circular piece of DNA called a plasmid expression vector, thus producing a recombinant expression vector. This recombinant vector is then put into bacterial cells, where the bacteria express the HGH gene. These transgenic bacteria can then be grown on an industrial scale. After bacterial growth ends, a huge number of cells are harvested and HGH is isolated. This method enables isolation of enough HGH to treat anyone who needs it.

Impact and Applications

One use of transgenic HGH is the treatment of acromegaly, dwarfism, and gigantism. The availability of large quantities of HGH has also led to other biomedical advances in growth and endocrinology. For example, growth hormone does not affect EPCs in tissue culture. Ensuing research, first with animal growth hormone and later with HGH, uncovered the EPC stimulant somatomedin. Somatomedin stimulates growth in other tissues as well and belongs to a protein group called insulin-like growth factors. Many researchers have concluded that the small size of women compared to men is caused by estrogen-diminished somatomedin action on EPCs. Estrogen, however, stimulates female reproductive system growth by interacting with other insulin-like growth factors.

Another interesting experiment involving HGH and genetic engineering is the production of rat-sized mice. This venture, accomplished by putting the HGH gene into a mouse chromosome, has important implications for understanding such mysteries as the basis for species specificity of growth hormones and maximum size control for all organisms. Hence, experiments with HGH and advancements in ge-

netic engineering technology have led to, and should continue to lead to, valuable insights into the study of growth and other aspects of life science.

—Sanford S. Singer

See also: Cloning; Dwarfism; Genetic Engineering: Historical Development; Genetics, Historical Development of; Prader-Willi and Angelman Syndromes; Turner Syndrome.

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- Ulijaszek, J. S., M. Preece, and S. J. Ulijaszek. *Cambridge Encyclopedia of Human Growth and Development Growth Standards*. New York: Cambridge University Press, 1998. Broadly discusses genetic growth anomalies in relation to environmental, physiological, social, economic, and nutritional influences on human growth.

Web Sites of Interest

ABC News Online, Standing Tall. http://abcnews.go.com/sections/living/goodmorningamerica/growthhormone_030619.html. A June, 2003, news report on children using human growth hormone to increase their stature.

National Institutes of Health, National Library of Medicine, Growth Disorders. <http://www.nlm.nih.gov/medlineplus/growthdisorders.html>. Information on all aspects of growth disorders and HGH treatment.

Huntington's Disease

Field of study: Diseases and syndromes

Significance: Huntington's disease is an autosomal dominant neurodegenerative disorder. The symptoms of this incurable, fatal condition include uncontrollable body movements and progressive dementia. The relevant gene contains a domain of repeating triplets composed of the nucleotides cytosine (C), adenine (A), and guanine (G). Mutation of this gene causes an increase in triplet number, triggering the dysfunction and death of certain neurons in the brain.

Key terms

CAG EXPANSION: a mutation-induced increase in the number of consecutive CAG nucleotide triplets in the coding region of a gene

GENETIC ANTICIPATION: progressively earlier onset of a hereditary disease in successive generations

POLYGLUTAMINE TRACT: in a protein, an amino acid sequence consisting exclusively of glutamine, encoded by repeating CAG triplets

Characteristics

Studying an extended New York family in 1872, Dr. George Huntington first documented the heritable malady that bears his name. Huntington's disease (HD) was originally known as Huntington's chorea because of its hallmark jerky involuntary movements (the term "chorea" comes from the Greek *choros*, meaning "dance"). Patients also experience marked cognitive and psychiatric decline. HD's gradual

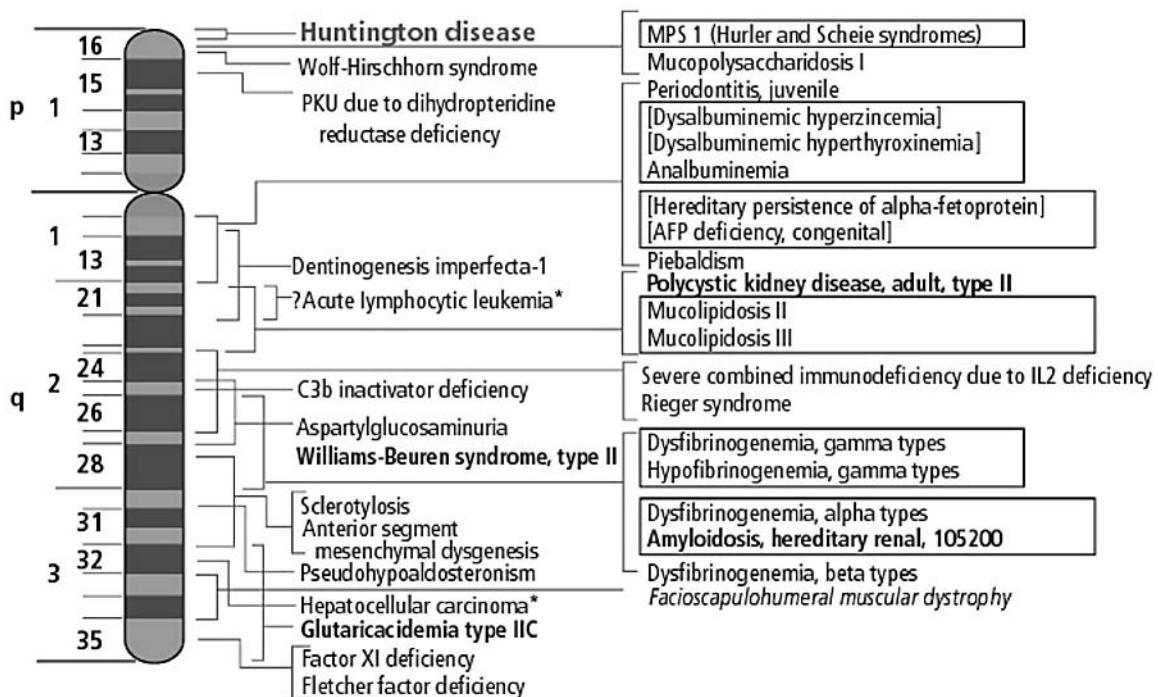
onset usually begins between ages thirty and forty, although its symptoms can first appear within an age range of two to eighty years. The disease typically progresses to death within fifteen or twenty years of diagnosis. HD affects about one in ten thousand people of European descent, and fewer than one in one million people in African and Japanese populations.

In HD, degeneration of neurons in specific brain regions occurs over time. Hardest hit is a particular subset of neurons in the striatum, a brain structure critical for movement control. Also affected is the frontal cortex, which is involved in cognitive processes. As the communication link between the striatum and cortex is broken through ongoing neuronal death, uncontrollable chorea as well as intellectual and psychiatric symptoms develop and worsen.

The HD Gene and Its Product

HD is inherited as a dominant mutation of a gene located on the short arm of chromosome 4. The cloning of the *HD* gene in 1993 provided major impetus to understanding its function. The *HD* gene encodes a 348 kDa cytoplasmic protein called huntingtin. Normally, the *HD* gene contains a stretch of repeating nucleotide triplets consisting of C (cytosine), A (adenine), and G (guanine). Healthy *HD* alleles contain anywhere from 9 to 35 CAG repeats. The CAG triplet encodes the amino acid glutamine; therefore, normal huntingtin contains a polyglutamine tract. Huntingtin is expressed throughout the brain (and indeed, the body); however, its regular function remains unclear. In neurons, huntingtin is thought to be important in counterbalancing programmed cell

Chromosome 4



The gene for Huntington's disease is located on chromosome 4. Huntington's is one of the rare single-gene disorders, clearly detected genetically. Other genetic disease conditions have been located to chromosome 4, also shown here. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>.)

death by promoting the expression of growth factors. Huntington may therefore help protect striatal neurons throughout life.

The HD Mutation

Mutant *HD* alleles contain an expansion of the CAG repeat. The magnitude of this expansion can range from 36 to more than 60 CAG repeats (rarely, as many as 250 repeats have been observed). There is an inverse relationship between repeat number and age of disease onset: Higher repeat numbers are usually linked to younger onset. People with 36-39 CAG repeats may never show disease symptoms, whereas people with 40-60 repeats usually develop HD in mid-adulthood, and those with more than 60 repeats often experience onset at less than twenty years of age.

Although original *HD* mutations clearly must occur, they are rare and of unknown cause. However, HD's inheritance patterns shed light on the mechanisms of CAG expansion. HD exhibits genetic anticipation: Affected members of successive generations may show earlier onset, particularly when the pathogenic allele is inherited paternally. It is thought that CAG expansion occurs during the repair of DNA strand breaks, when CAG loops are retained in the nucleotide sequence during gap repair. If this happens in reproductive cells (particularly sperm), a larger CAG expansion will be present in the offspring.

Consequences of CAG Expansion

The direct result of CAG expansion within the *HD* gene is that mutant huntingtin has a polyglutamine tract of variable but abnormally long length. Misfolding and aggregation of mutant huntingtin ensues. Cleavage of the mutant protein occurs, generating a fragment that can enter the nucleus. Visible cytoplasmic and nuclear huntingtin aggregates are a key pathological feature of the striatal neurons destined to die. This aggregation represents a different (albeit toxic) function for huntingtin. The aggregates contain not only mutant huntingtin but also several other critical proteins whose functions are effectively withheld. Because some of these sequestered proteins are transcription factors, transcriptional dysregulation

may affect the expression of a host of additional proteins. In fact, the expression of huntingtin itself (from the remaining normal *HD* allele) is significantly reduced. This diminution of the availability of normal huntingtin may also contribute to neuronal demise. However, it is still unknown why only certain neurons die despite huntingtin's ubiquitous expression.

Living with HD

The cloning of the *HD* gene has enabled direct genetic testing for the *HD* mutation. With a blood test, at-risk individuals can learn not only whether they carry the CAG expansion but also its length. Knowing one's carrier status and predicted age of onset can eliminate doubt and assist in making life plans, but the prospect of developing a fatal disease can be far more stressful than the uncertainty. This may explain why a relatively low percentage of those with a family history of HD have opted to be tested. Whenever testing is performed, it is accompanied by extensive counseling both before and after the results are known.

Current treatments for HD are palliative and include antidepressants and sedatives. Strategies now under study are aimed at preventing CAG expansion, counteracting the toxic effects of mutant huntingtin, and delivering neuroprotective agents to the brain. Another tactic is to replace the dying striatal neurons with transplanted fetal neurons or stem cells. This approach has shown some promise: Following striatal grafts, a small number of HD patients have experienced improvement in motor and cognitive function.

—Mary A. Nastuk

See also: Behavior; Biological Clocks; Blotting: Southern, Northern, and Western; Chromatin Packaging; Chromosome Walking and Jumping; DNA Replication; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Insurance; Pedigree Analysis; Prader-Willi and Angelman Syndromes; Repetitive DNA; Stem Cells.

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- Huntington's Disease Collaborative Research Group. "A Novel Gene Containing a Tri-nucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes." *Cell* 72, no. 6 (1993): 971-983. A benchmark study in which the *HD* gene was isolated and the nature of the mutation identified.
- Wexler, Alice. *Mapping Fate: A Memoir of Family, Risk, and Genetic Research*. Berkeley: University of California Press, 1996. The author's mother had HD, and her sister was part of the research group that cloned the *HD* gene. This account is striking for its immediacy, clarity, and accuracy.

Web Sites of Interest

- Hereditary Disease Foundation. <http://hd-foundation.org>. Site devoted mainly to Huntington's disease. Includes links to research articles, organizations, and news stories.
- Huntington's Disease Society of America. <http://www.hdsa.org>. The organization that supports research for therapies and a cure. This site offers information, support resources, publications, and ways of "getting help."

Hybridization and Introgession

Field of study: Population genetics
Significance: Hybridization and introgression are biological processes that are essential to creating genetic variation, and hence biodiversity, in plant and animal populations. These processes occur both in natural populations and in human-directed, controlled breeding programs.

Key terms

GENETICALLY MODIFIED ORGANISMS (GMOs): plants and animals in which techniques of

recombinant DNA have been used to introduce, remove, or modify specific parts of the genome of an organism

HYBRIDIZATION: the process of mating or crossing two genetically different individuals; the resultant progeny is called a hybrid

INTROGRESSION: the transfer of genes from one species to another or the movement of genes between species (or other well-marked genetic populations) mediated by backcrossing

TRANSGENE: a gene introduced into a cell or organism by means other than sexual

Definitions and Types

Hybridization and introgression are natural biological processes. Natural hybridization is common among plant and animal species. Hybridization generally refers to the mating between genetically dissimilar individuals; parents may differ in a few or many genes. They may come from different populations or races of the same taxonomic species (interspecific hybridization) or of different species (intergeneric hybridization). In nature, hybridization can occur only if there is no barrier to cross-breeding, or when the usual barrier breaks down. Hybridization produces new genetic combinations or genetic variability. Through artificial means (controlled pollination), hybridization of both cross-pollinated and self-pollinated plants can be accomplished. Plant breeding encompasses hybridization within a species as well as hybridization between species and even genera (wide crosses). The latter are important for generating genetic variability or for incorporating a desirable gene not available within a species. There are crossing barriers, however, for accomplishing interspecific and intergeneric crosses. Josef Gottlieb Kölreuter (c. 1761) was the first to report on hybrid vigor (heterosis) in interspecific crosses of various species of *Nicotiana*, concluding that cross-fertilization was generally beneficial and self-fertilization was not.

Introgession is the introduction of genes from one species or gene pool into another species or gene pool. Introgression follows hybridization and occurs when hybrids reproduce with members of one or both of the pa-

rental species that produced the hybrids. It usually involves transfer of a small amount of DNA from one species or genus to another. Both hybridization and introgression can cause rapid evolution, that is, speciation or extinction. When introgression occurs between a common species and a rare species, the rare species is frequently exterminated.

Scientific breakthroughs relative to species-specific molecular (DNA) markers allow quantitative assessment of introgression and hybridization in natural populations. A clear distinction among species is a prerequisite to guide efforts to conserve biodiversity.

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The world's second "cama," shown here with its mother, is a hybrid of a llama and a camel. (AP/Wide World Photos)

Reproductive Isolation Barriers

Isolation barriers can be divided into two types: (1) external and (2) internal. External barriers to genetic interchange between related populations prevent pollen of plants in one population from falling on stigmas of plants in another. A combination of barriers, such as geographical and ecological or ecological and seasonal (flowering time), is more common than individual barriers.

Internal barriers to genetic interchange between related populations operate through incompatibilities between physiological or cytological systems of plants from different

populations. They may (1) prevent the production of F_1 (first-generation) zygotes, even if the pollen from flowers in one population falls on stigmas of flowers in the other; (2) produce F_1 hybrids that are nonviable, weak, or sterile; or (3) cause hybrid breakdown in F_2 or later generations.

The promotion of natural hybridization and introgression has, across time, increased the genetic diversity available to farmers. Traditional farmers experiment with new varieties and breed plants purposely to create new strains. They generally plant experimental plots first and integrate new varieties into their main crops only when a variety has proven itself to be of value. This constant experimentation and breeding have created the diversity of crops on which we now depend.

Transgenic Crops and Controversy

Termed "gene flow," the movement of genes between closely related plant species is quite natural and has been occurring ever since flowering plants evolved. Hybrids that are the offspring resulting from the mating of related species may then mate

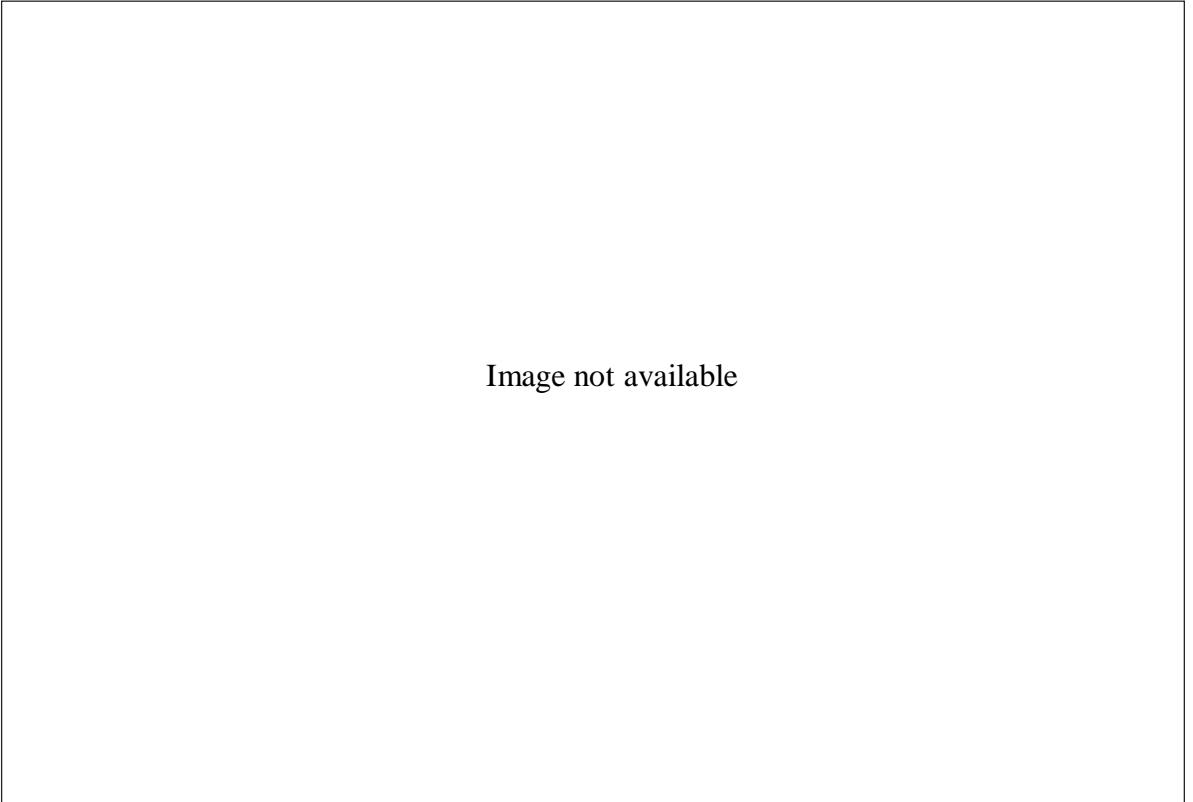


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One of the rarest of hybrids is the zebrass, a cross between a zebra and a donkey. (AP/Wide World Photos)

through pollen exchange with the wild-type (original) plants. Backcrossing, which is also called introgression, increases hybrids' biological fitness.

The term "transgenic" or "genetically modified organism" (GMO) has been applied to plants and animals in which techniques of recombinant DNA have been used to modify specific parts of the genome of an organism. When the procedure is successful, the resulting organism may stably express a novel protein, express a protein with novel properties, or carry a change in the regulation of some of its genes. Usually, such a change is designed to improve the ability of the organism to grow (for instance, by resisting pests or using nutrients more efficiently) or to improve the usefulness of the organism (by improving its nutritive value, using it to manufacture pharmaceutically important molecules, or employing it to carry out environmentally important processes such as digesting environmental toxins).

Hybridization and introgression may introduce novel adaptive traits. The subjects have raised controversy, because transgenes introduced into crops have the potential for spreading into related weeds or wild plants. Scientists have hypothesized that transgenes might move from the genetically modified crop plants to weeds. The possibility of spreading transgenes via introgression and bridging, from genetically modified crops to related weed species, is a concern; introduction of herbicide-resistant cultivars into commercial agriculture could lead to the creation of superweeds.

Some researchers believe that if herbicide-resistant genes were to become more common in weeds as a result of widespread use of herbicide-resistant crops, farmers who rely on herbicides to manage weeds would be forced to use greater amounts and a larger number of herbicides.

To "solve" problem of horizontal gene transfer, the producers of transgenic crops naturally turn to gene technology. They propose to re-

duce the risk of creating transgenic uncontrollable weeds and volunteer cultivars by linking herbicide-resistance genes to other genes that are harmless to the crop but damaging to a weed, such as genes that affect seed dormancy or prevent flowering in the next generation. Thus, if a weed did acquire an herbicide-resistance gene from a transgenic crop, its offspring would not survive to spread the herbicide resistance through the weed population. Several of the newly patented techniques sterilize seeds so that farmers cannot replant them. In addition, patent protection and intellectual property rights keep farmers from sharing and storing seeds. Thus, genetic seed sterility could increase seed industry profits; farmers would need to buy seed every season.

Maternal Inheritance

Most crops are genetically modified via insertion of genes into the nucleus. The genes can, therefore, spread to other crops or wild relatives by movement of pollen. By engineering tolerance to the herbicide glyphosate into the tobacco chloroplast genome, however, researchers not only have obtained high levels of transgene expression but also, because chloroplasts are inherited maternally in many species, have prevented transmission of the gene by pollen—closing a potential escape route for transgenes into the environment. Glyphosate is the most widely used herbicide in the world. It interferes with 5-enol-pyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme that is encoded by a nuclear gene and catalyzes a step in the biosynthesis of certain (aromatic) amino acids in the chloroplasts. Conventional strategies for producing glyphosate-tolerant plants are to insert, into the nucleus, an EPSPS gene from a plant or a glyphosate-tolerant bacterium (the bacterial gene is modified so that the enzyme is correctly targeted to the chloroplasts), or a gene that inactivates the herbicide.

Putting GMOs in Perspective

The prestigious Genetics Society of America has weighed in on the issue of GMOs. Part of its statement reads:

Every year, thousands of Americans become ill and die from food contamination. This is not a consequence of using GMOs, but instead reflects contamination from food-borne bacteria. “Natural” food supplements are widely used but are generally not well-defined, purified, or studied. Although recent reports of contamination of corn meal by GMOs not approved for human consumption led to several claims of allergic response, to date, none of those individuals has been shown to contain antibodies to the GM protein.

—Manjit S. Kang

See also: Artificial Selection; Biodiversity; Chromosome Theory of Heredity; Classical Transmission Genetics; Dihybrid Inheritance; Epistasis; Extrachromosomal Inheritance; Genetic Engineering: Agricultural Applications; Genetic Engineering: Risks; Genetically Modified (GM) Foods; Hardy-Weinberg Law; High-Yield Crops; Inbreeding and Assortative Mating; Incomplete Dominance; Lateral Gene Transfer; Polyploidy; Population Genetics; Quantitative Inheritance; Repetitive DNA; Transgenic Organisms.

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Hybridomas and Monoclonal Antibodies

Field of study: Immunogenetics

Significance: In 1975, Georges Köhler and Cesar Milstein reported that fusion of spleen cells from an immunized mouse with a cultured plasmacytoma cell line resulted in the formation of hybrid cells called hybridomas that secreted the antibody molecules that the spleen cells had been stimulated to produce. Clones of hybrid cells producing antibodies with a desired specificity are called monoclonal antibodies and can be used as a reliable and continuous source of that antibody. These well-defined and specific antibody reagents have a wide range of biological uses, including basic research, industrial applications, and medical diagnostics and therapeutics.

Key terms

ANTIBODY: a protein produced by plasma cells (matured B cells) that binds specifically to an antigen

ANTIGEN: a foreign molecule or microorganism that stimulates an immune response in an animal

ANTISERA: a complex mixture of heterogeneous antibodies that react with various parts of an antigen; each type of antibody protein in the mixture is made by a different type (clone) of plasma cell

PLASMACYTOMA: a plasma cell tumor that can be grown continuously in a culture

A New Way to Make Antibodies

Because of their specificity, antisera have long been used as biological reagents to detect or isolate molecules of interest. They have been useful for biological research, industrial separation applications, clinical assays, and immunotherapy. One disadvantage of conventional antisera is that they are heterogeneous collections of antibodies against a variety of antigenic determinants present on the antigen that has elicited the antibody response. In an animal from which antisera is collected, the mixture of antibodies changes with time so that the types and relative amounts of particular antibodies are different in samples taken at differ-

ent times. This variation makes standardization of reagents difficult and means that the amount of characterized and standardized antisera is limited to that available from a particular sample.

The publication of a report by Georges Köhler and Cesar Milstein in the journal *Nature* in 1975 describing production of the first monoclonal antibodies provided a method to produce continuous supplies of antibodies against specific antigenic determinants. Milstein's laboratory had been conducting basic research on the synthesis of immunoglobulin chains in plasma cells, mature B cells that produce large amounts of a single type of immunoglobulin. As a model system, they were using rat and mouse plasma cell tumors (plasmacytomas). Prior to 1975, Köhler and Milstein had completed a series of experiments in which they had fused rat and mouse plasmacytomas and determined that the light and heavy chains from the two species associate randomly to form the various possible combinations. In these experiments they used mutant plasmacytoma lines that would not grow in selective culture media, while the hybrid cells complemented each others' deficiencies and multiplied in culture.

After immunizing mice with sheep red blood cells (SRBC), Köhler and Milstein removed the spleen cells from the immunized mice and fused them with a mouse plasmacytoma cell line. Again, the selective media did not allow unfused plasmacytomas to grow, and unfused spleen cells lasted for only a short time in culture so that only hybrids between plasmacytoma cells and spleen cells grew as hybrids. These hybrid plasmacytomas have come to be called hybridomas.

Shortly after the two types of cells are fused by incubation with a fusing agent such as polyethylene glycol, they are plated out into a series of hundreds of small wells so that only a limited number of hybrids grow out together in the same well. Depending on the frequency of hybrids and the number of wells used, it is possible to distribute the cells so that each hybrid cell grows up in a separate cell culture well.

On the basis of the number of spleen cells that would normally be making antibodies

against SRBC after mice have been immunized with them, the investigators expected that one well in about 100,000 or more might have a clone of hybrid cells making antibody that reacted against this antigen. The supernatants (liquid overlying settled material) from hundreds of wells were tested, and the large majority were found to react with the immunizing antigen. Further work with other antigens confirmed that a significant fraction of hybrid cells formed with spleen cells of immunized mice produce antibodies reacting with the antigen recently injected into the mouse. The production of homogeneous antibodies from clones of hybrid cells thus became a practical way to obtain reliable supplies of well-defined immunological reagents.

The antibodies can be collected from the media in which the cells are grown, or the hybridomas can be injected into mice so that larger concentrations of monoclonal antibodies can be collected from fluid that collects in the abdominal cavity of the animals.

Specific Antibodies Against Antigen Mixtures

One advantage of separating an animal's antibody response into individual antibody components by hybridization and separation of cells derived from each fusion event is that antibodies that react with individual antigenic components can be isolated even when the mouse is immunized with a complex mixture of antigens. For example, human tumor cells injected into a mouse stimulate the production of many different types of antibodies. A few of these antibodies may react specifically with tumor cells or specific types of human cells, but, in a conventional antisera, these antibodies would be mixed with other antibodies that react with any human cell and would not be easily separated from them. If the tumor cells are injected and hybridomas are made and screened to detect antibodies that react with tumor cells and not with most normal cells, it is possible to isolate antibodies that are useful for detection and characterization of specific types of tumor cells. Similar procedures can also be used to make antibodies against a single protein after the mouse has been immunized with this protein

included in a complex mixture of other biological molecules such as a cell extract.

Following the first report of monoclonal antibodies, biologists began to realize the implications of being able to produce a continuous supply of antibodies with selected and well-defined reactivity patterns. There was discussion of "magic bullets" that would react specifically with and carry specific cytotoxic agents to tumor cells without adverse effects on normal cells. Biologists working in various experimental systems realized how specific and reliable sources of antibody reagent might contribute to their investigations, and entrepreneurs started several biotechnology companies to develop and apply monoclonal antibody methods. This initial enthusiasm was quickly moderated as some of the technical difficulties involved in production and use of these antibodies became apparent; with time, however, many of the projected advantages of these reagents have become a reality.

Monoclonal Reagents

A survey of catalogs of companies selling products used in biological research confirms that many of the conventional antisera commonly used as research reagents have been replaced with monoclonal antibodies. These products are advantageous to the suppliers, being produced in constant supply with standardized protocols from hybrid cells, and the users, who receive well-characterized reagents with known specificities free of other antibodies that could produce extraneous and unexpected reactions when used in some assay conditions. Antibodies are available against a wide range of biomolecules reflecting current trends in research; examples include antibodies against cytoskeletal proteins, protein kinases, and oncogene proteins, gene products involved in the transition of normal cells to cancer cells.

Immunologists were among the first to take advantage of monoclonal antibody technology. They were able to use them to "trap" the spleen cells making antibodies against small, well-defined molecules called haptens and to then characterize the antibodies produced by the hybridomas. This enabled them to define classes of antibodies made against specific anti-

genic determinants and to derive information about the structure of the antibody-binding sites and how they are related to the determinants they bind. Other investigators produced antibodies that reacted specifically against subsets of lymphocytes playing specific roles in the immune responses of animals and humans. These reagents were then used to study the roles that these subsets of immune cells play in responses to various types of antigens.

Antibodies that react with specific types of immune cells have also been used to modulate the immune response. For example, antibodies that react with lymphocytes that would normally react with a transplanted tissue or organ can be used to deplete these cells from the circulation and thus reduce their response against the transplanted tissue.

Monoclonal Antibodies as Diagnostic Reagents

Monoclonal antibodies have been used as both *in vitro* and *in vivo* diagnostic reagents. By the 1980's, many clinical diagnostic tests such as assays for hormone or drug levels relied upon antisera as detecting reagents. Antibodies reacting with specific types of bacteria and viruses have also been used to classify infections so that the most effective treatment can be determined. In the case of production of antibodies for typing microorganisms, it has frequently been easier to make type-specific monoclonal antibodies than it had been to produce antisera that could be used to identify the same microorganisms.

Companies supplying these diagnostic reagents have gradually switched over to the use of monoclonal antibody products, thus facilitating the standardization of the reactions and the protocols used for the clinical tests. The reproducibility of the assays and the reagents has made it possible to introduce some of these tests that depend upon measurement of concentrations of substances in urine as kits that can be used by consumers in their own homes. Kits have been made available for testing glucose levels of diabetics, for pregnancy, and for the presence of certain drugs.

Although the much-hoped-for "magic bullet" that would eradicate cancer has not been

found, there are several antibodies in use for tumor detection and for experimental forms of cancer therapy. Monoclonal antibodies that react selectively with cancer cells but not normal cells can be used to deliver cytotoxic molecules to the cancer cells. Monoclonal reagents are also used to deliver isotopes that can be used to detect the presence of small concentrations of cancer cells that would not normally be found until the tumors grew to a larger size.

Human Monoclonal Antibodies

The majority of monoclonal antibodies made against human antigens were mouse antibodies derived from the spleens of immunized mice. When administered to humans in clinical settings, the disadvantage of the animal origin of the antibodies soon became apparent. The human immune system recognized the mouse antibodies as foreign proteins and produced an immune response against them, limiting their usefulness. Even when the initial response to an antibody's administration was positive, the immune reaction against the foreign protein quickly limited its effectiveness. In an attempt to avoid this problem, human monoclonal antibodies have been developed using several methods. The first is the hybridization of human lymphocytes stimulated to produce antibodies against the antigen of interest with mouse plasmacytomas or later with human plasmacytoma cell lines. This method has been used successfully, although it is limited by the ability to obtain human B cells or plasma cells stimulated against specific antigens because it is not possible to give an individual a series of immunizations and then remove stimulated cells from the spleen. Limited success has resulted from the fusion of circulating lymphocytes from immunized individuals or fusion of lymphocytes that have been stimulated by the antigen in cell cultures. Investigators have reported some success in making antitumor monoclonal antibodies by fusing lymph node cells from cancer patients with plasmacytoma cell lines and screening for antibodies that react with the tumor cells.

There has also been some success at "humanizing" mouse antibodies using molecular genetic techniques. In this process, the portion

of the genes that make the variable regions of the mouse antibody protein that reacts with a particular antigen is spliced in to replace the variable region of a human antibody molecule being produced by a cultured human cell or human hybridoma. What is produced is a human antibody protein that has the binding specificity of the original mouse monoclonal antibody. When such antibodies are used for human therapy, the reaction against the injected protein is reduced compared to the administration of the whole mouse antibody molecules.

Another application of antibody engineering is the production of bispecific antibodies. This has been accomplished by fusing two hybridomas making antibodies against two different antigens. The result is an antibody that contains two types of binding sites and thus binds and cross-links two antigens, bringing them into close proximity to each other.

Recombinant Antibodies

Advances in molecular genetic techniques and in the characterization of the genes for the variable and constant regions of antibody molecules have made it possible to produce new forms of monoclonal antibodies. The generation of these recombinant antibodies is not dependent upon the immunizing of animals but on the utilization of combinations of antibody genes generated using the *in vitro* techniques of genetic engineering. Geneticists discovered that genes inserted into the genes for fibers expressed on the surface of bacterial viruses called bacteriophages are expressed and detectable as new protein sequences on the surface of the bacteriophage. Investigators working with antibody genes found that they could produce populations of bacteriophage expressing combinations of antibody-variable genes. Molecular genetic methods have made it possible to generate populations of bacteriophage expressing different combinations of antibody-variable genes with frequencies approaching the number present in an individual mouse or human immune system. The population of bacteriophage can be screened for binding to an antigen of interest, and the bacteriophage expressing combinations of variable regions binding

to the antigen can be multiplied and then used to generate recombinant antibody molecules in culture.

Researchers have also experimented with introducing antibody genes into plants, resulting in plants that produce quantities of the specific antibodies. Hybridomas or bacteriophages expressing specific antibodies of interest may be a potential source of the antibody gene sequences introduced into these plant antibody factories.

—Roger H. Kennett

See also: Allergies; Antibodies; Autoimmune Disorders; Burkitt's Lymphoma; Cancer; Genetic Engineering; Genetic Engineering: Medical Applications; Immunogenetics; Model Organism: *Mus musculus*; Oncogenes; Organ Transplants and HLA Genes; Synthetic Antibodies.

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Hypercholesterolemia

Field of study: Diseases and syndromes

Significance: *Hypercholesterolemia represents a significant risk factor for coronary artery disease and stroke. Diet as well as genetics influence the development of hypercholesterolemia.*

Key terms

APOLIPOPROTEIN B (APO-B): a protein essential for cholesterol transport

HIGH-DENSITY LIPOPROTEIN (HDL): a small, denser form of cholesterol, popularly known as the “good” cholesterol because it can transport cholesterol from tissues to the liver

LOW-DENSITY LIPOPROTEIN (LDL): the “bad” cholesterol that tends to deposit into the tissues, especially in the vessel walls

Cholesterol’s Role in the Body

Cholesterol is a steroid lipid, a type of fat molecule that is essential for life. It is an important component of cell membranes and is used by the body to synthesize various steroid hormones. When cooled, cholesterol is a waxy substance, which cannot dissolve in the bloodstream. It is transported in the bloodstream in complexes of cholesterol and protein called lipoproteins.

There are two different classes of lipoproteins in the bloodstream. Low-density lipoprotein (LDL) cholesterol is the “bad” cholesterol that tends to deposit into the tissues, especially in the vessel walls. High-density lipoprotein (HDL), a smaller, denser molecule, is the “good” cholesterol, because it can transport cholesterol from tissues to the liver.

About one tablespoon of cholesterol circu-

lates in the bloodstream, which is enough to meet the body’s needs. Cholesterol is present in animal-derived foods, but is also produced by the liver. The liver manufactures and regulates the amount of lipoproteins in the body. The normal range of total cholesterol is less than 200 milligrams per deciliter (mg/dl) of blood. A total cholesterol level between 200–240 mg/dl is borderline high, and a total cholesterol level above 240 mg/dl is considered high. The normal range of LDL cholesterol is less than 130 mg/dl, and the normal range of HDL cholesterol is greater than 35 mg/dl. Hypercholesterolemia is diagnosed when the total cholesterol level is higher than the normal range, and the term “hypercholesterolemia” is often used to refer to familial hypercholesterolemia as well.

Causes of Hypercholesterolemia

Hypercholesterolemia itself may be asymptomatic but can still be damaging to the vascular system. Excess amounts of cholesterol in the blood can build up along the walls of the arteries, which results in hardening and narrowing of the arteries, called atherosclerosis. Severe atherosclerosis can lead to a blockage of blood flow. Atherosclerosis in the heart causes cardiovascular disease (such as heart attacks). The result of atherosclerosis in the brain can be a stroke. Atherosclerosis can also occur in the extremities of the body, such as the legs, causing pain and blood clots.

Hypercholesterolemia occurs when the body is unable to use or eliminate excessive amounts of cholesterol. Several diseases can contribute to hypercholesterolemia, such as diabetes, thyroid disorders, and liver diseases. However, the most important cause of hypercholesterolemia is a combination of diet and genetic factors.

Cholesterol naturally exists in animal products, such as meats (particularly fatty meats), eggs, milk, cheese, liver, and egg yolks. Large intakes of these products can certainly increase one’s cholesterol level, not only because they have high concentrations of cholesterol itself but, more important, because they contain fats that prompt the body to make cholesterol. The genetic influence on hypercholesterolemia is also significant.

Genetics of Hypercholesterolemia

It is evident that hypercholesterolemia is more common among certain ethnic groups. Cholesterol levels in northern European countries are higher than those in southern Europe. Asians have lower cholesterol levels than Caucasians. A severe form of hereditary hypercholesterolemia called familial hypercholesterolemia typically does not respond to lifestyle changes. Thus, there is no doubt that genes play an important role in the occurrence of hypercholesterolemia.

Hypercholesterolemia is on the increase worldwide. People with hypercholesterolemia often develop coronary heart disease at a younger age than those in a general population as a result of increased LDL cholesterol levels (about two times higher than normal). In cases of extreme hypercholesterolemia (exceeding three or four times normal), high cholesterol levels can be detected in utero or at birth in cord blood. Individuals with extreme hypercholesterolemia usually develop the first cardiovascular event in childhood or adolescence and die by the age of thirty.

Familial hypercholesterolemia is the best understood genetically. It displays autosomal dominant inheritance, which means that either parent with hypercholesterolemia has a high probability of passing it on. This disorder results from defects of the LDL receptor, which ensures the proper movement of LDLs. Thus, dysfunction of this receptor causes increased levels of LDL in the blood. The LDL receptor gene, which is located on the short arm of human chromosome 19, is prone to a variety of mutations that affect LDL metabolism and movement.

Apolipoprotein B (Apo-B) is a protein essential for cholesterol transport. Apo-B can be affected by both diet and genetics. Individuals with one or more specific genotypes (the genetic constitution of an individual) have much greater changes in cholesterol levels in response to diet than do other genotypes.

The other genetic cause is mutations in the gene for the enzyme cholesterol 7-alpha hydroxylase (CYP7A1), which is essential for the

normal elimination of cholesterol in the blood. It initiates the primary conversion of cholesterol into bile acids in the liver. Mutations can cause an accumulation of cholesterol in the liver, as the primary route of converting cholesterol to bile acids is blocked. The liver responds to excessive cholesterol by reducing the number of receptors available to take up LDL from the blood, resulting in an accumulation of LDL in the blood.

Implications

Although genetics plays an important role, hypercholesterolemia is often the result of a combination of genetics and lifestyle. Consuming a healthy diet and exercising regularly can help to maintain an optimal cholesterol level and to reduce the risk of cardiovascular disease for people with either a good gene or a bad gene.

—*Kimberly Y. Z. Forrest*

See also: Alzheimer's Disease; Breast Cancer; Cancer; Heart Disease; Hereditary Diseases; Steroid Hormones.

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Web Site of Interest

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Icelandic Genetic Database

Fields of study: Bioinformatics; Techniques and methodologies

Significance: *Iceland is the first country to license the rights of an entire population's genetic code to a private company. The potential scientific and health care benefits of the Icelandic Genetic Database are considered significant. However, its creation has led to a worldwide debate concerning genetic research and its role in public health.*

Key terms

GENETIC DATABASE: a set of computerized records of individuals that contain their genetic information and medical histories

GENETIC PROFILE: a description of a person's genes, including any variations within them

INFORMED CONSENT: the right for a potential research subject to be adequately informed of the aims, methods, sources of funding, conflicts of interest, anticipated benefits, potential risks, discomforts involved in a procedure or trial, and the ability to withdraw consent, which should be in a written, signed document

PHARMACOGENOMICS: the study of how variations in the human genome affect responses to medications; can be used to find the most suitable patients for drug therapy trials or to match people with similar genetic profiles to the drugs most likely to work for them

POPULATION DATABASE: a database containing information on the individuals in a population, which can be defined by a variety of criteria, such as location (a state or country) or ethnicity

Why Iceland?

Icelanders have always displayed an intense interest in documenting their genealogical and medical histories. The complete family histories for more than 75 percent of all Icelanders who have ever lived are known. Although standardized recording of extensive and precise medical records became law in 1915, additional records date to the 1600's. These extensive written records of the Icelandic people are of high quality and unique in the world today.

History of the Database

In the mid-1970's, the Icelandic parliament considered collecting these records into a computer database. The idea was abandoned because of a lack of funding, concern over privacy, and inadequate technology. While working on identifying the gene for multiple sclerosis in 1994, Icelander physician and scientist Dr. Kári Stefánsson realized that Iceland's genealogical and medical records would greatly aid in the search for genes involved in complex but common diseases such as heart disease and diabetes. He also believed that since all Icelanders can trace their genetic roots to the same few founders, their genetic backgrounds would be very similar, making it easier and faster to identify the mutations causing diseases. He determined it was financially and technologically feasible to build a computer database integrating genealogical, medical, and genetic profiles for the first time. However, the genetic profiles of the Icelandic population had yet to be determined. Because Iceland has a nationalized health care system, permission of the Icelandic parliament was required.

With private financial backing, Stefánsson founded the company deCODE Genetics in 1996. Two years later, Iceland's parliament enacted the Act on a Health Sector Database for an Icelandic Genetic Database, awarding a twelve-year license exclusively to deCODE. The database immediately became the subject of intense ethical and medical debates. While this controversy continued, deCODE Genetics computerized the Icelandic genealogical records, created the genetic profiles of eight thousand Icelandic volunteers, and uploaded their genetic, medical, and genealogical records.

As of 2003, the Icelandic Genetic Database was not officially operating. Only the data for volunteers could be used until the database passed a government-ordered security test to ensure that the database would be accessible only to those with appropriate permission and the data had been encrypted and privatized.

Current Uses of the Database

From the very beginning, two different but

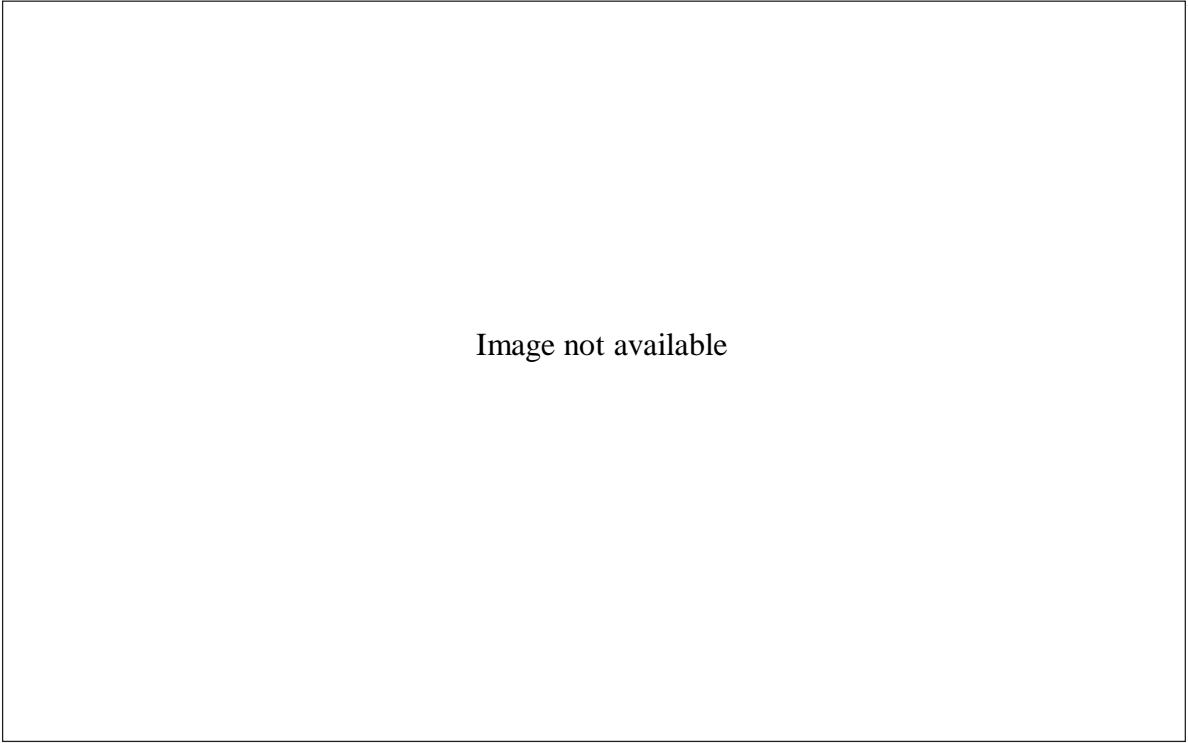


Image not available

Kári Steffánsson, founder of deCODE Genetics, speaking before the forty-first annual meeting of the American Society of Hematology in December, 1999. (AP/Wide World Photos)

interrelated objectives for the database were defined: (1) discovering the genes involved in complex diseases and (2) finding new drugs through pharmacogenomics to combat those same diseases once their genes were identified.

Working with volunteers, deCODE launched an initiative to discover genes involved in more than fifty common diseases, such as diabetes and asthma. Genes involved in all fifty diseases have been mapped at least to a chromosome. Three genes have been isolated, including one for schizophrenia. Based upon this early and rapid success, the company has entered into new pharmacogenomic partnerships with additional companies with the aim to discover drugs that can effectively counter the diseases that have been genetically mapped.

Potential Uses

Because the database will contain the information of the entire Icelandic people, it is also considered a population genetic database. Its data could be used not only to determine an in-

dividual's predisposition to a particular disease but also to predict diseases within the entire population of Iceland before they actually occur. This new form of medical intervention could be used to plan public health policies for groups of people. Predicting diseases is a significant departure from current public health practice, which develops treatment regimens only after a disease appears, not before. What began as a single country's genetic database has now grown into the recognition of the potential role of genetics in worldwide public health policy and planning.

Ethical Concerns

The Act on a Health Sector Database is silent on what data were to be used, how they would be used, informed consent issues, and the right to privacy. Heavily encrypting all the information in the database, removing all personal information that could identify patients individually, and security testing the database were a result of these privacy concerns.

Informed consent issues have created the most serious problems. The act presumes informed consent unless an individual "opts out," which many feel violates the intent of consent. Icelandic physicians have filed a lawsuit to clarify this issue, since Icelandic law requires that physicians guarantee full informed consent.

A second major concern is the licensing of Iceland's complete genetic profile to a company. Because Iceland has a nationalized health plan, medical records have always been considered a national resource. Many feel that Icelandic genetic records are also a national resource and should remain with the people. Related to this issue is concern that granting the rights to only a single company will prevent scientific research both in Iceland and elsewhere on any genes deCODE may identify.

Although controversial, the database continues to provide guidance and lessons for other nations in developing new genetic databases. Ethical, medical, and social issues first raised in Iceland have quickly become issues worldwide as population genetic databases proliferate. This, in turn, has resulted in an active debate on the role of genetic information in worldwide public health and whether it should be permitted to operate in all countries, if at all.

—Diane C. Rein

See also: Bioinformatics; Genetic Screening; Genetic Testing: Ethical and Economic Issues; Genomic Libraries; Genomics; Human Genome Project; Linkage Maps; Pedigree Analysis; Population Genetics.

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deCode Genetics. <http://www.decode.com>. Site of the company compiling the Icelandic Genetic Database.

Mapping the Icelandic Genome. <http://sunsite.berkeley.edu/biotech/iceland>. Site devoted to "the scientific, political, economic, religious, and ethical issues surrounding the deCode Project and its global implications."

Immunogenetics

Field of study: Immunogenetics

Significance: *Immunogenetics is primarily concerned with the major histocompatibility genes that identify self tissues, the genes in B lymphocytes that direct antibody synthesis, and the genes that direct the synthesis of T lymphocyte receptors. This same genetic control that directs immune cell embryonic*

development and activation from an antigenic challenge also explains the basis of organ transplant rejection, autoimmunity, allergies, immunodeficiency, and potential future therapies.

Key terms

APOPTOSIS: cell death that is programmed as a natural consequence of growth and development through normal cellular pathways or through signals from neighboring cells

CYTOKINES: soluble intercellular molecules produced by cells such as lymphocytes that can influence the immune response

DOWNTSTREAM: describes the left-to-right direction of DNA whose nucleotides are arranged in sequence with the 5' carbon on the left and the 3' on the right; the direction of RNA transcription of a genetic message with the beginning of a gene on the left and the end on the right

HAPLOTYPE: a sequential set of genes on a single chromosome inherited together from one parent; the other parent provides a matching chromosome with a different set of genes

TRANSPOSON: a sequence of nucleotides flanked by inverted repeats capable of being removed or inserted within a genome

Genes, B Cells, and Antibodies

The fundamental question that led to the development of immunogenetics relates to how scientists are able to make the thousands of specific antibodies that protect people from the thousands of organisms with which they come in contact. Macfarlane Burnet proposed the clonal selection theory, which states that an antigen (that is, anything not self, such as an invading microorganism) selects, from the thousands of different B cells, the receptor on a particular B cell that fits it like a key fitting a lock. That cell is activated to make a clone of plasma cells, producing millions of soluble antibodies with attachment sites identical to the receptor on that B-cell surface. The problem facing scientists who were interested in a genetic explanation for this capability was the need for more genes than the number that was believed to make up the entire human genome.

It was Susumu Tonegawa who first recog-

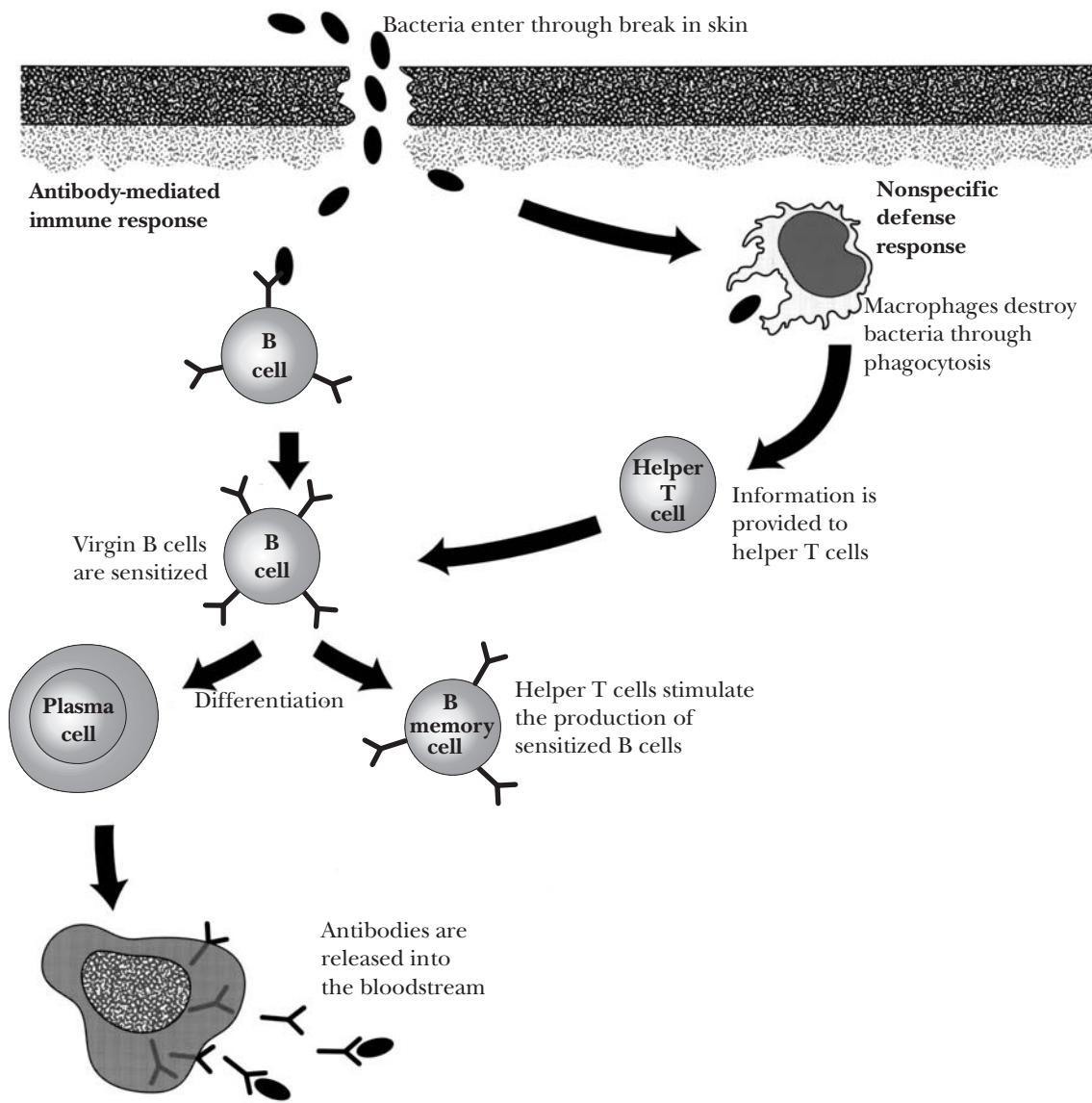
nized that a number of antibodies produced in the lifetime of a human did not have to have the equivalent number of physical genes on their chromosomes. From his work, it was determined that the genes responsible for antibody synthesis are arranged in tandem segments on specific chromosomes relating to specific parts of antibody structure. The amino acids that form the two light polypeptide chains and the two heavy polypeptide chains making up the IgG class of antibody are programmed by nucleotide sequences of DNA that exist on three different chromosomes. Light-chain genes are found on chromosomes 2 and 22. The specific nucleotide sequences code for light polypeptide chains, with half the chain having a constant amino acid sequence and the other half having a variable sequence. The amino acid sequences of the heavy polypeptide chains are constant over three-quarters of their length, with five basic sequences identifying five classes of human immunoglobulins: IgG, IgM, IgD, IgA, and IgE. The other quarter length has a variable sequence that, together with the variable sequence of the light chain, forms the antigen-binding site. The nucleotide sequence coding for the heavy chain is part of chromosome 14.

The actual light-chain locus is organized into sequences of nucleotides designated V, J, and C segments. The multiple options for the different V and J segments and mixing the different V and J segments cause the formation of many different DNA light-chain nucleotide sequences and the synthesis of different antibodies. The same type of rearrangement occurs between a variety of nucleotide sequences related to the V, D, and J segments of the heavy-chain locus. The recombination of segments appears to be genetically regulated by recombination signal sequences downstream from the variable segments and recombination activating genes that function during B-cell development. Genetic recombination is complete with the immature B cell committed to producing one kind of antibody. The diversity of antibody molecules is explained by the fact that the mRNA transcript coding for either the light polypeptide chain or the heavy polypeptide chain is formed containing exons transcribed from re-

combined gene segments during B-cell differentiation. The unique antigen receptor-binding site is formed when the variable regions of one heavy and one light chain come together during the formation of the completed antibody in the endoplasmic reticulum of the mature B cell. The B-cell antigen receptor is an attached surface antibody of the IgM class. Bind-

ing of the antigen to the specific B cell activates its cell division and the formation of a clone of plasma cells that produce a unique antibody. If this circulating B cell does not contact its specific antigen within a few weeks, it will die by apoptosis. During plasma cell formation, the class of antibody protein produced normally switches from IgM to IgG through the forma-

The Response of the Immune System to Bacterial Infection



tion of an mRNA transcript containing the exon nucleotide sequence made from IgG heavy-chain C segment rather than the heavy-chain C segment for IgM. The intervening nucleotide sequence of the IgM constant segment is deleted from the chromosome as an excised circle reminiscent of the transposon or plasmid excision process. The result of this switch is the formation of an IgG antibody having the same antigen specificity as the IgM antibody, because the variable regions of the light and heavy polypeptide chains remain the same. Although the activation and development of B cells by some antigens may not need T-cell involvement, it is believed that class switching and most B-cell activity are influenced by T-cell cytokines.

Major Histocompatibility Genes

In humans, the major histocompatibility genes encoding “self antigens” are also called the HLA complex and are located on chromosome 6. The nucleotides that compose this DNA complex encode for two sets of cell surface molecules designated MHC Class I and MHC Class II antigens. The Class I region contains loci *A*, *B*, and *C*, which encode for MHC Class I *A*, *B*, and *C* glycoproteins on every nucleated cell in the body. Because the *A*, *B*, and *C* loci comprise highly variable nucleotide sequences, numerous kinds of *A*, *B*, and *C* glycoproteins characterize humans. All people inherit MHC Class I *A*, *B*, and *C* genes as a haplotype from each of their parents. Children will have tissues with half of their Class I *A*, *B*, and *C* antigens like those of their mother and half like those of their father. Siblings could have tissue antigens identical or totally dissimilar based on their MHC I glycoproteins. Body surveillance by T lymphocytes involves T cells recognizing self glycoproteins. Cellular invasion by a virus or any other parasite results in the processing of antigen and its display in the cleft of the MHC Class I glycoprotein. T cytotoxic lymphocytes with T-cell receptors specific for the antigen-MHC I complex will attach to the antigen and become activated to clonal selection. Infected host cells are killed when activated cytotoxic T cells bind to the surface and release perforins, causing apoptosis.

MHC Class II genes are designated *DPα* and

β, *DQα* and *β*, and *DRα* and *β*. These genes encode for glycoprotein molecules that attach to the cell surface in *α* and *β* pairs. A child will inherit the six genes as a group or haplotype, three *α* and *β* glycoprotein gene pairs from each parent. The child will also have glycoprotein molecules made from combinations of the maternal and paternal *α* and *β* pairings during glycoprotein synthesis.

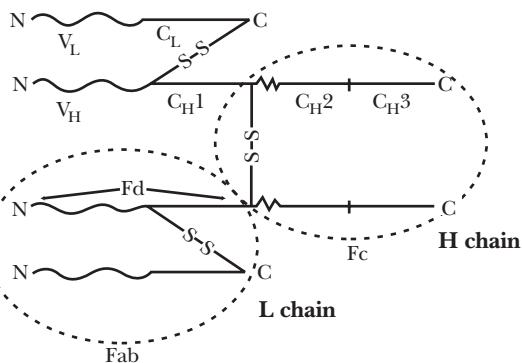
The Class II MHC molecules are found on the membranes of macrophages, B cells, and dendritic cells. These specialized cells capture antigens and attach antigen peptides to the three-dimensional grooves formed by combined *α* and *β* glycoprotein pairs. The antigen attached to the Class II groove is presented to the T helper cell, with the receptor recognizing the specific antigen in relation to the self antigen. The specific T helper cell forms a specific clone of effector cells and memory cells.

Genes, T Helper Cells, and T Cytotoxic Cells

The thousands of specific T-cell receptors (TCR) available to any specific antigen one might encounter in a lifetime are formed in the human embryonic thymus from progenitor T cells. The TCR comprises two dissimilar polypeptide chains designated *α* and *β* or *γ* and *δ*. They are similar in structure to immunoglobulins and MHC molecules, having regions of variable amino acid sequences and constant amino acid sequences arranged in loops called domains. This basic structural configuration places all three types of molecules in a chemically similar grouping designated the immunoglobulin superfamily. The genes of these molecules are believed to be derived from a primordial supergene that encoded the basic domain structure.

The exons encoding the *α* and *γ* polypeptides are designated V, J, and C gene segments in sequence and associate with recombination signal sequences similar to the immunoglobulin light-chain gene. The *β* and *δ* polypeptide genes are designated VDJ and C exon segments in sequence associating with recombination signal sequences similar to the immunoglobulin heavy-chain genes. Just as there are multiple forms for each of the immunoglobulin variable

The Structure of Immunoglobulin G



A Y-shaped model of the antibody immunoglobulin G (IgG). V indicates a region of variability that would permit recognition by a wide variety of antigens.

Source: After John J. Cebra's "The 1972 Nobel Prize for Physiology or Medicine," *Science*, 1972.

gene segments, so there are multiple forms for the variable TCR gene segments. Thymocytes, T-cell precursors in the thymus, undergo chance recombinations of gene segments. These genetic recombinations, as well as the chance combination of a completed α polypeptide with a completed β polypeptide, provide thousands of completed specific TCRs ready to be chosen by an invading antigen and to form a clone of either T helper cells or T cytotoxic cells.

Immunogenetic Disease

The HLA genes of the major histocompatibility complex identify every human being as distinct from all other things, including other human beings, because of the MHC Class I and Class II antigens. Surveillance of self involves B- and T-cell antigen recognition because of MHC self-recognition. How well individual human beings recognize self and their response to antigen in an adaptive immune response are determined by MHC haplotypes as well as the genes that make immunoglobulins and T-cell receptors. These same genes can explain a variety of disease states, such as autoimmunity, allergy, and immunodeficiency.

Because immunoglobulin structure and T-cell receptor formation are based on a mechanism of chance, problems involving self-recognition may occur. It is currently believed that thymocytes with completed T-cell receptors are protected from apoptosis when they demonstrate self-MHC molecule recognition. Alternatively, it is believed that thymocytes are also presented with self-antigens processed by specialized macrophages bearing MHC Class I and Class II molecules. Thymocytes reacting with high-affinity receptors to processed self-antigens undergo apoptosis. There also appears to be a negative selection process within the bone marrow that actively eliminates immature B cells with membrane bound auto-antibodies that react with self-antigens. In spite of these selective activities, it is believed that autoreactive T cells and B cells can be part of circulating surveillance, causing autoimmune disease of either single organs or multiple tissues.

It has long been recognized that autoimmune diseases occur in families, and there is growing evidence that an individual with a certain HLA haplotype has a greater risk for developing a particular disease. For example, ankylosing spondylitis develops more often in individuals with *HLA-B27* than in those with another *HLA-B* allele, and rheumatoid arthritis is associated with *DR1* and *DR4* alleles. Myasthenia gravis and multiple sclerosis are two neurological diseases caused by auto-antibodies, and there is evidence that they are related to restricted expression of T-cell variable genes. Genomic studies are providing evidence for the possibility that autoimmune induction occurs because of molecular mimicry between human host proteins and microbial antigens. Among the cross-reacting antigens that have been implicated are papillomavirus E2 and the insulin receptor, and poliovirus VP2 and the acetyl choline receptor.

The genetics of immunity also involves the study of defective genes that cause primary immunodeficiency infectious disease. The deficiency can result in a decrease in an adaptive immune response involving B cells, T cells, or both, as is the case with severe combined immunodeficiency disorder (SCID). There is evi-

dence that SCID can demonstrate either autosomal recessive or X-linked inheritance. One such defect has been located on the short arm of chromosome 11 and involves a mutation of recombination-activating genes that are necessary for the rearrangement of immunoglobulin gene segments and the T-cell receptor gene segments. The inability to recombine the VD and J variable segments prevents the development of active B cell and T cells with the variety of antigen receptors. SCID is essentially incompatible with life and characterized by severe opportunistic infections caused by even normally benign organisms.

Allergies are widely understood to have a genetic component, with the understanding that atopy, an abnormal IgE response, is common to certain families. There is evidence that children have a 30 percent chance of developing an allergic disease if one parent is allergic, while those children with two allergic parents have a 50 percent chance. The genetic control of IgE production can be related to T_{H2} lymphocyte cytokine stimulation of class switching from the constant segment of IgG to the constant segment of IgE on chromosome 14 in an antigen-selected cell undergoing clonal selection.

Immunogenetics and Treatment

Understanding the genetic basis for allergic reactions is resulting in novel approaches to protect against disease. Through genetic engineering, monoclonal mouse/human antibodies can be made that are able to react with serum IgE and down-regulate IgE production. Probably the greatest potential for therapy will parallel the human genome studies that are further elucidating the genetic relationship to immune defense, autoimmunity, and allergy. As science continues to identify those genes that provide protein receptors and messengers, the best drug therapies and molecular manipulation will be discovered.

—Patrick J. DeLuca

See also: Allergies; Antibodies; Autoimmune Disorders; Hybridomas and Monoclonal Antibodies; Organ Transplants and HLA Genes; Synthetic Antibodies.

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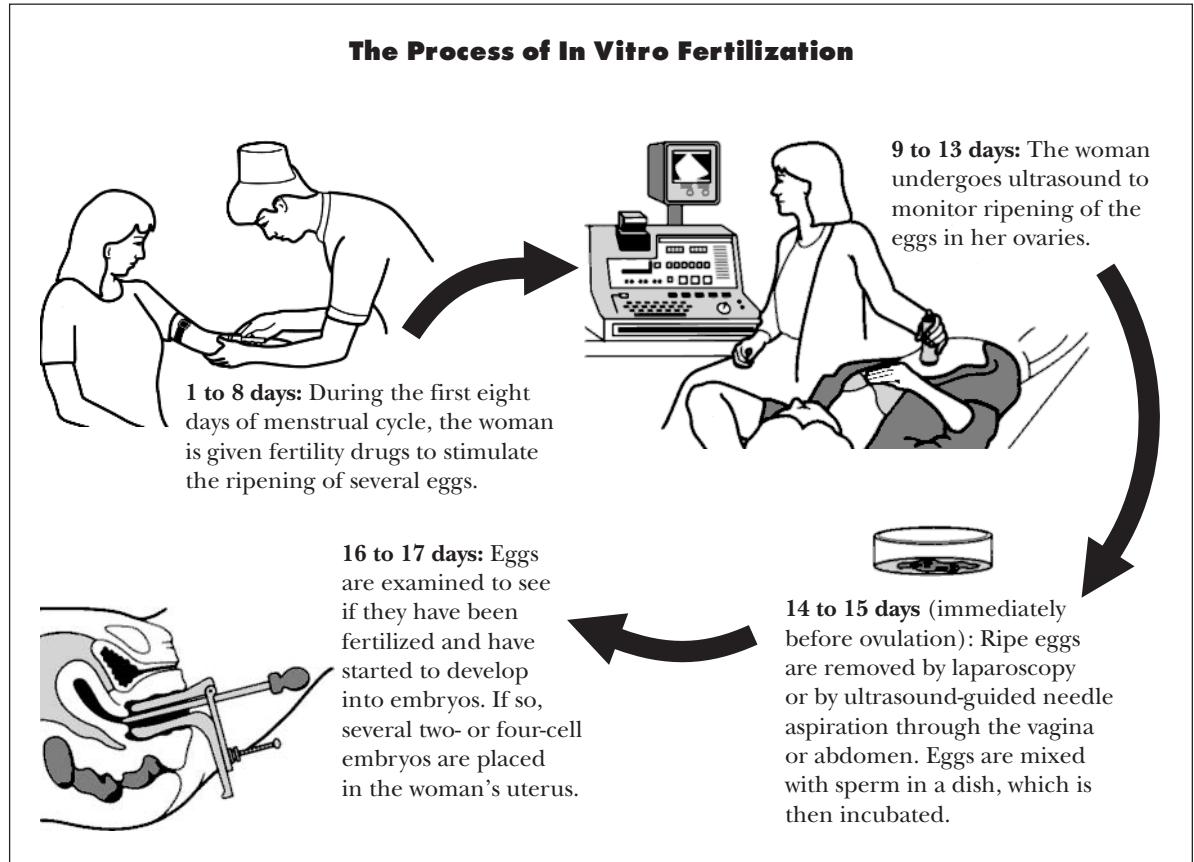
Web Sites of Interest

- American Society for Histocompatibility and Immunogenetics. <http://www.ashihla.org>. A nonprofit professional organization for immunologists, geneticists, molecular biologists, transplant surgeons, and pathologists, devoted to advancing the science and exchanging information.
- ImMunoGeneTics (IMGT) Database. <http://imgt.cines.fr:8104>. A database focusing on immunoglobulins, T-cell receptors, and MHC molecules of all vertebrates, including interactive tools.

In Vitro Fertilization and Embryo Transfer

Field of study: Human genetics and social issues

Significance: *The term “in vitro” designates a living process removed from an organism and isolated “in glass” for laboratory study. In vitro fertilization (IVF) is a process in which harvested eggs and sperm can be brought together artificially to form a zygote. The resulting zygote can be grown for a time in vivo, where it can be tested biochemically and genetically, if desired, after which it can be implanted in the uterus of the egg donor or a surrogate.*



(Hans & Cassidy, Inc.)

Key terms

DIPLOID: possessing a full complement of chromosome pairs, as in humans, who have 23 pairs of chromosomes for a total of 46

GAMETE: a germ cell; an egg (ovum or oocyte) or a sperm (spermatozoan)

HAPLOID: possessing a full complement of one of each type of chromosome; mature human gametes are haploid, with 23 chromosomes

SURROGATE: a female that carries an embryo derived from an egg from another female

ZYGOTE: the earliest stage in the development of an organism, just after fertilization

Natural Fertilization

Fertilization, the union of a male gamete (sperm) with a female gamete (ovum), is fundamentally a genetic process. Each of the gametes is haploid, containing half of the genetic

information needed for a living organism. Fertilization brings together these two sets, thereby producing a diploid zygote that will develop into an embryo.

Gametes are produced in the gonads (ovaries in females, testes in males) by a special type of cell division called meiosis. Instead of producing diploid daughter cells, as in mitosis, meiosis results in haploid cells. In humans, the natural place for fertilization is in a Fallopian tube of a woman, the channel through which an ovum travels to the uterus. A normal adult woman ovulates each month, releasing a single haploid ovum from one of her two ovaries. Ovulation is under hormonal control.

Sperm from the male's testis are deposited in the woman's vagina during sexual intercourse. Typically, men release hundreds of millions of sperm into the vagina when they ejaculate. From the vagina, these sperm travel

through the uterus and into each Fallopian tube in search of an ovum. During this trip, the sperm undergo changes called capacitation. To fuse with the ovum, a sperm must penetrate several surrounding barriers. After fusion of sperm and egg, the nuclear membranes of the two cells break down so that the paternal and maternal chromosomes can congregate in a single nucleus. The resulting zygote divides into two new diploid cells, the first cells of a genetically unique new being.

In Vitro Fertilization and Embryo Transfer

Fertilization can also take place artificially in laboratory culture dishes. Gametes are collected, brought together, and fertilized in a laboratory. After the zygote develops into an embryo, it can then be transferred to a uterus for continued development and eventual birth. This procedure can be done for many species, including humans. The first human conceived by in vitro fertilization (IVF), Louise Brown, was born on July 25, 1978, in England.

Nuclear Transplantation from Donor Eggs

For women who do not produce any viable oocytes because of permanent failure of the ovaries, options for having a child who contains genetic information from the mother are limited. Nuclear transfer into an enucleated donor egg could address this limitation. Since the 1980's, nuclei from relatively undifferentiated mammalian embryonic cells have been successfully transferred to donor eggs. In 1996 researchers at the Roslyn Institute in Scotland advanced nuclear transfer by taking a nucleus from an adult somatic cell and successfully transferring it into an enucleated egg. The result of this work was the birth of the first vertebrate cloned from an adult cell, Dolly the sheep. Since Dolly, nuclear transfer has been successfully performed in cows, pigs, cats, and mice.

Adult somatic cells contain essentially the same genetic information as the single fertilized egg that gave rise to the adult organism. However, unlike the fertilized egg, most adult somatic cells are terminally differentiated and have lost the ability to produce any type of cell in the body, as a fertilized egg can. Nuclear transfer takes a nucleus from an adult somatic cell and places it into an enucleated donor egg. In the environment of the egg, the DNA in the transferred nucleus can "dedifferentiate" and direct the production of a new individual. Because this technique does not involve fertilization, the new individual is considered a clone of the adult organism that contributed the nucleus.

Is the new individual produced really a clone of the adult? The enucleated egg contributes the environment that directs the unfolding of the genetic program that leads to the development of the new individual. Proteins called transcription factors control the expression of individual genes within the

DNA. These transcription factors are contributed by the enucleated, donor egg, and they determine what genes will be active, in what cells, and for how long. Proteins contributed by the donor egg will control the early embryonic divisions. The donor egg also contains RNA molecules that serve as templates to create the proteins needed for events in early embryogenesis, essential to the development of the new organism. These molecules will influence how that organism grows and develops and what genes are expressed by its cells.

The nucleus is not the only source of DNA in the animal cell. The donor egg contains organelles called mitochondria that contain their own DNA. Mitochondria reproduce by a process much like bacteria, copying their own DNA and dividing within the cell. All of the mitochondria in an organism produced by nuclear transfer into a donor egg will be derived from the donor egg, not from the cell that donated the nucleus. Mitochondria are responsible for cellular metabolism, and some metabolic diseases can be traced directly to mutations within mitochondrial DNA.

As might be anticipated, this reproductive technique raises ethical questions, as only one parent can contribute a nucleus to the donor egg. Moreover, it involves a great deal of manipulation *in vitro*, and some suggest that developmental problems can result from such manipulation. Nevertheless, in 2003, as the first "test-tube baby," Louise Brown, celebrated her twenty-fifth birthday, many remarked on how many children had been similarly brought into the world since 1978 and how common the technique had become as an alternative for infertile couples.

—Michele Arduengo

In humans IVF is usually used to overcome infertility caused by problems such as blocked Fallopian tubes or low sperm count. IVF is also done in veterinary medicine and for scientific research. IVF also makes genetic diagnoses easier and could eventually lead to more effective gene therapy. Mature sperm for IVF are easily obtained by masturbation. Mature ova are more difficult to obtain. The female is given gonadotropin hormones to stimulate her to superovulate (that is, to produce ten or more mature eggs rather than just one). Ova are later collected by inserting a small suction needle into her pelvic cavity. The ova are inseminated with laboratory-capacitated sperm. Two to four embryos are transferred into the uterus through a catheter. Excess embryos can be saved by a freezing procedure called cryopreservation. These may be thawed for later attempts at implantation should the first attempt fail or a second pregnancy be desired.

Impact and Applications

Technology such as the polymerase chain reaction (PCR) permits assessment of genetic information in the nucleus of a single cell, whether diploid or haploid. IVF gives physicians access to sperm, ova, and very early embryos. One or two cells can be removed from an eight-cell embryo without damaging the ability of the remaining cells to develop normally following embryo transfer. Thus IVF permits genetic diagnosis at the earliest stages of human development and even allows the possibility of gene therapy.

Preimplantation genetic diagnosis (PGD) is used clinically to help people with significant genetic risks to avoid giving birth to an abnormal child that might die in infancy or early childhood. If tests show that the embryo is free of genetic defects, it can be transferred to the uterus for implantation; if found defective it can be destroyed. PGD is successful in avoiding pregnancies with embryos that will develop cystic fibrosis, Huntington's disease, Lesch-Nyhan disease, Tay-Sachs disease, and other genetic abnormalities. Prior to the development of PGD, detection of genetic defects was possible only by prenatal diagnosis during pregnancy. If a defect is detected, termination of the preg-

nancy through elective abortion becomes an option. Not only does abortion represent a higher risk to the mother, it is an unacceptable choice for many people because of ethical and moral concerns.

Access to gametes prior to fertilization and to embryos prior to implantation also opens the possibility of gene therapy. Gene therapy in human embryos presents insurmountable ethical issues, at present, and has been banned pending more study. Genetic modification of the embryos of other species, especially those of commercial interest, carries no such ethical concerns and is routinely practiced.

IVF also opens the possibility of genetic cloning. Cloning is the process of creating multiple individuals with identical genetic characteristics. This can be accomplished by dividing an early embryo, allowing each group of cells to develop into a separate embryo. A few of these embryos can then be implanted, saving the others for future attempts, or all can be implanted, using several different females as surrogate mothers. Through the use of cryopreservation, these pregnancies could occur years apart. It is even possible to remove the nucleus from an isolated cell and replace it with a nucleus taken from an adult. The cell with the transplanted nucleus is able, using special procedures, to develop into an embryo that can be implanted. The offspring will be genetically identical to the adult source of the transplanted nucleus. Most people recognize cloning technology as inappropriate in human medicine, but it has acceptable applications in agriculture and veterinary medicine.

—Armand M. Karow, updated by Bryan Ness

See also: Amniocentesis and Chorionic Villus Sampling; Cloning; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Infertility; Prenatal Diagnosis; Stem Cells; Totipotency; Turner Syndrome.

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Web Sites of Interest

American Society for Reproductive Medicine. <http://www.asrm.org>. Site includes information on infertility and reproduction.

International Council on Infertility Information Dissemination. <http://www.inciid.org>. Site provides "fact sheets" on in vitro fertilization.

Inborn Errors of Metabolism

Field of study: Diseases and syndromes

Significance: *Inborn errors of metabolism are hereditary genetic defects found in varying frequencies in human populations. Diagnosis and cure of these genetic diseases is a continuing focus of medical research.*

Key terms

METABOLIC PATHWAY: enzyme-mediated reactions that are connected in a series

METABOLISM: the collection of biochemical reactions occurring in an organism

Early Observations

In 1902, Sir Archibald Garrod, a British physician, presented a classic paper in which he summarized his observations and analyses of a rather benign condition known as alkaptonuria. The condition is easily diagnosed because the major symptom is dark urine caused by the excretion of homogentisic acid. Since homogentisic acid is not normally found in urine and is a by-product of certain amino acids with particular ring structures, Garrod reasoned that individuals with alkaptonuria had a defect in the utilization of these amino acids. Garrod also noted that the condition is often found in two or more siblings and postulated that the occurrence of this condition may be explained by the mechanism of inheritance.

In 1908, in "Inborn Errors of Metabolism," Garrod extended his observations on alkaptonuria to other diseases such as albinism and cystinuria. In each case, he argued that the abnormal or disease condition was caused by a defect in metabolism that resulted in a block of an important metabolic pathway. He speculated that when such a pathway is blocked, there would be an accumulation of products that are not seen in normal individuals, or important substances would be missing or abnormal. Some of these abnormal metabolic events might be harmless, such as in alkaptonuria, but others could lead to serious disease. He traced the inheritance of these conditions and discovered that they could be passed on from one generation to the next. He was the first to use

the term “inborn errors of metabolism” to describe these conditions. Other investigators have studied more than three thousand additional diseases that can be included in this category. A few of these conditions occur at relatively high frequency in humans. In the U.S. Caucasian population, cystic fibrosis occurs in about 1 in 2,000 births. Some conditions, such as phenylketonuria (PKU), are seen at moderate frequency, about 1 in 10,000. Many of the inborn errors are rare, with frequencies less than 1 in 100,000. A generally accepted definition of an inborn error of metabolism is any condition with actual or potential health consequences that can be inherited in the fashion described by Gregor Mendel in the nineteenth century.

Malfunctioning Proteins and Enzymes

The biochemical causes of the inborn errors of metabolism were discovered many years af-

ter Garrod presented his ideas. In 1952, Von Gierke’s disease was found to be caused by the defective enzyme glucose-6 phosphatase. After this discovery, many inborn errors of metabolism were traced to defects in other enzymes. Enzymes are proteins that catalyze biochemical reactions. They are responsible for increasing the rates of reactions that occur in all cells. These reactions are important steps in metabolic pathways that are responsible for processes such as utilization of nutrients, generation of energy, cell division, and biosynthesis of substances that are needed by organisms. There are many metabolic pathways that can be affected if one of the enzymes in the pathway is missing or malfunctions. In addition to enzymes, defective proteins with other functions may also be considered as candidates for inborn errors of metabolism. For example, there are many types of defective hemoglobin, the protein responsible for oxygen transport.

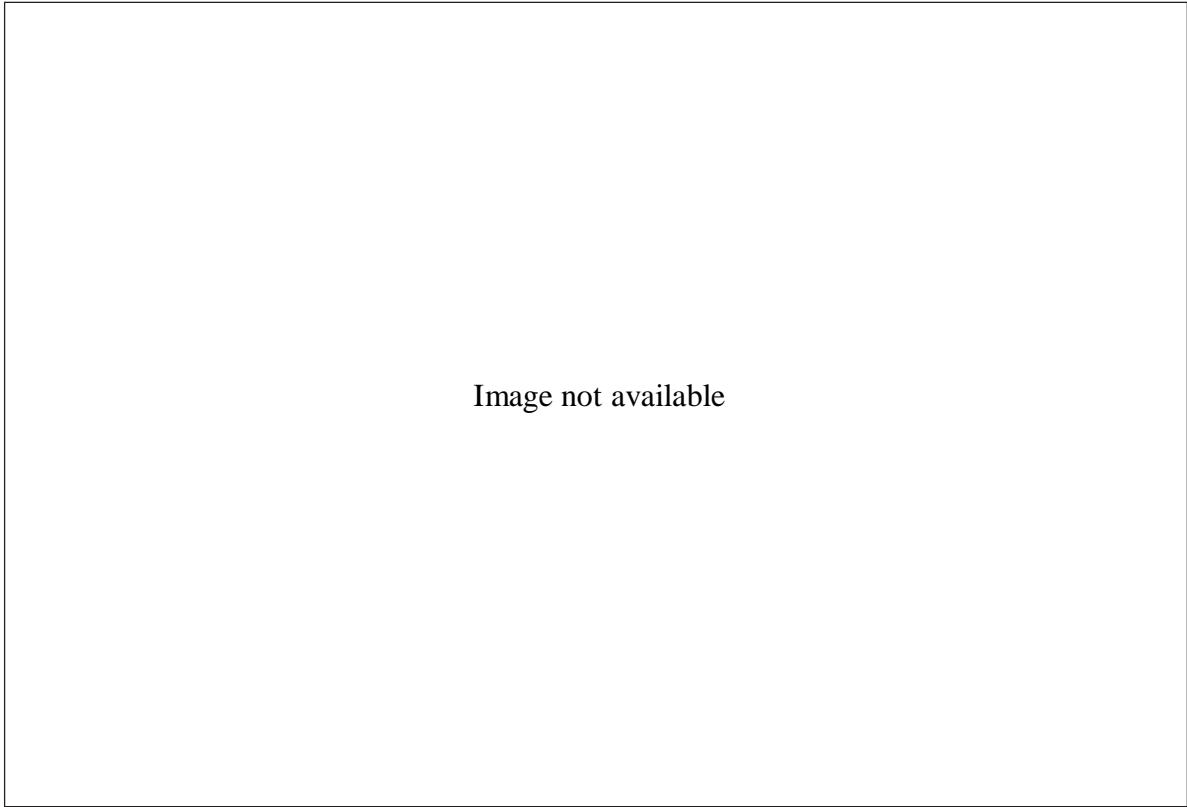


Image not available

Nine-year-old Andy Burgy in 2003. He suffers from an incurable inborn metabolic error known as epidermolysis bullosa, which makes his skin blister at the touch. (AP/Wide World Photos)

These defective hemoglobins are the causes of diseases such as sickle-cell disease and thalassemia.

Genetic Basis of Inborn Errors

The cause of these defects in enzymes and proteins has been traced to mutations in the genes that code for them. Alterations in the structure or nucleotide composition of DNA can have various consequences for the structure of the protein coded for by the DNA. Some of the genetic alterations affecting metabolism simply represent normal variation within the population and are asymptomatic. An example of such a genetic alteration is the ability of some individuals to experience a bitter taste after exposure to chemical derivatives of thiourea. Some asymptomatic variations may lead to complications after environmental conditions are changed. There are a few "inborn errors" that can be induced by certain drugs. Another class of alterations may be minor, with the resulting protein having some degree of function. Individuals with such alterations may live long lives but will occasionally experience a range of problems associated with their conditions. Depending on the exact nature of the mutation, some of the alterations in the resulting protein structure can lead to a completely nonfunctional protein or enzyme. Consequences of this type of mutation can be quite severe and may result in death.

Many of the inborn errors of metabolism are inherited as autosomal recessive traits. Individuals are born with two copies of the gene. If one copy is defective and the second copy is normal, enough functioning protein or enzyme can be made to prevent the individual from exhibiting any symptoms of the disease. Such individuals will be classified as carriers for the defect since they can pass on the defective gene to their offspring. About one in twenty Caucasians in the U.S. is a carrier for the cystic fibrosis gene, and about one in thirty individuals of Eastern Jewish descent carries the gene for the lethal Tay-Sachs disease. When an individual inherits two defective copies of the gene, the manifestations of the disease can be much more severe.

Some inborn errors of metabolism such as

Huntington's disease are manifested as dominant genetic traits. Only one copy of the defective gene is necessary for manifestations of the abnormal condition. There are some inborn errors of metabolism that are sex-linked. Diseases that involve mutations carried on the X chromosome may be severe in males because they have only one X chromosome but less severe or nonexistent in females because females carry two X chromosomes.

Diagnosis and Treatment

Significant progress has been made in the diagnosis of inborn errors of metabolism. Prior to 1980, much of the diagnosis for metabolic defects relied on symptoms detected during clinical examination. Biochemical tests are used to detect various substances that accumulate or are missing when an enzymatic defect is present. The commonly used screening for phenylketonuria (PKU) relies on detection of phenylketones in the blood of newborns. For cases in which the genetic defect is known, DNA can often be used for the purpose of genetic testing. Genetic counselors will help parents determine their chances of having a child with a severe defect when parents are identified as carriers. Small samples of cells can be used as a source of DNA, and such cells may even be obtained from amniotic fluid by amniocentesis. This allows diagnosis to be made prenatally. Some parents choose abortion when their fetus is diagnosed with a lethal or debilitating defect.

Although strides have been made in diagnosis, the problem of treatment still remains. For some inborn errors of metabolism such as phenylketonuria, dietary modification will often prevent the serious symptoms of the disease condition. Individuals with phenylketonuria must limit their intake of the amino acid phenylalanine during the critical stages of brain development, generally the first eight years of life. Treatment of other inborn errors may involve avoidance of certain environmental conditions. For example, individuals suffering from albinism, a lack of pigment production, must avoid the sun. For other inborn errors of metabolism, there are no simple cures on the horizon. Since the early 1990's, some medical pioneers have been involved in clinical trials of

gene therapy, an attempt to replace a defective gene by insertion of a normal, functioning version. Although theoretically promising, gene therapy has not met with significant success. In addition, there are many ethical issues raised when gene therapy trials are proposed before potential hazards have been completely eliminated. Nevertheless, scientists are looking more and more toward genetic cures to genetic problems such as those manifested as inborn errors of metabolism.

—Barbara Brennessel

See also: Amniocentesis and Chorionic Villus Sampling; Biochemical Mutations; Complementation Testing; Cystic Fibrosis; Genetic Screening; Genetic Testing; Genetics, Historical Development of; Hereditary Diseases; Huntington's Disease; Phenylketonuria (PKU); Tay-Sachs Disease.

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Web Sites of Interest

Children Living with Inherited Metabolic Diseases (CLIMB). <http://www.climb.org.uk>. A national British organization supporting families and research on a host of inherited metabolic disorders; includes information and links to sites on specific disorders.

Society for Inherited Metabolic Disorders. <http://www.simd.org>. A nonprofit professional organization promoting worldwide advancement of research and medical treatment of inherited disorders of metabolism. Includes a searchable database of detailed descriptions and diagnoses for specific inborn errors.

Inbreeding and Assortative Mating

Field of study: Population genetics

Significance: Most population genetic models assume that individuals mate at random. One common violation of this assumption is inbreeding, in which individuals are more likely to mate with relatives, resulting in inbreeding depression, a reduction in fitness. Another violation of random mating is assortative mating, or mating based on phenotype. Many traits of organisms, including pollination systems in plants and dispersal in animals, can be understood as mechanisms that reduce the frequency of inbreeding and the cost of inbreeding depression.

Key terms

ALLEL: any of a number of possible genetic variants of a particular gene locus

ASSORTATIVE MATING: mating that occurs when individuals make specific mate choices

based on the phenotype or appearance of others

HETEROZYGOTE: a diploid genotype that consists of two different alleles

HOMOZYGOTE: a diploid genotype that consists of two identical alleles

INBREEDING: mating between genetically related individuals

INBREEDING DEPRESSION: a reduction in the health and vigor of inbred offspring, a common and widespread phenomenon

RANDOM MATING: a mating system in which each male gamete (sperm) is equally likely to combine with any female gamete (egg)

Random Mating and the Hardy-Weinberg Law

Soon after the rediscovery of Gregor Mendel's rules of inheritance in 1900, British mathematician Godfrey Hardy and German physician Wilhelm Weinberg published a simple mathematical treatment of the effect of sexual reproduction on the distribution of genetic variation. Both men published their ideas in 1908 and showed that there was a simple relationship between allele frequencies and genotypic frequencies in populations. An allele is simply a genetic variant of a particular gene; for example, blood type in humans is controlled by a single gene with three alleles (*A*, *B*, and *O*). Every individual inherits one allele for each gene from both their mother and father and has a two-allele genotype. In the simplest case with only two alleles (for example, *A* and *a*), there are three different genotypes (*AA*, *Aa*, *aa*). The Hardy-Weinberg predictions specify the frequencies of genotypes (combinations of two alleles) in the population: how many will have two copies of the same allele (homozygotes such as *AA* and *aa*) or copies of two different alleles (heterozygotes such as *Aa*).

One important assumption that underlies the Hardy-Weinberg predictions is that gametes (sperm and egg cells) unite at random to form individuals or that individuals pair randomly to produce offspring. An example of the first case is marine organisms such as oysters that release sperm and eggs into the water; zygotes (fertilized eggs) are formed when a single sperm finds a single egg. Exactly which sperm cell and

which egg cell combine is expected to be unrelated to the specific allele each gamete is carrying, so the union is said to be random. In cases in which males and females form pairs and produce offspring, it is assumed that individuals find mates without reference to the particular gene under examination. In humans, people do not choose potential mates at random, but they do mate at random with respect to most genetic variation. For instance, since few people know (or care) about the blood type of potential partners, people mate at random with respect to blood-type alleles.

Inbreeding and assortative mating are violations of this basic Hardy-Weinberg assumption. For inbreeding, individuals are more likely to mate with relatives than with a randomly drawn individual (for outbreeding, the reverse is true). Assortative mating occurs when individuals make specific mate choices based on the phenotype or appearance of others. Each has somewhat different genetic consequences. When either occurs, the Hardy-Weinberg predictions are not met, and the relative proportions of homozygotes and heterozygotes are different from what is expected.

The Genetic Effects of Inbreeding

When relatives mate to produce offspring, the offspring may inherit an identical allele from each parent, because related parents share many of the same alleles, inherited from their common ancestors. The closer the genetic relationship, the more alleles two individuals will share. Inbreeding increases the number of homozygotes for a particular gene in a population because the offspring are more likely to inherit identical alleles from both parents. Inbreeding also increases the number of different genes in an individual that are homozygous. In either case, the degree of inbreeding can be measured by the level of homozygosity (the percentage or proportion of homozygotes relative to all individuals).

Inbreeding is exploited by researchers who want genetically uniform (completely homozygous) individuals for experiments: Fruit flies or mice can be made completely homozygous by repeated brother-sister matings. The increase in the frequency of homozygotes can be calcu-

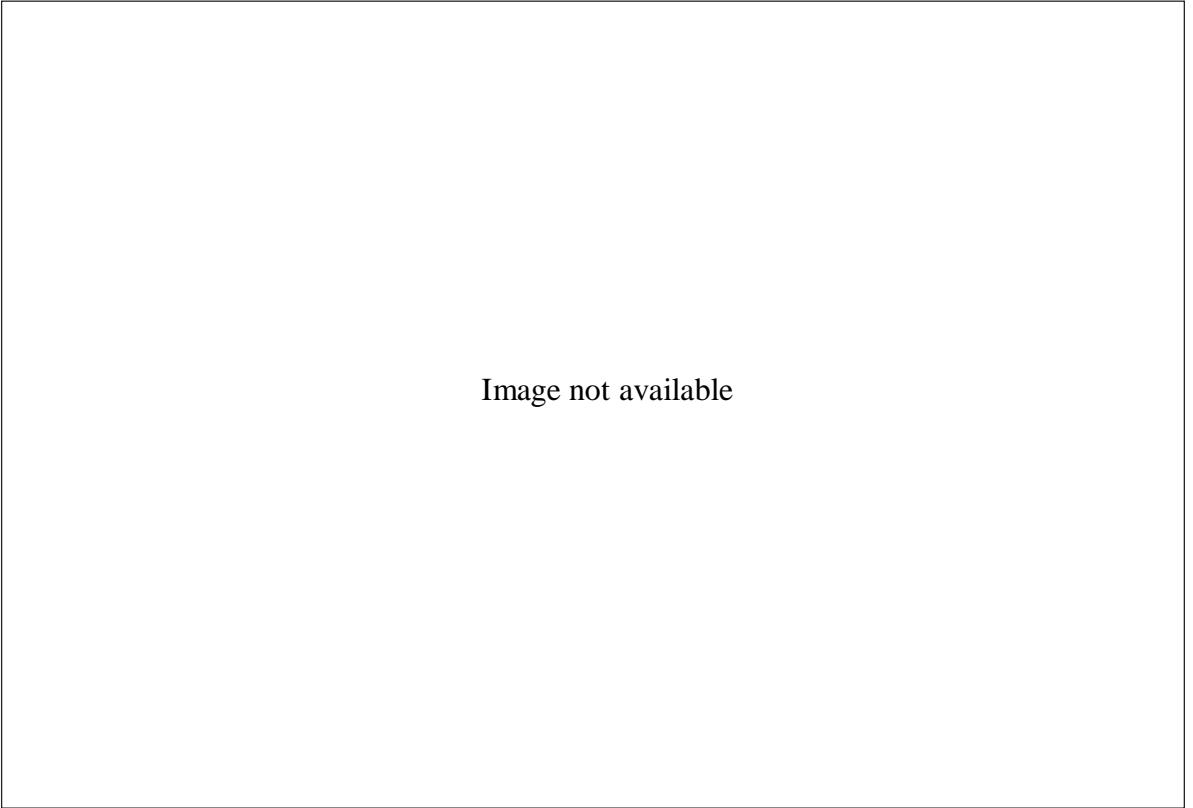


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Two children in the Indian state of Bihar in July, 2000. Many children in the area suffer from deformities. Activists blame uranium mining in the area, whereas government officials blame inbreeding, malnutrition, and unsanitary conditions. (AP/Wide World Photos)

lated for different degrees of inbreeding. Self-fertilization is the most extreme case of inbreeding, followed by sibling mating, and so forth. Sewall Wright pioneered computational methods to estimate the degree of inbreeding in many different circumstances. For self-fertilization, the degree of homozygosity increases by 50 percent each generation. For repeated generation of brother-sister matings, the homozygosity increases by about 20 percent each generation.

Inbreeding Depression

Inbreeding commonly produces inbreeding depression. This is characterized by poor health, lower growth rates, reduced fertility, and increased incidence of genetic diseases. Although there are several theoretical reasons why inbreeding depression might occur, the major effects are produced by uncommon and

deleterious recessive alleles. These alleles produce negative consequences for the individual when homozygous, but when they occur in a heterozygote, their negative effects are masked by the presence of the other allele. Because inbreeding increases the relative proportion of homozygotes in the population, many of these alleles are expressed, yielding reduced health and vigor. In some cases, the effects can be quite severe. For example, when researchers wish to create homozygous lines of the fruit fly *Drosophila melanogaster* by repeated brother-sister matings, 90 percent or more of the lines fail because of widespread genetic problems.

Assortative Mating

In assortative mating, the probability of particular pairings is affected by the phenotype of the individuals. In positive assortative matings, individuals are more likely to mate with others

of the same phenotype, while in negative assortative mating, individuals are more likely to mate with others that are dissimilar. In both cases, the primary effect is to alter the expected genotypic frequencies in the population from those expected under the Hardy-Weinberg law. Positive assortative mating has much the same effect as inbreeding and increases the relative frequency of homozygotes. Negative assortative mating, as expected, has the opposite effect and increases the relative proportion of heterozygotes. Positive assortative mating has been demonstrated for a variety of traits in humans, including height and hair color.

Impact and Applications

The widespread, detrimental consequences of inbreeding are believed to shape many aspects of the natural history of organisms. Many plant species have mechanisms developed through natural selection to increase outbreeding and avoid inbreeding. The pollen (male gamete) may be released before the ovules (female gametes) are receptive, or there may be a genetically determined self-incompatibility to prevent self-fertilization. In most animals, self-fertilization is not possible, and there are often behavioral traits that further reduce the probability of inbreeding. In birds, males often breed near where they were born, while females disperse to new areas. In mammals, the reverse is generally true, and males disperse more widely. Humans appear to be an exception among the mammals, with a majority of cultures showing greater movement by females. These sex-biased dispersal patterns are best understood as mechanisms to prevent inbreeding.

In humans, individuals are unlikely to marry others with whom they were raised. This prevents the potentially detrimental consequences of inbreeding in matings with close relatives. This has also been demonstrated in some birds. Domestic animals and plants may become inbred if careful breeding programs are not followed. Many breeds of dogs exhibit a variety of genetic-based problems (for example, hip problems, skull and jaw deformities, and nervous temperament) that are likely caused by inbreeding. Conservation biologists who manage endangered or threatened populations

must often consider inbreeding depression. In very small populations such as species maintained in captivity (zoos) or in isolated natural populations, inbreeding may be hard to avoid. Inbreeding has been blamed for a variety of health defects in cheetahs and Florida panthers.

—Paul R. Cabe

See also: Consanguinity and Genetic Disease; Genetic Load; Hardy-Weinberg Law; Heredity and Environment; Hybridization and Introgression; Lateral Gene Transfer; Mendelian Genetics; Natural Selection; Polyploidy; Population Genetics; Punctuated Equilibrium; Quantitative Inheritance; Sociobiology; Speciation.

Further Reading

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- Hedrick, Philip. *Genetics of Populations*. 2d ed. Boston: Jones and Bartlett, 2000. For those with quantitative experience in the field, this text integrates empirical and experimental approaches with theory, describing methods for estimating population genetics parameters as well as other statistical tools used for population genetics.
- Krebs, J., and N. Davies. *An Introduction to Behavioral Ecology*. Malden, Mass.: Blackwell, 1991. Discusses inbreeding avoidance and kin recognition.
- Laikre, Linda. *Genetic Processes in Small Populations: Conservation and Management Considerations with Particular Focus on Inbreeding and Its Effects*. Stockholm: Division of Population Genetics, Stockholm University, 1996. Aimed at conservation biologists and addresses the management of inbreeding in small populations. Illustrated.

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Incomplete Dominance

Field of study: Classical transmission genetics

Significance: *In most allele pairs, one allele is dominant and the other recessive; however, other relationships can occur. In incomplete dominance, one allele can only partly dominate or mask the other. Some very important human genes, such as the genes for pigmentation and height, show incomplete dominance of alleles.*

Key terms

ALLEL: one of the alternative forms of a gene

CODOMINANCE: the simultaneous expression of two different (heterozygous) alleles for a trait

COMPLETE DOMINANCE: expression of an allele for a trait in an individual that is heterozygous for that trait, determining the phenotype of the individual

HETEROZYGOUS: having two different alleles at a gene locus, often symbolized Aa or a^+a

HOMOZYGOUS: having two of the same alleles at a gene locus, often symbolized AA , aa , or a^+a^+

PHENOTYPE: the expression of a genotype, as observed in the outward appearance or biochemical characteristics of an organism

RECESSIVE TRAIT: a genetically determined trait that is expressed only if an organism receives the gene for the trait from both parents

Incomplete vs. Complete Dominance

Diploid organisms have two copies of each gene locus and thus two alleles at each locus. Each locus can have either a homozygous genotype (two of the same alleles, such as AA , aa , or a^+a^+) or a heterozygous genotype (two different alleles, such as Aa or a^+a). The phenotype of an organism that is homozygous for a particular gene is usually easy to predict. If a pea plant has two tall alleles of the height locus, the plant is tall; if a plant has two dwarf alleles of the height locus, it is small. The phenotype of a heterozygous individual may be harder to predict. In most circumstances, one of the alleles (the dominant) is able to mask or cover the other (the recessive). The phenotype is determined by the dominant allele, so a heterozygous pea plant, with one tall and one dwarf allele, will be tall. When Gregor Mendel delivered the results of his pea-plant experiments before the Natural Sciences Society in 1865 and published them in 1866, he reported one dominant and one recessive allele for each gene he had studied. Later researchers, starting with Carl Correns in the early 1900's, discovered alleles that did not follow this pattern.

When a red snapdragon or four-o'clock plant is crossed with a white snapdragon or four-o'clock, the offspring are neither red nor white. Instead, the progeny of this cross are pink. Similarly, when a chinchilla (gray) rabbit is crossed with an albino rabbit, the progeny are neither chinchilla nor albino but an intermediate shade called light chinchilla. This phenomenon is known as incomplete dominance, partial dominance, or semidominance.

If the flower-color locus of peas is compared with the flower-color locus of snapdragons, the differences and similarities can be seen. The two alleles in peas can be designated W for the purple allele and w for the white allele. Peas that are WW are purple, and peas that are ww are white. Heterozygous peas are Ww and appear purple. In other words, as long as one dominant allele is present, enough purple pigment is made to make the plant's flower color phenotype purple. In snapdragons, R is the red allele and r is the white allele. Homozygous RR plants have red flowers and rr plants have white flowers. The heterozygous Rr plants have the

same kind of red pigment as the *RR* plants but not enough to make the color red. Instead, the less pigmented red flower is designated as pink. Because neither allele shows complete dominance, other symbols are sometimes used. The red allele might be called c^R or C_1 , while the white allele might be called c^W or C_2 .

The Enzymatic Mechanism of Incomplete Dominance

To understand why incomplete dominance occurs, metabolic pathways and the role of enzymes must be understood. Enzymes are proteins that are able to increase the rate of chemical reactions in cells without the enzymes themselves being altered. Thus an enzyme can be used over and over again to speed up a particular reaction. Each different chemical reaction in a cell needs its own enzyme. Each enzyme is composed of one or more polypeptides, each of which is coded by a gene. Looking again at flower color in peas, the *W* allele codes for an enzyme in the biochemical pathway for production of purple pigment. Whenever a *W* allele is present, this enzyme is also present. The *w* allele has been changed (mutated) in some way so that it no longer codes for a functional enzyme. Thus *ww* plants have no functional enzyme and cannot produce any purple pigment. Since many biochemicals such as fibrous polysaccharides and proteins found in plants are opaque white, the color of a *ww* flower is white by default. In a *Ww* plant, there is only one copy of the allele for a functional enzyme. Since enzymes can be used over and over again, one copy of the functional allele produces sufficient enzyme to make enough pigment for the flower to appear purple. In snapdragons the *R* allele, like the *W* allele, codes for a functional enzyme, while the *r* allele does not. The difference is in the enzyme coded by the *R* allele. The snapdragon enzyme is not very efficient, which leads to a deficiency in the amount of red pigment. Flowers with the reduced amount of red pigment appear pink.

Phenotypic Ratios

Phenotypic ratios in the progeny from controlled crosses are also different than for simple Mendelian traits. For Mendelian traits,

crossing two heterozygous individuals will produce the following results: $Ww \times Ww \rightarrow \frac{1}{4}WW + \frac{1}{2}Ww + \frac{1}{4}ww$. Since both *WW* and *Ww* look the same, the $\frac{1}{4}WW$ and the $\frac{1}{2}Ww$ can be added together to give $\frac{3}{4}$ purple. In other words, when two heterozygotes are crossed, the most common result is to have $\frac{3}{4}$ of the progeny look like the dominant and $\frac{1}{4}$ look like the recessive—the standard 3:1 ratio. With incomplete dominance, each genotype has its own phenotype, so when two heterozygotes are crossed (for example, *Rr* \times *Rr*), $\frac{1}{4}$ of the progeny will be *RR* and look like the dominant (in this case red), $\frac{1}{4}$ will be *rr* and look like the recessive (in this case white), but $\frac{1}{2}$ will be *Rr* and have an intermediate appearance (in this case pink)—a 1:2:1 ratio.

Codominance

One type of inheritance that can be confused with incomplete dominance is codominance. In codominance, both alleles in a heterozygote are expressed simultaneously. Good examples are the *A* and *B* alleles of the human ABO blood system. ABO refers to chemicals, in this case short chains of sugars called antigens, that can be found on the surfaces of cells. Blood classified as A has *A* antigens on the surface, B blood has *B* antigens, and AB blood has both *A* and *B* antigens. (O blood has neither *A* nor *B* antigens on the surface.)

Genetically, individuals that are homozygous for the *A* allele, $I^A I^A$, have *A* antigens on their cells and are classified as type A. Those homozygous for the *B* allele, $I^B I^B$, have *B* antigens and are classified as type B. Heterozygotes for these alleles, $I^A I^B$, have both *A* and *B* antigens and are classified as type AB. This is called codominance because both alleles are able to produce enzymes that function. When both enzymes are present, as in the heterozygous $I^A I^B$ individual, both antigens will be formed. The progeny ratios are the same for codominance and incomplete dominance, because each genotype has its own phenotype.

Whether an allele is called completely dominant, incompletely dominant, or codominant often depends on how the observer looks at the phenotype. Consider two alleles of the hemoglobin gene: H^A (which codes for normal hemoglobin) and H^S (which codes for sickle-cell

hemoglobin). To the casual observer, both $H^A H^A$ homozygotes and $H^A H^S$ heterozygotes have normal-appearing blood. Only the $H^S H^S$ homozygote shows the sickling of blood cells that is characteristic of the disease. Thus H^A is dominant to H^S . Another observer, however, may note that under conditions of oxygen deprivation, the blood of heterozygotes does sickle. This looks like incomplete dominance. The phenotype is intermediate between never sickling, as seen in the normal homozygote, and frequently sickling, as seen in the $H^S H^S$ homozygote. A third way of observing, however, would be to look at the hemoglobin itself. In normal homozygotes, all hemoglobin is normal. In $H^S H^S$ homozygotes, all hemoglobin is abnormal. In the heterozygote, both normal and abnormal hemoglobin is present; thus, the alleles are codominant.

Incomplete Dominance and Polygenes

In humans and many other organisms, single characteristics are often under the genetic control of several genes. Many times these genes function in an additive manner so that a characteristic such as height is not determined by a single height gene with just two possible alternatives, as in tall and dwarf peas. There can be any number of these genes that determine the expression of a single characteristic, and very often the alleles of these genes show incomplete dominance.

Suppose one gene with an incompletely dominant allele determined height. Three genotypes of height could exist: HH , which codes for the maximum height possible (100 percent above the minimum height), Hh , which codes for 50 percent above the minimum height, and hh , which codes for the minimum height. If two height genes existed, there would be five possible heights: $AABB$ (maximum height); $AaBB$ or $AABb$ (75 percent above minimum); $AAbb$, $AaBb$, or $aaBB$ (50 percent above minimum); $Aabb$ or $aaBb$ (25 percent above minimum); and $aabb$ (minimum). If there were five genes involved in height, there would be $aabbccdee$ individuals with minimum height; $Aabbccdee$, $aaBbccdee$, and other individuals having genotypes with only one of the incompletely dominant alleles at 10 percent above the minimum;

$AAbbccdee$, $aaBbccDdee$, and other individuals with two incompletely dominant alleles at 20 percent above the minimum; all the way up to $AABBCCDDEE$ individuals that show the maximum (100 percent above the minimum) height. The greater the number of genes with incompletely dominant alleles that affect a phenotype, the more the distribution of phenotypes begins to look like a continuous distribution. Human skin, hair, and eye pigmentation phenotypes are also determined by the additive effects of several genes with incompletely dominant alleles.

Incomplete Dominance and Sex Linkage

In many organisms, sex is determined by the presence of a particular combination of sex chromosomes. Human females, for example, have two of the same kind of sex chromosomes, called X chromosomes, so that all normal human females have the XX genotype. Human males have two different sex chromosomes; thus, all normal human males have the XY genotype. The same situation is also seen in the fruit fly *Drosophila melanogaster*. When genes with incompletely dominant alleles are located on the X chromosome, only the female with her two X chromosomes can show incomplete dominance. The apricot (w^a) and white (w) alleles of the eye color gene in *D. melanogaster* are on the X chromosome, and w^a is incompletely dominant to w . Male flies can have either of two genotypes, $w^a Y$ or $w Y$, and appear apricot or white, respectively. Females have three possible genotypes: $w^a w^a$, $w^a w$, and $w w$. The first is apricot and the third is white, but the second genotype, $w^a w$, is an intermediate shade often called light apricot.

In birds and other organisms in which the male has two of the same kind of sex chromosomes and the female has the two different sex chromosomes, only the male can show incomplete dominance. A type of codominance can also be seen in genes that are sex linked. In domestic cats, an orange gene exists on the X chromosome. The alleles are orange (X^O) and not orange (X^+). Male cats can be either black (or any color other than orange, depending on other genes that influence coat color) when they are X^+Y , or they can be orange (or light or

ange) when they are X^OY . Females show those same colors when they are homozygous (X^+X^+ or X^OX^O) but show a tortoiseshell (or calico) pattern of both orange and not-orange hairs when they are X^+X^O .

—Richard W. Cheney, Jr., updated by Bryan Ness

See also: Biochemical Mutations; Complete Dominance; Dihybrid Inheritance; Epistasis; Mendelian Genetics; Monohybrid Inheritance; Multiple Alleles.

Further Reading

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Infertility

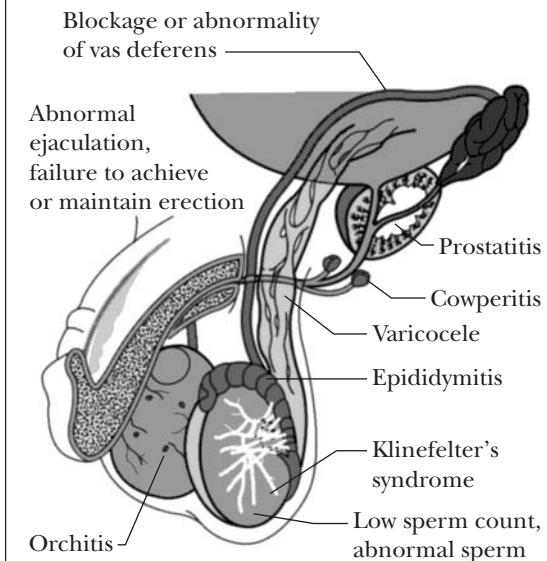
Field of study: Diseases and syndromes

Significance: *Infertility is a disease of the reproductive system that impairs the conception of children. About one in six couples in the United States is infertile. The risk that a couple's infertility may be caused by genetic problems such as abnormal sex chromosomes is approximately one in ten.*

Key terms

IN VITRO FERTILIZATION (IVF): a process in which harvested eggs and sperm are brought together artificially to form a zygote

Common Causes of Male Infertility



(Hans & Cassidy, Inc.)

SEX CHROMOSOMES: the chromosomes that determine the sex of an individual; females have two X chromosomes, while males have one X and one Y chromosome

A Reproductive Disease

Infertility is a disease of the reproductive system that impairs a couple's ability to have children. Sometimes infertility has a genetic cause. The conception of children is a complex process that depends upon many factors, including the production of healthy sperm by the man and healthy eggs by the woman, unblocked Fallopian tubes that allow the sperm to reach the egg, the sperm's ability to fertilize the egg when they meet, the ability of the fertilized egg (embryo) to become implanted in the woman's uterus, and sufficient embryo quality. If the pregnancy is to continue to full term, the embryo must be healthy, and the woman's hormonal environment must be adequate for its development. Infertility can result when one of these factors is impaired. Physicians define infertility as the inability to conceive a child after one year of trying.

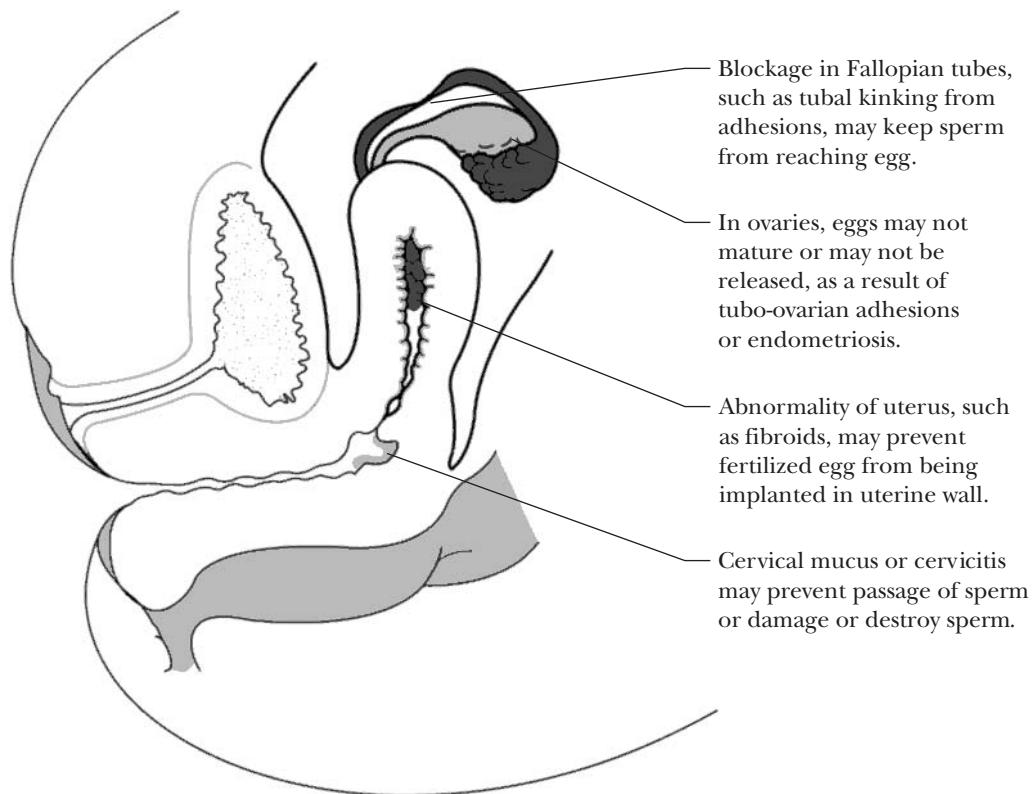
Genetic Causes of Infertility

The most common male infertility factors include conditions in which few or no sperm cells are produced. Sometimes sperm cells are malformed or die before they can reach the egg. A genetic disease such as a sex chromosome abnormality can also cause infertility in men. A genetic disorder may be caused by an incorrect number of chromosomes (having more or fewer than the normal forty-six chromosomes). Having a wrong arrangement of the chromosomes may also cause infertility. This situation occurs when part of the genetic material is lost or damaged. One such genetic disease is Klinefelter syndrome, which is caused by an extra X chromosome in males. The loss of a tiny piece of the male sex chromosome (the Y chromosome) may cause the most severe form of male

infertility: the complete inability to produce sperm. This form of infertility can arise from a deletion in one or more genes in the Y chromosome. Fertility problems can pass from father to son, especially in cases in which physicians use a single sperm from an infertile man to inseminate a woman's egg.

Female infertility may be caused by an irregular menstrual cycle, blocked Fallopian tubes, or birth defects in the reproductive system. One genetic cause of infertility in females is Turner syndrome. Most females with Turner syndrome lack all or part of one of their X chromosomes. The disorder may result from an error that occurs during division of the parent's sex cells. Infertility and short stature are associated with Turner syndrome. Other genetic disorders in females include trisomy X, tetrasomy

Common Causes of Female Infertility



X, and pentasomy. These syndromes are the female counterparts of Klinefelter syndrome and can be associated with mental retardation.

At least 60 percent of miscarriages or pregnancy losses are caused by chromosomal abnormalities. Most babies with these abnormalities would not survive even if they were born. Chromosomal problems are more common if the mother is older and has a history of requiring longer than a year to conceive. Men who are older or who have a history of being subfertile can also contribute to genetic abnormalities. After the age of thirty-five, the structure within a woman's eggs is more likely to become damaged. Men over the age of forty-five have an increased risk of damage to the structure of the chromosomes in their sperm.

Scientists believe that as their understanding of the genetic basis of infertility problems increases, new therapies will be developed to treat them. Most infertility cases are treated with drugs or surgery to repair the reproductive organs. No treatment is available to correct sex chromosomal abnormalities such as Turner syndrome. However, some women with Turner syndrome can have children. For women who cannot conceive, possible procedures include in vitro fertilization (fertilizing a woman's egg with sperm outside the body) and embryo transfer (moving the fertilized egg into a woman's uterus). Adoption is another option for infertile men and women.

—Fred Buchstein

See also: Amniocentesis and Chorionic Villus Sampling; Cloning; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; In Vitro Fertilization and Embryo Transfer; Prenatal Diagnosis; Stem Cells; Sterilization Laws; Totipotency; Turner Syndrome; X Chromosome Inactivation; XYY Syndrome.

Further Reading

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tersection of biology, the environment, and culture.

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Web Sites of Interest

American Association of Reproductive Medicine. <http://www.asrm.org>. Site includes information on infertility and reproduction.

International Council on Infertility Information Dissemination. <http://www.inciid.org>. Site provides "fact sheets" on in vitro fertilization.

National Institutes of Health, National Library of Medicine. <http://www.nlm.nih.gov/medlineplus/infertility.html>. Provides information on all aspects of infertility.

Insurance

Field of study: Bioethics; Human genetics and social issues

Significance: *Many social policy analysts and public health advocates worry that as genetic screening becomes more widely available, individuals considered high-risk may be denied health and life insurance coverage. On the other hand, if such information is withheld from insurance companies, individuals might purchase extra life insurance, causing insurance companies to unknowingly carry unacceptably high risks. Some kind of balance between appropriate disclosure and privacy rights will need to be established.*

Key terms

ALZHEIMER'S DISEASE: a degenerative brain disorder usually found among the elderly; sufferers gradually lose cognitive function and become unable to function independently

CHRONIC ILLNESS: an ongoing condition such as diabetes or hypertension

HIGH RISK: characterized by being likely to someday suffer from a particular disease or disabling condition

PREEEXISTING CONDITION: a disease or disorder that is diagnosed prior to a person's application for insurance coverage

High-Risk Individuals and Preexisting Conditions

As tests for a steadily increasing number of genetic defects are perfected, concern has grown among both health experts and the general public that negative results could lead to the denial of health insurance coverage to these high-risk individuals. The insurance industry has always been reluctant to insure people identified as being at high risk or who suffer from preexisting conditions, a reluctance that has intensified as health care costs have increased. For example, people with a family medical history of coronary artery disease have long been considered a higher risk than members of the general population. As a consequence, based on information provided through disclosures of family histories, these people occasionally have been denied health

insurance coverage or have been required to pay higher premiums.

Similarly, people who suffer from conditions such as diabetes or hypertension and who change jobs or insurance carriers occasionally discover that their new medical insurance will not pay for any treatment for medical conditions that had been diagnosed prior to obtaining the new insurance. Such "preexisting" conditions are considered ineligible for payment of benefits. While some insurance companies will put a time limit on the restrictions for coverage of preexisting conditions of a few months or a year, providing there are no active occurrences of the disorder, other insurers may exclude making any payments related to a preexisting condition for an indefinite period of time. A person with a chronic condition such as diabetes may discover that while a new insurer will pay for conditions unrelated to the diabetes, such as a broken leg, the individual will be solely responsible for any diabetes-related expenses for the remainder of his or her life. Alternately, the sufferer of a chronic condition may discover that health insurance is available, but only at a much higher premium.

Insurance and Genetic Screening

Insurance companies are just beginning to confront the problems of genetic tests for genetic predisposition to disease. In one court case, *Katskee v. Blue Cross Blue Shield of Nebraska* (1994), the plaintiff had been diagnosed with a 50 percent chance of developing breast and/or ovarian cancer. Consequently, she was seeking payment from her insurance company to cover the costs of prophylactic removal of her ovaries. Initially, the insurance company approved the surgery, but later it reversed that decision, saying that the plaintiff was not covered because her condition was not a "disease" or "bodily disorder." The suit occurred because the plaintiff proceeded with the surgery anyway and then looked to the courts to help her collect from her insurer. The first ruling was in favor of the insurance company but was reversed on appeal, the higher court considering a 50 percent predisposition as meeting the definition of a disease.

Two responses from the insurance industry

are possible as cases like these become more common. They could choose to cover prophylactic treatments as a way to cut long-term costs associated with development of the genetic disease, or they could choose to exclude such conditions under a "preexisting condition" argument. So far the trend has been toward trying to exclude treatment, by the latter approach, including considering the later development of the full-blown disease as a preexisting condition. This is a disturbing trend, as it would tend to discourage genetic screening, ruining the opportunity for preventive measures. If the courts decide to require that insurers must fund prophylactic or preventive treatments, then another quandary occurs: At what percentage predisposition will insurers be required to cover the costs? A predisposition of 50 percent seems like a reasonable number, but what about 45 percent, also high? Covering any level of predisposition would be unreasonable, as it would bankrupt the system, so a line must be drawn, but where? Much more information will be needed before such lines can be drawn without being arbitrary.

In the case of degenerative disorders such as Alzheimer's disease or Huntington's disease, for which there is no prophylactic treatment available, patients may live for many years following the initial diagnosis of the disease while they become progressively more helpless and eventually require extended hospitalization or custodial care. An insurance company that wrote plans to cover nursing-home care could decide to exclude people identified as carrying a gene putting them at risk of developing Alzheimer's disease. The insurance company's reasoning would be that because Alzheimer's sufferers may require many more years of custodial care than the average nursing-home resident, it would be unprofitable to insure known future Alzheimer's sufferers. Such people would be seen as simply being too high-risk.

A number of geneticists and other analysts have suggested that another inherent difficulty with genetic screening is that it opens the door for possible restriction of access to health insurance while not holding out any hope of a treatment or cure for the patient. It is now possible to detect the genetic markers for many condi-

tions for which no effective preventive treatment exists. Alzheimer's disease provides a particularly poignant example. As of the late 1990's, the connection between genes identified as appearing in some early-onset Alzheimer's disease patients and the disease itself was still unclear. People who underwent genetic screening to discover if they carried that particular genetic marker could spend many decades worrying needlessly about their own risk of developing Alzheimer's disease while knowing that there was no way to prevent it. At the same time, the identification of the genetic marker would have identified the patient as a high risk for medical insurance. Huntington's disease represents an even more serious case, in which the test is nearly 100 percent predictive. A positive test is essentially a guaranteed prediction of early death. Tests like these may provide no benefit to affected individuals, and may even cause harm if the information is freely available to insurers.

On the other hand, in some cases the benefits of genetic screening may outweigh its potential costs. For example, certain cancers have long been recognized as running in some families. Doctors routinely counsel women with a family history of breast cancer to have annual mammograms and even, in cases where the risk seems particularly high, to undergo prophylactic mastectomy or lumpectomy. The discovery of genetic markers for breast cancer suggests that women who are concerned that they are at higher-than-average risk for the disease can allay their fears through genetic screening rather than subjecting themselves to disfiguring surgery. Still, the very act of screening could become a double-edged sword. A positive test not only would confirm a woman's worst fears but also could result in her being denied high insurance coverage. Many patients with a high-risk family profile fear that even if the screening turns out negative, simply requesting the test will serve as a flag to health insurers, and they, too, will be assessed higher premiums or denied coverage based on their family histories.

In a climate of rising medical costs and efforts by both traditional insurance providers and health maintenance organizations to reduce expenses, many people feel there is good

reason to fear that genetic screening will serve primarily as a tool to restrict access to health insurance. In response to these concerns, a number of government studies have been undertaken to assess potential remedies. Some possible solutions include making the results of all genetic tests confidential, available to insurers and employers only with permission from the individual; passing laws that prohibit discrimination of individuals with genetic predispositions by insurers and employers; and universal health coverage with clearly defined guidelines based on extensive research. A number of states have already enacted laws prohibiting insurance companies from denying coverage to individuals with genetic defects. It is also possible that the Americans with Disabilities Act could be cited against genetic discrimination.

Impact of Medical Genomics

With the mapping of the human genome completed in 2003, it suddenly became clear that nearly all human disease—from complex chronic conditions such as cancer, Alzheimer's, and diabetes to the predisposition for infectious disease and even trauma—has some genetic basis. Although genome sequences are essentially the same among all individuals, what variation there is accounts for many of the differences in disease susceptibility and other health-related differences. All of this has made the drive to study human genomics as it affects human health a burgeoning new field, medical genomics, that promises to affect every medical field. The basis for this discipline will be data gleaned from large, well-designed and controlled clinical studies that are being developed and implemented in several nations to provide information on how genes influence a wide range of traits, from disease states to behavior.

Given the dangers outlined above, it would seem that such studies pose an increased concern regarding issues of privacy, discrepancies in access to health care, and even threats to individuals' jobs, as more and more employers have been forced to "self-insure"—essentially becoming insurers themselves and thus being forced to consider employees' health in deci-

sions of hiring and continued employment. However, in an op-ed piece for *The Wall Street Journal* (December 20, 2002), William R. Brody, president of The Johns Hopkins University, sounded an ominous yet potentially positive note. In view of the inevitable discovery that nearly all disease conditions have some genetic basis, he predicted that, given the difficulty private insurers will soon face in discriminating among conditions, they might also be facing their own demise as medical insurers:

If legislatures pass laws banning insurers from using genetic screening data, those companies will protect themselves by continually raising premiums to consumers. Some may even go bankrupt because purchasers of insurance will be the more knowledgeable in the transaction. Yet if we allow insurers to use genetic data, many more individuals will be left without coverage because they will be deemed too high-risk to warrant insurance at affordable prices. Given this conundrum, there is only one solution that can preserve the concept of health insurance: universal coverage.

Based on the concept of "community rating," such coverage would spread risk across a large group of individuals (now confined to smaller groups), and hence cost would spread across a national pool, allowing individual traits and hence risks to be diluted. Brody predicts that "that day is coming sooner than many people imagine."

—Nancy Farm Männikkö and Bryan Ness

See also: Aging; Alzheimer's Disease; Bioethics; Bioinformatics; Breast Cancer; Congenital Defects; Eugenics; Eugenics: Nazi Germany; Forensic Genetics; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Genomic Libraries; Genomics; Hereditary Diseases; Human Genetics; Icelandic Genetic Database; Prenatal Diagnosis; Race; Sickle-Cell Disease; Sterilization Laws.

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Intelligence

Field of study: Human genetics and social issues

Significance: *The study of the genetic basis of intelligence is one of the most controversial areas in human genetics. Researchers generally agree that mental abilities are genetically transmitted to some extent, but there is disagreement over the relative roles of genes and environment in the development of mental abilities. There is also disagreement over whether different mental abilities are products of a single ability known as intelligence and disagreement over how to measure intelligence.*

Key terms

DIZYGOTIC ORGANISM: an organism developed from two separate ova; fraternal twins are dizygotic

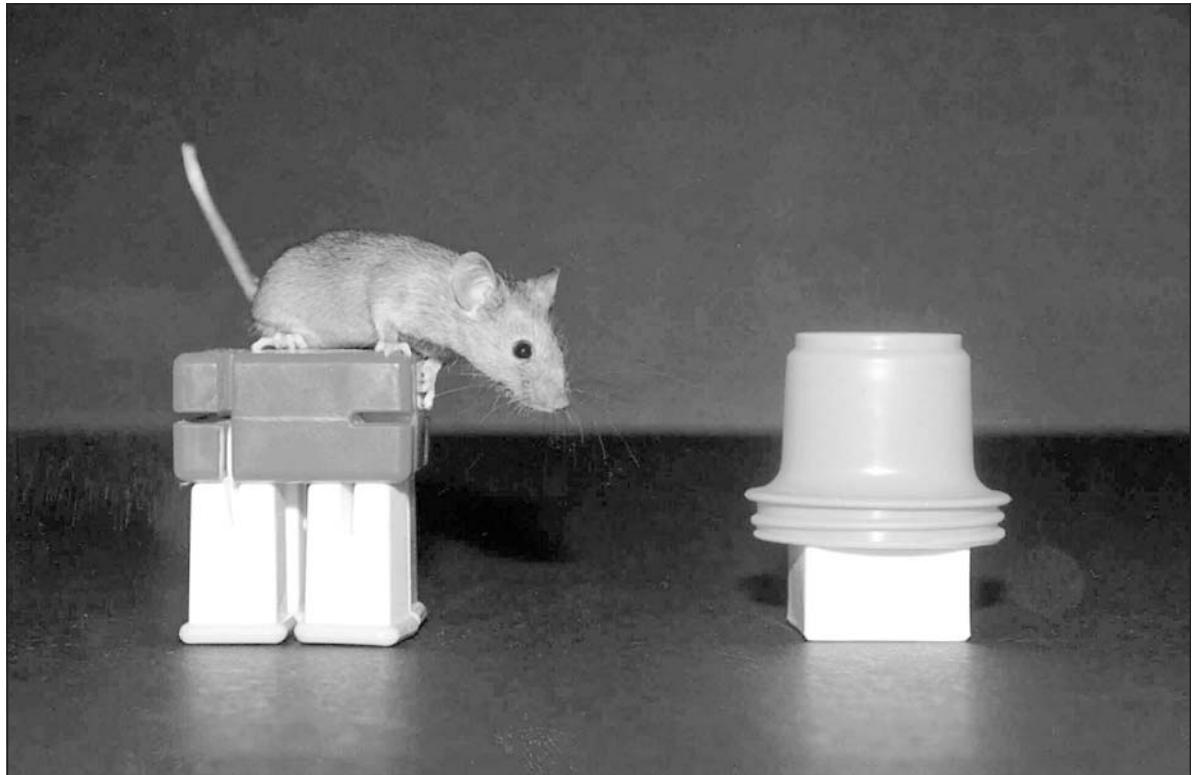
INTELLIGENCE QUOTIENT (IQ): the most common measure of intelligence; it is based on the view that there is a single capacity for complex mental work and that this capacity can be measured by testing

MONOZYGOTIC ORGANISM: developed from a single ovum (egg); identical twins are monozygotic because they originate in the womb from a single fertilized ovum that splits in two

PSYCHOMETRICIAN: one who measures intellectual abilities or other psychological traits

Evidence for Genetic Links to Intelligence

Much of the research into the connection between genes and intelligence has focused on attempting to determine the relative roles of biological inheritance and social influence in de-



A genetically engineered smart mouse performs a learning and memory test. Researchers hope to find causes and cures for Alzheimer's disease, and possibly ways to increase human intelligence. (AP/Wide World Photos)

veloping intelligence. Such attempts have usually involved a combination of four methods: associations of parental intelligence with the intelligence of offspring, associations of the intelligence of siblings (brothers and sisters), comparisons of dizygotic (fraternal) twins and monozygotic (identical) twins, and adoption studies.

To the extent that mental qualities are inherited, one should expect blood relatives to share these qualities with each other more than with nonrelatives. In an article published in 1981 in the journal *Science*, T. J. Bouchard, Jr., and Matt McGue examined studies that looked at statistical relationships of intellectual abilities among family members. These studies did reveal strong associations between mental capacities of parents and children and strong associations among the mental capacities of siblings. Further, if genes are involved in establishing mental abilities, one should expect that the more genes related people share, the more similar

they will be in intelligence. Studies have indicated that fraternal twins are only slightly more similar to each other than are nontwin siblings. Identical twins, developing from a single egg with identical genetic material, have even more in common. Bouchard and McGue found that there was an overlap of about 74 percent in the intellectual abilities of identical twins and an overlap of about 36 percent in the intellectual abilities of fraternal twins.

Family members may be similar because they live in similar circumstances, and identical twins may be similar because they receive nearly identical treatment. However, studies of adopted children show that the intellectual abilities of these children were more closely related to those of their biological parents than to those of their adoptive parents. Studies of identical twins who were adopted and raised apart from each other indicate that these twins have about 62 percent of their intellectual abilities in common.

Twin studies, in particular, have helped to establish that heredity is involved in a number of intellectual traits. Memory, number ability, perceptual skills, psychomotor skills, fluency in language use, and proficiency in spelling are only a few of the traits in which people from common genetic backgrounds tend to be similar to each other. However, psychometricians have not reached agreement on the extent to which mental abilities are products of genes rather than of environmental factors such as upbringing and opportunity. Some researchers estimate that only 40 percent of intellectual ability is genetic; others set the estimate as high as 80 percent.

It is important to keep in mind that even if most differences in mental abilities among human beings were caused by genetics, members of families would still show varied abilities. If, for example, there is a gene for high mathematical ability (gene *A*) and a gene for low mathematical ability (gene *a*), it is quite possible that a woman who has inherited each gene (*Aa*) from her parents will marry a man who has inherited each gene (*Aa*) from his parents. In this case, there is a 1 in 4 probability that they will have a child who is mathematically gifted (*AA*) and a 1 in 4 probability that they will have a child who is mathematically slow (*aa*). This example, although grossly simplified, gives an idea of the effect of variation in the genes inherited.

The Problem of Defining and Measuring Intelligence

Debates over genetic links to intelligence are complicated by the problem of precisely defining and accurately measuring intelligence. It may be that abilities to build houses, draw, play music, or understand complex mathematical procedures are inherited as well as learned. Which of these abilities, however, constitute intelligence? Because of this debate, some people, such as Harvard psychologist Howard Gardner, have argued that there is no single quality of intelligence but rather multiple forms of intelligence.

If there is no single ability that can be labeled "intelligence," this means that one cannot measure intelligence or determine the ex-

tent to which general intellectual ability may be genetic in character. Intelligence quotient (IQ), the measure of intelligence most commonly used to study genetic links to intellectual ability, is based on the view that there is a great deal of overlap among various mental traits. Although a given individual may be skilled at music or writing and poor at mathematics, on the average, people who are proficient in one area also tend to be talented in other areas. Propponents of IQ measures argue that this overlap exists because there is a single, underlying, general intelligence that affects how people score on tests of various kinds of mental abilities. The opponents of IQ measures counter that even if one can speak of intelligence rather than "intelligences," it is too complex to be reduced to one number.

Impact and Applications

The passing of mental abilities from parents to children by genetic inheritance is a politically controversial issue because genetic theories of intelligence may be used to justify existing social inequalities. Social and economic inequalities among racial groups, for example, have been explained as differences among groups in inherited intelligence levels. During the nineteenth century, defenders of slavery claimed that black slaves were by nature less intelligent than the white people who held them in slavery. After World War I, the Princeton University psychologist C. C. Brigham concluded from results of army IQ tests that southern European immigrants had lower levels of inherited intelligence than native-born Americans and that blacks had even more limited intelligence. White supremacists and segregationists used Brigham's results to justify limiting the access of blacks to higher education and other opportunities for advancement. In 1969, Berkeley psychologist Arthur R. Jensen touched off a storm of debate when he published an article that suggested that differences between black and white children in educational success were caused in part by genetic variations in mental ability.

Wealth and poverty, even within racial and ethnic groups, have been explained as consequences of inherited intelligence. Harvard psy-

chologist Richard Herrnstein and social critic Charles Murray have argued that American society has become a competitive, information-based society in which intellectual ability is the primary basis of upward mobility. They have maintained, furthermore, that much of intellectual ability is genetic in character and that people tend to marry and reproduce within their own social classes. Therefore, in their view, social classes also tend to be intellectual classes: a cognitive elite at the top of the Ameri-

can social system and a genetically limited lower class at the bottom.

Scientific truth cannot be established by accusing theories of being inconvenient for social policies of equal opportunity. Nevertheless, it is not clear that genetic differences in intelligence are necessarily connected to social status. Even those who believe that inherited intelligence affects social position generally recognize that social status is affected by many other factors such as parental wealth, ed-

Genetics and IQ

The genetics of intelligence continues to prompt controversy and often emotional debate centering on the relative roles of genetics and environment in shaping intelligence and multiple intelligence. The dictionary defines intelligence as the capacity to acquire knowledge, process information by reasoning, and make rational decisions. It follows that some individuals may have a greater facility for acquiring and analyzing information than others. Even the concept of multiple intelligence implies that some individuals are more intelligent than others. This is not to say, however, that individuals with a higher level of intelligence will always be more successful while individuals with lower levels of intelligence will always be failures. That is, the abilities conferred by higher levels of inherited intelligence are probabilistic rather than determinate and are shaped by many factors other than genetics alone.

Out of the enormous amount of debate certain facts have emerged about the inheritance of intelligence in humans. First, geneticists, behavioral geneticists, and neurobiologists have consistently demonstrated that there is a significant genetic contribution to intelligence, although the exact genes that code for intelligence have not yet been discovered. Embedded within this inheritance pattern, however, is the undeniable and at times substantial contribution of the social environment in development of intelligence in individuals.

The role of environment in shaping intelligence has also been consistently demonstrated to be a vital factor in shaping intelligence. This is most clearly shown where environmental factors adversely influence growth and development of the central nervous system. Low birth rate, anoxia, malnutrition, childhood trauma, income, occupation, parent sep-

aration, and divorce have all been shown to influence the development of intelligence by as much as 40 percent, leading some environmentalists and sociologists to claim that culture is the major factor in intelligence. It is precisely because of the influence of such environmental factors in shaping intelligence that performance gains can be increased somewhat.

Support for the genetic contribution to intelligence comes primarily from studies of identical and fraternal twins, siblings, and family groupings. For example, the correlation of intelligence between identical twins (monozygotic twins) reared together is consistently well over 0.8 (1.0 being the highest correlation), with highest scores measured at 0.86. Scores of fraternal (dizygotic) twins and siblings are lower but still higher than less closely related kin such as cousins and uncles. Furthermore, intelligence measures of adopted siblings show lower correlations compared to intelligence correlations between or among natural siblings. Thousands of such data have led most authorities to suggest that between 40 and 80 percent of an individual's intelligence is shaped by genetics.

The basic genetic mechanisms underlying these observations are not, as yet, well understood. Geneticists, behavioral geneticists, and neurobiologists argue that genes code for brain size, number of brain cells, and number of connections, all of which probably play roles in determining intellectual ability, though the relative contributions of each remain unclear. Further evidence for the role of genetics comes from cases of chromosomal deletions, trisomy, and other genetic abnormalities.

—Dwight G. Smith

ucational opportunity, and cultural attitudes.

It seems evident that there are genetic links to mental ability. At the same time, however, the extent to which genes shape intellectual capacities, whether these capacities should be combined into one dimension called intelligence, and the validity of measures of intelligence remain matters of debate. The scientific debate, moreover, is difficult to separate from social and political debates.

—Carl L. Bankston III

See also: Aging; Biological Determinism; Chromosome Mutation; Congenital Defects; Criminality; Developmental Genetics; Down Syndrome; Eugenics; Eugenics: Nazi Germany; Fragile X Syndrome; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; Heredity and Environment; Human Genetics; Human Growth Hormone; Klinefelter Syndrome; Nondisjunction and Aneuploidy; Phenylketonuria (PKU); Prader-Willi and Angelman Syndromes; Prenatal Diagnosis; Race; Twin Studies; X Chromosome Inactivation; XYY Syndrome.

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Web Site of Interest

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Klinefelter Syndrome

Field of study: Diseases and syndromes
Significance: *Klinefelter syndrome is a sex chromosome disorder in which males have an extra X chromosome. It accounts for ten out of every one thousand institutionalized mentally retarded adults in industrialized nations and is one of the more common chromosomal aberrations.*

Key terms

AZOOSPERMIA: the absence of spermatozoa from the semen

CYTOGENETICS: the study of chromosome number and structure, including identification of abnormalities

GYNecomastia: a condition characterized by abnormally large mammary glands in the male that sometimes secrete milk

HYPOGONADISM: a condition resulting in smaller than normal testicles in males

KARYOTYPE: a pictorial or verbal description of the chromosomes of a single cell

MOSAICISM: a condition in which an individual has two or more cell populations derived from the same fertilized ovum, or zygote, as in sex chromosome mosaics in which some cells contain the usual XY chromosome pattern and others contain extra X chromosomes

Definition and Diagnosis

Klinefelter syndrome is a relatively common genetic abnormality named after Harry Klinefelter, Jr., an American physician. The fundamental chromosomal defect associated with the syndrome is the presence of one or more extra X chromosomes. The normal human male karyotype (array of chromosomes) consists of twenty-two pairs of chromosomes, called autosomes, plus the XY pair, called sex chromosomes. The female also has twenty-two autosome pairs but with an XX pair in place of the XY pair for the sex chromosomes. Klinefelter syndrome affects 1 in every 500 to 600 men. The incidence is relatively high in the mentally retarded population.

Because individuals with Klinefelter syndrome have a Y chromosome, they are always

male. Sometimes Klinefelter syndrome is the result of mosaicism, with males having both normal (XY) karyotypes in some cells and abnormal karyotypes (usually with an extra X chromosome) in others. Individuals with sex chromosome complements of XXYY, XXXY, or XX can also be diagnosed with Klinefelter syndrome. Individuals with Klinefelter syndrome that have a sex chromosome complement of XX are male because although an entire Y chromosome is not present, a portion of a Y chromosome is often attached to another chromosome. This condition can sometimes be diagnosed by a careful karyotype analysis.

Signs and Symptoms

The classic type of Klinefelter syndrome usually becomes apparent at puberty, when the secondary sex characteristics develop. The testes fail to mature, causing primary hypogonadism. In this classic type, degenerative testicular changes begin that eventually result in irreversible infertility. Gynecomastia is often present, and it is usually associated with learning disabilities, mental retardation, and violent, antisocial behavior. Other common symptoms include abnormal body proportions (disproportionate height relative to arm span), chronic pulmonary disease, varicosities of the legs, and diabetes mellitus (which occurs in 8 percent of those afflicted with Klinefelter's). Another 18 percent exhibit impaired glucose tolerance. Most people affected also have azoospermia (no spermatozoa in the semen) and low testosterone levels. However, men with the mosaic form of Klinefelter syndrome may be fertile.

Congenital hypogonadism appears as delayed puberty. Men with hypogonadism experience decreased libido, erection dysfunction, hot sweats, and depression. Genetic testing and careful physical examination may reveal Klinefelter syndrome to be the reason for the primary complaint of infertility. Mental retardation is a frequent symptom of congenital chromosomal aberrations such as Klinefelter syndrome because of probable coincidental defective development of the central nervous system. Early spontaneous abortion is a common occurrence.

Treatment and Psychosocial Implications

Depending on the severity of the syndrome, treatment may include mastectomy to correct gynecomastia. Supplementation with testosterone may be necessary to induce the secondary sexual characteristics of puberty, although the testicular changes that lead to infertility cannot be prevented. Any mental retardation present is irreversible. Psychotherapy with sexual counseling is appropriate when sexual dysfunction causes emotional problems. In people with the mosaic form of the syndrome who are fertile, genetic counseling is vital because they may pass on this chromosomal abnormality. Therapists should encourage discussion of feelings of confusion and rejection that commonly accompany this disorder, and they should attempt to reinforce the victim's male identity. Hormonal therapy can provide some benefits, but both benefits and side effects of hormonal therapy should be made clear. Some men with Klinefelter syndrome are sociopathic; for this population, careful monitoring by probation officers or jail personnel can assist in identifying potential violent offenders, who can be offered psychological counseling.

—Lisa Levin Sobczak, updated by Bryan Ness

See also: Hereditary Diseases; Infertility; Intelligence; Mutation and Mutagenesis; Non-disjunction and Aneuploidy; X Chromosome Inactivation; XYY Syndrome.

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Web Sites of Interest

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Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

National Institute of Child Health and Human Development. <http://nichd.nih.gov>. Site includes a link to "Understanding Klinefelter Syndrome: A Guide for XXY Males and Their Families," which includes an introduction to the syndrome and more.

Knockout Genetics and Knockout Mice

Field of study: Genetic engineering and biotechnology

Significance: *In knockout methodology, a specific gene of an organism is inactivated, or “knocked out,” allowing the consequences of its absence to be observed and its function to be deduced. The technique, first and mostly applied to mice, permits the creation of animal models for inherited diseases and a better understanding of the molecular basis of physiology, immunology, behavior, and development. Knockout genetics is the study of the function and inheritance of genes using this technology.*

Key terms

EMBRYONIC STEM CELL: a cell derived from an early embryo that can replicate indefinitely in vitro and can differentiate into other cells of the developing embryo

GENOME: the total complement of genetic material for an organism

IN VITRO: a biological or biochemical process occurring outside a living organism, as in a test tube

IN VIVO: a biological or biochemical process occurring within a living organism

Knockout Methodology

Before knockout mice, transgenic animals had been generated in which “foreign” DNA was incorporated into their genomes in a largely haphazard fashion; such animals should more properly be referred to as “genetically modified.” In contrast, knockout technology targets a particular gene to be altered. Prior to the creation of transgenic animals, any genetic change resulted from spontaneous and largely random mutations. Individual variability and inherited diseases are the results of this natural phenomenon—as are, on a longer time frame, the evolutionary changes responsible for the variety of living species on the earth. Spontaneously generated animal models of human inherited diseases have been helpful in understanding mutations and developing treatments for them. However, these mutants

were essentially gifts of nature, and their discovery was largely serendipitous. In knockout mice, animal models are directly generated, expediting study of the pathology and treatment of inherited diseases.

In a knockout mouse, a single gene is selected to be inactivated in such a way that the nonfunctional gene is reliably passed to its progeny. Developed independently by Mario Capecchi at the University of Utah and Oliver Smithies of the University of North Carolina, the process is formally termed “targeted gene inactivation,” and, although simple in concept, it is operationally complex and technically demanding. It involves several steps *in vitro*: inactivating and tagging the selected gene, substituting the nonfunctional gene for the functional gene in embryonic stem cells, and inserting the modified embryonic stem cells into an early embryo. The process then requires transfer of that embryo to a surrogate mother, which carries the embryo to term, and selection of offspring that are carrying the inactive gene. It may require several generations to verify that the genetic modification is being dependably transmitted.

Usefulness of Knockout Mice

Knockout mice are important because they permit the function of a specific gene to be established, and, since mice and humans share 99 percent of the same genes, the results can often be applied to people. However, knockout mice are not perfect models in that some genes are specific to mice or humans, and similar genes can be expressed at different levels in the two species. Nevertheless, knockout mice are vastly superior to spontaneous mutants because the investigator selects the gene to be modified. Mice are predominantly used in this technology because of their short generation interval and small size; the short generation interval accelerates the breeding program necessary to establish pure strains, and the small size reduces the space and food needed to house and sustain them.

Knockout mice are, first of all, excellent animal models for inherited diseases, the study of which was the initial impetus for their creation. The Lesch-Nyhan syndrome, a neurological

disorder, was the focus of much of the early work with the knockout technology. The methodology has permitted the creation of previously unknown animal models for cystic fibrosis, Alzheimer's disease, and sickle-cell disease, which will stimulate research into new therapies for these diseases. Knockout mice have also been developed to study atherosclerosis, cancer susceptibility, and obesity, as well as immunity, memory, learning, behavior, and developmental biology.

Knockout mice are particularly appropriate for studying the immune system because immune-compromised animals can survive if kept isolated from pathogens. More than fifty genes are responsible for the development and operation of B and T lymphocytes, the two main types of cells that protect the body from infection. Knockout technology permits a system-

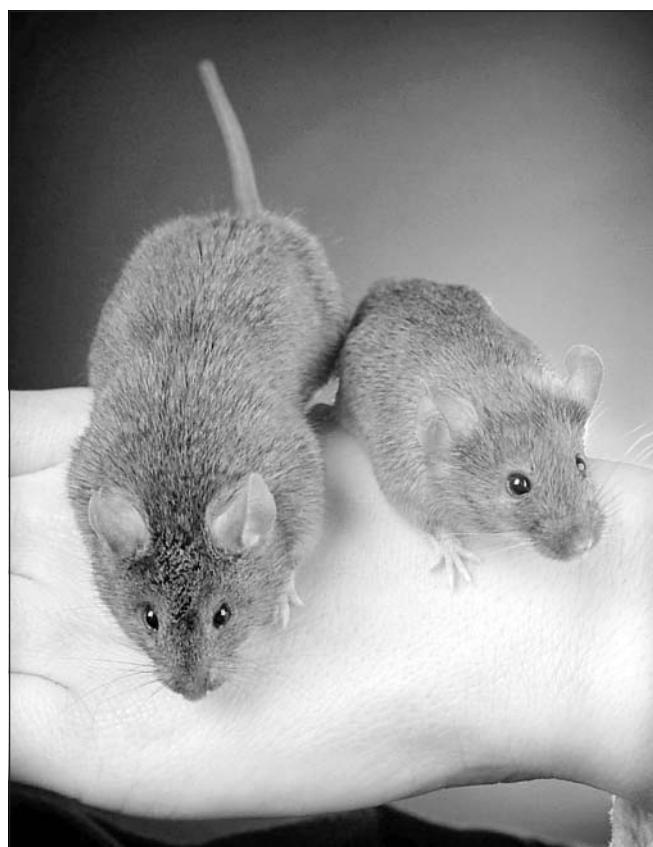
atic examination of the role played by these genes. It has also proven useful in understanding memory, learning, and behavior, as knockout mice with abnormalities in these areas can also survive if human intervention can compensate for their deficiencies. Knockout mice have been created that cannot learn simple laboratory tests, cannot remember symbols or smells, lack nurturing behavior, or exhibit extreme aggression, which have implications for the fields of education, psychology, and psychiatry.

