

FOURTH EDITION ESSENTIAL CELL BIOLOGY

ALBERTS · BRAY · HOPKIN · JOHNSON · LEWIS · RAFT · ROBERTS · WALTER



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Preface

In our world there is no form of matter more astonishing than the living cell: tiny, fragile, marvelously intricate, continually made afresh, yet preserving in its DNA a record of information dating back more than three billion years, to a time when our planet had barely cooled from the hot materials of the nascent solar system. Ceaselessly re-engineered and diversified by evolution, extraordinarily versatile and adaptable, the cell retains a complex core of self-replicating chemical machinery that is shared and endlessly repeated by every living organism on the face of the Earth—in every animal, every leaf, every bacterium in a piece of cheese, every yeast in a vat of wine.

Curiosity, if nothing else, should drive us to study cell biology; we need to understand cell biology to understand ourselves. But there are practical reasons, too, why cell biology should be a part of everyone's education. We are made of cells, we feed on cells, and our world is made habitable by cells. The challenge for scientists is to deepen our knowledge of cells and find new ways to apply it. All of us, as citizens, need to know something of the subject to grapple with the modern world, from our own health affairs to the great public issues of environmental change, biomedical technologies, agriculture, and epidemic disease.

Cell biology is a big subject, and it has links with almost every other branch of science. The study of cell biology therefore provides a great scientific education. However, as the science advances, it becomes increasingly easy to become lost in detail, distracted by an overload of information and technical terminology. In this book we therefore focus on providing a digestible, straightforward, and engaging account of only the essential principles. We seek to explain, in a way that can be understood even by a reader approaching biology for the first time, how the living cell works: to show how the molecules of the cell—especially the protein, DNA, and RNA molecules—cooperate to create this remarkable system that feeds, responds to stimuli, moves, grows, divides, and duplicates itself.

The need for a clear account of the essentials of cell biology became apparent to us while we were writing *Molecular Biology of the Cell* (*MBoC*), now in its fifth edition. *MBoC* is a large book aimed at advanced undergraduates and graduate students specializing in the life sciences or medicine. Many students and educated lay people who require an introductory account of cell biology would find *MBoC* too detailed for their needs. *Essential Cell Biology* (*ECB*), in contrast, is designed to provide the fundamentals of cell biology that are required by anyone to understand both the biomedical and the broader biological issues that affect our lives.

This fourth edition has been extensively revised. We have brought every part of the book up to date, with new material on regulatory RNAs, induced pluripotent stem cells, cell suicide and reprogramming, the human genome, and even Neanderthal DNA. In response to student feedback, we have improved our discussions of photosynthesis and DNA

repair. We have added many new figures and have updated our coverage of many exciting new experimental techniques—including RNAi, optogenetics, the applications of new DNA sequencing technologies, and the use of mutant organisms to probe the defects underlying human disease. At the same time, our “How We Know” sections continue to present experimental data and design, illustrating with specific examples how biologists tackle important questions and how their experimental results shape future ideas.

As before, the diagrams in *ECB* emphasize central concepts and are stripped of unnecessary details. The key terms introduced in each chapter are highlighted when they first appear and are collected together at the end of the book in a large, illustrated glossary.

A central feature of the book is the many questions that are presented in the text margins and at the end of each chapter. These are designed to provoke students to think carefully about what they have read, encouraging them to pause and test their understanding. Many questions challenge the student to place the newly acquired information in a broader biological context, and some have more than one valid answer. Others invite speculation. Answers to all the questions are given at the end of the book; in many cases these provide a commentary or an alternative perspective on material presented in the main text.

For those who want to develop their active grasp of cell biology further, we recommend *Molecular Biology of the Cell, Fifth Edition: A Problems Approach*, by John Wilson and Tim Hunt. Though written as a companion to *MBoC*, this book contains questions at all levels of difficulty and contains a goldmine of thought-provoking problems for teachers and students. We have drawn upon it for some of the questions in *ECB*, and we are very grateful to its authors.

The explosion of new imaging and computer technologies continues to provide fresh and spectacular views of the inner workings of living cells. We have captured some of this excitement in the new *Essential Cell Biology* website, located at www.garlandscience.com/ECB4-students. This site, which is freely available to anyone in the world with an interest in cell biology, contains over 150 video clips, animations, molecular structures, and high-resolution micrographs—all designed to complement the material in individual book chapters. One cannot watch cells crawling, dividing, segregating their chromosomes, or rearranging their surface without a sense of wonder at the molecular mechanisms that underlie these processes. For a vivid sense of the marvel that science reveals, it is hard to match the narrated movie of DNA replication. These resources have been carefully designed to make the learning of cell biology both easier and more rewarding.

Those who seek references for further reading will find them on the *ECB* student and instructor websites. But for the very latest reviews in the current literature, we suggest the use of web-based search engines, such as PubMed (www.ncbi.nlm.nih.gov) or Google Scholar (scholar.google.com).

As with *MBoC*, each chapter of *ECB* is the product of a communal effort, with individual drafts circulating from one author to another. In addition, many people have helped us, and these are credited in the Acknowledgments that follow. Despite our best efforts, it is inevitable that there will be errors in the book. We encourage readers who find them to let us know at science@garland.com, so that we can correct these errors in the next printing.

Acknowledgments

The authors acknowledge the many contributions of professors and students from around the world in the creation of this fourth edition. In particular, we are grateful to the students who participated in our focus groups; they provided invaluable feedback about their experiences using the book and our multimedia, and many of their suggestions were implemented in this edition.

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We also received detailed reviews from many instructors who used the third edition, and we would like to thank them for their contributions: Devavani Chatterjea, Macalester College; Frank Hauser, University of Copenhagen; Alan Jones, University of North Carolina at Chapel Hill; Eugene Mesco, Savannah State University; M. Scott Shell, University of California Santa Barbara; Grith Lykke Sørensen, University of Southern Denmark; Marta Bechtel, James Madison University; David Bourgaize, Whittier College; John Stephen Horton, Union College; Sieirn Lim, Nanyang Technological University; Satoru Kenneth Nishimoto, University of Tennessee Health Science Center; Maureen Peters, Oberlin College; Johanna Rees, University of Cambridge; Gregg Whitworth, Grinnell College; Karl Fath, Queens College, City University of New York; Barbara Frank, Idaho State University; Sarah Lundin-Schiller, Austin Peay State University; Marianna Patrauchan, Oklahoma State University; Ellen Rosenberg, University of British Columbia; Leslie Kate Wright, Rochester Institute of Technology; Steven H. Denison, Eckerd College; David Featherstone, University of Illinois at Chicago; Andor Kiss, Miami University; Julie Lively, Sewanee, The University of the South; Matthew Rainbow, Antelope Valley College; Juliet Spencer, University of San Francisco; Christoph Winkler, National University of Singapore; Richard Bird, Auburn University; David Burgess, Boston

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We are very grateful, too, to the readers who alerted us to errors they had found in the previous edition.

Many staff at Garland Science contributed to the creation of this book and made our work on it a pleasure. First of all, we owe a special debt to Michael Morales, our editor, who coordinated the whole enterprise. He organized the initial reviewing and the focus groups, worked closely with the authors on their chapters, urged us on when we fell behind, and played a major part in the design, assembly, and production of *Essential Cell Biology* student website. Monica Toledo managed the flow of chapters through the book development and production process, and oversaw the writing of the accompanying question bank. Lamia Harik gave editorial assistance. Nigel Orme took original drawings created by author Keith Roberts and redrew them on a computer, or occasionally by hand, with great skill and flair. To Matt McClements goes the credit for the graphic design of the book and the creation of the chapter-opener sculptures. As in previous editions, Emma Jeffcock did a brilliant job in laying out the whole book and meticulously incorporating our endless corrections. Adam Sendroff and Lucy Brodie gathered user feedback and launched the book into the wide world. Denise Schanck, the Vice President of Garland Science, attended all of our writing retreats and orchestrated everything with great taste and diplomacy. We give our thanks to everyone in this long list.

Last but not least, we are grateful, yet again, to our colleagues and our families for their unflagging tolerance and support.

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Resources for Instructors and Students

The teaching and learning resources for instructors and students are available online. The instructor's resources are password protected and available only to qualified instructors. The student resources are available to everyone. We hope these resources will enhance student learning, and make it easier for instructors to prepare dynamic lectures and activities for the classroom.

INSTRUCTOR RESOURCES

Instructor Resources are available on the Garland Science Instructor's Resource Site, located at www.garlandscience.com/instructors. The website provides access not only to the teaching resources for this book but also to all other Garland Science textbooks. Qualified instructors can obtain access to the site from their sales representative or by emailing science@garland.com.

Art of Essential Cell Biology, Fourth Edition

The images from the book are available in two convenient formats: PowerPoint® and JPEG. They have been optimized for display on a computer. Figures are searchable by figure number, figure name, or by keywords used in the figure legend from the book.

Figure-Integrated Lecture Outlines

The section headings, concept headings, and figures from the text have been integrated into PowerPoint presentations. These will be useful for instructors who would like a head start creating lectures for their course. Like all of our PowerPoint presentations, the lecture outlines can be customized. For example, the content of these presentations can be combined with videos and questions from the book or "Question Bank," in order to create unique lectures that facilitate interactive learning.

Animations and Videos

The 130+ animations and videos that are available to students are also available on the Instructor's Resource site in two formats. The WMV-formatted movies are created for instructors who wish to use the movies in PowerPoint presentations on Windows® computers; the QuickTime-formatted movies are for use in PowerPoint for Apple computers or Keynote® presentations. The movies can easily be downloaded to your computer using the "download" button on the movie preview page.

Question Bank

Written by Linda Huang, University of Massachusetts, Boston, and Cheryl D. Vaughan, Harvard University Division of Continuing Education, the revised and expanded question bank includes a variety of question formats: multiple choice, fill-in-the-blank, true-false, matching, essay, and challenging "thought" questions. There are approximately 60–70 questions per chapter, and a large number of the multiple-choice questions will be suitable for use with personal response systems (that is, clickers). The *Question Bank* was created with the philosophy that a good exam should do much more than simply test students' ability to memorize information; it should require them to reflect upon and integrate information as a part of a sound understanding. It provides a comprehensive sampling of questions that can be used either directly or as inspiration for instructors to write their own test questions.

References

Adapted from the detailed references of *Molecular Biology of the Cell*, and organized by the table of contents for *Essential Cell Biology*, the "References" provide a rich compendium of journal and review articles for reference and reading assignments. The "References" PDF document is available on both the instructor and student websites.

Medical Topics Guide

This document highlights medically relevant topics covered throughout the book, and will be particularly useful for instructors with a large number of premedical, health science, or nursing students.

Media Guide

This document overviews the multimedia available for students and instructors and contains the text of the voice-over narration for all of the movies.

Blackboard® and LMS Integration

The movies, book images, and student assessments that accompany the book can be integrated into Blackboard or other learning management systems. These resources are bundled into a "Common Cartridge" that facilitates bulk uploading of textbook resources into Blackboard and other learning management systems. The LMS Common Cartridge can be obtained on a DVD from your sales representative or by emailing science@garland.com.

STUDENT RESOURCES

The resources for students are available on the *Essential Cell Biology* Student Website, located at www.garlandscience.com/ECB4-students.

Animations and Videos

There are over 130 movies, covering a wide range of cell biology topics, which review key concepts in the book and illuminate the cellular microcosm.

Student Self-Assessments

The website contains a variety of self-assessment tools to help students.

- Each chapter has a multiple-choice quiz to test basic reading comprehension.
- There are also a number of media assessments that require students to respond to specific questions about movies on the website or figures in the book.
- Additional concept questions complement the questions available in the book.
- “Challenge” questions are included that provide a more experimental perspective or require a greater depth of conceptual understanding.

Cell Explorer

This application teaches cell morphology through interactive micrographs that highlight important cellular structures.

Flashcards

Each chapter contains a set of flashcards, built into the website, that allow students to review key terms from the text.

Glossary

The complete glossary from the book is available on the website and can be searched or browsed.

References

A set of references is available for each chapter for further reading and exploration.

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CHAPTER ONE

1

Cells: The Fundamental Units of Life

What does it mean to be living? Petunias, people, and pond scum are all alive; stones, sand, and summer breezes are not. But what are the fundamental properties that characterize living things and distinguish them from nonliving matter?

The answer begins with a basic fact that is taken for granted now, but marked a revolution in thinking when first established 175 years ago. All living things (or *organisms*) are built from **cells**: small, membrane-enclosed units filled with a concentrated aqueous solution of chemicals and endowed with the extraordinary ability to create copies of themselves by growing and then dividing in two. The simplest forms of life are solitary cells. Higher organisms, including ourselves, are communities of cells derived by growth and division from a single founder cell. Every animal or plant is a vast colony of individual cells, each of which performs a specialized function that is regulated by intricate systems of cell-to-cell communication.

Cells, therefore, are the fundamental units of life. Thus it is to *cell biology*—the study of cells and their structure, function, and behavior—that we must look for an answer to the question of what life is and how it works. With a deeper understanding of cells, we can begin to tackle the grand historical problems of life on Earth: its mysterious origins, its stunning diversity produced by billions of years of evolution, and its invasion of every conceivable habitat. At the same time, cell biology can provide us with answers to the questions we have about ourselves: Where did we come from? How do we develop from a single fertilized egg cell? How is each of us similar to—yet different from—everyone else on Earth? Why do we get sick, grow old, and die?

UNITY AND DIVERSITY OF CELLS

CELLS UNDER THE MICROSCOPE

THE PROKARYOTIC CELL

THE EUKARYOTIC CELL

MODEL ORGANISMS

In this chapter, we begin by looking at the great variety of forms that cells can show, and we take a preliminary glimpse at the chemical machinery that all cells have in common. We then consider how cells are made visible under the microscope and what we see when we peer inside them. Finally, we discuss how we can exploit the similarities of living things to achieve a coherent understanding of all forms of life on Earth—from the tiniest bacterium to the mightiest oak.

UNITY AND DIVERSITY OF CELLS

Cell biologists often speak of “the cell” without specifying any particular cell. But cells are not all alike; in fact, they can be wildly different. Biologists estimate that there may be up to 100 million distinct species of living things on our planet. Before delving deeper into cell biology, we must take stock: What does a bacterium have in common with a butterfly? What do the cells of a rose have in common with those of a dolphin? And in what ways do the plethora of cell types within an individual multicellular organism differ?

Cells Vary Enormously in Appearance and Function

Let us begin with size. A bacterial cell—say a *Lactobacillus* in a piece of cheese—is a few **micrometers**, or μm , in length. That’s about 25 times smaller than the width of a human hair. A frog egg—which is also a single cell—has a diameter of about 1 millimeter. If we scaled them up to make the *Lactobacillus* the size of a person, the frog egg would be half a mile high.

Cells vary just as widely in their shape (Figure 1–1). A typical nerve cell in your brain, for example, is enormously extended; it sends out its electrical signals along a fine protrusion that is 10,000 times longer than it is thick, and it receives signals from other nerve cells through a mass of shorter processes that sprout from its body like the branches of a tree (see Figure 1–1A). A *Paramecium* in a drop of pond water is shaped like a submarine and is covered with thousands of *cilia*—hairlike extensions whose sinuous beating sweeps the cell forward, rotating as it goes (Figure 1–1B). A cell in the surface layer of a plant is squat and immobile, surrounded

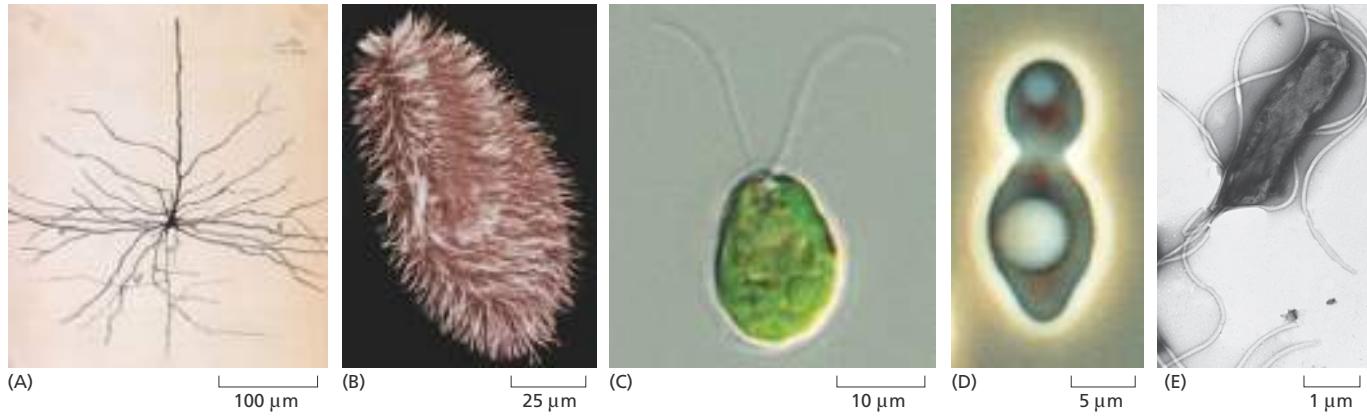


Figure 1–1 Cells come in a variety of shapes and sizes. Note the very different scales of these micrographs. (A) Drawing of a single nerve cell from a mammalian brain. This cell has a huge branching tree of processes, through which it receives signals from as many as 100,000 other nerve cells. (B) *Paramecium*. This protozoan—a single giant cell—swims by means of the beating cilia that cover its surface. (C) *Chlamydomonas*. This type of single-celled green algae is found all over the world—in soil, fresh water, oceans, and even in the snow at the top of mountains. The cell makes its food like plants do—via photosynthesis—and it pulls itself through the water using its paired flagella to do the breaststroke. (D) *Saccharomyces cerevisiae*. This yeast cell, used in baking bread, reproduces itself by a process called budding. (E) *Helicobacter pylori*. This bacterium—a causative agent of stomach ulcers—uses a handful of whiplike flagella to propel itself through the stomach lining. (A, copyright Herederos de Santiago Ramón y Cajal, 1899; B, courtesy of Anne Fleury, Michel Laurent, and André Adoutte; C, courtesy of Brian Piasecki; E, courtesy of Yutaka Tsutsumi.)

by a rigid box of cellulose with an outer waterproof coating of wax. A neutrophil or a macrophage in the body of an animal, by contrast, crawls through tissues, constantly pouring itself into new shapes, as it searches for and engulfs debris, foreign microorganisms, and dead or dying cells. And so on.

Cells are also enormously diverse in their chemical requirements. Some require oxygen to live; for others this gas is deadly. Some cells consume little more than air, sunlight, and water as their raw materials; others need a complex mixture of molecules produced by other cells.

These differences in size, shape, and chemical requirements often reflect differences in cell function. Some cells are specialized factories for the production of particular substances, such as hormones, starch, fat, latex, or pigments. Others are engines, like muscle cells that burn fuel to do mechanical work. Still others are electricity generators, like the modified muscle cells in the electric eel.

Some modifications specialize a cell so much that they spoil its chances of leaving any descendants. Such specialization would be senseless for a cell that lived a solitary life. In a multicellular organism, however, there is a division of labor among cells, allowing some cells to become specialized to an extreme degree for particular tasks and leaving them dependent on their fellow cells for many basic requirements. Even the most basic need of all, that of passing on the genetic instructions of the organism to the next generation, is delegated to specialists—the egg and the sperm.

Living Cells All Have a Similar Basic Chemistry

Despite the extraordinary diversity of plants and animals, people have recognized from time immemorial that these organisms have something in common, something that entitles them all to be called living things. But while it seemed easy enough to recognize life, it was remarkably difficult to say in what sense all living things were alike. Textbooks had to settle for defining life in abstract general terms related to growth, reproduction, and an ability to respond to the environment.

The discoveries of biochemists and molecular biologists have provided an elegant solution to this awkward situation. Although the cells of all living things are infinitely varied when viewed from the outside, they are fundamentally similar inside. We now know that cells resemble one another to an astonishing degree in the details of their chemistry. They are composed of the same sorts of molecules, which participate in the same types of chemical reactions (discussed in Chapter 2). In all organisms, genetic information—in the form of genes—is carried in DNA molecules. This information is written in the same chemical code, constructed out of the same chemical building blocks, interpreted by essentially the same chemical machinery, and replicated in the same way when an organism reproduces. Thus, in every cell, the long DNA polymer chains are made from the same set of four monomers, called *nucleotides*, strung together in different sequences like the letters of an alphabet to convey information. In every cell, the information encoded in the DNA is read out, or *transcribed*, into a chemically related set of polymers called RNA. A subset of these RNA molecules is in turn *translated* into yet another type of polymer called a protein. This flow of information—from DNA to RNA to protein—is so fundamental to life that it is referred to as the *central dogma* (Figure 1–2).

The appearance and behavior of a cell are dictated largely by its protein molecules, which serve as structural supports, chemical catalysts,

QUESTION 1–1

"Life" is easy to recognize but difficult to define. According to one popular biology text, living things:

1. Are highly organized compared to natural inanimate objects.
2. Display homeostasis, maintaining a relatively constant internal environment.
3. Reproduce themselves.
4. Grow and develop from simple beginnings.
5. Take energy and matter from the environment and transform it.
6. Respond to stimuli.
7. Show adaptation to their environment.

Score a person, a vacuum cleaner, and a potato with respect to these characteristics.

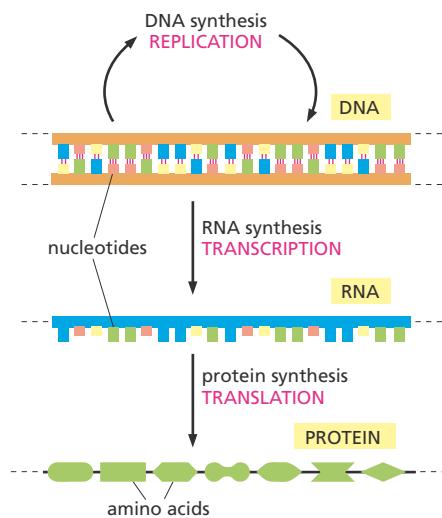


Figure 1–2 In all living cells, genetic information flows from DNA to RNA (transcription) and from RNA to protein (translation)—a sequence known as the **central dogma**. The sequence of nucleotides in a particular segment of DNA (a gene) is transcribed into an RNA molecule, which can then be translated into the linear sequence of amino acids of a protein. Only a small part of the gene, RNA, and protein are shown.



(A)



(B)



(C)



(D)

Figure 1–3 All living organisms are constructed from cells. A colony of bacteria, a butterfly, a rose, and a dolphin are all made of cells that have a fundamentally similar chemistry and operate according to the same basic principles. (A, courtesy of Janice Carr; C, courtesy of the John Innes Foundation; D, courtesy of Jonathan Gordon, IFAW.)

molecular motors, and so on. Proteins are built from *amino acids*, and all organisms use the same set of 20 amino acids to make their proteins. But the amino acids are linked in different sequences, giving each type of protein molecule a different three-dimensional shape, or *conformation*, just as different sequences of letters spell different words. In this way, the same basic biochemical machinery has served to generate the whole gamut of life on Earth (Figure 1–3). A more detailed discussion of the structure and function of proteins, RNA, and DNA is presented in Chapters 4 through 8.

If cells are the fundamental unit of living matter, then nothing less than a cell can truly be called living. Viruses, for example, are compact packages of genetic information—in the form of DNA or RNA—encased in protein but they have no ability to reproduce themselves by their own efforts. Instead, they get themselves copied by parasitizing the reproductive machinery of the cells that they invade. Thus, viruses are chemical zombies: they are inert and inactive outside their host cells, but they can exert a malign control over a cell once they gain entry.

All Present-Day Cells Have Apparently Evolved from the Same Ancestral Cell

A cell reproduces by replicating its DNA and then dividing in two, passing a copy of the genetic instructions encoded in its DNA to each of its daughter cells. That is why daughter cells resemble the parent cell. However, the copying is not always perfect, and the instructions are occasionally corrupted by *mutations* that change the DNA. For this reason, daughter cells do not always match the parent cell exactly.

Mutations can create offspring that are changed for the worse (in that they are less able to survive and reproduce), changed for the better (in that they are better able to survive and reproduce), or changed in a neutral way (in that they are genetically different but equally viable). The struggle for survival eliminates the first, favors the second, and tolerates the third. The genes of the next generation will be the genes of the survivors.

On occasion, the pattern of descent may be complicated by sexual reproduction, in which two cells of the same species fuse, pooling their DNA. The genetic cards are then shuffled, re-dealt, and distributed in new combinations to the next generation, to be tested again for their ability to promote survival and reproduction.

These simple principles of genetic change and selection, applied repeatedly over billions of cell generations, are the basis of **evolution**—the process by which living species become gradually modified and adapted to their environment in more and more sophisticated ways. Evolution offers a startling but compelling explanation of why present-day cells are so similar in their fundamentals: they have all inherited their genetic instructions from the same common ancestor. It is estimated that this ancestral cell existed between 3.5 and 3.8 billion years ago, and we must

QUESTION 1–2

Mutations are mistakes in the DNA that change the genetic plan from the previous generation. Imagine a shoe factory. Would you expect mistakes (i.e., unintentional changes) in copying the shoe design to lead to improvements in the shoes produced? Explain your answer.

suppose that it contained a prototype of the universal machinery of all life on Earth today. Through a very long process of mutation and natural selection, the descendants of this ancestral cell have gradually diverged to fill every habitat on Earth with organisms that exploit the potential of the machinery in an endless variety of ways.

Genes Provide the Instructions for Cell Form, Function, and Complex Behavior

A cell's **genome**—that is, the entire sequence of nucleotides in an organism's DNA—provides a genetic program that instructs the cell how to behave. For the cells of plant and animal embryos, the genome directs the growth and development of an adult organism with hundreds of different cell types. Within an individual plant or animal, these cells can be extraordinarily varied, as we discuss in Chapter 20. Fat cells, skin cells, bone cells, and nerve cells seem as dissimilar as any cells could be. Yet all these *differentiated cell types* are generated during embryonic development from a single fertilized egg cell, and all contain identical copies of the DNA of the species. Their varied characters stem from the way that individual cells use their genetic instructions. Different cells *express* different genes: that is, they use their genes to produce some proteins and not others, depending on their internal state and on cues that they and their ancestor cells have received from their surroundings—mainly signals from other cells in the organism.

The DNA, therefore, is not just a shopping list specifying the molecules that every cell must make, and a cell is not just an assembly of all the items on the list. Each cell is capable of carrying out a variety of biological tasks, depending on its environment and its history, and it selectively uses the information encoded in its DNA to guide its activities. Later in this book, we will see in detail how DNA defines both the parts list of the cell and the rules that decide when and where these parts are to be made.

CELLS UNDER THE MICROSCOPE

Today, we have the technology to decipher the underlying principles that govern the structure and activity of the cell. But cell biology started without these tools. The earliest cell biologists began by simply looking at tissues and cells, and later breaking them open or slicing them up, attempting to view their contents. What they saw was to them profoundly baffling—a collection of tiny and scarcely visible objects whose relationship to the properties of living matter seemed an impenetrable mystery. Nevertheless, this type of visual investigation was the first step toward understanding cells, and it remains essential in the study of cell biology.

Cells were not made visible until the seventeenth century, when the **microscope** was invented. For hundreds of years afterward, all that was known about cells was discovered using this instrument. *Light microscopes* use visible light to illuminate specimens, and they allowed biologists to see for the first time the intricate structure that underpins all living things.

Although these instruments now incorporate many sophisticated improvements, the properties of light itself set a limit to the fineness of detail they reveal. *Electron microscopes*, invented in the 1930s, go beyond this limit by using beams of electrons instead of beams of light as the source of illumination, greatly extending our ability to see the fine details of cells and even making some of the larger molecules visible individually. These and other forms of microscopy remain vital tools in the modern cell biology laboratory, where they continue to reveal new and sometimes surprising details about the way cells are built and how they operate.

The Invention of the Light Microscope Led to the Discovery of Cells

The development of the light microscope depended on advances in the production of glass lenses. By the seventeenth century, lenses were powerful enough to make out details invisible to the naked eye. Using an instrument equipped with such a lens, Robert Hooke examined a piece of cork and in 1665 reported to the Royal Society of London that the cork was composed of a mass of minute chambers. He called these chambers “cells,” based on their resemblance to the simple rooms occupied by monks in a monastery. The name stuck, even though the structures Hooke described were actually the cell walls that remained after the living plant cells inside them had died. Later, Hooke and his Dutch contemporary Antoni van Leeuwenhoek were able to observe living cells, seeing for the first time a world teeming with motile microscopic organisms.

For almost 200 years, such instruments—the first light microscopes—remained exotic devices, available only to a few wealthy individuals. It was not until the nineteenth century that microscopes began to be widely used to look at cells. The emergence of cell biology as a distinct science was a gradual process to which many individuals contributed, but its official birth is generally said to have been signaled by two publications: one by the botanist Matthias Schleiden in 1838 and the other by the zoologist Theodor Schwann in 1839. In these papers, Schleiden and Schwann documented the results of a systematic investigation of plant and animal tissues with the light microscope, showing that cells were the universal building blocks of all living tissues. Their work, and that of other nineteenth-century microscopists, slowly led to the realization that all living cells are formed by the growth and division of existing cells—a principle sometimes referred to as the *cell theory* (Figure 1–4). The implication that

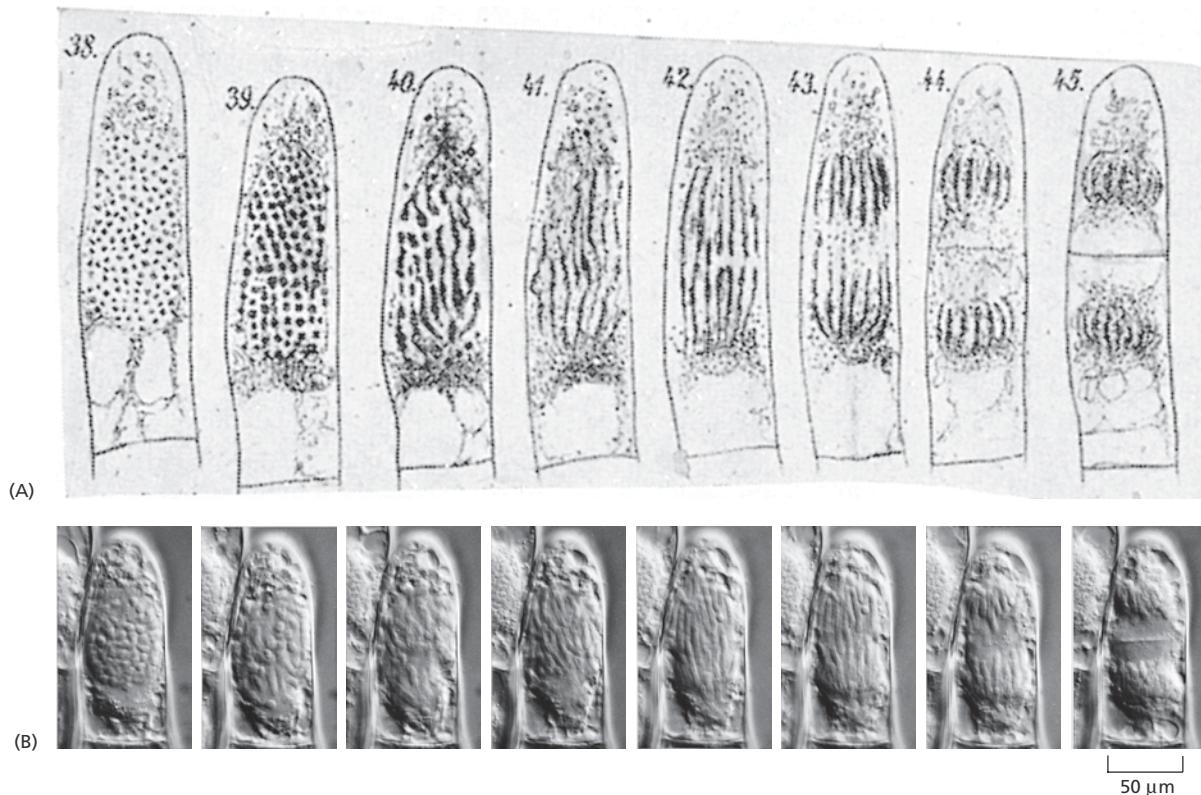


Figure 1–4 New cells form by growth and division of existing cells. (A) In 1880, Eduard Strasburger drew a living plant cell (a hair cell from a *Tradescantia* flower), which he observed dividing into two daughter cells over a period of 2.5 hours. (B) A comparable living plant cell photographed recently through a modern light microscope. (B, courtesy of Peter Hepler.)

living organisms do not arise spontaneously but can be generated only from existing organisms was hotly contested, but it was finally confirmed in the 1860s by an elegant set of experiments performed by Louis Pasteur.

The principle that cells are generated only from preexisting cells and inherit their characteristics from them underlies all of biology and gives the subject a unique flavor: in biology, questions about the present are inescapably linked to questions about the past. To understand why present-day cells and organisms behave as they do, we need to understand their history, all the way back to the misty origins of the first cells on Earth. Charles Darwin provided the key insight that makes this history comprehensible. His theory of evolution, published in 1859, explains how random variation and natural selection gave rise to diversity among organisms that share a common ancestry. When combined with the cell theory, the theory of evolution leads us to view all life, from its beginnings to the present day, as one vast family tree of individual cells. Although this book is primarily about how cells work today, we will encounter the theme of evolution again and again.

Light Microscopes Allow Examination of Cells and Some of Their Components

If you cut a very thin slice from a suitable plant or animal tissue and view it using a light microscope, you will see that the tissue is divided into thousands of small cells. These may be either closely packed or separated from one another by an *extracellular matrix*, a dense material often made of protein fibers embedded in a polysaccharide gel (**Figure 1–5**). Each cell is typically about 5–20 μm in diameter. If you have taken care of your specimen so that its cells remain alive, you will be able to see particles moving around inside individual cells. And if you watch patiently, you may even see a cell slowly change shape and divide into two (see Figure 1–4 and a speeded-up video of cell division in a frog embryo in **Movie 1.1**).

To see the internal structure of a cell is difficult, not only because the parts are small, but also because they are transparent and mostly colorless. One way around the problem is to stain cells with dyes that color particular components differently (see Figure 1–5). Alternatively, one can exploit the fact that cell components differ slightly from one another in

QUESTION 1–3

You have embarked on an ambitious research project: to create life in a test tube. You boil up a rich mixture of yeast extract and amino acids in a flask along with a sprinkling of the inorganic salts known to be essential for life. You seal the flask and allow it to cool. After several months, the liquid is as clear as ever, and there are no signs of life. A friend suggests that excluding the air was a mistake, since most life as we know it requires oxygen. You repeat the experiment, but this time you leave the flask open to the atmosphere. To your great delight, the liquid becomes cloudy after a few days and under the microscope you see beautiful small cells that are clearly growing and dividing. Does this experiment prove that you managed to generate a novel life-form? How might you redesign your experiment to allow air into the flask, yet eliminate the possibility that contamination is the explanation for the results? (For a ready-made answer, look up the classic experiments of Louis Pasteur.)

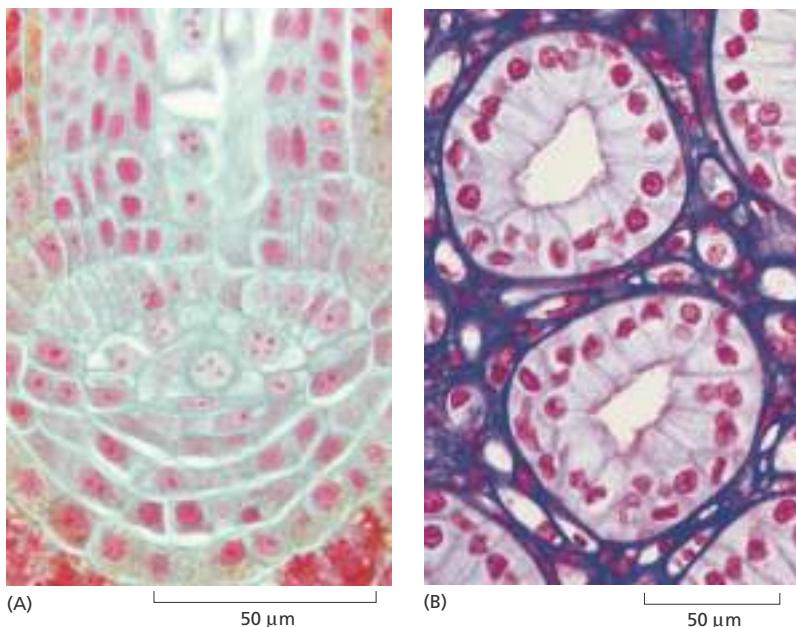


Figure 1–5 Cells form tissues in plants and animals. (A) Cells in the root tip of a fern. The nuclei are stained red, and each cell is surrounded by a thin cell wall (light blue). (B) Cells in the urine-collecting ducts of the kidney. Each duct appears in this cross section as a ring of closely packed cells (with nuclei stained red). The ring is surrounded by extracellular matrix, stained purple. (A, courtesy of James Mauseth; B, from P.R. Wheater et al., *Functional Histology*, 2nd ed. Edinburgh: Churchill Livingstone, 1987. With permission from Elsevier.)