Developmental biology has also benefited from knockout technology. Animals with minor developmental abnormalities can be studied with relative ease, whereas those with highly deleterious mutations may be maintained in the heterozygous state, with homozygotes generated only as needed for study. The generation of conditional knockouts is facilitating study of the genes responsible for controlling the development of various tissues (lung, heart, skeleton, and muscle) during embryonic development. These genes can be explored methodically with knockout technology.

By 1997, more than one thousand different knockout mice had been created worldwide. A primary repository for such animals is the nonprofit Jackson Laboratory in Bar Harbor, Maine, where more than two hundred so-called induced mutant strains are available to investigators. Other strains are available from the scientists who first derived them or commercial entities licensed to generate and sell them.

Double Knockouts, Conditional Knockouts, and Reverse Knockouts

Redundancy is fairly common in gene function: Often, more than one gene has responsibility for the same or similar activity *in vivo*. Eliminating one redundant gene may have little consequence because another gene can fulfill its function. This has led to the creation of double knockout mice, in which two specific genes are eliminated. Double knockouts are generated by crossing



On the left, a "knockout" mouse whose gene for growth/differentiation factor 8 (GDF 8) was removed from its genetic code has grown dramatically larger than the normal mouse (right). (AP/Wide World Photos)

two separate single knockout mice to produce double mutant offspring. Consequences of both mutations can then be examined simultaneously.

Some single knockout mice are deleteriously affected during embryonic development and do not survive to birth. This has led to the generation of conditional knockout mice, in which the gene is functional until a particular stage of life or tissue development triggers its inactivation. The approach is to generate animals with two mutations: The first is the addition of a new gene that causes a marked segment of a gene to be deleted in response to a temporal or tissue signal, and the second is to mark the gene that has been selected to be excised. In these animals, the latter gene remains functional until signaled to be removed.

Knockout methodology involves generation of loss-of-function or null mutations. Its reversal would permit the function of an inoperative gene to be restored. This reversal has been successfully accomplished in mice with the correction of the Lesch-Nyhan defect. Further experimentation may permit it to be applied to humans and other animals. Such targeted restoration of gene function would be the most direct way for gene therapy (the process of introducing a functional gene into an organism's cells) to cure inherited diseases.

—James L. Robinson

See also: Cloning; Developmental Genetics; Genetic Engineering; Genetic Engineering: Medical Applications; Genomics; Model Organism: *Caenorhabditis elegans*; Model Organism: *Mus musculus*; Model Organisms; Transgenic Organisms.

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Web Site of Interest

TBASE: The Transgenic/Targeted Mutation Database, Jackson Laboratory, Bar Harbor, Maine. <http://tbbase.jax.org>. Database of information about transgenic animals generated worldwide, searchable by species, technique, DNA construct, phenotype, laboratory. Features the "Knockout Model of the Month"—a discussion of new animal models—and a glossary.

Lactose Intolerance

Field of study: Diseases and syndromes

Significance: *Lactose intolerance is a common disorder associated with the digestion of milk sugar. It affects a large portion of the human population and creates unpleasant intestinal effects. Its understanding has led to the commercial availability of alternative products that supplement the lack of dairy products in the diet.*

Key terms

CONGENITAL DEFICIENCY: a deficiency that is attributed to inherited genetic causes often apparent at birth

GALACTOSEMIA: a disease attributed to the accumulation of galactose in the blood, caused by a lack of the enzyme that metabolizes galactose; galactosemia is not related to lactose intolerance, which is attributed to the lack of the lactase enzyme

LACTASE: an enzyme that breaks down lactose to the monosaccharides glucose and galactose in the small intestine during the metabolic process; its deficiency is responsible for the ill effects associated with lactose intolerance

LACTOSE: a sugar, also known as milk sugar, that constitutes 2 to 8 percent of milk content and makes up about 40 percent of an infant's diet

The Function of Lactose and Lactase

Milk is the primary source of nutrition for infants. One pint of cow's summer milk provides about 90 percent of the calcium, 30 to 40 percent of the riboflavin, 25 to 30 percent of the protein, and 10 to 20 percent of the calories needed daily. Lactose, also known as milk sugar, exists in the milk of humans, cows, and other mammals. About 7.5 percent of human milk consists of lactose, while cow's milk is about 4.5 percent lactose. This sugar is also one of the few carbohydrates exclusively associated with the animal kingdom; its biosynthesis takes place in the mammary tissue. It is produced commercially from whey, which is obtained as a by-product during the manufacture of cheese. Its so-called alpha form is used as an infant food. Its sweetness is about one-sixth that of sucrose (table sugar).

The metabolism (breaking down) of lactose to glucose and galactose takes place via a specific enzyme called lactase, which is produced by the mucosal cells of the small intestine. Because lactase activity is rate-limiting for lactose absorption, any deficiency in the enzyme is directly reflected in a diminished rate of the sugar absorption. This irregularity should not be confused with intolerance to milk resulting from a sensitivity to milk proteins such as beta-lactoglobulin.

Consequences of Lactase Deficiency

There are three types of lactase deficiency: inherited deficiency, secondary low-lactase activity, and primary low-lactase activity. In inherited lactase deficiency, the symptoms of intolerance develop very soon after birth, as indicated by the presence of lactose in the urine. Patients are recommended a lactose-free diet as well as the consumption of live-culture yogurt, which provides the enzyme beta-galactosidase that attacks the small amounts of lactose that may be in the diet. Beta-galactosidase preparations are also commercially available. Secondary low-lactase activity can be a side effect of peptic ulcer surgery or can occur for a variety of reasons. It may also be present during intestinal diseases such as colitis, gastroenteritis, kwashiorkor, and sprue. Individuals sometimes develop primary low-lactase activity as they get older. A large number of adults, estimated at almost 20 percent, gradually exhibit lactose intolerance, caused by the gradual inability to synthesize an active form of lactase. Susceptible individuals may start developing lactose intolerance as early as four years old.

As a result of lactose intolerance, relatively large quantities of the unhydrolyzed (unbroken) lactose pass into the large intestine, which causes the transfer of water from the interstitial fluid to the lumen by osmosis. At the same time, the intestinal bacteria produce organic acids as well as gases such as carbon dioxide, methane, and hydrogen, which lead to nausea and vomiting. The combined effect also produces cramps and abdominal pains.

Definitive diagnosis of the condition is established by an assay for lactase content in the in-

testinal mucosa. Such a test requires that the individuals drink 50 grams of lactose in 200 milliliters of water. Blood specimens are then taken after 30, 60, and 120 minutes for glucose analysis. An increase of blood glucose by 30 milligrams per deciliter is considered normal, while an increase of 20 to 30 milligrams per deciliter is borderline. A smaller increase indicates lactase deficiency. This test, however, may still show deficiency results with individuals who have a normal lactase activity.

Lactase deficiency displays remarkable genetic variations. The condition is more prevalent among infants of Middle Eastern, Asian (especially Chinese and Thai), and African descent (such as the Ibo, Yoruba, and other tribes in Nigeria and the Hausa in Sudan). On the other hand, Europeans (especially northern) appear to be statistically less susceptible to the deficiency. Similarly, the Fula tribe in Sudan raises the fulani breed of cattle, and the Eastern African Tussi, who own cattle in Rundi, appear to be rarely affected. It is estimated that 10 to 20 percent of American Caucasians and about 75 percent of African Americans are affected.

The ill effects disappear as long as the diet excludes milk altogether. Often people who exhibit partial lactose intolerance can still consume dairy products, including cheese and yogurt, if the food is processed or partially hydrolyzed. This may be accomplished by heating or partially fermenting milk. Some commercial products, such as Lactaid, are designed for lactose-intolerant people because they include the active form of the lactase enzyme in either liquid or tablet form.

—Soraya Ghayourmanesh

See also: Aging; Hereditary Diseases; Inborn Errors of Metabolism.

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Web Sites of Interest

American Gastroenterological Association. <http://www.gastro.org>. Site provides a guide to lactose intolerance, including discussion of causes, diagnostics, and treatment, and links to related resources.

National Institute of Diabetes & Digestive & Kidney Diseases. <http://www.niddk.nih.gov>. This arm of the National Institutes of Health offers resources and links to research on lactose intolerance.

Lamarckianism

Field of study: Evolutionary biology; History of genetics

Significance: Although some aspects of Lamarckianism have been discredited, the basic premises of nineteenth century French biologist Jean-Baptiste Lamarck's philosophy have become widely accepted tenets of evolutionary theory. Lamarckianism be-

came intellectually suspect following fraudulent claims by the Soviet scientist Trofim Lysenko that he could manipulate the heredity of plants by changing their environment; by the 1990's, however, scientists had become more willing to acknowledge the influence of Lamarckianism in evolutionary biology.

Key terms

ACQUIRED CHARACTERISTIC: a change in an organism brought about by its interaction with its environment

LYSENKOISM: a theory of transformation that denied the existence of genes

TRANSFORMIST THEORY OF EVOLUTION: a nineteenth century theory that animals gradually changed over time in response to their perceived needs

Lamarckianism Defined

The term “Lamarckianism” has for many years been associated with intellectually disreputable ideas in evolutionary biology. Originally formulated by the early nineteenth century French scientist Jean-Baptiste-Pierre-Antoine de Monet, chevalier de Lamarck (1744-1829), Lamarckianism had two components that were often misinterpreted by scholars and scientists. The first was the transformist theory that animals gradually changed over time in response to their perceived needs. Many critics interpreted this to mean that species could adapt by wanting to change—in other words, that giraffes gradually evolved to have long necks because they wanted to reach the leaves higher in the trees or that pelicans developed pouched beaks because they wanted to carry more fish. Where Lamarck had suggested only that form followed function—for example, that birds that consistently relied on seeds for food gradually transformed to have beaks that worked best for eating seeds—critics saw the suggestion of active intent or desire.

The second component of Lamarckianism, that changes in one generation of a species could be passed on to the next, also led to misinterpretations and

abuses of his ideas. In the most egregious cases, researchers in the late nineteenth and early twentieth centuries claimed that deliberate mutilations of animals could cause changes in succeeding generations—for example, they believed that if they cut the tails off a population of mice, succeeding generations would be born without tails. During the twentieth century, the Soviet agronomist Trofim Lysenko claimed to have achieved similar results in plants. Such claims have been thoroughly disproved.

Who Was Lamarck?

Such gross distortions of his natural philosophy would probably have appalled Lamarck. Essentially an eighteenth century intellectual, Lamarck was one of the last scientists who saw himself as a natural philosopher. He was born August 1, 1744, in Picardy, and as the youngest of eleven children was destined originally for the church. The death of his father in 1759 freed Lamarck to leave the seminary and enlist in the military, but an injury forced him to re-



Jean-Baptiste Lamarck (Library of Congress)

Lysenkoism

Although Lamarckian evolutionary theories never enjoyed wide acceptance, a century after Lamarck's death a Russian agronomist, Trofim Denisovich Lysenko (1898-1976), promoted similar theories of heritability of acquired characteristics. Lysenko, born in Ukraine, earned a doctorate in agricultural science from the Kiev Agricultural Institute in 1925.

Lysenko claimed that changing the environment in which plants grew made it possible to alter the fruit they bore, and those alterations would be present in the plants grown from their seed. Unlike Lamarck, who posited gradual change over many generations, Lysenko suggested that dramatic alterations were possible immediately. One of his more outlandish claims was that wheat grown under conditions suited for rye would yield rye seeds, a notion as biologically impossible as the idea that feeding cat food to a dog would result in its giving birth to kittens instead of puppies.

Lysenko's ideas were based on results achieved by an uneducated but successful horticulturalist, Ivan V. Michurin (1855-1935). Michurin developed hundreds of varieties of berries and fruit trees. He credited his achievements to inheritance of acquired characteristics rather than to selective breeding. Lysenko believed similar success was possible with cereal grains, primarily wheat, upon which the Soviet Union relied.

Lysenko used vernalization of wheat as proof that acquired characteristics were heritable. Vernalization involves forcing seeds into responding to the changing of seasons earlier than they would under natural conditions. Bulbs of tulips, for example, when refrigerated for a short time and then placed

in a warm environment sprout and bloom and can thereby be forced to blossom midwinter if desired. Lysenko claimed that seeds from vernalized wheat would sprout early without undergoing vernalization themselves. Several ensuing years of good wheat production seemed to validate Lysenko's claims.

Unfortunately for both Soviet science and Soviet agriculture, before it could become evident that Lysenko's seeming successes resulted from good growing conditions rather than from his theories, Lysenko proved more adept at politics than he was at biology. He and his supporters denounced Darwinian evolutionary theories as "bourgeois," contrary to the fundamental principles of Marxism and dialectical materialism as practiced in the Soviet Union. By politicizing science, Lysenko made it impossible for other Russian scientists to pursue research that contradicted Lysenko's pet theories. As director of the Institute of Genetics of the Academy of Sciences from 1940 to 1965, Lysenko wielded tremendous power within the Soviet scientific community. Scientists who challenged his theories not only risked losing their academic positions and research funding but also could be charged with crimes against the state. In the 1940's several of Lysenko's critics were found guilty of anti-Soviet activity, resulting in either their execution or exile to Siberian prison camps.

By the 1950's it was clear that Lysenko's theories did not work. Wheat production consistently failed to achieve promised yields. Agronomists quietly stopped using Lysenko's methods as Lysenko's influence faded, but Lysenko managed to retain his administrative positions for another decade.

—Nancy Farm Männikkö

sign his commission in 1768. He sampled a variety of possible vocations before deciding to pursue a career in science.

His early scientific work was in botany. He devised a system of classification of plants and in 1778 published a guide to French flowers. In 1779, at the age of thirty-five, Lamarck was elected to the Académie des Sciences. Renowned naturalist Georges-Louis Leclerc, comte de Buffon, obtained a commission for Lamarck to travel in Europe as a botanist of the king. In 1789, Lamarck obtained a position at the Jardin du Roi as keeper of the herbarium.

When the garden was reorganized as the Muséum National d'Histoire Naturelle in 1794, twelve professorships were created; Lamarck became a professor of what would now be called invertebrate zoology.

Lamarck demonstrated through his lectures and published works that he modeled his career on that of his mentor, Buffon. He frequently went beyond the strictly technical aspects of natural science to discuss philosophical issues, and he was not afraid to use empirical data as a basis for hypothesizing. Thus, he often speculated freely on the transformation of species.

Philosophie Zoologique (Zoological Philosophy), now considered his major published work, was issued in two volumes in 1809. In it, Lamarck elaborated upon his theories concerning the evolution of species through adaptation to changes in their environments. An essentially philosophical work, *Zoological Philosophy* is today remembered primarily for Lamarck's two laws:

First Law: In every animal which has not passed the limit of its development, a more frequent and continuous use of any organ gradually strengthens, develops and enlarges that organ and gives it a power proportional to the length of time it has been so used; while the permanent disuse of any organ imperceptibly weakens and deteriorates it, and progressively diminishes its functional capacity, until it finally disappears.

Second Law: All the acquisitions or losses wrought by nature on individuals, through the influence of the environment in which their race has long been placed, and hence through the influence of the predominant use or disuse of any organ; all these are preserved by reproduction to the new individuals which arise, provided that the acquired modifications are common to both sexes, or at least to the individuals which produce the young.

These two tenets constitute the heart of Lamarckianism.

During his lifetime, Lamarck's many books were widely read and discussed, particularly *Zoological Philosophy*. It is true Lamarck's ideas on the progression of life from simple forms to more complex forms in a great chain of being met with opposition, but that opposition was not universal. He was not the only "transformist" active in early nineteenth century science, and his influence extended beyond Paris. Whether or not Lamarck directly influenced Charles Darwin is a matter of debate, but it is known that geologist Charles Lyell read Lamarck, and Lyell in turn influenced Darwin.

Lamarckianism's fall into disrepute following Lamarck's death was prompted by social and political factors as well as scientific criteria. By the 1970's, after a century and a half of denigration, Lamarckianism began creeping back

into evolutionary theory and scientific discourse. Researchers in microbiology have described processes that have been openly described as Lamarckian, while other scholars began to recognize that Lamarck's ideas did indeed serve as an important influence in developing theories about the influence of environment on both plants and animals.

—Nancy Farm Männikkö

See also: Central Dogma of Molecular Biology; Chromosome Theory of Heredity; Classical Transmission Genetics; DNA Structure and Function; Evolutionary Biology; Genetic Code, Cracking of; Genetic Engineering: Historical Development; Genetics, Historical Development of; Genetics in Television and Films; Genomics; Human Genome Project; Mendelian Genetics.

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Frederick Griffith with pneumonia-causing bacteria in the 1920's. Griffith discovered the process of bacterial transformation, by which the organism acquires genetic material from its environment and expresses the traits contained on the DNA in its own cells. Bacteria may also acquire foreign genetic material by the process of transduction. In transduction a bacteriophage picks up a piece of host DNA from one cell and delivers it to another cell, where it integrates into the genome. This material may then be expressed in the same manner as any of the other of the host's genes. A third mechanism, conjugation, allows two bacteria that are connected by means of a cytoplasmic bridge to exchange genetic information.

With the development of molecular biology, evidence has been accumulated that supports the lateral movement of genes between prokaryotic species. In the case of *Escherichia coli*, one of the most heavily researched bacteria on the planet, there is evidence that as much as 20 percent of the organism's approximately 4,403 genes may have been transferred laterally into the species from other bacteria. This may explain the ability of *E. coli*, and indeed many other prokaryotic species, to adapt to new environments. It may also explain why in a given bacterial genus some members are pathogenic while others are not. Rather than evolving pathogenic traits, bacteria may have acquired genetic sequences from other organisms and then exploited their new abilities.

It is also now possible to screen the genomes of bacteria for similarities in genetic sequences and use this information to reassess previously established phylogenetic relationships. Once again, the majority of this work has been done in prokaryotic organisms, with the primary focus being on the relationship between the domains *Archaea* and *Bacteria*. Several researchers have detected evidence of lateral gene transfer between thermophilic bacteria and *Archaea* prokaryotes. Although the degree of gene transfer between these domains is under contention, there is widespread agreement that the transfer of genes occurred early in their evolutionary history. The fact that there was lateral gene transfer has complicated accurate determinations of divergence time and order.

Lateral Gene Transfer

Field of study: Population genetics

Significance: *Lateral gene transfer is the movement of genes between organisms. It is also sometimes called horizontal gene transfer. In contrast, vertical gene transfer is the movement of genes between parents and their offspring. Vertical gene transfer is the basis of the study of transmission genetics, while lateral gene transfer is important in the study of evolutionary genetics, as well as having important implications in the fields of medicine and agriculture.*

Key terms

GENE TRANSFER: the movement of fragments of genetic information, whole genes, or groups of genes between organisms

GENETICALLY MODIFIED ORGANISM (GMO): an organism produced by using biotechnology to introduce a new gene or genes, or new regulatory sequences for genes, into it for the purpose of giving the organism a new trait, usually to adapt the organism to a new environment, provide resistance to pest species, or enable the production of new products from the organism

TRANSPOSONS: mobile genetic elements that may be responsible for the movement of genetic material between unrelated organisms

Gene Transfer in Prokaryotes

The fact that genes may move between bacteria has been known since the experiments of

Gene Transfer in Eukaryotes

Although not as common as in prokaryotes, there is evidence of gene transfer in eukaryotic organisms as well. A mechanism by which gene transfer may be possible is the transposon. Barbara McClintock first proposed the existence of transposons, or mobile genetic elements, in 1948. One of the first examples of a transposon moving laterally between species was discovered in *Drosophila* in the 1950's. A form of transposon called a *P* element was found to have moved from *D. willistoni* to *D. melanogaster*. What is interesting about these studies is that the movement of the *P* element was enabled by a parasitic mite common to the two species. This suggests that parasites may play an important role in lateral gene transfer, especially in higher organisms. Furthermore, since the transposon may move parts of the host genome during transition, it may play a crucial role in gene transfer.

The completion of the Human Genome Project, and the technological advances in genomic processing that it developed, have allowed researchers to compare the human genome with the genomes of other organisms to look for evidence of lateral transfer. It is estimated that between 113 and 223 human genes may not be the result of vertical gene transfer but instead might have been introduced laterally from bacteria.

Implications

While the concept of lateral gene transfer may initially seem to be a concern only for evolutionary geneticists in their construction of phylogenetic trees, in reality the effects of lateral gene transfer pose concerns with regard to both medicine and agriculture, specifically in the case of transgenic plants.

Currently the biggest concern regarding lateral gene transfer is the unintentional movement of genes from genetically modified organisms (GMOs) into other plant species. Such transfer may occur by parasites, as appears to have occurred with *Drosophila* in animals, or by dispersal of pollen grains out of the treated field. This second possibility holds particular significance for corn growers, whose crop is

wind-pollinated. Genetically modified corn, containing the microbial insecticide *Bt*, may cross-pollinate with unintentional species, reducing the effectiveness of pest management strategies. In another case, the movement of herbicide resistant genes from a GMO to a weed species may result in the formation of a superweed.

On the beneficial side, lateral gene transfer may also play a part in medicine as part of gene therapy. A number of researchers are examining the possibility of using viruses, transposons, and other systems to move genes, or parts of genes, into target cells in the human body, where they may be therapeutic in treating diseases and disorders.

—Michael Windelspecht

See also: Archaea; Bacterial Genetics and Cell Structure; Evolutionary Biology; Gene Regulation: Bacteria; Gene Regulation: Eukaryotes; Gene Regulation: *Lac Operon*; Gene Regulation: Viruses; Hybridization and Introgression; Molecular Genetics; Transposable Elements.

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Linkage Maps

Field of study: Techniques and methodologies

Significance: *Linkage maps can be used to predict the outcome of genetic crosses involving linked genes and, more important, can be used to find the location of genes that are responsible for specific traits or genetic defects.*

Key terms

ALLELES: different forms of the same gene locus; in diploids there are two alleles at each locus

CROSSING OVER: an event early in meiosis in which homologous chromosomes exchange homologous regions

DIHYBRID: an organism that is heterozygous for both of two different gene loci

HOMOLOGOUS CHROMOSOMES: chromosomes that are structurally the same and contain the same loci, although the loci may each have different alleles

LOCUS (*pl. LOCI*): The specific region of a chromosome that contains a specific gene

MEIOSIS: cell division that reduces the chromosome number from two sets (diploid) to one set (haploid), ultimately resulting in the formation of gametes (eggs or sperm) or spores

Linkage and Crossing Over

When Gregor Mendel examined inheritance of two traits at a time, he found that the dihybrid parent (Aa or Bb) produced offspring with the four possible combinations of these alleles at equal frequencies: $\frac{1}{4}AB$, $\frac{1}{4}Ab$, $\frac{1}{4}aB$, and $\frac{1}{4}ab$. He called this pattern “independent assortment.” The discovery of meiosis explained the basis of independent assortment. If the A locus and the B locus are on nonhomologous chromosomes, then segregation of the alleles of one locus (A and a) will be independent of the segregation of the alleles of the other (B and b).

Even simple plants, animals, fungi, and protists have thousands of genes. The number of human genes is unknown, but with the completion of the human genome in 2003 it appeared that the actual number of protein-

coding genes was only about 21,000. Human beings have forty-six chromosomes in each cell (twenty-three from the mother and twenty-three from the father): twenty-two pairs of autosomal chromosomes plus two sex chromosomes (two X chromosomes in females and an X and a Y chromosome in males). Since humans have only twenty-four kinds of chromosomes, there must be less than a few thousand genes on the average human chromosome.

If two loci fail to show independent assortment, they are said to be linked and are therefore near one another on the same chromosome. For example, if the alleles A and B are on one chromosome and a and b are on the homologue of that chromosome, then the dihybrid (AB/ab) would form gametes with the combinations AB and ab more often than Ab and aB . How much more often? At one extreme, if there is no crossover between these two loci on the two homologous chromosomes, then $\frac{1}{2}$ of the gametes would be AB and $\frac{1}{2}$ would be ab . At the other extreme, if the two genes are so far apart on a large chromosome that crossover occurs between the loci almost every time meiosis occurs, they would assort independently, thus behaving like two loci on different nonhomologous chromosomes. When two genes are on the same chromosome but show no linkage, they are said to be “syntenic.”

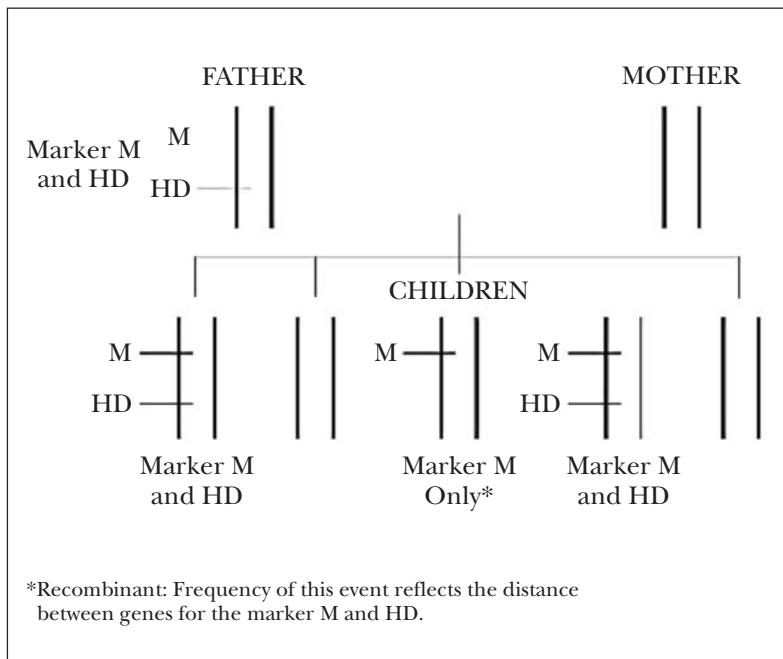
In the first stage of meiosis, homologous chromosomes pair tightly with one another (synapsis). At this stage of meiosis, each homologous chromosome is composed of two chromatids called sister chromatids, so there are four complete DNA molecules (a tetrad) present in the paired homologous chromosomes. A reciprocal exchange of pieces of two paired homologous chromosomes can produce new combinations of alleles between two linked loci if a crossover occurs in the right region. Chromosomes that display a new arrangement of alleles due to crossover are called recombinants. For example, a crossover in a dihybrid with AB on one chromosome and ab on its homologue could form Ab and aB recombinants. The average number of crossovers during a meiotic division differs from species to species and sometimes between the sexes of a single species. For example, crossover does not occur in male fruit

flies (*Drosophila melanogaster*), and it may occur slightly less often in human males than in females. Nevertheless, within a single sex of a single species, the number of crossovers during a meiotic division is fairly constant and many crossovers typically occur along the length of each pair of chromosomes.

Constructing the Maps

If two loci are very close together on the same chromosome, crossover between them will be rare, and thus recombinant gametes will also be rare. Conversely, crossover will occur more frequently between two loci that are farther apart on the same chromosome. This is true because the location for any particular crossover is random. This fact has been used to construct linkage maps (also called crossover maps or genetic maps) of the chromosomes of many species. The distances between loci on linkage maps are expressed as percent crossover. A crossover of 1 percent is equal to one centi-Morgan (cM). If two loci are 12 cM apart on a linkage map, a dihybrid will form approximately twelve recombinant gametes for every eighty-eight nonrecombinant gametes. Linkage maps are made by combining data from many different controlled crosses or matings. For instance, suppose that a cross between a dihybrid *AB*/*ab* individual and a homozygous *ab*/*ab* individual produced 81 *AB*/*ab* + 83 *ab*/*ab* progeny (non-crossover types) and 20 *Ab*/*ab* + 16 *aB*/*ab* progeny (crossover types). The map distance between these loci would be $100(20 + 16)/(81 + 83 + 20 + 16) = 18$ cM.

The table shows the frequency of recombinant gametes from test crosses of three different dihybrids, including the one already described:



As a result of crossing over, traits located on one chromosome may not be inherited together. Those traits that tend to be inherited together most often also tend to be those located near each other on the chromosome. Those traits that are more distant are more likely to cross over or recombine during the production of gametes (eggs and sperm) and therefore to be absent as a result of crossover. Genetic linkage maps can be constructed based on the frequency of these events. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

gene pair	cM
<i>a</i> and <i>b</i>	18
<i>a</i> and <i>c</i>	7

It is clear that the *C* locus must be between the other two loci on the linkage map. The absolute order, *ACB* or *BCA*, is arbitrarily defined by the first person who constructs a linkage map of a species.

<i>a</i>	<i>c</i>	<i>b</i>
7		11

In this example, the linkage map is exactly additive. In real experiments, linkage map distances are seldom exactly additive, because the longer the distance between two loci, the greater chance there will be for double crossovers.

overs to occur. Double crossovers give the same result as no crossover, and are therefore not detected. Thus, the greater the distance between two loci, the more the distance will be underestimated.

Once a large number of genes on the same chromosome have been mapped, the linkage map is redrawn with map positions rather than map distances. For example, if many other experiments provided more information about linked genes, the following linkage map might emerge:

<i>p</i>	<i>q</i>	<i>a</i>	<i>c</i>	<i>b</i>	<i>r</i>	<i>s</i>
0	6	14	21	32	39	49

The *A* and *C* loci are still 7 cM apart ($21 - 14 = 7$), and the other distances on the first map are also still the same.

Very detailed linkage maps have been constructed for some plants, animals, fungi, and protists that are of particular value to medicine, agriculture, industry, or scientific research. Among them are *Zea mays* (maize), *Drosophila melanogaster* (fruit fly), and *Saccharomyces cerevisiae* (baker's yeast). The linkage map of *Homo sapiens* (humans) is not very detailed because it is unethical and socially impossible to arrange all of the desired crosses that would be necessary to construct one. Other techniques have allowed the construction of very detailed physical maps of human chromosomes.

Genetic Linkage Maps and the Structure of Chromosomes

It should be emphasized that the linkage map is not a scale model of the physical chromosome. It is generally true that the relative order of genes on the linkage map and the physical chromosome map are the same. However, the relative distances between genes on the linkage map may not be proportionately the same on the physical map. Consider three loci

(*A*, *B*, and *C*) that are arranged in that order on the chromosome. Suppose that the *AB* distance on the physical map is exactly the same as the *BC* distance. If the crossover frequency between *A* and *B* is higher than between *B* and *C*, then the *AB* linkage map distance will be larger than the *BC* linkage map distance. It is common to find small discrepancies between linkage maps and physical maps all along the chromosome. Large discrepancies are usually limited to loci close to centromeres. Crossover frequencies are generally very low near centromeres, apparently due to the structural characteristics of centromeres. If two loci are on opposite sides of a centromere, they will appear farther apart on the physical map and much closer on the linkage map.

—James L. Farmer, updated by Bryan Ness

See also: Chromosome Structure; Chromosome Theory of Heredity; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Gene Families; Genomics; Mendelian Genetics; Mitosis and Meiosis; Model Organism: *Drosophila melanogaster*; Model Organism: *Neurospora crassa*.

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Mendelian Genetics

Field of study: Classical transmission genetics; History of genetics

Significance: *Gregor Mendel was a monk and a science teacher in Moravia when he wrote his famous paper about experimental crosses of pea plants. Little note was taken of it when it was published in 1866, but it provided concepts and methods that catalyzed the growth of modern genetics after 1900 and earned Mendel posthumous renown as the founder of the new science.*

Key terms

GAMETES: reproductive cells that unite during fertilization to form an embryo; in plants, the pollen cells and egg cells are gametes

HYBRID: a plant form resulting from a cross between two distinct varieties

INDEPENDENT ASSORTMENT: the segregation of two or more pairs of genes without any tendency for certain genes to stay together

SEGREGATION: the process of separating a pair of Mendelian hereditary elements (genes), one from each parent, and distributing them at random into the gametes

Early Life

Born Johann Mendel on July 22, 1822, the future teacher, monk, abbot, botanist, and meteorologist grew up in a village in Moravia, a province of the Austrian Empire that later became part of Czechoslovakia (1918) and the Czech Republic (1993). His parents were peasant farmers and belonged to the large, German-speaking minority in this predominantly Czech province. Like most places in Moravia, Mendel's hometown had two names: Hynčice in Czech and Heinzendorf in German.

Johann Mendel was an exceptional pupil, but no local schooling was available for him beyond the age of ten. In 1833, he persuaded his parents to send him to town to continue his education. They were reluctant to let him go because they could ill afford to dispense with his help on the farm or finance his studies. In 1838, Mendel's father was partially disabled in a logging accident, and Johann, then sixteen and still at school, had to support himself. He

earned just enough from tutoring to get by. At times, however, the pressure became too much for him. He suffered a breakdown in 1839 and returned home for several months to recuperate. He was to have several more of these stress-related illnesses, but no precise information is available about their causes and symptoms.

In 1840, Mendel completed *Gymnasium*, as the elite secondary schools were called, and entered the University of Olomouc for the two-year program in philosophy that preceded higher university studies. He had trouble supporting himself in Olomouc, perhaps because there was less demand for German-speaking tutors, and his Czech was not good enough for teaching. He suffered another breakdown in 1841 and retreated to Hynčice during spring exams.

That summer, Mendel decided once more against staying and taking over the farm. Since his father could not work, the farm was sold to his elder sister's husband. Johann's share of the proceeds was not enough to see him through



Gregor Mendel. (National Library of Medicine)

the Olomouc program, especially since he had to repeat a year because of the missed exams. However, his twelve-year-old sister sacrificed part of her future dowry so that he could continue. (He repaid her years later by putting her three sons through *Gymnasium* and university.)

Upon finishing at Olomouc in 1843, Mendel decided to enter the clergy. The priesthood filled his need for a secure position and held out possibilities for further learning and teaching, but Mendel did not seem to be called to it. Aided by a professor's recommendation, Mendel was accepted into the Augustinian monastery in Brno, the capital of Moravia, where he took the name Gregor. In 1847, after four years of preparation at the monastery, he was ordained a priest.

Priesthood and Teaching

The Brno monastery was active in the community and provided highly qualified instructors for *Gymnasia* and technical schools throughout Moravia. Several monks, including the abbot, were interested in science, and they had experimental gardens, a herbarium, a mineralogical collection, and an extensive library. Mendel found himself in learned company with opportunities for research in his spare time.

Unfortunately, Mendel's nerves failed him when he had to minister to the sick and dying. Assigned to a local hospital in 1848, he was so upset by it that he was bedridden himself within five months. However, his abbot was sympathetic and let him switch to teaching. A letter survives in which the abbot explains this decision to the bishop: "[Mendel] leads a retiring, modest and virtuous religious life . . . and he devotes himself diligently to scholarly pursuits. For pastoral duties, however, he is less suited, because at the sick-bed or at the sight of the sick or suffering he is seized by an insurmountable dread, from which he has even fallen dangerously ill."

Mendel taught Latin and Greek, German literature, math, and science as a substitute at the *Gymnasium* and was found to be very good at teaching. Therefore, he was sent to Vienna in 1850 to take the licensing examinations so that he could be promoted to a regular position.

These exams were very demanding and normally required more preparation than Mendel's two years at Olomouc. Mendel failed, but one examiner advised the abbot to let him try again after further study. The abbot took this advice and sent Mendel to study in Vienna for two years (1851–1853). There he took courses in biology, physics, and meteorology with some of the best-known scientists of his day, including physicist Christian Doppler and botanist Franz Unger.

For unknown reasons, Mendel returned to Moravia to resume substitute teaching and did not go to Vienna for the exams until 1856. This time he was too nervous to finish. After writing one essay, he fell ill and returned to Brno. Despite this failure, he was allowed to teach regular classes until 1868 even though he was technically only a substitute.

Scientific Work

During his teaching career, Mendel performed his famous experiments on peas in a garden at the monastery. He published the results in an 1866 article, which introduced fundamental concepts and methods of genetics. The first set of experiments involved fourteen varieties of pea plant, each with a single distinguishing trait. These traits made up seven contrasting pairs, such as seeds that were either round or wrinkled in outline or seed colors that were green or yellow. Upon crossing each pair, Mendel obtained hybrids identical to one parent variety. For example, the cross of round with wrinkled peas yielded only round peas; the cross of green with yellow peas yielded only yellow peas. He referred to traits that asserted themselves in the hybrids as "dominant." The others were "recessive" because they receded from view. The effect was the same regardless of whether he fertilized the wrinkled variety with pollen from the round or the round variety with pollen from the wrinkled. This indicated to Mendel that both pollen cells and egg cells contributed equally to heredity; this was a significant finding because the details of plant reproduction were still unclear.

Mendel next allowed the seven hybrids to pollinate themselves, and the recessive traits reappeared in the second generation. For in-

stance, the round peas, which were hybrids of round and wrinkled peas, yielded not only more round peas but also some wrinkled ones. Moreover, the dominant forms outnumbered the recessives three to one. Mendel explained the 3:1 ratio as follows. He used the symbols *A* for the dominant form, *a* for the recessive, and *Aa* for the hybrid. A hybrid, he argued, could produce two types of pollen cell, one containing some sort of hereditary element corresponding to trait *A* and the other an element corresponding to trait *a*. Likewise, it could produce eggs containing either *A* or *a* elements. This process of dividing up the hereditary factors among the gametes became known as segregation.

The gametes from the *Aa* hybrids could come together in any of four combinations: pollen *A* with egg *A*, pollen *A* with egg *a*, pollen *a* with egg *A*, and pollen *a* with egg *a*. The first three of these combinations all grew into plants with the dominant trait *A*; only the fourth produced the recessive *a*. Therefore, if all four combinations were equally common, one could expect an average of three plants exhibiting *A* for every one exhibiting *a*.

Allowing self-pollination to continue, Mendel found that the recessives always bred true. In other words, they only produced more plants with that same recessive trait; no dominant forms reappeared, not even in subsequent generations. Mendel's explanation was that the recessives could only have arisen from

the pollen *a* and egg *a* combination, which excludes the *A* element. For similar reasons, plants with the dominant trait bred true one-third of the time, depending on whether they were the pure forms from the pollen *A* and egg *A* combination or the hybrids from the pollen *A* and egg *a* or pollen *a* and egg *A* combinations.

Mendel's hereditary elements sound like the modern geneticist's genes or alleles, and Mendel usually receives credit for introducing the gene concept. Like genes, Mendel's elements were material entities inherited from both parents and transmitted to the gametes. They also retained their integrity even when recessive in a hybrid. However, it is not clear whether he pictured two copies of each element in every cell, one copy from each parent, and he certainly did not associate them with chromosomes.

In a second set of experiments, Mendel tested combinations of traits to see whether they would segregate freely or tend to be inherited together. For example, he crossed round, yellow peas with wrinkled, green ones. That cross first yielded only round, yellow peas, as could be expected from the dominance relationships. Then, in the second generation, all four possible combinations of traits segregated out: not only the parental round yellow and wrinkled green peas but also new round green and wrinkled yellow ones. Mendel was able to explain the ratios as before, based on equally

The Results of Mendel's Pea-Plant Experiments

<i>Parental characteristics</i>	<i>First generation</i>	<i>Second generation</i>	<i>Second generation ratio</i>
Round × wrinkled seeds	All round	5,474 round : 1,850 wrinkled	2.96 : 1
Yellow × green seeds	All yellow	6,022 yellow : 2,001 green	3.01 : 1
Gray × white seedcoats	All gray	705 gray : 224 white	3.15 : 1
Inflated × pinched pods	All inflated	882 inflated : 299 pinched	2.95 : 1
Green × yellow pods	All green	428 green : 152 yellow	2.82 : 1
Axial × terminal flowers	All axial	651 axial : 207 terminal	3.14 : 1
Long × short stems	All long	787 long : 277 short	2.84 : 1

likely combinations of hereditary elements coming together at fertilization. The free re-grouping of hereditary traits became known as independent assortment. In the twentieth century, it was found not to occur universally because some genes are linked together on the same chromosome.

Mendel's paper did not reach many readers. As a *Gymnasium* teacher and a monk in Moravia without even a doctoral degree, Mendel could not command the same attention as a university professor in a major city. Also, it was not obvious that the behavior of these seven pea traits illustrated fundamental principles of heredity. Mendel wrote to several leading botanists in Germany and Austria about his findings, but only Carl von Nägeli at the University of Munich is known to have responded, and even he was skeptical of Mendel's conclusions. Mendel published only one more paper on heredity (in 1869) and did little else to follow up his experiments or gain wider attention from scientists.

Mendel pursued other scientific interests as well. He was active in local scientific societies and was an avid meteorologist. He set up a weather station at the monastery and sent reports to the Central Meteorological Institute in Vienna. He also helped organize a network of weather stations in Moravia. He envisioned telegraph connections among the stations and with Vienna that would make weather forecasting feasible. In his later years, Mendel studied sunspots and tested the idea that they affected the weather. He also monitored the water level in the monastery well in order to test a theory that changes in the water table were related to epidemics. A common thread that ran through these diverse research interests was that they all involved counting or measuring, with the goal of discovering scientific laws behind the numerical patterns. His one great success was in explaining the pea data with his concepts of dominance, segregation, and independent assortment.

Mendel felt pleased and honored to be elected abbot in 1868, even though he had to give up teaching and most of his research. He did not have the heart to say good-bye to his pupils. Instead, he asked the school director to announce his departure and give his last month's

Mendel's Pea Plants

Dominant trait	Recessive trait
Round	Wrinkled
Yellow	Green
Purple flower	White flower
Inflated pod	Constricted pod
Green pod	Yellow pod
Axial flowers	Terminal flowers
Long stem	Short stem

Mendel evaluated the transmission of seven paired traits in his studies of garden peas. (Electronic Illustrators Group)

salary to the three neediest boys in the class. As abbot, Mendel had a reputation for generosity to the poor and to scientific and cultural institutions. He was also an efficient manager of the monastery and its extensive land holdings and a fierce defender of the monastery's interests. From 1874 on, he feuded with imperial authorities over a new tax on the monastery, which he refused to pay as long as he lived. Mendel's health failed gradually in the last years of his life. He had kidney problems and an abnormally fast heartbeat, the latter probably from nerves and nicotine. (A doctor recommended smoking to control his weight, and he developed a twenty-cigar-a-day habit.) He died January 6, 1884, of heart and kidney failure.

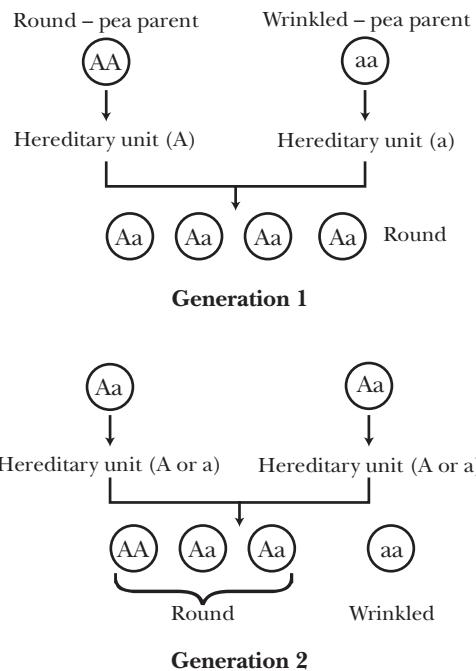
Impact and Applications

Years after Mendel's death, a scientific colleague remembered him saying, prophetically, "my time will come." It came in 1900, when papers by three different botanists reported experimental results that were similar to Mendel's and endorsed Mendel's long-overlooked explanations. This event became known as the rediscovery of Mendelism. By 1910, Mendel's theory had given rise to a whole new field of research, which was given the name "genetics." Mendel's hereditary elements were described more precisely as "genes" and were presumed to be located on the chromosomes. By the 1920's, the sex chromosomes were identified, the determination of sex was explained in Mendelian terms, and the arrangements of genes on chromosomes could be mapped.

The study of evolution was also transformed by Mendelian genetics, as Darwinians and anti-Darwinians alike had to take the new information about heredity into account. By 1930, it had been shown that natural selection could cause evolutionary change in a population by shifting the proportions of individuals with different genes. This principle of population genetics became a cornerstone of modern Darwinism.

Investigations of the material basis of heredity led to the discovery of the gene's DNA structure in 1953. This breakthrough marked the beginning of molecular genetics, which studies how genes are copied, how mutations occur,

Mendel's Law of Segregation



Mendel's law of segregation is demonstrated by an initial cross between true-breeding plants with round peas and plants with wrinkled peas. The round trait is dominant, and the wrinkled trait is recessive. The second generation consists of round-pea plants and wrinkled-pea plants produced in a ratio of 3:1.

and how genes exert their influence on cells. In short, all of modern genetics can trace its heritage back to the ideas and experiments of Gregor Mendel.

—Sander Gliboff

See also: Chloroplast Genes; Chromosome Structure; Chromosome Theory of Heredity; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Genetic Engineering: Historical Development; Genetics, Historical Development of; Incomplete Dominance; Linkage Maps; Monohybrid Inheritance; Natural Selection; Quantitative Inheritance.

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Web Site of Interest

MendelWeb. <http://www.mendelweb.org>. This site, designed for teachers and students, revolves around Mendel's 1865 paper and includes educational activities, images, interactive learning, and other resources.

Metafemales

Field of study: Diseases and syndromes

Significance: *Genetic defects are quite common in humans. The frequency of females born with XXX chromosomes, called multiple X or metafemale syndrome, generally varies between one in one thousand and one in fifteen hundred but may be less in some populations. Although most such females have normal appearance and sexual reproduction, this abnormality needs to be better understood so that the affected individuals' lives are bettered medically and socially.*

Key terms

AUTOSOMES: all chromosomes other than sex chromosomes in a cell nucleus

BARR BODY: named after its discoverer, Murray L. Barr, a dark-stained sex chromatin body in nuclei of females, which represents the inactivated X chromosome; the number of Barr bodies in any cell is generally one less than the number of X chromosomes

LYON HYPOTHESIS: proposed by Mary Lyon in 1962, a hypothesis that during development one of the two X chromosomes in normal mammalian females is inactivated at random; the inactivated X chromosome is a Barr body

MEIOSIS: the process by which gametes (sperm and eggs) are produced in sexually reproducing organisms

NONDISJUNCTION: the failure of homologous chromosomes to disjoin during meiosis I, or the failure of sister chromatids to separate and migrate to opposite poles during meiosis II

SEX CHROMOSOMES: the homologous pair of chromosomes that determines the sex of an individual; in humans, XX is female and XY is male; XX females produce one kind of gamete, X (homogametic sex), and XY males produce two kinds of gametes, X and Y (heterogametic sex)

History and Symptoms

In 1914, Calvin Blackman Bridges discovered nondisjunction of sex chromosomes in the fruit fly, *Drosophila melanogaster*. In 1925,

he proposed the genic or sex balance theory, which defined the relationship between sex chromosomes and autosomes (A) for sex determination. According to this theory, the following ratios of sex chromosomes and number of sets of autosomes determine what sex phenotype will emerge in humans. For example, XX + 2 sets of autosomes (2X:2A ratio = 1.0) = normal female; XY + 2 sets of autosomes (1X:2A ratio = 0.5) = normal male; and XXX + 2 sets of autosomes (3X:2A ratio = 1.5) = metafemale, or superfemale.

The term “metafemale” was first applied to the XXX (triple X) condition by Curt Stern around 1959. The frequency of metafemale phenotype in the general human population is approximately one in one thousand to fifteen hundred newborn girls. The XXX females are characterized by the presence of two Barr bodies in their cells. They have a total of 47 chromosomes instead of the normal complement of 46.

Metafemales have variable fertility, ranging from normal to sterile. They may be phenotypically normal but are often slightly taller than average, with longer legs. These individuals may have widely spaced nipples and a webbed neck. Studies have shown that most metafemales lead a normal sexual life and have normal children. In some cases, menstruation may begin at an older age, menstrual cycles may be irregular or temporarily interrupted, and menopause may begin earlier compared to normal XX women.

Genetic Cause

The basic causes of XXX females are best explained through meiosis, the cell division that halves the number of chromosomes in gametes, and nondisjunction. From a single human cell (46 chromosomes) designated for sexual reproduction, meiosis produces four cells, each with 23 chromosomes. Thus, normal human eggs carry one-half ($22A + 1X = 23$) of the total number of chromosomes ($44A + 2X = 46$). Occasionally, a mistake occurs during meiosis, called nondisjunction. Nondisjunction during meiosis I or meiosis II can produce eggs with 2X chromosomes ($22A + 2X = 24$). Usually the nondisjunction that gives rise to XXX fe-

males occurs in the female parent during meiosis I.

Fertilization of an egg carrying two X chromosomes by an X-bearing ($22A + 1X = 23$) sperm results in an individual with $44A + 3X = 47$ chromosomes, or a metafemale. The extra X chromosome is not usually transmitted to the children. Thus, metafemales can have normal children. Triple X, triplo-X, trisomy X, and 47 XXX are also the names given to the metafemale phenotype. This genetic condition has also been referred to as “extra X aneuploidy” or “multiple X syndrome.”

Social Issues

The IQ of metafemales is usually low normal to normal. In some studies, IQ was found to be lower by 30 points than that of their normal siblings; only a few had an IQ lower than 70. Language learning in XXX children is usually delayed. Emotional maturation may also be delayed. These delays in development are preventable by providing increased psychological, social, and motor stimulation both at home and at school. Tutoring is often needed at some time during development.

The 47 XXX condition can put some affected individuals at risk for speech disorders, learning disabilities, and neuro-motor deficits, which ultimately could lead to decreased psychosocial adaptation, especially during adolescence. One study found young females with 47 XXX to be less well adapted in both their teen and adult years; they described their lives as more stressful. On average, they experienced more work, social, and relationship problems than their siblings. Metafemales may encounter behavioral problems, including mild depression, conduct disorder, immature behavior, and socializing problems. Good parenting and a supportive home may assure a better social and behavioral development.

—Manjit S. Kang

See also: Behavior; Biological Clocks; Gender Identity; Hermaphrodites; Homosexuality; Human Genetics; Pseudohermaphrodites; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

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Web Site of Interest

Triplo-X Syndrome. <http://www.triplo-x.org>. A site that offers social support, a brief introduction to the syndrome, and links to related articles.

Miscegenation and Antimiscegenation Laws

Field of study: Bioethics; History of genetics; Human genetics and social issues

Significance: *Miscegenation is the crossing or hybridization of different races. As knowledge of the nature of human variability has expanded, clearly defining "race" has become increasingly difficult; the study of genetics reveals that the concept of race is primarily a social construct as opposed to a biological reality. Limited understanding of the biological and genetic effects of mating between races, as well as racial prejudice, played a major role in the development of the eugenics movement and the enactment of antimiscegenation laws in the first half of the twentieth century.*

Key terms

- EUGENICS:** the control of individual reproductive choices to improve the genetic quality of the human population
- HYBRIDIZATION:** the crossing of two genetically distinct species, races, or types to produce mixed offspring
- NEGATIVE EUGENICS:** preventing the reproduction of individuals who have undesirable genetic traits, as defined by those in control
- POSITIVE EUGENICS:** selecting individuals to reproduce who have desirable genetic traits, as seen by those in control
- RACE:** in the biological sense, a group of people who share certain genetically transmitted physical characteristics

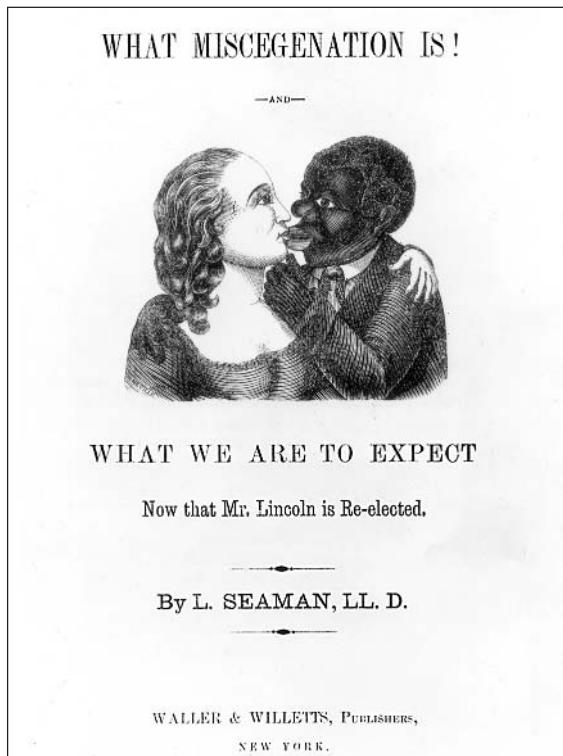
What Is a Race?

Implicit in most biological definitions of race is the concept of shared physical characteristics that have come from a common ancestor. Humans have long recognized and attempted to classify and categorize different kinds of people. The father of modern systematics, Carolus Linnaeus, described, in his system of binomial nomenclature, four races of humans: Africans (black), Asians (dark), Europeans (white), and Native Americans (red). Skin color in humans has been, without doubt, the primary feature used to classify people, although there is no single trait that can be used to do this. Skin color is used because it makes it very easy to tell groups of people apart. However, there are thousands of human traits. What distinguishes races are differences in gene frequencies for a variety of traits. The great majority of genetic traits are found in similar frequencies in people of different skin color. There may not be a single genetic trait that is always associated with people of one skin color while not appearing at all in people of another skin color. It is possible for a person to differ more from another person of the same skin color than from a person of a different skin color.

Many scientists think that the word "race" is not useful in human biology research. Scientific and social organizations, including the American Association of Physical Anthropologists and the American Anthropological Association, have deemed that racial classifications

are limited in their scope and utility and do not reflect the evolving concepts of human variability. It is of interest to note that subjects are frequently asked to identify their race in studies and surveys.

It is useful to point out the distinction between an "ethnic group" and a race. An ethnic group is a group of people who share a common social ancestry. Cultural practices may lead to a group's genetic isolation from other groups with a different cultural identity. Since members of different ethnicities may tend to marry only within their group, certain genetic traits may occur at different frequencies in the group than they do in other ethnic or racial groups, or the population at large.



The fear of interracial marriage during the 1860's is only too clear from the title page to this antimiscegenation tract, published after Emancipation near the end of the Civil War. At the time, Charles Darwin had recently published his theory of natural selection, which "social Darwinists" misapplied to justify antiracial social and business policies. Today geneticists can verify that all human beings, despite allelic variations such as skin color, share the same genetic heritage. (Library of Congress)

Miscegenation

Sir Francis Galton, a cousin of Charles Darwin, is often regarded as the father of eugenics. He asserted that humans could be selectively bred for favorable traits. In his 1869 book *Heredity Genius*, he set out to prove that favorable traits were inborn in people and concluded that

the average intellectual standard of the Negro race is some two grades below our own. That the average ability of the [ancient] Athenian race is, on the lowest possible estimate, very nearly two grades higher than our own—that is, about as much as our race is above that of the African Negro.

In spite of its scientific inaccuracy by today's standards, the work of Galton was widely accepted by political and scientific leaders of his time. Bertrand Russell even suggested that the United Kingdom should issue color-coded "procreation tickets" issued to individuals based on their status in society: "Those who dared breed with holders of a different colored ticket would face a heavy fine." These "scientific" findings, combined with social and racial stereotypes, led to the eugenics movement and its development in many countries, including England, France, Germany, Sweden, Canada, and the United States.

Laws were passed to restrict the immigration of certain ethnic groups into the United States. Between 1907 and 1940, laws allowing forcible sterilization were passed in more than thirty states. Statutes prohibiting and punishing interracial marriages were passed in many states and, even as late as 1952, more than half the states still had antimiscegenation laws. The landmark decision against antimiscegenation laws occurred in 1967 when the U.S. Supreme Court declared the Virginia law unconstitutional. The decision, *Loving v. Virginia*, led to the erosion of the legal force of the antimiscegenation laws in the remaining states.

Impact and Applications

In spite of antimiscegenation laws and societal and cultural taboos, interracial matings have been a frequent occurrence. Many coun-

tries around the world, including the United States, are now racially heterogeneous societies. Genetic studies indicate that perhaps 20 to 30 percent of the genes in most African Americans are a result of admixture of white genes from mixed matings since the introduction of slavery to the Americas more than three hundred years ago. Miscegenation has been widespread throughout the world, and there may not even be such a thing as a "pure" race. No adverse biological effects can be attributed to miscegenation.

—Donald J. Nash

See also: Biological Determinism; Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Genetic Engineering; Social and Ethical Issues; Heredity and Environment; Intelligence; Race; Sociobiology; Sterilization Laws.

Further Reading

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Brah, Avtar, and Annie E. Coombes, eds. *Hybridity and Its Discontents: Politics, Science, Culture*. New York: Routledge, 2000. Covers ideas on miscegenation and racial purity, engineering the future, cultural translation, and reconfiguring concepts of nation, community, and belonging. Illustrations, bibliography, index.

Moran, Rachel F. *Interracial Intimacy: The Regulation of Race and Romance*. Chicago: University of Chicago Press, 2001. Discusses antimiscegenation laws and the legal maintenance of racial boundaries; breaking through racial boundaries; judicial review; race and identity; children, custody, and adoption; the new multiracialism; and more.

Sollors, Werner, ed. *Interracialism: Black-White Intermarriage in American History, Literature, and Law*. New York: Oxford University Press, 2000. Collection of foundational writings on interracial marriage and its effects on racial identity and racial relations. Bibliography, index.

Yancey, George. "An Analysis of Resistance to Racial Exogamy." *Journal of Black Studies* 31 (May, 2001). A look at opposition to interracial marriage and at South Carolina's attempt in 1998 to legalize interracial marriage through state referendum.

Web Site of Interest

Cold Spring Harbor Laboratory, Image Archive on the American Eugenics Movement. <http://www.eugenicsarchive.org/eugenics>. Comprehensive and extensively illustrated site that covers the eugenics movement in the United States, including miscegenation and antimiscegenation laws.

Mitochondrial Diseases

Field of study: Diseases and syndromes

Significance: Mitochondrial genes are few in number but are necessary for animal cells to grow and survive. Mutations in these genes can result in age-related degenerative disorders and serious diseases of muscles and the central nervous system for which there is no generally effective treatment. Mitochondrial diseases are transmitted maternally and are usually associated with heteroplasmy, a state in which more than one type of gene arrangement, or genotype, occurs in the same individual.

Key terms

HETEROPLASMY: a mutation in which more than one set of gene products encoded by mitochondrial DNA (mtDNA) can be present in an individual organ or tissue type, a single cell, or a single mitochondrion

MATERNAL INHERITANCE: the transmission pattern characteristically shown by mitochondrial diseases and mutations in mtDNA, where changes that occur in the mother's genetic material are inherited directly by children of both sexes without masking or interference by the mtDNA of the father

MITOCHONDRIA: small structures, or organelles, enclosed by double membranes found outside the nucleus, in the cytoplasm of all higher cells, that produce chemical power

for the cells and harbor their own genetic material

MITOCHONDRIAL DNA (mtDNA): genetic material found uniquely in mitochondria, located outside the nucleus and therefore separate from the nuclear DNA

REPLICATIVE SEGREGATION: a mechanism by which individual mtDNAs carrying different mutations can come to predominate in any one mitochondrion

not. Organs also require different amounts of adenosine triphosphate (ATP), the cell's energy source produced in mitochondria. If the population of mutated mitochondria grows to outnumber the unmutated forms, most cells in a particular organ may appear diseased. This process has been called replicative segregation, and a mitochondrial disease is the result. Loss of mtDNA also occurs with increasing age, especially in the brain and heart.

Mitochondrial Genetics and Disease

The unique arrangement of subunits making up individual genes is highly mutable, and thousands of different arrangements, or genotypes, are cataloged in humans. A tiny number of genes in animal cells are strictly inherited from the maternal parent and are found in the mitochondria, located in the cell's cytoplasm, outside the nucleus, where most genetic information resides in nuclear DNA. Some variants in mitochondrial DNA (mtDNA) sequences can cause severe defects in sight, hearing, skeletal muscles, and the central nervous system. Symptoms of these diseases often include great fatigue. The diseases themselves are difficult to diagnose accurately, and they are currently impossible to treat effectively. New genetic screening methods based on polymerase chain reaction (PCR) technologies using muscle biopsies are essential for correct identification of these diseases.

A person normally inherits a single mtDNA type, but families are occasionally found in which multiple mtDNA sequences are present. This condition, called heteroplasmy, is often associated with mitochondrial disease. Heteroplasmy occurs in the major noncoding region of mtDNA without much impact, but if it exists in the genes that control the production of cellular energy, severe consequences result. Weak muscles and multiple organs are involved in most mitochondrial diseases, and there can be variable expression of a particular syndrome within the same family that may either increase or decrease with age. It is easiest to understand this problem by remembering that each cell contains a population of mitochondria, so there is the possibility that some mtDNAs will carry a particular mutation while others do

Particular Mitochondrial Diseases

Mitochondrial diseases show a simple pattern of maternal inheritance. The first mitochondrial disease identified was Leber's hereditary optic neuropathy (LHON), a condition associated with the sudden loss of vision when the optic nerve is damaged, usually occurring in a person's early twenties. The damage is not reversible. Biologists now know that LHON is caused by at least four specific mutations that alter the mitochondrial proteins ND1, ND4, and CytB. A second mitochondrial syndrome is myoclonic epilepsy with ragged-red fiber disease (MERRF), which affects the brain and muscles throughout the body. This disease, along with another syndrome called mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), is associated with particular mutations in mitochondrial transfer RNA (tRNA) genes that help produce proteins coded for by mtDNA. Finally, deletions and duplications of mtDNA are associated with Kearns-Sayre disease (affecting the heart, other muscles, and the cerebellum), chronic progressive external ophthalmoplegia (CPEO; paralysis of the eye muscles), rare cases of diabetes, heart deficiencies, and certain types of deafness. Some of these conditions have been given specific names, but others have not.

Muscles are often affected by mitochondrial diseases because muscle cells are rich in mitochondria. New treatments for these diseases are based on stimulating undamaged mtDNA in certain muscle precursor cells, called satellite cells, to fuse to damaged muscle cells and regenerate the muscle fibers. Others try to prevent damaged mtDNA genomes from replicating biochemically in order to increase the number of good mtDNAs in any one cell. This last

set of experiments has worked on cells in tissue culture but has not been used on humans. These approaches aim to alter the competitive ability of undamaged genes to exist in a cellular environment that normally favors damaged genes. Further advances in treatment will also require better understanding of the natural ability of mtDNA to undergo genetic recombination and DNA repair.

—Rebecca Cann

See also: Aging; Extrachromosomal Inheritance; Hereditary Diseases; Human Genetics; Mitochondrial Genes.

Further Reading

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- Raven, Peter H., and George B. Johnson. *Biology*. 6th ed. New York: W. H. Freeman/Worth, 1999. Helps clarify mitochondria and how they interact with a cell's nucleus. Illustrations, maps, index.

Web Site of Interest

United Mitochondrial Diseases Foundation. <http://www.umdf.org>. A support organization that promotes research offers support to affected individuals and families; the site explains the genetics of mitochondrial disorders and offers interactive medical advice.

Mitochondrial Genes

Field of study: Molecular genetics

Significance: *Mutations in mitochondrial genes have been shown to cause several human genetic diseases associated with a gradual loss of tissue function. Understanding the functions of mitochondrial genes and their nuclear counterparts may lead to the development of treatments for these*

debilitating diseases. Analysis of the mitochondrial DNA sequence of different human populations has also provided information relevant to the understanding of human evolution.

Key terms

- ADENOSINE TRIPHOSPHATE (ATP):** the molecule that serves as the major source of energy for the cell
- ATP SYNTHASE:** the enzyme that synthesizes ATP
- CYTOCHROMES:** proteins found in the electron transport chain
- ELECTRON TRANSPORT CHAIN:** a series of protein complexes that pump H⁺ ions out of the mitochondria as a way of storing energy that is then used by ATP synthase to make ATP
- MITOCHONDRIAL DNA (mtDNA):** genetic material found uniquely in mitochondria, located outside the nucleus and therefore separate from the nuclear DNA
- RIBOSOMES:** organelles that function in protein synthesis and are made up of a large and a small subunit composed of proteins and ribosomal RNA (rRNA) molecules
- SPACERS:** long segments of DNA rich in adenine-thymine (A-T) base pairs that separate exons and introns, although most of the spacer DNA is transcribed but is not translated messenger RNA (mRNA)

Mitochondrial Structure and Function

Mitochondria are membrane-bound organelles that exist in the cytoplasm of eukaryotic cells. Structurally, they consist of an outer membrane and a highly folded inner membrane that separate the mitochondria into several compartments. Between the two membranes is the intermembrane space; the innermost compartment bounded by the inner membrane is referred to as the "matrix." In addition to enzymes involved in glucose metabolism, the matrix contains several copies of the mitochondrial chromosome as well as ribosomes, transfer RNA (tRNA), and all the other necessary factors required for protein synthesis. Mitochondrial ribosomes are structurally different from the ribosomes located in the cytoplasm of the eukaryotic cell and, in fact, more closely resemble ribosomes from bacterial cells. This

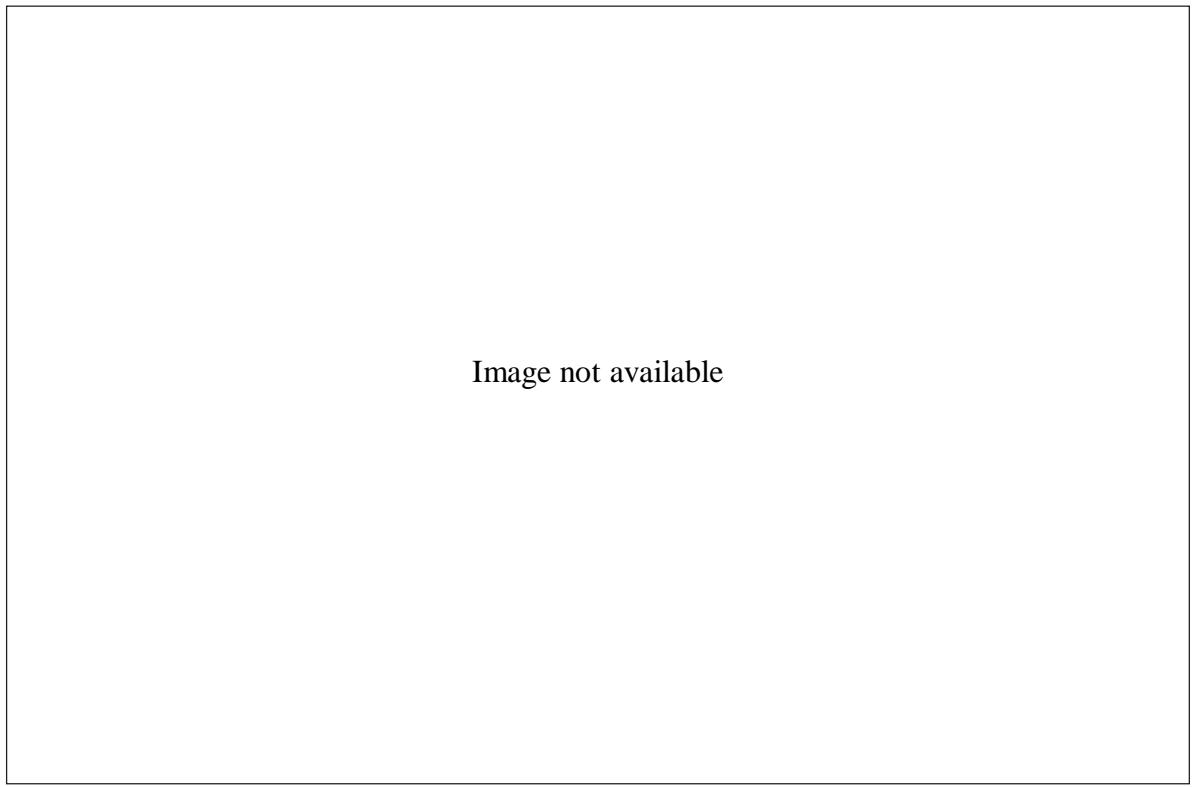


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At London's Natural History Museum in 1997, anthropologist Chris Stringer displays the nine-thousand-year-old skull of Cheddar Man (named for the southwestern English town), to whom he traced a modern relative by comparing DNA samples from the skull with samples from a living, forty-two-year-old schoolteacher. This is possible because mitochondrial DNA is passed unchanged from generation to generation down the maternal line. (AP/Wide World Photos)

similarity led to the endosymbiont hypothesis developed by Lynn Margulis, which proposes that mitochondria arose from bacteria that took up residence in the cytoplasm of the ancestor to eukaryotes.

Embedded in the inner mitochondrial membrane is a series of protein complexes that are known collectively as the “electron transport chain.” These proteins participate in a defined series of reactions that begin when energy is released from the breakdown of glucose and end when oxygen combines with 2H^+ ions to produce water. The net result of these reactions is the movement of H^+ ions (also called protons) from the matrix into the intermembrane space. This establishes a proton gradient in which the intermembrane space has a more positive charge and is more acidic than the matrix. Thus mitochondria act as tiny batteries that separate positive and negative charges in

order to store energy. Another protein that is embedded in the inner mitochondrial membrane is an enzyme called adenosine triphosphate (ATP) synthase. This enzyme allows the H^+ ions to travel back into the matrix. When this happens, energy is released that is then used by the synthase enzyme to make ATP. Cells use ATP to provide energy for all of the biological work they perform, including movement and synthesis of other molecules. The concept of linking the production of a proton gradient to ATP synthesis was developed by Peter Mitchell in 1976 and is referred to as the chemiosmotic hypothesis.

Mitochondrial Genes

The mitochondrial chromosome is a circular DNA molecule that varies in size from about 16,000 base pairs (bp) in humans to more than 100,000 base pairs in certain species of plants.

Despite these size differences, mitochondrial DNA (mtDNA) contains only a few genes that tend to be similar over a wide range of organisms. This discussion will focus on genes located on the human mitochondrial chromosome that has been completely sequenced. These genes fall into two broad categories: those that play a role in mitochondrial protein synthesis and those involved in electron transport and ATP synthesis.

Mitochondria have their own set of ribosomes that consist of a large and a small subunit. Each ribosomal subunit is a complex of ri-

bosomal RNA (rRNA) and proteins. Genes that play a role in mitochondrial protein synthesis include two rRNA genes designated 16S rRNA and 12S rRNA, indicating the RNA for the large and small subunits respectively. Also in this first category are genes for mitochondrial transfer RNA. Transfer RNA (tRNA) is an L-shaped molecule that contains the RNA anticodon at one end and an amino acid attached to the other end. The tRNA anticodon pairs with the codon of the messenger RNA (mRNA) and brings the correct amino acid into position to be added to the growing protein chain. Thus

The Diversity of mtDNA

The mitochondria of plants, animals, and fungi include their own DNA genomes, mitochondrial DNA (mtDNA). The mtDNA genome typically consists of a bacteria-like circular loop of DNA located in highly condensed structures called nucleoids within the mitochondrial matrix. However, the mtDNA of the yeast *Hansenula*, the protozoans *Tetrahymena* and *Paramecium*, and the alga *Chlamydomonas* are chainlike or linear rather than circular, while that of protozoan parasites such as *Trypanosoma*, *Leishmania*, and *Cryptosporidium* is organized into a network of several hundred maxicircles about 21–31 kilobase pairs (kb) long, interlocked with several thousand minicircles, each about 0.5–2.5 kb.

The size of each mtDNA varies greatly among organisms. Most animals have small mtDNA genomes ranging from about 6 to 20 kb, such as the 6-kb mtDNA genome of the protozoan parasite *Plasmodium falciparum*, which causes malaria, and the 14.3-kb mtDNA of free-living *Ascaris* roundworms. The mtDNA genome of humans is about 16.5 kb and comprises about 0.3 percent of the total genome. The mtDNA genomes of most plants and fungi are larger: The mtDNA of the yeast *Saccharomyces cerevisiae* is 86 kb, that of the common pea *Sativa* is 110 kb, that of the liverwort *Marchantia* is 186 kb, and that of the muskmelon *Cucumis melo* is a gigantic 2,400 kb. Much of the size variation is due to the presence of long segments of noncoding sequences embedded within the genome, which seem to be especially abundant in plants and fungi but not in animal mtDNA. More than half of the mtDNA of yeasts, for example, is formed by long segments of spacers, while another quarter consists of introns, interven-

ing sequences between segments consisting of functioning genes.

Despite the size differences, plant and animal mtDNA usually carry the same thirty-seven coding genes: twenty-two genes coding for transfer RNA molecules, two ribosomal RNA genes, and thirteen genes coding for proteins involved in mitochondrial respiration. Again, certain organisms differ. *Marchantia* mtDNA, for example, includes an additional sixteen genes that code for ribosomal proteins and twenty-nine genes that code for proteins of unknown function.

Translation of mtDNA is consistent with the universal genetic code, with notable departures. For example, both AGA and AGG specify the amino acid arginine in the universal genetic code but are stop codes in animal mtDNA. In ciliated protozoans the mtDNA code for glutamine is UAA and UAG, which specifies stop in the universal genetic code. In yeast the mtDNA codes CUU, CUA, CUC, and CUG specify the amino acid threonine instead of leucine, as specified by the universal genetic code. Presumably, all of these mtDNA coding departures from the universal genetic code result from mutations that occurred subsequent to the endosymbiotic incorporation of the original mitochondria into early eukaryotic cells.

Inheritance patterns of mtDNA differ for some plants and animals as well. In animals the mtDNA genome is transmitted primarily through the female egg to the offspring, but in *Chlamydomonas* algae and yeasts male and female gametes are nearly equal in size and contribute mtDNA genome to the offspring.

—Dwight G. Smith

the tRNA molecule serves as a bridge between the information in the mRNA molecule and the sequence of amino acids in the protein. Mitochondrial tRNAs are different from those involved in protein synthesis in the cytoplasm. In fact, cytoplasmic tRNAs would not be able to function on mitochondrial ribosomes, nor could mitochondrial tRNAs work with cytoplasmic ribosomes. Thus, mtDNA contains a complete set of twenty-two tRNA genes.

Genes involved in electron transport fall into the second category of mitochondrial genes. The electron transport chain is divided into a series of protein complexes, each of which consists of a number of different proteins, a few of which are encoded by mtDNA. The NADH dehydrogenase complex (called complex I) contains about twenty-two different proteins. In humans, only six of these proteins are encoded by genes located on the mitochondrial chromosome. Cytochrome *c* reductase (complex III) contains about nine proteins, including cytochrome *b*, which is the only one whose gene is located on mtDNA. Cytochrome oxidase (complex IV) contains seven proteins, three of which are encoded by mitochondrial genes. About sixteen different proteins combine to make up the mitochondrial ATP synthase, and only two of these are encoded by mtDNA.

All of the proteins not encoded by mitochondrial genes are encoded by genes located on nuclear chromosomes. In fact, more than 90 percent of the proteins found in the mitochondria are encoded by nuclear genes. These genes must be transcribed into mRNA in the nucleus, then the mRNA must be translated into protein on cytoplasmic ribosomes. Finally, the proteins are transported into the mitochondria where they function. By contrast, genes located on mtDNA are transcribed in the mitochondria and translated on mitochondrial ribosomes.

Impact and Applications

Any mutation occurring in a mitochondrial gene has the potential to reduce or prevent mitochondrial ATP synthesis. Because human cells are dependent upon mitochondria for their energy supply, the effects of these muta-

tions can be wide-ranging and debilitating, if not fatal. If the mutation occurs in a gene that plays a role in mitochondrial protein synthesis, the ability of the mitochondria to perform protein synthesis is affected. Consequently, proteins that are translated on mitochondrial ribosomes such as cytochrome *b* or the NADH dehydrogenase subunits cannot be made, leading to defects in electron transport and ATP synthesis. Mutations in mitochondrial tRNA genes, for example, have been shown to be the cause of several degenerative neuromuscular disorders. Genes involved in electron transport and ATP synthesis have a more directly negative effect when mutated. Douglas C. Wallace and coworkers identified a mutation within the NADH dehydrogenase subunit 4 gene, for example, that was the cause of a maternally inherited form of blindness and was one of the first mitochondrial diseases to be identified.

Of further interest is the study of nuclear genes that contribute to mitochondrial function. Included in this list of nuclear genes are those encoding proteins involved in mtDNA replication, repair, and recombination; enzymes involved in RNA transcription and processing; and ribosomal proteins and the accessory factors required for translation. It is presumed that a mutation in any of these genes could have negative effects upon the ability of the mitochondria to function. Understanding how nuclear genes contribute to mitochondrial activity is an essential part of the search for effective treatments for mitochondrial diseases.

Human evolutionary studies have also been affected by the understanding of mitochondrial genes and their inheritance. Researchers Allan C. Wilson and Rebecca Cann, knowing that mitochondria are inherited exclusively through the female parent, hypothesized that a comparison of mitochondrial DNA sequences in several human populations would enable them to trace the origins of the ancestral human population. These studies led to the conclusion that a female living in Africa about 200,000 years ago was the common ancestor for all modern humans; she is referred to as "mitochondrial Eve."

—Bonnie L. Seidel-Rogol

See also: Aging; Ancient DNA; Extrachromosomal Inheritance; Hereditary Diseases; Human Genetics; Mitochondrial Diseases; RNA World.

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Mitosis and Meiosis

Field of study: Cellular biology

Significance: *Mitosis is the process of cell division in multicellular eukaryotic organisms. Meiosis is the process of cell division that produces haploid gametes in sexually reproducing eukaryotic organisms.*

Key terms

BINARY FISSION: reproduction of a cell by division into two parts

CENTROMERE: a region on the chromosome where chromatids join

CHROMATID: one-half of a replicated chromosome

CYTOKINESIS: division of the cytoplasm to form new cells

DAUGHTER CELLS: cells resulting from the division of a parent cell

DIPLOID CELLS: cells containing two sets of homologous chromosomes

HAPLOID CELLS: cells containing one set of chromosomes; eggs and sperm are haploid cells

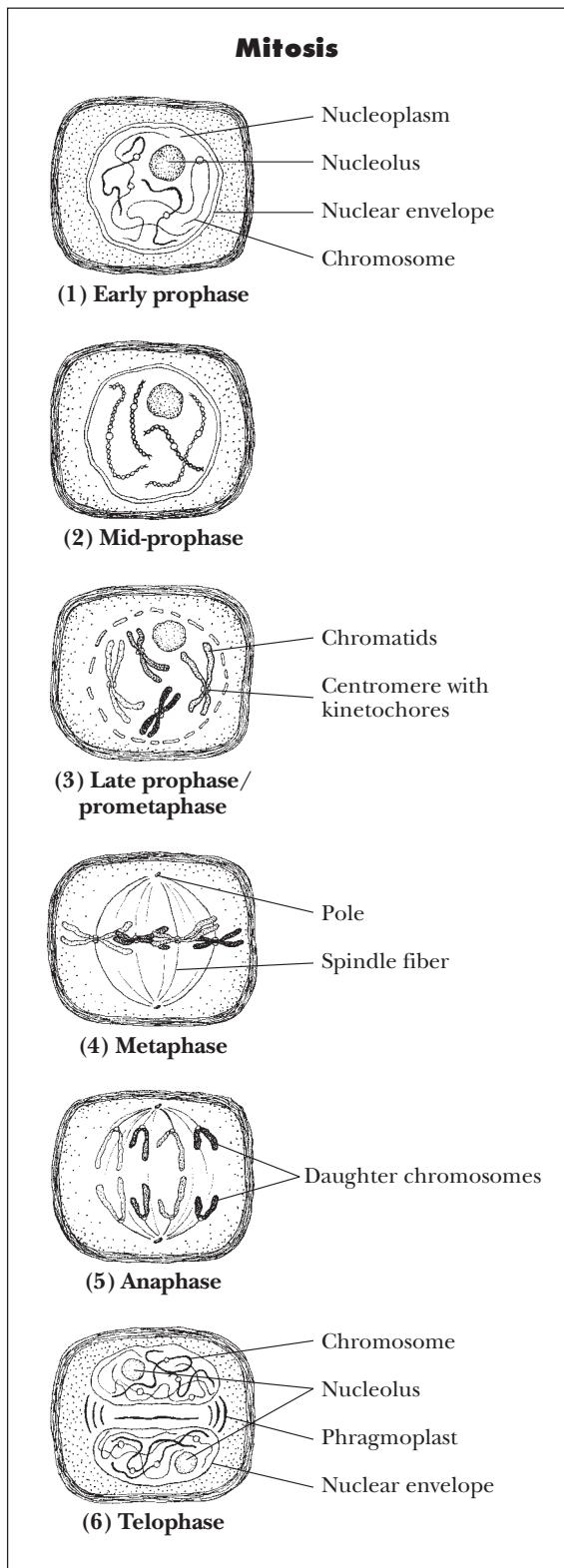
Cellular Reproduction

Organisms must be able to grow and reproduce. Prokaryotes, such as bacteria, duplicate DNA and divide by splitting in two, a process called binary fission. Cells of eukaryotes, including those of animals, plants, fungi, and protists, divide by one of two methods: mitosis or meiosis. Mitosis produces two cells, called daughter cells, with the same number of chromosomes as the parent cell, and is used to produce new somatic (body) cells in multicellular eukaryotes or new individuals in single-celled eukaryotes. In sexually reproducing organisms, cells that produce gametes (eggs or sperm) divide by meiosis, producing four cells, each with half the number of chromosomes possessed by the parent cell.

Chromosome Replication

All eukaryotic organisms are composed of cells containing chromosomes in the nucleus. Chromosomes are made of DNA and proteins. Most cells have two complete sets of chromosomes, which occur in pairs. The two chromosomes that make up a pair are homologous, and contain all the same loci (genes controlling the production of a specific type of product). These chromosome pairs are usually referred to as homologous pairs. An individual chromosome from a homologous pair is sometimes called a homolog. For example, typical lily cells contain twelve pairs of homologous chromosomes, for a total of twenty-four chromosomes. Cells that have two homologous chromosomes of each type are called diploid. Some cells, such as eggs and sperm, contain half the normal number of chromosomes (only one of each homolog) and are called haploid. Lily egg and sperm cells each contain twelve chromosomes.

DNA must replicate before mitosis or meiosis can occur. If daughter cells are to receive a



(Kimberly L. Dawson Kurnizki)

full set of genetic information, a duplicate copy of DNA must be available. Before DNA replication occurs, each chromosome consists of a single long strand of DNA called a chromatid. After DNA replication, each chromosome consists of two chromatids, called sister chromatids. The original chromatid acts as a template for making the second chromatid; the two are therefore identical. Sister chromatids are attached at a special region of the chromosome called the centromere. When mitosis or meiosis starts, each chromosome in the cell consists of two sister chromatids.

Mitosis and meiosis produce daughter cells with different characteristics. When a diploid cell undergoes mitosis, two identical diploid daughter cells are produced. When a diploid cell undergoes meiosis, four unique haploid daughter cells are produced. It is important for gametes to be haploid so that when an egg and sperm fuse, the diploid condition of the mature organism is restored.

Cellular Life Cycles

Mitosis and meiosis occur in the nuclear region of the cell, where all the cell's chromosomes are found. Nuclear control mechanisms begin cell division at the appropriate time. Some cells in an adult organism rarely divide by mitosis in adult organisms, while other cells divide constantly, replacing old cells with new. Meiosis occurs in the nuclei of cells that produce gametes. These specialized cells occur in reproductive organs, such as flower parts in higher plants.

Cells, like organisms, are governed by life cycles. The life cycle of a cell is called the cell cycle. Cells spend most of their time in interphase. Interphase is divided into three stages: first gap (G_1), synthesis (S), and second gap (G_2). During G_1 , the cell performs its normal functions and often grows in size. During the S stage, DNA replicates in preparation for cell division. During the G_2 stage, the cell makes materials needed to produce the mitotic apparatus and for division of the cytoplasmic components of the cell. At the end of interphase, the cell is ready to divide. Although each chromosome now consists of two sister chromatids, this is not apparent when viewed through a mi-

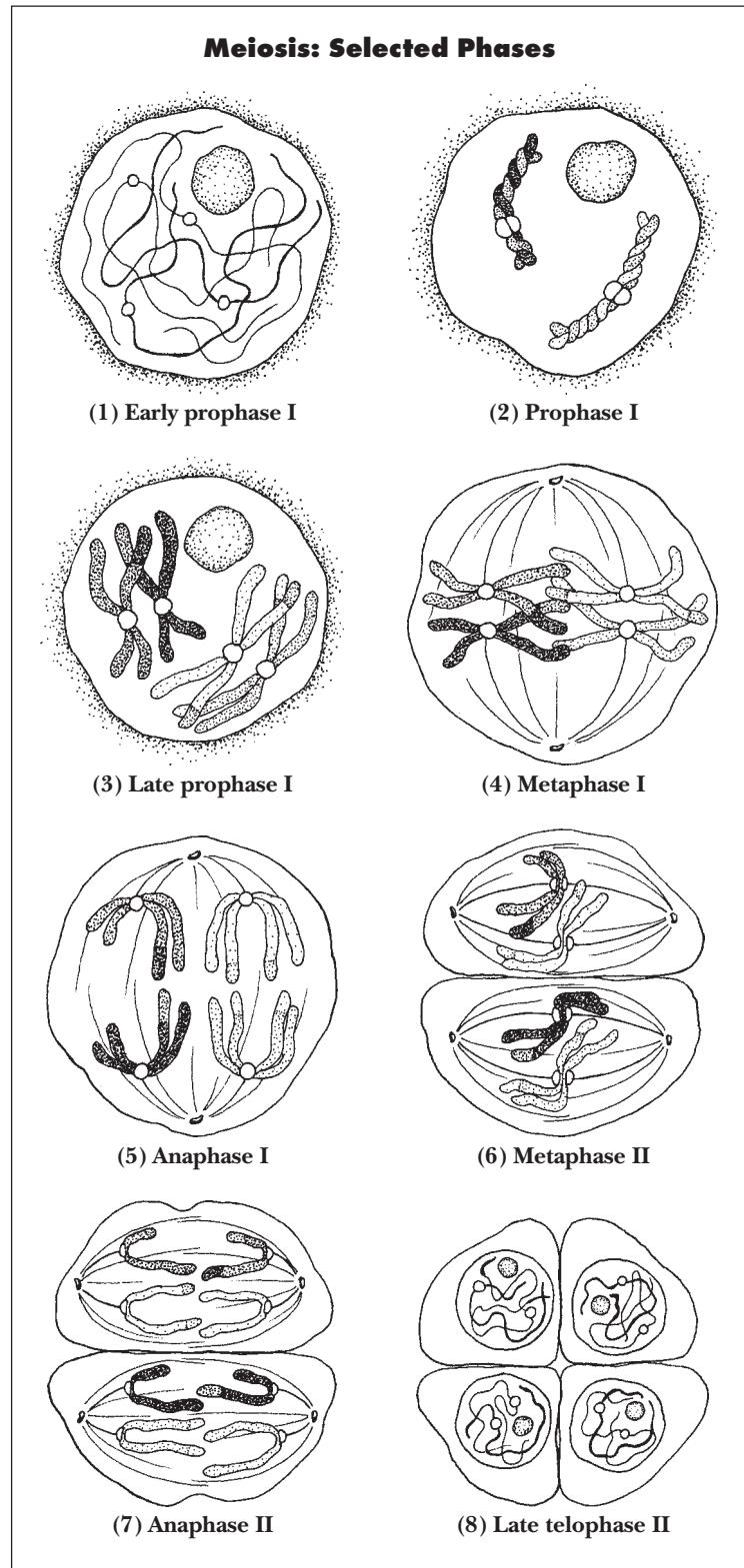
croscope; all the chromosomes are in a highly relaxed state and simply appear as a diffuse material called chromatin.

Mitosis

Mitosis consists of five stages: prophase, prometaphase, metaphase, anaphase, and telophase. Although certain events identify each stage, mitosis is a continuous process, and each stage gradually passes into the next. Identification of the precise state is therefore difficult at times.

During prophase, the chromatin becomes more tightly coiled and condenses into chromosomes that are clearly visible under a microscope, the nucleolus disappears, and the spindle apparatus begins to form in the cytoplasm. In prometaphase the nuclear envelope breaks down, and the spindle apparatus is now able to invade the nuclear region. Some of the spindle fibers attach themselves to a region near the centromere of each chromosome called the kinetochore. The spindle apparatus is the most obvious structure of the mitotic apparatus. The nuclear region of the cell has opposite poles, like the North and South Poles of the earth. Spindle fibers reach from pole to pole, penetrating the entire nuclear region.

During metaphase, the cell's chromosomes align in a region called the metaphase plate, with the sister chromatids oriented toward opposite poles. The metaphase plate traverses the cell, much like the equator passes through the center of the earth. Sister chromatids separate during anaphase. The sister chromatids of each chromosome split



(Kimberly L. Dawson Kurnizki)

apart, and the spindle fibers pull each sister chromatid (now a separate chromosome) from each pair toward opposite poles, much as a rope-tow pulls a skier up a mountain. Telophase begins as sister chromatids reach opposite poles. Once the chromatids have reached opposite poles, the spindle apparatus falls apart, and the nuclear membrane re-forms. Mitosis is complete.

Meiosis

Meiosis is a more complex process than mitosis and is divided into two major stages: meiosis I and meiosis II. As in mitosis, interphase precedes meiosis. Meiosis I consists of prophase I, metaphase I, anaphase I, and telophase I. Meiosis II consists of prophase II, metaphase II, anaphase II, and telophase II. In some cells, an interphase II occurs between meiosis I and meiosis II, but no DNA replication occurs.

During prophase I, the chromosomes condense, the nuclear envelope falls apart, and the spindle apparatus begins to form. Homologous chromosomes come together to form tetrads (a tetrad consists of four chromatids, two sister chromatids for each chromosome). The arms of the sister chromatids of one homolog touch the arms of sister chromatids of the other homolog, the contact points being called chiasmata. Each chiasma represents a place where the arms have the same loci, so-called homologous regions. During this intimate contact, the chromosomes undergo crossover, in which the chromosomes break at the chiasmata and swap homologous pieces. This process results in recombination (the shuffling of linked alleles, the different forms of genes, into new combinations), which results in increased variability in the offspring and the appearance of character combinations not present in either parent.

Tetrads align on the metaphase plate during metaphase I, and one spindle fiber attaches to the kinetochore of each chromosome. In anaphase I, instead of the sister chromatids separating, they remain attached at their centromeres, and the homologous chromosomes separate, each homolog from a tetrad moving toward opposite poles. Telophase I begins as the homologs reach opposite poles, and similar to telophase of mitosis, the spindle apparatus

falls apart, and a nuclear envelope re-forms around each of the two haploid nuclei. Because the number of chromosomes in each of the telophase I nucleus is half the number in the parent nucleus, meiosis I is sometimes called the reductional division.

Meiosis II is essentially the same as mitosis, dividing the two haploid nuclei formed in meiosis I. Prophase II, metaphase II, anaphase II, and telophase II are essentially identical to the stages of mitosis. Meiosis II begins with two haploid cells and ends with four haploid daughter cells.

Nuclear Division and Cytokinesis

Mitosis and meiosis result in the division of the nucleus. Nuclear division is nearly always coordinated with division of the cytoplasm. Cleaving of the cytoplasm to form new cells is called cytokinesis. Cytokinesis begins toward the middle or end of nuclear division and involves not just the division of the cytoplasm but also the organelles. In plants, after nuclear division ends, a new cell wall must be formed between the daughter nuclei. The new cell wall begins when vesicles filled with cell wall material congregate where the metaphase plate was located, producing a structure called the cell plate. When the cell plate is fully formed, cytokinesis is complete. Following cytokinesis, the cell returns to interphase. Mitotic daughter cells enlarge, reproduce organelles, and resume regular activities. Following meiosis, gametes may be modified or transported in the reproductive system.

Alternation of Generations

Meiotic daughter cells continue development only if they fuse during fertilization. Mitosis and meiosis alternate during the life cycles of sexually reproducing organisms. The life-cycle stage following mitosis is diploid, and the stage following meiosis is haploid. This process is called alternation of generations. In plants, the diploid state is referred to as the sporophyte generation, and the haploid stage as the gametophyte generation. In nonvascular plants, the gametophyte generation dominates the life cycle. In other words, the plants normally seen on the forest floor are made of haploid cells.

The sporophytes, which have diploid cells, are small and attached to the body of the gametophyte. In vascular plants, sporophytes are the large, multicellular individuals (such as trees and ferns) whereas gametophytes are very small and either are embedded in the sporophyte or are free-living, as are ferns. The genetic variation introduced by sexual reproduction has a significant impact on the ability of species to survive and adapt to the environment. Alternation of generations allows sexual reproduction to occur without changing the chromosome number characterizing the species.

—Joyce A. Corban and Randy Moore

See also: Cell Culture: Animal Cells; Cell Culture: Plant Cells; Cell Cycle, The; Cell Division; Cytokinesis; Gene Regulation: Eukaryotes; Polyploidy; Totipotency.

Further Reading

Alberts, Bruce, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts, and James D. Watson. *Molecular Biology of the Cell*. 4th ed. New York: Garland, 2002. The chapter “How Cells Are Studied” gives extensive information regarding study methods in cell biology. Light and electron microscopy are discussed as well as staining techniques and tissue culture.

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Campbell, Neil A., and Jane B. Reece. *Biology*. 6th ed. San Francisco: Benjamin Cummings, 2002. The chapter “Reproduction of Cells” provides extensive information regarding mitosis and the cell cycle. The phases of mitosis, the mitotic spindle, cytokinesis, control mechanisms, and abnormal cell division are discussed in detail. The chapter “Meiosis and Sexual Life Cycles” addresses the stages of meiosis, sexual life cycles, and a comparison of mitosis and meiosis. This text is intended for use in introductory biology and is very readable and informative.

John, Bernard. *Meiosis*. New York: Cambridge University Press, 1990. Review and discussion of meiosis, the antithesis of fertilization. Discusses the scheduling, mechanisms, biochemistry, and the genetic control of the events in meiosis.

Keeton, William T., and James L. Gould. *Biological Science*. 5th ed. New York: W. W. Norton, 1993. The chapter “Cellular Reproduction” discusses in detail the stages of mitosis and meiosis. Excellent diagrams allow visualization of cell division.

Model Organism: *Arabidopsis thaliana*

Field of study: Techniques and methodologies

Significance: *Arabidopsis thaliana*, also known as mouse-ear cress, can grow from seed to maturity and back to thousands of seeds again in about six weeks. Its short reproduction cycle and simple, low-cost cultivation allow genetic experiments with tens of thousands of plants and make it a popular and convenient organism to use as a model organism.

Key terms

BRASSICACEAE: the mustard family, a large, cosmopolitan family of plants with many wild species, some of them common weeds, including widely cultivated edible plants like cabbage, cauliflower, radish, rutabaga, turnip, and mustard

GENETIC MAP: a “map” showing distances between genes in terms of recombination frequency

Natural History

Although common as an introduction into America and Australia, *Arabidopsis thaliana* (often referred to simply by its genus name, *Arabidopsis*) is found in the wild throughout Europe, the Mediterranean, the East African highlands, and Eastern and Central Asia (which is probably where it originated). Since *Arabidopsis* is a low winter annual (standing about 1.5 decimeters), it flowers in disturbed habitats from

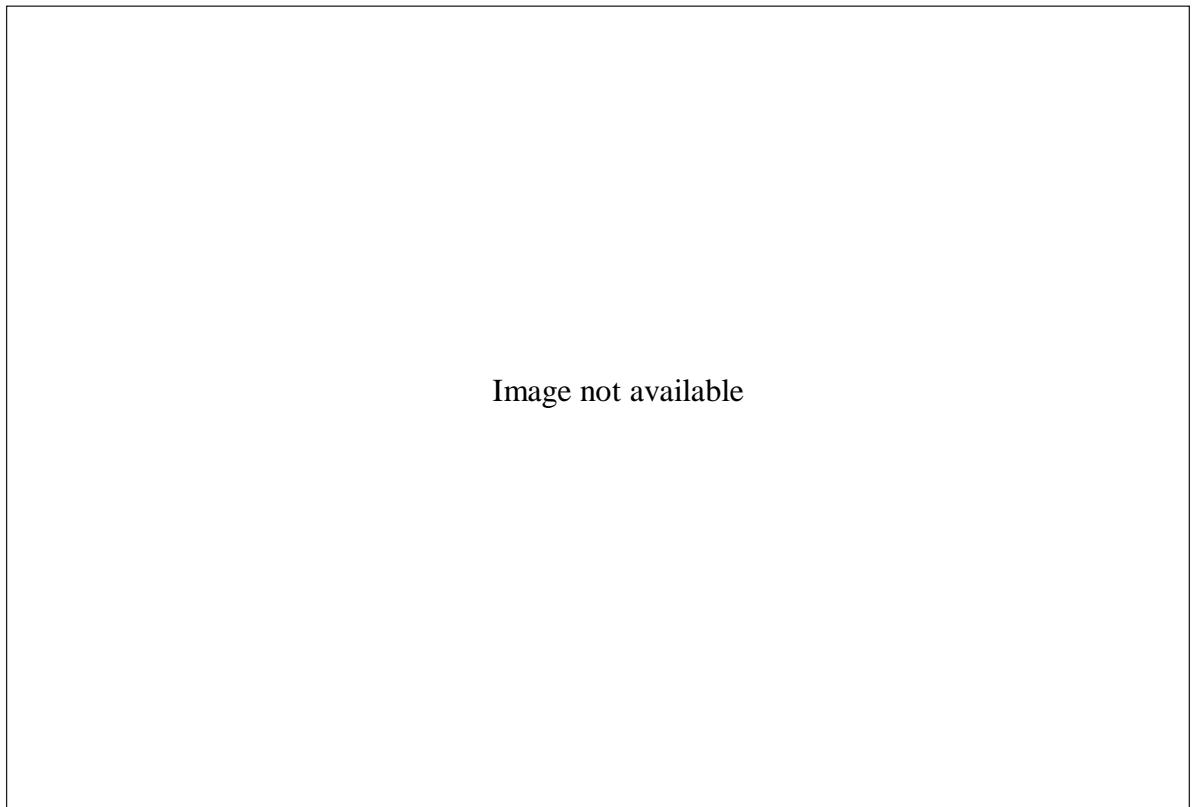


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Two specimens of mouse-ear cress, *Arabidopsis thaliana*. (AP/Wide World Photos)

March through May. *Arabidopsis* was first described by Johannes Thal (hence the *thaliana* as the specific epithet) in the sixteenth century in Germany's Harz Mountains, but he named it *Pilosella siliquosa* at the time. Undergoing systematic revisions and several name changes, the little plant was finally called *Arabidopsis thaliana* in 1842.

Several characteristics of *Arabidopsis* make it useful as a model organism: First, it has a short life cycle; it goes from seed to seed in only about three months, and each individual plant is prolific, yielding thousands of seeds. Second, the plants are small, easy to grow and to manipulate, so that many genetic screens can be done on petri dishes with a thousand seedlings examined inside just one dish. Also, the genome of *Arabidopsis* is relatively small, with 120 million base pairs (Mbp), 25,000 genes, and five chromosomes containing all the requisite information to encode an entire plant (similar to the functional complexity of the fruit fly *Drosophila*

melanogaster, long a favorite model organism among geneticists). Yet in comparison to the genome of corn (*Zea mays*), for example, *Arabidopsis* has a genome that is ten times smaller. Furthermore, *Arabidopsis* is easily transformed using the standard vector *Agrobacterium tumefaciens*. There are also a large number of mutant lines and genomic resources available for *Arabidopsis* at stock centers, and a cooperative multi-national research community of academic, government, and industry laboratories exists, all working with *Arabidopsis*.

History of Experimental Work with *Arabidopsis*

The earliest report of a mutant probably was made in 1873 by A. Braun, and Freidrich Laibach first compiled the unique characteristics of *Arabidopsis thaliana* as a model organism for genetics in 1943 (publishing the correct chromosome number of five much earlier, in 1907, later confirmed by other investigators).

Erna Reinholtz (a student of Laibach) submitted her thesis in 1945, published in 1947, on the first collection of X-ray-induced mutants. Peter Langridge established the usefulness of *Arabidopsis* in the laboratory in the 1950's, as did George Redei and other researchers, including J. H. van der Veen in the Netherlands, J. Veleminsky in Czechoslovakia, and G. Robbelin in Germany in the 1960's.

Maarten Koornneef and his coworkers published the first detailed genetic map for *Arabidopsis* in 1983. A genetic map allows researchers to observe approximate positions of heritable factors (genes and regulatory elements) on chromosomes. The 1980's saw the first steps in analysis of the genome of *Arabidopsis*. Tagged mutant collections were developed. Physical maps, with distances between genes in terms of DNA length, based on restriction fragment length polymorphisms (RFLPs), were also made in this time period. The physical maps allow genes to be located and characterized, even if their identities remained unknown.

The 1990's saw scientists outlining long-range plans for *Arabidopsis* through the Multi-national Coordinated *Arabidopsis* Genome Research Project, which called for genetic and physiological experimentation necessary to identify, isolate, sequence, and understand *Arabidopsis* genes. In the United States, the National Science Foundation (NSF), U.S. Department of Energy (DOE), and Agricultural Research Service (ARS) funded work done at Albany directed by Athanasios Theologis. NSF and DOE funds went also to Stanford, Philadelphia, and four other U.S. laboratories. Worldwide communication among laboratories and the creation of shared databases (particularly in the United States, Europe, and Japan) were established. Transformation methods have become much more efficient, and a large number of *Arabidopsis* mutant lines, gene libraries, and genomic resources have been made and are now available to the scientific community through public stock centers. The expression of multiple genes has been followed, too. Teresa Mozo provided the first comprehensive physical map of the *Arabidopsis* genome, published in 1999; she used overlapping fragments of cloned DNA. These fundamental data pro-

vide an important resource for map-based gene cloning and genome analysis. The *Arabidopsis* Genome Initiative, an international effort to sequence the complete *Arabidopsis* genome, was created in the mid-1990's, and the results of this massive undertaking were published on December 14, 2000, in *Nature*.

Comparative Genomics

With full sequencing of the genome of *Arabidopsis* completed, the first catalog of genes involved in the life cycle of a typical plant is now available, and the investigational emphasis has shifted to functional and comparative genomics. Scientists began looking at when and where specific genes are expressed in order to learn more about how plants grow and develop in general, how they survive in the changing environment, and how the gene networks are controlled or regulated. Potentially this research and work can lead to improved crop plants that are more nutritious, more resistant to pests and disease, less vulnerable to crop failure, and capable of producing higher yields with less damage to the natural environment. Since many more people die from malnutrition in the world than from diseases, the *Arabidopsis* genome takes on a much more important consideration than one might think. Of course, plants are fundamental to all ecosystems, and their energy input into those systems is essential and critical.

Already the genetic research on *Arabidopsis* has boosted production of staple crops such as wheat, tomatoes, and rice. The genetic basis for every economically important trait in plants—whether pest resistance, vegetable oil production, or even wood quality in paper products—is under intense scrutiny in *Arabidopsis*.

Although *Arabidopsis* is considered a weed throughout its ecological range on the planet, it is closely related to a number of vegetables, including broccoli, cabbage, brussels sprouts, and cauliflower, which are very important to humans nutritionally and economically. For example, a mutation observed in *Arabidopsis* has resulted in its floral structures assuming the basic shape of a head of cauliflower. This mutation in *Arabidopsis*, not surprisingly, is referred to simply as “cauliflower” and was isolated by

Martin Yanofsky's laboratory. The analogous gene from the cauliflower plant was examined, and it was discovered the cauliflower plant already had a mutation in this gene. From the study of *Arabidopsis*, therefore, researchers have uncovered why a head of cauliflower looks the way it does.

In plants there is an ethylene-signaling pathway (ethylene is a plant hormone) that regulates fruit ripening, plant senescence, and leaf abscission. The genes necessary for the ethylene-signaling pathway have been identified in *Arabidopsis*, including genes coding for the ethylene receptors. As expected, a mutation in these ethylene receptors would also cause the *Arabidopsis* plant to be unable to sense ethylene. Ethylene receptors have now been uncovered from other plant species from the knowledge gained from *Arabidopsis*. Harry Klee's laboratory, for example, has found a tomato mutation in the ethylene receptor, which prevents ripening. When the mutant *Arabidopsis* receptor is expressed in other plants, moreover, the transformed plants also exhibit this insensitivity to ethylene and the lack of ensuing processes associated with it. Therefore, the mechanism of ethylene perception would seem to be conserved in plants, and modifying ethylene receptors can induce change in a plant.

Advances in evolutionary biology and medicine are expected from *Arabidopsis* research, too. Robert Martienssen of Cold Spring Harbor Laboratory has referred to the completion of the *Arabidopsis* genome sequence as having major impact on human health as well as plant biology and agriculture. Surprisingly, some of the newly identified *Arabidopsis* genes are extremely similar or even identical to human genes linked to certain illnesses. No doubt there are many more mysteries to unravel with the proteome analysis of *Arabidopsis* (analysis of how proteins function in the plant), and the biological role of all the twenty-five thousand genes will keep scientists busy for some time to come. For example, this relatively "simple" little plant has surprised workers with its amazing genetic duplication where more than 70 percent of its DNA is copied at least once somewhere else on its genome.

—F. Christopher Sowers

See also: Cell Culture; Plant Cells; Extra-chromosomal Inheritance; Model Organisms.

Further Reading

- Bowman, John L. *Arabidopsis: An Atlas of Morphology and Development*. New York: Springer-Verlag, 1993. Contains images and descriptions of normal and mutant *Arabidopsis* plants.
- Russell, Peter J. *Genetics*. San Francisco, Calif.: Benjamin Cummings, 2002. Good genetic textbook with specific references to genetic duplications, genome sequences, homeotic genes, model organism considerations, and the regulation of development in *Arabidopsis*.
- Wilson, Zoe A. *Arabidopsis: A Practical Approach*. New York: Oxford University Press, 2000. Provides an introduction to techniques required for the use of *Arabidopsis* as an experimental system. Provides strategies for the identification, mapping, and characterization of mutants by microscopy, molecular cytogenetics, and gene expression analysis.

Web Site of Interest

The *Arabidopsis* Information Resource (TAIR). <http://www.arabidopsis.org>. The gateway to the *Arabidopsis* Genome Initiative (AGI), designed for the scientific community, consists of a searchable relational database with many different data types that can be viewed, analyzed, and downloaded. Also has pages for news, lab protocols, and links.

Model Organism: *Caenorhabditis elegans*

Field of study: Techniques and methodologies

Significance: *The roundworm Caenorhabditis elegans has helped scientists understand development of multicellular organisms. For their work using C. elegans to identify apoptosis, or programmed cell death, three scientists received the Nobel Prize. The C. elegans genome project has enabled scientists to develop much of the technology that was used to sequence the human genome. Re-*

search with this organism has also contributed to understanding genetics of the nervous system, aging, and even learning.

Key terms

CELL DIFFERENTIATION: a process during which a cell specifically expresses certain genes, ultimately adopting its final cell fate to become a specific type of cell, such as a neuron, or undergoing programmed cell death (apoptosis)

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

The Organism

The nematode *Caenorhabditis elegans* (*C. elegans*) has been the subject of intense analysis by biologists around the world. Nematodes, or roundworms, are simple metazoan animals that have cells specialized to form tissues and organs such as nerve tissue and digestive tissue. Analysis of genetic control of the events that lead to the formation of the tissues in *C. elegans* has revealed biological mechanisms that also control the differentiation of tissues and organs in more complex organisms such as humans.

Caenorhabditis elegans is a microscopic, 1-millimeter-long roundworm that lives in soils and eats bacteria from decaying materials. It belongs to the phylum *Nematoda* (the roundworms), which includes many significant plant and animal parasites. *Caenorhabditis elegans*, however, is free-living (nonparasitic) and does not cause any human diseases. It exists as two sexes, males (containing a single X chromosome) and hermaphrodites (containing two X chromosomes). Both male and hermaphrodite worms have five pairs of autosomal (non-sex) chromosomes. The hermaphrodites are self-fertile. They produce sperm first, which they store, and later “switch” gonads to begin producing eggs. These eggs may be fertilized by the hermaphrodite’s own sperm, or if the hermaphrodite mates with a male, sperm from the male will fertilize the eggs. A hermaphrodite that is not mated will lay approximately three

hundred fertilized eggs in the first four days of adulthood; hermaphrodites that mate with males will continue to lay eggs as long as sperm are present.

Caenorhabditis elegans eggs begin development within the uterus. They hatch as small L1 larvae and molt four times as they proceed through the easily recognizable larval stages of L2, L3, L4, and adult. The adult hermaphrodite is a little larger than the adult male and can be distinguished by the presence of fertilized eggs lined up in the uterus. The smaller males have specialized tails that contain structures for mating called copulatory spicules.

A Model Organism

Because of its small size and simple diet (bacteria), *C. elegans* is easily adapted to laboratory culture conditions. The worms are grown on small agar-filled petri plates that are seeded with *E. coli*. The worms live comfortably at room temperature, but elevating or lowering the temperature can speed up or slow down development, and changes in temperatures can even reveal conditional phenotypes of some genetic mutations.

One unmated hermaphrodite will produce three hundred progeny over the first four days of adulthood. Additionally, *C. elegans* has a short generation time of approximately three weeks. Obtaining large numbers of progeny allows thorough statistical analysis of the way a mutation is segregated within a population. Because researchers can screen large numbers of worms in a short period of time, extremely rare mutations are likely to be revealed. Genetically “pure” strains are also quickly produced.

Hermaphrodite genetics also provides advantages. Because hermaphrodites are self-fertile, getting homozygous mutations is not difficult. A hermaphrodite that is heterozygous for a given mutation (has one wild-type copy of a gene and one mutated copy of a gene) will produce progeny, one-fourth containing two mutated copies of the gene (homozygotes). Additionally, for researchers studying mutations that affect reproduction or mating behavior, having self-fertile hermaphrodites allows them to maintain mutations that affect processes such as sperm production. A hermaphrodite that

cannot make its own sperm can be mated to a wild-type male, and the mutation causing the defect can be maintained. This is not possible in organisms that are strictly male/female or that are strictly hermaphroditic.

Another strength of *C. elegans* is that the genetic strains can be frozen in liquid nitrogen and maintained indefinitely. Even fruit flies have to be constantly mated or “passaged” to maintain the genetic stocks for a laboratory. *Caenorhabditis elegans* strains are maintained in a central location, giving all scientists access to the same well-characterized genetic stocks.

Caenorhabditis elegans is a transparent worm, ideally suited for microscopic analysis. The origin and ultimate fate of every cell in the worm (the cell lineage) has been mapped and traced microscopically. Adult hermaphrodites have 959 somatic (non-sex) cell nuclei, and males

have 1,021. Because the entire cell lineage for the worm is known and the worm is transparent, researchers can use a laser to destroy a single, specific cell and observe how loss of one cell affects development of the worm. These kinds of studies have contributed to the understanding of how neurons find target cells and how one cell can direct the fate of another.

Embryonic Development: Asymmetric Divisions

Research on *C. elegans* has revealed how programmed genetic factors (autonomous development) and cell-cell interactions guide development of an organism from egg to adult. The very first division of the fertilized egg (zygote) in *C. elegans* is asymmetric (uneven) and creates the first difference in the cells of the organism that is reflected in the adult. This division pro-

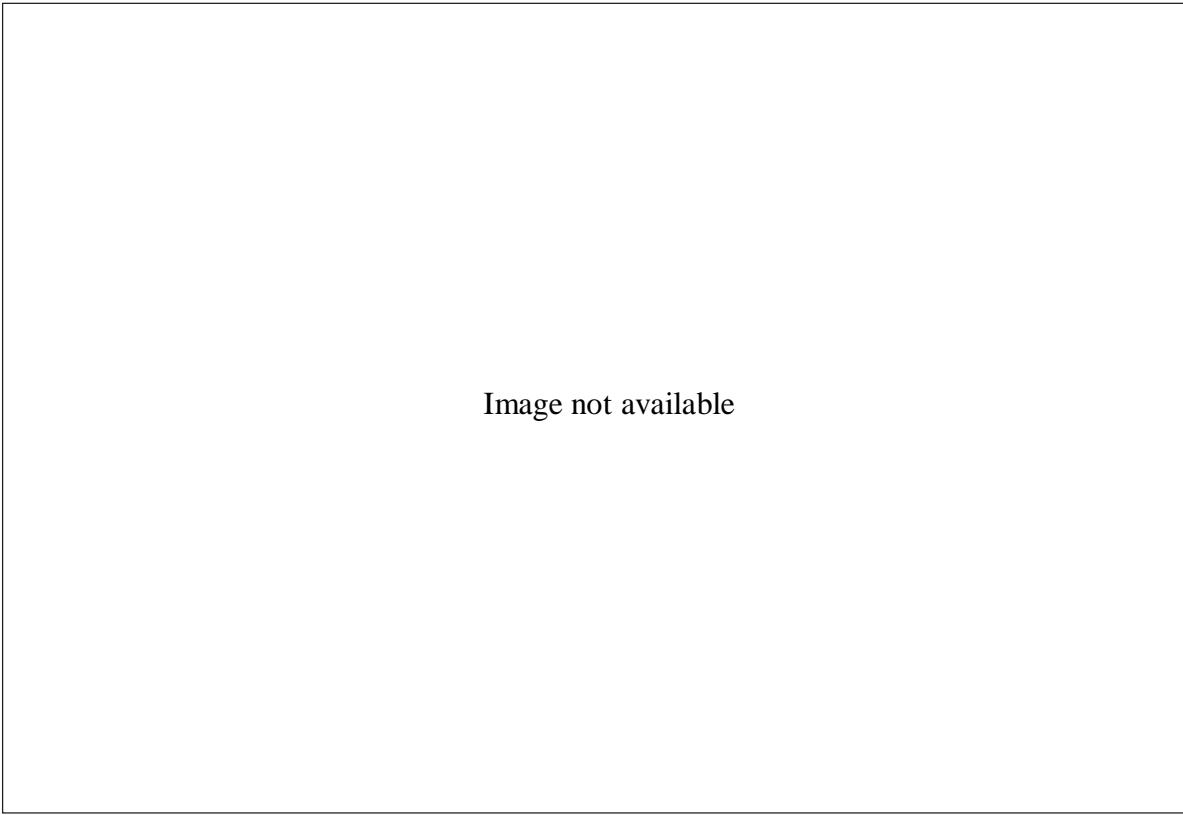


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H. Robert Horvitz points to an image of the nematode Caenorhabditis elegans. Working with this organism, he, Sydney Brenner, and John E. Sulston won the 2002 Nobel Prize in Physiology or Medicine for discovering genes regulating organ development and leading to apoptosis (programmed cell death)—discoveries with significant implications for cancer therapies. (AP/Wide World Photos)

duces two daughter cells called P and AB. AB is a large cell that gives rise to tissues such as muscle and digestive tract. P is a much smaller cell that ultimately produces the cells that become the gonads (sex cell-producing tissues). The difference in P and AB is determined by the segregation of small P granules in the cell. The location of these granules and the asymmetry of this initial division are determined by the point of entry of the sperm. Until the eight-cell stage, there is no genetic activity by the embryo; the first few divisions are directed by the maternal gene products. This is one example of how maternal gene products can influence the early development of an embryo.

Neural Development

One of the areas of later development that is particularly well understood in *C. elegans* is the development of the nervous system. The nervous system has been completely reconstructed with serial electron micrographs that reveal precisely how one neuron connects to another. Some neurons migrate to assume their final cell fate and function. These migrations are easily studied in the worm because of its transparency, and a single neuron can be visualized by marking it with green fluorescent protein. Many genes and their encoded proteins that have been identified as important for directing the growth, connectivity, and migration of *C. elegans* neurons are highly conserved in evolution and control axon guidance in the vertebrate spinal cord.

Apoptosis: Programmed Cell Death

The 2002 Nobel Prize in Physiology or Medicine was awarded to Sydney Brenner, H. Robert Horvitz, and John E. Sulston for identifying genetically controlled cell death in worms. Cell death is an important part of development in plants and animals. For instance, human embryos have webbing between fingers and toes. This webbing is composed of cells that die in the course of normal development before a human baby is born. The death of these cells occurs because of a genetic program in the cells, apoptosis. The genes that control apoptosis are highly conserved throughout evolution. Apoptosis also plays a role in cancer. Often cancer is

thought of as resulting from uncontrolled proliferation of cells, but it can also result when cells that should die during development fail to die. Scientists are looking at ways to specifically activate apoptosis in tumor cells in order to kill tumors. The clues for what genes to target for such treatments come from studies of the apoptosis pathway in organisms such as *C. elegans*.

A Molecular Tool

The first metazoan genome that was sequenced was *C. elegans*. Many of the technologies (automated machines, chemistries for isolating and preparing DNA) that were developed in the course of the *C. elegans* genome-sequencing project were directly applied to the human genome sequencing project, and many of the scientists involved in sequencing the *C. elegans* genome contributed expertise to the Human Genome Project as well.

The green fluorescent protein, which is a protein that was first used to trace neurons in *C. elegans*, is now used in experiments with a wide variety of organisms or cell cultures to follow specific cells or specific proteins during development. RNA interference, a technique that uses RNA specifically to knock out gene expression of a target gene, was first described in worms. This technique enables scientists to knock out gene expression at the RNA level rather than requiring laborious genetic engineering of DNA. This technique promises to be particularly useful for researchers working with human or other mammal cell culture systems.

Caenorhabditis elegans research identified the first presenilin, a class of proteins later implicated in Alzheimer's disease. Research on the worm has led to a greater understanding of certain proteins that are involved in cellular aging. Studies in *C. elegans* are even contributing to a better understanding of learning and behavior. Most *C. elegans* scientists are studying the worm because it provides a tool for answering many of the hows and whys of biology that cannot be answered easily in more complex systems. The answers to seemingly esoteric questions, such as how *C. elegans* sperm move, will shed light on fundamental biological processes shared by all organisms.

—Michele Arduengo

See also: Aging; Antisense RNA; Complementation Testing; Human Genome Project; Model Organism: *Chlamydomonas reinhardtii*; Model Organisms; Noncoding RNA Molecules.

Further Reading

Lewin, Benjamin. *Genes VII*. New York: Oxford University Press, 2001. Contains articles about many of the processes researched in the worm, including apoptosis.

Wood, W. B., et al. *The Nematode Caenorhabditis elegans*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1988. The first “worm book” contains an excellent overview of worm development and an introductory letter from Nobel laureate Sydney Brenner.

Web Site of Interest

Caenorhabditis elegans Web server. <http://elegans.swmed.edu>. Contains links to major worm labs around the world and to introductory information about the worm. Includes access to WormBase, a “repository of mapping, sequencing and phenotypic information.”

spond to genes but lack introns that are present in the actual genome

COSMID: a cloning vector, a hybrid of bacterial plasmid and bacteriophage vectors, that relies on bacteriophage capsules to infect bacteria; these are constructed with selectable markers from plasmids and two regions of lambda phage DNA known as cos (for cohesive end) sites

INSERTIONAL MUTAGENESIS: the generation of a mutant by inserting several nucleotides into a genome

MICROARRAY: a flat surface on which 10,000 to 100,000 tiny spots of DNA molecules fixed on glass or another solid surface are used for hybridization with a probe of fluorescent DNA or RNA

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

TRANSFORMATION: a change in both genotype and phenotype resulting from the uptake of exogenous DNA

The Organism

Chlamydomonas reinhardtii is the best-researched member of the green algal genus *Chlamydomonas* (Greek *chlamus*, a cloak, plus *monas*, solitary). *Chlamydomonas reinhardtii* is unicellular with a definite cell wall that consists of glycoproteins rich in the amino acid hydroxyproline. A large, solitary chloroplast folded into a cup shape dominates most of the cytoplasm. The presence of this chloroplast allows autotrophic growth, although *C. reinhardtii* is capable of using acetate as an external carbon source. A circular body that is prominent within the chloroplast is referred to as the pyrenoid. It is the site of carbohydrate synthesis during the light-independent reactions of photosynthesis. The chloroplast also contains a red eyespot with a rhodopsin-like pigmented photoreceptor, called the stigma, that permits phototaxis. *Chlamydomonas reinhardtii* cells display positive phototaxis (that is, swimming toward light) in moderate light and negative phototaxis in intense light.

The cell nucleus is visible with light micro-

Model Organism: *Chlamydomonas reinhardtii*

Field of study: Techniques and methodologies

Significance: *Chlamydomonas reinhardtii* is a unicellular green alga that has been extremely useful as a genetics model organism. It has a simple life cycle, is easily mutable, and is accessible for molecular genetic studies.

Key terms

BACTERIAL ARTIFICIAL CHROMOSOME (BAC): a vector used to clone large fragments of DNA (up to 500 kb) that can be readily inserted in a bacterium, such as *Escherichia coli*

COMPLEMENTARY DNA (cDNA): a DNA molecule that is synthesized using messenger RNA (mRNA) as a template and the enzyme reverse transcriptase; these molecules corre-

copy and predominates cross-sectional images in electron microscopy, along with the nucleolus. Electron microscopy also indicates sixteen or more chromosomes, which is consistent with the seventeen linkage groups defined by cytogenetic analysis. The cell's anterior end consists of two contractile vacuoles, and mitochondria are dispersed throughout the cytosol. Two long, whiplike flagella extend from basal bodies, which are also located at the anterior end of the cell. *Chlamydomonas reinhardtii* swims using a breaststroke motion. Internally the flagella consist of a central pair of microtubules surrounded by nine doublets. Each doublet consists of arms made of the protein dynein. The dynein interacts with adjacent doublets by pressing and sliding against the neighboring microtubule when adenosin triphosphate (ATP) is hydrolyzed. This brings about the flagellar beat and allows the organism to swim.

Chlamydomonas reinhardtii reproduces asexually by mitotic divisions. Parental cells can produce as many as sixteen progeny cells by successive divisions within the cell wall. Each progeny cell secretes a cell wall and generates flagella. The new cells escape by secreting autolytic enzymes that digest the parental cell wall.

Mating and Laboratory Analysis

The vegetative form of *C. reinhardtii* is haploid and exists as one of two genetically distinct mating types (mt^+) and (mt^-). When deprived of nitrogen, cells of each mating type differentiate into gametes. Gametes of opposite mating types come into contact with each other by way of their flagella. The gametes fuse, thereby forming a zygote. The zygote secretes a heavy wall and becomes a zygospor. Zygospores can remain dormant and viable in soils for several years. Light and nitrogen can bring about zygospor germination. Four biflagellated cells, known as zoospores, are released. In some strains, meiosis occurs prior to the release of zoospores, followed by a mitotic division. The result is the release of eight zoospores rather than four.

Cells of *C. reinhardtii* are easy to culture. They grow copiously in defined culture media under varying environmental conditions. Mating can be induced when cells of opposite

mating types are placed in a nitrogen-free medium. The zygote formed from such a mating can produce four unordered tetrads on appropriate media. Sometimes an additional mitotic event generates eight haploid products that are easy to recover. These features have made *C. reinhardtii* extremely useful as an experimental organism.

Mutagenesis and Transmission Genetics

Research in the 1950's led to the isolation of mutants displaying defects in the ability to photosynthesize. Since then mutants have been developed that affect every structure, function, and behavior of *C. reinhardtii*. Ultraviolet or chemical methods can be used to induce mutants. One of the first mutants isolated was resistant to the antibiotic streptomycin (designated sr). These cells are able to grow on media supplemented with streptomycin as well as media free of streptomycin. Wild-type cells (designated ss) are unable to grow on media containing the antibiotic. Reciprocal crosses with cells of these distinct phenotypes resulted in segregation patterns that departed significantly from Mendelian expectations. The sr phenotype was clearly transmitted only through mt^+ cells. Further study has shown that resistance passed through the mt^+ chloroplast. The chloroplast contains more than fifty copies of a circular, double-stranded DNA molecule. Uniparental inheritance has been demonstrated for the mitochondrial genome, too. This genome contains fewer genes than the chloroplast, but antibiotic resistant mutations have been generated, along with other types. It is interesting to note that mitochondrial inheritance of antibiotic resistance appears to be transmitted by way of mt^- cells.

Mutational analysis has elucidated aspects of nuclear inheritance, also. The mating type phenotype segregates in a 1:1 ratio in accordance with Mendelian principles. With the advent of molecular techniques, insertional mutagenesis has resulted in a wide array of mutants, including nonphotosynthetic, nonmotile, antibiotic resistant, herbicide resistant, and many more. This type of analysis has resulted in mapping nearly two hundred nuclear loci.

Molecular Analysis

Transformation of *C. reinhardtii* is relatively easy and can be carried out by mixing with DNA-coated glass beads or electroporation, that is, using a current to introduce the DNA into a cell. The frequency of transformation success is highest in wall-less mutants or cells whose walls have been removed prior to transformation. Both nuclear, mitochondrial, and chloroplast transformation studies have been performed, leading to the development of several molecular constructs that have been used to study gene expression. Cosmids and BAC libraries have been created for several markers in order to make the current molecular map of about 240 markers, each having an average spacing of 400 to 500 kb. These markers have been placed on the seventeen linkage groups mentioned previously.

Thus far, the greatest impact these molecular markers are having is in the study of photosynthesis. A chloroplast gene known as *Stt7* has been characterized using these methods. *Stt7* is required for activation of the major light-harvesting protein and interactions between photosystem I and photosystem II when light conditions change. Chloroplast and nuclear transformations have been used in conjunction with developmental mutants to study chloroplast biogenesis. This has increased researchers' understanding of the expression and regulation of many chloroplast genes. A cDNA library composed of many unique chloroplast genes is being constructed and their coding regions sequenced. These cDNAs are called expressed sequence tags (ESTs) and have proven extremely useful for identifying protein-coding genes in other organisms. Thousands of these cDNAs could be placed on pieces of glass the size of a microscope slide using microarray technology to monitor changes in gene expression of virtually the entire genome at the same time. Interactions between the nuclear genome and the chloroplast genome can be assessed in this manner as well.

—Stephen S. Daggett

See also: cDNA Libraries; Extrachromosomal Inheritance; Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Drosophila melanogaster*,

Model Organism: *Escherichia coli*; Model Organism: *Mus musculus*; Model Organism: *Neurospora crassa*; Model Organism: *Saccharomyces cerevisiae*; Model Organism: *Xenopus laevis*; Model Organisms.

Further Reading

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- _____. *The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use*. San Diego, Calif.: Academic Press, 1989. The ultimate guide to working with *Chlamydomonas* species, including a detailed look at the organism, a thorough literature review, and several protocols, some for teaching purposes.

Web Site of Interest

Chlamydomonas Genetics Center. <http://www.biology.duke.edu/chlamy>. Sponsored by the National Science Foundation, the clearinghouse for data on the genetics of this model organism, including the genome project and the nuclear, chloroplast, and mitochondrial genomes.

Model Organism: *Drosophila melanogaster*

Field of study: Techniques and methodologies

Significance: *Drosophila melanogaster* is the scientific name for a species of fruit fly whose study led scientists to discover many of the fundamental principles of the inheritance of traits. The first genetic map that assigned genes to specific chromosomes was developed for *Drosophila*. With advances in molecular technology, continued study

of *Drosophila* has led to a greater understanding of genetic control in early embryonic development.

Key terms

LINKED GENES: genes, and traits they specify, that are situated on the same chromosome and tend to be inherited together

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

SEX CHROMOSOMES: The X and Y chromosomes, which determine sex in many organisms; in *Drosophila*, a female carries two X chromosomes and a male carries one X and one Y chromosome

Early Studies of *Drosophila*

By the early 1900's, scientists had discovered chromosomes inside of cells and knew that they occurred in pairs, that one partner of each pair was provided by each parent during reproduction, and that fertilization restored the paired condition. This behavior of chromosomes paralleled the observations of Austrian botanist Gregor Mendel, first published in 1866, which showed that traits in pea plants segregated and were assorted independently during reproduction. This led geneticists Walter Sutton, Theodor Boveri, and their colleagues to propose, in 1902, the "chromosome theory of inheritance," which postulated that Mendel's traits, or "genes," existed on the chromosomes. However, this theory was not accepted by all scientists of the time.

Thomas Hunt Morgan was an embryologist at Columbia University in New York City, and he chose to study the chromosome theory and inheritance in the common fruit fly, *Drosophila melanogaster*. This organism was an ideal one for genetic studies because a single mating could produce hundreds of offspring, it developed from egg to adult in only ten days, it was inexpensively and easily kept in the laboratory, and it had only four pairs of chromosomes that were easily distinguished with a simple microscope. Morgan was the first scientist to keep large numbers of fly "stocks" (organisms with



Thomas Hunt Morgan, one of the most important biologists in classical transmission genetics, established the "Fly Room" at Columbia University in 1910, where for the next quarter century he and his students studied the genetics of the fruit fly. (© The Nobel Foundation)

particular characteristics), and his laboratory became known as the "fly room."

After one year of breeding flies and looking for inherited variations of traits, Morgan found a single male fly with white eyes instead of the usual red, the normal or wild-type color. When he bred this white-eyed male with a red-eyed female, his results were consistent with that expected for a recessive trait, and all the offspring had wild-type eyes. When he mated some of these offspring, he was startled to discover a different inheritance pattern than he expected from Mendel's experiments. In the case of this mating, half of the males and no females had white eyes; Morgan had expected half of all of the males and females to be white-eyed. After many more generations of breeding, Morgan was able to deduce that eye color in a fly was re-

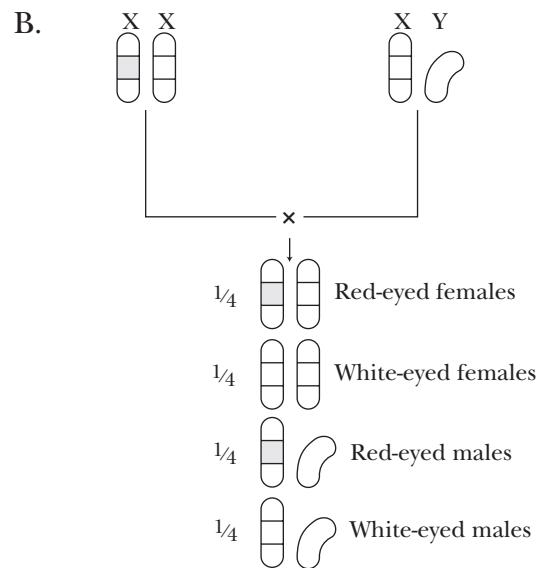
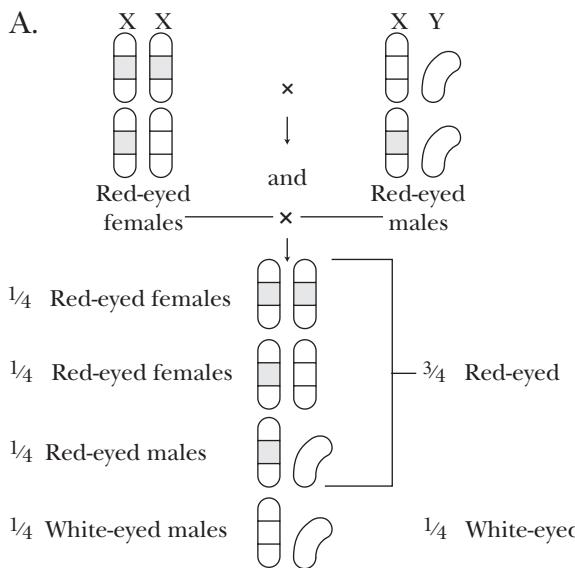
lated to its sex, and he located the eye-color gene to the X chromosome of the fruit fly. The X chromosome is one of the sex chromosomes. Because a female fly has two X chromosomes and a male has one X and one Y chromosome, and because the Y chromosome does not carry genes corresponding to those on the X chromosome, any gene on the male's X chromosome is expressed as a trait, even if it is normally recessive. This interesting and unusual example of the first mutant gene in flies was called a "sex-linked" trait because the trait was located on the X chromosome.

This important discovery attracted many students to Morgan's laboratory, and before long they found many other unusual inherited traits in flies and determined their inheritance patterns. One of the next major discoveries by members of the "fly lab" was that of genes existing on the same chromosome, information that was used to map the genes to individual chromosomes.

Linked Genes and Chromosome Maps

Many genes are located on each chromosome. Genes, and the traits they specify, that are situated on the same chromosome tend to be inherited together. Such genes are referred to as "linked" genes. Morgan performed a variety of genetic crosses with linked genes and developed detailed maps of the positions of the genes on the chromosomes based on his results. Morgan did his first experiments with linked genes in *Drosophila* that specified body color and wing type. In fruit flies, a brown body is the wild type and a black body is a mutant type. In wild flies wings are very long, while one mutant variant has short, crinkled wings referred to as "vestigial" wings. When Morgan mated wild-type females with black-bodied, vestigial-winged males, the next generation consisted of all wild-type flies. When he then mated females from this new generation with black-bodied, vestigial-winged males, most of the progeny were either brown and normal winged

Thomas Hunt Morgan's Experimental Work with *Drosophila*



Morgan's experiments discovered such results as the following: A. A red-eyed female is crossed with a white-eyed male. The red-eyed progeny interbreed to produce offspring in a $\frac{3}{4}$ red to $\frac{1}{4}$ white ratio. All the white-eyed flies are male. B. A white-eyed male is crossed with its red-eyed daughter, giving red-eyed and white-eyed males and females in equal proportions. (Electronic Illustrators Group)

or wild-type black and vestigial winged, in about equal proportions. A few of the offspring were either just black bodied (with wild-type wings) or vestigial winged (with wild-type body color), trait combinations found in neither parent. Because of the equal distribution of these mutant traits between males and females, Morgan knew the genes were not sex linked. Because the traits for body color and wing length generally seemed to be inherited together, he deduced that they existed on the same chromosome.

As Morgan and his students and colleagues continued their experiments on the inheritance of body color and wing length, they observed a small but consistent percentage of offspring with trait combinations not observed in either parent, referred to as nonparents. After repeating these experiments with many different linked genes, Morgan discovered that chromosomes exchange pieces during egg and sperm formation. This exchange of chromosome pieces occurs during a process called meiosis, which occurs in sexually reproducing organisms and results in the production of gametes, generally eggs and sperm. During meiosis, the homologous chromosomes pair tightly and may exchange pieces; since the homologous chromosomes contain genes for the same trait along their length, this exchange does not present any genetic problems. The eggs or sperm produced through meiosis contain one of each pair of chromosomes.

In some of Morgan's genetic crosses, flies carried one chromosome with alleles (alternate forms of a gene at a specific locus) for black bodies and vestigial wings. The homologous chromosome carried wild alleles for both traits. During meiosis, portions of the homologous chromosomes exchanged pieces, resulting in some flies receiving chromosomes carrying genes for black bodies and normal wings or brown bodies and vestigial wings. The exchange of chromosome pieces resulting in new combinations of traits in progeny is referred to as "recombination." Morgan's students and

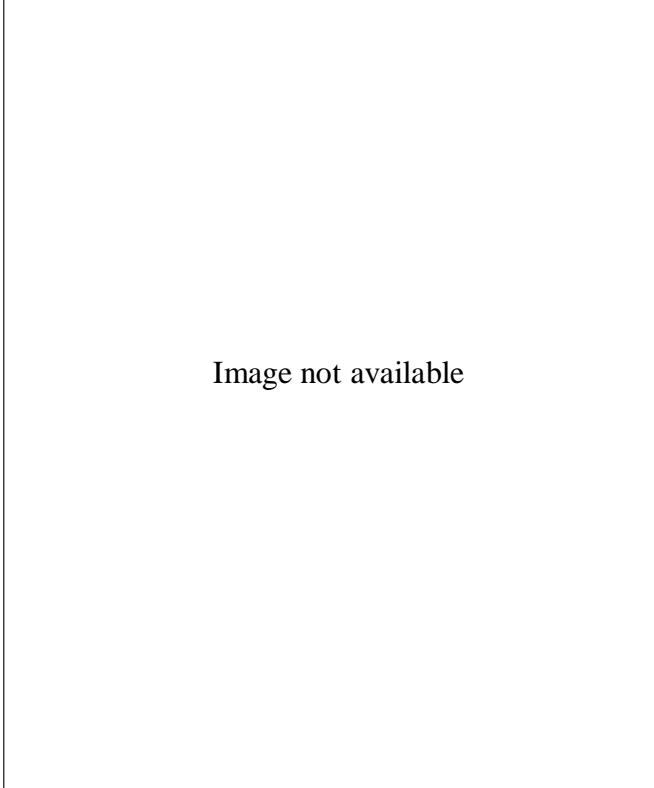


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Alfred H. Sturtevant (California Institute of Technology)

colleagues pursued many different traits that showed genetic recombination. In 1917, one of Morgan's students, Alfred Sturtevant, reasoned that the further apart two genes were on a chromosome, the more likely they were to recombine and the more progeny with new combinations of traits would be observed. Over many years of work, Sturtevant and his colleagues were able to collect recombination data and cluster all the then-known mutant genes into four groupings that corresponded to the four chromosomes of *Drosophila*. They generated the first linkage maps that located genes to chromosomes based on their recombination frequencies.

The chromosomes in the salivary glands of the larval stage of the fruit fly are particularly large. Scientists were able to isolate these chromosomes, stain them with dyes, and observe them under microscopes. Each chromosome had an identifying size and shape and highly detailed banding patterns. X rays and chemi-

cals were used to generate new mutations for study in *Drosophila*, and researchers realized that in many cases they could correlate a particular gene with a physical band along a chromosome. Also noted were chromosome abnormalities, including deletions of pieces, inversions of chromosome sections, and the translocation of a portion of one chromosome onto another chromosome. The pioneering techniques of linkage mapping through recombination of traits and physical mapping of genes to chromosome sections provided detailed genetic maps of *Drosophila*. Similar techniques have been used to construct gene maps of other organisms, including humans.

Control of Genes at the Molecular Level

This seminal genetic work on *Drosophila* was unparalleled in providing insights into the mechanisms of inheritance. Most of the inheritance patterns discovered in the fruit flies were found to be applicable to nearly all organisms. However, the usefulness of *Drosophila* as a research organism did not end with classical transmission genetics; it was found to provide equally valuable insight into the mechanisms of development at the level of DNA.

Drosophila were discovered to be ideal organisms to use in the study of early development. During its development in the egg, the *Drosophila* embryo orchestrates a cascade of events that results in the embryo having a polarity (a head and a tail), with segments between each end defined to become a particular body part in the adult. For example, the second segment of the thorax will support one pair of wings and one of the three pairs of legs. By studying many types of mutants that showed bizarre appearances as adults (for example, two sets of wings or legs replacing the normal antennae on the head), scientists were able to elucidate some of the mechanisms that control development in nearly all animals.

Developmental instructions from the mother fruit fly are sequestered in the egg. When the egg is fertilized, these instructions begin to "turn on" genes within the fertilized eggs that begin to establish the directionality and segment identity within the embryo. So many genes are involved in this process that a defect in a single one will truncate the rest of development, resulting in a severely mutated fly. It was found that conserved regions of DNA outside of the developmental genes received the signals to "turn on." Such sequences were found to be present in all animals studied. These control regions were termed "homeoboxes" after the homeotic genes that control the overall body plan of an organism in early development.

Many other aspects of *Drosophila* were found to be useful in understanding the structure and function of the DNA of all organisms. It was found that in *Drosophila*, large pieces of DNA will, under certain circumstances, pop out of the chromosome and reinsert themselves at another site. One such element, called a P element, was used by scientists to introduce nonfly DNA into the fruit fly embryo, thus providing information on how DNA is expressed in animals. This work also provided early clues into the successful creation of transgenic animals commonly used in research to study cancer and other diseases.

Impact and Applications

Genetic studies of *Drosophila melanogaster* have provided the world with a fundamental understanding of the mechanisms of inheritance. In addition to the inheritance modes shown by Mendel's studies of pea plants, fruit fly genetics revealed that some genes are sex linked in sexually reproducing animals. The research led to the understanding that while many genes are linked to a single chromosome, the linkage is not necessarily static, and that chromosomes can exchange pieces during recombination. The ease with which mutant fruit flies could be generated led to the development of detailed linkage maps for all the chromosomes and ultimately to the localization of genes to specific regions of chromosomes. With the advent of molecular techniques, it was discovered that *Drosophila* again provided a wealth of information in terms of mobile genetic elements and developmental studies. Although all of these breakthroughs were scientifically interesting in terms of the flies themselves, many of the breakthroughs helped identify fundamental principles consistent among all animals. Most of what is known about hu-

man genetics and genetic diseases has come from these pioneering studies with *Drosophila*.

Because of the sheer numbers of offspring from any mating of flies, their very short life cycle, and large numbers of traits that are easily observable, fruit flies have become an ideal system to screen for potential chemical carcinogens (cancer-causing agents) or mutagens (agents that cause mutations in DNA) in humans. Flies are exposed to the chemical in question and mated; then their offspring are analyzed for any abnormal appearances or behaviors, or for low numbers of offspring. Should a test substance cause any variation in the expected outcome of a cross, it is then subjected to more rigorous research in other organisms.

The versatile, easy-to-care-for, inexpensive fruit fly is often a fixture in classrooms around the world. Indeed, many geneticists have traced their passion to their first classroom encounters with fruit flies and the excitement of discovering the inheritance patterns for themselves. Flies are also routinely used in the study of neural pathways, learning patterns, behavior, and population genetics. Because of the ease of study and the volumes of information that have been compiled about its genetics, development, and behavior, *Drosophila* will continue to be an important model organism for biological study. The completion of the complete genome sequence of *Drosophila* should greatly increase the usefulness of this model organism, allowing an even more detailed understanding of its genetics.

—Karen E. Kalumuck

See also: Aging; Bioinformatics; Biological Clocks; Chemical Mutagens; Chromosome Mutation; Chromosome Theory of Heredity; Developmental Genetics; Genetics, Historical Development of; Homeotic Genes; Human Genome Project; Inbreeding and Assortative Mating; Incomplete Dominance; Lateral Gene Transfer; Linkage Maps; Metafemales; Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Escherichia coli*; Model Organism: *Mus musculus*; Model Organism: *Neurospora crassa*; Model Organism: *Saccharomyces cerevisiae*; Model Organism: *Xenopus laevis*; Model Organisms; Mutation and Mutation

genesis; Natural Selection; Noncoding RNA Molecules; Population Genetics.

Further Reading

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Web Sites of Interest

Drosophila Virtual Library. <http://www.ceolas.org/VL/fly>. Links to databases, labs, and other Web resources of interest to researchers.

FlyBase. <http://flybase.bio.indiana.edu:82>. A joint venture of the Berkeley and European Drosophila Genome Projects. Includes data from the *Drosophila* genome projects and a vast amount of other information—bibliographies, directories, descriptions of chromosomal aberrations, lists of *Drosophila* stocks, genome project data, images, and more.

Model Organism: *Escherichia coli*

Fields of study: Bacterial genetics; Techniques and methodologies

Significance: Through the study of the genetics of *Escherichia coli* biologists have come to understand the molecular-level regulation of gene expression and how genes direct routine activities of living cells. This understanding of the genetics of this bacterium has led to the extensive use of this organism in biotechnology. Such technology permits the introduction of foreign genes into the organism's cells, which may result in new bacterial strains capable of solving problems as diverse as environmental pollution, food and energy shortages, and the spread of diseases.

Key terms

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

OPERON: a genetic unit consisting of structural genes coding for amino acid chains; an operator gene controlling the transcriptional (message encoding) activity of the structural genes

REPLICATION: the process of DNA duplication

A Suitable Experimental Organism

Discovered in 1885 by Theodor Escherich, *Escherichia coli* is the most intensely studied bacterium in genetics. In fact, of the earth's living organisms, this bacterium is one of the better understood, and its use as a favorite experimental organism dates to the mid-twentieth century. Even before gaining a rich genetic history, the bacterium was selected for genetic research for several reasons: its ease of handling in experiments, its twenty-minute generation time, its single copy of each gene, and its meager genetic material. Results derived from genetic experiments with *E. coli* have significantly influenced the thinking of biologists, and the genetics of *E. coli* has provided evidence that explains mechanisms underlying important processes: *E. coli* chromosome organization; regulation of gene expression; DNA replication, transcription, and translation; mutation and DNA repair; biotechnology; and evolution.

The *E. coli* cell usually contains a single chromosome, although the cell's actual number of chromosomes depends on the bacterium's growth rate. Fast-growing *E. coli* have two to four chromosome copies per cell, while the slow-growing counterparts have one to two copies per cell. These multiple copies, however, are genetically identical, permitting *E. coli* to behave as haploids (cells containing a single chromosome). This chromosome, a dense cellular structure carrying hereditary information from generation to generation, consists of a single molecule of double-stranded DNA. The DNA, in a closed-circle form, is located in the nucleoid, a central region of the *E. coli* cell. The DNA of *Escherichia coli* is probably made up

of four million base pairs and carries 2,800 genes. These genes constitute 75 percent of the DNA molecule; the remaining DNA consists of regions between genes, such as the stretch of DNA acting as the unique origin of replication for the *E. coli* DNA molecule.

Packaging this DNA into the nucleoid is an important concept in *E. coli* genetics because the length of the bacterial chromosome containing the DNA is twelve hundred times that of the *E. coli* cell. The chromosome, therefore, is packaged in a highly compact form. This compact DNA consists of one hundred independent genetic segments, each having forty thousand base pairs (bp) of DNA containing extra twists, with the ends of each genetic segment held, presumably, by proteins. The extra-twisted DNA in one genetic segment is unaffected by events influencing extra twisting of DNA in other genetic segments. Such a structure forms because the DNA, a negatively charged molecule, associates with positively charged structural proteins.

About one-third of the 2,800 genes in *E. coli* have been located on the bacterium's chromo-

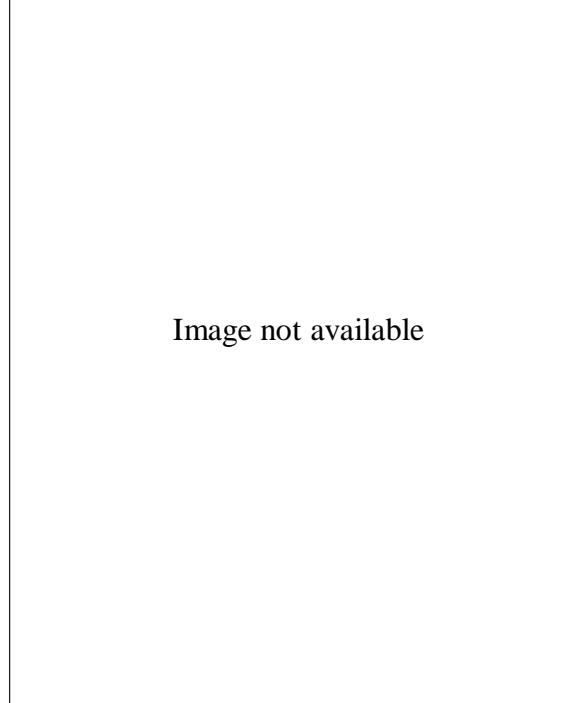
A large rectangular box with a thin black border, occupying the right half of the page. It is intended to hold an image of an *E. coli* cell, but the text "Image not available" is displayed instead.

Image not available

A single E. coli cell. (AP/Wide World Photos)

some using gene mapping (which determines the locations of genes along the chromosome) and recombinant DNA techniques such as DNA sequencing (which determines the order of the nucleotides in DNA). Of the located genes, 260 of them are organized into seventy-five operons, with the remaining 740 genes scattered, perhaps randomly, around the rest of the DNA molecule.

Regulation of Gene Expression

The genetics of *E. coli* reveals that 26 percent of its mapped genes are organized in transcriptional units (DNA segments containing message-encoding start and stop signals) called operons; these work to regulate gene expression. Operons, coordinately regulated units, often contain genes with related functions. Each regulated unit has a set of adjoining structural genes, a promoter for enzyme binding, and an operator for regulatory protein binding. If the genes encode enzymes involved in an anabolic pathway (in which chemical reactions form larger molecules from smaller ones), they are usually turned off in the presence of the pathway's end product. Alternatively, if the genes encode enzymes involved in a catabolic pathway (in which chemical reactions break down large molecules into smaller ones), they are often expressed in the presence of the enzymes' substrates (molecules whose actions are increased).

The genetics of the lactose (*lac*) and the tryptophan (*trp*) operons were unraveled using *E. coli*. This earned the bacterium a place in history for helping to explain the regulation of gene expression. The *lac* operon consists of three structural genes—Z, Y, and A—that encode beta-galactosidase, beta-galactoside permease, and beta-galactoside transacetylase, respectively. Other operon components are the promoter and the operator adjoining the Z gene. The regulator gene has its own promoter and adjoins the operon. The *lac* operon is an example of an inducible system because the operon's three structural genes are transcribed (put into message code) only in the presence of lactose. In the absence of lactose, the *lac* repressor (a protein product of the regulator gene) binds to the operator and prevents RNA

polymerase from initiating operon transcription.

In contrast to the *lac* operon, the *trp* operon is a repressible system, in which the production of an enzyme stops with the addition of the end product of the enzyme reaction. Transcription of the operon's five structural genes, which encode enzymes involved in tryptophan production, is repressed in the presence of tryptophan. A second regulatory mechanism, called attenuation, also controls the system.

Based on the genetics of *E. coli*, biologists know that operon function may change if fused to a new operator. French molecular biologist François Jacob's research team showed this for the structural genes of the *lac* operon. The team used DNA fragments carrying parts of the *lac-pur* region, but with an added deletion that eliminated the *lac* operator and part of the Z gene. These modified DNA fragments were inserted into *E. coli* that were unable to produce the enzymes permease and acetylase. The functional lac enzymes produced by the modified particles were no longer activated by lactose. Such enzymes were instead under control of the deactivated purine operator. As a result, excessive purine caused repression of galactoside permease and acetylase. In *E. coli*, gene expression can be regulated at different levels, but transcriptional regulation is the most common.

DNA Replication, Transcription, and Translation

Early in the study of *E. coli*, Matthew Meselson and Franklin Stahl determined how DNA duplicates itself in the bacterium. They grew the organism, across several generations, in culture media containing nucleotides enriched with nitrogen 15 (a heavier isotope of nitrogen), which would be incorporated into all newly synthesized strands of DNA. Then some of these cells with nitrogen 15 enriched DNA were transferred to media containing nucleotides containing normal nitrogen 14, so that all newly synthesized strands of DNA would then contain nitrogen 14 rather than nitrogen 15. After allowing enough time for the cells to divide once, they isolated their DNA and then used cesium chloride density-gradient centrifugation to characterize their results. Their

Sequencing the *E. coli* Genome

As part of the Human Genome Project (begun in 1990), several model organisms were selected for sequencing. Such direct DNA sequence information could be correlated with the extensive data available from classical and molecular genetics. Not only would it provide a means for identifying similar genes in the human genome; it would also provide a means for comparative genomics, that is, to identify similar genes among both model organisms and sequence data from related organisms. The latter is useful to explore the evolution of specific genes and evolutionary relatedness of organisms. Consequently, the sequencing of the *Escherichia coli*, the prokaryotic organism most studied genetically, biochemically, and physiologically, was of high priority. Due to efforts led by Frederick Blattner at the University of Wisconsin, along with colleagues at four other institutions, the six-year project resulted in the complete genomic sequence of *E. coli* K12 (strain MG1655), published on September 5, 1997, in the journal *Science*; the final corrected sequence was updated in October, 2001.

Although there are many different strains of *E. coli*, strain MG1655 was chosen because it is a well-established, stable laboratory strain. The sequencing of a second laboratory strain, W3110, was completed by a consortium of Japanese researchers. The *E. coli* MG1655 genome consists of 4,639,221 base pairs, a number slightly higher than estimated from earlier studies. Of these, 87.8 percent are found in protein-coding genes, 0.8 percent in stable RNA sequences, 0.7 percent in noncoding repeats, and approximately 11 percent in regulatory and other sequences. One difference between eukaryote and prokaryote genomes is the large amount of noncoding sequences

in the former and the relative lack of such sequences in the latter. This was borne out by the *E. coli* sequence: The genome analysis indicates that there are 4,405 genes, including 4,286 protein-coding sequences, about 50 percent more than originally predicted. Only about one-third of these represent well-characterized proteins. There are also 7 ribosomal RNA (rRNA) operons and 86 transfer RNA (tRNA) genes.

While *E. coli* is a normal inhabitant of the human gut, the average person associates the name *E. coli* with strain O157:H7, a human pathogen causing intestinal hemorrhaging and resulting in about five hundred deaths per year in the United States. Strain O157:H7 has acquired two toxin genes from a related bacterium, *Shigella dysenteriae*, often found in cattle. The complete sequence of O157:H7 was completed in January, 2001, and provides interesting comparisons. Its genome is 5,528,455 base pairs, with 5,416 genes of which 1,387 are not found in *E. coli* MG1655. These new genes include those for virulence factors, alternative metabolic capacities, and new prophages. Moreover, O157:H7 lacks 528 genes found in *E. coli* MG1655. These marked differences lead some to believe that O157:H7 is actually a different species, having evolutionarily diverged from standard *E. coli* about 4.5 million years ago. This example of comparative genomics illustrates its potential as a powerful tool for medical and other applications.

Sequencing of other strains of *E. coli*, particularly pathogenic strains, is ongoing under the aegis of the *E. coli* Genome Project, based at the University of Wisconsin.

—Ralph R. Meyer

findings, verified through autoradiography several years later by John Cairns, showed that in *E. coli*, DNA duplicates itself semiconservatively. This means that in *E. coli*, the strands of the DNA double helix separate and form a Y-shaped replication fork where DNA duplication begins. Proteins stabilize the unwound helix and assist in relaxing the coiling tension created ahead of the duplication activity. A new, complementary strand of DNA, duplicated in *E. coli* at the rate of thirty thousand nucleotides per minute, is produced on each of the two paren-

tal template (guide) strands. The DNA duplication process results in two double-stranded DNA molecules, each having one strand from the parent molecule and one newly produced strand. This semiconservative mechanism ensures the faithful copying of the genetic information at each *E. coli* cell division.

During the message-encoding process (transcription), the genic message (RNA transcript) is created step by step, using the DNA template. The template is read in one direction, while RNA is produced in the opposite direction.

The process includes initiation, elongation, and termination phases. The transcription initiation site is signaled by the promoter (a short nucleotide sequence recognized by an RNA polymerase). During elongation, RNA polymerase migrates along the DNA molecule, melting and unwinding the double helix as it moves and sequentially attaching ribonucleotides to one end of the growing RNA molecule. Base pairing to the template strand of the gene determines the identity of the ribonucleotide added to each position. By a complex signal, transcription is terminated shortly after the ends of genes. As a result of the process, a gene-complementary, single-stranded RNA molecule (messenger RNA, or mRNA) is created.

Like the message-encoding process, the message-decoding process (translation) consists of initiation, elongation, and termination. In *E. coli*, the small subunit of a ribosome (the cell's interior structure for protein production) attaches to the ribosome-binding site of an mRNA, resulting in an initiation complex. In elongation, the large subunit of the ribosome attaches to the initiation complex, creating two different binding sites for transfer RNA (tRNA), the amino acid transporter. Ribosomes use mRNA-coded information to take amino acids brought by tRNA and assemble them, on ribosomes, into protein.

Mutation and DNA Repair

In the genetics of *E. coli*, phenotypes resulting from changes in the DNA can occur because of either mutation (a change in the nucleotide sequence of a gene) or recombination (a process leading to new combinations of genes on a chromosome). These new combinations can occur following transfer of chromosomal genes from one bacterial cell to another by transformation (in which a recipient cell acquires genes from free DNA in the medium), transduction (in which a virus carries DNA from donor to recipient cell), or conjugation (in which two bacterial cells make contact and exchange DNA). Transposon and insertion elements, both found in *E. coli*, may also change phenotypes. A transposon is a mobile DNA segment containing genes for inserting DNA into the chromosome and for moving the element

to other chromosome locations. Insertion elements, the simplest transposable elements in *E. coli*, contain only genes for mobilizing the elements and inserting them into chromosomes at new locations.

In *E. coli*, as in all other organisms, many chemical and physical agents cause structural changes in DNA. Consequently, mechanisms are needed for repairing such damaged DNA. Such repair mechanisms exist in *E. coli*, although they are complicated and require many different proteins. The three main types of repair mechanisms in *E. coli* are direct repair (the reversal of a structural change), excision repair (in which appropriate enzymes recognize and "label" a damaged nucleotide, excise it, fill in the gap, and seal the strand), and mismatch repair (in which enzymes recognize the mismatch nucleotide and either label it or repair it directly). The parental strand is distinguished from the newly created daughter strand by tagging the parental strand with methyl groups attached to adenines occurring within specific sequences. Such modified adenines act as labels for the parent strand, enabling the repair enzymes to recognize which strand should be repaired at a mismatch position.

Biotechnology

To test a genetic hypothesis, the genetic history of the organism involved must be well known so that the genetic background of the parents used in the experimental crosses is known. The genetics of *E. coli* provides geneticists with such an experimental organism. As a result, *E. coli* is used extensively in biotechnology. In this industry, a foreign gene inserted into the bacterium may be replicated and sometimes translated in the same manner as the native bacterial DNA, producing a foreign gene product. *Escherichia coli* can accept foreign DNA derived from any organism because the genetic code is nearly universal. As an example, genetic mapping of a free-living, nitrogen-fixing bacterium showed that seventeen genes involved in nitrogen fixation are clustered on one portion of the chromosome. Biologists transferred this gene cluster to a plasmid—a circular, independently replicating DNA molecule—and introduced the plasmid into *E. coli*.

cells, which then produced the enzyme nitrogenase and fixed nitrogen.

A significant breakthrough occurred when a synthetic gene coding for somatostatin, an antigrowth hormone important in the treatment of different human growth disorders, was fused with the start of the *lacZ'* gene contained within a cloning vector (a self-duplicating DNA molecule containing inserted, foreign DNA). Cells of *E. coli* transformed with this recombinant plasmid were able to transcribe the fused gene, recognizing the *lac* promoter as its binding site. The mRNA was then translated by ribosomes that recognized the *lac* ribosome binding sequence. The resulting fused protein was cleaved with cyanogen bromide, which cuts amino acid chains specifically at methionines, resulting in pure-form somatostatin.

Implications for Evolution

The genetics of *E. coli* provides evidence for punctuated equilibrium caused by the appearance of rare, beneficial mutations. This evidence involved studies that measured changes in cell size over three thousand generations of bacteria in a constant environment. During the studies, periods of stagnancy were interrupted by periods of rapid change. The changes in cell size may be the result of direct selection for a rare, beneficial mutation that caused increased cell size. This mutation swept through the population, producing a change in cell size in one hundred generations or less.

Impact and Applications

Geneticists use model organisms for their research. Their favorite organisms, such as *E. coli*, have qualities that make them well suited for genetic experimentation—a rich genetic history, a short life cycle, production of large progeny from a mating, ease in handling, and genetic variation among the individuals in the population. Added to the much that was already known about *E. coli* was the completion of the complete sequence of the chromosome of *E. coli* in 1995. The quantity of genetics involving *E. coli* is a testament to the bacterium's suitability as an experimental organism for testing genetic hypotheses. The hypotheses tested using this experimental organism have contrib-

uted in a revolutionary way to the understanding of significant scientific concepts and to the understanding of the genetics of organisms more complex than bacteria, such as humans.

In addition, recombinant DNA technology (techniques for constructing, studying, and using DNA created in a test tube), which uses *E. coli* extensively, is used in all areas of basic genetics research to investigate genetic circumstances. Many biotechnology companies owe their existence to recombinant DNA technology—and to *E. coli*—as they seek to clone and manipulate genes for the production of commercial products, the improvement of plant and animal agriculture, the development of diagnostic tools for genetic diseases, and the development of new or more effective pharmaceuticals.

—Robert Haynes

See also: Antibodies; Archaea; Bacterial Genetics and Cell Structure; Bacterial Resistance and Super Bacteria; Blotting: Southern, Northern, and Western; Chromosome Theory of Heredity; Cloning Vectors; DNA Isolation; DNA Repair; Emerging Diseases; Gene Families; Gene Regulation: *Lac Operon*; Gene Regulation: Viruses; Genetic Code; Genetic Engineering; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Medical Applications; Genetics, Historical Development of; Human Genome Project; Human Growth Hormone; Model Organisms; Noncoding RNA Molecules; Plasmids; Proteomics; Restriction Enzymes; Shotgun Cloning; Synthetic Genes; Transposable Elements.

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- National Institutes of Health, Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/genbank/genbanksearch.html>. For information on *E. coli*, see accession number U00096.

Model Organism: *Mus musculus*

Field of study: Techniques and methodologies

Significance: *Model organisms allow geneticists to investigate how genes affect organismal and cellular function. The mouse is an ideal organism for genetic research because of its size, life span, and*

litter size. It shares many similarities with humans and is useful for modeling complex phenomena such as cancer and development.

Key terms

- EMBRYONIC STEM CELLS:** cultured cells derived from an early embryo
- GENOMICS:** the study of the entire DNA content of an organism, called its genome
- INBREEDING:** the process of mating brothers and sisters to create genetically identical offspring
- MODEL ORGANISM:** an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population
- PHENOTYPE:** an observable trait
- TRANSGENICS:** the technique of modifying an organism by introducing new DNA into its chromosomes

History of Mice in Genetic Research

The use of mice in genetic research had its origin in the efforts of mouse fanciers, who raised mice as pets and developed numerous strains with distinct coat colors. Researchers in the late 1800's who were trying to determine the validity of Gregor Mendel's laws of heredity in animals found the existence of domesticated mice with distinct coat colors to be an ideal choice for their experiments. Through the work of early mouse geneticists such as Lucien Cuénot and others, Mendel's ideas were validated and expanded.

Development of Inbred Strains

As genetic work on mice continued into the 1900's, a number of mouse facilities were created, including the Bussey Institute at Harvard University. One member of the institute, Clarence Little, carried out a set of experiments that would help establish the utility of mice in scientific research. Little mated a pair of mice and then mated the offspring with each other. He continued this process for many generations. After a number of generations of inbreeding, Little's mice lost all genetic variation and became genetically identical. These mice, named DBA mice, became the first strain of in-

bred mice and marked an important contribution to mouse research. In an experiment using inbred DBA mice, any difference displayed by two mice could not be due to genetic variation and had to be from the result of the experiment. Through inbreeding, genetic variation was removed as a variable. Also, through careful crossing and selection of different inbred strains, populations of mice that differed by only a few genes could be created. Geneticists could then examine the effects of these genes knowing that all other genes were the same. The creation of inbred mice allowed geneticists to study genes in a carefully controlled way.

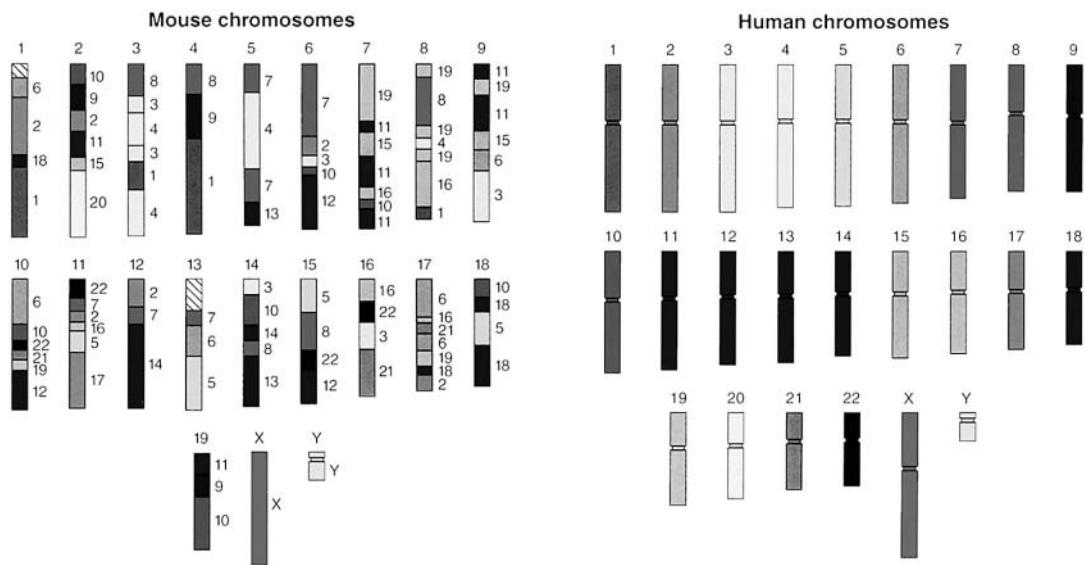
The first use of inbred mice was in the study of cancer. As inbred strains of mice were created, it was noticed that certain strains had a tendency to develop cancer at a very high frequency. Some of these strains developed tumors that were very similar to those found in

human cancers. These mice became some of the first mouse models used to study a human disease.

Unique Aspects of the Mouse Model

The ability of mice to acquire cancer illustrates why the mouse is a unique and valuable tool for research. Although mice are not as easy to maintain as other model organisms, they are vertebrates and thus share a number of physiological and developmental similarities with humans. They can be used to model processes, such as those involved in cancer and skeletal development, that do not exist in simpler organisms. In this capacity, mice represent a balance between the need for an animal with developmental complexity and the need for an animal with a quick generation time that is easily bred and raised. Other organisms, such as chimpanzees, may more closely resemble hu-

Mouse and Human Genetic Similarities



Courtesy Lisa Stubbs
Oak Ridge National Laboratory

One of the most amazing discoveries in genetics is that very different organisms can have very similar genomes. This figure from the Human Genome Program, for example, shows the similarities between the genes of mice and those of human beings. Approximately 80 to 90 percent of the genes in humans have a counterpart in the mouse. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

mans, but their lengthy generation time and small litter size make them difficult to use for the many and repeated experiments needed for genetic research.

The use of the mouse model has advanced considerably since the early 1900's. Initially, geneticists relied on the random occurrence of natural mutations to generate mice with traits that mirrored aspects of human biology and disease. Careful crossbreeding and the use of inbred strains allowed the trait to be isolated and maintained. Although this process was slow and tedious, a large number of inbred strains were identified. Later, it was discovered that X rays and other chemicals could increase the rate of mutation, leading to an increase in the rate at which mice with interesting traits could be found. However, the discovery of a mouse strain that modeled a particular human disease was still a matter of chance.

It was the advent of molecular biology that removed this element of chance and brought the mouse to its full prominence as a model organism. Molecular biology provided a mechanistic understanding of gene function and offered tools that allowed for the direct manipulation of genes.

Transgenic Mice

The technique of transgenics allows geneticists to create mice that carry specific mutations in specific genes. Using recombinant DNA technology, a geneticist can construct a piece of DNA containing a mutant form of a chosen gene, then use the mutated gene to modify the existing DNA of mouse embryonic stem cells. These modified embryonic stem cells can be combined with a normal mouse embryo to form a transgenic embryo that can be implanted into the uterus of a female mouse. The transgenic mouse that is born from this process carries in every tissue a mixture of normal cells and cells with the specific DNA alteration introduced by the researcher. Careful crossing of the transgenic mouse with mice of the same inbred strain can then be done to create a new line of mice that carry the DNA alteration in all cells. These mice will then express a phenotype that results directly from the modified gene. Transgenics has allowed geneticists

to custom design mice to display the genetic defects they desire.

In the era of genomics, transgenic mice have become a powerful tool in the effort to understand the function of human genes. Since the complete sequences of the mouse and human genomes are known, it is possible to compare the genes of mice and humans directly. Approximately 80 to 90 percent of the genes in humans have a counterpart in the mouse. Using transgenics to target genes in the mouse that are similar to humans can help geneticists understand their functions. However, care must be used in drawing comparisons. There are a number of examples of mouse genes that carry out functions different from their human counterparts. Despite this concern, comparison of mouse and human genes has provided tremendous insight into the function of the human genome.

Economic and Ethical Considerations

The demand for mice in research has resulted in a \$100 million industry devoted to the maintenance and development of mouse models. Companies specializing in mice have developed thousands of inbred strains for use in research. The economic impact of mice has led to patents on transgenic mice and has caused controversy over who has the right to own a particular mouse strain. Also, the extensive use of mice in research (25 million mice in the year 2000) has raised concerns by some for the welfare of mice and questions about the ethics of using them in research.

Research Using the Mouse Model

The study of cancer was the first area of research to benefit from the use of mice. Early mouse geneticists were able to learn about the genetic and environmental factors that influenced the development of cancer. Today's cancer research relies heavily on the mouse model as a way of determining how genes affect the interaction between cancer and the body. Understanding the function of tumor-suppressor genes such as *p53* has come in part from the use of transgenic mice. Mice have also been important in investigating the role of the immune system and angiogenesis in tumor progression.

Mouse work in cancer also made contributions to immunology, which relies heavily on the mouse as a model of an intact immune system. Inbred strains of mice with defective immune systems have been developed to help geneticists understand the role of the immune system in disease progression and transplant rejection. Mice have also been instrumental in studying how genes in pathogenic microorganisms allow the microbes to cause disease. The mouse model has been used to understand how diseases like cholera and anthrax are able to infect and cause damage.

The study of many genetic diseases, such as sickle-cell disease and phenylketonuria (PKU), has benefited from the existence of mouse models that mimic the disease. The genetic components of such complex phenomena as heart disease and obesity are also being elucidated using the mouse model.

Developmental biology has relied heavily on the mouse to determine how gene expression leads to the formation of multicellular organisms. Work that has shown the role homeo-genes play in determining mammalian body structure and how genes affect development of organs has been done in mouse models.

The mouse has also proven to be a valuable model in investigating the effects of various genes on brain development and function. Mouse models have provided insights into the way the brain develops and functions, as well as genetic contributions to complex behaviors. Genes have been identified that play roles in complex behaviors such as raising young and predisposition toward addiction.

—Douglas H. Brown

See also: Altruism; Chromosome Theory of Heredity; Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Drosophila melanogaster*; Model Organism: *Escherichia coli*; Model Organism: *Neurospora crassa*; Model Organism: *Saccharomyces cerevisiae*; Model Organism: *Xenopus laevis*; Model Organisms.

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Web Sites of Interest

Mouse Atlas and Gene Expression Database. <http://www3.oup.co.uk/nar/database/summary/20>. The Medical Research Council and the University of Edinburgh sponsor the site free on the Web; the data are also available for a fee on CD-ROM. This ongoing project is intended to evolve into the premier source for three-dimensional images on morphology, gene expression, and mutant phenotypes in mouse development. The initial digital embryo images are mounted, accessible through a controlled vocabulary linked to the images. Developmental geneticists will be able to synthesize information from many sources.

Mouse Genome Informatics, Jackson Laboratory, Bar Harbor, Maine. <http://www.informatics.jax.org>. A center for mutant mouse research, providing access to genetic maps, phenotypes, gene expression data, and sequence information. Includes the Mouse Genome Database, the Gene Expression Database, and the Mouse Genome Sequence Project.

Model Organism: *Neurospora crassa*

Field of study: Techniques and methodologies

Significance: *Neurospora crassa* is a bread mold with a relatively small genome, allowing this organism to be studied by causing mutations in its genes and observing the effects of these mutations.

*Such studies are important to the understanding of genetics and genetically related disease, particularly because *N. crassa* is eukaryotic and more similar to human DNA than it is to bacteria and viruses.*

Key terms

ASCOMYCETES: organisms of the phylum *Ascomycota*, a group of fungi known as the sac fungi, which are characterized by a saclike structure, the ascus

AUXOTROPHIC STRAIN: a mutant strain of an organism that cannot synthesize a substance required for growth and therefore must have the substance supplied in the growth medium

CYTOGENETICS: the study of normal and mutated chromosomes and their behavior

DIPLOID CELL: a cell that contains two copies of each chromosome

HAPLOID CELL: a cell that contains one copy of each chromosome

MINIMAL MEDIUM: an environment that contains the simplest set of ingredients that the microorganism can use to produce all the substances required for reproduction and growth

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle (allowing the production of several generations in a short space of time), and genetic variation between individuals in the population

The Beginning of Biochemical Genetics

Neurospora crassa was first used in genetic experiments by Carl Lindegren in the 1930's. He was able to isolate several morphological mutant strains and create the first "linkage maps" showing where genes are located on chromosomes. This research determined some of the basic principles of "crossing over" during meiosis. Crossing over is the exchange of genes between homologous chromosome pairs by the breaking and reunion of the chromosome. Lindegren was able to show that crossing over occurs before the separation of the homologous pair, between the second and fourth chromatids. *Neurospora crassa* was used as a model organism in the investigation of crossing-over

mechanisms because the four products of meiosis (later duplicated by mitosis to produce eight spores) are arranged in the organism's saclike ascus in a way that exactly reflects the orientation of the four chromatids of each tetrad at the metaphase plate in the first meiotic division. The products of meiosis line up in order and therefore are more easily studied in this organism.

One Gene, One Enzyme

In 1941 George Beadle and Edward Tatum published a paper establishing biochemical genetics as an experimental science. They introduced a procedure for isolating an important class of lethal mutations in an organism, namely, those for blocking the synthesis of essential biological substances. These were expressed in the organism as new nutritional requirements.

By supplying a variety of compounds in the nutrient medium and seeing which allowed various mutant strains to grow and which did not, Beadle and Tatum saw that they could deduce the sequence of biochemical reactions in cells that make necessary compounds, such as amino acids. They concluded that the function of a gene is to direct the formation of a particular enzyme which regulates a chemical event. A mutation can alter a gene so that it no longer produces the normal enzyme, resulting in a physical symptom, such as the need for nutritional supplements. Beadle and Tatum proposed that, in general, each gene directs the formation of one enzyme.

These mutation studies promoted understanding of the biochemistry of gene expression and promoted the use of fungi in genetic experiments. In 1958, Beadle and Tatum were awarded the Nobel Prize in Physiology or Medicine for their discovery that the characteristic function of the gene was to control the synthesis of a particular enzyme.

The Organism

The orange bread mold *Neurospora crassa*, a multicellular lower eukaryote, is the best characterized of the filamentous fungi. Filamentous fungi are a group of fungi with a microscopic, stalklike structure called the mycelium.

They grow on substances of plant or animal origins and reproduce via spores. This group of organisms has importance in agriculture, medicine, and the environment because they are so abundant and are able to proliferate very quickly. It is therefore easy and cheap to reproduce them rapidly. Moreover, the widespread availability of *Neurospora crassa* in nature makes genetic population studies more feasible. Because it can be grown in large quantity, experiments are easier to conduct and their results are more easily analyzed.

Neurospora crassa is a filamentous ascomycete that has ascii; an ascus is a saclike structure inside of which four or eight ascospores develop during reproduction. In the *N. crassa* ascii, one round of mitosis usually follows meiosis and leaves eight nuclei (new daughter cells). These nuclei eventually become eight ascospores (sexual spores produced by ascomycetes). After the ascospores are formed within the ascus, they are released and germinate to form a new haploid mycelium.

A Model Organism

Geneticists use a variety of organisms in their research. Because it is haploid (containing half the chromosomal material of the parent cell), genotypic changes in *N. crassa* (mutations in genes) are directly observed through the changes in the phenotype (physical characteristics), because only one gene determines physical characteristics. The small size of the genome is a result of a unique feature of *N. crassa*: It has very little repeated DNA. The lack of repetitive DNA is also valuable to researchers when parts of the genome are amplified or sequenced.

Neurospora crassa has been extensively used for genetic research, resulting in hundreds of published articles. They include research on gene expression and effects of external factors, metabolic studies, and genomic mapping experiments. A large number of mutants have been characterized, providing the foundation for many genetic experiments.

Repeat-Induced Point (RIP) Mutations

By using recombinant DNA methods, researchers can study *N. crassa* using a technique

known as repeat-induced point (RIP) mutations, the creation of point mutations of a single base pair in specific genes. RIP detects duplications of gene-sized segments and creates repeated point mutations. RIP specifically changes a GC (guanine-cytosine) pair to an AT (adenine-thymine) pair. Repeated sequences are heavily mutated by RIP in the period between fertilization (the time when the sperm comes into contact with the egg) and karyogamy (fusion of the haploid cells to form diploid cells). After the mutation, the altered sequence is methylated (a CH₃, or methyl, group is attached). The methyl group serves as a tag so the mutations can be easily identified. RIP mutations usually indicate a crossing over during meiosis. RIP mutations cause inactivations of duplicate genes, whose functions are then more easily detected.

Sequencing and Linkage

Large-scale sequencing of the *N. crassa* genome has been initiated for several linkage groups (genes that are located on the same chromosomes). Early in the sequencing of the *N. crassa* genome, it became apparent that its genome contains many unique genes. These genes and others have been sorted into linkage groups. There are many maps available for *N. crassa*. The largest group is that at the Whitehead Institute Center for Genome Research under the Fungal Genome Initiative. Restriction fragment length polymorphism (RFLP) maps show the restriction site for a particular restriction endonuclease. Linkage maps show the distribution and linkage of genes throughout the *N. crassa* genome. These maps are particularly important when a researcher is interested in recombinant DNA research.

—Leah C. Nesbitt, James N. Robinson, and Massimo D. Bezoari

See also: Chromosome Theory of Heredity; Complementation Testing; Extrachromosomal Inheritance; Genetics, Historical Development of; Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Drosophila melanogaster*; Model Organism: *Escherichia coli*; Model Organism: *Mus musculus*; Model Organism: *Saccharomyces cerevisiae*; Model

Organism: *Xenopus laevis*; Model Organisms; One Gene-One Enzyme Hypothesis.

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- Thancker, Paul D. "Understanding Fungi Through Their Genomes." *Bioscience* 53, no. 1 (January, 2003): 10-15. Useful for students and researchers.

Web Sites of Interest

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Whitehead Institute for Biomedical Research. <http://www-genome.wi.mit.edu>. One of the major gateways to genomics research, soft-

ware, and sequencing databases; provides access to one of the largest collections of linkage maps for *Neurospora* under the Fungal Genome Initiative.

Model Organism: *Saccharomyces cerevisiae*

Field of study: Techniques and methodologies

Significance: *Saccharomyces cerevisiae* is a highly tractable yeast organism that was the first eukaryote to have its DNA completely sequenced. Yeast genetic research has been at the forefront of scientists' efforts to identify the genes and processes required for cell growth and division and is now an important tool for nonyeast research to identify proteins that physically interact with one another in the cell.

Key terms

ASCUS: the cellular structure that results from meiosis in yeast, containing four recombinant spores that are fully capable of growing into haploid yeast cells

BUDDING: the asexual method of duplication used by yeast to create a clone of the original cell

DIPLOID CELL: a cell that contains two copies of each chromosome

HAPLOID CELL: a cell that contains one copy of each chromosome

MATING TYPE: one of two types of yeast cell, depending on a soluble factor that each cell secretes

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

The Organism

Saccharomyces cerevisiae (*S. cerevisiae*, or baker's yeast) has been used for millennia to provide leavening to bread products. Yeast is a simple, one-celled eukaryote with six thousand genes on sixteen chromosomes. It was the first eukaryote to have its entire DNA sequenced.

Yeast produce offspring using two different methods, a sexual life cycle and an asexual life cycle. In the asexual life cycle, the yeast cell produces the next generation by a process called budding. All genetic components of the mother cell are duplicated and a small “bud” begins to grow from the mother cell. The bud continues to grow until it is nearly the size of the mother cell. The DNA and other duplicated cellular components are then partitioned into the new bud. The cells undergo cytokinesis and are now separate entities able to grow and continue reproducing independently of one another.

To produce offspring that are not clones of the mother cell, yeast use a sexual life cycle. A yeast cell exists stably as either a diploid or a haploid organism, but only the haploid organism is able to mate and exchange genetic information. Haploid yeast contain either the *MATa* or *MATalpha* gene. These genes produce soluble factors that distinguish them as one of two mating types. An “a” cell (*MATa*) and an “alpha” cell (*MATalpha*) mate by sequentially fusing their cell walls, their cytoplasms, and finally their nuclei. This diploid cell now contains two copies of each chromosome that can undergo recombination during meiosis. When all environmental signals are ideal, the diploid yeast will undergo meiosis, allowing exchange and recombination of genetic information brought to the diploid by both haploid cells. The result of meiosis is an ascus that contains four recombinant spores that will grow into haploid yeast cells when environmental conditions are ideal.

A Model Organism

Researchers choose yeast as a model organism to study specific areas of interest for many different reasons. *Saccharomyces cerevisiae* is non-pathogenic to humans, allowing manipulation in a laboratory with little or no containment required. At a temperature of 30 degrees Celsius (86 degrees Fahrenheit), the yeast population can double in ninety minutes, allowing many experiments to be completed in one day. Among the primary reasons for selection of yeast as a model system is that they offer the possibility of studying the genes and proteins that are required for basic growth functions

and cellular division. Yeast use many of the same genes and proteins to govern the same processes that animal and plant cells use for growth and division. Each single cell has to take in nutrients, grow, and pass along information to its progeny. In many ways, yeast can be considered a simplified version of a plant or animal cell, in that it lacks all the genes that provide the determinants that are expressed as differences between plants and animals. Another important reason for using yeast is that yeast is amenable to investigation using both genetic and biochemical approaches. This allows for correlation of findings from both approaches and a better understanding of a specific process or activity.

Yeast is also ideal for use as a model system due to at least four well-established techniques and procedures. First, genetics in yeast takes advantage of well-established auxotrophic markers. These markers are usually mutations in biosynthetic pathways that are used to synthesize required cellular components such as amino acids and nucleotides. By using these marker genes, researchers can follow genes and their associated chromosomes from one generation to the next.

Second, yeast is readily transformed by plasmids that function as artificial chromosomes. All that is needed is an auxotrophic marker to follow the plasmid through succeeding generations, a yeast origin of replication to allow replication of the plasmid DNA, and a region into which the gene of interest can be inserted in the plasmid DNA. This allows the researcher to move genes easily from yeast strain to yeast strain and quickly examine the effect of the gene in combination with many other genes.

Third, yeast is easily mutated by chemicals and can be grown in a small space, which allows the researcher quickly to identify mutations in genes that result in a specific phenotype. For example, to define all the genes in the adenine biosynthetic pathway, a researcher would mutate a yeast strain with one of many available mutagenic chemicals, resulting in changes within the DNA. The mutated yeast strains would then be checked to see if the strain was able to grow on media lacking adenine. All of the strains mutant for growth on adenine

would be collected and could identify a number of genes involved in the adenine biosynthetic pathway. Further research could establish whether each of these mutations in the yeast identified one gene or many genes.

Fourth, yeast is the model system of choice when examining and identifying proteins that interact with one another in the cell. This technique is called the two-hybrid system.

Two-Hybrid System

The two-hybrid system takes advantage of scientists' understanding of transcription at the *GAL1* gene in yeast. The promoter region of *GAL1* contains a binding site for the Gal4p transcription factor. When the cell is grown on the sugar galactose, Gal4p binds to the promoter of *GAL1* and activates transcription of the *GAL1* gene. Gal4p can be essentially divided into two functional regions: one region that binds to DNA and another region that activates transcription.

The two-hybrid system uses the *GAL1*-Gal4p transcription system to identify previously unknown proteins that interact with a protein of interest. The system consists of a reporter gene under the control of the *GAL1* promoter and two plasmids that produce fusions with the Gal4p transcription factor. The first plasmid contains a gene of interest fused to a DNA-binding domain. This plasmid expresses a protein that is able to bind to the DNA-binding site in the *GAL1* promoter of the reporter gene. This plasmid is unable to activate transcription of the reporter gene, since the Gal4p fragment does not contain the information to activate transcription. The second plasmid is provided from a collection of plasmids that consist of unknown or random genes fused to the transcription activation domain of Gal4p. This plasmid by itself is unable to bind to the DNA-binding site in the *GAL1* promoter and thus is unable to activate transcription of the reporter gene. If both plasmids contain genes whose protein products physically interact in the cell, the complex is able to bind to the DNA-binding region of the *GAL1* promoter, and since the activation domain of Gal4p is also present in this complex, activation of the reporter gene will occur. The production of the reporter gene

serves as a signal that both of the gene products interact in the cell. The yeast strain containing the active reporter gene is then selected and further examined to determine the unknown DNA that resides on the second plasmid by sequence analysis.

Research and Implications

The years of work on yeast as a model system have provided many insights into how genes and their protein products interact to coordinate the many cellular mechanisms that take place in all cells from simple yeast to complicated humans. It is impossible to exhaustively list the different areas of research currently being examined or completely list the new understandings that have come to light through the use of the *S. cerevisiae* model system. Every major area of cellular research has at one time or another used yeast to ask some of the more difficult questions that could not be asked in other systems. Work in yeast has aided identification of genes and elucidated the mechanism of many different areas of research, including cell cycle regulation, mechanisms of signal transduction, the process of secretion, replication of DNA, transcription of DNA, translation of messenger RNA into proteins, biosynthetic pathways of amino acids and other basic building blocks of cells, and regulation and progression of cells through mitosis and meiosis. Despite all these advances, there is still much to learn from yeast and it will continue to provide information for years to come.

—John R. Geiser

See also: Cloning Vectors; Extrachromosomal Inheritance; Linkage Maps; Model Organisms; Noncoding RNA Molecules; Plasmids.

Further Reading

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Model Organism: *Xenopus laevis*

Field of study: Techniques and methodologies

Significance: *Xenopus laevis*, the African clawed frog, has been used widely in the field of developmental biology. By following the development of this unique organism, scientists have identified and now understand the role of many genes in frog development, providing insight into vertebrate development.

Key terms

EMBRYOLOGY: the study of developing embryos

FATE MAP: a map created by following the adult fate of embryonic cells

MODEL ORGANISM: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population

TRANSGENIC ANIMAL: an animal that contains a gene not normally expressed in its genome

The Organism

The African clawed frog, *Xenopus laevis*, is in the class *Amphibia*, order *Anura*, suborder *Opisthocheila*, family *Pipidae*, and genus *Xenopus*. This genus includes five other species that inhabit silt-filled ponds throughout much of

southern Africa. Members of this species share a distinctive habitat and morphology. The organism's name alone provides insight into its structure and habitats: The root *xeno* stems from Greek for "strange," while *pus* is from the Greek for "foot" and *laevis* is Latin for "slippery." *Xenopus laevis* is entirely aquatic, a feature that makes it unique among the other members of the genus, feeding and breeding under water. It is believed that they evolved from terrestrial anurans, organisms that are aquatic as tadpoles but are terrestrial as adults. Migration across land from pond to pond has been observed but is limited by distance and time of year (occurring during the rainy season) because out of water the frogs will dry out and die within a day. In instances of extreme drought, adult frogs will bury themselves in the mud and wait until the next rainfall.

Xenopus laevis is mottled greenish-brown on its dorsal surface and yellowish-white on its ventral surface. In appearance, these frogs are flattened dorsoventrally with dorsally oriented eyes as adults. The members of the genus are collectively known as platannas from the word "plathander," meaning flat-handed. Three toes of the hind limbs are clawed, and a line of specialized sensory organs (the lateral line organs) is found on both the dorsal and ventral surfaces and encircles the eyes. The breeding season for *X. laevis* depends on temperature and rainfall. The tadpoles are herbivorous, feeding on algae, whereas the adults are carnivorous, feeding on worms, crustaceans, and other creatures living in the mud.

A Model Organism

A model organism is defined as one that breeds quickly, is easily managed in the laboratory, and has large numbers of offspring or broods. *Xenopus laevis* meets these requirements nicely. An interesting feature of this organism is its responsiveness to human chorionic gonadotropin, a hormone secreted by the placenta and present in the urine of pregnant women. When exposed to the hormone, female frogs will spawn (lay eggs). As a result of this phenomenon, *X. laevis* was once used as an indicator in human pregnancy tests, whereby the female frogs were injected with human fe-

male urine. At present, researchers take advantage of this phenomenon to produce large numbers of offspring by injecting frogs with the hormone. Another characteristic that makes *X. laevis* a good model organism is that it is hardy and can survive in captivity for long periods of time with relatively low mortality rates.

A final requirement for an animal model to be useful is that research on the animal should add to the understanding of biological principles in other organisms. *Xenopus laevis* is widely used in the field of developmental biology. For many decades, amphibian embryologists used salamander embryos, such as *Triturus*, and embryos of the frog *Rana* species. As mentioned above, amphibian embryos have several advantages over other organisms: Amphibian embryos are large, can be obtained in large numbers, and can be maintained easily and inexpensively in the laboratory. However, one disadvantage of traditional amphibian species is that they are seasonal breeders. As a result, investigators cannot conduct experiments throughout the year on most amphibians. *Xenopus laevis* is a notable exception, because it can be induced to breed year-round.

As the fertilized *X. laevis* zygote develops, the yolk-laden cytoplasm, known as the vegetal pole, is oriented downward by gravity. The rest of the cytoplasm, termed the animal pole, orients itself upward. The animal pole is the main portion of the cell, giving rise to the embryo proper. Cell division, or cleavage of cells, in the animal pole increases the number of cells greatly. Movement and migration of these cells, under the influences of interactions with neighboring cells, give rise to a multilaminar embryo that includes the ectoderm (which gives rise to skin and nervous system), the mesoderm (which gives rise to muscle), and the endoderm (which gives rise to many of the “tubes” of the organism, such as the intestines and the respiratory tract).

By following embryos from the very earliest stages, researchers have been able to create “fate maps” of fertilized eggs, which can be used to predict adult derivatives of specific regions in a developing embryo. Early researchers introduced many different techniques to

create these kinds of maps. One technique involves destroying single cells during early development and following the development of the embryo to see what tissue is altered. Other methods include transplantation of individual cells or small groups of cells into a host organism and following the fate of the transplanted tissue.

Genetic Manipulation in *Xenopus*

Much of what is known today about the interactions between cells in developing vertebrate embryos has come from *X. laevis*. The early work of embryologists Hans Spemann and Pieter Nieuwkoop has been supported with molecular techniques, and many genes have been identified that control nearly every aspect of *Xenopus* development. A few examples include the *Xenopus Brachury* gene (*Xbra*), which is involved in the establishment of the dorsal-ventral axis; *Xenopus ventral* (*vent1*), which aids in the differentiation of ventral mesoderm and epidermal structures; and *Xenopus nodal-related 1* (*Xnr1*), a gene that is responsible for the specification of the left-right axis.

Xenopus embryos possess a number of advantages that have allowed investigators to study many aspects of developmental biology. One of the struggles that early researchers faced was the lack of dependable techniques for creating transgenic embryos to study the functions and role of individual genes. One can isolate and clone the genes of *Xenopus* and inject RNA into zygotes. RNA, however, is an unstable molecule and relatively short-lived. Therefore, the study of molecular events in the embryo after the period when the embryonic genes are turned on remained problematic. Attempts to inject cloned DNA to be expressed in the embryo were complicated by the fact that it does not integrate into the frog genomic chromosomes during cleavage. Exogenous DNA is then unequally distributed in embryonic cells and, therefore, is always expressed in random patterns. In 1996, Kristen L. Kroll and Enrique Amaya developed a technique to make stable transgenic *Xenopus* embryos. This technique has the potential to boost the utility of *Xenopus* tremendously. One significant advantage of us-

ing transgenic frogs over transgenic mice is that one can produce first-generation transgenics, making it unnecessary to wait until the second generation to examine the effects of the exogenous gene on development.

The transgenic technique has several steps, and each step is full of problems. Because exogenous DNA is not incorporated into the zygotic genome, Kroll and Amaya decided to attempt to introduce them into sperm nuclei. Sperm nuclei are treated with the enzyme lysolecithin to remove the plasma membrane prior to incubation with the linearized DNA plasmid containing the exogenous gene. The sperm nuclei are then incubated with restriction enzyme to introduce nicks in the nuclear DNA. The nicks facilitate incorporation of the plasmid DNA. The nuclei are then placed in an interphase egg extract, which causes the nuclei to swell as if they were male pronuclei. This technique has been used in many laboratories to introduce into the frog genes that are not normally expressed, allowing the researcher to study the function of these genes.

The National Institutes of Health is supporting the Trans-NIH *Xenopus* Initiative, specifically developed to support research in the areas of genomics and genetics in *Xenopus* research. While there is still much to be learned from this unique organism, it is clear that the advantages of this animal model far outweigh the disadvantages. With continued work in laboratories around the world, scientists may soon fully understand the genetics involved in vertebrate development. *Xenopus laevis* is ideally suited to provide critical breakthroughs in embryonic body patterning and cell fate determination, later development and the formation of organs, and cell biological and biochemical processes.

—Steven D. Wilt

See also: Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Drosophila melanogaster*; Model Organism: *Escherichia coli*; Model Organism: *Mus musculus*; Model Organism: *Neurospora crassa*; Model Organism: *Saccharomyces cerevisiae*; Model Organisms; Noncoding RNA Molecules; Totipotency.

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- National Institutes of Health. Trans-NIH *Xenopus* Initiative. <http://www.nih.gov/science/models/xenopus>. This site keeps researchers aware of NIH's plans regarding support of the genomic and genetic needs for *Xenopus* research.
- Xenbase: A *Xenopus* Web Resource. <http://www.xenbase.org>. A "database of information pertaining to the cell and developmental biology of the frog, *Xenopus*" with genomic information, directories, methods, links to databases and electronic journals, conference announcements.

Model Organisms

Field of study: Techniques and methodologies

Significance: *Due to evolutionary relationships between organisms, different organisms share similar, evolutionarily conserved genes and mechanisms of inheritance. This similarity between different species allows researchers to use model organisms to examine general genetic principles that are applicable to a wide variety of living organisms, including human beings. Findings from studies on model organisms not only reveal information about the influence of genetics on basic biology but also provide important insights into the role of genetics in human health and disease.*

Key terms

HOMOLOGY: similarity resulting from descent from a common evolutionary ancestor

MODEL ORGANISM: a species used for genetic analysis because of characteristics that make it desirable as a research organism and because of similarity to other organisms

Why Models?

Genetics research seeks to understand how genetic information is transmitted from one generation to the next and how this information influences the structure, function, development, and behavior of cells and organisms. However, the sheer number of different species and even greater diversity of cell types make the examination of every organism or type of cell impossible. Instead, researchers choose to investigate how genes influence function in a relatively small number of species. They then apply what they learn from these species to other organisms. Those species that are most commonly studied are called model organisms because they serve as models for researchers' understanding of gene function in other organisms.

Basic activities required for cells to survive are retained in virtually all organisms. Genes that have a common evolutionary origin and thus carry out a similar function are said to have homology. For example, many of the same genes used to repair damaged DNA molecules

in the bacterial cell *Escherichia coli* are retained in multicellular, eukaryotic organisms. Thus, much of what is known about genetic control of DNA repair in human cells has been learned by studying homologous genes in the relatively simple *E. coli*. Model organisms provide practical systems in which to ask important genetic questions.

Selection of Model Organisms

Scientific researchers choose which model organisms to study based on the presence of characteristics that make an organism useful for investigating a particular question. Because of the extensive number of questions being asked in biological research, a tremendous number of species are used as model organisms. However, virtually all model organisms fulfill three basic criteria:

- (1) they are relatively easy to grow and maintain
- (2) they reproduce rapidly
- (3) they are of reasonably small size

Geneticists add other criteria to their selection of model organisms, including the use of species for which many mutant forms have been isolated, into which mutations can be easily introduced, and for which techniques have been developed that allow for DNA introduction, isolation, and manipulation. Increasingly, model organisms are those whose genomes have been or will be completely sequenced, allowing for easier isolation and characterization of selected genes and subsequent analysis of gene function. Finally, the model organism must have enough similarity to other organisms that it can be used to ask interesting questions. Many model organisms are used to address questions that help scientists to better understand human cellular and genetic activities. Other model organisms are selected because they provide important information about pathogenic organisms, such as bacteria or viruses, or about economically significant organisms, such as agriculturally important species.

Some Commonly Used Model Organisms

Arguably the first model organism utilized by a geneticist was the garden pea, used by

Gregor Mendel to elucidate how particular traits are transmitted from generation to generation. The patterns of inheritance described by Mendel for the garden pea are applicable to all diploid, sexually reproducing organisms, making the pea a model organism for studying gene transmission. Many other organisms have subsequently been exploited to investigate all aspects of genetic influence on cell function. Prokaryotic cells, particularly the intestinal bacterium *Escherichia coli*, have provided important insights into basic cellular activities, ranging from DNA synthesis to protein translation to secretion of extracellular material. As unicellular eukaryotic cells, the brewer's yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe* have provided models for eukaryotic cell function, including how genes regulate cell division, how proteins are targeted to particular locations in cells, and how specific genes are turned on and off under specific conditions.

Multicellular model species are used to reveal how genes influence the interactions between cells, as well as the organization and function of the whole organism. The fruit fly *Drosophila melanogaster* has been used since the early twentieth century to investigate the association of particular traits with specific chromosomes and was the first organism in which sex-linked inheritance was described. *Drosophila* has also been used to study developmental and behavioral genetics, providing important insights into the role genes play in determining the organizational pattern of developing embryos and in influencing how organisms behave.

More recently, genetic examination of the roundworm *Caenorhabditis elegans* has provided further insights into the role of genes in generating developmental patterns. Some of these insights resulted in the awarding of the 2002 Nobel Prize in Physiology or Medicine to Sydney Brenner, H. Robert Horvitz, and John E. Sulston for their work on apoptosis, or "programmed cell death," in *C. elegans* and its applicability to investigations of apoptosis in other organisms, including humans.

Genetic analysis of plants is also performed using model organisms, the most important of

which is the mustard plant *Arabidopsis thaliana*, whose small genome, rapid generation time, and prolific seed production make it useful for studying plant inheritance patterns, flower generation, genetic responses to stress and pathogen attack, and developmental patterning, among other important plant activities.

Model organisms are also critical for enhancing our understanding of vertebrate genetics. The African clawed frog *Xenopus laevis* and zebrafish *Danio rerio* are used to study basic vertebrate developmental patterns and the organization of specific cell types into tissues and organs. The primary model organism for analysis of mammalian gene function is the house mouse, *Mus musculus*. The generation of thousands of mouse mutants, the ability to perform targeted knockouts of specific mouse genes, and the completion of DNA sequencing of the mouse genome have made the mouse a useful model for examining the role of genes in virtually all aspects of mammalian biology. In addition, the regions of DNA encoding genes in mice and humans are approximately 85 percent identical, making the mouse important not only for studying basic human biology but also as a model for understanding genetic influences on human health and disease.

—Kenneth D. Belanger

See also: Model Organism: *Arabidopsis thaliana*; Model Organism: *Caenorhabditis elegans*; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Drosophila melanogaster*; Model Organism: *Escherichia coli*; Model Organism: *Mus musculus*; Model Organism: *Neurospora crassa*; Model Organism: *Saccharomyces cerevisiae*; Model Organism: *Xenopus laevis*.

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Web Site of Interest

Genetics Society of America. <http://www.genetics-gsa.org>. Click on "Model Organisms" for links to Web pages on more than two dozen model organisms.

Molecular Clock Hypothesis

Fields of study: Evolutionary biology; Molecular genetics

Significance: *The molecular clock hypothesis (MCH) predicts that amino acid changes in proteins and nucleotide changes in DNA are approximately constant over time. When first proposed, it was immediately embraced by many evolutionists as a way to determine the absolute age of evolutionary lineages. After more protein sequences were analyzed, however, many examples were inconsistent with the MCH. The theory has generated a great deal of controversy among evolutionists, and although it is now generally accepted that many genes do not change at constant rates, methods are still being developed to determine the ages of lineages based on amino acid and nucleotide substitutions.*

Key terms

CODON: a three-letter nucleotide sequence in RNA or DNA that codes for a specific amino acid; a gene is composed of a long string of codons

INTRON: an intervening sequence in a eukaryotic gene (generally there are several to many

per gene) that must be removed when it is transcribed into messenger RNA (mRNA); introns are assumed to have no function and therefore mutations in them are often considered neutral

NEUTRAL MUTATION: a mutation in a gene which is considered to have no effect on the fitness of the organism

PHYLOGENY: often called an evolutionary tree, the branching patterns that show evolutionary relationships, with the taxa on the ends of the branches

TAXON (pl. TAXA): a general term used by evolutionists to refer to a type of organism at any taxonomic rank in a classification of organisms

History

In 1962 Émile Zuckerkandl and Linus Pauling published evidence that the rate of amino acid substitution in proteins is constant over time. In 1965, after several protein sequences (cytochrome c, hemoglobin, and fibrinopeptides) seemed to show this pattern, they proposed the molecular clock hypothesis (MCH). According to their hypothesis, mutations leading to changes in the amino acid sequence of a protein should occur at a constant rate over time, rather than per generation, as previously assumed. In other words, if the sequence of cytochrome c were determined 1,000,000 years ago, 500,000 years ago, and in the present, the rate of amino acid substitution would be the same between the first two samples as it would be between the second and third. To state this more accurately, they considered the rate approximately constant, which means that one protein may display some variation, but if the average rates of change for several were considered as a group, they would be constant.

Importance of the Molecular Clock Hypothesis

The evolutionary importance of the MCH was almost immediately apparent. Paleontologists had long determined the ages of fossils using radioactive dating techniques, but determining the date of a fossil was not the same as determining how long ago flowering plants diverged (evolved from) the other vascular

plants, for example. Using the MCH, researchers could compare the amino acid sequences of a protein in a flowering plant and another vascular plant, and if the substitution rate (that is, substitutions per unit of time) was known, they could determine how long ago these two plants diverged. The MCH held great promise for solving many of the questions about when various groups of organisms diverged from their common ancestors. To “calibrate” the clock—that is, to determine the rate of amino acid substitutions—all that was needed were the sequences of some taxa and a reliable age for fossils considered to represent the common ancestor to the taxa. Once this clock had been calibrated, other taxa that might not be as well represented in the fossil record could be studied, and their time of divergence could be determined as well.

As more data accumulated through the next twenty years, it was discovered that amino acid substitutions in many proteins were not as clocklike as hoped. Rates over time seemed to slow down and speed up, and there was no predictable pattern to the changes. In fact, the same proteins in different evolutionary lineages often “ticked” at a different rate.

The Neutral Theory

During the time that more and more proteins were being sequenced, DNA sequencing gradually began to dominate. One of the theories about why the MCH did not seem to be working was that protein sequences were constrained by natural selection. The intensity of natural selection has always been assumed to vary over time, and if this is true, then amino acid substitution rates should also increase and decrease as some kind of function of the pressure exerted by natural selection. DNA sequences were quickly hailed as the solution to this problem. In 1968, Motoo Kimura proposed the neutral theory, in which he proposed that any nucleotide substitution in DNA that occurred in a noncoding region, or that did not change the amino acid sequence in the gene’s product, would be unaffected by natural selection. He suggested that because of this, neutral mutations (nucleotide substitutions) would be free to take place without being weeded out by selection.

The strength of the neutral theory was that, unlike mutations that affect the amino acid sequence, neutral mutations should occur at a constant rate over time. Therefore, Kimura predicted that the MCH would be valid for neutral mutations. Most eukaryotic genomes are riddled with sequences, like introns or highly repetitive DNA, that have no apparent function and can therefore be assumed to be prone to neutral mutations. Even within the coding regions (exons) of expressed genes, the third position of many codons can be changed without affecting the amino acid for which it codes. A number of evolutionists expressed skepticism concerning the neutral theory, arguing that there is probably no truly neutral mutation.

As DNA sequences poured in, much the same story emerged as for protein sequences. Whether or not neutral mutations exist, nucleotide substitutions that were assumed to be neutral turned out to tick no better. In the 1980’s the controversy over the MCH reached its height, and most evolutionists were forced to conclude that very few genes, or neutral sequences, behaved like a clock. Even those that did behave like clocks did not tick at the same rate in all lineages, and even worse, some genes ticked more or less steadily in some lineages and very erratically in others. Comparisons among the many amino acid and nucleotide sequences revealed another surprise: Amino acid sequences tended, on average, to be more reliable than nucleotide sequences.

Beyond the Molecular Clock

Since the 1980’s, the MCH has fallen into disfavor among most evolutionists, but attempts to use amino acid and nucleotide sequences to estimate evolutionary ages are still being made. In a few cases, often in closely related taxa, the MCH works, but other approaches are used more often. Many of these approaches attempt to take into account the highly variable substitution rates among different lineages and over time. Rather than using a single protein or DNA sequence, as was attempted when the MCH was first developed, they use several in the same analysis. Data analysis relies on complex, and sometimes esoteric,

statistical algorithms that often require considerable computational power.

In some ways, the research community is in disarray when it comes to post-MCH methods. There are several alternative approaches, and some that represent blended approaches, and agreement is far from being achieved. It is hoped that as more data are collected and analyzed, a coherent approach will be developed.

See also: Ancient DNA; DNA Sequencing Technology; Evolutionary Biology; Natural Selection; Punctuated Equilibrium; Repetitive DNA.

—Bryan Ness

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Molecular Genetics

Field of study: Molecular genetics

Significance: *Molecular genetics is the branch of genetics concerned with the central role that molecules, particularly the nucleic acids DNA and RNA, play in heredity. The understanding of molecular genetics is at the heart of biotechnology, which has had a tremendous impact on medicine, agriculture, forensics, and many other fields.*

Key terms

DNA: deoxyribonucleic acid, a long-chain macromolecule, made of units called nucleotides and structured as a double helix joined by weak hydrogen bonds, which forms genetic material for most organisms

GENOME: the assemblage of the genetic information of an organism or of one of its organelles

REPLICATION: the process by which one DNA molecule is converted to two DNA molecules identical to the first

RNA: ribonucleic acid, the macromolecule in the cell that acts as an intermediary between the genetic information stored as DNA and the manifestation of that genetic information as proteins

TRANSCRIPTION: the process of forming an RNA molecule according to instructions contained in DNA

TRANSLATION: the process of forming proteins according to instructions contained in an RNA molecule

Identity and Structure of Genetic Material

Molecular genetics is the branch of genetics that deals with the identity of the molecules of heredity, their structure and organization, how these molecules are copied and transmitted, how the information encrypted in them is decoded, and how the information can change from generation to generation. In the late 1940's and early 1950's, scientists realized that the materials of heredity were nucleic acids. DNA was implicated as the substance extracted from a deadly strain of pneumococcal bacteria that could transform a mild strain into a lethal one and as the substance injected into bacteria by viruses as they start an infection. RNA was shown to be the component of a virus that determined what kind of symptoms of infection appeared on tobacco leaves.

The nucleic acids are made up of nucleotides linked end to end to produce very long molecules. Each nucleotide has sugar and phosphate parts and a nitrogen-rich part called a base. Four bases are commonly found in each DNA and RNA. Three, adenine (A), guanine (G), and cytosine (C), are found in both DNA

and RNA, while thymine (T) is normally found only in DNA and uracil (U) only in RNA. In the double-helical DNA molecule, two strands are helically intertwined in opposite directions. The nucleotide strands are held together in part by interactions specific to the bases, which “pair” perpendicular to the sugar-phosphate strands. The structure can be envisioned as a ladder. The A and T bases pair with each other, and G and C bases pair with each other, forming “rungs”; the sugar-phosphates, joined end to end, form the “sides” of the ladder. The entire molecule twists and bends in on itself to form a compact whole. An RNA molecule is essentially “half” of this ladder, split down the middle. RNA molecules generally adopt less regular structures but may also require pairing between bases.

DNA and RNA, in various forms, serve as the molecules of heredity. RNA is the genetic material that some viruses package in viral particles. One or several molecules of RNA may make up the viral information. The genetic material of most bacteria is a single circle of double-helical DNA, the circle consisting of from slightly more than 500,000 to about 5 million nucleotide pairs. In eukaryotes such as humans, the DNA genetic material is organized into multiple linear DNA molecules, each one the essence of a morphologically recognizable and genetically identifiable structure called a chromosome.

In each organism, the DNA is closely associated with proteins. Proteins are made of one or more polypeptides. Polypeptides are linear polymers, like nucleic acids, but the units linked end to end are amino acids rather than nucleotides. More than twenty kinds of amino acids make up polypeptides. Proteins are generally smaller than DNA molecules and assume a variety of shapes. Proteins contribute to the biological characteristics of an organism in many ways: They are major components of structures both inside (membranes and fibers) and outside (hair and nails) the cell; as enzymes, they initiate the thousands of chemical reactions that cells use to get energy and build new cells; and they regulate the activities of cells. Histone proteins pack eukaryotic nuclear DNA into tight bundles called nucleosomes. Further coiling and looping of nucleosomes results in

the compact structure of chromosomes. These can be seen with help of a microscope. The complex of DNA and protein is called chromatin.

The term “genome” denotes the roster of genes and other DNA of an organism. Most eukaryotes have more than one genome. The principal genome is the genome of the nucleus that controls most of the activities of cells. Two organelles, the mitochondria (which produce energy by oxidizing chemicals) and the plastids (such as chloroplasts, which convert light to chemical energy in photosynthesis) have their own genomes. The organelle genomes have only some of the genes needed for their functioning. The others are present in the nuclear genome. Nuclear genomes have many copies of some genes. Some repeated sequences are organized tandemly, one after the other, while others are interspersed with unique sequences. Some repeated sequences are genes present in many copies, while others are DNAs of unknown function.

Copying and Transmission of Genetic Nucleic Acids

James Watson and Francis Crick’s double-helical structure for DNA suggested to them how a faithful copy of a DNA could be made. The strands would pull apart. One by one, the new nucleotide units would then arrange themselves by pairing with the correct base on the exposed strands. When zipped together, the new units make a new strand of DNA. The process, called DNA replication, makes two double-helical DNAs from one original one. Each daughter double-helical DNA has one old and one new strand. This kind of replication, called semiconservative replication, was confirmed by an experiment by Matthew Meselson and Franklin Stahl.

Enzymes cannot copy DNA of eukaryotic chromosomes completely to each end of the DNA strands. This is not a problem for bacteria, whose circular genomes do not have ends. To keep the ends from getting shorter with each cycle of replication, eukaryotic chromosomes have special structures called telomeres at their ends that are targets of a special DNA synthesis enzyme.

When a cell divides, each daughter cell must get one and only one complete copy of the mother cell's DNA. In most bacterial chromosomes, this DNA synthesis starts at only one place, and that starting point is controlled so that the number of starts equals the number of cell fissions. In eukaryotes, DNA synthesis begins at multiple sites, and each site, once it has begun synthesis, does not begin another round until after cell division. When DNA has been completely copied, the chromosomes line up for distribution to the daughter cells. Protein complexes called kinetochores bind to a special region of each chromosome's DNA called the "centromere." Kinetochores attach to microtubules, fibers that provide the tracks along which the chromosomes move during their segregation into daughter cells.

Gene Expression, Transcription, and Translation

DNA is often dubbed the blueprint of life. It is more accurate to describe DNA as the computer tape of life's instructions because the DNA information is a linear, one-dimensional series of units rather than a two-dimensional diagram. In the flow of information from the DNA tape to what is recognized as life, two steps require the decoding of nucleotide sequence information. The first step, the copying of the DNA information into RNA, is called transcription, an analogy to medieval monks sitting in their cells copying, letter by letter, old Latin manuscripts. The letters and words in the new version are the same as in the old but are written with a different hand and thus have a slightly different appearance. The second step, in which amino acids are polymerized in response to the RNA information, is called translation. Here, the monks take the Latin words and find English, German, or French equivalents. The product is not in the nucleotide language but in the language of polypeptide sequences. The RNAs that direct the order of amino acids are called messenger RNAs (mRNAs) because they bring instructions from the DNA to the ribosome, the site of translation.

Multicellular organisms consist of a variety of cells, each with a particular function. Cells

also respond to changes in their environment. The differences among cell types and among cells in different environmental conditions are caused by the synthesis of different proteins. For the most part, regulation of which proteins are synthesized and which are not occurs by controlling the synthesis of the mRNAs for these proteins. Genes can have their transcription switched on or switched off by the binding of protein factors to a segment of the gene that determines whether transcription will start or not. An important part of this gene segment is the promoter. It tells the transcription apparatus to start RNA synthesis only at a particular point in the gene.

Not all RNAs are ready to function the moment their synthesis is over. Many RNA transcripts have alternating exon and intron segments. The intron segments are taken out with splicing of the end of one exon to the beginning of the next. Other transcripts are cut at several specific places so that several functional RNAs arise from one transcript. Eukaryotic mRNAs get poly-A tails (about two hundred nucleotide units in which every base is an A) added after transcription. A few RNAs are edited after transcription, some extensively by adding or removing U nucleotides in the middle of the RNA, others by changing specific bases.

Translation occurs on particles called ribosomes and converts the sequence of nucleotide residues in mRNA into the sequence of amino acid residues in a polypeptide. Since protein is created as a consequence of translation, the process is also called protein synthesis. The mRNA carries the code for the order of insertion of amino acids in three nucleotide units called codons. Failure of the ribosome to read nucleotides three at a time leads to shifts in the frame of reading the mRNA message. The frame of reading mRNA is set by starting translation only at a special codon.

Transfer RNA (tRNA) molecules actually do the translating. There is at least one tRNA for each of the twenty common amino acids. Anticodon regions of the tRNAs each specifically pair with only a specific subset of mRNA codons. For each amino acid there is at least one enzyme that attaches the amino acid to the

correct tRNA. These enzymes are thus at the center of translation, recognizing both amino acid and nucleotide residues.

The ribosomes have sites for binding of mRNA, tRNA, and a variety of protein factors. Ribosomes also catalyze the joining of amino acids to the growing polypeptide chain. The protein factors, usually loosely bound to ribosomes, assist in the proper initiation of polypeptide chains, in the binding of amino acid-bearing tRNA to the ribosome, and in moving the ribosome relative to the mRNA after each additional step. Three steps in translation use biochemical energy: attaching the amino acid to the tRNA, binding the amino acyl tRNA to the ribosome-mRNA complex, and moving the ribosome relative to the mRNA.

Protein Processing and DNA Mutation

The completed polypeptide chain is processed in one or more ways before it assumes its role as a mature protein. The linear string of amino acid units folds into a complex, three-dimensional structure, sometimes with the help of other proteins. Signals in some proteins' amino acid sequences direct them to their proper destinations after they leave the ribosomes. Some signals are removable, while others remain part of the protein. Some newly synthesized proteins are called polyproteins because they are snipped at specific sites, giving several proteins from one translation product. Finally, individual amino acid units may get other groups attached to them or be modified in other ways.

The DNA information can be corrupted by reaction with certain chemicals, some of which are naturally occurring while others are present in the environment. Ultraviolet and ionizing radiation can also damage DNA. In addition, the apparatus that replicates DNA will make a mistake at low frequency and insert the wrong nucleotide.

Collectively, these changes in DNA are called DNA damage. When DNA damage goes unrepaired before the next round of copying of the DNA, mutations (inherited changes in nucleotide sequence) result. Mutations may be substitutions, in which one base replaces another. They may also be insertions or deletions

of one or more nucleotides. Mutations may be beneficial, neutral, or harmful. They are the targets of the natural selection that drives evolution. Since some mutations are harmful, survival of the species requires that they be kept to a low level.

Systems that repair DNA are thus very important for the accurate transmission of the DNA information tape. Several kinds of systems have evolved to repair damaged DNA before it can be copied. In one, enzymes directly reverse the damage to DNA. In a second, the damaged base is removed, and the nucleotide chain is split to allow its repair by a limited resynthesis. In a third, a protein complex recognizes the DNA damage, which results in incisions in the DNA backbone on both sides of the damage. The segment containing the damage is removed, and the gap is filled by a limited resynthesis. In still another, mismatched base pairs, such as those that result from errors in replication, are recognized, and an incision is made some distance away from the mismatch. The entire stretch from the incision point to past the mismatch is then resynthesized. Finally, the molecular machinery that exchanges DNA segments, the recombination machinery, may be mobilized to repair damage that cannot be handled by the other systems.

Invasion and Amplification of Genes

Mutation is only one way that genomes change from generation to generation. Another way is via the invasion of an organism's genome by other genomes or genome segments. Bacteria have evolved restriction modification systems to protect themselves from such invasions. The gene for restriction encodes an enzyme that cleaves DNA whenever a particular short sequence of nucleotides is present. It does not recognize that sequence when it has been modified with a methyl group on one of its bases. The gene for modification encodes the enzyme that adds the methyl group. Thus the bacterium's own DNA is protected. However, DNA that enters the cell from outside, such as by phage infection or by direct DNA uptake, is not so protected and will be targeted for degradation by the restriction enzyme. Despite restriction, transfer of genes from one species

to another (horizontal, or lateral, gene transfer) has occurred.

As far as is known, restriction modification systems are unique to bacteria. Gene transfer from bacteria to plants occurs naturally in diseases caused by bacteria of the *Agrobacterium* genus. As part of the infection process, these bacteria transfer a part of their DNA containing genes, only active in plants, into the plant genome. Studies with fungi and higher plants suggest that eukaryotes cope with gene invasion by inactivating the genes (gene silencing) or their transcripts (cosuppression).

Another way that genomes change is by duplications of gene-sized DNA segments. When the environment is such that the extra copy is advantageous, the cell with the duplication survives better than one without the duplication. Thus genes can be amplified under selective pressure. In some tissues, such as salivary glands of dipteran insects and parts of higher plant embryos, there is replication of large segments of chromosomes without cell division. Monster chromosomes result.

Genomes also change because of movable genetic elements. Inversions of genome segments occur in bacteria and eukaryotes. Other segments can move from one location in the genome to another. Some of these movements appear to be rare, random events. Others serve particular functions and are programmed to occur under certain conditions. One kind of mobile element, the retrotransposon, moves into new locations via an RNA intermediate. The element encodes an enzyme that makes a DNA copy of the element's RNA transcript. That copy inserts itself into other genome locations. The process is similar to that used by retroviruses to establish infection in cells. Other mobile elements, called transposons or transposable elements, encode a transposase enzyme that inserts the element sequence, or a copy of it, into a new location. When that new location is in or near a gene, normal functioning of that gene is disturbed.

The production of genes for antibodies (an important part of a human's immune defense system) is a biological function that requires gene rearrangements. Antibody molecules consist of two polypeptides called light and heavy

chains. In most cells in the body, the genes for light chains are in two separated segments, and those for heavy chains are in three. During the maturation of cells that make antibodies, the genes are rearranged, bringing these segments together. The joining of segments is not precise. The imprecision contributes to the diversity of possible antibody molecules.

Cells of baker's or brewer's yeast (*Saccharomyces cerevisiae*) have genes specifying their sex, or mating type, in three locations. The information at one location, the expression locus, is the one that determines the mating type of the cell. A copy of this information is in one of the other two sites, while the third has the information specifying the opposite mating type. Yeast cells switch mating types by replacing the information at the expression locus with information from a storage locus. Mating-type switching and antibody gene maturation are only two examples of programmed gene rearrangements known to occur in a variety of organisms.

Genetic Recombination

Recombination occurs when DNA information from one chromosome becomes attached to the DNA of another. When participating chromosomes are equivalent, the recombination is called homologous. Homologous recombination in bacteria mainly serves a repair function for extreme DNA damage. In many eukaryotes, recombination is essential for the segregation of chromosomes into gamete cells during meiosis. Nevertheless, aspects of the process are common between bacteria and eukaryotes. Starting recombination requires a break in at least one strand of the double-helical DNA. In the well-studied yeast cells, a double-strand break is required. Free DNA ends generated by breaks invade the double-helical DNA of the homologous chromosome. Further invasion and DNA synthesis result in a structure in which the chromosomes are linked to one another. This structure, called a half-chiasma, is recognized and resolved by an enzyme system. Resolution can result in exchange so that one end of one chromosome is linked to the other end of the other chromosome and vice versa. Resolution can also result in restoration of the original linkage. In the latter case, the DNA

around the exchange point may be that of the other DNA. This is known as gene conversion.

Impact and Applications

Molecular genetics is at the heart of biotechnology, or genetic engineering. Its fundamental investigation of biological processes has provided tools for biotechnologists. Molecular cloning and gene manipulation in the test tube rely heavily on restriction enzymes, other nucleic-acid-modifying enzymes, and extrachromosomal DNA, all discovered during molecular genetic investigation. The development of nucleic acid hybridization, which allows the identification of specific molecular clones in a pool of others, required an understanding of DNA structure and dynamics. The widely used polymerase chain reaction (PCR), which can amplify minute quantities of DNA, would not have been possible without discoveries in DNA replication. Genetic mapping, a prelude to the isolation of many genes, was sped along by molecular markers detectable with restriction enzymes or the PCR. Transposable elements and the transferred DNA of *Agrobacterium*, because they often inactivate genes when they insert in them, were used to isolate the genes they inactivate. The inserted elements served as tags or handles by which the modified genes were pulled out of a collection of genes.

The knowledge of the molecular workings of genes gained by curious scientists has allowed other scientists to intervene in many disease situations, provide effective therapies, and improve biological production. Late twentieth century scientists rapidly developed an understanding of the infection process of the acquired immunodeficiency syndrome (AIDS) virus. The understanding, built on the skeleton of existing knowledge, has helped combat this debilitating disease. Molecular genetics has also led to the safe and less expensive production of proteins of industrial, agricultural, and pharmacological importance. The transfer of DNA from *Agrobacterium* to plants has been exploited in the creation of transgenic plants. These plants offer a new form of pest protection that provides an alternative to objectionable pesticidal sprays and protects against pathogens for which no other protection is available. Recombinant insulin

and recombinant growth hormone are routinely given to those whose conditions demand them. Through molecular genetics, doctors have diagnostic kits that can, with greater rapidity, greater specificity, and lower cost, determine whether a pathogen is present. Finally, molecular genetics has been used to identify genes responsible for many inherited diseases of humankind. Someday medicine may correct some of these diseases by providing a good copy of the gene, a strategy called gene therapy.

—Ulrich Melcher

See also: Ancient DNA; Antisense RNA; Biochemical Mutations; Central Dogma of Molecular Biology; Chemical Mutagens; Chloroplast Genes; Chromatin Packaging; DNA Isolation; DNA Repair; DNA Structure and Function; Gene Families; Genetic Code; Genetic Code, Cracking of; Genome Size; Genomics; Molecular Clock Hypothesis; Mutation and Mutagenesis; Noncoding RNA Molecules; Oncogenes; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; Proteomics; Pseudogenes; Repetitive DNA; Restriction Enzymes; Reverse Transcriptase; RNA Isolation; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World; Signal Transduction; Steroid Hormones; Telomeres; Transposable Elements; Tumor-Suppressor Genes.

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Max Planck Institute for Molecular Genetics. <http://www.molgen.mpg.de>. Research institute focuses on molecular mechanisms of DNA replication, recombination, protein synthesis, and ribosome structure, and offers educational information and history.

Monohybrid Inheritance

Field of study: Classical transmission genetics

Significance: Humans and other organisms show a number of different patterns in the inheritance and expression of traits. For many inherited characteristics, the pattern of transmission is monohybrid inheritance, in which a trait is determined by one pair of alleles at a single locus. An understanding of monohybrid inheritance is critical for understanding the genetics of many medically significant traits in humans and economically significant traits in domestic plants and animals.

Key terms

ALLELLE: one of the pair of possible alternative forms of a gene that occurs at a given site or locus on a chromosome

DOMINANT GENE: the controlling member of a pair of alleles that is expressed to the exclusion of the expression of the recessive member

RECESSIVE GENE: an allele that can only be expressed when the controlling or dominant allele is not present

Mendel and Monohybrid Inheritance

The basic genetic principles first worked out and described by Gregor Mendel in his classic experiments on the common garden pea have been found to apply to many inherited traits in all sexually reproducing organisms, including humans. Until the work of Mendel, plant and animal breeders tried to formulate laws of inheritance based upon the principle that characteristics of parents would be blended in their offspring. Mendel's success came about because he studied the inheritance of contrasting or alternative forms of one phenotypic trait at a time. The phenotype of any organism includes not only all of its external characteristics but also all of its internal structures, extending even into all of its chemical and metabolic functions. Human phenotypes would include characteristics such as eye color, hair color, skin color, hearing and visual abnormalities, blood disorders, susceptibility to various diseases, and muscular and skeletal disorders.

Mendel experimented with seven contrasting traits in peas: stem height (tall vs. dwarf), seed form (smooth vs. wrinkled), seed color (yellow vs. green), pod form (inflated vs. constricted), pod color (green vs. yellow), flower color (red vs. white), and flower position (axial vs. terminal). Within each of the seven sets, there was no overlap between the traits and thus no problem in classifying a plant as one or the other. For example, although there was some variation in height among the tall plants and some variation among the dwarf plants, there was no overlap between the tall and dwarf plants.

Mendel's first experiments crossed parents that differed in only one trait. Matings of this type are known as monohybrid crosses, and the rules of inheritance derived from such matings yield examples of monohybrid inheritance. These first experiments provided the evidence for the principle of segregation and the principle of dominance. The principle of segregation refers to the separation of members of a gene pair from each other during the formation of gametes (the reproductive cells: sperm in males and eggs in females). It was Mendel who first used the terms "dominant" and "recessive." It is of interest to examine his words and to realize how appropriate his definitions are today: "Those characters which are transmitted entire, or almost unchanged by hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the dominant and those which become latent in the process recessive." The terms dominant and recessive are used to describe the characteristics of a phenotype, and they may depend on the level at which a phenotype is described. A gene that acts as a recessive for a particular external trait may turn out not to be so when its effect is measured at the biochemical or molecular level.

An Example of Monohybrid Inheritance

The best way of describing monohybrid inheritance is by working through an example. Although any two people obviously differ in many genetic characteristics, it is possible, as Mendel did with his pea plants, to follow one trait governed by a single gene pair that is separate and independent of all other traits. In ef-

fect, by doing this, the investigator is working with the equivalent of a monohybrid cross. In selecting an example, it is best to choose a trait that does not produce a major health or clinical effect; otherwise, the clear-cut segregation ratios expected under monohybrid inheritance might not be seen in the matings.

Consider the trait of albinism, a phenotype caused by a recessive gene. Albinism is the absence of pigment in the hair, skin, and eyes. Similar albino genes have been found in many animals, including mice, buffalo, bats, frogs, and rattlesnakes. Since the albino gene is recessive, the gene may be designated with the symbol *c* and the gene for normal pigmentation as *C*. Thus a mating between a homozygous normal person (*CC*) and a homozygous albino person (*cc*) would be expected to produce children who are heterozygous (*Cc*) but phenotypically normal, since the normal gene is dominant to the albino gene. Only normal genes, *C*, would be passed on by the normally pigmented parent, and only albino genes, *c*, would be passed on by the albino parent. If there was a mating between two heterozygous people (*Cc* and *Cc*), the law of segregation would predict that each parent would produce two kinds of gametes: *C* and *c*. The resulting progeny would be expected to appear at a ratio of 1 *CC*: 2 *Cc*: 1 *cc*. Since *C* is dominant to *c*, $\frac{3}{4}$ of the progeny would be expected to have normal pigment, and $\frac{1}{4}$ would be expected to be albino. There are three genotypes (*CC*, *Cc*, and *cc*) and two phenotypes (normal pigmentation and albino). By following the law of segregation and taking account of the dominant gene, it is possible to determine the types of matings that might occur and to predict the types of children that would be expected (see the table "Phenotype Predictions: Albino Children").

Because of dominance, it is not always possible to tell what type of mating has occurred. For example, in matings 1, 2, and 4 in the table, the parents are both normal in each case. Yet in mating 4, $\frac{1}{4}$ of the offspring are expected to be albino. A complication arises when it is realized that in mating 4 the couple might not produce any offspring that are *cc*; in that case, all offspring would be normal. Often, because of the small number of offspring in humans and

Phenotype Predictions: Albino Children

Parents	Phenotypes	Offspring Expected
1. AA × AA	Normal × Normal	All AA (Normal)
2. AA × Aa	Normal × Normal	½ AA, ½ Aa (All Normal)
3. AA × aa	Normal × Albino	All Aa (Normal)
4. Aa × Aa	Normal × Normal	¼ AA, ½ Aa, ¼ aa (¾ Normal, ¼ Albino)
5. Aa × aa	Normal × Albino	½ Aa, ½ aa (½ Normal, ½ Albino)
6. aa × aa	Albino × Albino	All aa (Albino)

other animals, the ratios of offspring expected under monohybrid inheritance might not be realized. Looking at the different matings and the progeny that are expected, it is easy to see how genetics can help to explain not only why children resemble their parents but also why children do not resemble their parents.

Modification of Basic Mendelian Inheritance

After Mendel's work was rediscovered early in the twentieth century, it soon became apparent that there were variations in monohybrid inheritance that apparently were not known to Mendel. Mendel studied seven pairs of contrasting traits, and in each case, one gene was dominant and one gene was recessive. For each trait, there were only two variants of the gene. It is now known that other possibilities exist. For example, other types of monohybrid inheritance include codominance (in which both genes are expressed in the heterozygote) and sex linkage (an association of a trait with a gene on the X chromosome). Nevertheless, the law of segregation operates in these cases as well, making it possible to understand inheritance of the traits.

Within a cell, genes are found on chromosomes in the nucleus. Humans have forty-six chromosomes. Each person receives half of the chromosomes from each parent, and it is convenient to think of the chromosomes in pairs. Examination of the chromosomes in males and females reveals an interesting difference. Both sexes have twenty-two pairs of what are termed

"autosomes" or "body chromosomes." The difference in chromosomes between the two sexes occurs in the remaining two chromosomes. The two chromosomes are known as the sex chromosomes. Males have an unlike pair of sex chromosomes, one designated the X chromosome and the other, smaller one designated the Y chromosome. Females, on the other hand,

have a pair of like sex chromosomes, and these are similar to the X chromosome of the male. Although the Y chromosome does not contain many genes, it is responsible for male development. A person without a Y chromosome would undergo female development. Since genes are located on chromosomes, the pattern of transmission of the genes demonstrates some striking differences from that of genes located on any of the autosomes. For practical purposes, "sex linked" usually refers to genes found on the X chromosome since the Y chromosome contains few genes. Although X-linked traits do not follow the simple pattern of transmission of simple monohybrid inheritance as first described by Mendel, they still conform to his law of segregation. Examination of a specific example is useful to understand the principle.

The red-green color-blind gene is X-linked and recessive, since females must have the gene on both X chromosomes in order to exhibit the trait. For males, the terms "recessive" and "dominant" really do not apply since the male has only one X chromosome (the Y chromosome does not contain any corresponding genes) and will express the trait whether the gene is recessive or dominant. An important implication of this is that X-linked traits appear more often in males than in females. In general, the more severe the X-linked recessive trait is from a health point of view, the greater the proportion of affected males to affected females.

If the color-blind gene is designated *cb* and the normal gene *Cb*, the types of mating and offspring expected may be set up as they were

for the autosomal recessive albino gene. In the present situation, the X and Y chromosomes will also be included, remembering that the *Cb* and *cb* genes will be found only on the X chromosome and that any genotype with a Y chromosome will result in a male. (See the table "Phenotype Predictions: Color Blindness.")

"Carrier" females are heterozygous females who have normal vision but are expected to pass the gene to half their sons, who would be color blind. Presumably, the carrier female would have inherited the gene from her father, who would have been color blind. Thus, in some families the trait has a peculiar pattern of transmission in which the trait appears in a woman's father, but not her, and then may appear again in her sons.

Impact and Applications

The number of single genes known in humans has grown dramatically since Victor McKusick published the first *Mendelian Inheritance in Man* catalog in 1966. In the first catalog, there were 1,487 entries representing loci identified by Mendelizing phenotypes or by cellular and molecular genetic methods. In the 1994 catalog, the number of entries had grown to 6,459. Scarcely a day goes by without a news re-

port or story in the media involving an example of monohybrid inheritance. Furthermore, genetic conditions or disorders regularly appear as the theme of a movie or play. An understanding of the principles of genetics and monohybrid inheritance provides a greater appreciation of what is taking place in the world, whether it is in the application of DNA fingerprinting in the courtroom, the introduction of disease-resistant genes in plants and animals, the use of genetics in paternity cases, or the description of new inherited diseases.

Perhaps it is in the area of genetic diseases that knowledge of monohybrid inheritance offers the most significant personal applications. Single-gene disorders usually fall into one of the four common modes of inheritance: autosomal dominant, autosomal recessive, sex-linked dominant, and sex-linked recessive. Examination of individual phenotypes and family histories allows geneticists to determine which mode of inheritance is likely to be present for a specific disorder. Once the mode of inheritance has been identified, it becomes possible to determine the likelihood or the risk of occurrence of the disorder in the children. Since the laws governing the transmission of Mendelian traits are so well known, it is possible to pre-

Phenotype Predictions: Color Blindness

Parents	Phenotypes	Offspring Expected
1. $X^{Cb}X^{Cb} \times X^{Cb}Y$	Normal × Normal	$X^{Cb}X^{Cb}$ normal female $X^{Cb}Y$ normal male
2. $X^{Cb}X^{Cb} \times X^{cb}Y$	Normal × Color blind	$X^{Cb}X^{cb}$ normal female $X^{Cb}Y$ normal male
3. $X^{Cb}X^{cb} \times X^{Cb}Y$	Normal × Normal	$X^{Cb}X^{Cb}$ $X^{Cb}X^{cb}$ $\frac{1}{2}$ normal females, $\frac{1}{2}$ carrier females $X^{Cb}Y$ $X^{Cb}Y$ $\frac{1}{2}$ normal males, $\frac{1}{2}$ color-blind males
4. $X^{Cb}X^{cb} \times X^{cb}Y$	Normal × Color blind	$X^{Cb}X^{cb}$ $X^{cb}X^{cb}$ $\frac{1}{2}$ carrier females, $\frac{1}{2}$ color-blind females $X^{Cb}Y$ $X^{cb}Y$ $\frac{1}{2}$ normal males, $\frac{1}{2}$ color-blind males
5. $X^{cb}X^{cb} \times X^{Cb}Y$	Color blind × Normal	$X^{Cb}X^{cb}$ carrier females $X^{cb}Y$ color-blind males
6. $X^{cb}X^{cb} \times X^{cb}Y$	Color blind × Color blind	$X^{cb}X^{cb}$ color-blind females $X^{cb}Y$ color-blind males

dict with great accuracy when a genetic condition will affect a specific family member. In many cases, testing may be done prenatally or in individuals before symptoms appear. As knowledge of the human genetic makeup increases, it will become even more essential for people to have a basic knowledge of how Mendelian traits are inherited.

—Donald J. Nash

See also: Albinism; Classical Transmission Genetics; Complete Dominance; Dihybrid Inheritance; Epistasis; Hereditary Diseases; Incomplete Dominance; Mendelian Genetics; Multiple Alleles.

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Multiple Alleles

Field of study: Classical transmission genetics

Significance: Alleles are alternate forms of genes at the same locus. When three or more variations of a gene exist in a population, they are referred to as multiple alleles. The human ABO blood groups provide an example of multiple alleles.

Key terms

BLOOD TYPE: one of the several groups into which blood can be classified based on the presence or absence of certain molecules called antigens on the red blood cells

CODOMINANT ALLELES: two contrasting alleles that are both fully functional and fully expressed when present in an individual

DOMINANT ALLELE: an allele that masks the expression of another allele that is considered recessive to it

RECESSIVE ALLELE: an allele that will be exhibited only if two copies of if are present

The Discovery of Alleles and Multiple Alleles

Although Gregor Mendel, considered to be the father of genetics, did not discover multiple alleles, an understanding of his work is necessary to understand their role in genetics. In the 1860's, Mendel formulated the earliest concepts of how traits or characteristics are passed from parents to their offspring. His work on pea plants led him to propose that there are two factors, since renamed "genes," that cause each trait that an individual possesses. A particular form of the gene, called the "dominant" form, will enable the characteristic to occur whether the offspring inherits one or two copies of that allele. The alternate form of the gene, or allele, will be exhibited only if two copies of this allele, called the "recessive" form, are present. For example, pea seeds will be yellow if two copies of the dominant, yellow-causing gene are present and will be green if two copies of the recessive gene are present. However, since yellow is dominant to green, an individual plant with one copy of each allele will be as yellow as a plant possessing two yellow genes. Men-

Mendel discovered only two alternate appearances, called phenotypes, for each trait he studied. He found that violet is the allele dominant to white in causing flower color, while tall is the allele dominant to short in creating stem length.

Early in the twentieth century, examples of traits with more than an either/or phenotype caused by only two possible alleles were found in a variety of organisms. Coat color in rabbits is a well-documented example of multiple alleles. Not two but four alternative forms of the gene for coat color exist in rabbit populations, with different letters used to designate those colors. The gene producing color is labeled *c*; thus, *c^d* produces full, dark color, *c^{ch}* produces mixed colored and white hairs, *c^h* produces white on the body but black on the paws, and *c* creates a pure white rabbit. It is important to note that although three or more alternative forms can exist in a population, each individual organism can only possess two, acquiring only one from each of its parents. What, then, of Mendel's principle of one allele being dominant to the other? In the rabbit color trait, *c^d* is dominant to the *c^{ch}*, which is dominant to *c^h*, with *c*, the gene for pure white, recessive to the other three.

If mutation can create four possible color alleles, is it not also possible that successive mutations might cause a much larger number of multiple alleles? Numerous examples exist of genes with many alleles. For example, sickle-cell disease, and related diseases called thalassemias, are all caused by mutations in one of the two genes that code for the two protein subunits of hemoglobin, the protein that carries oxygen in the blood. Dozens of different types of thalassemia exist, all caused by mutations in the same gene.

Blood Types

One of the earliest examples of multiple alleles discovered in humans concerns the ABO blood type system. In 1900, the existence of four blood types (A, B, AB, and O) was discovered. The study of pedigrees (the family histo-

The Relationship Between Genotype and Blood Type

Genotype	Blood Type	Comments
AA	A	These two genotypes produce identical blood types.
AO	A	
BB	B	These two genotypes produce identical blood types.
BO	B	
AB	AB	Both dominant alleles are expressed.
OO	O	With no dominant alleles, the recessive allele is expressed.

ries of many individuals) revealed by 1925 that these four blood types were caused by multiple alleles. The alleles are named *I^A*, *I^B*, and *I^O*, or simply *A*, *B*, and *O*. Both *A* and *B* are dominant to *O*. However, *A* and *B* are codominant to each other. Thus, if both are present, both are equally seen in the individual. A person with two *A* alleles or an *A* and an *O* has type A blood. Someone with two *B* alleles or a *B* and an *O* has type B. Two *O* alleles result in type O blood. Because *A* and *B* are codominant, the individual with one of each allele is said to have type AB blood.

To say people are "type A" means that they have an antigen (a glycoprotein or protein-sugar molecule) of a particular type embedded in the membrane of all red blood cells. The presence of an *A* allele causes the production of an enzyme that transfers the sugar galactosamine to the glycoprotein. The *B* allele produces an enzyme that attaches a different sugar, called galactose, and the *O* allele produces a defective enzyme that cannot add any sugar. Because of codominance, people with type AB blood have both antigens on their red blood cells.

Transfusion with blood from a donor with a different blood type than the recipient can cause death, due to the potential presence of *A* or *B* antibodies in the recipient's blood. Antibodies are chemical molecules in the plasma (the liquid portion of the blood). If, by error, type A blood is given to a person with type B blood, the recipient will produce antibodies

against the type A red blood cells, which will attach to them, causing them to agglutinate, or form clumps. By this principle, a person with type O blood can donate it to people with any blood type, because their blood cells have neither an A nor a B antigen. Thus, people with type O blood are often referred to as universal donors because no antibodies will be formed against type O blood red blood cells. Likewise, people with type AB blood are often referred to as universal recipients because they have both types of antigens and therefore will not produce antibodies against any of the blood types. Medical personnel must carefully check the blood type of both the recipient and the donated blood to avoid agglutination and subsequent death.

Blood types have been used to establish paternity because a child's blood type can be used to determine what the parents' blood types could and could not be. Since a child receives one allele from each parent, certain men can be eliminated as a child's potential father if the alleles they possess could not produce the combination found in the child. However, this proves only that a particular person could be the father, as could millions of others who possess that blood type; it does not prove that a particular man is the father. Modern methods of analyzing the DNA in many of the individual's genes now make the establishment of paternity a more exact science.

Impact and Applications

The topic of multiple alleles has implications for many human disease conditions. One of these is cystic fibrosis (CF), the most common deadly inherited disease afflicting Caucasians. Characterized by a thick mucus buildup in lungs, pancreas, and intestines, it frequently brings about death by age twenty. Soon after the gene that causes CF was found in 1989, geneticists realized there may be as many as one hundred multiple alleles for this gene. The extent of the mutation in these alternate genes apparently causes the great variation in the severity of symptoms from one patient to another.

The successful transplantation of organs is also closely linked to the existence of multiple alleles. A transplanted organ has antigens on

its cells that will be recognized as foreign and destroyed by the recipient's antibodies. The genes that build these cell-surface antigens, called human leukocyte antigen (HLA), occur in two main forms. HLA-A has nearly twenty different alleles, and HLA-B has more than thirty. Since any individual can only have two of each type, there are an enormous number of possible combinations in the population. Finding donors and recipients with the same or a very close combination of HLA alleles is a very difficult task for those arranging successful organ transplantation.

Geneticists are coming to suspect that multiple alleles, once thought to be the exception to the rule, may exist for the majority of human genes. If this is so, the study of multiple alleles for many disease-producing genes should shed more light on why the severity of so many genetic diseases varies so widely from person to person.

—Grace D. Matzen, updated by Bryan Ness

See also: Complementation Testing; Cystic Fibrosis; Organ Transplants and HLA Genes; Population Genetics.

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Mutation and Mutagenesis

Field of study: Molecular genetics

Significance: *A mutation is a heritable change in the structure or composition of DNA. Depending on the function of the altered DNA segment, the effect of a mutation can range from undetectable to causing major deformities and even death. Mutation is a natural process by which new genetic diversity is produced. However, chemical pollutants and radiation can increase mutation rates and have a serious effect on health.*

Key terms

GENE POOL: all of the genes carried by all members of a population of organisms; the ge-

netic diversity in the gene pool provides the variation that allows adaptation to new conditions

GERMINAL MUTATION: a mutation in gamete-forming (germinal) tissue, which can be passed from a parent to its offspring

MUTAGEN: a chemical or physical agent that causes an increased rate of mutation

MUTAGENESIS: the process of a heritable change occurring in a gene, either spontaneously or in response to a mutagen

MUTATION RATE: the probability of a heritable change occurring in the genetic material over a given time period, such as a cell division cycle or a generation

PHENOTYPE: the observable effects of a gene; phenotypes include physical appearance, biochemical activity, cell function, or any other measurable factor

SOMATIC MUTATION: a mutation that occurs in a body cell and produces a group of mutant cells but is not transmitted to the next generation

WILD TYPE: the normal genetic makeup of an organism, as it occurs in nature (the wild); a mutation alters the phenotype of a wild-type trait to produce a mutant phenotype

Definitions

A mutation is any change in the genetic material that can be inherited by the next generation of cells or progeny. A mutation can occur at any time in the life of any cell in the body. If a mutation occurs in the reproductive tissue, the change can be passed to an offspring in the egg or sperm. That new mutation may then affect the development of the offspring and be passed on to later generations. However, if the mutation occurs in cells of the skin, muscle, blood, or other body (somatic) tissue, the new mutation will only be passed on to other body cells when that cell divides. This can produce a mosaic patch of cells carrying the new genetic change. Most of these are undetectable and have no effect on the carrier. An important exception is a somatic mutation that causes the affected cell to lose control of the cell cycle and divide uncontrollably, resulting in cancer. Many environmental chemicals and agents that cause mutations (such as X rays and ultraviolet

radiation) therefore also cause cancer.

Mutation also has an important, beneficial role in natural populations of all organisms. The ability of a species to adapt to changes in its environment, combat new diseases, or respond to new competitors is dependent on genetic diversity in the population's gene pool. Without sufficient resources of variability, a species faced by a serious new stress can become extinct. The reduced population sizes in rare and endangered species will result in reduced genetic diversity and a loss of the capacity to respond to selection pressures. Zoo breeding programs often take data on genetic diversity into account when planning the captive breeding of endangered species. The creation of new agricultural crops or of animal breeds with economically desirable traits also depends on mutations that alter development in a useful way. Therefore, mutation can have both damaging and beneficial effects.

The Role of Mutations in Cell Activity and Development

The genetic information in a cell is encoded in the sequence of subunits, the nucleotides, that make up the DNA molecule. A mutation is a change in the cell's genetic makeup, and it can range from changing just a single nucleotide in the DNA molecule to altering long pieces of DNA. To appreciate how such changes can affect an organism, it is important to understand how information is encoded in DNA and how it is translated to produce a specific protein. There are four different nucleotides in the DNA molecule: adenine (A), guanine (G), thymine (T), and cytosine (C). The DNA molecule is composed of two strands linked together by a sequence of base pairs (bp). An adenine on one strand pairs with a thymine on the other (A-T), and a guanine on one strand pairs with a cytosine on the other (G-C). When a gene is activated, one of the two strands is used as a model, or template, for the synthesis of a single-stranded molecule called messenger RNA (mRNA). The completed mRNA molecule is then transported out of the nucleus, and it binds with ribosomes (small structures in the cytoplasm of the cell), where a protein is made using the mRNA's nucleotide sequence as its

coded message. The nucleotides are read on the ribosome in triplets, with three adjacent nucleotides (called a codon) corresponding to one of the twenty amino acids found in protein.

Thus the sequence of nucleotides eventually determines the order of amino acids that are linked together to form a specific protein. The amino acid sequence in turn determines how the protein will work, either as a structural part of a cell or as an enzyme that will catalyze a specific biochemical reaction. A gene is often 1,000 bp or longer, so there are many points at which a genetic change can occur. If a mutation occurs in an important part of the gene, even the change of a single amino acid can cause a major change in protein function. Sickle-cell disease is a good example of this. In sickle-cell disease a base-pair substitution in the DNA causes the sixth codon in the mRNA to change from GAG to GUG. When this modified mRNA is used to create a protein, the amino acid valine is substituted for the normal glutamic acid in the sixth position in a string of 146 amino acids. This small change causes the protein to form crystals and thus deform cells when the amount of available oxygen is low. Since this protein is one of the parts of the oxygen-carrying hemoglobin molecule in red blood cells, this single DNA nucleotide change has potentially severe consequences for an affected individual.

Types of Mutation

Because they can be so diverse, one way to organize mutations is to describe the kind of molecular or structural change that has occurred. There are three broad classes of mutation. “Genomic mutations” are changes in the number of chromosomes in a cell. Inheriting an extra chromosome, as in Down syndrome, is an example of a genomic mutation. “Chromosome mutations” are changes in the structure of a chromosome and can include the loss, gain, or altered order of a series of genes. “Gene mutations” are genetic changes limited to an individual gene or the adjacent regions that control its activity during development. Thus, the amount of genetic information affected by a mutation can vary from a single gene to hundreds of them. Genes also vary in

the severity of their effects. Some are undetectable in the carrier, some cause small defects or even beneficial changes in the function of a protein, while others can produce major changes in several different developmental processes at the same time.

Gene mutations are sometimes called point mutations because their genetic effects are limited to a single point, or gene, on a chromosome that can carry up to several thousand different genes. The simplest kind of point mutation is a base substitution, in which one base pair is replaced by another (for example, the replacement of an A-T base pair at one point in the DNA molecule by a C-G base pair). This can change a codon triplet so that a different amino acid is placed in the protein at that point. This often changes the function of the protein, at least in minor ways. However, some base substitutions are silent. Since several different triplets can code for the same amino acid, not all base changes will result in an amino acid substitution.

Another common kind of gene mutation called a “frame shift” can have a much larger effect on protein structure. A frame-shift mutation occurs when a nucleotide is added to, or lost from, the DNA strand when it is duplicated during cell division. Since translation of the mRNA is done by the ribosomes adding one amino acid to the growing protein for every three adjacent nucleotides, adding or deleting one nucleotide will effectively shift that reading frame so that all following triplets are different. By analogy, one can consider the following sentence of three-letter words: THE BIG DOG CAN RUN FAR. If a base (for example, a letter X, in this analogy) is added at the end of the second triplet, the “sentence” will still read three letters at a time during translation and the meaning will be completely altered. THE BIX GDO GCA NRU NFA R. In a cell, a nonfunctional protein is produced unless the frame shift is near the terminal end of the gene.

Environmental agents such as ultraviolet (UV) radiation can affect DNA and base pairing. Certain UV wavelengths, for example, cause some DNA nucleotides to pair abnormally. Gene mutations have also been traced to the movement of transposable DNA elements.

Transposable elements were first discovered by Barbara McClintock while studying chromosome breakage and kernel traits in maize. Now they are known from many organisms, including humans. Transposable elements are small DNA segments that can become inserted into a chromosome and later excised and change their position. If one becomes inserted in the middle of a gene, it effectively separates the gene into two widely spaced fragments. In the fruit fly (*Drosophila melanogaster*), in which spontaneous mutations have been studied in detail at the DNA level, as many as half of the spontaneous mutations in certain genes have been traced to transposable elements.

There are four major kinds of chromosome mutations. A chromosome deletion or deficiency is produced when two breaks occur in the chromosome but are repaired by leaving out the middle section. For example, if the sections of a chromosome are labeled with the letters ABCDEFGHIJKLMNOP and chromosome breaks occur at F-G and at K-L, the broken chromosome can be erroneously repaired by enzymes that link the ABCDEF fragment to the LMN fragment. The genes in the unattached middle segment, GHIJK, will be lost from the chromosome. Losing these gene copies can affect many different developmental processes and even cause the death of the organism. Chromosome breaks and other processes can also cause some genes to be duplicated in the chromosome (for example, ABCDEFGHDEFG HIJKLMNOP). A third kind of chromosome mutation, an inversion, changes the order of the genes when the segment between two chromosomal breaks is reattached backward (for example, ABCDJIHGFEKLMN). Finally, chromosome segments can be moved from one kind of chromosome to another in a structural change called a translocation. Some examples of heritable Down syndrome are caused by this type of chromosome mutation.

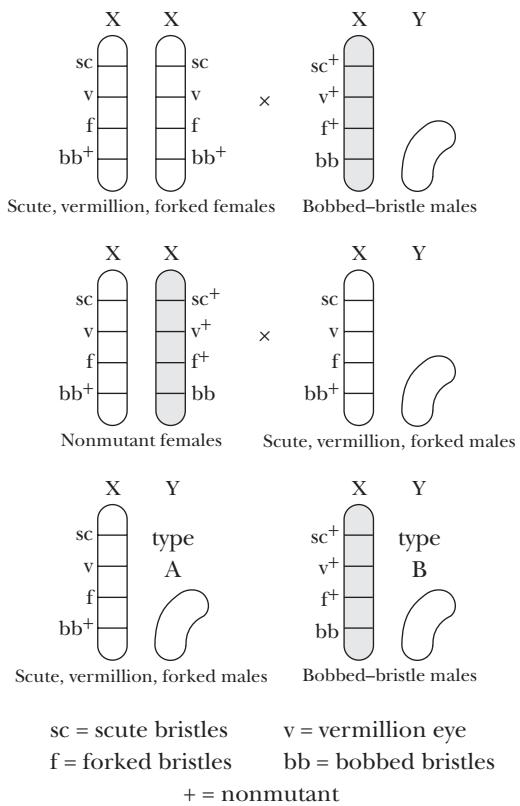
Genomic mutations are a large factor in the genetic damage that occurs in humans. Whole chromosomes can be lost or gained by errors during cell division. In animals, almost all examples of chromosome loss are so developmentally severe that the individual cannot survive to birth. On the other hand, since extra

chromosomes provide an extra copy of each of their genes, the amount of each protein they code for is unusually high, and this, too, can create biochemical abnormalities for the organism. In humans, an interesting exception is changes in chromosome number that involve the sex-determining chromosomes, especially the X chromosome (the Y is relatively silent in development). Since normal males have one X and females have two, the cells in females inactivate one of the X chromosomes to balance gene dosage. This dosage compensation mechanism can, therefore, also come into operation when one of the X chromosomes is lost or an extra one is inherited because of an error in cell division. The resulting conditions, such as Turner syndrome and Klinefelter syndrome, are much less severe than the developmental problems associated with other changes in chromosome number.

Mutation Rate

A mutation is any heritable change in the genetic material, but there are several different ways one can look at genetic change. For example, errors can occur when the DNA molecule is being duplicated during cell division. In simple organisms such as bacteria, about one thousand nucleotides are added to the duplicating DNA molecule each second. The speed is not as great in plants and animals, but errors still occur when mispairing between A and T or between C and G nucleotides occurs. DNA breaks are also common. These kinds of genetic change can be classified as "genetic damage." Some mutations are spontaneous, caused by changes that occur in the process of normal cell biochemistry. Other damage that can be traced to environmental factors changes bases, causes mispairing, or breaks DNA strands. Fortunately, almost all of this initial genetic damage is repaired by enzymes that recognize and correct errors in nucleotide pairing or DNA strand breaks. It is the unrepaired genetic damage that appears as new mutations. One of the first geneticists to design experiments to measure mutation rate was Hermann Müller, who received the Nobel Prize for his work on mutagenesis, including the discovery that X rays cause mutations.

Induction of Mutations by X Rays



If, for a given fly and its descendants, an induced or spontaneous lethal mutation occurs in the paternal X chromosome (shaded), no third-generation males of type B will result. If a spontaneous lethal mutation occurs in an original maternal X chromosome, then no third-generation males of type A will result.

The experiments by Müller provide a useful example of the kind of experimental design that can be used to measure mutation rates. Müller focused on new mutations (lethals) on the X chromosome of *Drosophila* that could cause the carrier to die. Since a male has only one X chromosome, a lethal mutation on that chromosome causes death. A living male *Drosophila* must, therefore, have no lethal mutations on his X chromosome. If a male *Drosophila* is treated with an agent such as X irradiation or certain chemicals, new lethal mutations can be detected when he is mated with special genetic strains of females. His X chromosomes are

eventually passed on to male descendants. If a new lethal mutation exists on a specific X chromosome, all males that inherit that chromosome copy will die during development. Spontaneous mutation rates measured by this technique average about 1×10^{-5} for each gene. In other words, there is a probability of about 1 in 100,000 that a mutation will occur in a particular gene each generation. This is a very low probability for a specific gene, but when it is multiplied for all of the genes in an animal or plant, it is likely that a new mutation has occurred somewhere on the chromosomes of an organism each generation.

Spontaneous mutation rates vary to some extent from one gene to another and from one organism to another, but one major source of variation in mutation rate comes from external agents that act on the DNA to increase damage or inhibit repair. One of the most widely used techniques for measuring the mutagenic activity of a chemical was developed in the 1970's by Bruce Ames. The Ames test uses bacteria that have a mutation that makes them unable to produce the amino acid histidine. These bacteria cannot survive in culture unless they are given histidine in the medium. To test whether a chemical increases the mutation rate, it is mixed with a sample of these bacteria, and they are placed on a medium without histidine. Any colonies that survive represent bacteria in which a new mutation has occurred to reverse the original defect (a back-mutation). Since many chemicals that cause mutations also cause cancer, this quick and inexpensive test is now used worldwide to screen potential carcinogenic, or cancer-causing, agents.

Mutation rates in mice are measured by use of the specific-locus test. In this test, wild-type male mice are mated with females that are homozygous for up to seven visible, recessive mutations that cause changes in coat color, eye color, and shape of the ear. If no mutations occur in any of the seven genes in the germ cells of the male, the male offspring will all be wild type in appearance. However, a new mutation in any of the seven genes will yield a progeny with a mutant phenotype (for example, a new coat color). The same cross can also be used to identify new mutations in females. Since mice

are mammals, they are a close model system to humans. Thus, results from mutation studies in mice have helped identify agents that are likely to be mutagenic in humans.

The Use of Mutations to Study Development

Mutations offer geneticists a powerful tool to analyze development. By understanding the way development is changed by a mutation, one can determine the role the normal gene plays. Although most people tend to think of mutations as causing some easily visible change in the appearance of a plant or animal (such as wrinkled pea seeds or white mouse fur), most mutations are actually lethal when present in two copies (homozygous). These lethal mutations affect some critical aspect of cell structure or other fundamental aspect of development or function. Genes turn on and off at specific times during development, and by studying the abnormalities that begin to show when a lethal

mutation carrier dies, a geneticist can piece together a picture of the timing and role of important gene functions.

Another useful insight comes from mutations with effects that vary. For example, many mutations have phenotypic effects that depend on the conditions, such as temperature, in which the individual develops. An interesting example of such temperature sensitivity is the fur color of Siamese cats. The biochemical pathway for pigmentation is active in cool temperatures but is inactivated at warmer body temperature. For this reason, a Siamese cat will only be pigmented in the cooler parts such as the tips of the ears and tail. Gene interactions like this allow geneticists to study the conditions under which the protein coded by a mutant gene works.

It would be a mistake, however, to think that all mutations have large phenotypic effects. Many complex traits are produced by many genes working together and are affected by

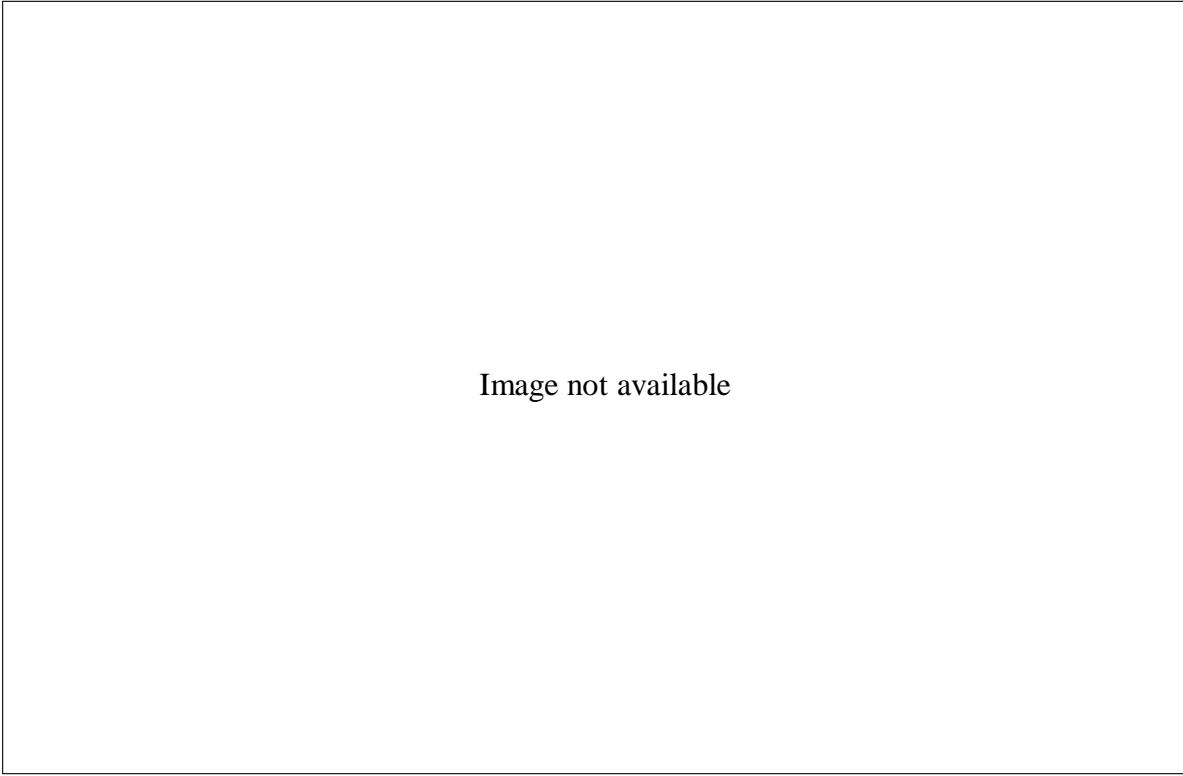


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The coats of Siamese cats are darker at their extremities as the result of a mutation that is affected by body temperature. (AP/Wide World Photos)

environmental variables such as temperature. These are called quantitative traits because they are measured on some kind of scale, such as size, number, or intensity. The mutations that affect quantitative traits are not different, except perhaps in the magnitude of their individual effects, from other kinds of gene mutation. Mutations in quantitative traits are a major source of heritable variation on which natural and artificial selection can act to change a phenotype.

Impact and Applications

It will probably never be possible to eliminate all mutation events because many mutations are caused by small errors in normal DNA duplication when cells divide. Learning how mutations affect cell division and cell function can help one to understand processes such as cancer and birth defects that can often be traced to genetic change. Some explanations of processes such as aging have focused on mutation in somatic cells. Mutation is also the source of genetic variation in natural populations, and the long-term survival of a species depends on its ability to draw on this variation to adapt to new environmental conditions.

Two aspects of mutagenesis will continue to grow in importance. First, environmental and human-made mutagens will continue to be a source of concern as technological advances occur. Many scientists are working to monitor and correct potential mutagenic hazards. Second, geneticists are beginning to use molecular tools, such as transposable elements and the techniques of genetic engineering, to produce preplanned genetic changes. Directed mutagenesis of DNA offers a way to correct preexisting genetic defects or alter phenotypes in planned ways. Mutation is, therefore, both a source of problems and a source of promise.

—James N. Thompson, Jr.
—R. C. Woodruff

See also: Biochemical Mutations; Cancer; Cell Cycle, The; Central Dogma of Molecular Biology; Chemical Mutagens; Chromosome Mutation; Classical Transmission Genetics; Complementation Testing; Congenital Disorders; Consanguinity and Genetic Disease; Cystic Fibrosis; Extrachromosomal Inheritance; Genetic Load; Hereditary Diseases; Huntington's Disease; Inborn Errors of Metabolism; Mitochondrial Genes; Molecular Genetics; Oncogenes; Phenylketonuria (PKU); Transposable Elements; Tumor-Suppressor Genes.

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Natural Selection

Fields of study: Evolutionary biology; Population genetics

Significance: *Natural selection is the mechanism proposed by Charles Darwin to account for biological evolutionary change. Using examples of artificial selection as analogies, he suggested that any heritable traits that allow an advantage in survival or reproduction to an individual organism would be “naturally selected” and increase in frequency until the entire population had the trait. Selection, along with other evolutionary forces, influences the changes in genetic and morphological variation that characterize biological evolution.*

Key terms

ADAPTATION: the evolution of a trait by natural selection, or a trait that has evolved as a result of natural selection

ARTIFICIAL SELECTION: selective breeding of desirable traits, typically in domesticated organisms

FITNESS: an individual’s potential for natural selection as measured by the number of offspring of that individual relative to those of others

GROUP SELECTION: selection in which characteristics of a group not attributable to the individuals making up the group are favored

Natural Selection and Evolution

In 1859, English naturalist Charles Darwin published *On the Origin of Species by Means of Natural Selection*, in which he made two significant contributions to the field of biology: First, he proposed that biological evolution can occur by “descent with modification,” with a succession of minor inherited changes in a lineage leading to significant change over many generations; and second, he proposed natural selection as the primary mechanism for such change. (This was also proposed independently by Alfred R. Wallace and was presented with Darwin in the form of a joint research paper some years earlier.) Darwin reasoned that if an individual organism carried traits that allowed it to have some advantage in survival or reproduction, then those traits would be car-

ried by its offspring, which would be better represented in future generations. In other words, the individuals carrying those traits would be “naturally selected” because of the advantages of the traits. For example, if a small mammal happened to have a color pattern that made it more difficult for predators to see, it would have a better chance of surviving and reproducing. The mammal’s offspring would share the color pattern and the advantage over differently patterned members of the same species. Over many generations, the proportion of individuals with the selected pattern would increase until it was present in every member of the species, and the species would be said to have evolved the color pattern trait.

Natural selection is commonly defined as “survival of the fittest,” although this is often misinterpreted to mean that individuals who are somehow better than others will survive while the others will not. As long as the traits convey some advantage in reproduction so that the individual’s offspring are better represented in the next generation, then natural selection is occurring. The advantage may be a better ability to survive, or it may be something else, such as the ability to produce more offspring.

For natural selection to lead to evolutionary change, the traits under selection must be heritable, and there must be some forms of the traits that have advantages over other forms (variation). If the trait is not inherited by offspring, it cannot persist and become more common in later generations. Darwin recognized this, even though in his time the mechanisms of heredity and the sources of new genetic variation were not understood. After the rediscovery of Gregor Mendel’s principles of genetics in the early years of the twentieth century, there was not an immediate integration of genetics into evolutionary biology. In fact, it was suggested that genetic mutation might be the major mechanism of evolution. This belief, known as Mendelism, was at odds with Darwinism, in which natural selection was the primary force of evolution. However, with the “modern synthesis” of genetics and evolutionary theory in the 1940’s and 1950’s, Mendelian genetics

was shown to be entirely compatible with Darwinian evolution. With this recognition, the role of mutation in evolution was relegated to the source of variation in traits upon which natural selection can act.

The potential for natural selection of an organism is measured by its “fitness.” In practice, the fitness of an individual is some measure of the representation of its own offspring in the next generation, often relative to other individuals. If a trait has evolved as a result of natural selection, it is said to be an “adaptation.” The term “adaptation” can also refer to the process of natural selection driving the evolution of such a trait. There are several evolutionary forces in addition to selection (for example, genetic drift, migration, and mutation) that can influence the evolution of a trait, though the process is called adaptation as long as selection is involved.

Population Genetics and Natural Selection

Population geneticists explore the actual and theoretical changes in the genetic composition of natural or hypothetical populations. Not surprisingly, a large part of the theoretical and empirical work in the field has concentrated on the action of natural selection on genetic variation in a population. Ronald A. Fisher and J. B. S. Haldane were the primary architects of selection theory beginning in the 1930’s, and Theodosius Dobzhansky was a pioneer in the detection of natural selection acting on genetic variants in populations of *Drosophila melanogaster* (fruit flies).

The most basic mathematical model of genes in a population led to the Hardy-Weinberg law, which predicts that there would be no change in the genetic composition of a population in the absence of any evolutionary forces such as natural selection. However, models that include selection show that it can have specific influences on a population’s genetic variation. In such models, the fitness of an organism’s genotype is represented by a fitness coefficient (or the related selection coefficient), in which the genotype with the highest fitness is assigned a value of 1, and the remaining genotypes are assigned values relative to the highest fitness. A fitness coefficient of 0 represents a lethal genotype (or, equivalently, one that is incapable of reproduction).

The simplest models of selection include the assumption that a genotype’s fitness does not change with time or context and demonstrate three basic types of selection, defined by how selection acts on a distribution of varying forms of a trait (where extreme forms are rare and average forms are common). These three types are directional selection (in which one extreme is favored), disruptive selection (in which both extremes are favored), and stabilizing selection (in which average forms are favored). The first two types (with the first probably being the most common) can lead to substantial genetic change and thus evolution, though in the process genetic variation is depleted. The third type maintains variation but does not result in much genetic change. These results create a problem: Natural populations generally have substantial genetic variation, but most selection is expected to deplete it. The problem has led population geneticists to explore the role of other forces working in place of, or in conjunction with, natural selection and to study more complex models of selection. Examples include models that allow a genotype to be more or less fit if it is more common (frequency-dependent selection) or that allow many genes to interact in determining a genotype’s fitness (multilocus selection). Despite the role of other forces, selection is considered an important and perhaps complex mechanism of genetic change.

Detecting and Measuring Fitness

Although a great amount of theoretical work on the effects of selection has been done, it is also important to relate theoretical results to actual populations. Accordingly, there has been a substantial amount of research on natural and laboratory populations to measure the presence and strength of natural selection. In practice, selection must be fairly strong for it to be distinguished from the small random effects that are inherent in natural processes.

Ideally, a researcher would measure the total selection on organisms over their entire life cycles, but in some cases this may be too difficult or time-consuming. Also, a researcher may be interested in discovering what specific parts of

the life cycle selection influences. For these reasons, many workers choose to measure components of fitness by breaking down the life cycle into phases and looking for fitness differences among individuals at some or all of them. These components can differ with different species but often include fertility selection (differences in the number of gametes produced), fecundity selection (differences in the number of offspring produced), viability selection (differences in the ability to survive to reproductive age), and mating success (differences in the ability to successfully reproduce). It is often found in such studies that total lifetime fitness is caused primarily by fitness in one of these components, but not all. In fact, it may be that genotypes can have a disadvantage in one component but still be selected with a higher overall fitness because of greater advantages in other components.

There are several empirical methods for detection and measurement of fitness. One relatively simple way is to observe changes in gene or genotype frequencies in a population and fit the data on the rate of change to a model of gene-frequency change under selection to yield an estimate of the fitness of the gene or genotype. The estimate is more accurate if the rate of mutation of the genes in question is taken into account. In the famous example of “industrial melanism,” it was observed that melanic (dark-colored) individuals of the peppered moth *Biston betularia* became more common in Great Britain in the late nineteenth century, corresponding to the increase in pollution that came with the Industrial Revolution. It was suggested that the melanic moths were favored over the lighter moths because they were camouflaged on tree trunks where soot had killed the lichen and were therefore less conspicuous to bird predators. Although it is now known that the genetics of melanism are more complex, early experiments suggested that there was a single locus with a dominant melanic allele and a recessive light allele; the data from one hundred years of moth samples were used to infer that light moths have two-thirds the survival ability of melanic moths. Later studies also showed that peppered moths do not rest on tree trunks, calling into question

the role of bird predation in the selection process. Nevertheless, selection of some sort is still considered the best explanation for the changes observed in peppered moth populations, even though the selective factor responsible is not known.

Later, a second method of fitness measurement was applied to the peppered moth using a mark-recapture experiment. In such an experiment, known quantities of marked genotypes are released into nature and collected again some time later. The change in the proportion of genotypes in the recaptured sample provides a way to estimate their relative fitnesses. In practice, this method has a number of difficulties associated with making accurate and complete collections of organisms in nature, but the fitness measure of melanic moths by this method was in general agreement with that of the first method. A third method of measuring fitness is to measure deviations from the genotype proportions expected if a population is in Hardy-Weinberg equilibrium. This method can be very unreliable if deviations are the result of something other than selection.

Units of Selection

Darwin envisioned evolution by selection on individual organisms, but he also considered the possibility that there could be forms of selection that would not favor the survival of the individual. He noted that in many sexual species, one sex often has traits that are seemingly disadvantageous but may provide some advantage in attracting or competing for mates. For instance, peacocks have a large, elaborately decorated tail that is energetically costly to grow and maintain and might be a burden when fleeing from predators. However, it seems to be necessary to attract and secure a mate. Darwin, and later Fisher, described how such a trait could evolve by sexual selection if the female evolves a preference for it, even if natural selection would tend to eliminate it.

Other researchers have suggested that in some cases selection may act on biological units other than the individual. Richard Dawkins's *The Selfish Gene* (1976) popularized the idea that selection may be acting directly on genes

and only indirectly on the organisms that carry them. This distinction is perhaps only a philosophical one, but there are specific cases in which genes are favored over the organism, such as the “segregation distorter” allele in *Drosophila* that is overrepresented in offspring of heterozygotes but lethal in homozygous conditions.

The theory of kin selection was developed to explain the evolution of altruistic behavior such as self-sacrifice. In some bird species, for example, an individual will issue a warning call against predators and subsequently be targeted by the predator. Such behavior, while bad for the individual, can be favored if those benefiting from it are close relatives. While the individual may perish, relatives that carry the genes for the behavior survive and altruism can evolve. Kin selection is a specific type of group selection in which selection favors attributes of a group rather than an individual. It is not clear whether group selection is common in evolution or limited to altruistic behavior.

Impact and Applications

The development of theories of selection and the experimental investigation of selection have always been intertwined with the field of evolutionary biology and have led to a better understanding of the history of biological change in nature. More recently, there have been medical applications of this knowledge, particularly in epidemiology. The specific mode of action of a disease organism or other parasite is shaped by the selection pressures of the host it infects. Selection theory can aid in the understanding of cycles of diseases and the response of parasite populations to antibiotic or vaccination programs used to combat them.

Although the idea of natural selection as a mechanism of biological change was suggested in the nineteenth century, artificial selection in the form of domestication of plants and animals has been practiced by humans for many thousands of years. Early plant and animal breeders recognized that there was variation in many traits, with some variations being more desirable than others. Without a formal understanding of genetics, they found that by choosing and breeding individuals with the desired

traits, they could gradually improve the lineage. Darwin used numerous examples of artificial selection to illustrate biological change and argued that natural selection, while not necessarily as strong or directed, would influence change in much the same way. It is important to make a clear distinction between the two processes: Breeders have clear, long-term goals in mind in their breeding programs, but there are no such goals in nature. There is only the immediate advantage of the trait to the continuation of the lineage. The application of selection theory to more recent breeding programs has benefited human populations in the form of new and better food supplies.

—Stephen T. Kilpatrick

See also: Altruism; Ancient DNA; Artificial Selection; Classical Transmission Genetics; Evolutionary Biology; Genetic Code; Genetic Code, Cracking of; Genetics, Historical Development of; Hardy-Weinberg Law; Human Genetics; Lamarckianism; Mendelian Genetics; Molecular Clock Hypothesis; Mutation and Mutagenesis; Population Genetics; Punctuated Equilibrium; Repetitive DNA; RNA World; Sociobiology; Speciation; Transposable Elements.

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Web Site of Interest

Writings of Charles Darwin. <http://pages.britishlibrary.net/charles.darwin>. Resource for Darwin's writings, a bibliography, and biographical material. Includes online version of Darwin's *On the Origin of Species by Means of Natural Selection* (1859).

Neural Tube Defects

Field of study: Diseases and syndromes

Significance: Neural tube defects are a category of birth defects that usually result from the failure of the neural tube to close properly during gestational development. Many neural tube defects can be prevented through folic acid supplementation and avoidance of other risk factors. However, because the neural tube closes during the first gestational

month, preventive measures must be instituted prior to pregnancy. Therefore, prevention of neural tube defects depends on the planning or expectation of pregnancies while initiating positive lifestyle changes.

Key terms

ANENCEPHALUS: a neural tube defect characterized by the failure of the cerebral hemispheres of the brain and the cranium to develop normally

ETIOLOGY: the cause or causes of a disease or disorder

MULTIFACTORIAL: characterized by a complex interaction of genetic and environmental factors

NEURAL TUBE: the embryonic precursor to the spinal cord and brain that normally closes at small openings, or neuropores, by the twenty-eighth day of gestation

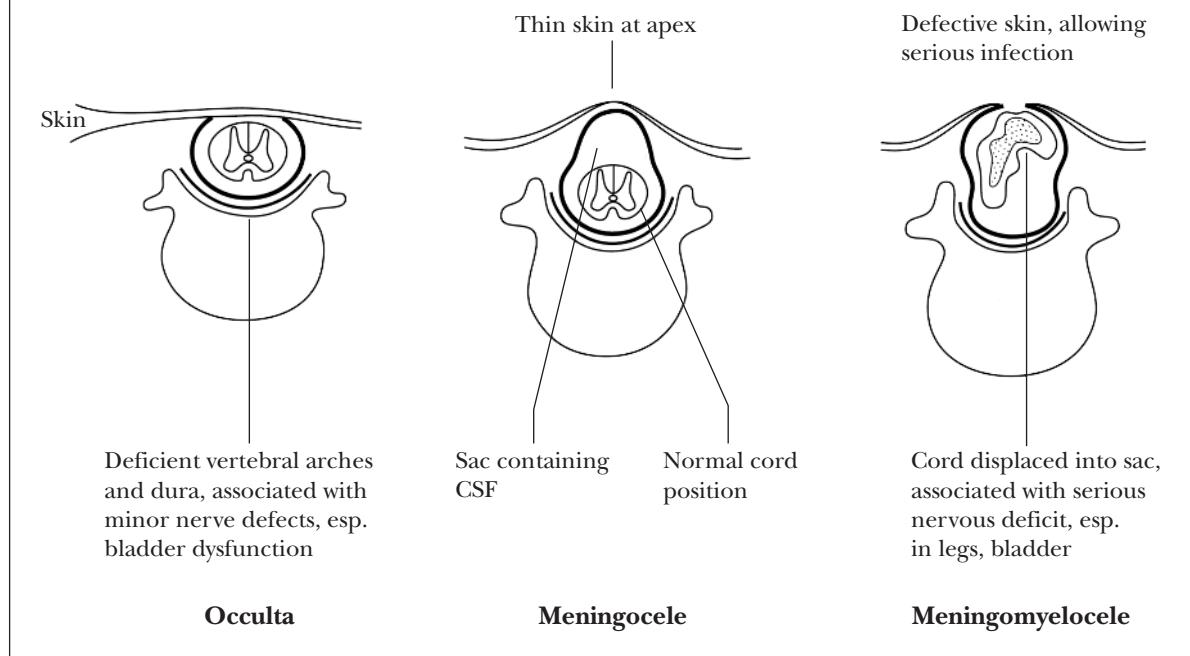
SPINA BIFIDA: a neural tube defect that usually results from the failure of the posterior neuropore to close properly during gestation

Formation of the Neural Tube

Neural tube defects represent congenital defects that have long been prevalent in human populations. Documented cases of anencephalus and spina bifida have been found among the skeletal remains of the ancient Egyptians and prehistoric Native Americans. In the contemporary world, spina bifida remains one of the most common birth defects. Yet despite their prevalence and antiquity, some questions concerning the causes of neural tube defects remain unanswered.

Neural tube defects result from a disruption in the formation or closure of the neural tube, which, during embryonic development, differentiates into the brain and spinal cord. The neural tube develops first out of the neural plate. The borders of the neural plate are folded, forming the neural groove. The neural groove becomes progressively deeper, placing the two folds in opposition. Final development of the neural tube occurs as the dorsal folds fuse along the midline. Closure of this structure begins around the third gestational week,

Types of Spina Bifida



Spina bifida is among the most common neural tube disorders. (Hans & Cassidy, Inc.)

beginning at its midportion and ending at the anterior and posterior neuropores around the twenty-fifth and twenty-seventh gestational days, respectively.

Classification of Neural Tube Defects

Disruption in the formation and closure of the posterior neuropores is associated with spina bifida. Spina bifida occulta is generally unaccompanied by protrusion of the spinal cord or its coverings through the open, unfused arches of the vertebrae. On the other hand, much more severe conditions, categorized as spina bifida cystica, result in the herniation of neural tissues and the formation of cystic swelling. One form of spina bifida cystica, meningocele, is marked by the protrusion of both the meninges and the spinal cord through the unfused vertebral arch. In the most severe cases, portions of the spinal cord and nerve roots are encased in the walls of the sac, damaging and hindering normal neurological functioning and development. In such instances, the severity of the neurological dysfunction depends on the location of the lesion along the vertebral column, as nerves below the defect are adversely affected. Meningocele, a more moderate manifestation of spina bifida cystica, is encountered four to five times less frequently than meningocele. Unlike the latter condition, the cystic sacs of meningoceles are made up solely of meninges and spinal fluid. This factor, coupled with the lack of involvement of the spinal cord, generally affords a more favorable prognosis, although some sensory and motor deficits may persist after surgery.

Anencephalus, which results from the disruption of the anterior neuropore, is the most devastating and severe of all the neural tube defects. Infants born with this birth defect are lacking significant areas of their brain and skull. The region normally occupied by the cerebral hemispheres consists of a formless mass of highly vascular connective tissue, while most of the bones of the skull are simply absent. Many anencephalic infants are stillborn; most die soon after birth.

Encephalocele, like anencephalus, is believed to result from defective closure of the anterior neuropore. In these conditions, a saclike

protrusion of neural tissue occurs through an opening along the midline of the skull. The prognosis and outcome of infants born with encephalocele depends upon the size of the lesion and the extent to which neural tissues are involved.

Prevalence-at-Birth Rates and Causes

Prevalence-at-birth rates of neural tube defects show substantial geographic and temporal variation. Historically, some of the highest prevalence-at-birth rates have been documented in the British Isles and range from as high as 4.5 in 1,000 births in Belfast, Ireland, to as low as 1.5 in 1,000 births in London, England. In the United States, the highest rates of neural tube defects have historically occurred in northeastern states. Rates of neural tube defects are declining in most areas of the world, although regional outbreaks, marked by higher birth prevalence rates, have been reported and are generally unexplained. Typically, rates in the contemporary United States average around 1 to 2 in 1,000 births, and the risk of having an infant with a neural tube defect increases by about 2 percent if a couple has previously had a child with such a defect.

Among the most important risk factors are those relating to the diet and health status of prospective mothers. Also, there are indications that excessive elevation of a woman's body temperature during early pregnancy, through hot baths or recreational hot tubs, may increase her chances of having an infant with a neural tube defect. A number of studies have suggested that women who give birth to infants with neural tube defects have lower health status and poorer diets than other women. Inadequate levels of folate appear to place women at greater risk of having an infant with a neural tube defect. Doctors now recommend that women planning a pregnancy supplement their diets with folate, although any woman planning to become pregnant should first consult her doctor before taking any supplement. Tests for alpha-fetoprotein in the mother's blood during the prenatal period can help detect the presence of a neural tube defect in the developing fetus.

—*Mary K. Sandford*

See also: Amniocentesis and Chorionic Villus Sampling; Congenital Defects; Developmental Genetics; Prenatal Diagnosis.

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Web Sites of Interest

Medline Plus. <http://www.nlm.nih.gov/medlineplus/neuraltubedefects.html>. Medline, sponsored by the National Institutes of Health, is one of the first stops for any medical question; this page provides descriptions and links to clinical trials and other resources for neural tube defects.

Spina Bifida Association of America. <http://www.sbaa.org>. Offers information, fact sheets, testimonials, a clinic directory, copious links to other resources, and more.

Noncoding RNA Molecules

Field of study: Molecular genetics

Significance: *Although less familiar than mRNA, tRNA, and rRNA, noncoding RNA molecules (ncRNAs) play many roles—including some that have not yet been elucidated—in normal cellular functions. The existence of ncRNAs has been known since the 1960's, but it was not until the last decade of the twentieth century that their significance and functions began to be understood.* The functions of the many ncRNAs so far discovered include roles in DNA replication, post-transcriptional control of gene expression, processing of other RNAs, and mRNA stability.

Key terms

cDNA LIBRARY: a collection of clones produced from all the RNA molecules in the cells of a particular organism, often from a single tissue

CLONE: a culture of bacteria, usually *Escherichia coli*, whose cells contain a recombinant plasmid

CODON: a three-letter nucleotide sequence in RNA or DNA that codes for a specific amino acid; a gene is composed of a long string of codons

INTRON: an intervening sequence in a eukaryotic gene (generally there are several to many per gene) which must be removed when it is transcribed into messenger RNA (mRNA); introns are assumed to have no function and therefore mutations in them are often considered neutral

SPLICOSOME: a complex assemblage of proteins and RNA in the nucleus of cells that cuts out introns and splices the exons of a maturing mRNA

Definition

Noncoding RNAs (ncRNAs) include any RNA that is not messenger RNA (mRNA), ribosomal RNA (rRNA), or transfer RNA (tRNA). The discovery of the first ncRNAs in the 1960's occurred because they were expressed in such high numbers. At the time, RNA was considered to function only as a means to express a gene, all three of the main types of RNA being intimately involved in this process. Many of the ncRNAs discovered over the next twenty years were also discovered fortuitously, before any speculation about their possible functions was even considered. Once transcription and processing of mRNAs was elucidated, many of the ncRNAs were considered leftover fragments representing the introns that had been cut out of pre-mRNAs. At the same time it was discovered that some of the ncRNAs were involved in the process of intron removal and exon splicing.

ing. Systematic searches for ncRNAs did not begin until the later 1990's and, once undertaken, revealed a veritable universe of ncRNAs, ranging from very short sequences of less than 100 nucleotides to some around 100,000 nucleotides, and possibly more. For a system considered so well understood, the entry of so many new players has added a whole new layer of complexity to the study of genetics.

Types of ncRNA and Their Occurrence

Researchers have now identified ncRNAs in essentially all organisms, from bacteria to humans. In bacteria they tend to be smaller and in most cases are called small RNA (sRNA). Although the most common name for noncoding RNAs in other organisms is ncRNA, they also have gone by the name "small non-messenger RNA" (snmRNA). After these general names, there is a collection of names for ncRNAs that have particular characteristics or functions, and the list of names will probably grow as new ncRNAs are discovered. Some newly discovered ncRNAs cannot be assigned a function.

ncRNAs Involved in RNA Processing

Because almost all eukaryotic genes contain intervening sequences, called introns, that in-

terrupt the coding sequence of the gene, when an RNA is first transcribed it cannot be translated without being processed. Processing involves removal of the introns and the splicing of the remaining fragments, the exons, which contain the coding sequence of the gene. The cellular "machine" that does this job is the spliceosome. It is a complex assemblage of proteins and small RNAs. The proteins and RNAs are grouped together into several particles called small nuclear ribonucleoproteins (snRNPs, pronounced "snurps" by geneticists). The RNA component of snRNPs are small nuclear RNAs (snRNAs), the best known being U1 snRNA. Several different snRNAs are now known, and they are components of the several snRNPs that come together to make a functional spliceosome.

Subsequent discoveries revealed that snRNPs, and thus snRNAs, were involved in other types of RNA processing. Some are involved in polyadenylation, the addition of adenine nucleotides to the 3' end of mRNAs to make what is called a poly-A tail. Histone protein mRNAs are known to lack poly-A tails, but in *Xenopus* (the African clawed frog), snRNPs are still involved in properly finishing the 3' end. A final role for some is maturation of rRNA transcripts, whose spacer RNA sequences must be removed. All of these functions verge on being enzyme-like.

A complex related to snRNPs was first found in bacteria and has now been found in all groups of organisms. It contains proteins and RNA and is called ribonuclease P (RNase P); it is involved in the processing of tRNA and some rRNAs. Experiments have shown that the RNA component can catalyze the required reactions, even without the protein component, making it the first clear-cut "ribozyme," an RNA with catalytic properties. Several types of ncRNA are now known to act as ribozymes, and this ability prompted the evolutionary community to propose that early "life" was RNA-based rather than protein and DNA-based.

ncRNAs Involved in RNA Modification

RNA modification by small nucleolar RNAs (snoRNAs) has been best studied in *Saccharomyces cerevisiae* (yeast). Mature rRNAs must

Types of ncRNA

Type of ncRNA	Abbreviation
guide RNA	gRN
heterogeneous nuclear RNA	hnRNA
micro-RNA	miRNA
small cytoplasmic RNA	scRN
small interfering RNA	siRNA
small non-messenger RNA	snmRNA
small nuclear RNA	snRNA
small nucleolar RNA	snoRNA
small temporal RNA	stRNA
transfer messenger RNA	tmRNA

—Bryan Ness

have some of their ribose sugars methylated, and although their exact role in the process has not yet been completely defined, snoRNAs are involved. They bind to rRNAs in small regions where they have complementary base sequences and somehow direct methylation. Other snoRNAs are involved in pseudouridylation (that is, conversion of some of the uracil nucleotides in rRNA to pseudouracil, a modified nucleotide) of rRNA. The enzyme that actually performs the pseudouridylation is not known. Many eukaryotes have snoRNAs, and recently snoRNA homologs (a homolog is a molecule that is similar to another) have been found in *Archaea*, but not yet in *Bacteria*.

Not as well known are guide RNAs (gRNAs), discovered in some protists. They also modify rRNA, by guiding the insertion or deletion of uracil nucleotides. The details of the process are not well understood, but the mechanism involves complementary base pairing between the rRNA and a gRNA, much like that seen with snoRNAs. It is possible that, as more studies are undertaken, gRNAs will be found in other types of organisms.

ncRNAs Affecting mRNA Stability and Translation

Another type of ncRNA that has been known for some time is small interfering RNA (siRNA). A type of antisense RNA, siRNAs have base sequences complementary to the coding, or “sense,” region of an mRNA. By binding to an mRNA, a siRNA is able to block translation, and there is evidence that it also tags the mRNA for degradation. This type of genetic control is often called post-transcriptional gene silencing, or RNAi (RNA interference). Another ncRNA, micro-RNA (miRNA), also seems to target specific mRNAs for degradation. Both of these types represent very small RNA molecules of generally fewer than thirty nucleotides.

Apparently targeting specific mRNAs for degradation in bacteria are sRNAs, the mechanism being somewhat uncertain. Another function of sRNAs in bacteria is activation of certain mRNAs by preventing formation of an inhibitory structure in the mRNA. Another ncRNA, simply called OxyS RNA, represses translation by interfering with ribosome binding.

A final ncRNA, originally believed to be found only in the nematode *Caenorhabditis elegans*, is small temporal RNA (stRNA). The stRNAs block translation of specific mRNAs after translation has begun, but apparently they do not tag the mRNA for degradation. They are now believed to represent a subset of miRNA with slightly different properties. Their size is typically between twenty-one and twenty-five nucleotides, and screening of a variety of other organisms suggests that they may be more widespread than first assumed.

Other Specialized ncRNAs

A variety of other ncRNAs carry out more specialized functions, some just beginning to be understood. Gene silencing is a very important component of normal development. As cells become differentiated and specialized, they must express certain genes, and the remaining genes must be silenced. One form of silencing is called imprinting, whereby certain alleles from an allele pair are silenced, often those received from only one sex. A large ncRNA (a little longer than 100,000 nucleotides) called *Air* is responsible for silencing the paternal alleles in a small autosomal gene cluster. How it does this is still being studied.

In human females, one of the X chromosomes (females have two) must be inactivated so the genes on it will not be expressed. This inactivation, called Lyonization after the discoverer of the phenomenon, Mary Lyon, occurs during development on a random basis in each cell, so that the X chromosome subjected to deactivation is randomly determined. An ncRNA called *Xist* plays a central part in this process. It is a large RNA of 16,500 nucleotides and is transcribed from genes on both X chromosomes. It is inherently unstable but somehow becomes stable and binds all over one of the two X chromosomes. The X chromosome that gets coated with *Xist* is then inactivated, and the only gene it transcribes thereafter is the *Xist* gene. Transcription of *Xist* ceases on the active X chromosome.

A type of ncRNA called transfer messenger RNA (tmRNA) is involved in resuming translation at ribosomes that have stalled. When a stalled ribosome is encountered, a tmRNA first

acts as a tRNA charged with the amino acid alanine. The stalled polypeptide is transferred to the alanine on the tmRNA. Then translation continues, but now the tmRNA acts as the mRNA, instead of the mRNA the ribosome was initially translating. A termination codon is soon reached and the amino acids that were added based on the tmRNA code act as a tag for enzymes in the cytoplasm to break it down. This allows those ribosomes that would normally remain tied up with an mRNA they cannot complete translating to be recycled for translating another mRNA.

The Future of ncRNA Research

Most of the ncRNAs described above were unknown until the 1980's, and some of them were only discovered in the 1990's. What appeared to be a relatively simple picture of genetic control in cells has now gained many, previously hidden, layers involving all manner of RNAs, ranging from a mere 20 nucleotides to 100,000 nucleotides or so in length. Some are suggesting that this glimpse is just the tip of the iceberg and that continued research will reap increasingly complex interactions among RNAs and between RNAs and proteins. Genomics, the study of the DNA sequence of genomes, has been a hot field for some time, but now it looks as if "RNomeics" is beginning to steal the show.

Some strides have already been made in RNomeics with surveys of cDNA libraries for ncRNA sequences, especially some of the smaller ones that were long thought merely to be leftover scraps from other processes. For example, one study in 2001, which included a survey of a mouse-brain cDNA library, revealed 201 potential novel, small ncRNAs. In a 2003 survey of a cDNA library from *Drosophila melanogaster* (fruit fly), sixty-six potential novel ncRNAs were discovered. Judging by the large numbers of candidate ncRNAs showing up in what are essentially first-time surveys, many more may remain to be found. There could potentially be thousands of ncRNA genes. What is surprising is that many of these ncRNA genes are being found in spacer regions and introns, places that were once considered useless junk. With so much now being found in these re-

gions, many geneticists have become ever more cautious in calling any DNA sequence junk DNA.

Because the field of RNomeics is in its infancy and the functions of many of the ncRNAs are just barely understood, it may be premature to predict specific medical applications, but certainly the potential is there. The population of ncRNAs in a cell, in some sense, resembles a complex set of switches that turn genes on and off—before they are transcribed, while they are being transcribed, or even once translation has begun. Once these switches are better understood, researchers may be able to exploit the system with artificially produced RNAs. Geneticists will probably also discover that a number of diseases that appeared to have unexplained genetic behavior will find the solutions in ncRNA.

—Bryan Ness

See also: cDNA Libraries; Central Dogma of Molecular Biology; DNA Structure and Function; RNA Structure and Function; RNA Transcription and mRNA Processing.

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other textbooks. Various chapters include discussion of ncRNAs.

Storz, Gisela. "An Expanding Universe of Non-coding RNAs." *Science* 296 (2002): 1260-1263. A fairly complete overview of the various kinds of ncRNA, along with as much as is known about many of them.

Nondisjunction and Aneuploidy

Fields of study: Cellular biology; Diseases and syndromes

Significance: *Nondisjunction is the faulty disjoining of replicated chromosomes during mitosis or meiosis, which causes an alteration in the normal number of chromosomes (aneuploidy). Nondisjunction is a major cause of Down syndrome and various sex chromosome anomalies. Understanding the mechanisms associated with cell division may provide new insight into the occurrence of these aneuploid conditions.*

Key terms

MEIOSIS: a series of two nuclear divisions that occur in gamete formation in sexually reproducing organisms

MITOSIS: nuclear division of chromosomes, usually accompanied by cytoplasmic division; two daughter cells are formed with identical genetic material

Background

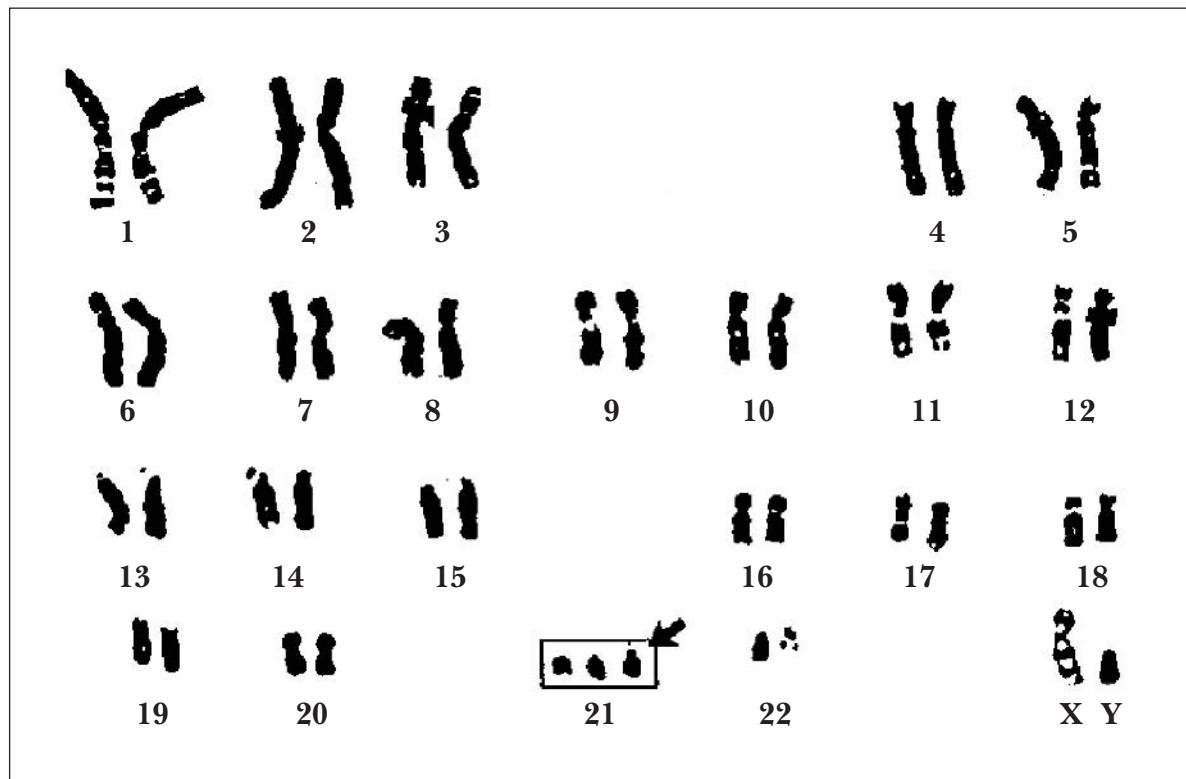
Each cell in multicellular organisms contains all the hereditary information for that individual, in the form of DNA. In eukaryotes, DNA is packaged in rodlike structures called chromosomes, and any given species has a characteristic chromosome number. There are typically two of each kind of chromosome, which is referred to as being diploid. In humans (*Homo sapiens*), there are forty-six chromosomes; in corn (*Zea mays*), there are twenty chromosomes. A haploid cell has half the number of chromosomes as a diploid cell of the same species, which constitutes one of each kind of chromosome. One set of chromosomes

is contributed to a new individual by each parent in sexual reproduction through the egg and sperm, which are both haploid. Thus, a fertilized egg will contain two sets of chromosomes and will be diploid.