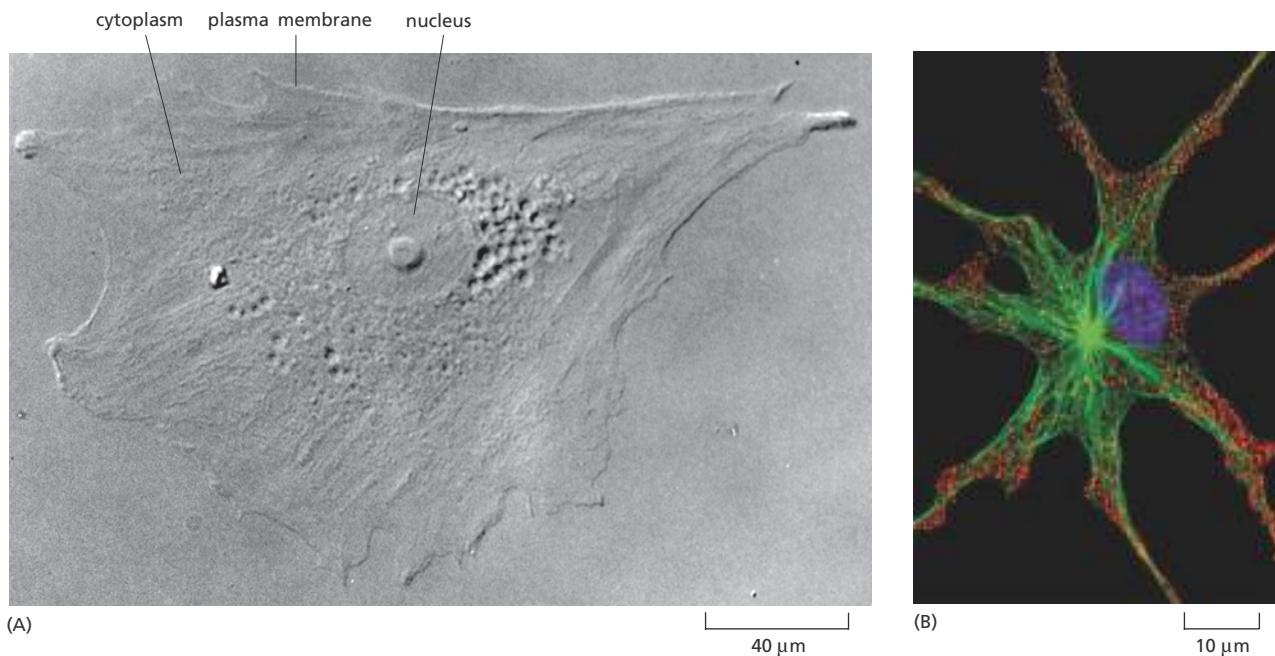


Figure 1–6 Some of the internal structures of a living cell can be seen with a light microscope. (A) A cell taken from human skin and grown in culture was photographed through a light microscope using interference-contrast optics (see Panel 1–1, pp. 10–11). The nucleus is especially prominent. (B) A pigment cell from a frog, stained with fluorescent dyes and viewed with a confocal fluorescence microscope (see Panel 1–1). The nucleus is shown in purple, the pigment granules in red, and the microtubules—a class of filaments built from protein molecules in the cytoplasm—in green. (A, courtesy of Casey Cunningham; B, courtesy of Stephen Rogers and the Imaging Technology Group of the Beckman Institute, University of Illinois, Urbana.)

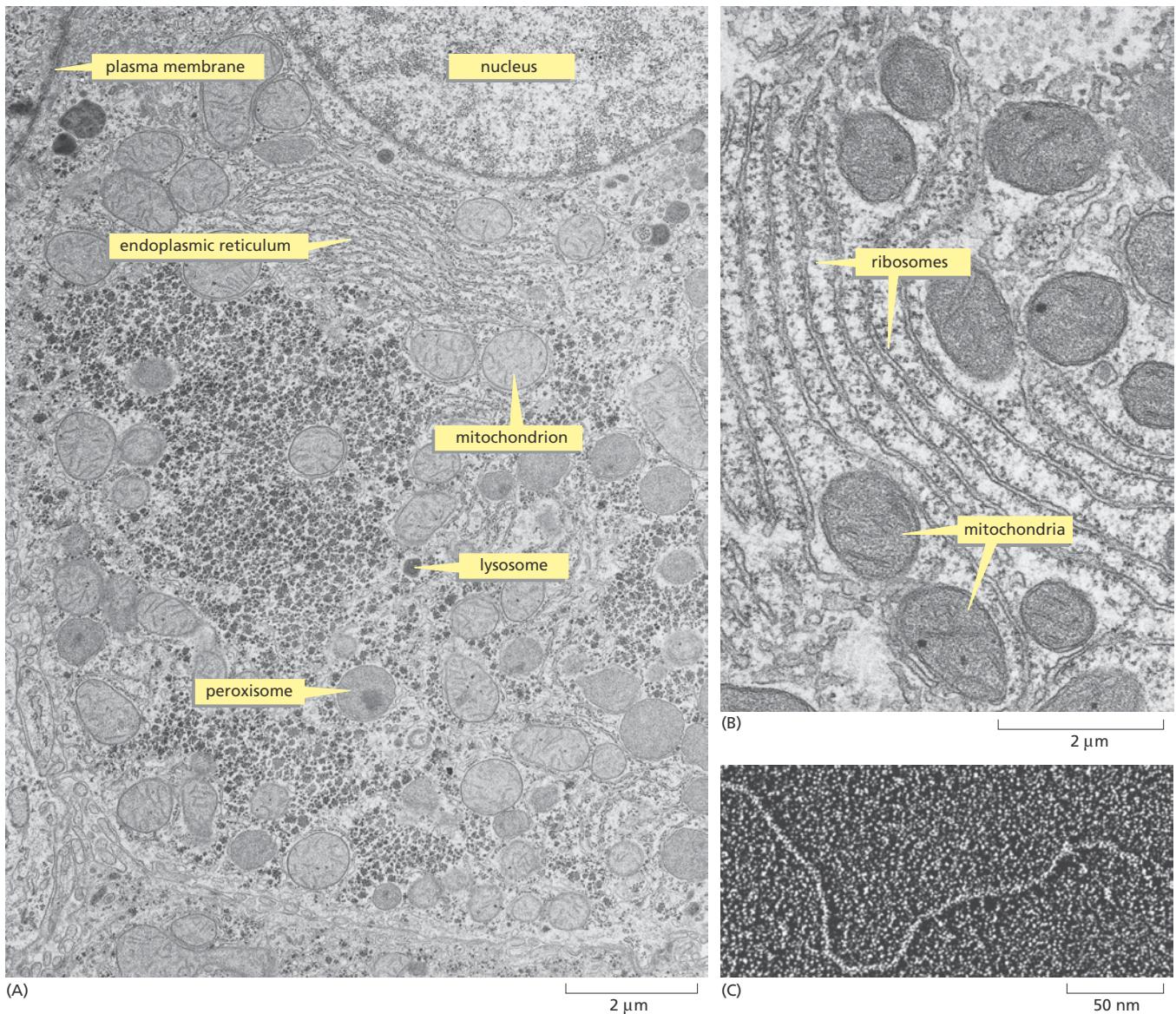
refractive index, just as glass differs in refractive index from water, causing light rays to be deflected as they pass from the one medium into the other. The small differences in refractive index can be made visible by specialized optical techniques, and the resulting images can be enhanced further by electronic processing.

The cell thus revealed has a distinct anatomy (**Figure 1–6A**). It has a sharply defined boundary, indicating the presence of an enclosing membrane. A large, round structure, the *nucleus*, is prominent in the middle of the cell. Around the nucleus and filling the cell's interior is the *cytoplasm*, a transparent substance crammed with what seems at first to be a jumble of miscellaneous objects. With a good light microscope, one can begin to distinguish and classify some of the specific components in the cytoplasm, but structures smaller than about 0.2 μm—about half the wavelength of visible light—cannot normally be resolved; points closer than this are not distinguishable and appear as a single blur.

In recent years, however, new types of **fluorescence microscopes** have been developed that use sophisticated methods of illumination and electronic image processing to see fluorescently labeled cell components in much finer detail (**Figure 1–6B**). The most recent super-resolution fluorescence microscopes, for example, can push the limits of resolution down even further, to about 20 nanometers (nm). That is the size of a single **ribosome**, a large macromolecular complex composed of 80–90 individual proteins and RNA molecules.

The Fine Structure of a Cell Is Revealed by Electron Microscopy

For the highest magnification and best resolution, one must turn to an **electron microscope**, which can reveal details down to a few nanometers. Cell samples for the electron microscope require painstaking preparation. Even for light microscopy, a tissue often has to be *fixed* (that is, preserved by pickling in a reactive chemical solution), supported by *embedding* in a solid wax or resin, cut or *sectioned* into thin slices, and *stained* before it is viewed. For electron microscopy, similar procedures are required, but the sections have to be much thinner and there is no possibility of looking at living, wet cells.



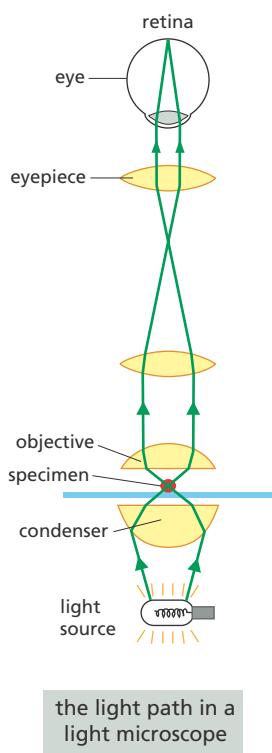
When thin sections are cut, stained, and placed in the electron microscope, much of the jumble of cell components becomes sharply resolved into distinct **organelles**—separate, recognizable substructures with specialized functions that are often only hazily defined with a light microscope. A delicate membrane, only about 5 nm thick, is visible enclosing the cell, and similar membranes form the boundary of many of the organelles inside (Figure 1–7A, B). The membrane that separates the interior of the cell from its external environment is called the **plasma membrane**, while the membranes surrounding organelles are called *internal membranes*. All of these membranes are only two molecules thick (as discussed in Chapter 11). With an electron microscope, even individual large molecules can be seen (Figure 1–7C).

The type of electron microscope used to look at thin sections of tissue is known as a *transmission electron microscope*. This is, in principle, similar to a light microscope, except that it transmits a beam of electrons rather than a beam of light through the sample. Another type of electron microscope—the *scanning electron microscope*—scatters electrons off the surface of the sample and so is used to look at the surface detail of cells and other structures. A survey of the principal types of microscopy used to examine cells is given in **Panel 1–1** (pp. 10–11).

Figure 1–7 The fine structure of a cell can be seen in a transmission electron microscope. (A) Thin section of a liver cell showing the enormous amount of detail that is visible. Some of the components to be discussed later in the chapter are labeled; they are identifiable by their size and shape. (B) A small region of the cytoplasm at higher magnification. The smallest structures that are clearly visible are the ribosomes, each of which is made of 80–90 or so individual large molecules. (C) Portion of a long, threadlike DNA molecule isolated from a cell and viewed by electron microscopy. (A and B, courtesy of Daniel S. Friend; C, courtesy of Mei Lie Wong.)

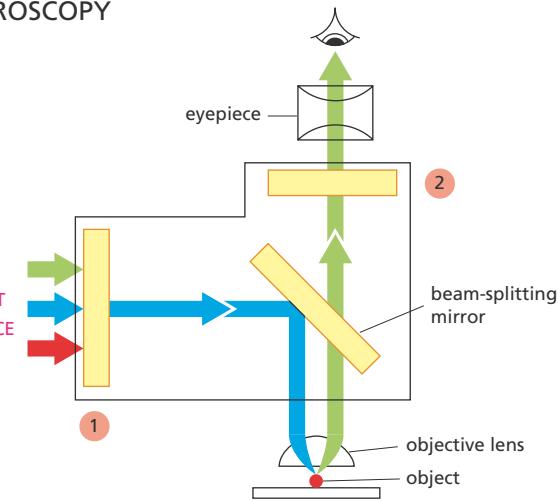


THE LIGHT MICROSCOPE

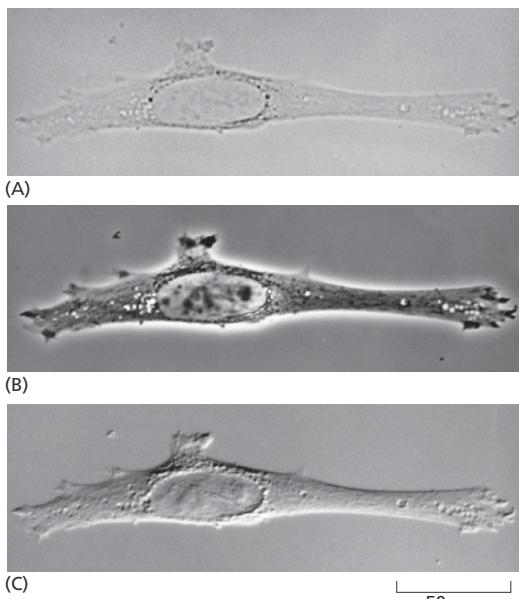


The light microscope allows us to magnify cells up to 1000 times and to resolve details as small as $0.2\text{ }\mu\text{m}$ (a limitation imposed by the wavelike nature of light, not by the quality of the lenses). Three things are required for viewing cells in a light microscope. First, a bright light must be focused onto the specimen by lenses in the condenser. Second, the specimen must be carefully prepared to allow light to pass through it. Third, an appropriate set of lenses (objective and eyepiece) must be arranged to focus an image of the specimen in the eye.

FLUORESCENCE MICROSCOPY



Fluorescent dyes used for staining cells are detected with the aid of a *fluorescence microscope*. This is similar to an ordinary light microscope except that the illuminating light is passed through two sets of filters. The first (1) filters the light before it reaches the specimen, passing only those wavelengths that excite the particular fluorescent dye. The second (2) blocks out this light and passes only those wavelengths emitted when the dye fluoresces. Dyed objects show up in bright color on a dark background.

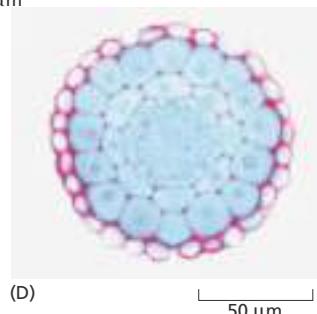


LOOKING AT LIVING CELLS

The same unstained, living animal cell (fibroblast) in culture viewed with (A) straightforward (bright-field) optics; (B) phase-contrast optics; (C) interference-contrast optics. The two latter systems exploit differences in the way light travels through regions of the cell with differing refractive indexes. All three images can be obtained on the same microscope simply by interchanging optical components.

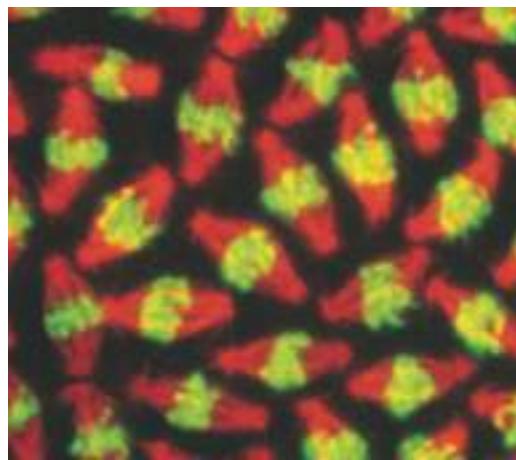
FIXED SAMPLES

Most tissues are neither small enough nor transparent enough to examine directly in the microscope. Typically, therefore, they are chemically fixed and cut into very thin slices, or *sections*, that can be mounted on a glass microscope slide and subsequently stained to reveal different components of the cells. A stained section of a plant root tip is shown here (D). (Courtesy of Catherine Kidner.)



FLUORESCENT PROBES

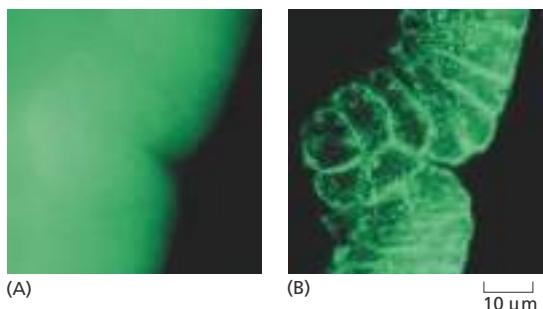
Dividing nuclei in a fly embryo seen with a fluorescence microscope after staining with specific fluorescent dyes.



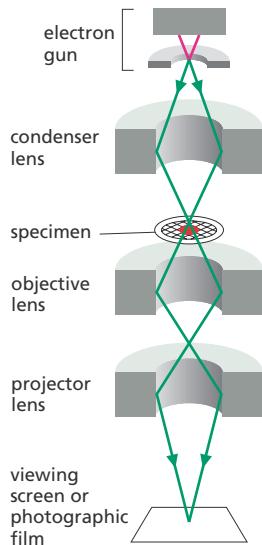
Fluorescent dyes absorb light at one wavelength and emit it at another, longer wavelength. Some such dyes bind specifically to particular molecules in cells and can reveal their location when examined with a fluorescence microscope. An example is the stain for DNA shown here (green). Other dyes can be coupled to antibody molecules, which then serve as highly specific and versatile staining reagents that bind selectively to particular large molecules, allowing us to see their distribution in the cell. In the example shown, a microtubule protein in the mitotic spindle is stained red with a fluorescent antibody. (Courtesy of William Sullivan.)

CONFOCAL MICROSCOPY

A confocal microscope is a specialized type of fluorescence microscope that builds up an image by scanning the specimen with a laser beam. The beam is focused onto a single point at a specific depth in the specimen, and a pinhole aperture in the detector allows only fluorescence emitted from this same point to be included in the image. Scanning the beam across the specimen generates a sharp image of the plane of focus—an *optical section*. A series of optical sections at different depths allows a three-dimensional image to be constructed. An intact insect embryo is shown here stained with a fluorescent probe for actin filaments. (A) Conventional fluorescence microscopy gives a blurry image due to the presence of fluorescent structures above and below the plane of focus. (B) Confocal microscopy provides an optical section showing the individual cells clearly. (Courtesy of Richard Warn and Peter Shaw.)



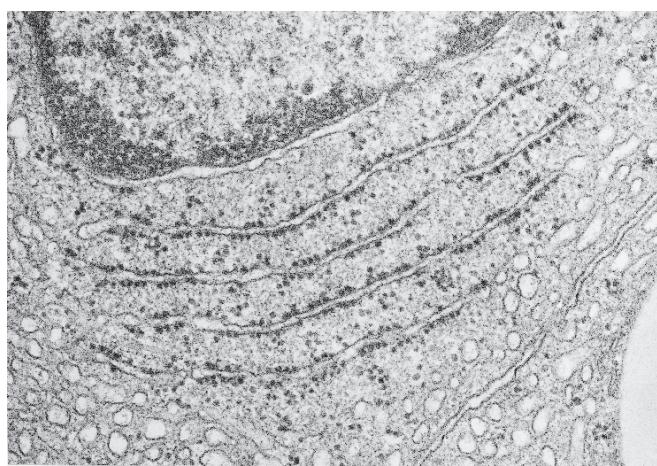
TRANSMISSION ELECTRON MICROSCOPY



Courtesy of Philips Electron Optics, with permission from FEI Co.

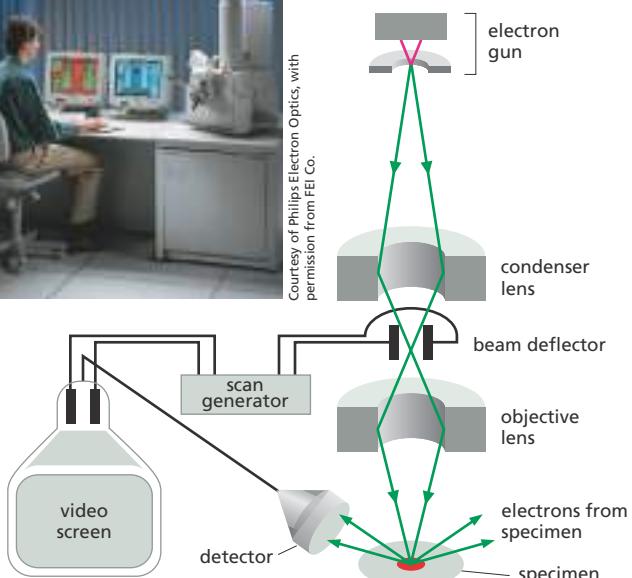


The electron micrograph below shows a small region of a cell in a piece of testis. The tissue has been chemically fixed, embedded in plastic, and cut into very thin sections that have then been stained with salts of uranium and lead. (Courtesy of Daniel S. Friend.)



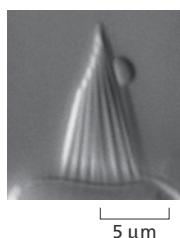
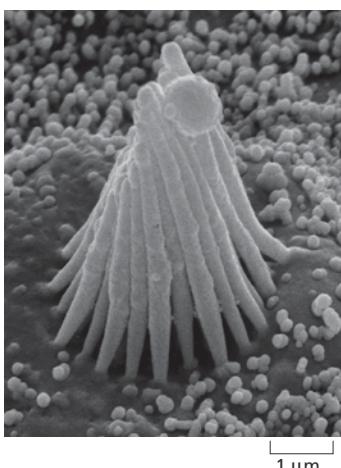
The transmission electron microscope (TEM) is in principle similar to a light microscope, but it uses a beam of electrons instead of a beam of light, and magnetic coils to focus the beam instead of glass lenses. The specimen, which is placed in a vacuum, must be very thin. Contrast is usually introduced by staining the specimen with electron-dense heavy metals that locally absorb or scatter electrons, removing them from the beam as it passes through the specimen. The TEM has a useful magnification of up to a million-fold and can resolve details as small as about 1 nm in biological specimens.

SCANNING ELECTRON MICROSCOPY



Courtesy of Philips Electron Optics, with permission from FEI Co.

In the scanning electron microscope (SEM), the specimen, which has been coated with a very thin film of a heavy metal, is scanned by a beam of electrons brought to a focus on the specimen by magnetic coils that act as lenses. The quantity of electrons scattered or emitted as the beam bombards each successive point on the surface of the specimen is measured by the detector, and is used to control the intensity of successive points in an image built up on a video screen. The microscope creates striking images of three-dimensional objects with great depth of focus and can resolve details down to somewhere between 3 nm and 20 nm, depending on the instrument.



Scanning electron micrograph of stereocilia projecting from a hair cell in the inner ear (left). For comparison, the same structure is shown by light microscopy, at the limit of its resolution (above). (Courtesy of Richard Jacobs and James Hudspeth.)

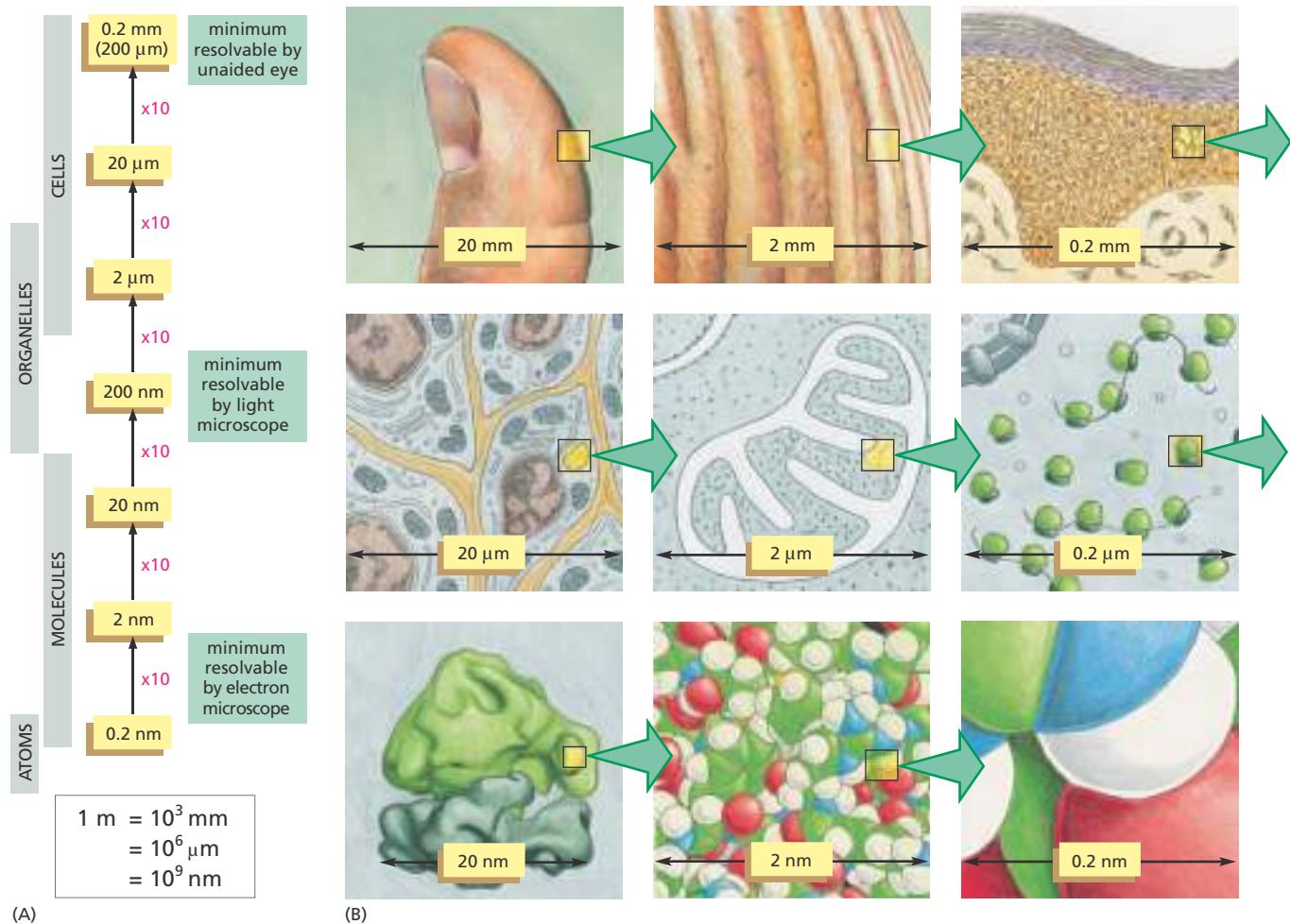


Figure 1–8 How big is a cell and its components? (A) The sizes of cells and of their component parts, plus the units in which they are measured. (B) Drawings to convey a sense of scale between living cells and atoms. Each panel shows an image that is magnified by a factor of 10 compared to its predecessor—producing an imaginary progression from a thumb, to skin, to skin cells, to a mitochondrion, to a ribosome, and ultimately to a cluster of atoms forming part of one of the many protein molecules in our bodies. Note that ribosomes are present inside mitochondria (as shown here), as well as in the cytoplasm. Details of molecular structure, as shown in the last two panels, are beyond the power of the electron microscope.

Even the most powerful electron microscopes, however, cannot visualize the individual atoms that make up biological molecules (Figure 1–8). To study the cell’s key components in atomic detail, biologists have developed even more sophisticated tools. A technique called X-ray crystallography, for example, is used to determine the precise three-dimensional structure of protein molecules (discussed in Chapter 4).

THE PROKARYOTIC CELL

Of all the types of cells revealed by the microscope, *bacteria* have the simplest structure and come closest to showing us life stripped down to its essentials. Indeed, a bacterium contains essentially no organelles—not even a nucleus to hold its DNA. This property—the presence or absence of a nucleus—is used as the basis for a simple but fundamental classification of all living things. Organisms whose cells have a nucleus are called **eukaryotes** (from the Greek words *eu*, meaning “well” or “truly,” and *karyon*, a “kernel” or “nucleus”). Organisms whose cells do not have a nucleus are called **prokaryotes** (from *pro*, meaning “before”). The terms

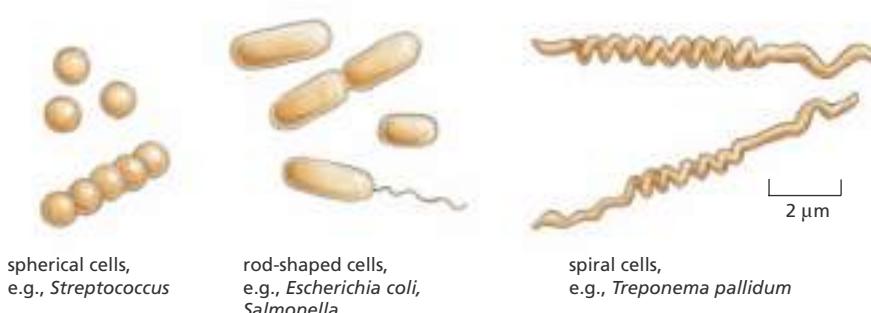


Figure 1–9 Bacteria come in different shapes and sizes. Typical spherical, rodlike, and spiral-shaped bacteria are drawn to scale. The spiral cells shown are the organisms that cause syphilis.

"bacterium" and "prokaryote" are often used interchangeably, although we will see that the category of prokaryotes also includes another class of cells, the *archaea* (singular *archaeon*), which are so remotely related to bacteria that they are given a separate name.

Prokaryotes are typically spherical, rodlike, or corkscrew-shaped (Figure 1–9). They are also small—generally just a few micrometers long, although there are some giant species as much as 100 times longer than this. Prokaryotes often have a tough protective coat, or cell wall, surrounding the plasma membrane, which encloses a single compartment containing the cytoplasm and the DNA. In the electron microscope, the cell interior typically appears as a matrix of varying texture, without any obvious organized internal structure (Figure 1–10). The cells reproduce quickly by dividing in two. Under optimum conditions, when food is plentiful, many prokaryotic cells can duplicate themselves in as little as 20 minutes. In 11 hours, by repeated divisions, a single prokaryote can give rise to more than 8 billion progeny (which exceeds the total number of humans presently on Earth). Thanks to their large numbers, rapid growth rates, and ability to exchange bits of genetic material by a process akin to sex, populations of prokaryotic cells can evolve fast, rapidly acquiring the ability to use a new food source or to resist being killed by a new antibiotic.

Prokaryotes Are the Most Diverse and Numerous Cells on Earth

Most prokaryotes live as single-celled organisms, although some join together to form chains, clusters, or other organized multicellular structures. In shape and structure, prokaryotes may seem simple and limited, but in terms of chemistry, they are the most diverse and inventive class of cells. Members of this class exploit an enormous range of habitats, from hot puddles of volcanic mud to the interiors of other living cells, and they vastly outnumber all eukaryotic organisms on Earth. Some are aerobic, using oxygen to oxidize food molecules; some are strictly anaerobic and are killed by the slightest exposure to oxygen. As we discuss later in this

QUESTION 1–4

A bacterium weighs about 10^{-12} g and can divide every 20 minutes. If a single bacterial cell carried on dividing at this rate, how long would it take before the mass of bacteria would equal that of the Earth (6×10^{24} kg)? Contrast your result with the fact that bacteria originated at least 3.5 billion years ago and have been dividing ever since. Explain the apparent paradox. (The number of cells N in a culture at time t is described by the equation $N = N_0 \times 2^{t/G}$, where N_0 is the number of cells at zero time and G is the population doubling time.)

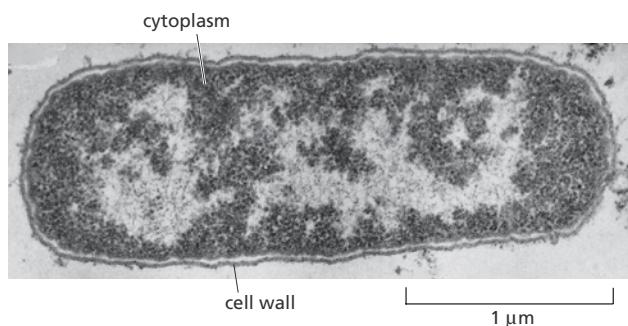


Figure 1–10 The bacterium *Escherichia coli* (*E. coli*) has served as an important model organism. An electron micrograph of a longitudinal section is shown here; the cell's DNA is concentrated in the lightly stained region. (Courtesy of E. Kellenberger.)

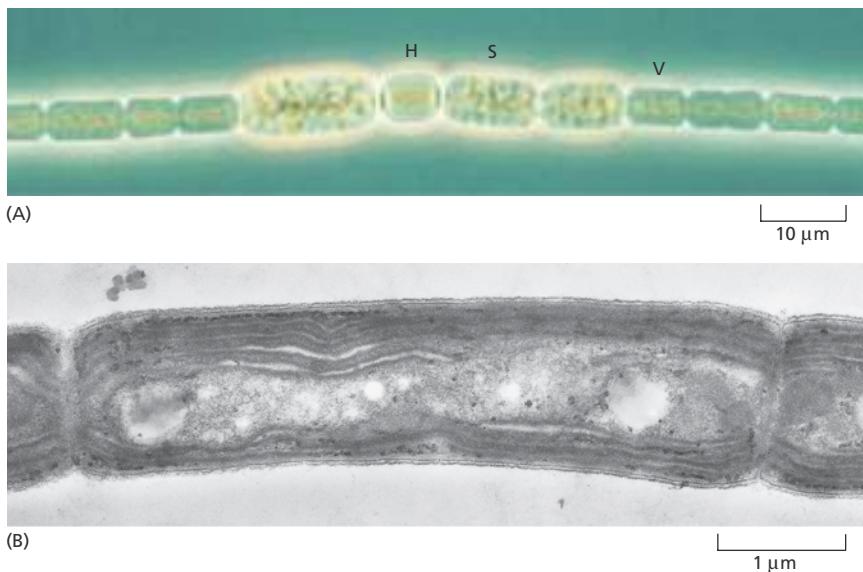


Figure 1–11 Some bacteria are photosynthetic. (A) *Anabaena cylindrica* forms long, multicellular filaments. This light micrograph shows specialized cells that either fix nitrogen (that is, capture N₂ from the atmosphere and incorporate it into organic compounds; labeled H), fix CO₂ through photosynthesis (labeled V), or become resistant spores (labeled S). (B) An electron micrograph of a related species, *Phormidium laminosum*, shows the intracellular membranes where photosynthesis occurs. These micrographs illustrate that even some prokaryotes can form simple multicellular organisms. (A, courtesy of David Adams; B, courtesy of D.P. Hill and C.J. Howe.)

chapter, *mitochondria*—the organelles that generate energy in eukaryotic cells—are thought to have evolved from aerobic bacteria that took to living inside the anaerobic ancestors of today's eukaryotic cells. Thus our own oxygen-based metabolism can be regarded as a product of the activities of bacterial cells.

Virtually any organic, carbon-containing material—from wood to petroleum—can be used as food by one sort of bacterium or another. Even more remarkably, some prokaryotes can live entirely on inorganic substances: they can get their carbon from CO₂ in the atmosphere, their nitrogen from atmospheric N₂, and their oxygen, hydrogen, sulfur, and phosphorus from air, water, and inorganic minerals. Some of these prokaryotic cells, like plant cells, perform *photosynthesis*, using energy from sunlight to produce organic molecules from CO₂ (Figure 1–11); others derive energy from the chemical reactivity of inorganic substances in the environment (Figure 1–12). In either case, such prokaryotes play a unique and fundamental part in the economy of life on Earth: other living things depend on the organic compounds that these cells generate from inorganic materials.