A karyotype is a drawing or picture that displays the number and physical appearance of the chromosomes from a single cell. A normal human karyotype contains twenty-two pairs of autosomes (chromosomes that are not sex chromosomes) and one pair of sex chromosomes. Females normally possess two X chromosomes in their cells, one inherited from each parent. Males have a single X chromosome, inherited from the mother, and a Y chromosome, inherited from the father.

The many cells of a multicellular organism are created as the fertilized egg undergoes a series of cell divisions. In each cell division cycle, the chromosomes are replicated, and, subsequently, one copy of each chromosome is distributed to two daughter cells through a process called mitosis. When gametes (eggs or sperm) are produced in a mature organism, a different type of nuclear division occurs called meiosis. Gametes contain one set of chromosomes instead of two. When two gametes join (when a sperm cell fertilizes an egg cell), the diploid chromosome number for the species is restored, and, potentially, a new individual will form with repeated cell divisions.

When replicated chromosomes are distributed to daughter cells during mitosis or meiosis, each pair of chromosomes is said to disjoin from one another (disjunction). Occasionally, this process fails. When faulty disjoining (nondisjunction) of replicated chromosomes occurs, a daughter cell may result with one or more chromosomes than normal or one or more fewer than normal. This alteration in the normal number of chromosomes is called aneuploidy. One chromosome more than normal is referred to as a "trisomy." For example, Down syndrome is caused by trisomy 21 in humans. One chromosome fewer than normal is called monosomy. Turner syndrome in humans is an example of monosomy. Turner's individuals are women who have only one X chromosome in their cells, whereas human females normally have two X chromosomes. When



A karyotype is a picture that displays the number and physical appearance of the chromosomes from a single cell. This karyotype shows the trisomy at chromosome 21 that results in Down syndrome. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

nondisjunction occurs in the dividing cells of a mature organism or a developing organism, a portion of the cells of the organism may be aneuploid. If nondisjunction occurs in meiosis during gamete formation, then a gamete will not have the correct haploid chromosome number. If that gamete joins with another, the resulting embryo will be aneuploid. Examples of human aneuploid conditions occurring in live births include Down syndrome (trisomy 21), Edwards' syndrome (trisomy 18), Patau syndrome (trisomy 13), metafemale (more than two X chromosomes), Klinefelter syndrome (XXY), and Turner syndrome (XO). Most aneuploid embryos do not survive to birth.

Causes of Nondisjunction

There are both environmental and genetic factors associated with nondisjunction in plants and animals. Environmental factors that

may induce nondisjunction include physical factors such as heat, cold, maternal age, and ionizing radiation, in addition to a wide variety of chemical agents.

In humans, it is well established that increased maternal age is a cause of nondisjunction associated with the occurrence of Down syndrome. For mothers who are twenty years of age, the incidence of newborns with Down syndrome is 0.4 in 1,000 newborns. For mothers over forty-five years of age, the incidence of newborns with Down syndrome is 17 in 1,000 newborns. While it is clear that increased maternal age is linked to nondisjunction, it is not known what specific physiological, cellular, or molecular mechanisms or processes are associated with this increased nondisjunction. While nondisjunction in maternal meiosis may be the major source of trisomy 21 in humans, paternal nondisjunction in sperm formation does occur and may result in aneuploidy.

In a study conducted by Karl Sperling and colleagues published in the *British Medical Journal* (July 16, 1994), low-dose radiation in the form of radioactive fallout from the Chernobyl nuclear accident (April, 1986) was linked to a significant increase in trisomy 21 in West Berlin in January, 1987: twelve births of trisomy 21 compared to the expected two or three births. This study suggests that, at least under certain circumstances, ionizing radiation may affect the occurrence of nondisjunction. Researchers have shown that ethanol (the alcohol in alcoholic beverages) causes nondisjunction in mouse-egg formation, suggesting a similar possibility in humans. Other researchers have found that human cells in tissue culture (cells growing on nutrient media) had an increased occurrence of nondisjunction if the media was deficient in folic acid. This implies that folic acid may be necessary for normal chromosome segregation or distribution during cell division.

Scientists know from genetics research that mutations (changes in specific genes) in the fruit fly result in the occurrence of nondisjunction. This genetic component of nondisjunction is further supported by the observation that an occasional family gives birth to more than one child with an aneuploid condition. In these instances, it is likely that genetic factors are contributing to repeated nondisjunction.

Impact and Applications

There are several reasons scientists are devoting research efforts to understanding the consequences of nondisjunction and aneuploidy. First, at least 15 to 20 percent of all recognized human pregnancies end in spontaneous abortions. Of these aborted fetuses, between 50 and 60 percent are aneuploid. Second, of live births, 1 in 700 is an individual with Down syndrome. Mental retardation is a major symptom in individuals with Down syndrome. Thus, nondisjunction is one cause of mental retardation. Finally, aneuploidy is common in cancerous cells. Scientists do not know whether nondisjunction is part of the multistep process of tumor formation or whether aneuploidy is a consequence of tumor growth. Continued re-

search into the mechanics of cell division and the various factors that influence that process will increase the understanding of the consequences of nondisjunction and possibly provide the means to prevent its occurrence.

—Jennifer Spies Davis

See also: Chromosome Theory of Heredity; Down Syndrome; Hereditary Diseases; Klinefelter Syndrome; Metafemales; Polyploidy; Turner Syndrome; XYY Syndrome.

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Oncogenes

Fields of study: Molecular genetics; Viral genetics

Significance: *Oncogenes are a group of genes originally identified in RNA tumor viruses and later identified in many types of human tumors. The discovery of oncogenes has revolutionized the understanding of cancer genetics and contributed to the development of a model of cancer as a multi-stage genetic disorder. The identification of these abnormally functioning genes in many types of human cancer has also provided new molecular targets for therapeutic intervention.*

Key terms

PROTO-ONCOGENES: cellular genes that carry out specific steps in the process of cellular proliferation; as a consequence of mutation or deregulation, these genes may be converted into cancer-causing genes

RETROVIRUS: a virus that converts its RNA genome into a DNA copy that integrates into the host chromosome

The Discovery of Oncogenes

The discovery of oncogenes has been closely linked to the study of the role of a group of RNA tumor viruses, retroviruses (*Retroviridae*), in the etiology of many animal cancers. In the early part of the twentieth century, Peyton Rous identified a virus (called Rous sarcoma virus after its discoverer) capable of inducing tumors called sarcomas in chickens. Many other RNA tumor viruses capable of causing tumor formation in animals or experimental systems were later discovered, which led to a search for specific viral genes responsible for the cancer-causing properties of these viruses.

The identification of these cancer-causing genes (oncogenes) awaited developments in the area of recombinant DNA technology and molecular genetics, which ultimately facilitated the molecular analysis of this group of genes. These analyses revealed that viral oncogenes were actually cellular genes that were incorporated into the genetic material of the RNA tumor virus during the process of infection. The acquisition of these host-cell genes

was responsible for the cancer-causing properties of these viruses. The first oncogene discovered was the *src* gene of the Rous sarcoma virus. Subsequently, at least thirty different oncogenes were discovered in avian and mammalian RNA tumor viruses. Each of these oncogenes has a cellular counterpart that is the presumed origin of the viral gene; with the exception of the Rous sarcoma virus, the incorporation of the host-cell gene into the virus, involving a process called transduction, results in the loss of viral genes, generating a defective virus.

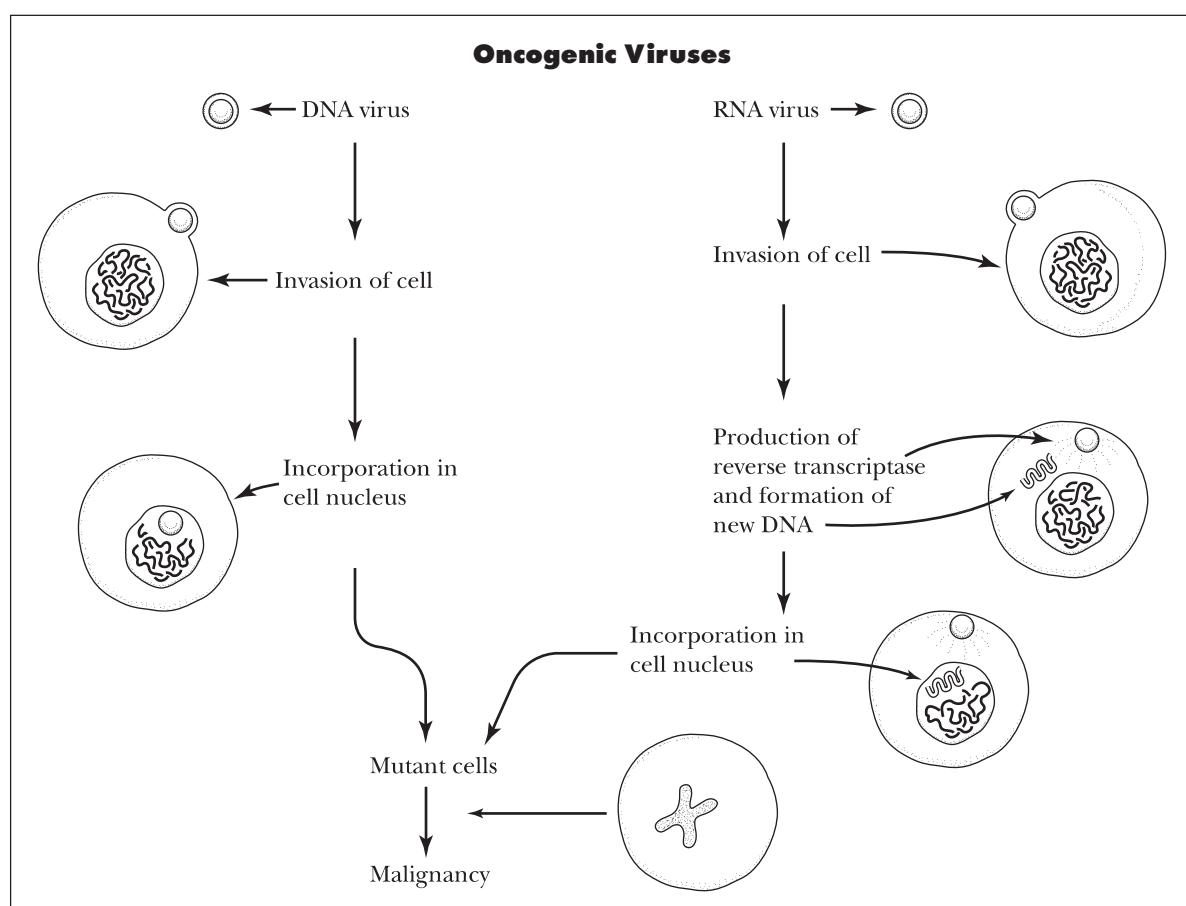
In addition to the oncogenes originally identified in viruses, more than fifty oncogenes have been identified in malignant tumors as part of chromosomal rearrangements or the amplification or mutations of specific genes. The first genetic rearrangement linked to a specific type of human malignancy involved the "Philadelphia" chromosome in patients with chronic myelogenous leukemia (CML). This chromosome represents a shortened version of chromosome 22, which results from an exchange of genetic material between chromosomes 9 and 22 (called a reciprocal translocation). Subsequent molecular analyses showed that the oncogene *abl*, originally identified in a mammalian RNA tumor virus, was translocated to chromosome 22 in CML patients. Additional human malignancies involving translocated oncogenes previously identified in RNA tumor viruses have been identified, notably the oncogene *myc* in patients with Burkitt's lymphoma, a disease primarily found in parts of Africa.

Additional genetic rearrangements represent amplification of existing oncogenes (segments of genetic material duplicated many times in genetically unstable tumor cells). These gene amplifications may be associated with the presence of multiple copies of genetic segments along a chromosome, designated as homogeneously staining regions (HSRs), or may appear in the form of minichromosomes containing the amplified genes, termed double-minutes (DMs). For example, late-stage neuroblastomas often contain numerous double-minute chromosomes containing amplified copies of the *N-myc* gene.

The Properties of Oncogenes

The first dramatic evidence linking oncogenes with cancer was provided by studies of the *sis* oncogene of simian sarcoma virus, which proved to be an altered form of the mammalian platelet derived growth factor (PDGF). Growth factors are proteins that bind to receptors on target cells to initiate an intracellular signaling cascade, which results in cellular proliferation. This seminal discovery led to the development of the proto-oncogene model. This model states that oncogenes are derived from normal host genes called proto-oncogenes, which encode gene products involved in controlling cell division. If proto-oncogene expression is altered by mutation or deregulation, they may disrupt the normal control of cell division, resulting in unregulated cellular proliferation, a hallmark of malignancy.

Subsequent analyses of oncogene activities and the structure and function of the cellular proto-oncogenes from which they are derived have provided strong evidence for this model. Viral and cellular oncogenes with functions affecting every step in the control of the cell have been identified. In addition to altered growth factors such as *sis*, researchers have also identified altered growth factor receptors such as the epidermal growth factor receptor (*erb-b*), elements of the intracellular signal cascade (*src* and *ras*), nuclear transcriptional activators (*myc*), cell-cycle regulators called cyclin-dependent kinases (cdk's), and cell death inhibitors (*bc12*) in human tumors of diverse tissue origin. Each of these oncogenic gene products represents an altered form of normal cellular genes that participate in cell-division pathways. Numerous mutations in proto-oncogenes have



(Electronic Illustrators Group)

been identified, including single base changes (point mutations), gene truncations, gene amplifications, and gene rearrangements resulting from exchanges between different chromosomes called translocations.

One of the most dramatic discoveries involved a comparative analysis of the structures of the normal and oncogene forms of the *ras* proto-oncogene isolated from human bladder carcinomas. Surprisingly, a single-base change was sufficient to convert a normal cellular gene to a cancer-causing gene. The observed mutations were localized to regulatory regions of the *ras* gene product, resulting in its permanent activation. Molecular analyses of many other oncogenes have shown that the observed mutations fall into several categories: nucleotide base changes that result in gene products whose functions are not subject to normal inhibitory processes, overproduction of gene products caused by gene amplification or translocation, and loss of regulatory components caused by gene translocation or truncation. The generalized consequence of these mutations is to convert normal cellular gene products important in cell division to dominant, unregulated gene products that cause the inappropriate stimulation of cell division.

Interestingly, most tumors analyzed show the involvement of multiple oncogenes and tumor-suppressor genes (another class of otherwise normal genes that are modified in some manner). Studies of tumor development in human colorectal carcinomas in which it is possible to identify discrete stages of tumor development have indicated a progressive increase in the number and types of cellular oncogenes at successive stages of tumor development. From these studies, a model of oncogenesis has emerged in the form of a multistage disorder characterized by the successive accumulation of mutations in specific cellular oncogenes and tumor-suppressor genes, which results in the inability to regulate cellular proliferation.

Impact and Applications

The identification of oncogenes has provided enormous amounts of information on the cellular mechanisms responsible for the loss of growth control in cancer cells. In addi-

tion, these dysfunctional gene products represent potential targets for therapeutic applications. Research studies have been directed toward the design of inhibitors of specific oncogenes such as *ras* and *erb-b* in order to block the effects of oncogenes in malignant cells. Additional molecular targets include overexpressed oncogenes that stimulate cellular proliferation or blood vessel formation (angiogenesis), processes critical to tumor establishment. The advantages of these approaches include better targeting of cancer cells, as well as a potential decrease in side effects as compared to conventional chemotherapy. Structural abnormalities in oncogene products may be used in the development of monoclonal antibodies directed against these dysfunctional proteins. Toxins may also be linked to the antibodies to generate immunotoxins whose cell-killing activities directly target malignant cells. Malignant melanoma (skin cancer) has been the focus of many of these targeted approaches directed against specific abnormal gene products. Successful clinical applications will most likely combine approaches involving cytotoxic drugs and inhibitors targeting multiple sites of oncogene dysfunction in the cancer cell.

—Sarah Crawford Martinelli

See also: Aging; Blotting: Southern, Northern, and Western; Breast Cancer; Burkitt's Lymphoma; Cancer; Cell Culture: Animal Cells; Cell Cycle, The; Gene Therapy; Genetics, Historical Development of; Human Genetics; Hybridomas and Monoclonal Antibodies; Repetitive DNA; RNA Transcription and mRNA Processing; Tumor-Suppressor Genes.

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American Society of Clinical Oncology. <http://www.asco.org>. Searchable site on oncogenes and molecular oncology.

dational to understanding the molecular basis of gene action. Today, with a more detailed understanding of how genes work, geneticists consider the original hypothesis an oversimplification and have reformulated it as the "one gene-one polypeptide" hypothesis. Even in its new form, however, there are exceptions.

Key terms

MESSENGER RNA (mRNA) PROCESSING: chemical modifications that alter messenger RNAs, often resulting in more than one gene product formed from the same gene

METABOLIC PATHWAY: a series of enzyme-catalyzed reactions leading to the complete breakdown or synthesis of a particular biological molecule

POLYPEPTIDE: a complex molecule encoded by the genetic code and composed of amino acids; one or more of which compose a protein

POST-TRANSLATIONAL MODIFICATION: chemical alterations to proteins that alter their properties as enzymes

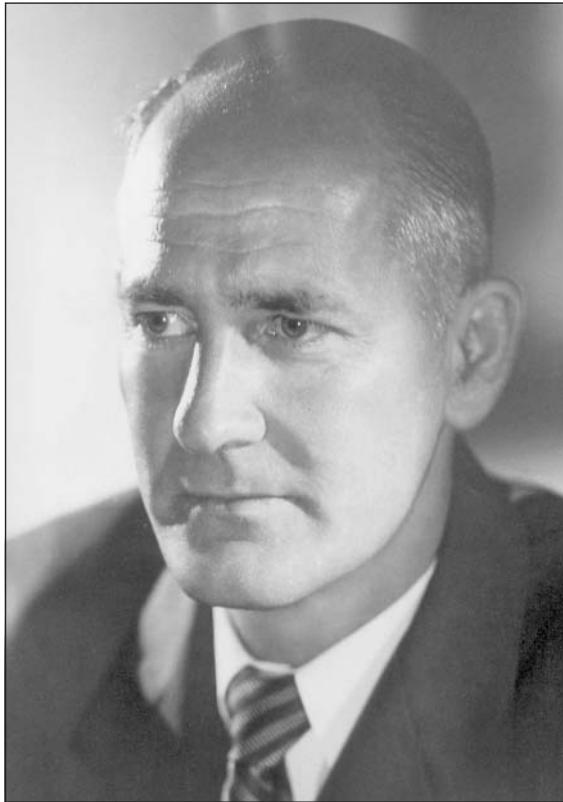
Genetics Meets Biochemistry

In the early part of the twentieth century, genetics was becoming an established discipline, but the relationship between genes and how they are expressed as phenotypes was not yet understood. Biochemistry was also in its infancy, particularly the study of the enzyme-catalyzed chemical reactions of metabolic pathways. In 1902, a British medical doctor named Archibald Garrod brought genetics and biochemistry together in the discovery that a human disease called alkaptonuria, which causes individuals with the disease to accumulate a black pigment in their urine—was inherited as a recessive trait. Equally important, however, was Garrod's observation that alkaptonurics were unable to metabolize alkapton, the molecule responsible for the black pigmentation, an intermediate in the degradation of amino acids. Garrod's conclusion was that people with alkaptonuria lack the enzyme that normally degrades alkapton. Because it thus appeared that a defective gene led to an enzyme deficiency, Garrod predicted that genes form enzymes. This statement was the precursor of what came to be known as the one gene-one enzyme hypothesis.

One Gene-One Enzyme Hypothesis

Field of study: History of Genetics; Molecular genetics

Significance: *The formulation of the one gene-one enzyme hypothesis in 1941, which simply states that each gene gives rise to one enzyme, was foun-*



George Wells Beadle. (© The Nobel Foundation)

Formation of the Hypothesis

Garrod's work went largely ignored until 1941, when George Beadle and Edward Tatum, geneticists at Stanford University, used bread mold (*Neurospora crassa*) to test and refine Garrod's theory. Wild-type *Neurospora* grows well on minimal media containing only sugar, ammonia, salts, and biotin, because it can biosynthesize all other necessary biochemicals. Beadle and Tatum generated mutants that did not grow on minimal media but instead grew only when some other factor, such as an amino acid, was included. They surmised that the mutant molds lacked specific enzymes involved in biosynthesis. With several such mutants, Beadle and Tatum demonstrated that mutations in single genes often corresponded to disruptions of single enzymatic steps in biosynthetic metabolic pathways. They concluded that each enzyme is controlled by one gene, a relationship they called the "one gene-one enzyme hypothesis." This time, the scientific community

took notice, awarding a Nobel Prize in Physiology or Medicine to Beadle and Tatum in 1958, and the hypothesis served as the basis for biochemical genetics for the next several years.

Modifications to the Hypothesis

The one gene-one enzyme hypothesis was accurate in predicting many of the findings in biochemical genetics after 1941. It is now known that DNA genes are often transcribed into messenger RNAs (mRNAs), which in turn are translated into polypeptides, many of which form enzymes. Thus, the basic premise that genes encode enzymes still holds. On the other hand, Beadle and Tatum had several of the details wrong, and today the hypothesis should be restated as follows: Most genes encode information for making one polypeptide.

There are at least three reasons that the original one gene-one enzyme hypothesis does not accurately explain biologists' current understanding of gene expression. First of all, enzymes are often formed from more than one



Edward Lawrie Tatum. (© The Nobel Foundation)

polypeptide, each of which is the product of a different gene. For example, the enzyme ATP synthase is composed of at least seven different polypeptides, all encoded by separate genes. Thus, the one-to-one ratio of genes to enzymes implied by the hypothesis is clearly incorrect. This fact was recognized early and led to the theory's reformulation as the "one gene-one polypeptide" hypothesis. However, even this newer version of the hypothesis has since been shown to be inaccurate.

Second, several important genes do not encode enzymes. For example, some genes encode transfer RNAs (tRNAs), which are required for translating mRNAs. Thus, clearly even the one gene-one polypeptide hypothesis is insufficient, since tRNAs are not polypeptides.

Finally, further deviation from the original one gene-one enzyme hypothesis is required when one considers that several modifications to RNAs and polypeptides occur after gene transcription, and can do so in more than one way. Thus, a single gene can give rise to more than one mRNA, and potentially to numerous different polypeptides with varying properties. Post-transcriptional variation in gene expression occurs first during RNA processing, when the polypeptide-encoding regions of mRNA are spliced together. It is important to note that the exact splicing pattern can vary depending on the exact needs of the cell. One example of a gene that undergoes differential mRNA processing leading to two dramatically different phenotypes is the fruit fly gene sex-lethal (*sxl*). A long version of *sxl* mRNA is generated in developing male flies and a shorter one in female flies. Because the *sxl* protein regulates sexual development, mutant female flies that mistakenly splice *sxl* mRNA display male sexual characteristics.

Like differential mRNA processing, post-translational protein modification varies by cellular context, allowing a single gene to generate more than one kind of enzyme. However, unlike mRNA processing, protein modification is often reversible. For example, liver cells responding to insulin will chemically modify some of their enzymes by way of a process called signal transduction, thereby changing their en-

zymatic properties, often essentially making them into different enzymes. Once insulin is no longer present, the cell can undo the modifications, returning the enzymes back to their original forms.

—Stephen Cessna

See also: Complementation Testing; Genetics, Historical Development of; Model Organism: *Neurospora crassa*; Signal Transduction.

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Organ Transplants and HLA Genes

Field of study: Immunogenetics

Significance: *Organ transplantation has saved the lives of countless people. Although the success rate for organ transplantation continues to improve, many barriers remain, including an inadequate supply of donor organs and the phenomenon of transplant rejection. Transplant rejection is caused by an immune response by the recipient to molecules on the transplanted organs that are coded for by the human leukocyte antigen (HLA) gene complex.*

Key terms

ALLELES: the two alternate forms of a gene at the same locus on a pair of homologous chromosomes

ANTIGENS: molecules recognized as foreign to the body by the immune system, including molecules associated with disease-causing organisms (pathogens)

HISTOCOMPATIBILITY ANTIGENS: molecules expressed on transplanted tissues that are recognized as foreign by the immune system, causing rejection of the transplant; the most important histocompatibility antigens in vertebrates are coded for by a cluster of genes called the major histocompatibility complex (MHC)

LOCUS (*pl.* LOCI): the location of a gene on a chromosome

POLYMORPHISM: the presence of many different alleles for a particular locus in individuals of the same species

Transplantation

The replacement of damaged organs by transplantation was one of the great success stories of modern medicine in the latter decades of the twentieth century. During the 1980's, the success rates for heart and kidney transplants showed marked improvement and, most notably, the one-year survival for pancreas and liver transplants rose from 20 percent and 30 percent to 70 percent and 75 percent, respectively. These increases in organ survival were largely attributable to improvements in two aspects of the transplantation protocol that directly reduced tissue rejection: the development of more accurate methods of tissue typing that allowed better tissue matching of donor and recipient, and the discovery of more effective and less toxic antirejection drugs. In fact, these changes helped make transplantation procedures so common by the 1990's that the low number of donor organs became a major limiting factor in the number of lives saved by this procedure.

Rejection and the Immune Response

The rejection of transplanted tissues is associated with genetic differences between the donor and recipient. Transplants of tissue within

the same individual, called autografts, are never rejected. Thus the grafting of blood vessels transplanted from the leg to an individual's heart during bypass operations are never in danger of being rejected. On the other hand, organs transplanted between genetically distinct humans tend to undergo clinical rejection within a few days to a few weeks after the procedure. During the rejection process, the transplanted tissue is gradually destroyed and loses its function. When examined under the microscope, tissue undergoing rejection is observed to be infiltrated with a variety of cells, causing its destruction. These infiltrating cells are part of the recipient's immune system, which recognizes molecules on the transplant as foreign to the body and responds to them as they would to a disease-causing, pathogenic organism.

The human immune response is a complex system of cells and secreted proteins that has evolved to protect the body from invasion by pathogens. Immune mechanisms are directed against molecules or parts of molecules called antigens. The ultimate function of the immune response is to recognize pathogen-associated antigens as foreign to the body and to eliminate and destroy the organism, thus resolving the disease. On the other hand, the immune response is prevented, under most circumstances, from attacking the antigens expressed on the tissues of the body in which they originate. The ability to distinguish between self and foreign antigens is critical to protecting the body from pathogens and to the maintenance of good health.

A negative consequence of the ability of the immune system to discriminate between self and foreign antigens is the recognition and destruction of transplants. The antigens associated with transplants are recognized as foreign in the same fashion as pathogen-associated antigens, and many of the same immune mechanisms used to kill pathogens are responsible for the destruction of the transplant. The molecules on the transplanted tissues recognized by the immune system are called histocompatibility antigens. The term "histocompatibility" refers to the fact that transplanted organs are often not compatible with the body of a genetically distinct recipient. All vertebrate animals have a cluster of genes that code for the most

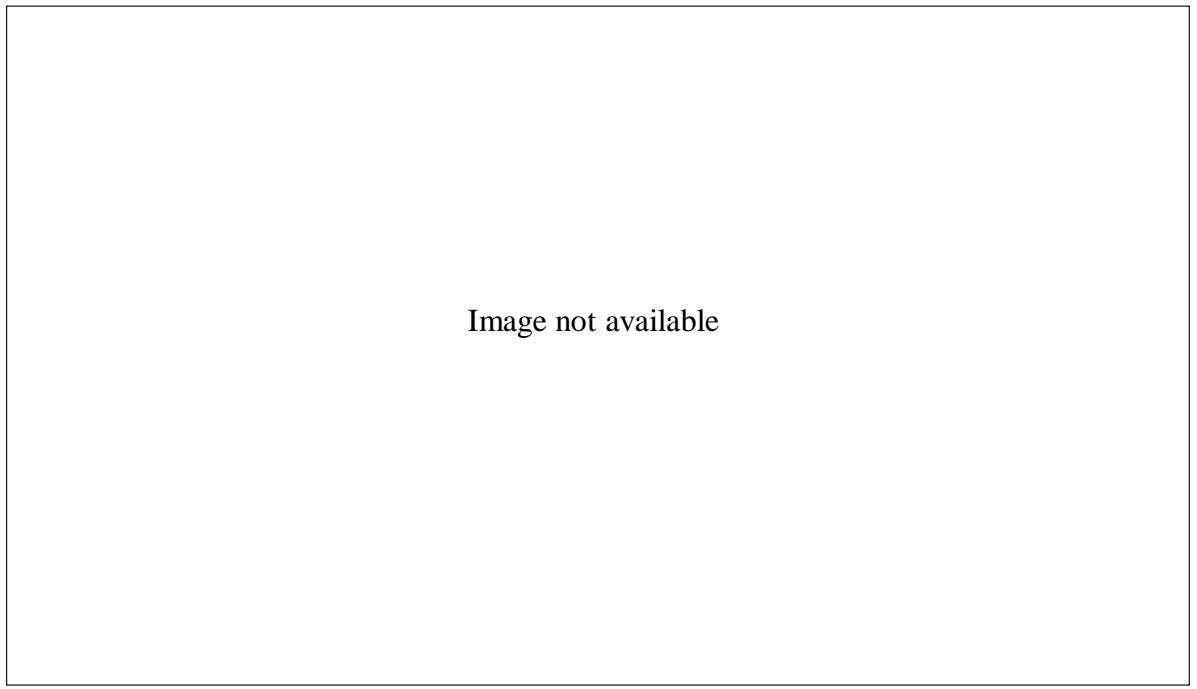


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A two-week-old piglet in April, 2002, one of three that were the first to be cloned from both human and pig cells. Normal pigs have been sources of human “replacement parts” (such as heart valves) from some time. The hope is that organs from pigs with human genes will be more easily accepted by the human body after transplantation. (AP/Wide World Photos)

important histocompatibility antigens, called the major histocompatibility complex (MHC).

MHC Polymorphism, HLA Genes, and Tissue Typing

Each MHC locus is highly polymorphic, meaning that many different alleles exist within a population (members of a species sharing a habitat). The explanation for the polymorphism of histocompatibility antigens is related to the actual function of these molecules within the body. Clearly, histocompatibility molecules did not evolve to induce the rejection of transplants, despite the fact that this characteristic led to their discovery and name.

Histocompatibility molecules function by regulating immunity against foreign antigens. Each allele codes for a protein that allows the immune response to recognize a different set of antigens. Many pathogens, including the viruses associated with influenza and acquired immunodeficiency syndrome (AIDS), undergo genetic mutations that lead to changes in their antigens, making it more difficult for the body

to make an immune response to the virus. The existence of multiple MHC alleles in a population, therefore, ensures that some individuals will have MHC alleles allowing them to mount an immune response against a particular pathogen. If an entire population lacked these alleles, their inability to respond to certain pathogens could threaten the very existence of the species. The disadvantage of MHC polymorphism, however, is the immune response to the donor’s histocompatibility antigens that causes organ rejection.

The human leukocyte antigen (HLA) gene complex is located on chromosome 6 in humans. Six important histocompatibility antigens are coded for by the HLA complex: the A, B, C, DR, DP, and DQ alleles. Differences in HLA antigens between the donor and recipient are determined by tissue typing. For many years, tissue typing was performed using antibodies specific to different HLA alleles. Antibodies are proteins secreted by the cells of the immune system that are used in the laboratory to identify specific antigens. As scientists began

to clone the genes for the most common HLA alleles in the 1980's and 1990's, however, it appeared that direct genetic analysis would eventually replace or at least supplement these procedures.

Fewer differences in these antigens between donor organ and recipient mean a better prognosis for transplant survival. Therefore, closely related individuals who share many of their histocompatibility alleles are usually preferred as donors. When a family member is not available, the process of finding a donor is problematic. Worldwide computer databases are used to match potential donors with recipients, who are placed on a waiting list based on the severity of their disease.

Immunosuppressive Antirejection Drugs

Perhaps the most important medical breakthrough responsible for the increased success of organ transplantation occurred in the last two decades of the twentieth century. This breakthrough involved the discovery and successful use of antirejection drugs, most of which act by suppressing the immune response to the transplanted tissue. Immunosuppressive drugs are usually given in high doses for the first few weeks after transplantation or during a rejection crisis, but the dosage of these drugs is usually reduced thereafter to avoid their toxic effects.

Cyclosporine is by far the most effective of these drugs and has largely been responsible for the increased efficacy of liver, pancreas, lung, and heart transplantation procedures. In spite of its successes, cyclosporine has limitations in that it can cause kidney damage when given in high doses. Azathioprine, associated with bone marrow toxicity, was largely supplanted by the introduction of the less toxic cyclosporine. However, azathioprine has been used as part of a combined cyclosporine-azathioprine regimen. This practice allows the reduction of both the cyclosporine and azathioprine dosages, reducing the toxicity of both drugs. The search for more effective and less toxic antirejection drugs continues. Individuals receiving immunosuppressive therapy have other concerns in addition to the toxicity of the drugs themselves. As these individuals will have an impaired ability to mount an immune re-

sponse to pathogens, their susceptibility to a variety of diseases will be increased. Thus transplant recipients must take special precautions to avoid exposure to potential pathogens, especially when receiving high doses of the drugs.

—James A. Wise

See also: Animal Cloning; Bacterial Genetics and Cell Structure; Bioethics; Biological Weapons; Cancer; Cloning; Cloning: Ethical Issues; Diabetes; Gene Therapy: Ethical and Economic Issues; Genetic Engineering: Historical Development; Genetics, Historical Development of; Heart Disease; Huntington's Disease; Hybridomas and Monoclonal Antibodies; Immunogenetics; In Vitro Fertilization and Embryo Transfer; Model Organism: *Mus musculus*; Model Organism: *Xenopus laevis*; Multiple Alleles; Paternity Tests; Polymerase Chain Reaction; Prion Diseases: Kuru and Creutzfeldt-Jakob Syndrome; Race; Sickle-Cell Disease; Stem Cells; Synthetic Antibodies; Totipotency; Transgenic Organisms; Xenotransplants.

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Parthenogenesis

Field of study: Genetic engineering and biotechnology

Significance: *Parthenogenesis is the development of unfertilized eggs, which produces individuals that are genetically alike and allows rapid expansion of a population of well-adapted individuals into a rich environment. This clonal reproduction strategy is used by a number of species for rapid reproduction under very favorable conditions, and it appears to offer a selective advantage to individuals living in disturbed habitats.*

Key terms

ADAPTIVE ADVANTAGE: increased fertility in offspring as a result of passing on favorable genetic information

DIPLOID: having two sets of homologous chromosomes

FERTILIZATION: the fusion of two cells (egg and sperm) in sexual reproduction

HAPLOID: having one set of chromosomes

MEIOSIS: nuclear division that reduces the chromosome number from diploid to haploid in the production of the sperm and the egg

ZYGOTE: the product of fertilization in sexually reproducing organisms

The Nature of Parthenogenesis

Parthenogenesis is derived from two Greek words that mean “virgin” (*parthenos*) and “origin” (*genesis*) and describes a form of reproduction in which females lay diploid eggs (containing two sets of chromosomes) that develop into new individuals without fertilization—there is no fusion of a sperm nucleus with the egg nucleus to produce the new diploid individual. This is a form of clonal reproduction because all of the individuals are genetically identical to the mother and to each other. The mechanisms of parthenogenesis do not show any single pattern and have evolved independently in different groups of organisms. In some organisms, such as rotifers and aphids, parthenogenesis alternates with normal sexual reproduction. When there is a rich food source, such as new rose bushes emerging in the early spring, aphids re-

produce by parthenogenesis; late in the summer, however, as the food source is decreasing, sexually reproducing females appear. The same pattern has been observed in rotifers, in which a decrease in the quality of the food supply leads to the appearance of females that produce haploid eggs by normal meiosis that require fertilization for development. The strategy appears to involve the clonal production of large numbers of genetically identical individuals that are well suited to the environment when the conditions are favorable and the production of a variety of different types, by the recombination that occurs during normal meiosis and the mixing of alleles from two individuals in sexual reproduction, when the conditions are less favorable. In social insects, such as bees, wasps, and ants, parthenogenesis is a major factor in sex determination, although it may not be the only factor. In these insects, eggs that develop by parthenogenesis remain haploid and develop into males, while fertilized eggs develop into diploid, sexually reproducing females.

In algae and some forms of plants, parthenogenesis also allows rapid reproduction when conditions are favorable. In citrus, seed development by parthenogenesis maintains the favorable characteristics of each plant. For this reason, most commercial citrus plants are propagated by asexual means, such as grafting. Parthenogenesis has also been induced in organisms that do not show the process in natural populations. In sea urchins, for example, development can be induced by mechanical stimulation of the egg or by changes in the chemistry of the medium. Even some vertebrate eggs have shown signs of early development when artificially stimulated, but haploid vertebrate cells lack all of the information required for normal development, so such “zygotes” cease development very early.

Parthenogenesis in Vertebrates

Parthenogenesis has been observed in vertebrates such as fish, frogs, and lizards. In these parthenogenetic populations, all the individuals are females, so reproduction of the clone is restricted to parthenogenesis. Parthenoge-

netic fish often occur in populations along with sexually reproducing individuals. The parthenogenetic forms produce diploid eggs that develop without fertilization; in rare cases, however, fertilization of a parthenogenetic egg gives rise to a triploid individual that has three sets of chromosomes rather than the normal two sets (two from the diploid egg and one from the sperm). In some groups, penetration of a sperm is necessary to activate development of the zygote, but the sperm nucleus is not incorporated into the zygote.

Evidence indicates that in each of these vertebrate situations, the parthenogenetic populations have resulted from a hybridization between two different species. The parthenogenetic forms always occur in regions where the two parental species overlap in their distribution, often an area that is not the most favorable habitat for either species. The hybrid origin has been confirmed by the demonstration that the animals have two different forms of an enzyme that have been derived from the two different species in the region. Genetic identity has also been confirmed using skin graft studies. In unrelated organisms, skin grafts are quickly rejected because of genetic incompatibilities; clonal animals, on the other hand, readily accept grafts from related donors. Parthenogenetic fish from the same clone accept grafts that confirm their genetic identity, but rejection of grafts by other parthenogenetic forms from different populations shows that they are different clones and must have a different origin. This makes it possible to better understand the structure of the populations and helps in the study of the origins of parthenogenesis within those populations. Comparisons using nuclear and mitochondrial DNA also allow the determination of species origin and the maternal species of the parthenogenetic form since the mitochondria are almost exclusively transmitted through the vertebrate egg. Within the hybrid, a mechanism has originated that allows the egg to develop without fertilization, although, as already noted, penetration by a sperm may be required to activate development in some of the species.

The advantage of parthenogenesis appears to be the production of individuals that are geneti-

cally identical. Since the parthenogenetic form may, at least in vertebrates, be a hybrid, it is heterozygous at most of its genetic loci. This provides greater variation that may provide the animal with a greater range of responses to the environment. Maintaining this heterozygous genotype may give the animals an advantage in environments where the parental species are not able to reproduce successfully and may be a major reason for the persistence of this form of reproduction. Many vertebrate parthenogenetic populations are found in disturbed habitats, so their unique genetic composition may allow for adaptation to these unusual conditions.

Mechanisms of Development

The mechanisms of diploid egg development are as diverse as the organisms in which this form of reproduction is found. In normal meiosis, the like chromosomes of each pair separate at the first division and the copies of each chromosome separate at the second division (producing four haploid cells). During the meiotic process in the egg, three small cells (the polar bodies), each with one set of chromosomes, are produced, and one set of chromosomes remains as the egg nucleus. In parthenogenetic organisms, some modification of this process occurs that results in an egg nucleus with two sets of chromosomes—the diploid state. In some forms, the first meiotic division does not occur, so two chromosome sets remain in the egg following the second division. In other forms, one of the polar bodies fuses back into the cell so that there are two sets of chromosomes in the final egg. In another variation, there is a replication of chromosomes after the first division, but no second division takes place in the egg, so the chromosome number is again diploid. In all of these mechanisms, the genetic content of the egg is derived from the mother's genetic content, and there is no contribution to the genetic content from male material.

The situation may be even more complex, however, because some hybrid individuals may retain the chromosomal identity of one species by a selective loss of the chromosomes of the other species during meiosis. The eggs may carry the chromosomes of one species but the

mitochondria of the other species. The haploid eggs must be fertilized, so these individuals are not parthenogenetic, but their presence in the population shows how complex reproductive strategies can be and how important it is to study the entire population in order to understand its dynamics fully: A single population may contain individuals of the two sexual species, true parthenogenetic individuals, and triploid individuals resulting from fertilization of a diploid egg.

—D. B. Benner

See also: Totipotency.

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Patents on Life-Forms

Field of study: Bioethics; Human genetics and social issues

Significance: In 1980, the U.S. Supreme Court upheld the right to patent a live, genetically altered organism. The decision was opposed by many scientists and theologians who believed that such organisms would pose a threat to the future of humanity. Although "legally" settled, the debate has continued, opponents arguing that patenting life-forms and DNA sequences imposes too great a cost and greatly inconveniences genetic research.

Key term

PATENT: a grant made by the government that gives the creator or inventor the sole right to make, use, or sell that invention for a specific period of time, usually seventeen years in the United States

Patent on Life-Form Upheld

On June 16, 1980, the U.S. Supreme Court voted 5 to 4 that living organisms could be patented under federal law. The case involved Ananda M. Chakrabarty, a scientist who, while working for General Electric in 1972, had created a new form of bacteria, *Pseudomonas* *originosa*, which could break down crude oil, and, therefore, could be used to clean up oil spills. Chakrabarty filed for a patent, but an examiner for the Patent Office rejected the application on the ground that living things are not patentable subject matter under existing patent law. Commissioner of Patents and Trademarks Sidney A. Diamond supported this view. Federal patent law provided that a patent could be issued only to a person who invented or discovered any new and useful "manufacture" or "composition of matter." The U.S. Court of Customs and Patent Appeals reversed that decision in 1979, concluding that the fact that microorganisms are alive has no legal significance. It held that a live, human-made bacterium is a patentable item since the microorganism was manufactured by crossbreeding four existing strains of bacteria and had never existed in nature.

Writing for the majority, Chief Justice Warren Burger upheld the patent appeals court judgment, making a distinction between the new bacterium and "laws of nature, physical phenomena and abstract ideas," which are not patentable. In the Court majority's view, Chakrabarty had invented a form of life that did not exist in the natural world, so it could not be considered part of nature. Instead, it was a product of human "ingenuity and research" that deserved patent protection. Items not patentable include new minerals that are discovered in the earth or a new species of plant found in a distant forest. These things occur naturally and are not created by humans. Burger also stressed that physicist Albert Einstein could not have patented his formula $E = mc^2$, since it is a law of nature, nor could Sir Isaac Newton have received a patent for the law of gravity. Discoveries such as these are part of the natural world and cannot be owned by a single individual.

Chakrabarty, on the other hand, had not found an unknown, natural species, nor had he discovered a law of nature. His new bacterium had a distinctive name and was developed in

the laboratory for a specific purpose. None of the characteristics of the new organism could be found in nature. His discovery, Burger re-emphasized, was patentable because he had created it.

Opposition to the Ruling

The Court majority refused to consider arguments made in friend-of-the-court briefs filed by opponents of genetic engineering. The briefs were presented by groups representing scientists, including several Nobel Prize winners, and religious organizations. One brief suggested that genetic research posed a dangerous and serious threat to the future of humanity and should, therefore, be prohibited. Possible dangers included the spread of pollution and disease by newly created bacteria, none of which would have any natural enemies. Other threats involved the possible loss of genetic diversity, if, for instance, only the "best" form of laboratory-created plant seeds were grown. Research into human genetics could lead to newly designed gene material that could be used to build a "master race," thereby devaluing other human lives. Justice Burger concluded, however, that humans could be trusted not to create such horrible things. Quoting William Shakespeare's *Hamlet*, the chief justice asserted that it is sometimes better "to bear those ills we have than to fly to others that we know not of." People can try to guess what genetic manipulation could lead to, but it would also be a good idea to expect good things from science rather than "a gruesome parade of horribles." Besides, he then said, it did not matter whether a patent was granted in this case; in either case, scientific research would continue into the nature of genes.

The People's Business Commission, a non-profit educational foundation, had argued that granting General Electric and Chakrabarty a patent would give corporations the right "to own the processes of life in the centuries to come" through genetic manipulation. Chief Justice Burger wrote that the Court was "without competence to entertain these arguments." They did not have enough information available to determine whether to ignore such fears "as fantasies generated by fear of the unknown"

or accept them. Such a determination was not the responsibility of the Court, however. Questions of the morality of genetic research and manipulation were better left to Congress and the political process. How to proceed in these matters could only be resolved "after the kind of investigation, examination, and study that legislative bodies can provide and courts cannot."

Justice William J. Brennan, Jr., presented a brief dissenting opinion. He noted that Congress had twice, in 1930 and 1970, permitted new types of plants to be patented. However, those laws made no mention of bacteria. Thus, Brennan argued, Congress had indicated that only plants could receive patents and that the legislators had thus clearly indicated that other life-forms were excluded from the patent process. The Court majority rejected this view, arguing that Congress had not specifically excluded other life-forms.

Developments Since 1980

Since the patenting of the petroleum-eating bacteria, a variety of other genetically modified (GM) organisms have been patented, including pest-resistant crop plants and numerous types of "knockout" mice used by many researchers. The controversy around such patents initially calmed, but more recent developments have rekindled the flames. Since the advent of the Human Genome Project, the sequencing of genomes has accelerated exponentially. Because many sequences might contain valuable genes or markers, companies and nonprofit organizations began patenting the sequences.

After much debate, the patenting of DNA sequences has been allowed, and although the guidelines are still being fine-tuned, the general rule is that any "distinctive" DNA sequence can be patented. Those opposed to the patenting of DNA sequences say that it will impede research and even the development of useful medical applications. Ethicists argue that no one should have a right to patent DNA sequences, which represent the very basis of life. Some scientists have pushed for more restrictive rules, such as that a sequence cannot be patented unless there is clear evidence that the

sequence codes for a useful product or would likely lead to a specific application.

At present it is too early to predict the final outcome of the push for patenting DNA sequences. There is still debate and there is a large backlog of sequences for which patents are still pending. A survey of medical testing laboratories in 2003 found that a number of labs either no longer used certain tests or did not plan to develop them when licensing fees were required for permission to use a relevant DNA sequence. Overwhelmingly, the labs surveyed saw patenting of DNA sequences as having a negative effect on the development of affordable clinical genetic tests.

—Leslie V. Tischauer, updated by Bryan Ness

See also: Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genetic Engineering: Social and Ethical Issues; Human Genetics; Human Genome Project; Hybridization and Introgression; Model Organism: *Mus musculus*; Transgenic Organisms.

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Paternity Tests

Field of study: Human genetics and social issues

Significance: Establishing paternity can be important for establishing legal responsibility for child support, health insurance, veterans' and social security benefits, and legal access to medical records. It may also affect a child's future as it relates to inherited diseases.

Key terms

FORENSIC GENETICS: the use of genetic tests and principles to resolve legal questions

HUMAN LEUKOCYTE ANTIGENS (HLA): antigens produced by a cluster of genes that play a critical role in the outcome of transplants; because they are made up of a large number of genes, they are used in individual identification and the matching of parents and offspring

PATERNITY EXCLUSION: the indication, through genetic testing, that a particular man is not the biological father of a particular child

Genetic Principles of Paternity Testing

The basic genetic principles utilized in paternity testing have remained the same from the first applications of ABO blood groups to applications of DNA fingerprinting. Available tests may positively exclude a man from being a child's biological father. Evidence supporting paternity, however, cannot be considered conclusive. Ultimately, a court must decide whether a man is determined to be the legal father based on all lines of evidence.

The genetic principles can be illustrated with a very simple example that uses ABO blood types. The four blood groups (A, B, AB, and O) are controlled by three pairs of genes. In the example, however, only three of the blood groups will be used to demonstrate the range of matings with the possible children for each of them (see the table headed "Blood Types, Genes, and Possible Offspring").

Example 1: A man is not excluded.

Mother: A

Child: A

Putative Father: AB

It can be seen that the mothers in matings 1 and 4 satisfy the condition of the mother being A and possibly having a child being A. Mating 4 satisfies the condition of a father being AB, the mother A, and a possible child being A. Results indicate that the putative father could be the father. He is not excluded.

Example 2: A man is excluded.

Mother: A

Child: A

Putative Father: B

Again, it is seen that the mothers in matings 1, 4, and 7 satisfy the condition of the mother being A and possibly having a child being A.

Mating 7 satisfies the condition of a father being B and the mother A, but mating 7 cannot produce a child being A. The putative father cannot be the father, and he is excluded.

DNA Fingerprinting

After the initial use of ABO blood groups in paternity testing, it became apparent that there were many cases in which the ABO phenotypes did not permit exclusion. Other blood group systems have also been used, including the MN and Rh groups. As more blood groups are utilized, the probability of exclusion (or nonexclusion) increases. Paternity tests have not been restricted to blood groups alone; tissue types and serum enzymes have also been used.

The most powerful tool developed has been DNA testing. DNA fingerprinting was developed in England by Sir Alec Jeffreys. DNA is extracted from white blood cells and broken down into fragments by bacterial enzymes (restriction endonucleases). The fragments are separated by size, and specific fragments are identified. Each individual has a different DNA profile, but the profiles of parents and children have similarities in greater proportion than those between unrelated people. Also, frequencies of different fragments tend to vary among ethnic groups. It is possible not only to exclude

Blood Types, Genes, and Possible Offspring

Mating Number	Genes of Parents		Blood Type of Parents		Possible Children	
	Father	Mother	Father	Mother	Genes	Blood Type
1	AA	AA	A	A	AA	A
2	AA	AB	A	AB	AA or AB	A or AB
3	AA	BB	A	B	AB	AB
4	AB	AA	AB	A	AA or AB	A or AB
5	AB	AB	AB	AB	AA, AB, or BB	A, AB, or B
6	AB	BB	AB	B	AB or BB	AB or B
7	BB	AA	B	A	AB	AB
8	BB	AB	B	AB	AB or BB	AB or B
9	BB	BB	B	B	BB	B

someone who is not the biological father but also to determine actual paternity with a probability approaching 100 percent.

Impact and Applications

The personal, social, and economic implications involved in paternity testing have far-reaching consequences. Blood-group analysis is cheaper but less consistent than DNA testing. Paternity can often be excluded but rarely proven with the same degree of accuracy that DNA testing provides. Human leukocyte antigen (HLA) testing can also be used but suffers from many of the same problems as blood-group analysis. The development of DNA testing after 1984 revolutionized the field of paternity testing. DNA fingerprinting has made decisions on paternity assignments virtually 100 percent accurate. The same technique has also been applied in cases of individual identification, and results have helped to release people who have been falsely imprisoned as well as convict other people with the analysis of trace evidence.

—Donald J. Nash

See also: DNA Fingerprinting; Forensic Genetics; Repetitive DNA.

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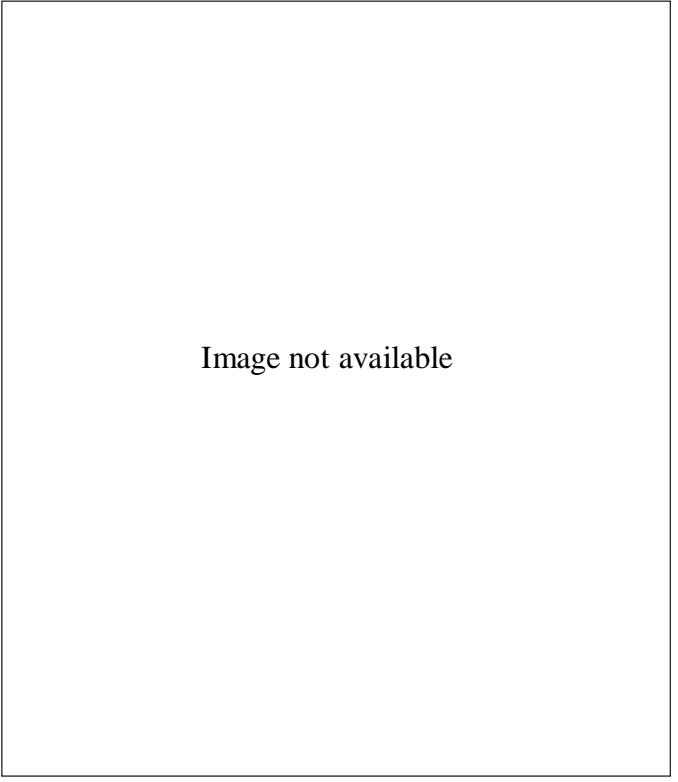


Image not available

At Paternity Testing Corporation in January, 2003, a technician drops a specimen into a dish with lysis solution to extract DNA for a paternity test.
 (AP/Wide World Photos)

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Weir, Bruce S. *Human Identification: The Use of DNA Markers*. New York: Kluwer Academic, 1995. Discussion includes the debates over using DNA profiles to identify paternity. Bibliography.

Web Sites of Interest

Earl's Forensic Page. <http://members.aol.com/EarlNMeyer/DNA.html>. Summarizes how DNA fingerprinting works and its use in

crime investigations and in determining paternity.

National Newborn Screening and Genetics Resource Center. <http://genes-r-us.uthscsa.edu>. Site serves as a resource for information on genetic screening, including paternity testing.

Pedigree Analysis

Fields of study: Population genetics; Techniques and methodologies

Significance: *Charts called pedigrees are used to represent the members of a family and to indicate which individuals have particular inherited traits. A pedigree is built of shapes connected by lines. Pedigrees are used by genetic counselors to help families determine the risk of genetic disease and are used by research scientists in determining how traits are inherited.*

Key terms

ALLELES: alternate forms of a gene locus, some of which may cause disease

AUTOSOMAL TRAIT: a trait that typically appears just as frequently in either sex because an autosomal chromosome, rather than a sex chromosome, carries the gene

DOMINANT ALLELE: an allele that is expressed even when only one copy (instead of two) is present

HEMIZYGOUS: the human male is considered to be hemizygous for X-linked traits, because he has only one copy of X-linked genes

HETEROZYGOUS CARRIERS: individuals who have one copy of a particular recessive allele that is expressed only when present in two copies

HOMOZYGOTE: an organism that has identical alleles at the same locus

RECESSIVE ALLELE: an allele that is expressed only when there are two copies present

X-LINKED TRAIT: a trait caused by a gene carried on the X chromosome, which has different patterns of inheritance in females and males because females have two X chromosomes while males have only one

Overview and Definition

Pedigree analysis involves the construction of family trees that can be used to trace inheritance of a trait over several generations. It is a graphical representation of the appearance of a particular trait or disease in related individuals along with the nature of the relationships.

Standardized symbols are used in pedigree charts. Males are designated by squares, females by circles. Symbols for individuals affected by a trait are shaded, while symbols for unaffected individuals are not. Heterozygous carriers are indicated by shading of half of the symbol, while carriers of X-linked recessive traits have a dot in the middle of the symbol. Matings are indicated by horizontal lines linking the mated individuals. The symbols of the individuals who are offspring of the mated individuals are linked to their parents by a vertical line intersecting with the horizontal mating line.

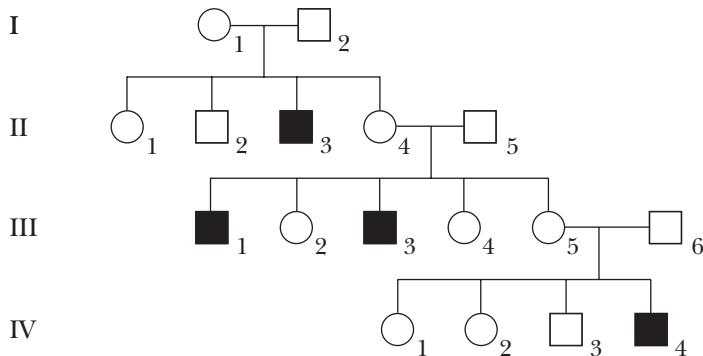
The classic way to determine the mode of inheritance of a trait is to conduct experimental matings of large numbers of individuals. Such experimental matings between humans are not possible, so it is necessary to infer the mode of inheritance of traits in humans through the use of pedigrees. Large families with good historical records are the easiest to analyze. Once a pedigree is established, it can be used to determine the likely mode of inheritance of a particular trait and, if the mode of inheritance can be determined with certainty, to determine the risk of the trait's appearing in offspring.

Typical Pedigrees

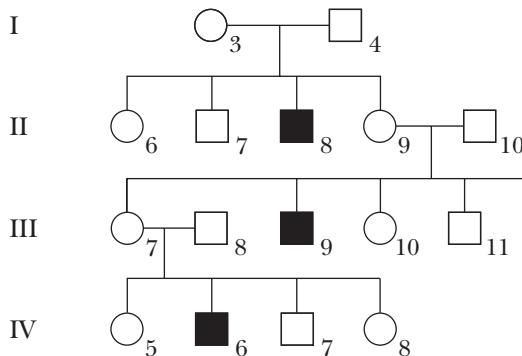
There are four common modes of inheritance detected using pedigree analysis: autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive. Autosomal traits are governed by genes found on one of the autosomes (chromosomes 1-22), while the genes that cause X-linked traits are found on the X chromosome. Males and females are equally likely to be affected by autosomal traits, whereas X-linked traits are never passed on from father to son and all affected males in a family received the mutant allele from their mothers.

Examples of Pedigrees

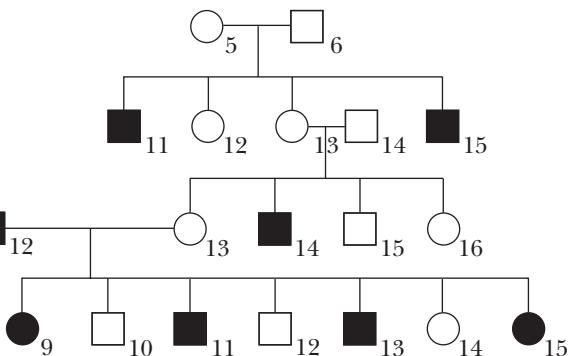
Family A



Family B



Family C



Typical pedigree charts for three families: Roman numerals indicate generations. Squares denote male individuals; circles, female individuals; white or blank individuals are “normal” phenotype; black denotes “affected” phenotype. The charts read like a family tree, with “mother” and “father” at the top and vertical lines denoting offspring; individuals connected only by horizontal lines are mates that have entered the genetic line from outside (“in-laws” in the case of humans). Family A provides an example of a sex-linked recessive trait. Families B and C (joined at 12 and 13) give examples of autosomal traits and how they can resemble sex-linked recessive traits sometimes—and hence the reason for using large families when constructing pedigrees. (Bryan Ness)

The pattern of autosomal dominant inheritance is perhaps the easiest type of Mendelian inheritance to recognize in a pedigree. A trait that appears in successive generations, and is found only among offspring where at least one of the parents is affected, is normally due to a dominant allele.

If neither parent has the characteristic phenotype displayed by the child, the trait is recessive. For recessive traits, on average, the recur-

rence risk to the unborn sibling of an affected individual is one in four. The majority of X-linked traits are recessive. The hallmark of X-linked recessive inheritance is that males are much more likely to be affected than females, because males are hemizygous, that is, they possess only one X chromosome, while females have two X chromosomes. Therefore, a recessive trait on the X chromosome will be expressed in all males who possess that X chro-

mosome, while females with one affected X chromosome will be asymptomatic carriers unless their other X chromosome also carries the recessive trait. The trait or disease is typically passed from an affected grandfather through his carrier daughters to half of his grandsons.

X-linked dominant traits are rare but distinctive. All daughters of an affected male and a normal female are affected, while all sons of an affected male and a normal female are normal. For matings between affected females and normal males, the risk of having an affected child is one in two, regardless of the sex of the child. Males are usually more severely affected than females. The trait may be lethal in males. In the general population, females are more likely to be affected than males, even if the disease is not lethal in males.

Usefulness

Pedigrees are important both for helping families identify the risk of transmitting an inherited disease and as starting points for searching for the genes responsible for inherited diseases. Mendelian ratios do not apply in individual human families because of the small size. Pooling of families is possible; in the United States, the Mormons and the Amish have kept good records that have aided genetic studies.

However, even using large, carefully constructed records, pedigrees can be difficult to construct and interpret for several reasons. Tracing family relationships can be complicated by adoption, children born out of wedlock, blended families, and assisted reproductive technologies that result in children who may not be genetically related to their parents. Additionally, people are sometimes hesitant to supply information because they are embarrassed by genetic conditions that affect behavior or mental stability.

Many traits do not follow clear-cut Mendelian ratios. Extensions and exceptions to Mendel's laws that can confound efforts to develop a useful pedigree are numerous. In diseases with variable expressivity, some of the symptoms of the disease are always expressed but may range from very mild to severe. In autosomal dominant diseases with incomplete

penetrance, some individuals who possess the dominant allele may not express the disease phenotype at all. Some traits have a high recurrent mutation rate. An example is achondroplasia (a type of dwarfism), in which 85 percent of cases are due to new mutations, where both parents have a normal phenotype. Traits due to multifactorial inheritance have variable expression as a result of interactions of the genes involved with the environment. Early-acting lethal alleles can lead to embryonic death and a resulting dearth of expected affected individuals. Pleiotropy is the situation in which a single gene controls several functions and therefore has several effects; it can result in different symptoms in different affected individuals. Finally, one trait can have a different basis of inheritance in different families. For example, mutations in any one of more than four hundred different genes can result in hereditary deafness.

Modern Applications

Genetic counseling is one of the key areas in which pedigrees are employed. A genetic counseling session usually begins with the counselor taking a family history and sketching a pedigree with paper and pencil, followed by use of a computer program to create an accurate pedigree. The Human Genome Project has accelerated the number of genetic disorders that can be detected by heterozygote and prenatal screening. A large part of the genetic counselor's job is to determine for whom specific genetic tests are appropriate.

Although genetic tests for many disorders are now available, the genes involved in many other disorders have yet to be identified. Therefore most human gene mapping utilizes molecular DNA markers, which reflect variation at noncoding regions of the DNA near the affected gene, rather than biochemical, morphological, or behavioral traits. A DNA marker is a piece of DNA of known size, representing a specific locus, that comes in identifiable variations. These allelic variations segregate according to Mendel's laws, which means it is possible to follow their transmission as one would any gene's transmission. If a particular allelic variant of the DNA marker is found in individuals with a

particular phenotype, the DNA marker can be used to develop a pedigree. The DNA from all available family members is examined and the pedigree is constructed using the presence of the DNA marker rather than phenotypic categories. This method is particularly useful for late-onset diseases such as Huntington's disease, whose victims may not know they carry the deleterious allele until they are in their forties or fifties, well past reproductive years. Although using DNA markers is a powerful method, crossover in the chromosome between the marker and the gene can cause an individual to be normal but still have the marker that suggests presence of the mutant allele. Thus, for all genetic tests there is a small percentage of false positive and false negative results, which must be factored into the advice given during genetics counseling.

—Lisa M. Sardinia

See also: Artificial Selection; Classical Transmission Genetics; Complete Dominance; Eugenics; Genetic Counseling; Homosexuality; Incomplete Dominance; Multiple Alleles.

Further Reading

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several case studies and numerous problems.

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Penetrance

Field of study: Population genetics

Significance: *Penetrance is a measure of how frequently a specific genotype results in the same, predictable phenotype. Such variable expression of the same genotype is the result of different genetic backgrounds and the effects of variations in the environment. Geneticists desire 100 percent penetrance for desirable genes that offer disease resistance but reduced penetrance and low expressivity for others that may contribute to human diseases.*

Key terms

EXPRESSIVITY: the degree to which a phenotype is expressed, or the extent of expression of a phenotype

PHENOTYPE: the physical appearance or biochemical and physiological characteristics of an individual, which is determined by both heredity and environment

Gene Expression and Environment

Gene expression results in a chemical product (protein) with a specific function. The genotype (genetic makeup, or gene) and environmental conditions determine the phenotype of an individual.

Penetrance and Expressivity

Gene expression is dependent upon environmental factors and may be modified, enhanced, silenced, and/or timed by the regulatory mechanisms of the cell in response to internal and external forces. A range of phenotypes can result from a genotype in response to different environments; the phenomenon is called "norms of reaction" or "phenotypic plasticity." Norms of reaction represent the expres-

sion of phenotypic variability in individuals of a single genotype.

The question of which is more important in the formation of an organism, nature (genotype) or nurture (environment), has been debated for centuries. The answer is that it depends. The genotype defines phenotypic potential. The environment works on the plasticity of expression to produce different phenotypes from similar genotypes.

Penetrance is the proportion of individuals with a specific genotype who display a defined phenotype. Some individuals may not express a gene if modifiers, epistatic genes, or suppressors are also present in the genome. Penetrance is the likelihood, or probability, that a condition or disease phenotype will, in fact, appear when a given genotype is present. If every person carrying a gene for a dominantly inherited disorder has the mutant phenotype, then the gene is said to have 100 percent penetrance. If

only 30 percent of those carrying the mutant allele exhibit the mutant phenotype, the penetrance is 30 percent. Sometimes an individual with a certain genotype fails to express the expected phenotype, and then the allele is said to be nonpenetrant in the individual. If the phenotype is expressed to any degree, the genotype is penetrant.

Given a particular phenotypic trait and a genotype, penetrance can be expressed as the probability of the phenotype given the genotype. For example, penetrance can be the probability of round seeds, a phenotype, given the genotype G ; it can also be the probability of wrinkled seeds, another phenotype, given the genotype G . One could label the specific phenotype of interest as P_i (P_i might refer to either the round or wrinkled seeds) and the specific genotype among many possibilities as G_j . The penetrance would then be the probability of P_i given G_j . These penetrances can all be ex-

Penetrance vs. Expressivity

Complete Penetrance:



Variable Penetrance:



Variable Expressivity (100% Penetrance):



Variable Penetrance and Expressivity:



pressed using the mathematical notation of conditional probabilities as follows:

- Case 1: $\text{Pr}(\text{round} | G)$
- Case 2: $\text{Pr}(\text{wrinkled} | G)$
- Case 3: $\text{Pr}(P | G)$

A 100 percent penetrance means that all individuals who possess a particular genotype express the phenotype (common in all homozygous lethal genes). Tay-Sachs disease shows complete, or 100 percent, penetrance, as all homozygotes for this allele develop the disease and die.

An allele, *Fu*, in mice causes fusion in the tail in heterozygotes, *Fufu*, and extremely fused and abnormal tails in the homozygotes, *FuFu*. From testcross matings of *Fufu* × *fufu*, 87 fused-tailed mice and 129 nonfused-tailed mice resulted. Genetic analyses of the 129 nonfused-tailed mice revealed that 22 were genotypically *Fufu*. The number of fused-tailed mice was 87 and the number of mice with the *Fufu* genotype but nonfused tails was 22. The total number of fused-tailed mice expected was $(87 + 22) = 109$. Therefore, penetrance was calculated at $87/109 = 0.798$.

Expressivity

Whereas penetrance describes the frequency that a genotype is expressed as a specified phenotype, expressivity describes the range of variation in the phenotype when expression is observed. Expressivity is variation in allelic expression when the allele is penetrant. Not all traits are expressed 100 percent of the time even though the allele is present. Expressivity is the range of variation in a phenotype; it refers to the degree of expression of a given trait or combination of traits that is associated with a gene. Affected individuals may have severe or mild symptoms; they may have symptoms that show up in one organ or combination of organs in one individual but not in the same locations in other individuals.

Phenotype may be altered by heterogeneity of other genes that affect the expression of a particular locus in question, or by environmental influence. Variable expressivity is a common feature of a variety of cancers. The lower the penetrance, the fewer number of individuals

will be affected. In humans, the dominant allele *P* produces polydactyly—extra toes and/or fingers. Matings between two normal appearing parents sometimes produce offspring with polydactyly. The parent with the *Pp* genotype exhibits reduced penetrance for the *P* allele.

—Manjit S. Kang

See also: Hereditary Diseases; Pedigree Analysis.

Further Reading

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Phenylketonuria (PKU)

Field of study: Diseases and syndromes

Significance: *Phenylketonuria is a relatively common genetic disease affecting about one in every ten thousand newborn babies. If the disease is not detected and treatment is not begun within the first few weeks of life, the child will develop various neurological symptoms including retardation. If the disease is detected shortly after birth and dietary treatment is instituted, symptoms characteristic of the disease usually will not develop.*

Key terms

PHENYLALANINE: an essential amino acid that accumulates in those affected by PKU; phenylalanine and tyrosine, another essential amino acid, can be converted into various compounds such as melanin, epinephrine, norepinephrine, and dopamine

PHENYLALANINE HYDROXYLASE: the enzyme that converts phenylalanine into tyrosine; in those affected by PKU, this enzyme is defective or missing

PHENYL PYRUVIC ACID: a compound derived from phenylalanine that accumulates in those affected by PKU

TYROSINE: an essential amino acid that can be derived from phenylalanine; it can be converted into various compounds such as melanin, epinephrine, norepinephrine, and dopamine

Discovery of PKU

Phenylketonuria, or PKU, was discovered in 1934 by Asbjørn Følling in Norway. Følling discovered that the urine of retarded children turned green when ferric chloride, a chemical used to detect ketones in the urine of diabetics, was added. The urine of diabetics normally turns purple or burgundy with the addition of ferric chloride. Følling conducted further investigations and discovered that the substance responsible for turning urine green upon addition of ferric chloride was phenylpyruvic acid. Følling discovered that the origin of phenylpyruvic acid was the amino acid phenylalanine.

Symptoms and Effects on Metabolism

Common characteristics of untreated patients with PKU are mental retardation, light-colored skin, hyperactivity, schizophrenia, tremors, and eczema.

PKU also has major metabolic effects. In people with normal metabolisms, phenylalanine, an essential amino acid, must be consumed in the diet. Phenylalanine is either incorporated into the body's proteins or converted by the enzyme phenylalanine hydroxylase into tyrosine, another amino acid. Tyrosine is either incorporated into protein or converted into other important biological molecules, such as dopamine, epinephrine, norepinephrine, and melanin. Alternatively, tyrosine can be completely metabolized and eliminated from the body.

People with PKU cannot metabolize phenylalanine into tyrosine at normal rates. Normally, blood phenylalanine concentrations are between 2 and 6 milligrams per deciliter (mg/dl), but in PKU phenylalanine accumulates to 20

mg/dl or more. Since phenylalanine cannot be properly converted into tyrosine, melanin, dopamine, norepinephrine, and epinephrine, there is a deficiency of those important compounds, which probably contributes to the development of symptoms characteristic of the disease. The high levels of phenylalanine may also interfere with the transport of other important amino acids into the brain. Since several amino acids use the same transport system as phenylalanine, phenylalanine is preferentially transported at the expense of the others. This may also contribute to the development of symptoms characteristic of PKU. Additionally, since phenylalanine cannot be metabolized normally, it is metabolized into abnormal compounds such as phenylpyruvic acid, which further contributes to the development of PKU symptoms.

The PKU Gene

The gene responsible for PKU encodes the information for the liver enzyme phenylalanine hydroxylase (PAH), which catalyzes the conversion of phenylalanine to tyrosine. The disease-causing mutant PKU gene is recessive. Thus, in order for a person to have PKU he or she must inherit two copies of the mutant gene. Approximately one in every fifty people in the United States is a heterozygous carrier for the disease. About one in every ten thousand newborn babies has the disease. African Americans have a much lower incidence of PKU than do Caucasian Americans. In certain other populations, such as in Ireland, the incidence of the disease is much higher.

The PKU gene was isolated in 1992, and soon afterward it was discovered that there is no one type of PKU mutation. Instead, the disease can be caused by a variety of defects affecting the PKU gene. Many of these defects are "point" mutations resulting in single base-pair changes in the DNA which lead to amino acid substitutions in the *PAH* gene. Other defects include base-pair changes leading to splicing defects in *PAH* messenger RNA (mRNA), deletions resulting in one or more missing amino acids in PAH, and insertions resulting in mRNA reading frame shifts. More than four hundred mutations have been found in the PKU gene.

The variety of different defects in the PKU gene leads to variability in the activity of PAH and the severity of the disease.

PKU Screening

In 1957 Willard Centerwall introduced ferric chloride as a screening technique by impregnating babies' diapers with ferric chloride. If the babies' urine contained phenylpyruvic acid, the diaper would turn green. Since the test was reliable only after the baby was several weeks old and after brain damage may already have occurred, a new, more reliable and more sensitive test was needed.

Robert Guthrie developed a more sensitive test. In the Guthrie test, bacteria are grown on an agar medium that contains an inhibitor of growth that can be overcome by exogenously added phenylalanine. If a small piece of filter paper containing blood is placed on the agar medium with the bacteria, the phenylalanine in the blood leaches out of the filter paper and stimulates growth of the bacteria. The extent of the growth around the filter paper is directly proportional to the amount of phenylalanine in the blood. Guthrie published his procedure in 1961. In 1963 Massachusetts became the first state to legislate mandatory PKU screening of all newborns. It is now mandatory in all fifty states.

Treatment

The treatment of choice for PKU is dietary or nutritional intervention. PKU babies placed on very low phenylalanine diets show normal cognitive development. The PKU diet eliminates high-protein foods, which are replaced with low-phenylalanine foods and supplemented with a nutritional formula. In 1954, Horst Bickel was the first to treat PKU with diet therapy.

It is recommended that dietary intervention begin as soon as possible after birth and continue for life. It is especially important that pregnant PKU women adhere closely to the diet, or their babies will be mentally retarded. Studies have shown that if children or adults are taken off the diet, some PKU symptoms may develop.

—Charles L. Vigue

See also: Biochemical Mutations; Genetic Screening; Genetic Testing; Hereditary Diseases; Inborn Errors of Metabolism; Model Organism: *Mus musculus*.

Further Reading

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National PKU News. This newsletter, published in Seattle, Washington, three times per year, provides the latest information about PKU.

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Web Site of Interest

National Organization for Rare Disorders. <http://www.rarediseases.org>. Searchable site by type of disorder. Includes background information on PKU, a list of other names for the disease, and a list of related organizations.

Plasmids

Field of study: Molecular genetics

Significance: *Plasmids are DNA molecules that exist separately from the chromosome. Plasmids exist in a commensal relationship with their host and may provide the host with new abilities. They are used in genetic research as vehicles for carrying genes. In the wild, they promote the exchange of genes and contribute to the problem of antibiotic resistance.*

Key terms

COMMENSALISM: a relationship in which two organisms rely on each other for survival

GENE: a region of DNA containing instructions for the manufacture of a protein

TRANSPOSON: a piece of DNA that can copy itself from one location to another

Plasmid Structure

The structure of plasmids is usually circular, although linear forms do exist. Their size ranges from a few thousand base pairs to hundreds of thousands of base pairs. They are found primarily in bacteria but have also been found in fungi, plants, and even humans.

In its commensal relationship with its host, the plasmid can be thought of as a molecular parasite whose primary function is to maintain itself within its host and to spread itself as widely as possible to other hosts. The majority of genes that are present on a plasmid will be dedicated to this function. Researchers have discovered that despite the great diversity of plasmids, most of them have similar genes, dedicated to this function. This relative simplicity of plasmids makes them ideal models of gene function, as well as useful tools for molecular biology. Genes of interest can be placed on a plasmid, which can easily be moved in and out of cells. Using plasmids isolated from the wild, molecular biologists have designed many varieties of artificial plasmids, which have greatly facilitated research in molecular biology.

Plasmid Replication

To survive and propagate, a plasmid must be able to copy itself, or replicate. The genes that direct this process are known as the replication genes. These genes do not carry out all the functions of replication, but instead coopt the host's replication machinery to replicate the plasmid. Replication allows the plasmid to propagate by creating copies of itself that can be passed to each daughter cell when the host divides. In this manner, the plasmid propagates along with the host.

A second function of the replication genes is to control the copy number of the plasmid. The number of copies of a plasmid that exist inside a host can vary considerably. Plasmids can exist at a very low copy number (one or two copies per cell) or at a higher copy number, with dozens of copies per cell. Adjusting the copy number is an important consideration for a plasmid. Plasmid replication is an expensive process that consumes energy and resources of the host cell. A plasmid with a high copy

number can place a significant energy drain on its host cell. In environments where the nutrient supply is low, a plasmid-bearing cell may not be able to compete successfully with other, non-plasmid-containing cells. Wild plasmids often exist at a low copy number, or create a high copy number for only a brief period of time.

Plasmid Partitioning

Because the presence of a plasmid is expensive in terms of energy, a cell harboring a plasmid will grow more slowly than a similar cell with no plasmid. This can cause a problem for a plasmid if it fails to partition properly during its host's division. If the plasmid does not partition properly, then one of the host's daughter cells will not contain a plasmid. Since this cell does not have to spend energy replicating a plasmid, it will gain an ability to grow faster, as will all of its offspring. In such a situation, the population of non-plasmid-containing cells could outgrow the population of plasmid-containing cells and use up all the nutrients in the environment. To avoid this problem, plasmids have evolved strategies to prevent improper partitioning. One strategy is for the plasmid to contain partitioning genes. Partitioning genes encode proteins that actively partition plasmids into each daughter cell during the host cell's division. Active partitioning greatly reduces the errors in partitioning that might occur if partitioning were left to chance.

A second strategy that plasmids use to prevent partitioning errors is the plasmid addition system. In this strategy, genes on the plasmid direct the production of both a toxin and an antidote. The antidote protein is very unstable and degrades quickly, but the toxin is quite stable. As long as the plasmid is present, the cytoplasm of the cell will be full of toxin and antidote. Should a daughter cell fail to receive a plasmid during division, the residual antidote and toxin present in the cytoplasm from the mother cell will begin to degrade, since there is no longer a plasmid present to direct the synthesis of either toxin or antidote. Since the antidote is very unstable, it will degrade first, leaving only toxin, which will kill the cell.

Plasmid Transfer Between Cells

Propagation of plasmids can occur through the spread of plasmids from parent cells to their offspring (referred to as vertical transfer), but propagation can also occur between two different cells (referred to as horizontal transfer). Many plasmids are able to transfer themselves from one host to another through the process of conjugation. Conjugal plasmids contain a collection of genes that direct the host cell that contains them to attach to other cells and transfer a copy of the plasmid. In this manner, the plasmid can spread itself to other hosts and is not limited to spreading itself only to the descendants of the original host cell.

One of the first plasmids to be identified was discovered because of its ability to conjugate. This plasmid, known as the F plasmid, or F factor, is a plasmid found in the bacterium *Escherichia coli*. Cells harboring the F plasmid are designated F⁺ cells and can transfer their plasmid to other *E. coli* cells that do not contain the F plasmid (called F⁻ cells).

Conjugal plasmids can be very specific and transfer only between closely related members of the same species (such as the F plasmid), or they can be very promiscuous and allow transfer between unrelated species. An extreme example of cross-species transfer is the Ti plasmid of the bacterial species *Agrobacterium tumefaciens*. The Ti plasmid is capable of transferring part of itself from *A. tumefaciens* into the cells of dicotyledonous plants. Plant cells that receive parts of the Ti plasmid are induced to grow and form a tumorlike structure, called a gall, that provides a hospitable environment for *A. tumefaciens*.

Host Benefits from Plasmids

In most commensal relationships, there is an exchange of benefits between the two partners. The same is true for plasmids and their hosts. In many cases, plasmids provide their host cells with a collection of genes that enhance the ability of the host cell to survive. Enhancements include the ability to metabolize a wider range of materials for food and the ability to survive in hostile environments. One particular hostile environment in which plasmids can provide

the ability to survive is the human body. A number of pathogenic microorganisms gain their ability to inhabit the human body, and thus cause disease, from genes contained on plasmids. An example of this is *Bacillus anthracis*, the agent that causes anthrax. Many of the genes that allow this organism to cause disease are contained on one of two plasmids, called pXO1 and pXO2. *Yersinia pestis*, the causative agent of bubonic plague, also gains its disease-causing ability from plasmids.

R Factors

Another example of plasmids conferring on their hosts the ability to survive in a hostile environment is antibiotic resistance. Plasmids known as R factors contain genes that make their bacterial hosts resistant to antibiotics. These R factors are usually conjugal plasmids, so they can move easily from cell to cell. Because the antibiotic resistance genes they carry are usually parts of transposons, they can readily copy themselves from one piece of DNA to another. Two different R factors that happened to be together in one cell could exchange copies of each other's antibiotic resistance genes. A number of R factors exist that contain multiple antibiotic resistance genes. Such plasmids can result in the formation of "multi-drug resistant" (MDR) strains of pathogenic bacteria, which are difficult to treat. There is much evidence to suggest that the widespread use of antibiotics has contributed to the development of MDR pathogens, which are emerging as an important health concern.

Role of Plasmids in Evolution

Through conjugation, plasmids can transfer genetic information from one species of bacterial cell to another. During its stay in a particular host, a plasmid may acquire some of the chromosomal genes of the host, which it then carries to a new host by conjugation. These genes can then be transferred from the plasmid to the chromosome of the new host. If the new host and the old host are different species, this gene transfer can result in the introduction of new genes, and thus new traits, into a cell. Bacteria, being asexual, produce daughter cells

that are genetically identical to their parent. The existence of conjugal plasmids, which allow for the transfer of genes between bacterial species, may represent an important mechanism by which bacteria generate diversity and create new species.

—Douglas H. Brown

See also: Anthrax; Antisense RNA; Archaea; Bacterial Genetics and Cell Structure; Bacterial Resistance and Super Bacteria; Biopesticides; Biopharmaceuticals; Blotting: Southern, Northern, and Western; Cloning; Cloning Vectors; DNA Sequencing Technology; Emerging Diseases; Extrachromosomal Inheritance; Gene Regulation: Bacteria; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Genome Size; Genomics; High-Yield Crops; Human Growth Hormone; Immunogenetics; Model Organism: *Chlamydomonas reinhardtii*; Model Organism: *Escherichia coli*; Model Organism: *Saccharomyces cerevisiae*; Model Organism: *Xenopus laevis*; Noncoding RNA Molecules; Polymerase Chain Reaction; Proteomics; Shotgun Cloning; Transgenic Organisms; Transposable Elements.

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Polygenic Inheritance

Fields of study: Classical transmission genetics

Significance: *Polygenically inherited traits—characterized by the amount of some attribute that they possess but not by their presence or absence—are central to plant and animal breeding, medicine, and evolutionary biology. Most of the economically important traits in plants and animals—for example, yield and meat production—are polygenic in nature. Quantitative genetic principles are applied to improve such traits.*

Key terms

HERITABILITY: the proportion of the total observed variation for a trait attributable to heredity or genes

MERIC TRAIT: traits that are counted, such as number of trichomes or bristles

QUANTITATIVE TRAIT: a trait, such as human height or weight, that shows continuous variation in a population and can be measured; also called a metric trait

QUANTITATIVE TRAIT LOCI (QTLs): genomic regions that condition a quantitative trait, generally identified via DNA-based markers

THRESHOLD TRAITS: characterized by discrete classes at an outer scale but exhibiting continuous variation at an underlying scale; for example, diabetes, schizophrenia, and cancer

Discovery of Polygenic Inheritance

Soon after the rediscovery of Gregor Mendel's laws of inheritance in 1900, Herman Nilsson-Ehle, a Swedish geneticist, showed in 1909 how multiple genes with small effects could collectively affect a continuously varying character. He crossed dark, red-grained wheat with white-grained wheat and found the progeny with an intermediate shade of red. Upon crossing the progeny among themselves, he obtained grain colors ranging from dark red to white. He could classify the grains into five groups in a symmetric ratio of 1:4:6:4:1, with the extreme phenotypes being one-sixteenth dark red and one-sixteenth white. This suggested two-gene segregation. For a two-gene

($n = 2$) model, the number and frequency of phenotypic classes ($2n + 1 = 5$) can be determined by expanding the binomial $(a + b)^4$, where a represents number of favorable alleles and b represents number of nonfavorable alleles.

Subsequently, Nilsson-Ehle crossed a different variety of red-grained wheat with white-grained wheat. He found that one-sixty-fourth of the plants produced dark red kernels and one-sixty-fourth produced white kernels. There were a total of seven phenotypic (color) classes instead of five. The segregation ratio corresponded to three genes: $(a + b)^6 = 1a^6 + 6a^5b^1 + 15a^4b^2 + 20a^3b^3 + 15a^2b^4 + 6a^1b^5 + 1b^6$. Here, a^6 means that one of sixty-four individuals possessed six favorable alleles, $20a^3b^3$ means that twenty of sixty-four individuals had three favorable and three nonfavorable alleles, and b^6 means that one individual had six nonfavorable alleles. An assumption was that each of the alleles had an equal, additive effect. These experiments led to what is known as the multiple-factor hypothesis, or polygenic inheritance (Kenneth Mather coined the terms "polygenes" and "polygenic traits"). Around 1920, Ronald Aylmer Fisher, Sewall Green Wright, and John Burdon Sanderson Haldane developed methods of quantitative analysis of genetic effects.

Polygenic traits are characterized by the amount of some attribute that they possess but not by presence or absence, as is the case with qualitative traits that are controlled by one or two major genes. Environmental factors generally have little or no effect on the expression of a gene or genes controlling a qualitative trait, whereas quantitative traits are highly influenced by the environment and genotype is poorly represented by phenotype. Genes controlling polygenic traits are sometimes called minor genes.

Examples and Characteristics of Polygenic Traits

Quantitative genetics encompasses analyses of traits that exhibit continuous variation caused by polygenes and their interactions among themselves and with environmental factors. Such traits include height, weight, and some genetic defects.

Diabetes and cancer are considered to be threshold traits because all individuals can be classified as affected or unaffected (qualitative). They are also continuous traits because severity varies from nearly undetectable to extremely severe (quantitative). Because it is virtually impossible to determine the exact genotype for such traits, it is difficult to control defects with a polygenic mode of inheritance.

Detection of Genes Controlling Polygenic Traits

The detection of genes controlling polygenic traits is challenging and complex because:

- (1) The expression of genes controlling such traits is modified by fluctuations in environmental and/or management factors.
- (2) A quantitative trait is usually a composite of many other traits, each influenced by many genes with variable effects.
- (3) Effects of allele substitution are small because many genes control the trait.
- (4) Expression of an individual gene may be modified by the expression of other genes and environment.

Polygenic traits are best analyzed with statistical methods, the simplest of which are estimation of arithmetic mean, standard error, variance, and standard deviation. Two populations can have the same mean, but their distribution may be different. Thus, one needs information on variances for describing the two populations more fully. From variances, effects of genes can be ascertained in the aggregate rather than as individual genes.

The issues in quantitative genetics are not only how many and which genes control a trait but also how much of what is observed (phenotype) is attributable to genes (heritability) and how much to the environment. The concept of heritability in the broad sense is useful for quantitative traits, but heritability itself does not give any clues to the total number of genes involved. If heritability is close to 1.0, the variance for a trait is attributable entirely to genetics, and when it is close to zero, the population's phenotype is due entirely to the variation in the underlying environment. Environmental effects mask or modify genetic effects.

Distribution or frequency of different classes in segregating populations—for example, F₂—may provide an idea about the number of genes, particularly if the gene number is small (say, three to four). Formulas have been devised to estimate the number of genes conditioning a trait, but these estimates are not highly reliable. Genes controlling quantitative traits can be estimated via use of chromosomal translocations or other cytogenetic procedures. The advent of molecular markers, such as restriction fragment length polymorphisms, has made it easier and more reliable to pinpoint the location of genes on chromosomes of a species of interest. With much work in a well-characterized organism, these polygenes can be mapped to chromosomes as quantitative trait loci.

—Manjit S. Kang

See also: Congenital Defects; Genetic Engineering; Hereditary Diseases; Neural Tube Defects; Pedigree Analysis; Plasmids; Quantitative Inheritance.

Further Reading

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Key terms

DNA POLYMERASE: an enzyme that copies or replicates DNA; it uses a single-stranded DNA as a template for synthesis of a complementary new strand and requires an RNA primer or a small section of double-stranded DNA to initiate synthesis

MOLECULAR CLONING: the process of splicing a piece of DNA into a plasmid, virus, or phage vector to obtain many identical copies of that DNA

The Development of the Polymerase Chain Reaction

The polymerase chain reaction (PCR) was developed by Kary B. Mullis in the mid-1980's. The technique revolutionized molecular genetics and the study of genes. One of the difficulties in studying genes is that a specific gene can be one of approximately twenty-one thousand genes in a complex genome. To obtain the number of copies of a specific gene needed for accurate analysis required the time-consuming techniques of molecular cloning and detection of specific DNA sequences. The polymerase chain reaction changed the science of molecular genetics by allowing huge numbers of copies of a specific DNA sequence to be produced without the use of molecular cloning. The tremendous significance of this discovery was recognized by the awarding of the 1993 Nobel Prize in Chemistry to Mullis for the invention of the PCR method. (The 1993 prize was also awarded to Michael Smith, for work on oligonucleotide-based, site-directed mutagenesis and its development for protein studies.)

Polymerase Chain Reaction

Fields of study: Genetic engineering and biotechnology; Molecular genetics; Techniques and methodologies

Significance: *Polymerase chain reaction (PCR) is the in vitro (in the test tube) amplification of specific nucleic acid sequences. In a few hours, a single piece of DNA can be copied one billion times. Because this technique is simple, rapid, and very sensitive, it is used in a very wide range of applications, including forensics, disease diagnosis, molecular genetics, and nucleic acid sequencing.*

How Polymerase Chain Reaction Works

PCR begins with the creation of a single-stranded DNA template to be copied. This is done by heating double-stranded DNA to temperatures near boiling (about 94 to 99 degrees Celsius, or about 210 degrees Fahrenheit). This is followed by the annealing (binding of a complementary sequence) of pairs of oligonucleotides (short nucleic acid molecules about ten to twenty nucleotides long) called primers. Because DNA polymerase requires a double-stranded region to prime (initiate) DNA synthesis, the starting point for DNA synthesis is

specified by the location at which the primer anneals to the template. The primers are chosen to flank the DNA to be amplified. This annealing is done at a lower temperature (about 30-65 degrees Celsius, or about 86-149 degrees Fahrenheit). The final step is the synthesis by DNA polymerase of a new strand of DNA complementary to the template starting from the primers. This step is carried out at temperatures about 65-75 degrees Celsius (149-167 degrees Fahrenheit). These three steps are repeated many times (for many cycles) to amplify the template DNA. The time for each of the three steps is typically one to two minutes. If, in each cycle, one copy is made of each of the strands of the template, the number of DNA molecules produced doubles each cycle. Because of this doubling, more than one million copies of the template DNA are made at the end of twenty cycles.

The PCR reaction is made more efficient by the use of heat-stable DNA polymerases, isolated from bacteria that live at very high temperatures in hot springs or deep-sea vents, and by the use of a programmable water bath (called a thermal cycler) to change the temperatures of samples quickly to each of the temperatures needed in each of the steps of a cycle.

Impact and Applications

PCR is extremely rapid. One billion copies of a specific DNA can be made in a few hours. It is also extremely sensitive. It is possible to copy a single DNA molecule. Great care must be taken to avoid contamination, however, for even trace contaminants can readily be amplified by this method.

PCR is a useful tool for many different applications. It is used in basic research to obtain DNA for sequencing and other analyses. PCR is

Image not available

A technician performs polymerase chain reaction testing of anthrax samples. (AP/Wide World Photos)

used in disease diagnosis, in prenatal diagnosis, and to match donor and recipient tissues for organ transplants. Because a specific sequence can be amplified greatly, much less clinical material is needed to make a diagnosis. The assay is also rapid, so results are available sooner. PCR is used to detect pathogens, such as the causative agents for Lyme disease or for acquired immunodeficiency syndrome (AIDS), that are difficult to culture. PCR can even be used to amplify DNA from ancient sources such as mummies, bones, and other museum specimens. PCR is an important tool in forensic investigations. Target DNA from trace amounts of biological material such as semen, blood, and hair roots can be amplified. There are probes for regions of human DNA that show hypervariability in the population and therefore make good markers to identify the source of the DNA. PCR can therefore be used to evaluate evidence at the scene of a crime, help identify missing people, and resolve paternity cases.

—Susan J. Karcher

See also: Ancient DNA; Anthrax; Bioinformatics; Blotting; Southern, Northern, and Western; Central Dogma of Molecular Biology; Cloning Vectors; DNA Fingerprinting; DNA Sequencing Technology; Forensic Genetics; Genetic Engineering; Historical Development; Human Genome Project; In Vitro Fertilization and Embryo Transfer; Mitochondrial Diseases; Molecular Genetics; Paternity Tests; Repetitive DNA; RFLP Analysis; RNA Isolation; Swine Flu.

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Polyploidy

Field of study: Population genetics

Significance: *Polyploids have three or more complete sets of chromosomes in their nuclei instead of the two sets found in diploids. Polyploids are especially common in plants, with some examples also existing in animals, and have a prominent role in*

the evolution of species. Some tissues of diploid organisms are polyploid, while the remaining cells in the organism are diploid.

Key terms

ALLOPOLYPLOID: a type of polyploid species that contains genomes from more than one ancestral species

ANEUPLOID: a cell or an organism with one or more missing or extra chromosomes; the opposite is “euploid,” a cell with the normal chromosome number

AUTOPOLYPLOID: a type of polyploid species that contains more than two sets of chromosomes from the same species

HOMOLOGOUS CHROMOSOMES: chromosomes that are structurally the same and have the same gene loci, although they may have different alleles (alternative forms of a gene) at many of their shared loci

The Formation of Polyploidy

Most animals are diploid, meaning that they have two homologous sets of chromosomes in their cells; and their gametes (eggs and sperm) are haploid, that is, having one set of chromosomes. Plants, a variety of single-celled eukaryotes, and some insects have individual or parts of an individual’s life cycle when they are haploid. In any case, when there are more than two sets of homologous chromosomes, the cell or organism is considered polyploid. A triploid organism has three sets of homologous chromosomes, a tetraploid has four sets, a dodecaploid has twelve sets, and there are organisms known to have many more than a dozen sets of homologous chromosomes.

How polyploids are formed in nature is still debated. Regardless of what theory is accepted, the first step certainly involves a failure during cell division, in either meiosis or mitosis. For example, if cytokinesis (division of the cytoplasm) fails at the conclusion of meiosis II, the daughter cells will be diploid. If, by chance, a diploid sperm fertilizes a diploid egg, the resulting zygote will be tetraploid. Although polyploidy might occur this way, biologists have proposed an alternative model involving a triploid intermediate stage.

The triploid intermediate model has been

applied primarily to plants, in which polyploidy is better studied. Hybrids between two species are often sterile, but occasionally a diploid gamete from one of the species joins with a normal haploid gamete from the other species, which produces a triploid hybrid. Triploids are also sterile, for the most part, but do produce a small number of gametes, many of which are diploid. This makes the probability that two diploid gametes will join, to form a tetraploid, much higher. This hypothesis is supported by the discovery of triploid hybrid plants that do produce a small number of viable gametes. This type of polyploid, formed as a result of hybridization between two species, is called an allopolyploid. Allopolyploids are typically fertile and represent a new species.

Polyploidy can also occur within a single species, without hybridization, in which case it is called an autopolyploid. Autopolyploids can form in the same way as allopolyploids, but they can also occur as the result of a failure in cell division in a bud. If a cell in the meristematic region (a rapidly dividing group of cells at the tip of a bud) completes mitosis but not cytokinesis, it will be a tetraploid cell. All daughter cells from this cell will also be tetraploid, so that any flowers borne on this branch will produce diploid gametes. If the plant is self-compatible, it can then produce tetraploid offspring from these flowers. Autopolyploids are often a little larger and more robust than the diploids that produce them, but they are often so similar they cannot be easily distinguished. An autopolyploid, when formed, represents a new species but is not generally recognized as such unless it looks different enough physically from diploids.

The Genetics of Polyploids

A polyploid has more copies of each gene than a diploid. For example, a tetraploid has four alleles at each locus, which means tetraploids can contain much more individual variability than diploids. This has led some evolutionists to suggest that polyploids should have higher fitness than the diploids from which they came. With more variation, the individual would be preadapted to a much wider range of conditions. Because there are so many extra

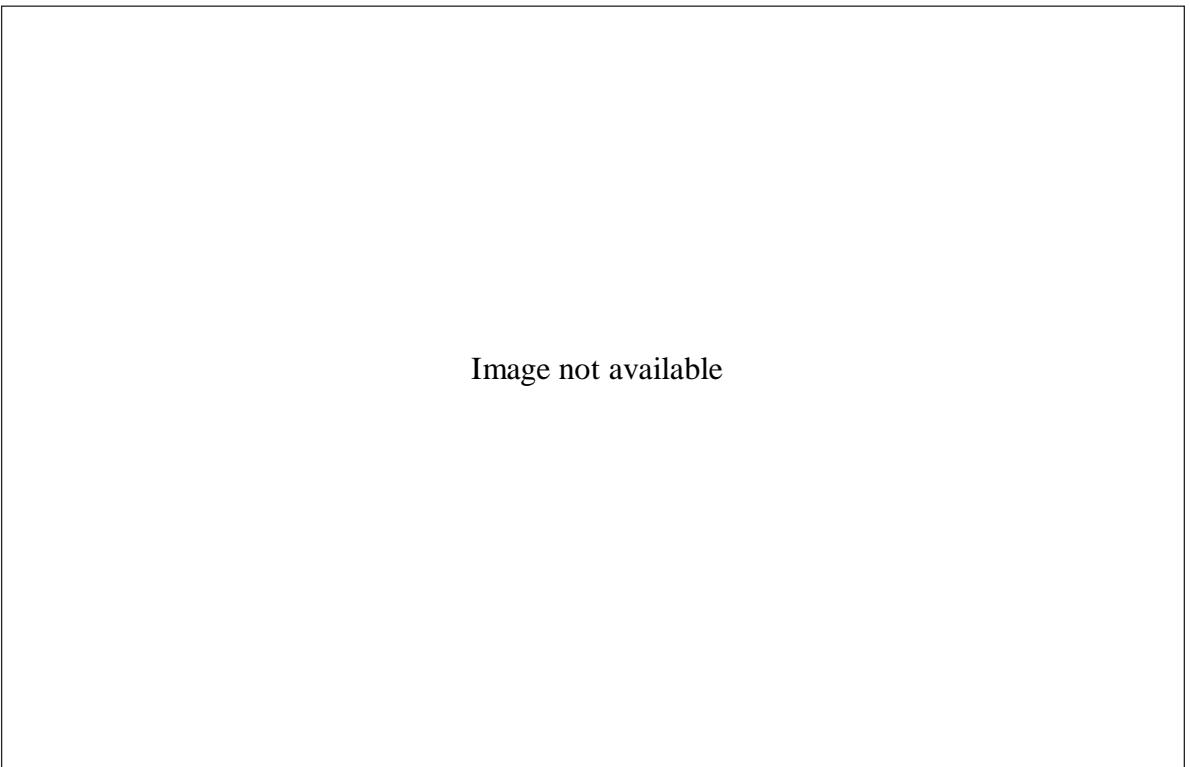


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Wheat is one of many important polyploid crops. (AP/Wide World Photos)

copies of genes, a certain amount of gene silencing (loss of genes through mutation or other processes) occurs, with no apparent detriment to the plant.

The pairing behavior of chromosomes in polyploids is also unique. In a diploid, during meiosis, homologous chromosomes associate in pairs. In an autotetraploid there are four homologous chromosomes of each type which associate together in groups of four. In an allotetraploid, the chromosomes from the two species from which they are derived are commonly not completely homologous and do not associate together. Consequently, the pairs of homologous chromosomes from one parent species associate together in pairs, as do the chromosomes from the other parent species. For this reason, sometimes allopolyploids are referred to as amphidiploids, because their pairing behavior looks the same as it does in a diploid. This is also why an allopolyploid is fertile (because meiosis occurs normally), but a hybrid between two diploids commonly is not,

because the chromosomes from the two species are unable to pair properly.

Polyplid Plants and Animals

In the plant kingdom, it is estimated by some that 95 percent of pteridophytes (plants, including ferns, that reproduce by spores) and perhaps as many as 80 percent of angiosperms (flowering plants that form seeds inside an ovary) are polyploid, although there is high variability in its occurrence among families of angiosperms. In contrast, polyploidy is uncommon in gymnosperms (plants that have naked seeds that are not within specialized structures). Extensive polyploidy is observed in chrysanthemums, in which chromosome numbers range from 18 to 198. The basic chromosome number (haploid or gamete number of chromosomes) is 9. Polyploids from triploids (with 27 chromosomes) to 22-ploids (198 chromosomes) are observed. The stonecrop *Sedum suaveolens*, which has the highest chromosome number of any angiosperm, is believed to be

about 80-ploid (720 chromosomes). Many important agricultural crops, including wheat, corn, sugarcane, potatoes, coffee, apples, and cotton, are polyploid.

Polyploid animals are less common than polyploid plants but are found among some groups, including crustaceans, earthworms, flatworms, and insects such as weevils, sawflies, and moths. Polyploidy has also been observed in some vertebrates, including tree frogs, lizards, salamanders, and fish. It has been suggested that the genetic redundancy observed in vertebrates may be caused by ancestral polyploidy.

Polyploidy in Tissues

Most plants and animals contain particular tissues that are polyploid or polytene, while the rest of the organism is diploid. Polyploidy is observed in multinucleate cells and in cells that have undergone endomitosis, in which the chromosomes condense but the cell does not undergo nuclear or cellular division. For example, in vertebrates, liver cells are binucleate and therefore tetraploid. In addition, in humans, megakaryocytes can have polyploidy levels of up to sixty-four. A megakaryocyte is a giant bone-marrow cell with a large, irregularly lobed nucleus that is the precursor to blood platelets. A megakaryocyte does not circulate, but forms platelets by budding. A single megakaryocyte can produce three thousand to four thousand platelets. A platelet is an enucleated, disk-shaped cell in the blood that has a role in blood coagulation. In polytene cells, the replicated copies of the chromosomal DNA remain associated to produce giant chromosomes that have a continuously visible banding pattern. The trophoblast cells of the mammalian placenta are polytene.

Importance of Polyploids to Humans

Most human polyploids die as embryos or fetuses. In a few rare cases, a polyploid infant is born that lives for a few days. In fact, polyploidy is not tolerated in most animal systems. Plants, on the other hand, show none of these problems with polyploidy. Some crop plants are much more productive because they are polyploid. For example, wheat (*Triticum aestivum*) is

an allohexaploid and contains chromosome sets that are derived from three different ancient types. Compared to the species from which it evolved, *T. aestivum* is far more productive and produces larger grains of wheat. *Triticum aestivum* was not developed by humans but appears to have arisen by a series of chance events in the past, humans simply recognizing the better qualities of *T. aestivum*. Another fortuitous example involves three species of mustard that have given rise to black mustard, turnips, cabbage, broccoli, and several other related crops, all of which are allotetraploids.

Polyploids may be induced by the use of drugs such as colchicine, which halts cell division. Because of the advantages of the natural polyploids used in agriculture, many geneticists have experimented with artificially producing polyploids to improve crop yields. One prime example of this approach is *Triticale*, which represents an allopolyploid produced by hybridizing wheat and rye. Producing artificial polyploids often produces a new variety that has unexpected negative characteristics, so that only a few such polyploids have been successful. Nevertheless, research on polyploidy continues.

—Susan J. Karcher, updated by Bryan Ness

See also: Cell Division; Cytokinesis; High-Yield Crops; Gene Families; Genome Size; Hereditary Diseases; Nondisjunction and Aneuploidy.

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NEUTRAL THEORY OF EVOLUTION: Motoo Kimura's theory that nucleotide substitutions in the DNA often have no effect on fitness, and thus changes in allele frequencies in populations are caused primarily by genetic drift

The Hardy-Weinberg Law

The branch of genetics called population genetics is based on the application of nineteenth century Austrian botanist Gregor Mendel's principles of inheritance to genes in a population. (Although, for some species, "population" can be difficult to define, the term generally refers to a geographic group of interbreeding individuals of the same species.) Mendel's principles can be used to predict the expected proportions of offspring in a cross between two individuals of known genotypes, where the genotype describes the genetic content of an individual for one or more genes. An individual carries two copies of all chromosomes (except perhaps for the sex chromosomes, as in human males) and therefore has two copies of each gene. These two copies may be identical or somewhat different. Different forms of the same gene are called alleles. A genotype in which both alleles are the same is called a homozygote, while one in which the two alleles are different is a heterozygote. Although a single individual can carry no more than two alleles for a particular gene, there may be many alleles of a gene present in a population.

Population Genetics

Field of study: Population genetics

Significance: *Population genetics is the study of how genes behave in populations. It is concerned with both theoretical and experimental investigations of changes in genetic variation caused by various forces; therefore, the field has close ties to evolutionary biology. Population genetics models can be used to explore the evolutionary histories of species, make predictions about future evolution, and predict the behavior of genetic diseases in human populations.*

Key terms

ALLELLE: one of the different forms of a particular gene (locus)

FITNESS: a measure of the ability of a genotype or individual to survive and reproduce compared to other genotypes or individuals

GENE POOL: all of the alleles in all the gametes of all the individuals in a population

GENETIC DRIFT: random changes in genetic variation caused by sampling error in small populations

GENOTYPE: the pair of alleles carried by an individual for a specific gene locus

HARDY-WEINBERG LAW: a mathematical model that predicts, under particular conditions, that allele frequencies will remain constant over time, with genotypes in specific predictable proportions

MODERN SYNTHESIS: the merging of the Darwinian mechanisms for evolution with Mendelian genetics to form the modern fields of population genetics and evolutionary biology

It would be essentially impossible to track the inheritance patterns of every single mating pair in a population, in essence tracking all the alleles in the gene pool. However, by making some simplifying assumptions about a population, it is possible to predict what will happen to the gene pool over time. Working independently in 1908, the British mathematician Godfrey Hardy and the German physiologist Wilhelm Weinberg were the first to formulate a simple mathematical model describing the behavior of a gene (locus) with two alleles in a population. In this model, the numbers of each allele and of each genotype are not represented as actual numbers but as proportions (known as allele frequencies and genotype frequencies,

respectively) so that the model can be applied to any population regardless of its size. By assuming Mendelian inheritance of alleles, Hardy and Weinberg showed that allele frequencies in a population do not change over time and that genotype frequencies will change to specific proportions, determined by the allele frequencies, within one generation and remain at those proportions in future generations. This result is known as the Hardy-Weinberg law, and the stable genotype proportions predicted by the law are known as Hardy-Weinberg equilibrium. It was shown in subsequent work by others that the Hardy-Weinberg law remains true in more complex models with more than two alleles and more than one locus.

In order for the Hardy-Weinberg law to work, certain assumptions about a population must be true:

- (1) the gene pool must be infinite in size;
- (2) mating among individuals (or the fusion of gametes) must be completely random;
- (3) there must be no new mutations;
- (4) there must be no gene flow (that is, no alleles should enter or leave the population; and
- (5) there should be no natural selection.

Since real populations cannot meet these conditions, it may seem that the Hardy-Weinberg model is too unrealistic to be useful, but, in fact, it can be useful. First, the conditions of a natural population may be very close to Hardy-Weinberg assumptions, so the Hardy-Weinberg law may be approximately true for at least some populations. Second, if genotypes in a population are not in Hardy-Weinberg equilibrium, it is an indication that one or more of these assumptions is not met. The Hardy-Weinberg law has been broadly expanded, using sophisticated mathematical modeling, and with adequate data can be used to determine why a population's allele and genotype frequencies are out of Hardy-Weinberg equilibrium.

Genetic Variation and Mathematical Modeling

Sampling and genetic analyses of real populations of many different types of organisms reveal that there is usually a substantial amount

of genetic variation, meaning that for a fairly large proportion of genes (loci) that are analyzed, there are multiple alleles, and therefore multiple genotypes, within populations. For example, in the common fruit fly *Drosophila melanogaster* (an organism that has been well studied genetically since the very early 1900's), between one-third and two-thirds of the genes that have been examined by protein electrophoresis have been found to be variable. Genetic variation can be measured as allele frequencies (allelic variation) or genotype frequencies (genotypic variation). A major task of population geneticists has been to describe such variation, to try to explain why it exists, and to predict its behavior over time.

The Hardy-Weinberg law predicts that if genetic variation exists in a population, it will remain constant over time, with genotypes in specific proportions. However, the law cannot begin to explain natural variation, since genotypes are not always found in Hardy-Weinberg proportions, and studies that involve sampling populations over time often show that genetic variation can be changing. The historical approach to explaining these observations has been to formulate more complex mathematical models based on the simple Hardy-Weinberg model that violate one or more of the implicit Hardy-Weinberg conditions.

Beginning in the 1920's and 1930's, a group of population geneticists, working independently, began exploring the effects of violating Hardy-Weinberg assumptions on genetic variation in populations. In what has become known as the "modern synthesis," Ronald A. Fisher, J. B. S. Haldane, and Sewall Wright merged Darwin's theory of natural selection with Mendel's theory of genetic inheritance to create a field of population genetics that allows for genetic change. They applied mathematics to the problem of variation in populations and were eventually able to incorporate what happens when each, or combinations, of the Hardy-Weinberg assumptions are violated.

Assortative Mating and Inbreeding

One of the implicit conditions of the Hardy-Weinberg model is that genotypes form mating pairs at random. In most cases mates are not se-

lected based on genotype. Unless the gene in question has some direct effect on mate choice, mating with respect to that gene is random. However, there are conditions in natural populations in which mating is not random. For example, if a gene controls fur color and mates are chosen by appropriate fur color, then the genotype of an individual with respect to that gene will determine mating success. For this gene, then, mating is not random but rather “assortative.” Positive assortative mating means that individuals tend to choose mates with genotypes like their own, while negative assortative mating means that individuals tend to choose genotypes different than their own.

Variation in a population for a gene subject to assortative mating is altered from Hardy-Weinberg expectations. Although allele frequencies do not change, genotype frequencies are altered. With positive assortative mating, the result is higher proportions of homozygotes and fewer heterozygotes, while the opposite is true when assortative mating is negative. Sometimes random mating in a population is not possible because of the geographic organization of the population or general mating habits. Truly random mating would mean that any individual can mate with any other, but this is nearly impossible because of gender differences and practical limitations. In natural populations, it is often the case that mates are somewhat related, even closely related, because the population is organized into extended family groups whose members do not (or cannot, as in plants) disperse to mate with members of other groups. Mating between relatives is called inbreeding. Because related individuals tend to have similar genotypes for many genes, the effects of inbreeding are much like those of positive assortative mating for many genes. The proportions of homozygotes for many genes tend to increase. Again, this situation has no effect on allelic variation, only genotypic variation. Clearly, the presence of nonrandom mating patterns cannot by themselves explain the majority of patterns of genetic variation in natural populations but can contribute to the action of other forces, such as natural selection.

Migration and Mutation

In the theoretical Hardy-Weinberg population, there are no sources of new genetic variation. In real populations, alleles may enter or leave the population, a process called migration or “gene flow” (a more accurate term, since migration in this context means not only movement between populations but also successful reproduction to introduce alleles in the new population). Also, new alleles may be introduced by mutation, the change in the DNA sequence of an existing allele to create a new one, as a result of errors during DNA replication or the inexact repair of DNA damage from environmental influences such as radiation or mutagenic chemicals. Both of these processes can change both genotype frequencies and allele frequencies in a population. If the tendency to migrate is associated with particular genotypes, a long period of continued migration tends to push genotype and allele frequencies toward higher proportions of one type (in general, more homozygotes) so that the overall effect is to reduce genetic variation. However, in the short term, migration may enhance genetic variation by allowing new alleles and genotypes to enter. The importance of migration depends on the particular population. Some populations may be relatively isolated from others so that migration is a relatively weak force affecting genetic variation, or there may be frequent migration among geographic populations. There are many factors involved, not the least of which is the ability of members of the particular species to move over some distance.

Mutation, because it introduces new alleles into a population, acts to increase genetic variation. Before the modern synthesis, one school of thought was that mutation might be the driving force of evolution, since genetic change over time coming about from continual introduction of new forms of genes seemed possible. In fact, it is possible to develop simple mathematical models of mutation that show resulting patterns of genetic variation that resemble those found in nature. However, to account for the rates of evolution that are commonly observed, very high rates of mutation are required. In general, mutation tends to be quite

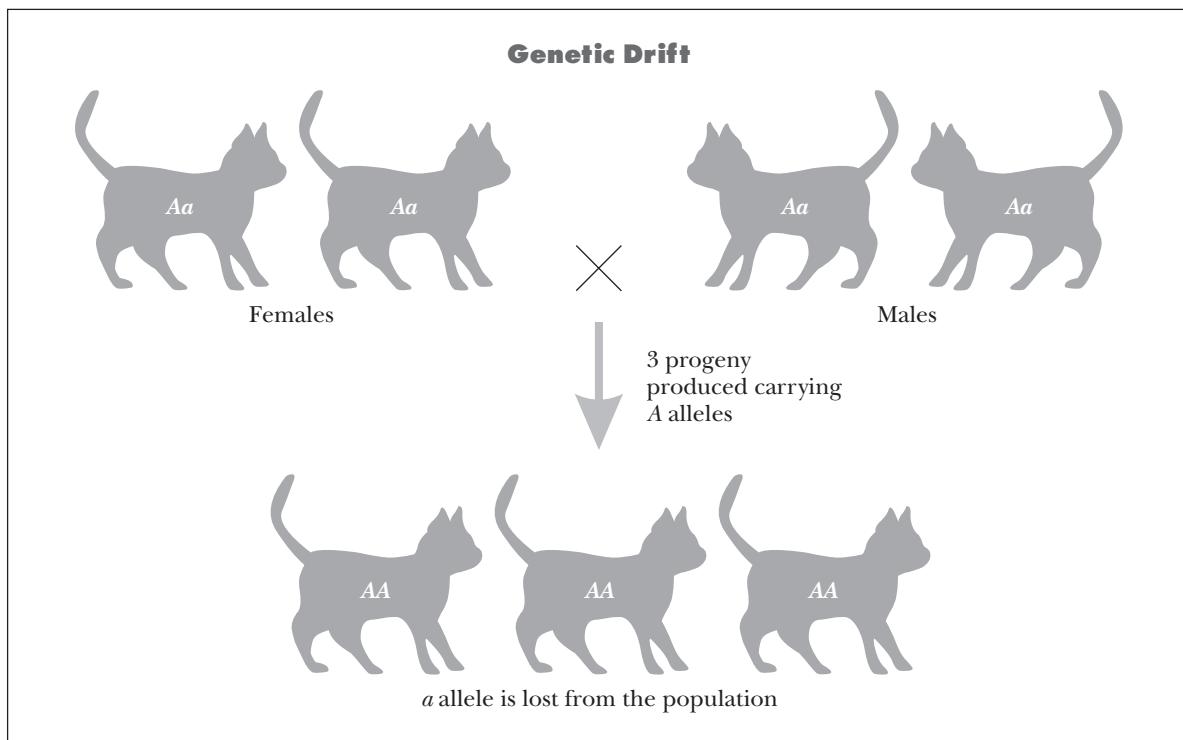
rare, making the hypothesis of evolution by mutation alone unsatisfactory.

The action of mutation in conjunction with other forces, such as selection, may account for the low-frequency persistence of clearly harmful alleles in populations. For example, one might expect that alleles that can result in genetic diseases (such as cystic fibrosis) would be quickly eliminated from human populations by natural selection. However, low rates of mutation can continually introduce these alleles into populations. In this “mutation-selection balance,” mutation tends to introduce alleles while selection tends to eliminate them, with a net result of continuing low frequencies in the population.

Genetic Drift

Real populations are not, of course, infinite in size, though some are large enough that this Hardy-Weinberg condition is a useful approximation. However, many natural populations are small, and any population with less than about one thousand individuals will vary ran-

domly in the pattern of genetic variation from generation to generation. These random changes in allele and genotype frequencies are called genetic drift. The situation is analogous to coin tossing. With a fair coin, the expectation is that half of the tosses will result in heads and half in tails. On average, this will be true, but in practice a small sample will not show the expectation. For example, if a coin is tossed ten times, it is unlikely that the result will be exactly five heads and five tails. On the other hand, with a thousand tosses, the results will be closer to half and half. This higher deviation from the expected result in small samples is called a sampling error. In a small population, there is an expectation of the pattern of genetic variation based on the Hardy-Weinberg law, but sampling error during the union of sex cells to form offspring genotypes will result in random deviations from that expectation. The effect is that allele frequencies increase or decrease randomly, with corresponding changes in genotype frequencies. The smaller the population, the greater the sam-



In this small population, the a allele has disappeared as a result of random chance and is lost to future generations.

pling error and the more pronounced genetic drift will be.

Genetic drift has an effect on genetic variation that is similar to that of other factors. Over the long term, allele frequencies will drift until all alleles have been eliminated but one, eliminating variation. (For the moment, ignore the action of other forces that increase variation.) Over a period of dozens of generations, however, drift can allow variation to be maintained, especially in larger populations in which drift is minimal.

In the early days of population genetics, the possibility of genetic drift was recognized but often considered to be a minor consideration, with natural selection as a dominant force. Fisher in particular dismissed the importance of genetic drift, engaging over a number of years in a published debate with Wright, who always felt that drift would be important in small populations. Beginning in the 1960's with the acquisition of data on DNA-level population variation, the role of drift in natural populations became more recognized. It appears to be an especially strong force in cases in which a small number of individuals leave the population and migrate to a new area where they establish a new population. Large changes can occur, especially if the number of migrants is only ten or twenty. This type of situation is now referred to as a founder effect.

Natural Selection

Natural selection in a simple model of a gene with two alleles in a population can be easily represented by assuming that genotypes differ in their ability to survive and produce offspring. This ability is called fitness. In applying natural selection to a theoretical population, each genotype is assigned a fitness value between zero and one. Typically, the genotype in a population that is best able to survive and can, on average, produce more offspring than other genotypes is assigned a fitness value of one, and genotypes with lower fitness are assigned fitnesses with fractional values relative to the high-fitness genotype.

The study of this simple model of natural selection has revealed that it can alter genetic variation in different ways, depending on which

genotype has the highest fitness. In the simple one-gene, two-allele model, there are three possible genotypes: two homozygotes and one heterozygote. If one homozygote has the highest fitness, it will be favored, and the genetic composition of the population will gradually shift toward more of that genotype (and its corresponding allele). This is called directional selection. If both homozygotes have higher fitness than the heterozygote (disruptive selection), one or the other will be favored, depending on the starting conditions. Both of these situations will decrease genetic variation in the population, because eventually one allele will prevail. Although each of these types of selection (particularly directional) may be found for genes in natural populations, they cannot explain why genetic variation is present, and is perhaps increasing, in nature.

Heterozygote advantage, in which the heterozygote has higher fitness than either homozygote, is the other possible situation in this model. In this case, because the heterozygote carries both alleles, both are expected to be favored together and therefore maintained. This is the only condition in this simple model in which genetic variation may be maintained or increased over time. Although this seems like a plausible explanation for the observed levels of natural variation, studies in which fitness values are measured almost never show heterozygote advantage in genes from natural populations. As a general explanation for the presence of genetic variation, this simple model of selection is unsatisfactory.

Studies of more complex theoretical models of selection (for example, those with many genes and different forms of selection) have revealed conditions that allow patterns of variation very similar to those observed in natural populations, and in some cases it seems clear that natural selection is a major factor determining patterns of genetic change. However, in many cases, selection does not seem to be the most important factor or even a factor at all.

Experimental Population Genetics and the Neutral Theory

Population genetics has always been a field in which the understanding of theory is ahead

of empirical observation and experimental testing, but these have not been neglected. Although Fisher, Haldane, and Wright were mainly theorists, there were other architects of the modern synthesis who concentrated on testing theoretical predictions in natural populations. Beginning in the 1940's, for example, Theodosius Dobzhansky showed in natural and experimental populations of *Drosophila* species that frequency changes and geographic patterns of variation in chromosome variants are consistent with the effects of natural selection.

Natural selection was the dominant hypothesis for genetic changes in natural populations for the first several decades of the modern synthesis. In the 1960's, new techniques of molecular biology allowed population geneticists to examine molecular variation, first in proteins and later, with the use of restriction enzymes in the 1970's and DNA sequencing in the 1980's and 1990's, in DNA sequences. These types of studies only confirmed that there is a large amount of genetic variation in natural populations, much more than can be attributed only to natural selection. As a result, Motoo Kimura proposed the "neutral theory of evolution," the idea that most DNA sequence differences do not have fitness differences and that population changes in DNA sequences are governed mainly by genetic drift, with selection playing a minor role. This view, although still debated by some, was mostly accepted by the 1990's, although it was recognized that evolution of proteins and physical traits may be governed by selection to a greater extent.

Impact and Applications

The field of population genetics is a fundamental part of the modern field of evolutionary biology. One possible definition of evolution would be "genetic change in a population over time," and population geneticists try to describe patterns of genetic variation, document changes in variation, determine their theoretical causes, and predict future patterns. These types of research have been valuable in studying the evolutionary histories of organisms for which there are living representatives, including humans.

In addition to the scientific value of understanding evolutionary history better, there are more immediate applications of such work. In conservation biology, data about genetic variation in a population can help to assess its ability to survive in the future. Data on genetic similarities between populations can aid in decisions about whether they can be considered as the same species or are unique enough to merit preservation.

Population genetics has had an influence on medicine, particularly in understanding why "disease genes," while clearly harmful, persist in human populations. The field has also affected the planning of vaccination protocols to maximize their effectiveness against parasites, since a vaccine-resistant strain is a result of a rare allele in the parasite population. In the 1990's it began to be recognized that effective treatments for medical conditions would need to take into account genetic variation in human populations, since different individuals might respond differently to the same treatment.

—Stephen T. Kilpatrick, updated by Bryan Ness

See also: Artificial Selection; Behavior; Consanguinity and Genetic Disease; Emerging Diseases; Evolutionary Biology; Genetic Load; Genetics, Historical Development of; Hardy-Weinberg Law; Heredity and Environment; Hybridization and Introgression; Inbreeding and Assortative Mating; Lateral Gene Transfer; Natural Selection; Polyploidy; Punctuated Equilibrium; Quantitative Inheritance; Sociobiology; Speciation.

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Prader-Willi and Angelman Syndromes

Field of study: Diseases and syndromes

Significance: Both Prader-Willi and Angelman syndromes are caused by errors at the same site in the long arm of chromosome 15 (15q11-q13), but the clinical outcomes of these errors are markedly different, because the chromosomes containing the errors come from different parents. Thus, these syndromes offer a striking example of the concept known as parental imprinting.

Key terms

DELETION: loss of a portion of a chromosome, which may be very small or very large

DISOMY: a case in which both copies of a chromosome come from a single parent, rather than (as is usual) one being maternal and one being paternal

FLUORESCENT IN SITU HYBRIDIZATION (FISH): an extremely sensitive assay for determining the presence of deletions on chromosomes, which uses a fluorescence-tagged segment of DNA that binds to the DNA region being studied

IMPRINTING: marking a chromosome so that there are differences between maternal and paternal inheritance

TRANSLOCATION: the removal of a portion of a chromosome which is then attached to the end of another chromosome; may involve loss of control for several genes

Symptoms

Angelman syndrome (AS) was first described in 1965 by Dr. Harry Angelman, who described three children with a stiff, jerky gait, absent speech, excessive laughter, and seizures. Newer reports include severe mental retardation and a characteristic face that is small with a large mouth and prominent chin. These characteristics give rise to the alternate name for the syndrome, that being "happy puppet syndrome." The syndrome is fairly rare, with an incidence estimated to be between one in fifteen thousand to one in thirty thousand. It is usually not recognized at birth or in infancy, since the

developmental problems are nonspecific during this period.

Prader-Willi syndrome (PWS), by comparison, is characterized by mental retardation, hypotonia (decreased muscle tone), skin picking, short stature, cryptorchidism (small or undescended testes), and hyperphagia (overeating leading to severe obesity). Delayed motor and language development are common, as is intellectual impairment (the average IQ is about 70). The syndrome was first described by Doctors Andrea Prader, Alexis Labhart, and Heinrich Willi in 1956. Like Angelman syndrome, PWS has a fairly low incidence, estimated at one in fifteen thousand. Neither condition is race-specific, and neither is considered to be a familial disease.

The primary cause of both syndromes appears to be a small deletion on the long arm of chromosome 15 (del 15q11-q13). The deleted area is estimated to be about 4 million base pairs (bp), small by molecular standards but large enough to contain several genes. This area of chromosome 15 is known to contain several genes that are activated or inactivated depending on the chromosome's parent of origin (that is, a gene may be turned on in the chromosome inherited from the mother but turned off in the chromosome inherited from the father). This parent-specific activation is referred to as genetic imprinting. It is now known that the deletions causing AS appear in the chromosome inherited from the mother, while those causing PWS occur in the chromosome inherited from the father. Since the genes of only one chromosome are active at a time, any disruption (deletion) in the active chromosome will lead to the effects seen in one of these syndromes.

Genetic Basis of AS

In 1997 a gene within the AS deletion region called *UBE3A* was found to be mutated in approximately 5 percent of AS individuals. These mutations can be as small as a single base pair. This gene codes for a protein/enzyme called a ubiquitin protein ligase, and *UBE3A* is believed to be the causative gene in AS. All mechanisms known to cause AS appear to cause inactivation or absence of this gene. *UBE3A* is an enzymatic

component of a complex protein degradation system termed the ubiquitin-proteasome pathway. This pathway is located in the cytoplasm of all cells. The pathway involves a small protein molecule (ubiquitin) that can be attached to proteins, thereby causing them to be degraded. In the normal brain, *UBE3A* inherited from the father is almost completely inactive, so the maternal copy performs most of the ubiquitin-producing function. Inheritance of a *UBE3A* mutation from the mother causes AS; inheritance of the mutation from the father has no apparent effect on the child. In some families, AS caused by a *UBE3A* mutation can occur in more than one family member.

Another cause of AS (3 percent of cases) is paternal uniparental disomy (UPD). In this case a child inherits both copies of chromosome 15 from the father, with no copy inherited from the mother. Even though there is no deletion or mutation, the child is still missing the active *UBE3A* gene because the paternally derived chromosomes only have brain-inactivated *UBE3A* genes.

A fourth class of AS individuals (3-5 percent) have chromosome 15 copies inherited from both parents, but the copy inherited from the mother functions in the same way as a paternally inherited one would. This is referred to as an "imprinting defect." Some individuals may have a very small deletion of a region known as the imprinting center (IC), which regulates the activity of *UBE3A* from a distant location. The mechanism for this is not yet known.

While there are several genetic mechanisms for AS, all of them lead to the typical clinical features found in AS individuals, although minor differences in incidence of features may occur between each group.

Genetic Basis of PWS

The primary genes involved in PWS are *SNRPN*, a gene that encodes the small ribonucleotide polypeptide SmN that is found in the fetal and adult brain, and *ZFN127*, a gene that encodes a zinc-finger protein of unknown function. *SNRPN* is involved in messenger RNA (mRNA) processing, an intermediate step between DNA transcription and protein formation. A mouse model of PWS has been devel-

oped with a large deletion that includes the *SNRPN* region and the PWS imprinting center and shows a phenotype similar to that of infants with PWS.

It is probable that the hypothalamic problems (such as overeating) associated with PWS might result from a loss of *SNRPN*. The production of this protein is found mainly in the hypothalamic regions of the brain and in the olfactory cortex. Thus, disruption of hypothalamic functions such as satiety are a likely result of this defect. Prader-Willi syndrome is the most common genetic cause of obesity. In addition to its role in satiety, the hypothalamus regulates growth, sexual development, metabolism, body temperature, pigmentation, and mood—all functions that are affected in those with PWS.

PWS may also be caused by uniparental disomy, as seen in AS. However, in PWS both copies of chromosome 15 are derived from the mother instead of from the father.

As mentioned above, the imprinting center may be involved in at least some cases of both syndromes. This chromosome 15 IC is about 100 kilobase pairs (kb) long and includes exon 1 of the *SNRPN* gene. Mutations in this area appear to prevent the paternal-to-maternal imprinting switch in the AS families and prevents the maternal-to-paternal switch in PWS families. Therefore, it is possible that the IC is needed to regulate alternate RNA splicing in the *SNRPN* gene transcripts.

Genetic Diagnosis

The usual chromosome studies carried out during prenatal diagnosis are interpreted as normal in fetuses with AS and PWS syndromes, since the small abnormalities on chromosome 15 are not detected by this type of study. Likewise, fetal ultrasound offers no help in detecting physical abnormalities related to AS or PW, since the affected fetus is well formed. Amniotic fluid volume and alpha-feto protein levels also appear normal.

Specialized chromosome 15 FISH studies are needed to determine the presence of either syndrome resulting from chromosomal deletions. Testing for parent-specific methylation imprints at the 15q11-q13 locus detects more

than 95 percent of cases. For cases caused by uniparental disomy, polymerase chain reaction (PCR) testing can be used.

Relevance to Geneticists

Few examples of known parental imprinting occur in the human, so AS and PWS provide rare opportunities for geneticists and biologists to study this important phenomenon. Examples of nonhuman parental imprinting are well known, but the genetic and biochemical mechanisms have not been established. Detailing the IC for chromosome 15 will be key to understanding how imprinting occurs and how the effects of AS and PWS are manifested.

The suggestion has been made that PWS (and therefore disruption of the IC) may also, at least in some cases, have an environmental trigger. A high association of PWS with fathers employed in hydrocarbon-related occupations (such as factory workers, lumbermen, machinists, chemists, and mechanics) at the time of conception has been reported by one investigative team. This is an area that needs further exploration.

—Kerry L. Cheesman

See also: Amniocentesis and Chorionic Villus Sampling; Chromosome Structure; Congenital Defects; Down Syndrome; Fragile X Syndrome; Hereditary Diseases; Human Growth Hormone; Huntington's Disease; Intelligence; Polymerase Chain Reaction; Prenatal Diagnosis.

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Web Sites of Interest

National Organization for Rare Disorders. <http://www.rarediseases.org>. Searchable site by type of disorder. Includes background information on Prader-Willi and Angelman syndrome, a list of other names for the disorder, and a list of related organizations.

Prader-Willi Syndrome Association. <http://www.pwsusa.org>. This site offers background information on the syndrome, a research/medical section, links, and more.

Prenatal Diagnosis

Field of study: Human genetics and social issues

Significance: Tests ranging from ultrasound and maternal blood tests to testing fetal cells from the amniotic fluid or placenta are performed to detect genetic disorders that the fetus may have. Although tests may show the absence of specific genetic defects, the detection of a genetic defect can produce an ethical dilemma for the parents and their physician.

Key terms

AMNIOTIC FLUID: the liquid that surrounds the developing fetus

NEURAL TUBE: the embryonic structure that becomes the brain and spinal cord

PLACENTA: an organ composed of both fetal and maternal tissue through which the fetus is nourished

TRISOMY: the presence of three copies (instead of two) of a particular chromosome in a cell

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An eight-cell human embryo. (AP/Wide World Photos)

The Eight-Cell Stage

Preimplantation genetic diagnosis (PGD) has been used since 1988 to screen for genetic disorders. The most common type of PGD involves embryo biopsy at the 6-8 cell stage after fertilization has occurred in vitro. This early form of prenatal diagnosis is typically performed on day 3 embryos. One to two blastomeres (cells) are removed from the embryo (either by aspiration or by extrusion) using a fine glass needle. The biopsied embryo is then returned to culture, where the lost cells are replenished. Genetic testing is then carried out on the biopsied cells using either a technique known as fluorescent in situ hybridization (FISH) or a second technique known as fluorescent polymerase chain reaction (PCR). The FISH technique can be used to determine the presence of chromosomes 13, 16, 18, 21, 22, X, and Y. Aneuploidies (abnormal numbers of chromosomes) involving these chromosomes account for the majority of first-trimester miscarriages and for 95 percent of all postnatal chromosomal abnormalities. PCR involves amplification of DNA and allows diagnosis of single-gene diseases.

By enabling very early diagnosis of these abnormalities, PGD allows physicians to determine which embryos are most likely to be chromosomally normal prior to placement in the uterus. This increases the probability of a successful pregnancy and a healthy baby. Genetic testing generally takes only six to eight hours to complete, so that intrauterine transfer of the chromosomally normal embryo can take place within one day. If more normal embryos are obtained than one wishes to implant, the extra embryos may be preserved for future use by cryopreservation. The survival rate of frozen embryos is thought to be about 50 percent.

While this early form of prenatal diagnosis allows the elimination of many embryos carrying major genetic defects prior to implantation, it is still recommended that followup prenatal diagnosis (using chorionic villus sampling or amniocentesis) be done on resulting pregnancies. It must be realized that in order to employ preimplantation diagnostic testing, a couple must undergo in vitro fertilization even if they are fertile. This is an expensive, time-consuming process, and generally results in only a 20 percent pregnancy rate per cycle. For couples with fertility problems, this is an easy path to choose as they try to ensure implantation of normal, healthy embryos leading to healthy babies.

This technique has been used increasingly by couples who have a history of genetic disorders. In the past, couples who had a history of genetic abnormalities could decide (1) not to have children, (2) become pregnant and knowingly risk and accept abnormalities, or (3) become pregnant and rely on chorionic villus sampling or amniocentesis to diagnose genetic problems and terminate (abort) problem pregnancies.

To date, PGD has been used to detect cases of cystic fibrosis, Tay-Sachs disease, beta-thalassemia, Huntington's disease, myotonic dystrophy, X-linked disorders, and aneuploidies such as trisomy 13, 18, or 21, Turner syndrome, or Klinefelter syndrome. The number of detectable genetic defects has greatly increased since 1988. In that time, hundreds of healthy children have been born to parents undergoing preimplantation diagnosis.

—Robin Kamienny Montvilo

Prenatal Testing

Prenatal testing is administered to a large number of women, and the tests are becoming more informative. Some of the tests are only mildly invasive to the mother, but others involve obtaining fetal cells. Some are becoming routine for all pregnant women; others are offered only when an expectant mother meets a certain set of criteria. Some physicians will not offer the testing (especially the more invasive procedures) unless the parents have agreed that they will abort the fetus if the testing re-

veals a major developmental problem, such as Down syndrome or Tay-Sachs disease. Others will order testing without any such guarantees, believing that test results will give the parents time to prepare themselves for a special-needs baby. The test results are also used to determine if additional medical teams should be present at the delivery to deal with a newborn who is not normal and healthy. Most often, prenatal testing is offered if the mother is age thirty-five or older, if a particular disorder is present in relatives on one or both sides of the

family, or if the parents have already produced one child with a genetic disorder.

Maternal Blood Tests and Ultrasound

Screening maternal blood for the presence of alpha fetoprotein (AFP) is offered to pregnant women who are about eighteen weeks into a pregnancy. Although AFP is produced by the fetal liver, some will cross the placenta into the mother's blood. Elevated levels of AFP can indicate an open neural tube defect (such as spina bifida), although it can also indicate twins. Unusual AFP findings are usually followed up by ultrasound examination of the fetus.

Other tests of maternal blood measure the amounts of two substances that are produced by the fetal part of the placenta: hCG and UE3. Lower-than-average levels of AFP and UE3, combined with a higher-than-average amount of hCG, increases the risk that the woman is carrying a Down syndrome (trisomy 21) fetus. For example, a nineteen-year-old woman has a baseline risk of conceiving a fetus with Down syndrome of 1 in 1,193. When blood-test results show low AFP and UE3 along with high hCG, the probability of Down syndrome rises to 1 in 145.

During an ultrasound examination, harmless sound waves are bounced off the fetus from an emitter placed on the surface of the mother's abdomen or in her vagina. They are used to make a picture of the fetus on a television monitor. Measurements on the monitor can often be used to determine the overall size, the head size, and the sex of the fetus, and whether all the arms and legs are formed and of the proper length. Successive ultrasound tests will indicate if the fetus is growing normally. Certain ultrasound findings, such as shortened long bones, may indicate an increased probability for a Down syndrome baby. Because Down syndrome is a highly variable condition, normal ultrasound findings do not guarantee that the child will be born without Down syndrome. Only a chromosome analysis can determine this for certain.

Amniocentesis, Karyotyping, and FISH

Amniocentesis is the process of collecting fetal cells from the amniotic fluid. Fetal cells col-

lected by amniocentesis can be grown in culture; then the fluid around the cells is collected and analyzed for enzymes produced by the cells. If an enzyme is missing (as in the case of Tay-Sachs disease), the fetus may be diagnosed with the disorder before it is born. Because disorders such as Tay-Sachs disease are untreatable and fatal, a woman who has had one Tay-Sachs child may not wish to give birth to another. Early diagnosis of a second Tay-Sachs fetus would permit her to have a therapeutic abortion.

Chromosomes in the cells obtained by amniocentesis may be stained to produce a karyotype. In a normal karyotype, the chromosomes will be present in pairs. If the fetus has Down syndrome (trisomy 21), there will be three copies of chromosome 21. Other types of chromosome abnormalities that also appear in karyotypes are changes within a single chromosome. If a chromosome has lost a piece, it is said to contain a deletion. Large deletions will be obvious when a karyotype is analyzed because the chromosome will appear smaller than normal. Sometimes the deletion is so small that it is not visible on a karyotype.

If chromosome analysis is needed early in pregnancy before the volume of amniotic fluid is large enough to permit amniocentesis, the mother and doctor may opt for chorionic villus sampling (CVS). The embryo produces finger-like projections (villi) into the uterine lining. Because these projections are produced by the embryo, their cells will have the same chromosome number as the rest of the embryonic cells. After growing in culture, the cells may be karyotyped in the same way as those obtained by amniocentesis. Both amniocentesis and CVS carry risks of infection and miscarriage. Normally these procedures are not offered unless the risk of having an affected child is found to be greater than the risk of complications from the procedures.

If the doctor is convinced that the fetus has a tiny chromosomal defect that is not visible on a karyotype, it will then be necessary to probe (or "FISH") the fetal chromosomes; the initials "FISH" stand for "fluorescent in situ hybridization." A chromosome probe is a piece of DNA that is complementary to DNA within a gene. Complementary pieces of DNA will stick to-

gether (hybridize) when they come in contact. The probe also has an attached molecule that will glow when viewed under fluorescent light. A probe for a particular gene will stick to the part of the chromosome where the gene is located and make a glowing spot. If the gene is not present because it has been lost, no spot will appear. Probes have been developed for many individual genes that cause developmental abnormalities when they are deleted from the chromosomes.

Cells obtained by amniocentesis can be probed in less time than it takes to grow and prepare them for karyotyping. Probes have been developed for the centromeres of the chromosomes that are frequently present in extra copies, such as 13, 18, 21, X, and Y. Y chromosomes that have been probed appear as red spots, X chromosomes as green spots, and number 18 chromosomes as aqua spots. A second set of probes attached to other cells from the same fetus will cause number 13 chromo-

somes to appear as green spots and number 21 chromosomes to appear as red spots. Cells from a girl with trisomy 21 would have two green spots and two aqua spots, but no red spot when the first set of probes is used. Some other cells from the same girl will show two red spots, but three green ones, when the second set of probes is used.

More recently, tests for many more genetic defects using advanced molecular genetics tests have been developed. DNA can be isolated from fetal cells, obtained by one of the methods already described, which is then probed for single gene defects. Hundreds of potential genetic defects can be detected in this way, although only a few such tests are generally available. Another barrier to their use is their high cost. Costs will likely drop in the future as the tests are perfected and are used more widely. These same tests may be performed on the parents to determine whether they are carriers of certain genetic diseases.

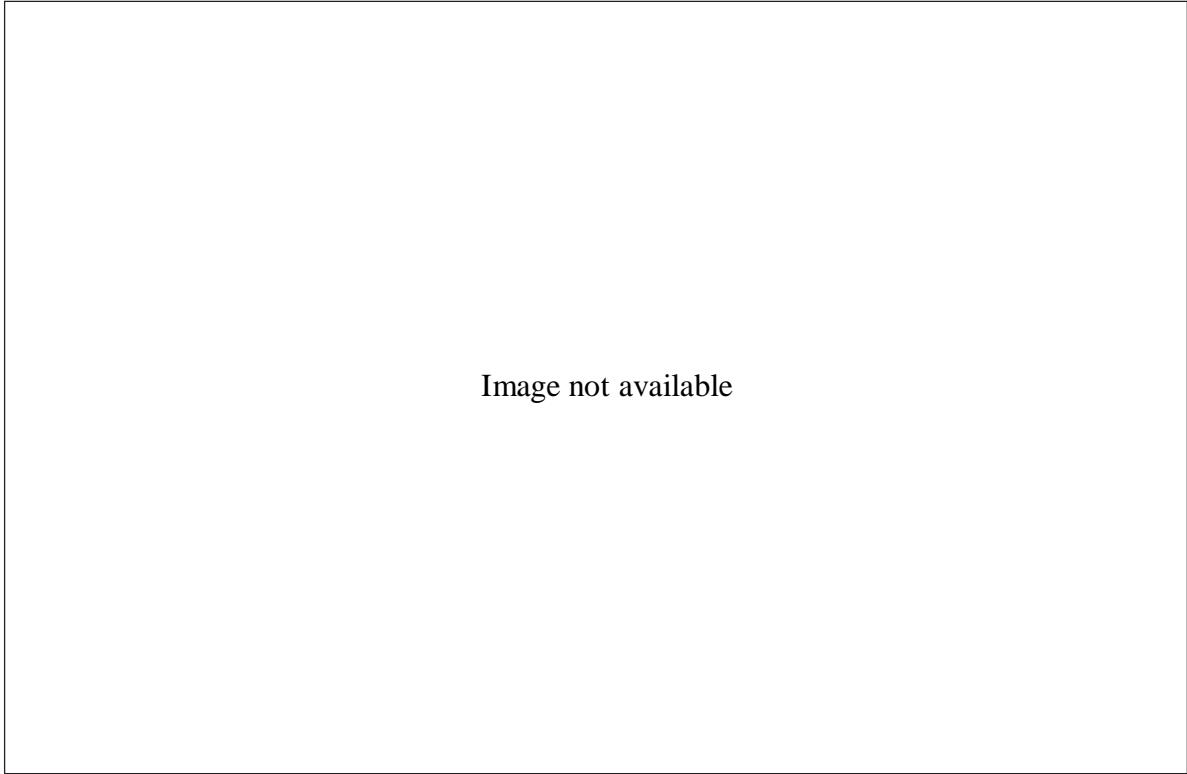


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Yury Verlinsky (right), known for his cutting-edge work in prenatal testing, and Ridvan Seckin Ozen examine human chromosomes at the Reproductive Genetics Institute in Chicago. (AP/Wide World Photos)

Impact and Applications

Until the development of prenatal techniques, pregnant women had to wait until delivery day to find out the sex of their child and whether or not the baby was normal. Now much more information is available to both the woman and her doctor weeks before the baby is due. Even though tests are not available for all possible birth defects, normal blood tests, karyotypes, or FISH can be very comforting. On the other hand, abnormal test results give the parents definite information about birth defects, as opposed to the possibilities inherent in a statement of risk. The parents must decide whether to continue the pregnancy. If they do, they must then cope with the fact that they are not going to have a normal child. When properly administered, the test results are explained by a genetic counselor who is also equipped to help the parents deal with the strong emotions that bad news can produce. Genetic testing also has far-reaching implications. If insurance companies pay for the prenatal testing, they receive copies of the results. Information about genetic abnormalities could cause the insurance companies to deny claims arising from treatment of the newborn or to deny insurance to the individual later in life.

—Nancy N. Shontz, updated by Bryan Ness

See also: Albinism; Amniocentesis and Chorionic Villus Sampling; Burkitt's Lymphoma; Color Blindness; Congenital Defects; Consanguinity and Genetic Disease; Cystic Fibrosis; Down Syndrome; Dwarfism; Fragile X Syndrome; Gender Identity; Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Heart Disease; Hemophilia; Hereditary Diseases; Hermaphrodites; Human Genetics; Human Genome Project; Huntington's Disease; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Klinefelter Syndrome; Metafemales; Monohybrid Inheritance; Neural Tube Defects; Pedigree Analysis; Phenylketonuria (PKU); Polymerase Chain Reaction; Prader-Willi and Angelman Syndromes; Pseudohermaphrodites; RFLP Analysis; Sickle-Cell Disease; Tay-Sachs Disease; Testicular Feminization Syndrome; Thalidomide and Other Teratogens; Turner Syndrome; XYY Syndrome.

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Web Sites of Interest

Association of Women's Health, Obstetric, and Neonatal Nurses. <http://www.awhonn.org>. Offers pages for education and practice resources as well as legal policy.

March of Dimes. <http://www.marchofdimes.com>. This site is searchable by keyword and includes information on the basics of amniocentesis and chorionic villus sampling and articles on how the two procedures relate to genetics.

National Newborn Screening and Genetics Resource Center. <http://genes-r-us.uthscsa.edu>. Site serves as a resource for information on genetic screening.

Key terms

DEMENTIA: mental deterioration ranging from forgetfulness and disorientation to complete unresponsiveness

PRION: short for "proteinaceous infectious particle," an element consisting mainly of protein and generally lacking nucleic acid (DNA and RNA), which is often the causative agent behind various spongiform encephalopathies

Causes, Symptoms, and Treatment

Kuru and Creutzfeldt-Jakob syndrome, degenerative diseases of the human central nervous system, are among a group of diseases that also affect cattle (mad cow disease) and sheep (scrapie). They have been classified in several ways, including "slow-virus" infections (because of the extremely long incubation period between contact and illness) and "spongiform encephalopathies" (because of the large holes seen in the brain after death). However, a virus that may cause such a disease has never been found, and the body does not respond to the disease as an infection. The only clue to the cause is the accumulation of a transmissible, toxic protein known as a prion; therefore, these disorders are now known simply as "prion diseases."

Creutzfeldt-Jakob syndrome is rare: Approximately 250 people die from it yearly in the United States. It usually begins in middle age with symptoms that include rapidly progressing dementia, jerking spastic movements, and visual problems. Within one year after the symptoms begin, the patient is comatose and paralyzed, and powerful seizures affect the entire body. Death occurs shortly thereafter. The initial symptom of the illness (rapid mental deterioration) is similar to other disorders; therefore, diagnosis is difficult. No typical infectious agent (bacteria or viruses) can be found in the blood or in the fluid that surrounds the brain and spinal cord. X rays and other scans are normal. There is no inflammation, fever, or antibody production. Brain wave studies are, however, abnormal, and at autopsy, the brain is found to have large holes and massive protein deposits in it.

Kuru is found among the Fore tribe of Papua New Guinea. Until the early 1960's, more than

Prion Diseases: Kuru and Creutzfeldt-Jakob Syndrome

Field of study: Diseases and syndromes

Significance: *Kuru and Creutzfeldt-Jakob syndrome are rare, fatal diseases of the brain and spinal cord. Nerve cell death is caused by the accumulation of a protein called a "prion" that appears to be a new infectious agent that interferes with gene expression in nerve cells. Understanding these diseases has far-reaching implications for the study of other degenerative mental disorders.*

The Discovery of Prions

In 1972, Stanley B. Prusiner, then a resident in neurology at the University of California School of Medicine at San Francisco, lost a patient to Creutzfeldt-Jakob disease. He resolved to learn more about the condition. He read that it and related diseases, scrapie and kuru, could be transmitted by injecting extracts from diseased brain into the brains of healthy animals. At the time, the diseases were thought to be caused by a slow-acting virus, but it had not been identified. He was intrigued by a study from the laboratory of Tikvah Alper that suggested that the scrapie agent lacked nucleic acid. When he started his own lab in 1974, Prusiner decided to pursue the nature of the infectious agent.

He and his associates determined to purify the causative agent in scrapie-infected brains and, by 1982, had a highly purified preparation. They subjected it to extensive analysis, and all of their results indicated that it indeed lacked DNA or RNA and that it consisted mainly, if not exclusively, of protein. The infectivity was lost when treated with procedures that denatured protein, but not when treated with those detrimental to nucleic acids. He named the agent a "prion," an abbreviation for "proteinaceous infectious particle." Shortly afterward, he showed that it consisted of a single protein. This was a highly unorthodox discovery because all pathogens studied to date contained nucleic acid. Skeptics were convinced that a very small amount of nucleic acid must be contaminating the prions, although the limits on detection showed that it contained fewer than one

hundred nucleotides and would have to be smaller than any known virus.

Prusiner and his collaborators subsequently learned that the gene for the prion protein was found in chromosomes of hamsters, mice, humans, and all other mammals that have been examined. Furthermore, most of the time these animals make the prion protein without getting sick—a startling observation. Prusiner and his team subsequently showed that the prion protein existed in two forms, one harmless and the other leading to disease. The latter proved to be highly resistant to degradation by proteolytic enzymes and accumulated in the brain tissue of affected animals and people. In infectious disease, the harmful form of the prions appears to convert the harmless form to the harmful form, although the mechanism is not understood. In inherited disease, mutations in the prion may cause it to adopt the harmful form spontaneously or after some unknown signal, leading eventually to the disease state. While questions remain, research since the 1980's has established the involvement of prions in various spongiform encephalopathies.

In 1997, Prusiner was awarded the Nobel Prize in Physiology or Medicine for his pioneering discovery of prions and their role in various neurological diseases. The Nobel Committee also noted his perseverance in pursuing an unorthodox hypothesis in the face of major skepticism.

—James L. Robinson

one thousand Fore died of Kuru each year. Anthropologists recording their customs described their practice of eating the brains of their dead relatives in order to gain the knowledge they contained. Clearly, some infectious agent was being transmitted during this ritual. Such cannibalism has since stopped, and Kuru has declined markedly. Kuru, like Creutzfeldt-Jakob syndrome, shows the same spongiform changes and protein deposits in the brain after death. Similarly, early symptoms include intellectual deterioration, spastic movements, and visual problems. Within a year, the patient becomes unresponsive and dies.

The outbreak of "mad cow" disease in the mid-1990's in Great Britain led to widespread

fear. Thousands of cattle were killed to prevent human consumption of contaminated beef. The cows were infected by supplemental feedings tainted by infected sheep meat. Animal-to-human transmission of these diseases appears to occur, and research has shown that human-to-animal infection is possible as well.

Both Kuru and Creutzfeldt-Jakob syndrome, as well as the animal forms, have no known treatment or cure. Because of the long incubation period, decades may pass before symptoms appear, but once they do, the central nervous system is rapidly destroyed, and death comes quickly. It is likely that many more people die of these disorders than is known because they are so rarely diagnosed.

Properties of Prions

Most of the research on prion diseases has focused on scrapie in sheep. It became clear that the infectious particle had novel properties: It was not a virus as had been suspected, nor did the body react to it as an invader. It was discovered that this transmissible agent was an abnormal version of a common protein, which defied medical understanding. This protein is normally secreted by nerve cells and is found on their outer membranes. Its gene is on chromosome 20 in humans. The transmissible, infectious fragment of the prion somehow disrupts the nerve cell, causing it to produce the abnormal fragment instead of the normal protein. This product accumulates to toxic levels in the tissue and fluid of the brain and spinal cord over many years, finally destroying the central nervous system.

Prion infection appears to occur from exposure to infected tissues or fluids. Transmission has occurred accidentally through nerve tissue transplants and neurosurgical instruments. Prions are not affected by standard sterilization techniques; prevention requires careful handling of infected materials and extended autoclaving of surgical instruments (for at least one hour) or thorough rinsing in chlorine bleach. The agent is not spread by casual contact or air, and isolating the patient is not necessary.

Other human degenerative nervous system diseases whose causes remain unclear also show accumulations of proteins to toxic levels. Alzheimer's disease is the best-studied example, and it is possible that a process similar to that in prion diseases is at work. The discovery of prions has far-reaching implications for genetic and cellular research. Scientists have already learned a startling fact: Substances as inert as proteins and far smaller than viruses can act as agents of infections.

—Connie Rizzo

See also: Alzheimer's Disease; Huntington's Disease.

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Web Site of Interest

National Organization for Rare Disorders. <http://www.rarediseases.org>. Searchable site by type of disorder. Includes background information on Creutzfeldt-Jakob syndrome, a list of other names for the disorder, and a list of related organizations.

Protein Structure

Field of study: Molecular genetics

Significance: *Proteins have three-dimensional structures that determine their functions, and slight changes in overall structure may significantly alter their activity. Correlation of protein structure and function can provide insights into cellular metabolism and its many interconnected processes. Because most diseases result from improper protein function, advances in this field could lead to effective molecular-based disease treatments.*

Key terms

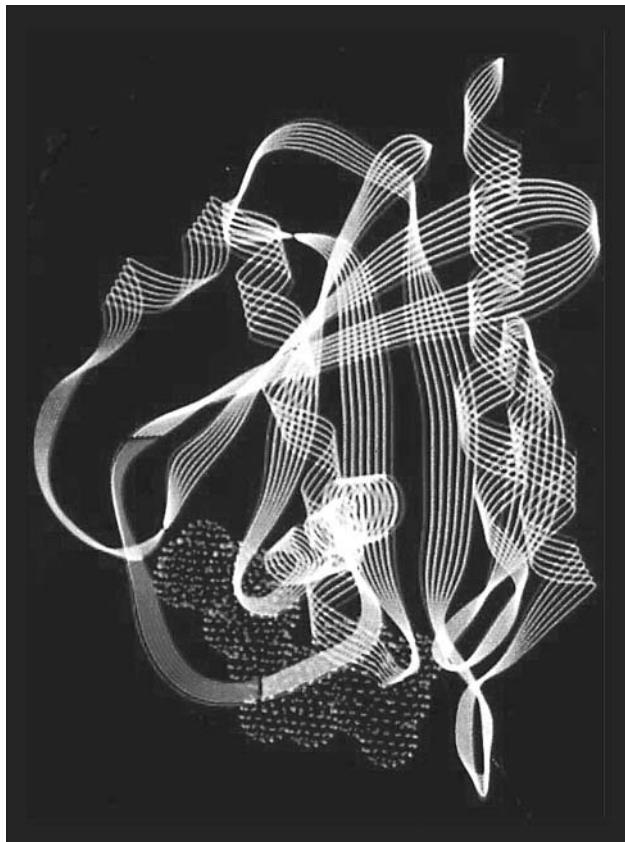
AMINO ACID: the basic subunit of a protein; there are twenty commonly occurring amino acids, any of which may join together by chemical bonds to form a complex protein molecule

ENZYMES: proteins that are able to increase the rate of chemical reactions in cells without being altered in the process

HYDROGEN BOND: a weak bond that helps stabilize the folding of a protein

POLYPEPTIDE: a chain of amino acids joined by chemical bonds

R GROUP: a functional group that is part of an



A three-dimensional image of the ras protein. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

amino acid that gives each amino acid its unique properties

Protein Structure and Function

Proteins consist of strings of individual subunits called amino acids that are chemically bonded together with peptide bonds. Once amino acids are bonded, the resulting molecule is called a polypeptide. The properties and arrangement of the amino acids in the polypeptide cause it to fold into a specific shape or conformation that is required for proper protein function. Proteins have been called the "workhorses" of the cell because they perform most of the activities encoded in the genes of the cell. Proteins function by binding to other molecules, frequently to other proteins. The precise three-dimensional shape of a protein determines the specific molecules it will be

able to bind to, and for many proteins binding is specific to just one other specific type of molecule.

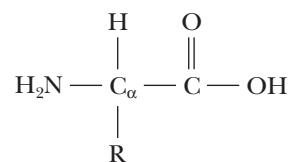
In 1973, Christian B. Anfinsen performed experiments that showed that the three-dimensional structure of a protein is determined by the sequence of its amino acids. He used a protein called ribonuclease (RNase), an enzyme that degrades RNA in the cell. The ability of ribonuclease to degrade RNA is dependent upon its ability to fold into its proper three-dimensional shape. Anfinsen showed that if the enzyme was completely unfolded by heat and chemical treatment (at which time it would not function), it formed a linear chain of amino acids. Although there were 105 possible conformations that the enzyme could take upon refolding, it would refold into the single correct functional conformation upon removal of heat and chemicals. This established that the amino acid sequences of proteins, which are specified by the genes of the cell, carry all of the information necessary for proteins to fold into their proper three-dimensional shapes.

To understand protein conformation better, it is helpful to analyze the underlying levels of structure that determine the final three-dimensional shape. The primary structure of a polypeptide is the simplest level of structure and is, by definition, its amino acid sequence. Because primary structure of polypeptides ultimately determines all succeeding levels of structure, knowing the primary structure should theoretically allow scientists to predict the final three-dimensional structure. Building on a detailed knowledge of the structure of many proteins, scientists can now develop computer programs that are able to predict three-dimensional shape with some degree of accuracy, but much more research will be required to increase the accuracy of these methods.

Primary Protein Structure

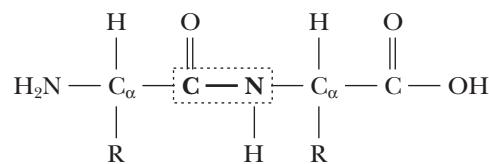
There are twenty naturally occurring amino acids that are commonly found in proteins, and each of these has a common structure consisting of a nitrogen-containing amino group ($-\text{NH}_2$), a carboxyl group ($-\text{COOH}$), a hydrogen atom (H), and a unique functional group referred to as an R group, all bonded to a

central carbon atom (known as the alpha carbon, or C_α) as shown in the following figure:



The uniqueness of each of the twenty amino acids is determined by the R group. This group may be as simple as a hydrogen atom (in the case of the amino acid glycine) or as complex as a ring-shaped structure (as found in the amino acid phenylalanine). It may be charged, either positively or negatively, or it may be uncharged.

Cells join amino acids together to form peptides (strings of up to ten amino acids), poly-peptides (strings of ten to one hundred amino acids), or proteins (single or multiple polypeptides folded and oriented to one another so they are functional). The amino acids are joined together by covalent bonds, called peptide bonds (in the box in the following figure), between the carbon atom of the carboxyl group of one amino acid ($-\text{COOH}$) and the nitrogen atom of the amino group ($-\text{NH}_2$) of the next adjacent amino acid:



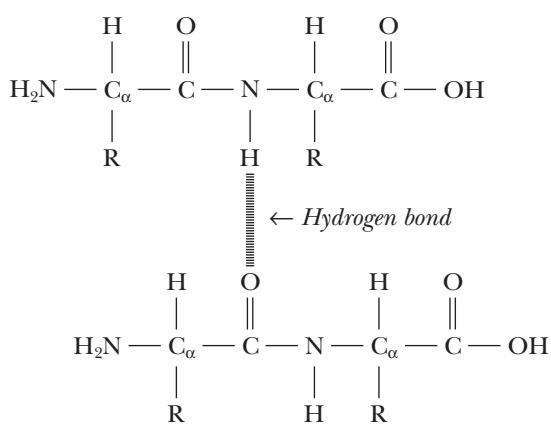
During the formation of the peptide bond, a molecule of water (H_2O) is lost (an $-\text{OH}$ from the carboxyl group and an $-\text{H}$ from the amino group), so this reaction is also called a dehydration synthesis. The result is a dipeptide (a peptide made of two amino acids joined by a peptide bond) that has a “backbone” of nitrogens and carbons ($\text{N}—\text{C}_\alpha—\text{C}—\text{N}—\text{C}_\alpha—\text{C}$) with other elements and R groups protruding from the backbone. An amino acid may be joined to the growing peptide chain by formation of a

peptide bond between the carbon atom of the free carboxyl group (on the right of the preceding figure) and the nitrogen atom of the amino acid being added. The end of a polypeptide with an exposed carboxyl group is called the C-terminal end, and the end with an exposed amino group is called the N-terminal end.

The atoms and R groups that protrude from the backbone are capable of interacting with each other, and these interactions lead to higher-order secondary, tertiary, and quaternary structures.

Secondary Structure

The next level of structure is secondary structure, which involves the formation of hydrogen bonds between the oxygen atoms in carboxyl groups with the hydrogen atoms of amino groups from different parts of the polypeptide. Hydrogen bonds are weak bonds that form between atoms that have a very strong attraction for electrons (such as oxygen or nitrogen), and a hydrogen atom that is bound to another atom with a very strong attraction for electrons. Secondary structure does not involve the formation of bonds with R groups or atoms that are parts of R groups, but involves bonding just between amino and carboxyl groups that are in the peptide bonds making up the backbone of polypeptides.



These hydrogen bonds between backbone molecules lead to the formation of two major types of structures: alpha helices and beta-

pleated sheets. An alpha helix is a rigid structure shaped very much like a telephone cord; it spirals around as the oxygen of one amino acid of the chain forms a hydrogen bond with the hydrogen atom of an amino acid five amino acids away on the protein strand. The rigidity of the structure is caused by the large number of hydrogen bonds (individually weak but collectively strong) and the compactness of the helix that forms. Many alpha helices are found in proteins that function to maintain cell structure.

Beta sheets are formed by hydrogen bonding between amino acids in different regions (often very far apart on the linear strand) of a polypeptide. The shape of a beta-pleated sheet may be likened to the bellows of an accordion or a sheet of paper that has been folded multiple times to form pleats. Because of the large number of hydrogen bonds in them, beta sheets are also strong structures, and they form planar regions that are often found at the bottom of “pockets” inside proteins to which other molecules attach.

In addition to alpha helices or beta-pleated sheets, other regions of the protein may have no obvious secondary structure; these regions are said to have a “random coil” shape. It is the combinations of random coils, alpha helices, and beta sheets that form the secondary structure of the protein.

Tertiary Structure

The final level of protein shape (for a single polypeptide or simple protein) is called tertiary structure. Tertiary structure is caused by the numerous interactions of R groups on the amino acids and of the protein with its environment, which is usually aqueous (water based). Various R groups may either be attracted to and form bonds with each other, or they may be repelled from each other. For example, if an R group has an overall positive electrical charge, it will be attracted to R groups with a negative charge but repelled from other positively charged R groups. For a polypeptide with one hundred amino acids, if amino acid number 6 is negatively charged, it could be attracted to a positively charged amino acid at position 74, thus bringing two ends of the protein that are

linearly distant into close proximity. Many of these attractions lead to the formation of hydrogen, ionic, or covalent bonds. For example, sulfur is contained in the R groups of a few of the amino acids, and sometimes a disulfide bond (a covalent bond) will be formed between two of these. It is the arrangement of disulfide bonds in hair proteins that gives hair its physical properties of curly versus straight. Hair permanent treatments actually break these disulfide bonds and then reform them when the hair is arranged as desired. Many other R groups in the protein will also be attracted to or repelled from each other, leading to an overall folded shape that is most stable. In addition, because most proteins exist in an aqueous environment in the cell, most proteins are folded such that their amino acids with hydrophilic R groups (R groups attracted to water) are on the outside, while their amino acids with hydrophobic R groups (R groups repelled from water) are tucked away in the interior of the protein.

Quaternary Structure

Many polypeptides are nonfunctional until they physically associate with another polypeptide, forming a functional unit made up of two or more subunits. Proteins of this type are said to have quaternary structure. Quaternary structure is caused by interactions between the R groups of amino acids of two different polypeptides. For example, hemoglobin, the oxygen-carrying protein found in red blood cells, functions as a tetramer, with four polypeptide subunits.

Because secondary, tertiary, and quaternary interactions are caused by the R groups of the specific amino acids, the folding is ultimately dictated by the amino acid sequence of the protein. Although there may be numerous possible final conformations that a polypeptide could take, it usually assumes only one of these, and this is the conformation that leads to proper protein function. Many polypeptides are capable of folding into their final conformation spontaneously. More complex ones may need the assistance of other proteins, called chaperones, to help in the folding process.

Impact and Applications

The function of a protein may be altered by changing its shape, because proper function is dependent on proper conformation. Many genetic defects are detrimental because they represent a mutation that results in a change in protein structure. Changes in protein conformation are also an integral part of metabolic control in cells. Normal cellular processes are controlled by “turning on” and “turning off” proteins at the appropriate time. A protein’s activity may be altered by attaching a molecule or ion to that protein that results in a change of shape. Because the shape is caused by R group interactions, binding of a charged ion such as calcium to the protein will alter these interactions and thus alter the shape and function of the protein. One molecular “on/off” switch that is used frequently within a cell involves the attachment or removal of a phosphate group to or from a protein. Attachment of a phosphate will significantly alter the shape of the protein by repelling negatively charged amino acids and attracting positively charged amino acids, which will either activate the protein to perform its function (turn it on) or deactivate it (turn it off).

Cancer and diseases caused by bacterial or viral infections are often the result of nonfunctional proteins that have been produced with incorrect shapes or that cannot be turned on or off by a molecular switch. The effects may be minor or major, depending upon the protein, its function, and the severity of the structural deformity. Understanding how a normal protein is shaped and how it is altered in the disease process allows for the development of drugs that may block the disease. This may be accomplished by blocking or changing the effect of the protein of interest or by generating drugs or therapies that mimic the normal functioning of the protein. Thus, understanding protein structure is essential for understanding proper protein function and for developing molecular-based disease treatments.

—Sarah Lea McGuire, updated by Bryan Ness

See also: Central Dogma of Molecular Biology; DNA Repair; DNA Replication; DNA Structure and Function; Genetic Code; Genetic Code, Cracking of; Molecular Genetics;

Protein Synthesis; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World; Synthetic Genes.

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Protein Synthesis

Field of study: Molecular genetics

Significance: Cellular proteins can be grouped into two general categories: proteins with a structural function that contribute to the three-dimensional organization of a cell, and proteins with an enzymatic function that catalyze the biochemical reactions required for cell growth and function. Understanding the process by which proteins are synthesized provides insight into how a cell organizes itself and how defects in this process can lead to disease.

Key terms

AMINO ACID: the basic subunit of a protein; there are twenty commonly occurring amino acids, any of which may join together by chemical bonds to form a complex protein molecule

PEPTIDE BOND: the chemical bond between amino acids in protein

POLYPEPTIDE: a linear molecule composed of amino acids joined together by peptide bonds; all proteins are functional polypeptides

RNA: ribonucleic acid, that molecule that acts as the messenger between genes in DNA and their protein product, directing the assembly of proteins; as an integral part of ribosomes, RNA is also involved in protein synthesis

TRANSLATION: the process of forming proteins according to instructions contained in an RNA molecule

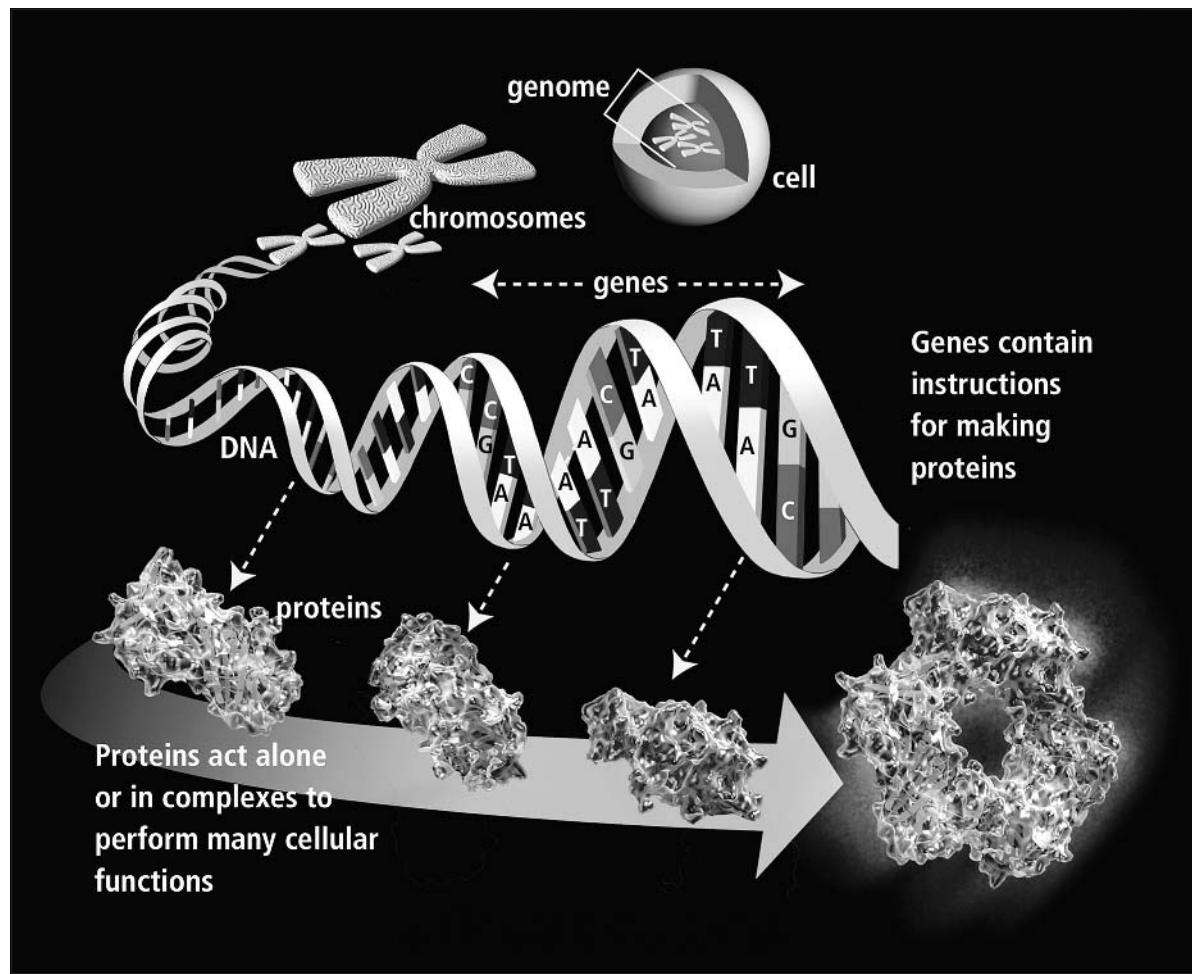
The Flow of Information from Stored to Active Form

The cell can be viewed as a unit that assembles resources from the environment into biochemically functional molecules and organizes these molecules in three-dimensional space in a way that allows cellular growth and replication. In order to carry out this organizational process, a cell must have a biosynthetic means to assemble resources into useful molecules, and it must contain the information required to produce the biosynthetic and structural machinery. DNA serves as the stored form of this

information, whereas protein is its active form. Although there are thousands of different proteins in cells, they either serve a structural role or are enzymes that catalyze the biosynthetic reactions of a cell. Following the discovery of the structure of DNA in 1953 by James Watson and Francis Crick, scientists began to study the process by which the information stored in this molecule is converted into protein.

Proteins are linear, functional molecules composed of a unique sequence of amino acids. Twenty different amino acids are used as the protein building blocks. Although the information for the amino acid sequence of each protein is present in DNA, protein is not synthesized directly from this source. Instead,

RNA serves as the intermediate form from which proteins are synthesized. RNA plays three roles during protein synthesis. Messenger RNA (mRNA) contains the information for the amino acid sequence of a protein. Transfer RNAs (tRNAs) are small RNA molecules that serve as adapters that decipher the coded information present within an mRNA and bring the appropriate amino acid to the polypeptide as it is being synthesized. Ribosomal RNAs (rRNAs) act as the engine that carries out most of the steps during protein synthesis. Together with a specific set of proteins, rRNAs form ribosomes that bind the mRNA, serve as the platform for tRNAs to decode an mRNA, and catalyze the formation of peptide bonds between



The fundamental steps in protein synthesis. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

amino acids. Each ribosome is composed of two subunits: a small (or 40s) and a large (or 60s) subunit, each of which has its own function. The “s” in 40s and 60s is an abbreviation for Svedberg units, which are a measure of how quickly a large molecule or complex molecular structure sediments (or sinks) to the bottom of a centrifuge tube while being centrifuged. The larger the number, the larger the molecule.

Like all RNA, mRNA is composed of just four types of nucleotides: adenine (A), guanine (G), cytosine (C), and uracil (U). Therefore, the information in an mRNA is contained in a linear sequence of nucleotides that is converted into a protein molecule composed of a linear sequence of amino acids. This process is referred to as “translation,” since it converts the “language” of nucleotides that make up an mRNA into the “language” of amino acids that make up a protein. This is achieved by a three-letter genetic code in which each amino acid in a protein is specified by a three-nucleotide sequence in the mRNA called a codon. The four possible “letters” means that there are sixty-four possible three-letter “words.” As there are only twenty amino acids used to make proteins, most amino acids are encoded by several different codons. For example, there are six different codons (UCU, UCC, UCA, UCG, AGU, and AGC) that specify the amino acid serine, whereas there is only one codon (AUG) that specifies the amino acid methionine. The mRNA, therefore, is simply a linear array of codons (that is, three-nucleotide “words” that are “read” by tRNAs together with ribosomes). The region within an mRNA containing this sequence of codons is called the coding region.

Before translation can occur in eukaryotic cells, mRNAs undergo processing steps at both ends to add features that will be necessary for translation (These processing steps do not occur in prokaryotic cells.) Nucleotides are structured such that they have two ends, a 5' and a 3' end, that are available to form chemical bonds with other nucleotides. Each nucleotide present in an mRNA has a 5' to 3' orientation that gives a directionality to the mRNA so that the RNA begins with a 5' end and finishes in a 3' end. The ribosome reads the coding region of an mRNA in a 5' to 3' direction. Following the

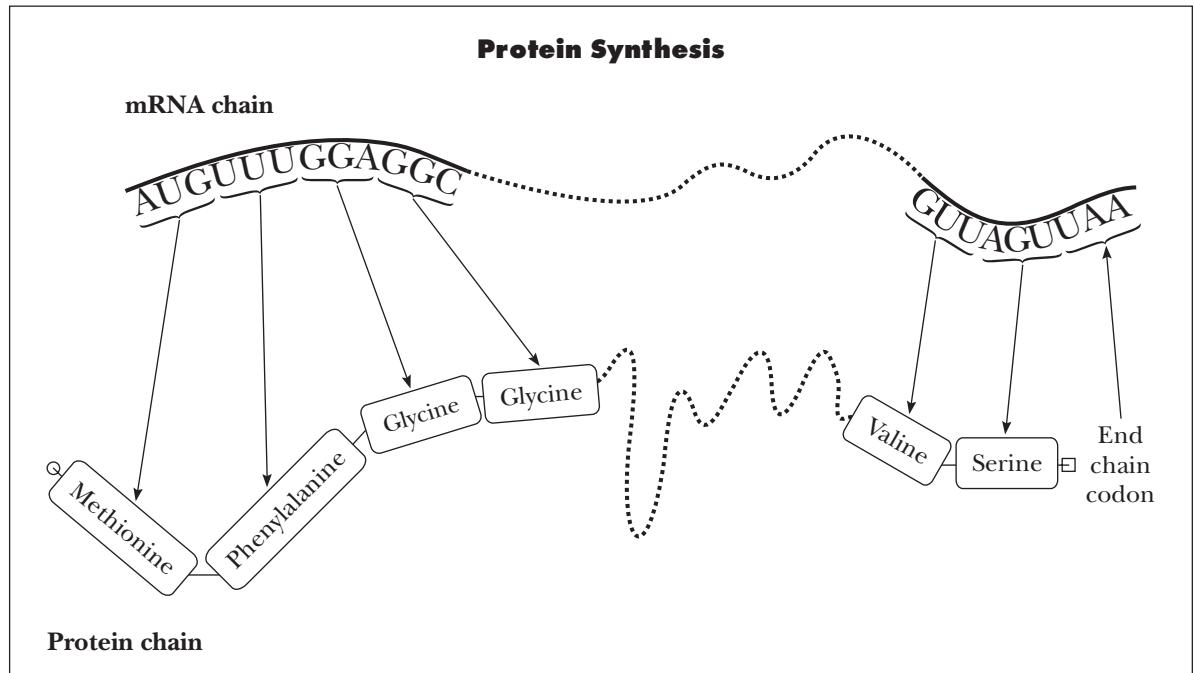
synthesis of an mRNA from its DNA template, one guanine is added to the 5' end of the mRNA in an inverted orientation and is the only nucleotide in the entire mRNA present in a 3' to 5' orientation. It is referred to as the cap. A long stretch of adenosine is added to the 3' end of the mRNA to make what is called the poly-A tail.

Typically, mRNAs have a stretch of nucleotide sequence that lies between the cap and the coding region. This is referred to as the leader sequence and is not translated. Therefore, a signal is necessary to indicate where the coding region initiates. The codon AUG usually serves as this initiation codon; however, other AUG codons may be present in the coding region. Any one of three possible codons (UGA, UAG, or UAA) can serve as stop codons that signal the ribosome to terminate translation. Several accessory proteins assist ribosomes in binding mRNA and help carry out the required steps during translation.

The Translation Process: Initiation

Translation occurs in three phases: initiation, elongation, and termination. The function of the 40s ribosomal subunit is to bind to an mRNA and locate the correct AUG as the initiation codon. It does this by binding close to the cap at the 5' end of the mRNA and scanning the nucleotide sequence in its 5' to 3' direction in search of the initiation codon. Marilyn Kozak identified a certain nucleotide sequence surrounding the initiator AUG of eukaryotic mRNAs that indicates to the ribosome that this AUG is the initiation codon. She found that the presence of an A or G three nucleotides prior to the AUG and a G in the position immediately following the AUG were critical in identifying the correct AUG as the initiation codon. This is referred to as the “sequence context” of the initiation codon. Therefore, as the 40s ribosomal subunit scans the leader sequence of an mRNA in a 5' to 3' direction, it searches for the first AUG in this context and may bypass other AUGs not in this context.

Nahum Sonenberg demonstrated that the scanning process by the 40s subunit can be impeded by the presence of stem-loop structures present in the leader sequence. These form



Protein synthesis is directed by messenger RNA (mRNA). The order of the amino acids in the protein chain is controlled by the order of the bases in the mRNA chain. It takes a codon of three bases to specify one amino acid.

from base pairing between complementary nucleotides present in the leader sequence. Two nucleotides are said to be complementary when they join together by hydrogen bonds. For instance, the nucleotide (or base) A is complementary to U, and these two can form what is called a “base pair.” Likewise, the nucleotides C and G are complementary. Several accessory proteins, called eukaryotic initiation factors (eIFs), aid the binding and scanning of 40s subunits. The first of these, eIF4F, is composed of three subunits called eIF4E, eIF4A, and eIF4G. The protein eIF4E is the subunit responsible for recognizing and binding to the cap of the mRNA. The eIF4A subunit of eIF4F, together with another factor called eIF4B, functions to remove the presence of stem-loop structures in the leader sequence through the disruption of the base pairing between nucleotides in the stem loop. The protein eIF4G is the large subunit of eIF4F, and it serves to interact with several other proteins, one of which is eIF3. It is this latter initiation factor that the 40s subunit first associates with during its initial binding to an mRNA.

Through the combined action of eIF4G and eIF3, the 40s subunit is bound to the mRNA, and through the action of eIF4A and eIF4B, the mRNA is prepared for 40s subunit scanning. As the cellular concentration of eIF4E is very low, mRNAs must compete for this protein. Those that do not compete well for eIF4E will not be translated efficiently. This represents one means by which a cell can regulate protein synthesis. One class of mRNA that competes poorly for eIF4E encodes growth-factor proteins. Growth factors are required in small amounts to stimulate cellular growth. Sonnenberg has shown that the overproduction of eIF4E in animal cells leads to a reduction in the competition for this protein, and mRNAs such as growth-factor mRNAs that were previously poorly translated when the concentration of eIF4E was low are now translated at a higher rate when eIF4E is abundant. This in turn results in the overproduction of growth factors, which leads to uncontrolled growth, a characteristic typical of cancer cells.

A protein that specifically binds to the poly-A tail at the 3' end of an mRNA is called the poly-

A-binding protein (PABP). Discovered in the 1970's, the only function of this protein was thought to be to protect the mRNA from attack at its 3' end by enzymes that degrade RNA. Daniel Gallie demonstrated another function for PABP by showing that the PABP-poly-A-tail complex was required for the function of the eIF4F-cap complex during translation initiation. The idea that a protein located at the 3' end of an mRNA should participate in events occurring at the opposite end of an mRNA seemed strange initially. However, RNA is quite flexible and is rarely present in a straight, linear form in the cellular environment. Consequently, the poly-A tail can easily approach the cap at the 5' end. Gallie showed that PABP interacts with eIF4G and eIF4B, two initiation factors that are closely associated with the cap, through protein-to-protein contacts. The consequence of this interaction is that the 3' end of an mRNA is held in close physical proximity to its cap. The interaction between these proteins stabilizes their binding to the mRNA, which in turn promotes protein synthesis. Therefore, mRNAs can be thought of as adopting a circular form during translation that looks similar to a snake biting its own tail. This idea is now widely accepted by scientists.

One additional factor, called eIF2, is needed to bring the first tRNA to the 40s subunit. Along with the initiator tRNA (which decodes the AUG codon specifying the amino acid methionine), eIF2 aids the 40s subunit in identifying the AUG initiation. Once the 40s subunit has located the initiation codon, the 60s ribosomal subunit joins the 40s subunit to form the intact 80s ribosome. (Svedberg units are not additive; therefore, a 40s and 60s unit joined together do not make a 100s unit.) This marks the end of the initiation phase of translation.

The Translation Process: Elongation and Termination

During the elongation phase, tRNAs bind to the 80s ribosome as it passes over the codons of the mRNA, and the amino acids attached to the tRNAs are transferred to the growing polypeptide. Binding of the tRNAs to the ribosome is assisted by an accessory protein called eukary-

otic elongation factor 1 (eEF1). A codon is decoded by the appropriate tRNA through base pairing between the three nucleotides that make up the codon in the mRNA and three complementary nucleotides within a specific region (called the anticodon) within the tRNA. The tRNA binding sites in the 80s ribosome are located in the 60s subunit. The ribosome moves over the coding region one codon at a time, or in steps of three nucleotides, in a process referred to as "translocation." When the ribosome moves to the next codon to be decoded, the tRNA containing the appropriate anticodon will bind tightly in the open site in the 60s subunit (the A site). The tRNA that bound to the previous codon is present in a second site in the 60s subunit (the P site). Once a new tRNA has bound to the A site, the ribosomal RNA itself catalyzes the formation of a peptide bond between the growing polypeptide and the new amino acid. This results in the transfer of the polypeptide attached to the tRNA present in the P site to the amino acid on the tRNA present in the A site. A second elongation factor, eEF2, catalyzes the movement of the ribosome to the next codon to be decoded. This process is repeated one codon at a time until a stop codon is reached.

The termination phase of translation begins when the ribosome reaches one of the three termination or stop codons. These are also referred to as "nonsense" codons as the cell does not produce any tRNAs that can decode them. Accessory factors, called release factors, are also required to assist this stage of translation. They bind to the empty A site in which the stop codon is present, and this triggers the cleavage of the bond between the completed protein from the last tRNA in the P site, thereby releasing the protein. The ribosome then dissociates into its 40s and 60s subunits, the latter of which diffuses away from the mRNA. The close physical proximity of the cap and poly-A tail of an mRNA maintained by the interaction between PABP and the initiation factors (eIF4G and eIF4B) is thought to assist the recycling of the 40s subunit back to the 5' end of the mRNA to participate in a subsequent round of translation.

Impact and Applications

The elucidation of the process and control of protein synthesis provides a ready means by which scientists can manipulate these processes in cells. In addition to infectious diseases, insufficient dietary protein represents one of the greatest challenges to world health. The majority of people living today are limited to obtaining their dietary protein solely through the consumption of plant matter. Knowledge of the process of protein synthesis may allow molecular biologists to increase the amount of protein in important crop species. Moreover, most plants contain an imbalance in the amino acids needed in the human diet that can lead to disease. For example, protein from corn is poor in the amino acid lysine, whereas the protein from soybeans is poor in methionine and cysteine. Molecular biologists may be able to correct this imbalance by changing the codons present in plant genes, thus improving this source of protein for those people who rely on it for life.

—Daniel R. Gallie

See also: Central Dogma of Molecular Biology; DNA Repair; DNA Replication; DNA Structure and Function; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Protein Structure; RNA Structure and Function; RNA Transcription and mRNA Processing; RNA World; Synthetic Genes.

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Proteomics

Fields of study: Molecular genetics; Techniques and methodologies

Significance: *The study of proteomics and its relationship to genomics currently focuses on the vast family of gene-regulating proteins. These polypeptides and their functions affect the expression of various genetically related diseases, such as Alzheimer's and cancer. By focusing on the interrelated groups of regulator functions, geneticists are learning the connections between structure, abundance within the cell, and how each protein relates to expression.*

Key terms

CHROMATOGRAPHY: a separation technique involving a mobile solvent and a stationary, adsorbent phase

MASS SPECTROSCOPY: a method of analyzing molecular structure in which sample molecules are ionized and the resulting fragmented particles are passed through electric and magnetic fields to a detector

PERIPHERAL PROTEINS: proteins of the chromosome that do not directly affect transcription

PROTEIN FOLDING STRUCTURE: the three-dimensional structure of proteins created by the folding of linked amino acids upon each other; this structure is held together by intermolecular forces, such as hydrogen bonds and ionic attractions

PROTEIN MARKER: a sequence of DNA that chemically attracts a particular regulatory protein sequence or structure

REGULATORS: proteins that control the transcription of a gene

SENILE PLAQUES: protein sections that are no longer functional and clutter the intercellular space of the brain, disrupting proper processes

TRANSCRIPTION: the process by which mRNA is formed using DNA as a template

TRANSLATION: the process of building a protein by bonding amino acids according to the mRNA marker present

What Is “Proteomics”?

Historically, much of the focus in genetic research has been on genes and completion of the Human Genome Project. More recently, the focus has shifted to a new and related topic, the proteome. Proteins are known to perform most of the important functions of cells. Therefore, proteomics is, essentially, the study of proteins in an organism and, most important, their function. There are many aspects to the understanding of protein function, including where a particular protein is located in the cell, what modifications occur during its activity, what ligands may bind to it, and its activity. Researchers are seeking to identify all the proteins made in a given cell, tissue, or organism and determine how those proteins interact with metabolites, with themselves, and with nucleic acids. By studying proteomics, scientists hope to uncover underlying causes of disease at the cellular level, invent better methods of diagnosis, and discover new, more efficient medicines for the treatment of disease.

Proteomics has moved to the forefront of molecular research, especially in the area of drug research. Neither the structure nor the function of a gene can be predicted from the DNA sequence alone. Although genes code for proteins, there is a large difference between the number of messenger (mRNA) molecules transcribed from DNA and the number of proteins in a cell. In addition, two hundred known modifications occur during the stages between transcription and post-translation, including phosphorylation, glycosylation, proteolytic processing, deamidation, sulfation, and nitration. Other factors that affect the expression of proteins include aging, stress, environmental forces, and drugs. In addition, changes to the sequence of amino acids may occur during or after translation.

Methods of Proteomic Research

In order to study the functions of a protein, it must be separated from other proteins or contaminants, purified, and structurally characterized. These are the major tasks facing researchers in the field.

In order to obtain a sufficient quantity of a particular protein for study, the coding plas-

mid can be injected into *Escherichia coli* bacteria and the cells will translate the protein multiple times. Alternatively, it must be extracted from biological tissues. The desired polypeptide must then be separated from cells or tissues that may contain thousands of unique proteins. This can be accomplished by homogenizing the tissue, extracting the proteins with solvents or by centrifugation, and further purifying the protein by various means, including high-pressure liquid chromatography (HPLC, separation by solubility differences) and two-dimensional (2-D) gel electrophoresis (separation of molecules by charge and molecular mass).

Structural characterization begins with establishing the order of linked amino acids in the protein. This can be accomplished by the classical techniques of using proteases to fragment the protein chemically and then analyzing the fragments by separation and spectroscopic analysis. The molecular mass of small polypeptides can be investigated by employing several techniques involving mass spectrometry (MS). Sequentially coupled mass spectrometers (the “tandem” MS/MS techniques) are being used to analyze the amino acid sequence and molecular masses of isolated larger polypeptides. These MS/MS analyses are sometimes added to a separation method, such as HPLC, to analyze mixtures of polypeptides.

Historically, Linus Pauling used analytical data from X-ray diffraction (or crystallography) to determine the three-dimensional, helical structure of proteins. The method is still being used to investigate the structures of proteins and ligand-protein complexes. Such studies may lead to significant improvements in the design of medicinal drugs. One significant drawback to analyzing protein structure by X-ray diffraction, however, is that the method requires a significant quantity (approximately 1 milligram) of the protein. Transmission electron microscopy (TEM), which uses electron beams to produce images and diffraction patterns from extremely small samples or regions of a sample, is therefore often preferable. The TEM method may involve auxiliary techniques to analyze data, including enhancement of images by means of computer software.

Although such methods provide valuable information in analyzing the structure of proteins, they suffer from the loss of spatial information that occurs when tissues are homogenized, when the protein is obtained from a manufactured, bacterial environment, or when it is otherwise isolated. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a complementary method of analysis that does not yield structural information but provides protein profiles from intact tissue, allowing comparison of diseased versus normal tissue.

Large databases of mass spectroscopic data are being assembled to assist in future identification of known proteins. Further databases of proteome information include particular molecular masses, charges, and, in some cases, connections to the genes regulated or the parent gene of the peptide in question. Scientists hope to relate regulators and the complex web of peripheral proteins that affect the function of each gene.

Challenges and Limitations of Current Methods

The amount of data being obtained by proteomics research poses a problem in organizing and processing the information obtained on proteins. The Human Proteome Organization (HUP) and the European Bioinformatics Institute (EBI) are two organizations whose purposes include the management and organization of proteomics information and databases, and the facilitation of the advancement of this scientific endeavor.

Analyzing MS data from proteins and relating the complex array of proteins within a single cell to the linear genetic material of DNA present challenges to researchers that they are tackling through computer algorithms, programs, and databases. The SWISS-PROT database, for example, is an annotated protein-sequence database maintained by the Swiss Bioinformatics Institute.

Other obstacles to relating proteins to parent genes include the loss of quaternary structure during separation and the presence of post-translation processing, which can alter the amino acid sequence to the extent that it be-

comes almost unrecognizable from the parent gene. A lack of protein amplification methods—techniques that would produce more copies of a protein to aid in study—requires sensitive analysis methods and increasingly strong detectors. Currently new methods are being developed, but the limit of study is as large as 1 nanometer.

Disease

Proteins often act as markers for disease. As researchers study proteins, they have found that disease may be characterized by some proteins that are being overproduced, not being produced at all, or being produced at inappropriate times. As the correlation of proteins to disease becomes clearer, better diagnostic tests and drugs are being explored. For example, Alzheimer's disease and Down syndrome are associated with a common protein fragment as the major extracellular protein component of senile plaques.

Researchers are investigating changes in protein expression in heart disease and heart failure, and several hundred cardiac proteins have already been identified. The study of proteomics in immunological diseases has revealed that there is a connection between the human neutrophil α -defensins (HNPs) and human immunodeficiency virus, HIV-1. HNPs are small, cysteine-rich, cationic antimicrobial proteins that are stored in the azurophilic granules of neutrophils and released during phagocytosis to kill ingested foreign microbes. To date, the three most abundant forms of the protein have been implicated in suppressing HIV-1 in vivo.

Similarly, cancer is being studied to find a roster of proteins that are present in cancerous cells but not in normal cells. A joint effort from the National Cancer Institute and the Food and Drug Administration is searching for the differences between cancerous and normal cells, and also for protein "markers."

Possible Future Directions

Although proteomics is a relatively new area of genetic research, the importance of the sugar coatings of proteins and cells is gaining attention, under the name "glycomics." This

area of study has arisen because of the many roles of sugar coatings in important cellular functions, including the immunological recognition sites, barriers, and sites for attack by pathogens.

—Audrey Krumbach, Kayla Williams, and Massimo D. Bezoari

See also: Bioinformatics; Genomics; Human Genetics; Human Genome Project; Protein Structure; Protein Synthesis.

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Link, Andrew J., ed. *2-D Proteome Analysis Protocols*. Nashville, Tenn.: Vanderbilt University Medical Center. Practical proteomics, presenting techniques with step-by-step instructions for laboratory researchers. Fifty-five chapters prepared by more than seventy specialists.

Modern Drug Discovery (October, 2002). The entire issue is devoted to proteomics, with many interesting articles on methods of research, Web sites, and computer-assisted methods of data analysis.

Web Sites of Interest

Cambridge Healthtech Institute. <http://www.genomicglossaries.com/content/proteomics.asp>. This site provides a useful glossary of technical terms used in proteomics, as well as many links to related sites.

Human Proteomics Organization. <http://www.hupo.org>. HUPO works to consolidate regional proteome organizations into a worldwide group, conduct scientific and educational activities, and disseminate knowledge about both the human proteome and model organisms.

Pseudogenes

Field of study: Molecular genetics

Significance: *Pseudogenes are DNA sequences derived from partial copies, mutated complete copies of functional genes, or normal copies of a gene that has lost its control sequences and therefore cannot be transcribed. They may originate by gene duplication or retrotransposition. They are apparently nonfunctional regions of the genome that may evolve at a maximum rate, free from the evolutionary constraints of natural selection.*

Key terms

INTRONS: noncoding segments of DNA within a gene that are removed from pre-messenger RNA (pre-mRNA) as a part of the process of producing a mature mRNA

LONG INTERSPersed SEQUENCES (LINES): long repeats of DNA sequences scattered throughout a genome

NEUTRAL THEORY OF MOLECULAR EVOLUTION: the theory that most DNA sequence evolution is a result of mutations that are neutral with respect to the fitness of the organism

RETROTRANSPOSON (RETROPOSON): a DNA sequence that is transcribed to RNA and reverse transcribed to a DNA copy able to insert itself at another location in the genome

REVERSE TRANSCRIPTASE: an enzyme, isolated from retroviruses, that synthesizes a DNA strand from an RNA template

SHORT INTERSPersed SEQUENCES (SINES): short repeats of DNA sequences scattered throughout a genome

Definition and Origin

Pseudogenes are DNA sequences that resemble genes but are not correctly transcribed or translated to a functional polypeptide. If a functional gene is duplicated so that there are two nonhomologous copies of it in the genome, one of the copies can retain the code for the original polypeptide product, while the other is free from such constraints, since one copy of the gene is sufficient to produce the protein. Because mutations in one copy do not destroy the gene's function, they may be retained, and the unneeded copy can evolve

more quickly. It may change to produce a different, functional polypeptide (and effectively become a new gene), or it may remain nonfunctional as a pseudogene. There are two types of pseudogenes, defined by how they were produced: nonprocessed and processed.

Nonprocessed Pseudogenes

Nonprocessed (or duplicated) pseudogenes arise when a portion of the original gene is duplicated, with portions necessary for proper functioning missing or altered or when the complete original gene is duplicated. They can be identified by the presence of introns and may have mutations in the promoter that prevent transcription or the correct removal of introns, or they may have other mutations (such as premature stop codons) within exons that result in translation of a nonfunctional polypeptide. A series of tandem duplications of a gene can result in clustered gene families, which can include expressed genes, expressed pseudogenes (which are transcribed but produce no functional polypeptide), and nonexpressed pseudogenes that are not transcribed. The alpha-globulin and beta-globulin clusters are examples of such gene families. Other examples of nonprocessed pseudogenes include members of the immunoglobulin (Ig) and major histocompatibility complex (MHC) gene families.

Processed Pseudogenes

Processed pseudogenes originate from transcribed RNA copies of genes that are copied back to DNA by the enzyme reverse transcriptase. Processed pseudogenes are usually integrated into the genome in a different location from the original gene. Reverse transcriptase is an enzyme produced by retroviruses, which have RNA genomes that are reverse transcribed to DNA when the viruses infect host cells. Retrotransposons, which are related to retroviruses, are DNA sequences that transpose or duplicate themselves by reverse transcription of a transcribed RNA copy of the sequence.

Often, retrotransposons will carry along a copy of the surrounding host DNA, resulting in the duplication of that sequence—a processed

pseudogene. Because the introns of a gene are removed from the RNA transcript, processed pseudogenes are not exact copies of the original DNA sequence; the introns are missing. Copies of protein-coding genes copied by this mechanism are members of a type of repetitive DNA called LINES (for long interspersed sequences) and exist in multiple copies scattered around the genome, each up to several thousand base pairs in length. Short processed pseudogenes are members of another class of repetitive DNA called SINES (short interspersed sequences of up to several hundred base pairs in length) and result from the retrotransposon-mediated copying of tRNA or rRNA genes. SINES of this type are sometimes very abundant in genomes because they may have internal promoters, so that they are more easily transcribed, and therefore transposed. The most prominent of SINES are those that are members of the *Alu* family, which occur an average of once every six thousand base pairs in the human genome.

Pseudogenes and Neutral Evolution

In 2003, Japanese researchers reported the discovery of a mouse pseudogene that is involved in regulating the expression of its related “functional” gene. This discovery suggested that at least some pseudogenes may have important functions. Although pseudogenes are very commonly found across genomes, most do not appear to serve any function, and until further research uncovers more functional pseudogenes this assumption appears warranted. Their abundance can be explained by the tendency of duplicated sequences to be further copied. Retrotransposition increases the number of copies of processed pseudogenes, and gene duplication leading to unprocessed pseudogenes favors mechanisms that generate additional copies, leading to clustered gene families. Natural selection does not tend to eliminate these additional copies because their presence does not harm the organism as long as there is at least one functional copy of the original gene. In other words, pseudogenes are selectively neutral.

Because of their selective neutrality, pseudo-

genes are especially useful for estimating neutral mutation rates in genomes. The neutral theory of evolution predicts that, because of the constraints of selection, functional regions of the genome (such as the exons, or coding sequences, of genes) will evolve more slowly than less critical sequences, such as introns, or non-functional sequences like pseudogenes. The number of nucleotide differences between homologous sequences of related species can be used to calculate estimates of evolutionary rates, and such estimates support the neutral theory: the greatest rates of divergence occur within pseudogenes. Using comparisons from several pseudogenes, researchers can establish the baseline neutral mutation rate for a group of species.

—Stephen T. Kilpatrick

See also: Gene Families; Repetitive DNA.

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medical evaluation and appropriate medical intervention to help ensure a healthy, well-adjusted life.

Key terms

AMBIGUOUS GENITALIA: external sexual organs that are not clearly male or female

GENOTYPE: the sum total of the genes present in an individual

GONADS: organs that produce reproductive cells and sex hormones, for example, testes in males and ovaries in females

KARYOTYPE: the number and kind of chromosomes present in every cell of the body (normal female karyotype is 46,XX and normal male karyotype is 46,XY)

PHENOTYPE: the physical appearance and physiological characteristics of an individual, which depends on the interaction of genotype and environment

Normal Fetal Development

Prior to nine weeks gestational age, a male and a female fetus have identical external genitalia (sexual organs) consisting of a phallus and labioscrotal folds. The phallus develops into a penis in males and a clitoris in females; labioscrotal folds become the scrotum in males and the labial folds in females. Early in development, the gonads can develop into either testes or ovaries. In a fetus with a normal male karyotype (46,XY), the primitive gonads become testes, which produce testosterone. Testosterone in turn causes enlargement of the primitive phallus into a penis. It is the presence of the Y chromosome, and in particular a small, sex-determining region of the Y chromosome termed the *SRY* locus, that drives the formation of the testes. The presence of the *SRY* locus appears to be essential for development of a normal male.

Pseudohermaphroditism

A true hermaphrodite is born with both ovarian and testicular tissue. A male pseudohermaphrodite has a 46,XY karyotype with either female genitalia or ambiguous genitalia (but only testicular tissue); a female pseudohermaphrodite has a 46,XX karyotype with either male genitalia or ambiguous genitalia (but

Pseudohermaphrodites

Field of study: Diseases and syndromes

Significance: *Pseudohermaphrodites are individuals born with either ambiguous genitalia or external genitalia that are the opposite of their chromosomal sex. These individuals need a thorough*

only ovarian tissue). Ambiguous genitalia typically consist of a small, abnormally shaped, phalluslike structure, often with hypospadias (in which urine comes from the base of the penis instead of the tip) and abnormal development of the labioscrotal folds (not clearly a scrotum or labia). A vaginal opening may be present.

Most cases of pseudohermaphroditism result from abnormal exposure to increased or decreased amounts of sex hormones during embryonic development. The most common cause of female pseudohermaphroditism is exposure of a female fetus to increased levels of testosterone during the first half of pregnancy. Maternal use of anabolic steroids can cause this condition, but the most common genetic cause of increased testosterone exposure is congenital adrenal hyperplasia (CAH). CAH results from an abnormality in the enzymatic pathways of the fetus that make both cortisol (a stress hormone) and the sex steroids (such as testosterone). At several points in these pathways, there may be a nonfunctioning enzyme that results in too little production of cortisol and too much production of the sex steroids. This will result in partial masculinization of the external genitalia of a female embryo. Females with CAH are usually born with an enlarged clitoris (often mistakenly thought to be a penis) and partial fusion of the labia. Males can also have CAH, but the excess testosterone does not affect their genital development since a relatively high level of testosterone exposure is a normal part of their development.

The most common causes of male pseudohermaphroditism are abnormalities of testosterone production or abnormalities in the testosterone receptor at the cellular level. One example is a deficiency in 5-alpha-reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT). When there is a deficiency of this enzyme, there will be a deficiency of DHT, which is the hormone primarily responsible for masculinization of external genitalia. A male who lacks DHT will have female-appearing external genitalia or ambiguous genitalia at birth. Often these individuals are reared as females, but at puberty they will masculinize because of greatly increased pro-

duction of testosterone. These individuals may actually develop into nearly normal-appearing males. Abnormalities of the testosterone receptor can also result in a range of different conditions in affected males, from normal female appearance (a totally defective receptor) to ambiguous genitalia (partially defective receptor) in a 46,XY male. These individuals will not masculinize at puberty because no matter how much testosterone or DHT they produce, their bodies cannot respond to the hormones.

Both male and female pseudohermaphroditism can result from chromosomal abnormalities. The absence or dysfunction of the *SRY* locus produces an individual with normal female genitalia but a 46,XY karyotype. Individuals with a 46,XX karyotype who have the *SRY* locus transposed to one of their X chromosomes will have a normal male appearance.

Impact and Applications

Some forms of pseudohermaphroditism are life threatening, and so early diagnosis is imperative. Both males and females with CAH are at risk for sudden death caused by low cortisol levels and other hormone deficiencies. Early diagnosis is relatively easy in affected females since their genital abnormalities are noticeable at birth. Affected males are often not recognized until they have a life-threatening event, which usually occurs in the first two weeks of life. Treatment of CAH consists of appropriate hormone supplementation that, if instituted early in life, can help prevent serious problems. CAH is inherited in an autosomal recessive manner, so parents of an affected individual have a 25 percent chance of having another affected child with each pregnancy.

The sex of rearing of a child with ambiguous genitalia is usually determined by the child's type of pseudohermaphroditism. Typically, sex of rearing will be based on the chromosomal sex of the child. These children may need sex hormone supplementation or surgery to assist in developing gender-appropriate genitalia. Children with pseudohermaphroditism with normal-appearing genitalia at birth may not be recognized until puberty, when abnormal masculinization or feminization may occur.

These individuals need medical evaluation and karyotype determination to guide the proper medical treatment.

—Patricia G. Wheeler

See also: Biological Clocks; Gender Identity; Hermaphrodites; Homosexuality; Human Genetics; Metafemales; RNA Transcription and mRNA Processing; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

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Web Sites of Interest

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperpla-

sia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

National Organization for Rare Disorders (NORD). <http://www.rarediseases.org>. Offers information and articles about rare genetic conditions and diseases, including XYY syndrome, in several searchable databases.

PUNCTUATED EQUILIBRIUM

Fields of study: Evolutionary biology; Population genetics

Significance: *Punctuated equilibrium is a model of evolutionary change in which new species originate abruptly and then exist through a long period of stasis. This model is important as an explanation of the stepwise pattern of species change seen in the fossil record.*

Key terms

ALLOPATRIC SPECIATION: a theory that suggests that small parts of a population may become genetically isolated and develop differences that would lead to the development of a new species

HETEROCHRONY: a change in the timing or rate of development of characters in an organism relative to those same events in its evolutionary ancestors

PHYLETIC GRADUALISM: the idea that evolutionary change proceeds by a progression of tiny changes, adding up to produce new species over immense periods of time

Evolutionary Patterns

Nineteenth century English naturalist Charles Darwin viewed the development of new species as occurring slowly by a shift of characters within populations, so that a gradual transition from one species to another took place. This is now generally referred to as

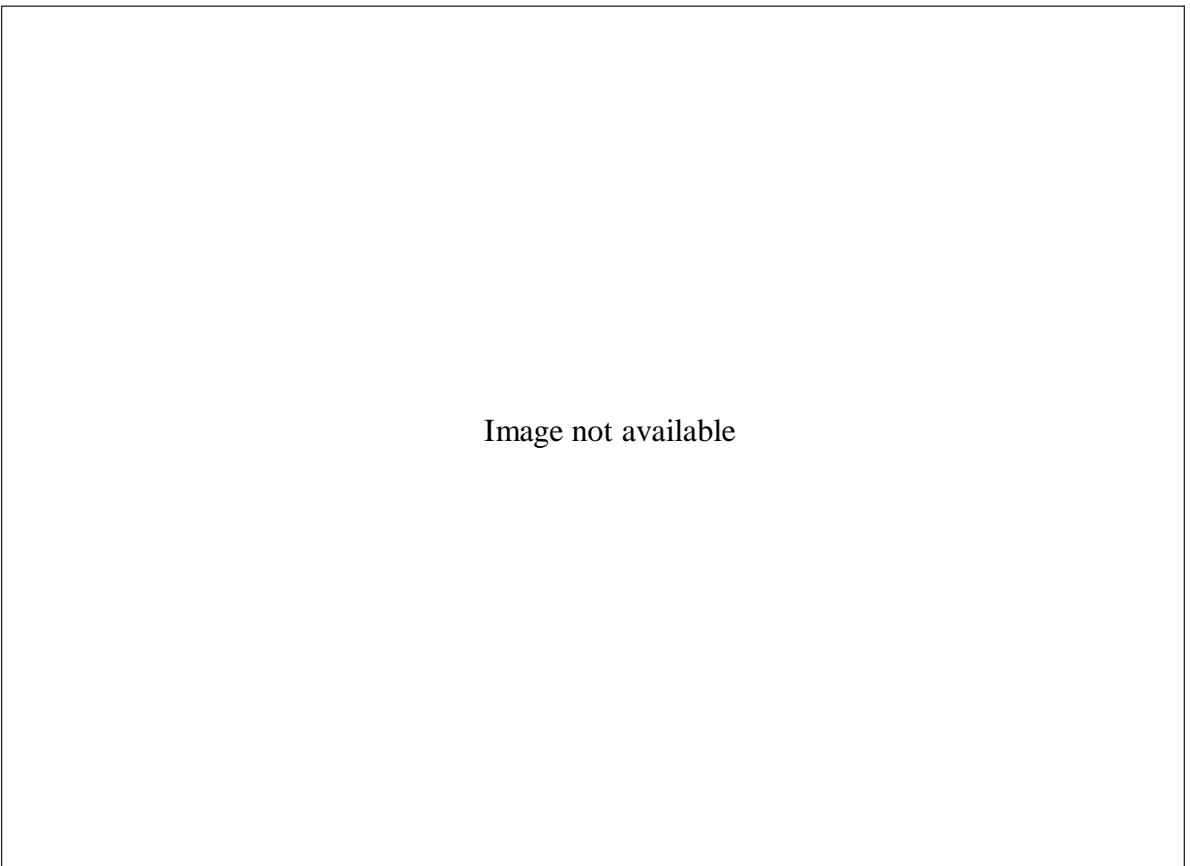


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Stephen Jay Gould in his office on the Harvard campus in 1997. Together with Niles Eldredge, Gould developed the theory of punctuated equilibrium to explain gaps in the fossil record. (AP/Wide World Photos)

phytic gradualism. A number of examples from the fossil record were put forward to support this view, particularly that of the horse, in which changes to the feet, jaws, and teeth seem to have progressed in one direction over a long period of time. Peter Sheldon in 1987 documented gradual change in eight lineages of trilobites over a three-million-year period in the Ordovician period of Wales. Despite these, and other, examples (some of which have been reinterpreted), it is clear that the fossil record more commonly shows a picture of populations that are stable through time but are separated by abrupt morphological breaks. This pattern was recognized by Darwin but was attributed by him to the sketchy and incomplete nature of the fossil record. So few animals become fossilized, and conditions for fossilization are so rare, that he felt only a fragmentary

sampling of gradual transitions was present, giving the appearance of abrupt change.

One hundred years later, the incompleteness of the fossil record no longer seemed convincing as an explanation. In 1972, Niles Eldredge and Stephen Jay Gould published their theory of the evolutionary process, called by them "punctuated equilibrium." This model explains the lack of intermediates by suggesting that evolutionary change occurs only in short-lived bursts in which a new species arises abruptly from a parent species, often with relatively large morphological changes, and thereafter remains more or less stable until its extinction.

The Process of Punctuated Equilibrium

A number of explanations have been put forward to show how this process might take

place. One of these, termed allopatric speciation, was first proposed by Ernst Mayr in 1963. He pointed out that a reproductive isolating mechanism is needed to provide a barrier to gene flow and that this could be provided by geographic isolation. Allopatric or geographical isolation could result when the normal range of a population of organisms is reduced or fragmented. Parts of the population become separated in peripheral isolates, and if the population is small, it may become modified rapidly by natural selection or genetic drift, particularly if it is adapting to a new environment. This type of process is commonly called the founder effect, because it is the characteristics of the small group of individuals that will overwhelmingly determine the possible characteristics of their descendants. As the initial members of the peripheral isolate may be few in number, it might take only a few generations for the population to have changed enough to become reproductively isolated from the parent population. In the fossil record, this will be seen as a period of stasis representing the parent population, followed by a rapid morphological change as the peripheral population is isolated from it and then replaces it, either competitively or because it has become extinct or has moved to follow a shifting habitat. Because this is thought to take place rapidly in small populations, fossilization potential is low, and unequivocal examples are not common in the fossil record. However, in 1981, Peter Williamson published a well-documented example from the Tertiary period of Lake Turkana in Kenya, which showed episodes of stasis and rapid change in populations of freshwater mollusks. The increases in evolutionary rate were apparently driven by severe environmental change that caused parts of the lake to dry up.

Punctuated changes may also have taken place because of heterochrony, which is a change in the rate of development or timing of appearance of ancestral characters. Paedomorphosis, for example, would result in the retention of juvenile characters in the adult, while its opposite, peramorphosis, would result in an adult morphologically more advanced than its ancestor. Rates of development could

be affected by a mutation, perhaps resulting in the descendant growing for much longer than the ancestral form, thus producing a giant version. These changes would be essentially instantaneous and thus would show as abrupt changes of species in the fossil record.

Impact and Applications

The publication of the idea of punctuated equilibrium ignited a storm of controversy that still persists. It predicts that speciation can be very rapid, but more important, it is consistent with the prevalence of stasis over long periods of time so often observed in the fossil record. Species had long been viewed as flexible and responsive to the environment, but fossil species showed no change over long periods despite a changing environment. Biologists have thus had to review their ideas about the concept of species and the processes that operate on them. Species are now seen as real entities that have characteristics that are more than the sum of their component populations. Thus the tendency of a group to evolve rapidly or slowly may be intrinsic to the group as a whole and not dependent on the individuals that compose it. This debate has helped show that the fossil record can be important in detecting phenomena that are too large in scale for biologists to observe.

—David K. Elliott

See also: Artificial Selection; Consanguinity and Genetic Disease; Evolutionary Biology; Genetic Load; Hardy-Weinberg Law; Inbreeding and Assortative Mating; Molecular Clock Hypothesis; Natural Selection; Population Genetics; Speciation.

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Quantitative Inheritance

Field of study: Population genetics

Significance: *Quantitative inheritance involves metric traits. These traits are generally associated with adaptation, reproduction, yield, form, and function. They are thus of great importance to evolution, conservation biology, psychology, and especially to the improvement of agricultural organisms.*

Key terms

GENOTYPE: the genetic makeup of an organism at all loci that affect a quantitative trait

HERITABILITY: the proportion of phenotypic differences among individuals that are a result of genetic differences

METRIC TRAITS: traits controlled by multiple genes with small individual effects and continuously varying environmental effects, resulting in continuous variation in a population

PHENOTYPE: the observed expression of a genotype that results from the combined effects of the genotype and the environment to which the organism has been exposed

The Genetics Underlying Metric Traits

An understanding of the genetics affecting metric traits came with the unification of the Mendelian and biometrical schools of genetics early in the 1900's. The statistical relationships involved in inheritance of metric traits such as height of humans were well known in the late 1800's. Soon after that, Gregor Mendel's breakthrough on particulate inheritance, obtained from work utilizing traits such as colors and shapes of peas, was rediscovered. However, some traits did not follow Mendelian inheritance patterns. As an example, Francis Galton crossed pea plants having uniformly large seeds with those having uniformly small seeds. The seed size of the progeny was intermediate. However, when the progeny were mated among themselves, seed size formed a distribution from small to large with many intermediate sizes.

How could particulate genetic factors explain a continuous distribution? The solution

was described early in the twentieth century when Swedish plant breeder Herman Nilsson-Ehle crossed red and white wheat. The resulting progeny were light red in color. When matings were made within the progeny, the resulting kernels of wheat ranged in color from white to red. He was able to categorize the wheat into five colors: red, intermediate red, light red, pink, and white. Intermediate colors occurred with greater frequency than extreme colors. Nilsson-Ehle deduced that particulate genetic factors (now known as alleles) were involved, with red wheat inheriting four red alleles, intermediate red inheriting three red alleles, light red inheriting two red alleles, pink inheriting one red allele, and white inheriting no red alleles. These results were consistent with Mendel's findings, except that two sets of factors (now known as loci) were controlling this trait rather than the single locus observed for the traits considered by Mendel. Further, these results could be generalized to account for additional inheritance patterns controlled by more than two loci. Quantitative inheritance was mathematically described by British statistician and geneticist Ronald A. Fisher.

Under many circumstances, the environment also modifies the expression of traits. A combination of many loci with individually small effects alone would produce a rough bell-shaped distribution for a quantitative trait. Environmental effects are continuous and are independent of genetic effects. Environmental effects blur the boundaries of the genetic categories and can make it difficult or impossible to identify the effects of individual loci for many quantitative traits. The distribution of phenotypes, reflecting combined genetic and environmental effects, is typically a smooth, bell-shaped curve.

Genetic and environmental effects jointly influence the value of most metric traits. The relative magnitudes of genetic and environmental effects are measured using heritability statistics. Although essentially equivalent, heritability has several practical definitions. One definition states that heritability is equal to the proportion of observed differences among or

ganisms for a trait due to genetic differences. For example, if one-quarter of the differences among cows for the amount of milk they produce are caused by differences among their genotypes, the heritability of milk production is 25 percent. The remaining 75 percent of differences among the animals are attributed to environmental effects. An alternative definition is that heritability is equal to the proportion of differences among sets of parents that are passed on to their progeny. For example, if the average height of a pair of parents is 8 inches (20 centimeters) more than the mean of their population and the heritability of height is 50 percent, their progeny would be expected to average 4 inches (10 centimeters) taller than their peers in the population.

Fundamental Relationships of Quantitative Genetics

Two relationships are fundamental to the understanding and application of quantitative genetics. First, there is a tendency for likeness among related individuals. Although similarities of human stature and facial appearance within families are familiar to most people, similar relationships hold for such traits in all organisms. Correlation among relatives exists for such diverse traits as blood pressure, plant height, grain yield, and egg production. These correlations are caused by relatives sharing a portion of genes in common. The more closely the individuals are related, the greater the proportion of genes that are shared. Identical twins share all their genes, and full brothers and sisters or parent and offspring are expected to share one-half their genes. This relationship is commonly utilized in the improvement of agricultural organisms. Individuals are chosen to be parents based on the performance of their relatives. For example, bulls of dairy breeds are chosen to become widely used as sires based on the milk-producing ability of their sisters and daughters.

The second fundamental relationship is that, in organisms that do not normally self-fertilize, vigor is depressed in progeny that result from the mating of closely related individuals. This effect is known as inbreeding depression. It may be the basis of the social taboos

regarding incestuous relationships in humans and for the dispersal systems for some other species of mammals such as wolves. Physiological barriers have evolved to prevent fertilization between close relatives in many species of plants. Some mechanisms function as an anatomical inhibitor to prevent union of pollen and ova from the same plant; in maize, for example, the male and female flower are widely separated on the plant. Indeed, in some species such as asparagus and holly trees, the sexes are separated in different individuals; thus all seeds must consequently result from cross-pollination. In other systems, cross-pollination is required for fertile seeds to result. The pollen must originate from a plant genetically different from the seed parent. These phenomena are known as self-incompatibility and are present in species such as broccoli, radishes, some clovers, and many fruit trees.

The corollary to inbreeding depression is hybrid vigor, a phenomenon of improved fitness that is often evident in progeny resulting from the mating of individuals less related than the average in a population. Hybrid vigor has been utilized in breeding programs to achieve remarkable productivity of hybrid seed corn as well as crossbred poultry and livestock. Hybrid vigor results in increased reproduction and efficiency of nutrient utilization. The mule, which results from mating a male donkey to a female horse, is a well-known example of a hybrid that has remarkable strength and hardiness compared to the parent species, but which is, unfortunately, sterile.

Quantitative Traits of Humans

Like other organisms, many traits of humans are quantitatively inherited. Psychological characteristics, intelligence quotient (IQ), and birth weight have been studied extensively. The heritability of IQ has been reported to be high. Other personality characteristics such as incidence of depression, introversion, and enthusiasm have been reported to be highly heritable. Musical ability is another characteristic under some degree of genetic control. These results have been consistent across replicated studies and are thus expected to be reliable; however, some caution must be exercised when consid-

ering the reliability of results from individual studies. Most studies of heritability in humans have involved likeness of twins reared together and apart. The difficulty in obtaining such data results in a relatively small sample size, at least relative to similar experiments in animals. An unfortunate response to studies of quantitative inheritance in humans was the eugenics movement.

Birth weight of humans is of interest because it is both under genetic control and subject to influence by well-known environmental factors, such as smoking by the mother. Birth weight is subject to stabilizing selection, in which individuals with intermediate values have the highest rates of survival. This results in genetic pressure to maintain the average birth weight at a relatively constant value.

Quantitative Characters in Agricultural Improvement

The ability to meet the demand for food by a growing world population is dependent upon continuously increasing agricultural productivity. Reserves of high-quality farmland have nearly all been brought into production, and a sustainable increase in the harvest of fish is likely impossible. Many countries that struggle to meet the food demands of their populations are too poor to increase agricultural yields through increased inputs of fertilizer and chemicals. Increased food production will, therefore, largely depend on genetic improvement of the organisms produced by farmers worldwide.

Most characteristics of economic value in agriculturally important organisms are quantitatively inherited. Traits such as grain yield, baking quality, milk and meat production, and efficiency of nutrient utilization are under the influence of many genes as well as the production environment. The task of breeders is not only to identify organisms with superior genetic characteristics but also to identify those breeds and varieties well adapted to the specific environmental conditions in which they will be produced. The type of dairy cattle that most efficiently produces milk under the normal production circumstances in the United States, which includes high health status, unlimited

access to high-quality grain rations, and protection from extremes of heat and cold, may not be ideal under conditions in New Zealand in which cattle are required to compete with herdmates for high-quality pasture forage. Neither of these animals may be ideal under tropical conditions where extremely high temperatures, disease, and parasites are common.

Remarkable progress has been made in many important food crops. Grain yield has responded to improvement programs. Development of hybrid corn increased yield several-fold over the last few decades of the twentieth century. Development of improved varieties of small grains resulted in an increased ability of many developing countries to be self-sufficient in food production. Grain breeder Norman Borlaug won the Nobel Peace Prize in 1970 for his role in developing grain varieties that contributed to the Green Revolution.

Can breeders continue to make improvements in the genetic potential for crops, livestock, and fish to yield enough food to support a growing human population? Tools of biotechnology are expected to increase the rate at which breeders can make genetic change. Ultimately, the answer depends upon the genetic variation available in the global populations of food-producing organisms and their wild relatives. The potential for genetic improvement of some species has been relatively untapped. Domestication of fish for use in aquaculture and utilization of potential crop species such as amaranth are possible food reserves. Wheat, corn, and rice provide a large proportion of the calories supporting the world population. The yields of these three crop species have already benefited from many generations of selective breeding. For continued genetic improvement, it is critical that variation not be lost through the extinction of indigenous strains and wild relatives of important food-producing organisms.

Impact and Applications

Molecular genetics and biotechnology have also added new tools for analyzing the genetics of quantitative traits. In any organism that has had its genome adequately mapped, genetic markers can be used to determine the number of loci involved in a particular trait. In carefully

constructed crosses geneticists look for statistical correlations between markers and the trait of interest. When a high correlation is found, the marker is said to represent a quantitative trait locus (QTL). Often a percentage effect for each QTL can be determined and because the location of markers is typically known, the potential location of the gene can also be inferred (that is, somewhere near the marker). A good understanding of the QTLs involved in the expression of a quantitative trait can help determine the best way to improve the organism.

Although QTLs are much easier to discover in organisms where controlled crosses are possible, studies have also been carried out in humans. In humans, geneticists must rely on whatever matings have happened, and due to ethical limitations, cannot set up specific crosses. Studies in humans have attempted to quantify the number of QTLs responsible for such things as IQ and various physical traits. One study even purported to show that homosexuality is genetically based. Although there is some support for such studies, much contro-

versy surrounds them, and ethicists continue to worry that conclusions from such research will be used in a new wave of eugenics. In spite of the risk of misusing an improved understanding of human quantitative traits, human biology and medicine stand to benefit.

—William R. Lamberson, updated by Bryan Ness

See also: Artificial Selection; Biofertilizers; Consanguinity and Genetic Disease; Epistasis; Genetic Load; Hardy-Weinberg Law; Hybridization and Introgression; Inbreeding and Assortative Mating; Mendelian Genetics; Polygenic Inheritance; Population Genetics; Speciation; Twin Studies.

Further Reading

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Race

Field of study: Human genetics and social issues

Significance: Humans typically have been categorized into a small number of races based on common traits, ancestry, and geography. Knowledge of human genomic diversity has increased awareness of ambiguities associated with traditional racial groups. The sociopolitical consequences of using genetics to devalue certain races are profound, and based on the available data, are completely baseless.

Key terms

EUGENICS: a movement concerned with the improvement of human genetic traits, predominantly by the regulation of mating

HUMAN GENOME DIVERSITY PROJECT: an extension of the Human Genome Project in which DNA of native people around the world is collected for study

POPULATION: a group of geographically localized, interbreeding individuals

RACE: a collection of geographically localized populations with well-defined genetic traits

History of Racial Classification

Efforts to classify humans into a number of distinct types date back at least to the ancient Greeks. Applying scientific principles to separate people into races has been a goal for more than two centuries. In 1758, the founder of biological classification, Swedish botanist Carolus Linnaeus, arranged humans into four principal races: *Americanus*, *Europeus*, *Asiaticus*, and *Afer*. Although geographic location was his primary organizing factor, Linnaeus also described the races according to subjective traits such as temperament. Despite his use of archaic criteria, Linnaeus did not give superior status to any of the races.

Johann Friedrich Blumenbach, a German naturalist and admirer of Linnaeus, developed a classification with lasting influence. Many of his contemporaries believed that different groups of humans arose separately in several regions of the world. Blumenbach, on the other hand, strongly believed in one form of human and believed that physical variations among

races were chiefly caused by differences in environment. Therefore, his scheme sought to show a gradual change in bodily appearance, all deviating from an original type. Blumenbach maintained that the original forms, which he named "Caucasian," were those primarily of European ancestry. His final classification, published in 1795 in *On the Natural Variety of Mankind*, consisted of five races: Caucasian, Malay, Ethiopian, American, and Mongolian. Two races directly radiated from the Caucasians: the Malay and the American. The Malay (Pacific islanders) then generated the Ethiopian (Africans), while the American (from the New World) gave rise to the Mongolian (East Asians). The fifth race, the Malay, was added to Linnaeus's classification to show a step-by-step change from the original body type.

After Linnaeus and Blumenbach, many variations of their categories were formulated, chiefly by biologists and anthropologists. Classification "lumpers" combined people into only a few races (for example, black, white, and Asian). "Splitters" separated the traditional groups into many different races. One classification scheme divided all Europeans into Alpine, Nordic, and Mediterranean races. Others split Europeans into ten different races. No one scheme of racial classification came to be accepted throughout the scientific community.

Genetic Diversity Among Races

The genetic components of a population are produced by three primary factors: natural selection, nonadaptive genetic change, and mating between neighboring populations. The first two factors may result in differences between populations, and reproductive isolation, either voluntary or because of geographic isolation, perpetuates the distinctions. Natural selection refers to the persistence of genetic traits favorable in a specific environment. For example, a widely held assumption concerns skin color, primarily a result of the pigment melanin. Melanin offers some shielding from ultraviolet solar rays. According to this theory, people living in regions with concentrated ultraviolet exposure have increased melanin synthesis and,

therefore, dark skin color conferring additional protection against skin cancer. Individuals with genes for increased melanin have enhanced survival rates and reproductive opportunities. The reproductive opportunities produce offspring that inherit those same genes for increased melanin. This process results in a higher percentage of the population with elevated melanin production genes. Therefore, genes coding for melanin production are favorable and persist in these environments.

The second factor contributing to the genetic makeup of a population is nonadaptive genetic change. This process involves random genetic mutations. Mutations are changes resulting in modified forms of the same gene. For example, certain genes are responsible for eye color. Individuals contain alternate forms of these genes, or alleles, which result in observed differences in eye color. Alleles resulting from nonadaptive genetic change may remain in the population because of their neutral nature. In other words, they are not harmful or beneficial. Because these traits are impartial to environmental influences, they may endure from generation to generation. Different populations will spontaneously produce, persist, and delete them. Genetic difference between populations caused by these random mutations and isolation is called genetic drift.

The third factor, mating between individuals from neighboring groups, tends to merge traits from several populations. This genetic mixing often results in offspring with blended characteristics and only moderate variations between adjacent groups.

Several studies have compared the overall genetic complement of various human populations. On average, any two people of the same or a different race diverge genetically by a mere 0.2 percent. It is estimated that only 0.012 percent contributes to traditional racial variations. Hence, most of the genetic dissimilarities between a person of African descent and a person of European descent are also different between two individuals with the same ancestry. The genes do not differ. It is the proportion of individuals expressing a specific allele of a gene that varies from population to population.

Upon closer examination, it was found that

Africa is unequaled with respect to cumulative genetic diversity. If overall genetic distinctness is evaluated, numerous races are found in Africa, Khoisan Africans of southern Africa being the most distinct. According to one theory, the remainder of the human species (including Asians, Europeans, and aboriginal Australians) corresponds to only one other race.

Conflicts Concerning Definitions of Race

Linnaeus developed a scientific system of classification that is fundamentally still in use. This approach involves separating all organisms first into broad groups based on general characteristics. These large groups are broken down further into smaller and smaller groups, each subdivision containing individuals with more similarities. For example, humans are found within the large kingdom containing all types of animals. Animals are separated based on the formation of a backbone. Of those animals containing a backbone, humans are placed into a set with all mammals and then further cataloged with other primates. Each succeeding classification unit contains individuals more alike, since the characteristics used to define each subdivision are more specific. Eventually, all organisms are placed into a species category. Humans belong in the species *Homo sapiens*. By definition, a sexually reproducing species contains all individuals that can mate and produce fertile offspring. Race is analogous to a more specific unit, the subspecies, a fundamentally distinct subgroup within one species.

For a racial or subspecies classification scheme to be objective and biologically meaningful, researchers must decide carefully which heritable characteristics (passed to future generations genetically) will define, or separate, the races. Several principles are considered. First, the discriminating traits must be discrete. In other words, differences among races must be distinguishable, not continually changing by small degrees between populations. Second, everyone placed within a specific race must possess the selected trait's defining variant. Features used to describe a race must agree. This means that all of the selected characteristics are found consistently in each member. For example, if blue eyes and brown hair are cho-

sen as defining characteristics, everyone designated as belonging to that race must share both of those characteristics. Individuals placed in other races should not exhibit this particular combination. The purpose of using these characteristics is to distinguish groups. Consequently, if traits are shared by members of two or more races, their defining value is poor. Third, individuals of the same race must have descended from a common ancestor, unique to those people. Many shared characteristics present in individuals of a race may be traced to that ancestor by heredity. Based on the preceding defining criteria (selection of discrete traits, agreement of traits, and common ancestry), pure representatives of each racial category should be detectable.

Many researchers maintain that traditional races do not conform to accepted scientific

principles of subspecies classification. For example, the traits used to define traditional human races are rarely discrete. Skin color, a prominent characteristic employed, is not a well-defined trait. Approximately five genes influence skin color significantly, but fifty or so likely contribute. Pigmentation in humans results from a complex series of biochemical pathways regulated by amounts of enzymes (molecules that control chemical reactions) and enzyme inhibitors, along with environmental factors. Like most complex traits involving many genes, human skin color varies on a continuous gradation. From lightest to darkest, all intermediate pigmentations are represented. Color may vary widely even within the same family. The boundary between black and white is an arbitrary, human-made border, not one imposed by nature.

From DNA to Humans

DNA Codes for ~80,000 different proteins in trillions of cells

CGTTCTCTATTAACA...

GCAAGAGATAATTGT...

3 billion DNA subunits in the cell nucleus

YGG-00-0482

On average, any two people of the same or a different race diverge genetically by a mere 0.2 percent, and only 0.012 percent contribute to traditional racial variations. Allelic variations account for most of the superficial differences perceived as race. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

In addition, traditional defining racial characteristics, such as skin color and facial characteristics, are not found in all members of a race; they are not in agreement. For example, many Melanesians, indigenous to Pacific islands, have pigmentation as dark as any human but are not classified as "black." Another example concerns unclassifiable populations. For example, many individuals native to India have Caucasoid facial features and very dark skin, yet live in Asia. When traditional racial characteristics are examined closely, many groups are left with no conventional race. No "pure" genetic representatives of any traditional race exist.

Common ancestry, or evolutionary relationships, must also be considered. Genetic studies have shown that Africans do not belong to a single "black" heritage. In fact, several lineages are found in Africa. An even greater variance is found in African Americans. Besides a diverse African ancestry, it is estimated that, on average, 20 to 30 percent of African American heritage is European or Native American. Yet all black Americans are consolidated into one race.

The true diversity found in humans is not patterned according to accepted standards of the subspecies. Only at extreme geographical distances are notable differences found. However, "in-between" populations have always been in existence because of mating, and therefore gene flow, between neighboring groups. Consequently, human populations in close proximity have more genetic similarities than distant populations. It is the population itself that best illustrates the pattern of human diversity. Well-defined genetic borders between human populations are not observed, and racial boundaries in classification schemes are often formed arbitrarily.

Theories of Human and Racial Evolution

Advances in DNA technology have greatly aided researchers in their quest to reconstruct the history of *Homo sapiens* and its various subgroups. Analysis of human DNA has been performed on both nuclear and mitochondrial DNA. Mitochondria are organelles responsible for generating cellular energy. Each mitochondrion contains a single, circular DNA molecule accounting for approximately 0.048 percent of the entire genetic complement. In 1987, geneticist Rebecca L. Cann compared mitochondrial DNA from many populations: African, Asian, Caucasian, Australian, and New Guinean. Agreeing with other mitochondrial and nuclear DNA studies, the results indicated that Africans were the most genetically variable by a significant extent. The results suggested to Cann that Africa was the root of all humankind and that humans first arose there 100,000 to 200,000 years ago. Several lines of research, including DNA analysis of humanoid fossils, provide evidence for this theory.

Many scientists are using genetic markers to decipher the migrations that fashioned past and present human populations. For example, DNA comparisons revealed three Native American lineages. Some scientists believe one migration crossed the Bering Strait, most likely from Mongolia. Only after further migration throughout the Americas were the three American Indian lineages formed. Another theory states that three separate Asian migrations occurred, each bringing a different lineage. Another example is the South African Lemba community. DNA analysis gives credence to their claim as one of the lost tribes of Israel. Considering the cumulative evidence, many scientists regard a more correct depiction of human populations to be a roughly inverted version of Blumenbach's. Asians arose from Africans, and Europeans are Asian and North African hybrids. However, interpretations of DNA analyses are, almost inevitably, controversial. Multiple theories abound and are revised as additional research is performed.

Sociopolitical Implications

Race is often portrayed as a natural, biological division, the result of geographic isolation and adaptation to local environment. However, confusion between biological and cultural classification obscures perceptions of race. When individuals describe themselves as "black," "white," or "Hispanic," for example, they are usually describing cultural heredity as well as biological similarities. The relative importance of perceived cultural affiliations or genetics

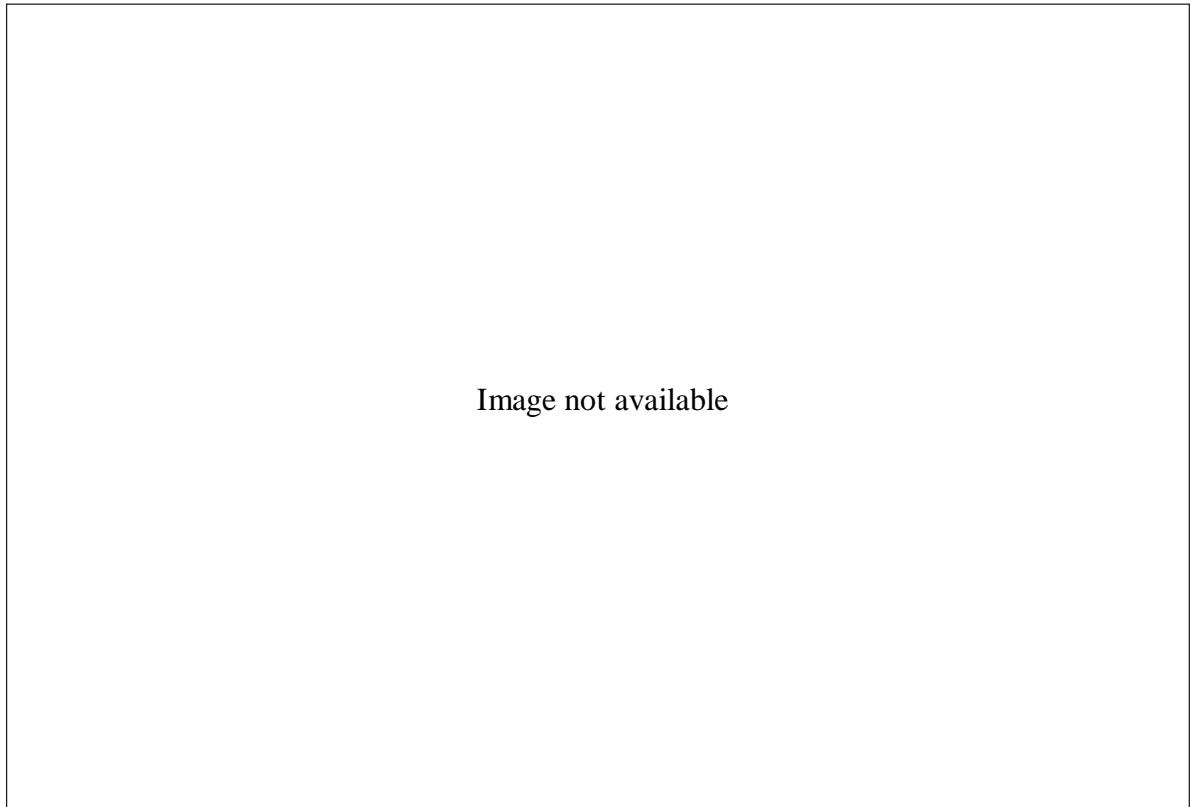


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Descendants of Sally Hemings, an African American slave of Thomas Jefferson who is known by DNA evidence to have had children by him, pose at Jefferson's home, Monticello, during a July, 2003, reunion. (AP/Wide World Photos)

varies depending on the circumstances. Examples illustrating the ambiguities are abundant. Nearly all people with African American ancestry are labeled black, even if they have a white parent. In addition, dark skin color designates one as belonging to the black race, including Africans and aboriginal Australians, who have no common genetic lineage. State laws, some on the books until the late 1960's, required a "Negro" designation for anyone with one-eighth black heritage (one black great-grandparent).

Unlike biological boundaries, cultural boundaries are sharp, repeatedly motivating discrimination, genocide, and war. The frequent use of biology to devalue certain races and excuse bigotry has profound implications for individuals and society. In the early and mid-twentieth century, the eugenics movement, advocating the genetic improvement of the human species, translated into laws against

interracial marriage, sterilization programs, and mass murder. Harmful effects include accusations of deficiencies in intelligence or moral character based on traditional racial classification.

The frequent use of biology to devalue certain races and excuse bigotry has profound implications for individuals and society. Blumenbach selected Caucasians (who inhabit regions near the Caucasus Mountains, a Russian and Georgian mountain range) as the original form of humans because in his opinion they were the most beautiful. All other races deviated from this ideal and were, therefore, less beautiful. Despite Blumenbach's efforts not to demean other groups based on intelligence or moral character, the act of ranking in any form left an ill-fated legacy.

Many scientists are attempting to reconcile the negativities associated with racial studies. The Human Genome Diversity Project, a global

undertaking, has requested that researchers collect and store DNA from indigenous populations around the world. These samples will be available to all qualified scientists. Results of the studies may include gene therapy treatments and greater success with organ transplantation. A more thorough understanding of the genetic diversity and unity in the species *Homo sapiens* will as a result be possible.

—Stacie R. Chismark

See also: Biological Determinism; Eugenics; Eugenics: Nazi Germany; Evolutionary Biology; Genetic Engineering; Social and Ethical Issues; Heredity and Environment; Intelligence; Miscegenation and Antimiscegenation Laws; Sociobiology; Sterilization Laws.

Further Reading

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Cavalli-Sforza, Luigi L., et al. *The History and Geography of Human Genes*. Princeton, N.J.: Princeton University Press, 1996. Often referred to as a "genetic atlas," this volume contains fifty years of research comparing heritable traits, such as blood groups, from more than one thousand human populations. Illustrations, maps, bibliography, index.

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single intelligence and that cognitive ability is shaped through education. Bibliography, index.

Fraser, Steven, ed. *The "Bell Curve" Wars: Race, Intelligence, and the Future of America*. New York: Basic Books, 1995. Brief, critical response to the book by Herrnstein and Murray by scholars from a variety of disciplines and backgrounds. Bibliography.

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Graves, Joseph L., Jr. *The Emperor's New Clothes: Biological Theories of Race at the Millennium*. New Brunswick, N.J.: Rutgers University Press, 2001. Argues for a more scientific approach to debates about race, one that takes human genetic diversity into account. Illustrations, bibliography, index.

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Valencia, Richard R., and Lisa A. Suzuki. *Intelligence Testing and Minority Students: Foundations, Performance Factors, and Assessment Issues*. Thousand Oaks, Calif.: Sage, 2000. Historical and multicultural perspective on intelli-

gence and its often assumed relation with socioeconomic status, home environment, test bias, and heredity. Illustrations, bibliography, index.

Web Sites of Interest

Genetics and Identity Project. http://www.bioethics.umn.edu/genetics_and_identity. Project looks at the ways genetic research affects racial, ethnic, and familial identities.

Human Genome Project Research Institute, Minorities, Race, and Genomics. http://www.ornl.gov/techresources/human_genome/elsi/minorities.html. Site provides information on race and genetic research, particularly on how that research affects minority communities.

National Academies Press, Evaluating Human Genetic Diversity. <http://www.nap.edu>. A free, downloadable book on human genetic diversity, which includes the chapter “Human Rights and Human Genetic-Variation Research.”

VARIABLE NUMBER TANDEM REPEAT (VNTR): a type of DNA sequence in which a short sequence is repeated over and over; chromosomes from different individuals frequently have different numbers of the basic repeat, and if many of these variants are known, the sequence is termed a hypervariable

Types of Repetitive DNA

The nuclear genomes of eukaryotes are characterized by repetitive DNA elements consisting of nucleotide sequences that vary in length and base composition and that are localized to a particular region of the genome or dispersed throughout the genome (for example, on different chromosomes). Some repetitive DNA elements are found in the genome a few times, whereas others may be repeated millions or billions of times; thus, the percentage of the total genome represented by repetitive DNA varies widely among taxa.

There are two major classes of tandem repetitive DNAs (TR-DNAs): those that are localized to a particular region (or regions) of the genome and those that are dispersed throughout the genome. TR-DNAs are composed of repeating units that are oriented in “head-to-tail” arrays. The repetitive units of an array may include genes, promoters, and intergenic spacers or repeats of simple nucleotide sequences. For example, in the kangaroo rat the simple sequence AAG is repeated 2.4 billion times.

Localized TR-DNA is often composed of members of multigene families. For example, in humans there are 350 copies of the ribosomal RNA (rRNA) genes on five different chromosomes that occur as tandemly repeated arrays. Transfer RNA (tRNA) and immunoglobulin genes represent other examples of multigene families that are tandemly repeated. However, most localized TR-DNA consists of simple, noncoding repetitive DNA sequences that often, but not always, can be found in heterochromatic or centromeric regions.

Dispersed TR-DNA sequences are scattered throughout the genome and can be divided into two major groups: short interspersed elements (SINEs) and long interspersed repeats (LINEs).

Repetitive DNA

Field of study: Molecular genetics

Significance: *Eukaryotic nuclei contain repetitive DNA elements of different origin, which constitute between 20 and 90 percent of the genome depending on the species. The presence and type of repetitive DNA elements have provided insights into gene flow, forensic investigations, biomedicine, and genomic mapping.*

Key terms

NUCLEOTIDE: the basic unit of DNA, consisting of a five-carbon sugar, a nitrogen-containing base, and a phosphate group

POLYMORPHISM: the presence of many different alleles for a particular locus in individuals of the same species

RETROTRANSPOSITION: a subset of the replicative transposable elements that transpose through an RNA intermediate

TANDEM REPETITIVE DNA (TR-DNA): DNA are composed of repeating units that are oriented in “head-to-tail” arrays

Origin and Evolution of Dispersed DNA Elements

SINEs are nonviral retropseudogenes that were derived from genes encoding small, untranslated RNAs (for example, tRNAs). The RNA transcript was reverse transcribed into DNA and then was inserted into the genome. In their current state, although they resemble the genes they were from which they derived, they no longer function properly. SINEs are also examples of transposable elements capable of “jumping” from one locus to another via an RNA intermediate.

The best-characterized SINEs in humans are highly repetitive *Alu* sequences, so named because they are cleaved multiple times by the endonuclease *Alu*I, derived from the bacterium *Arthrobacter luteus*. Between 500,000 and 1 million *Alu* copies are scattered across the human genome, each approximately three hundred nucleotides in length. *Alu* sequences may constitute as much as 5 percent of the human genome.

LINEs are derived from a viral ancestor and are also capable of transposition. The most common LINE element in humans, constituting 5 percent of the human genome, is termed L1. There are about 200,000 copies of L1 in each diploid cell. Full-length, functional (that is, transpositionally competent) L1 elements are approximately 6 kilobase pairs (kb) in length, but most copies of L1 are truncated at the 5' end and incapable of moving. Full-length L1 copies contain two protein-coding regions, or open reading frames (ORFs): ORF-1 and ORF-2. ORF-1 encodes an RNA-binding protein, and ORF-2 codes for reverse transcriptase.

Classification of Simple Tandem Repeats

Simple sequences that are tandemly repeated are classified into four major groups based on three characteristics: the number of nucleotides in the repetitive unit, the number of times the unit is repeated, and whether or not the element is localized or scattered across the genome. Satellite DNA is composed of basic units, ranging from two to hundreds of nucleotides in length, that are repeated more than one thousand times. Satellite DNA represents an example of a localized simple repeat

that is typically found in centromeric regions. Units of between nine and one hundred nucleotides that are tandemly repeated ten to one hundred times and scattered throughout the genome are known as minisatellites. Microsatellites are also dispersed elements composed of short repeats of a basic unit one to six nucleotides in length that is tandemly repeated ten to one hundred times at each locus. The most common microsatellite loci in humans are di-nucleotide arrays of $(CA)_N$. However, on average there is at least one tri- or tetranucleotide microsatellite locus per 10 kb of human genomic DNA. Finally, the basic unit of dispersed *Alu* sequences is one to five nucleotides in length, and this unit is repeated ten to forty times per locus.

Polymorphism at Loci Composed of Simple Tandem Repeats

For purposes of convenience, the four groups of simple tandem repeats discussed above (satellite DNA, minisatellites, microsatellites, and *Alu* sequences) are sometimes collectively referred to as variable number tandem repeats (VNTRs).

Separate VNTR loci are thought of as alleles; therefore, in humans each VNTR locus will be represented by two alleles, one paternal and the other maternally inherited. All VNTR loci exhibit high rates of mutation. For these reasons, VNTR loci are highly polymorphic, that is, there are a large number of alleles at any given locus. This polymorphism can be assayed using laboratory techniques such as polymerase chain reaction (PCR) or Southern blotting to examine the differences in the lengths of the alleles (repetitive elements) at a particular locus.

Length differences at VNTR loci arise as a result of mispairing of repeats during replication, mitosis, or meiosis theoretically resulting in the loss or gain of one to many of the repeat units. Empirical studies and computer-based modeling experiments have demonstrated that each mutation usually increases or decreases the number of repeated units of an allele in a “one-step” manner. In other words, most mutations result in the loss or gain of only one repeated unit.

The multiallelic variation that arises through variation in repeat copy number provides genetic markers useful for many different applications. For example, under conditions of random mating and because of high mutation rates at VNTR loci, most individuals within the human population are heterozygous at any selected VNTR locus. This observation directly led to the origin of DNA fingerprinting (or DNA profiling), which is now considered admissible forensic evidence in many judicial systems worldwide. Length variation of VNTRs creates a powerful tool for identity analysis (for example, paternity testing) and is routinely used by population geneticists to examine gene flow among populations. In the fields of genomics and biomedicine, VNTR loci are useful genetic landmarks for mapping the location of other genes of interest, that is, those with a particular function or others implicated in disease.

Are Interspersed Repeated Elements “Junk” DNA?

Repeated DNA elements were once believed to be “selfish” or “junk” DNA, concerned only with their own proliferation within the host cell’s genome. Recent studies, however, reveal that repetitive elements interact with the genome with profound evolutionary consequences. For example, satellite DNA found near the centromere may play a role in assembling and fusing chromosomal microtubules during cell division. It is also now clear that transposable genetic elements such as SINES, LINEs, and *Alu* sequences may have played a significant role in the evolution of particular proteins. For example, *Alu* elements flanking the primordial human growth hormone gene are responsible for the evolution of a relatively new member of the gene family, the chorionic somatomammotropin gene. Transposable repeated elements may have contributed substantially to the origin of new gene functions by initiating a copy of an existing gene (which, over time, can acquire a different function) or by creating “composite” genes composed of domains from two or more previously unrelated genes.

Transposable Elements and Human Disease

Retrotranspositions of LINEs and SINES into coding or noncoding genomic DNAs represent major insertional mutations. The effects of such insertions vary but are usually deleterious, leading to debilitating human diseases. Among a growing list of diseases known in some cases to be caused by the insertion of LINEs or SINES are Duchenne muscular dystrophy, Glanzmann thrombasthenia, hemophilia, hypercholesterolemia, neurofibromatosis, Sandhoff disease, and Tay-Sachs disease. Translocation of repeated sequences has also been demonstrated to “turn on” tumorogenic oncogenes (for example, one type of colon cancer).

Other studies have shown that “unstable” minisatellite, microsatellite, and *Alu* loci can also cause disease. In short, there seems to be a threshold number of repeats of the basic nucleotide unit that can be accommodated at a given locus. When this threshold is exceeded by overamplification of the basic repeated unit, serious diseases may arise. Among those diseases attributed to overamplification of tandem repeats of simple sequences are fragile X syndrome and Huntington’s disease.

—J. Craig Bailey

See also: Aging; Anthrax; Chromosome Structure; Chromosome Walking and Jumping; DNA Fingerprinting; Gene Families; Genome Size; Genomics; Human Genetics; Model Organism: *Neurospora crassa*; Molecular Clock Hypothesis; Pseudogenes; RFLP Analysis; Telomeres.

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Restriction Enzymes

Fields of study: Genetic engineering and biotechnology; Molecular genetics

Significance: *Restriction enzymes are bacterial enzymes capable of cutting DNA molecules at specific nucleotide sequences. Discovery of these enzymes was a pivotal event in the development of genetic engineering technology, and they are routinely and widely used in molecular biology.*

Key terms

ENZYME: a molecule, usually a protein, that is used by cells to facilitate and speed up a chemical reaction

METHYLATION: the process of adding a methyl chemical group (one carbon atom and three hydrogen atoms) to a particular molecule, such as a DNA nucleotide

NUCLEASE: a type of enzyme that breaks down the sugar-phosphate backbone of nucleic acids such as DNA and RNA

NUCLEOTIDES: the building blocks of nucleic acids, composed of a sugar, a phosphate group, and nitrogen-containing bases

Discovery and Role of Restriction Enzymes in Bacteria

Nucleases are a broad class of enzymes that destroy nucleic acids by breaking the sugar-phosphate backbone of the molecule. Until 1970, the only known nucleases were those that destroyed nucleic acids nonspecifically—that is, in a random fashion. For this reason, these enzymes were of limited usefulness for working with nucleic acids such as DNA and RNA. In 1970, molecular biologist Hamilton Smith discovered a type of nuclease that could fragment DNA molecules in a specific and therefore pre-

dictable pattern. This nuclease, *HindII*, was the first restriction endonuclease or restriction enzyme. Smith was working with the bacterium *Haemophilus influenzae* (*H. influenzae*) when he discovered this enzyme, which was capable of destroying DNA from other bacterial species but not the DNA of *H. influenzae* itself. The term “restriction” refers to the apparent role these enzymes play in destroying the DNA of invading bacteriophages (bacterial viruses), while leaving the bacterial cell’s own DNA untouched. A bacterium with such an enzyme was said to “restrict” the host range of the bacteriophage.

As more restriction enzymes from a wide variety of bacterial species were discovered in the 1970’s, it became increasingly clear that these enzymes could be useful for creating and manipulating DNA fragments in unique ways. What was not clear, however, was how these enzymes were able to distinguish between bacteriophage DNA and the bacterial cell’s own DNA. A chemical comparison between DNA that could and could not be fragmented revealed that the DNA molecules differed slightly at the restriction sites (the locations the enzyme recognized and cut). Nucleotides at the restriction site were found to have methyl (CH_3) groups attached to them, giving this phenomenon the name DNA methylation.

The conclusion was that the methylation somehow protected the DNA from attack, and this could account for Smith’s observation that *H. influenzae* DNA was not destroyed by its own restriction enzyme; presumably the enzyme recognized a specific methylation pattern on the DNA molecule and left it alone. Foreign DNA (from another species, for example) would not have the correct methylation pattern, or it might not be methylated at all, and could therefore be fragmented by the restriction enzyme. Hence, restriction enzymes are now regarded as part of a simple yet effective bacterial defense mechanism to guard against foreign DNA, which can enter bacterial cells with relative ease.

Mechanism of Action

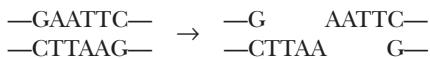
To begin the process of cleaving a DNA molecule, a restriction enzyme must first recognize

the appropriate place on the molecule. The recognition site for most restriction enzymes involves a short, usually four- to six-nucleotide, palindromic sequence. A palindrome is a word or phrase that reads the same backward and forward, such as “Otto” or “madam”; in terms of DNA, a palindromic sequence is one that reads the same on each strand of DNA but in opposite directions. *EcoRI* (derived from the bacterium *Escherichia coli*) is an example of an enzyme that has a recognition site composed of nucleotides arranged in a palindromic sequence:



If the top sequence is read from left to right or the bottom sequence is read from right to left, it is always GAATTC.

An additional consideration in the mechanism of restriction enzyme activity is the type of cut that is made. When a restriction enzyme cuts DNA, it is actually breaking the “backbone” of the molecule, consisting of a chain of sugar and phosphate molecules. This breakage occurs at a precise spot on each strand of the double-stranded DNA molecule. The newly created ends of the DNA fragments are then informally referred to as “sticky ends” or “blunt ends.” These terms refer to whether single-stranded regions of DNA are generated by the cutting activity of the restriction enzyme. For example, the enzyme *EcoRI* is a “sticky end” cutter; when the cuts are made at the recognition site, the result is:



The break in the DNA backbone is made just after the G in each strand; this helps weaken the connections between the nucleotides in the middle of the site, and the DNA molecule splits into two fragments. The single-stranded regions, where the bases TTAA are not paired with their complements (AATT) on the other

The Action of Restriction Enzymes

Part of Duplex with Bonds Broken by Chosen RE

...xxCTATA GxxxxxCTATA GxxxCTATA G...

...xxG ATATCxxxxx GATATCxxx GATATC...

↓
RE

...xxCTATA GxxxxxCTATA GxxxCTATA G...

...xxG ATATCxxxxx GATATCxxx GATATC...

DNA Fragments with Sticky Ends

A restriction enzyme (RE) breaks part of a duplex into fragments with “sticky ends.” Each x denotes an unspecified base in a nucleotide unit.

strand, are called overhangs; however, the bases in one overhang are still capable of pairing with the bases in the other overhang as they did before the DNA strands were cut. The ends of these fragments will readily stick to each other if brought close together (hence the name “sticky ends”).

Enzymes that create blunt ends make a flush cut and do not leave any overhangs, as demonstrated by the cutting site of the enzyme *AluI*:

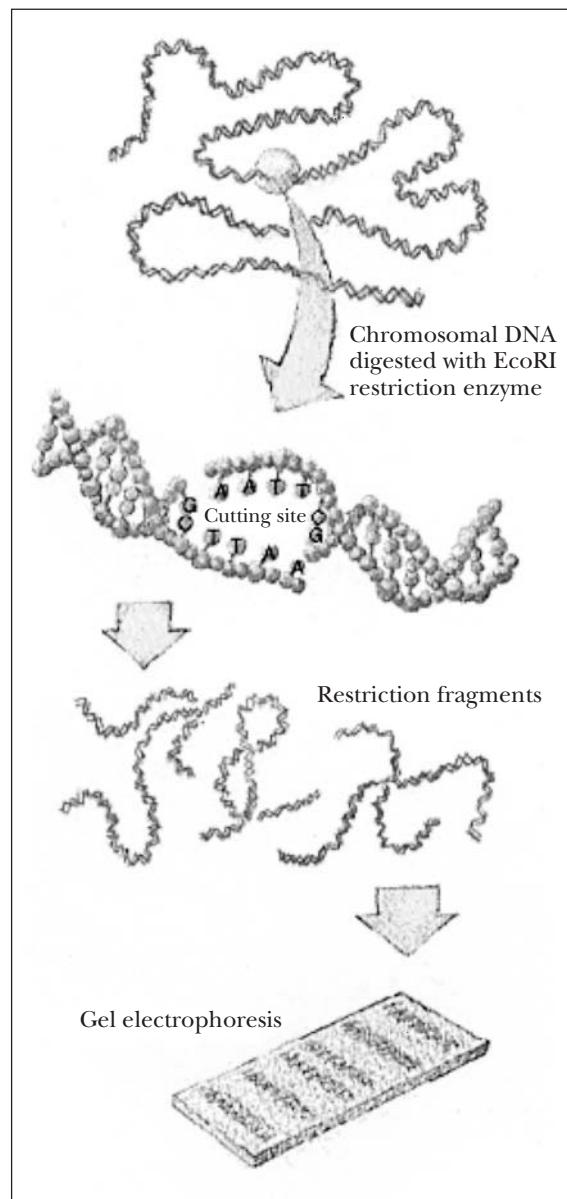


Because of the lack of overhanging single-strand regions, these two DNA fragments will not readily rejoin. In practice, either type of restriction enzyme may be used, but enzymes that produce sticky ends are generally favored over blunt-end-cutting enzymes because of the ease with which the resulting fragments can be rejoined.

Impact and Applications

It is no exaggeration to say that the entire field of genetic engineering would have been impossible without the discovery and wide-

spread use of restriction enzymes. On the most basic level, restriction enzymes allow scientists to create recombinant DNA molecules (hybrid molecules containing DNA from different sources, such as humans and bacteria). No matter what the source, DNA molecules can be cut with restriction enzymes to produce fragments that can then be rejoined in new combinations



The process of cutting DNA with restriction enzymes. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

with DNA fragments from other molecules. This technology has led to advances such as the production of human insulin by bacterial cells such as *Escherichia coli*.

The DNA of most organisms is relatively large and complex; it is usually so large, in fact, that it becomes difficult to manipulate and study the DNA of some organisms, such as humans. Restriction enzymes provide a convenient way to cut large DNA molecules very specifically into smaller fragments that can then be used more easily in a variety of molecular genetics procedures.

Another area of genetic engineering that is possible because of restriction enzymes is the production of restriction maps. A restriction map is a diagram of a DNA molecule showing where particular restriction enzymes cut the molecule and the molecular sizes of fragments that are generated. The restriction sites can then be used as markers for further study of the DNA molecule and to help geneticists locate important genetic regions. Use of restriction enzymes has also revealed other interesting and useful markers of the human genome, called restriction fragment length polymorphisms (RFLP). RFLP refers to changes in the size of restriction fragments caused by mutations in the recognition site for a particular restriction enzyme. More specifically, the recognition site is mutated so that the restriction enzyme no longer cuts there; the result is one long fragment where, before the mutation, there would have been two shorter fragments. These changes in fragment length can then be used as markers for the region of DNA in question. Because they result from mutations in the DNA sequence, they are inherited from one generation to the next. Thus these mutations have been a valuable tool for molecular biologists in producing a map of human DNA and for those scientists involved in “fingerprinting” individuals by means of their DNA.

—Randall K. Harris, updated by Bryan Ness

See also: Bacterial Genetics and Cell Structure; Bioinformatics; Biopharmaceuticals; Blotting: Southern, Northern, and Western; Cloning; Cloning Vectors; DNA Fingerprinting; Forensic Genetics; Gender Identity; Genetic Engineering; Genetic Engineering;

Historical Development; Genetic Engineering; Social and Ethical Issues; Genomic Libraries; Model Organism: *Xenopus laevis*; Molecular Genetics; Population Genetics; RFLP Analysis; Shotgun Cloning; Synthetic Genes.

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Reverse Transcriptase

Fields of study: Genetic engineering and biotechnology; Molecular genetics

Significance: *Retroviruses infect eukaryotic cells, using reverse transcriptases (RTs) to turn their RNA genomes to DNA that enables their host to use the DNA to make new virus particles. Retroviral DNA, often dormant for years before new virus particles are released, can be oncogenic, giving infected cells high incidences of cancer. Purified RTs are used to make RNA into DNA for biotechnology.*

Key terms

DEOXYRIBONUCLEOSIDE TRIPHOSPHATE (dNTP): one of four monomers (dATP, dCTP, dGTP, dTTP) incorporated into DNA

DNA POLYMERASE: an enzyme that catalyzes the formation of a DNA strand using a template DNA or RNA molecule as a guide

PRIMER: A short piece of single-stranded DNA that can hybridize to denatured DNA and

provide a start point for extension by a DNA polymerase

PROOFREADING ACTIVITY: enzyme activity in DNA polymerase that fixes errors made in copying templates

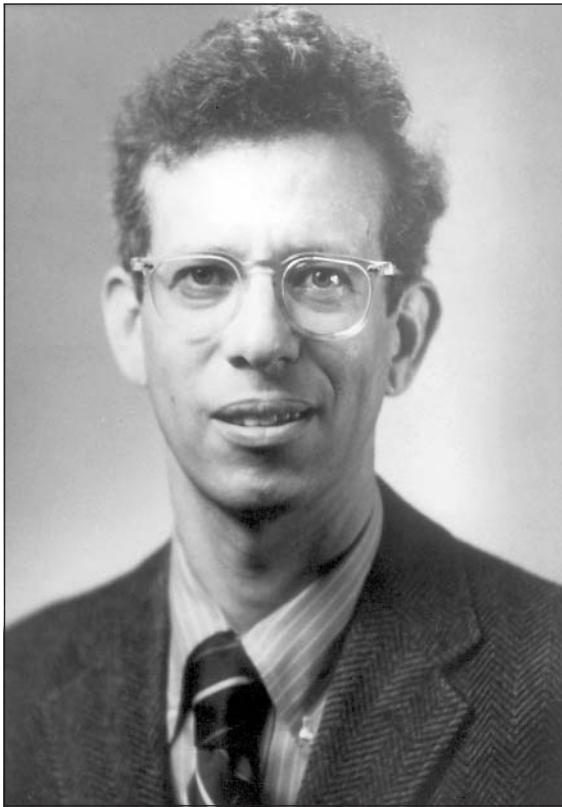
RETROVIRUSES: viruses that possess RNA genomes with genetic information that flows from RNA to host DNA via reverse transcriptases

Genetic Information Flow and Retroviruses

The central dogma of molecular genetics states that information flow is from DNA to RNA to proteins. RNA polymerase transcribes RNA using a DNA template. For structural genes, the transcribed RNA is a messenger RNA (mRNA), which is used by ribosomes to produce a protein. To maintain and reproduce its DNA, an organism uses RNA to make DNA, via DNA polymerase. It was long believed by geneticists that there were no exceptions to the central dogma.

Some viruses, retroviruses, possess RNA genomes with genetic information flow from RNA to DNA (via reverse transcriptases), and back, before translation. Retroviruses have been isolated from cancers and cancer tissue cultures from birds, rodents, primates, and humans, and some retroviruses cause a high incidence of certain cancers. Flow of retroviral genetic information from RNA to DNA was proposed in 1964 by Howard Temin (1934-1994) for Rous sarcoma virus. Temin, along with David Baltimore, jointly received the Nobel Prize in Physiology or Medicine in 1975 for independently discovering the enzyme reverse transcriptase (RT).

Rous sarcoma virus causes tumors in birds. Temin's hypothesis was based on effects of nucleic acid synthesis inhibitors on replication of the virus. First, the process was inhibited by actinomycin D, an inhibitor of DNA-dependent RNA synthesis. Furthermore, DNA synthesis inhibition by cytosine arabinoside, early after infection, stopped viral replication. Therefore, a DNA intermediate seemed involved in viral replication. The expected process was termed reverse transcription because RNA becomes DNA instead of DNA becoming RNA.



Howard M. Temin. (© The Nobel Foundation)

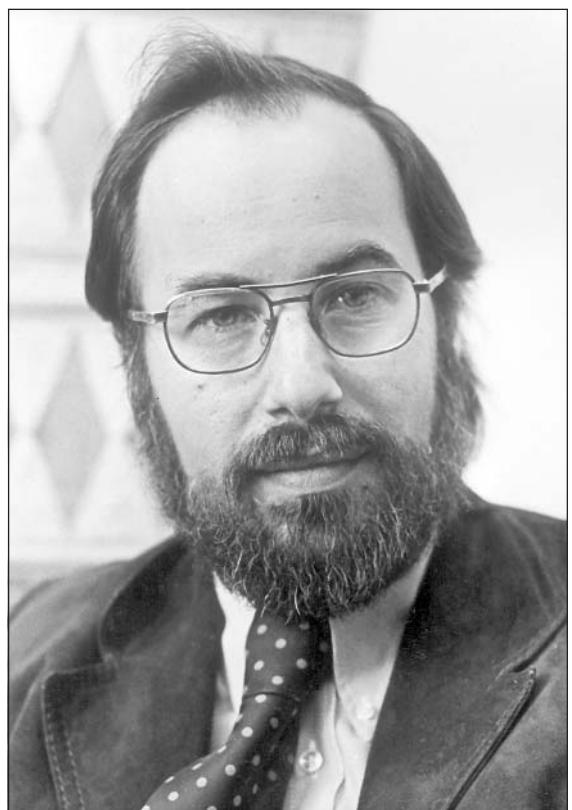
RT Discovery and Properties

Retrovirus infection begins with injection of RT and single-stranded RNA into host cells. RT (an RNA-dependent DNA polymerase) causes biosynthesis of viral DNA using an RNA template from the retrovirus HIV (human immunodeficiency virus), the causative agent of acquired immunodeficiency syndrome (AIDS). RTs have been purified from many retroviruses. Avian, murine, and human RTs have been studied most. All have ribonuclease H (RNase H) activity on the same protein as polymerase activity. Ribonuclease H degrades RNA strands of DNA-RNA hybrids. A nuclease that degrades DNA is later involved in retrovirus DNA integration into host cell DNA. Most biochemical properties of purified RTs are common to them and other DNA polymerases. For example, all require the following for DNA synthesis: a primer on which synthesis begins, a template which is copied, and a supply of the four dNTPs.

An RT converts a retroviral single-stranded RNA genome to “integrated double-stranded DNA” as follows: First a hybrid (DNA-RNA) duplex is made from viral RNA, as an antiparallel DNA strand is produced. The RNA-directed DNA polymerase activity of RT is primed by host cell transfer RNA, which binds to the viral RNA. Then, the viral RNA strand is destroyed by RNase H, and the first DNA strand now becomes the template for synthesis of a second antiparallel DNA strand. Resultant duplex DNA is next integrated into a host cell chromosome, where it is immediately used to make virus particles or, alternatively, it takes up residence in the host cell’s genome, remaining unused—often for years—until it is activated and causes cancer or production of new viruses.

Importance of Reverse Transcriptases

RTs can use almost any RNA template for DNA synthesis. Low RT template specificity allows RT to be used to make DNA copies of a



David Baltimore. (© The Nobel Foundation)

wide variety of RNAs in vitro. This has been very useful in molecular biology, especially in production of exact DNA copies of purified RNAs. Once the copies are made by RT, they can be cloned into bacterial expression vectors, where mass quantities of the gene product can be produced. It has also been shown that RT activity takes part in making telomeres (protective chromosome ends). Telomere formation and maintenance are essential cell processes, related to life span and deemed important to understanding cancer.

RTs are also important in treatment of acquired immunodeficiency syndrome (AIDS). The drugs most useful for AIDS treatment are RT inhibitors such as zidovudine, didanosine, zalcitabine, and stavudine. RTs are also associated with the difficulty in maintaining successful long-term AIDS treatment, due to rapid development of resistant HIV in individual AIDS patients. The resistance is postulated to be due to RT's lack of a proofreading component. Inadequate proofreading in sequential replication of HIV viral particles from generation to generation is believed to cause the rapid mutation of the viral genome.

—Sanford S. Singer

See also: cDNA Libraries; Central Dogma of Molecular Biology; Model Organism: *Chlamydomonas reinhardtii*; Pseudogenes; Repetitive DNA; RNA Isolation; RNA Structure and Function; RNA World; Shotgun Cloning.

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RFLP Analysis

Field of study: Techniques and methodologies

Significance: *RFLP analysis was the first simple method available for distinguishing individuals based on DNA sequence differences. The conceptual basis for this technique is still widely used in genetics, although RFLP analysis has been largely supplanted by other, faster and more powerful techniques for the comparison of genetic differences.*

Key terms

GEL ELECTROPHORESIS: a method for separating DNA molecules by size by applying electric current to force DNA through a matrix of agarose, which inhibits the migration of larger DNA fragments more than small DNA fragments

RESTRICTION ENZYMES: proteins that recognize specific DNA sequences and then cut the DNA, normally at the same sequence recognized by the enzymes

SOUTHERN BLOTTING: a method for transferring DNA molecules from an agarose gel to a nylon membrane; once the DNA is on the membrane, it is incubated with a DNA containing an identifiable label and is then used to detect similar or identical DNA sequences on the membrane

The Procedure

Restriction fragment length polymorphism (RFLP) analysis is a method for distinguishing individuals and analyzing relatedness, based on genetic differences. RFLP analysis relies on small DNA sequence differences that lead to the loss or gain of restriction enzyme sites in a chromosome or to the change in size of a DNA fragment bracketed by restriction enzyme sites. These sequence differences lead to a different pattern of bands on a gel (reminiscent of a bar code) that varies from individual to individual.

RFLP analysis starts with the isolation of DNA. Typically, DNA isolation requires the use of detergents, protein denaturants, RNA degrading enzymes, and alcohol precipitation to separate the DNA from the other cellular components. This DNA could be isolated from a blood sample provided by an individual, from evidence left at the scene of a crime, or from other sources of cells or tissues.

The purified DNA is then digested with a molecular “scissors” called a restriction enzyme. Restriction enzymes recognize and cut precise sequences, typically six base pairs in length. If one base pair is changed in that recognition sequence, the enzyme will not cut the DNA at that point. On the other hand, if a sequence that is not recognized by a restriction enzyme is altered by mutation, so that it now is recognized, the DNA will be cleaved at that point. In other cases, the DNA sequences recognized by the restriction enzymes themselves are not changed, but the length of DNA between two restriction enzyme sites differs between individuals. These types of mutations occur with enough regularity that often even two closely related individuals will have some detectable differences in the sizes of DNA fragments produced from restriction enzyme digestion.

Once the DNA has been digested with a restriction enzyme, it is separated by size in an agarose gel. At this point, the DNA appears, to the eye, to be a smear of molecules of all sizes, and it is not generally possible to differentiate the DNAs from different individuals at this stage. The size-fractionated DNA is next transferred to a nylon membrane in a process called Southern blotting. The result of the transfer is

that the location and arrangement of the DNA fragments in the gel is maintained on the membrane, but the DNA is now single-stranded (critical for the next step in the process) and much easier to handle.

The final step in the process is to detect specific DNA fragments on the membrane. This is done by using a DNA fragment that is labeled to act as a probe, to home in on and identify similar DNA sequences on the membrane. Before use, the probe is made single-stranded, so it can bind to the single-stranded DNA on the membrane. The probe DNA can be labeled with radioactivity, in which case it is detected using X-ray film. The probe DNA can also be labeled with molecules that are bound by proteins, and the proteins can then be detected either directly or indirectly.

In a case in which a restriction enzyme site has been added or removed, the probe is normally a DNA fragment that is found in only one location in the genome. In cases in which one is looking at the size of fragments bracketed by restriction enzyme sites, the probe DNA is normally a DNA molecule that is found in several sites in the genome, and the DNA fragments that are identified in this analysis are ones that tend to vary between individuals. In many cases, the probe DNA binds to regions of DNA that consist of variable number tandem repeats (VNTRs). The number of VNTRs tends to vary between different individuals and, consequently, these sequences are useful for identification.

Applications

One of the earliest uses of this technique in clinical medicine was in the prenatal diagnosis of sickle-cell disease. Previous work had shown that many individuals with the disease had a mutation in their DNA that eliminated a restriction enzyme site in a gene encoding a hemoglobin protein. This information was used to develop a diagnostic RFLP procedure. A section of the hemoglobin gene is used as a probe. The size of restriction enzyme fragments identified is different in individuals who have sickle-cell disease (and therefore have two mutant alleles) compared with individuals who carry either one mutant allele or have two unmutated

hemoglobin alleles. This method allowed for the identification of affected fetuses using DNA from cells isolated from amniotic fluid (a much simpler and safer procedure than the previous method of diagnosis, which required isolating fetal red blood cells).

Another widely reported use of RFLP analysis has been in forensic science. RFLP methods have been critical in helping to identify criminals, and these methods have also helped exonerate innocent people. The first application of RFLP in forensic analysis was in the case of the murders of two young girls in England, in 1983 and 1986. Initially, a seventeen-year-old boy confessed to the murders. RFLP analysis, using DNA from the crime scene, indicated that he was not the murderer. After extensive investigation, including RFLP analysis of DNA from more than forty-five hundred men, a suspect was identified. Confronted with the evidence, the suspect pleaded guilty to both murders and was jailed for life. Since then, RFLP analysis has been used in thousands of criminal cases. Other forensic applications of RFLP include its use as evidence in court cases involving paternity determinations and its role in identifying the bodies of missing persons who otherwise could not be identified.

In addition to the clinical and forensic applications described above, RFLP analysis has been used in many subdisciplines of biology since the early 1980's. The applications of RFLP analysis range from the conservation of endangered species to the identification of strains of bacteria associated with disease outbreaks to basic research involving the classification of organisms.

Although RFLP analysis has been widely used since its inception, it is increasingly being displaced by polymerase chain reaction (PCR) methods, which typically are much faster and require much less DNA. RFLP analysis was, however, an important step in the introduction of modern DNA analysis into the biology laboratory and the courtroom. The guiding principle behind RFLP analysis—identifying individuals, strains, and species, based on DNA sequence differences—is still a part of more recently developed techniques.

—Patrick G. Guilfoile

See also: Blotting; Southern, Northern, and Western; Chromosome Theory of Heredity; DNA Fingerprinting; Gender Identity; Genetic Engineering; Genetic Testing; Model Organism: *Arabidopsis thaliana*; Paternity Tests; Polymerase Chain Reaction; Prenatal Diagnosis; Restriction Enzymes.

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RNA Isolation

Field of study: Molecular genetics

Significance: All cells in an organism or population of organisms of the same species contain the same (or nearly the same) set of genes. Therefore, understanding which genes are expressed under different conditions is critical to answering many questions in biology, including how cells differentiate into tissues, how cells respond to different environments, and which genes are expressed in tumor cells. The starting point for answering those questions is RNA isolation.

Key terms

- CDNA LIBRARY:** a set of copies, or clones, of all or nearly all mRNA molecules produced by cells of an organism
- COMPLEMENTARY DNA (cDNA):** also called copy DNA, DNA that copies RNA molecules, made using the enzyme reverse transcriptase
- MICROARRAY ANALYSIS:** a method, requiring isolated RNA, that allows simultaneous determination of which of thousands of genes are transcribed (expressed) in cells
- REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR):** a technique, requiring isolated RNA, for quickly determining if a gene or a small set of genes are transcribed in a population of cells
- RNA:** ribonucleic acid, the macromolecule in the cell that acts as an intermediary between the genetic information stored as DNA and the manifestation of that genetic information as proteins
- RNASES:** ribonucleases, or cellular enzymes that catalyze the breakdown of RNA

Cell Lysis

RNA isolation is a difficult proposition. RNA has a short life span in cells (as short as minutes in bacteria), and it is somewhat chemically unstable. In addition, enzymes that degrade RNA (RNases) are widespread in the environment, further complicating the task of separating intact RNA from other molecules in the cell.

The first step in RNA isolation is rapidly breaking open cells under conditions where RNA will not be degraded. One method involves freezing cells immediately in liquid nitrogen, then grinding the cells in liquid nitrogen in order to prevent any RNA degradation. Other methods involve lysing cells in the presence of strong protein denaturants so that any RNases present in the cell or the environment will be rapidly inactivated. The difficulty of the cell lysis step depends substantially on the type of cell involved. Bacterial and fungal cells are typically much more difficult to break open than cells from mammals. As a consequence, it is often more difficult to isolate intact RNA from bacteria and fungi.

Protein Denaturation and Further Purification

The next step in RNA isolation is to denature all proteins from the cell, to ensure that RNases will be inactive. In many cases, this is done at the same time as cell lysis. RNases are among the most resilient enzymes known, capable of being boiled or even autoclaved, yet retaining the ability to cleave RNA once they cool down. Consequently, the RNA next needs to be separated from RNases and other proteins to ensure that it will remain intact.

The separation of RNA from the rest of the macromolecules in the cell can be accomplished in a number of ways. One of the older methods for purifying RNA uses ultracentrifugation in very dense cesium chloride solutions. During high-speed centrifugation, these solutions create a gradient, with the greatest density at the bottom of the tube. RNA is the densest macromolecule in the cell, so it forms a pellet in the bottom of the ultracentrifuge tube. A more recently developed technique for RNA purification involves the use of columns that bind RNA but not other macromolecules. The columns are washed to remove impurities, such as DNA and proteins, and then the RNA is eluted from the column matrix. Another, more recently developed technique is based on the observation that, at an appropriate pH (level of acidity), RNA partitions into the water phase of a water-organic mixture. DNA and proteins either are retained at the boundary of the water-organic mixture or are dissolved in the organic phase.

Once the RNA is isolated, it needs to be handled carefully to ensure that it will not be degraded. Normally this involves re-suspending the RNA in purified water, adding an alcohol solution, and storing it at -70 or -80 degrees Celsius (-94–112 degrees Fahrenheit). The purified RNA can then be used in a variety of techniques that help determine which genes are being transcribed in particular cells or tissues. These techniques include RT-PCR, northern hybridization, microarray analysis, and the construction of cDNA libraries.

Special RNA Isolation Procedures

In some cases, a geneticist wants to isolate only RNA from the cytoplasm of the cell, since

RNA from the nucleus may be more heterogeneous. In this case, cells are lysed using a gentle detergent that disrupts the cytoplasmic membrane, without disturbing the nuclear membrane. Centrifugation is used to separate the nuclei from the cytoplasm, and then the cytoplasmic RNA is further purified as described above.

For some procedures, such as RT-PCR, the RNA sometimes needs to be further purified to ensure that no contaminating DNA is present. In this case, the RNA sample may be treated with the enzyme DNase I, which destroys DNA but leaves RNA intact.

For other procedures, like cDNA library construction, the RNA is often purified to remove ribosomal RNA (rRNA), transfer RNA (tRNA), and other stable RNAs, since the majority of RNA in the cell (typically more than 90 percent) is rRNA and tRNA. In this case, the RNA solution is treated by incubating it with single-stranded DNA containing a chain of eighteen to twenty thymine nucleotides, either on a column or in solution. Messenger RNA (mRNA) from eukaryotes contains runs of twenty to two hundred adenine nucleotides that bind to the single-stranded DNA and allow the mRNA to be purified away from the stable RNAs.

Like most techniques in genetics, RNA isolation methods have improved greatly over the years. With advances in methods for studying gene expression such as microarray analysis, isolating intact RNA is a technique that is more critical than ever in the modern genetics laboratory.

—Patrick G. Guilfoile

See also: cDNA Libraries; DNA Isolation; DNA Structure and Function; Polymerase Chain Reaction; Reverse Transcriptase; RNA Structure and Function; RNA Transcription and mRNA Processing.

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RNA Structure and Function

Field of study: Molecular genetics

Significance: Ribonucleic acid (RNA), a molecule that plays many roles in the storage and transmission of genetic information, exists in several forms, each with its own unique function. RNA acts as the messenger between genes in the DNA and their protein product, directing the assembly of proteins. RNA is also an integral part of ribosomes, the site of protein synthesis, and some RNAs have been shown to have catalytic properties. Understanding the structure and function of RNA is important to a fundamental knowledge of genetics; in addition, many developing medical therapies will undoubtedly utilize special RNAs to combat genetic diseases.

Key terms

MESSENGER RNA: a type of RNA that carries genetic instructions, copied from genes in DNA, to the ribosome to be decoded during translation

RETROVIRUS: a special type of virus that carries its genetic information as RNA and converts it into DNA that integrates into the cells of the virus's host organism

RIBOSOMAL RNA: a type of RNA that forms a major part of the structure of the ribosome

RIBOSOMES: organelles that function in protein synthesis and are made up of a large and a small subunit composed of proteins and ribosomal RNA (rRNA) molecules

RIBOZYME: an RNA molecule that can function catalytically as an enzyme

TRANSCRIPTION: the synthesis of an RNA molecule directed by RNA polymerase using a DNA template

TRANSFER RNA: a form of RNA that acts to decode genetic information present in mRNA, carries a particular amino acid, and is vital to translation

TRANSLATION: the synthesis of a protein molecule directed by the ribosome using information provided by an mRNA

The Chemical Nature of RNA

Ribonucleic acid (RNA) is a complex biological molecule that is classified along with DNA as a nucleic acid. Chemically, RNA is a polymer (long chain) consisting of subunits called ribonucleotides linked together by phosphodiester bonds. Each ribonucleotide consists of three parts: the sugar ribose (a five-carbon simple sugar), a negatively charged phosphate group, and a nitrogen-containing base. There are four types of ribonucleotides, and the differences among them lie solely in which of four possible bases each contains. The four bases are adenine (A), guanine (G), cytosine (C), and uracil (U).

The structures of DNA and RNA are very similar, with the following differences. The sugar found in the nucleotide subunits of DNA is deoxyribose, which differs slightly from the ribose found in the ribonucleotides of RNA. In addition, while DNA nucleotides also contain four possible bases, there is no uracil in DNA; instead, DNA nucleotides contain a different base called thymine (T). Finally, while DNA exists as a double-stranded helix in nature, RNA is almost always single-stranded. Like DNA, a single RNA strand has a 5'-to-3' polarity. These numbers are based on which carbon atom is exposed at the end of the polymer, each of the carbon atoms being numbered around the sugar molecule.

The Folding of RNA Molecules

The function of an RNA molecule is determined by its nucleotide sequence, which represents information derived from DNA. This nucleotide sequence is called the primary structure of the molecule. Many RNAs also have an important secondary structure, a three-dimensional shape that is also important for the function of the molecule. The secondary structure is determined by hydrogen bonding between parts of the RNA molecule that are complementary. Complementary pairing is always between A and U ribonucleotides and C and G ribonucleotides. Hydrogen bonding results in double-stranded regions in the secondary structure.

Since RNA is single-stranded, it was recognized shortly after the discovery of some of its major roles that its capacity for folding is great and that this folding might play an important part in the functioning of the molecule. Base pairing often represents local interactions, and a common structural element is a “hairpin loop” or “stem loop.” A hairpin loop is formed when two complementary regions are separated by a short stretch of bases so that when they fold back and pair, some bases are left unpaired, forming the loop. The net sum of these local interactions is referred to as the RNA’s secondary structure and is usually important to an understanding of how the RNA works. All transfer RNAs (tRNAs), for example, are folded into a secondary structure that contains three stem loops and a fourth stem without a loop, a structure resembling a cloverleaf in two dimensions.

Finally, local structural elements may interact with other elements in long-range interactions, causing more complicated folding of the molecule. The full three-dimensional structure of a tRNA molecule from yeast was finally confirmed in 1978 by several groups independently, using X-ray diffraction. In this process, crystals of a molecule are bombarded with X rays, which causes them to scatter; an expert can tell by the pattern of scattering how the different atoms in the molecule are oriented with respect to one another. The cloverleaf arrangement of a tRNA undergoes further folding so that the entire molecule takes on a roughly L-

shaped appearance in three dimensions. An understanding of the three-dimensional shape of an RNA molecule is crucial to understanding its function. By the late 1990's, the three-dimensional structures of many tRNAs had been worked out, but it had proven difficult to do X-ray diffraction analyses on most other RNAs because of technical problems. More advanced computer programs and alternate structure-determining techniques are enabling research in this field to proceed.

Synthesis and Stability of RNA

RNA molecules of all types are continually being synthesized and degraded in a cell; even the longest-lasting ones exist for only a day or two. Shortly after the structure of DNA was established, it became clear that RNA was synthesized using a DNA molecule as a template, and the mechanism was worked out shortly thereafter. The entire process by which an RNA molecule is constructed using the information in DNA is called transcription. An enzyme called RNA polymerase is responsible for assembling the ribonucleotides of a new RNA complementary to a specific DNA segment (gene). Only one strand of the DNA is used as a template (the sense strand), and the ribonucleotides are initially arranged according to the base-pairing rules. A DNA sequence called the "promoter" is a site RNA polymerase can bind initially and allows the process of RNA synthesis to begin. At the appropriate starting site, RNA polymerase begins to assemble and connect the nucleotides according to the complementary pairing rules, such that for every A nucleotide in the DNA, RNA polymerase incorporates a U ribonucleotide into the RNA being assembled. The remaining pairing rules stipulate that a T in DNA denotes an A in RNA and that a C in DNA represents a G in RNA (and vice versa). This process continues until another sequence, called a "terminator," is reached. At this point, the RNA polymerase stops transcription, and a new RNA molecule is released.

Much attention is rightfully focused on transcription, since it controls the rate of synthesis of each RNA. It has become increasingly clear, however, that the amount of RNA in the cell at a given time is also strongly dependent on RNA

stability (the rate at which it is degraded). Every cell contains several enzymes called ribonucleases (RNases) whose job it is to cut up RNA molecules into their ribonucleotides subunits. Some RNAs last only thirty seconds, while others may last up to a day or two. The signals regulating RNA degradation are being studied, and although much has been learned, many details remain unclear. It is important to remember that both the rates of synthesis (transcription) and degradation ultimately determine the amount of functional RNA in a cell at any given time.

Three Classes of RNA

While all RNAs are produced by transcription, several classes of RNA are created, and each has a unique function. By the late 1960's, three major classes of RNAs had been identified, and their respective roles in the process of protein synthesis had been identified. In general, protein synthesis refers to the assembly of a protein using information encoded in DNA, with RNA acting as an intermediary to carry information and assist in protein building. In 1956, Francis Crick, one of the scientists who had discovered the double-helical structure of DNA, referred to this information flow as the "central dogma," a term that continues to be used, although exceptions to it are now known.

A messenger RNA (mRNA) carries a complementary copy of the DNA instructions for building a particular protein. In eukaryotes it typically represents the information from a single gene and carries the information to a ribosome, the site of protein synthesis. The information must be decoded to make a protein. Nucleotides are read in groups of three (called codons). In addition, mRNAs contain signals that tell a ribosome where to start and stop translating.

Ribosomal RNA (rRNA) is part of the structure of the ribosome. Four different rRNAs interact with many proteins to form functional ribosomes that direct the events of protein synthesis. One of the rRNAs interacts with mRNA to orient it properly so translation can begin at the correct location. Another rRNA acts to facilitate the transfer of the growing polypeptide from one tRNA to another (peptidyl transferase activity).

Transfer RNA (tRNA) serves the vital role of decoding the genetic information. There are at least twenty and usually more than forty different tRNAs in a cell. On one side, tRNAs contain an “anticodon” loop, which can base-pair with mRNA codons according to their sequence and the base-pairing rules. On the other side, each contains an amino acid binding site, with the appropriate amino acid for its anticodon. In this way, tRNAs recognize the codons and supply the appropriate amino acids. The process continues until an entire new polypeptide has been constructed.

The attachment of the correct amino acids is facilitated by a group of enzymes called tRNA amino acyl synthetases. Each type of tRNA has a corresponding synthetase that facilitates the attachment of the correct amino acid to the amino acid binding site. The integrity of this process is crucial to translation; if only one tRNA is attached to an incorrect amino acid, the resulting proteins will likely be nonfunctional.

Split Genes and mRNA Processing in Eukaryotes

In bacterial genes, there is a colinearity between the segment of a DNA molecule that is transcribed and the resulting mRNA. In other words, the mRNA sequence is complementary to its template and is the same length, as would be expected. In the late 1970’s, several groups of scientists made a seemingly bizarre discovery regarding mRNAs in eukaryotes (organisms whose cells contain a nucleus, including all living things that are not bacteria): The sequences of mRNAs isolated from eukaryotes were not collinear with the DNA from which they were transcribed. The coding regions of the corresponding DNA were interrupted by seemingly random sequences that served no apparent function. These “introns,” as they came to be known, were apparently transcribed along with the coding regions (exons) but were somehow removed before the mRNA was translated. This completely unexpected observation led to further investigations that revealed that mRNA is extensively processed, or modified, after its transcription in eukaryotes.

After a eukaryotic mRNA is transcribed, it

contains several to many introns and is referred to as immature, or a “pre-mRNA.” Before it can become mature and functional, three major processing events must occur: splicing, the addition of a 5’ cap, and a “tail.” The process of splicing is complex and occurs in the nucleus with the aid of “spliceosomes,” large complexes of RNAs and proteins that identify intervening sequences and cut them out of the pre-mRNA. In addition, spliceosomes rejoin the exons to produce a complete, functional mRNA. Splicing must be extremely specific, since a mistake causing the removal of even one extra nucleotide could change the final protein, making it nonfunctional. During splicing, capping and the addition of a poly-A tail take place. A so-called cap, which consists of a modified G nucleotide, is added to the beginning (5’ end) of the pre-mRNA by an unconventional linkage. The cap appears to function by interacting with the ribosome, helping to orient the mature mRNA so that translation begins at the proper end. A tail, which consists of many A nucleotides (often two hundred or more), is attached to the 3’ end of the pre-mRNA. This so-called poly-A tail, which virtually all eukaryotic mRNAs contain, seems to be one factor in determining the relative stability of an mRNA. These important steps must be performed after transcription in eukaryotes to produce a functional mRNA.

Other Important Classes of RNA and Specialized Functions

The traditional roles of RNA in protein synthesis were originally considered its only roles. RNA in general, while considered an important molecule, was thought of as a “helper” in translation. This all began to change in 1982, when the molecular biologists Thomas Cech and Sidney Altman, working independently and with different systems, reported the existence of RNA molecules that had catalytic activity. This means that RNA molecules can function as enzymes; until this time, it was believed that all enzymes were protein molecules. The importance of these findings cannot be overstated, and Cech and Altman ultimately shared the 1989 Nobel Prize in Chemistry for the discovery of these RNA enzymes, or “ribozymes.”

Both of these initial ribozymes catalyzed reactions that involved the cleavage of other RNA molecules—that is, they acted as nucleases. Subsequently, many ribozymes have been found in various organisms, from bacteria to humans. Some of them are able to catalyze different types of reactions, and there are new ones reported every year. Thus ribozymes are not a mere curiosity but play an integral role in the molecular machinery of many organisms. Their discovery also gave rise to the idea that at one point in evolutionary history, molecular systems composed solely of RNA, performing many roles, existed in an “RNA world.”

At around the same time as these momentous discoveries, still other classes of RNAs were being discovered, each with its own specialized functions. In 1981, Jun-ichi Tomizawa discovered RNA interference (RNAi), the first example of what would become another major class of RNAs, the “antisense RNAs” or “interference RNAs.” The RNAs in this group are complementary to a target molecule (usually an mRNA) and can bind to that target via complementary base pairing. RNAi binding usually plays a regulatory role, often acting to prevent translation of the relevant mRNA to modulate the expression of the protein for which it codes. Most of these antisense RNAs are encoded by the same gene as their target, but a group called the “transencoded antisense RNAs” actually have their own genes, which are separate and distinct from their target molecule’s gene. This is especially significant because the complementarity between antisense RNA and the target is often not perfect, resulting in interesting interactions with unique structural features. The prototype of this class of RNAs, *micF* RNA, was discovered in 1983 by Masayori Inouye and subsequently characterized by Nicholas Delibas. An understanding of the binding of this special type of antisense RNA to its target will provide insights into RNA-RNA interactions that may be vital for use in genetic therapy. Research on RNAi molecules continues, and many new insights into genetic control after transcription have been gained.

Another major class of RNAs, the small nuclear RNAs (snRNAs), was also discovered in the early 1980’s. Molecular biologist Joan Steitz

was working on the autoimmune disease systemic lupus when she began to characterize the snRNAs. There are six different snRNAs, now called U1-U6 RNAs. These RNAs exist in the nucleus of eukaryotic cells and play a vital role in mRNA splicing. They associate with proteins in the spliceosome, forming so-called ribonucleoprotein complexes (snRNPs, pronounced “snurps”), and play a prominent role in detecting proper splice sites and directing the protein enzymes to cut and paste at the proper locations.

It has been known since the late 1950’s that many viruses contain RNA, and not DNA, as their genetic material. This is another fascinating role for RNA. The viruses that cause influenza, polio, and a host of other diseases are RNA viruses. Of particular note are a class of RNA viruses known as retroviruses. Retroviruses, which include human immunodeficiency virus (HIV), the virus that causes acquired immunodeficiency syndrome (AIDS) in humans, use a special enzyme called reverse transcriptase to make a DNA copy of their RNA when they enter a cell. The DNA copy is inserted into the DNA of the host cell, where it is referred to as a “provirus,” and never leaves. This discovery represents one of the exceptions to the central dogma. In the central dogma, RNA is always made from DNA, and retroviruses have reversed this flow of information. Clearly, understanding the structures and functions of the RNAs associated with these viruses will be important in attempting to create effective treatments for the diseases associated with them.

An additional role of RNA was noted during the elucidation of the mechanism of DNA replication. It was found that a small piece of RNA, called a “primer,” must be laid down by the enzyme primase, an RNA polymerase, before DNA polymerase can begin. RNA primers are later removed and replaced with DNA. Also, it is worth mentioning that the universal energy-storing molecule of all cells, adenosine triphosphate (ATP), is in fact a version of the RNA nucleotide containing adenine (A).

Impact and Applications

The discovery of the many functions of RNA, especially its catalytic ability, has radically

changed the understanding of the functioning of genetic and biological systems and has revolutionized the views of the scientific community regarding the origin of life. The key to understanding how RNA can perform all of its diverse functions lies in elucidating its many structures, since structure and function are inseparable. Much progress has been made in establishing the structures of hundreds of RNA molecules; several methods, including advanced computer programs, are making it easier to predict and analyze RNA structure. Three-dimensional modeling is much more difficult, and while the three-dimensional structures of several RNAs have been worked out, much work remains.

In terms of basic research and genetic engineering, the discovery of antisense RNAs and ribozymes has facilitated many procedures, providing insight at the molecular level of genetic processes that would have been difficult to obtain without this knowledge and the tools it has made available. Additionally, plants, bacteria, and animals have been genetically engineered to alter the expression of some of their genes, in many cases making use of the new RNA technology. An example is the genetically engineered tomato, which does not ripen until it is treated at the point of sale. This tomato was created by inserting an antisense RNA gene; when it is expressed, it inactivates the mRNA that codes for the enzyme involved in production of the ripening hormone.

Although success in human gene therapy has been limited, the usage of retroviruses to introduce ribozymes, antisense RNAs, or a combination of both into genetically defective cells offers great promise for the future in fighting a wide variety of diseases, from AIDS and cancer to cystic fibrosis and sickle-cell disease. One thing is clear: RNA will play an important role in increasing the understanding of genetics and in the revolution of gene therapy. RNA is one of the most structurally interesting and functionally diverse of all the biological molecules.

—Matthew M. Schmidt, updated by Bryan Ness

See also: Ancient DNA; Antisense RNA; Chromosome Structure; DNA Isolation; DNA Repair; DNA Replication; DNA Structure and

Function; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Noncoding RNA Molecules; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; Repetitive DNA; RNA Isolation; RNA Transcription and mRNA Processing; RNA World.

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RNA Transcription and mRNA Processing

Field of study: Molecular genetics

Significance: Translation of messenger RNA molecules (mRNAs) occurs even while transcription is taking place in prokaryotes. In eukaryotes the process is much more complex, with transcription occurring in the nucleus, followed by multiple pro-

cessing steps before a mature mRNA is ready to be translated. All of these extra steps are required for mRNAs to be transported out of the nucleus and for recognition by ribosomes in the cytoplasm.

Key terms

MESSENGER RNA (mRNA): the form of RNA that contains the coding instructions used to make a polypeptide by ribosomes

RNA POLYMERASE: the enzyme that transcribes RNA using a strand of DNA as a template

TRANSCRIPTION: the process that converts DNA code into a complementary strand of RNA (mRNA) containing code that can be interpreted by ribosomes

TRANSLATION: the process, mediated by ribosomes, in which the genetic code in an mRNA is used to produce a polypeptide, the ultimate product of structural genes

RNA Polymerase

Transcription is the process whereby the directions for making a protein are converted from DNA-based instructions to RNA-based instructions. This step is required in the process of expressing a gene as a polypeptide, because ribosomes, which assemble polypeptides, can read only RNA-based messages. Although transcription is complicated and involves dozens of enzymes and proteins, it is much simpler in prokaryotes than in eukaryotes. Because prokaryotes lack a nucleus, transcription and translation are linked processes both occurring in the cytoplasm. In eukaryotes, transcription and translation occur as completely separate processes, transcription occurring in the nucleus and translation occurring in the cytoplasm. (It is now known that some translation also occurs in the nucleus, but apparently only a small amount, probably less than 10 percent of the translation occurring in a cell.)

In eukaryotes there are three different types of RNA polymerase that transcribe RNA using a strand of DNA as a template (there is a single type of RNA polymerase in prokaryotes). Two of them, called RNA polymerase I (pol I) and RNA polymerase III (pol III), specialize in transcribing types of RNA that are functional products themselves, such as ribosomal RNA (rRNA) and transfer RNA (tRNA). These

RNAs are involved in translation. RNA polymerase II (pol II) transcribes RNA from structural genes, that is, genes that code for polypeptides. Pol II therefore is the primary RNA polymerase and the one that will be the focus of this article when discussing transcription in eukaryotes.

Transcription in Prokaryotes

The first step in transcription is for RNA polymerase to identify the location of a gene. In prokaryotes many genes are clustered together in functional groups called operons. For example, the lactose (*lac*) operon contains three genes, each coding for one of the enzymes needed to metabolize the sugar lactose. At the beginning of each operon are two control sequences, the operator and the promoter. The promoter is where RNA polymerase binds, in preparation for transcription. The operator is a control region that determines whether RNA polymerase will be able to bind to the promoter. The operator interacts with other proteins that determine when the associated operon should be expressed. They do this by either preventing RNA polymerase from binding to the promoter or by assisting it to bind.