Plants, too, can capture energy from sunlight and carbon from atmospheric CO₂. But plants unaided by bacteria cannot capture N₂ from the atmosphere, and in a sense even plants depend on bacteria for photosynthesis. It is almost certain that the organelles in the plant cell that



Figure 1–12 A sulfur bacterium gets its energy from H₂S. *Beggiaotoa*, a prokaryote that lives in sulfurous environments, oxidizes H₂S to produce sulfur and can fix carbon even in the dark. In this light micrograph, yellow deposits of sulfur can be seen inside both of the cells. (Courtesy of Ralph W. Wolfe.)

perform photosynthesis—the *chloroplasts*—have evolved from photosynthetic bacteria that long ago found a home inside the cytoplasm of a plant cell ancestor.

The World of Prokaryotes Is Divided into Two Domains: Bacteria and Archaea

Traditionally, all prokaryotes have been classified together in one large group. But molecular studies reveal that there is a gulf within the class of prokaryotes, dividing it into two distinct *domains* called the **bacteria** and the **archaea**. Remarkably, at a molecular level, the members of these two domains differ as much from one another as either does from the eukaryotes. Most of the prokaryotes familiar from everyday life—the species that live in the soil or make us ill—are bacteria. Archaea are found not only in these habitats, but also in environments that are too hostile for most other cells: concentrated brine, the hot acid of volcanic springs, the airless depths of marine sediments, the sludge of sewage treatment plants, pools beneath the frozen surface of Antarctica, and in the acidic, oxygen-free environment of a cow's stomach where they break down cellulose and generate methane gas. Many of these extreme environments resemble the harsh conditions that must have existed on the primitive Earth, where living things first evolved before the atmosphere became rich in oxygen.

THE EUKARYOTIC CELL

Eukaryotic cells, in general, are bigger and more elaborate than bacteria and archaea. Some live independent lives as single-celled organisms, such as amoebae and yeasts (**Figure 1–13**); others live in multicellular assemblies. All of the more complex multicellular organisms—including plants, animals, and fungi—are formed from eukaryotic cells.

By definition, all eukaryotic cells have a nucleus. But possession of a nucleus goes hand-in-hand with possession of a variety of other organelles, most of which are membrane-enclosed and common to all eukaryotic organisms. In this section, we take a look at the main organelles found in eukaryotic cells from the point of view of their functions, and we consider how they came to serve the roles they have in the life of the eukaryotic cell.

The Nucleus Is the Information Store of the Cell

The **nucleus** is usually the most prominent organelle in a eukaryotic cell (**Figure 1–14**). It is enclosed within two concentric membranes that form the *nuclear envelope*, and it contains molecules of DNA—extremely long polymers that encode the genetic information of the organism. In the light microscope, these giant DNA molecules become visible as individual **chromosomes** when they become more compact before a cell divides into two daughter cells (**Figure 1–15**). DNA also carries the genetic information in prokaryotic cells; these cells lack a distinct nucleus not because they lack DNA, but because they do not keep their DNA inside a nuclear envelope, segregated from the rest of the cell contents.

Figure 1–13 Yeasts are simple free-living eukaryotes. The cells shown in this micrograph belong to the species of yeast, *Saccharomyces cerevisiae*, used to make dough rise and turn malted barley juice into beer. As can be seen in this image, the cells reproduce by growing a bud and then dividing asymmetrically into a large mother cell and a small daughter cell; for this reason, they are called budding yeast.

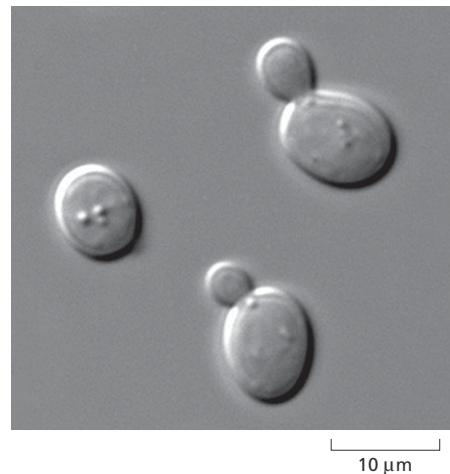
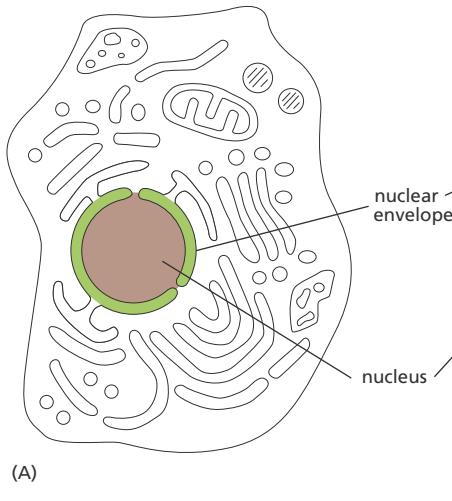
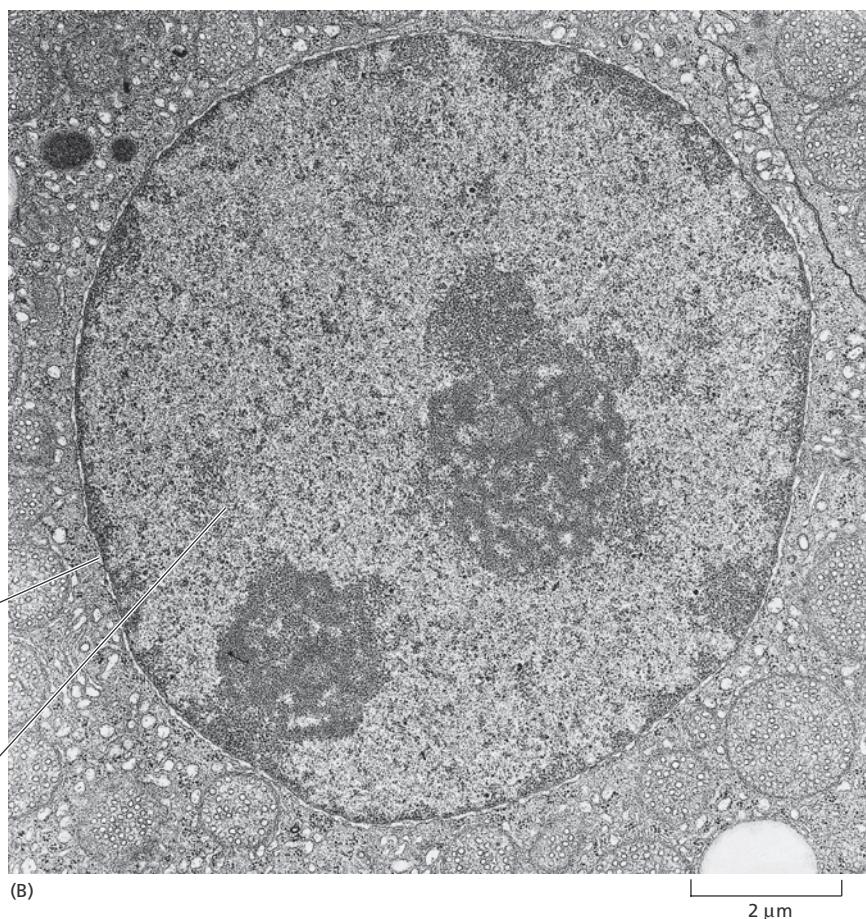


Figure 1–14 The nucleus contains most of the DNA in a eukaryotic cell. (A) This drawing of a typical animal cell shows its extensive system of membrane-enclosed organelles. The nucleus is colored brown, the nuclear envelope is green, and the cytoplasm (the interior of the cell outside the nucleus) is white. (B) An electron micrograph of the nucleus in a mammalian cell. Individual chromosomes are not visible because at this stage of the cell's growth its DNA molecules are dispersed as fine threads throughout the nucleus. (B, courtesy of Daniel S. Friend.)



(A)



(B)

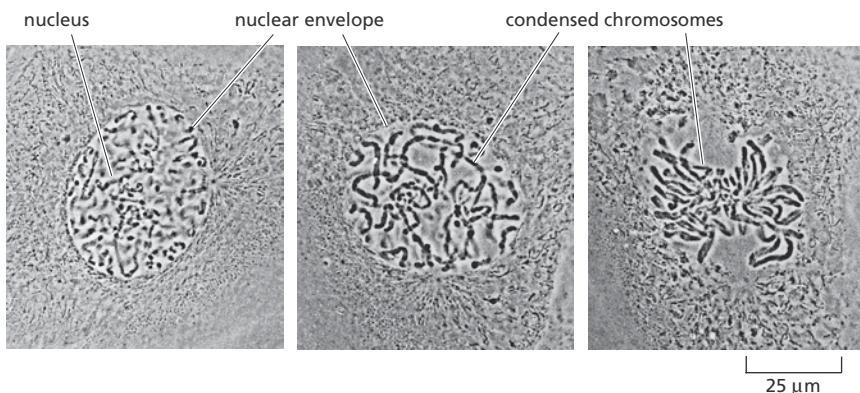
2 μm

Mitochondria Generate Usable Energy from Food to Power the Cell

Mitochondria are present in essentially all eukaryotic cells, and they are among the most conspicuous organelles in the cytoplasm (see Figure 1–7B). In a fluorescence microscope, they appear as worm-shaped structures that often form branching networks (Figure 1–16). When seen with an electron microscope, individual mitochondria are found to be enclosed in two separate membranes, with the inner membrane formed into folds that project into the interior of the organelle (Figure 1–17).

Microscopic examination by itself, however, gives little indication of what mitochondria do. Their function was discovered by breaking open cells and then spinning the soup of cell fragments in a centrifuge; this

Figure 1–15 Chromosomes become visible when a cell is about to divide. As a eukaryotic cell prepares to divide, its DNA molecules become progressively more compacted (condensed), forming wormlike chromosomes that can be distinguished in the light microscope. The photographs show three successive steps in this process in a cultured cell from a newt's lung; note that in the last micrograph on the right, the nuclear envelope has broken down. (Courtesy of Conly L. Rieder.)

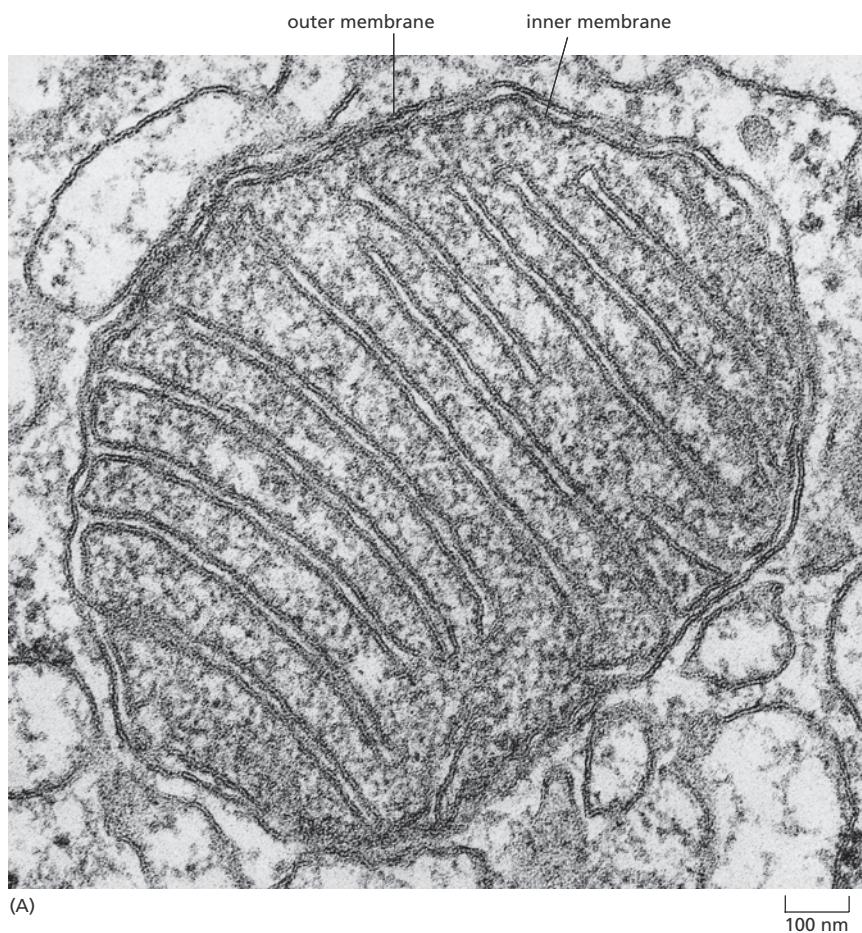


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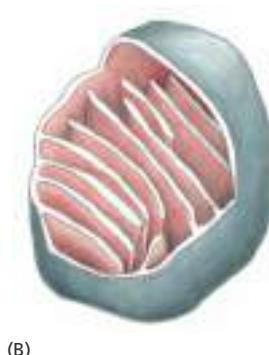
Figure 1–16 Mitochondria can be variable in shape and size. This budding yeast cell, which contains a green fluorescent protein in its mitochondria, was viewed in a super-resolution confocal fluorescence microscope. In this three-dimensional image, the mitochondria are seen to form complex branched networks. (From A. Egner et al., *Proc. Natl Acad. Sci. USA* 99:3370–3375, 2002. With permission from the National Academy of Sciences.)



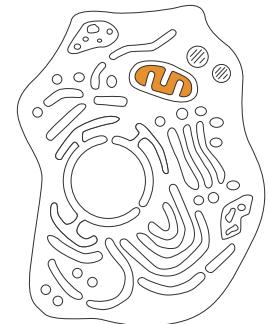
separates the organelles according to their size and density. Purified mitochondria were then tested to see what chemical processes they could perform. This revealed that mitochondria are generators of chemical energy for the cell. They harness the energy from the oxidation of food molecules, such as sugars, to produce *adenosine triphosphate*, or ATP—the basic chemical fuel that powers most of the cell's activities. Because the mitochondrion consumes oxygen and releases carbon dioxide in the course of this activity, the entire process is called *cellular respiration*—essentially, breathing on a cellular level. Without mitochondria, animals, fungi, and plants would be unable to use oxygen to extract the energy they need from the food molecules that nourish them. The process of cellular respiration is considered in detail in Chapter 14.



(A)



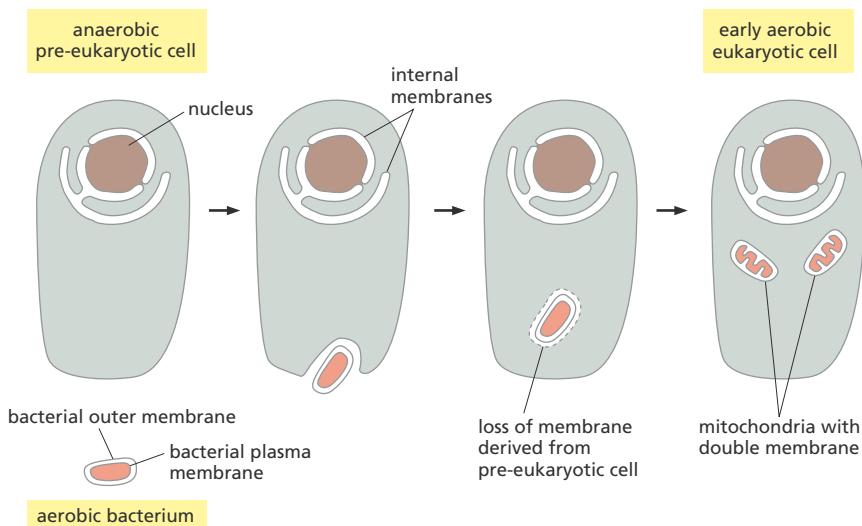
(B)



(C)

Figure 1–17 Mitochondria have a distinctive structure. (A) An electron micrograph of a cross section of a mitochondrion reveals the extensive infolding of the inner membrane. (B) This three-dimensional representation of the arrangement of the mitochondrial membranes shows the smooth outer membrane (gray) and the highly convoluted inner membrane (red). The inner membrane contains most of the proteins responsible for cellular respiration—one of the mitochondrion's main functions—and it is highly folded to provide a large surface area for this activity. (C) In this schematic cell, the interior space of the mitochondrion is colored orange. (A, courtesy of Daniel S. Friend.)

Figure 1–18 Mitochondria most likely evolved from engulfed bacteria. It is virtually certain that mitochondria originate from bacteria that were engulfed by an ancestral pre-eukaryotic cell and survived inside it, living in symbiosis with their host. Note that the double membrane of present-day mitochondria is thought to have been derived from the plasma membrane and outer membrane of the engulfed bacterium.



Mitochondria contain their own DNA and reproduce by dividing in two. Because they resemble bacteria in so many ways, they are thought to have been derived from bacteria that were engulfed by some ancestor of present-day eukaryotic cells (Figure 1–18). This evidently created a *symbiotic* relationship in which the host eukaryote and the engulfed bacterium helped one another to survive and reproduce.

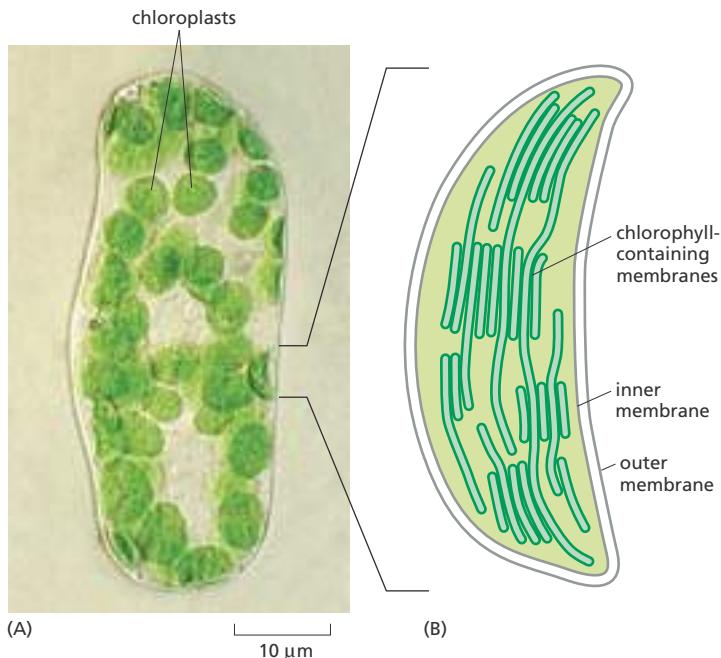
Chloroplasts Capture Energy from Sunlight

Chloroplasts are large, green organelles that are found only in the cells of plants and algae, not in the cells of animals or fungi. These organelles have an even more complex structure than mitochondria: in addition to their two surrounding membranes, they possess internal stacks of membranes containing the green pigment *chlorophyll* (Figure 1–19).

Chloroplasts carry out **photosynthesis**—trapping the energy of sunlight in their chlorophyll molecules and using this energy to drive the manufacture of energy-rich sugar molecules. In the process, they release

Figure 1–19 Chloroplasts in plant cells capture the energy of sunlight.

(A) A single cell isolated from a leaf of a flowering plant, seen in the light microscope, showing many green chloroplasts. (B) A drawing of one of the chloroplasts, showing the inner and outer membranes, as well as the highly folded system of internal membranes containing the green chlorophyll molecules that absorb light energy. (A, courtesy of Preeti Dahiya.)



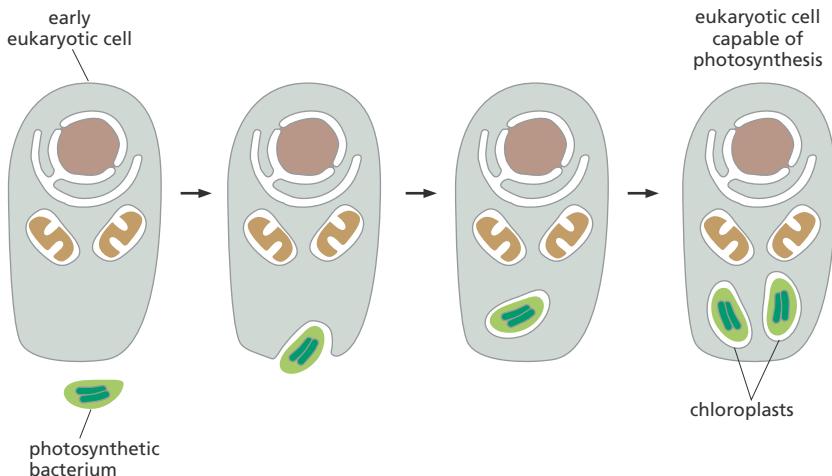


Figure 1–20 Chloroplasts almost certainly evolved from engulfed photosynthetic bacteria. The bacteria are thought to have been taken up by early eukaryotic cells that already contained mitochondria.

oxygen as a molecular by-product. Plant cells can then extract this stored chemical energy when they need it, by oxidizing these sugars in their mitochondria, just as animal cells do. Chloroplasts thus enable plants to get their energy directly from sunlight. And they allow plants to produce the food molecules—and the oxygen—that mitochondria use to generate chemical energy in the form of ATP. How these organelles work together is discussed in Chapter 14.

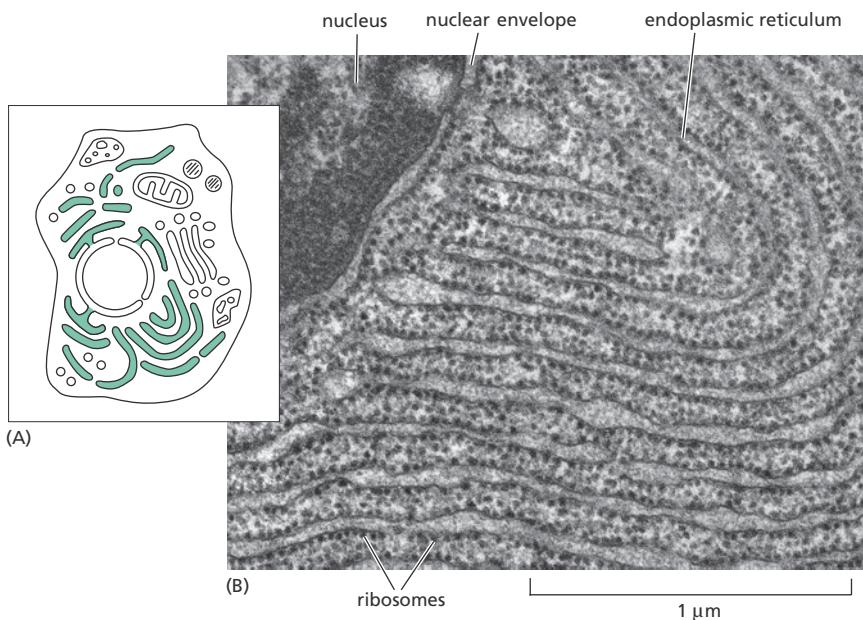
Like mitochondria, chloroplasts contain their own DNA, reproduce by dividing in two, and are thought to have evolved from bacteria—in this case, from photosynthetic bacteria that were engulfed by an early eukaryotic cell (Figure 1–20).

Internal Membranes Create Intracellular Compartments with Different Functions

Nuclei, mitochondria, and chloroplasts are not the only membrane-enclosed organelles inside eukaryotic cells. The cytoplasm contains a profusion of other organelles that are surrounded by single membranes (see Figure 1–7A). Most of these structures are involved with the cell's ability to import raw materials and to export both the useful substances and waste products that are produced by the cell.

The *endoplasmic reticulum (ER)* is an irregular maze of interconnected spaces enclosed by a membrane (Figure 1–21). It is the site where most cell-membrane components, as well as materials destined for export from the cell, are made. This organelle is enormously enlarged in cells that are specialized for the secretion of proteins. Stacks of flattened, membrane-enclosed sacs constitute the *Golgi apparatus* (Figure 1–22), which modifies and packages molecules made in the ER that are destined to be either secreted from the cell or transported to another cell compartment. *Lysosomes* are small, irregularly shaped organelles in which intracellular digestion occurs, releasing nutrients from ingested food particles and breaking down unwanted molecules for either recycling within the cell or excretion from the cell. Indeed, many of the large and small molecules within the cell are constantly being broken down and remade. *Peroxisomes* are small, membrane-enclosed vesicles that provide a safe environment for a variety of reactions in which hydrogen peroxide is used to inactivate toxic molecules. Membranes also form many different types of small *transport vesicles* that ferry materials between one membrane-enclosed organelle and another. All of these membrane-enclosed organelles are sketched in Figure 1–23A.

Figure 1–21 The endoplasmic reticulum produces many of the components of a eukaryotic cell. (A) Schematic diagram of an animal cell shows the endoplasmic reticulum (ER) in green. (B) Electron micrograph of a thin section of a mammalian pancreatic cell shows a small part of the ER, of which there are vast amounts in this cell type, which is specialized for protein secretion. Note that the ER is continuous with the membranes of the nuclear envelope. The black particles studding the particular region of the ER shown here are ribosomes, structures that translate RNAs into proteins. Because of its appearance, ribosome-coated ER is often called “rough ER” to distinguish it from the “smooth ER,” which does not have ribosomes bound to it. (B, courtesy of Lelio Orci.)



A continual exchange of materials takes place between the endoplasmic reticulum, the Golgi apparatus, the lysosomes, and the outside of the cell. The exchange is mediated by transport vesicles that pinch off from the membrane of one organelle and fuse with another, like tiny soap bubbles budding from and rejoining larger bubbles. At the surface of the cell, for example, portions of the plasma membrane tuck inward and pinch off to form vesicles that carry material captured from the external medium into the cell—a process called *endocytosis* (Figure 1–24). Animal cells can

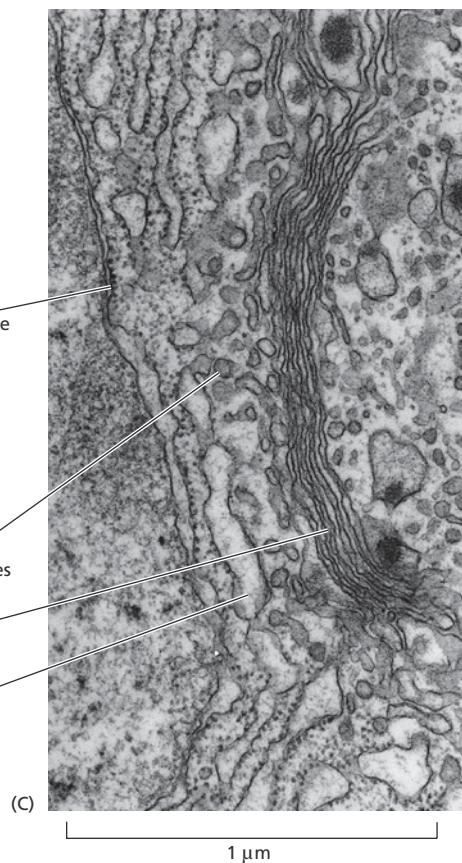
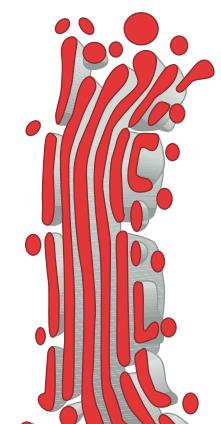
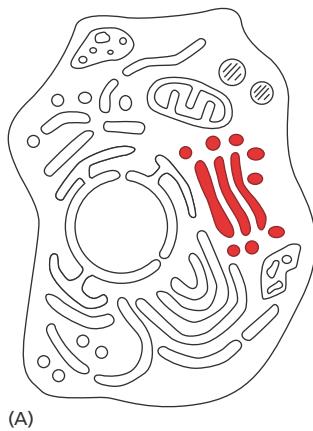


Figure 1–22 The Golgi apparatus is composed of a stack of flattened discs.

(A) Schematic diagram of an animal cell with the Golgi apparatus colored red. (B) More realistic drawing of the Golgi apparatus. Some of the vesicles seen nearby have pinched off from the Golgi stack; others are destined to fuse with it. Only one stack is shown here, but several can be present in a cell. (C) Electron micrograph that shows the Golgi apparatus from a typical animal cell. (C, courtesy of Brij J. Gupta.)

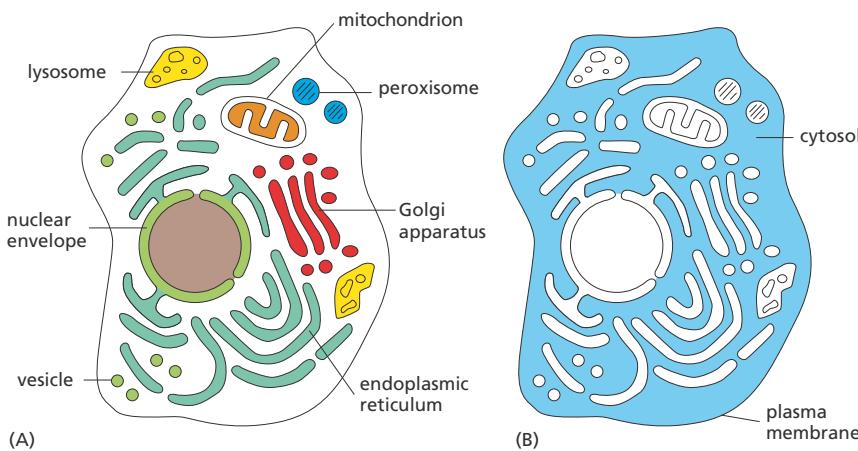


Figure 1–23 Membrane-enclosed organelles are distributed throughout the eukaryotic cell cytoplasm. (A) The membrane-enclosed organelles, shown in different colors, are each specialized to perform a different function. (B) The cytoplasm that fills the space outside of these organelles is called the cytosol (colored blue).

engulf very large particles, or even entire foreign cells, by endocytosis. In the reverse process, called *exocytosis*, vesicles from inside the cell fuse with the plasma membrane and release their contents into the external medium (see Figure 1–24); most of the hormones and signal molecules that allow cells to communicate with one another are secreted from cells by exocytosis. How membrane-enclosed organelles move proteins and other molecules from place to place inside the cell is discussed in detail in Chapter 15.

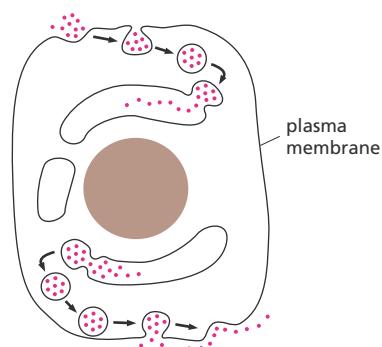
The Cytosol Is a Concentrated Aqueous Gel of Large and Small Molecules

If we were to strip the plasma membrane from a eukaryotic cell and then remove all of its membrane-enclosed organelles, including the nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, chloroplasts, and so on, we would be left with the **cytosol** (see Figure 1–23B). In other words, the cytosol is the part of the cytoplasm that is not contained within intracellular membranes. In most cells, the cytosol is the largest single compartment. It contains a host of large and small molecules, crowded together so closely that it behaves more like a water-based gel than a liquid solution (Figure 1–25). The cytosol is the site of many chemical reactions that are fundamental to the cell's existence. The early steps in the breakdown of nutrient molecules take place in the cytosol, for example, and it is here that most proteins are made by ribosomes.

The Cytoskeleton Is Responsible for Directed Cell Movements

The cytoplasm is not just a structureless soup of chemicals and organelles. Using an electron microscope, one can see that in eukaryotic cells the cytosol is criss-crossed by long, fine filaments. Frequently, the filaments are seen to be anchored at one end to the plasma membrane or to radiate out from a central site adjacent to the nucleus. This system of protein filaments, called the **cytoskeleton**, is composed of three major filament types (Figure 1–26). The thinnest of these filaments are the *actin filaments*; they are abundant in all eukaryotic cells but occur in especially large numbers inside muscle cells, where they serve as a central part of the machinery responsible for muscle contraction. The thickest filaments in the cytosol are called *microtubules*, because they have the form of minute hollow tubes. In dividing cells, they become reorganized into a spectacular array that helps pull the duplicated chromosomes in opposite

IMPORT BY ENDOCYTOSIS



EXPORT BY EXOCYTOSIS

Figure 1–24 Eukaryotic cells engage in continual endocytosis and exocytosis. They import extracellular materials by endocytosis and secrete intracellular materials by exocytosis.



Figure 1–25 The cytoplasm is stuffed with organelles and a host of large and small molecules. This schematic drawing, which extends across two pages and is based on the known sizes and concentrations of molecules in the cytosol, shows how crowded the cytoplasm is. Proteins are blue, membrane lipids are yellow, and ribosomes and DNA are pink. The panorama begins on the far left at the plasma membrane, moves through the endoplasmic reticulum, Golgi apparatus, and a mitochondrion, and ends on the far right in the nucleus. (Courtesy of D. Goodsell.)

QUESTION 1–5

Suggest a reason why it would be advantageous for eukaryotic cells to evolve elaborate internal membrane systems that allow them to import substances from the outside, as shown in Figure 1–24.

directions and distribute them equally to the two daughter cells (Figure 1–27). Intermediate in thickness between actin filaments and microtubules are the *intermediate filaments*, which serve to strengthen the cell. These three types of filaments, together with other proteins that attach to them, form a system of girders, ropes, and motors that gives the cell its mechanical strength, controls its shape, and drives and guides its movements (Movie 1.2 and Movie 1.3).

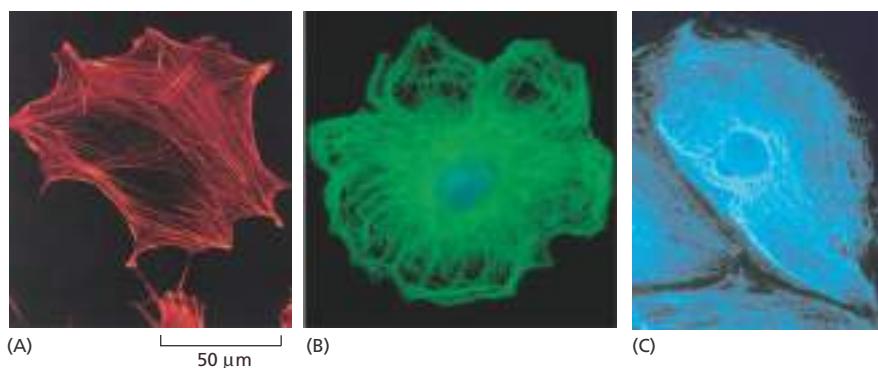
Because the cytoskeleton governs the internal organization of the cell as well as its external features, it is as necessary to a plant cell—boxed in by a tough wall of extracellular matrix—as it is to an animal cell that freely bends, stretches, swims, or crawls. In a plant cell, for example, organelles such as mitochondria are driven in a constant stream around the cell interior along cytoskeletal tracks (Movie 1.4). And animal cells and plant cells alike depend on the cytoskeleton to separate their internal components into two daughter cells during cell division (see Figure 1–27).

The cytoskeleton's role in cell division may be its most ancient function. Even bacteria contain proteins that are distantly related to those of eukaryotic actin filaments and microtubules, forming filaments that play a part in prokaryotic cell division. We examine the cytoskeleton in detail in Chapter 17, discuss its role in cell division in Chapter 18, and review how it responds to signals from outside the cell in Chapter 16.

The Cytoplasm Is Far from Static

The cell interior is in constant motion. The cytoskeleton is a dynamic jungle of protein ropes that are continually being strung together and taken apart; its filaments can assemble and then disappear in a matter of minutes. *Motor proteins* use the energy stored in molecules of ATP to trundle along these tracks and cables, carrying organelles and proteins throughout the cytoplasm, and racing across the width of the cell in seconds. In addition, the large and small molecules that fill every free space in the cell are swept to and fro by random thermal motion, constantly colliding with one another and with other structures in the cell's crowded cytoplasm (Movie 1–5).

Figure 1–26 The cytoskeleton is a network of protein filaments that criss-crosses the cytoplasm of eukaryotic cells. The three major types of filaments can be detected using different fluorescent stains. Shown here are (A) actin filaments, (B) microtubules, and (C) intermediate filaments. (A, courtesy of Simon Barry and Chris D'Lacey; B, courtesy of Nancy Kedersha; C, courtesy of Clive Lloyd.)





Of course, neither the bustling nature of the cell's interior nor the details of cell structure were appreciated when scientists first peered at cells in a microscope; our knowledge of cell structure accumulated slowly. A few of the key discoveries are listed in **Table 1–1**. In addition, **Panel 1–2** summarizes the differences between animal, plant, and bacterial cells.

Eukaryotic Cells May Have Originated as Predators

Eukaryotic cells are typically 10 times the length and 1000 times the volume of prokaryotic cells, although there is huge size variation within each category. They also possess a whole collection of features—a cytoskeleton, mitochondria, and other organelles—that set them apart from bacteria and archaea.

When and how eukaryotes evolved these systems remains something of a mystery. Although eukaryotes, bacteria, and archaea must have diverged from one another very early in the history of life on Earth (discussed in Chapter 14), the eukaryotes did not acquire all of their distinctive features at the same time (**Figure 1–28**). According to one theory, the ancestral eukaryotic cell was a predator that fed by capturing other cells. Such a way of life requires a large size, a flexible membrane, and a cytoskeleton to help the cell move and eat. The nuclear compartment may have evolved to keep the DNA segregated from this physical and chemical hurly-burly, so as to allow more delicate and complex control of the way the cell reads out its genetic information.

Such a primitive cell, with a nucleus and cytoskeleton, was most likely the sort of cell that engulfed the free-living, oxygen-consuming bacteria that were the likely ancestors of the mitochondria (see Figure 1–18). This partnership is thought to have been established 1.5 billion years ago, when the Earth's atmosphere first became rich in oxygen. A subset of

QUESTION 1–6

Discuss the relative advantages and disadvantages of light and electron microscopy. How could you best visualize (a) a living skin cell, (b) a yeast mitochondrion, (c) a bacterium, and (d) a microtubule?

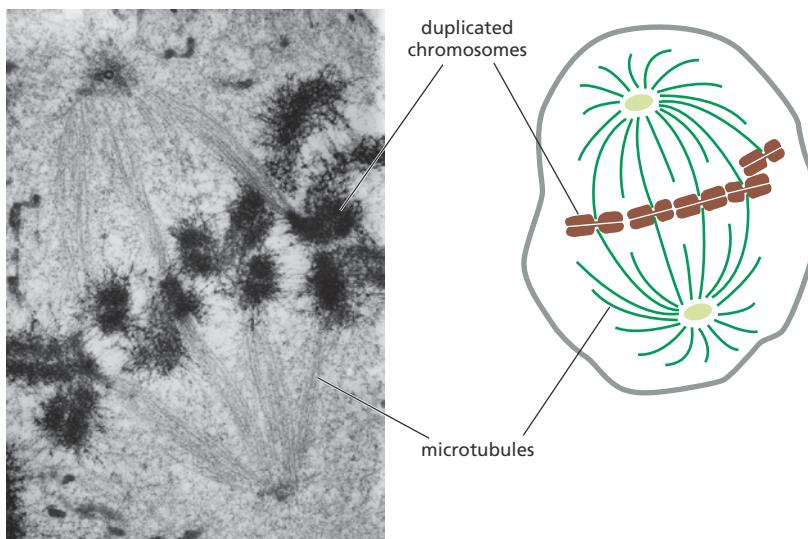


Figure 1–27 Microtubules help distribute the chromosomes in a dividing cell.

When a cell divides, its nuclear envelope breaks down and its DNA condenses into visible chromosomes, each of which has duplicated to form a pair of conjoined chromosomes that will ultimately be pulled apart into separate cells by microtubules. In the transmission electron micrograph (left), the microtubules are seen to radiate from foci at opposite ends of the dividing cell. (Photomicrograph courtesy of Conly L. Rieder.)

TABLE 1–1 HISTORICAL LANDMARKS IN DETERMINING CELL STRUCTURE

1665	Hooke uses a primitive microscope to describe small chambers in sections of cork that he calls "cells."
1674	Leeuwenhoek reports his discovery of protozoa. Nine years later, he sees bacteria for the first time.
1833	Brown publishes his microscopic observations of orchids, clearly describing the cell nucleus.
1839	Schleiden and Schwann propose the cell theory, stating that the nucleated cell is the universal building block of plant and animal tissues.
1857	Kölliker describes mitochondria in muscle cells.
1879	Flemming describes with great clarity chromosome behavior during mitosis in animal cells.
1881	Cajal and other histologists develop staining methods that reveal the structure of nerve cells and the organization of neural tissue.
1898	Golgi first sees and describes the Golgi apparatus by staining cells with silver nitrate.
1902	Boveri links chromosomes and heredity by observing chromosome behavior during sexual reproduction.
1952	Palade, Porter, and Sjöstrand develop methods of electron microscopy that enable many intracellular structures to be seen for the first time. In one of the first applications of these techniques, Huxley shows that muscle contains arrays of protein filaments—the first evidence of a cytoskeleton.
1957	Robertson describes the bilayer structure of the cell membrane, seen for the first time in the electron microscope.
1960	Kendrew describes the first detailed protein structure (sperm whale myoglobin) to a resolution of 0.2 nm using X-ray crystallography. Perutz proposes a lower-resolution structure for hemoglobin.
1965	Christian de Duve and his colleagues use a cell-fractionation technique to separate peroxisomes, mitochondria, and lysosomes from a preparation of rat liver.
1968	Petran and collaborators make the first confocal microscope.
1970	Frye and Edidin use fluorescent antibodies to show that plasma membrane molecules can diffuse in the plane of the membrane, indicating that cell membranes are fluid.
1974	Lazarides and Weber use fluorescent antibodies to stain the cytoskeleton.
1994	Chalfie and collaborators introduce green fluorescent protein (GFP) as a marker to follow the behavior of proteins in living cells.

these cells later acquired chloroplasts by engulfing photosynthetic bacteria (see Figure 1–20). The likely history of these endosymbiotic events is illustrated in Figure 1–28.

That single-celled eukaryotes can prey upon and swallow other cells is borne out by the behavior of many of the free-living, actively motile

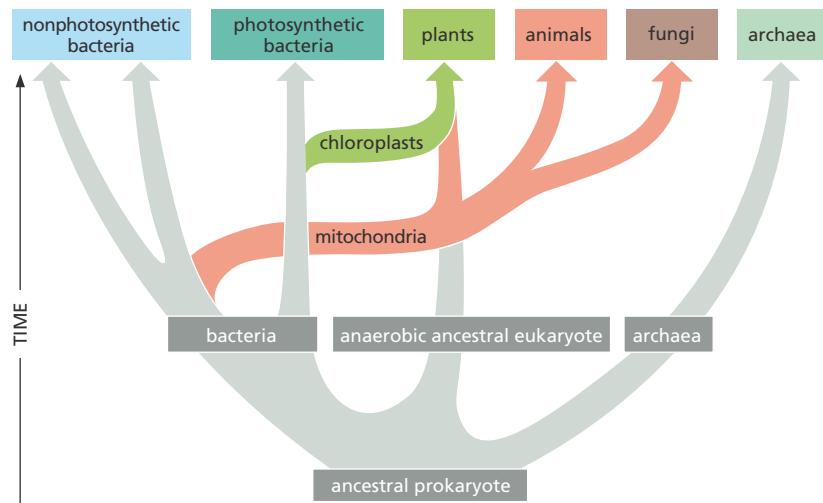


Figure 1–28 Where did eukaryotes come from? The eukaryotic, bacterial, and archaeal lineages diverged from one another very early in the evolution of life on Earth. Some time later, eukaryotes are thought to have acquired mitochondria; later still, a subset of eukaryotes acquired chloroplasts. Mitochondria are essentially the same in plants, animals, and fungi, and therefore were presumably acquired before these lines diverged.

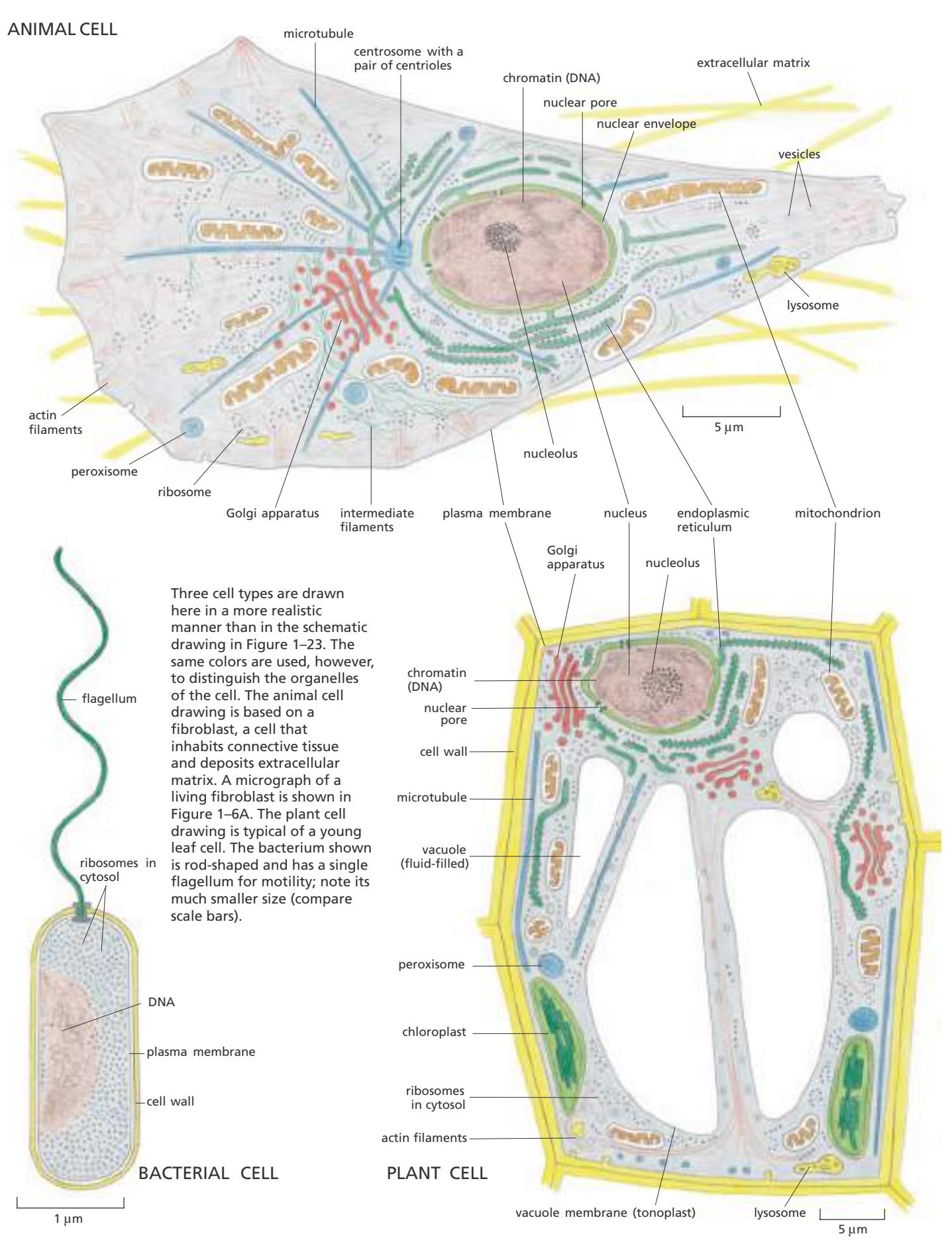
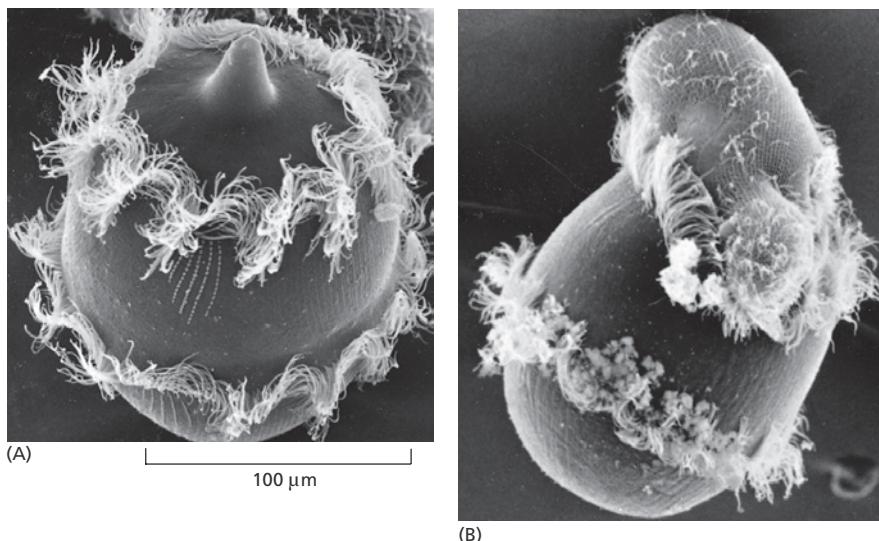


Figure 1–29 One protozoan eats another.

(A) The scanning electron micrograph shows *Didinium* on its own, with its circumferential rings of beating cilia and its “snout” at the top. (B) *Didinium* is seen ingesting another ciliated protozoan, a *Paramecium*. (Courtesy of D. Barlow.)



microorganisms called **protozoans**. *Didinium*, for example, is a large, carnivorous protozoan with a diameter of about 150 μm —roughly 10 times that of the average human cell. It has a globular body encircled by two fringes of cilia, and its front end is flattened except for a single protrusion rather like a snout (Figure 1–29A). *Didinium* swims at high speed by means of its beating cilia. When it encounters a suitable prey, usually another type of protozoan, it releases numerous small, paralyzing darts from its snout region. *Didinium* then attaches to and devours the other cell, inverting like a hollow ball to engulf its victim, which can be almost as large as itself (Figure 1–29B).

Not all protozoans are predators. They can be photosynthetic or carnivorous, motile or sedentary. Their anatomy is often elaborate and includes such structures as sensory bristles, photoreceptors, beating cilia, stalk-like appendages, mouthparts, stinging darts, and musclemlike contractile bundles (Figure 1–30). Although they are single cells, protozoans can be as intricate and versatile as many multicellular organisms. Much remains to be learned about fundamental cell biology from studies of these fascinating life-forms.

MODEL ORGANISMS

All cells are thought to be descended from a common ancestor, whose fundamental properties have been conserved through evolution. Thus knowledge gained from the study of one organism contributes to our understanding of others, including ourselves. But certain organisms are easier than others to study in the laboratory. Some reproduce rapidly and are convenient for genetic manipulations; others are multicellular but transparent, so that one can directly watch the development of all their internal tissues and organs. For reasons such as these, large communities of biologists have become dedicated to studying different aspects of the biology of a few chosen species, pooling their knowledge to gain a deeper understanding than could be achieved if their efforts were spread over many different species. Although the roster of these representative organisms is continually expanding, a few stand out in terms of the breadth and depth of information that has been accumulated about them over the years—knowledge that contributes to our understanding of how all cells work. In this section, we examine some of these **model organisms** and review the benefits that each offers to the study of cell biology and, in many cases, to the promotion of human health.

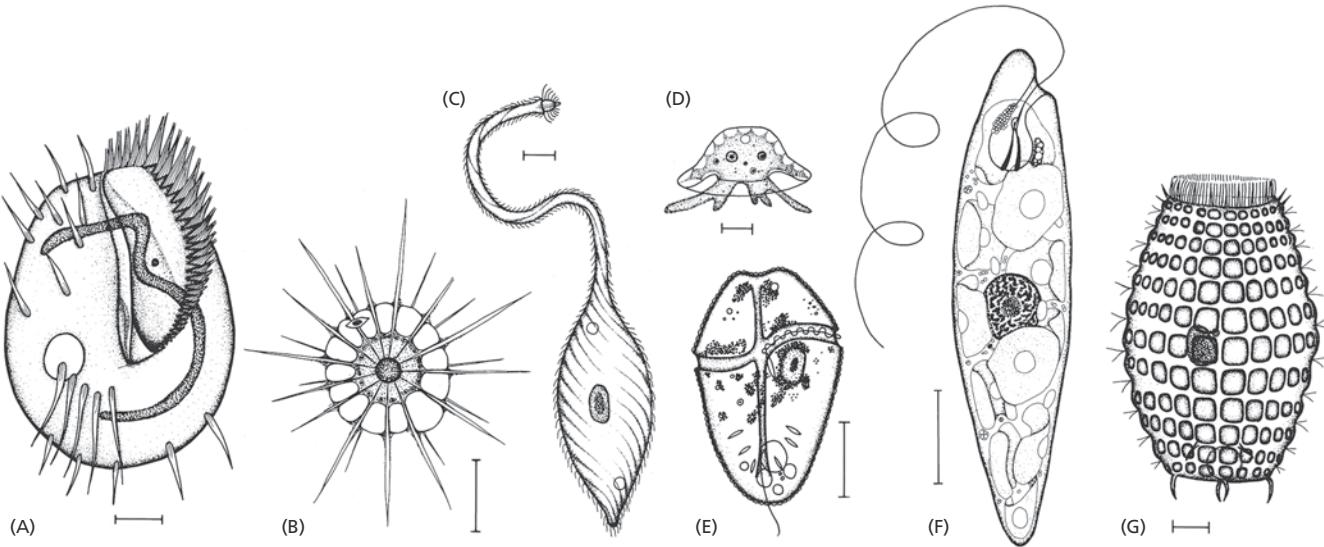


Figure 1–30 An assortment of protozoans illustrates the enormous variety within this class of single-celled microorganisms. These drawings are done to different scales, but in each case the scale bar represents 10 µm. The organisms in (A), (C), and (G) are ciliates; (B) is a heliozoan; (D) is an amoeba; (E) is a dinoflagellate; and (F) is a euglenoid. To see the latter in action, watch [Movie 1.6](#). (From M.A. Sleigh, *The Biology of Protozoa*. London: Edward Arnold, 1973. With permission from Edward Arnold.)

Molecular Biologists Have Focused on *E. coli*

In molecular terms, we understand the workings of the bacterium *Escherichia coli*—*E. coli* for short—more thoroughly than those of any other living organism (see Figure 1–10). This small, rod-shaped cell normally lives in the gut of humans and other vertebrates, but it also grows happily and reproduces rapidly in a simple nutrient broth in a culture bottle.

Most of our knowledge of the fundamental mechanisms of life—including how cells replicate their DNA and how they decode these genetic instructions to make proteins—has come from studies of *E. coli*. Subsequent research has confirmed that these basic processes occur in essentially the same way in our own cells as they do in *E. coli*.

Brewer's Yeast Is a Simple Eukaryotic Cell

We tend to be preoccupied with eukaryotes because we are eukaryotes ourselves. But human cells are complicated and reproduce relatively slowly. To get a handle on the fundamental biology of eukaryotic cells, it is often advantageous to study a simpler cell that reproduces more rapidly. A popular choice has been the budding yeast *Saccharomyces cerevisiae* (Figure 1–31)—the same microorganism that is used for brewing beer and baking bread.

S. cerevisiae is a small, single-celled fungus that is at least as closely related to animals as it is to plants. Like other fungi, it has a rigid cell wall, is relatively immobile, and possesses mitochondria but not chloroplasts. When nutrients are plentiful, *S. cerevisiae* reproduces almost as rapidly as a bacterium. Yet it carries out all the basic tasks that every eukaryotic cell must perform. Genetic and biochemical studies in yeast have been crucial to understanding many basic mechanisms in eukaryotic cells, including the cell-division cycle—the chain of events by which the nucleus and all the other components of a cell are duplicated and parceled out to create two daughter cells. The machinery that governs cell division has been

QUESTION 1–7

Your next-door neighbor has donated \$100 in support of cancer research and is horrified to learn that her money is being spent on studying brewer's yeast. How could you put her mind at ease?

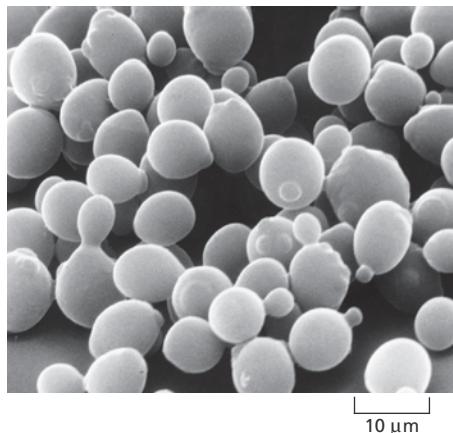


Figure 1–31 The yeast *Saccharomyces cerevisiae* is a model eukaryote. In this scanning electron micrograph, a few yeast cells are seen in the process of dividing, which they do by budding. Another micrograph of the same species is shown in Figure 1–13. (Courtesy of Ira Herskowitz and Eric Schabatach.)

so well conserved over the course of evolution that many of its components can function interchangeably in yeast and human cells (see **How We Know**, pp. 30–31). Darwin himself would no doubt have been stunned by this dramatic example of evolutionary conservation.

Arabidopsis Has Been Chosen as a Model Plant

The large multicellular organisms that we see around us—both plants and animals—seem fantastically varied, but they are much closer to one another in their evolutionary origins, and more similar in their basic cell biology, than the great host of microscopic single-celled organisms. Whereas bacteria, archaea, and eukaryotes separated from each other more than 3 billion years ago, plants, animals, and fungi diverged only about 1.5 billion years ago, and the different species of flowering plants less than 200 million years ago.

The close evolutionary relationship among all flowering plants means that we can gain insight into their cell and molecular biology by focusing on just a few convenient species for detailed analysis. Out of the several hundred thousand species of flowering plants on Earth today, molecular biologists have focused their efforts on a small weed, the common wall cress *Arabidopsis thaliana* (Figure 1–32), which can be grown indoors in large numbers: one plant can produce thousands of offspring within 8–10 weeks. Because genes found in *Arabidopsis* have counterparts in agricultural species, studying this simple weed provides insights into the development and physiology of the crop plants upon which our lives depend, as well as into the evolution of all the other plant species that dominate nearly every ecosystem on Earth.



Model Animals Include Flies, Fish, Worms, and Mice

Multicellular animals account for the majority of all named species of living organisms, and the majority of animal species are insects. It is fitting, therefore, that an insect, the small fruit fly *Drosophila melanogaster* (Figure 1–33), should occupy a central place in biological research. In fact, the foundations of classical genetics were built to a large extent on studies of this insect. More than 80 years ago, genetic analysis of the fruit fly provided definitive proof that genes—the units of heredity—are carried on chromosomes. In more recent times, *Drosophila*, more than any other organism, has shown us how the genetic instructions encoded in DNA molecules direct the development of a fertilized egg cell (or zygote) into an adult multicellular organism containing vast numbers of different cell types organized in a precise and predictable way. *Drosophila* mutants with body parts strangely misplaced or oddly patterned have provided the key to identifying and characterizing the genes that are needed to make a properly structured adult body, with gut, wings, legs, eyes, and all the other bits and pieces in their correct places. These genes—which are copied and passed on to every cell in the body—define how each cell will behave in its social interactions with its sisters and cousins, thus controlling the structures that the cells can create. Moreover, the genes

Figure 1–32 *Arabidopsis thaliana*, the common wall cress, is a model plant. This small weed has become the favorite organism of plant molecular and developmental biologists. (Courtesy of Toni Hayden and the John Innes Centre.)



Figure 1–33 *Drosophila melanogaster* is a favorite among developmental biologists and geneticists. Molecular genetic studies on this small fly have provided a key to the understanding of how all animals develop. (Courtesy of E.B. Lewis.)

responsible for the development of *Drosophila* have turned out to be amazingly similar to those of humans—far more similar than one would suspect from outward appearances. Thus the fly serves as a valuable model for studying human development and disease.

Another widely studied organism is the nematode worm *Caenorhabditis elegans* (Figure 1–34), a harmless relative of the eelworms that attack the roots of crops. Smaller and simpler than *Drosophila*, this creature develops with clockwork precision from a fertilized egg cell into an adult that has exactly 959 body cells (plus a variable number of egg and sperm cells)—an unusual degree of regularity for an animal. We now have a minutely detailed description of the sequence of events by which this occurs—as the cells divide, move, and become specialized according to strict and predictable rules. And a wealth of mutants are available for testing how the worm’s genes direct this developmental ballet. Some 70% of human genes have some counterpart in the worm, and *C. elegans*, like *Drosophila*, has proved to be a valuable model for many of the developmental processes that occur in our own bodies. Studies of nematode development, for example, have led to a detailed molecular understanding of *apoptosis*, a form of programmed cell death by which surplus cells are disposed of in all animals—a topic of great importance for cancer research (discussed in Chapters 18 and 20).

Another organism that is providing molecular insights into developmental processes, particularly in vertebrates, is the zebrafish. Because this

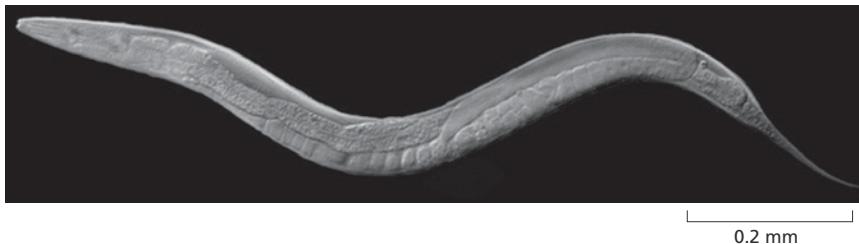


Figure 1–34 *Caenorhabditis elegans* is a small nematode worm that normally lives in the soil. Most individuals are hermaphrodites, producing both sperm and eggs (the latter of which can be seen along the underside of the animal). *C. elegans* was the first multicellular organism to have its complete genome sequenced. (Courtesy of Maria Gallegos.)

LIFE'S COMMON MECHANISMS

All living things are made of cells, and all cells—as we have discussed in this chapter—are fundamentally similar inside: they store their genetic instructions in DNA molecules, which direct the production of RNA molecules, which in turn direct the production of proteins. It is largely the proteins that carry out the cell's chemical reactions, give the cell its shape, and control its behavior. But how deep do these similarities between cells—and the organisms they comprise—really run? Are parts from one organism interchangeable with parts from another? Would an enzyme that breaks down glucose in a bacterium be able to digest the same sugar if it were placed inside a yeast cell or a cell from a lobster or a human? What about the molecular machines that copy and interpret genetic information? Are they functionally equivalent from one organism to another? Insights have come from many sources, but the most stunning and dramatic answer came from experiments performed on humble yeast cells. These studies, which shocked the biological community, focused on one of the most fundamental processes of life—cell division.

Division and discovery

All cells come from other cells, and the only way to make a new cell is through division of a preexisting one. To reproduce, a parent cell must execute an orderly sequence of reactions, through which it duplicates its contents and divides in two. This critical process of duplication and division—known as the *cell-division cycle*, or *cell cycle* for short—is complex and carefully controlled. Defects in any of the proteins involved can be devastating to the cell.

Fortunately for biologists, this acute reliance on crucial proteins makes them easy to identify and study. If a protein is essential for a given process, a mutation that results in an abnormal protein—or in no protein at all—can prevent the cell from carrying out the process. By isolating organisms that are defective in their cell-division cycle, scientists have worked backward to discover the proteins that control progress through the cycle.

The study of cell-cycle mutants has been particularly successful in yeasts. Yeasts are unicellular fungi and are popular organisms for such genetic studies. They are eukaryotes, like us, but they are small, simple, rapidly reproducing, and easy to manipulate genetically. Yeast mutants that are defective in their ability to complete cell division have led to the discovery of many genes that control the cell-division cycle—the so-called *Cdc* genes—and have provided a detailed understanding of how these genes, and the proteins they encode, actually work.

Paul Nurse and his colleagues used this approach to identify *Cdc* genes in the yeast *Schizosaccharomyces pombe*, which is named after the African beer from which it was first isolated. *S. pombe* is a rod-shaped cell, which grows by elongation at its ends and divides by fission into two, through the formation of a partition in the center of the rod. The researchers found that one of the *Cdc* genes they had identified, called *Cdc2*, was required to trigger several key events in the cell-division cycle. When that gene was inactivated by a mutation, the yeast cells would not divide. And when the cells were provided with a normal copy of the gene, their ability to reproduce was restored.

It's obvious that replacing a faulty *Cdc2* gene in *S. pombe* with a functioning *Cdc2* gene from the same yeast should repair the damage and enable the cell to divide normally. But what about using a similar cell-division gene from a different organism? That's the question the Nurse team tackled next.

Next of kin

Saccharomyces cerevisiae is another kind of yeast and is one of a handful of model organisms biologists have chosen to study to expand their understanding of how cells work. Also used to brew beer, *S. cerevisiae* divides by forming a small bud that grows steadily until it separates from the mother cell (see Figures 1–13 and 1–31). Although *S. cerevisiae* and *S. pombe* differ in their style of division, both rely on a complex network of interacting proteins to get the job done. But could the proteins from one type of yeast substitute for those of the other?

To find out, Nurse and his colleagues prepared DNA from healthy *S. cerevisiae*, and they introduced this DNA into *S. pombe* cells that contained a mutation in the *Cdc2* gene that kept the cells from dividing when the temperature was elevated. And they found that some of the mutant *S. pombe* cells regained the ability to proliferate when warm. If spread onto a culture plate containing a growth medium, the rescued cells could divide again and again to form visible colonies, each containing millions of individual yeast cells (**Figure 1–35**). Upon closer examination, the researchers discovered that these “rescued” yeast cells had received a fragment of DNA that contained the *S. cerevisiae* version of *Cdc2*—a gene that had been discovered in pioneering studies of the cell cycle by Lee Hartwell and colleagues.

The result was exciting, but perhaps not all that surprising. After all, how different can one yeast be from another? A more demanding test would be to use DNA from a more distant relative. So Nurse's team repeated the experiment, this time using human DNA. And the results were the same. The human equivalent of the

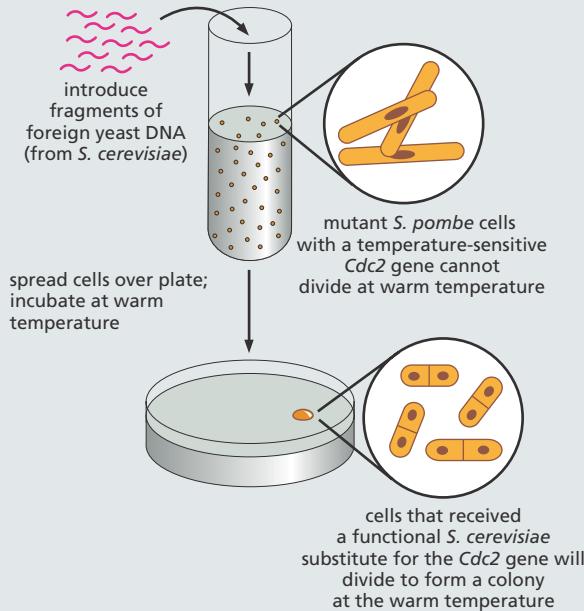


Figure 1–35 *S. pombe* mutants defective in a cell-cycle gene can be rescued by the equivalent gene from *S. cerevisiae*.

DNA is collected from *S. cerevisiae* and broken into large fragments, which are introduced into a culture of mutant *S. pombe* cells dividing at room temperature. We discuss how DNA can be manipulated and transferred into different cell types in Chapter 10. These yeast cells are then spread onto a plate containing a suitable growth medium and are incubated at a warm temperature, at which the mutant Cdc2 protein is inactive. The rare cells that survive and proliferate on these plates have been rescued by incorporation of a foreign gene that allows them to divide normally at the higher temperature.

S. pombe *Cdc2* gene could rescue the mutant yeast cells, allowing them to divide normally.

Gene reading

This result was much more surprising—even to Nurse. The ancestors of yeast and humans diverged some 1.5 billion years ago. So it was hard to believe that these

two organisms would orchestrate cell division in such a similar way. But the results clearly showed that the human and yeast proteins are functionally equivalent. Indeed, Nurse and colleagues demonstrated that the proteins are almost exactly the same size and consist of amino acids strung together in a very similar order; the human Cdc2 protein is identical to the *S. pombe* Cdc2 protein in 63% of its amino acids and is identical to the equivalent protein from *S. cerevisiae* in 58% of its amino acids (Figure 1–36). Together with Tim Hunt, who discovered a different cell-cycle protein called cyclin, Nurse and Hartwell shared a 2001 Nobel Prize for their studies of key regulators of the cell cycle.

The Nurse experiments showed that proteins from very different eukaryotes can be functionally interchangeable and suggested that the cell cycle is controlled in a similar fashion in every eukaryotic organism alive today. Apparently, the proteins that orchestrate the cycle in eukaryotes are so fundamentally important that they have been conserved almost unchanged over more than a billion years of eukaryotic evolution.

The same experiment also highlights another, even more basic, point. The mutant yeast cells were rescued, not by direct injection of the human protein, but by introduction of a piece of human DNA. Thus the yeast cells could read and use this information correctly, indicating that, in eukaryotes, the molecular machinery for reading the information encoded in DNA is also similar from cell to cell and from organism to organism. A yeast cell has all the equipment it needs to interpret the instructions encoded in a human gene and to use that information to direct the production of a fully functional human protein.

The story of Cdc2 is just one of thousands of examples of how research in yeast cells has provided critical insights into human biology. Although it may sound paradoxical, the shortest, most efficient path to improving human health will often begin with detailed studies of the biology of simple organisms such as brewer's or baker's yeast.

human	...FGLARAFGIPIRVYTHEVVTLWYRSPEVLLGSARYSTPVDIWSIGTIFAELATKLPLFHGDSEIDQLFRIPRALGTPNNEVWPEEVESLQDYKNTFP...
<i>S. pombe</i>	...FGLARSFGVPLRNYTHEIVTLWYRAPEVLLGSRHYSTGVDIWSVGCIFAENIRRSPLFPGDSEIDEIFKIPQVLGTPNEEVWPGVTLLQDYKSTFP...
<i>S. cerevisiae</i>	...FGLARAFGVPLRAYTHEIVTLWYRAPEVLLGGKQYSTGVDTWSIGCIFAEHCNRLPIFSGDSEIDQIFKIPRVLGTPNEAIWPDIVYLPDFKPSFP...

Figure 1–36 The cell-division-cycle proteins from yeasts and human are very similar in their amino acid sequences. Identities between the amino acid sequences of a region of the human Cdc2 protein and a similar region of the equivalent proteins in *S. pombe* and *S. cerevisiae* are indicated by green shading. Each amino acid is represented by a single letter.

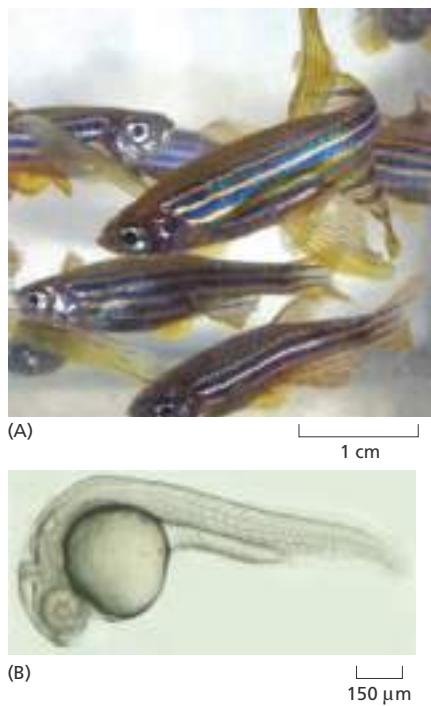


Figure 1-37 Zebrafish are popular models for studies of vertebrate development. (A) These small, hardy, tropical fish are a staple in many home aquaria. But they are also ideal for developmental studies, as their transparent embryos (B) make it easy to observe cells moving and changing their characters in the living organism as it develops. (A, courtesy of Steve Baskauf; B, from M. Rhinn et al., *Neural Dev.* 4:12, 2009. With permission from BioMed Central Ltd.)

creature is transparent for the first 2 weeks of its life, it provides an ideal system in which to observe how cells behave during development in a living animal (**Figure 1-37**).

Mammals are among the most complex of animals, and the mouse has long been used as the model organism in which to study mammalian genetics, development, immunology, and cell biology. Thanks to modern molecular biological techniques, it is now possible to breed mice with deliberately engineered mutations in any specific gene, or with artificially constructed genes introduced into them. In this way, one can test what a given gene is required for and how it functions. Almost every human gene has a counterpart in the mouse, with a similar DNA sequence and function. Thus, this animal has proven an excellent model for studying genes that are important in both human health and disease.