RNA polymerase recognizes promoters by the specific base-pair sequences they contain. Assuming all conditions are correct, RNA polymerase binds to the promoter, along with another protein called the sigma factor (σ). The beginning of genes are detected with the aid of σ . Transcription begins at a leader sequence a little before the beginning of the first gene and continues until RNA polymerase reaches a termination signal. If the operon contains more than one gene, all of the genes are transcribed into a single long mRNA, each gene separated from its neighbors by a spacer region. The mRNA is put together by pairing ribonucleotides with their complementary nucleotides in the DNA template. In place of thymine (T), RNA uses uracil (U); otherwise the same bases are present in RNA and DNA, the others being adenine (A), guanine (G), and cytosine (C). The pairing relationships are as follows, the DNA base listed first in each pair: A-U, T-A, G-C, and C-G.

RNA polymerase catalyzes the joining of

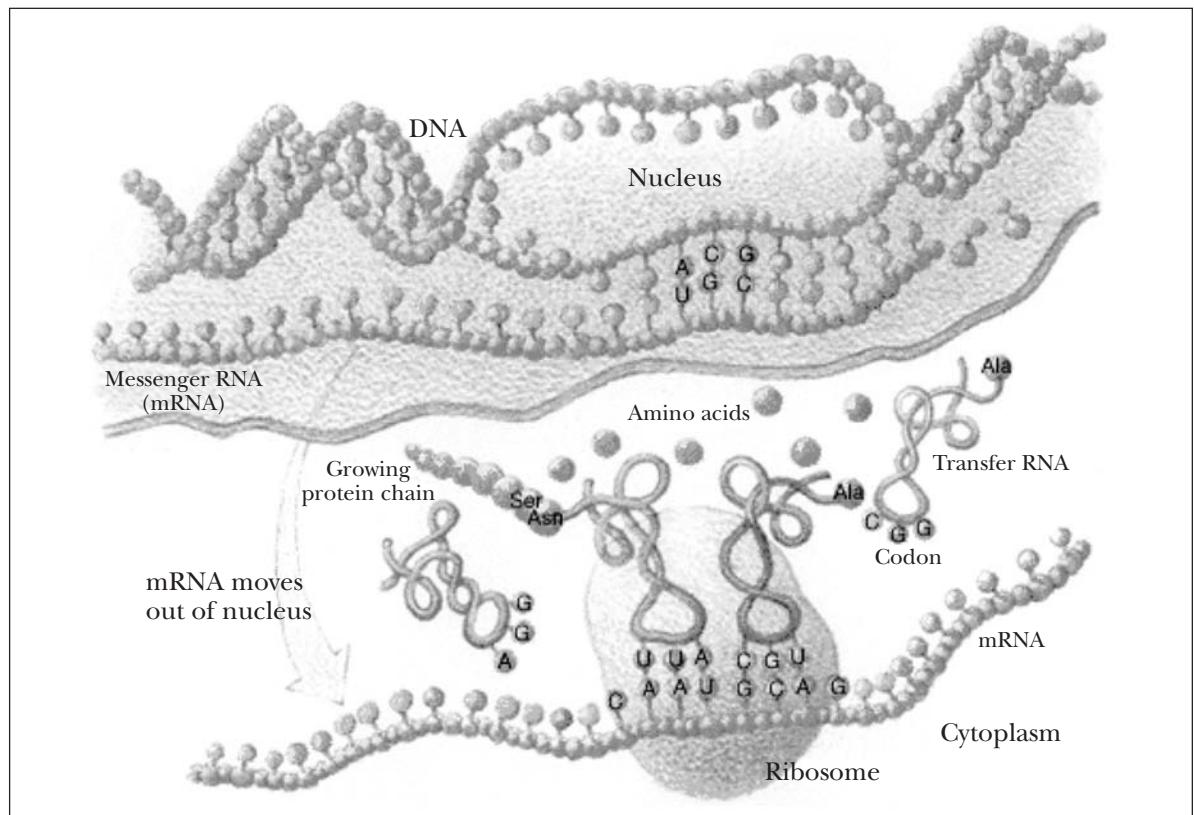
ribonucleotides as they pair with the DNA template. Each mRNA is constructed beginning at the 5' end (the phosphate end) and ending with the 3' end (the hydroxyl end). Even while transcription is taking place, ribosomes begin binding to the mRNA to begin translation. As soon as RNA polymerase has completed transcribing the genes of an operon, it releases from the DNA and soon binds to another promoter to begin the process all over again.

Transcription in Eukaryotes

Transcription in eukaryotes differs from the process in prokaryotes in the following major ways: (1) genes are transcribed individually instead of in groups; (2) DNA is complexed with many proteins and is highly compacted, and therefore must be “unwound” to expose its

promoters; (3) transcription occurs in a separate compartment (the nucleus) from translation, most of which occurs in the cytoplasm; and (4) initially transcription results in a pre-messenger RNA (pre-mRNA) molecule that must be processed before it emerges as a mature mRNA ready for translation. Additionally, mRNAs are much longer-lived in eukaryotes.

The first step in transcription is for RNA polymerase to find a gene that needs to be transcribed. Only genes occurring in regions of the DNA that have been unwound are prepared for potential transcription. RNA polymerase binds to an available promoter, which is located just before a gene and has a region in it called the TATA box (all promoters have the consensus sequence TATAAAA in them). RNA polymerase is unable to bind to the promoter without



A depiction of the process of RNA transcription. Messenger RNA (mRNA) moves the DNA's template or instructions for protein synthesis (genetic code, or arrangement of bases) from the cell nucleus out into the cytoplasm, where it binds to a ribosome, the cell's “protein factory.” Transfer RNA molecules then synthesize amino acids by linking to a codon on the mRNA and transferring the resulting amino acid to a growing chain of amino acids, the protein molecule. (U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

assistance from over a dozen other proteins, including a TATA-binding protein, several transcription factors, activators, and coactivators. There are other DNA sequences further upstream than the promoter that control transcription too, thus accounting for the fact that some genes are transcribed more readily, and therefore more often, than others.

Once RNA polymerase has bound to the promoter, it begins assembling an RNA molecule complementary to the DNA code in the gene. It starts by making a short leader sequence, then transcribes the gene, and finishes after transcribing a short trailer sequence. Transcription ends when RNA polymerase reaches a termination signal in the DNA. The initial product is a pre-mRNA molecule which is much longer than the mature mRNA will be.

mRNA Processing in Eukaryotes

Pre-mRNAs must be processed before they can leave the nucleus and be translated at a ribosome. Three separate series of reactions play a part in producing a mature mRNA: (1) intron removal and exon splicing, (2) 5' capping, and (3) addition of a poly-A tail. Not all transcripts require all three modifications, but most do.

The reason pre-mRNAs are much longer than their respective mature mRNAs has to do with the structure of genes in the DNA. The coding sequences of almost all eukaryotic genes are interrupted with noncoding regions. The noncoding regions are called introns, because they represent “intervening” sequences, and the coding regions are called exons. For an mRNA to be mature it must have all the introns removed and all the exons spliced together into one unbroken message. Special RNA/protein complexes called small nuclear ribonucleoprotein particles, or snRNPs (pronounced as “snurps” by geneticists), carry out this process. The RNAs in the snRNPs are called small nuclear RNAs or snRNAs. Several snRNPs grouped together form a functional splicing unit called a spliceosome. Spliceosomes are able to recognize short signal sequences in pre-mRNA molecules that identify the boundaries of introns and exons. When a spliceosome has found an intron, it binds correctly, and through formation of a lariat-shaped structure, it cuts

the intron out and splices the exons that were on each side of the intron to each other. Genes may have just a few introns, or they may have a dozen or more. Why eukaryotes have introns at all is still an open question, as introns, in general, appear to have no function.

While intron removal and exon splicing are taking place, both ends of maturing mRNAs must also be modified. At the 5' end (the end with an exposed phosphate) an enzyme adds a modified guanosine nucleotide called 7-methylguanosine. This special nucleotide is added so that ribosomes in the cytoplasm can recognize the correct end of mRNAs, and it probably also prevents the 5' end of mRNAs from being degraded.

At the 3' end of maturing mRNAs another enzyme, called polyadenylase, adds a string of adenine nucleotides. Polyadenylase actually recognizes a special signal in the trailer sequence, at which it cuts and then adds the adenines. The result is what is called a poly-A tail. Initially geneticists did not understand the function of poly-A tails, but now it appears that they protect mRNAs from enzymes in the cytoplasm that could break them down. Essentially, poly-A tails are the main reason mRNAs in eukaryotes survive so much longer than mRNAs in prokaryotes.

Once the modifications have been completed, mRNAs are ready to be exported from the nucleus and will now travel through nuclear pores and enter the cytoplasm, where awaiting ribosomes will translate them, using the RNA code to build polypeptides.

Transcription and Disease

Ordinarily transcription works like a well-oiled machine, and only the right genes are transcribed at the right time so that just the right amount of protein product is produced. Unfortunately, due to the great complexity of the system, problems can occur that lead to disease. It has been estimated that about 15 percent of all genetic diseases may be due to improper intron removal and exon splicing in pre-mRNA molecules. Improper gene expression accounts for many other diseases, including many types of cancer.

Beta-thalassemia, a genetic disorder causing

Cooley's anemia, is caused by a point mutation (a change in a single nucleotide) that changes a cutting and splicing signal. As a result, the mature mRNA has an extra piece of intron, making the mRNA longer and causing a reading frame shift. A reading frame shift causes everything from the mutation forward to be skewed, so that the code no longer codes for the correct amino acids. Additionally, as in the case of Cooley's anemia, a reading frame shift often introduces a premature stop codon. The gene involved codes for the beta chain of hemoglobin, the protein that carries oxygen in the blood, and this mutation results in a shortened polypeptide that does not function properly.

A single point mutation in a splicing site can have even more far-reaching consequences. In 2000, researchers in Italy discovered an individual who was genetically male (having one X and one Y chromosome) but was phenotypically female. She had no uterus or ovaries and only superficial external female anatomy, making her a pseudohermaphrodite. This condition can be caused by defects either in androgen production or in the androgen receptor. In this case, the defect was a simple point mutation in the androgen receptor gene that led to one intron being retained in the mature mRNA. Within the intron was a stop codon, which meant when the mRNA was translated, a shorter, nonfunctional polypeptide was formed. The subject did show a very small response to androgen, so apparently some of the pre-mRNAs were being cut and spliced correctly, but not enough to produce the normal male phenotype.

The same kinds of mutations as those discussed above can lead to cancer, but mutations that change the level of transcription of proto-oncogenes can also lead to cancer. Proto-oncogenes are normal genes involved in regulating the cell cycle, and when these genes are overexpressed they become oncogenes (cancer-causing genes). Overexpression of proto-oncogenes leads to overexpression of other genes, because many proto-oncogenes are transcription factors, signal proteins that interact with molecules controlling intracellular growth and growth factors released by cells to stimulate other cells to divide.

Overexpression can occur when there is a mutation in one of the control regions upstream from a gene. For example, a mutation in the promoter sequence could cause a transcription factor, and thus RNA polymerase, to bind more easily, leading to higher transcription rates. Other control regions, such as enhancer sequences, often far removed from the gene itself, may also affect transcription rates.

Anything that causes the transcription process to go awry will typically have far-reaching consequences. Geneticists are just beginning to understand some of the underlying errors behind a host of genetic diseases, and it should be no surprise that some of them involve how genes are transcribed. Knowing what the problem is, unfortunately, does not usually point to workable solutions. When the primary problem is an excessive rate of transcription, specially designed antisense RNA molecules (RNA molecules that are complementary to mRNA molecules) might be designed that will bind to the overexpressed mRNAs and disable them. This approach is still being tested. In the case of point mutations that derail the cutting and splicing process, the only solution may be gene therapy, a technique still not considered technically possible and not expected to be feasible for some time to come.

—Bryan Ness

See also: Ancient DNA; Antisense RNA; Cancer; Chromosome Structure; DNA Isolation; DNA Repair; DNA Replication; DNA Structure and Function; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Noncoding RNA Molecules; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; Pseudohermaphrodites; Repetitive DNA; RNA Isolation; RNA Structure and Function; RNA World.

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RNA World

Fields of study: Evolutionary biology; Molecular genetics

Significance: *The RNA world is a theoretical time in the early evolution of life, during which RNA molecules played important genetic and enzymatic roles that were later taken over by molecules of DNA and proteins. Ideas about RNA's ancient functions have led to new concepts of the origin of life and have important implications in the use of gene therapy to treat diseases.*

Key term

RIBOSOMAL RNA (rRNA): a type of RNA that forms a major part of the structure of the ribosome

RIBOSOME: an organelle that functions in protein synthesis, composed of a large and a small subunit composed of proteins and ribosomal RNA molecules

RIBOZYME: an RNA molecule that can function catalytically as an enzyme

The Central Dogma and the Modern Genetic World

Soon after the discovery of the double-helical structure of DNA in 1953 by James Watson and Francis Crick, Crick proposed an idea regarding information flow in cells that he called the "central dogma of molecular biology." Crick correctly predicted that in all cells, information flows from DNA to RNA to protein. DNA was known to be the genetic material, the "library" of genetic information, and it had been clear for some time that the enzymes that actually did the work of facilitating chemical reactions were invariably protein molecules. The discovery of three classes of RNA during the 1960's seemed to provide the link between the DNA instructions and the protein products.

In the modern genetic world, cells contain three classes of RNA that act as helpers in the synthesis of proteins from information stored in DNA, a process called translation. A messenger RNA (mRNA) is "transcribed" from a segment of DNA (a gene) that contains information about how to build a particular protein and carries that information to the cellular site of protein synthesis, the ribosome. Ribosomal RNAs (rRNAs) interacting with many proteins make up the ribosome, whose major job is to coordinate and facilitate the protein-building procedure. Transfer RNAs (tRNAs) act as decoding molecules, reading the mRNA information and correlating it with a specific amino acid. As the ribosome integrates the functions of all three types of RNA, polypeptides are built one amino acid at a time. These polypeptides, either singly or in aggregations, can then function as enzymes, ultimately determining the capabilities and properties of the cell in which they act.

While universally accepted, the central dogma led many scientists to question how this complex, integrated system came about. It seemed to be a classic "chicken and egg" dilemma: Proteins could not be built without instructions from DNA, but DNA could not replicate and maintain itself without help from protein enzymes. The two seemed mutually dependent upon each other in an inextricable way. An understanding of the origins of the modern genetic system seemed far away.

The Discovery of Ribozymes

In 1983, a discovery was made that seemed so radical it was initially rejected by most of the scientific community. Molecular biologists Thomas Cech and Sidney Altman, working independently and in different systems, announced the discovery of RNA molecules that possessed catalytic activity. This meant that RNA itself can function as an enzyme, obliterating the idea that only proteins could function catalytically.

Cech had been working with the protozoan *Tetrahymena*. In most organisms except bacteria, the coding portions of DNA genes (exons) are interrupted by noncoding sequences (introns), which are transcribed into mRNA but which must be removed before translation. Protein enzymes called nucleases are usually responsible for cutting out the introns and joining together the exons in a process called splicing. The molecule with which Cech was working was an rRNA that contained introns but could apparently remove them and rejoin the coding regions without any help. It was a self-splicing RNA molecule, which clearly indicated its enzymatic capability. Altman was working with the enzyme ribonuclease (RNase) P in bacteria, which is responsible for cutting mature tRNA molecules out of an immature RNA segment. RNase P thus also acts as a nuclease. It was known for some time that RNase P contains both a protein and an RNA constituent, but Altman was ultimately able to show that it was the RNA rather than the protein that actually catalyzed the reaction.

The importance of these findings cannot be overstated, and Cech and Altman ultimately shared the 1989 Nobel Prize in Chemistry for the discovery of these RNA enzymes, or ribozymes (joining the terms "ribonucleic acid" and "enzymes"). Subsequently, many ribozymes have been found in various organisms, from bacteria to humans. Some of them are able to catalyze different types of reactions, and new ones are periodically reported. Ribozymes have thus proven to be more than a mere curiosity, playing an integral role in the molecular machinery of many organisms.

At around the same time as these important discoveries, still other functions of RNA were

being identified. While perhaps not as dramatic as the ribozymes, antisense RNAs, small nuclear RNAs, and a variety of others further proved the versatility of RNA. While understanding the roles of ribozymes and other unconventional RNAs is important to the understanding of genetic functioning in present-day organisms, these discoveries were more intriguing to many scientists interested in the origin and evolution of life. In a sense, the existence of ribozymes was a violation of the central dogma, which implied that information was ultimately utilized solely in the form of proteins. While the central dogma was not in danger of becoming obsolete, a clue had been found that might possibly allow a resolution, at least in theory, to questions about whether the DNA or the protein came first. The exciting answer: perhaps neither.

The RNA World Theory and the Origin of Life

Given that RNA is able to store genetic information (as it certainly does when it functions as mRNA) and the new discovery that it could function as an enzyme, there was no longer any need to invoke the presence of either DNA or protein as necessities in the first living system. The first living molecule would have to be able to replicate itself without any help, and just such an "RNA replicase" has been proposed as the molecule that eventually led to life as it is now known. Like the self-splicing intron of *Tetrahymena*, this theoretical ribozyme could have worked on itself, catalyzing its own replication. This RNA would therefore have functioned as both the genetic material and the replication enzyme, allowing it to make copies of itself without the need for DNA or proteins. Biologist Walter Gilbert coined the term "RNA world" for this interesting theoretical period dominated by RNA. Modern catalytic RNAs can be thought of as molecular fossils that remain from this period and provide clues about its nature.

How might this initial RNA have come into being in the first place? Biologist Aleksandr Oparin predicted in the late 1930's that if simple gases thought to be present in Earth's early atmosphere were subjected to the right condi-

tions (energy in the form of lightning, for example), more complex organic molecules would be formed. His theory was first tested in 1953 and was resoundingly confirmed. A mixture of methane, ammonia, water vapor, and hydrogen gas was energized with high-voltage electricity, and the products were impressive: several amino acids and aldehydes, among other organic molecules. Subsequent experiments have been able to produce ribonucleotide bases. It seems reasonable, then, that nucleotides could have been present on the early Earth and that their random linkage could lead to the formation of an RNA chain.

After a while, RNA molecules would have found a way to synthesize proteins, which are able to act as more efficient and diverse enzymes than ribozymes by their very nature. Why are proteins better enzymes than ribozymes? Since RNA contains only four bases that are fundamentally similar in their chemical properties, the range of different configurations and functional capabilities is somewhat limited as opposed to proteins. Proteins are constructed of twenty different amino acids whose functional groups differ widely in terms of their chemical makeup and potential reactivity. It is logical to suppose, therefore, that proteins eventually took over most of the roles of RNA enzymes because they were simply better suited to doing so. Several of the original or efficient ribozymes would have been retained, and those are the ones that can be observed today.

How could a world composed strictly of RNAs, however, be able to begin protein synthesis? While it seems like a tall order, scientists have envisioned an early version of the ribosome that was composed exclusively of RNA. Biologist Harry Noller reported in the early 1990's that the activity of the modern ribosome that is responsible for catalyzing the formation of peptide bonds between amino acids is in fact carried out by rRNA. This so-called peptidyl-transferase activity had always been attributed to one of the ribosomal proteins, and rRNA had been envisioned as playing a primarily structural role. Noller's discovery that the large ribosomal RNA is actually a ribozyme allows scientists to picture a ribosome working in roughly the same way that modern ones do,

without containing any proteins. As proteins began to be synthesized from the information in the template RNAs, they slowly began to assume some of the RNA roles and probably incorporated themselves into the ribosome to allow it to function more efficiently.

The transition to the modern world would not be complete without the introduction of DNA as the major form of the genetic material. RNA, while well suited to diverse roles, is actually a much less suitable genetic material than DNA for a complex organism (even one only as complex as a bacterium). The reason for this is that the slight chemical differences between the sugars contained in the nucleotides of RNA and DNA cause the RNA to be more reactive and much less chemically stable; this is good for a ribozyme but clearly bad if the genetic material is to last for any reasonable amount of time. Once DNA initially came into existence, therefore, it is likely that the relatively complex organisms of the time quickly adopted it as their genetic material; shortly thereafter, it became double-stranded, which facilitated its replication immensely. This left RNA, the originator of it all, relegated to the status it enjoys today; molecular fossils exist that uncover its former glory, but it functions mainly as a helper in protein synthesis.

This still leaves the question of how DNA evolved from RNA. At least two protein enzymes were probably necessary to allow this process to begin. The first, ribonucleoside diphosphate reductase, converts RNA nucleotides to DNA nucleotides by reducing the hydroxyl group located on the 2' carbon of ribose. Perhaps more important, the enzyme reverse transcriptase would have been necessary to transcribe RNA genomes into corresponding DNA versions. Examples of both of these enzymes exist in the modern world.

Some concluding observations are in order to summarize the evidence that RNA and not DNA was very likely the first living molecule. No enzymatic activity has ever been attributed to DNA; in fact, the 2' hydroxyl group that RNA possesses and DNA lacks is vital to RNA's ability to function as a ribozyme. Furthermore, ribose is synthesized much more easily than deoxyribose under laboratory conditions. All mod-

ern cells synthesize DNA nucleotides from RNA precursors, and many other players in the cellular machinery are RNA-related. Important examples include adenosine triphosphate (ATP), the universal cellular energy carrier, and a host of coenzymes such as nicotinamide adenine dinucleotide (NAD), derived from B vitamins and vital in energy metabolism.

Impact and Applications

The discovery of ribozymes and the other interesting classes of RNAs has dramatically altered the understanding of genetic processes at the molecular level and has provided compelling evidence in support of exciting new theories regarding the origin of life and cellular evolution. The RNA world theory, first advanced as a radical and unsupported hypothesis in the early 1970's, has gained almost universal acceptance by scientists. It is the solution to the evolutionary paradox that has plagued scientists since the discovery and understanding of the central dogma: Which came first, DNA or proteins? Since they are inextricably dependent upon each other in the modern world, the idea of the RNA world proposes that, rather than one giving rise to the other, they are both descended from RNA, that most ancient of genetic and catalytic molecules. Unfortunately, the RNA world model is not without its problems.

In the mid- to late 1990's, several studies on the stability of ribose, the sugar portion of ribonucleotides, showed that it breaks down relatively easily, even in neutral solutions. A study of the decay rate of ribonucleotides at different temperatures also caused some concern for the RNA world theory. Most current scenarios see life arising in relatively hot conditions, at least near boiling, and the instability of ribonucleotides at these temperatures would not allow for the development of any significant RNA molecules. Ribonucleotides are much more stable at 0 degrees Celsius (32 degrees Fahrenheit), but evidence for a low-temperature environment for the origin of life is limited. Consequently, some evolutionists are suggesting that the first biological entities might have relied on something other than RNA, and that the RNA world was a later development. Therefore, although the RNA world seems like a plausible

model, another model is now needed to establish the precursor to the RNA world.

Apart from origin-of-life concerns, the discoveries that led to the RNA world theory are beginning to have a more practical impact in the fields of industrial genetic engineering and medical gene therapy. The unique ability of ribozymes to find particular sequences and initiate cutting and pasting at desired locations makes them powerful tools. Impressive uses have already been found for these tools in theoretical molecular biology and in the genetic engineering of plants and bacteria. Most important to humans, however, are the implications for curing or treating genetically related disease using this powerful new RNA-based technology.

Gene therapy, in general, is based on the idea that any faulty, disease-causing gene can theoretically be replaced by a genetically engineered working replacement. While theoretically a somewhat simple idea, in practice it is technically very challenging. Retroviruses may be used to insert DNA into particular target cells, but the results are often not as expected; the new genes are difficult to control or may have adverse side effects. Molecular biologist Bruce Sullenger pioneered a new approach to gene therapy, which seeks to correct the genetic defect at the RNA level. A ribozyme can be engineered to seek out and replace damaged sequences before they are translated into defective proteins. Sullenger has shown that this so-called trans-splicing technique can work in nonhuman systems and, in 1996, began trials to test his procedure in humans.

Many human diseases could be corrected using gene therapy technology of this kind, from inherited defects such as sickle-cell disease to degenerative genetic problems such as cancer. Even pathogen-induced conditions such as acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), could be amenable to this approach. It is ironic and gratifying that an understanding of the ancient RNA world holds promise for helping scientists to solve some of the major problems in the modern world of DNA-based life.

—Matthew M. Schmidt, updated by Bryan Ness

See also: Ancient DNA; Antisense RNA; Chromosome Structure; DNA Isolation; DNA Repair; DNA Replication; DNA Structure and Function; Genetic Code; Genetic Code, Cracking of; Molecular Genetics; Noncoding RNA Molecules; One Gene-One Enzyme Hypothesis; Protein Structure; Protein Synthesis; Repetitive DNA; RNA Isolation; RNA Structure and Function; RNA Transcription and mRNA Processing.

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Shotgun Cloning

Field of study: Genetic engineering and biotechnology

Significance: *Shotgun cloning is the random insertion of a large number of different DNA fragments into cloning vectors. A large number of different recombinant DNA molecules are generated, which are then introduced into host cells, often bacteria, and amplified. Because a large number of different recombinant DNAs are generated, there is a high likelihood one of the clones contains a fragment of DNA of interest.*

Key terms

CLONING VECTOR: a plasmid or virus into which foreign DNA can be inserted to amplify the number of copies of the foreign DNA

MARKER: a gene that encodes an easily detected product that is used to indicate that foreign DNA is in an organism

RECOMBINANT DNA: a novel DNA molecule formed by the joining of DNAs from different sources

RESTRICTION ENDONUCLEASE: an enzyme that recognizes a specific nucleotide sequence in a piece of DNA and causes cleavage of the DNA; often simply called a restriction enzyme

Recombinant DNA Cloning and Shotgun Cloning

Before the development of recombinant DNA cloning, it was very difficult to study DNA sequences. Cloning a DNA fragment allows a researcher to obtain large amounts of that specific DNA sequence to analyze without interference from the presence of other DNA sequences. There are many uses for a cloned DNA fragment. For example, a DNA fragment can be sequenced to determine the order of its nucleotides. This information can be used to determine the location of a gene and the amino acid sequence of the gene's protein product. Cloned pieces of DNA are also useful as DNA probes. Because DNA is made of two strands that are complementary to each other, a cloned piece of DNA can be used to probe for copies of the same or similar DNA sequences in

other samples. A cloned gene can also be inserted into an expression vector where it will produce the gene's protein product.

Shotgun cloning begins with the isolation of DNA from the organism of interest. In separate test tubes, the DNA to be cloned and the cloning vector DNA are digested (cut) with a restriction endonuclease that cuts the vector in just one location and the foreign DNA many times. Many restriction endonucleases create single-stranded ends that are complementary, so the end of any DNA molecule cut with that endonuclease can join to the end of any other DNA cut with the same endonuclease. When the digested vector and foreign DNA are mixed, they join randomly and are then sealed using DNA ligase, an enzyme that seals the small gap between two pieces of DNA. This creates recombinant DNA molecules composed of a copy of the vector and a random copy of foreign DNA. The recombinant DNA molecules are then introduced into host cells where the cloning vector can replicate each time the cell divides, which is approximately every twenty minutes in the case of *Escherichia coli*. The resulting collection of clones, each containing a potentially different fragment of foreign DNA, called a genomic library. If a large collection of clones is produced, it is likely that every part of the genome from which the DNA came will be represented somewhere in the genomic library.

The presence of the cloning vector in host cells is determined by selecting for a marker gene in the cloning vector. Most vectors have two marker genes, and often both are different antibiotic resistance genes. A common example is the plasmid pBR322, which has a tetracycline and an ampicillin resistance gene. A restriction endonuclease cuts once somewhere in the tetracycline resistance gene, and if a foreign DNA fragment becomes incorporated, the resulting recombinant plasmid will have a nonfunctional tetracycline resistance gene. A bacterial cell transformed with a recombinant plasmid will therefore be resistant to ampicillin, but will be sensitive to tetracycline. Many plasmids will not incorporate any foreign DNA and will be nonrecombinant. Cells that are

transformed with a nonrecombinant plasmid will be resistant to both tetracycline and ampicillin. After the bacterial cells have been transformed, they are grown on a medium with ampicillin. The only cells that will survive will be those that have received a plasmid vector. To determine which cells have received a recombinant plasmid, the colonies are carefully transferred onto a new medium that has both ampicillin and tetracycline. On this medium, only cells with nonrecombinant plasmids will survive. Thus, colonies that grew on the first media, but not on the second, contain recombinant plasmids. Cells from these colonies are collected and grown, each in a separate tube, and these constitute a genomic library.

Once a genomic library has been produced, the DNA fragments contained in it can be screened and analyzed in various ways. Using the right techniques, specific genes can be found, which can then be used in future analyses and experiments.

Alternatives to Shotgun Cloning

In shotgun cloning, many different DNA fragments from an organism are cloned, and then the specific DNA clone of interest is identified. The number of clones can be reduced, making the search easier, if the DNA of interest is known to be in a restriction endonuclease fragment of a specific size. DNA can be size-selected before cloning using gel electrophoresis, in which an electric current carries DNA fragments through the pores or openings of an agarose gel. DNA migrates through the gel based on DNA fragment size, with the smaller fragments traveling more rapidly than the larger fragments. DNA of a specific size range can be isolated from the gel and then used for cloning. Finally, to clone a piece of DNA known to code for a protein, scientists can use an enzyme called reverse transcriptase to make DNA copies (called a complementary DNA or cDNA) of isolated messenger RNA (mRNA). The cDNA is then cloned, in a similar manner to that already discussed, to produce what is called a cDNA library. One of the advantages of this approach is that the number of clones is greatly reduced.

—Susan J. Karcher, updated by Bryan Ness

See also: cDNA Libraries; Cloning; Cloning Vectors; Genomic Libraries; Genomics; Human Genome Project; Restriction Enzymes.

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Sickle-Cell Disease

Field of study: Diseases and syndromes

Significance: *Sickle-cell disease is a treatable hereditary blood disease that occurs mainly among people of African, Caribbean, and Mediterranean descent, which has led to concerns, particularly in the United States, that it might be used as a surrogate for discrimination against particular racial groups. It is one of the most well documented exam-*

ples of an evolutionary process known as heterozygote advantage, an important means by which genetic variability is preserved.

Key terms

HEMOGLOBIN: a molecule made up of two alpha and two beta amino acid chains whose precise chemical and structural properties normally allow it to bind with oxygen in the lungs and transport it to other parts of the body

HETEROZYGOUS: having two different forms (alleles) of the same gene (locus), each inherited from a different parent

HOMOZYGOUS: having the same allele from both parents

Genetics and Early Research

Sickle-cell disease, also known as sickle-cell anemia, is a hereditary blood disease found primarily among people of African, Caribbean, and Mediterranean descent. Studies of the incidence of the disease in families led to recognition that the illness is manifested only in individuals who receive the sickle-cell allele from both parents. In most circumstances, individuals who inherit the sickle-cell allele from only one parent display no symptoms of the disease; however, they are carriers of the sickle-cell gene and may pass it on to their children.

In 1910, James B. Herrick, a Chicago physician, first described the characteristically “sickle” or bent appearance of the red blood cells after which the disease is named in blood taken from an anemic patient. In the mid-1930’s, Linus Pauling, working with graduate student Charles Coryell, demonstrated that hemoglobin undergoes a dramatic structural change as it combines and releases oxygen. Upon learning that red blood cells from sickle-cell disease patients only assume their characteristic form in the oxygen-deprived venous blood system, Pauling proposed in 1949 that sickle-cell disease was the result of a change in

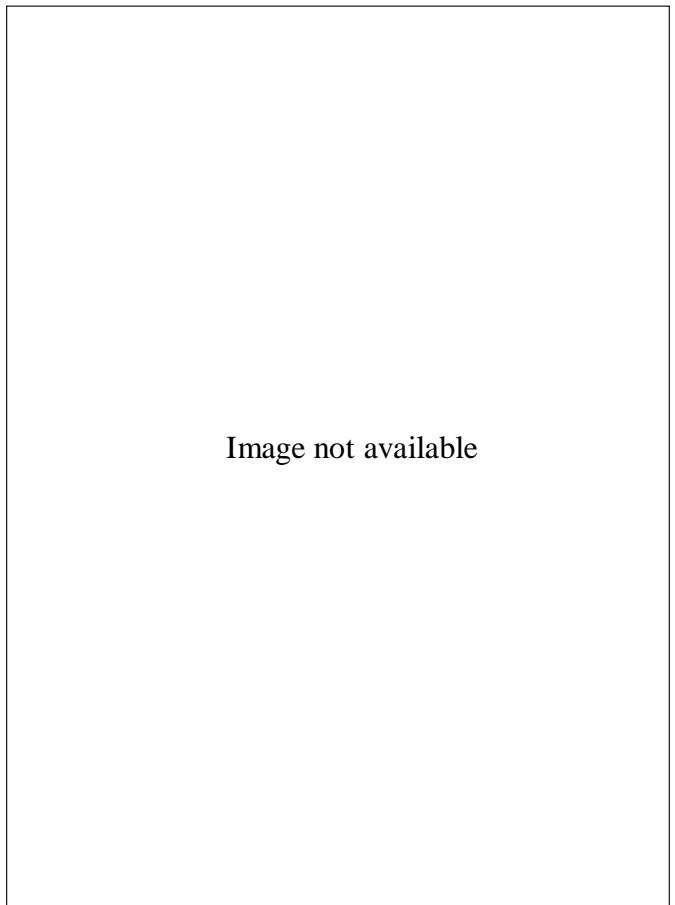


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Linus Pauling (right), with George Beadle, c. 1952. (California Institute of Technology)

the normal amino acid sequence of hemoglobin that interferes with its binding properties.

Three years later, while working with another graduate student, Harvey Itano, Pauling isolated normal hemoglobin and sickle-cell hemoglobin from an individual with anemia using a technique known as electrophoresis. They conducted this investigation by loading hemoglobin onto a paper medium and subjecting it to an electrical current, the presumption being that if the two molecules differed in overall electrical charge, one would migrate along the path of the current faster than the other. In this way, Pauling and Itano established that normal and sickle-cell hemoglobins differ in their respective electrical charges, and people who are heterozygous for the sickle-cell gene have hemoglobin of both types.

In the mid-1950's, Vernon Ingram approached the problem using a more sophisticated version of Pauling's procedure. Ingram first treated hemoglobin of the two types with an enzyme (trypsin) that broke the complex hemoglobin molecules into smaller polypeptides and then used electrophoretic techniques on the resulting polypeptides to determine precisely where in their respective amino acid sequences the two hemoglobins differed from one another. Ingram was able to show that normal and sickle-cell hemoglobin differ by only a single amino acid out of a total of more than three hundred: Where the normal hemoglobin gene codes for glutamic acid in the sixth position of the beta-globin, the sickle-cell gene substitutes another amino acid (usually lysine). Ingram's work provided proof of Pauling's earlier proposal, making sickle-cell disease the very first example of a genetic disease being traced to its precise origin at the molecular level.

Physiological Basis, Symptoms, and Treatments

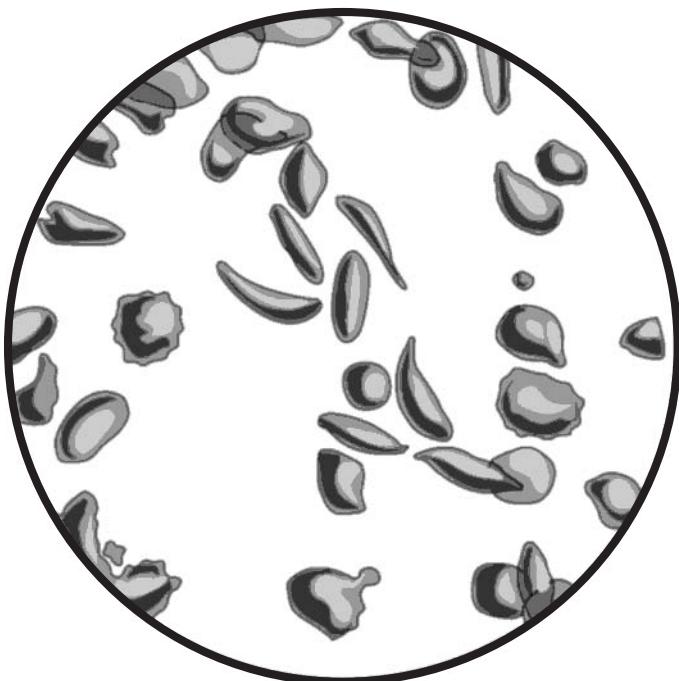
This substitution of lysine for glutamic acid in the beta chain of the hemoglobin molecule has a profound effect on its biological properties under conditions of oxygen deprivation. Hemoglobin coded for by the sickle-cell gene causes the beta chains of the hemoglobin to stick to one another as long, rigid rods and consequently deforms the normally smooth, donut-shaped appearance of the red blood cell to a characteristic sickle shape that prevents it from squeezing through tiny blood capillaries.

Symptoms of the disease appear about six months after birth, when the last of fetal hemoglobin, a type of hemoglobin that increases the oxygen supply of blood, leaves the infant's body. The severity of the illness varies widely among individuals. Some develop severe anemia as deformed red blood cells are removed more rapidly from the bloodstream (an average "life" of seventeen versus forty days).

They may also experience periodic bouts of severe pain ("pain crises"), strokes, and blindness, all thought to be the direct result of sickled cells clogging blood vessels and thereby depriving tissues of oxygen. Heterozygous carriers of the gene normally display no symptoms, although some have been known to become ill under extreme circumstances, such as high altitudes.

A great deal of progress has been made in the diagnosis and treatment of sickle-cell disease. This includes both a variety of pain management therapies and the use of antibiotics such as penicillin to prevent infections. Although there is no cure, several promising experimental therapies for this disease are under investigation, including the use of bone marrow transplants (transplants of the mast cells that give rise to red blood cells from people not having the disease) and

Sickle-Cell Anemia



The red blood cells are sickle-shaped rather than round, which causes blockage of capillaries. (Hans & Cassidy, Inc.)

hydroxyurea, a chemical thought to stimulate the production of fetal hemoglobin.

Attention to and funding for research on sickle-cell disease has increased since World War II, although misinformation about the disease persists. Many have raised concerns that carriers of the disease are discriminated against, both by potential employers and insurance companies. Several organizations were established in the United States in the early 1970's to promote education, treatment, and research for the disease, including Howard University's Center for Sickle-Cell Disease, founded by Ronald B. Scott in 1972. Today, forty U.S. states, the District of Columbia, Puerto Rico, and the Virgin Islands, were screening newborns for the sickle-cell trait.

Evolutionary Significance

In most cases, hereditary diseases with such negative consequences as those associated with sickle-cell disease are kept at low frequencies in populations by natural selection; that is, individuals who carry genes for hereditary diseases are less likely to survive and reproduce than those who carry the normal form of the gene. The continued presence of defective genes in a population therefore reflects the action of chance mutations. Yet the sickle-cell gene is much more common than one would expect if its frequency in a population was caused by mutation alone.

In some areas that are associated with a high incidence of malaria, such as the equatorial belt of Africa, some tribes have been found to have frequencies of the sickle-cell gene as high as 40 percent. This curious correlation between high frequencies of the sickle-cell gene and areas where malaria is common led Anthony C. Alison to suggest, in 1953, that the sickle-cell gene provides an advantage in such environments. Malaria is a deadly, mosquito-borne disease caused by a microscopic parasite, *Plasmodium vivax*, which uses human red blood cells as hosts for part of its life cycle. People who have normal hemoglobin are vulnerable to the disease, and people who are homozygous for the sickle-cell gene in malaria-infested regions die quite early in life because of anemia and other complications. However,

when the red cells of people who are heterozygous for the sickle-cell gene are invaded by the malarial parasite, the red cells adhere to blood vessel walls, become deoxygenated, and assume the sickled shape, prompting both their destruction and that of their parasitic invader. This provides the heterozygous carrier with a natural resistance to malaria and explains the relatively high frequency of the sickle-cell gene in such environments. Sickleg-cell disease thus represents a particularly well-documented example of a selective process known as heterozygote advantage, in which individuals heterozygous for a given gene have a greater probability of surviving or reproducing than either homozygote. This is an important phenomenon from an evolutionary standpoint because it provides a mechanism by which genetic diversity in a population may be preserved.

—David Wijss Rudge

See also: Amniocentesis and Chorionic Villus Sampling; Biopharmaceuticals; Genetic Engineering; Genetic Screening; Genetic Testing; Hardy-Weinberg Law; Incomplete Dominance; Mutation and Mutagenesis; RFLP Analysis; Shotgun Cloning.

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Web Sites of Interest

Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including sickle-cell disease.

Sickle Cell Information Center. <http://www.sinfo.org>. This site provides news and research updates, worldwide resource links, and an interactive link for children.

ing. The protein products of these genes allow cells to communicate with each other in order to coordinate their metabolism, movements, and reproduction. Failure of cells to communicate properly can lead to cancer, defects in embryological development, and many other disorders.

Key terms

CELL CYCLE: the orderly sequence of events by which a cell grows, duplicates its chromosomal DNA, and partitions the DNA into two new cells

CELL SIGNALING: communication between cells that occurs most commonly when one cell releases a specific "signaling" molecule that is received by another cell

RECEPTORS: molecules in target cells that bind specifically to a particular signaling molecule

TARGET CELL: the cell that receives and responds to a signaling molecule

Signal Transduction Pathways

Signal transduction can occur by a number of different, often complex, sequences of molecular events called signal transduction pathways, which result in several kinds of target cell response, including the turning on of genes, the activation of metabolic pathways, and effects on the cell cycle. Among the signaling molecules found in higher organisms are hormones, local mediators that produce local physiological effects, growth factors that act locally to promote growth, and survival factors that act locally to repress cell suicide (apoptosis). Growth factors and survival factors are particularly important during embryological development, when they orchestrate the changes in cell types, positions, and numbers that give rise to the new organism.

Types of Receptors

Most signal transduction pathways begin with the binding of signaling molecules to specific receptors in target cells. Signaling molecules are often referred to as receptor ligands. The binding of the ligand to its receptor initiates a signal transduction pathway. A cell can respond to a particular signaling molecule only if it possesses a receptor for it.

Signal Transduction

Field of study: Molecular genetics

Significance: *Signal transduction consists of all of the molecular events that occur between the arrival of a signaling molecule at a target cell and its response. A significant proportion of the genome in animals consists of genes involved in cell signal-*

Receptors are protein molecules. There are two categories of them, based on location in the cell: receptors that are intracellular and receptors that are anchored in the cell's surface membrane. The membrane-anchored receptors can be further divided based on the steps of the signal transduction pathway that they initiate: receptors that bind to and activate GTP-binding proteins (G proteins), receptors that are enzymes, and receptors that are ion channels. Receptors that are channels bind neurotransmitters or hormones and increase or decrease the flow of specific ions into the cell, leading to a physiological response by the cell. These receptors generally do not have a direct effect on gene expression (although changes in a cell's calcium ion concentrations can influence gene expression). Each of the other receptor types stands at the head of a signal transduction pathway that is characteristic for each receptor type and can lead to gene expression. In what follows, some of the more common transduction pathways that can lead to gene expression are described.

Intracellular Receptors

Intracellular receptors include the receptors for lipid-soluble hormones such as steroid hormones. Some of these receptors are in the cell's cytoplasm and some are in the nucleus. Hormone molecules enter the cell by first diffusing across the membrane and then binding to the receptor. Before the hormones enter the cell, the receptors are attached to "chaperone" proteins, which hold the receptor in a configuration that allows hormone binding but prevents it from binding to DNA. Hormone molecules displace these chaperone molecules, enabling the receptor to bind to DNA. If the receptor is a cytoplasmic receptor, the hormone-receptor complex is first transported into the nucleus, where it binds to a specific DNA nucleotide sequence called a hormone response element (HRE) that is part of the promoter of certain genes. In most cases the receptors bind as dimers; that is, two hormone-receptor complexes bind to the same HRE. The receptor-hormone complex functions as a transcription factor, promoting transcription of the gene and production of a protein that the cell was

not previously producing. The hormone hydrocortisone, for example, triggers the synthesis of the enzymes aminotransferase and tryptophan oxygenase. A single hormone such as hydrocortisone can turn on synthesis of two or more proteins if each of the genes for the proteins contains an HRE. In some cases, when hormone-receptor complexes bind to an HRE, they suppress transcription rather than promote it.

G Protein-Binding Receptors

Many hormones, growth factors, and other signaling molecules bind to membrane receptors that can associate with and activate heterotrimeric G proteins when a signaling molecule is bound to the receptor. Heterotrimeric G proteins are a family of proteins that are present on the cytoplasmic surface of the cell membrane. Many cell types in the body contain one or more of these family members, and different cell types contain different ones. All heterotrimeric G proteins are made up of three subunits: the alpha, beta, and gamma subunits. The alpha subunit has a binding site for GTP or GDP (hence the name G proteins) and is the principal part of the protein that differs from one heterotrimeric G protein family member to another. When the receptor is empty (no signal molecule attached), these G proteins have GDP bound to the alpha subunit and the G protein is not bound to the receptor.

However, when a signaling molecule binds to the receptor, the cytoplasmic domain of the receptors changes shape so that it now binds to the G protein. In binding to the receptor, the G protein also changes shape, causing GDP to leave and GTP to bind instead. Simultaneously, the alpha subunit detaches from the beta-gamma subunit and both the alpha subunit and the beta-gamma subunit detach from the receptor. The alpha subunit or the beta-gamma subunit (depending upon the particular G-protein family member involved and the cell type) then activates (or with some G-protein family members, inhibits) one of several enzymes, most commonly adenylate cyclase or phospholipase C. Alternatively, they can open or close a membrane ion channel, altering the

electrical properties of the cell; for example, potassium ion channels in heart muscle cells can be opened by G proteins in response to the neurotransmitter acetylcholine.

In cases where adenylate cyclase or phospholipase C is activated, these enzymes catalyze reactions that produce molecules called second messengers, which, through a series of steps, activate proteins that lead to a physiological response (such as contraction of smooth muscle), a biochemical response (such as glycogen synthesis) or a genetic response (such as activating a gene).

Activation of adenylate cyclase causes it to catalyze the conversion of adenosine triphosphate (ATP) to the second messenger cyclic adenosine monophosphate (cAMP), which in turn activates a protein called protein kinase A which, in some cells, moves into the nucleus and phosphorylates and activates transcription factors such as CREB (CRE-binding protein). CREB binds to a specific DNA sequence in the promoter of certain genes called the CRE (cAMP-response element), as well as to other transcription factors, to activate transcription of the gene. In other cells, protein kinase A activates enzymes or other proteins involved in physiological or metabolic responses.

Activation of phospholipase C catalyzes the breakdown of a glycolipid component of the cell membrane called phosphatidylinositol bisphosphate (PIP₂) into two second messengers, inositol triphosphate (IP₃) and diacylglycerol (DAG). DAG activates a protein called protein kinase C (PK-C), which in turn activates other proteins, leading to various cell responses, including, in certain cells of the immune system, activation of transcription factors which turn on genes involved in the body's immune response to infection. IP₃ causes the release of calcium ions stored in the endoplasmic reticulum. These ions bind and activate the protein calmodulin, which activates a variety of proteins, leading in most cases to a physiological response in the cell.

Catalytic Receptors

Catalytic receptors are receptors that function as enzymes, catalyzing specific reactions in the cell. The part of the receptor that is in the

cytoplasm (the cytoplasmic domain) has catalytic capability. Binding of a signaling molecule to the external domain of the receptor activates the catalytic activity of the cytoplasmic domain. There are several kinds of catalytic receptors based on the type of reaction they catalyze; these include receptor tyrosine phosphatases, receptor guanylate cyclases, receptor serine/threonine kinases, and receptor tyrosine kinases. Receptor tyrosine kinases (RTKs) are the most common of these.

RTKs are the receptors for many growth factors and at least one hormone. For example, they are the receptors for fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), and insulin. RTKs play a role in regulating many fundamental processes, such as cell metabolism, the cell cycle, cell proliferation, cell migration, and embryonic development. In most cases, when a ligand binds to this type of receptor, a conformational (shape) change occurs in the receptor so that it binds to another identical receptor-ligand complex to produce a double or dimeric receptor. The dimeric receptor then catalyzes a cytoplasmic reaction in which several tyrosine amino acids in the cytoplasmic domain of the receptor itself are phosphorylated. The phosphorylated tyrosines then function as docking sites for several other proteins, each of which can initiate one of the many branches of the RTK signal transduction pathway, leading to the various cell responses. One of the major branches of the RTK pathway that in many cases results in gene expression begins with the binding of the G protein ras (ras is not one of the trimeric G proteins discussed above) to the activated RTK receptor via adapter proteins. Binding of ras to the adapter proteins activates it by allowing it to bind GTP instead of GDP. Activated ras then phosphorylates the enzyme MEK, which phosphorylates and activates an enzyme of the MAP kinase family. In cases where this enzyme is MAP kinase itself, the enzyme dimerizes, moves into the nucleus, and activates genes, usually many genes, by phosphorylating and activating their transcription factors.

Signal Transduction and the Cell Cycle

The biochemical machinery that produces the cell cycle consists of several cyclins whose concentrations rise and fall throughout the cell cycle. Cyclins activate cyclin-dependent kinases (cdk's), which activate the proteins that carry out the events of each stage of the cell cycle. In higher organisms, control of the cell cycle is carried out primarily by growth factors. In the absence of growth factors, many cells will stop at a point in the cell cycle known as the G₁ checkpoint and cease dividing. The cell cycle is started when the cells are exposed to a growth factor. For example, some growth factors start cell division by binding to a membrane receptor and initiating the RTK/MAP kinase signal transduction pathway. The activated transcription factor that results from this pathway activates a gene called *myc*. The protein that is produced from this gene is itself a transcription factor, which activates the cyclin D gene, which produces cyclin D, an important component of the cell cycle biochemical machinery. Cyclin D activates cyclin-dependent kinase 4 (cdk4), which drives the cell into the G₁ phase of the cell cycle. cdk4 also causes an inhibiting molecule called pRB to be removed from a transcription factor for the cyclin E gene. Cyclin E is then produced and activates cyclin-dependent kinase 2 (cdk2), which drives the cell into the S phase of the cell cycle, during which chromosomal DNA is replicated, leading to cell division by mitosis.

Signal Transduction and Cancer

Cancer is caused primarily by uncontrolled cell proliferation. Since many signal transduction pathways lead to cell proliferation, it is not surprising that defects in these pathways can lead to cancer. For example, as described above, many growth factors promote cell proliferation by activating the RTK/MAP kinase signal transduction pathway. In that pathway a series of proteins is activated (ras, MAP kinase, and so on). If a mutation occurred in the gene for one of these, ras for example, such that the mutant ras protein is always activated rather than being activated only when it binds to the receptor, then the cell would always be dividing and cancerous growth could result. Another

example would be if the gene for pRB that binds to and inhibits the cyclin E transcription factor were mutated such that the pRB could never bind to the transcription factor; then the cell would divide continuously. Mutations in both ras and pRB are in fact known to cause cancer in humans.

—Robert Chandler

See also: Burkitt's Lymphoma; Cancer; Cell Cycle, The; Cell Division; DNA Replication; Gene Regulation: Bacteria; Model Organism: *Saccharomyces cerevisiae*; Oncogenes; One Gene–One Enzyme Hypothesis; Steroid Hormones; Tumor-Suppressor Genes.

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- Lodish, Harvey, et al. *Molecular Cell Biology*. New York: W. H. Freeman, 2000. One of the standard textbooks in the field of cell biology. Chapter 20 provides detailed information on signal transduction.

Smallpox

Fields of study: Diseases and syndromes;

Viral genetics

Significance: *Smallpox is a poxvirus disease of humans existing in two forms; the more virulent and frequently lethal form is Variola major, and a milder form is Variola minor. Smallpox is very contagious, requiring strict quarantine measures and aggressive vaccination programs to contain and eradicate outbreaks. Although smallpox was eradicated globally in 1977, at least two research stocks exist, and there is concern that clandestine-held stocks of the virus may be used as weapons of bioterrorism.*

Key terms

BIOTERRORISM: the use of living organisms as instruments or weapons of terror, such as the deliberate introduction of smallpox, anthrax, or other diseases into civilian populations

POXVIRUS: any of the family of viruses that produces pustules on the surface of the skin

TRANSMISSIBILITY: the rate at which a disease spreads from primary to secondary cases

Definitions

Smallpox is a member of the *Poxviridae* family of viruses, which are the largest and most complex of all known viruses. Poxviruses are named for the characteristic rash or pox lesions that occur during most infections. The poxviruses include a number of familiar diseases such as smallpox, cowpox, rabbitpox, sheppox, and fowlpox. Two subfamilies of poxviruses are recognized based on their hosts. The orthopoxvirus subfamily comprises viruses that affect vertebrates and includes smallpox; the poxviruses of the subfamily parapoxviruses infect invertebrates, primarily insects. There are two types of variola, the poxvirus that causes smallpox: *Variola major* causes the more virulent and lethal form of smallpox in humans, and *V. minor* causes a milder form of smallpox. Both varieties infect only humans and monkeys. Other names or synonyms for smallpox include alastrim, amaas, Kaffir mil pox, West Indian modified smallpox, and para-smallpox.

History and Symptoms

Historically one of the most devastating and lethal of all human diseases, smallpox is named for the small pustules that occur as a rash over the skin of the victim. Smallpox symptoms include a rash that spreads over the entire body, high fever, chills, aches and pains, and vomiting. The most lethal form, black or hemorrhagic smallpox, results in death within two to six days. The fatality rate varies with health and previous exposure of the local population but ranges from 30 to 90 percent.

Humans have had a long and unfortunate history of association with smallpox. The disease apparently originated in India and spread westward into the Middle East and Northern Africa several thousand years ago. An Egyptian mummy of the Twentieth Dynasty shows the characteristic scarring associated with smallpox. Warriors returning from the Crusades brought the disease back with them. In the following centuries smallpox became endemic throughout much of Europe and became a rite of passage for much of the population—those who contracted smallpox and survived were marked by its scars throughout life. In time, the population built up a partial immunity to the disease. Smallpox was carried by Europeans to the New World and to Australia during the Age of Exploration. It was spread to the immunologically defenseless Amerindians of North America and Aboriginals of Australia with devastating effect and may have contributed to the ease of European settlement following the decimation of tribal peoples in both areas, as it caused widespread death and devastation among the indigenous populations and was at least partly responsible for the depopulation of natives in the newly discovered lands. Before its eradication, smallpox was endemic throughout the world, with major centers of the disease in Africa, Asia, and the Middle East.

Genetics of Smallpox

The poxviruses are the largest and most complex of all the viruses that have so far been identified in animals. The variola virus that causes smallpox has a brick-shaped outer envelope and a dumbbell-shaped core that contains the smallpox genome. The smallpox genome is

composed of linear, double-stranded DNA containing more than two hundred genes. Chemically, the smallpox virion consists of 90 percent protein, 3 percent DNA, and 5 percent lipid. The DNA genome codes for several hundred polypeptides, including several transcriptases responsible for replication of the virus within the cells of the host.

Replication of smallpox begins when the virus attaches to the surface of a host cell. After binding to receptors on the plasma membrane of the host cell, the host cell passes the virus into the cytoplasm by endocytosis. Once inside the cell, the virus becomes trapped in a lysosome vesicle in the cytoplasm. The first step in removing its viral coat probably occurs at this stage, as host cell enzymes dissolve the viral envelope. The viral core, containing the DNA, then exits the lysosome and enters the cytoplasm, where the viral genome can be ex-

pressed. One of the first steps involves the production of enzymes that degrade the proteins of the viral core, which releases the naked viral DNA into the cytoplasm. Additional transcription takes place, initially producing structural proteins and enzymes, including DNA polymerase, which promotes the replication of the viral DNA. Finally, the late messenger RNA (mRNA) is transcribed, producing additional structural proteins and assembly enzymes that complete virion construction. During viral replication, most host-cell protein synthesis is blocked, because transport of host-cell mRNA molecules through the nuclear envelope into the cytoplasm is prevented.

Newly completed viroids exit the host cell through microvilli on the cell surface or fuse with the cell membrane, after which they exit the cell by the process of exocytosis. Once in the tissue, fluids, and bloodstream, the newly

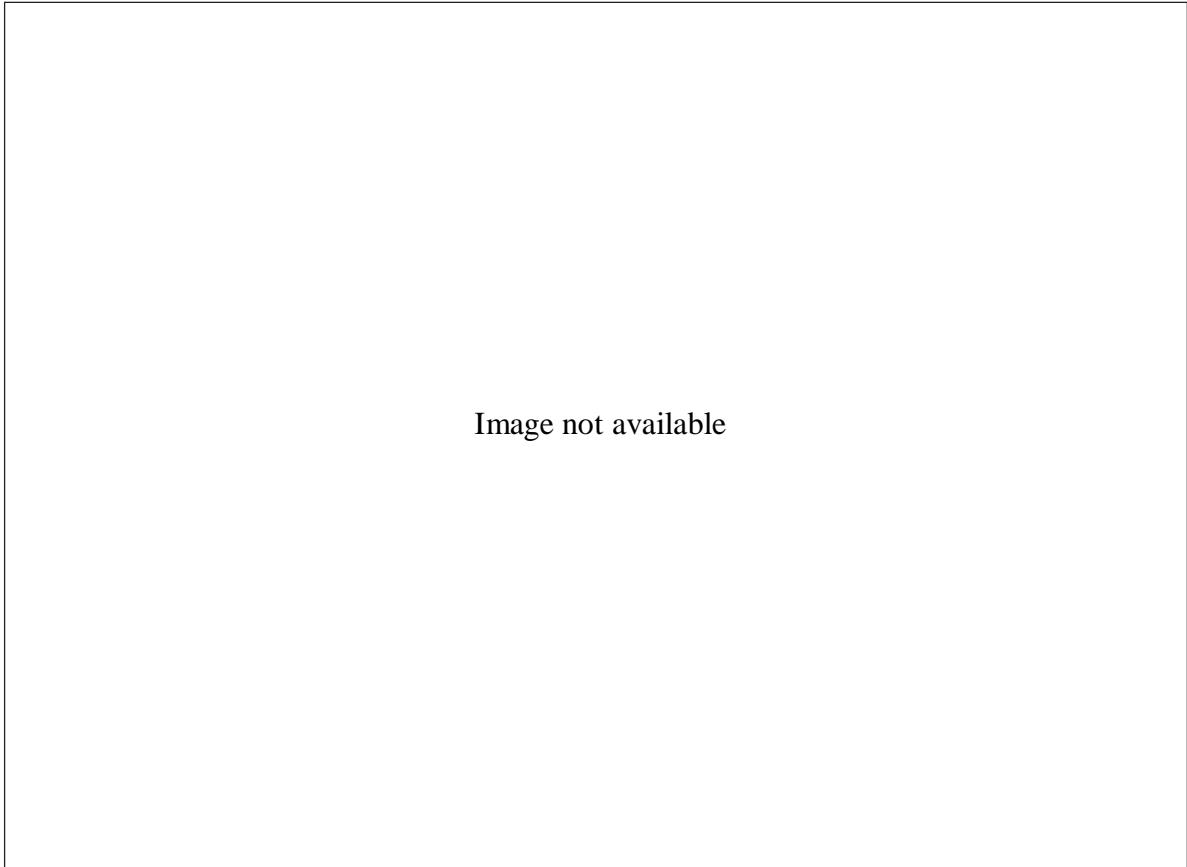


Image not available

A man with advanced smallpox in 1941. (AP/Wide World Photos)

released and highly infectious viral particles can invade and replicate in other host cells.

Transmission

Smallpox is transmitted from one human to another, either by direct contact or via droplets released into the air during sneezing and coughing fits. The virus does not live long outside the human body and does not reproduce outside the human body. No natural animal carriers of variola other than monkeys, which are also susceptible, are known for the smallpox disease. In extremely rare cases smallpox is transmitted by carriers that are themselves immune to the disease but can transmit the disease to others. Still, only a few droplets settling on another person are sufficient to transmit smallpox. Because of the virulence and mode of transmission, public health regulations specify decontamination procedures. Living quarters, bedding, clothes, and other articles of infected persons must be thoroughly cleansed by heat or with formaldehyde, or destroyed altogether.

Pathogenesis and Symptoms

Infection occurs when the variola virus enters the respiratory mucosa of the nasal or pharyngeal region of the upper respiratory tract of humans. Apparently, only a few viral particles are needed to produce an infection. After a few hours or a few days, the virus migrates to and invades cells in the lymph nodes of the nasopharyngeal region, where it enters the cells, following which rapid reproduction occurs. After a few days, it enters the bloodstream, a condition called viremia. At this time symptoms of smallpox appear. The virus spreads into lymph nodes, spleen, and bone marrow, where reproduction continues rapidly. By the eighth day of infection, the virus is contained in white blood cells or leukocytes, which transmit it to the small blood vessels in the dermis of the skin as well as in the mucosa that lines the mouth and pharynx.

Following an incubation period of about two weeks (the range is between seven and seventeen days), symptoms appear, including high fever, headache, nausea, malaise, and often backache. Accompanying these symptoms is a

rash that begins in the mouth and spreads across the face, forearms, trunk, and legs. The rash is first confined to a reddish or purplish swelling of the blood vessels but soon becomes pustular as little round nodules appear on the surface of the body. If the patient recovers, the pustules crust over and the resultant scabs eventually split, which causes scarring of the face.

Death occurs within a few days following the appearance of the rash, most commonly from toxemia caused by variola antigens and various immune complexes circulating in the blood. In some cases the disease is followed by encephalitis. Smallpox fatalities typically occur because of complications such as pneumonia, septicemia, and nephritis (kidney failure). Survivors often suffer from general scarring, ulcers, scarring of the cornea leading to blindness, and skin abscesses. Treatment of survivors with chemotherapy has reduced the severity of many of these complications.

The considerably less virulent form of smallpox, *Variola minor*, produces a much less severe illness characterized by fever, chills, and a milder rash. The same conditions are sometimes seen in patients who have previously been vaccinated or even as a response to vaccinations.

Treatment and Control

Despite decades of research, there is no specific treatment for smallpox other than bed rest and application of antibiotics to prevent secondary infections. Therefore, only prevention of spread by quarantine of infected persons prevents epidemics. Immediate recognition of the disease remains the strongest control measure, followed by vaccination of all health care personnel and others that may come in contact with infected persons.

Widespread and aggressive inoculation programs conducted during the first half of the twentieth century eradicated smallpox from most regions of the world, including North America, Eurasia, and Oceania, largely as a result of the success of the vaccination process originally developed by Edward Jenner. By 1967 smallpox was found only in thirty-three countries and had an annual infection rate of

10 million to 15 million cases. In that year the World Health Organization (WHO) initiated a campaign to eliminate smallpox completely as a human disease, concentrating in Africa, India, and Indonesia. The last case of smallpox in Asia was reported in Bangladesh in 1975 and the last known smallpox victim was recorded in Somalia in October, 1977. Eradication was considered accomplished by 1979. The cost of the eradication campaign was \$150 million.

Most researchers conclude that the effective eradication of smallpox was made possible for several reasons: (1) smallpox cases could be quickly and positively identified, (2) there are no natural carriers that serve as disease reservoirs, (3) humans were the only carriers, (4) individuals who survived did not continue to harbor the virus, and (5) the smallpox vaccine proved highly effective.

Recombinant DNA Technology and Vaccinia Viruses

Vaccinia viruses can absorb comparatively large amounts of foreign DNA without losing their ability to replicate, giving rise to the idea that they may provide a vehicle for providing immunity for other viral diseases of humans. One of several ongoing investigations involves insertion of 22-25 kilobase pairs into vaccinia. Experiments using this technique have produced vaccinia strains that encode surface proteins (antigens) of a number of important viruses, including influenza, hepatitis B, and herpesvirus. One possible outcome of these recombinant DNA experiments is the production of vaccinia strains that can serve as vaccines for several viral diseases simultaneously.

Smallpox as a Bioterrorism Weapon

Since its official eradication in 1979, only two stocks of smallpox officially remain; one stock is held at the Centers for Disease Control in Atlanta, Georgia, and the other is kept at VECTOR, Novosibirsk, in central Russia. However, there remains the possibility that clandestine stocks still exist, and these stocks may serve as potential bioterrorism weapons, either to be used against military or civilian populations or to be mounted as international threats. The use of smallpox as a bioterrorism weapon

would be classed as an international crime, but prevention of its use is difficult unless all existing stocks can be identified and destroyed.

Smallpox is a potential bioterrorism weapon because of its transmissibility, its known lethality, and the general lack of immunity of much of the global population. Because of its bioterrorism potential, research is now centered on rapid identification methods that enable the early detection of smallpox as well as aggressive vaccination programs for individuals most at risk, who have been identified as health care workers. In addition, smallpox vaccinations were reinstated in 2002 for some U.S. military personnel and some health care workers, essentially those considered at highest risk. The vaccine is made from live but weakened vaccinia virus that is pricked into the skin. The characteristic blister scabs over within three weeks. During this time it is possible to transmit the virus to other parts of the body and to other people. Reactions to the vaccine range from a mild soreness around the vaccination site to more severe effects that may include brain inflammation and a rare and progressive bacterial inflammation called vaccinia that is sometimes fatal. For these reasons, mass vaccinations of the general public have been discouraged.

See also: Anthrax; Bacterial Resistance and Super Bacteria; Biological Weapons; Emerging Diseases; Gene Regulation: Viruses; Hereditary Diseases; Viral Genetics.

—Dwight G. Smith

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World Health Organization. *Future Research on Smallpox Virus Recommended*. Geneva, Switzerland: World Health Organization Press, 1999. This press release emphasizes the need for smallpox research in light of its potential use as a weapon in the bioterrorism arsenal.

Web Sites of Interest

National Organization for Rare Disorders. <http://www.rarediseases.org>. Searchable site by type of disorder. Includes background information on smallpox and a list of related resources.

Centers for Disease Control. <http://www.bt.cdc.gov/agent/smallpox/index.asp>. The CDC's Web page on smallpox includes information on the disease and posts the latest on smallpox vaccines.

Sociobiology

Fields of study: History of genetics; Human genetics and social issues; Population genetics

Significance: *Sociobiology attempts to explain social interactions among members of animal species from an evolutionary perspective. The application of the principles of sociobiology to human social behavior initiated severe criticism and accusations of racism and sexism.*

Key terms

ALTRUISM: the capacity of one individual to behave in a way that benefits another individual of the same species at some cost to the actor

EUSOCIALITY: an extreme form of altruism and kin selection in which most members of the society do not reproduce but rather feed and protect their relatives

KIN SELECTION: a special type of altruistic behavior in which the benefactor is related to the actor

RECIPROCAL ALTRUISM: a type of altruism in which the benefactor may be expected to return the favor of the actor

SOCIETY: a group of individuals of the same species in which members interact in relatively complex ways

History

Sociobiology is best known from the works of Edward O. Wilson, especially his 1975 book *Sociobiology: The New Synthesis*. This work both synthesized the concepts of the field and initiated the controversy over the application of sociobiological ideas to humans. However, the concepts and methods of sociobiology did not start with Wilson; they can be traced to Charles Darwin and others who studied the influence of genetics and evolution on behavior. Sociobiologists attempt to explain the genetics and evolution of social activity of all types, ranging from flocking in birds and herd formation in mammals to more complex social systems such as eusociality. "The new synthesis" attempted to apply genetics, population biology, and evolutionary theory to the study of social systems.

When sociobiological concepts were applied to human sociality, many scientists, especially social scientists, feared a return to scientific theories of racial and gender superiority. They rebelled vigorously against such ideas. Wilson was vilified by many of these scientists, and some observers assert that the term "sociobiology" generated such negative responses that scientists who studied in the field began using other names for it. At least one scientific journal dropped the word "sociobiology" from its title, perhaps in response to its negative connotations. However, the study of sociobiological phenomena existed in the social branches of animal behavior and ethology long before the term was coined. Despite the criticism, research has continued under the name sociobiology as well as other names, such as "behavioral ecology."

Sociobiology and the Understanding of Altruism

Sociobiologists have contributed to the understanding of a number of aspects of social behavior, such as altruism. Illogical in the face of evolutionary theory, apparently altruistic acts can be observed in humans and other animal groups. Darwinian evolution holds that the organism that leaves the largest number of mature offspring will have the greatest influence on the characteristics of the next generation. Under this assumption, altruism should disappear from the population as each individual seeks to maximize its own offspring production. If an individual assists another, it uses energy, time, and material it might have used for its own survival and reproduction and simultaneously contributes energy, time, and material to the survival and reproductive effort of the recipient. As a result, more members of the next generation should be like the assisted organism than like the altruistic one. Should this continue generation after generation, altruism would decrease in the population and selfishness would increase. Yet biologists have cataloged a number of altruistic behaviors.

When a prairie dog "barks," thus warning others of the presence of a hawk, the prairie dog draws the hawk's attention. Should it not just slip into its burrow, out of the hawk's reach?

When a reproductively mature acorn woodpecker stays with its parents to help raise the next generation, the woodpecker is bypassing its own reproduction for one or more years. Should it not leave home and attempt to set up its own nest and hatch its own young? Eusocial species, such as honeybees and naked mole rats, actually have many members who never reproduce; they work their entire lives to support and protect a single queen, several reproductive males, and their offspring. It would seem that all these altruistic situations should produce a decrease in the number of members of the next generation carrying altruistic genes in favor of more members with "selfish" genes.

Sociobiologists have reinterpreted some of these apparently altruistic acts as camouflaged selfishness. The barking prairie dog, for example, may be notifying the hawk that it sees the predator, that it is close to its burrow and cannot be caught; therefore, the hawk would be better off hunting someone else. Perhaps the young acorn woodpecker learns enough from the years of helping to make its fewer reproductive years more successful than its total reproductive success without the training period.

It is difficult, however, to explain the worker honeybee this way. The worker bee never gets an opportunity to reproduce. Sociobiologists explain this and other phenomena by invoking kin selection. Since the worker bees are closely related to the queen (as sisters or daughters), to reproductive males, and to other workers they help feed and protect, they share a large number of genes with them. If they help raise enough brothers and sisters (especially males and queens) to more than make up for the offspring they do not produce themselves, they will actually increase the proportion of individuals similar to themselves more than if they "selfishly" reproduced.

The prairie dog's behavior might be explained this way as well. The organisms the prairie dog is warning are primarily relatives. By warning them, the prairie dog helps preserve copies of its own genes in its relatives. If the cost of the behavior (an occasional barking prairie dog being captured by a hawk because the warning call drew the hawk's attention) is more than compensated for by the number of

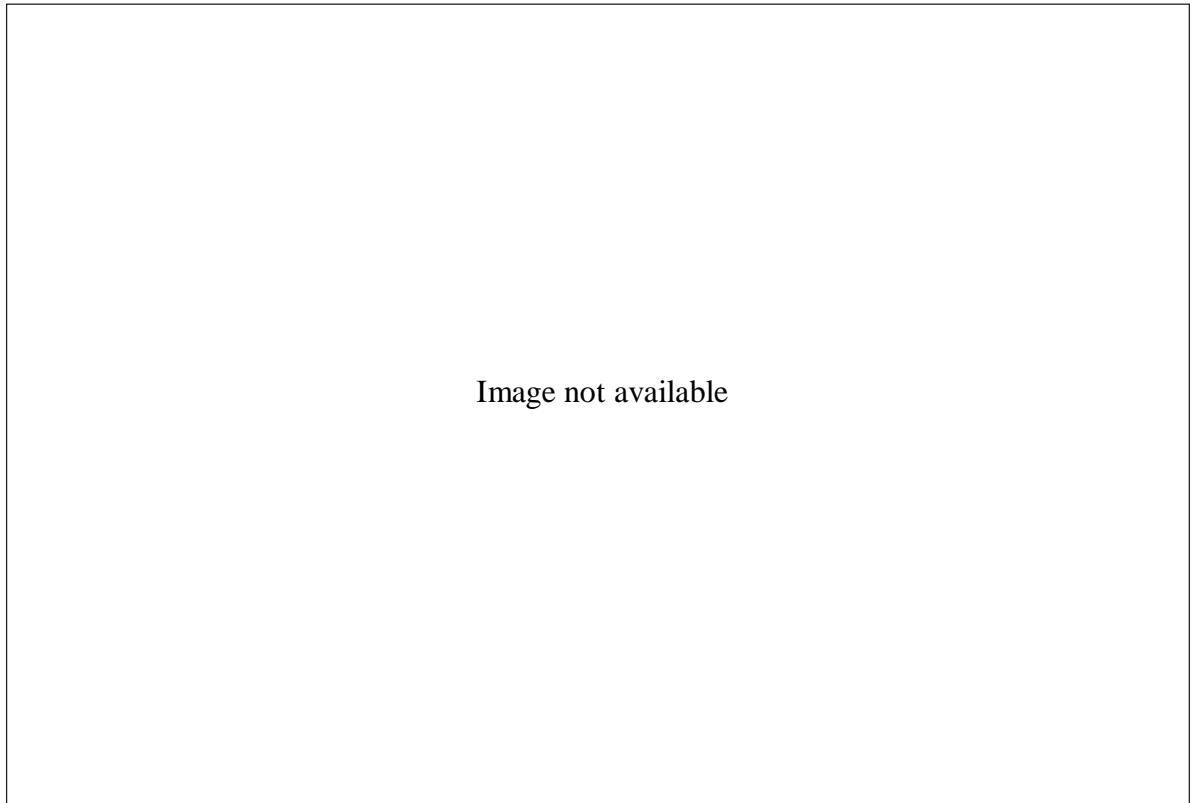


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Edward O. Wilson's studies of insect behavior influenced his controversial theories of sociobiology. (AP/Wide World Photos)

relatives saved from the hawk by the warning, kin selection will preserve the behavior. The helper acorn woodpecker's behavior may be explained in similar ways, not as an altruistic act but as a selfish act to favor copies of the helper's genes in its relatives. Another explanation of altruism set forth by sociobiologists is reciprocity or reciprocal altruism: If the prairie dog is sometimes warned by others and returns the favor by calling out a warning when it sees a predator, the prairie dog town will be safer for all prairie dogs.

Opposition to the Application of Sociobiology to Humans

Wilson's new synthesis attempted to incorporate biology, genetics, population biology, and evolution into the study and explanation of social behavior. When the analyses turned to human sociality, critics feared that they would lead back to the sexist, racist, and determinist viewpoints of the early twentieth century. The

argument over the relative importance of heredity or environment (nature or nurture) in determining individual success had been more or less decided in favor of the environment, at least by social scientists. Poor people were not poor because they were inherently inferior but because the environment they lived in did not give them an equal chance. Black, Hispanic, and other minority people were not inordinately represented among the poor because they were genetically inferior but because their environment kept them from using their genetic capabilities.

Sociobiologists entered the fray squarely on the side of an appreciable contribution from genetic and evolutionary factors. Few, if any, said that the environment was unimportant in the molding of racial, gender, and individual characteristics; rather, sociobiologists claimed that the genetic and evolutionary history of human individuals and groups played an important role in determining their capabilities, just

as they do in other animals. Few, if any, claimed that this meant that one race, gender, or group was superior to another. However, many (if not all) sociobiologists were accused of promoting racist, sexist, and determinist ideas with their application of sociobiological concepts to humans.

Extremists on both sides of the question have confused the issues. Such extremists range from opponents of sociobiological ideas who minimize genetic or evolutionary influence on the human cultural condition to sociobiologists who minimize the role of environmental influences. In at least some minds, extremists in the sociobiological camp have done as much damage to sociobiology as its most ardent opponents. Sociobiology (by that or another name) will continue to contribute to the understanding of the social systems of animals and humans. The biological, genetic, and evolutionary bases of human social systems must be studied. The knowledge obtained may prove to be as enlightening as has sociobiology's contribution to the understanding of social systems in other animals.

—Carl W. Hoagstrom

See also: Aggression; Alcoholism; Altruism; Behavior; Biological Clocks; Biological Determinism; Criminality; Developmental Genetics; Eugenics; Gender Identity; Genetic Engineering: Medical Applications; Genetic Engineering: Social and Ethical Issues; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Heredity and Environment; Homosexuality; Human Genetics; Inbreeding and Assortative Mating; Intelligence; Klinefelter Syndrome; Knockout Genetics and Knockout Mice; Miscegenation and Antimiscegenation Laws; Natural Selection; Twin Studies; XYY Syndrome.

Further Reading

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Segerstråle, Ullica. *Defenders of the Truth: The Battle for Science in the Sociobiology Debate and Beyond*. New York: Oxford University Press, 2000. Addresses Wilson's *Sociobiology* and the ensuing debates on determinism versus free will, nature versus nurture, adaptationism versus environmentalism, and others. Bibliography, index.

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cussions of sociobiological perspectives on war and other forms of conflict, marital relations, and utopia. Illustrations, bibliography, index.

Wilson, Edward O. "The Biological Basis of Morality." *Atlantic Monthly*, April, 1998. Wilson argues that ethical and moral reasoning comes not from outside human nature, as if God-given, but from human nature itself in an ever-changing world.

_____. *On Human Nature*. Cambridge, Mass.: Harvard University Press, 1978. A look at the significance of biology and genetics on how we understand human behaviors, including aggression, sex, and altruism and the institution of religion.

_____. *Sociobiology: The New Synthesis*. Cambridge, Mass.: Belknap Press of Harvard University Press, 1975. The text that brings together Wilson's theories on the genetic, biological, and evolutionary basis of social systems.

Web Site of Interest

The Open Directory Project, Sociobiology. <http://dmoz.org/science/biology/sociobiology>. Comprehensive list of sites devoted to sociobiology, including links to sites covering the science of sociobiology.

Speciation

Field of study: Population genetics

Significance: *Speciation, the biological formation of new species, has produced the wide variety of living things on earth. Although speciation can be caused by other forces or events, natural selection is considered the primary mechanism promoting speciation.*

Key terms

ALLOPATRIC SPECIATION: the genetic divergence of populations caused by separation from each other by a geographic barrier such as a mountain range or an ocean

POPULATION: a group of organisms of the same species in the same place at the same time

and thus potentially able to mate; populations are the basic unit of speciation

REPRODUCTIVE ISOLATING MECHANISM: a characteristic that prevents an individual of one species from interbreeding (hybridizing) with a member of another species

SPECIES: a class of organisms with common attributes; individuals are usually able to produce fertile offspring only when mating with members of their own species

SYMPATRIC SPECIATION: the genetic divergence of populations that are not separated geographically

Species Concepts

Before the time of Charles Darwin, physical appearance was the only criterion for classifying an organism. This "typological species concept" was associated with the idea that species never change (fixity of species). This way of defining a species causes problems when males and females of the same species look different (as with peacocks and peahens) or when there are several different color patterns among members of a species (as with many insects). Variability within species, whether it is a visible part of their anatomy, an invisible component of their biochemistry, or another characteristic such as behavior, is an important element in understanding how species evolve.

The "biological species concept" uses reproduction to define a species. It states that a species is composed of individuals that can mate and produce fertile offspring in nature. This concept cannot be used to classify organisms such as bacteria, which do not reproduce sexually. It also cannot be used to classify dead specimens or fossils. This definition emphasizes the uniqueness of each individual (variability) in sexually reproducing species. For example, in the human species (*Homo sapiens*), there are variations in body build, hair color and texture, ability to digest milk sugar (lactose), and many other anatomical, biochemical, and behavioral characteristics. All of these variations are the result of genetic mutations, or changes in genes.

According to evolutionary scientist Ernst Mayr, to a "population thinker," variation is reality and type is an abstraction or average; to a

“typological thinker,” variation is an illusion and type is the reality. Typological thinking is similar to typecasting or stereotyping, and it cannot explain the actual variability seen in species, just as stereotyping does not recognize the variability seen in people. Additional definitions, such as the “evolutionary species concept,” include the continuity of a species’ genes through time or other factors not addressed by the biological species concept.

Isolation and Divergence of Populations

Species are composed of unique individuals that are nevertheless similar enough to be able to mate and produce fertile offspring. However, individuals of a species are infrequently in close enough proximity to be able to choose a mate from all opposite-sex members of the same species. Groups of individuals of the same species that are at least potential mates because of proximity are called populations.

The basic type of speciation in most sexually reproducing organisms is believed to be “allopatric,” in which geographic isolation (separation) of the species into two or more populations is followed by accumulation of differences (divergence) between the populations that eventually prevent them from interbreeding. These differences are caused primarily by natural selection of characteristics advantageous to populations in different environments. If both populations were in identical environments after geographic isolation, they would be much less likely to diverge or evolve into new species.

Another type of speciation is “sympatric,” in which populations are not separated geographically, but reproduction between them cannot occur (reproductive isolation) for some other reason. For example, one population may evolve a mutation that makes the fertilized egg (zygote) resulting from interbreeding with the other population incapable of surviving. Another possibility is a mutation that changes where or when individuals are active so that members of the different populations never encounter one another.

Darwin thought that divergence, and thus speciation, occurred gradually by the slow accumulation of many small adaptations “se-

lected” by the environment. More recently, it has been recognized that a very small population, or even a “founder” individual, may be the genetic basis of a new species that evolves more rapidly. This process, called genetic drift, is essentially random. For example, which member of an insect species is blown to an island by a storm is not determined by genetic differences from other members of the species but by a random event (in this case, the weather). This individual (or small number of individuals) is highly unlikely to contain all of the genetic diversity of the entire species. Thus the new population begins with genetic differences that may be enhanced by its new environment. Speciation proceeds according to the allopatric model, but faster. However, extinction of the new population may also occur.

Plants are able to form new species by hybridization (crossbreeding) more often than are animals. When plants hybridize, post-mating incompatibility between the chromosomes of the parents and the offspring may immediately create a new, fertile species rather than a sterile hybrid, as in animals such as the mule. A frequent method of speciation in plants is polyploidy, in which two or more complete sets of chromosomes end up in the offspring. (Usually, one complete set is made up of half of each parent’s chromosomes.)

Many species reproduce asexually (without the exchange of genes between individuals that defines sexual reproduction). These include bacteria and some plants, fish, salamanders, insects, rotifers, worms, and other animals. In spite of the fact that reproductive isolation has no meaning in these organisms, they are species whose chromosomes and genes differ from those of their close relatives.

Impact and Applications

Environmentalists and scientists recognize that the biodiversity created by speciation is essential to the functioning of the earth’s life-support systems for humans as well as other species. Some practical benefits of biodiversity include medicines, natural air and water purification, air conditioning, and food.

The impact of understanding the genetic basis of evolving species cannot be underesti-

mated. Artificial selection (in which humans decide which individuals of a species survive and reproduce) of plants has produced better food crops (for example, modern corn from teosinte) and alleviated hunger in developing nations by creating new varieties of existing species (for example, rice). Hybridization of animals has resulted in mules and beefaloes for the farm (both of which are sterile hybrids rather than species). Artificial selection of domesticated animals has produced the many breeds of horses, dogs, and cats (each of which is still technically one species). Genetic engineering promises to create crops that resist pests, withstand frost or drought, and contain more nutrients. Finally, understanding the genetics of the evolving human species has broad implications for curing disease and avoiding birth defects.

—Barbara J. Abraham

See also: Artificial Selection; Evolutionary Biology; Hardy-Weinberg Law; Hybridization and Introgression; Lateral Gene Transfer; Natural Selection; Polyploidy; Population Genetics; Punctuated Equilibrium.

Further Reading

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Giddings, L. V., Kenneth Y. Kaneshiro, and Wyatt W. Anderson, eds. *Speciation, and the Founder Principle*. General principles of speciation among both plants and animals, with emphasis on the founder principle, covered by seventeen internationally known geneticists.

Mayr, Ernst. *One Long Argument: Charles Darwin and the Genesis of Modern Evolutionary Thought*. Cambridge, Mass.: Harvard University Press, 1991. Includes a chapter ("How Species Originate") that points out that Darwin's explanation of speciation was limited by his lack of understanding of the origin of genetic variation (mutation and recombination). Illustrations, bibliography, index.

Stem Cells

Fields of study: Cellular biology; Human genetics and social issues

Significance: *Stem cells, which can be manipulated to create unlimited amounts of specialized tissue, may be used to treat a variety of diseases and injuries that have destroyed a patient's cells, tissues, or organs. Stem cells could also be used to gain a better understanding of how genetics works in the early stages of cell development and may play a role in the testing and development of drugs.*

Key terms

ADULT STEM CELL: an undifferentiated cell found among differentiated cells in a tissue or organ of an adult organism

BLASTOCYST: a preimplantation embryo consisting of a hollow ball of two layers of cells

CELL DIFFERENTIATION: the process whereby a precursor cell produces progeny that are capable of expressing a different set of genes

EMBRYONIC STEM CELL: an undifferentiated cell derived from the inner cell mass of a blastocyst

MULTIPOTENCY: the ability of cells to form progeny that can differentiate into one of the different types of cells that form the living organism

PLURIPOTENCY: the ability of a cell to give rise to all the differentiated cell types in an embryo

TOTIPOTENCY: the ability of a single cell to express the full genome in the cells to which it gives rise by cell division

Types of Stem Cells

Stem cells are defined by their ability to renew themselves, their lack of differentiation, and their ability to diversify into other cell types. There are three major classes of stem cells: totipotent, pluripotent, and multipotent. Totipotent cells can differentiate to become all of the cells that make up an embryo, all of the extraembryonic tissues, and all of the post-embryonic tissues and organs. Pluripotent cells have the potential to become almost all of the tissues found in an embryo but are not capable of giving rise to supporting cells and tissues.

Multipotent cells are specialized stem cells capable of giving rise to one class of cells.

A fertilized egg, or zygote, is totipotent. The zygote first divides into two cells about one day after fertilization and becomes an embryo. The embryonic cells remain totipotent for about four days after fertilization. At that point, the embryo consists of about eight cells. As the cells of the embryo continue to divide, they form a hollow sphere. The approximately fifty to one hundred cells on the inner side of the sphere are pluripotent and will continue developing to form the embryo, while the cells on the outer surface will give rise to the extraembryonic tissues, such as the placenta and the umbilical cord.

Multipotent stem cells are found in a variety of tissues in adult mammals and are sometimes referred to as adult stem cells. They are specialized stem cells that are committed to giving rise to cells that have a particular function.

Identities of some multipotent stem cells have been confirmed. Hematopoietic stem cells give rise to all the types of blood cells. Mesenchymal stem cells in the bone marrow give rise to a variety of cell types: bone cells, cartilage cells, fat cells, and other kinds of connective tissue cells such as those in tendons. Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of nonneuronal cells, astrocytes and oligodendrocytes. Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and the epidermis.

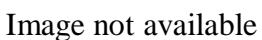
Stem cells in adult mammalian tissues are rare and difficult to isolate. There is considerable debate concerning the plasticity of stem cells in adults. Plasticity is the ability of multipotent cells to exhibit pluripotency, such as the capacity of hematopoietic stem cells to differentiate into neurons.

Behavior in Cell Culture

During the 1980's researchers first established in vitro culture conditions that allowed embryonic stem cells to divide without differentiating. Embryonic stem cells are relatively easy to grow in culture but appear to be genetically unstable; mice cloned from embryonic stem cells by nuclear transfer suffered many genetic defects as a result of the genetic instability of the embryonic stem cells. As embryonic stem cells divide in culture, they lose the tags that tell an imprinted gene to be either turned on or turned off during development. Researchers have found that even clones made from sister stem cells show differences in their gene expression. However, these genetic changes, while having defined roles in fetal development, may have little significance in therapeutic uses, because the genes involved do not serve a critical role in adult differentiated cells.

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Embryonic stem cells in laboratory bottles, 2001. (AP/Wide World Photos)

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Paraplegic protesters in Washington, D.C., staged this mock “hanging in the balance” in April, 2002, to dramatize the need for embryonic stem cell research and to urge Congress to allow it. Embryonic stem cell research was subsequently limited to an existing number of cell lines already being investigated. (AP/Wide World Photos)

Unlike embryonic stem cells, adult stem cells do not divide prolifically in culture. When these stem cells do divide in culture, their division is unlike that of most cells. Generally, when a cell divides in culture, the two daughter cells produced are identical in appearance as well as in patterns of gene expression. However, when stem cells divide in culture, at least one of the daughter cells retains its stem cell culture while the other daughter cell is frequently a transit cell destined to produce a terminally differentiated lineage. The genes expressed in a stem cell and a transit cell are significantly different. Therefore a culture of adult stem cells may become heterogeneous in a short time.

Potential Therapeutic Issues

Although stem cells have significant use as models for early embryonic development, an-

other major research thrust has been for therapeutic uses. Stem cell therapy has been limited almost exclusively to multipotent stem cells obtained from umbilical cord blood, bone marrow, or peripheral blood. These stem cells are most commonly used to assist in hematopoietic (blood) and immune system recovery following high-dose chemotherapy or radiation therapy for malignant and nonmalignant diseases such as leukemia and certain immune and genetic disorders. For stem cell transplants to succeed, the donated stem cells must repopulate or engraft the recipient’s bone marrow, where they will provide a new source of essential blood and immune system cells.

In addition to the uses of stem cells in cancer treatment, the isolation and characterization of stem cells and in-depth study of their molecular and cellular biology may help scientists under-

stand why cancer cells, which have certain properties of stem cells, survive despite very aggressive treatments. Once the cancer cell's ability to renew itself is understood, scientists can develop strategies for circumventing this property.

Research efforts are under way to improve and expand the use of stem cells in treating and potentially curing human diseases. Possible therapeutic uses of stem cells include treatment of autoimmune diseases such as muscular dystrophy, multiple sclerosis, and rheumatoid arthritis; repair of tissues damaged during stroke, spinal cord injury, or myocardial infarction; treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS, commonly called Lou Gehrig's disease) and numerous neurological conditions such as Parkinson's, Huntington's, and Alzheimer's diseases; and replacement of insulin-secreting cells in diabetics.

Stem cells may also find use in the field of gene therapy, where a gene that provides a

missing or necessary protein is introduced into an organ for a therapeutic effect. One of the most difficult problems in gene therapy studies has been the loss of expression (or insufficient expression) following introduction of the gene into more differentiated cells. Introduction of the gene into stem cells to achieve sufficient long-term expression would be a major advance. In addition, the stem cell is clearly a more versatile target cell for gene therapy, since it can be manipulated to become theoretically any tissue. A single gene transfer into a pluripotent stem cell could enable scientists to generate stem cells for blood, skin, liver, or even brain targets.

Ethical Issues Concerning Use

Stem cell research, particularly embryonic stem cell research, has unleashed a storm of controversy. One primary controversy surrounding the use of embryonic stem cells is based on the belief by opponents that a fertil-

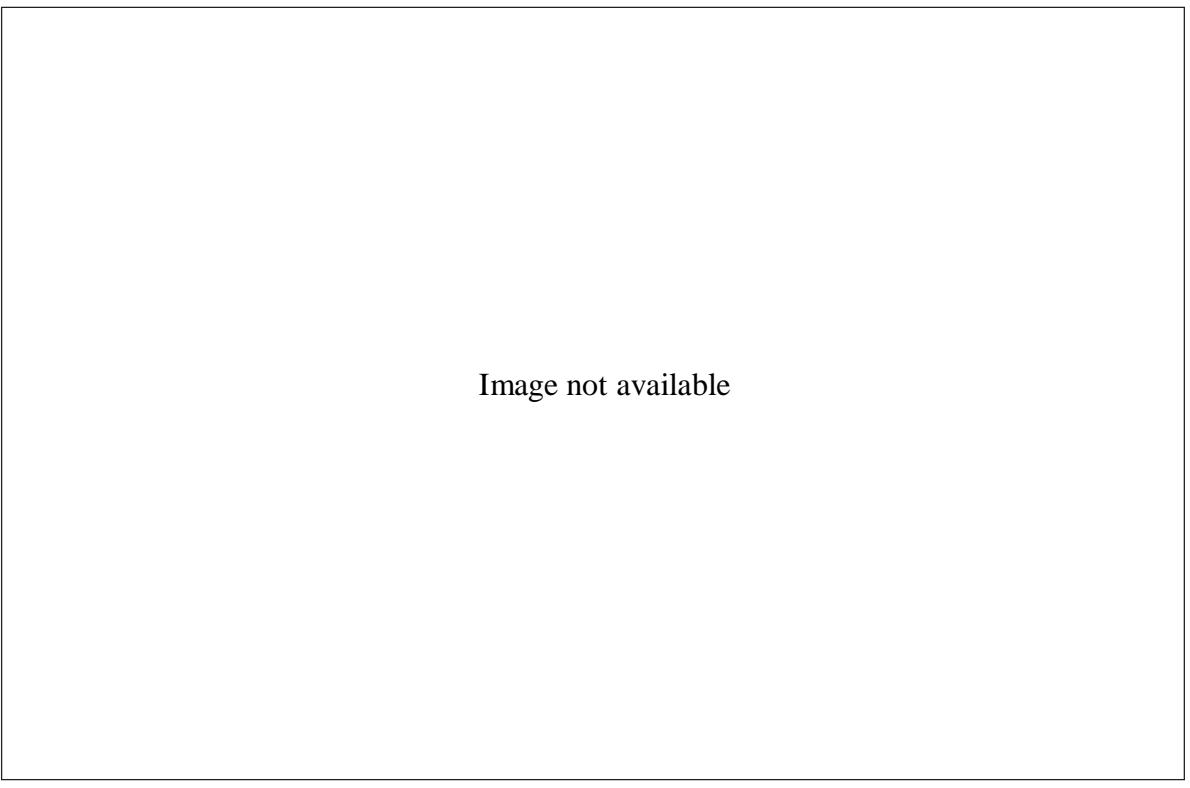


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A group of pro-life demonstrators urge a ban on embryonic stem cell research, seeing such investigations as tantamount to baby killing. (AP/Wide World Photos)

ized egg is fundamentally a human being with rights and interests that need to be protected. Those who oppose stem cell research do not want fetuses and fertilized eggs used for research purposes. Others accept the special status of an embryo as a potential human being yet argue that the respect due to the embryo increases as it develops and that this respect, in the early stages in particular, may properly be weighed against the potential benefits arising from the proposed research.

Another ethical issue concerns the method by which embryonic stem cells are obtained. Embryonic stem cells are isolated from two sources: surplus embryos produced by in vitro fertilization and embryos produced by somatic cell nuclear transfer (SCNT), often referred to as therapeutic cloning. In SCNT, genetic material from a cell in an adult's body is fused with an enucleated egg cell. With the right conditions, this new cell can then develop into an embryo from which stem cells could be harvested. Opponents argue that therapeutic cloning is the first step on the slippery slope to reproductive cloning, the use of SCNT to create a new adult organism. Proponents maintain that producing stem cells by SCNT using genetic material from the patient will eliminate the possibility of rejection when the resulting stem cells are returned to the patient.

Legal Status

On August 9, 2001, President George W. Bush announced that federal funds could be used to support research using the sixty human embryonic stem cell lines that had been derived before that date. However, there were no restrictions placed on the types of research that could be conducted on mouse embryonic stem cell lines and no federal law or policy prohibiting the private sector from isolating stem cells from human embryos. Several states have introduced legislation to encourage research on stem cells taken from human embryos.

As of March, 2003, neither reproductive cloning nor therapeutic cloning was forbidden by law in the United States. Congress was debating competing legislation; one bill proposed to ban both types of cloning, while an alternative proposal would ban only reproductive cloning.

A number of states already have laws that ban human cloning for reproductive purposes, while a small number of states forbid cloning of embryos for stem cells as well.

—Lisa M. Sardinia

See also: Aging; Alzheimer's Disease; Autoimmune Disorders; Biochemical Mutations; Bioethics; Cancer; Cell Culture: Animal Cells; Cell Culture: Plant Cells; Cell Cycle, The; Cell Division; Cloning; Cloning: Ethical Issues; Cloning Vectors; Cystic Fibrosis; Developmental Genetics; Eugenics; Eugenics: Nazi Germany; Gene Therapy; Gene Therapy: Ethical and Economic Issues; Genetic Engineering: Medical Applications; Huntington's Disease; In Vitro Fertilization and Embryo Transfer; Infertility; Knockout Genetics and Knockout Mice; Model Organism: *Mus musculus*; Organ Transplants and HLA Genes; Totipotency; Transgenic Organisms.

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Marshak, Daniel R., Richard L. Gardner, and David Gottlieb, eds. *Stem Cell Biology*. Cold Spring Harbor, N.Y.: Cold Spring Harbor

Laboratory Press, 2002. Contains papers on early embryonic development, cell cycle controls, embryonal carcinoma cells as embryonic stem cells, stem cells of human adult bone marrow, intestinal epithelial stem cells, and much more, designed for researchers new to the field of stem cell biology.

Rao, Mahendra S., ed. *Stem Cells and CNS Development*. Totowa, N.J.: Humana Press, 2001. Collection of papers on neural stem cells, including multipotent cells in both embryos and adults, transplant therapy, drug and gene discovery, and much more. Designed for scientists.

Web Site of Interest

National Institutes of Health, Stem Cell Information. <http://stemcells.nih.gov>. Government site covering stem cell basics, the science of stem cell research, and links to related resources.

Sterilization Laws

Field of study: Human genetics and social issues

Significance: *Forced sterilization for eugenic reasons became legal throughout much of the United States and many parts of the world during the first half of the twentieth century. Though sterilization is an ineffective mechanism for changing the genetic makeup of a population, sterilization laws remain in effect in many states in the United States and other countries throughout the world.*

Key terms

NEGATIVE EUGENICS: the effort to improve the human species by discouraging or eliminating reproduction among those deemed to be socially or physically unfit

POSITIVE EUGENICS: the effort to encourage more prolific breeding among "gifted" individuals

STERILIZATION: an operation to make reproduction impossible; in tubal ligation, doctors sever the Fallopian tubes so that a woman cannot conceive a child

The Eugenics Movement and Sterilization Laws

The founder of the eugenics movement is considered to be Sir Francis Galton, who carried out extensive genetic studies of human traits. He thought that the human race would be improved by encouraging humans with desirable traits (such as intelligence, good character, and musical ability) to have more children than those people with less desirable traits (positive eugenics). With the development of Mendelian genetics shortly after the beginning of the twentieth century, research on improving the genetic quality of plants and animals was in full swing. Success with plants and domestic animals made it inevitable that interest would develop in applying those principles to the improvement of human beings. As some human traits became known to be under the control of single genes, some geneticists began to claim that all sorts of traits (including many behavioral traits and even social characteristics and preferences) were under the control of a single gene with little regard for the possible impact of environmental factors.

The Eugenics Record Office at Cold Springs Harbor, New York, was set up by Charles Davenport to gather and collate information on human traits. The eugenics movement became a powerful political force that led to the creation and implementation of laws restricting immigration and regulating reproduction. Some geneticists and politicians reasoned that since mental retardation and other "undesirable" behavioral and physical traits were affected by genes, society had an obligation and a moral right to restrict the reproduction of individuals with "bad genes" (negative genetics).

The state of Indiana passed the first sterilization law in 1907, which permitted the involuntary sterilization of inmates in state institutions. Inmates included not only "imbeciles," "idiots," and others with varying degrees of mental retardation (described as "feeble-minded") but also people who were committed for behavioral problems such as criminality, swearing, and slovenliness. By 1911, similar laws had been passed in six states, and, by the end of the 1920's, twenty-four states had similar sterilization laws. Although not necessarily strictly en-

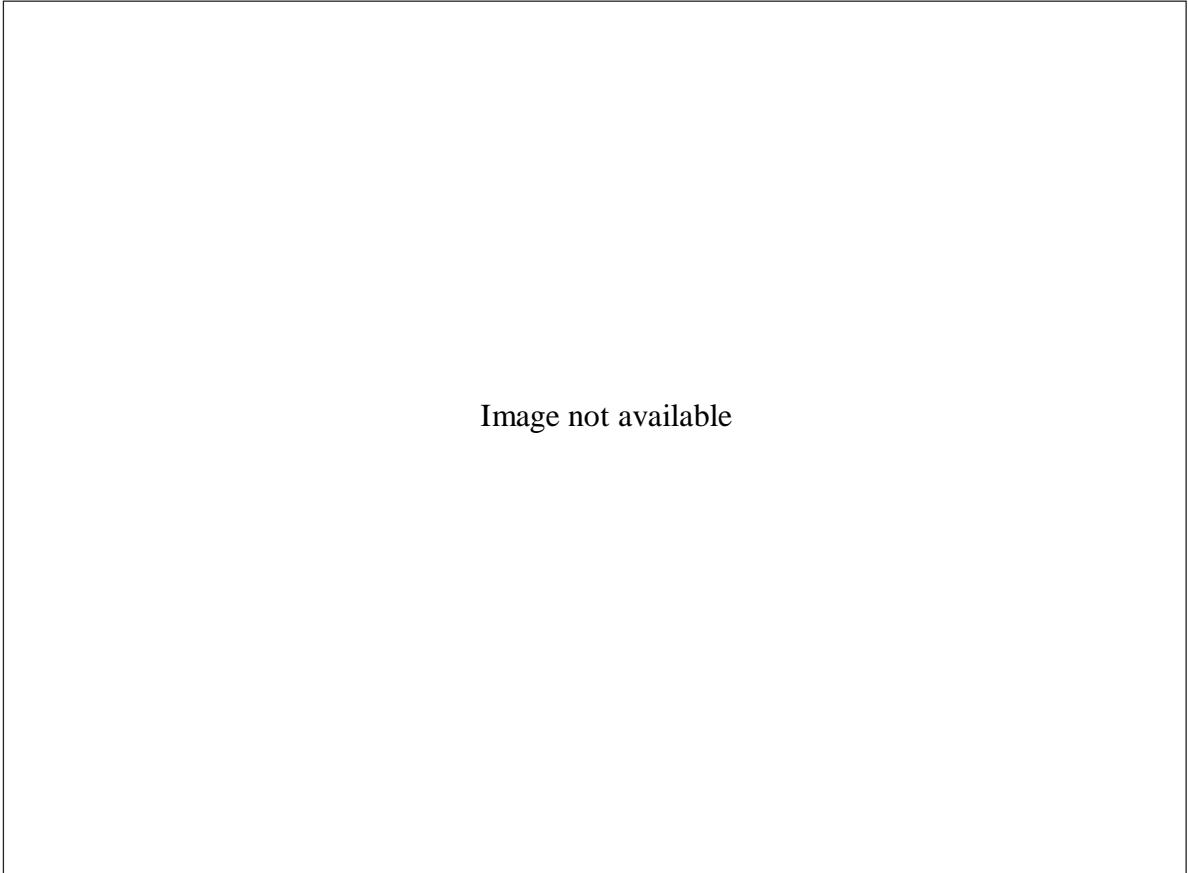


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In 1927, the U.S. Supreme Court, in its *Buck v. Bell* decision, supported the eugenic principle that states could use involuntary sterilization to eliminate genetic defects from the population. The result was the sterilization of more than sixty thousand mainly young people deemed to be weak, "feeble-minded," or otherwise genetically inferior. Two sterilized residents of Lynchburg, Virginia, where many such sterilizations occurred, observe a historical marker that commemorates the tragic decision. (AP/Wide World Photos)

forced, twenty-two states currently have sterilization laws on the books.

The U.S. Supreme Court, in its 1927 *Buck v. Bell* decision, supported the eugenic principle that states could use involuntary sterilization to eliminate genetic defects from the population. The vote of the Court was eight to one. The court's reasoning went as follows:

We have seen more than once that the public welfare may call upon the best citizens for their lives. It would be strange if it could not call upon those who already sap the strength of the state for these lesser sacrifices, often not felt to be such by those concerned, in order to prevent our being swamped with incompetence. It is better for all the world, if instead of waiting to execute de-

generate offspring for crime, or to let them starve for their imbecility, society can prevent those who are manifestly unfit from continuing their kind. The principle that sustains compulsory vaccination is broad enough to cover cutting the Fallopian tubes.

Ironically, the sterilization laws of the United States and Canada served as models for the eugenics movement in Nazi Germany in its program to ensure so-called racial purity and superiority.

Impact and Applications

Two problems associated with eugenics are the subjective nature of deciding which traits are desirable and determining who should de-

cide. These concerns aside, the question of whether there is a sound scientific basis for the desire to manipulate the human gene pool remains. Does the sterilization of individuals who are mentally retarded or who have some other mental or physical defect improve the human genetic composition? Involuntary sterilization of affected individuals would quickly reduce the incidence of dominant genetic traits. Individuals who were homozygous for recessive traits would also be eliminated. However, most harmful recessive genes are carried by individuals who appear normal and, therefore, would not be "obvious" for sterilization purposes. These "normal" people would continue to pass the "bad" gene on to the next generation, and a certain number of affected people would again be born. It would take an extraordinary number of generations to significantly reduce the frequency of harmful genes.

Although the number of involuntary sterilizations in the United States is now minimal, the impact sterilization laws had on the population through 1960 was far-reaching, as nearly sixty thousand people were sterilized. Other countries also had laws that allowed forced sterilizations, with many programs continuing into the 1970's. The province of Alberta, Canada, sterilized three thousand people before its law was repealed. Another sixty thousand were sterilized in Sweden. The story of sterilization and "euthanasia" in Germany needs no retelling. With the ability to decipher the human genome and implement improved genetic testing procedures, a danger exists that new programs of eugenics and involuntary sterilization might once again emerge.

—Donald J. Nash

See also: Criminity; Eugenics; Eugenics: Nazi Germany; Hardy-Weinberg Law; Miscegenation and Antimiscegenation Laws; Prion Diseases; Kuru and Creutzfeldt-Jakob Syndrome; Race.

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Web Sites of Interest

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University of Vermont, Vermont Eugenics: A Documentary History. <http://www.uvm.edu/~eugenics/sterilizationdl.html>. A listing of original documents related to sterilization and eugenics in the United States, including a statement from the American Eugenics Society (1926) and related newspaper articles.

Steroid Hormones

Fields of study: Developmental genetics; Molecular genetics

Significance: Steroid hormones—hormones containing a steroid ring derived from cholesterol—are important for many processes that control sex determination, reproduction, behavior, and metabolism. Mutations in the genes that produce or

regulate the action of specific steroid hormones may lead to infertility, sterility, sex determination, osteoporosis, autoimmune diseases, heart abnormalities, and breast, uterine, and prostate cancer.

Key terms

ANABOLIC STEROIDS: drugs derived from androgens and used to enhance performance in sports

ANDROGENS: steroid hormones that cause masculinization

ESTROGENS: steroid hormones that produce female characteristics

GLUCOCORTICOIDS: steroid hormones that respond to stress and maintain sugar, salt, and body fluid levels

HORMONES: chemical messengers produced by endocrine glands and secreted into the blood

MINERALOCORTICOIDS: a group of steroid hormones important for maintenance of salt and water balance

PROGESTINS: steroid hormones important for pregnancy and breast development

TESTOSTERONE: the principal androgen, produced by the testes and responsible for male secondary sexual characteristics

Steroid Hormone Characteristics and Function

Steroid hormones represent a group of hormones that all contain a characteristic “steroid” ring structure. This steroid ring is derived from cholesterol, and cholesterol is the starting material for the production of different steroid hormones. Steroid hormones, like other types of hormones, are secreted by endocrine glands into the bloodstream and travel throughout the body before having an effect. All steroid hormones, although specific for the regulation of certain genes, function in a similar manner. Because steroid hormones are derived from cholesterol, they have the unique ability to diffuse through a cell’s outer plasma membrane. Inside the cell, the steroid hormone binds to its specific receptor in the cytoplasm. Upon binding, the newly formed hormone-receptor complex relocates to the nucleus. In the nucleus, the hormone-receptor complex binds to the DNA in the promoter region of certain

genes at specific nucleotide sequences termed hormone-responsive elements. The binding of the hormone-receptor complex to hormone-responsive elements causes the increased production of transcription and protein production in most cases. In some instances, binding to a specific hormone-responsive element will stop the production of proteins that are usually made in the absence of the hormone.

There are two types (sex steroid and adrenal steroid) and five classes of steroid hormones. The sex steroid hormones include the androgens, estrogens, and progestins and are produced by the male testes (androgens) and female ovaries. Adrenal steroid hormones include glucocorticoids and mineralocorticoids and are produced by the adrenal glands.

Sex Steroid Hormones

Sex steroid hormone genes are responsible for determining the sex and development of males and females. Androgens are a group of steroid hormones that cause masculinization. The principal androgen is testosterone, which is produced by the testes and is responsible for male secondary sexual characteristics (growth of facial and pubic hair, deepening of voice, sperm production). Estrogens are sex steroid hormones produced in the ovaries and cause feminization. In addition, estrogens control calcium content in the bones, modulate other hormones produced in the ovary, modify sexual behavior, regulate growth of secondary sex characteristics (menstrual periods, breast development, pubic hair) and are essential for pregnancy to occur. The most potent estrogen is 17-beta estradiol. Progestins, including progesterone, are also sex steroid hormones. Progesterone is important for proper breast development and normal and healthy pregnancies; it functions in the mother to alter endometrial cells so the embryo can implant. The loss of progesterone at the end of a pregnancy aids in the beginning of uterine contractions.

Anabolic steroids are drugs derived from the male steroid hormone testosterone and were developed in the late 1930’s to treat hypogonadism in men, a condition that results in insufficient testosterone production by the testes. During this same period, scientists discovered

that anabolic steroids also increased the muscle mass in animals. These findings led to the use of anabolic steroids by bodybuilders, weight-lifters, and other athletes to increase muscle mass and enhance performance. Anabolic steroid use can seriously affect the long-term health of an individual and in women results in masculinization.

Adrenal Steroid Hormones

Adrenal steroid hormones are secreted from the adrenal cortex and are important for many bodily functions, including response to stress, maintenance of blood sugar levels, fluid balance, and electrolytes. The glucocorticoids represent one class of adrenal steroid hormone. The most important, cortisol, performs critically important functions; it helps to maintain blood pressure and can decrease the response of the body's immune system. Cortisol can also elevate blood sugar levels and helps to control the amount of water in the body. Elevated cortisol helps the body respond to stress. The glucocorticoids cortisone and hydrocortisone are used as anti-inflammatory drugs to control itching, swelling, pain, and other inflammatory reactions. Prednisone and prednisolone, also members of the glucocorticoid class of hormones, are the broadest anti-inflammatory and immunosuppressive medications available.

The second class of adrenal steroid hormones is the mineralocorticoids, including aldosterone, which helps maintain salt and water balance and increases blood pressure. Aldosterone is crucial for retaining sodium in the kidney, salivary glands, sweat glands, and colon.

Genetic Defects Affecting Sex Steroid Hormones

Defects in the genes involved in the production of sex steroid hormones can have serious consequences. Mutations in the androgen receptor, the receptor for testosterone, result in testicular feminization syndrome. In this syndrome, the individual has the genes of a male (XY) but develops, behaves, and appears female. Other gene defects in androgen biosynthesis often result in sterility. Genetic defects in estrogen receptors or estradiol biosynthesis lead to infertility. Reduced levels of estradiol

have also been linked to bone loss (osteoporosis) and infertility, whereas excessive levels are associated with an increased risk of breast and uterine cancer. Similarly, genetic mutations in the progesterone production pathway or the progesterone receptor are associated with infertility. In addition, bone loss is one of the most serious results of progesterone deficiency, made worse by inappropriate diet and lack of exercise.

Genetic Defects Affecting Adrenal Steroid Hormones

Genetic abnormalities in adrenal steroid hormone biosynthesis are known to cause hypertension in some cases of congenital adrenal hyperplasia (CAH). In people with this condition, hypertension usually accompanies a characteristic phenotype with abnormal sexual differentiation. CAH is a family of autosomal recessive disorders of adrenal steroidogenesis. Each disorder has a specific pattern of hormonal abnormalities resulting from a deficiency of one of the enzymes necessary for cortisol synthesis. The most common form of CAH is 21-hydroxylase deficiency; however, in all forms, cortisol production is impaired, which results in an increase in adrenocorticotropin and the overproduction of androgen steroids.

There are two major forms of 21-hydroxylase deficiency. Classic CAH deficiency results in masculinized girls that are born with genital ambiguity and may possess both female and male genitalia. Nonclassic 21-hydroxylase deficiency does not produce ambiguous genitalia in female infants but may result in premature puberty, short stature, menstrual irregularities or lack of a menstrual cycle, and infertility. Familial glucocorticoid deficiency (FGD) is an extremely rare, genetic autosomal recessive condition in which a part of the adrenal glands are destroyed. These changes result in very low levels of cortisol. Although this disease is easily treatable if recognized, when left untreated it is often fatal or can lead to severe mental disability.

Recently, the genetic basis of four forms of severe hypertension transmitted on an autosomal basis has been determined. All of these conditions are characterized by salt-sensitive

increases in blood pressure, indicating an increased mineralocorticoid effect. The four disorders—aldosteronism, mineralocorticoid excess syndrome, activating mutation of the mineralocorticoid receptor, and Liddle syndrome—are a consequence of either abnormal biosynthesis, abnormal metabolism, or abnormal action of steroid hormones and the development of hypertension. Adrenal insufficiency is known as Addison's disease and causes death within two weeks unless treated. Classical Addison's disease results from a loss of both cortisol and aldosterone secretion as a result of the near total or total destruction of both adrenal glands.

—Thomas L. Brown

See also: Aggression; Allergies; Autoimmune Disorders; Behavior; Cancer; Gender Identity; Heart Disease; Hermaphrodites; Human Genetics; Metafemales; Pseudohermaphrodites; Testicular Feminization Syndrome; X Chromosome Inactivation; XYY Syndrome.

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Swine Flu

Fields of study: Diseases and syndromes; Viral genetics

Significance: *The swine flu outbreak of 1918 was the most lethal worldwide epidemic known to humankind. Constant genetic changes in the influenza virus that caused the disease keep alive the potential for such another pandemic to occur in the future.*

Key terms

ANTIGENIC DRIFT: minor changes in the H and N proteins of the influenza virus

ANTIGENIC SHIFT: the acquisition by a strain of influenza virus of a different H and/or N protein

HEMAGLUTTININ (H): a protein necessary for entry of the influenza virus into a host cell

NEURAMINIDASE (N): a protein necessary for exit of the influenza virus from an infected cell

PANDEMIC: a worldwide outbreak of a particular disease

Virus Structure and Replication

Swine flu is a respiratory disease of humans caused by the H1N1 subtype of influenza A virus. Various types of influenza A virus can be found in humans, birds, swine, and other animals. Human disease is spread most commonly

person to person and, rarely, from animal to person. Although some antiviral drugs are available, treatment involves mainly supportive therapy. Vaccination prevents disease, but the genetic nature of the virus requires that vaccinations must be given annually to be effective. Advances in genetic technology are helping scientists to understand why the swine flu virus caused such a devastating epidemic in 1918 and to develop treatment and prevention strategies that will be effective against future potential epidemics of swine flu.

The swine flu virus is composed of eight segments of RNA surrounded by a lipid envelope. Embedded in the envelope are two proteins essential for viral replication, known as hemagglutinin (H) and neuraminidase (N). The infectious cycle begins when hemagglutinin binds to the surface of the host cell. Next, the viral envelope fuses with the host cell mem-

brane and the RNA is released. Inside the host cell viral RNA is replicated and new proteins are synthesized. Newly assembled virus particles bud through the host cell membrane to acquire their envelopes. Neuraminidase keeps the new virus particles from sticking to the dying host cell so they are free to infect surrounding cells. The cycle continues until either the host is dead or the host's immune system stops the spread of the virus. The majority of the host's immune response to infection is directed against the H and N proteins.

Type A influenza viruses are continuously changing in the amino acid sequence of their H and N proteins by a process called antigenic drift. These changes occur because mistakes are made during the replication of the viral RNA that codes for these proteins. Changes that result in H and N proteins that are no longer recognized by the host's immune system al-

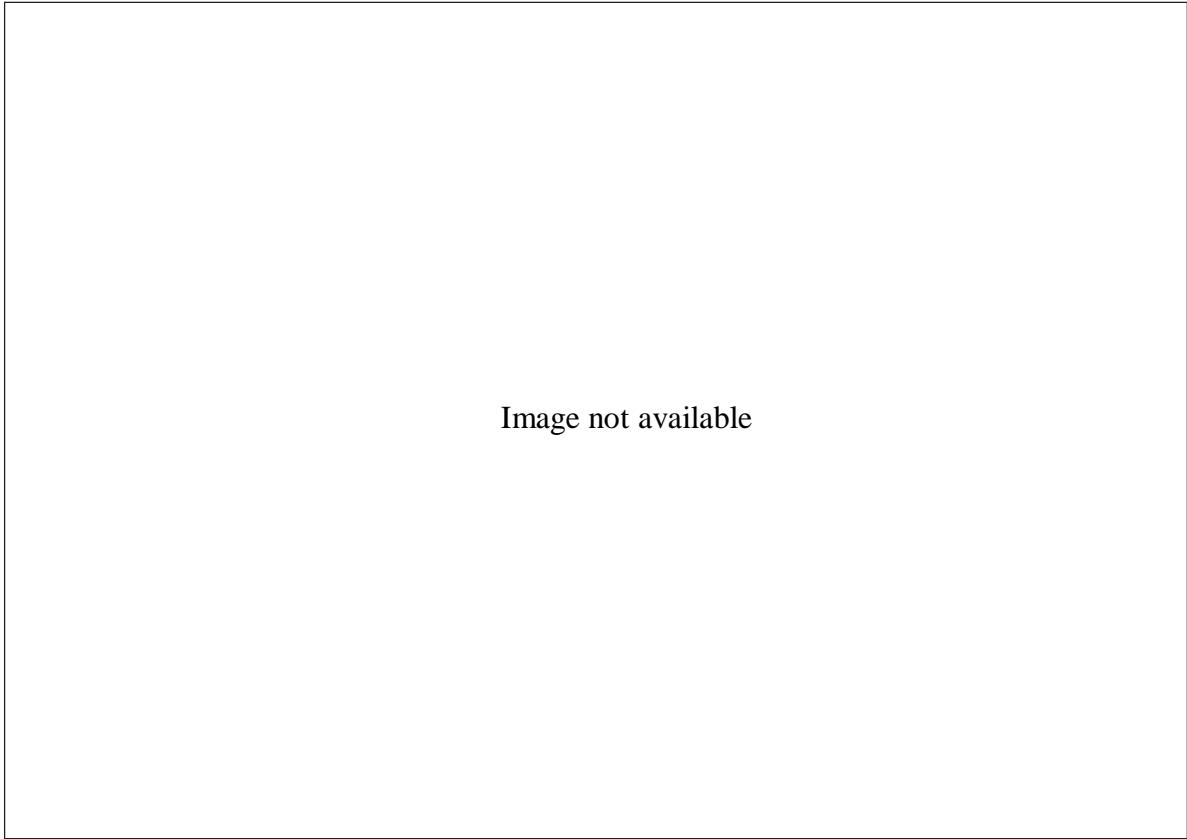


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Victims of the 1918 influenza pandemic line an emergency hospital facility at Fort Riley, Kansas, near where the virus is thought to have originated. The pandemic caused at least 20 million deaths worldwide. (AP/Wide World Photos)

low that strain of virus to become prevalent over the strains of virus that are recognized. Antigenic drift is the reason that influenza vaccines are effective only for the year in which they are made. By the following year, the prevalent viruses have changed enough that the population is no longer immune to them.

Type A influenza viruses also change abruptly on a more sporadic basis due to antigenic shift. One type of antigenic shift, called reassortment, occurs when two different strains of influenza virus infect the same host cell. Because the genome is segmented, it is possible for RNA segments from one virus to get mixed up with RNA segments from the second virus when the new virus particles are made. Thus, a new virus containing genes from both viruses can arise. The influenza pandemics of so-called Asian flu in 1957 and Hong Kong flu in 1968 were a result of reassortment. A second type of antigenic shift occurs when an animal influenza virus jumps directly into the human population as occurred in the 1997 avian flu and the 1999 A(H9N2) outbreaks.

History

In 1918, an epidemic of swine flu killed more than 500,000 people in the United States and between 20 million and 50 million people worldwide—more than any other disease in such a short period of time in the history of humankind. After the influenza virus was isolated in 1933, scientists used blood tests to determine that a type A(H1N1) virus had caused the pandemic. In 1976, a second outbreak of A(H1N1) influenza was discovered in the United States in both humans and pigs (hence the name swine flu). The United States mobilized a massive vaccination program, but the predicted epidemic never followed.

Advances in genetic technology have enabled scientists to study RNA from the actual virus that caused the 1918 epidemic. Influenza genes were recovered from samples of the lung tissue of three victims by reverse transcription and polymerase chain reaction. A few genes have been sequenced and compared to known sequences of viral RNA from more recent outbreaks of influenza. It is not yet clear, however, what made the 1918 strain of virus so deadly.

Vaccinations

Constant genetic changes in influenza viruses dictate the development of new vaccines every year. The Global Influenza Surveillance Network, an arm of the World Health Organization (WHO), monitors viruses circulating in humans and identifies new strains, recommending annually a vaccine that targets the three most prevalent strains in circulation. Current research looks for vaccines that would be effective against all strains of influenza, so that new vaccines would not need to be developed each year or at least could be produced more quickly in case of a pandemic.

Future

Influenza experts agree that another pandemic is likely to happen. If a new strain of flu virus appears after antigenic shift against which the human population has no immunity, and that strain can cause illness and spread easily from person to person, an influenza pandemic can occur. Continuous global surveillance of influenza outbreaks, accompanied by full exchanges of information by national governments and their health agencies, is the key to identifying and preventing another pandemic. Advances in genetic technology will help solve the mystery of the 1918 swine flu and make improvements in vaccines and antiviral drugs that could help minimize an epidemic if another one occurs.

—Vicki J. Isola

See also: Antibodies; Bacterial Genetics and Cell Structure; Bacterial Resistance and Super Bacteria; Down Syndrome; Emerging Diseases; Gene Regulation: Viruses; Human Genome Project; Organ Transplants and HLA Genes; Restriction Enzymes; RNA Structure and Function; Smallpox.

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Web Site of Interest

National Center for Infectious Diseases. <http://www.cdc.gov/ncidod/diseases/flu/fluivirus.htm>. This page on influenza provides basic information on the various forms of the virus, its effects, and treatments.

Synthetic Antibodies

Field of study: Immunogenetics

Significance: *Synthetic antibodies are artificially produced replacements for natural human antibodies. They are used to treat a variety of illnesses and promise to be an important part of medical technology in the future.*

Key terms

ANTIBODY: a protein molecule that binds to a substance in order to remove, destroy, or deactivate it

ANTIGEN: the substance to which an antibody binds

B CELLS: white blood cells that produce antibodies

MONOCLONAL ANTIBODIES: identical antibodies produced by identical B cells

The Development of Antibody Therapy

Natural antibodies are protein molecules produced by white blood cells known as B cells in response to the presence of foreign substances. A specific antibody binds to a specific substance, known as an antigen, in a way that renders it harmless or allows it to be removed from the body or destroyed. A person will produce antibodies naturally upon exposure to harmless versions of an antigen, a process

known as active immunization. Active immunization was the first form of antibody therapy to be developed and is used to prevent diseases such as measles and polio.

The oldest method of producing therapeutic antibodies outside the human body is known as passive immunization. This process involves exposing an animal to an antigen so that it develops antibodies to it. The antibodies are separated from the animal's blood and administered to a patient. Passive immunization is used to treat diseases such as rabies and diphtheria. A disadvantage of antibodies derived from animal blood is the possibility that the patient may develop an allergic reaction. Because the animal's antibodies are foreign substances, the patient's own antibodies may treat them as antigens, leading to fever, rash, itching, joint pain, swollen tissues, and other symptoms. Antibodies derived from human blood are much less likely to cause allergic reactions than antibodies from the blood of other animals. This led researchers to seek a way to develop synthetic human antibodies.

A major breakthrough in the search for synthetic antibodies was made in 1975 by Cesar Milstein and Georges Köhler. They developed a technique that allowed them to produce a specific antibody outside the body of a living animal. This method involved exposing an animal to an antigen, causing it to produce antibodies. Instead of obtaining the antibodies from the animal's blood, they obtained B cells from the animal's spleen. These cells are then combined with abnormal B cells known as myeloma cells. Unlike normal B cells, myeloma cells can reproduce identical copies of themselves an unlimited number of times. The normal B cells and the myeloma cells fuse to form cells known as hybridoma cells. Hybridoma cells are able to reproduce an unlimited number of times and are able to produce the same antibodies as the B cells. Those hybridoma cells that produce the desired antibody are separated from the others and allowed to reproduce. The antibodies produced this way are known as monoclonal antibodies.

Because human B cells do not normally form stable hybridoma cells with myeloma cells, B cells from mice are usually used. Be-

cause mouse antibodies are not identical to human antibodies, they may be treated as antigens by the patient's own antibodies, leading to allergic reactions. During the 1980's and 1990's, researchers began to develop methods of producing synthetic antibodies that were similar or identical to human antibodies. An antibody consists of a variable region, which binds to the antigen, and a constant region. The risk of allergic reactions can be reduced by combining variable regions derived from mouse hybridoma cells with constant regions from human cells. The risk can be further reduced by identifying the exact sites on the mouse variable region that are necessary for binding and integrating these sites into human variable regions. This method produces synthetic antibodies that are very similar to human antibodies.

Other methods exist to produce synthetic antibodies that are identical to human antibodies. A species of virus known as the Epstein-Barr virus can be used to change human B cells in such a way that they will fuse with myeloma cells to form stable hybridoma cells that produce human antibodies. Another method involves using genetic engineering to produce mice with B cells that produce human antibodies rather than mouse antibodies. One of the most promising techniques involves creating a "library" of synthetic human antibodies. This is done by using the polymerase chain reaction (PCR) to produce multiple copies of the genetic material within B cells. This genetic material contains the information that results in the production of proteins that come together to form antibodies. By causing these proteins to be produced and allowing them to combine at random, researchers are able to produce mil-

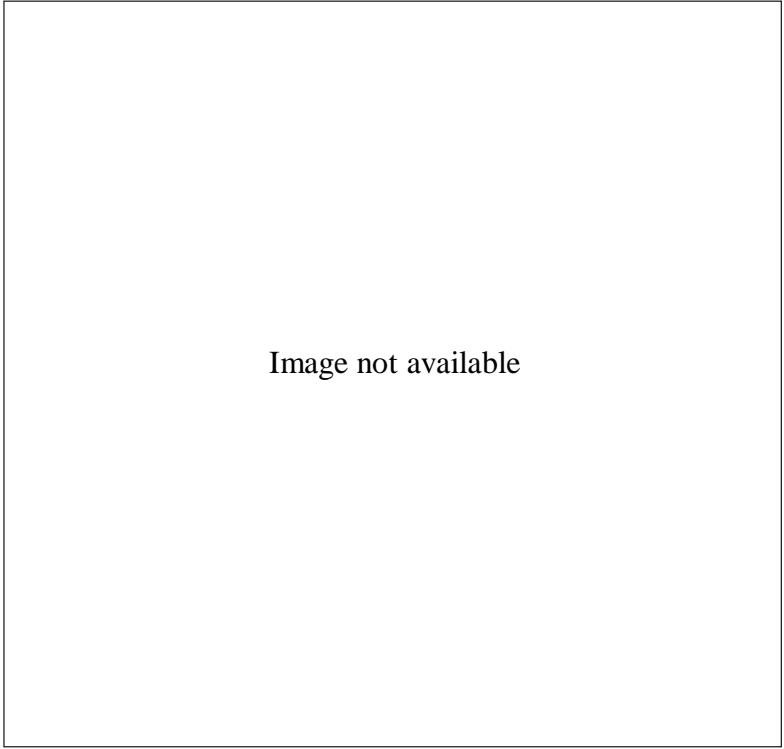
A black and white photograph showing a person's hands wearing gloves, holding a small glass vial and a thin glass tube. They are performing a precise procedure, likely injecting a liquid into a small, translucent animal embryo, possibly a chick embryo, held in a dish.

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At Onogen Therapeutics in Burlingame, California, a technician injects a chicken embryo with stem cells of another chicken embryo to which human antibodies have been added in order to make additional antibodies for pharmaceutical use. (AP/Wide World Photos)

lions of different antibodies. The antibodies are then tested to detect those that bind to selected antigens.

Impact and Applications

Some synthetic antibodies are used to help prevent the rejection of transplanted organs. An antibody that binds to the heart drug digoxin can be used to treat overdoses of that drug. Antibodies attached to radioactive isotopes are used in certain diagnostic procedures. Synthetic antibodies have also been used in patients undergoing a heart procedure known as a percutaneous transluminal coronary angioplasty (PTCA). The use of a particular synthetic antibody has been shown to reduce the risk of having one of the blood vessels that supply blood to the heart shut down during or after a PTCA. Researchers also hope to develop synthetic antibodies to treat acquired immunodeficiency syndrome (AIDS) and sep-

tic shock, a syndrome caused by toxic substances released by certain bacteria.

The most active area of research involving synthetic antibodies in the 1990's was in the treatment of cancer. On November 26, 1997, the U.S. Food and Drug Administration approved a synthetic antibody for use in non-Hodgkin's lymphoma, a cancer of the white blood cells. It was the first synthetic antibody approved for use in cancer therapy.

—Rose Secrest

See also: Allergies; Anthrax; Antibodies; Autoimmune Disorders; Biopharmaceuticals; Blotting: Southern, Northern, and Western; Burkitt's Lymphoma; Cancer; Central Dogma of Molecular Biology; Cloning; Diabetes; Diphtheria; Genetic Engineering: Historical Development; Genetic Engineering: Industrial Applications; Hybridomas and Monoclonal Antibodies; Immunogenetics; Molecular Genetics; Multiple Alleles; Oncogenes; Organ Transplants and HLA Genes; Prion Diseases; Kuru and Creutzfeldt-Jakob Syndrome; Transgenic Organisms.

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Synthetic Genes

Field of study: Genetic engineering and biotechnology

Significance: *Synthetic genes have been shown to function in biological organisms. Scientists hope that it will prove possible to restore normal func-*

tion in diseased humans, animals, and plants by replacing defective natural genes with appropriately modified synthetic genes.

Key terms

RESTRICTION ENZYME: an enzyme that cleaves, or cuts, DNA at specific sites with sequences recognized by the enzyme; also called restriction endonucleases

REVERSE TRANSCRIPTION: the synthesis of DNA from RNA

A Brief History

In 1871, Swiss physician Johann Friedrich Miescher reported that the chief constituent of the cell nucleus was nucleoprotein, or nuclein. Later it was established that the nuclei of bacteria contained little or no protein, so the hereditary material was named nucleic acid. At the end of the nineteenth century, German biochemist Albrecht Kossel identified the four nitrogenous bases: the purines adenine (A) and guanine (G) and the pyrimidines cytosine (C) and uracil (U). In the 1920's, Phoebus A. Levene and others indicated the existence of two kinds of nucleic acid: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA); the latter contains thymine (T) instead of uracil.

The chemical identity of genes began to unfold in 1928, when Frederick Griffith discovered the phenomenon of genetic transformation. Oswald Avery, Colin MacLeod, and Maclyn McCarty (in 1944) and Alfred Hershey and Martha Chase (in 1952) demonstrated that DNA was the hereditary material. Following the elucidation of the structure of DNA in 1953 by James Watson and Francis Crick, pioneering efforts by several scientists led to the eventual synthesis of a gene. The successful enzymatic synthesis of DNA in vitro (in the test tube) in 1956, by Arthur Kornberg and colleagues, and that of RNA by Marianne Grunberg-Manago and Severo Ochoa also contributed to the development of synthetic genes. In 1961, Marshall Nirenberg and Heinrich Matthaei synthesized polyphenylalanine chains using a synthetic messenger RNA (mRNA). In 1965, Robert W. Holley and colleagues determined the complete sequence of alanine transfer RNA (tRNA) isolated from yeast. The interpretation of the

genetic code by several groups of scientists throughout the 1960's was also clearly important.

In 1970, Har Gobind Khorana, along with twelve associates, synthesized the first gene: the gene for an alanine tRNA in yeast. There were no automatic DNA synthesizers available then. In 1976, Khorana's group synthesized the tyrosine suppressor tRNA gene of *Escherichia coli* (*E. coli*). The *lac* operator gene (twenty-one nucleotides long) was also synthesized, introduced into *E. coli*, and demonstrated to be functional. It took ten years to synthesize the first gene; by the mid-1990's, gene machines could synthesize a gene in hours.

Gene Synthesis

Protein engineering is possible by making targeted changes in a DNA sequence to produce a different product (protein) polypeptide with different properties, such as stress tolerance. The process of targeting a specific change in the nucleotide sequence (site-directed mutagenesis) allows the correlation of gene structure with protein function. Rapid sequencing with modern capillary DNA sequencers facilitates determination of the order of nucleotides that make up a gene in a matter of hours.

Once the sequence of a gene is known, it can be synthesized from nucleotides using gene machines. A gene machine is simply a chemical synthesizer made up of tubes, valves, and pumps that bonds nucleotides together in the right order under the direction of a computer. An intelligent person with a minimum of training can produce synthetic genes. A gene may be isolated from an organism using restriction enzymes (any of the several enzymes found in bacteria that serve to chop up the DNA of invading viruses), or it may be made on a gene machine. For example, the chymosin gene (an

enzyme used in cheese making) in calves can be synthesized from its known nucleotide sequence instead of isolating it from calf DNA using restriction enzymes. Alternatively, chymosin mRNA can be obtained from calf stomach cells, which can be transformed into DNA through reverse transcription.

New or modified genes may be manufactured to obtain a desired product. Gene synthesis, coupled with automated rapid sequencing and protein analysis, has yielded remarkable dividends in medicine and agriculture. Genetic engineers are designing new proteins from scratch to learn more about protein function and architecture. With synthetic genes, the process of mutagenesis can be explored in greater depth. It is possible to produce various alterations at will in the nucleotide sequence of a gene and observe their effects on protein function. Such studies carry the potential to unravel many biochemical and genetic pathways that could be the key to a better understanding of health and disease.

—Manjit S. Kang

See also: Biopharmaceuticals; Cell Culture: Plant Cells; Cloning; Cloning Vectors; DNA Sequencing Technology; Gene Therapy; Genetic Engineering; Protein Synthesis; Restriction Enzymes; Reverse Transcriptase; Synthetic Antibodies.

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Tay-Sachs Disease

Field of study: Diseases and syndromes

Significance: *Tay-Sachs disease (TSD) is a lethal disease inherited as an autosomal recessive disorder. Affected children are normal at birth, and symptoms are usually noticed by six months of age, after which they progressively worsen; the child usually dies at or before four years of age. There is no cure for this severe disorder of the nervous system, but an understanding of the genetic nature of the disorder has led to effective population screening, prenatal diagnosis, and genetic counseling.*

Key terms

GENETIC SCREENING: the testing of individuals for a disease-causing gene

HEXOSAMINIDASE A (HEX A): a lysosomal enzyme, the absence of which leads to Tay-Sachs disease

LYSOSOME: an organelle or structure in the cytoplasm of a cell that contains enzymes involved in the breakdown of metabolic products

PRENATAL DIAGNOSIS: the identification of a gene or disease in an embryo or fetus

Symptoms of Tay-Sachs Disease

Tay-Sachs disease (TSD) is an inherited birth defect that is named after Warren Tay, an English ophthalmologist, and Bernard Sachs, an American neurologist, who first described the disorder. TSD is one of the lysosomal storage disorders, as are Hurler's syndrome, Hunter's syndrome, Gaucher disease, and Fabry disease. Lysosomes are organelles found in the cytoplasm of cells and contain many enzymes that digest the cell's food and waste. TSD is caused by the lack of the enzyme hexosaminidase A (Hex A), which facilitates the breakdown of fatty substances and gangliosides in the brain and nerve cells. When Hex A is sufficiently lacking, as in TSD, gangliosides accumulate in the body and eventually lead to the destruction of the nervous system.

Children with TSD appear normal at birth and up to six months of age. During this time, they may show an exaggerated startle response to sound. Shortly after six months, more obvi-

ous symptoms appear. The child may show poor head control and an involuntary back-and-forth movement of the eyes. Also distinctive of TSD is a "cherry red spot" on the retina of the eye, first described by Tay, that usually appears after one year of age as atrophy of the optic nerve head occurs. The symptoms are progressive, and the child loses all the motor and mental skills developed to that point. Convulsions, increased motor tone, and blindness develop as the disease progresses. The buildup of storage material in the brain causes the head to enlarge, and brain weight may be 50 percent greater than normal at the time of death. There is no cure for TSD, and death usually occurs between two and four years of age, with the most common cause of death being pneumonia.

There are several forms of Tay-Sachs disease in addition to the classical, or infant, form already described. There is a juvenile form in which similar symptoms appear between two and five years of age, with death occurring around age fifteen. A chronic form of TSD has symptoms beginning at age five that are far milder than those of the infant and juvenile forms. Late-onset Tay-Sachs disease (LOTS) is a rare form in which there is some residual Hex A activity so that symptoms appear later in life and the disease progresses much more slowly.

Genetics of Tay-Sachs Disease

All forms of TSD are inherited as autosomal recessive disorders. One of the interesting features of TSD, as is true of some other genetic disorders, is its variation across ethnic groups. The Ashkenazi Jewish population, ancestors of most of the Jewish people in the United States, is a group of Jews of Eastern European descent. This group has a high incidence of TSD, about 1 in 3,600. Approximately one in thirty Ashkenazi Jews is a heterozygote (a person who carries one copy of the gene but does not show symptoms), compared to a figure of perhaps one in three hundred for the rest of the world's population. It is possible to screen the population and identify heterozygous individuals by means of a blood plasma assay that detects differences in Hex A activity. If two people are

carriers of the gene, they have a one-quarter chance of having a child with TSD. If one or both individuals are not carriers, they can be reassured that their child will not have TSD. If both people are carriers, once pregnancy ensues, prenatal diagnosis can determine whether the developing fetus is affected. In cases of a positive diagnosis, couples can be counseled regarding therapeutic pregnancy termination.

Impact and Applications

Although much has been learned about the genetics of the Tay-Sachs gene and the protein deficiency that causes the disease, there is still no cure. Nevertheless, TSD provides an excellent example of how the medical community can assist a susceptible population in confronting an incurable genetic disease. The effective screening of populations at risk for TSD and prenatal detection of fetuses with TSD have served to dramatically reduce the overall incidence of this terrible disease.

—Donald J. Nash

See also: Genetic Counseling; Genetic Screening; Genetic Testing; Genetic Testing: Ethical and Economic Issues; Hereditary Diseases; In Vitro Fertilization and Embryo Transfer; Inborn Errors of Metabolism; Penetrance; Prenatal Diagnosis; Repetitive DNA.

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Dolan DNA Learning Center, Your Genes Your Health. <http://www.ygyh.org>. Sponsored by the Cold Spring Harbor Laboratory, this site, a component of the DNA Interactive Web site, offers information on more than a dozen inherited diseases and syndromes, including Tay-Sachs disease.

Telomeres

Field of study: Cellular biology

Significance: Telomeres, the ends of the arms of chromosomes of eukaryotes, become shorter as organisms age. They are thought to act biologically to slow chromosome shortening, which can lead to cell death caused by the loss of genes and may be related to aging and diseases such as cancer.

Key terms

EUKARYOTE: a unicellular or multicellular organism with cells that contain a membrane-bound nucleus, multiple chromosomes, and membrane-bound organelles

PROKARYOTE: a unicellular organism with a single chromosome and lacking a nucleus or any other membrane-bound organelles

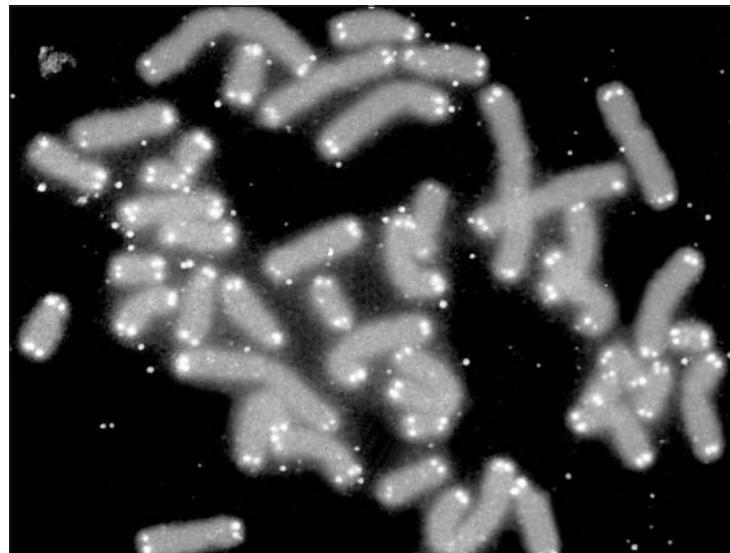
Eukaryotic Chromosomes and Telomeres

The DNA of bacteria and other related simple organisms (prokaryotes) consists of one double-stranded DNA molecule. Structurally and functionally, the prokaryotic chromosome contains one copy of most genes as well as DNA regions that control expression of these genes. Prokaryotic gene expression depends primarily upon a cell's moment-to-moment needs. An entire prokaryotic chromosome, its genome,

usually encodes about one thousand genes.

The genomes of eukaryotes are much more complex and may include 100,000 or more genes. The number of chromosomes in different types of eukaryotes can range from just a few to several hundred. Each of these huge DNA molecules is linear rather than the circular molecule of the type seen in prokaryotes. In addition, many individual segments of the DNA of eukaryotes exist in multiple copies. For example, about 10 percent of the DNA of a eukaryote consists of "very highly repetitive segments" (VRS's), units that are less than ten deoxyribonucleotides long that are repeated up to several million times per cell. DNA segments that are several hundred deoxyribonucleotide units long represent about 20 to 25 percent of the DNA. They are repeated one thousand times or more per cell. The rest of the eukaryote DNA (from 65 to 70 percent of the total) consists of larger segments repeated once or a few times, the genes, and the DNA regions that control the expression of the genes.

Much of the repetitive DNA, called satellite DNA, does not seem to be involved in coding for proteins or RNAs involved in making proteins. Telomeres are part of this DNA and consist of pieces of DNA that are several thousand deoxyribonucleotide units long, found at both chromosome ends. They are believed to act to stabilize the ends of chromosomes and protect them from exonuclease enzymes that degrade DNA from the ends. Researchers have concluded this for two reasons. First, the enzymes that make two chromosomes every time a cell reproduces are unable to operate at the chromosome ends. Hence, the repeated reproduction of a eukaryote cell and its DNA will lead to the creation of shorter and shorter chromosomes, a process that can cause cell death when essential genes are lost. Second, as organisms age, the telomeres of their cells become shorter and shorter.



Telomeres appear as the lightened tips of the chromosomes shown here. (Robert Moyzis, University of California, Irvine, CA; U.S. Department of Energy Human Genome Program, <http://www.ornl.gov/hgmis>)

Telomerase Enzymes

When chromosomes are replicated in preparation for cell division, the internal segments are replicated by a complex process involving the enzymes primase and DNA polymerase. Primase lays down a small segment of RNA on the template strand of DNA, and DNA polymerase uses the primer to start replication. Making the end of a linear chromosome is a problem, however, because primers cannot consistently be produced at the very ends of the chromosomes. Consequently, with each cell division a small portion of the ends of newly replicated chromosomes is single-stranded and is trimmed off by exonucleases. This problem is solved by enzymes known as telomerases, which add telomeres to eukaryote chromosomes. Each telomerase contains a nucleic acid component (RNA) about 150 ribonucleotides long. This is equivalent to 1.5 copies of the appropriate repeat in the DNA telomere to be made. The enzyme uses this piece of RNA to make the desired DNA strand of the telomere. How the telomerase in any given species identifies the correct length of telomere repeat for a specific chromosome is not clearly understood, nor is the exact mechanism by which the DNA strand is made.

Telomerase activity can be lost in certain strains of simple eukaryotes, such as protozoa. When this happens to a given cell line, each cell division leads to the additional shortening of its telomeres. This process continues for a fixed number of cell divisions; it then ends with the death of the telomerase-deficient cell line.

A related observation has been made in humans. It has been shown that when human fibroblasts are grown in tissue culture, telomere length is longest when cells are obtained from young individuals. They are shorter in cells taken from the middle-aged, and very short in cells taken from the aged. Similar observations have been made with the fibroblasts from other

higher eukaryotes as well as with other human cell types. In contrast, the process of telomere shortening does not happen when germ-cell lines—which in the whole organism produce sperm and ova—are grown in tissue culture. This suggests a basis for differences in longevity of the germ cells and the somatic cells that make up other human tissues.

Impact and Applications

The discovery and study of telomeres and telomerases produced new insights into DNA synthesis, the number of times a cell can reproduce, and the aging process. The circular DNA of bacteria (which are prokaryotes) allows them

Telomere Length in Clones

Dolly the sheep, the first mammal to be cloned from adult cells, was born on July 5, 1996. While Dolly ushered in a new era of mammalian cloning, her tenure as the cloning community's lovable mascot was, quite literally, short-lived. Dolly was euthanized on February 14, 2003, after being diagnosed with a progressive lung disease; she had already been suffering from debilitating arthritis. While Dolly's health problems could have resulted from "natural causes," both ailments are more characteristic of much older sheep. Sheep normally live to an age of about twelve, Dolly was only half that.

Dolly's early demise was actually foreshadowed in 1999, when the group which cloned her reported that Dolly's telomeres were shorter than expected for a sheep of her age. Dolly's telomeres were about the length one would expect if her cells had been six years old on the day she was born (Dolly was cloned from a six-year-old ewe). Since telomere length acts as a "molecular clock" that determines the age of a cell, researchers had hoped that this clock would somehow be "reset" upon transfer of an adult nucleus to a host ovum.

While clearly not the case for Dolly, this resetting of telomere length has been demonstrated in cloned cows. In 2000, Robert Lanza and colleagues reported that cloned cows had longer-than-normal telomeres. Will these "super cows" be able to live appreciably longer than normal cows? Only time will tell, since cows have a normal life span of about twenty years. What accounts for the difference between Dolly and these cloned cows? Subsequent research has shown

that the type of cell used in the cloning process may be an important factor. In 2002, Norikazu Miyashita and colleagues reported that cows cloned from mammary gland cells (like Dolly) had shorter-than-normal telomeres, clones obtained from skin fibroblasts (connective tissue precursors, those used by Lanza) had longer-than-normal telomeres, and clones obtained from muscle cells showed no significant differences in telomere length.

The telomere length of clones may also be species-specific. Teruhiko Wakayama and colleagues cloned mice sequentially for six generations but saw no difference in telomere length in any of the clones produced. Mice, however, are known to have extremely long telomeres to begin with; also, unlike the somatic cells of cows or sheep, many of the somatic cells of mice are known to express the telomerase enzyme.

More research is necessary to understand exactly why certain animal clones are produced with shortened telomeres and others are not. Currently, our lack of knowledge on the subject remains one of the more compelling reasons not to attempt to clone a human at this time. A human clone produced with unusually short telomeres may, like Dolly, meet with an untimely death. In fact, patients with a human genetic disease called Hutchinson-Gilford progeria have skin fibroblasts with greatly reduced telomere lengths; persons affected with this disease live to an average age of about thirteen years.

—James S. Godde

to undergo many more cycles of reproduction than the somatic cells of the eukaryotes. The linear eukaryote chromosome may have evolved because such DNA molecules were too large to survive as circular molecules given their rigidity and fragility. In addition, the observation of telomere shortening in simple and complex eukaryotes raises the fascinating possibility that the life spans of organisms may be related to the conservation of telomeres associated with the replication of these structures by telomerases.

The role of telomere length in longevity is uncertain, but apparently significant. Cells grown in cell culture typically divide only a predictable number of times, and once this limit is reached they can no longer divide. At the same time, telomere length shortens with each division. Sometimes cells in culture will go through what is called a "crisis," after which they become "immortalized" and are able to divide an indefinite number of times. Immortal cells also actively express telomerases and maintain constant telomere lengths. Cancer cells typically exhibit these same characteristics. A better understanding of telomeres and telomerase expression might provide insights into aging and cancer, leading to a potential cure for cancer and age-related diseases.

—Sanford S. Singer

See also: Aging; Animal Cloning; Chromosome Mutation; Chromosome Structure; Cloning Vectors; DNA Replication; Molecular Genetics; Reverse Transcriptase.