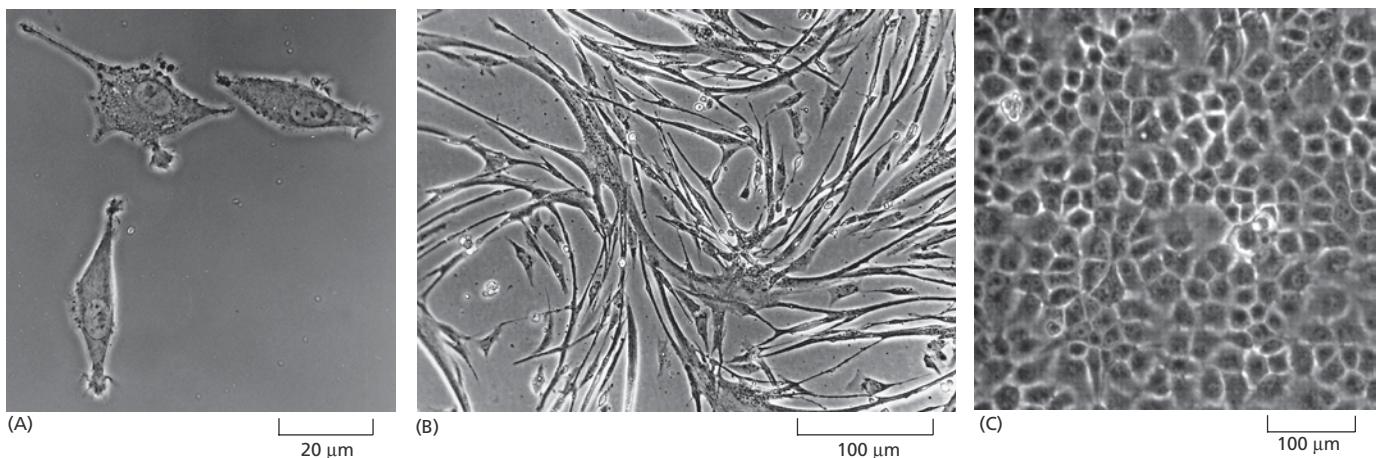
Biologists Also Directly Study Human Beings and Their Cells

Humans are not mice—or fish or flies or worms or yeast—and so we also study human beings themselves. Like bacteria or yeast, our individual cells can be harvested and grown in culture, where we can study their biology and more closely examine the genes that govern their functions. Given the appropriate surroundings, most human cells—indeed, most cells from animals or plants—will survive, proliferate, and even express specialized properties in a culture dish. Experiments using such cultured cells are sometimes said to be carried out *in vitro* (literally, “in glass”) to contrast them with experiments on intact organisms, which are said to be carried out *in vivo* (literally, “in the living”).

Although not true for all types of cells, many types of cells grown in culture display the differentiated properties appropriate to their origin: fibroblasts, a major cell type in connective tissue, continue to secrete collagen; cells derived from embryonic skeletal muscle fuse to form muscle fibers, which contract spontaneously in the culture dish; nerve cells extend axons that are electrically excitable and make synapses with other nerve cells; and epithelial cells form extensive sheets, with many of the properties of an intact epithelium (**Figure 1-38**). Because cultured cells are maintained in a controlled environment, they are accessible to study in ways that are often not possible *in vivo*. For example, cultured cells can be exposed to hormones or growth factors, and the effects that these signal molecules have on the shape or behavior of the cells can be easily explored.

In addition to studying human cells in culture, humans are also examined directly in clinics. Much of the research on human biology has been driven by medical interests, and the medical database on the human species is enormous. Although naturally occurring mutations in any given human gene are rare, the consequences of many mutations are well documented. This is because humans are unique among animals in that they report and record their own genetic defects: in no other species are billions of individuals so intensively examined, described, and investigated.

Nevertheless, the extent of our ignorance is still daunting. The mammalian body is enormously complex, being formed from thousands of



billions of cells, and one might despair of ever understanding how the DNA in a fertilized mouse egg cell makes it generate a mouse rather than a fish, or how the DNA in a human egg cell directs the development of a human rather than a mouse. Yet the revelations of molecular biology have made the task seem eminently approachable. As much as anything, this new optimism has come from the realization that the genes of one type of animal have close counterparts in most other types of animals, apparently serving similar functions (Figure 1–39). We all have a common evolutionary origin, and under the surface it seems that we share the same molecular mechanisms. Flies, worms, fish, mice, and humans thus provide a key to understanding how animals in general are made and how their cells work.

Comparing Genome Sequences Reveals Life's Common Heritage

At a molecular level, evolutionary change has been remarkably slow. We can see in present-day organisms many features that have been preserved through more than 3 billion years of life on Earth—about one-fifth of the age of the universe. This evolutionary conservatism provides the foundation on which the study of molecular biology is built. To set the scene for the chapters that follow, therefore, we end this chapter by considering a little more closely the family relationships and basic similarities among all living things. This topic has been dramatically clarified in the past few years by technological advances that have allowed us to determine the complete genome sequences of thousands of organisms, including our own species (as discussed in more detail in Chapter 9).

The first thing we note when we look at an organism's genome is its overall size and how many genes it packs into that length of DNA. Prokaryotes carry very little superfluous genetic baggage and, nucleotide-

Figure 1–38 Cells in culture often display properties that reflect their origin.

(A) Phase-contrast micrograph of fibroblasts in culture. (B) Micrograph of cultured myoblasts, some of which have fused to form multinucleate muscle cells that spontaneously contract in culture. (C) Cultured epithelial cells forming a cell sheet. **Movie 1.7** shows a single heart muscle cell beating in culture. (A, courtesy of Daniel Zicha; B, courtesy of Rosalind Zalin; C, from K.B. Chua et al., *Proc. Natl Acad. Sci. USA* 104:11424–11429, 2007, with permission from the National Academy of Sciences.)

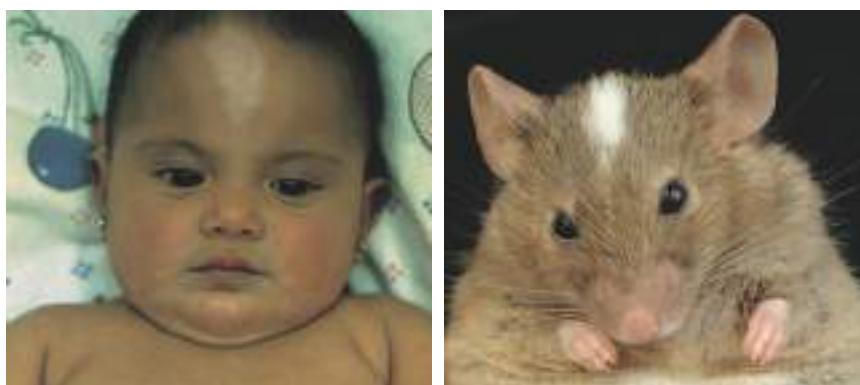
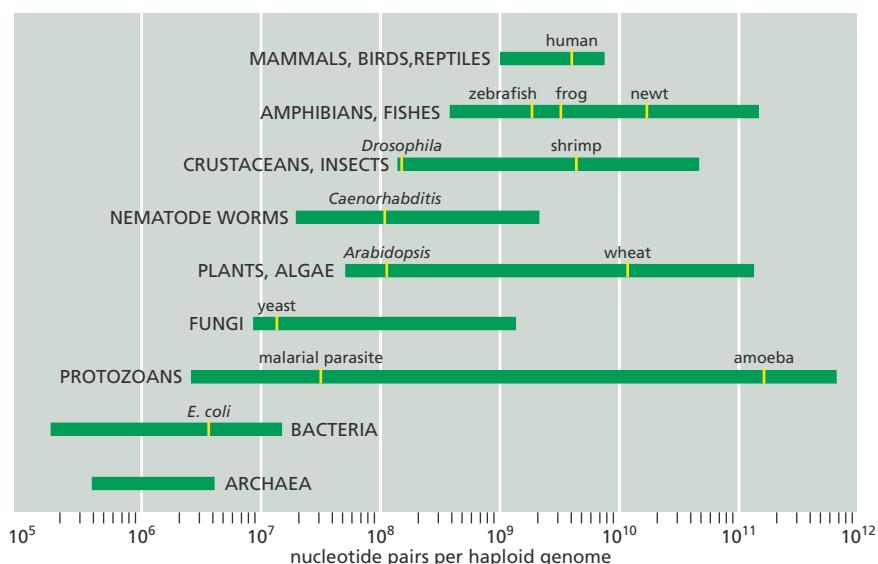


Figure 1–39 Different species share similar genes. The human baby and the mouse shown here have similar white patches on their foreheads because they both have defects in the same gene (called *Kit*), which is required for the development and maintenance of some pigment cells. (Courtesy of R.A. Fleischman, from *Proc. Natl Acad. Sci. USA* 88:10885–10889, 1991. With permission from the National Academy of Sciences.)

Figure 1–40 Organisms vary enormously in the size of their genomes. Genome size is measured in nucleotide pairs of DNA per haploid genome, that is, per single copy of the genome. (The body cells of sexually reproducing organisms such as ourselves are generally diploid: they contain two copies of the genome, one inherited from the mother, the other from the father.) Closely related organisms can vary widely in the quantity of DNA in their genomes (as indicated by the length of the green bars), even though they contain similar numbers of functionally distinct genes. (Adapted from T.R. Gregory, 2008, Animal Genome Size Database: www.genomesize.com)



for-nucleotide, they squeeze a lot of information into their relatively small genomes. *E. coli*, for example, carries its genetic instructions in a single, circular, double-stranded molecule of DNA that contains 4.6 million nucleotide pairs and 4300 genes. The simplest known bacterium contains only about 500 genes, but most prokaryotes have genomes that contain at least 1 million nucleotide pairs and 1000–8000 genes. With these few thousand genes, prokaryotes are able to thrive in even the most hostile environments on Earth.

The compact genomes of typical bacteria are dwarfed by the genomes of typical eukaryotes. The human genome, for example, contains about 700 times more DNA than the *E. coli* genome, and the genome of an amoeba contains about 100 times more than ours (Figure 1–40). The rest of the model organisms we have described have genomes that fall somewhere in between *E. coli* and human in terms of size. *S. cerevisiae* contains about 2.5 times as much DNA as *E. coli*; *Drosophila* has about 10 times more DNA per cell than yeast; and mice have about 20 times more DNA per cell than the fruit fly (Table 1–2).

TABLE 1–2 SOME MODEL ORGANISMS AND THEIR GENOMES

Organism	Genome size* (nucleotide pairs)	Approximate number of genes
<i>Homo sapiens</i> (human)	3200×10^6	30,000
<i>Mus musculus</i> (mouse)	2800×10^6	30,000
<i>Drosophila melanogaster</i> (fruit fly)	200×10^6	15,000
<i>Arabidopsis thaliana</i> (plant)	220×10^6	29,000
<i>Caenorhabditis elegans</i> (roundworm)	130×10^6	21,000
<i>Saccharomyces cerevisiae</i> (yeast)	13×10^6	6600
<i>Escherichia coli</i> (bacteria)	4.6×10^6	4300

*Genome size includes an estimate for the amount of highly repeated DNA sequence not in genome databases.

In terms of gene numbers, however, the differences are not so great. We have only about six times as many genes as *E. coli*. Moreover, many of our genes—and the proteins they encode—fall into closely related family groups, such as the family of hemoglobins, which has nine closely related members in humans. Thus the number of fundamentally different proteins in a human is not very many times more than in a bacterium, and the number of human genes that have identifiable counterparts in the bacterium is a significant fraction of the total.

This high degree of “family resemblance” is striking when we compare the genome sequences of different organisms. When genes from different organisms have very similar nucleotide sequences, it is highly probable that both descended from a common ancestral gene. Such genes (and their protein products) are said to be **homologous**. Now that we have the complete genome sequences of many different organisms from all three domains of life—archaea, bacteria, and eukaryotes—we can search systematically for homologies that span this enormous evolutionary divide. By taking stock of the common inheritance of all living things, scientists are attempting to trace life’s origins back to the earliest ancestral cells.

Genomes Contain More Than Just Genes

Although our view of genome sequences tends to be “gene-centric,” our genomes contain much more than just genes. The vast bulk of our DNA does not code for proteins or for functional RNA molecules. Instead, it includes a mixture of sequences that help regulate gene activity, plus sequences that seem to be dispensable. The large quantity of regulatory DNA contained in the genomes of eukaryotic multicellular organisms allows for enormous complexity and sophistication in the way different genes are brought into action at different times and places. Yet, in the end, the basic list of parts—the set of proteins that the cells can make, as specified by the DNA—is not much longer than the parts list of an automobile, and many of those parts are common not only to all animals, but also to the entire living world.

That DNA can program the growth, development, and reproduction of living cells and complex organisms is truly amazing. In the rest of this book, we will try to explain what is known about how cells work—by examining their component parts, how these parts work together, and how the genome of each cell directs the manufacture of the parts the cell needs to function and to reproduce.

ESSENTIAL CONCEPTS

- Cells are the fundamental units of life. All present-day cells are believed to have evolved from an ancestral cell that existed more than 3 billion years ago.
- All cells are enclosed by a plasma membrane, which separates the inside of the cell from its environment.
- All cells contain DNA as a store of genetic information and use it to guide the synthesis of RNA molecules and proteins.
- Cells in a multicellular organism, though they all contain the same DNA, can be very different. They turn on different sets of genes according to their developmental history and to signals they receive from their environment.
- Animal and plant cells are typically 5–20 μm in diameter and can be seen with a light microscope, which also reveals some of their internal components, including the larger organelles.

- The electron microscope reveals even the smallest organelles, but specimens require elaborate preparation and cannot be viewed while alive.
- Specific large molecules can be located in fixed or living cells with a fluorescence microscope.
- The simplest of present-day living cells are prokaryotes: although they contain DNA, they lack a nucleus and other organelles and probably resemble most closely the ancestral cell.
- Different species of prokaryotes are diverse in their chemical capabilities and inhabit an amazingly wide range of habitats. Two fundamental evolutionary subdivisions are recognized: bacteria and archaea.
- Eukaryotic cells possess a nucleus and other organelles not found in prokaryotes. They probably evolved in a series of stages, including the acquisition of mitochondria by engulfment of aerobic bacteria and (for plant cells) the acquisition of chloroplasts by engulfment of photosynthetic bacteria.
- The nucleus contains the genetic information of the eukaryotic organism, stored in DNA molecules.
- The cytoplasm includes all of the cell's contents outside the nucleus and contains a variety of membrane-enclosed organelles with specialized functions: mitochondria carry out the final oxidation of food molecules; in plant cells, chloroplasts perform photosynthesis; the endoplasmic reticulum and the Golgi apparatus synthesize complex molecules for export from the cell and for insertion in cell membranes; lysosomes digest large molecules.
- Outside the membrane-enclosed organelles in the cytoplasm is the cytosol, a very concentrated mixture of large and small molecules that carry out many essential biochemical processes.
- The cytoskeleton is composed of protein filaments that extend throughout the cytoplasm and are responsible for cell shape and movement and for the transport of organelles and other large molecular complexes from one location to another.
- Free-living, single-celled eukaryotic microorganisms are complex cells that can swim, mate, hunt, and devour other microorganisms.
- Animals, plants, and some fungi consist of diverse eukaryotic cell types, all derived from a single fertilized egg cell; the number of such cells cooperating to form a large multicellular organism such as a human runs into thousands of billions.
- Biologists have chosen a small number of model organisms to study closely, including the bacterium *E. coli*, brewer's yeast, a nematode worm, a fly, a small plant, a fish, a mouse, and humans themselves.
- The simplest known cell is a bacterium with about 500 genes, but most cells contain significantly more. The human genome has about 25,000 genes, which is only about twice as many as a fly and six times as many as *E. coli*.

KEY TERMS

archaeon	eukaryote	nucleus
bacterium	evolution	organelle
cell	fluorescence microscope	photosynthesis
chloroplast	genome	plasma membrane
chromosome	homologous	prokaryote
cytoplasm	micrometer	protein
cytoskeleton	microscope	protozoan
cytosol	mitochondrion	ribosome
DNA	model organism	RNA
electron microscope		

QUESTIONS

QUESTION 1–8

By now you should be familiar with the following cellular components. Briefly define what they are and what function they provide for cells.

- A. cytosol
- B. cytoplasm
- C. mitochondria
- D. nucleus
- E. chloroplasts
- F. lysosomes
- G. chromosomes
- H. Golgi apparatus
- I. peroxisomes
- J. plasma membrane
- K. endoplasmic reticulum
- L. cytoskeleton

QUESTION 1–9

Which of the following statements are correct? Explain your answers.

- A. The hereditary information of a cell is passed on by its proteins.
- B. Bacterial DNA is found in the cytosol.
- C. Plants are composed of prokaryotic cells.
- D. All cells of the same organism have the same number of chromosomes (with the exception of egg and sperm cells).
- E. The cytosol contains membrane-enclosed organelles, such as lysosomes.
- F. The nucleus and mitochondria are surrounded by a double membrane.
- G. Protozoans are complex organisms with a set of specialized cells that form tissues, such as flagella, mouthparts, stinging darts, and leglike appendages.

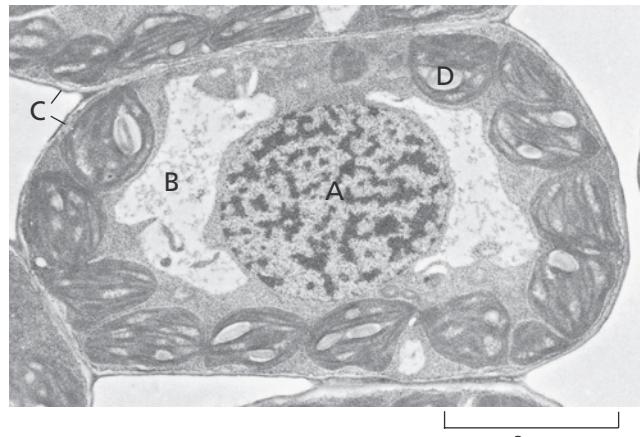
H. Lysosomes and peroxisomes are the sites of degradation of unwanted materials.

QUESTION 1–10

To get a feeling for the size of cells (and to practice the use of the metric system), consider the following: the human brain weighs about 1 kg and contains about 10^{12} cells. Calculate the average size of a brain cell (although we know that their sizes vary widely), assuming that each cell is entirely filled with water (1 cm^3 of water weighs 1 g). What would be the length of one side of this average-sized brain cell if it were a simple cube? If the cells were spread out as a thin layer that is only a single cell thick, how many pages of this book would this layer cover?

QUESTION 1–11

Identify the different organelles indicated with letters in the electron micrograph of a plant cell shown below. Estimate the length of the scale bar in the figure.



? μm

QUESTION 1–12

There are three major classes of filaments that make up the cytoskeleton. What are they, and what are the differences in

their functions? Which cytoskeletal filaments would be most plentiful in a muscle cell or in an epidermal cell making up the outer layer of the skin? Explain your answers.

QUESTION 1–13

Natural selection is such a powerful force in evolution because cells with even a small proliferation advantage quickly outgrow their competitors. To illustrate this process, consider a cell culture that contains 1 million bacterial cells that double every 20 minutes. A single cell in this culture acquires a mutation that allows it to divide faster, with a generation time of only 15 minutes. Assuming that there is an unlimited food supply and no cell death, how long would it take before the progeny of the mutated cell became predominant in the culture? (Before you go through the calculation, make a guess: do you think it would take about a day, a week, a month, or a year?) How many cells of either type are present in the culture at this time? (The number of cells N in the culture at time t is described by the equation $N = N_0 \times 2^{t/G}$, where N_0 is the number of cells at zero time and G is the generation time.)

QUESTION 1–14

When bacteria are grown under adverse conditions, i.e., in the presence of a poison such as an antibiotic, most cells grow and proliferate slowly. But it is not uncommon that the growth rate of a bacterial culture kept in the presence of the poison is restored after a few days to that observed in its absence. Suggest why this may be the case.

QUESTION 1–15

Apply the principle of exponential growth of a culture as described in Question 1–13 to the cells in a multicellular organism, such as yourself. There are about 10^{13} cells in your body. Assume that one cell acquires a mutation that allows it to divide in an uncontrolled manner (i.e., it becomes a cancer cell). Some cancer cells can proliferate with a generation time of about 24 hours. If none of the cancer cells died, how long would it take before 10^{13} cells in your body would be cancer cells? (Use the equation $N = N_0 \times 2^{t/G}$, with t , the time, and G , the length of each generation. Hint: $10^{13} \approx 2^{43}$.)

QUESTION 1–16

Discuss the following statement: "The structure and function of a living cell are dictated by the laws of physics and chemistry."

QUESTION 1–17

What, if any, are the advantages in being multicellular?

QUESTION 1–18

Draw to scale the outline of two spherical cells, one a bacterium with a diameter of 1 μm , the other an animal cell with a diameter of 15 μm . Calculate the volume, surface area, and surface-to-volume ratio for each cell. How would the latter ratio change if you included the internal membranes of the cell in the calculation of surface area (assume internal membranes have 15 times the area of the plasma membrane)? (The volume of a sphere is given by $4\pi r^3/3$ and its surface by $4\pi r^2$, where r is its radius.) Discuss the following hypothesis: "Internal membranes allowed bigger cells to evolve."

QUESTION 1–19

What are the arguments that all living cells evolved from a common ancestor cell? Imagine the very early days of evolution of life on Earth. Would you assume that the primordial ancestor cell was the first and only cell to form?

QUESTION 1–20

In Figure 1–25, proteins are blue, nucleic acids are pink, lipids are yellow, and polysaccharides are green. Identify the major organelles and other important cellular structures shown in this slice through a eukaryotic cell.

QUESTION 1–21

Looking at some pond water under the microscope, you notice an unfamiliar rod-shaped cell about 200 μm long. Knowing that some exceptional bacteria can be as big as this or even bigger, you wonder whether your cell is a bacterium or a eukaryote. How will you decide? If it is not a eukaryote, how will you discover whether it is a bacterium or an archaeon?



CHAPTER TWO

2

Chemical Components of Cells

It is at first sight difficult to accept that living creatures are merely chemical systems. Their incredible diversity of form, their seemingly purposeful behavior, and their ability to grow and reproduce all seem to set them apart from the world of solids, liquids, and gases that chemistry normally describes. Indeed, until the nineteenth century, it was widely believed that animals contained a vital force—an “animus”—that was responsible for their distinctive properties.

We now know that there is nothing in living organisms that disobeys chemical or physical laws. However, the chemistry of life is indeed a special kind. First, it is based overwhelmingly on carbon compounds, the study of which is known as *organic chemistry*. Second, it depends almost exclusively on chemical reactions that take place in a watery, or *aqueous*, solution and in the relatively narrow range of temperatures experienced on Earth. Third, it is enormously complex: even the simplest cell is vastly more complicated in its chemistry than any other chemical system known. Fourth, it is dominated and coordinated by collections of enormous *polymeric molecules*—chains of chemical **subunits** linked end-to-end—whose unique properties enable cells and organisms to grow and reproduce and to do all the other things that are characteristic of life. Finally, the chemistry of life is tightly regulated: cells deploy a variety of mechanisms to make sure that all their chemical reactions occur at the proper place and time.

Because chemistry lies at the heart of all biology, in this chapter, we briefly survey the chemistry of the living cell. We will meet the molecules from which cells are made and examine their structures, shapes, and chemical properties. These molecules determine the size, structure, and functions

CHEMICAL BONDS

SMALL MOLECULES IN CELLS

MACROMOLECULES IN CELLS

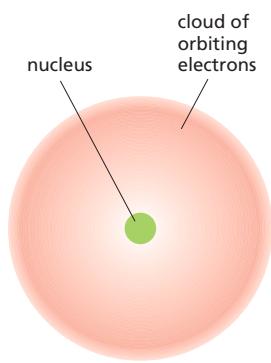


Figure 2–1 An atom consists of a nucleus surrounded by an electron cloud. The dense, positively charged nucleus contains most of the atom's mass. The much lighter and negatively charged electrons occupy space around the nucleus, as governed by the laws of quantum mechanics. The electrons are depicted as a continuous cloud, as there is no way of predicting exactly where an electron is at any given instant. The density of shading of the cloud is an indication of the probability that electrons will be found there. The diameter of the electron cloud ranges from about 0.1 nm (for hydrogen) to about 0.4 nm (for atoms of high atomic number). The nucleus is very much smaller: about 5×10^{-6} nm for carbon, for example.

of living cells. By understanding how they interact, we can begin to see how cells exploit the laws of chemistry and physics to survive, thrive, and reproduce.

CHEMICAL BONDS

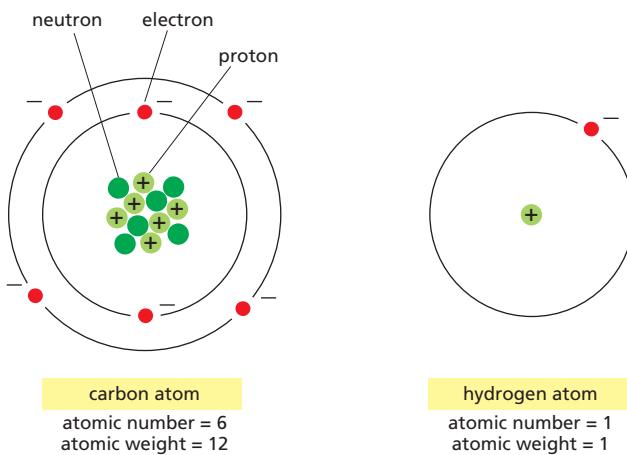
Matter is made of combinations of *elements*—substances such as hydrogen or carbon that cannot be broken down or interconverted by chemical means. The smallest particle of an element that still retains its distinctive chemical properties is an *atom*. The characteristics of substances other than pure elements—including the materials from which living cells are made—depend on which atoms they contain and the way these atoms are linked together in groups to form *molecules*. To understand living organisms, therefore, it is crucial to know how the chemical bonds that hold atoms together in molecules are formed.

Cells Are Made of Relatively Few Types of Atoms

Each **atom** has at its center a dense, positively charged nucleus, which is surrounded at some distance by a cloud of negatively charged **electrons**, held there by electrostatic attraction to the nucleus (Figure 2–1). The nucleus consists of two kinds of subatomic particles: **protons**, which are positively charged, and **neutrons**, which are electrically neutral. The number of protons present in an atom's nucleus determines its *atomic number*. An atom of hydrogen has a nucleus composed of a single proton; so hydrogen, with an atomic number of 1, is the lightest element. An atom of carbon has six protons in its nucleus and an atomic number of 6 (Figure 2–2). The electric charge carried by each proton is exactly equal and opposite to the charge carried by a single electron. Because the whole atom is electrically neutral, the number of negatively charged electrons surrounding the nucleus is equal to the number of positively charged protons that the nucleus contains; thus the number of electrons in an atom also equals the atomic number. All atoms of a given element have the same atomic number, and we will see shortly that it is this number that dictates each atom's chemical behavior.

Neutrons have essentially the same mass as protons. They contribute to the structural stability of the nucleus—if there are too many or too few, the nucleus may disintegrate by radioactive decay—but they do not alter the chemical properties of the atom. Thus an element can exist in several physically distinguishable but chemically identical forms, called *isotopes*, each having a different number of neutrons but the same number of protons. Multiple isotopes of almost all the elements occur naturally.

Figure 2–2 The number of protons in an atom determines its atomic number. Schematic representations of an atom of carbon and an atom of hydrogen are shown. The nucleus of every atom except hydrogen consists of both positively charged protons and electrically neutral neutrons; the atomic weight equals the number of protons plus neutrons. The number of electrons in an atom is equal to the number of protons, so that the atom has no net charge. In contrast to Figure 2–1, the electrons are shown here as individual particles. The concentric black circles represent in a highly schematic form the “orbits” (that is, the different distributions) of the electrons. The neutrons, protons, and electrons are in reality minute in relation to the atom as a whole; their size is greatly exaggerated here.



including some that are unstable—and thus radioactive. For example, while most carbon on Earth exists as the stable isotope carbon 12, with six protons and six neutrons, also present are small amounts of an unstable isotope, carbon 14, which has six protons and eight neutrons. Carbon 14 undergoes radioactive decay at a slow but steady rate, which allows archaeologists to estimate the age of organic material.

The **atomic weight** of an atom, or the **molecular weight** of a molecule, is its mass relative to that of a hydrogen atom. This is essentially equal to the number of protons plus neutrons that the atom or molecule contains, because the electrons are so light that they contribute almost nothing to the total mass. Thus the major isotope of carbon has an atomic weight of 12 and is written as ^{12}C . The unstable carbon isotope just mentioned has an atomic weight of 14 and is written as ^{14}C . The mass of an atom or a molecule is generally specified in *daltons*, one dalton being an atomic mass unit approximately equal to the mass of a hydrogen atom.

Atoms are so small that it is hard to imagine their size. An individual carbon atom is roughly 0.2 nm in diameter, so that it would take about 5 million of them, laid out in a straight line, to span a millimeter. One proton or neutron weighs approximately $1/(6 \times 10^{23})$ gram. As hydrogen has only one proton—thus an atomic weight of 1—1 gram of hydrogen contains 6×10^{23} atoms. For carbon—which has six protons and six neutrons, and an atomic weight of 12—12 grams contain 6×10^{23} atoms. This huge number, called **Avogadro's number**, allows us to relate everyday quantities of chemicals to numbers of individual atoms or molecules. If a substance has a molecular weight of M , M grams of the substance will contain 6×10^{23} molecules. This quantity is called one *mole* of the substance (**Figure 2–3**). The concept of mole is used widely in chemistry as a way to represent the number of molecules that are available to participate in chemical reactions.

There are about 90 naturally occurring elements, each differing from the others in the number of protons and electrons in its atoms. Living organisms, however, are made of only a small selection of these elements, four of which—carbon (C), hydrogen (H), nitrogen (N), and oxygen (O)—constitute 96% of an organism's weight. This composition differs markedly from that of the nonliving inorganic environment on Earth (**Figure 2–4**) and is evidence of a distinctive type of chemistry.

The Outermost Electrons Determine How Atoms Interact

To understand how atoms come together to form the molecules that make up living organisms, we have to pay special attention to the atoms' electrons. Protons and neutrons are welded tightly to one another in an atom's nucleus, and they change partners only under extreme conditions—during radioactive decay, for example, or in the interior of the sun or of a nuclear reactor. In living tissues, only the electrons of an atom undergo rearrangements. They form the accessible part of the atom and specify the rules of chemistry by which atoms combine to form molecules.

Electrons are in continuous motion around the nucleus, but motions on this submicroscopic scale obey different laws from those we are familiar with in everyday life. These laws dictate that electrons in an atom can exist only in certain discrete regions of movement—roughly speaking, in discrete orbits. Moreover, there is a strict limit to the number of electrons that can be accommodated in an orbit of a given type, a so-called *electron shell*. The electrons closest on average to the positive nucleus are attracted most strongly to it and occupy the inner, most tightly bound shell. This innermost shell can hold a maximum of two electrons. The second shell is farther away from the nucleus, and can hold up to eight

A **mole** is X grams of a substance, where X is the molecular weight of the substance. A mole will contain 6×10^{23} molecules of the substance.

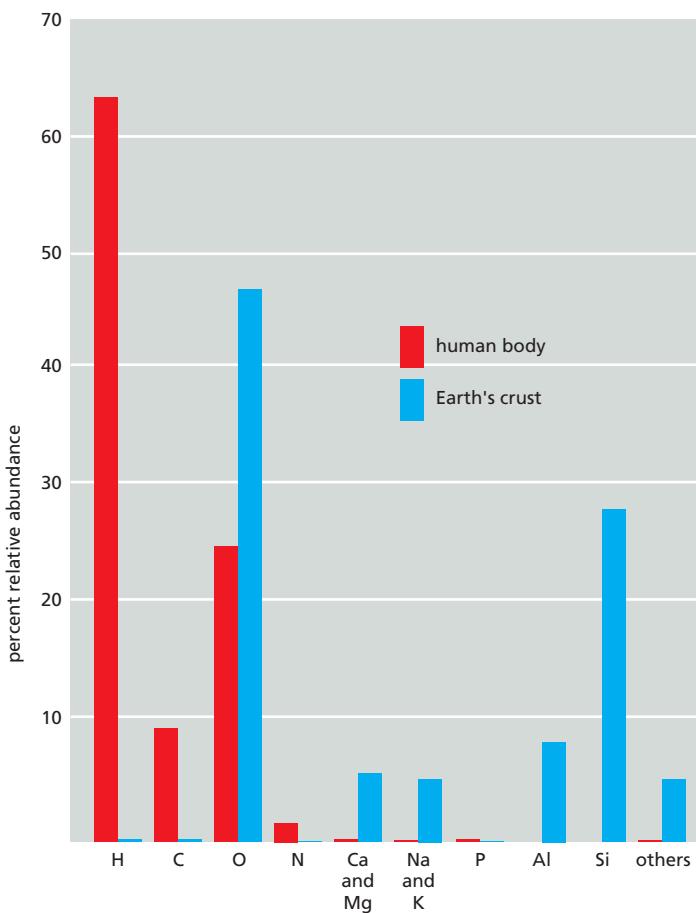
1 mole of carbon weighs 12 g
1 mole of glucose weighs 180 g
1 mole of sodium chloride weighs 58 g

A **one molar solution** has a concentration of 1 mole of the substance in 1 liter of solution. A 1 M solution of glucose, for example, contains 180 g/l, and a one millimolar (1 mM) solution contains 180 mg/l.

The standard abbreviation for gram is g; the abbreviation for liter is L.

Figure 2–3 What's a mole? Some sample calculations of moles and molar solutions.

Figure 2–4 The distribution of elements in the Earth's crust differs radically from that in a living organism. The abundance of each element is expressed here as a percentage of the total number of atoms present in a biological or geological sample, including water. Thus, for example, more than 60% of the atoms in the human body are hydrogen atoms, and nearly 30% of the atoms in the Earth's crust are silicon atoms (Si). The relative abundance of elements is similar in all living things.



QUESTION 2–1

A cup of water, containing exactly 18 g, or 1 mole, of water, was emptied into the Aegean Sea 3000 years ago. What are the chances that the same quantity of water, scooped today from the Pacific Ocean, would include at least one of these ancient water molecules? Assume perfect mixing and an approximate volume for the world's oceans of 1.5 billion cubic kilometers ($1.5 \times 10^9 \text{ km}^3$).

electrons. The third shell can also hold up to eight electrons, which are even less tightly bound. The fourth and fifth shells can hold 18 electrons each. Atoms with more than four shells are very rare in biological molecules.

The arrangement of electrons in an atom is most stable when all the electrons are in the most tightly bound states that are possible for them—that is, when they occupy the innermost shells, closest to the nucleus. Therefore, with certain exceptions in the larger atoms, the electrons of an atom fill the shells in order—the first before the second, the second before the third, and so on. An atom whose outermost shell is entirely filled with electrons is especially stable and therefore chemically unreactive. Examples are helium with 2 electrons (atomic number 2), neon with $2 + 8$ electrons (atomic number 10), and argon with $2 + 8 + 8$ electrons (atomic number 18); these are all inert gases. Hydrogen, by contrast, has only one electron, which leaves its outermost shell half-filled, so it is highly reactive. The atoms found in living organisms all have outermost shells that are incompletely filled, and they are therefore able to react with one another to form molecules (Figure 2–5).

Because an incompletely filled electron shell is less stable than one that is completely filled, atoms with incomplete outer shells have a strong tendency to interact with other atoms so as to either gain or lose enough electrons to achieve a completed outermost shell. This electron exchange can be achieved either by transferring electrons from one atom to another or by sharing electrons between two atoms. These two strategies generate the two types of **chemical bonds** that bind atoms to one another: an *ionic bond* is formed when electrons are donated by one atom to another, whereas a *covalent bond* is formed when two atoms share a pair of electrons (Figure 2–6).

An H atom, which needs only one more electron to fill its only shell, generally acquires it by sharing—forming one covalent bond with another atom. The other most common elements in living cells—C, N, and O, which have an incomplete second shell, and P and S, which have an incomplete third shell (see Figure 2-5)—generally share electrons and achieve a filled outer shell of eight electrons by forming several covalent bonds. The number of electrons an atom must acquire or lose (either by sharing or by transfer) to attain a filled outer shell determines the number of bonds the atom can make.

Because the state of the outer electron shell determines the chemical properties of an element, when the elements are listed in order of their atomic number we see a periodic recurrence of elements with similar properties: an element with, say, an incomplete second shell containing one electron will behave in much the same way as an element that has filled its second shell and has an incomplete third shell containing one electron. The metals, for example, have incomplete outer shells with just one or a few electrons, whereas, as we have just seen, the inert gases have full outer shells. This arrangement gives rise to the *periodic table* of the elements, outlined in **Figure 2–7**, which shows elements found in living organisms highlighted in color.

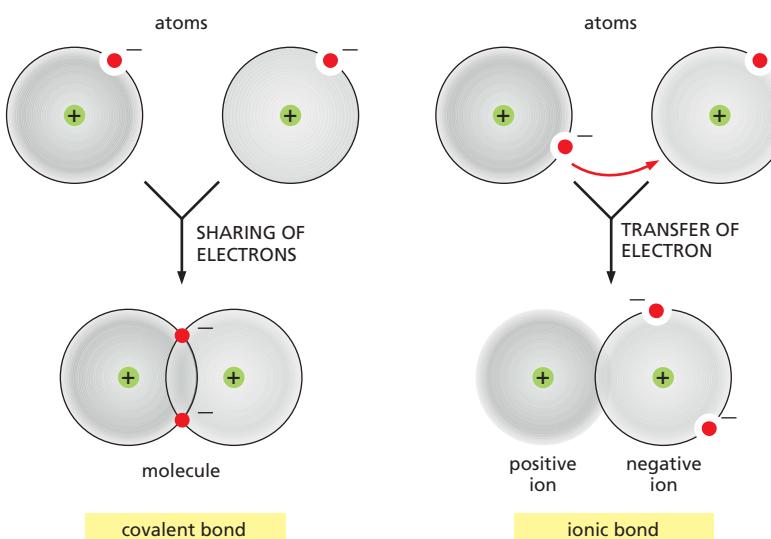


Figure 2–5 An element's chemical reactivity depends on how its outermost electron shell is filled. All of the elements commonly found in living organisms have outermost shells that are not completely filled with electrons (red) and can thus participate in chemical reactions with other atoms. Inert gases (yellow), in contrast, have completely filled outermost shells and are thus chemically unreactive.

QUESTION 2-2

A carbon atom contains six protons and six neutrons.

- A. What are its atomic number and atomic weight?
 - B. How many electrons does it have?
 - C. How many additional electrons must it add to fill its outermost shell? How does this affect carbon's chemical behavior?
 - D. Carbon with an atomic weight of 14 is radioactive. How does it differ in structure from nonradioactive carbon? How does this difference affect its chemical behavior?

Figure 2–6 Atoms can attain a more stable arrangement of electrons in their outermost shell by interacting with one another. A covalent bond is formed when electrons are shared between atoms. An ionic bond is formed when electrons are transferred from one atom to the other. The two cases shown represent extremes; often, covalent bonds form with a partial transfer (unequal sharing of electrons), resulting in a polar covalent bond, as we discuss shortly.

Figure 2–7 The chemistry of life is predominantly the chemistry of lighter elements. When ordered by their atomic number into a periodic table, elements fall into groups that show similar properties based on the number of electrons each element possesses in its outer shell. Atoms in the same vertical column must gain or lose the same number of electrons to attain a filled outer shell, and they thus behave similarly. Thus, both magnesium (Mg) and calcium (Ca) tend to give away the two electrons in their outer shells to form ionic bonds with atoms such as chlorine (Cl) that need extra electrons to complete their outer shells.

The four elements highlighted in red constitute 99% of the total number of atoms present in the human body and about 96% of our total weight. An additional seven elements, highlighted in blue, together represent about 0.9% of the total number of atoms. Other elements, shown in green, are required in trace amounts by humans. It remains unclear whether those elements shown in yellow are essential in humans or not.

The atomic weights shown here are those of the most common isotope of each element.

Covalent Bonds Form by the Sharing of Electrons

All of the characteristics of a cell depend on the molecules it contains. A **molecule** is a cluster of atoms held together by **covalent bonds**, in which electrons are shared rather than transferred between atoms. The shared electrons complete the outer shells of the interacting atoms. In the simplest possible molecule—a molecule of hydrogen (H_2)—two H atoms, each with a single electron, share their electrons, thus filling their outermost shells. The shared electrons form a cloud of negative charge that is densest between the two positively charged nuclei. This electron density helps to hold the nuclei together by opposing the mutual repulsion between their positive charges that would otherwise force them apart. The attractive and repulsive forces are in balance when the nuclei are separated by a characteristic distance, called the *bond length* (Figure 2–8).

Whereas an H atom can form only a single covalent bond, the other common atoms that form covalent bonds in cells—O, N, S, and P, as well as the all-important C—can form more than one. The outermost shells of these atoms, as we have seen, can accommodate up to eight electrons, and they form covalent bonds with as many other atoms as necessary to reach this number. Oxygen, with six electrons in its outer shell, is most stable when it acquires two extra electrons by sharing with other atoms, and it therefore forms up to two covalent bonds. Nitrogen, with five outer electrons, forms a maximum of three covalent bonds, while carbon, with four outer electrons, forms up to four covalent bonds—thus sharing four pairs of electrons (see Figure 2–5).

When one atom forms covalent bonds with several others, these multiple bonds have definite orientations in space relative to one another, reflecting the orientations of the orbits of the shared electrons. Covalent bonds between multiple atoms are therefore characterized by specific bond angles, as well as by specific bond lengths and bond energies (Figure 2–9). The four covalent bonds that can form around a carbon

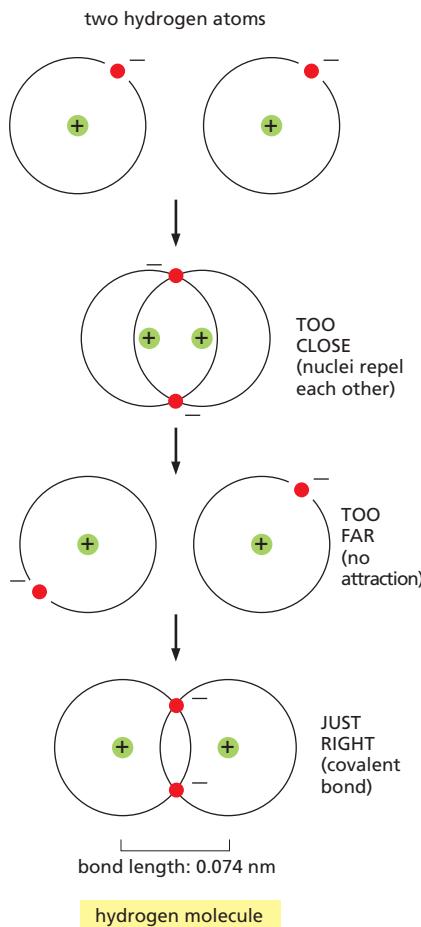


Figure 2–8 The hydrogen molecule is held together by a covalent bond.

Each hydrogen atom in isolation has a single electron, which means that its first (and only) electron shell is incompletely filled. By coming together, the two atoms are able to share their electrons, so that each obtains a completely filled first shell, with the shared electrons adopting modified orbits around the two nuclei. The covalent bond between the two atoms has a definite length—0.074 nm, which is the distance between the two nuclei. If the atoms were closer together, the positive nuclei would repel each other; if they were farther apart, they would not be able to share electrons as effectively.

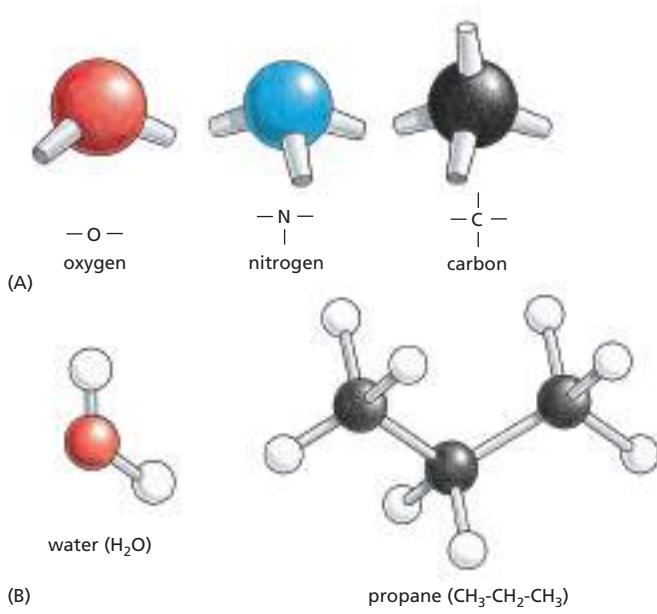


Figure 2-9 Covalent bonds are characterized by particular geometries.

(A) The spatial arrangement of the covalent bonds that can be formed by oxygen, nitrogen, and carbon. (B) Molecules formed from these atoms therefore have a precise three-dimensional structure defined by the bond angles and bond lengths for each covalent linkage. A water molecule, for example, forms a "V" shape with an angle close to 109° .

In these ball-and-stick models, the different colored balls represent different atoms, and the sticks represent the covalent bonds. The colors traditionally used to represent the different atoms—black for carbon, white for hydrogen, blue for nitrogen, and red for oxygen—were established by the chemist August Wilhelm Hofmann in 1865, when he used a set of colored croquet balls to build molecular models for a public lecture on "the combining power of atoms."

atom, for example, are arranged as if pointing to the four corners of a regular tetrahedron. The precise orientation of the covalent bonds around carbon produces the three-dimensional geometry of organic molecules.

There Are Different Types of Covalent Bonds

Most covalent bonds involve the sharing of two electrons, one donated by each participating atom; these are called *single bonds*. Some covalent bonds, however, involve the sharing of more than one pair of electrons. Four electrons can be shared, for example, two coming from each participating atom; such a bond is called a *double bond*. Double bonds are shorter and stronger than single bonds and have a characteristic effect on the three-dimensional geometry of molecules containing them. A single covalent bond between two atoms generally allows the rotation of one part of a molecule relative to the other around the bond axis. A double bond prevents such rotation, producing a more rigid and less flexible arrangement of atoms (Figure 2-10). This restriction has a major influence on the three-dimensional shape of many macromolecules. Panel 2-1 (pp. 66–67) reviews the covalent bonds commonly encountered in biological molecules.

Some molecules contain atoms that share electrons in a way that produces bonds that are intermediate in character between single and double bonds. The highly stable benzene molecule, for example, is made up of a ring of six carbon atoms in which the bonding electrons are evenly distributed (although the arrangement is sometimes depicted as an alternating sequence of single and double bonds, as shown in Panel 2-1).

When the atoms joined by a single covalent bond belong to different elements, the two atoms usually attract the shared electrons to different degrees. Covalent bonds in which the electrons are shared unequally in this way are known as *polar covalent bonds*. A **polar** structure (in the electrical sense) is one in which the positive charge is concentrated toward one end of the molecule (the positive pole) and the negative charge is concentrated toward the other end (the negative pole). Oxygen and nitrogen atoms, for example, attract electrons relatively strongly, whereas an H atom attracts electrons relatively weakly (because of the relative differences in the positive charges of the nuclei of C, O, N, and H). Thus the

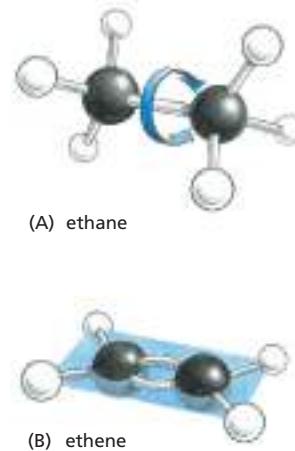


Figure 2-10 Carbon–carbon double bonds are shorter and more rigid than carbon–carbon single bonds.

(A) The ethane molecule, with a single covalent bond between the two carbon atoms, shows the tetrahedral arrangement of the three single covalent bonds between each carbon atom and its three attached H atoms. The CH_3 groups, joined by a covalent C–C bond, can rotate relative to one another around the bond axis. (B) The double bond between the two carbon atoms in a molecule of ethene (ethylene) alters the bond geometry of the carbon atoms and brings all the atoms into the same plane; the double bond prevents the rotation of one CH_2 group relative to the other.

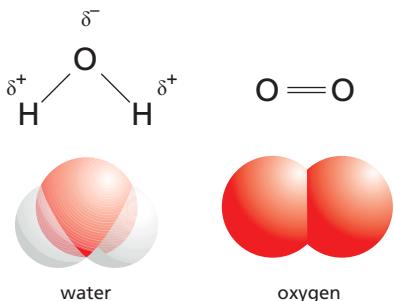


Figure 2-11 In polar covalent bonds, the electrons are shared unequally. Comparison of electron distributions in the polar covalent bonds in a molecule of water (H_2O) and the nonpolar covalent bonds in a molecule of oxygen (O_2). In H_2O , electrons are more strongly attracted to the oxygen nucleus than to the H nucleus, as indicated by the distributions of the partial negative (δ^-) and partial positive (δ^+) charges.

covalent bond between O and H, O–H, or between N and H, N–H, is polar (**Figure 2–11**). An atom of C and an atom of H, by contrast, attract electrons more equally. Thus the bond between carbon and hydrogen, C–H, is relatively nonpolar.

Covalent Bonds Vary in Strength

We have already seen that the covalent bond between two atoms has a characteristic length that depends on the atoms involved. A further crucial property of any chemical bond is its strength. *Bond strength* is measured by the amount of energy that must be supplied to break the bond, usually expressed in units of either kilocalories per mole (kcal/mole) or kilojoules per mole (kJ/mole). A kilocalorie is the amount of energy needed to raise the temperature of 1 liter of water by 1°C. Thus, if 1 kilocalorie of energy must be supplied to break 6×10^{23} bonds of a specific type (that is, 1 mole of these bonds), then the strength of that bond is 1 kcal/mole. One kilocalorie is equal to about 4.2 kJ, which is the unit of energy universally employed by physical scientists and, increasingly, by cell biologists as well.

To get an idea of what bond strengths mean, it is helpful to compare them with the average energies of the impacts that molecules continually undergo owing to collisions with other molecules in their environment—their thermal, or heat, energy. Typical covalent bonds are stronger than these thermal energies by a factor of 100, so they are resistant to being pulled apart by thermal motions. In living organisms, they are normally broken only during specific chemical reactions that are carefully controlled by highly specialized protein catalysts, called *enzymes*.

When water is present, covalent bonds are much stronger than ionic bonds. In ionic bonds, electrons are transferred rather than shared, as we now discuss.

Ionic Bonds Form by the Gain and Loss of Electrons

Ionic bonds are usually formed between atoms that can attain a completely filled outer shell most easily by donating electrons to—or accepting electrons from—another atom, rather than by sharing them. For example, returning to Figure 2–5, we see that a sodium (Na) atom can achieve a filled outer shell by giving up the single electron in its third shell. By contrast, a chlorine (Cl) atom can complete its outer shell by gaining just one electron. Consequently, if a Na atom encounters a Cl atom, an electron can jump from the Na to the Cl, leaving both atoms with filled outer shells. The offspring of this marriage between sodium, a soft and intensely reactive metal, and chlorine, a toxic green gas, is table salt (NaCl).

When an electron jumps from Na to Cl, both atoms become electrically charged **ions**. The Na atom that lost an electron now has one less electron than it has protons in its nucleus; it therefore has a net single positive charge (Na^+). The Cl atom that gained an electron now has one more electron than it has protons and has a net single negative charge (Cl^-). Because of their opposite charges, the Na^+ and Cl^- ions are attracted

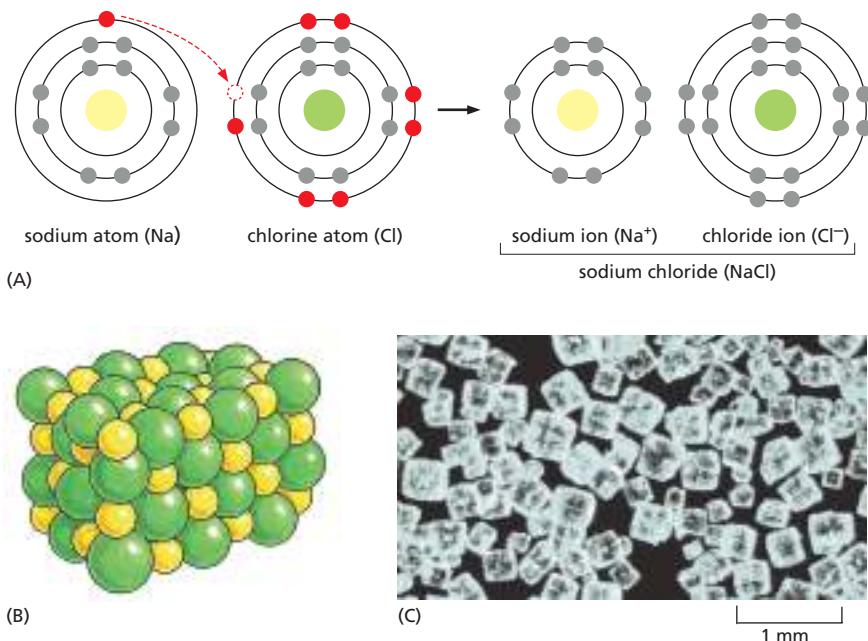


Figure 2–12 Sodium chloride is held together by ionic bonds. (A) An atom of sodium (Na) reacts with an atom of chlorine (Cl). Electrons of each atom are shown in their different shells; electrons in the chemically reactive (incompletely filled) outermost shells are shown in red. The reaction takes place with transfer of a single electron from sodium to chlorine, forming two electrically charged atoms, or ions, each with complete sets of electrons in their outermost shells. The two ions have opposite charge and are held together by electrostatic attraction. (B) The product of the reaction between sodium and chlorine, crystalline sodium chloride, contains sodium and chloride ions packed closely together in a regular array in which the charges are exactly balanced. (C) Color photograph of crystals of sodium chloride.

to each other and are thereby held together by an ionic bond (Figure 2–12A). Ions held together solely by ionic bonds are generally called *salts* rather than molecules. A NaCl crystal contains astronomical numbers of Na^+ and Cl^- packed together in a precise three-dimensional array with their opposite charges exactly balanced: a crystal only 1 mm across contains about 2×10^{19} ions of each type (Figure 2–12B and C).

Because of the favorable interaction between ions and water molecules (which are polar), many salts (including NaCl) are highly soluble in water. They dissociate into individual ions (such as Na^+ and Cl^-), each surrounded by a group of water molecules. Positive ions are called *cations*, and negative ions are called *anions*. Small inorganic ions such as Na^+ , Cl^- , K^+ , and Ca^{2+} play important parts in many biological processes, including the electrical activity of nerve cells, as we discuss in Chapter 12.

Noncovalent Bonds Help Bring Molecules Together in Cells

In aqueous solution, ionic bonds are 10–100 times weaker than the covalent bonds that hold atoms together in molecules. But this weakness has its place: much of biology depends on specific but transient interactions between one molecule and another. These associations are mediated by **noncovalent bonds**. Although noncovalent bonds are individually quite weak, their energies can sum to create an effective force between two molecules.

The ionic bonds that hold together the Na^+ and Cl^- ions in a salt crystal (see Figure 2–12) are a form of noncovalent bond called an *electrostatic attraction*. Electrostatic attractions are strongest when the atoms involved are fully charged, as are Na^+ and Cl^- . But a weaker electrostatic attraction also occurs between molecules that contain polar covalent bonds (see Figure 2–11). Polar covalent bonds are thus extremely important in biology because they allow molecules to interact through electrical forces. Any large molecule with many polar groups will have a pattern of partial positive and negative charges on its surface. When such a molecule encounters a second molecule with a complementary set of charges, the two will be attracted to each other by electrostatic attraction—even

QUESTION 2–4

What, if anything, is wrong with the following statement: "When NaCl is dissolved in water, the water molecules closest to the ions will tend to preferentially orient themselves so that their oxygen atoms face the sodium ions and face away from the chloride ions"? Explain your answer.

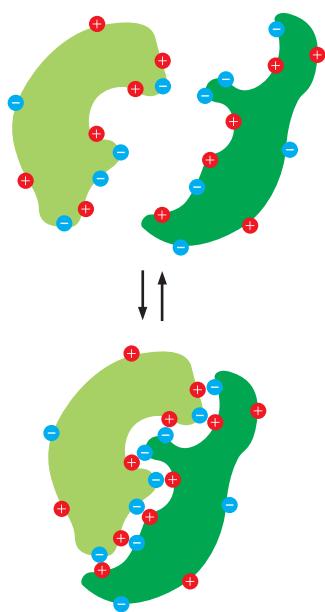


Figure 2-13 A large molecule, such as a protein, can bind to another protein through complementary charges on the surface of each molecule. In the aqueous environment of a cell, the many individual electrostatic attractions shown would help the two proteins stay bound to each other.

though water greatly reduces the attractiveness of these charges in most biological settings. When present in large numbers, however, weak non-covalent bonds on the surfaces of large molecules can promote strong and specific binding (**Figure 2-13**).

Hydrogen Bonds Are Important Noncovalent Bonds For Many Biological Molecules

Water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment. Life on Earth is thought to have begun in the ocean. Thus the properties of water have put a permanent stamp on the chemistry of living things.

In each molecule of water (H_2O), the two H atoms are linked to the O atom by covalent bonds. The two H-O bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive. Consequently, there is an unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and negative charge on the O (see Figure 2-11). When a positively charged region of one water molecule (that is, one of its H atoms) comes close to a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can establish a weak bond called a **hydrogen bond** (**Figure 2-14**). These bonds are much weaker than covalent bonds and are easily broken by random thermal motions. Thus each bond lasts only an exceedingly short time. But the combined effect of many weak bonds is far from trivial. Each water molecule can form hydrogen bonds through its two H atoms to two other water molecules, producing a network in which hydrogen bonds are being continually broken and formed. It is because of these interlocking hydrogen bonds that water at room temperature is a liquid—with a high boiling point and high surface tension—and not a gas. Without hydrogen bonds, life as we know it could not exist. The biologically significant properties of water are reviewed in **Panel 2-2** (pp. 68–69).

Hydrogen bonds are not limited to water. In general, a hydrogen bond can form whenever a positively charged H atom held in one molecule by a polar covalent linkage comes close to a negatively charged atom—typically an oxygen or a nitrogen—belonging to another molecule (see Figure 2-14). Hydrogen bonds can also occur between different parts of a single large molecule, where they often help the molecule fold into a particular shape. The length and strength of hydrogen bonds and of ionic bonds are compared to those of covalent bonds in **Table 2-1**.

Molecules, such as alcohols, that contain polar bonds and that can form hydrogen bonds mix well with water. As mentioned previously, molecules carrying positive or negative charges (ions) likewise dissolve readily in water. Such molecules are termed **hydrophilic**, meaning that they are

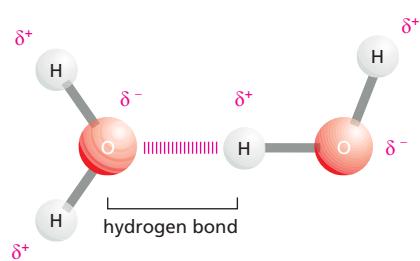


Figure 2-14 A hydrogen bond can form between two water molecules. These bonds are largely responsible for water's life-sustaining properties—including its ability to exist as a liquid at the temperatures inside the typical mammalian body.

TABLE 2-1 LENGTH AND STRENGTH OF SOME CHEMICAL BONDS

Bond type	Length* (nm)	Strength (kcal/mole)	
		in vacuum	in water
Covalent	0.10	90 [377]**	90 [377]
Noncovalent: ionic bond	0.25	80 [335]	3 [12.6]
Noncovalent: hydrogen bond	0.17	4 [16.7]	1 [4.2]

*The bond lengths and strengths listed are approximate, because the exact values will depend on the atoms involved.

**Values in brackets are kJ/mole. 1 calorie = 4.184 joules.

"water-loving." A large proportion of the molecules in the aqueous environment of a cell fall into this category, including sugars, DNA, RNA, and a majority of proteins. **Hydrophobic** ("water-fearing") molecules, by contrast, are uncharged and form few or no hydrogen bonds, and they do not dissolve in water.

Hydrocarbons are important hydrophobic cell constituents (see Panel 2–1, pp. 66–67). In these molecules, the H atoms are covalently linked to C atoms by nonpolar bonds. Because the H atoms have almost no net positive charge, they cannot form effective hydrogen bonds to other molecules. This makes the hydrocarbon as a whole hydrophobic—a property that is exploited by cells, whose membranes are constructed largely from *lipid molecules* that have long hydrocarbon tails. Because lipids do not dissolve in water, they can form the thin membrane barriers that keep the aqueous interior of the cell separate from the surrounding aqueous environment, as we discuss later.

Some Polar Molecules Form Acids and Bases in Water

One of the simplest kinds of chemical reaction, and one that has profound significance in cells, takes place when a molecule possessing a highly polar covalent bond between a hydrogen and another atom dissolves in water. The hydrogen atom in such a bond has given up its electron almost entirely to the companion atom, so it exists as an almost naked positively charged hydrogen nucleus—in other words, a *proton* (H^+). When the polar molecule becomes surrounded by water molecules, the proton will be attracted to the partial negative charge on the oxygen atom of an adjacent water molecule (see Figure 2–11); this proton can dissociate from its original partner and associate instead with the oxygen atom of the water molecule, generating a **hydronium ion** (H_3O^+) (Figure 2–15A). The reverse reaction also takes place very readily, so one has to imagine an equilibrium state in which billions of protons are constantly flitting to and fro between one molecule and another in an aqueous solution.

Substances that release protons when they dissolve in water, thus forming H_3O^+ , are termed **acids**. The higher the concentration of H_3O^+ , the more acidic the solution. H_3O^+ is present even in pure water, at a concentration of 10^{-7} M, as a result of the movement of protons from one water molecule to another (Figure 2–15B). By tradition, the H_3O^+ concentration

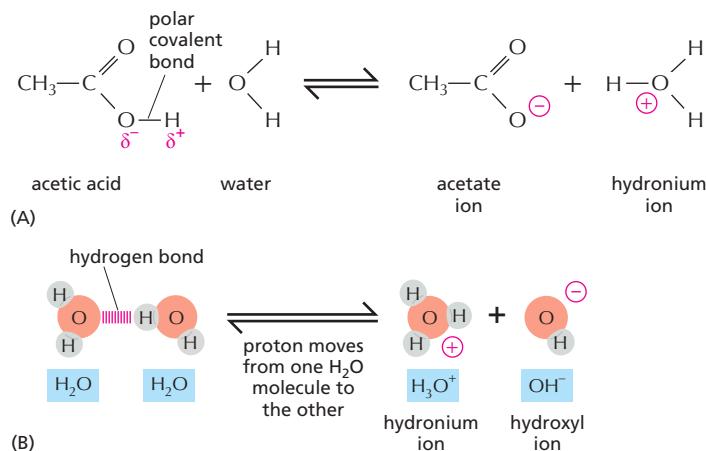


Figure 2–15 Protons move continuously from one water molecule to another

in aqueous solutions. (A) The reaction that takes place when a molecule of acetic acid dissolves in water. At pH 7, nearly all of the acetic acid molecules are present as acetate ions. (B) Water molecules are continually exchanging protons with each other to form hydronium and hydroxyl ions. These ions in turn rapidly recombine to form water molecules.

is usually referred to as the H⁺ concentration, even though most protons in an aqueous solution are present as H₃O⁺. To avoid the use of unwieldy numbers, the concentration of H⁺ is expressed using a logarithmic scale called the **pH scale**, as illustrated in Panel 2–2. Pure water has a pH of 7.0 and is thus neutral—that is, neither acidic (pH < 7) nor basic (pH > 7).

Acids are characterized as being strong or weak, depending on how readily they give up their protons to water. Strong acids, such as hydrochloric acid (HCl), lose their protons easily. Acetic acid, on the other hand, is a weak acid because it holds on to its proton more tightly when dissolved in water. Many of the acids important in the cell—such as molecules containing a carboxyl (COOH) group—are weak acids (see Panel 2–2, pp. 68–69). Their tendency to give up a proton with some reluctance is a useful characteristic, as it renders the molecules sensitive to changes in pH in the cell—a property that can be exploited to regulate function.

Because protons can be passed readily to many types of molecules in cells, thus altering the molecules' character, the H⁺ concentration inside a cell (the pH) must be closely controlled. Acids—especially weak acids—will give up their protons more readily if the H⁺ concentration is low and will tend to accept them back if the concentration is high.

The opposite of an acid is a **base**, which includes any molecule that accepts a proton when dissolved in water. Just as the defining property of an acid is that it raises the concentration of H₃O⁺ ions by donating a proton to a water molecule, so the defining property of a base is that it raises the concentration of hydroxyl (OH[−]) ions by removing a proton from a water molecule. Thus sodium hydroxide (NaOH) is basic (the term *alkaline* is also used) because it dissociates in aqueous solution to form Na⁺ ions and OH[−] ions; because it does so readily, NaOH is called a strong base. Weak bases—which have a weak tendency to accept a proton from water—however, are actually more important in cells. Many biologically important weak bases contain an amino (NH₂) group, which can generate OH[−] by taking a proton from water: $\text{--NH}_2 + \text{H}_2\text{O} \rightarrow \text{--NH}_3^+ + \text{OH}^-$ (see Panel 2–2, pp. 68–69).

Because an OH[−] ion combines with a proton to form a water molecule, an increase in the OH[−] concentration forces a decrease in the H⁺ concentration, and vice versa. A pure solution of water thus contains an equal concentration (10^{−7} M) of both ions, rendering it neutral (pH 7). The interior of a cell is also kept close to neutral by the presence of **buffers**: mixtures of weak acids and bases that can adjust proton concentrations around pH 7 by releasing protons (acids) or taking them up (bases). This give-and-take keeps the pH of the cell relatively constant under a variety of conditions.

SMALL MOLECULES IN CELLS

Having looked at the ways atoms combine to form small molecules and how these molecules behave in an aqueous environment, we now examine the main classes of small molecules found in cells and their biological roles. Amazingly, we will see that a few basic categories of molecules, formed from a handful of different elements, give rise to all the extraordinary richness of form and behavior displayed by living things.

A Cell Is Formed from Carbon Compounds

If we disregard water, nearly all the molecules in a cell are based on carbon. Carbon is outstanding among all the elements in its ability to form large molecules; silicon—an element with the same number of electrons in its outer shell—is a poor second. Because a carbon atom is small and

QUESTION 2–5

- A. Are there any H₃O⁺ ions present in pure water at neutral pH (i.e., at pH = 7.0)? If so, how are they formed?
- B. If they exist, what is the ratio of H₃O⁺ ions to H₂O molecules at neutral pH? (Hint: the molecular weight of water is 18, and 1 liter of water weighs 1 kg.)

has four electrons and four vacancies in its outer shell, it can form four covalent bonds with other atoms (see Figure 2–9). Most importantly, one carbon atom can join to other carbon atoms through highly stable covalent C–C bonds to form chains and rings and hence generate large and complex molecules with no obvious upper limit to their size. The small and large carbon compounds made by cells are called **organic molecules**. By contrast, all other molecules, including water, are said to be **inorganic**.

Certain combinations of atoms, such as the methyl ($-\text{CH}_3$), hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), carbonyl ($-\text{C=O}$), phosphoryl ($-\text{PO}_3^{2-}$), and amino ($-\text{NH}_2$) groups, occur repeatedly in organic molecules. Each such **chemical group** has distinct chemical and physical properties that influence the behavior of the molecule in which the group occurs, including whether the molecule tends to gain or lose protons and with which other molecules it will interact. Knowing these groups and their chemical properties greatly simplifies understanding the chemistry of life. The most common chemical groups and some of their properties are summarized in Panel 2–1 (pp. 67–68).

Cells Contain Four Major Families of Small Organic Molecules

The small organic molecules of the cell are carbon compounds with molecular weights in the range 100–1000 that contain up to 30 or so carbon atoms. They are usually found free in solution in the cytosol and have many different roles. Some are used as *monomer* subunits to construct the cell's giant polymeric *macromolecules*—its proteins, nucleic acids, and large polysaccharides. Others serve as energy sources, which are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways. Many have more than one role in the cell—acting, for example, as both a potential subunit for a macromolecule and as an energy source. The small organic molecules are much less abundant than the organic macromolecules, accounting for only about one-tenth of the total mass of organic matter in a cell. As a rough guess, there may be a thousand different kinds of these small organic molecules in a typical animal cell.

All organic molecules are synthesized from—and are broken down into—the same set of simple compounds. Both their synthesis and their breakdown occur through sequences of simple chemical changes that are limited in variety and follow step-by-step rules. As a consequence, the compounds in a cell are chemically related, and most can be classified into a small number of distinct families. Broadly speaking, cells contain four major families of small organic molecules: the *sugars*, the *fatty acids*, the *amino acids*, and the *nucleotides* (Figure 2–16). Although many compounds present in cells do not fit into these categories, these four families of small organic molecules, together with the macromolecules made by linking them into long chains, account for a large fraction of a cell's mass (Table 2–2).

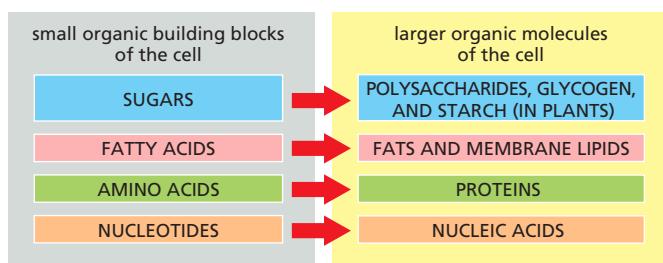


Figure 2–16 Sugars, fatty acids, amino acids, and nucleotides are the four main families of small organic molecules in cells. They form the monomeric building blocks, or subunits, for larger organic molecules, including most of the macromolecules and other molecular assemblies of the cell. Some, like the sugars and the fatty acids, are also energy sources.

TABLE 2–2 THE CHEMICAL COMPOSITION OF A BACTERIAL CELL

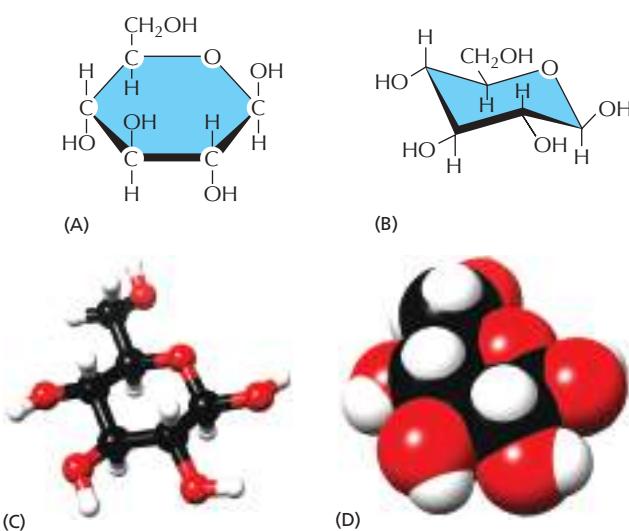
	Percent of total cell weight	Approximate number of types of each class of molecule
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	300
Phospholipids	2	4*
Macromolecules (nucleic acids, proteins, and polysaccharides)	24	3000

*There are four classes of phospholipids, each of which exists in many varieties.

Sugars Are Both Energy Sources and Subunits of Polysaccharides

The simplest **sugars**—the *monosaccharides*—are compounds with the general formula $(\text{CH}_2\text{O})_n$, where n is usually 3, 4, 5, or 6. Sugars, and the larger molecules made from them, are also called *carbohydrates* because of this simple formula. Glucose, for example, has the formula $\text{C}_6\text{H}_{12}\text{O}_6$ (Figure 2–17). The formula, however, does not fully define the molecule: the same set of carbons, hydrogens, and oxygens can be joined together by covalent bonds in a variety of ways, creating structures with different shapes. Thus glucose can be converted into a different sugar—mannose or galactose—simply by switching the orientations of specific $-\text{OH}$ groups relative to the rest of the molecule (Panel 2–3, pp. 70–71). Each of these sugars, moreover, can exist in either of two forms, called the *D*-form and the *L*-form, which are mirror images of each other. Sets of molecules with the same chemical formula but different structures are called *isomers*, and mirror-image pairs of such molecules are called *optical isomers*. Isomers are widespread among organic molecules in general, and they play a

Figure 2–17 The structure of glucose, a monosaccharide, can be represented in several ways. (A) A structural formula in which the atoms are shown as chemical symbols, linked together by solid lines representing the covalent bonds. The thickened lines are used to indicate the plane of the sugar ring and to show that the $-\text{H}$ and $-\text{OH}$ groups are not in the same plane as the ring. (B) Another kind of structural formula that shows the three-dimensional structure of glucose in the so-called “chair configuration.” (C) A ball-and-stick model in which the three-dimensional arrangement of the atoms in space is indicated. (D) A space-filling model, which, as well as depicting the three-dimensional arrangement of the atoms, also gives some idea of their relative sizes and of the surface contours of the molecule (Movie 2.1). The atoms in (C) and (D) are colored as in Figure 2–9: C, black; H, white; O, red. This is the conventional color coding for these atoms and will be used throughout this book.



major part in generating the enormous variety of sugars. A more complete outline of sugar structures and chemistry is presented in Panel 2–3.