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Testicular Feminization Syndrome

Field of study: Diseases and syndromes

Significance: *The sex of a baby is usually determined at conception by the sex chromosomes, but other genetic events can alter the outcome. One such condition is testicular feminization syndrome, which causes a child with male chromosomes to be born with feminized genitals. Information gained from the study of this and similar conditions is being used to challenge the validity of sex-determination tests for athletes.*

Key terms

ANDROGEN RECEPTORS: molecules in the cytoplasm of cells that join with circulating male hormones

ANDROGENS: hormones that promote male body characteristics

DIFFERENTIATION: the process of changing from an unspecialized condition to a final specialized one

PHENOTYPE: the expressed characteristics, both physical and physiological, of an individual

SEX DETERMINATION: events that cause an embryo to become male or female

Development of Testicular Feminization Syndrome

Introductory biology courses teach that a fertilized egg that receives two X chromosomes at conception will be a girl, whereas a fertilized egg that receives an X and a Y chromosome will become a boy. However, other factors can also affect the development of a person's gender. Gender development in mammals begins at conception with the establishment of chromosomal sex (the presence of XX or XY chromosomes). Even twelve weeks into development, male and female embryos have the same external appearance. Internal structures for both sexes are also similar. However, the machinery has been set in motion to cause the external genitals to become male or female, with corresponding internal structures of the appropriate sex. The baby is usually born with the proper phenotype to match its chromosomal sex. However, development of the sex organs is controlled by several genes. This leaves a great deal of room for developmental errors to occur.

The primary gene involved in sex determination is carried on the Y chromosome. It is responsible for converting the early unisex gonads into testes. Once formed, the testes then produce the balance of androgen and estrogen that pushes development in the direction of the male phenotype. In the absence of this gene, the undetermined gonads become ovaries, and the female phenotype emerges. Therefore, the main cause of sex determination is not XX or XY chromosomes, but rather the presence or absence of the gene that promotes testis differentiation.

In order for the male hormones to have an influence on the development of the internal and external reproductive structures, the cells of those structures must receive a signal that

they are part of a male animal. The androgens produced by the testes are capable of entering a cell through the cell membrane. Inside the cell, the androgens attach to specific protein receptor molecules (androgen receptors). Attachment causes the receptors to move from the cytoplasm into the nucleus of the cell. Once in the nucleus, the receptor-steroid complexes bind to DNA near genes that are designed to respond to the presence of these hormones. The binding event is part of the process that turns on specific genes—in this case, the genes that direct the process of building male genitals from the unisex embryonic structures as well as those that suppress the embryonic female uterus and tubes present in the embryo's abdomen.

In cases of testicular feminization, androgen receptors are missing from male cells. This is the result of a recessive allele located on the X chromosome. Because normal males have only one X, the presence of a recessive allele on that X will result in no production of the androgen receptor in that individual. The developing embryo is producing androgen in the testes; without the receptor molecules, however, the cells of the genitals are unable to sense the androgen and respond to it. For this reason, the disorder is sometimes known by an alternate name: androgen-insensitivity syndrome. The cells of the genitals are still capable of responding to estrogen from the testes. As a result, the genitals become feminized: labia and clitoris instead of a scrotum and penis, and a short, blind vagina. To the obstetrician and parents, the baby appears to be a perfect little girl. An internal examination would show the presence of testes rather than ovaries and the lack of a uterus and Fallopian tubes, but there would normally be no reason for such an examination.

Impact and Applications

Several events may lead to the diagnosis of this condition. The attempted descent of the testes into a nonexistent scrotum will cause pain that may be mistaken for the pain of a hernia; the presence of testes in the apparent girl will be discovered when the child undergoes repair surgery. In other cases, the child may seek

medical help in the mid-teen years because she does not menstruate. Exploratory surgery would then reveal the presence of testes and the absence of a uterus. As a general rule, the testes are left in the abdomen until after puberty because they are needed as a source of estrogen to promote the secondary sex characteristics, such as breast development. Without this estrogen, the girl would remain childlike in body form. After puberty, the testes are usually removed because they have a tendency to become cancerous.

As a result of its phenotypic sex, an infant with testicular feminization is normally raised as a girl whose only problem is an inability to bear children. If the girl has athletic ability, however, other problems may arise. Since 1966, female Olympic athletes have had to submit to a test for the presence of the correct chromosomal sex. In the past, this has meant microscopic examination of cheek cells to count X chromosomes. In 1992, this technique was replaced by a test for the Y chromosome. Individuals who fail the "sex test," including those with testicular feminization syndrome, cannot compete against other women. Proponents argue that androgens aid muscle development, and the extra testosterone produced by the testes of a normal male would provide an unfair physical advantage. However, because people with testicular feminization syndrome are lacking androgen receptors, their muscle development would be unaffected by the extra androgen produced by the testes, and thus they would not be any stronger than well-conditioned women.

—Nancy N. Shontz

See also: Fragile X Syndrome; Gender Identity; Hereditary Diseases; Hermaphrodites; Klinefelter Syndrome; Metafemales; Pseudohermaphrodites; Steroid Hormones; XYY Syndrome.

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Web Sites of Interest

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

Thalidomide and Other Teratogens

Field of study: Diseases and syndromes

Significance: *Teratogenesis is the development of defects in the embryo or fetus caused by exposure to chemicals, radiation, or other environmental conditions. Thalidomide, a sedative whose ingestion by pregnant women led to the birth of abnormal babies in the late 1950's and early 1960's, is one of the more publicized examples of a chemical teratogen.*

Key terms

CONGENITAL DEFECT: a defect or disorder that occurs during prenatal development

PEROMELIA: the congenital absence or malformation of the extremities caused by abnormal development of the limb bud from about the fourth to the eighth week after conception; the ingestion of thalidomide by pregnant women can cause this disorder in fetuses

Teratogenesis and Its Causes

Teratogenesis is the development of structural or functional abnormalities in an embryo or fetus due to the presence of a toxic chemical or other environmental factor. The term is derived from the Greek words *teras* (monster) and *genesis* (birth). The phenomenon is usually attributed to exposure of the mother to some causative agent during the early stages of pregnancy. These may include chemicals, excessive radiation exposure, viral infections, or drugs.

Image not available

This three-year-old girl was born without arms in 1962 to a German mother who had taken thalidomide during her pregnancy. (AP/Wide World Photos)

Approximately 3 percent of the developmental abnormalities are attributed to drugs. Drugs that are taken by the father may be teratogenic only if they damage the chromosomes of a spermatozoan that then joins with the egg to form a zygote.

For many centuries, the impression that malformed babies were conceived as a result of the intercourse between humans and devils or animals dominated society. Seventeenth century English physiologist William Harvey attributed teratogenesis to embryonic development. In the nineteenth century, the French brothers Étienne and Isidore Geoffroy Saint-Hilaire outlined a systematic study on the science of teratology. In the United States, the importance of teratogens was first widely covered during the 1940's, when scientists discovered that pregnant women who were affected by German measles (rubella) often gave birth to babies that had one or more birth defects. In the 1940's and 1950's, the consumption of diethylstilbestrol (DES) before the ninth week of gestation to prevent miscarriage was found to produce cancer in the developing fetus. Animal studies have also shown that defective offspring result from the use of hallucinogens such as lysergic acid diethylamide (LSD).

A broader definition of teratogenesis may include other minor birth defects that are more likely to be genetically linked, such as clubfoot, cleft lip, and cleft palate. These defects can often be treated in a much more effective way than those caused by toxic substances. Clubfoot, for example, which can be detected by the unusual twisted position of one or both feet, may be treated with surgery and physical therapy within the first month after birth. Brachydactyly (short digits) in rabbits has been linked to a recessive gene that causes a local breakdown of the circulation in the developing bud of the embryo, which is followed by necrosis (tissue death) and healing. In more extreme cases of agenesis, such as limb absence, a fold of amnion (embryonic membrane) was found to cause strangulation of the limb. Agenesis has been observed with or-

gans such as kidneys, bladders, testicles, ovaries, thyroids, and lungs. Other genetic teratogenic malformations include anencephaly (absence of brain at birth), microcephaly (small-size head), hydrocephaly (large-size head caused by accumulation of large amounts of fluids), spina bifida (failure of the spine to close over the spinal cord), cleft palate (lack of fusion in the ventral laminae), and hermaphroditism (presence of both male and female sexual organs).

Thalidomide and Its Impact

Thalidomide resembles glutethimide in its sedative action. Laboratory studies of the late 1950's and early 1960's had shown thalidomide to be a safe sedative for pregnant women. As early as 1958, the West German government made the medicine available without prescription. Other Western European countries followed, with the medicine available only upon physician's prescription. It took several years for the human population to provide the evidence that laboratory animals could not. German physician Widukind Lenz established the role of thalidomide in a series of congenital defects. He proved that administration of the drug during the first twelve weeks of the mother's pregnancy led to the development of phocomelia, a condition characterized by peromelia (the congenital absence or malformation of the extremities caused by the abnormal formation and development of the limb bud from about the fourth to the eighth week after conception), absence or malformation of the external ear, fusion defects of the eye, and absence of the normal openings of the gastrointestinal system of the body.

The United States escaped the thalidomide tragedy to a great extent because of the efforts of Frances O. Kelsey, M.D., of the U.S. Food and Drug Administration (FDA). She had serious doubts about the drug's safety and was instrumental in banning the approval of thalidomide for marketing in the United States. Other scientists such as Helen Brooke Taussig, a pioneer of pediatric cardiology and one of the physicians who outlined the surgery on babies with the Fallot (blue baby) syndrome, played a key role in preventing the approval of thalidomide by the FDA. It is estimated that about

seven thousand births were affected by the ingestion of thalidomide.

The thalidomide incident made all scientists more skeptical about the final approval of any type of medicine, especially those likely to be used during pregnancy. The trend intensified the fight against any chemicals that might affect the fetus during the first trimester, when it is particularly vulnerable to teratogens. Alcohol and tobacco drew many headlines in the media in the 1990's. Both have been shown to create congenital problems in mental development and learning abilities. At the same time, regulation of new FDA-approved medicine became much stricter, and efforts to study the long-term effects of various pharmaceuticals increased. Surprisingly, thalidomide itself has been used successfully in leprosy cases and, in conjunction with cyclosporine, to treat cases of the immune reaction that appears in many bone-marrow transplant patients. There is also a movement to use thalidomide in the treatment of acquired immunodeficiency syndrome (AIDS).

In addition to drugs, many other agents can affect fetal development. Essentially, any factor with the potential to cause DNA mutations has a high probability of being teratogenic. Consequently, early in pregnancy, women are advised to limit their exposure to a variety of potential teratogens, such as excess radiation, toxic chemicals, tobacco, alcohol, and other drugs. Prevention might even include work reassignment to limit or eliminate the woman's normal exposure to teratogens. Unfortunately, teratogenesis can occur early in the pregnancy, before the woman is even aware that she is pregnant. Prevention by avoidance is therefore essential.

—*Soraya Ghayourmanesh, updated by Bryan Ness*

See also: Congenital Defects; Prenatal Diagnosis.

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Web Site of Interest

Teratology Society. <http://www.teratology.org>.

The Teratology Society is a multidisciplinary scientific society founded in 1960, the members of which study the causes and biological processes leading to abnormal development and birth defects at the fundamental and clinical level, and appropriate measures for prevention.

Totipotency

Field of study: Cellular biology

Significance: *Totipotency is the ability of a living cell to express all of its genes to regenerate a whole new individual. Totipotent cells from plants have been used in tissue-culture techniques to produce improved plant materials that are pathogen-free and disease-resistant. Totipotent cells from animals are now being used to clone mammals, although ethical questions remain over whether cloning a human should be done.*

Key terms

MULTIPOTENT CELL: a stem cell capable of forming multiple differentiated tissues

PARTHENOGENESIS: asexual reproduction from a single egg without fertilization by sperm

PLURIPOTENT CELL: a stem cell that forms all types of differentiated tissues

UNIPOTENT CELL: a stem cell that forms only one differentiated tissue

Egg and Sperm Cells

In plants and animals, a whole organism is sexually reproduced from a zygote, a product of fusion between egg and sperm. Zygotes are totipotent. A zygote in the seed of a plant or in the uterus of a mammal divides by mitosis and has the potential to produce more cells, called embryonic cells, before developing into an adult individual. During embryonic cell divi-

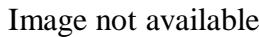
sion, the cells begin to differentiate. Once differentiated, these specialized cells still possess all the genetic materials inherited from the zygote. Differentiated cells express or use some of their genes (not all) to produce their own specific proteins. For example, epidermal cells in human beings produce fibrous proteins called keratin to protect the skin, and red blood cells produce hemoglobin to help transport oxygen. Due to the differences in gene expression, differentiated cells have their own distinct structures and functions, and some differentiated cells are totipotent.

A whole organism can be asexually reproduced from a single egg without the sperm by a process called parthenogenesis. This occurs naturally in some insects, snakes, lizards, and amphibians, as well as in some plants. In this type of reproduction, the haploid chromosomes within an unfertilized egg duplicate, and the embryo develops as if the egg had been fertilized. The pseudo-fertilized eggs are totipotent and generate all female individuals. The females can reproduce under favorable environmental conditions without waiting for a mate. Like in vitro fertilization, parthenogenesis is used as a technique to create an embryo in the laboratory. Chromosomal duplication is induced in the egg cell to reproduce female individuals. However, no parthenogenic mammals had been developed.

It may become possible to produce males through a process called androgenesis. In the laboratory, the haploid chromosomes from one sperm may be induced to duplicate. As in animal cloning, the duplicated chromosomes, which are diploid, can be implanted into an enucleated egg cell (a cell from which the nucleus has been removed). Although androgenesis holds some promise, so far it has not produced normal embryos.

Cell Differentiation

Cell differentiation is a process whereby genetically identical cells become different or specialized for their specific functions. During differentiation, enzymes and other polypeptides, including other large molecules, are synthesized. Ribosomes and other cell structures are assembled. Differentiated cells express only

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DNA is removed by pipette suction from a mammalian egg cell to prepare an enucleated egg cell for androgenesis. (AP/Wide World Photos)

some of their genes to make enzymes and other proteins.

Tissue differentiation is usually triggered by mitosis, followed by cytokinesis. Then differentiation occurs in the daughter cells. Often the two daughter cells have different structures and functions, but both retain the same genes. For example, the epidermal cell mitotically divides to produce one large and one small cell on the root surface; the large one maintains the role of epidermal cell as a root covering, whereas the small one becomes the root hair.

Totipotent Cells in Plant-Tissue Culture

Cuttings of plants and tissue-culture techniques have proven that many plant cells are totipotent. Tissue culture, however, helps to identify what specific type of cell is totipotent, because the technique uses a very small piece of known tissue. For example, if pith tissues from tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), and other dicot stems are cut off and cultured aseptically on an agar medium with proper nutrients and hormones, a clump

of unspecialized and loosely arranged cells, called a callus, is formed. Each cell from the callus begins to divide and differentiate, forming a multicellular embryoid. One test tube can accommodate thousands of cells, and each embryoid has the potential to become a complete plantlet. Plantlets can be transplanted into the soil to develop into adult plants.

The phloem tissues from the roots of carrots (*Daucus* species) also exhibit totipotency. Cells in pollen grains of tobacco are totipotent, and they produce haploid plants. Using meristem tissues of shoot and root tips, the cells regenerate new plants that are free of viruses, bacteria, and fungi. Pathogen elimination is possible because vascular tissues (xylem and phloem), in which viruses move, do not reach the root or shoot apex. The protoplasts (cells without cell walls) from mesophyll cells of the leaf regenerate new plants.

Plant Hormones

Totipotency of plant cells is enhanced by the presence of hormones, such as auxins and

cytokinins, in the culture media. Addition of auxins influences the expression of genes and causes physiological and morphological changes in plants. Addition of cytokinins promotes cell division, cytokinesis, and organ formation. If these are present in the proper ratio, callus from many plant species can be made to develop into an entire new plant. If the cytokinin-to-auxin ratio is high, cells in the callus divide and give rise to the development of buds, stems, and leaves. If the cytokinin-to-auxin ratio is low, root formation is favored. Totipotency of some plant cells is promoted by the addition of coconut water to the culture media—an indication that coconut water has the right proportion of cytokinin and auxin to regenerate an entire plant.

Importance of Totipotency in Plants

Clonal propagation of plants using tissue culturing is used commercially to mass-produce numerous ornamentals, vegetables, and forest trees. A major use of pathogen-free plants is for the storage of germ plasm and for transport of plant materials into different countries. It is also possible to generate plants with desirable traits, such as resistance to herbicides and environmental stressors or tolerance of soil salinity, soil acidity, and heavy-metal toxicity. It is easier to select resistant or tolerant plants from a thousand cells than from a thousand plants.

Somatic Cells in Animal Cloning

Animals are more difficult to reproduce asexually than plants are. Somatic cells of animals become totipotent when used as donor cells in cloning. The first successful animal cloned was a frog, *Xenopus laevis*. This cloning involved the use of a nucleus from the intestinal epithelial cells of a tadpole and an egg cell from a mature frog. In the laboratory, the nucleus from the egg cell was removed (enucleated) by micropipette. The tadpole's nucleus (the donor cell) was inserted into the enucleated frog's egg cell. The nuclei-injected egg cell underwent a series of embryonic developmental stages, including the blastula stage, developing into tadpoles that later died before becoming adults.

Cloning of Dolly the sheep (*Ovis* species) used the mammary cell of a six-year-old ewe as the donor cell. It was injected into the enucleated sheep's egg cell. Cloning a mammal requires a surrogate mother. The blastula stage of embryo was developed in vitro and was implanted into a surrogate mother. After five months, a lamb was born. The lamb was genetically identical to the sheep from which the mammary cell was taken. Today, cloning has been done by scientists to produce other animals, including cattle, pigs, monkeys, cats, and dogs. Cloning a human seems possible, but there are so many ethical and moral questions whether it should be done or not.

Stem Cells in Animal Cloning

Stem cells exhibit totipotency because they can generate new types of tissues. Some sources of stem cells are the blastocyst (the immature embryo), the fetus, the placenta, bone marrow, blood, skeletal muscle, and brain. Because there is no proof yet whether a single embryonic stem cell has the ability to regenerate into a complete individual, stem cells are generally only partially totipotent. A unipotent stem cell can form only one differentiated tissue. A multipotent stem cell can form multiple differentiated tissues. For example, stem cells from blood can form platelets, white blood cells, or red blood cells. The stem cells from skeletal muscle can form smooth muscle, cardiac muscle, bone, or cartilage. A pluripotent stem cell from embryo, brain, or bone marrow has the ability to develop all types of differentiated tissues of the body. For example, brain stem cells can be turned into all tissue types, including brain, muscles, blood cells, and nerves.

—Domingo M. Jariel

See also: Cell Culture: Plant Cells; Stem Cells.

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Transgenic Organisms

Field of study: Genetic engineering and biotechnology

Significance: *Implanting genes from one organism into the genome of another enables scientists to study basic genetic mechanisms and inherited diseases and to create plants and animals with traits that are beneficial to humans.*

Key terms

GENOME: the complete genetic material carried by an individual

PLASMID: a circular piece of bacterial DNA that is often used as a vector

TRANSFORMATION: integration of foreign DNA into a cell

TRANSGENE: the foreign gene incorporated into a cell's DNA during transformation

VECTOR: a carrier molecule that introduces foreign genetic materials into a cell

Engineering Organisms

Domestication and selective breeding of animals and plants began before recorded history. In fact, historians propose, the shaping of organisms to fit human needs contributed to the rise of settled, complex culture. Until late in the twentieth century, farmers and scientists could breed novel strains only from closely related species or subspecies because the DNA had to be compatible in order to produce offspring that in turn were fertile.

In the late 1970's and early 1980's, molecular biologists learned how to surpass the limitations of selective breeding. They invented procedures for combining the DNA of species as distantly related as plants and animals. Organisms produced by such means are termed transgenic. This branch of genetic engineering made it possible to design novel organisms for genetic and biochemical research and for medical, agricultural, and ecological innovations. Commercial use of transgenic organisms also created worldwide controversy because of their potential threat to human health and the environment.

Transgenesis is much like gene therapy in that both transform cells for a specific purpose. However, whereas gene therapy targets only certain cells in order to cure a defect in them, transgenesis seeks to produce an entirely modified organism by incorporating the transgene into all the cells of the mature organism and changing the genome. This is done by transforming not only the somatic (body) cells of the host organism but also the germ cells, so that when the organism reproduces, the transgene will pass to the next generation. Transgenes perform their alterations by blocking the function of a host gene, by replacing the host gene with one that codes for a variant protein, or by introducing an additional gene.

Transgenic Animals

In 1978, yeast cells were the first to be transformed by insertion of foreign DNA, followed by mouse cells in 1979. Mouse embryos were transformed in 1980, which later led to the development of a "supermouse" that grew much larger than ordinary mice because it had received the gene for human growth hormone. Most of these transformations came after microinjection of DNA directly into cells. Later, scientists were able to deliver foreign genes into hosts by several other methods: incorporating them into retroviruses and then infecting target cells; electroinfusion, whereby an electric current passed the foreign DNA through the relatively flimsy animal cell wall; biolistics, a means of mechanically shooting a DNA bullet into cells; and conveying the DNA into an ovum aboard sperm. Two methods, de-

veloped at first for mice, are particularly successful in growing genetically modified animals after transformation. The first entails injecting transformed embryonic stem cells into a blastocyst (an early spherical form of an embryo). In the second, the DNA is inserted into the pronucleus of a freshly fertilized egg. The blastocyst or egg is then implanted into a foster mother for gestation.

The first complex transgenic animals were intended for genetic research. After disabling a specific gene, scientists could study its effect on the appearance, metabolic processes, and health of the mature animal. By 2003 thousands of genes had been tested. Also, research with mice transformed with human DNA enabled scientists to identify genes associated with breast and prostate cancers, cystic fibrosis, Alzheimer's disease, and severe combined im-

munodeficiency disorder (SCID). In 2001 the first transgenic primate, a rhesus monkey, was born, potentially supplying a research model genetically much more similar to humans than mice are.

Beginning in the late 1990's, transgenic animals were developed for production of proteins that can be used in pharmaceutical drugs to treat human disease. Accordingly, they have become known as "pharm animals." Lactating transgenic mice make tissue plasminogen activator in their milk. Similarly, transgenic sheep supply blood coagulation factor IX and alpha¹-antitrypsin, transgenic pigs produce human hemoglobin, and transgenic cows make human lactoferrin. Scientists have also developed transgenic pigs that may supply tissue and organs for transplantation into humans without tissue rejection.

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These two rhesus monkeys were born from cloned embryos in 1996. (AP/Wide World Photos)

Transgenic Plants

Plant cells present greater difficulties for transformation because their cell walls are sturdier than animal cell walls. Microinjection and biolistics are possible but tricky and slow. A breakthrough for plant transgenesis came in 1983, when three separate teams of scientists used plasmids as vectors (carrier molecules) to infect plants with foreign DNA. The achievement came about because of research into plant tumors caused by crown gall disease. The pathogen, the soil bacterium *Agrobacterium tumefaciens*, caused the disease by ferrying bits of its own DNA into the genome of plants via plasmids, circular bits of extranuclear DNA. Scientists found that they could take the same plasmid, cut out bits of its DNA with enzymes and insert transgenes, and then use the altered plasmids as vectors to transform plants. Subsequently, scientists discovered that liposomes can be vectors. A liposome is a tiny ball of lipids that binds readily to a cell wall, opens a passage, and delivers any DNA that has been put inside it.

A great variety of transgenic plants have been designed for agriculture to produce genetically modified (GM) foods. The first to be marketed was a strain of tomato that ripened slowly so that it gained flavor by staying longer on the vine and remained ripe longer on super-

market shelves. This Flavr Savr tomato was not a commercial success, however. Corn, cotton, soybeans, potatoes, and papayas received a gene from the bacterium *Bacillus thuringiensis* (*Bt*) that enables them to make a caterpillar-killing toxin; these are frequently referred to as *Bt* crops. Other crops have been made resistant to herbicides so that weeds can be easily killed without harming the food plants. Similarly, some transgenic crops tolerate salty or aluminum-rich soil, have less impact on the land because they require less water or tillage, or produce a high yield.

Like transgenic animals, some transgenic crops promise to deliver pharmaceuticals at lower costs and more conveniently than factory-made drugs. GM bananas and potatoes contain vaccines for protection against diarrheal diseases, such as cholera, and hepatitis B. In 2000, scientists reported invention of rice and wheat strains that produce anti-cancer antibodies. Golden rice, a transgenic strain that contains vitamin A, was developed to ward off blindness from vitamin A deficiency, which is a problem in countries that subsist largely on rice. Another strain has elevated iron levels to combat anemia. In a bid to reduce the health risk from smoking, a tobacco company developed a strain free of nicotine.

The Debate over Transgenesis

Transgenic organisms offer great benefits to humankind: deeper understanding of the genetic component in disease and aids in diagnosis; new, cheaper, more easily produced drugs; and crops that could help alleviate the growing hunger in the world. Yet during the 1990's protests against transgenesis began that are as contentious as any since the controversy over the pesticide DDT during the 1960's.

Some opponents object to the very fact that organisms are modified strictly for human benefit. They find such manipulations of life's essential code blasphemous and arrogant, or at the very least unethical and reckless. Furthermore, animal rights groups regard the production of transgenic pharm and research animals cruel and in violation of the natural rights of other species.

The greater portion of opponents, however,

are concerned with specific dangers that transgenic organism may pose. Many consumers, most noticeably those in Europe, worry that GM foods contain hidden health risks. After transgenes were found to escape from crops and become part of wild plants, environmentalists proposed that there could be unforeseen and harmful ecological consequences, especially in the destruction of natural species and reduction of biodiversity.

Even those who welcome the creation of transgenic animals and plants are concerned about the legal and social effects. Principally, because biotechnology corporations can patent transgenic organisms, they potentially have great influence on agribusiness, perhaps to the detriment of small farmers and consumers.

—Roger Smith

See also: Antibodies; Biopesticides; Biopharmaceuticals; Genetic Engineering; Genetic Engineering: Agricultural Applications; Genetic Engineering: Medical Applications; Genetic Engineering: Risks; Genetic Engineering: Social and Ethical Issues; Genetically Modified (GM) Foods; Genomics; Human Growth Hormone; Hybridization and Introgression; Knockout Genetics and Knockout Mice; Lateral Gene Transfer; Model Organism: *Drosophila melanogaster*; Model Organism: *Mus musculus*; Model Organism: *Xenopus laevis*; Molecular Genetics; Viroids and Virusoids.

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Web Sites of Interest

Oak Ridge National Laboratory. Transgenic and Targeted Mutant Animal Database. <http://www.ornl.gov/TechResources/Trans/hmepg.html>. A searchable professional database about lines of genetically modified animals, methods used to create them and descriptions of the modified DNA, the expression of transgenes, and how transgenes are named.

TBASE: The Transgenic/Targeted Mutation Database, Jackson Laboratory, Bar Harbor, Maine. <http://tbase.jax.org>. Database of information about transgenic animals generated worldwide, searchable by species, technique, DNA construct, phenotype, laboratory. Features the "Knockout Model of the Month"—a discussion of new animal models—and a glossary.

Transgenic Crops: An Introduction and Resource Guide <http://www.colostate.edu/programs/lifesciences/transgeniccrops>. This richly illustrated site provides information about the history of plant breeding, the making of transgenic plants, government regulations, and risks and concerns. Also available in Spanish.

University of Michigan. Transgenic Animal Model Core. <http://www.med.umich.edu/>

tamc. A professional Web site for researchers seeking a host animal to test transgenes. However, it contains much useful general information about transgenics (especially transgenic rats), vectors, and laboratory procedures. With links and a photo gallery.

Transposable Elements

Fields of study: Bacterial genetics; Molecular genetics

Significance: *Transposable elements are discrete DNA sequences that have evolved the means to move (transpose) within the chromosomes. Transposition results in mutation and potentially large-scale genome rearrangements. Transposable elements contribute to the problem of multiple antibiotic resistance by mobilizing the genes of pathogenic bacteria for antibiotic resistance.*

Key terms

COMPOSITE TRANSPOSON: a transposable element that contains genes other than those required for transposition

RESISTANCE PLASMID (R PLASMID): a small, circular DNA molecule that replicates independently of the bacterial host chromosome and encodes a gene for antibiotic resistance

SELFISH DNA: a DNA sequence that has no apparent purpose for the host that spreads by forming additional copies of itself within the genome

TRANSPOSASE: an enzyme encoded by a transposable element that initiates transposition by cutting specifically at the ends of the element and randomly at the site of insertion

Jumping Genes

Transposable elements are DNA sequences that are capable of moving from one chromosomal location to another in the same cell. In some senses, transposable elements have been likened to intracellular viruses. The first genetic evidence for transposable elements was described by Barbara McClintock in the 1940's. She was studying the genetics of the pigmentation of maize (corn) kernels and realized that

Barbara McClintock

Though best known for her research on mobile genetic elements (for which she won the first unshared Nobel Prize in Physiology or Medicine awarded to a woman), Barbara McClintock's contributions to the field of genetics were many. McClintock's career in genetics spanned the development of the field itself. While an undergraduate at Cornell University (from which she earned her bachelor of science degree in 1923), she was invited to participate in a graduate course in genetics, then a fledgling discipline. She continued at Cornell as a graduate student (earning her Ph.D. in 1927), combining her interests in the microscopic internal structure of the cell (cytology) with the transmission of heritable traits (genetics).

McClintock's keen observational skills and her holistic approach to science allowed her to make significant advancements. It had only recently been established that the chromosomes (visible under the microscope) were the carriers of Gregor Mendel's "factors," or genes. McClintock used information gleaned from characteristics of the corn plant, *Zea mays* (maize), in conjunction with changes in its

chromosomes to elucidate many aspects of genetic control. Her first major contribution was the identification and naming of the maize chromosomes. Shortly after, using cytological markers on the chromosomes, McClintock and graduate student Harriet Creighton demonstrated the correlation between patterns of inheritance and chromosomal crossover—the exchange of material between chromosomes. Breeding experiments focusing on "linkage groups" allowed McClintock to associate each of corn's ten chromosomes with the genes they carry.

At that point, Lewis Stadler, who was studying the mutagenic effects of X rays, sent some irradiated corn to McClintock. McClintock demonstrated that the resultant broken chromosomes can fuse into a ring, and she then hypothesized the existence of the telomere, a protective stabilizing structure at the end of the chromosome. At the University of Missouri, McClintock observed the ability of such broken chromosomes to go through a series of breakages and fusions (the breakage-fusion-bridge cycle) and identified concomitant chromosomal inversions and deletions. She ultimately discovered that certain genes could transfer from cell to cell and between chromosomes, thereby influencing the color patterns in the leaves and kernels of corn.

In 1941, McClintock moved to Cold Spring Harbor, New York, where she would remain. Doing much of her work before the discovery of the double helical structure of DNA, she rejected the simplistic one-way flow from DNA to RNA to protein outlined in the central dogma of molecular biology, seeking instead an explanation for the spatial and temporal variation in gene expression needed to link genetics to developmental and evolutionary change. Her own work showed that both the location and direction of genetic material, as well as the presence of other "controlling elements," had important effects on the expression of the gene.

In addition to this better-known work, McClintock identified the chromosomes of the bread mold *Neurospora* and described its meiotic cycle. She also headed a study aimed at conserving indigenous corn varieties in the Americas. In recognition of her place as one of the most distinguished scientists of the twentieth century, *The Barbara McClintock Papers* are available through the National Library of Medicine through its "Profiles in Science" Web site.



Barbara McClintock. (© The Nobel Foundation)

—Lee Anne Martínez

the patterns of inheritance were not following Mendelian laws. Furthermore, she surmised that insertion and excision of genetic material were responsible for the genetic patterns she observed. McClintock was recognized for this pioneering work with a Nobel Prize in Physiology or Medicine in 1983. It was not until the 1960's that the jumping genes that McClintock postulated were isolated and characterized. The first transposable elements to be well characterized were found in the bacteria *Escherichia coli* but have subsequently been found in the cells of many bacteria, plants, and animals.

Transposable elements are discrete DNA sequences that encode a transposase, an enzyme that catalyzes transposition. Transposition refers to the movement within a genome. The borders of the transposable element are defined by specific DNA sequences; often the sequences at either end of the transposable element are inverted repeats of one another. The transposase enzyme cuts the DNA sequences at the ends of the transposable element to initiate transposition and cuts the DNA at the insertion site. The site for insertion of the transposable element is not specific. Therefore, transposition results in random insertion into chromosomes and often results in mutation and genome rearrangement. In many organisms, transposition accounts for a significant fraction of all mutation. Although the details of the mechanism may vary, there are two basic mechanisms of transposition: conservative and replicative. In conservative transposition, the transposable element is excised from its original site and inserted at another. In replicative transposition, a copy of the transposable element is made and is inserted in a new location. The original transposable element remains at its initial site.

A subset of the replicative transposable elements includes the retrotransposons. These elements transpose through an RNA intermediate. Interestingly, their DNA sequence and organization are similar to those of retroviruses. It is likely that either retroviruses evolved from retrotransposons by gaining the genes to produce the proteins for a viral coat or retrotransposons evolved from retroviruses that lost the genes for a viral coat. This is one of the rea-

sions that transposons are likened to viruses. Viruses can be thought of as transposons that gained the genes for a protein coat and thus the ability to leave one cell and infect others; conversely, transposons can be thought of as intracellular viruses.

Genetic Change and Selfish DNA

Transposition is a significant cause of mutation for many organisms. When McClintock studied the genetic patterns of maize kernel pigmentation, she saw the results of insertion and excision of transposable elements into and out of the pigment genes. Subsequently, it has been well established that mutations in many organisms are the result of insertion of transposable elements into and around genes. Transposition sometimes results in deletion mutations as well. Occasionally the transposase will cut at one end of the transposable element but skip the other end, cutting the DNA further downstream. This can result in a deletion of the DNA between the end of the transposable element and the cut site.

In addition to these direct results, it is believed that transposable elements may be responsible for large-scale rearrangements of chromosomes. Genetic recombination, the exchange of genetic information resulting in new combinations of DNA sequences, depends upon DNA sequence homology. Normally, recombination does not occur between nonhomologous chromosomes or between two parts of the same chromosome. However, transposition can create small regions of homology (the transposable element itself) spread throughout the chromosomes. Recombination occurring between homologous transposable elements can create deletions, inversions, and other large-scale rearrangements of chromosomes.

Scientists often take advantage of transposable elements to construct mutant organisms for study. The random nature of insertion ensures that many different genes can be mutated, the relatively large insertion makes it likely that there will be a complete loss of gene function, and the site of insertion is easy to locate to identify the mutated region.

Biologists often think of natural selection as working at the level of the organism. DNA se-

quences that confer a selective advantage to the organism are increased in number as a result of the increased reproductive success of the organisms that possess those sequences. It has been said that organisms are simply DNA's means of producing more DNA. In 1980, however, W. Ford Doolittle, Carmen Sapienza, Leslie Orgel, and Francis Crick elaborated on another kind of selection that occurs among DNA sequences within a cell. In this selection, DNA sequences are competing with each other to be replicated. DNA sequences that spread by forming additional copies of themselves will increase relative to other DNA sequences. There is selection for discrete DNA sequences to evolve the means to propagate themselves. One of the key points is that this selection does not work at the level of the organism's phenotype. There may be no advantage for the organism to have these DNA sequences. In fact, it may be that there is a slight disadvantage to having many of these DNA sequences. For this reason, DNA sequences that are selected because of their tendency to make additional copies of themselves are referred to as "selfish" DNA. Transposable elements are often cited as examples of selfish DNA.

Composite Transposons and Antibiotic Resistance

Some transposable elements have genes unrelated to the transposition process located between the inverted, repeat DNA sequences that define the ends of the element. These are referred to as composite transposons. Very frequently, bacterial composite transposons contain a gene that encodes resistance to antibiotics. The consequence is that the antibiotic resistance gene is mobilized: It will jump along with the rest of the transposable element to new DNA sites. Composite transposons may be generated when two of the same type of transposable elements end up near each other and flanking an antibiotic resistance gene. If mutations occurred to change the sequences at the "inside ends" of the transposable elements, the transposase would then only recognize and cut at the two "outside end" sequences to cause everything in between to be part of a new composite transposon.

Resistance to antibiotics is a growing public health problem that threatens to undo much of the progress that the antibiotic revolution made against infectious disease. Transposition of composite transposons is part of the problem. Transposition can occur between any two sites within the same cell, including between the chromosome and plasmid DNA. Plasmids are small, circular DNA molecules that replicate independently of the bacterial host chromosome. Resistance plasmids (R plasmids) are created when composite transposons carrying an antibiotic resistance gene insert into a plasmid. What makes this particularly serious is that some plasmids encode fertility factors (genes that promote the transfer of the plasmid from one bacteria to another). This provides a mechanism for rapid and widespread antibiotic resistance whenever antibiotics are used. The great selective pressure exerted by antibiotic use results in the spread of R plasmids throughout the bacterial population. This, in turn, increases the opportunities for composite transposon insertion into R plasmids to create multiple drug-resistant R plasmids. The first report of multiple antibiotic resistance caused by R plasmids was in Japan in 1957 when strains of *Shigella dysenteriae*, which causes dysentery, became resistant to four common antibiotics all at once. Some R plasmids encode resistance for up to eight different antibiotics, which often makes treatment of bacterial infection difficult. Furthermore, some plasmids are able to cause genetic transfer between bacterial species, limiting the usefulness of many antibiotics.

—Craig S. Laufer

See also: Antisense RNA; Archaea; Bacterial Resistance and Super Bacteria; Immunogenetics; Lateral Gene Transfer; Model Organism: *Escherichia coli*; Molecular Genetics; Mutation and Mutagenesis; Plasmids; Repetitive DNA.

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mal form of an oncogene, called a proto-oncogene, is involved in regulating the cell cycle

p53 GENE: a tumor-suppressor gene, implicated in many types of cancer

Discovery of Tumor-Suppressor Genes

The existence of genes that play critical roles in cell cycle regulation by inhibiting cell division was predicted by several lines of evidence. In vitro studies involving the fusion of normal and cancer cell lines were often observed to result in suppression of the malignant phenotype, suggesting that normal cells contained inhibitors that could reprogram the abnormal growth behavior in the cancer cell lines. In addition, studies by Alfred Knudsen on inherited and noninherited forms of retinoblastoma, a childhood cancer associated with tumor formation in the eye, suggested that the inactivation of recessive genes as a consequence of mutation could result in the loss of function of inhibitory gene products critical to cell division control. With the advent of molecular methods of genetic analysis, the gene whose inactivation is responsible for retinoblastoma was identified and designated *Rb*.

Additional tumor-suppressor genes were identified by studies of DNA tumor viruses whose cancer-causing properties were found to result, in part, from the ability of specific viral gene products to inactivate host cell inhibitory gene products involved in cell cycle regulation. By inactivating these host cell proteins, the tumor virus removes the constraints on viral and cellular proliferation. The most important cellular gene product to be identified in this way is the *p53* protein, named after its molecular weight. Genetic studies of human malignancies have implicated mutations in the *p53* gene in up to 75 percent of tumors of diverse tissue origin, including an inherited disorder called Li-Fraumeni syndrome, associated with many types of cancer. In addition, studies of other rare inherited malignancies have led to the identification of many other recessive genes whose inactivation contributes to oncogenic or cancer-causing mechanisms. Included in this list are the *BRCA1* and *BRCA2* genes in breast cancer, the *NF1* gene in neurofibromatosis, the

Tumor-Suppressor Genes

Field of study: Molecular genetics

Significance: *Molecular analysis of tumor-suppressor genes has provided important information on mechanisms of cell cycle regulation and patterns of growth control in normal dividing cells and cancer cells. Tumor-suppressor genes represent cell cycle control genes that inhibit cell division and initiate cell death processes in abnormal cells. Mutations in these genes have been identified in many types of human cancer and play a critical role in the genetic destabilization and loss of growth control characteristic of malignancy.*

Key terms

CELL CYCLE: a highly regulated series of events critical to the initiation of cell division processes

ONCOGENES: a mutated or improperly expressed gene that can cause cancer; the nor-

p16 gene in melanoma, and the *APC* gene in colorectal carcinoma. Each of these genes has also been implicated in nonhereditary cancers.

The Properties of Tumor-Suppressor Genes

Molecular analyses of the genetic and biochemical properties of tumor-suppressor genes have suggested that these gene products play critical but distinct roles in regulating processes involved in cellular proliferation. The *Rb* gene product represents a prototype tumor-suppressor gene that blocks progression of the cell cycle and cell division by binding to transcription factors in its active form. In order for cell division to occur in response to growth factor stimulation, elements of the signal cascade inactivate *Rb*-mediated inhibition by a mechanism involving the addition of phosphate to the molecule, a reaction called phosphorylation. Loss of *Rb* function as a consequence of mutation removes the brakes on this form of inhibitory control; the cell division machinery proceeds regardless of appropriate initiation by growth factors or other stimuli.

The *p53* tumor-suppressor gene product is a DNA-binding protein that regulates the expression of specific genes in response to genetic damage or other abnormal events that may occur during cell cycle progression. In response to *p53* activation, the cell may arrest the process of cell division (by indirectly blocking *Rb* inactivation) to repair genetic damage before proceeding further along the cell cycle; alternatively, if the damage is too great, the *p53* gene product may initiate a process of cell death called apoptosis. The loss of *p53* activity in the cell as a consequence of mutation results in genetic destabilization and the failure of cell death mechanisms to eliminate damaged cells from the body; both events appear to be critical to late-stage oncogenic mechanisms.

Impact and Applications

The discovery of tumor-suppressor genes has revealed the existence of inhibitory mechanisms critical to the regulation of cellular proliferation. Mutations that destroy the functional activities of these gene products cause the loss of growth control characteristic of can-

cer cells. Taken together, research on the patterns of oncogene activation and the loss of tumor-suppressor gene function in many types of human malignancy suggest a general model of oncogenesis. Molecular analyses of many tumors show multiple genetic alterations involving both oncogenes and tumor-suppressor genes, suggesting that oncogenesis (development of cancer) requires unregulated stimulation of cellular proliferation pathways along with a loss of inhibitory activities that operate at cell cycle checkpoints.

With respect to clinical applications, restoration of *p53* tumor-suppressor gene function by gene therapy appears to result in tumor regression in some experimental systems; however, much more work needs to be done in this area to achieve clinical relevance. More important, research on the mechanism of action of standard chemotherapeutic drugs suggests that cytotoxicity may be caused by *p53*-induced cell death; the absence of functional *p53* in many tumors may account for their resistance to chemotherapy. Promising research suggests that it may be possible to elicit cell death in tumor cells lacking functional *p53* gene product in response to chemotherapy. The clinical significance of activating these *p53*-independent cell death mechanisms may be extraordinary.

—Sarah Crawford Martinelli

See also: Aging; Breast Cancer; Cancer; Cell Cycle, The; Cell Division; DNA Repair; Human Genetics; Human Genome Project; Model Organism: *Mus musculus*; Oncogenes.

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American Cancer Society. <http://www.cancer.org>. Site has searchable information on tumor suppressor genes.

Key terms

ESTROGENS: hormones or chemicals that stimulate the development of female sexual characteristics and control the female reproductive cycle

GROWTH HORMONE: a chemical that plays a key role in promoting growth in body size

KARYOTYPE: a laboratory analysis that confirms the diagnosis of Turner syndrome by documenting the absence or abnormality of one of the two X chromosomes normally found in women

SEX CHROMOSOMES: the chromosomes that control sexual determination during the development of males and females; females have two X chromosomes and males have one X and one Y chromosome

SYNDROME: a set of features or symptoms often occurring together and believed to stem from the same cause

Discovery of Turner Syndrome

Henry H. Turner, an eminent clinical endocrinologist, is credited with first describing Turner syndrome. In 1938, he published an article describing seven patients, ranging in age from fifteen to twenty-three years, who exhibited short stature, a lack of sexual development, arms that turned out slightly at the elbows, webbing of the neck, and low posterior hairline. He did not know what caused this condition. In 1959, C. E. Ford discovered that a chromosomal abnormality involving the sex chromosomes caused Turner syndrome. He found that most girls with Turner syndrome did not have all or part of one of their X chromosomes and argued that this missing genetic material accounted for the physical findings associated with the condition.

Turner syndrome begins at conception. The disorder results from an error during meiosis in the production of one of the parents' sex cells, although the exact cause remains unknown. Girls suspected of having Turner syndrome, usually because of their short stature, usually undergo chromosomal analysis. A simple blood test and laboratory analysis called a karyotype are done to document the existence of an abnormality.

Shortness is the most common characteris-

Turner Syndrome

Field of study: Diseases and syndromes

Significance: *Turner syndrome is one of the most common genetic problems in women, affecting 1 out of every 2,000 to 2,500 women born. Short stature, infertility, and incomplete sexual development are the characteristics of this condition.*

tic of Turner syndrome. The incidence of short stature among women with Turner syndrome is virtually 100 percent. Women who have this condition are, on average, 4 feet 8 inches (1.4 meters) tall. The cause of the failure to grow is unclear. However, growth-promoting therapy with growth hormones has become standard. Most women with the syndrome also experience ovarian failure. Since the ovaries normally produce estrogen, women with Turner syndrome lack this essential hormone. This deficit results in infertility and incomplete sexual development. Cardiovascular disorders are the single source of increased mortality in women with this condition. High blood pressure is common.

Other physical features often associated with Turner syndrome include puffy hands and feet at birth, a webbed neck, prominent ears, a small jaw, short fingers, a low hairline at the back of the neck, and soft fingernails that turn up at the ends. Some women with Turner syndrome have a tendency to become overweight. Many women will exhibit only a few of these distinctive features, and some may not show any of them. This condition does not affect general intelligence. Girls with Turner syndrome follow a typical female developmental pattern with unambiguous female gender identification. However, another possible symptom is poor spatial perception abilities. For example, women with this condition may have difficulty driving, recognizing subtle social clues, and solving nonverbal mathematics problems; they may also suffer from clumsiness and attention-deficit disorder.

Treatments and Therapies

No treatment is available to correct the chromosome abnormality that causes this condition. However, injections of human growth hormone can restore most of the growth deficit. Unless they undergo hormone replacement therapy, girls with Turner syndrome will not menstruate or develop breasts and pubic hair. In addition to estrogen replacement therapy, women with Turner syndrome are often advised to take calcium and exercise regularly. Although infertility cannot be altered, pregnancy may be made possible through in vitro

fertilization (fertilizing a woman's egg with sperm outside the body) and embryo transfer (moving the fertilized egg into a woman's uterus). Individuals with Turner syndrome can be healthy, happy, and productive members of society.

Nevertheless, because of its relative rarity, a woman with Turner syndrome may never meet another individual with this condition and may suffer from self-consciousness, embarrassment, and poor self-esteem. The attitudes of parents, siblings, and relatives are important in helping develop a strong sense of identity and self-worth. The Turner Syndrome Society of the United States is a key source of information and support groups. Advances in chromosomal analysis have proved helpful in the diagnosis and management of Turner syndrome. In addition, new developments in hormonal therapy for short stature and ovarian failure, combined with advances in in vitro fertilization, have significantly improved the potential for growth, sexual development, and fertility for afflicted individuals.

—Fred Buchstein

See also: Amniocentesis and Chorionic Villus Sampling; Dwarfism; Hereditary Diseases; Infertility; Klinefelter Syndrome; Mutation and Mutagenesis; Nondisjunction and Aneuploidy; X Chromosome Inactivation; XYY Syndrome.

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Web Sites of Interest

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. A guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

National Institute of Child Health and Human Development. <http://turners.nichd.nih.gov>. Site provides information on the genetic and clinical features of the syndrome.

The Turner Syndrome Society of the United States. <http://www.turner-syndrome-us.org>. The main national support organization, offering resources and information.

Twin Studies

Field of study: Techniques and methodologies

Significance: *Studies of twins are widely considered to be the best way to determine the relative contributions of genetic and environmental factors to the development of human physical and psychological characteristics.*

Key terms

DIZYGOTIC: developed from two separate zygotes; fraternal twins are dizygotic because they develop from two separate fertilized ova (eggs)

MONOZYGOTIC: developed from a single zygote; identical twins are monozygotic because they develop from a single fertilized ovum that splits in two

ZYGOSITY: the degree to which two individuals are genetically similar

ZYGOTE: a cell formed from the union of a sperm and an ovum

The Origin of Twin Studies

Sir Francis Galton, an early pioneer in the science of genetics and a founder of the theory of eugenics, conducted some of the earliest systematic studies of human twins in the 1870's. Galton recognized the difficulty of identifying the extent to which human traits are biologically inherited and the extent to which traits are produced by diet, upbringing, education, and other environmental influences. Borrowing a phrase from William Shakespeare, Galton called this the "nature vs. nurture" problem. Galton reasoned that he could attempt to find an answer to this problem by comparing similarities among people who obviously shared a great deal of biological inheritance, with similarities among people sharing less biological inheritance. Twins offered the clearest example of people who shared common biological backgrounds.

Galton contacted all of the twins he knew and asked them to supply him with the names of other twins. He obtained information on ninety-four sets of twins. Of these, thirty-five sets were very similar, people who would today

be called identical twins. These thirty-five pairs reported that people often had difficulty telling them apart. Using questionnaires and interviews, Galton compared the thirty-five identical pairs with the other twins. He found that the identical twins were much more similar to one another in habits, interests, and personalities, as well as in appearance. They were even much more alike in physical health and susceptibility to illness. The one area in which all individuals seemed to differ markedly was in handwriting.

Modern Twin Studies

Since Galton's time, researchers have discovered how biological inheritance occurs, and this has made possible an understanding of why twins are similar. It has also enabled researchers to make more sophisticated use of twins in studies that address various aspects of the nature vs. nurture problem. Parents pass

their physical traits to their children by means of genes in chromosomes. Each chromosome carries two genes (called alleles) for every hereditary trait. One allele comes from the father and one comes from the mother. Any set of full brothers and sisters will share many of the same alleles, since all of their genes come from the same parents. However, brothers and sisters usually also differ substantially; each zygote (ovum, or egg, fertilized by a sperm cell) will combine alleles from the father and the mother in a unique manner, so different zygotes will develop into unique individuals. Even when two fertilized eggs are present at the same time, as in the case of dizygotic or fraternal twins, the two will have different combinations of genes from the mother and the father.

Identical twins are an exception to the rule of unique combinations of genes. Identical twins develop from a single zygote, a cell created by one union of egg and sperm. There-

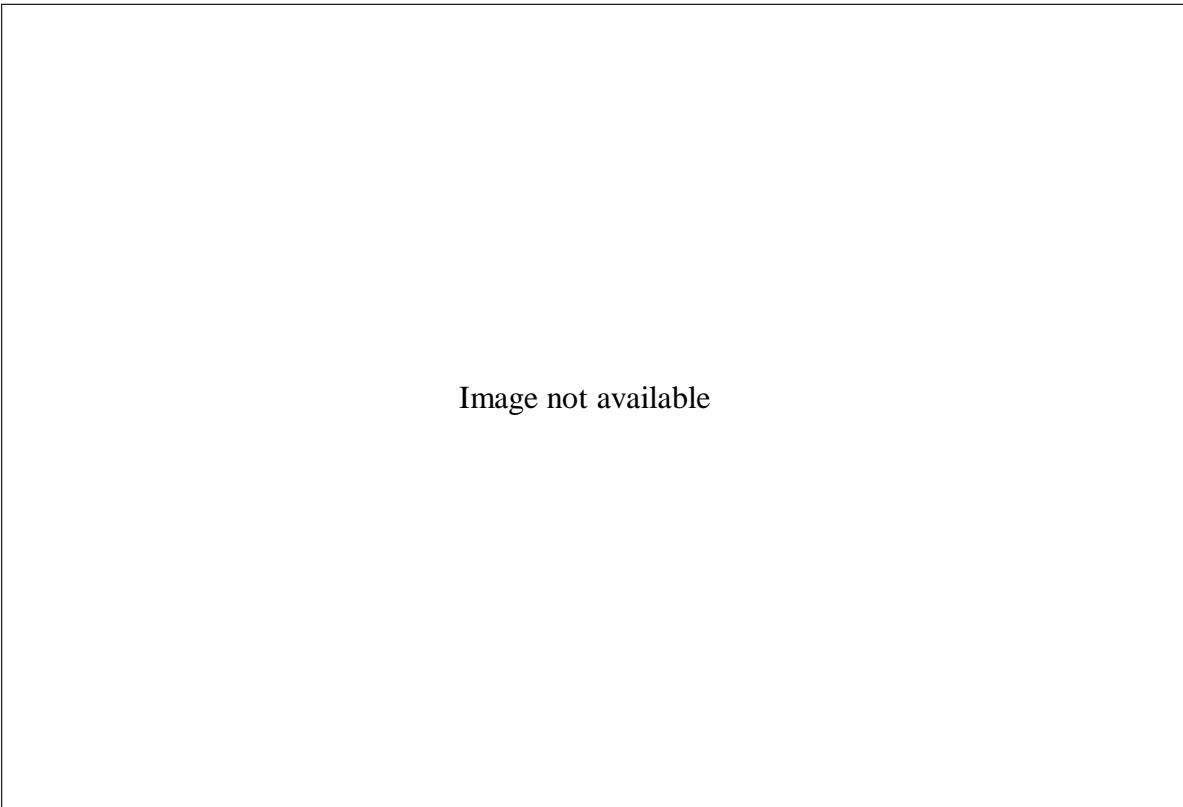


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In the background, the co-director of the Twins Reared Apart project, Nancy Segal, with twins Mark Newman and Gerald Levey, separated at birth. (AP/Wide World Photos)

fore, monozygotic twins (from one zygote) will normally have the same genetic makeup. Differences between genetic twins, researchers argue, must therefore be produced by environmental factors following birth.

The ideal way to conduct twin studies is to compare monozygotic twins who have been reared apart from each other in vastly different types of families or environments. This is rarely possible, however, because the number of twins separated at birth and adopted is relatively small. For this reason, researchers in most twin studies use fraternal twins as a comparison group, since the major difference between monozygotic and dizygotic twins is that the former are genetically identical. Statistical similarities among monozygotic twins that are not found among dizygotic twins are therefore believed to be caused by genetic inheritance.

Researchers use several types of data on twins to estimate the extent to which human characteristics are the consequence of genetics. One of the main sources for twin studies is the Minnesota Twin Registry. In the 1990's, this registry consisted of about 10,500 twins in Minnesota. They were found in Minnesota birth records from the years 1936 through 1955, and they were located and recruited by mail between 1985 and 1990. A second major source of twin studies is the Virginia Twin Registry. This is a register of twins constructed from a systematic review of public birth records in the Commonwealth of Virginia. A few other states also maintain records of twins. Some other organizations, such as the American Association of Retired Persons (AARP), keep records of twins who volunteer to participate and make these records available to researchers.

Zygosity, or degree of genetic similarity between twins, is usually measured by survey questions about physical similarity and by how often other people mistake one twin for the other. In some cases, zygosity may be determined more rigorously through analysis of DNA samples.

Problems with Twin Studies

Although twin studies are one of the best available means for studying genetic influences in human beings, there are a number of problems with this approach. Although twin studies

assume that monozygotic twins are biologically identical, some critics have claimed that there are reasons to question this assumption. Even though these twins tend to show greater uniformity than other people, developmental differences may emerge even in the womb after the splitting of the zygote.

Twins who show a great physical similarity may also be subject to environmental similarities so that traits believed to be caused by genetics may, in fact, be a result of upbringing. Some parents, for example, dress twins in matching clothing. Even when twins grow up in separate homes without being in contact with each other, their appearances and mannerisms may evoke the same kinds of responses from others. Physical attractiveness, height, and other characteristics often affect how individuals are treated by others so that the biologically based resemblances of twins can lead to common experiences.

Finally, critics of twin studies point out that twins constitute a special group of people and that it may be difficult to apply findings from twin studies to the population at large. Some studies have indicated that intelligence quotient (IQ) scores of twins, on average, are about five points below IQ scores in the general population, and twins may differ from the general population in other respects. It is conceivable that genetics plays a more prominent role in twins than in most other people.

Impact and Applications

Twin studies have provided evidence that a substantial amount of human character and behavior may be genetically determined. In 1976, psychologists John C. Loehlin and Robert C. Nichols published their analyses of the backgrounds and performances of 850 sets of twins who took the 1962 National Merit Scholarship test. Results showed that identical twins showed greater similarities than fraternal twins in abilities, personalities, opinions, and ambitions. A careful examination of backgrounds indicated that these similarities could not be explained by the similar treatment received by identical twins during upbringing.

Later twin studies continued to provide evidence that genes shape many areas of human

life. Monozygotic twins tend to resemble each other in probabilities of developing mental illnesses, such as schizophrenia and depression, suggesting that these psychological problems are partly genetic in origin. A 1996 study published in the *Journal of Personality and Social Psychology* used a sample from the Minnesota Twin Registry to establish that identical twins are similar in probabilities of divorce. A 1997 study in the *American Journal of Psychiatry* indicated that there is even a great resemblance between twins in intensity of religious faith. Twin studies have offered evidence that homosexual or heterosexual orientation may be partly a genetic matter, although researcher Scott L. Hershberger has found that the genetic inheritance of sexual orientation may be greater among women than among men.

—Carl L. Bankston III

See also: Aging; Animal Cloning; Behavior; Cloning: Ethical Issues; Diabetes; DNA Fingerprinting; Gender Identity; Genetic Testing; Genetics in Television and Films; Heredity and Environment; Homosexuality; Intelligence; Prenatal Diagnosis; Quantitative Inheritance.

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Web Site of Interest

- Minnesota Twin Family Study. <http://www.psych.umn.edu/psylabs/mtfs>. Site of an ongoing research study into the genetic and environmental factors of psychological development. Includes the discussion, "What's Special About Twins to Science?"

Viral Genetics

Field of study: Viral genetics

Significance: *The composition and structures of virus genomes are more varied than any identified in the entire bacterial, botanical, or animal kingdoms. Unlike the genomes of all other cells, which are composed of DNA, virus genomes may contain their genetic information encoded in either DNA or RNA. Viruses cannot replicate on their own but must instead use the reproductive machinery of host cells to reproduce themselves.*

Key terms

CAPSID: the protective protein coating of a virus particle

RIBOSOME: a cytoplasmic organelle that serves as the site for amino acid incorporation during the synthesis of protein

VIRIONS: mature infectious virus particles

What Is a Virus?

Viruses are submicroscopic, obligate intracellular parasites. This definition differentiates viruses from all other groups of living organisms. There exists more biological diversity within viruses than in all other known life-forms combined. This is the result of viruses successfully parasitizing all known groups of living organisms. Viruses have evolved in parallel with other species by capturing and using genes from infected host cells for functions that they require to produce their progeny, to enhance their escape from their host's cells and immune system, and to survive the intracellular and extracellular environment. At the molecular level, the composition and structures of virus genomes are more varied than any others identified in the entire bacterial, botanical, or animal kingdoms. Unlike the genomes of all other cells composed of DNA, virus genomes may contain their genetic information encoded in either DNA or RNA. The nucleic acid comprising a virus genome may be single-stranded or double-stranded and may occur in a linear, circular, or segmented configuration.

The Need for a Host

It must be understood that virus particles

themselves do not grow or undergo division. Virus particles are produced from the assembling of pre-formed components, whereas other agents actually grow from an increase in the integrated sum of their components and reproduce by division. The reason is that viruses lack the genetic information that encodes the apparatus necessary for the generation of metabolic energy or for protein synthesis (ribosomes). The most critical interaction between a virus and a host cell is the need of the virus for the host's cellular apparatus for nucleic acid and for the synthesis of proteins. No known virus has the biochemical or genetic potential to generate the energy necessary for producing all biological processes. Viruses depend totally on a host cell for this function.

Viruses are therefore not living in the traditional sense, but they nevertheless function as living things; they do replicate their own genes. Inside a host cell, viruses are "alive," whereas outside the host they are merely a complex assemblage of metabolically inert chemicals—basically a protein shell. Therefore, while viruses have no inner metabolism and cannot reproduce on their own, they carry with them the means necessary to get into other cells and then use those cells' own reproductive machinery to make copies of themselves. Viruses thrive at the host cell's expense.

Replication

The sole goal of a virus is to replicate its genetic information. The type of host cell infected by a virus has a direct effect on the process of replication. For viruses of prokaryotes (bacteria, primarily), reproduction reflects the physical simplicity of the host cell. For viruses with eukaryotic host cells (plants and animals), reproduction is more complex. The coding capacity of the genome forces the virus to choose a reproductive strategy. The strategy might involve near-total reliance on the host cell, resulting in a compact genome encoded for only a few essential proteins (+), or could involve a large, complex virus genome encoded with nearly all the information necessary for replication, relying on the host cell only for energy

and ribosomes. Those viruses with an RNA genome plus messenger RNAs (mRNAs) have no need to enter the nucleus of their host cell, although during replication many often do. DNA genome viruses mostly replicate in the host cell's nucleus, where host DNA is replicated and the biochemical apparatus required for this process is located. Some DNA viruses (poxviruses) have evolved to contain the biochemical capacity to replicate in their host's cytoplasm, with a minimal need for the host cell's other functions.

Virus replication involves several stages carried out by all types of viruses, including the onset of infection, replication, and release of mature virions from an infected host cell. The stages can be defined in eight basic steps: attachment, penetration, uncoating, replication, gene expression, assembly, maturation, and release.

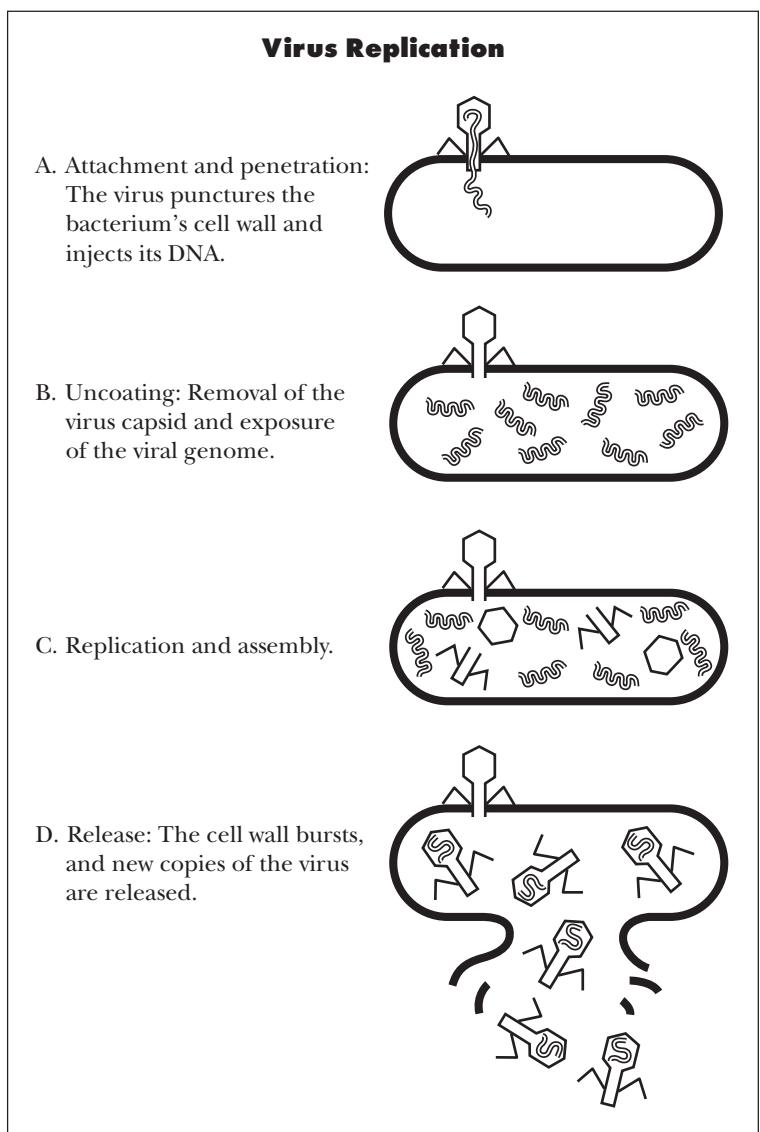
The first stage, attachment, occurs when a virus interacts with a host cell and attaches itself—binds with a virus-attachment protein (anitreceptor)—to a cellular receptor molecule in the cell membrane. The receptor may be a protein or a carbohydrate residue. Some complex viruses, such as herpesviruses, use more than one receptor and therefore have alternate routes of cellular invasion.

Shortly after attachment the target cell is penetrated. Cell penetration is usually an energy-dependent process, and the cell must be metabolically active for penetration to occur. The virus bound to the cellular receptor molecule is translocated across the cell membrane by the receptor and is engulfed by the cell's cytoplasm.

Uncoating occurs after penetration and results in the com-

plete or partial removal of the virus capsid and the exposure of the virus genome as a nucleo-protein complex. This protein complex can be a simple RNA genome or can be highly complex, as in the case of a retrovirus containing a diploid RNA genome responsible for converting a virus RNA genome into a DNA provirus.

How a virus replicates and the resulting expression of its genes depends on the nature of its genetic materials. Control of gene expression is a vital element of virus replication. Viruses use the biochemical apparatus of their



(Electronic Illustrators Group)

infected host cells to express their genetic information as proteins and do this by using the appropriate biochemical language recognized by the host cell. Viruses include double-stranded DNA viruses such as papoviruses, poxviruses, and herpesviruses; single-stranded sense DNA viruses such as parvoviruses; double-stranded RNA reoviruses; single-stranded sense RNA viruses such as flaviviruses, togaviruses, and caliciviruses; single-stranded antisense RNA such as filoviruses and bunyaviruses; single-stranded sense RNA with DNA intermediate retroviruses; and double-stranded DNA with RNA intermediate-like hepadnaviruses.

During assembly, the basic structure of the virus particle is formed. Virus proteins anchor themselves to the cellular membrane, and, as virus proteins and genome molecules reach a critical concentration, assembly begins. The result is that a genome is stuffed into a completed protein shell. The process of maturation prepares the virus particle for infecting subsequent cells and usually involves the cleavage of proteins to form matured products or conformational structural changes.

For most viruses, release is a simple matter of breaking open the infected cell and exiting. The breakage normally occurs through a physical interaction of proteins against the inner surface of the host cell membrane. A virus may also exit a cell by budding. Budding involves the creation of a lipoprotein envelope around the virion prior to the virion's being extruded out through the cell membrane.

—Randall L. Milstein

See also: DNA Structure and Function; Gene Regulation: Viruses; Genetic Engineering; Hybridomas and Monoclonal Antibodies; Oncogenes; Organ Transplants and HLA Genes; RNA Structure and Function; Viroids and Virusoids.

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Viroids and Virusoids

Field of study: Viral genetics

Significance: *Viroids are naked strands of RNA, 270 to 380 nucleotides long, that are circular and do not code for any proteins. However, some viroids are catalytic RNAs (ribozymes), able to cleave and ligate themselves. In spite of their simplicity, they are able to cause disease in susceptible plants, many of them economically important. Virusoids are similar to viroids, except that they require a helper virus to infect a plant and reproduce.*

Key terms

RNA POLYMERASE: an enzyme that catalyzes the joining of ribonucleotides to make RNA using DNA or another RNA strand as a template

RNASE: an enzyme that catalyzes the cutting of an RNA molecule

General Characteristics of Viroids and Virusoids

Viroids, and some virusoids, are circular, single-stranded RNA molecules, which normally appear as rods but when denatured by heating appear as closed circles. The rod-shaped structure is formed by extensive base pairing within the RNA molecule, and the secondary structure is divided into five structural domains. One domain is called the pathogenicity (P) domain, because differences among variant strains of the same species of viroid seem to correlate with differences in pathogenicity. Virusoids may also comprise linear RNA or, rarely, double-stranded circular RNA.

The difference between viroids and virusoids is in their mode of transmission. Viroids have no protective covering of any kind and are no more than the RNA that makes up their genetic material. They depend on breaks in a plant's epidermis or can travel with pollen or ovules to gain entry. Virusoids, also known as satellite RNAs, are packaged in the protein coat of other plant viruses, referred to as helpers, and are therefore dependent on the other virus.

Viroids are typically divided into two groups based on the nature of their RNA molecule. Group A is the smallest group, and their RNA has the ability to self-cleave. These include the avocado sunblotch and peach latent mosaic viroids. Group B contains all the other viroids, and their RNA is not capable of self-cleavage. Species in group B include the potato spindle tuber, coconut cadang, tomato plant macho, and citrus bent leaf viroids.

Virusoids are less well studied than viroids and, although more diverse, are most similar to group B viroids in that they cannot self-cleave. Examples include the tomato black ring virus viroid, the peanut stunt virus viroid, and the tobacco ringspot virus viroid. Because so little is known about virusoids, the remainder of this article will focus on viroids.

Viroid Pathogenesis

If infected leaves are homogenized in a blender and passed through an "ultrafilter" fine enough to exclude bacteria, the infection is easily transmitted to another plant by painting some of the filtrate on a leaf. Even billion-

fold dilutions of the filtrate retain the ability to cause infection, suggesting that it is being replicated. RNase destroys infectivity, suggesting that the genetic material (RNA) is exposed to the medium, unlike viruses, which have a protective protein coat. When isolated from other cell components, an absorbance spectrum shows that viroids are pure nucleic acid, lacking a protein coat.

Although viroids are structurally simple and do not code for any proteins, they still cause disease. Although the molecular mechanisms of viroid pathogenesis are unknown, it is clear that the pathogenesis domain (P domain) is primarily responsible.

Changes in the sequence of nucleotides in the P domain have been correlated with pathogenicity. Some research suggests that the pathogenicity of a viroid strain is related to the resistance of the P domain to heat denaturation, with stability of this region being inversely related to severity. However, some evidence suggests that this may not be entirely true. In a series of nucleotide substitutions introduced by researchers into the P region of an intermediate strain (that is, intermediate in pathogenicity) of potato spindle tuber viroid (PSTVd), four showed viroid infectivity and pathogenicity that were the same as those of a previously reported severe strain of PSTVd. Altogether, eight different mutant strains were analyzed, and resistance to denaturation and PSTVd pathogenicity were not correlated in all cases.

Research is under way to understand how viroids move from cell to cell and traverse the cytoplasm to the nucleus, where many viroids replicate. There is evidence that a possible interaction might involve viroid RNA activating an RNA-activated protein kinase in response to a nucleotide sequence similar to that of the normal RNA activator. Protein kinases are integral to intracellular signaling pathways that control many aspects of cell metabolism. Once researchers understand the signals that viroids use to get around, it may be possible to devise treatments against them. A better understanding of the process may also shed light on normal biochemical communication pathways in plant cells.

Viroid Replication

Viroids replicate by a rolling circle mechanism, a method also used by some viruses. The original strand is referred to as the “+ strand,” and complementary copies of it are called “– strands.” Type A and B viroids replicate slightly differently. In type A viroids, the circular + strand is replicated by RNA-dependent RNA polymerase to form several linear copies of the RNA – strand connected end to end. Site-specific self-cleavage produces individual – strands later circularized by a host RNA ligase. Each – strand is finally copied by the RNA polymerase to make several linear copies of + strand RNA. Cleavage of this last strand makes individual RNA + strands, which are then circularized. Self-cleavage in viroids represents one of the cases in which RNA acts as an enzyme. The RNA forms a “hammerhead” structure that enzymatically cleaves the longer RNAs at just the right sites.

Replication of type B viroids is apparently mediated by normal host DNA-dependent RNA polymerase, which mistakes the viroid RNA for DNA. The overall process is similar to what happens with type A viroids, except that the – strand is not cleaved but instead is copied directly, yielding a + strand that is cleaved by host RNase to form individual copies that are ligated to become circular.

Economic Impact of Viroids

Genetically engineered plants in the future might make proteins that would essentially confer immunity by preventing viroids from entering the nucleus. With no access to the nucleus, a viroid would be incapable of replicating, effectively preventing the damage normally associated with viroid infection. Currently, no such transgenic plants exist, and viroids can reduce agricultural productivity if outbreaks are not checked quickly. The typical treatment is simply to destroy the affected plants, as there is no cure.

Although predominantly negative, viroids may have some potentially positive benefits. They have already been used in unique ways to study plant genetics, and they may provide insights into how plant proteins and nucleic acids move in and out of cell nuclei. It may also be

possible to harness the benefits of viroid infection for certain agricultural applications, such as dwarfing citrus trees. Considerably more will need to be learned about viroids before they can be adequately controlled or used for human benefit.

—Bryan Ness

See also: DNA Structure and Function; Gene Regulation: Viruses; Genetic Engineering; Hybridomas and Monoclonal Antibodies; Oncogenes; Organ Transplants and HLA Genes; RNA Structure and Function; Viral Genetics.

Further Reading

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Hammond, R. W. “Analysis of the Virulence Modulating Region of Potato Spindle Tuber Viroid (PSTVd) by Site-Directed Mutagenesis.” *Virology* 187 (1992): 654-662. Report of experimental results on the potato spindle tuber viroid.

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Owens, R. A., W. Chen, Y. Hu, and Y-H. Hsu. “Suppression of Potato Spindle Tuber Viroid Replication and Symptom Expression by Mutations, Which Stabilize the Pathogenicity Domain.” *Virology* 208, no. 2 (1995): 554-564. Aimed at researchers.

Wassenegger, M., S. Heimes, L. Reidel, and H. L. Sanger. “RNA-Directed *De Novo* Methylation of Genomic Sequences in Plants.” *Cell* 76 (1994): 567-576. Report on an experiment involving viroids that demonstrated the possibility that a mechanism of de novo methylation of genes might exist that can be targeted in a sequence-specific manner by their own mRNA.

X Chromosome Inactivation

Field of study: Developmental genetics
Significance: *Normal females have two X chromosomes, and normal males have one X chromosome. In order to compensate for the potential problem of doubling of gene products in females, one X chromosome is randomly inactivated in each cell.*

Key terms

BARR BODY: a highly condensed and inactivated X chromosome visible in female cells as a darkly staining spot in a prepared microscope slide

DOSAGE COMPENSATION: an equalization of gene products that can occur whenever there are more or fewer genes for specific traits than normal

MOSAIC: an individual possessing cells with more than one type of genetic constitution

SEX CHROMOSOMES: the X and Y chromosomes; females possess two X chromosomes, while males possess one X and one Y chromosome

The History of X Chromosome

Inactivation

In 1961, Mary Lyon hypothesized that gene products were found in equal amounts in males and females because one of the X chromosomes in females became inactivated early in development. This hypothesis became known as the Lyon hypothesis, and the process became known as Lyonization, or X chromosome inactivation. Prior to this explanation, it was recognized that females had two X chromosomes and males had only one X chromosome, yet the proteins encoded by genes on the X chromosomes were found in equal amounts in females and males because of dosage compensation.

The principles of inheritance dictate that individuals receive half of their chromosomes from their fathers and the other half from their mothers at conception. Therefore, a female possesses two different X chromosomes (one from each parent). In addition to hypothesizing the inactivation of one X chromosome in each cell, the Lyon hypothesis also implies that the event occurs randomly. In any individual,

approximately one-half of the paternal X chromosomes and one-half of the maternal X chromosomes are inactivated. Thus, females display a mosaic condition since half of their cells express the X chromosome genes inherited from the father and half of their cells express the X chromosome genes inherited from the mother. In fact, this situation can be seen in individuals who inherit an allele for a different form of a protein from each parent: Some cells express one parent's protein form, while other cells express the other parent's protein form.

Prior to Lyon's hypothesis, it was known that a densely staining material could be seen in cells from females that was absent in cells from males. This material was termed a "Barr body," after Murray Barr. Later, it was shown that Barr bodies were synonymous with the inactivated X chromosome. Other observations led scientists to understand that the number of Barr bodies in a cell always represented one less than the number of X chromosomes in the cell. For example, one Barr body indicated the presence of two X chromosomes, and two Barr bodies indicated the presence of three X chromosomes.

Clinical Significance

The significance of Barr bodies became apparent with the observation that females lacking one Barr body or possessing more than one Barr body developed an abnormal appearance. Particularly intriguing were females with Turner syndrome. These females possess only one X chromosome per cell, a condition that is not analogous to normal females, who possess only one functional X chromosome per cell as a result of inactivation. The difference in the development of a Turner syndrome female and a normal female lies in the fact that both X chromosomes are active in normal females during the first few days of development. After this period, inactivation occurs randomly in each cell, as hypothesized by Lyon. In cases in which inactivation is not random, individuals may have a variety of developmental problems. Therefore, there is apparently a critical need for both X chromosomes to be active in females in early development for normal development to occur.

It is equally important that there not be more than two X chromosomes present during this early development. Females possessing three X chromosomes, and therefore two Barr bodies, are sometimes called superfemales or metafemales because of a tendency to be taller than average. These females are also two to ten times more likely to suffer from mild to moderate mental retardation.

The same phenomenon has been observed in males who possess Barr bodies. Barr bodies are not normally present in males because they have only one X chromosome. The presence of Barr bodies indicates the existence of an extra X chromosome that has become inactive. Just as in females, extra X chromosomes are also expressed in early development, and abnormal amounts of gene products result in abnormal physical characteristics and mental retardation. Males with Klinefelter syndrome have two X chromosomes and a Y chromosome. In cases in which males have more than two X chromosomes, the effects are even more remarkable.

Mechanism of X Inactivation

While it has been apparent since the 1960's that X inactivation is required for normal female development, the mechanism has been elusive. Only with the development of techniques to study the molecular events of the cell and its chromosomes has progress been made in understanding the process of inactivation. One process involved in turning off a gene (thus "shutting down" the process of transcription) is the alteration of one of the molecules of DNA known as cytosine. By adding a methyl group to the cytosine, the gene cannot produce the RNA necessary to make a protein. It is thought that this methyl group blocks the proteins that normally bind to the DNA so that transcription cannot occur. When methyl groups are removed from cytosines, the block is removed and transcription begins. This is a common means of regulating transcription of genes. Methylation is significantly higher in the inactivated X chromosome than in the activated X chromosome. As the genes on the chromosome become inactive, the chromosome condenses into the tightly packed mass

known as the Barr body. However, the process of methylation alone cannot entirely account for inactivation.

A region on the X chromosome called the X inactivation center (XIC) is considered the control center for X inactivation. In this region is a gene called the X inactivation specific transcripts (*XIST*) gene. At the time of its discovery, this gene was the only gene known to be functional in an inactivated chromosome. It produces an RNA that remains inside the nucleus.

Evidence in humans supports the hypothesis that the *XIST* gene is turned on and begins to make its RNA when the egg is fertilized. Studies with mice have shown that RNA is produced, at first, in low levels and from both X chromosomes. It has been shown in mice, but not humans, that prior to inactivation, *Xist* (lower-cased when referring to mouse genes) RNA is localized at the XIC site only, thus suggesting a potential role prior to actual inactivation of the chromosome. At this point, one X chromosome will begin to increase its production of *XIST* RNA; shortly thereafter, *XIST* RNA transcription from the other X chromosome ceases. It is not clear how *XIST* RNA initiates the process of inactivation and condensing of the inactive chromosome, but *XIST* RNA binds along the entire length of the inactive X chromosome in females. These results suggest that inactivation spreads from the XIC region toward the end of the chromosome and that *XIST* RNA is required to maintain an inactive state. If a mouse's *Xist* gene is mutated and cannot produce its RNA, inactivation of that X chromosome is blocked. Other studies have suggested that a product from a nonsex chromosome may interact with the XIC region, causing it to remain active. As expected, but not explained, the *XIST* gene is repressed, or expresses *XIST* RNA at only very low levels, in males with only one X chromosome.

No difference has been detected between maternally and paternally expressed *XIST* genes in humans. This has led scientists to suspect that *XIST* gene RNA may not be responsible for determining which X chromosome becomes inactivated. It is also not clear how the cell knows how many X chromosomes are pres-

ent. The search for other candidates for these roles is under way. Finally, there are a few genes besides the *XIST* gene that are also active on the inactive X chromosome. How they escape the inactivation process and why this is necessary are also questions that must be resolved.

—Linda R. Adkison

See also: Fragile X Syndrome; Gender Identity; Hermaphrodites; Infertility; Klinefelter Syndrome; Metafemales; Pseudohermaphrodites; Testicular Feminization Syndrome; Turner Syndrome; XYY Syndrome.

Further Reading

- Erbe, Richard W. "Single-Active-X Principle." *Scientific American Medicine* 2, section 9:IV (1995). Reviews the significance of gene dosage compensation in humans.
- Latham, Keith E. "X Chromosome Imprinting and Inactivation in the Early Mammalian Embryo." *Trends in Genetics*, April, 1996. Discussion of observations on embryos with sex chromosomes from only one parent.
- "X in a Cage." *Discover* 15 (March, 1994). Summarizes a mechanism for X chromosome inactivation.

Web Sites of Interest

Intersex Society of North America. <http://www.isna.org>. The society is "a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or physiology which differs from cultural ideals of male and female)." Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on "patient resources."

Xenotransplants

Field of study: Genetic engineering and biotechnology

Significance: *Xenotransplants are transplants of organs or cellular tissue between different species of animals, such as between pigs and humans. Although initial research in xenotransplantation focused primarily on transplanting organs such as hearts and kidneys, molecular biologists have also become interested in transplanting small amounts of cellular tissue or genetic material as part of therapeutic treatments. In addition, molecular biologists believe it may be possible to manipulate the DNA of animals in such a way to make their organs less prone to rejection if used in humans.*

Key terms

ANTI-REJECTION MEDICATION: drugs developed to counteract the natural immune system's reaction to transplanted organs

REJECTION: refusal of a patient's body to accept a transplanted organ

History

The idea of xenotransplants is actually quite old. During the eighteenth century, for example, transfusions of sheep's blood were believed to be therapeutic for certain human illnesses. Scientists also speculated about using animal organs to replace failing human ones. Until the mid-twentieth century, however, the human body's immune system prevented any successful xenotransplants from taking place. As the science of organ transplants between humans progressed, researchers became increasingly interested in experimenting with using animals as donors. Organ transplantation became an accepted medical treatment in humans, but there would never be enough donor organs available to treat every patient who could benefit from the procedure: As the demand for transplant surgery grew, the pool of available donor organs shrank in relation. As a result, one of the ethical dilemmas inherent in human organ transplant is that, almost always, in order for one person to receive a transplant, another person must die. Bone marrow and kidney transplants are among the few exceptions.

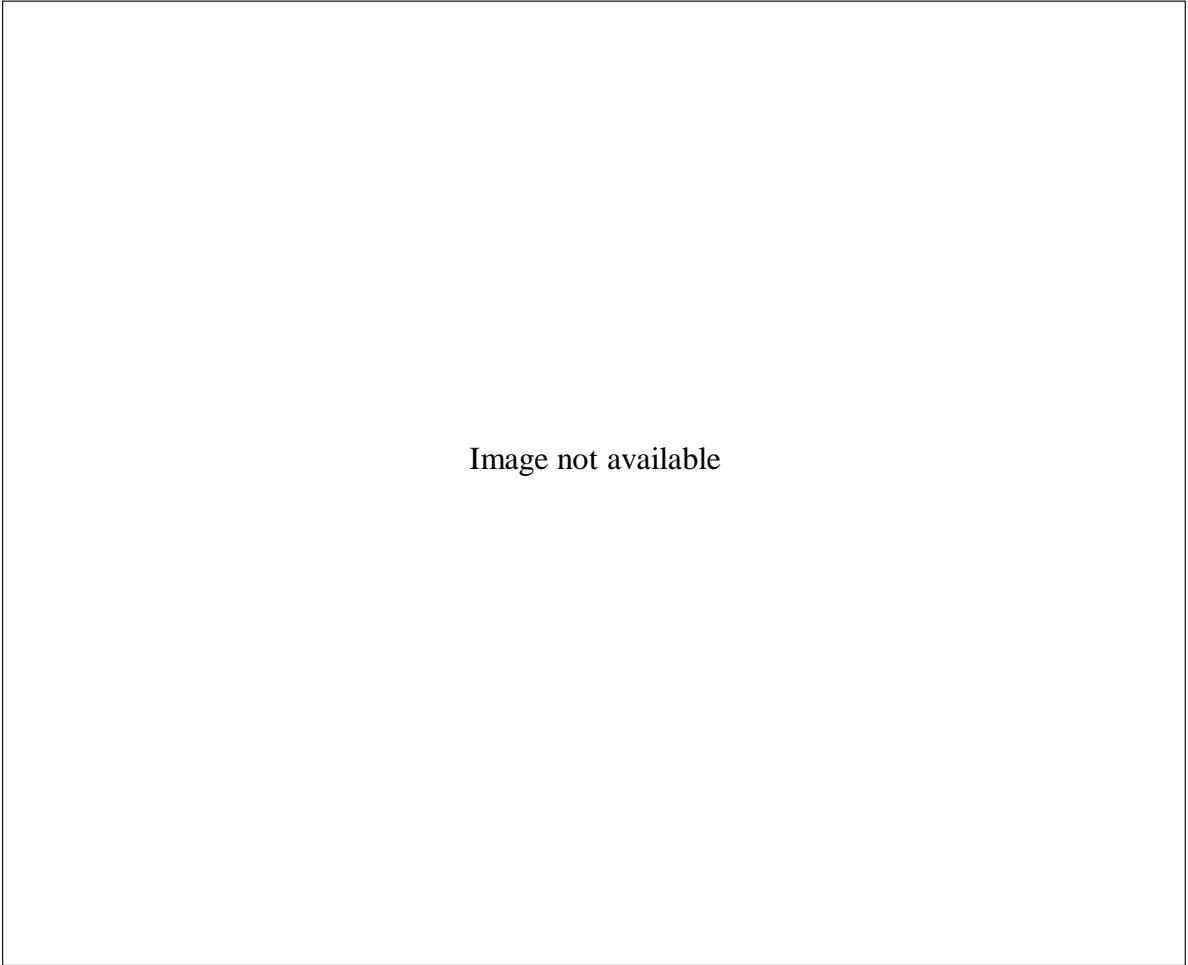
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A human ear grows on the body of a mouse, engineered in Shanghai, China, in 1997. Human cells were used to grow the ear and then were inserted into the mouse body. (AP/Wide World Photos)

tions; the donor usually can donate bone marrow or one kidney and still survive. Nevertheless, for most organs the dilemma remains.

Researchers then suggested that organs could be harvested from compatible animals, eliminating both the need to wait for a compatible human donor and the shortage of usable organs. This is one form of xenotransplant, and it could eliminate the shortage of donor organs.

Early research focused on potential donor animals that were similar to humans, that is, primates such as baboons and chimpanzees. Perhaps the most publicized example of such a xenotransplant was the 1984 Baby Fay case, in which doctors in California transplanted a ba-

boon heart into a newborn human infant who otherwise had no chance for survival. The infant survived for several days before succumbing to complications. Researchers quickly learned that primates were not ideal candidates for organ donation; their organs were small in comparison to those of humans. Chimpanzee kidneys, for example, are too small to perform adequately in an adult human.

Researchers then turned their attention to pigs as possible donors. Swine make ideal donor candidates because they are physically large enough to have organs that can sustain humans, have a short gestation cycle, produce large litters of offspring, and, because they are routinely raised for meat production, are

viewed as expendable by the general public. By using a combination of selective breeding and genetic manipulation, researchers hope to develop swine whose organs will be less susceptible to rejection by the human body. Pig heart valves are already routinely used in humans, with an estimated sixty thousand implanted annually. The use of larger organs has been less successful; transplant recipients experienced hyper-acute rejection. That is, their bodies immediately reacted to the foreign tissue by shutting off the flow of blood to it.

Ethical and Medical Concerns

Xenotransplantation presents a number of ethical and medical dilemmas. One major concern is the possibility that a virus, harmless to the donor animal, is transmitted to the human host and then proves fatal. Scientists worry that a potentially deadly disease epidemic could re-

sult from using organs or other tissue from either swine or primates. While many researchers are confident that careful screening of donor animals would eliminate or minimize such risks, critics remain convinced that it is possible a virus could lie dormant and undetected in animals, causing problems only after the transplants occurred. Baboons, for example, carry a virus that has the potential to cause cancer in humans.

In addition to the medical issues raised, many bioethicists question the morality of using animals as a source of "spare parts" for humans. They are particularly troubled by the idea of possibly genetically altering a species such as swine in order to make their organs more compatible with human hosts. Proponents of xenotransplants counter these arguments by noting that humans have selectively bred animals for various purposes for thousands of years to eliminate certain characteristics while enhancing others. In addition, animals such as swine are already routinely slaughtered for human consumption.

Finally, there is the problem of human perceptions. While many people support the idea of xenotransplants on the genetic or cellular level, they are less enthusiastic about possible organ transplants. That is, while a majority of people surveyed said they would have no problem accepting a xenotransplant if it were part of gene therapy, far fewer were interested in possibly receiving a pig's heart if the need arose. If researchers do achieve successful xenotransplants using such organs, however, public perceptions could change. It is easy to question a medical procedure when it is still theoretical; it becomes much more difficult to do so after it becomes a reality.

—Nancy Farm Männikkö

See also: Animal Cloning; Cancer; Cloning; Cloning: Ethical Issues; Gene Therapy: Ethical and Economic Issues; Genetic Engineering: Historical Development; Heart Disease; Immunogenetics; In Vitro Fertilization and Embryo Transfer; Model Organism: *Mus musculus*; Model Organism: *Xenopus laevis*; Organ Trans-

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Animal rights advocates and other activists protest the use of animals as "spare parts" factories for human organs in this demonstration in Munich, Germany, in November, 2000. (AP/Wide World Photos)

plants and HLA Genes; Stem Cells; Synthetic Antibodies; Totipotency; Transgenic Organisms

Further Reading

- Bassett, Pamela. *Emerging Markets in Tissue Engineering: Angiogenesis, Soft and Hard Tissue Regeneration, Xenotransplant, Wound Healing, Biomaterials, and Cell Therapy*. Southborough, Mass.: D & MD Reports, 1999. Looks at xenotransplants from an economic perspective and discusses the potential for growth for biomedical firms entering the field.
- Bloom, E. T., et al. "Xenotransplantation: The Potential and the Challenges." *Critical Care Nurse* 19 (April 1999): 76-83. Looks at the potential impact of xenotransplantation on patient care as well as the effects it might have on nursing responsibilities.
- Cruz, J., et al. "Ethical Challenges of Xenotransplantation." *Transplant Proceedings* 32 (December, 2000): 2687. Members of a medical transplant team discuss questions about potential moral contradictions in using animal organs in human patients.
- Daar, A. S. "Xenotransplants: Proceed with Caution." *Nature* 392 (March 5, 1998): 11. Sounds a warning about some of the potential risks involved in xenotransplants.
- Persson, M. D., et al. "Xenotransplantation Public Perceptions: Rather Cells than Organs." *Xenotransplantation* 10 (2003): 72-79. Describes a public opinion survey done to gauge the public's willingness to support xenotransplant research and implementation.
- "U.S. Decides Close Tabs Must Be Kept on Xenotransplants." *Nature* 405 (June 8, 2000): 606-607. Discussion of federal regulations regarding xenotransplant research.

Key terms

- ANEUPLOIDY:** possession of one or a few more or less than the normal number of chromosomes
- GENETIC SCREENING:** a medical technique that uses either fetal or adult cells to directly view the chromosomes of an individual to detect abnormalities in number or structure
- MEIOSIS:** cell division that produces sperm and egg cells having half the original number of chromosomes
- NONDISJUNCTION:** abnormal separation of chromosome pairs or duplicates during cell division, resulting in one daughter cell receiving an extra chromosome (or chromosomes) and the other daughter cell receiving a complementary number, less than normal
- SEX CHROMOSOMES:** the X and Y chromosomes, which determine the gender of an individual

Causes and Effects of XYY Syndrome

All normal human cells contain forty-six chromosomes consisting of twenty-three pairs; one member of each pair is contributed by the female parent and one by the male. Of these forty-six chromosomes, two chromosomes, designated X and Y, are known as the sex chromosome pair. Individuals with an XX pair are female, while those with an XY pair are male. Unlike the other twenty-two chromosome pairs, the X and Y chromosomes are strikingly different from each other in both size and function. While the Y chromosome is primarily concerned with maleness, the X chromosome contains information important to both genders.

During formation of sperm and eggs in the testes and ovaries, respectively, a unique form of nuclear division, known as meiosis (or reductional division), occurs during cell division that halves the chromosome number from forty-six to twenty-three. Sperm and eggs are thus carrying only one member of each pair of chromosomes, and the original number will be restored during fertilization. Because females only have the XX pair, their eggs can only have an X chromosome, while the male, having the XY pair, produces sperm bearing an X or a Y chromosome.

A common genetic error during sperm or

XYY Syndrome

Field of study: Diseases and syndromes

Significance: *XYY syndrome occurs at fertilization and represents one of several human sex chromosome abnormalities. The resulting XYY male bears an extra Y chromosome that is associated with tall stature and possible intelligence and behavioral problems.*

egg production is known as nondisjunction, which is the improper division of chromosomes between the daughter cells. Nondisjunction in the production of either gamete can result, at fertilization, in embryos without the normal forty-six chromosomes. XYY syndrome is one of several of these aneuploid conditions that involve the sex chromosome pair. While Klinefelter syndrome (an XXY male) and Turner syndrome (an X female) are more widely studied and recognized genetic diseases, the XYY male occurs with a frequency of 1 in 1,000 male births in the United States. Caused by a YY-bearing sperm fertilizing a normal X-bearing egg, the XYY embryo develops along a seemingly normal route and, unlike most other sex chromosome diseases, is not apparent at birth. In fact, identification of this disorder requires genetic testing or screening and is often discovered accidentally as a consequence of results from another genetic test. The only physical clue is unusually tall stature; otherwise, an affected male will be normal in appearance. The XYY male is also fertile, unlike those with aneuploidies involving other combinations of sex chromosomes, which usually result in sterility.

Behavioral and Research Implications

Interest in the association between aggression and the Y chromosome began in the years following World War II. Both psychologists and geneticists began intensive scrutiny of the genes that were located on the male sex chromosome. Men with multiple copies of the Y chromosome thus became the subjects of much of this research. Genetic links to violent, aggressive, and even criminal behavior were found, although many argued that below-average intelligence played a greater role. Many males with XYY syndrome do perform lower than average on standard intelligence tests and have a greater incidence of behavioral problems. The majority, however, lead normal lives and are indistinguishable from XY males.

The controversy surrounding this research began with a study at Harvard University that began in the early 1960's and ended in 1973 because of pressure from both public and scientific communities. The researchers screened all boys born at a Boston hospital, identifying

those with sex chromosomal abnormalities. Because the parents of XYY boys were told of their children's genetic makeup and the possibility of lower intelligence and bad behavior, critics claimed that the researchers had biased the parents against their sons, causing the parents to treat the children differently. The environment would thus play a greater role than genetics in their behavior. Subsequent research has shown that the original hypothesis is at least partially accurate. There is a disproportionately large number of XYY males in prison populations, and they are usually of subaverage intelligence compared to other prisoners. It must be emphasized, however, that the majority of XYY males show neither low intelligence nor criminal behavior.

Scientists, doctors, geneticists, and psychologists now agree that the extra Y chromosome does cause above normal height, reading and math difficulties, and, in some cases, severe acne, but the explanation of the high prevalence of XYY men in prison populations has changed its focus from genes to environment. Large body size during childhood, adolescence, and early adulthood will no doubt cause people to treat these individuals differently, and they may in turn have learned to use their size defensively. Aggressive behavior, coupled with academic difficulties, may lead to further problems. Clearly, however, the majority of XYY males do well. The issue would be much easier to resolve if a YY or Y male existed, but because lack of an X chromosome results in spontaneous miscarriage, no YY or Y male embryo could ever survive.

—Connie Rizzo

See also: Aggression; Behavior; Criminality; Intelligence; Klinefelter Syndrome; Metafemales; Nondisjunction and Aneuploidy; Steroid Hormones; Testicular Feminization Syndrome; X Chromosome Inactivation.

Further Reading

Mader, Sylvia S. *Human Reproductive Biology*. 3d ed. New York: McGraw-Hill, 2000. Provides an excellent introduction to cell division, genetics, and sex from fertilization through birth. Illustrations, bibliography, index.
Tamarin, Robert H. *Principles of Genetics*. Bos-

ton: McGraw-Hill, 2002. A well-written reference text on genetics with complete discussions on aneuploidy, the sex chromosomes, genes, and abnormalities; it also includes a thorough reading list. Illustrations, maps (some color), bibliography, index.

Web Sites of Interest

Intersex Society of North America. <http://www.isna.org>. The society is “a public awareness, education, and advocacy organization which works to create a world free of shame, secrecy, and unwanted surgery for intersex people (individuals born with anatomy or

physiology which differs from cultural ideals of male and female).” Includes links to information on such conditions as clitoromegaly, micropenis, hypospadias, ambiguous genitals, early genital surgery, adrenal hyperplasia, Klinefelter syndrome, androgen insensitivity, and testicular feminization.

Johns Hopkins University, Division of Pediatric Endocrinology, Syndromes of Abnormal Sex Differentiation. <http://www.hopkinsmedicine.org/pediatricendocrinology>. Site provides a guide to the science and genetics of sex differentiation, including a glossary. Click on “patient resources.”

Biographical Dictionary of Important Geneticists

Altman, Sidney (1939-): Won the 1989 Nobel Prize in Chemistry, with Thomas R. Cech. Working independently Altman and Cech discovered that RNA, like proteins, can act as a catalyst; moreover, they found that when ribosomal RNA participates in translation of messenger RNA (mRNA) and the synthesis of polypeptides, it acts as a catalyst in some steps.

Anfinsen, Christian B. (1916-1995): Won the 1972 Nobel Prize in Chemistry. Anfinsen, studying the three-dimensional structure of the enzyme ribonuclease, proved that its conformation was determined by the sequence of its amino acids and that to construct a complete enzyme molecule no separate structural information was passed on from the DNA in the cell's nucleus.

Arber, Werner (1929-): First to isolate enzymes that modify DNA and enzymes that cut DNA at specific sites. Such restriction enzymes were critical in the developing field of molecular biology. Arber was awarded the 1978 Nobel Prize in Physiology or Medicine.

Aristotle (384-322 B.C.E.): Greek philosopher and scientist. Aristotle's *De Generatione* was devoted in part to his theories on heredity. Aristotle believed the semen of the male contributes a form-giving principle (*eidos*), while the menstrual blood of the female is shaped by the *eidos*. The philosophy implied it was the father only who supplied form to the offspring.

Auerbach, Charlotte (1899-1994): German-born geneticist who fled to England following the rise of the Nazi Party. Demonstrated that the mutations produced by mustard gases and other chemicals in *Drosophila* (fruit flies) were similar to those induced by X rays, suggesting a common mechanism.

Avery, Oswald Theodore (1877-1955): Immunologist and biologist who determined DNA to be the genetic material of cells. Avery's early work involved classification of the pneumococci, the common cause of pneu-

monia in the elderly. In 1944, he reported that the genetic information in these bacteria is DNA.

Bailey, Catherine (1921-): By applying methods of selective breeding, developed new varieties of fruits.

Baltimore, David (1938-): Along with Howard Temin, Baltimore isolated the enzyme RNA-directed DNA polymerase (reverse transcriptase), demonstrating the mechanism by which RNA tumor viruses can integrate their genetic material into the cell chromosome. Baltimore was awarded the 1975 Nobel Prize in Physiology or Medicine.

Barr, Murray Llewellyn (1908-1995): Canadian geneticist who discovered the existence of the Barr (Barr's) body, an inactive X chromosome found in cells from a female. The existence or absence of the body has been used in determining the sex of the individual from whom the cell originated.

Bateson, William (1861-1926): Plant and animal geneticist who popularized the earlier work of Gregor Mendel. In his classic *Mendel's Principles of Heredity* (1909), Bateson introduced much of the modern terminology used in the field of genetics. Bateson suggested the term "genetics" (from the Greek word meaning "descent") to apply to the field of the study of heredity.

Beadle, George Wells (1903-1989): Beadle's studies of the bread mold *Neurospora* demonstrated that the function of a gene is to encode an enzyme. Beadle and Edward Tatum were awarded the 1958 Nobel Prize in Physiology or Medicine for their one gene-one enzyme hypothesis.

Beckwith, Jonathan R. (1935-): Determined role of specific genes in regulating bacterial cell division. During the 1960's, he was among the first to isolate a specific gene. Beckwith is also known as a social activist in his arguments for the use of science for improvement of society.

Bell, Julia (1879-1979): British geneticist who applied statistical analysis in understanding

hereditary medical disorders of the nervous system and limbs.

Benacerraf, Baruj (1920-): Won the 1980 Nobel Prize in Physiology or Medicine, with Jean Dausset and George D. Snell. Benacerraf, Dausset, and Snell each explained the genetic components of the major histocompatibility complex (MHC), the key to a person's immune system, and how the system produces antibodies to such a wide variety of foreign molecules and pathogens, such as viruses, fungi, and bacteria.

Berg, Paul (1926-): Developed DNA recombination techniques for insertion of genes in chromosomes. The techniques became an important procedure in understanding gene function and for the field of genetic engineering. Berg was awarded the 1980 Nobel Prize in Chemistry.

Bishop, John Michael (1936-): Determined that oncogenes, genetic information initially isolated from RNA tumor viruses, actually originate in normal host cells. Bishop was awarded the 1989 Nobel Prize in Physiology or Medicine for his discovery.

Bluhm, Agnes (1862-1943): German physician whose controversial theories on improvement of the "Race" through eugenics and fertility selection provided a basis for Nazi race theories. Among other aspects of her theory was the use of enforced sterilization.

Boring, Alice Middleton (1883-1955): Confirmed existing theories of the chromosomal basis of heredity. Her professional career consisted primarily in serving as a biology teacher to students in China between the world wars.

Borlaug, Norman (1914-): Won the 1970 Nobel Prize in Peace. Borlaug was a key figure in the Green Revolution in agriculture. Working as a geneticist and plant physiologist in a joint Mexican-American program, he developed strains of high-yield, short-strawed, disease-resistant wheat. His goal was to increase crop production and alleviate world hunger.

Botstein, David (1942-): Developed methods of localized mutagenesis for understanding the relationship between the structure and function of proteins. His development

of a linkage map involving human genes contributed to the progress of the Human Genome Project.

Boyer, Herbert W. (1926-): His isolation of restriction enzymes that produced a staggered cut on the DNA allowed for creation of so-called "sticky ends," which allowed DNA from different sources or species to be spliced together.

Brenner, Sydney (1927-): Molecular geneticist whose observations of mutations in nematodes (long, unsegmented worms) helped in understanding the design of the nervous system. Brenner was among the first to clone specific genes. He was awarded the Nobel Prize in Physiology or Medicine in 2002.

Brown, Michael S. (1941-): By studying the role of cell receptors in uptake of lipids from the blood, Brown discovered the genetic defect in humans associated with abnormally high levels of cholesterol. He was awarded the 1985 Nobel Prize in Physiology or Medicine.

Burnet, Frank Macfarlane (1899-1985): Proposed a theory of clonal selection to explain regulation of the immune response. Burnet was awarded the 1960 Nobel Prize in Physiology or Medicine.

Cairns, Hugh John (1922-): British virologist whose investigations of rates and mechanisms of DNA replication helped to lay the groundwork in studying the replication process.

Carroll, Christiane Mendrez (1937-1978): French geneticist and paleontologist, most noted for her taxonomic interpretations of early reptiles.

Cech, Thomas R. (1947-): Won the 1989 Nobel Prize in Chemistry, with Sidney Altman. Working independently, Cech and Altman discovered that RNA, like proteins, can act as a catalyst; moreover, Cech found that when ribosomal RNA participates in translation of mRNA and the synthesis of polypeptides, it acts as a catalyst in some steps.

Chargaff, Erwin (1905-2002): Determined that the DNA composition in a cell is characteristic of that particular organism. His discovery

of base ratios, in which the concentration of adenine is equal to that of thymine, and guanine to that of cytosine, provided an important clue to the structure of DNA.

Cohen, Stanley N. (1935-): Developed the techniques for transfer of DNA between species, a major factor in the process of genetic engineering.

Collins, Francis Sellers (1950-): In 1989, Collins identified the gene that, when mutated, results in the genetic disease cystic fibrosis. Collins was instrumental in the identification of a number of genes associated with genetic diseases.

Correns, Carl Erich (1864-1935): German botanist who confirmed Gregor Mendel's laws through his own work on the garden pea. Correns was one of several geneticists who rediscovered Mendel's work in the early 1900's.

Crick, Francis Harry Compton (1916-): Along with James Watson, Crick determined the double-helix structure of DNA. Crick was awarded the Nobel Prize in Physiology or Medicine in 1962.

Darlington, Cyril (1903-1981): British geneticist who demonstrated changes in chromosomal patterns which occur during meiosis, leading to an understanding of chromosomal distribution during the process. He also described a role played by crossing over, or genetic exchange, in changes of patterns.

Darwin, Charles Robert (1809-1882): Naturalist whose theory of evolution established natural selection as the basis for descent with modification, more commonly referred to as evolution. His classic work on the subject, *On the Origin of Species by Means of Natural Selection* (1859), based on his five-year voyage during the 1830's on the British ship HMS *Beagle*, summarized the studies and observations that initially led to the theory. Darwin's pangenesis theory, first noted in *The Variation of Animals and Plants Under Domestication* (1868), later became the basis for the concept of the gene.

Darwin, Erasmus (1731-1802): British physician, inventor, and writer. In his classic *Zoonomia*, he advanced a theory of the role of the environment on genetic changes in organisms. A similar theory was later devel-

oped by Jean-Baptiste Lamarck. Darwin was the grandfather of both Charles Darwin and Francis Galton.

Dausset, Jean (1916-): Won the 1980 Nobel Prize in Physiology or Medicine, with Baruj Benacerraf and George D. Snell. Dausset, Benacerraf, and Snell each explained the genetic components of the major histocompatibility complex (MHC), the key to a person's immune system, and how the system produces antibodies to such a wide variety of foreign molecules and pathogens, such as viruses, fungi, and bacteria.

Delbrück, Max (1906-1981): A leading figure in the application of genetics to bacteriophage research, and later, with *Phycomyces*, a fungal organism. His bacteriophage course, taught for decades at Cold Spring Harbor, New York, provided training for a generation of biologists. He was awarded the 1969 Nobel Prize in Physiology or Medicine.

Demerec, Milislav (1895-1966): Croatian-born geneticist who was among the scientists who brought the United States to the forefront of genetics research. Demerec's experiments, based on the genetics of corn, addressed the question of what a gene represents. His work with bacteria included the determination of mechanisms of antibiotic resistance, as well as the existence of operons, closely linked genes which are coordinately regulated. Demerec was director of the biological laboratories in Cold Spring Harbor, New York, for many years among the most important sites of genetic research.

De Vries, Hugo (1848-1935): Dutch botanist whose hypothesis of intracellular pangenesis postulated the existence of pangenesis, factors which determined characteristics of a species. De Vries established the concept of mutation as a basis for variation in plants. In 1900, de Vries was one of several scientists who rediscovered Mendel's work.

Dobzhansky, Theodosius (1900-1975): Russian-born American geneticist who established evolutionary genetics as a viable discipline. His book *Genetics and the Origin of Species* (1937) represented the first application of Mendelian theory to Darwinian evolution.

Dulbecco, Renato (1914-): Among the first to study the genetics of tumor viruses. Dulbecco was awarded the 1975 Nobel Prize in Physiology or Medicine.

Ferguson, Margaret Clay (1863-1951): Plant geneticist whose use of *Petunia* as a model helped explain life cycles of various plants. Also noted for her description of the life cycle of pine trees.

Fink, Gerald R. (1940-): Isolation of specific mutants in yeast allowed the use of genetics in understanding biochemical mechanisms in that organism.

Fisher, Ronald Aylmer (1890-1962): British biologist whose application of statistics provided a means by which use of small sampling size could be applied to larger interpretations. Fisher's breeding of small animals led to an understanding of genetic dominance. He later applied his work to the study of inheritance of blood types in humans.

Franklin, Rosalind Elsie (1920-1958): British crystallographer whose X-ray diffraction studies helped confirm the double-helix nature of DNA. Franklin's work, along with that of Maurice Wilkins, was instrumental in confirming the structure of DNA as proposed by James Watson and Francis Crick. Franklin's early death precluded her receiving a Nobel Prize for her research.

Galton, Francis (1822-1911): British scientist who was an advocate of eugenics, the belief that human populations could be improved through "breeding" of desired traits. Galton was also the first to observe that fingerprints were unique to the individual.

Garnjobst, Laura Flora (1895-1977): Following her training under Nobel laureate Edward Tatum at both Stanford and Yale, Garnjobst spent her career in the study of genetics of the mold *Neurospora*.

Garrod, Archibald Edward (1857-1936): Applying his work on alkaptonuria, Garrod proposed that some human diseases result from a lack of specific enzymes. His theory of inborn errors of metabolism, published in 1908, established the genetic basis for certain hereditary diseases.

Gartner, Carl Friedrich von (1772-1850): Ger-

man plant biologist and geneticist. Though Gartner did not generalize as to the significance of his work, his results provided the experimental basis for questions later developed by Gregor Mendel and Charles Darwin.

Giblett, Eloise (1921-): Discoverer of numerous genetic markers useful in defining blood groups and serum proteins. In the 1970's, Giblett discovered that certain immunodeficiency diseases result from the absence of certain enzymes necessary for immune cell development.

Gilbert, Walter (1932-): Developed method of sequencing DNA. With Paul Berg and Frederick Sanger, awarded the 1980 Nobel Prize in Chemistry.

Gilman, Alfred G. (1941-): Discovered the role of "G" proteins in regulating signal transduction in eukaryotic cells. With Martin Rodbell, won the Nobel Prize in Physiology or Medicine for 1994.

Goldschmidt, Richard B. (1878-1958): German-born geneticist who proposed that the chemical makeup of the chromosome determines heredity rather than the quantity of genes. He theorized that large mutations, or "genetic monsters," were important in generation of new species.

Goldstein, Joseph L. (1940-): Won the 1985 Nobel Prize in Physiology or Medicine, with Michael S. Brown. Brown and Goldstein conducted extensive research in the regulation of cholesterol in humans. They showed that in families with a history of high cholesterol, individuals who carry two copies of a mutant gene (homozygotes) have cholesterol levels several times higher than normal and those who have one mutant gene (heterozygotes) have levels about double normal. Their discoveries proved invaluable in managing heart disease and other cholesterol-related ailments.

Griffith, Frederick (1877-1941): British microbiologist who in 1928 reported the existence of a "transforming principle," an unknown substance that could change the genetic properties of bacteria. In 1944, Oswald Avery determined the substance to be DNA, three years after Griffith was killed during the German bombing of London.

Gruhn, Ruth (1907-1988): German geneticist who applied mathematical principles in the breeding of poultry and pigs.

Haeckel, Ernst Heinrich (1834-1919): German zoologist whose writings were instrumental in the dissemination of Charles Darwin's theories. Haeckel's "biogenetic law," since discarded, stated that "ontogeny repeats phylogeny," suggesting that embryonic development mirrors the evolutionary relationship of organisms.

Haldane, John Burdon Sanderson (1892-1964): British physiologist and geneticist who proposed that natural selection, and not mutation per se, was the driving force of evolution. Haldane was the first to determine an accurate rate of mutation for human genes, and he later demonstrated the genetic linkage of hemophilia and color blindness.

Hanafusa, Hidesaburo (1929-): Japanese-born scientist who played a key role in elucidating the role of oncogenes found among the RNA tumor viruses in transforming mammalian cells.

Hanawalt, Philip C. (1931-): In 1963, discovered the existence of a repair mechanism associated with DNA replication in bacteria. He later found a similar mechanism in eukaryotic cells. Hanawalt's later work included development of the technique of site mutagenesis in gene mapping.

Hardy, Godfrey Harold (1877-1947): British mathematician who, along with Wilhelm Weinberg, developed the Hardy-Weinberg law of population genetics. In a 1908 letter to the journal *Science*, Hardy used algebraic principles to confirm Mendel's theories as applied to populations, an issue then currently in dispute.

Hartwell, Leland H. (1939-): Discovered genes that regulate the movement of eukaryotic cells through the cell cycle. With Tim Hunt and Sir Paul Nurse, won the Nobel Prize in Physiology or Medicine in 2001.

Haynes, Robert Hall (1931-1998): Canadian molecular biologist who carried out much of the early work in the understanding of DNA repair mechanisms.

Hershey, Alfred Day (1908-1997): Molecular biologist who played a key role in under-

standing the replication and genetic structure of viruses. His experiments with Martha Chase confirmed that DNA carried the genetic information in some viruses. Hershey was awarded the 1969 Nobel Prize in Physiology or Medicine.

Herskowitz, Ira (1946-2003): His studies of gene conversion pathways in yeast led to an understanding of gene switching in control of mating types.

Hertwig, Paula (1889-1983): German embryologist who studied the effects of radiation on embryonic development of fish and animals.

Hippocrates (c. 460-377 B.C.E.): Greek physician who proposed the earliest theory of inheritance. Hippocrates believed that "seed material" was carried by body humors to the reproductive organs.

Hogness, David S. (1925-): One of the first to clone a gene from *Drosophila* (fruit flies). His technique of chromosomal "walking" allowed for the isolation of any known gene based on its ability to mutate. Also involved in identification of homeotic genes, genes which regulate development of body parts.

Holley, Robert William (1922-1993): Determined the sequence of nucleotide bases in transfer RNA (tRNA), the molecule that carries amino acids to ribosomes for protein synthesis. Holley's work provided a means for demonstrating the reading of the genetic code. He was awarded the Nobel Prize in Physiology or Medicine in 1968.

Horvitz, H. Robert (1947-): Harvard neurobiologist whose study of cell regulation in the nematode *Caenorhabditis* led to the discovery of genes that regulate cell death during embryonic development. With Sydney Brenner and John Sulston, he was awarded the Nobel Prize in Physiology and Medicine in 2002.

Hunt, R. Timothy (1943-): Discovered the existence and role of proteins called cyclins, which regulate the cell cycle in eukaryotic cells. With Leland Hartwell and Sir Paul Nurse, won the Nobel Prize in Physiology or Medicine in 2001.

Jacob, François (1920-): French geneticist and molecular biologist who, along with

Jacques Monod, elucidated a mechanism of gene and enzyme regulation in bacteria. The Jacob-Monod theory of gene regulation became the basis for understanding a wide range of genetic processes; they were awarded the 1965 Nobel Prize in Physiology or Medicine.

Jeffreys, Sir Alec (1950-): British biochemist who discovered the existence of introns in mammalian genes. His study of the pattern of repeat sequences in DNA was shown to be characteristic of individuals, and became the theoretical basis for DNA fingerprinting and DNA profiles.

Johannsen, Wilhelm L. (1857-1927): Danish botanist who introduced the term "genes," derived from "pangenes," factors suggested by Hugo de Vries to determine hereditary characteristics in plants. Johannsen also introduced the concepts of phenotype and genotype to distinguish between physical and hereditary traits.

Kenyon, Cynthia J. (1976-): Discovered the role of specific genes in regulation of cell migration and the aging process in the nematode *Caenorhabditis*, helping to clarify similar processes in more highly evolved eukaryotic organisms.

Khorana, Har Gobind (1922-): Developed methods for investigating the structure of DNA and deciphering the genetic code. Khorana synthesized the first artificial gene in the 1960's. He was awarded the Nobel Prize in Physiology or Medicine in 1968.

King, Helen Dean (1869-1955): By selective breeding of rodents, developed a method for production of inbred strains of animals for laboratory studies. The methodology was later applied to development of more desirable breeds of horses.

Klug, Aaron (1759-1853): Won the 1982 Nobel Prize in Chemistry. Klug used X-ray crystallography to investigate biochemical structures, especially that of viruses. He was able to link the assembly of viral protein subunits with specific sites on viral RNA, which helped in fighting viruses that cause disease in plants and, more basically, in understanding the mechanism of RNA transfer of genetic information. He also determined the

structure of transfer RNA (tRNA), which has a shape similar to that of a bent hair pin.

Knight, Thomas Andrew (1759-1853): Plant biologist who first recognized the usefulness of the garden pea for genetic studies because of its distinctive traits. Knight was the first to characterize dominant and recessive traits in the pea, though, unlike Gregor Mendel, he never determined the mathematical relationships among his crosses.

Kölreuter, Josef Gottlieb (1733-1806): A fore-runner of Gregor Mendel, Kölreuter demonstrated the sexual nature of plant fertilization, in which characteristics were derived from each member of the parental generation in equivalent amounts.

Kornberg, Arthur (1918-): Carried out the first purification of DNA polymerase, the enzyme that replicates DNA. His work on the synthesis of biologically active DNA in a test tube culminated with his being awarded the 1959 Nobel Prize in Physiology or Medicine.

Kossel, Albrecht (1853-1927): Won the 1910 Nobel Prize in Physiology or Medicine. Isolated and described molecular constituents of the cell's nucleus, notably cytosine, thymine, and uracil. These molecules later proved to be constituents of the codons in DNA and RNA. Thus, Kossel's research prepared the way for understanding the biochemistry of genetics.

Lamarck, Jean-Baptiste (1744-1829): French botanist and evolutionist who introduced many of the earliest concepts of inheritance. Lamarck proposed that hereditary changes occur as a result of an organism's needs; his theory of inherited characteristics, since discredited, postulated that organisms transmit acquired characteristics to their offspring.

Leder, Philip (1934-): Along with Marshal Nirenberg, identified the genetic code words for amino acids. His later work has involved the transplantation of human oncogenes into mice, for the purpose of studying the effects of such genes in development of cancer.

Lederberg, Joshua (1925-): Established the occurrence of sexual reproduction in

bacteria. Lederberg demonstrated that genetic manipulation of the DNA during bacterial conjugation could be used to map bacterial genes. He was awarded the 1958 Nobel Prize in Physiology or Medicine.

Levene, Phoebus Aaron (1869-1940): American biochemist who determined the components found in DNA and RNA. Levene described the presence of ribose sugar in RNA and of 2'-deoxyribose in DNA, thereby differentiating the two molecules. He also identified the nitrogen bases found in nucleic acid, though he was never able to determine the acid's molecular structure.

Lewis, Edward B. (1918-): Through the use of X-ray-induced mutations in *Drosophila* (fruit flies), Lewis was able to discover and map genes that regulate embryonic development. Among Lewis's discoveries was the existence of homeotic genes, genes that regulate development of body parts. Along with Christiane Nüsslein-Volhard and Eric Wieschaus, awarded the Nobel Prize in Physiology or Medicine in 1995.

Linnaeus, Carolus (1707-1778): Swedish naturalist and botanist most noted for establishing the modern method for classification of plants and animals. In his *Philosophia Botanica* (1751; *The Elements of Botany*, 1775), Linnaeus proposed that variations in plants or animals are induced by environments such as soil.

Luria, Salvador E. (1912-1991): A pioneer in understanding replication and genetic structure in viruses. The Luria-Delbrück fluctuation test, developed by Luria and Max Delbrück, demonstrated that genetic mutations precede environmental selection. Luria was awarded the 1969 Nobel Prize in Physiology or Medicine.

Lwoff, André (1902-1994): French biochemist and protozoologist. Lwoff's early work demonstrated that vitamins function as components of living organisms. He is best known for demonstrating that the genetic material of bacteriophage can become part of the host bacterium's DNA, a process known as lysisogeny. Lwoff was awarded the 1965 Nobel Prize in Physiology or Medicine.

Lyon, Mary Frances (1925-): British cyto-

geneticist who proposed what became known as the Lyon hypothesis, that only a single X chromosome is active in a cell. Any other X chromosomes are observed as Barr bodies.

McClintock, Barbara (1902-1992): Demonstrated the existence in plants of transposable elements, or transposons, genes that "jump" from one place on a chromosome to another. The process was discovered to be widespread in nature. McClintock was awarded the 1983 Nobel Prize in Physiology or Medicine.

Macklin, Madge Thurlow (1893-1962): Developed a method to apply statistical analysis to understanding congenital diseases in human families. Her arguments were used to introduce genetics as a component of the curriculum in medical schools. Her support of eugenics for improvement of humans later made her views controversial.

McKusick, Victor A. (1921-): Cataloged and indexed many of the genes responsible for disorders that are passed in Mendelian fashion.

Margulis, Lynn (1938-): Developed the endosymbiont theory, which suggests that internal eukaryotic organelles, such as mitochondria and chloroplasts, originated as free-living prokaryotic ancestors. She proposed that free-living bacteria became incorporated in a larger, membrane-bound structure and developed a symbiotic relationship within the larger cell.

Mendel, Johann Gregor (1822-1884): The "father of genetics," Mendel was an Austrian monk whose studies on the transmission of traits in the garden pea established the mathematical basis of inheritance. Mendel's pioneering theories, including such fundamental genetic principles as the law of segregation and the law of independent assortment, were published in 1866 but received scant attention until the beginning of the twentieth century.

Meselson, Matthew Stanley (1930-): Demonstrated the nature of DNA replication, in which the two parental DNA strands are separated, each passing into one of the two daughter molecules. Also noted as a social activist.

Meyerowitz, Elliot M. (1951-): Discovered roles played by specific genes in differentiation of the plant *Arabidopsis*, as well as genes which regulate flowering in plants.

Miescher, Johann Friedrich (1844-1895): In 1869, Miescher discovered and purified DNA from cell-free nuclei obtained from white blood cells and gave the name "nuclein" to the extract. The substance was later known as nucleic acid.

Mintz, Beatrice (1921-): Noted for studies of the role of gene control in differentiation of cells and disease in humans. Developed a mouse model for the understanding of melanoma development in humans.

Monod, Jacques Lucien (1910-1976): French geneticist and molecular biologist who with François Jacob demonstrated a method of gene regulation in bacteria that came to be known as the Jacob-Monod model. Jacob and Monod were jointly awarded the 1965 Nobel Prize in Physiology or Medicine.

Moore, Stanford (1913-1982): Won the 1972 Nobel Prize in Chemistry, with William H. Stein. Moore and Stein supplemented Alfinsen's research by identifying the sequence of amino acids in ribonuclease, a clue to the structure of the gene responsible for it.

Morgan, Lilian Vaughan (1870-1952): Discovered the attached X and ring X chromosomes in *Drosophila* (fruit flies). Later contributed to studying the effects of polio vaccines in primates. Married to Thomas Hunt Morgan.

Morgan, Thomas Hunt (1866-1945): Considered the father of modern genetics, an embryologist whose studies of fruit flies (*Drosophila melanogaster*) established the existence of genes on chromosomes. Through his selective breeding of flies, Morgan also established concepts such as gene linkage, sex-linked characteristics, and genetic recombination. Won the 1933 Nobel Prize in Physiology or Medicine.

Müller, Hermann Joseph (1890-1967): Geneticist and colleague of Thomas Hunt Morgan. Muller's experimental work with fruit flies established the gene as the site of mutation. His work with X rays demonstrated a means

of artificially introducing mutations into an organism. Won the 1946 Nobel Prize in Physiology or Medicine.

Mullis, Kary Banks (1944-): Devised the polymerase chain reaction (PCR), a method for duplicating small quantities of DNA. The PCR procedure became a major tool in research in the fields of genetics and molecular biology. Mullis was awarded the 1993 Nobel Prize in Chemistry.

Nathans, Daniel (1928-1999): Applied the use of restriction enzymes to the study of genetics. Nathans developed the first genetic map of SV40, among the first DNA viruses shown to transform normal cells into cancer. Nathans was awarded the 1978 Nobel Prize in Physiology or Medicine.

Neel, James Van Gundia (1915-2000): Considered to be the father of human genetics. Among his discoveries was the recognition of the genetic basis of sickle-cell disease. He was also noted for his study of the after-effects of radiation on survivors of the atomic attack on Hiroshima and Nagasaki in World War II. He was the first to propose what was referred to as the thrifty-gene hypothesis, the idea that potentially lethal genes may have been beneficial to the human population earlier in evolution.

Nelson, Oliver Evans, Jr. (1920-2001): During the 1950's, carried out the first structural analysis of a gene in higher plants (corn), at the same time confirming the existence of transposable elements. His later work demonstrated the genetic significance of enzymatic defects in maize.

Neufeld, Elizabeth F. (1928-): French-born biochemist who found that many mucopolysaccharide storage diseases resulted from the absence of certain metabolic enzymes. Her work opened the way for prenatal diagnosis of such diseases.

Nirenberg, Marshall Warren (1927-): Molecular biologist who was among the first to decipher the genetic code. He later demonstrated the process of ribosome binding in protein synthesis and carried out the first cell-free synthesis of protein. Nirenberg was awarded the 1968 Nobel Prize in Physiology or Medicine.

Nurse, Sir Paul M. (1949-): British scientist who discovered the role of chemical modification (phosphorylation) in regulation of the cell cycle. With Tim Hunt and Leland Hartwell, he was awarded the Nobel Prize in Physiology or Medicine in 2001.

Nüsslein-Volhard, Christiane (1942-): German biologist whose genetic studies in *Drosophila* (fruit flies) led to the discovery of genes that regulate body segmentation in the embryo. Along with Edward Lewis and Eric Wieschaus, won the Nobel Prize in Physiology or Medicine in 1995.

Ochoa, Severo (1905-1993): Won the 1959 Nobel Prize in Physiology or Medicine, with Arthur Kornberg. Ochoa and Kornberg isolated enzymes involved in the synthesis of DNA and RNA, representing the first steps in decoding the biochemical instructions preserved in the structure of genes.

Olson, Maynard V. (1943-): Studied base-pair polymorphisms in the human genome and their significance to evolution. In 1987, with David Burke, Olson developed a new type of cloning vector, artificial chromosomes, that filled the need created by the Human Genome Project to clone very large insert DNAs (hundreds of thousands to millions of base pairs in length).

Pauling, Linus (1901-1994): American chemist who received the Nobel Prize in Chemistry in 1954 for his work on the nature of the chemical bond and the 1962 Nobel Peace Prize for his antinuclear activism. His 1950's investigations of protein structure contributed to the determination of the structure of DNA.

Punnett, Reginald C. (1875-1967): English biologist who collaborated with William Bateson in a series of important breeding experiments that confirmed the principles of Mendelian inheritance. Punnett also introduced the Punnett square, the standard graphical method of depicting hybrid crosses.

Rhabar, Shemooil (1929-): Iranian director at the University of Tehran, who became known as the most important immunologist in the Muslim world.

Roberts, Richard J. (1943-): Discovered that genes in eukaryotic cells and animal vi-

ruses are often discontinuous, with intervening sequences between segments of genetic material. With Philip Sharp, Roberts received the Nobel Prize in Physiology or Medicine in 1993.

Rodbell, Martin (1925-1998): Discovered the role of membrane-bound "G" proteins in regulation of signal transduction in eukaryotic cells. With Alfred Gilman, awarded the Nobel Prize in Physiology or Medicine in 1994.

Rowley, Janet (1925-): Cytogeneticist who developed the staining techniques for observation of cell structures. She demonstrated the role of chromosomal translocation as the basis for chronic myeloid leukemia, the first example of translocation as a cause of cancer.

Rubin, Gerald M. (1950-): Major figure in developing a structure/functional relationship of genes in *Drosophila* (fruit flies) through the use of insertion mutagenesis to inactivate specific genes.

Russell, Elizabeth Shull (1913-2001): Contributed to the understanding of the role played by specific genes in creating coat variations in animals. Her later work involved the identification of genetic defects in the aging process and in the development of diseases such as muscular dystrophy.

Russell, William (1910-2003): A pioneer in the genetic effects of radiation at Oak Ridge National Laboratory whose testing of mice led to standards for acceptable levels of human exposure to radiation. Winner of the 1976 Fermi Award.

Sager, Ruth (1918-1997): During the 1950's, demonstrated the existence of nonchromosomal heredity, also known as cytoplasmic inheritance, and hence the role of cytoplasmic genes in organelle development. Later involved in study of tumor suppressor and breast cancer genes.

Sageret, Augustin (1763-1851): French botanist who discovered the ability of different traits to segregate independently in plants.

Sanger, Frederick (1918-): Determined the method for sequencing DNA. His method separated the strands of DNA and then rebuilt them in stages that allowed the

terminal nucleotides to be identified. This made it possible to sequence the entire genomes of organisms. With Paul Berg and Walter Gilbert, Sanger received the 1980 Nobel Prize in Chemistry.

Sharp, Phillip A. (1944-): Discovered that genes in eukaryotic cells or animal viruses are discontinuous, with segments divided by sections separated by intervening sequences of genetic material. With Richard Roberts, received the Nobel Prize in Physiology or Medicine in 1993.

Simpson, George Gaylord (1902-1984): American paleontologist who applied population genetics to the study of the evolution of animals. Simpson was instrumental in establishing a neo-Darwinian theory of evolution (the rejection of Lamarck's inheritance of acquired characteristics) during the early twentieth century.

Singer, Maxine (1931-): Applied the use of the newly discovered restriction enzymes in formation of recombinant DNA. Singer is most noted as a "voice of calm" in the debate over genetic research, emphasizing the application of such research, and the self-policing of scientists carrying out such work.

Smith, Hamilton Othanel (1931-): Pioneered the purification of restriction enzymes, winning the 1978 Nobel Prize in Physiology or Medicine, with Werner Arber and Daniel Nathans. Arber and the team of Nathans and Smith separately described the restriction-modification system by studying bacteria and bacteriophages; the system involves the action of site-specific endonuclease and other enzymes that cleave DNA into segments.

Smith, Michael (1932-): Won the 1995 Nobel Prize in Chemistry. Smith developed site-directed mutagenesis, a means for reconfiguring genes in order to create altered proteins with distinct properties. Smith's genetic engineering tool made it possible to treat genetic disease and cancer and to create novel plant strains.

Snell, George D. (1903-1996): Snell's discovery of the H-2 histocompatibility complex, which regulates the immune response in

mice, led to the later discovery of the equivalent HLA complex in humans. Awarded the Nobel Prize in Physiology or Medicine in 1980.

Sonneborn, Tracy Morton (1905-1981): Discovered crossbreeding and mating types in paramecia, integrating the genetic principles as applied to multicellular organisms with single-celled organisms such as protozoa.

Spemann, Hans (1869-1941): Won the 1935 Nobel Prize in Physiology or Medicine. By transplanting bits of one embryo into a second, viable embryo, Spemann compiled evidence that an "organizer center" directs the development of an embryo and that different parts of the organizer governed distinct portions of the embryo. His experiments provided clues to the genetic control of growth from the earliest stages of an organism.

Spencer, Herbert (1820-1903): English philosopher influenced by the work of Charles Darwin. Spencer proposed the first general theory of inheritance, postulating the existence of self-replicating units within the individual which determine the traits. Spencer is more popularly known as the source of the notion of "survival of the fittest" as applied to natural selection.

Stanley, Wendell Meredith (1904-1971): American biochemist who was the first to crystallize a virus (tobacco mosaic virus), demonstrating its protein nature. Stanley was later a member of the team that determined the amino acid sequence of the TMV protein. Stanley spent the last years of his long career studying the relationship of viruses and cancer.

Stein, William H. (1911-1980): Won the 1972 Nobel Prize in Chemistry, with Stanford Moore. Stein and Moore supplemented Alfinsen's research by identifying the sequence of amino acids in ribonuclease, a clue to the structure of the gene responsible for it.

Stevens, Nettie Maria (1861-1912): Discovered the existence of the specific chromosomes that determine sex, now known as the X and Y chromosomes. Described the existence of

chromosomes as paired structures within the cell.

Strobell, Ella Church (1862-1920): Developed the technique of photomicroscopy for analysis of chromosomal theory.

Sturtevant, Alfred Henry (1891-1970): Colleague of Thomas Hunt Morgan and among the pioneers in application of the fruit fly (*Drosophila*) in the study of genetics. In 1913, Sturtevant constructed the first genetic map of a fruit fly chromosome. His work became a major factor in chromosome theory. In the 1930's, his work with George Beadle led to important observations of meiosis.

Sulston, John E. (1942-): Developed first map of cell lineages in the model nematode *Caenorhabditis*, leading to the discovery of the first gene associated with programmed cell death. Sulston was also part of the team that sequenced the worm's genome. With Sydney Brenner and H. Robert Horvitz, he was awarded the Nobel Prize in Physiology or Medicine in 2002.

Sutton, Walter Stanborough (1877-1916): Biologist and geneticist who demonstrated the role of chromosomes during meiosis in gametes, and demonstrated their relationship to Mendel's laws. Sutton observed that chromosomes form homologous pairs during meiosis, with one member of each pair appearing in gametes. The particular member of each pair was subject to Mendel's law of independent assortment.

Tammes, Jantine (1871-1947): Dutch geneticist who demonstrated that the inheritance of continuous characters, traits that have a range of expression, could be explained in a Mendelian fashion. She developed a multiple allele hypothesis that helped explain some of the data.

Tan Jiazhen (C. C. Tan; 1909-): Considered the father of Chinese genetics. In a career spanning more than seven decades, Tan studied genetic structure and variation in a wide range of organisms. His most important work involved the study of evolution of genetic structures in *Drosophila* (fruit flies), as well as the concept of mosaic dominance in the beetle.

Tatum, Edward Lawrie (1909-1975): Along

with George Beadle, Tatum demonstrated that the function of a gene is to encode an enzyme. Beadle and Tatum were awarded the 1958 Nobel Prize in Physiology or Medicine for their one gene-one enzyme hypothesis.

Temin, Howard Martin (1934-1994): Proposed that RNA tumor viruses replicate by means of a DNA intermediate. Temin's theory, initially discounted, became instrumental in revealing the process of infection and replication by such viruses. He later isolated the replicating enzyme, the RNA-directed DNA polymerase (reverse transcriptase). He was awarded the 1975 Nobel Prize in Physiology or Medicine, along with David Baltimore, for this work.

Todd, Alexander Robertus (1907-1997): Won the 1957 Nobel Prize in Chemistry. As part of wide-ranging research in organic chemistry, Todd revealed how ribose and deoxyribose bond to the nitrogenous bases on one side of a nucleotide unit and to the phosphate group on the other side. These discoveries provided necessary background for work by others that explained the structure of the DNA molecule.

Tonegawa, Susumu (1939-): Discovered the role of genetic rearrangement of DNA in lymphocytes, which plays a key role in generation of antibody diversity. In 1987, awarded the Nobel Prize in Physiology or Medicine.

Varmus, Harold Elliot (1939-): Elucidated the molecular mechanisms by which retroviruses (RNA tumor viruses) transform cells. Varmus was awarded the 1989 Nobel Prize in Physiology or Medicine.

Waelsch, Salome Gluecksohn (1907-): Studied the role genes play in abnormal cell differentiation and congenital abnormalities. Born in Germany, she fled to the United States from the Nazis in 1933. Her 1938 publication of the role of genes in the T (tailless) phenotype in mice is considered a genetic classic.

Waterston, Robert (1943-): Identified many of the genes that regulate muscle development in the nematode *Caenorhabditis*, as well as contributing to the sequence of the

genome. His sequencing work was also applied in the Human Genome Project led by Francis Collins.

Watson, James Dewey (1928-): Along with Francis Crick, determined the double-helix structure of DNA. Together with Crick and Maurice Wilkins, Watson was awarded the 1962 Nobel Prize in Physiology or Medicine for their work in determining the structure of DNA.

Weinberg, Robert Allan (1942-): Molecular biologist who isolated the first human oncogene, the *ras* gene, associated with a variety of cancers, including those of the colon and brain. Weinberg later isolated the first tumor suppressor gene, the retinoblastoma gene. Weinberg is considered among the leading researchers in understanding the role played by oncogenes in development of cancer.

Weinberg, Wilhelm (1862-1937): German obstetrician who demonstrated that hereditary characteristics of humans such as multiple births and genetic diseases were subject to Mendel's laws of heredity. The mathematical application of such characteristics, published simultaneously (and independently) by Godfrey Hardy, became known as the Hardy-Weinberg equilibrium. The equation demonstrates that dominant genes do not replace recessive genes in a population; gene frequencies would not change from one generation to the next if certain criteria such as random mating and lack of natural selection were met.

Weismann, August (1834-1914): German zoologist noted for his chromosome theory of heredity. Weismann proposed that the source of heredity is in the nucleus only and that inheritance is based on transmission of a chemical or molecular substance from one generation to the next. Weismann's theory, which rejected the inheritance of acquired characteristics, came to be called neo-Darwinism. Though portions of Weismann's theory were later disproved, the nature of the chromosome was subsequently demonstrated by Thomas Hunt Morgan and his colleagues.

Wieschaus, Eric F. (1947-): Wieschaus's studies of genetic control in *Drosophila* (fruit

flies) led to the discovery of genes that regulate cell patterns and shape in the embryo. Along with Edward Lewis and Christiane Nüsslein-Volhard, he was awarded the Nobel Prize in Physiology or Medicine in 1995.

Wilkins, Maurice Hugh Frederick (1916-): Studies on the X-ray diffraction patterns exhibited by DNA confirmed the double-helix structure of the molecule. Wilkins was a colleague of Rosalind Franklin, and it was their work that confirmed the nature of DNA as proposed by Watson and Crick. Wilkins was awarded the Nobel Prize for Physiology and Medicine in 1962, along with Watson and Crick.

Wilmut, Ian (1944-): Scottish embryologist and leader of a research team at the Roslin Institute near Edinburgh. In 1996, Wilmut and his colleagues succeeded in cloning an adult sheep, Dolly, the first adult mammal to be successfully produced by cloning.

Wilson, Edmund Beecher (1856-1939): His study of chromosomes in collaboration with Nettie Stevens led to the discovery of the X and Y chromosomes, playing a key role in the foundation of modern genetics. His later work involved the study of development and differentiation of the fertilized egg.

Witkin, Evelyn Maisel (1921-): Through her studies of induced or spontaneous mutations in bacterial DNA, discovered processes of enzymatic repair of DNA.

Woese, Carl R. (1928-): Based on his studies of ribosomal RNA differences in prokaryotes and eukaryotes, proposed that all life-forms exist in one of three domains: Bacteria, Archaea ("ancient" bacteria), and Eukarya (eukaryotic organisms, from microscopic plants to large animals). Woese expanded his theory in arguing that the Archaea represent the earliest form of life on Earth, and that they later formed a branch which became the eukaryotes.

Wright, Sewall (1889-1988): Discovered genetic drift of genetic traits. The "Sewall Wright" effect, the random drift of characters in small populations, was explained by the random loss of genes, even in the absence of natural selection.

Yanofsky, Charles (1925-): Confirmed that the genetic code involved groups of three bases by demonstrating colinearity of the bases and amino acid sequences. He applied this work in demonstrating similar colinearity of mutations in the tryptophan operon and changes in amino acid sequences.

Zinder, Norton (1928-): With Joshua Lederberg, discovered the role of bacteriophage in transduction, the movement of genes from one host to another by means of viruses. Zinder was also noted for his discovery of RNA bacteriophage and his work on the molecular genetics of such agents.

—Richard Adler

Nobel Prizes for Discoveries in Genetics

Physiology or Medicine

- 1910 **Albrecht Kossel (German)** isolated and described molecular constituents of the cell's nucleus, notably cytosine, thymine, and uracil. These molecules later proved to be constituents of the codons in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Thus, Kossel's research prepared the way for understanding the biochemistry of genetics.
- 1933 **Thomas Hunt Morgan (American)**. Experimenting with the fruit fly *Drosophila melanogaster*, Morgan discovered that the mechanism for the Mendelian laws of heredity lies in the chromosomes inside the nucleus of cells and that specific genes on the chromosomes govern specific somatic traits in the flies. Morgan confirmed the accuracy of Mendel's laws and ended a controversy over their physiological source.
- 1935 **Hans Spemann (German)**. By transplanting bits of one embryo into a second, viable embryo, Spemann compiled evidence that an "organizer center" directs the development of an embryo and that different parts of the organizer governed distinct portions of the embryo. His experiments provided clues to the genetic control of growth from the earliest stages of an organism.
- 1946 **Hermann J. Muller (American)**. Muller proved that X rays damage genes by altering their structure: radiation-induced mutation. Consequently, X rays also modify the structure of chromosomes. The mutations most often produce recessive and harmful traits in the irradiated organism.
- 1958 **George Beadle and Edward Tatum (both American)**. In research on the fungus *Neurospora crassa*, Beadle and Tatum found that biotin was essential to cultivating certain mutant strains of the fungus; this fact demonstrated that genes regulate the synthesis of specific cellular chemicals, one or more of these genes being mutated in the biotin-independent strain.
- 1958 **Joshua Lederberg (American)**. Lederberg showed that the bacterium *Escherichia coli*, although not able to reproduce sexually, is capable of genetic recombination between chromosomes from different cells through a process called conjugation.
- 1959 **Severo Ochoa (Spanish) and Arthur Kornberg (American)**. Ochoa and Kornberg isolated enzymes involved in the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), representing the first steps in decoding the biochemical instructions preserved in the structure of genes.
- 1962 **Francis Crick (British), James Watson (American), and Maurice Wilkins (British)**. Using X-ray diffraction analysis and molecular modeling, Wilkins, Crick, and Watson found that deoxyribonucleic acid (DNA) is structured in a double helix. They were able to identify the specific three-dimensional structure that is the basis for the ability of DNA to be replicated and transcribed.
- 1965 **François Jacob and Jacques Monod (both French)**. Studying enzyme action, Jacob and Monod proved that messenger ribonucleic acid (mRNA) carries instructions from the nucleus to ribosomes, where molecules are assembled for use in the body, and they distinguished structural genes from regulatory genes.
- 1965 **André Lwoff (French)**. Lwoff proposed that viral deoxyribonucleic acid (DNA) can become active after invading cells and cause the cells to divide out of control, producing cancerous tumors.
- 1968 **Robert W. Holley (American), Har Gobind Khorana (Indian), and Marshall W. Nirenberg (American)**. Working separately, Holley, Khorana, and Nirenberg deciphered

- the genetic code in ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Their work anticipated DNA sequencing and genetic engineering.
- 1969 **Max Delbrück (German), Alfred D. Hershey (American), and Salvador E. Luria (Italian).** In joint studies of bacteriophages and their bacterial hosts, Delbrück, Hershey, and Luria described the conformation of bacteriophage deoxyribonucleic acid (DNA), showed that different strains exchange genetic information, and proved that bacterial DNA mutated to confer protection from attack, demonstrating that bacterial heredity is based on genetic exchange. The discovery explained why bacteria gradually become resistant to pharmaceuticals.
- 1975 **David Baltimore and Howard M. Temin (both American).** Working separately, Baltimore and Temin discovered reverse transcriptase, the enzyme that inserts viral deoxyribonucleic acid (DNA) into cellular DNA, which can cause cancer. They also identified retroviruses, a class of virus that includes the human immunodeficiency virus (HIV) that causes acquired immunodeficiency syndrome (AIDS).
- 1975 **Renato Dulbecco (Italian).** Dulbecco described how tumor viruses cause cellular transformation in somatic cells by suppressing the regulatory system that controls division; the cells then divide out of control.
- 1978 **Werner Arber (Swiss), Daniel Nathans (American), and Hamilton O. Smith (American).** Arber and the team of Nathans and Smith separately described the restriction-modification system by studying bacteria and bacteriophages; the system involves the action of site-specific endonuclease and other enzymes that cleave deoxyribonucleic acid (DNA) into segments.
- 1980 **Baruj Benacerraf (Venezuelan), Jean Dausset (French), and George D. Snell (American).** Benacerraf, Dausset, and Snell each explained the genetic components of the major histocompatibility complex (MHC), the key to a person's immune system, and how the system produces antibodies to such a wide variety of foreign molecules and pathogens, such as viruses, fungi, and bacteria.
- 1983 **Barbara McClintock (American)** investigated the genetics of maize (corn) and discovered a new mechanism of gene modification: Some "jumping genes" (now called transposable elements or transposons) move to new sites on chromosomes and either suppress nearby structural genes or inactivate suppressor genes. The discovery was a major breakthrough in understanding novel, non-Mendelian types of genetic variation.
- 1985 **Michael S. Brown and Joseph L. Goldstein (both American).** Brown and Goldstein conducted extensive research in the regulation of cholesterol in humans. They showed that in families with a history of high cholesterol, individuals who carry two copies of a mutant gene (homozygotes) have cholesterol levels several times higher than normal and those who have one mutant gene (heterozygotes) have levels about double normal. Their discoveries proved invaluable in managing heart disease and other cholesterol-related ailments.
- 1987 **Susumu Tonegawa (Japanese)** explained the diversity of antibodies by showing that the antigen-sensitive part of each antibody is created by segments of three genes; since the segments from each gene can vary in length, the possible combinations from three genes can produce billions of distinct antibodies.
- 1989 **J. Michael Bishop and Harold E. Varmus (both American).** Bishop and Varmus discovered that oncogenes (genes that play a role in initiating cancer) originate in normal cells and control cellular growth and are not solely derived from retroviruses, as previously thought. Their work greatly influenced subsequent studies of tumor development.

- 1993 **Richard J. Roberts (British) and Phillip A. Sharp (American).** Roberts and Sharp separately studied the relationship between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). They discovered that portions of a human gene can be divided among several DNA segments, called introns, separated by noncoding segments called exons. This discovery became important to genetic engineering and to understanding the mechanism for hereditary diseases.
- 1995 **Edward B. Lewis (American).** Lewis found that an array of master genes governs embryo development.
- 1995 **Christiane Nüsslein-Volhard (German) and Eric F. Wieschaus (American).** Nüsslein-Volhard and Wieschaus worked together to extend Lewis's investigations into the genetic control of embryo development through studies of fruit flies. They isolated more than five thousand participating genes and distinguished four types of "master" control genes: gap, pair-rule, segment polarity, and even-skipped.
- 2001 **Leland H. Hartwell (American), R. Timothy Hunt (British), and Paul M. Nurse (British).** Hartwell, Hunt, and Nurse conducted research on the regulation of cell cycles. Hartwell identified a class of genes that controls the cycle, including a gene that initiates it. Nurse cloned and described the genetic model of a key regulator, cyclin dependent kinase, while Hunt discovered cyclins, a class of regulatory proteins.
- 2002 **Sydney Brenner (American)** used the transparent nematode *Caenorhabditis elegans* to establish a simple model organism for studying how genes control the development of organs.
- 2002 **John E. Sulston (British)** studied cell division and cell lineages in *Caenorhabditis elegans* following Brenner's methods. He demonstrated that genetic control of specific lineages includes programmed cell death, called apoptosis, as part of the regulatory process, and he isolated the protein that degrades the deoxyribonucleic acid (DNA) of dead cells.
- 2002 **H. Robert Horvitz (American).** Using Brenner's *Caenorhabditis elegans* model, Horvitz discovered the first two "death genes" which instigate cell death. He further found that another gene helps protect cells from cell death.

Chemistry

- 1957 **Alexander Robertus Todd (British).** As part of wide-ranging research in organic chemistry, Todd revealed how ribose and deoxyribose bond to the nitrogenous bases on one side of a nucleotide unit and to the phosphate group on the other side. These discoveries provided necessary background for work by others that explained the structure of the deoxyribonucleic acid (DNA) molecule.
- 1972 **Christian B. Anfinsen (American).** Anfinsen, studying the three-dimensional structure of the enzyme ribonuclease, proved that its conformation was determined by the sequence of its amino acids and that to construct a complete enzyme molecule no separate structural information was passed on from the deoxyribonucleic acid (DNA) in the cell's nucleus.
- 1972 **Stanford Moore and William H. Stein (both American).** Moore and Stein supplemented Alfinisen's research by identifying the sequence of amino acids in ribonuclease, a clue to the structure of the gene responsible for it.
- 1980 **Paul Berg (American).** Berg invented procedures for removing a gene from a chromosome of one species and inserting it into the chromosome of an entirely different species, enabling him to study how the genetic information of the contributing organism interacts with host's deoxyribonucleic acid (DNA). The recombinant DNA technology, sometimes called gene splicing, became fundamental to the genetic engineering of transgenic species.