Monosaccharides can be linked by covalent bonds—called glycosidic bonds—to form larger carbohydrates. Two monosaccharides linked together make a disaccharide, such as sucrose, which is composed of a glucose and a fructose unit. Larger sugar polymers range from the *oligosaccharides* (trisaccharides, tetrasaccharides, and so on) up to giant *polysaccharides*, which can contain thousands of monosaccharide units. In most cases, the prefix *oligo-* is used to refer to molecules made of a small number of monomers, typically 2 to 10 in the case of oligosaccharides. Polymers, in contrast, can contain hundreds or thousands of subunits.

The way sugars are linked together illustrates some common features of biochemical bond formation. A bond is formed between an –OH group on one sugar and an –OH group on another by a **condensation reaction**, in which a molecule of water is expelled as the bond is formed. The sub-units in other biological polymers, including nucleic acids and proteins, are also linked by condensation reactions in which water is expelled. The bonds created by all of these condensation reactions can be broken by the reverse process of **hydrolysis**, in which a molecule of water is consumed (Figure 2–18).

Because each monosaccharide has several free hydroxyl groups that can form a link to another monosaccharide (or to some other compound), sugar polymers can be branched, and the number of possible polysaccharide structures is extremely large. For this reason, it is much more difficult to determine the arrangement of sugars in a complex polysaccharide than to determine the nucleotide sequence of a DNA molecule or the amino acid sequence of a protein, in which each unit is joined to the next in exactly the same way.

The monosaccharide *glucose* has a central role as an energy source for cells. It is broken down to smaller molecules in a series of reactions, releasing energy that the cell can harness to do useful work, as we explain in Chapter 13. Cells use simple polysaccharides composed only of glucose units—principally *glycogen* in animals and *starch* in plants—as long-term stores of glucose, held in reserve for energy production.

Sugars do not function exclusively in the production and storage of energy. They are also used, for example, to make mechanical supports. The most abundant organic molecule on Earth—the *cellulose* that forms plant cell walls—is a polysaccharide of glucose. Another extraordinarily abundant organic substance, the *chitin* of insect exoskeletons and fungal cell walls, is also a polysaccharide—in this case, a linear polymer of a sugar derivative called *N*-acetylglucosamine (see Panel 2–3, pp. 70–71). Other polysaccharides, which tend to be slippery when wet, are the main components of slime, mucus, and gristle.

Smaller oligosaccharides can be covalently linked to proteins to form *glycoproteins*, or to lipids to form *glycolipids* (Panel 2–4, pp. 72–73), which are both found in cell membranes. The sugar side chains attached to glycoproteins and glycolipids in the plasma membrane are thought to help protect the cell surface and often help cells adhere to one another. Differences in the types of cell-surface sugars form the molecular basis for different human blood groups.

Fatty Acid Chains Are Components of Cell Membranes

A **fatty acid** molecule, such as *palmitic acid*, has two chemically distinct regions. One is a long hydrocarbon chain, which is hydrophobic and not very reactive chemically. The other is a carboxyl (–COOH) group,

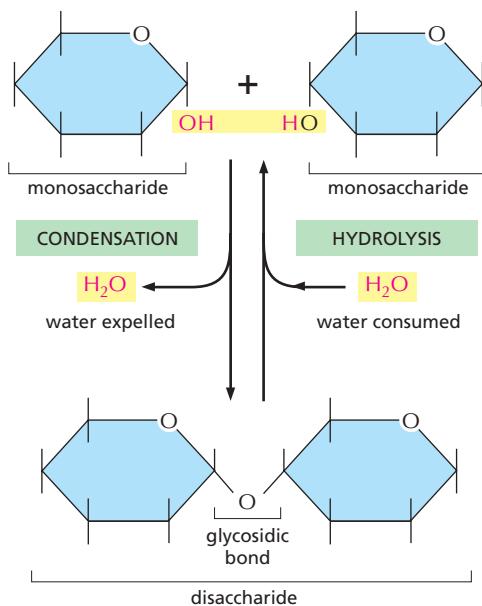
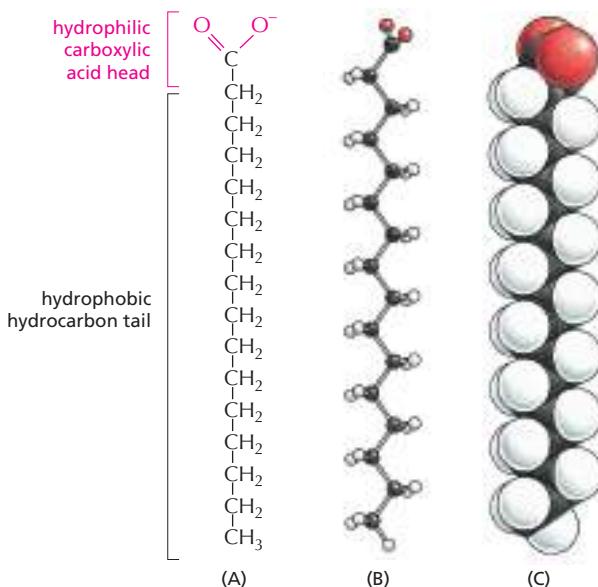


Figure 2–18 Two monosaccharides can be linked by a covalent glycosidic bond to form a disaccharide. This reaction belongs to a general category of reactions termed *condensation reactions*, in which two molecules join together as a result of the loss of a water molecule. The reverse reaction (in which water is added) is termed *hydrolysis*.

Figure 2–19 Fatty acids have both hydrophobic and hydrophilic components. The hydrophobic hydrocarbon chain is attached to a hydrophilic carboxylic acid group. Different fatty acids have different hydrocarbon tails. Palmitic acid is shown here. (A) Structural formula, showing the carboxylic acid head group in its ionized form, as it exists in water at pH 7. (B) Ball-and-stick model. (C) Space-filling model ([Movie 2.2](#)).



which behaves as an acid (carboxylic acid): in an aqueous solution, it is ionized (-COO^-), extremely hydrophilic, and chemically reactive ([Figure 2–19](#)). Almost all the fatty acid molecules in a cell are covalently linked to other molecules by their carboxylic acid group (see Panel 2–4, pp. 72–73). Molecules—such as fatty acids—that possess both hydrophobic and hydrophilic regions are termed *amphipathic*.

The hydrocarbon tail of palmitic acid is *saturated*: it has no double bonds between its carbon atoms and contains the maximum possible number of hydrogens. Some other fatty acids, such as oleic acid, have *unsaturated* tails, with one or more double bonds along their length. The double bonds create kinks in the hydrocarbon tails, interfering with their ability to pack together, and it is the absence or presence of these double bonds that accounts for the difference between hard (saturated) and soft (polyunsaturated) margarine. Fatty acid tails are also found in cell membranes, where the tightness of their packing affects the fluidity of the membrane. The many different fatty acids found in cells differ only in the length of their hydrocarbon chains and in the number and position of the carbon–carbon double bonds (see Panel 2–4).

Fatty acids serve as a concentrated food reserve in cells: they can be broken down to produce about six times as much usable energy, weight for weight, as glucose. Fatty acids are stored in the cytoplasm of many cells in the form of fat droplets composed of *triacylglycerol* molecules—compounds made of three fatty acid chains covalently joined to a glycerol molecule ([Figure 2–20](#), and see Panel 2–4). Triacylglycerols are the animal fats found in meat, butter, and cream, and the plant oils such as corn oil and olive oil. When a cell needs energy, the fatty acid chains can be released from triacylglycerols and broken down into two-carbon units. These two-carbon units are identical to those derived from the breakdown of glucose, and they enter the same energy-yielding reaction pathways, as described in Chapter 13.

Fatty acids and their derivatives, including triacylglycerols, are examples of **lipids**. Lipids are loosely defined as molecules that are insoluble in water but soluble in fat and organic solvents such as benzene. They typically contain long hydrocarbon chains, as in the fatty acids, or multiple linked aromatic rings, as in the **steroids** (see Panel 2–4).

The most unique function of fatty acids is in the formation of the **lipid bilayer**, which is the basis for all cell membranes. These thin sheets,

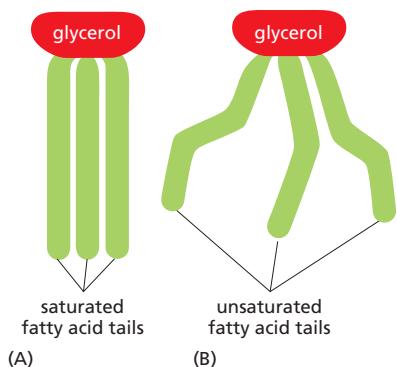


Figure 2–20 The properties of fats depend on the length and saturation of the fatty acid chains they carry. Fatty acids are stored in the cytoplasm of many cells in the form of droplets of *triacylglycerol* molecules made of three fatty acid chains joined to a glycerol molecule. (A) Saturated fats are found in meat and dairy products. (B) Plant oils, such as corn oil, contain unsaturated fatty acids, which may be monounsaturated (containing one double bond) or polyunsaturated (containing multiple double bonds); this is why plant oils are liquid at room temperature. Although fats are essential in the diet, saturated fats are not: they raise the concentration of cholesterol in the blood, which tends to clog the arteries, increasing the risk of heart attacks and strokes.

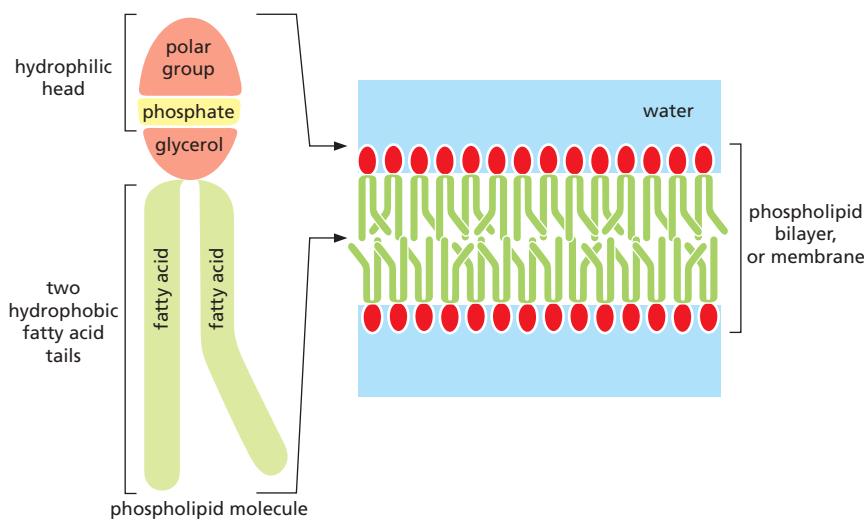


Figure 2–21 Phospholipids can aggregate to form cell membranes. Phospholipids are composed of two hydrophobic fatty acid tails joined to a hydrophilic head. In an aqueous environment, the hydrophobic tails pack together to exclude water, forming a lipid bilayer, with the hydrophilic heads of the phospholipid molecules on the outside, facing the aqueous environment, and the hydrophobic tails on the inside.

which enclose all cells and surround their internal organelles, are composed largely of *phospholipids* (Figure 2–21).

Like triacylglycerols, most phospholipids are constructed mainly from fatty acids and glycerol. In these phospholipids, however, the glycerol is joined to two fatty acid chains, rather than to three as in triacylglycerols. The remaining -OH group on the glycerol is linked to a hydrophilic phosphate group, which in turn is attached to a small hydrophilic compound such as choline (see Panel 2–4, pp. 72–73). With their two hydrophobic fatty acid tails and a hydrophilic, phosphate-containing head, phospholipids are strongly amphipathic. This characteristic amphipathic composition and shape gives them different physical and chemical properties from triacylglycerols, which are predominantly hydrophobic. In addition to phospholipids, cell membranes contain differing amounts of other lipids, including *glycolipids*, which contain one or more sugars instead of a phosphate group.

Thanks to their amphipathic nature, phospholipids readily form membranes in water. These lipids will spread over the surface of water to form a monolayer, with their hydrophobic tails facing the air and their hydrophilic heads in contact with the water. Two such molecular layers can readily combine tail-to-tail in water to form the phospholipid sandwich that is the lipid bilayer (see Chapter 11).

Amino Acids Are the Subunits of Proteins

Amino acids are small organic molecules with one defining property: they all possess a carboxylic acid group and an amino group, both linked to their α -carbon atom (Figure 2–22). Each amino acid also has a side chain attached to its α -carbon. The identity of this side chain is what distinguishes one amino acid from another.

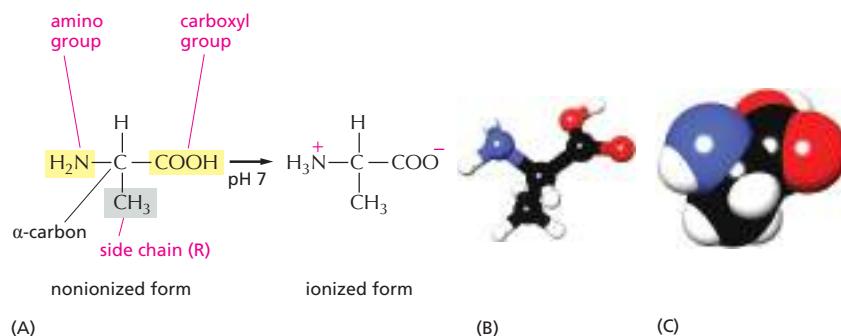


Figure 2–22 All amino acids have an amino group, a carboxyl group, and a side chain (R) attached to their α -carbon atom. In the cell, where the pH is close to 7, free amino acids exist in their ionized form; but, when they are incorporated into a polypeptide chain, the charges on their amino and carboxyl groups disappear. (A) The amino acid shown is alanine, one of the simplest amino acids, which has a methyl group (CH_3) as its side chain. (B) A ball-and-stick model and (C) a space-filling model of alanine. In (B) and (C), the N atom is blue.

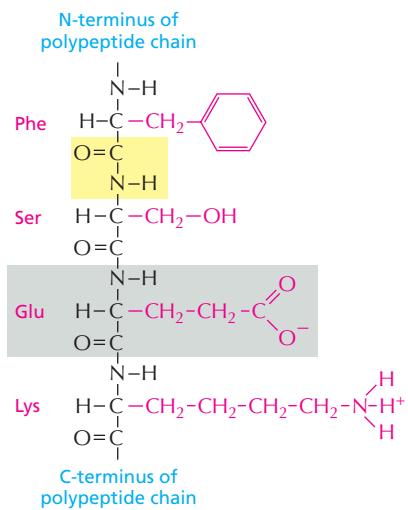


Figure 2-23 Amino acids in a protein are held together by peptide bonds.

bonds. The four amino acids shown are linked together by three peptide bonds, one of which is highlighted in yellow. One of the amino acids, glutamic acid, is shaded in gray. The amino acid side chains are shown in red. The two ends of a polypeptide chain are chemically distinct. One end, the N-terminus, is capped by an amino group, and the other, the C-terminus, ends in a carboxyl group. The sequence of amino acids in a protein is abbreviated using either a three-letter or a one-letter code, and the sequence is always read from the N-terminus (see Panel 2-5, pp. 74–75). In the example given, the sequence is Phe-Ser-Glu-Lys (or FSEK).

Cells use amino acids to build **proteins**—polymers made of amino acids, which are joined head-to-tail in a long chain that folds up into a three-dimensional structure that is unique to each type of protein. The covalent bond between two adjacent amino acids in a protein chain is called a *peptide bond*; the chain of amino acids is also known as a *polypeptide*. Peptide bonds are formed by condensation reactions that link one amino acid to the next. Regardless of the specific amino acids from which it is made, the polypeptide always has an amino (NH_2) group at one end—its *N-terminus*—and a carboxyl (COOH) group at its other end—its *C-terminus* (Figure 2-23). This difference in the two ends gives a polypeptide a definite directionality—a structural (as opposed to electrical) polarity.

Twenty types of amino acids are commonly found in proteins, each with a different side chain attached to the α -carbon atom (Panel 2-5, pp. 74–75). The same 20 amino acids are found in all proteins, whether they hail from bacteria, plants, or animals. How this precise set of 20 amino acids came to be chosen is one of the mysteries surrounding the evolution of life; there is no obvious chemical reason why other amino acids could not have served just as well. But once the selection had been locked into place, it could not be changed, as too much chemistry had evolved to exploit it. Switching the types of amino acids used by cells would require a living creature to retool its entire metabolism to cope with the new building blocks.

Like sugars, all amino acids (except glycine) exist as optical isomers in D- and L-forms (see Panel 2-5). But only L-forms are ever found in proteins (although D-amino acids occur as part of bacterial cell walls and in some antibiotics, and D-serine is used as a signal molecule in the brain). The origin of this exclusive use of L-amino acids to make proteins is another evolutionary mystery.

The chemical versatility that the 20 standard amino acids provide is vitally important to the function of proteins. Five of the 20 amino acids—including lysine and glutamic acid, shown in Figure 2-23—have side chains that can form ions in solution and can therefore carry a charge. The others are uncharged. Some amino acids are polar and hydrophilic, and some are nonpolar and hydrophobic (see Panel 2-5). As we discuss in Chapter 4, the collective properties of the amino acid side chains underlie all the diverse and sophisticated functions of proteins.

Nucleotides Are the Subunits of DNA and RNA

DNA and RNA are built from subunits called **nucleotides**. **Nucleosides** are made of a nitrogen-containing ring compound linked to a five-carbon sugar, which can be either ribose or deoxyribose (Panel 2-6, pp. 76–77). Nucleotides are nucleosides that contain one or more phosphate groups attached to the sugar, and they come in two main forms: those containing ribose are known as *ribonucleotides*, and those containing deoxyribose are known as *deoxyribonucleotides*.

QUESTION 2-6

Why do you suppose only L-amino acids and not a random mixture of the L- and D-forms of each amino acid are used to make proteins?

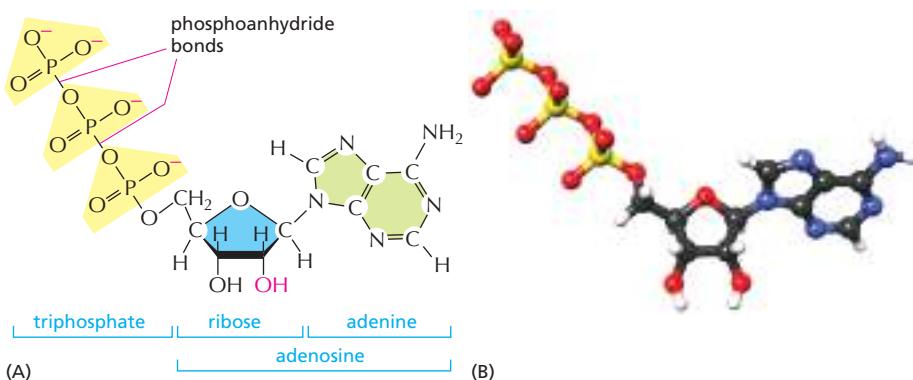


Figure 2–24 Adenosine triphosphate (ATP) is a crucially important energy carrier in cells. (A) Structural formula, in which the three phosphate groups are shaded in yellow. (B) Ball-and-stick model (Movie 2.3). In (B), the P atoms are yellow.

The nitrogen-containing rings of all these molecules are generally referred to as *bases* for historical reasons: under acidic conditions, they can each bind an H^+ (proton) and thereby increase the concentration of OH^- ions in aqueous solution. There is a strong family resemblance between the different nucleotide bases. *Cytosine* (C), *thymine* (T), and *uracil* (U) are called *pyrimidines*, because they all derive from a six-membered pyrimidine ring; *guanine* (G) and *adenine* (A) are *purines*, which bear a second, five-membered ring fused to the six-membered ring. Each nucleotide is named after the base it contains (see Panel 2–6, pp. 76–77).

Nucleotides can act as short-term carriers of chemical energy. Above all others, the ribonucleotide **adenosine triphosphate**, or **ATP** (Figure 2–24), participates in the transfer of energy in hundreds of metabolic reactions. ATP is formed through reactions that are driven by the energy released by the breakdown of foodstuffs. Its three phosphates are linked in series by two *phosphoanhydride bonds* (see Panel 2–6). Rupture of these phosphate bonds releases large amounts of useful energy. The terminal phosphate group in particular is frequently split off by hydrolysis (Figure 2–25). In many situations, transfer of this phosphate to other molecules releases energy that drives energy-requiring biosynthetic reactions. Other nucleotide derivatives serve as carriers for the transfer of other chemical groups. All of this is described in Chapter 3.

Nucleotides also have a fundamental role in the storage and retrieval of biological information. They serve as building blocks for the construction

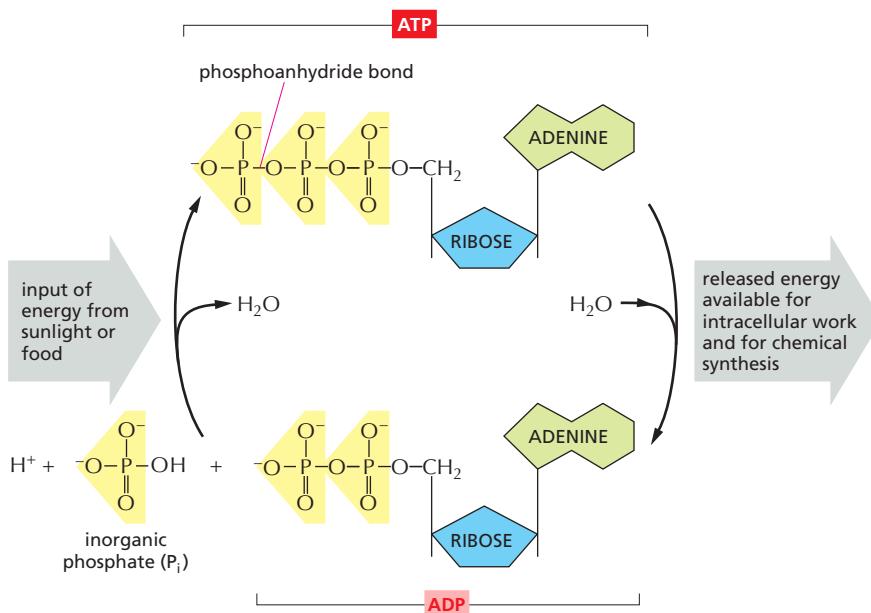


Figure 2–25 ATP is synthesized from ADP and inorganic phosphate, and it releases energy when it is hydrolyzed back to ADP and inorganic phosphate. The energy required for ATP synthesis is derived from either the energy-yielding oxidation of foodstuffs (in animal cells, fungi, and some bacteria) or the capture of light (in plant cells and some bacteria). The hydrolysis of ATP provides the energy to drive many processes inside cells. Together, the two reactions shown form the ATP cycle.

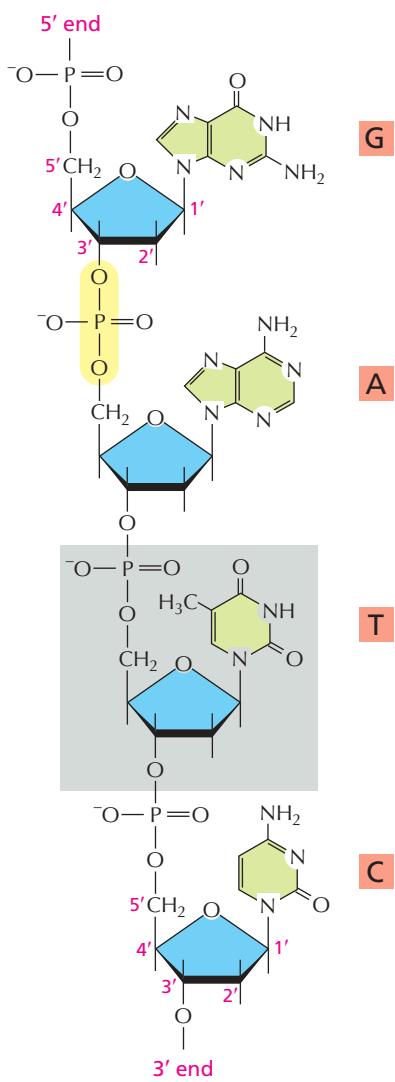


Figure 2–26 A short length of one chain of a deoxyribonucleic acid (DNA) molecule shows the covalent phosphodiester bonds linking four consecutive nucleotides. Because the bonds link specific carbon atoms in the sugar ring—known as the 5' and 3' atoms—one end of a polynucleotide chain, the 5' end, has a free phosphate group and the other, the 3' end, has a free hydroxyl group. One of the nucleotides, thymine (T), is shaded in gray, and one phosphodiester bond is highlighted in yellow. The linear sequence of nucleotides in a polynucleotide chain is commonly abbreviated by a one-letter code, and the sequence is always read from the 5' end. In the example illustrated, the sequence is GATC.

of *nucleic acids*—long polymers in which nucleotide subunits are linked by the formation of covalent *phosphodiester bonds* between the phosphate group attached to the sugar of one nucleotide and a hydroxyl group on the sugar of the next nucleotide (Figure 2–26). Nucleic acid chains are synthesized from energy-rich nucleoside triphosphates by a condensation reaction that releases inorganic pyrophosphate during phosphodiester bond formation (see Panel 2–6, pp. 76–77).

There are two main types of nucleic acids, which differ in the type of sugar contained in their sugar-phosphate backbone. Those based on the sugar ribose are known as **ribonucleic acids**, or **RNA**, and contain the bases A, G, C, and U. Those based on deoxyribose (in which the hydroxyl at the 2' position of the ribose carbon ring is replaced by a hydrogen) are known as **deoxyribonucleic acids**, or **DNA**, and contain the bases A, G, C, and T (T is chemically similar to the U in RNA; see Panel 2–6). RNA usually occurs in cells in the form of a single-stranded polynucleotide chain, but DNA is virtually always in the form of a double-stranded molecule: the DNA double helix is composed of two polynucleotide chains that run in opposite directions and are held together by hydrogen bonds between the bases of the two chains (Panel 2–7, pp. 78–79).

The linear sequence of nucleotides in a DNA or an RNA molecule encodes genetic information. The two nucleic acids, however, have different roles in the cell. DNA, with its more stable, hydrogen-bonded helices, acts as a long-term repository for hereditary information, while single-stranded RNA is usually a more transient carrier of molecular instructions. The ability of the bases in different nucleic acid molecules to recognize and pair with each other by hydrogen-bonding (called *base-pairing*)—G with C, and A with either T or U—underlies all of heredity and evolution, as explained in Chapter 5.

MACROMOLECULES IN CELLS

On the basis of weight, macromolecules are by far the most abundant of the organic molecules in a living cell (Figure 2–27). They are the principal building blocks from which a cell is constructed and also the components that confer the most distinctive properties on living things. Intermediate in size and complexity between small organic molecules and organelles, **macromolecules** are constructed simply by covalently linking small organic **monomers**, or *subunits*, into long chains, or **polymers** (Figure 2–28 and **How We Know**, pp. 60–61). Yet they have many unexpected properties that could not have been predicted from their simple constituents. For example, it took a long time to determine that the nucleic acids DNA and RNA store and transmit hereditary information (see *How We Know*, pp. 174–176).

Proteins are especially versatile and perform thousands of distinct functions in cells. Many proteins act as enzymes that catalyze the chemical

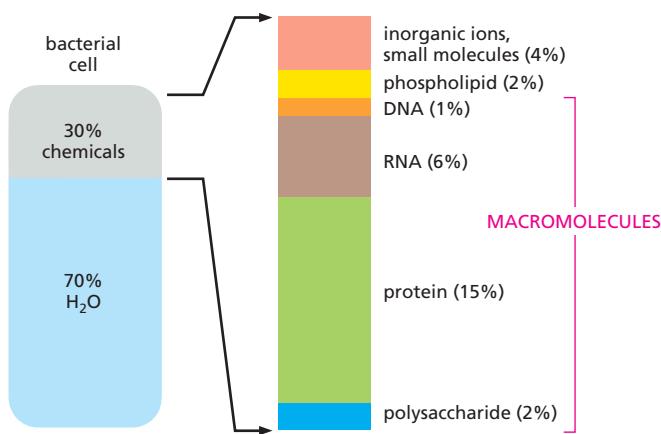


Figure 2–27 Macromolecules are abundant in cells. The approximate composition (by mass) of a bacterial cell is shown. The composition of an animal cell is similar.

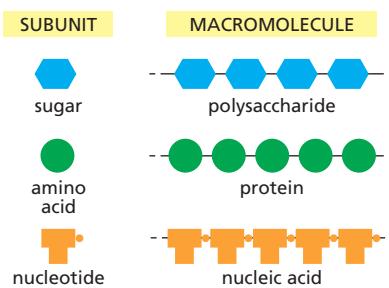


Figure 2–28 Polysaccharides, proteins, and nucleic acids are made from monomeric subunits. Each macromolecule is a polymer formed from small molecules (called monomers or subunits) that are linked together by covalent bonds.

reactions that take place in cells. For example, an enzyme in plants, called ribulose bisphosphate carboxylase, converts CO₂ to sugars, thereby creating most of the organic matter used by the rest of the living world. Other proteins are used to build structural components: tubulin, for example, self-assembles to make the cell's long, stiff microtubules (see Figure 1–27B), and histone proteins assemble into spool-like structures that help wrap up the cell's DNA in chromosomes. Yet other proteins, such as myosin, act as molecular motors to produce force and movement. We examine the molecular basis for many of these wide-ranging functions in later chapters. Here, we consider some of the general principles of macromolecular chemistry that make all of these activities possible.

Each Macromolecule Contains a Specific Sequence of Subunits

Although the chemical reactions for adding subunits to each polymer are different in detail for proteins, nucleic acids, and polysaccharides, they share important features. Each polymer grows by the addition of a monomer onto one end of the polymer chain via a condensation reaction, in which a molecule of water is lost with each subunit added (Figure 2–29). In all cases, the reactions are catalyzed by specific enzymes, which ensure that only the appropriate monomer is incorporated.

The stepwise polymerization of monomers into a long chain is a simple way to manufacture a large, complex molecule, because the subunits are added by the same reaction performed over and over again by the same set of enzymes. In a sense, the process resembles the repetitive operation of a machine in a factory—with some important differences. First, apart from some of the polysaccharides, most macromolecules are made from a set of monomers that are slightly different from one another; for example, proteins are constructed from 20 different amino acids (see Panel 2–5, pp. 74–75). Second, and most important, the polymer chain is not assembled at random from these subunits; instead the subunits are added in a particular order, or **sequence**.

The biological functions of proteins, nucleic acids, and many polysaccharides are absolutely dependent on the particular sequence of subunits in the linear chains. By varying the sequence of subunits, the cell can make an enormous diversity of the polymeric molecules. Thus, for a protein chain 200 amino acids long, there are 20²⁰⁰ possible combinations (20 × 20 × 20 × 20... multiplied 200 times), while for a DNA molecule

QUESTION 2–7

What is meant by “polarity” of a polypeptide chain and by “polarity” of a chemical bond? How do the meanings differ?

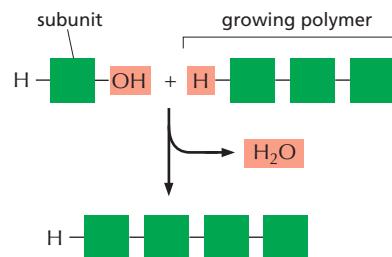


Figure 2–29 Macromolecules are formed by adding subunits to one end. In a condensation reaction, a molecule of water is lost with the addition of each monomer to one end of the growing chain. The reverse reaction—the breakdown of the polymer—occurs by the addition of water (hydrolysis). See also Figure 2–18.

WHAT ARE MACROMOLECULES?

The idea that proteins, polysaccharides, and nucleic acids are large molecules that are constructed from smaller subunits, linked one after another into long molecular chains, may seem fairly obvious today. But this was not always the case. In the early part of the twentieth century, few scientists believed in the existence of such biological polymers built from repeating units held together by covalent bonds. The notion that such “frighteningly large” macromolecules could be assembled from simple building blocks was considered “downright shocking” by chemists of the day. Instead, they thought that proteins and other seemingly large organic molecules were simply heterogeneous aggregates of small organic molecules held together by weak “association forces” (**Figure 2–30**).

The first hint that proteins and other organic polymers are large molecules came from observing their behavior in solution. At the time, scientists were working with various proteins and carbohydrates derived from food-stuffs and other organic materials—albumin from egg whites, casein from milk, collagen from gelatin, and cellulose from wood. Their chemical compositions seemed simple enough: like other organic molecules, they contained carbon, hydrogen, oxygen, and, in the case of proteins, nitrogen. But they behaved oddly in solution, showing, for example, an inability to pass through a fine filter.

Why these molecules misbehaved in solution was a puzzle. Were they really giant molecules, composed of an unusual number of covalently linked atoms? Or were they more like a colloidal suspension of particles—a big, sticky hodgepodge of small organic molecules that associate only loosely?

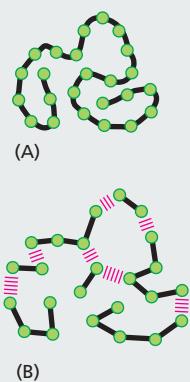


Figure 2–30 What might an organic macromolecule look like? Chemists in the early part of the twentieth century debated whether proteins, polysaccharides, and other apparently large organic molecules were (A) discrete particles made of an unusually large number of covalently linked atoms or (B) a loose aggregation of heterogeneous small organic molecules held together by weak forces.

One way to distinguish between the two possibilities was to determine the actual size of one of these molecules. If a protein such as albumin were made of molecules all identical in size, that would support the existence of true macromolecules. Conversely, if albumin were instead a miscellaneous conglomeration of small organic molecules, these should show a whole range of molecular sizes in solution.

Unfortunately, the techniques available to scientists in the early 1900s were not ideal for measuring the sizes of such large molecules. Some chemists estimated a protein’s size by determining how much it would lower a solution’s freezing point; others measured the osmotic pressure of protein solutions. These methods were susceptible to experimental error and gave variable results. Different techniques, for example, suggested that cellulose was anywhere from 6000 to 103,000 daltons in mass (where 1 dalton is approximately equal to the mass of a hydrogen atom). Such results helped to fuel the hypothesis that carbohydrates and proteins were loose aggregates of small molecules rather than true macromolecules.

Many scientists simply had trouble believing that molecules heavier than about 4000 daltons—the largest compound that had been synthesized by organic chemists—could exist at all. Take hemoglobin, the oxygen-carrying protein in red blood cells. Researchers tried to estimate its size by breaking it down into its chemical components. In addition to carbon, hydrogen, nitrogen, and oxygen, hemoglobin contains a small amount of iron. Working out the percentages, it appeared that hemoglobin had one atom of iron for every 712 atoms of carbon—and a minimum weight of 16,700 daltons. Could a molecule with hundreds of carbon atoms in one long chain remain intact in a cell and perform specific functions? Emil Fischer, the organic chemist who determined that the amino acids in proteins are linked by peptide bonds, thought that a polypeptide chain could grow no longer than about 30 or 40 amino acids. As for hemoglobin, with its purported 700 carbon atoms, the existence of molecular chains of such “truly fantastic lengths” was deemed “very improbable” by leading chemists.

Definitive resolution of the debate had to await the development of new techniques. Convincing evidence that proteins are macromolecules came from studies using the ultracentrifuge—a device that uses centrifugal force to separate molecules according to their size (see Panel 4–3, pp. 164–165). Theodor Svedberg, who designed the machine in 1925, performed the first studies. If a protein were really an aggregate of smaller molecules, he

reasoned, it would appear as a smear of molecules of different sizes when sedimented in an ultracentrifuge. Using hemoglobin as his test protein, Svedberg found that the centrifuged sample revealed a single, sharp band with a molecular weight of 68,000 daltons. His results strongly supported the theory that proteins are true macromolecules (Figure 2–31).

Additional evidence continued to accumulate throughout the 1930s, as other researchers began to prepare crystals of pure protein that could be studied by X-ray diffraction. Only molecules with a uniform size and shape can form highly ordered crystals and diffract X-rays in such a way that their three-dimensional structure can be determined, as we discuss in Chapter 4. A heterogeneous suspension could not be studied in this way.

We now take it for granted that large macromolecules carry out many of the most important activities in living cells. But chemists once viewed the existence of such polymers with the same sort of skepticism that a zoologist might show on being told that “In Africa, there are elephants that are 100 meters long and 20 meters tall.” It took decades for researchers to master the techniques required to convince everyone that molecules ten times larger than anything they had ever encountered were a cornerstone of biology. As we shall see throughout this book, such a labored pathway to discovery is not unusual, and progress in science is often driven by advances in technology.