- 1980 **Walter Gilbert (American) and Frederick Sanger (British).** Gilbert and Sanger independently developed methods for determining the sequence of nucleic acids in DNA, thus decoding the genetic information. Gilbert's method cuts DNA into small units that reveals their structure when exposed to specific chemicals; Sanger's method separates the strands of DNA and then rebuilds them in stages that allow the terminal nucleotides to be identified. Their methods later made it possible to sequence the entire genomes of organisms.
- 1982 **Aaron Klug (British).** Klug used X-ray crystallography to investigate biochemical structures, especially that of viruses. He was able to link the assembly of viral protein subunits with specific sites on viral ribonucleic acid (RNA), which helped in fighting viruses that cause disease in plants and, more basically, in understanding the mechanism of RNA transfer of genetic information. He also determined the structure of transfer RNA (tRNA), which has a shape similar to that of a bent hair pin.
- 1989 **Sidney Altman (Canadian) and Thomas R. Cech (American).** Working independently Altman and Cech discovered that ribonucleic acid (RNA), like proteins, can act as a catalyst; moreover, Cech found that when ribosomal RNA participates in translation of mRNA and the synthesis of polypeptides, it acts as a catalyst in some steps.
- 1995 **Kary B. Mullis (American).** Mullis invented polymerase chain reaction (PCR), a method for swiftly making millions of copies of deoxyribonucleic acid (DNA). PCR soon became an important tool in genetic engineering, DNA fingerprinting, and medicine.
- 1995 **Michael Smith (British).** Smith developed site-directed mutagenesis, a means for reconfiguring genes in order to create altered proteins with distinct properties. Smith's genetic engineering tool made it possible to treat genetic disease and cancer and to create novel plant strains.
- Peace**
- 1970 **Norman Borlaug (American).** Borlaug was a key figure in the “green revolution” of agriculture. Working as a geneticist and plant physiologist in a joint Mexican-American program, he developed strains of high-yield, short-strawed, disease-resistant wheat. His goal was to increase crop production and alleviate world hunger.

—Roger Smith

Time Line of Major Developments in Genetics

- 12,000 B.C.E. Humans begin domesticating plants and animals, the earliest form of artificial selection. Domestication involves selective breeding for certain traits. This form of “genetic engineering” allows for transition from hunter-gatherer societies to agrarian civilizations.
- c. 323 B.C.E. Aristotle theorizes about the nature of species, reproduction, and hybrids.
- 1651 William Harvey publishes *Exercitationes de generatione animalium* (*Anatomical Exercitations, Concerning the Generation of Living Creatures*, 1653), in which he suggests that all living things must originate in an egg.
- 1677 Antoni van Leeuwenhoek describes sperm and eggs and collects evidence that helps disprove the theory of spontaneous generation.
- 1691-1694 German botanist Rudolph Jacob Camerarius establishes the existence of sex in plants.
- 1759 Kaspar Friedrich Wolff publishes his epigenesis hypothesis, which states that the complex structures of chickens develop from initially homogeneous, structureless areas of the embryo. Many questions remain before this new hypothesis can be validated; other researchers focus their efforts on the sea squirt, a simpler organism with fewer differentiated tissues.
- 1760 Josef Gottlieb Kölreuter conducts studies on fertilization and hybridization, discovering the principle of incomplete dominance and laying the groundwork for later hybridizers.
- 1798 Thomas Robert Malthus publishes *An Essay on the Principle of Population*, in which he analyzes population growth and relates it to the struggle for existence, setting the stage for evolutionary theory.
- 1798 Edward Jenner develops vaccination. Jenner used the cowpox virus as a vaccine to induce immunity against the genetically and structurally similar, but lethal, virus that causes smallpox in humans.
- 1809 Jean-Baptiste Lamarck publishes *Philosophie zoologique* (*Zoological Philosophy*, 1914), in which he sets forth his laws of evolution, particularly his law of acquired characteristics. Although his notion that acquired traits are individually passed to the next generation was later disproved in favor of natural selection, Lamarck’s book makes the link between evolution and inherited traits that lays a foundation for later evolutionary theory.
- 1838 G. J. Mulder precipitates a fibrous material from cells. He calls this material “protein” and believes it is the most important of the known components of living matter.

- 1850 Theodore Schwann, Matthias Jakob Schleiden, and Rudolph Virchow recognize that tissues are made up of cells. The cell theory contradicts the prevailing view of “vitalism,” which states that no single part of an organism is alive (it was thought properties of living matter were somehow shared by the whole organism). The new theory considers the cell to be the basic and most fundamental unit of life.
- 1855 Alfred Russel Wallace publishes *On the Law Which Has Regulated the Introduction of New Species*; later, in 1858, he sends Charles Darwin a manuscript, “On the Tendency of Varieties to Depart Indefinitely from the Original Type.” Today Wallace is recognized as having developed the theory of natural selection along with Darwin.
- 1857 Louis Pasteur begins research into fermentation. His “pasteurization” process is originally proposed as a means of preserving beer and wines. Through his work, Pasteur makes the important discovery that “life must be derived from life.”
- 1859 Charles Darwin publishes *On the Origin of Species by Means of Natural Selection*, in which he sets forth his theory of natural selection. The actual mechanism of evolution is not understood at the time. Once genetics was studied as a discipline, it became clear that genetics and evolution are intimately associated. Genetic theories would later explain and prove the theory of evolution.
- 1862 The Organic Act establishes the U.S. Department of Agriculture (USDA). As one of its functions, the USDA is responsible for the collection of new and valuable seeds and plants and the distribution of them to agriculturists. The preservation and dissemination of agriculturally important plants was a necessity for maintaining and increasing the world’s food supply.
- 1866 Ernst Haeckel develops the hypothesis that hereditary information is transmitted by the cell nucleus.
- 1866 Gregor Mendel, an Austrian monk, publishes a paper titled “Experiments in Plant Hybridization.” Working with garden peas, Mendel used a systematic approach to study heredity, forming the theories of segregation and independent assortment. Although his work lies unnoticed for more than thirty years, it will eventually be rediscovered and become the foundation for the discipline of genetics.
- 1869 Francis Galton publishes *Hereditary Genius*, on the heredity of intelligence, which lays the foundation for the eugenics movement.
- 1869 Friedrich Miescher isolates “nuclein” from the nuclei of white blood cells. This substance is later found to be the nucleic acids DNA and RNA.
- 1875 Oskar Hertwig, a student of Ernst Haeckel, demonstrates the fertilization of an ovum in a sea urchin, thus establishing one of the basic principles of sexual reproduction: the union of egg and sperm cells.
- 1880 Walter Fleming first describes mitosis, one of the two major processes of cell division in higher organisms (the other being meiosis). This discovery is key to the understanding of inheritance, since microscopic observations of dividing cells helped early researchers connect Mendelian genetics with cellular biology.

- 1883 Galton founds the field of eugenics with the publication of *Inquiries into Human Faculty and Its Development*. The notion that the human species can be improved by selective breeding helps perpetuate racism and provides a scientific rationale for subsequent “ethnic cleansing” programs such as those of the Nazi Party fifty years later.
- 1883 Wilhelm Roux theorizes that mitosis must result in equal sharing of all chromosomal particles by the daughter cells and describes the process, but his work is generally ignored.
- 1883 E. van Beneden studies the processes of meiosis and fertilization in the parasitic worm *Ascaris*. Van Beneden was the first to observe that the chromosome number in somatic, or body, cells is twice the number that exist in gametes, or sex cells. He also realized that when fertilization occurs (the combination of two gametes, the egg from the female and the sperm from the male), the chromosome number of somatic cells is established.
- 1883 The first absolutely pure yeast culture (yeast propagated from a single cell) is introduced at Denmark’s Carlsberg Brewery. The ability to propagate and maintain pure strains of organisms—genetically identical strains, or clones—will prove pivotal to future genetic research.
- 1886 August Weismann publishes *The Germ-Plasm: A Theory of Heredity*, in which he maintains that only the “germ cells” (eggs and sperm), not somatic cells, can transmit hereditary information and changes from one generation to the next; he disproved the Lamarckian notion of “acquired” characteristics.
- 1887-1890 Theodor Boveri investigates and describes chromosomes and their behavior, noting that they are preserved through the process of cell division and that sperm and egg contribute equal numbers of chromosomes.
- 1888-1889 Émile Maupas describes the relationship of conjugation (genetic recombination) and senescence.
- 1889 Richard Altman renames “nuclein” (isolated by Miescher in 1869) “nucleic acid.”
- 1896 Edmund B. Wilson publishes *The Cell in Development and Heredity*, in which he discusses the role of cells and chromosomes in inherited traits.
- 1897 Eduard Buchner shows that organic chemical transformations can be performed by cell extracts. He discovers that yeast extracts can convert glucose to ethyl alcohol. Buchner’s was one of the first in vitro experiments. Performing such experiments outside the body allowed researchers to control conditions and to observe the effects of individual variables.
- 1899 The Royal Horticultural Society holds a meeting in Chiswick, London, in which William Bateson calls for research on discontinuous variations. The meeting later is renamed the First International Congress of Genetics, still held annually as of 2003.
- 1900 Hugo de Vries, Erich Tschermark von Seysenegg, and Carl Correns independently rediscover and reproduce Mendel’s work. Mendel’s theories provided a framework

- for other researchers. Studies in cytology, cellular biology, plant hybridization, and biochemistry support Mendel's assertions.
- 1900 Karl Landsteiner discovers human blood groups.
- 1901 Clarence McClung describes the role of the X chromosome in determining sex.
- 1902 Lucien Cuénot, William Bateson, and others begin to confirm Mendelian inheritance in animals.
- 1902 Austrian botanist Gottlieb Haberlandt completes the cell theory with his idea of totipotency: Cells must contain all of the genetic information necessary to create an entire, multicellular organism. Therefore, every plant cell is capable of developing into an entire plant.
- 1902 William Ernest Castle, director of the Bussey Institute at Harvard University, and his students begin research into mouse genetics. His laboratory produces some of the most influential mammalian geneticists of the twentieth century, including L. C. Dunn, Clarence Little, Sewall Wright, and George Snell.
- 1902 Theodor Boveri recognizes the correlation between Mendel's laws of inheritance and current studies of cellular biology; he deduces the haploid nature of sperm and egg cells (that each had equal amounts of hereditary information) and determines, by experimenting with sea urchin sperm and egg cells, that each must contribute half the total number of chromosomes to offspring for their normal development.
- 1903 Working independently of Boveri, Walter Sutton comes to similar conclusions using grasshoppers. Both Boveri and Sutton have formed the chromosomal theory of heredity. Mendel's notions of segregation and independent assortment coincided with Sutton's observations of how chromosomes segregated during cell division. This provided a cellular explanation for Mendel's observations.
- 1903 P. A. T. Levene establishes the distinction between DNA and RNA, showing that the thymine in DNA is replaced by uracil in RNA.
- 1905 William Bateson, E. R. Saunders, and R. C. Punnett discover the phenomenon of gene linkage when they observe a violation of the Mendelian rule of independent assortment, noting two traits that do not assort independently. Instead, these genes are carried, or linked, on the same chromosome. Bateson also coins the term "genetics" to describe the science of heredity.
- 1905 Nettie Stevens and Edmund Wilson independently describe the behavior of sex chromosomes. Their observations provide the first direct evidence to support the chromosomal theory of heredity.
- 1905-1933 The eugenics movement grows in popularity. It influences social policies and immigration and sterilization laws in the United States and other countries. The idea that human traits, notably behavior, are governed by simple genetic rules was used to discriminate against the "mentally deficient," immigrants from specific countries, and

- even the poor and homeless. The U.S. eugenics movement effectively ended after the theory became associated with the policies of Nazi Germany.
- 1908 Sir Archibald Garrod proposes that some human diseases are “inborn errors of metabolism.” By studying the inheritance of human disorders, Garrod provides the first evidence of a specific relationship between genes and enzymes.
- 1908 George Shull self-pollinates plants for many generations to produce pure-breeding lines. Donald Jones performs similar experiments to increase productivity. These two researchers develop the scientific basis of modern agricultural genetics.
- 1908 Godfrey Hardy and Wilhelm Weinberg discover mathematical relationships between genotypic and phenotypic frequencies in populations. Known as the Hardy-Weinberg law, the rules governing these mathematical relationships help researchers understand the dynamics of population genetics and the evolution of species.
- 1909 Wilhelm Johannsen, working on the statistical analysis of continuous variation, expands the modern genetic vocabulary, coining the terms “gene,” “genotype,” and “phenotype.”
- 1909 Hermann Nilsson-Ehle describes another violation of Mendelian inheritance. His studies with kernel color in wheat indicate this is a polygenic trait. This was one of the first demonstrations that many genes could influence a single trait. Depending on the alleles, each gene contributes to the trait in an additive fashion. Other examples of polygenic inheritance include skin color and height in humans.
- 1909 Carl Correns discovers another class of exceptions to Mendelian inheritance, one of the first examples of extranuclear inheritance. The notion that other cellular organelles besides the nucleus carry DNA was not recognized for decades. However, Correns’s experiments in the plant *Mirabilis jalapa* showed inheritance of leaf color via the DNA in the chloroplasts.
- 1910-1928 Thomas Hunt Morgan clearly establishes the chromosomal theory of heredity after investigating a white-eyed fruit fly and finding that the trait does not segregate exactly according to Mendelian principles, but rather is influenced by the sex of the fly. This fly experiment becomes the cornerstone upon which theories of Mendelian, chromosomal, and sexual inheritance are built into a cohesive whole. Morgan also establishes the “Fly Room” at Columbia University, where he and his students will conduct groundbreaking experiments using *Drosophila* for the next quarter century. He will win the Nobel Prize in Physiology or Medicine in 1933.
- 1910 Albrecht Kossel wins the 1910 Nobel Prize in Physiology or Medicine for earlier work isolating and describing molecular constituents of the cell’s nucleus, notably cytosine, thymine, and uracil.
- 1911 Peyton Rous produces cell-free extracts from chicken tumors that, when injected, can induce tumors in other chickens. The tumor-producing agent in the extract is later found to be a virus. Thus, Rous has discovered a link between cancer and viruses. He wins the Nobel Prize in Physiology or Medicine in 1966.

- 1913 Alfred H. Sturtevant, a student of Morgan, constructs the first gene maps of chromosomes. Maps indicate the order of genes as they exist physically on the chromosome. Knowledge of gene locations on chromosomes provided insights into inheritance, genetic diseases, and the function and regulation of DNA. In addition, isolation of specific genes often required knowledge of their chromosomal location.
- 1913 Eleanor Carothers reports her discovery of the chromosomal basis of independent assortment. By examining grasshopper chromosomes, Carothers observed the behavior of the X chromosome, responsible for sex determination, during cell division. These observations corresponded with Mendel's principle of independent assortment.
- 1914 Calvin Blackman Bridges uses the phenomenon of primary nondisjunction (a fault in cell division resulting in the failure of chromosomes to separate during metaphase I) to prove that genes are carried on chromosomes.
- 1915 *The Mechanism of Mendelian Heredity*, by Morgan, Sturtevant, Muller, and Bridges, is published, establishing *Drosophila* as a model organism for genetics research and describing fundamentals of gene mapping.
- 1916 Research on the major histocompatibility complex begins with Clarence Little and E. E. Tyzzer's experiments transplanting tumors between mice.
- 1917 Félix d'Herelle discovers bacteriophages, viruses that infect bacteria. Bacteriophages played an important role in early genetics research, including confirmation that DNA is the hereditary material. Bacteriophages also became important in recombinant DNA applications.
- 1917 O. Winge publishes "The Chromosomes: Their Number and General Importance," which for the first time describes the relationship between chromosome doubling and allopolyploidy in plants.
- 1922-1932 In what would become known as the "modern synthesis," Ronald A. Fisher, J. B. S. Haldane, Sewall Wright, and S. S. Chetverikov independently publish papers on evolution, Mendelian inheritance, and natural selection, merging Darwin's theory of natural selection with Mendel's theory of genetic inheritance to create a field of population genetics that allows for genetic change through genetic drift. Haldane develops quantitative methods of studying the effects of selection, identifying the number of generations needed to alter gene frequencies for recessive and dominant traits, autosomal and sex-linked genes, and haploid and diploid organisms.
- 1925-1926 A. H. Sturtevant describes the position effect: An inversion may place a gene in another location in the chromosome, removing the gene from its regulatory elements and altering its expression. He also provides genetic proof of inversion.
- 1927 Hermann J. Muller, another student of Morgan, uses X rays to induce mutations in organisms. The ability to mutate DNA was a powerful tool to determine the function of specific genes. Muller receives the Nobel Prize in Physiology or Medicine in 1946.

- 1928 Frederick Griffith uses the bacterium that causes pneumonia to initiate his investigations into the “transforming principle,” or transformation. The hereditary material has not yet been identified, but Griffith’s experiments indicate that the transforming principle is DNA. Although not absolute proof, his experiment contributes significantly to the field and sparks ideas in other researchers.
- 1929 Clarence Little helps found the Jackson Laboratory in Bar Harbor, Maine, which will become one of the most influential genetics research institutions in North America, particularly in mouse (mammalian) genetics.
- 1931 Barbara McClintock and Harriet Creighton discover physical exchange between chromosomes in corn, a process known as “crossing over.” Curt Stern uses a similar approach in the study of the X chromosome in *Drosophila*. Crossing over, or recombination, will be vital to mapping genes on chromosomes and to understanding inheritance involving linkage.
- 1932 Sewall Wright describes the relationship of genetic drift and evolution.
- 1933 Theophilus Painter discovers polytene chromosomes in *Drosophila* salivary glands. These special chromosomes, resulting from numerous rounds of DNA replications without separation, are large, with distinct banding patterns. They are used extensively in mapping genes to specific regions of the chromosome.
- 1933 Less than 1 percent of all the agricultural land in the Corn Belt has hybrid corn growing on it. However, by 1943, hybrids cover more than 78 percent of the same land. Techniques used to produce crops with desired properties rely heavily on an understanding of genetics. Through the process of producing hybrids, researchers attempt to breed the best traits of several varieties into one. This time-consuming and inexact process is to be superseded by the techniques of recombinant DNA technology.
- 1934 John Desmond Bernal examines protein structure by using X-ray crystallography.
- 1935 Ronald Aylmer publishes statistical analyses of Mendel’s work. He finds errors in Mendel’s interpretation of his data for a series of experiments. Aylmer does not dispute Mendel’s theories but instead implies that an assistant was ultimately responsible for the error.
- 1935 Hans Spemann wins the 1935 Nobel Prize in Physiology or Medicine. By transplanting bits of one embryo into a second, viable embryo, Spemann compiled evidence that an “organizer center” directs the development of an embryo and that different parts of the organizer governed distinct portions of the embryo. His experiments provided clues to the genetic control of growth from the earliest stages of an organism.
- 1937 Theodosius Dobzhansky publishes *Genetics and the Origin of Species*. He shows that, in natural and experimental populations of *Drosophila* species, frequency changes and geographic patterns of variation in chromosome variants are consistent with the effects of natural selection.

- 1939 R. J. Gautheret demonstrates the first successful culture of isolated plant tissues as a continuously dividing callus tissue.
- 1940 Karl Landsteiner and A. S. Wiener describe the Rh blood groups.
- 1941 George Wells Beadle and Edward Tatum, working with a bread mold, *Neurospora*, publish results indicating that genes mediate cellular chemistry through the production of specific enzymes: the “one gene-one enzyme” experiment. This establishes the use of “simple” organisms as model systems to study genetics. Beadle and Tatum will receive the Nobel Prize in Physiology or Medicine in 1958.
- 1943 The Rockefeller Foundation, in collaboration with the Mexican government, initiates the Mexican Agricultural Program, the first use of plant breeding in foreign aid.
- 1944 Oswald T. Avery, Colin MacLeod, and Maclyn McCarty purify DNA and identify it as the “transforming principle” of Frederick Griffith’s work. Although this experiment provides solid evidence that DNA is the hereditary material, most scientists still do not accept the notion.
- 1945 R. D. Owen conducts studies with two sets of cattle twins which demonstrate that their blood antigens could have come only from the opposite sires. These findings suggest the reciprocal passage of ancestral red blood cells. Owen’s work has significant implications for immunology.
- 1945 Max Delbrück, Salvador Luria, and Alfred Hershey work on bacteriophage as a model system to study the mechanism of heredity. Delbrück organizes a course at Cold Spring Harbor, New York, to introduce researchers to the methods of working with bacteriophage. His course will be taught for twenty-six years, helping countless researchers to understand the use of model organisms in genetic investigations. Delbrück, Luria, and Hershey later share the 1969 Nobel Prize in Physiology or Medicine.
- 1946 Joshua Lederberg and Edward Tatum discover genetic recombination (conjugation) in bacteria, leading them to believe that bacteria, like eukaryotes, have a sexual reproductive cycle. This discovery forces researchers to realize that bacteria are genetic organisms, similar to the eukaryotes studied at the time. Lederberg wins the Nobel Prize in Physiology or Medicine in 1958; Tatum and George Beadle will also share in the 1958 prize, for their work with cellular chemistry, enzymes, and genetics.
- 1949 Linus Pauling proposes that sickle-cell disease is the result of a change in the normal amino acid sequence of hemoglobin that interferes with its binding properties. His later investigations into protein structure help determine the structure of DNA. He receives the Nobel Prize in Chemistry in 1954.
- 1950 Barbara McClintock first describes the theory that DNA is mobile and that certain of its elements can insert into different regions on the chromosome. The technical name for this phenomenon is transposition, and the genes affected are casually dubbed “jumping genes” and, more properly, transposable elements or transposons. McClintock’s ideas were far ahead of her time. While most scientists were

- still trying to determine just how DNA works, McClintock is turning the field upside down. Her work will not be accepted until more evidence of transposons surfaces decades later. She will win the Nobel Prize in Physiology or Medicine in 1983.
- 1950 Erwin Chargaff discovers consistent one-to-one ratios of adenine to thymine and of guanine to cytosine in DNA. These four chemicals are the basic building blocks of DNA. Chargaff's observations become an important clue in determining the exact structure of DNA.
- 1951 Maurice Wilkins and Rosalind Franklin obtain X-ray diffraction photographs of DNA. These data indicate the exact shape of the DNA molecule; joined with Chargaff's data, these photographs begin to bring DNA into focus.
- 1952 Joshua and Esther Lederberg and Norton Zinder discover transduction, the transfer of genetic information by viruses. Using *Escherichia coli* and a bacteriophage called *P1*, the Lederbergs and Zinder are able to show that transduction can be used to map genes to the bacterial chromosome.
- 1952 Alfred Hershey and Martha Chase use bacteriophage and a blender to identify the transforming principle as DNA. They are able to show that DNA, and not protein, is responsible for transforming organisms. This experiment forms the conclusive piece of evidence confirming that DNA is the hereditary material.
- 1952 Investigations into bacteriophage by Salvador Luria and M. L. Human, and independently J. J. Weigle, lay the groundwork for the discovery of restriction endonucleases.
- 1953 The three-dimensional structure of DNA is outlined by James Watson and Francis Crick in a 900-word manuscript published in *Nature*, "Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid." This elegant and concise paper describes the structure of DNA and provides insight into its function. Watson and Crick, along with Maurice Wilkins, will win the Nobel Prize in Physiology or Medicine in 1962. Rosalind Franklin, who with Wilkins delineated the shape of DNA, did not share in the prize, having died several years earlier of cancer, almost certainly caused by her work with X rays.
- 1954 The first whole plant is regenerated, or cloned, from a single adult plant cell by W. H. Muir and colleagues.
- 1956 J. H. Tjio and A. Levan determine the chromosome number in humans to be forty-six. Until that time, the chromosome number was thought to be forty-eight. The advances that Tjio and Levan pioneered were instrumental in obtaining good chromosome preparations, allowing for significant advances in the field of cytogenetics.
- 1956-1958 Arthur Kornberg purifies the enzyme DNA polymerase from *Escherichia coli*. This is the enzyme responsible for DNA replication, making it possible to synthesize DNA. Kornberg, along with Severo Ochoa, wins the Nobel Prize in Physiology or Medicine in 1959.

- 1957 Heinz Fraenkel-Conrat and B. Singer show that tobacco mosaic virus contains RNA—the first concrete evidence that RNA, in addition to DNA, serves as the genetic material.
- 1957 In a landmark address to the British Society of Experimental Biology titled “On Protein Synthesis,” Francis Crick articulates both the sequence hypothesis (the order of bases on a section of DNA codes for an amino acid sequence on a protein) and the “central dogma” of molecular genetics (genetic information moves from DNA to RNA to proteins, but not from proteins back to DNA).
- 1957 Alexander Robertus Todd wins the 1957 Nobel Prize in Chemistry. As part of wide-ranging research in organic chemistry, Todd revealed how ribose and deoxyribose bond to the nitrogenous bases on one side of a nucleotide unit and to the phosphate group on the other side. These discoveries provide a foundation for work by others that explains the structure of the DNA molecule.
- 1958 Matthew Meselson and Frank Stahl determine that DNA replicates in a semi-conservative manner: Each strand of the molecule serves as a template for the synthesis of a new, complementary strand.
- 1959 Jérôme Lejeune discovers that Down syndrome is caused by the presence of an extra chromosome. This was the first evidence that genetic disorders could be the result of changes in chromosome number, as opposed to changes in individual genes inherited in a Mendelian fashion.
- 1960's Mitochondria—extranuclear organelles that are the site for ATP synthesis—are discovered to have their own DNA that is passed down maternally. In 1967, Lynn Margulis, resurrecting a theory proposed by Ivan Wallin in the 1920's, proposes that mitochondria in eukaryotic cells may have evolved from a symbiotic relationship between bacteria (prokaryotes) and ancient eukarotes.
- 1961 Sol Spiegelman and Benjamin Hall discover that single-stranded DNA will hydrogen bond to its complementary RNA. The discovery of the ability of DNA and RNA to form an association contributed greatly to the study of genes and their organization.
- 1961 Working initially with Johann Matthaei, biochemist Marshall Nirenberg discovers the first sequence of three bases of DNA that codes for an amino acid and “cracks” the genetic code. H. Gobind Khorana and Robert W. Holley extend the work and elucidate how the sequence of amino acids in a protein is encoded by the sequence of nucleic acids in a gene. Nirenberg, Khorana, and Holley receive the Nobel Prize in Physiology or Medicine in 1968.
- 1961 Jacques Monod, François Jacob, Sydney Brenner, and Francis Crick discover “messenger” RNA, reporting that it is the mechanism that carries the information from DNA to create proteins. This missing link between the genetic material of DNA and proteins was critical to the understanding of protein synthesis and hence gene expression. Monod and Jacob win the 1965 Nobel Prize in Physiology or Medicine for this work.

- 1962 Mary Lyon hypothesizes that during development, one of the two X chromosomes in normal mammalian females is inactivated at random. The inactivated X chromosome is called a Barr body, and her hypothesis is known as the Lyon hypothesis.
- 1962 Werner Arber finds bacteria that are resistant to infection by bacteriophage. It appears that some cellular enzymes destroy phage DNA, while others modify the bacterial DNA to prevent self-destruction. Several years later, Arber, Stuart Linn, Matthew Meselson, and Robert Yuan isolate the first restriction endonuclease and identify the modification of bacterial DNA as methylation. By this time, scientists are looking at how DNA regulates, and is regulated by, cellular activities in a new discipline, molecular genetics. Arber wins the Nobel Prize in Physiology or Medicine in 1978.
- 1964 Robin Holliday proposes a model for the recombination of DNA. Although recombination, or crossing over, is not a new idea, the molecular mechanism behind the exchange of genetic information between DNA strands was not known. Holliday's model, widely accepted, explains the phenomenon.
- 1964 John Gurden transfers nuclei from adult toad cells into toad eggs. F. C. Steward grows single adult cells from a carrot into fully formed, normal plants. These experiments produced viable organisms, ushering in the era of cloning.
- 1964 The International Rice Research Institute introduces new strains of rice that double the yield of previous strains. This marks the beginning of the Green Revolution, which sought to enable all nations to grow sufficient quantities of food to sustain their own populations. The "father" of this movement, Norman Borlaug, will win the Nobel Peace Prize in 1970 for his role in developing high-yield grain varieties.
- 1965 Sydney Brenner and colleagues discover stop codons.
- 1965 André Lwoff shares the Nobel Prize in Physiology or Medicine with Monod and Jacob. Lwoff earlier demonstrated that the genetic material of bacteriophage can become part of the host bacterium's DNA, a process known as lysogeny.
- 1966 Victor McKusick publishes the first catalog of single genes responsible for traits, *Mendelian Inheritance in Man*, which will appear in many subsequent editions.
- 1967 Mary Weiss and Howard Green improve the process of gene mapping by using somatic cell hybridization.
- 1967 DNA ligase, the enzyme that joins DNA molecules, is discovered.
- 1968 Reiji Okazaki reports the discovery of short fragments of RNA later known as Okazaki fragments, showing the discontinuous synthesis of the lagging DNA strand.
- 1970 M. Mandel and A. Higa discover a method to increase the efficiency of bacterial transformation. They make the cells "competent" to take up DNA by treating bacteria with calcium chloride and then heat-shocking the cells. Introducing foreign DNA into cells was a key to the success of recombinant DNA methods.

- 1970 H. Gobind Khorana and twelve associates synthesize the first gene: the gene for an alanine transfer RNA in yeast.
- 1970 David Baltimore and Howard Temin independently discover reverse transcriptase, an enzyme used by viruses to convert their RNA into DNA. The reverse transcriptase enzyme becomes a key tool in genetic engineering, for which Baltimore and Temin will win the 1975 Nobel Prize in Physiology or Medicine.
- 1970 Hamilton O. Smith isolates the first restriction endonuclease that cuts at a specific DNA sequence—the first “site-specific restriction enzyme.” Daniel Nathans uses this enzyme to create a restriction map of the virus SV40. The use of restriction enzymes, those that cut DNA, allowed for the detailed mapping and analysis of genes. It also was pivotal for recombinant DNA techniques, including the production of transgenic organisms. Nathans and Smith win the Nobel Prize in Physiology or Medicine in 1978 for their work on restriction enzymes.
- 1972 Paul Berg is the first to create a recombinant DNA molecule. He shows that restriction enzymes can be used to cut DNA in a predictable manner and that these DNA fragments can be joined together with fragments from different organisms. He is awarded the Nobel Prize in Physiology or Medicine in 1980.
- 1972 Stanford Moore and William H. Stein win the 1972 Nobel Prize in Chemistry for earlier work identifying the sequence of amino acids in ribonuclease, a clue to the structure of the gene responsible for it.
- 1973 Joseph Sambrook and other researchers at Cold Spring Harbor improve the method of separating DNA fragments based on size, a technique called agarose gel electrophoresis. This method makes it possible to achieve an accurate interpretation of the information in DNA.
- 1973 Stanley Cohen and Herbert Boyer develop recombinant DNA technology by producing the first recombinant plasmid in bacteria. Plasmids—small, circular pieces of DNA—occur naturally in bacteria. Using the newly discovered tools of molecular biology, Cohen and Boyer inserted a new, or “foreign,” piece of DNA into an existing plasmid and had it propagate in a bacterial cell.
- Feb., 1975 The Asilomar Conference is held in response to increasing concerns over safety and ethics of genetic engineering. Convening in Pacific Grove, California, under the auspices of the National Institutes of Health, 140 prominent international researchers and academicians, including Nobel laureate Phillip A. Sharp, air their opinions about recombinant DNA experimentation and advocate adoption of ethical guidelines. NIH later issues guidelines for recombinant DNA research to minimize potential hazards if genetically altered bacteria were released into the environment. The guidelines will be relaxed by 1981.
- 1975 Mary-Claire King and Allan Wilson report, based on results of a survey of protein and nucleic acid studies, that the average human protein is more than 99 percent identical to that of chimpanzees, which is confirmed by later research. The question of why two species that are so different can be as genetically similar as sibling species of other organisms remains open but is assumed to be a function of

- gene regulation as well as those relatively few mutations that make human DNA different.
- 1975 Edward Southern develops a method for transferring DNA from an agarose gel to a solid membrane. This technique, known as Southern blotting, becomes one of the most important methods used to identify cloned genes.
- 1975 Renato Dulbecco, David Baltimore, and Howard Temin receive the Nobel Prize in Physiology or Medicine for their work on the interaction between tumor viruses and the genetic material of the cell. Dulbecco applied phage genetic techniques to the study of animal viruses.
- 1976 Herbert Boyer and Robert Swanson form Genentech, a company devoted to the development and promotion of biotechnology and applications of genetical engineering.
- 1976 Susumu Tonegawa discovers the genetic principles for generation of antibody diversity. Tonegawa identified a novel mode of regulation of the genetic material. The genomic DNA of immune cells is actually cut and rejoined in different combinations. This explains how millions of different antibodies can be produced from a very small number of genes. Tonegawa wins the Nobel Prize in Physiology or Medicine in 1987.
- 1977 Allan Maxam and Walter Gilbert develop a method to determine the sequence of a piece of DNA. At the same time, Frederick Sanger develops a different method, the chain termination (dideoxy) sequencing method. It becomes possible, and relatively simple, to determine the exact sequence of adenine, guanine, thymine, and cytosine in any DNA molecule. Although both the Gilbert-Maxam and the Sanger methods are effective, the Sanger method becomes the dominant technique because it does not involve toxic chemicals. Gilbert and Sanger both receive the Nobel Prize in Physiology or Medicine in 1980.
- 1977 James Alwine develops the Northern blotting technique, which expands the basic blotting technique introduced by Southern to allow analysis of RNA and proteins.
- 1977 The U.S. Court of Customs and Patent Appeals rules that an inventor can patent new forms of microorganisms. The first patent granted for a recombinant organism, an oil-eating bacterium, is awarded in 1980. The legality and ethics of patenting recombinant organisms and other biological systems are highly controversial.
- 1977 Herbert Boyer synthesizes the human hormone somatostatin in *Escherichia coli*—the first successful use of recombinant DNA to produce a substance from the gene of a higher organism. Before, the first isolation of mammalian somatostatin required a half million sheep brains to produce 5 milligrams of the hormone. Now, with the use of recombinant DNA, only two gallons of bacterial culture are required to produce the same amount.
- 1977 Phillip A. Sharp and Richard Roberts discover that portions of a human gene can be divided among several DNA segments, called introns, separated by noncoding segments called exons. This discovery becomes important to genetic engineering and

to understanding the mechanism for hereditary diseases. Sharp and Roberts win the 1993 Nobel Prize in Physiology or Medicine.

- 1978 Herbert Boyer discovers a synthetic version of the human insulin gene and inserts it into *Escherichia coli* bacteria. The bacteria serve as cloning vectors to maintain and replicate large amounts of human insulin. This application of recombinant DNA technology to produce human insulin for diabetics becomes the foundation for future industrial and medical applications of genetic engineering.
- 1978 P. C. Steptoe and R. G. Edwards successfully use in vitro fertilization and artificial implantation in humans. Louise Brown, the first "test-tube baby," is born July 25. The process gives hope to many childless couples who, prior to this development, have been unable to conceive. It also raises concerns from ethicists and others over the potential effects on both the individual child's long-term health and social implications.
- 1980 A team headed by David Botstein measures "restriction fragments" and finds that the length of such fragments often varies in individuals. Such variation, or "restriction fragment length polymorphism" (RFLP), is used to allow rapid discovery of the location of many human genes and genetic differences among individuals.
- 1980 The first transgenic mouse is created by J. W. Gordon.
- June 16, 1980 The U.S. Supreme Court votes 5-4 that living organisms can be patented under federal law, and Ananda M. Chakrabarty receives the first patent for a genetically engineered organism, a form of bacteria, *Pseudomona originosa*, that can decompose crude oil for use in cleaning up oil spills.
- 1980 George Snell, Baruj Benacerraf, and Jean Dausset win the Nobel Prize in Physiology or Medicine for their discovery of and work on the major histocompatibility complex (MHC), the key to a person's immune system, and how the system produces antibodies to such a wide variety of foreign molecules and pathogens, such as viruses, fungi, and bacteria.
- 1981 J. Michael Bishop and Harold Varmus discover that oncogenes (genes that play a role in initiating cancer) originate in normal cells as genes that control cellular growth and are not solely derived from retroviruses, as previously thought. Their work greatly influences subsequent studies of tumor development. Varmus and Bishop win the Nobel Prize in Physiology or Medicine in 1989.
- 1982 The first genetically engineered product, human insulin, dubbed Humulin, is approved for sale by the U.S. government. The production of pharmaceuticals through recombinant DNA technology is becoming a driving force behind both the drug industry and agriculture.
- 1982 Aaron Klug wins a 1982 Nobel Prize in Chemistry. Klug used X-ray crystallography to investigate biochemical structures, especially that of viruses. He was able to link the assembly of viral protein subunits with specific sites on viral RNA, which helped in fighting viruses that cause disease in plants and, more basically, in understanding the mechanism of RNA transfer of genetic information. He also determined

- the structure of transfer RNA (tRNA), which has a shape similar to that of a bent hairpin.
- 1983 Nancy Wexler, Michael Conneally, and James Gusella determine the chromosomal location of the gene for Huntington's disease. Although close, they are unable to locate the gene itself; it will be discovered ten years later.
- 1983 Thomas Cech and Sidney Altman independently discover catalytic RNA. The idea that RNA can have an enzymatic function changes researchers' views on the role of this molecule, leading to important new theories about the evolution of life. Cech and Altman win the 1989 Nobel Prize in Chemistry.
- 1983 Bruce Cattanach provides evidence of genomic imprinting in mice. The phenomenon of imprinting is the modification of genes in male and female gametes. This leads to differential expression of these genes in the embryo after fertilization. Imprinting represents another exception to the rules of Mendelian inheritance.
- 1983 John Sulston, Sydney Brenner, and H. Robert Horvitz describe the cell lineage of the nematode *Caenorhabditis elegans*. The fixed developmental pattern of this small worm provides researchers with insights into how cells determine their own fates and how they influence the fates of neighboring cells. Sulston, Brenner, and Horvitz win the 2002 Nobel Prize in Physiology or Medicine.
- 1983-1984 William Bender's laboratory isolates and characterizes the molecular details of *Drosophila* homeotic genes. William McGinnis and J. Weiner discover that the base sequences of the homeotic genes they examined contain nearly the same sequence in the terminal 180 bases. They term the conserved 180-base sequence a "homeobox." These regulatory genes direct the development of body parts during gestation of most animals.
- 1983-1985 Kary B. Mullis invents the polymerase chain reaction (PCR). This revolutionary method of copying DNA from extremely small amounts of material changes the way molecular research is done in only a few short years. It also becomes important in medical diagnostics and forensic analysis. Mullis wins the Nobel Prize in Chemistry in 1995.
- 1984 The Plant Gene Expression Center, a collaborative effort between academia and the U.S. Department of Agriculture, is established to research plant molecular biology, sequence plant genomes, and develop genetically modified plants.
- 1984 Alec Jeffreys is the first to use DNA in identifying individuals. This technique, popularly known as "DNA fingerprinting," makes identification of individuals and construction of genetic relationships virtually indisputable.
- 1984 More than twenty-five scientists collaborate to isolate the gene that causes cystic fibrosis. As a result of technological advances, the identification, isolation, and sequencing of genes is becoming commonplace. Among the notable discoveries are genes implicated in Alzheimer's disease, diabetes, and even complex conditions such as cancer and heart disease.

- 1985 Michael S. Brown and Joseph L. Goldstein win the Nobel Prize in Physiology or Medicine for their work on the regulation of cholesterol in humans. They showed that in families with a history of high cholesterol, individuals who carry two copies of a mutant gene (homozygotes) have cholesterol levels several times higher than normal, and those who have one mutant gene (heterozygotes) have levels about double normal.
- 1985-1987 Robert Sinsheimer, Renato Dulbecco, and Charles DeLisi begin investigating the possibility of sequencing the entire human genome. DeLisi, head of the Department of Energy's Office of Health and Environmental Research, seeks federal funding. After the invention of automated sequencing (see below), the National Research Council and later the Office of Technology Assessment support the idea.
- 1986 Leroy Hood, a biologist at the California Institute of Technology, invents the automated sequencer, the most important advance in DNA sequencing technology since Gilbert and Sanger developed their sequencing methods in the 1970's. Automated sequencing replaces the use of dangerous radioactive labels for identifying the four DNA bases with colored fluorescent dyes. Each of the four DNA bases is coded with a different dye color to eliminate the need to run several reactions. Laser and computer technology are integrated at the end stage to gather data. The result is safer, more accurate, and much faster sequencing.
- 1986 The first release of a genetically modified crop, genetically engineered tobacco plants, is approved by the Environmental Protection Agency.
- 1987 Frostban, a genetically engineered bacterium designed to prevent freezing, is tested on strawberries in California. These bacteria are freely released outdoors, where it is hoped they will grow on the strawberries and prevent the fruit from being destroyed by frost late in the growing season. The environmental release of recombinant organisms is an important and controversial step in the application of genetic engineering.
- 1987 Calgene receives a patent for a DNA sequence that extends the shelf life of tomatoes.
- 1987 Carol Greider and Elizabeth Blackburn, using the model organism *Tetrahymena* (a protozoan), report evidence that telomeres are regenerated through an enzyme with an RNA component. Based on the action of DNA polymerase, telomeres (located at the tips of chromosomes) should become shorter during each round of cell division. Another enzyme, called telomerase, is found to be necessary to maintain the telomeres. Research in this field sparks interest in the possibility that declining levels of telomerase may contribute to aging and that the inappropriate expression of this enzyme in cells may be a factor in cancer.
- 1988 The Human Genome Organization (HUGO) is founded to coordinate and collect data from international efforts to sequence the human genome.
- 1988 The Food and Drug Administration (FDA) approves the sale of recombinant TPA (tissue plasminogen activator) as a treatment for blood clots. TPA shows promise in helping victims recovering from heart attack and stroke.

- 1989 Francis Collins, Lap-Chee Tsui, and researchers at Toronto's Hospital for Sick Children discover the *CF* gene, which codes the cystic fibrosis transmembrane conductance regulator (CFTR) protein.
- 1990 Gene therapy for severe combined immunodeficiency disorder (SCID) is tested in clinical trials, with promising if not completely successful results.
- 1990 The Human Genome Project begins, initially headed by James Watson, under the auspices of the National Institutes of Health, National Center for Human Genome Research. The project is to be completed by the year 2005. The ambitious project is designed to sequence the entire human genome in order to identify genes involved in biochemical processes such as disease pathology. Also included as part of the Human Genome Project is the sequencing of many model organisms.
- 1990 At the Plant Gene Expression Center, biologist Michael Fromm announces the use of a high-speed "gene gun" to transform corn. Gene guns are used to inject genetic material directly into cells via DNA-coated microparticles.
- 1990 The first human undergoes gene therapy. The patient is a four-year-old girl who was born without a functioning immune system as a result of a faulty gene that makes an enzyme called ADA (adenosine deaminase).
- 1991 J. Craig Venter of the National Institutes of Health demonstrates the use of automated sequencing and expressed sequence tags (ESTs)—cloned sequences of complementary DNA (cDNA) molecules stored in "libraries"—to identify genes and their functions rapidly and accurately.
- 1992 One of the first major accomplishments of the Human Genome Project is to publish a low-resolution linkage map of the entire human genome.
- 1993 The mutation that causes Huntington's disease is found, ten years after its chromosomal location was first identified. Fifty-eight scientists collaborated on the project.
- 1993 Gene therapy cures a mouse of cystic fibrosis.
- 1994 The Food and Drug Administration (FDA) approves the bovine hormone known as BST or BGH. The hormone is made from recombinant bacteria containing the bovine gene for BST. When injected into cows, the hormone increases milk production by up to 20 percent. Many supermarkets and manufacturers of dairy products refuse to carry or use milk from BST-injected cows, uncertain of what long-term effects this recombinant drug might have on the food chain.
- 1994 The Food and Drug Administration (FDA) gives approval for the marketing of the Flavr Savr tomato. This genetically altered tomato can be ripened on the vine before being picked and transported. Because the ripening process takes longer, the tomatoes do not rot on their way to the market.
- 1994 Alfred G. Gilman and Martin Rodbell receive the Nobel Prize in Physiology or Medicine for discovering the role of "G" proteins in regulating signal transduction in eukaryotic cells.

- 1995 A mutation in the gene *BRCA1*, found by Mark Skolnick and others, is implicated in breast cancer. More than any other gene previously identified, this discovery has wide potential for assessing cancer risk.
- 1995 J. Craig Venter of The Institute for Genome Research (TIGR) announces completion of the first DNA sequence of a nonviral, self-replicating, free-living organism, the bacterium *Haemophilus influenzae*, using “whole-genome random sequencing,” nicknamed “shotgun” sequencing. This method, which precludes the need for a preliminary physical map of the genome, speeds the sequencing of other organisms significantly.
- 1995 Completion of the sequence of the smallest known bacterium, *Mycoplasma genitalium*, identifies the minimum number of genes required for independent life.
- 1995 Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric Wieschaus win the Nobel Prize in Physiology or Medicine for their work on the genetic control of early development in *Drosophila*. These researchers took the fruit fly, a model organism from the age of classical genetics, into the age of molecular biology and discovered how genetics controls development. The same developmental mechanisms appear to be at work in other organisms, including humans.
- 1995 Nüsslein-Volhard completes a genetic mutation project involving zebra fish. Repeating her earlier work with *Drosophila*, Nüsslein-Volhard used similar techniques to begin an intensive study of development in a vertebrate system. This involved screening thousand of mutants to determine if any had developmental defects.
- 1995 Michael Smith wins the 1995 Nobel Prize in Chemistry for developing “site-directed mutagenesis,” a means of reconfiguring genes in order to create altered proteins with distinct properties. Smith’s technique makes it possible to treat genetic disease and cancer and to create novel plant strains.
- 1996 Kristen L. Kroll and Enrique Amaya create a technique to make stable transgenic *Xenopus* (frog) embryos.
- 1996 A group of more than six hundred researchers sequences the DNA of the yeast *Saccharomyces cerevisiae*, the first eukaryotic organism to be sequenced.
- 1997 Ian Wilmut at the Roslin Institute in Scotland announces the successful cloning of a sheep. The clone is named Dolly, the first vertebrate cloned from the cell of an adult vertebrate. It is hoped that successful cloning of a mammal will allow for easier and cheaper development and propagation of transgenic animals.
- 1997 The United Nations Educational, Scientific, and Cultural Organization (UNESCO) adopts the Universal Declaration on the Human Genome and Human Rights.
- 1997 The genomic sequence of the bacterium *Escherichia coli* is reported by Frederick Blattner and colleagues. Although *E. coli* is not the first complete bacterial sequence reported, because of the importance of *E. coli*, the event represents a critical step forward.

- 1998 The genome of the bacterium *Mycobacterium tuberculosis* is sequenced.
- 1998 Celera Genomics is founded by former National Institutes of Health researcher J. Craig Venter. Its mission is to sequence the human genome in the private sector, using fast-working automated sequencers.
- 1998 The genome of the nematode *Caenorhabditis elegans* is the first genome of a multicellular organism to be completely sequenced.
- 1999 Laboratory tests suggest that the pollen of corn bioengineered to release the pesticide *Bacillus thuringiensis* (*Bt*) endangers monarch butterfly caterpillars. Although later evidence calls the finding into question, it prompts controversy over the safety of transgenic plants.
- Sept., 1999 The first human death attributable to gene therapy during clinical trial is reported when an eighteen-year-old participant in a trial on gene therapy for hereditary ornithine transcarbamylase (OTC) deficiency dies of multiorgan failure caused by a severe immunological reaction to the disarmed adenovirus vector used in the trial.
- Sept., 1999 Celera Genomics sequences the full genome of *Drosophila* and reports the results the following year in the May 24 issue of *Science*. Of the fly's 13,601 genes, many are shown to be closely related to human genes.
- Dec. 1, 1999 The first human chromosome, chromosome 22, is completely sequenced.
- Jan. 28, 2000 At a meeting in Montreal, Canada, the United Nations Convention on Biological Diversity approves the Cartegena Protocol on Biosafety, which sets the criterion internationally for patenting genetically modified organisms, including agricultural products.
- 2000 Chromosome 21, the smallest human chromosome, is completely sequenced; it is the second human chromosome to be completed.
- 2000 It is estimated that more than two-thirds of the processed foods in U.S. markets contain genetically modified ingredients, primarily soybeans or corn.
- 2000 The environmental organization Friends of the Earth reveals that StarLink, a genetically engineered corn variety meant only for animal fodder, has contaminated the human food supply. The news ignites public debate over the use of genetically modified food crops.
- Dec. 13, 2000 At a press conference, a team of more than three hundred scientists from throughout the world announce that they have sequenced the genome of a plant for the first time. The plant is the model organism *Arabidopsis thaliana*.
- 2001 The third and fourth human chromosomes, chromosomes 20 and 14, are completely sequenced.
- Feb., 2001 The first working drafts of the human genome sequence are published in *Science* (which reports the results from the private company Celera Genomics, headed by

J. Craig Venter) and in *Nature* (reporting the results from the publicly funded Human Genome Project). The relatively low number of human genes, estimated to be about 30,000, makes it necessary to revise the “one gene-one enzyme” hypothesis, since it appears that a single gene can encode more than one protein. The principle is therefore renamed the “one gene-one polypeptide” hypothesis. The paper published in *Science* notes that the DNA of all human beings is 99.9 percent the same, which redefines the notion of human “races” as primarily a social, rather than a biological, construct.

- 2001 Researchers complete the genomic sequence for rice, *Oryza sativa*.
- Nov., 2001 Scientists report that genetic material from transgenic corn has mysteriously turned up in the genome of native corn species near Oaxaca, Mexico. Mexico banned transgenic crops three years earlier, and the closest known crop was located beyond the range of windborne pollen. The report raises concerns about the unintended ecological consequences of transgenic-wild hybrids, which could create problems such as “superweeds.”
- 2001 Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse win the 2001 Nobel Prize in Physiology or Medicine for their research on the regulation of cell cycles. Hartwell identified a class of genes that controls the cycle, including a gene that initiates it. Nurse cloned and described the genetic model of a key regulator, cyclin dependent kinase (cdk), and Hunt discovered cyclins, a class of regulatory proteins.
- Dec., 2002 The mouse genome sequence is completed, using the shotgun method; it is compared with the draft of the human genome and found to be very similar; both organisms have about 30,000 genes and about 2,000 non-gene, or “junk DNA,” regions.
- Jan., 2003 A company called Clonaid announces the births of several babies they claim are the result of human cloning but later fails to produce any scientific evidence that the babies are clones. The apparent hoax, initially a media event, energizes the public debate over human cloning and its ramifications.
- Feb., 2003 Dolly, the first vertebrate cloned from an adult cell, is euthanized after suffering advanced arthritis and lung disease. Researchers speculate about whether clones age prematurely as a result of the shortened telomere length of the chromosomes in the adult cells from which they are cloned.
- Feb. 27, 2003 The U.S. House of Representatives passes the Human Prohibition Cloning Act of 2003, banning the cloning of human beings; the bill goes to the Senate.
- April, 2003 The Human Genome Project completes its mission two years ahead of schedule: The entire human genome has now been sequenced.

—Nancy Morvillo, updated by Christina J. Moose

Glossary

A: the abbreviation for adenine, a purine nitrogenous base found in the structure of both DNA and RNA.

acentric chromosome: a chromosome that does not have a centromere and that is unable to participate properly in cell division; often the result of a chromosomal mutation during recombination.

acquired characteristic: a change in an individual organism brought about by its interaction with its environment.

acrocentric chromosome: a chromosome with its centromere near one end. *See also* metacentric chromosome and telocentric chromosome.

activator: a protein that binds to DNA, thus increasing the expression of a nearby gene.

active site: the region of an enzyme that interacts with a substrate molecule; any alteration in the three-dimensional shape of the active site usually has an adverse effect on the enzyme's activity.

adaptation: the evolution of a trait by natural selection, or a trait that has evolved as a result of natural selection.

adaptive advantage: increased reproductive potential in offspring as a result of passing on favorable genetic information.

adenine (A): a purine nitrogenous base found in the structure of both DNA and RNA.

adenosine triphosphate (ATP): the major energy molecule of cells, produced either through the process of cellular respiration or fermentation; it is also a component of DNA and RNA.

adult stem cell: an undifferentiated cell found among differentiated cells in a tissue or organ of an adult organism.

agarose: a chemical substance derived from algae and used to create gels for the electrophoresis of nucleic acids.

aggression: behavior directed toward causing harm to others.

Agrobacterium tumefaciens: a species of bacteria that causes disease in some plants and is able to transfer genetic information, in the form

of Ti plasmids, into plant cells; modified Ti plasmids can be used to produce transgenic plants.

albinism: the absence of pigment such as melanin in eyes, skin, hair, scales, or feathers or of chlorophyll in plant leaves and stems.

albino: a genetic condition in which an individual does not produce the pigment melanin in the skin; other manifestations of the trait may be seen in the pigmentation of the hair or eyes; albino individuals occur in many animals and plants and are due to the absence of a variety of different pigments.

alcoholism: a medical diagnosis given when there is repeated use of alcohol over the course of at least a year, despite the presence of negative consequences; tolerance, withdrawal, uncontrolled use, unsuccessful efforts to quit, considerable time spent getting or using the drug, and a decrease in other important activities because of the use are part of this condition.

algorithm: a mathematical rule or procedure for solving a specific problem.

alkaptonuria: a genetic disorder, first characterized by geneticist Archibald Garrod, in which a compound called homogentisic acid accumulates in the cartilage and is excreted in the urine of affected individuals, turning both of these black (the name of the disorder literally means "black urine"); the specific genetic defect involves an inability to process by-products of phenylalanine and tyrosine metabolism.

allele: a form of a gene at a locus; each locus in an individual's chromosomes has two alleles, which may be the same or different.

allele frequency: the proportion of all the genetic variants at a locus within a population of organisms.

allergy: an abnormal immune response to a substance that does not normally provoke an immune response or that is not inherently dangerous to the body (such as plant pollens, dust, or animal dander).

allopatric speciation: a model of speciation in

which parts of a population may become geographically isolated, effectively preventing interbreeding, and over time may develop differences that lead to reproductive isolation and the development of a new species.

allopolyploid: a type of polyploid species that contains genomes from more than one ancestral species.

altruism: behavior that benefits others at the evolutionary (reproductive) cost of the altruist.

Alu sequence: a repetitive DNA sequence of unknown function, approximately three hundred nucleotides long, scattered throughout the genome of primates; the name comes from the presence of recognition sites for the restriction endonuclease Alu I in these sequences.

Alzheimer's disease: a degenerative brain disorder, usually found among the elderly, characterized by brain lesions leading to loss of memory, personality changes, and deterioration of higher mental functions.

amber codon: a stop codon (UAG) found in messenger RNA (mRNA) molecules that signals termination of translation.

ambiguous genitalia: external sexual organs that are not clearly male or female.

Ames test: a test devised by molecular biologist Bruce Ames for determining the mutagenic or carcinogenic properties of various compounds based on their ability to affect the nutritional characteristics of the bacterium *Salmonella typhimurium*.

amino acid: a nitrogen-containing compound used as the building block of proteins (polypeptides); in nature, there are twenty amino acids that can be used to build proteins.

aminoacyl tRNA: a transfer RNA (tRNA) molecule with an appropriate amino acid molecule attached; in this form, the tRNA molecule is ready to participate in translation.

amniocentesis: a procedure in which a small amount of amniotic fluid containing fetal cells is withdrawn from the amniotic sac surrounding a fetus; fetal cells, found in the fluid, are then tested for the presence of genetic abnormalities.

amniotic fluid: the fluid in which the fetus is immersed during pregnancy.

amyloid plaques: protein deposits in the brain formed by fragments from amyloid precursor proteins; amyloid plaques are characteristic of Alzheimer's disease.

anabolic steroids: drugs derived from androgens and inappropriately used to enhance performance in sports.

anabolism: the part of the cell's metabolism concerned with synthesis of complex molecules and cell structures.

anaphase: the third phase in the process of mitosis; in anaphase, sister chromatids separate at the centromere and migrate toward the poles of the cell.

anaphylaxis: a severe, sometimes fatal allergic reaction often characterized by swelling of the air passages, leading to inability to breathe.

ancient DNA: DNA isolated from archaeological artifacts or fossils; it is typically extensively degraded.

androgen receptors: molecules in the cytoplasm of cells that join with circulating male hormones.

androgens: steroid hormones that cause masculinization.

anencephalus: a neural tube defect characterized by the failure of the cerebral hemispheres of the brain and the cranium to develop normally.

aneuploid: a cell or individual with one or a few missing or extra chromosomes.

angstrom: a unit of measurement equal to one ten-millionth of a millimeter; a DNA molecule is 20 angstroms wide.

animal cloning: animal cloning is the process of generating a genetic duplicate of an animal starting with one of its differentiated cells.

annealing: the process by which two single-stranded nucleic acid molecules are converted into a double-stranded molecule through hydrogen bonding between complementary base pairs.

anthrax: an acute bacterial disease caused by *Bacillus anthracis* that affects animals and humans and that is especially deadly in its pulmonary form.

antibiotic: any substance produced naturally by a microorganism that inhibits the growth of other microorganisms; antibiotics are im-

- portant in the treatment of bacterial infections.
- antibody:** an immune protein (immunoglobulin) that specifically recognizes an antigen; produced by B cells of the immune system.
- anticodon:** the portion of a transfer RNA (tRNA) molecule that is complementary in sequence to a codon in a messenger RNA (mRNA) molecule; because of this complementarity, the tRNA molecule can bind briefly to mRNA during translation and direct the placement of amino acids in a polypeptide chain.
- antigen:** any molecule that is capable of being recognized by an antibody molecule or of provoking an immune response.
- antigenic drift or shift:** minor changes in the H and N proteins of the influenza virus that enable the virus to evade the immune system of a potential host.
- antioxidant:** a molecule that preferentially reacts with free radicals, thus keeping them from reacting with other molecules and causing cellular damage.
- antiparallel:** a characteristic of the Watson-Crick double-helix model of DNA, in which the two strands of the molecule can be visualized as oriented in opposite directions; this characteristic is based on the orientation of the deoxyribose molecules in the sugar-phosphate backbone of the double helix.
- antirejection medication:** drugs developed to counteract the human body's natural immune system's reaction to transplanted organs.
- antisense:** a term referring to any strand of DNA or RNA that is complementary to a coding or regulatory sequence, for example, the strand opposite the coding strand (the sense strand) in DNA is called the antisense strand.
- antisense RNA:** a small RNA molecule that is complementary to the coding region of a messenger RNA (mRNA) and when bound to the mRNA prevents it from being translated.
- antitoxin:** a vaccine containing antibodies against a specific toxin.
- Apo-B:** a protein essential for cholesterol transport.

- apoptosis:** cell "suicide" occurring after a cell is too old to function properly, as a response to irreparable genetic damage, or as a function of genetic programming; apoptosis prevents cells from developing into a cancerous state and is a natural event during many parts of organismal development.
- Archaea:** the domain of life that includes diverse prokaryotic organisms distinct from the historically familiar Bacteria and which often require severe conditions for growth, such as high temperatures, high salinity, or lack of oxygen.
- artificial selection:** selective breeding of desirable traits, typically in domesticated organisms.
- ascomycetes:** organisms of the phylum *Ascomycota*, a group of fungi known as the sac fungi, which are characterized by a saclike structure, the ascus.
- ascospore:** a haploid spore produced by meiosis in ascomycete fungi.
- ascus:** a reproductive structure, found in ascomycete fungi, that contains ascospores.
- asexual reproduction:** reproduction of cells or organisms without the transfer or reassortment of genetic material; results in offspring that are genetically identical to the parent.
- assortative mating:** mating that occurs when individuals make specific mate choices based on the phenotype or appearance of others.
- ATP:** See adenosine triphosphate.
- ATP synthase:** the enzyme that synthesizes ATP.
- autoimmune disorders:** chronic diseases that arise from a breakdown of the immune system's ability to distinguish between the body's own cells (self) and foreign substances, leading to an individual's immune system attacking the body's own organs or tissues.
- autoimmune response:** an immune response of an organism against its own cells.
- automated fluorescent sequencing:** a modification of dideoxy termination sequencing which uses fluorescent markers to identify the terminal nucleotides, allowing the automation of sequencing.
- autopolyploid:** a type of polyploid species that contains more than two sets of chromosomes from the same species.

autosomal dominant allele: an allele of a gene (locus) on one of the nonsex chromosomes that is always expressed, regardless of the form of the other allele at the same locus.

autosomal recessive allele: an allele of a gene (locus) that will be expressed only if there are two identical copies at the same locus.

autosomal trait: a trait that typically appears just as frequently in either sex because an autosomal chromosome, rather than a sex chromosome, carries the gene.

autosomes: non-sex chromosomes; humans have forty-four autosomes.

auxotrophic strain: a mutant strain of an organism that cannot synthesize a substance required for growth and therefore must have the substance supplied in the growth medium.

azoospermia: the absence of spermatozoa in the semen.

B cells: a class of white blood cells (lymphocytes) derived from bone marrow and responsible for antibody-directed immunity.

B-DNA: the predominant form of DNA in solution and in the cell; a right-handed double helix most similar to the Watson-Crick model. *See also Z-DNA.*

B lymphocytes: *See B cells.*

B memory cells: descendants of activated B cells that are long-lived and that synthesize large amounts of antibodies in response to a subsequent exposure to the antigen, thus playing an important role in secondary immunity.

***Bacillus thuringiensis* (Bt):** a species of bacteria that produces a toxin deadly to caterpillars, moths, beetles, and certain flies.

backcross: a cross involving offspring crossed with one of the parents. *See also cross.*

bacterial artificial chromosomes (BACs): cloning vectors used to clone large DNA fragments (up to 500 kb) that can be readily inserted in a bacterium, such as *Escherichia coli*.

bacteriophage: a virus that infects bacterial cells; often simply called a phage.

baculovirus: a type of virus that is capable of causing disease in a variety of insects.

Barr body: a darkly staining structure primarily present in female cells, believed to be an in-

active X chromosome; used as a demonstration of the Lyon hypothesis.

base: a chemical subunit of DNA or RNA that encodes genetic information; in DNA, the bases are adenine (A), cytosine (C), guanine (G), and thymine (T); in RNA, thymine is replaced by uracil (U).

base pair (bp): often used as a measure of the size of a DNA fragment or the distance along a DNA molecule between markers; both the singular and plural are abbreviated bp.

base pairing: the process by which bases link up by hydrogen bonding to form double-stranded molecules of DNA or loops in RNA; in DNA, adenine (A) always pairs with thymine (T), and cytosine (C) pairs with guanine (G); in RNA, uracil (U) replaces thymine.

beta-amyloid peptide: the main constituent of the neuritic plaques in the brains of Alzheimer's patients.

bidirectional replication: a characteristic of DNA replication involving synthesis of DNA in both directions away from an origin of replication.

binary fission: cell division in prokaryotes in which the plasma membrane and cell wall grow inward and divide the cell in two.

biochemical pathway: the steps in the production or breakdown of biological chemicals in cells; each step usually requires a specific enzyme.

bioethics: the study of human actions and goals in a framework of moral standards relating to use and abuse of biological systems.

bioinformatics: the application of information technology to the management of biological information to organize data and extract meaning; a hybrid discipline that combines elements of computer science, information technology, mathematics, statistics, and molecular genetics.

biological clocks: genetically and biochemically based systems that regulate the timing and/or duration of biological events in an organism; examples of processes controlled by biological clocks include circadian rhythms, cell cycles, and migratory restlessness.

biological determinism: the concept that all

- characteristics of organisms, including behavior, are determined by the genes the organism possesses; it is now generally accepted that the characteristics of organisms are determined both by genes and environment.
- biological weapon (BW):** a delivery system or “weaponization” of such pathological organisms as bacteria and viruses to cause disease and death in people, animals, or plants.
- biometry:** the measurement of biological and psychological variables.
- biopesticides:** chemicals or other agents derived from or involving living organisms that can be used to control the population of a pest species.
- bioremediation:** biologic treatment methods to clean up contaminated water and soils.
- biotechnology:** the use of biological molecules or organisms in industrial or commercial products and techniques.
- bioterrorism:** use of organisms as instruments or weapons of terror; for example, deliberate introduction of smallpox, anthrax, or other diseases in civilian populations.
- blastocyst:** a preimplantation embryo consisting of a hollow ball of two layers of cells.
- blood type:** one of the several groups into which blood can be classified based on the presence or absence of certain molecules called antigens on the red blood cells.
- blotting:** the transfer of nucleic acids or proteins separated by gel electrophoresis onto a filter paper, which allows access by molecules that will interact with only one specific sequence or molecule.
- BRCA1 and BRCA2 genes:** the best known examples of genes associated with inherited breast cancers.
- Bt toxin:** a toxic compound naturally synthesized by bacterium *Bacillus thuringiensis*, which kills insects.
- C:** the abbreviation for cytosine, a pyrimidine nitrogenous base found in the structure of both DNA and RNA.
- C terminus:** the end of a polypeptide with an amino acid that has a free carboxyl group.
- C-value:** the characteristic genome size for a species.
- CAG expansion:** a mutation-induced increase

- in the number of consecutive CAG nucleotide triplets in the coding region of a gene.
- callus:** a group of undifferentiated plant cells growing in a clump.
- cAMP:** See cyclic adenosine monophosphate.
- cancer:** a disease in which there is unrestrained growth and reproduction of cells, loss of contact inhibition, and, eventually, metastasis (the wandering of cancer cells from a primary tumor to other parts of the body); invasion of various tissues and organs by cancer cells typically leads to death.
- capsid:** the protective protein coating of a virus particle.
- carcinogen:** any physical or chemical cancer-causing agent.
- carrier:** a healthy individual who has one normal allele and one defective allele for a recessive genetic disease.
- catabolism:** the part of the cell’s metabolism concerned with the breakdown of complex molecules, usually as an energy-generating mechanism.
- catabolite repression:** a mechanism of operon regulation involving an enzyme reaction’s product used as a regulatory molecule for the operon that encodes the enzyme; a kind of feedback inhibition.
- cDNA:** See complementary DNA.
- cDNA library:** a collection of clones produced from all the RNA molecules in the cells of a particular organism, often from a single tissue. *See also* complementary DNA.
- cell culture:** growth and maintenance of cells or tissues in laboratory vessels containing a precise mixture of nutrients and hormones.
- cell cycle:** the various growth phases of a cell, which include (in order) G1 (gap phase 1), S (DNA synthesis), G2 (gap phase 2), and M (mitosis).
- cell differentiation:** a process during which a cell becomes specialized as a specific type of cell, such as a neuron, or undergoes programmed cell death (apoptosis).
- cell line:** a cell culture maintained for an indefinite time.
- cell signaling:** communication between cells that occurs most commonly when one cell releases a specific “signaling” molecule that is received and recognized by another cell.

centiMorgan (cM): a unit of genetic distance between genes on the same chromosome, equal to a recombination frequency of 1 percent; also called a map unit, since these distances can be used to construct genetic maps of chromosomes.

central dogma: a foundational concept in modern genetics stating that genetic information present in the form of DNA can be converted to the form of messenger RNA (or other types of RNA) through transcription and that the information in the form of mRNA can be converted into the form of a protein through translation.

centriole: a eukaryotic cell structure involved in cell division, possibly with the assembly or disassembly of the spindle apparatus during mitosis and meiosis; another name for this organelle is the microtubule organizing center (MTOC).

centromere: a central region where a pair of chromatids are joined before being separated during anaphase of mitosis or meiosis; also, the region of the chromatids where the microtubules of the spindle apparatus attach.

checkpoint: the time in the cell cycle when molecular signals control entry to the next phase.

chemical mutagens: chemicals that can directly or indirectly create mutations in DNA.

chiasma (pl. chiasmata): the point at which two homologous chromosomes exchange genetic material during the process of recombination; the word literally means “crosses,” which refers to the appearance of these structures when viewed with a microscope.

chi-square analysis: a nonparametric statistical analysis of data from an experiment to determine how well the observed data correlate with the expected data.

chloroplast: the cellular organelle in plants responsible for photosynthesis.

chloroplast DNA (cpDNA): circular DNA molecules found in multiple copies in chloroplasts; they contain some of the genes required for chloroplast functions.

cholera: an intestinal disease caused by the bacteria *Vibrio cholerae* which is often spread by water contaminated with human waste.

chorionic villus sampling: a procedure in which fetal cells are obtained from an embryonic structure called the chorion and analyzed for the presence of genetic abnormalities in the fetus.

chromatid: one half of a chromosome that has been duplicated in preparation for mitosis or meiosis; each chromatid is connected to its sister chromatid by a centromere.

chromatin: the form chromosomes take when not undergoing cell division; a complex of fibers composed of DNA, histone proteins, and nonhistone proteins.

chromatography: a separation technique involving a mobile solvent and a stationary, adsorbent phase.

chromosome: the form in which genetic material is found in the nucleus of a cell; composed of a single DNA molecule that is extremely tightly coiled, usually visible only during the processes of mitosis and meiosis.

chromosome jumping: similar to chromosome walking, but involving larger fragments of DNA and thus resulting in faster analysis of longer regions of DNA. *See also* chromosome walking.

chromosome map: a diagram showing the locations of genes on a particular chromosome; generated through analysis of linkage experiments involving those genes.

chromosome mutation: a change in chromosome structure caused by chromosome breakage followed by improper rejoining; examples include deletions, insertions, inversions, and translocations.

chromosome puff: an extremely unwound or uncoiled region of a chromosome indicative of a transcriptionally active region of the chromosome.

chromosome theory of inheritance: a concept, first proposed by geneticists Walter Sutton and Theodor Boveri, that genes are located on chromosomes and that the inheritance and movement of chromosomes during meiosis explain Mendelian principles on the cellular level.

chromosome walking: a molecular genetics technique used for analysis of long DNA fragments; the name comes from the technique of using previously cloned and charac-

terized fragments of DNA to “walk” into uncharacterized regions of the chromosome that overlap with these fragments. *See also* chromosome jumping.

circadian rhythm: a cycle of behavior, approximately twenty-four hours long, that is expressed independent of environmental changes.

cirrhosis: a disease of the liver, marked by the development of scar tissue that interferes with organ function, that can result from chronic alcohol consumption.

cistron: a unit of DNA that is equivalent to a gene; it encodes a single polypeptide.

clinical trial: an experimental research study used to determine the safety and effectiveness of a medical treatment or drug.

clone: a molecule, cell, or organism that is a perfect genetic copy of another.

cloning: the technique of making a perfect genetic copy of an item such as a DNA molecule, a cell, or an entire organism.

cloning vector: a DNA molecule that can be used to transport genes of interest into cells, where these genes can then be copied.

codominance: a genetic condition involving two alleles at a locus in a heterozygous organism; each of these alleles is fully expressed in the phenotype of the organism.

codon: a group of three nucleotides in messenger RNA (mRNA) that represent a single amino acid in the genetic code; this is mediated through binding of a transfer RNA (tRNA) anticodon to the codon during translation.

color blindness: an inherited condition in people whose eyes lack one or more of the three color receptors.

complementary base pairing: hydrogen bond formation in DNA and RNA that occurs only between cytosine and guanine (in both DNA and RNA) or between adenine and thymine (in DNA) or adenine and uracil (in RNA).

complementary DNA (cDNA): a DNA molecule that is synthesized using messenger RNA (mRNA) as a template and which is catalyzed by the enzyme reverse transcriptase.

complementation testing: performing a cross between two individuals with the same phe-

notype to determine whether or not the mutations occur within the same gene.

composite transposon: a transposable element that contains genes other than those required for transposition.

concerted evolution: a process in which the members of a gene family evolve together.

concordance: the presence of a trait in both members of a pair of twins.

cones: the light-sensitive structures in the retina that are the basis for color vision.

congenital defect: a defect or disorder that occurs during prenatal development.

conjugation: a form of genetic transfer among bacterial cells involving the F pilus.

consanguine: of the same blood or origin; in genetics, the term implies the sharing of genetic traits or characteristics from the same ancestors (as cousins, for example).

consensus sequence: a sequence with no or only slight differences commonly found in DNA molecules from various sources, implying that the sequence has been actively conserved and plays an important role in some genetic process.

cosmid: a cloning vector partially derived from genetic sequences of lambda, a bacteriophage; cosmids are useful in cloning relatively large fragments of DNA.

cross: the mating of individuals to produce offspring by sexual reproduction.

crossing over: the exchange of genetic material between two homologous chromosomes during prophase I of meiosis, providing an important source of genetic variation; also called “recombination” or crossover.

cultivar: a variety of plant developed through controlled breeding techniques.

cyclic adenosine monophosphate (cAMP): an important cellular molecule involved in cell signaling and regulation pathways.

cyclins: a group of eukaryotic proteins with characteristic patterns of synthesis and degradation during the cell cycle; part of an elaborate mechanism of cell cycle regulation, and a key to the understanding of cancer.

cystic fibrosis: the most common recessive lethal inherited disease among Caucasians in the United States and the United Kingdom.

cytogenetics: the study of chromosome num-

- ber and structure, including identification of abnormalities.
- cytokines:** soluble intercellular molecules produced by cells such as lymphocytes that can influence the immune response.
- cytokinesis:** the division of the cytoplasm, typically occurring in concert with nuclear division (mitosis or meiosis).
- cytoplasmic inheritance:** *See* extranuclear inheritance.
- cytosine (C):** a pyrimidine nitrogenous base found in the structure of both DNA and RNA.
- cytoskeleton:** the structure, composed of microtubules and microfilaments, that gives shape to a eukaryotic cell, enables some cells to move, and assists in such processes as cell division.
- dalton:** a unit of molecular weight equal to the mass of a hydrogen atom; cellular molecules such as proteins are often measured in terms of a kilodalton, equal to 1,000 daltons.
- daughter cells:** cells that result from cell division.
- deamination:** the removal of an amino group from an organic molecule.
- degenerate:** refers to a property of the genetic code via which two or more codons can code for the same amino acid.
- deletion:** a type of chromosomal mutation in which a genetic sequence is lost from a chromosome, usually through an error in recombination.
- denaturation:** changes in the physical shape of a molecule caused by changes in the immediate environment, such as temperature or pH level; denaturation usually involves the alteration or breaking of various bonds within the molecule and is important in DNA and protein molecules.
- deoxyribonucleic acid (DNA):** the genetic material found in all cells; DNA consists of nitrogenous bases (adenine, guanine, cytosine, and thymine), sugar (deoxyribose), and phosphate.
- deoxyribose:** a five-carbon sugar used in the structure of DNA.
- diabetes:** a syndrome in which the body cannot metabolize glucose appropriately.
- diakinesis:** a subphase of prophase I in meiosis in which chromosomes are completely condensed and position themselves in preparation for metaphase.
- dicentric chromosome:** a chromosome with two centromeres, usually resulting from an error of recombination.
- dideoxy termination sequencing:** *See* Sanger sequencing.
- differentiation:** the series of changes necessary to convert an embryonic cell into its final adult form, usually with highly specialized structures and functions.
- dihybrid:** an organism that is hybrid for each of two genes—for example, *AaBb*; when two dihybrid organisms are mated, the offspring will appear in a 9:3:3:1 ratio with respect to the traits controlled by the two genes.
- diphtheria:** an acute bacterial disease caused by *Corynebacterium diphtheriae*; symptoms are primarily the result of a toxin released by the bacteria.
- diploid:** a cell or organism with two complete sets of chromosomes, usually represented as $2N$, where N stands for one set of chromosomes; for example, humans have two sets of twenty-three chromosomes in their somatic cells, making them diploid.
- diplotene:** a subphase of prophase I in meiosis in which synapsed chromosomes begin to move apart and the chiasmata are clearly visible.
- discontinuous replication:** replication on the lagging strand of a DNA molecule, resulting in the formation of Okazaki fragments. *See also* Okazaki fragments.
- discontinuous variation:** refers to a set of related phenotypes that are distinct from one another, with no overlapping.
- disjunction:** the normal division of chromosomes that occurs during meiosis or mitosis; the related term “nondisjunction” refers to problems with this process.
- disomy:** a case in which both copies of a chromosome come from a single parent, rather than (as is usual) one being maternal and one being paternal.
- dizygotic:** developed from two separate zygotes; fraternal twins are dizygotic because they develop from two separate fertilized ova (eggs).

DNA: See deoxyribonucleic acid.

DNA fingerprinting: a DNA test used by forensic scientists to aid in the identification of criminals or to resolve paternity disputes which involves looking at known, highly variable DNA sequences; more correctly called DNA genotyping.

DNA footprinting: a molecular biology technique involving DNA-binding proteins that are allowed to bind to DNA; the DNA is then degraded by DNases, and the binding sites of the proteins are revealed by the nucleotide sequences protected from degradation.

DNA gyrase: a bacterial enzyme that reduces tension in DNA molecules that are being unwound during replication; a type of cellular enzyme called a topoisomerase.

DNA library: a collection of cloned DNA fragments from a single source, such as a genome, chromosome, or set of messenger RNA (mRNA) molecules; most common examples are genomic and cDNA libraries.

DNA ligase: a cellular enzyme used to connect pieces of DNA together; important in genetic engineering procedures.

DNA polymerase: the cellular enzyme responsible for making new copies of DNA molecules through replication of single-stranded DNA template molecules or, more rarely, using an RNA template molecule as in the case of RNA-dependent DNA polymerase or reverse transcriptase.

DNA replication: synthesis of new DNA strands complementary to template strands resulting in new double-stranded DNA molecules comprising the old template and the newly synthesized strand joined by hydrogen bonds; described as a semiconservative process in that half (one strand) of the original template is retained and passed on.

DNase: refers to a class of enzymes, deoxyribonucleases, which specifically degrade DNA molecules.

domain: the highest-level division of life, sometimes called a superkingdom.

dominant: an allele or a trait that will mask the presence of a recessive allele or trait.

dosage compensation: an equalization of gene products that can occur whenever there are

more or fewer genes for specific traits than normal.

double helix: a model of DNA structure proposed by molecular biologists James Watson and Francis Crick; the major features of this model are two strands of DNA wound around each other and connected by hydrogen bonds between complementary base pairs.

down-regulation: generally used in reference to gene expression and refers to reducing the amount that a gene is transcribed and/or translated; up-regulation is the opposite.

Down syndrome: a genetic defect caused by possession of an extra copy of chromosome 21; symptoms include mental retardation, mongoloid facial features, and premature aging.

downstream: in relation to the left-to-right direction of DNA whose nucleotides are arranged in sequence with the 5' carbon on the left and the 3' on the right, downstream is to the right.

drug resistance: a phenomenon in which pathogens no longer respond to drug therapies that once controlled them; resistance can arise by recombination, by mutation, or by several methods of gene transfer, and is made worse by misuse of existing drugs.

duplication: a type of chromosomal mutation in which a chromosome region is duplicated because of an error in recombination during prophase I of meiosis; thought to play an important role in gene evolution.

dwarfism: the condition of adults of short stature who are less than 50 inches in height, which can be caused by genetic factors, endocrine malfunction, acquired conditions, or growth hormone deficiency; many dwarfs prefer to be called “little people.”

E. coli: See *Escherichia coli*.

electron transport chain: a series of protein complexes that use high-energy electrons to do work such as pumping H⁺ ions out of the mitochondrial matrix into the intermembrane space as a way of storing energy that is then used by ATP synthase to make ATP.

electrophoresis: See gel electrophoresis.

embryo: the term for a complex organism (particularly humans) during its earliest period of development, the stage of development that begins at fertilization and ends with the eighth week of development, after which the embryo is called a fetus.

embryology: the study of developing embryos.

embryonic stem cell: a cell derived from an early embryo that can replicate indefinitely *in vitro* and can differentiate into other cells of the developing embryo.

emerging disease: a disease whose incidence in humans or other target organisms has increased.

endemic: prevalent and recurring in a particular geographic region; for example, an organism that is specific to a particular region is characterized as endemic to that region.

endocrine gland: a gland that secretes hormones into the circulatory system.

endonuclease: an enzyme that degrades a nucleic acid molecule by breaking phosphodiester bonds within the molecule.

endosymbiotic hypothesis: a hypothesis stating that mitochondria and chloroplasts were once free-living bacteria that entered into a symbiotic relationship with early pre-eukaryotic cells; structural and genetic similarities between these organelles and bacteria provide support for this hypothesis.

enhancer: a region of a DNA molecule that facilitates the transcription of a gene, usually by stimulating the interaction of RNA polymerase with the gene's promoter.

enzyme: a protein that acts as a catalyst to speed up or facilitate a specific biochemical reaction in a cell.

epigenesis: the formation of differentiated cell types and specialized organs from a single, homogeneous fertilized egg cell without any preexisting structural elements.

epistasis: a genetic phenomenon in which a gene at one locus influences the expression of a second gene at another locus, usually by masking the effect of the second gene; however, only one trait is being controlled by these two genes, so epistasis is characterized by modified dihybrid ratios.

equational division: refers to meiosis II, in which the basic number of chromosomes

types remains the same although sister chromatids are separated from one another; after equational division occurs, functional haploid gametes are present.

***Escherichia coli*:** a bacterium widely studied in genetics research and extensively used in biotechnological applications.

estrogens: steroid hormones or chemicals that stimulate the development of female sexual characteristics and control the female reproductive cycle.

ethidium bromide: a chemical substance that inserts itself (intercalates) into the DNA double helix; when exposed to ultraviolet light, ethidium bromide fluoresces, making it useful for the visualization of DNA molecules in molecular biology techniques.

etiology: the cause or causes of a disease or disorder.

euchromatin: chromatin that is loosely coiled during interphase; thought to contain transcriptionally active genes.

eugenics: a largely discredited field of genetics that seeks to improve humankind by selective breeding; can be positive eugenics, in which individuals with desirable traits are encouraged or forced to breed, or negative eugenics, in which individuals with undesirable traits are discouraged or prevented from breeding.

eukaryote: a cell with a nuclear membrane surrounding its genetic material (a characteristic of a true nucleus) and a variety of subcellular, membrane-bound organelles; eukaryotic organisms include all known organisms except bacteria, which are prokaryotic. *See also* prokaryote.

euploid: the normal number of chromosomes for a cell or organism.

eusociality: an extreme form of altruism and kin selection in which most members of the society do not reproduce but rather feed and protect their relatives; bees, for example, are eusocial.

euthanasia: the killing of suffering individuals; sometimes referred to as “mercy” killing.

exogenous gene: a gene produced or originating from outside an organism.

exon: a protein-coding sequence in eukaryotic genes, usually flanked by introns.

exonuclease: an enzyme that degrades a nucleic acid molecule by breaking phosphodiester bonds at either end of the molecule.

expressed sequence tags (EST): an STS (sequence tagged site) that has been derived from a cDNA library.

expression vector: a DNA cloning vector designed to allow genetic expression of inserted genes via promoters engineered into the vector sequence.

expressivity: the degree to which a genotype is expressed as a phenotype.

extranuclear inheritance: inheritance involving genetic material located in the mitochondria or chloroplasts of a eukaryotic cell; also known as maternal inheritance (because these organelles are generally inherited from the mother) and cytoplasmic inheritance (because the organelles are found in the cell's cytoplasm rather than its nucleus).

extreme halophiles: microorganisms that require extremely high salt concentrations for optimal growth.

F pilus: also called the fertility pilus; a reproductive structure found on the surface of some bacterial cells that allows the cells to exchange plasmids or other DNA during the process of conjugation.

F₁ generation: first filial generation; offspring produced from a mating of P (parental) generation individuals.

F₂ generation: second filial generation; offspring produced from a mating of F₁ generation individuals.

fate map: a description of the adult fate of embryonic cells.

fertilization: the fusion of two cells (egg and sperm) in sexual reproduction.

fitness: a measure of the ability of a genotype or individual to survive and reproduce; when fitness is compared to other genotypes or individuals it is called relative fitness.

fluorescent in situ hybridization (FISH): an extremely sensitive assay for determining the presence of deletions on chromosomes, which uses a fluorescence-tagged segment of DNA that binds to the DNA region being studied.

foreign DNA: DNA taken from a source other than the host cell that is joined to the DNA of the cloning vector; also known as "insert DNA."

forensic genetics: the use of genetic tests and principles to resolve legal questions.

formylmethionine (fMet): the amino acid used to start all bacterial proteins; it is attached to the initiator transfer RNA (tRNA) molecule.

frameshift mutation: a DNA mutation involving the insertion or deletion of one of several nucleotides that are not in multiples of three, resulting in a shift of the codon reading frame; usually produces nonfunctional proteins. *See also* open reading frame; reading frame.

fraternal twins: twins that develop and are born simultaneously but are genetically unique, being produced from the fertilization of two separate eggs; a synonymous term is "dizygotic twins."

free radical: *See* oxygen free radical.

G: the abbreviation for guanine, a purine nitrogenous base found in the structure of both DNA and RNA.

G₀: a point in the cell cycle at which a cell is no longer progressing toward cell division; can be considered a "resting" stage.

G₁ checkpoint: a point in the cell cycle at which a cell commits either to progressing toward cell division (by replicating its DNA and eventually engaging in mitosis) or to entering the G₀ phase, thereby withdrawing from the cell cycle either temporarily or permanently.

gamete: a sex cell, either sperm or egg, containing half the genetic material of a normal cell.

gel electrophoresis: a technique of molecular biology in which biological molecules are placed into a gel-like matrix (such as agarose or polyacrylamide) and then subjected to an electric current; using this technique, researchers can separate molecules of varying sizes and properties.

GenBank: a comprehensive, annotated collection of publicly available DNA sequences maintained by the National Center for

Biotechnology Information and available through its Web site.

gene: a portion of a DNA molecule containing the genetic information necessary to produce a molecule of messenger RNA (via the process of transcription) that can then be used to produce a protein (via the process of translation); also includes regions of DNA that are transcribed to RNA that does not get translated, but carries out other roles in the cell.

gene expression: the combined biochemical processes, called “transcription” and “translation,” that convert the linearly encoded information in the bases of DNA into the three-dimensional structures of proteins.

gene families: multiple copies of the same or similar genes in the same genome; the copies can be identical and tandemly repeated, or they may differ slightly and be scattered on the same or different chromosomes.

gene flow: movement of alleles from one population to another by the movement of individuals or gametes.

gene frequency: the occurrence of a particular allele present in a population, expressed as a percentage of the total number of alleles present for the locus.

gene pool: the complete assortment of genes present in the gametes of the members of a population that are eligible to reproduce.

gene silencing: any form of genetic regulation in which the expression of a gene is completely repressed, either by preventing transcription (pre-transcriptional gene silencing) or after a messenger RNA (mRNA) has been transcribed (post-transcriptional gene silencing).

gene therapy: any procedure to alleviate or treat the symptoms of a disease or condition by genetically altering the cells of the patient.

gene transfer: the movement of fragments of genetic information, whole genes, or groups of genes between organisms.

genetic code: the correspondence between the sequence of nucleotides in DNA or messenger RNA (mRNA) molecules and the amino acids in the polypeptide a gene codes for.

genetic counseling: a discipline concerned with analyzing the inheritance patterns of a

particular genetic defect within a given family, including the determination of the risk associated with the presence of the genetic defect in future generations and options for treatment of existing genetic defects.

genetic drift: chance fluctuations in allele frequencies within a population, resulting from random variation in the number and genotypes of offspring produced by different individuals.

genetic engineering: a term encompassing a wide variety of molecular biology techniques, all concerned with the modification of genetic characteristics of cells or organisms to accomplish a desired effect.

genetic load: the average number of the recessive deleterious (lethal or sublethal) alleles in individuals in a population.

genetic map: a “map” showing distances between genes in terms of recombination frequency; using DNA sequence data a physical map with distance in base pairs can also be produced.

genetic marker: a distinctive DNA sequence that shows variation in the population and can therefore potentially be used for identification of individuals and for discovery of disease genes.

genetic screening: the testing of individuals for disease-causing genes or genetic disease.

genetic testing: the use of the techniques of genetics research to determine a person’s risk of developing, or status as a carrier of, a disease or other disorder.

genetically modified (GM) foods: foods produced through the application of recombinant DNA technology, whereby genes from the same or different species are transferred and expressed in crops that do not naturally harbor those genes.

genetically modified organism (GMO): an organism produced by using biotechnology to introduce a new gene or genes, or new regulatory sequences for genes, into it for the purpose of giving the organism a new trait, usually to adapt the organism to a new environment, provide resistance to pest species, or enable the production of new products from the organism. *See also* transgenic organism.

genetics: an area of biology involving the scientific study of heredity.

genome: all of the DNA in the nucleus or in one of the organelles, such as a chloroplast or mitochondrion.

genomic imprinting: a genetic phenomenon in which the phenotype associated with a particular allele depends on which parent donated the allele.

genomic library: a collection of clones that includes the entire genome of a single species as fragments ligated to vector DNA.

genomics: that branch of genetics dealing with the study of genetic sequences, including their structure and arrangement.

genotype: the genetic characteristics of a cell or organism, expressed as a set of symbols representing the alleles present at one or more loci.

germ cells: reproductive cells such as eggs and sperm.

germ-line gene therapy: a genetic modification in gametes or fertilized ova so all cells in the organism will have the change which potentially can be passed on to offspring.

germ-line mutation: a heritable change in the genes of an individual's reproductive cells, often linked to hereditary diseases.

gonad: an organ that produces reproductive cells and sex hormones; termed ovaries in females and testes in males.

Green Revolution: the introduction of scientifically bred or selected varieties of grain (such as rice, wheat, and maize) that, with high enough inputs of fertilizer and water, can greatly increase crop yields.

guanine (G): a purine nitrogenous base found in the structure of both DNA and RNA.

H substance: a carbohydrate molecule on the surface of red blood cells; when modified by certain monosaccharides, this molecule provides the basis of the ABO blood groups.

haplodiploidy: a system of sex determination in which males are haploid (developing from unfertilized eggs) and females are diploid.

haploid: refers to a cell or an organism with one set of chromosomes; usually represented as the N number of chromosomes, with $2N$

standing for the diploid number of chromosomes.

haplotype: a sequential set of genes on a single chromosome inherited together from one parent; the other parent provides a matching chromosome with a different set of genes.

Hardy-Weinberg law: a concept in population genetics stating that, given an infinitely large population that experiences random mating without mutation or any other such affecting factor, the frequency of particular alleles will reach a state of equilibrium, after which their frequency will not change from one generation to the next.

HeLa cells: the first human tumor cells shown to form a continuous cell line; they were derived from a cervical cancer tumor removed from a woman known as Henrietta Lacks.

helicase: a cellular enzyme that breaks hydrogen bonds between the strands of the DNA double helix, thus unwinding the helix and facilitating DNA replication.

helper T cells: a class of white blood cells (lymphocytes) derived from bone marrow that prompts the production of antibodies by B cells in the presence of an antigen.

hemizygous: characterized by having a gene present in a single copy, such as any gene on the X chromosome in a human male.

hemoglobin: a molecule made up of two alpha and two beta amino acid chains whose precise chemical and structural properties normally allow it to bind with oxygen in the lungs and transport it to other parts of the body.

hemophilia: an X-linked recessive disorder in which an individual's blood does not clot properly because of a lack of blood-clotting factors; as in all X-linked recessive traits, the disease is most common in males, the allele for the disease being passed from mother to son.

heredity: the overall mechanism by which characteristics or traits are passed from one generation of organisms to the next; genetics is the scientific study of heredity.

heritability: a proportional measure of the extent to which differences among organisms within a population for a particular charac-

- ter result from genetic rather than environmental causes (a measure of nature versus nurture).
- hermaphrodite:** an individual who has both male and female sex organs.
- heterochromatin:** a highly condensed form of chromatin, usually transcriptionally inactive.
- heterochrony:** a change in the timing or rate of development of characters in an organism relative to those same events in its evolutionary ancestors.
- heteroduplex:** a double-stranded molecule of nucleic acid with each strand from a different source, formed either through natural means such as recombination or through artificial means in the laboratory.
- heterogametic sex:** the particular sex of an organism that produces gametes containing two types of sex chromosome; in humans, males are the heterogametic sex, producing sperm that can carry either an X chromosome or a Y chromosome.
- heterogeneous nuclear RNA (hnRNA):** an assortment of RNA molecules of various types found in the nucleus of the cell and in various stages of processing prior to their export to the cytoplasm.
- heterozygote:** an individual with two different alleles at a gene locus.
- heterozygous:** composed of two alleles that are different, for example *Aa*; synonymous with “hybrid.”
- histones:** specialized proteins in eukaryotic cells that bind to DNA molecules and cause them to become more compact; thought to be involved in regulation of gene expression as well.
- HLA:** *See* human leukocyte antigens.
- hnRNA:** *See* heterogeneous nuclear RNA.
- holandric:** refers to a trait passed from father to son via a sex chromosome such as the Y chromosome in human males.
- homeobox:** a DNA sequence encoding a highly basic protein known as a homeodomain; a homeodomain functions as a transcription factor and is thought to help regulate major events in the embryonic development of higher organisms.
- homeotic gene:** a gene that helps determine body plan early in development; the products of homeotic genes are transcription factors that control the expression of other genes.
- homogametic sex:** the particular sex of an organism that produces gametes containing only one type of sex chromosome; in humans, females are the homogametic sex, producing eggs with X chromosomes.
- homologous:** refers to chromosomes that are identical in terms of types of genes present and the location of the centromere; because of their high degree of similarity, homologous chromosomes can synapse and recombine during prophase I of meiosis.
- homology:** similarity resulting from descent from a common evolutionary ancestor.
- homozygote:** an individual with two identical alleles at a gene locus.
- homozygous:** characterized by a genotype composed of two alleles at the same locus that are the same, for example *AA* or *aa*; synonymous with “purebred.”
- Human Genome Project:** a multi-year genetic research endeavor to sequence the entire human genome, as well as the genomes of related organisms; the human genome sequence was officially completed in 2003.
- human leukocyte antigens (HLA):** molecules found on the surface of cells that allow the immune system to differentiate between foreign, invading cells and the body’s own cells.
- hybrid:** any cell or organism with genetic material from two different sources, through either natural processes such as sexual reproduction or more artificial processes such as genetic engineering.
- hybridization:** a process of base pairing involving two single-stranded nucleic acid molecules with complementary sequences; the extent to which two unrelated nucleic acid molecules will hybridize is often used as a way to determine the amount of similarity between the sequences of the two molecules.
- hybridoma:** a type of hybrid cancer cell created by artificially joining a cancer cell with an antibody-producing cell; hybridomas have useful applications in immunological research.
- hydrogen bond:** a bond formed between molecules containing hydrogen atoms with posi-

tive charges and molecules containing atoms such as nitrogen or oxygen that can possess a negative charge; a relatively weak but important bond in nature that, among other things, connects water molecules, allows DNA strands to base-pair, and contributes to the three-dimensional shape of proteins.

identical twins: a pair of genetically identical offspring that develop from a single fertilized egg; also known as monozygotic twins.

immune system: the system in the body that normally responds to foreign agents by producing antibodies and stimulating antigen-specific lymphocytes, leading to destruction of these agents.

in vitro: literally, “in glass”; an event occurring in an artificial setting such as in a test tube, as opposed to inside a living organism.

in vivo: literally, “in the living”; an event occurring in a living organism, as opposed to an artificial setting.

inborn error of metabolism: a genetic defect in one of a cell’s metabolic pathways, usually at the level of an enzyme, that causes the pathway to malfunction; results in phenotypic alterations at the cellular or organismal level.

inbreeding: mating between genetically related individuals.

inbreeding depression: a reduction in the health and vigor of offspring from closely related individuals, a common and widespread phenomenon among nonhuman organisms.

inclusive fitness: an individual’s total genetic contribution to future generations, comprising both direct fitness, which results from individual reproduction, and indirect fitness, which results from the reproduction of close relatives.

incomplete dominance: a phenomenon involving two alleles, neither of which masks the expression of the other; instead, the combination of the alleles in the heterozygous state produces a new phenotype that is usually intermediate to the phenotypes produced by either allele alone in the homozygous state.

independent assortment: a characteristic of

standard Mendelian genetics referring to the random assortment or shuffling of alleles and chromosomes that occurs during meiosis I; independent assortment is responsible for the offspring ratios observed in Mendelian genetics.

inducer: a molecule that activates some bacterial operons, usually by interacting with regulatory proteins bound to the operator region.

induction: a process in which a cell or group of cells signals an adjacent cell or group of cells to pursue a different developmental pathway and so become differentiated from neighboring cells.

informed consent: the right of patients to know the risks of medical treatment and to determine what is done to their bodies, including the right to accept or refuse treatment based on this information.

initiation codon: also called the “start codon,” a codon, composed of the nucleotides AUG, that signals the beginning of a protein-coding sequence in a messenger RNA (mRNA) molecule; in the genetic code, AUG always represents the amino acid methionine.

insert DNA: *See* foreign DNA.

insertion sequence: a small, independently transposable genetic element.

intelligence quotient (IQ): the most common measure of intelligence; it is based on the view that there is a single capacity for complex mental work and that this capacity can be measured by testing.

intercalary deletion: a type of chromosome deletion in which DNA has been lost from within the chromosome (as opposed to a terminal deletion involving a region of DNA lost from the end of the chromosome).

interference: in genetic linkage, a mathematical expression that represents the difference between the expected and the observed number of double recombinant offspring; this can be a clue to the physical location of linked genes on the chromosome.

interphase: the period of the cell cycle in which the cell is preparing to divide, consisting of two distinct growth phases (G_1 and G_2) separated by a period of DNA replication (S phase).

introgression: the transfer of genes from one species to another or the movement of genes between species (or other well-marked genetic populations) mediated by backcrossing.

intron: an intervening sequence within eukaryotic DNA, transcribed as part of a messenger RNA (mRNA) precursor but then removed by splicing before the mRNA molecule is translated; introns are thought to play an important role in the evolution of genes.

inversion: a chromosomal abnormality resulting in a region of the chromosome where the normal order of genes is reversed.

isotope: an alternative form of an element with a variant number of neutrons in its atomic nucleus; isotopes are frequently radioactive and are important tools for numerous molecular biology techniques.

jumping: *See* chromosome jumping

junk DNA: a disparaging (and now known to be inaccurate) characterization of the noncoding DNA content of a genome.

karyokinesis: division of a cell's nuclear contents, as opposed to cytokinesis (division of the cytoplasm). *See also* cytokinesis.

karyotype: the complete set of chromosomes possessed by an individual, usually isolated during metaphase and arranged by size and type as a method of detecting chromosomal abnormalities.

kilobase (kb): a unit of measurement for nucleic acid molecules, equal to 1,000 bases or nucleotides.

kinase: an enzyme that catalyzes phosphate addition to molecules.

kinetochore: a chromosome structure found in the region of the centromere and used as an attachment point for the microtubules of the spindle apparatus during cell division.

Klinefelter syndrome: a human genetic disorder in males who possess an extra X chromosome; Klinefelter males have forty-seven chromosomes instead of the normal forty-six and suffer from abnormalities such as sterility, body feminization, and mental retardation.

knockout: the inactivation of a specific gene within a cell (or whole organism, as in the case of knockout mice) to determine the effects of loss of function of that gene.

lactose: a disaccharide that is an important part of the metabolism of many bacterial species; lactose metabolism in these species is genetically regulated via the *lac* operon.

lagging strand: in DNA replication, the strand of DNA being synthesized in a direction opposite to that of replication fork movement; this strand is synthesized in a discontinuous fashion as a series of Okazaki fragments later joined together. *See also* Okazaki fragments.

Lamarckianism: the theory, originally proposed by Jean-Baptiste Lamarck, that traits acquired by an organism during its lifetime can be passed on to offspring.

lambda (λ) phage: a bacteriophage that infects bacteria and then makes multiple copies of itself by taking over the infected bacteria's cellular machinery.

lateral gene transfer: the movement of genes between organisms; also called horizontal gene transfer.

leading strand: in DNA replication, the strand of DNA being synthesized in the same direction as the movement of the replication fork; this strand is synthesized in a continuous fashion.

leptotene: a subphase of prophase I of meiosis in which chromosomes begin to condense and become visible.

lethal allele: an allele capable of causing the death of an organism; a lethal allele can be recessive (two copies of the allele are required before death results) or dominant (one copy of the allele produces death).

leucine zipper: an amino acid sequence, found in some DNA-binding proteins, characterized by leucine residues separated by sets of seven amino acids; two molecules of this amino acid sequence can combine via the leucine residues and "zip" together, creating a structure that can then bind to a specific DNA sequence.

linkage: a genetic phenomenon involving two or more genes inherited together because they are physically located on the same chro-

mosome; Gregor Mendel's principle of independent assortment does not apply to linked genes, but genotypic and phenotypic variation is possible through crossing over.

linkage mapping: a form of genetic mapping that uses recombination frequencies to estimate the relative distances between linked genes.

locus (*pl. loci*): the specific location of a particular gene on a chromosome.

lymphocytes: sensitized cells of the immune system that recognize and destroy harmful agents via antibody and cell-mediated responses that include B lymphocytes from the bone marrow and T lymphocytes from the thymus.

Lyon hypothesis: a hypothesis stating that one X chromosome of the pair found in all female cells must be inactivated in order for those cells to be normal; the inactivated X chromosome is visible by light microscopy and stains as a Barr body.

lysis: the breaking open of a cell.

lysogeny: a viral process involving repression and integration of the viral genome into the genome of the host bacterial cell.

major histocompatibility complex (MHC): a group of molecules found on the surface of cells, allowing the immune system to differentiate between foreign, invading cells and the body's own cells; in humans, this group of molecules is called HLA (human leukocyte antigens). *See also* human leukocyte antigens.

map unit: *See* centiMorgan.

maternal inheritance: *See* extranuclear inheritance.

Maxam-Gilbert sequencing: a method of base-specific chemical degradation to determine DNA sequence; this method has largely been supplanted by the Sanger method. *See also* Sanger sequencing.

meiosis: a process of cell division in which the cell's genetic material is reduced by half and sex cells called gametes are produced; important as the basis of sexual reproduction.

melanism: the opposite of albinism, a condition that leads to the overproduction of melanin.

melting: a term sometimes used to describe the denaturation of a DNA molecule as it is heated in solution; as the temperature rises, hydrogen bonds between the DNA strands are broken until the double-strand molecule has been completely converted into two single-strand molecules.

Mendelian genetics: the genetics of traits that show simple inheritance patterns; based on the work of Gregor Mendel, a nineteenth century monk who studied the genetics of pea plants.

messenger RNA (mRNA): a type of RNA molecule containing the genetic information necessary to produce a protein through the process of translation; produced from the DNA sequence of a gene in the process of transcription.

metabolic pathway: a series of enzyme-catalyzed reactions leading to the breakdown or synthesis of a particular biological molecule.

metabolism: the collection of biochemical reactions occurring in an organism.

metacentric chromosome: a chromosome with the centromere located at or near the middle of the chromosome. *See also* acrocentric chromosome; telocentric chromosome.

metafemale: a term used to describe *Drosophila* (fruit fly) females that have more X chromosomes than sets of autosomes (for example, a female that has two sets of autosomes and three X chromosomes); also used in reference to human females with more than two X chromosomes.

metaphase: the second phase in the process of mitosis, involving chromosomes lined up in the middle of the cell on a line known as the equator.

methylation: the process of adding a methyl chemical group (one carbon atom and three hydrogen atoms) to a particular molecule, such as to the base portion of a nucleotide in a DNA nucleotide.

metric trait: *See* quantitative trait.

microarray: a flat surface on which 10,000 to 100,000 tiny spots of short DNA molecules (oligonucleotides) are fixed and are used to detect the presence of DNA or RNA molecules that are homologous to the oligonucleotides.

microsatellite DNA: a type of variable number tandem repeat (VNTR) in which the repeated motif is 1 to 6 base pairs; also called a simple sequence repeat (SSR) or a short tandem repeat (STR).

microtubule: a cell structure involved in the movement and division of chromosomes during mitosis and meiosis; part of the cell's cytoskeleton, microtubules can be rapidly assembled and disassembled.

microtubule organizing center (MTOC): *See* centriole.

minimal media: an environment that contains the simplest set of ingredients that a microorganism can use to produce all the substances required for reproduction and growth.

minisatellite DNA: a type of variable number tandem repeat (VNTR) in which the repeated motif is 12 to 500 base pairs in length.

miscegenation: sexual activity or marriage between members of two different human races.

mismatch repair: a cellular DNA repair process in which improperly base-paired nucleotides are enzymatically removed and replaced with the proper nucleotides.

missense mutation: a DNA mutation that changes an existing amino acid codon in a gene to some other amino acid codon; depending on the nature of the change, this can be a harmless or a serious mutation (for example, sickle-cell disease in humans is the result of a missense mutation).

mitochondrial genome (mtDNA): DNA found in mitochondria, which contains some of the genes that code for proteins involved in energy metabolism; it is a circular molecule similar in structure to the genome of bacteria.

mitochondrion: the organelle responsible for production of ATP through the process of cellular respiration in a eukaryotic cell; sometimes referred to as the "powerhouse of the cell."

mitosis: a process of cell division in which a cell's duplicated genetic material is evenly divided between two daughter cells, so that each daughter cell is genetically identical to the original parent cell.

model organism: an organism well suited for genetic research because it has a well-known genetic history, a short life cycle, and genetic variation between individuals in the population.

modern synthesis: the merging of the Darwinian mechanisms for evolution with Mendelian genetics to form the modern fields of population genetics and evolutionary biology; also called the neo-Darwinian synthesis.

molecular clock hypothesis: a hypothesis that predicts that amino acid changes in proteins and nucleotide changes in DNA are approximately constant over time.

molecular cloning: the process of splicing a piece of DNA into a plasmid, virus, or phage vector to obtain many identical copies of that DNA.

molecular genetics: the branch of genetics concerned with the central role that molecules, particularly the nucleic acids DNA and RNA, play in heredity.

monoclonal antibodies: identical antibodies (having specificity for the same antigen) produced by a single type of antibody-producing cell, either a B cell or a hybridoma cell line; important in various types of immunology research techniques.

monoculture: the agricultural practice of growing the same cultivar on large tracts of land.

monohybrid: an organism that is hybrid with respect to a single gene (for example, *Aa*); when two monohybrid organisms are mated, the offspring will generally appear in a 3:1 ratio involving the trait controlled by the gene in question.

monosomy: a genetic condition in which one chromosome from a homologous chromosome pair is missing, producing a $2n-1$ genotype; usually causes significant problems in the phenotype of the organism.

monozygotic: developed from a single zygote; identical twins are monozygotic because they develop from a single fertilized ovum that splits in two.

morphogen: a protein or other molecule made by cells in an egg that creates a concentration gradient affecting the developmental fate of surrounding cells by altering their

- gene expression or their ability to respond to other morphogens.
- morphogenesis:** the induction and formation of organized body parts or organs.
- mosaicism:** a condition in which an individual has two or more cell populations derived from the same fertilized ovum, or zygote, as in sex chromosome mosaics, in which some cells contain the usual XY chromosome pattern and others contain extra X chromosomes.
- mRNA:** *See* messenger RNA.
- mtDNA:** *See* mitochondrial genome.
- MTOC:** *See* centriole.
- multifactorial:** characterized by a complex interaction of genetic and environmental factors.
- multiple alleles:** a genetic phenomenon in which a particular gene locus is represented by more than two alleles in a population; the greater the number of alleles, the greater the genetic diversity.
- mutagen:** any chemical or physical substance capable of increasing mutations in a DNA sequence.
- mutant:** a trait or organism different from the normal, or wild-type, trait or organism seen commonly in nature; mutants can arise either through expression of particular alleles in the organism or through spontaneous or intentional mutations in the genome.
- mutation:** a change in the genetic sequence of an organism, usually leading to an altered phenotype.
- N terminus:** the end of a polypeptide with an amino acid that has a free amino group.
- natural selection:** a process involving genetic variation on the genotypic and phenotypic levels that contributes to the success or failure of various species in reproduction; thought to be the primary force behind evolution.
- negative eugenics:** improving human stocks through the restriction of reproduction by individuals with inferior traits or who are known to carry alleles for inferior traits.
- neural tube:** the embryonic precursor to the spinal cord and brain, which normally closes at small openings, or neuropores, by the twenty-eighth day of gestation in humans.

- neurotransmitter:** a chemical that carries messages between nerve cells.
- neutral mutation:** a mutation in a gene, or some other portion of the genome, that is considered to have no effect on the fitness of the organism.
- neutral theory of evolution:** Motoo Kimura's theory that nucleotide substitutions in the DNA often have no effect on fitness, and thus changes in allele frequencies in populations are caused primarily by genetic drift.
- nondisjunction:** refers to the improper division of chromosomes during anaphase of mitosis or meiosis, resulting in cells with abnormal numbers of chromosomes and sometimes seriously altered phenotypes.
- nonhistone proteins:** a heterogeneous group of acidic or neutral proteins found in chromatin that may be involved with chromosome structure, chromatin packaging, or the control of gene expression.
- nonsense codon:** another term for a termination or stop codon (UAA, UAG, or UGA).
- nonsense mutation:** a DNA mutation that changes an existing amino acid codon in a message to one of the three termination, or stop, codons; this results in an abnormally short protein that is usually nonfunctional.
- Northern blot:** a molecular biology procedure in which a labeled single-stranded DNA probe is exposed to cellular RNA immobilized on a filter; under the proper conditions, the DNA probe will seek out and bind to its complementary sequence in the RNA molecules if such a sequence is present.
- nuclease:** an enzyme that degrades nucleic acids by breaking the phosphodiester bond that connects nucleosides.
- nucleic acid:** the genetic material of cells, found in two forms: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA); composed of repeating subunits called nucleotides.
- nucleocapsid:** a viral structure including the capsid, or outer protein coat, and the nucleic acid of the virus.
- nucleoid:** a region of a prokaryotic cell containing the cell's genetic material.
- nucleolus:** a eukaryotic organelle located in

the nucleus of the cell; the site of ribosomal RNA (rRNA) synthesis.

nucleoside: a building block of nucleic acids, composed of a sugar (deoxyribose or ribose) and one of the nitrogenous bases: adenine (A), cytosine (C), guanine (G), thymine (T), or uracil (U).

nucleosome: the basic unit molecule of chromatin, composed of a segment of a DNA molecule that is bound to and wound around histone molecules; DNA with nucleosomes appears as beads on a string when viewed by electron microscopy.

nucleotide: a building block of nucleic acids, composed of a sugar (deoxyribose or ribose), one of the nitrogenous bases (adenine, cytosine, guanine, thymine, or uracil) and one or more phosphate groups.

nucleus: the “control center” of eukaryotic cells, where the genetic material is separated from the rest of the cell by a membrane; site of DNA replication and transcription.

nullisomy: a genetic condition in which both members of a homologous chromosome pair are absent; usually, embryos with this type of genetic defect are not viable.

ochre codon: a stop codon (UAA) found in messenger RNA (mRNA) molecules that signals termination of translation.

Okazaki fragments: short DNA fragments, approximately two thousand or fewer bases in length, produced during discontinuous replication of the “lagging” strand of a DNA molecule.

oligonucleotide: a short molecule of DNA, generally fewer than twenty bases long and usually synthesized artificially; an important tool for numerous molecular biology procedures, including site-directed mutagenesis.

oncogene: any gene capable of stimulating cell division, thereby being a potential cause of cancer if unregulated; found in all cells and in many cancer-causing viruses.

oogenesis: the process of producing eggs in a sexually mature female organism; another term for meiosis in females.

opal codon: a stop codon (UGA) found in messenger RNA (mRNA) molecules; signals termination of translation.

open reading frame (ORF): a putative protein-coding DNA sequence, marked by a start codon at one end and a stop codon at the other end.

operator: a region of a bacterial operon serving as a control point for transcription of the operon; a regulatory protein of some type usually binds to the operator.

operon: a genetic structure found only in bacteria, whereby a set of genes are controlled together by the same control elements; usually these genes have a common function, such as the genes of the lactose operon in *Escherichia coli* for the metabolism of lactose.

oxygen free radical: a highly reactive form of oxygen in which a single oxygen atom has a free, unpaired electron; free radicals are common by-products of chemical reactions.

P generation: parental generation; the original individuals mated in a genetic cross.

pachytene: a subphase of prophase I in meiosis in which tetrads become visible.

palindrome: in general, a word that reads the same forwards and backwards (such as the words “noon” and “racecar”); in genetics, a DNA sequence that reads the same on each strand of the DNA molecule, although in opposite directions because of the antiparallel nature of the double helix; most DNA palindromes serve as recognition sites for restriction endonucleases.

pandemic: a worldwide outbreak of a disease.

paracentric inversion: an inversion of a chromosome’s sequence that does not involve the centromere, taking place on a single arm of the chromosome.

parthenogenesis: production of an organism from an unfertilized egg.

paternal: coming from the father.

pedigree: a diagram of a particular family, showing the relationships between all members of the family and the inheritance pattern of a particular trait or genetic defect; especially useful for research into human traits that may otherwise be difficult to study.

penetrance: a quantitative term referring to the percentage of individuals with a certain genotype that also exhibit the associated phenotype.

peptide bond: a bond found in proteins; occurs between the carboxyl group of one amino acid and the amino group of the next, linking them together.

pericentric inversion: an inversion of a chromosome's sequence involving the centromere.

pharmacogenomics: the branch of human medical genetics that evaluates how an individual's genetic makeup influences his or her response to drugs.

phenotype: the physical appearance or biochemical and physiological characteristics of an individual, which is determined by both heredity and environment.

phenotypic plasticity: the ability of a genotype to produce different phenotypes when exposed to different environments.

phosphodiester bond: in DNA, the phosphate group connecting one nucleoside to the next in the polynucleotide chain.

photoreactivation repair: a cellular enzyme system responsible for repairing DNA damage caused by ultraviolet light; the system is activated by light.

phylogeny: often called an evolutionary tree, the branching patterns that show evolutionary relationships, with the taxa on the ends of the branches.

pilus: a hairlike reproductive structure possessed by some species of bacterial cells that allows them to engage in a transfer of genetic material known as conjugation.

plasmid: a small, circular DNA molecule commonly found in bacteria and responsible for carrying various genes, such as antibiotic resistance genes; important as a cloning vector for genetic engineering.

pleiotropy: a genetic phenomenon in which a single gene has an effect on two or more traits.

-ploid, -ploidy: a suffix that refers to a chromosome set; humans have two sets of chromosomes and are referred to as being diploid, whereas some plants may have four sets, called tetraploid. Other terms include "autopolyploid" and "polyploidy." *See also* allopolyploid; aneuploid; autopolyploid; diploid; euploid; haplodiploidy; haploid; polyploid; triploid.

pluripotency: the ability of a cell to give rise to all the differentiated cell types in an embryo.

point mutation: a DNA mutation involving a single nucleotide.

polar body: a by-product of oogenesis used to dispose of extra, unnecessary chromosomes while preserving the cytoplasm of the developing ovum.

polycistronic: characterizing messenger RNA (mRNA) molecules that contain coding sequences for more than one protein, common in prokaryotic cells.

polygenic inheritance: expression of a trait depending on the cumulative effect of multiple genes; human traits such as skin color, obesity, and intelligence are thought to be examples of polygenic inheritance.

polymerase: a cellular enzyme capable of creating a phosphodiester bond between two nucleotides, producing a polynucleotide chain complementary to a single-stranded nucleic acid template; the enzyme DNA polymerase is important for DNA replication, and the enzyme RNA polymerase is involved in transcription.

polymerase chain reaction (PCR): a technique of molecular biology in which millions of copies of a single DNA sequence can be artificially produced in a relatively short period of time; important for a wide variety of applications when the source of DNA to be copied is either scarce or impure.

polymorphism: the presence of many different alleles for a particular locus in individuals of the same species.

polypeptide: a single chain of amino acids connected to one another by peptide bonds; all proteins are polypeptides, but a protein may comprise one or more polypeptide molecules.

polyploid: a cell or organism that possesses multiple sets of chromosomes, usually more than two.

polysome: a group of ribosomes attached to the same messenger RNA (mRNA) molecule and producing the same protein product in varying stages of completion.

population: a group of organisms of the same species in the same place at the same time

and thus potentially able to mate; populations are the basic unit of speciation.

population genetics: the study of how genes behave in populations; often a highly mathematical branch of genetics in which evolutionary processes are modeled.

positive eugenics: selecting individuals to reproduce who have desirable genetic traits, as seen by those in control.

post-translational modification: chemical alterations to proteins after they have been produced at a ribosome that alters their properties.

prenatal testing: testing that is done during pregnancy to examine the chromosomes or genes of a fetus to detect the presence or absence of a genetic disorder.

primer: a short nucleic acid molecule used as a beginning point for the enzyme DNA polymerase as it replicates a single-stranded template.

prion: an infectious agent composed solely of protein; thought to be the cause of various human and animal diseases characterized by neurological degeneration, including scrapie in sheep, mad cow disease in cattle, and Creutzfeldt-Jakob disease in humans.

probe: in genetics research, typically a single-stranded nucleic acid molecule or antibody that has been labeled in some way, either with radioactive isotopes or fluorescent dyes; this molecule is then used to seek out its complementary nucleic acid molecule or protein target in a variety of molecular biology techniques such as Southern, Northern, or Western blotting.

product rule: a rule of probability stating that the probability associated with two simultaneous yet independent events is the product of the events' individual probabilities.

prokaryote: a cell that lacks a nuclear membrane (and therefore has no true nucleus) and membrane-bound organelles; bacteria are the only known prokaryotic organisms.

promoter: a region of a gene that controls transcription of that gene; a physical binding site for RNA polymerase.

prophase: the first phase in the process of mitosis or meiosis, in which the nuclear membrane disappears, the spindle apparatus be-

gins to form, and chromatin takes on the form of chromosomes by becoming shorter and thicker.

propositus: the individual in a human pedigree who is the focus of the pedigree, usually by being the first person who came to the attention of the geneticist.

protein: a biological molecule composed of amino acids linked together by peptide bonds; used as structural components of the cell or as enzymes; the term "protein" can refer to a single chain of amino acids or to multiple chains of amino acids functioning in a concerted way, as in the molecule hemoglobin.

proteomics: the study of which proteins are expressed in different types of cells, tissues, and organs during normal and abnormal conditions.

proto-oncogene: a gene, found in eukaryotic cells, that stimulates cell division; ordinarily, expression of this type of gene is tightly controlled by the cell, but in cancer cells, proto-oncogenes have been converted into oncogenes through alteration or elimination of controlled gene expression.

pseudodominance: a genetic phenomenon involving a recessive allele on one chromosome that is automatically expressed because of the deletion of its corresponding dominant allele on the other chromosome of the homologous pair.

pseudogenes: DNA sequences derived from partial copies, mutated complete copies, or normal copies of functional genes that have lost their control sequences and therefore cannot be transcribed; may originate by gene duplication or retrotransposition and are apparently nonfunctional regions of the genome that may evolve at a maximum rate, free from the evolutionary constraints of natural selection.

pseudohermaphrodite: individual born with either ambiguous genitalia or external genitalia that are the opposite of the chromosomal sex.

punctuated equilibrium: a model of evolutionary change in which new species originate abruptly and then exist through a long period of stasis; important as an explanation of

- the stepwise pattern of species change seen in the fossil record.**
- purine:** either of the nitrogenous bases adenine or guanine; used in the structure of nucleic acids.
- pyrimidine:** any of the nitrogenous bases cytosine, thymine, or uracil; used in the structure of nucleic acids.
- quantitative trait:** a trait, such as human height or weight, that shows continuous variation in a population and can be measured; also called a metric trait.
- quantitative trait loci (QTLs):** genomic regions that affect a quantitative trait, generally identified via DNA-based markers.
- reaction norm:** the relationship between environment and phenotype for a given genotype.
- reading frame:** refers to the manner in which a messenger RNA (mRNA) sequence is interpreted as a series of amino acid codons by the ribosome; because of the triplet nature of the genetic code, a typical messenger RNA (mRNA) molecule has three possible reading frames, although usually only one of these will actually code for a functional protein.
- receptors:** molecules to which signaling molecules bind in target cells.
- recessive:** a term referring to an allele or trait that will only be expressed if another, dominant, trait or allele is not also present.
- reciprocal cross:** a mating that is the reverse of another with respect to the sex of the organisms that possess certain traits; for example, if a particular cross were tall male \times short female, then the reciprocal cross would be short male \times tall female.
- reciprocal translocation:** a two-way exchange of genetic material between two nonhomologous chromosomes, resulting in a wide variety of genetic problems depending on which chromosomes are involved in the translocation.
- recombinant DNA:** DNA molecules that are the products of artificial recombination between DNA molecules from two different sources; important as a foundation of genetic engineering.

- recombination:** an exchange of genetic material, usually between two homologous chromosomes; provides one of the foundations for the genetic reassortment observed during sexual reproduction.
- reductional division:** refers to meiosis I, in which the amount of genetic material in the cell is reduced by half through nuclear division; it is at this stage that the diploid cell is converted to an essentially haploid state.
- reductionism:** the explanation of a complex system or phenomenon as merely the sum of its parts.
- replication:** the process by which a DNA or RNA molecule is enzymatically copied.
- replicon:** a region of a chromosome under control of a single origin of replication.
- replisome:** a multiprotein complex that functions at the replication fork during DNA replication; it contains all the enzymes and other proteins necessary for replication, including DNA polymerase.
- repressor:** a protein molecule capable of preventing transcription of a gene, usually by binding to a regulatory region close to the gene.
- resistance plasmid (R plasmid):** a small, circular DNA molecule that replicates independently of the bacterial host chromosome and encodes a gene for antibiotic resistance.
- restriction endonuclease:** a bacterial enzyme that cuts DNA molecules at specific sites; part of a bacterial cell's built-in protection against infection by viruses; an important tool of genetic engineering.
- restriction enzyme:** See restriction endonuclease.
- restriction fragment length polymorphism (RFLP):** a genetic marker, consisting of variations in the length of restriction fragments in DNA from individuals being tested, allowing researchers to compare genetic sequences from various sources; used in a variety of fields, including forensics and the Human Genome Project.
- retrotransposon (retroposon):** a DNA sequence that is transcribed to RNA and reverse transcribed to a DNA copy able to insert itself at another location in the genome.
- retrovirus:** a virus that carries reverse transcript-

tase that converts its RNA genome into a DNA copy that integrates into the host chromosome.

reverse transcriptase: a form of DNA polymerase, discovered in retroviruses, that uses an RNA template to produce a DNA molecule; the name indicates that this process is the reverse of the transcription process occurring naturally in the cell.

reverse-transcriptase polymerase chain reaction (RT-PCR): a technique, requiring isolated RNA, for quickly determining if a gene or a small set of genes are transcribed in a population of cells.

RFLP analysis: *See* restriction fragment length polymorphism.

Rh factor: a human red-blood-cell antigen, first characterized in rhesus monkeys, that contributes to blood typing; individuals can be either Rh positive (possessing the antigen on their red blood cells) or Rh negative (lacking the antigen).

ribonucleic acid (RNA): a form of nucleic acid in the cell used primarily for genetic expression through transcription and translation; in structure, it is virtually identical to DNA, except that ribose is used as the sugar in each nucleotide and the nitrogenous base thymine is replaced by uracil; present in three major forms in the cell: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

ribose: a five-carbon sugar used in the structure of ribonucleic acid (RNA).

ribosomal RNA (rRNA): a type of ribonucleic acid in the cell that constitutes some of the structure of the ribosome and participates in the process of translation.

ribosome: a cellular structure, composed of ribosomal RNA (rRNA) and proteins, that is the site of translation.

ribozyme: an RNA molecule that can function catalytically as an enzyme.

RNA: *See* ribonucleic acid.

RNA interference (RNAi): an artificial technique using small, interfering RNAs that cause gene silencing by binding to the part of a messenger RNA (mRNA) to which they are complementary, thus blocking translation.

RNA polymerase: the cellular enzyme required for making an RNA copy of genetic information contained in a gene; an integral part of transcription.

RNase: refers to a group of enzymes, ribonucleases, capable of specifically degrading RNA molecules.

rRNA: *See* ribosomal RNA.

Sanger sequencing: also known as dideoxy termination sequencing, a method using nucleotides that are missing the 3' hydroxyl group in order to terminate the polymerization of new DNA at a specific nucleotide; the most common sequencing method, used almost exclusively.

segregation: a characteristic of Mendelian genetics, resulting in the division of homologous chromosomes into separate gametes during the process of meiosis.

semiconservative replication: a characteristic of DNA replication, in which every new DNA molecule is actually a hybrid molecule, being composed of a parental, preexisting strand and a newly synthesized strand.

sex chromosome: a chromosome carrying genes responsible for determination of an organism's sex; in humans, the sex chromosomes are designated X and Y.

sex-influenced inheritance: inheritance in which the expression of autosomal traits is influenced or altered relative to the sex of the individual possessing the trait; pattern baldness is an example of this type of inheritance in humans.

sex-limited inheritance: inheritance of traits expressed in only one sex, although these traits are usually produced by non-sex-linked genes (that is, they are genes located on autosomes instead of sex chromosomes).

sexual reproduction: reproduction of cells or organisms involving the transfer and reassortment of genetic information, resulting in offspring that can be phenotypically and genotypically distinct from either of the parents; mediated by the fusion of gametes produced during meiosis.

Shine-Dalgarno sequence: a short sequence in prokaryotic messenger RNA (mRNA) molecules complementary to a sequence in the

prokaryotic ribosome; important for proper positioning of the start codon of the mRNA relative to the P site of the ribosome.

short interspersed sequences (SINES): short repeats of DNA sequences scattered throughout a genome.

shotgun cloning: a technique by which random DNA fragments from an organism's genome are inserted into a collection of vectors to produce a library of clones, which can then be used in a variety of molecular biology procedures.

sigma factor: a molecule that is part of RNA polymerase molecules in bacterial cells; allows RNA polymerase to select the genes that will be transcribed.

signal transduction: all of the molecular events that occur between the arrival of a signaling molecule at a target cell and its response; typically involves a cascading series of reactions that can eventually determine expression of many dozens of genes.

single nucleotide polymorphism (SNP): differences at the individual nucleotide level among individuals.

site-directed mutagenesis: a molecular genetics procedure in which synthetic oligonucleotide molecules are used to induce carefully planned mutations in a cloned DNA molecule.

small nuclear RNA (snRNA): small, numerous RNA molecules found in the nuclei of eukaryotic cells and involved in splicing of messenger RNA (mRNA) precursors to prepare them for translation.

snRNA: See small nuclear RNA.

snRNP: See small nuclear ribonucleoprotein.

sociobiology: the study of social structures, organizations, and actions in terms of underlying biological principles.

solenoid: a complex, highly compacted DNA structure consisting of many nucleosomes packed together in a bundle.

somatic mutation: a mutation occurring in a somatic, or nonsex, cell; because of this, somatic mutations cannot be passed to the next generation.

Southern blot: a molecular biology technique in which a labeled single-stranded DNA probe is exposed to denatured cellular DNA

immobilized on a filter; under the proper conditions, the DNA probe will seek out and bind to its complementary sequence among the cellular DNA molecules, if such a sequence is present.

speciation: the process of evolutionary change that leads to the formation of new species.

species: a group of organisms that can interbreed with one another but not with organisms outside the group; generally, members of a particular species share the same gene pool; defining a species is still controversial and remains a debated concept.

spermatogenesis: the process of producing sperm in a sexually mature male organism; another term for meiosis in males.

spindle apparatus: a structure, composed of microtubules and microfilaments, important for the proper orientation and movement of chromosomes during mitosis and meiosis; appears during prophase and begins to disappear during anaphase.

spliceosome: a complex of nuclear RNA and protein molecules responsible for the excision of introns from messenger RNA (mRNA) precursors before they are translated.

SRY: the sex-determining region of the Y chromosome; a gene encoding a protein product called testis determining factor (TDF), responsible for conversion of a female embryo to a male embryo through the development of the testes.

stem cell: an undifferentiated cell that retains the ability to give rise to other, more specialized cells.

sum rule: a rule of probability theory stating that the probability of either of two mutually exclusive events occurring is the sum of the events' individual probabilities.

supercoil: a complex DNA structure in which the DNA double helix is itself coiled into a helix; usually observed in circular DNA molecules such as bacterial plasmids.

sympatric speciation: the genetic divergence of populations, not separated geographically, that eventually results in formation of new species.

synapsis: the close association of homologous chromosomes occurring during early pro-

phase I of meiosis; during synapsis, recombination between these chromosomes can occur.

T: the abbreviation for thymine, a pyrimidine nitrogenous base found in the structure of DNA; in RNA, thymine is replaced by uracil.

Taq polymerase: DNA polymerase from the bacterium *Thermus aquaticus*; an integral component of polymerase chain reaction.

tautomerization: a spontaneous internal rearrangement of atoms in a complex biological molecule that often causes the molecule to change its shape or its chemical properties.

taxon (pl. taxa): a general term used by evolutionists to refer to a type of organism at any level in a classification of organisms.

telocentric chromosome: a chromosome with a centromere at the end. *See also* acrocentric chromosome; metacentric chromosome.

telomere: the end of a eukaryotic chromosome, protected and replaced by the cellular enzyme telomerase.

telophase: the final phase in the process of mitosis or meiosis, in which division of the cell's nuclear contents has been completed and division of the cell itself occurs.

template: a single-stranded DNA molecule (or RNA molecule) used to create a complementary strand of nucleic acid through the activity of a polymerase.

teratogen: any chemical or physical substance, such as thalidomide, that creates birth defects in offspring.

testcross: a mating involving an organism with a recessive genotype for desired traits crossed with an organism that has an incompletely determined genotype; the types and ratio of offspring produced allow geneticists to determine the genotype of the second organism.

tetrad: a group of four chromosomes formed as a result of the synapsis of homologous chromosomes that takes place early in meiosis.

tetranucleotide hypothesis: a disproven hypothesis, formulated by geneticist P. A. Levene, stating that DNA is a structurally simple molecule composed of a repeating

unit known as a tetranucleotide (composed, in turn, of equal amounts of the bases adenine, cytosine, guanine, and thymine).

thermal cycler: a machine that can rapidly heat and cool reaction tubes; used for performing PCR reactions.

theta structure: an intermediate structure in the bidirectional replication of a circular DNA molecule; the name comes from the resemblance of this structure to the Greek letter theta.

thymine (T): a pyrimidine nitrogenous base found in the structure of DNA; in RNA, thymine is replaced by uracil.

thymine dimer: a pair of thymine bases in a DNA molecule connected by an abnormal chemical bond induced by ultraviolet light; prevents DNA replication in the cell unless it is removed by specialized enzymes.

topoisomerases: cellular enzymes that relieve tension in replicating DNA molecules by introducing single- or double-stranded breaks into the DNA molecule; without these enzymes, replicating DNA becomes progressively more supercoiled until it can no longer unwind, and DNA replication is halted.

totipotent: the ability of a cell to produce an entire adult organism through successive cell divisions and development; as cells become progressively differentiated, they lose this characteristic.

trait: a phenotypic characteristic that is heritable.

transcription: the cellular process by which genetic information in the form of a gene in a DNA molecule is converted into the form of a messenger RNA (mRNA) molecule; dependent on the enzyme RNA polymerase.

transcription factor: a protein that is involved in initiation of transcription but is not part of the RNA polymerase.

transduction: DNA transfer between cells, with a virus serving as the genetic vector.

transfer RNA (tRNA): a type of RNA molecule necessary for translation to occur properly; provides the basis of the genetic code, in which codons in a messenger RNA (mRNA) molecule are used to direct the sequence of amino acids in a polypeptide; contains a

binding site for a particular amino acid and a region complementary to a messenger RNA (mRNA) codon (an anticodon).

transformation: the process by which a normal cell is converted into a cancer cell; also refers to the change in phenotype accompanying entry of foreign DNA into a cell, such as in bacterial cells being used in recombinant DNA procedures.

transgenic organism: an organism possessing one or more genes from another organism, such as mice that possess human genes; important for the study of genes in a living organism, especially in the study of mutations within these genes. *See also* genetically modified organism (GMO).

transition mutation: a DNA mutation in which one pyrimidine (cytosine or thymine) takes the place of another, or a purine (adenine or guanine) takes the place of another.

translation: the cellular process by which genetic information in the form of a messenger RNA (mRNA) molecule is converted into the amino acid sequence of a protein, using ribosomes and RNA molecules as accessory molecules.

translocation: the movement of a chromosome segment to a nonhomologous chromosome as a result of an error in recombination; also refers to the movement of a messenger RNA (mRNA) codon from the A site of the ribosome to the P site during translation.

transposable element: *See* transposon.

transposon: a DNA sequence capable of moving to various places in a chromosome, discovered by geneticist Barbara McClintock; transposons are thought to be important as mediators of genetic variability in both prokaryotes and eukaryotes.

transversion: a DNA mutation in which a pyrimidine (cytosine or thymine) takes the place of a purine (adenine or guanine), or vice versa.

triploid: possessing three complete sets of chromosomes, or $3N$; important in the development of desirable characteristics in the flowers or fruit of some plants; triploids are often sterile.

trisomy: a genetic condition involving one chromosome of a homologous chromosome

pair that has been duplicated in some way, giving rise to a $2N+1$ genotype and causing serious phenotypic abnormalities; a well-known example is trisomy 21, or Down syndrome, in which the individual possesses three copies of chromosome 21 instead of the normal two copies.

tRNA: *See* transfer RNA.

tumor-suppressor genes: any of a number of genes that limit or halt cell division under certain circumstances, thereby preventing the formation of tumors in an organism; two well-studied examples are the retinoblastoma gene and the *p53* gene; mutations in tumor suppressor genes can lead to cancer.

Turner syndrome: a human genetic defect in which an individual has only forty-five chromosomes, lacking one sex chromosome; the sex chromosome present is an X chromosome, making these individuals phenotypically female, although with serious abnormalities such as sterility and anatomical defects.

uracil (U): a pyrimidine nitrogenous base found in the structure of RNA; in DNA, uracil is replaced by thymine.

variable number tandem repeat (VNTR): a repetitive DNA sequence of approximately fifty to one hundred nucleotides; important in the process of forensic identification known as DNA fingerprinting.

vector: a DNA molecule, such as a bacterial plasmid, into which foreign DNA can be inserted and then transported into a cell for further manipulation; important in a wide variety of recombinant DNA techniques.

virions: mature infectious virus particles.

viroids: naked strands of RNA, 270 to 380 nucleotides long, that are circular and do not code for any proteins that are able to cause disease in susceptible plants, many of them economically important. *See also* virusoid.

virus: a microscopic infectious particle composed primarily of protein and nucleic acid; bacterial viruses, or bacteriophages, have been important tools of study in the history of molecular genetics.

virusoids: similar to viroids, microscopic infec-

tious particles composed primarily of protein and nucleic acid; unlike viroids, virusoids are packaged in the protein coat of other plant viruses, referred to as helpers, and are therefore dependent on the other virus. *See also* viroid.

VNTR: *See* variable number tandem repeats.

walking: *See* chromosome walking.

Western blot: a molecular biology technique involving labeled antibodies exposed to cellular proteins immobilized on a filter; under the proper conditions, the antibodies will seek out and bind to the proteins for which they are specific, if such proteins are present.

wild-type: a trait common in nature; usually contrasted with variants of the trait, which are known as mutants.

wobble hypothesis: a concept stating that the anticodon of a transfer RNA (tRNA) molecule is capable of interacting with more than one messenger RNA (mRNA) codon by virtue of the inherent flexibility present in the third base of the anticodon; first proposed by molecular biologist Francis Crick.

X linkage: a genetic phenomenon involving a gene located on the X chromosome; the typical pattern of X linkage involves recessive alleles, such as that for hemophilia, which exert their effects when passed from mother to son and are more likely to be exhibited by males than females.

X-ray diffraction: a method for determining the structure of molecules which infers structure by the way crystals of molecules scatter X rays as they pass through.

xenotransplants: transplants of organs or cellular tissue between different species of animals, such as between pigs and humans.

Y linkage: a genetic phenomenon involving a gene located on the Y chromosome; as a result, such a condition can be passed only from father to son.

yeast artificial chromosome (YAC): a cloning vector that has been engineered with all of the major genetic characteristics of a eukaryotic chromosome so that it will behave as such during cell division; YACs are used to clone extremely large DNA fragments from eukaryotic cells and are an integral part of the Human Genome Project.

Z-DNA: a zigzag form of DNA in which the strands form a left-handed helix instead of the normal right-handed helix of B-DNA; Z-DNA is known to be present in cells and is thought to be involved in genetic regulation. *See also* B-DNA.

zinc finger: an amino acid sequence, found in some DNA-binding proteins, that complexes with zinc ions to create polypeptide “fingers” that can then wrap around a specific portion of a DNA molecule.

zygote: a diploid cell produced by the union of a male gamete (sperm) with a female gamete (egg); through successive cell divisions, the zygote will eventually give rise to the adult form of the organism.

zygotene: a subphase of prophase I of meiosis involving synapsis between homologous chromosomes.

—Randall K. Harris, updated by Bryan Ness

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BACTERIAL GENETICS

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Web Sites

The sites listed below were visited by the editors of Salem Press in May of 2003. Because URLs frequently change or are moved, their accuracy cannot be guaranteed; however, long-standing sites—such as those of university departments, national organizations, and government agencies—generally maintain links when sites move or otherwise may upgrade their offerings and hence remain useful. Sites with an “N/A” affiliation are, to our knowledge, unallied, mounted by an individual, or “uncredited.” —Roger Smith

General Genetics

BioMedNet

Genetics Gateway

<http://reviews.bmn.com/?subject=Genetics>

A repository of research articles for scientists, links to databases, and news and feature articles written for general readers on all aspects of genetics. Users must register, for which there is no charge, although access to the full text of articles and databases requires a fee in some cases.

Dolan DNA Learning Center, Cold Spring Harbor Laboratory
Gene Almanac
<http://www.dnalc.org>

An online science center devoted to public education in genetics at high school and college levels, this is the best entry point to the Web for newcomers to genetics. It provides information on DNA science, genetics and medicine, and biotechnology through interactive features, animated tutorials, and downloads. With an extensive list of links.

Genetics Society of America
Home Page
<http://www.genetics-gsa.org>

Although dedicated to subscribers who are professional geneticists, the society's Web site supports genetics education for all ages and offers a history of the organization and short position statements on evolution and genetically modified organisms. There are also links to databases and related Web sites.

N/A
Kimball's Biology Pages
<http://biology-pages.info>

A collection of clear, well-illustrated explanations of topics in biology by university professor John W. Kimball, including all aspects of genetics and biotechnology, with news updates. A reliable place to start for those new to genetics.

MedBioWorld
Genetics, Genomics & Biotechnology
<http://www.medbioworld.com>

A list of links to the major journals in genetics, many of which allow free access to abstracts, some articles, and news postings. A fast search vehicle for the most recent information about a topic in genetics.

National Public Radio
The DNA Files
<http://www.dnafiles.org/home.html>

The text from an award-winning public radio series, including programs about the human genome, genetics and ecology, genetics and medicine, biotechnology, and the genetics of identity. Lucid, in-depth treatments for the nonscientist.

Nature Publishing Group
genetics@nature.com
<http://www.nature.com/genetics>

Part of the Web site for *Nature*, Britain's premier science journal. Written for educated general readers, the sections offer news and recently published articles, commentary, and an encyclopedia of life sciences. There are links to specialty journals concerning topics in genetics and biotechnology. Some articles are accessible by the general public, but full use of the site requires a subscription.

Netspace**MendelWeb**

<http://www.mendelweb.org>

An educational site for teachers and students concerning the origins of classical genetics and elementary plant science. It reproduces early publications by such pioneers as Gregor Mendel and William Bateson, accompanied by commentaries and reference resources.

Rutgers University**Morgan**

<http://morgan.rutgers.edu/>

[MorganWebFrames/How_to_use/
HTU_intro.html](MorganWebFrames/How_to_use/HTU_intro.html)

A multimedia tutorial for advanced high school students or beginning college students. Its six levels review basic principles in genetics with particular attention to molecular interactions.

**U.S. Department of Energy Office of Science
Virtual Library on Genetics**

[http://www.ornl.gov/TechResources/
Human_Genome/genetics.html](http://www.ornl.gov/TechResources/Human_Genome/genetics.html)

A comprehensive catalog of Web site links, arranged by subject, pertaining to the Human Genome Project. The links lead to gene and chromosome databases and specific information on genetics, bioinformatics, and genetic disorders.

University of Massachusetts**DNA Structure**

<http://molvis.sdsc.edu/dna/index.htm>

An interactive, animated tutorial on the molecular composition and structure of DNA for high school students and college freshmen. It can be downloaded and is available in Spanish, German, and Portuguese.

University of Utah**Genetic Science Learning Center**

<http://gslc.genetics.utah.edu>

Designed for students, this site posts essays on the basics of DNA, genetic disorders, cloning, stem cells, and genetic testing. It also describes simple experiments, such as how to extract DNA material.

Bioinformatics**Bioinformatics.org****Home Page**

<http://bioinformatics.org>

The site belongs to an international organization dedicated to the exchange of genetic information and includes online databases and analysis tools, software, explanations of frequently asked questions about bioinformatics, and news postings.

N/A**Earl's Forensic Page**

[http://members.aol.com/EarlNMeyer/
DNA.html](http://members.aol.com/EarlNMeyer/DNA.html)

With a variety of illustrations, Earl Meyer summarizes how genetic fingerprinting works and its use in crime investigations and in determining paternity.

European Bioinformatics Institute**The Path to Knowledge**

<http://www.ebi.ac.uk>

A research center, this institute maintains databases concerning nucleic acids, protein sequences, and macromolecular structures. Also posts news, events, and descriptions of ongoing scientific projects.

**Technical University of Denmark
Center for Biological Sequencing Analysis**

<http://www.cbs.dtu.dk>

The Center conducts basic research in bioinformatics and here offers its sequencing analysis databases, genome atlases, analysis tools, and news. Primarily meant for researchers and university students.

Biotechnology**Bio-Link****Educating the Bio-Link Workforce**

<http://Bio-Link.org>

Intended for technicians, this site offers information and instruction covering recent advances in biotechnology, as well as a virtual laboratory and library, news postings, and details about regional education centers.

Carolina

Biotechnology and Genetics
<http://www.carolina.com/biotech>

A rich resource designed for teachers and students. It offers a newsletter, workshops, articles, classroom activities, and videos, all focusing on the use of biotechnology and laboratory techniques.

Dow AgroSciences

Plant Genetics and Biotechnology
<http://www.dowagro.com/homepage/index.htm>

This corporate Web site concerns the marketing of its agricultural biotechnology. Given that bias, it contains news releases, descriptions of products, data sheets, articles and position statements, and a media kit of interest to general readers.

The Hastings Center

Home Page
<http://www.thehastingscenter.org>

The Hastings Center, an independent non-profit organization, specializes in bioethics, particularly in health care and biotechnology. Its site contains news postings, articles on bioethics and different aspects of genetics science, and announcements of events and publications.

**National Institutes of Health
National Center for Biotechnology
Information**

<http://www.ncbi.nlm.nih.gov>

This Web site for the main health agency of the United States contains links to the various specialized institutes under its umbrella as well as public databases in genomics and sequencing, articles and handbooks on a wide range of biotechnology topics, and more than a dozen types of free software for analyzing genetic data. Primarily intended for researchers, but with the public in mind—the site includes a science primer and other resources for educators, students, and other nonspecialists.

Genomics

Göteborg University, Sweden
RatMap, the Rat Genome Database
<http://ratmap.gen.gu.se>

A professional database of information on approximately six thousand rat genes, their positions on chromosomes, pertinent nomenclature, and gene functions.

**Johns Hopkins University
The Genome Database**

<http://gdbwww.gdb.org>

The official central storage center for gene-mapping data compiled in the Human Genome Initiative, an international effort to decode and analyze human DNA. Intended for scientists, the site presents information in three categories: regions, maps, and variations of the human genome.

**Lawrence Berkeley National Laboratory
Human Genome Sequencing Department**

<http://www-hgc.lbl.gov>

The department is part of the Joint Genome Institute, which includes genome laboratories at Lawrence Livermore and Los Alamos. The site describes its directed sequencing method, explains its work on sequencing the human genome and the genome of the fruit fly *Drosophila*, and provides access to sequencing archives.

**Massachusetts Institute of Technology
Whitehead Institute for Biomedical Research**

<http://www-genome.wi.mit.edu>

The institute's home page affords access to news of genomics research, software, and sequencing databases, all intended for scientists and university students. However, its information about the Human Genome Project has general background articles, photos, and a video that will be of interest to nonspecialists.

National Center for Biotechnology

Information
GeneMap '99
<http://www.ncbi.nlm.nih.gov/genemap99>

Starting with a general introduction to the human genome and the process of gene mapping, this site provides charts of the known genes on each chromosome, articles about the Human Genome Project and gene-related medical research, and links to other genome sites and databases.

National Human Genome Research Institute
Home Page
<http://www.genome.gov>

In addition to information for researchers, this site contains a comprehensive introduction to the Human Genome Project, a glossary of genetic terms, fact sheets, multimedia education kits, and links to online education resources, all for the general public.

New York University/Bell Atlantic/Center for Advanced Technology
The Student Genome Project
<http://www.cat.nyu.edu/sgp/parent.html>

Uses interactive multimedia and three-dimensional technology to present tutorials and games related to the human genome and genetics for middle school and high school students. Also provides news about New York-based science events and links.

Sanger Institute, Wellcome Trust
Home Page
<http://www.sanger.ac.uk>

This research institute is dedicated to genomics. Accordingly, the site offers news updates, a searchable database, explanations of gene sequencing and computer software aids, and descriptions of genomics research projects. All information is intended for scientists.

U.S. Department of Energy Biological and Environmental Research Program
Human Genome Research
http://www.er.doe.gov/production/ober/hug_top.html

This site describes the Department of Energy's contribution to the Human Genome Project with pages containing a history, a time line, project information, an essay on the science of genetics, and abstracts of recent research. There is also a section of links designed for young students and science teachers, as well as information about fellowships and research opportunities.

Medicine and Genetics
American College of Medical Genetics
Home Page
<http://www.acmg.net>

Designed for physicians, this site also contains information for patients about treatments, research, standards for gene therapy, and health, along with news articles and links to related sites.

Genethon
Gene Therapies Research and Applications Center
http://www.genethon.fr/php/index_us.php

Supported by the French Muscular Dystrophy Association, Genethon sponsors research in genetic and cellular therapies for rare diseases. This site discusses research methods, the organization's services, and, in a section accompanied by computer graphics, the theory of gene therapy.

National Center for Biotechnology Information
Online Mendelian Inheritance in Man
<http://www.ncbi.nlm.nih.gov/Omim>

The Online Mendelian Inheritance in Man (OMIM) is a catalog of human genes and genetic disorders for scientists. The site also offers maps of genes and diseases, statistical summaries, and links to similar sites devoted to medical literature and biotechnology.

National Fragile X Foundation
Xtraordinary Accomplishments
<http://www.nfxf.org>

Provides extensive general information about fragile X syndrome, a cause of inherited mental impairments, and advises care-givers on testing, medical treatment, education, and life-planning.

National Organization for Rare Disorders (NORD).
Home Page
<http://www.rarediseases.org>.

Offers a very useful and long list of genetically related disorders and diseases, each of which links to a fact sheet that ends with a list of organizations for additional information. Also posts articles about rare genetic conditions and diseases, accessible through searchable databases.

National Society of Genetic Counselors
Society Home Page
<http://www.nsge.org>

Although much of this site is devoted to society members, it has a search engine for locating genetic counselors in the United States and a newsroom with press releases and fact sheets about the counseling services.

The University of Nottingham
OMNI
<http://omni.ac.uk/subject-listing/QH426.html>

This gateway to information about biomedicine has links to sites devoted to the science, applications, and ethics of medical genetics.

Transgenics
Center for Life Sciences, Colorado State
University
Transgenic Crops: An Introduction and
Resource Guide
[http://www.colostate.edu/programs/
lifesciences/TransgenicCrops/](http://www.colostate.edu/programs/lifesciences/TransgenicCrops/)

The purpose of this site is to provide balanced information about the technology and safety issues involved in genetically modified food crops. Compiled by genetics researchers. With links, a history of plant breeding, and glossary. Also in Spanish.

National Academy of Sciences
Transgenic Plants and World Agriculture

<http://www.nap.edu/html/transgenic/>

An online, downloadable pamphlet published in July, 2000, by a consortium of leading research societies around the world. It assesses the need to modify crops genetically in order to feed the increasing world population and then discusses examples of the technology, its safety, effects on the environment, funding sources, and intellectual property issues.

Oak Ridge National Laboratory
Transgenic and Targeted Mutant Animal
Database
[http://www.ornl.gov/TechResources/Trans/
hmepg.html](http://www.ornl.gov/TechResources/Trans/hmepg.html)

A searchable professional database about lines of genetically modified animals, methods used to create them, and descriptions of the modified DNA, the expression of transgenes, and how transgenes are named.

University of Michigan
Transgenic Animal Model Core
<http://www.med.umich.edu/tamc>

A professional Web site for researchers seeking a host animal to test transgenes. However, it contains much useful general information about transgenics (especially transgenic rats), vectors, and laboratory procedures. With links and a photo gallery.

—Roger Smith

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