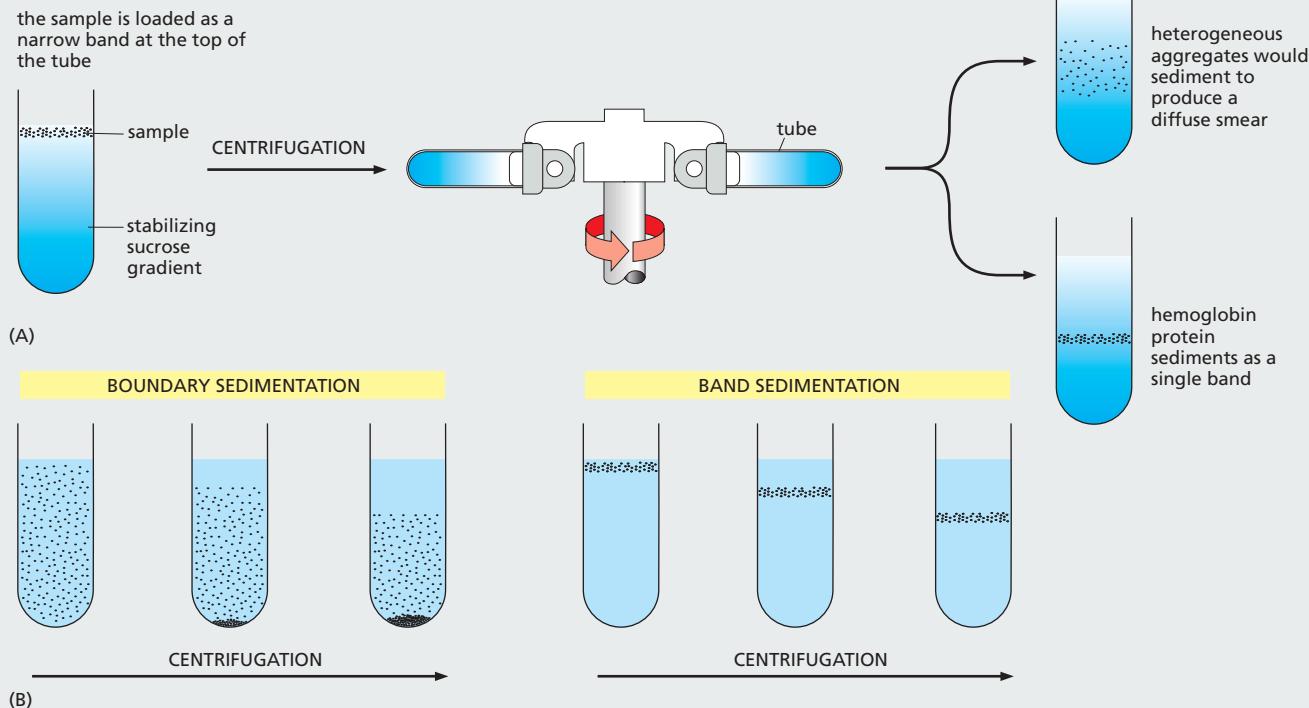


Figure 2–31 The ultracentrifuge helped to settle the debate about the nature of macromolecules. In the ultracentrifuge, centrifugal forces exceeding 500,000 times the force of gravity can be used to separate proteins or other large molecules. (A) In a modern ultracentrifuge, samples are loaded in a thin layer on top of a gradient of sucrose solution formed in a tube. The tube is placed in a metal rotor that is rotated at high speed. Molecules of different sizes sediment at different rates, and these molecules will therefore move as distinct bands in the sample tube. If hemoglobin were a loose aggregate of heterogeneous peptides, it would show a broad smear of sizes after centrifugation (top tube). Instead, it appears as a sharp band with a molecular weight of 68,000 daltons (bottom tube). Although the ultracentrifuge is now a standard, almost mundane, fixture in most biochemistry laboratories, its construction was a huge technological challenge. The centrifuge rotor must be capable of spinning centrifuge tubes at high speeds for many hours at constant temperature and with high stability; otherwise convection occurs in the sedimenting solution and ruins the experiment. In 1926, Svedberg won the Nobel Prize in Chemistry for his ultracentrifuge design and its application to chemistry. (B) In his actual experiment, Svedberg filled a special tube in the centrifuge with a homogeneous solution of hemoglobin; by shining light through the tube, he then carefully monitored the moving boundary between the sedimenting protein molecules and the clear aqueous solution left behind (so-called boundary sedimentation). The more recently developed method shown in (A) is a form of band sedimentation.

10,000 nucleotides long (small by DNA standards), with its four different nucleotides, there are $4^{10,000}$ different possibilities—an unimaginably large number. Thus the machinery of polymerization must be subject to a sensitive control that allows it to specify exactly which subunit should be added next to the growing polymer end. We discuss the mechanisms that specify the sequence of subunits in DNA, RNA, and protein molecules in Chapters 6 and 7.

Noncovalent Bonds Specify the Precise Shape of a Macromolecule

Most of the single covalent bonds that link together the subunits in a macromolecule allow rotation of the atoms they join; thus the polymer chain has great flexibility. In principle, this allows a single-chain macromolecule to adopt an almost unlimited number of shapes, or **conformations**, as the polymer chain writhes and rotates under the influence of random thermal energy. However, the shapes of most biological macromolecules are highly constrained because of weaker, noncovalent bonds that form between different parts of the molecule. In many cases, these weaker interactions ensure that the polymer chain preferentially adopts one particular conformation, determined by the linear sequence of monomers in the chain. Most protein molecules and many of the RNA molecules found in cells fold tightly into one highly preferred conformation in this way (**Figure 2–32**). These unique conformations—shaped by evolution—determine the chemistry and activity of these macromolecules and dictate their interactions with other biological molecules.

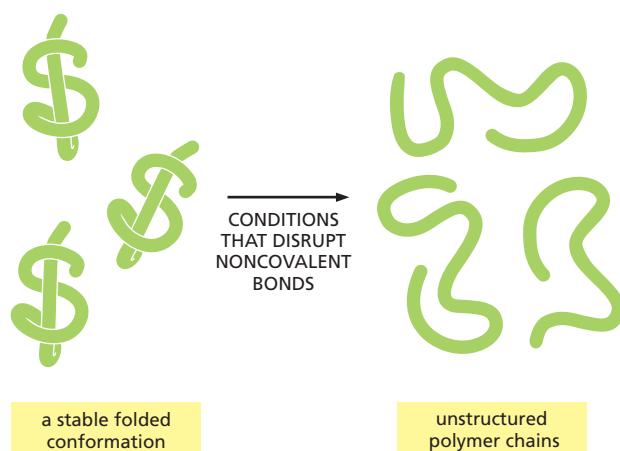
The noncovalent bonds important for the structure and function of macromolecules include two types described earlier: *electrostatic attractions* and *hydrogen bonds* (see Panel 2–7, pp. 78–79). Electrostatic attractions, although strong on their own, are quite weak in water because the charged or partially charged (polar) groups involved in the attraction are shielded by their interactions with water molecules and various inorganic ions present in the aqueous solution. Electrostatic attractions, however, are very important in biological systems. An enzyme that binds a positively charged substrate will often use a negatively charged amino acid side chain to guide its substrate into the proper position.

Earlier, we described the importance of hydrogen bonds in determining the unique properties of water. They are also very important in the folding of a polypeptide chain and in holding together the two strands of a double-stranded DNA molecule.

QUESTION 2–8

In principle, there are many different, chemically diverse ways in which small molecules can be linked to form polymers. For example, the small molecule ethene ($\text{CH}_2=\text{CH}_2$) is used commercially to make the plastic polyethylene (...– $\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$...). The individual subunits of the three major classes of biological macromolecules, however, are all linked by similar reaction mechanisms, i.e., by condensation reactions that eliminate water. Can you think of any benefits that this chemistry offers and why it might have been selected in evolution?

Figure 2–32 Most proteins and many RNA molecules fold into a particularly stable three-dimensional shape, or **conformation**. This shape is directed mostly by a multitude of weak, noncovalent intramolecular bonds. If the folded macromolecules are subjected to conditions that disrupt noncovalent bonds, the molecule becomes a flexible chain that loses both its conformation and its biological activity.



A third type of noncovalent interaction results from **van der Waals attractions**, which are a form of electrical attraction caused by fluctuating electric charges that arise whenever two atoms come within a very short distance of each other. Although van der Waals attractions are weaker than hydrogen bonds, in large numbers they play an important role in the attraction between macromolecules with complementary shapes. All of these noncovalent bonds are reviewed in Panel 2–7, pp. 78–79.

Another important noncovalent interaction is created by the three-dimensional structure of water, which forces together the hydrophobic portions of dissolved molecules in order to minimize their disruptive effect on the hydrogen-bonded network of water molecules (see Panel 2–7 and Panel 2–2, pp. 68–69). This expulsion from the aqueous solution generates what is sometimes thought of as a fourth kind of noncovalent bond, called a **hydrophobic interaction**. Such interactions hold together phospholipid molecules in cell membranes, for example, and they also play a crucial part in the folding of protein molecules into a compact globular shape.

Noncovalent Bonds Allow a Macromolecule to Bind Other Selected Molecules

As we discussed earlier, although noncovalent bonds are individually weak, they can add up to create a strong attraction between two molecules when these molecules fit together very closely, like a hand in a glove, so that many noncovalent bonds can occur between them (see Panel 2–7). This form of molecular interaction provides for great specificity in the binding of a macromolecule to other small and large molecules, because the multipoint contacts required for strong binding make it possible for a macromolecule to select just one of the many thousands of different molecules present inside a cell. Moreover, because the strength of the binding depends on the number of noncovalent bonds that are formed, associations of almost any strength are possible.

Binding of this type makes it possible for proteins to function as enzymes. It can also stabilize associations between any macromolecules, as long as their surfaces match closely (Figure 2–33 and Movie 2.4). Noncovalent bonds thereby allow macromolecules to be used as building blocks for the formation of much larger structures. For example, proteins often bind

QUESTION 2–9

Why could covalent bonds not be used in place of noncovalent bonds to mediate most of the interactions of macromolecules?

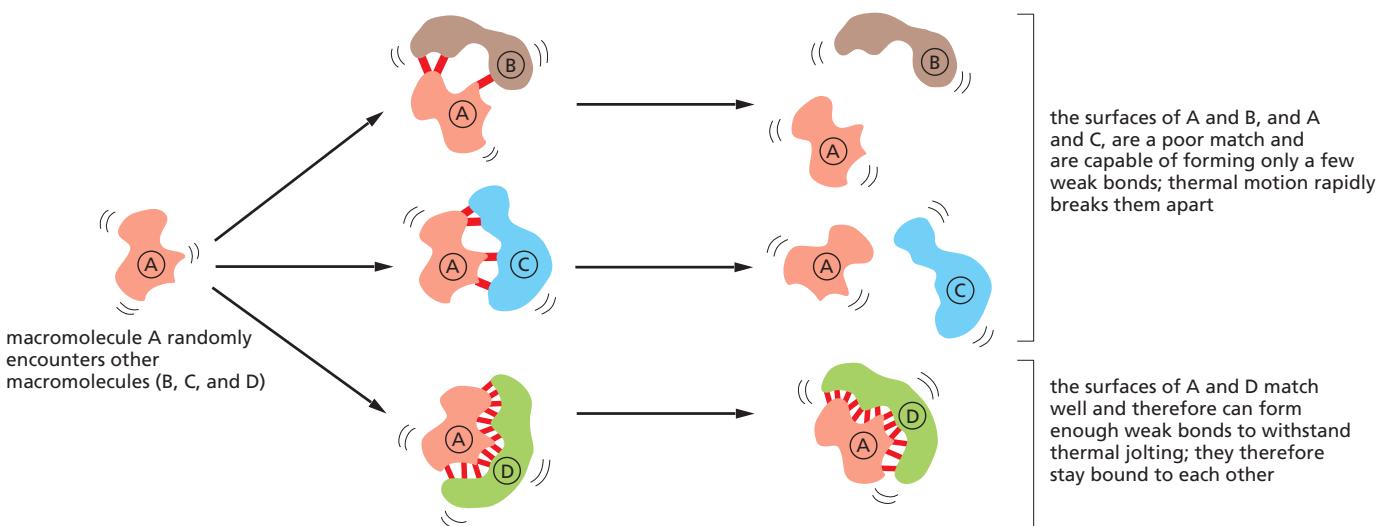


Figure 2–33 Noncovalent bonds mediate interactions between macromolecules. They can also mediate interactions between a macromolecule and small molecules (not shown).

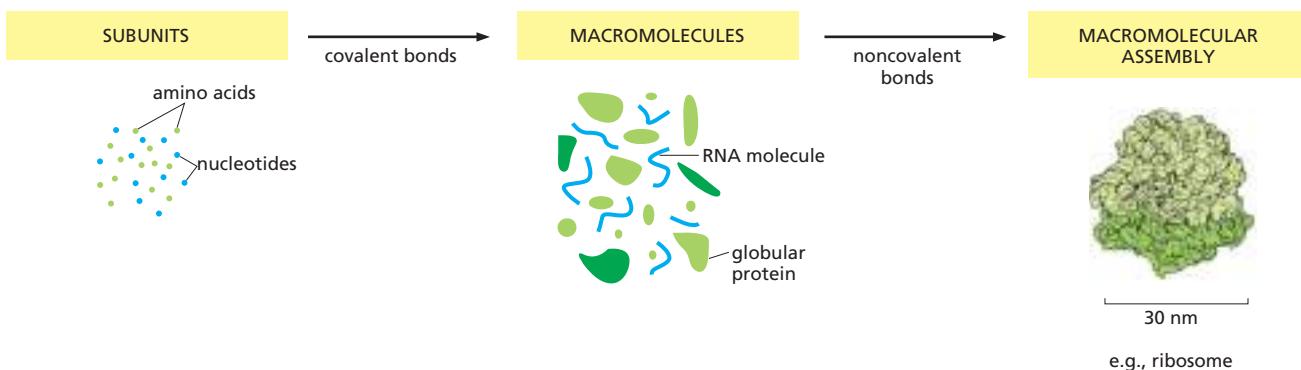


Figure 2–34 Both covalent bonds and noncovalent bonds are needed to form a macromolecular assembly such as a ribosome. Covalent bonds allow small organic molecules to join together to form macromolecules, which can assemble into large macromolecular complexes via noncovalent bonds. Ribosomes are large macromolecular machines that synthesize proteins inside cells. Each ribosome is composed of about 90 macromolecules (proteins and RNA molecules), and it is large enough to see in the electron microscope (see Figure 7–31). The subunits, macromolecules, and the ribosome here are shown roughly to scale.

together into multiprotein complexes that function as intricate machines with multiple moving parts, carrying out such complex tasks as DNA replication and protein synthesis (**Figure 2–34**). In fact, noncovalent bonds account for a great deal of the complex chemistry that makes life possible.

ESSENTIAL CONCEPTS

- Living cells obey the same chemical and physical laws as nonliving things. Like all other forms of matter, they are made of atoms, which are the smallest unit of a chemical element that retain the distinctive chemical properties of that element.
- Cells are made up of a limited number of elements, four of which—C, H, N, O—make up about 96% of a cell's mass.
- Each atom has a positively charged nucleus, which is surrounded by a cloud of negatively charged electrons. The chemical properties of an atom are determined by the number and arrangement of its electrons: it is most stable when its outer electron shell is completely filled.
- A covalent bond forms when a pair of outer-shell electrons is shared between two adjacent atoms; if two pairs of electrons are shared, a double bond is formed. Clusters of two or more atoms held together by covalent bonds are known as molecules.
- When an electron jumps from one atom to another, two ions of opposite charge are generated; these ions are held together by mutual attraction forming a noncovalent ionic bond.
- Living organisms contain a distinctive and restricted set of small carbon-based (organic) molecules, which are essentially the same for every living species. The main categories are sugars, fatty acids, amino acids, and nucleotides.
- Sugars are a primary source of chemical energy for cells and can also be joined together to form polysaccharides or shorter oligosaccharides.
- Fatty acids are an even richer energy source than sugars, but their most essential function is to form lipid molecules that assemble into cell membranes.
- The vast majority of the dry mass of a cell consists of macromolecules—mainly polysaccharides, proteins, and nucleic acids (DNA

and RNA); these macromolecules are formed as polymers of sugars, amino acids, or nucleotides, respectively.

- The most diverse and versatile class of macromolecules are proteins, which are formed from 20 types of amino acids that are covalently linked by peptide bonds into long polypeptide chains.
- Nucleotides play a central part in energy-transfer reactions within cells; they are also joined together to form information-containing RNA and DNA molecules, each of which is composed of only four types of nucleotides.
- Protein, RNA, and DNA molecules are synthesized from subunits by repetitive condensation reactions, and it is the specific sequence of subunits that determines their unique functions.
- Four types of weak noncovalent bonds—hydrogen bonds, electrostatic attractions, van der Waals attractions, and hydrophobic interactions—enable macromolecules to bind specifically to other macromolecules or to selected small molecules.
- The same four types of noncovalent bonds between different regions of a polypeptide or RNA chain allow these chains to fold into unique shapes (conformations).

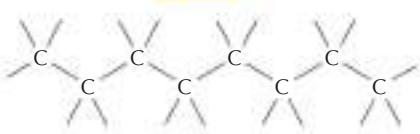
KEY TERMS

acid	inorganic molecule
amino acid	ion
atom	ionic bond
atomic weight	lipid
ATP	lipid bilayer
Avogadro's number	macromolecule
base	molecule
buffer	molecular weight
chemical bond	monomer
chemical group	noncovalent bond
condensation reaction	nucleotide
conformation	organic molecule
covalent bond	pH scale
DNA	polar
electron	polymer
electrostatic attraction	protein
fatty acid	proton
hydrogen bond	RNA
hydrolysis	sequence
hydronium ion	subunit
hydrophilic	sugar
hydrophobic	van der Waals attractions
hydrophobic interactions	

CARBON SKELETONS

Carbon has a unique role in the cell because of its ability to form strong covalent bonds with other carbon atoms. Thus carbon atoms can join to form:

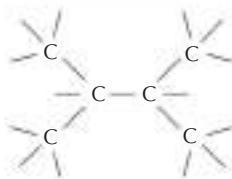
chains



also written as



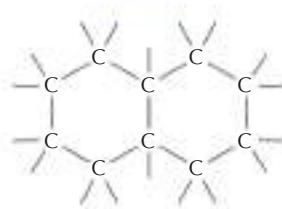
branched trees



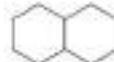
also written as



rings



also written as



COVALENT BONDS

A covalent bond forms when two atoms come very close together and share one or more of their outer-shell electrons. Each atom forms a fixed number of covalent bonds in a defined spatial arrangement.

SINGLE BONDS: two electrons shared per bond

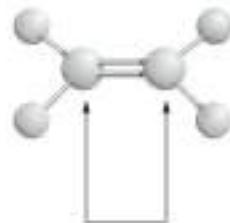


DOUBLE BONDS: four electrons shared per bond



The precise spatial arrangement of covalent bonds influences the three-dimensional structure and chemistry of molecules.

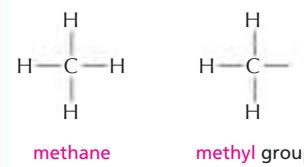
In this review panel, we see how covalent bonds are used in a variety of biological molecules.



Atoms joined by two or more covalent bonds cannot rotate freely around the bond axis. This restriction has a major influence on the three-dimensional shape of many macromolecules.

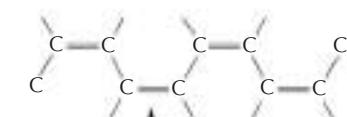
C–H COMPOUNDS

Carbon and hydrogen together make stable compounds (or groups) called **hydrocarbons**. These are nonpolar, do not form hydrogen bonds, and are generally insoluble in water.

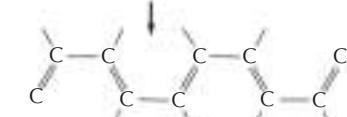


ALTERNATING DOUBLE BONDS

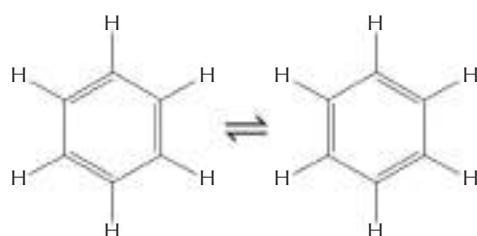
A carbon chain can include double bonds. If these are on alternate carbon atoms, the bonding electrons move within the molecule, stabilizing the structure by a phenomenon called **resonance**.



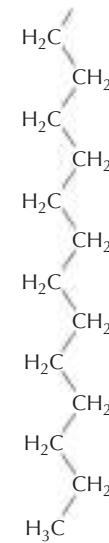
the truth is somewhere between these two structures



Alternating double bonds in a ring can generate a very stable structure.



often written as

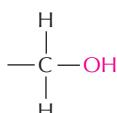


part of the hydrocarbon "tail" of a fatty acid molecule

C-O COMPOUNDS

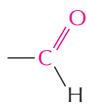
Many biological compounds contain a carbon covalently bonded to an oxygen. For example,

alcohol



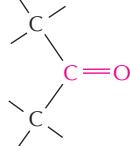
The $-OH$ is called a **hydroxyl** group.

aldehyde



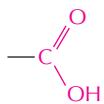
The $C=O$ is called a **carbonyl** group.

ketone



The $C=O$ is called a **carbonyl** group.

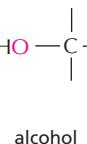
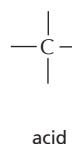
carboxylic acid



The $-COOH$ is called a **carboxyl** group. In water, this loses an H^+ ion to become $-COO^-$.

esters

Esters are formed by combining an acid and an alcohol.



acid

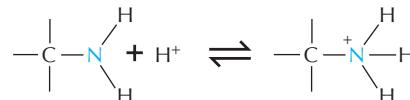
alcohol

ester

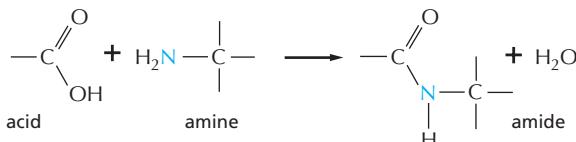
C-N COMPOUNDS

Amines and amides are two important examples of compounds containing a carbon linked to a nitrogen.

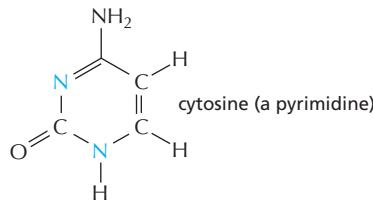
Amines in water combine with an H^+ ion to become positively charged.



Amides are formed by combining an acid and an amine. Unlike amines, amides are uncharged in water. An example is the peptide bond that joins amino acids in a protein.



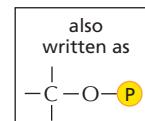
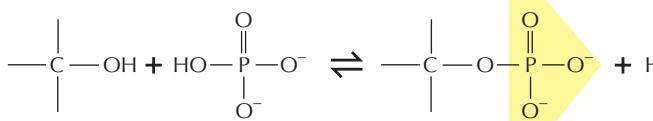
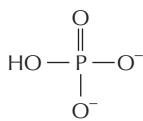
Nitrogen also occurs in several ring compounds, including important constituents of nucleic acids: purines and pyrimidines.



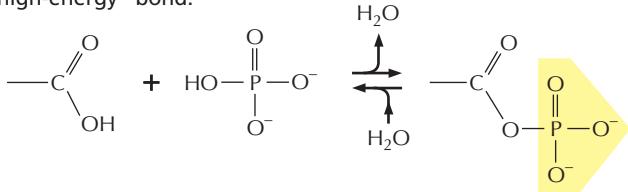
PHOSPHATES

Inorganic phosphate is a stable ion formed from phosphoric acid, H_3PO_4 . It is also written as P_i .

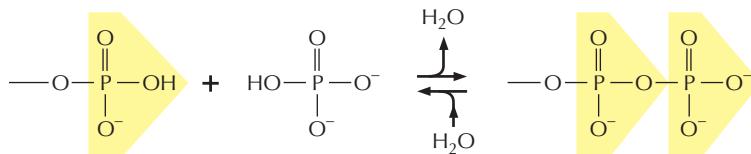
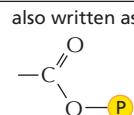
Phosphate esters can form between a phosphate and a free hydroxyl group. **Phosphate groups** are often covalently attached to proteins in this way.



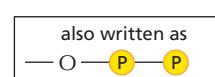
The combination of a phosphate and a carboxyl group, or two or more phosphate groups, gives an acid anhydride. Because compounds of this type release a large amount of energy when hydrolyzed in the cell, they are often said to contain a "high-energy" bond.



high-energy acyl phosphate bond (carboxylic-phosphoric acid anhydride) found in some metabolites



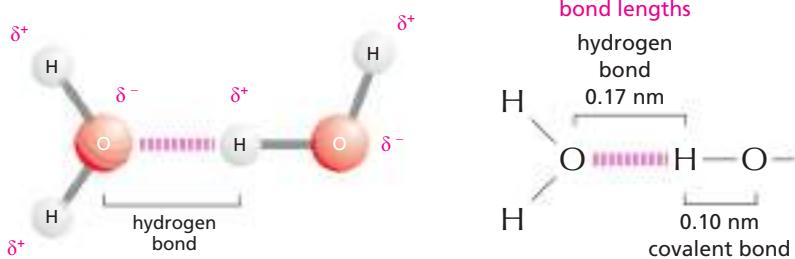
high-energy phosphoanhydride bond found in molecules such as ATP



HYDROGEN BONDS

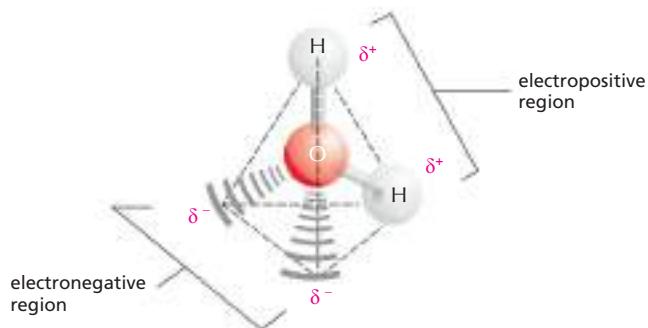
Because they are polarized, two adjacent H₂O molecules can form a noncovalent linkage known as a **hydrogen bond**. Hydrogen bonds have only about 1/20 the strength of a covalent bond.

Hydrogen bonds are strongest when the three atoms lie in a straight line.



WATER

Two atoms connected by a covalent bond may exert different attractions for the electrons of the bond. In such cases, the bond is **polar**, with one end slightly negatively charged (δ^-) and the other slightly positively charged (δ^+).



Although a water molecule has an overall neutral charge (having the same number of electrons and protons), the electrons are asymmetrically distributed, making the molecule polar. The oxygen nucleus draws electrons away from the hydrogen nuclei, leaving the hydrogen nuclei with a small net positive charge. The excess of electron density on the oxygen atom creates weakly negative regions at the other two corners of an imaginary tetrahedron. [On these pages](#), we review the chemical properties of water and see how water influences the behavior of biological molecules.

WATER STRUCTURE

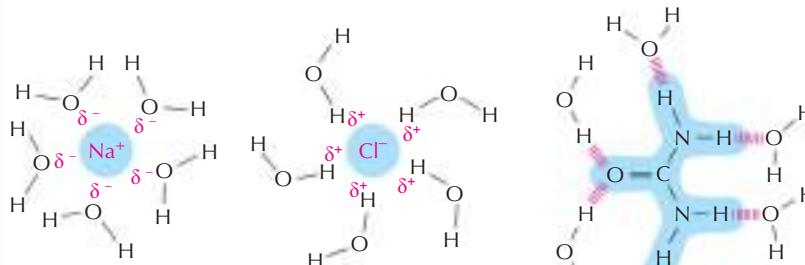
Molecules of water join together transiently in a hydrogen-bonded lattice.



The cohesive nature of water is responsible for many of its unusual properties, such as high surface tension, high specific heat, and high heat of vaporization.

HYDROPHILIC MOLECULES

Substances that dissolve readily in water are termed **hydrophilic**. They include ions and polar molecules that attract water molecules through electrical charge effects. Water molecules surround each ion or polar molecule and carry it into solution.

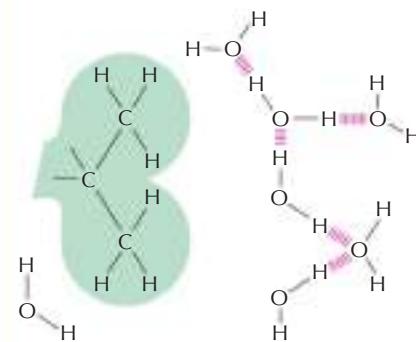


Ionic substances such as sodium chloride dissolve because water molecules are attracted to the positive (Na⁺) or negative (Cl⁻) charge of each ion.

Polar substances such as urea dissolve because their molecules form hydrogen bonds with the surrounding water molecules.

HYDROPHOBIC MOLECULES

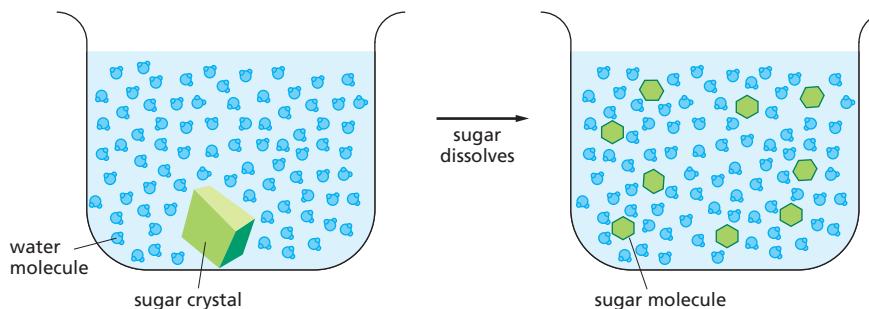
Substances that contain a preponderance of nonpolar bonds are usually insoluble in water and are termed **hydrophobic**. Water molecules are not attracted to such hydrophobic molecules and so have little tendency to surround them and bring them into solution.



Hydrocarbons, which contain many C-H bonds, are especially hydrophobic.

WATER AS A SOLVENT

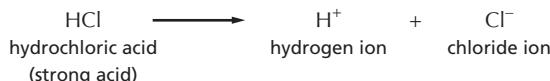
Many substances, such as household sugar (sucrose), **dissolve** in water. That is, their molecules separate from each other, each becoming surrounded by water molecules.



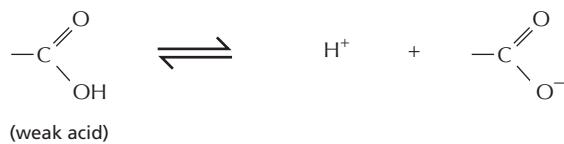
When a substance dissolves in a liquid, the mixture is termed a **solution**. The dissolved substance (in this case sugar) is the **solute**, and the liquid that does the dissolving (in this case water) is the **solvent**. Water is an excellent solvent for hydrophilic substances because of its polar bonds.

ACIDS

Substances that release hydrogen ions (protons) into solution are called **acids**.



Many of the acids important in the cell are not completely dissociated, and they are therefore **weak acids**—for example, the carboxyl group ($-\text{COOH}$), which dissociates to give a hydrogen ion in solution.



Note that this is a reversible reaction.

pH

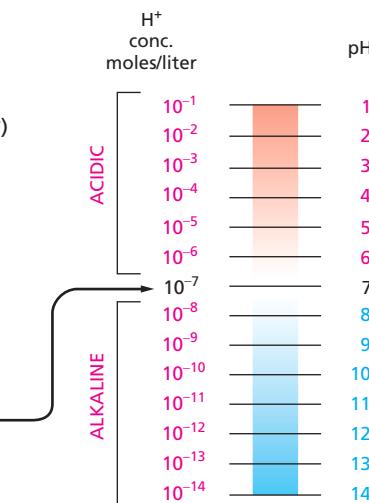
The acidity of a solution is defined by the concentration of hydronium ions (H_3O^+) it possesses, generally abbreviated as H^+ . For convenience, we use the pH scale, where

$$\text{pH} = -\log_{10}[\text{H}^+]$$

For pure water

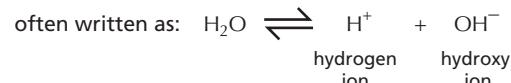
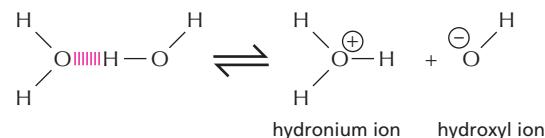
$$[\text{H}^+] = 10^{-7} \text{ moles/liter}$$

$$\text{pH} = 7.0$$



HYDROGEN ION EXCHANGE

Positively charged hydrogen ions (H^+) can spontaneously move from one water molecule to another, thereby creating two ionic species.



Because the process is rapidly reversible, hydrogen ions are continually shuttling between water molecules. Pure water contains equal concentrations of hydronium ions and hydroxyl ions (both 10^{-7} M).

BASES

Substances that reduce the number of hydrogen ions in solution are called **bases**. Some bases, such as ammonia, combine directly with hydrogen ions.



Other bases, such as sodium hydroxide, reduce the number of H^+ ions indirectly, by making OH^- ions that then combine directly with H^+ ions to make H_2O .



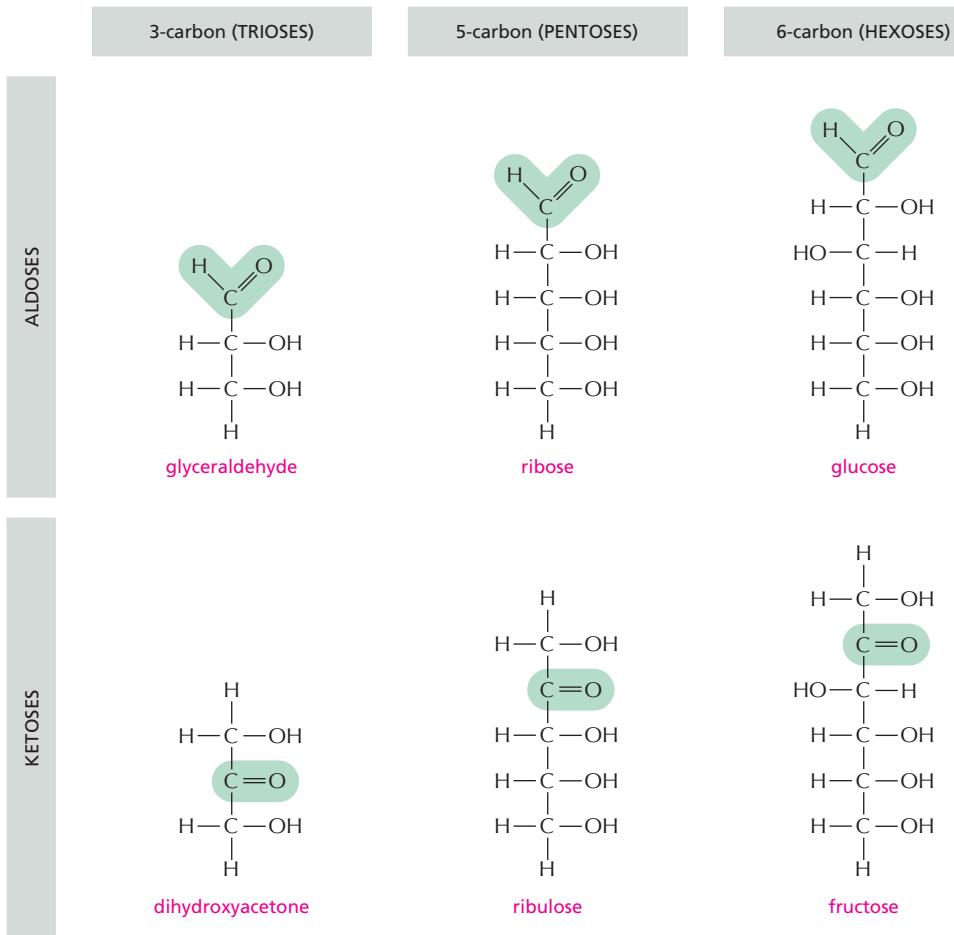
Many bases found in cells are partially associated with H^+ ions and are termed **weak bases**. This is true of compounds that contain an amino group ($-\text{NH}_2$), which has a weak tendency to reversibly accept an H^+ ion from water, thereby increasing the concentration of free OH^- ions.



PANEL 2–3 AN OUTLINE OF SOME OF THE TYPES OF SUGARS

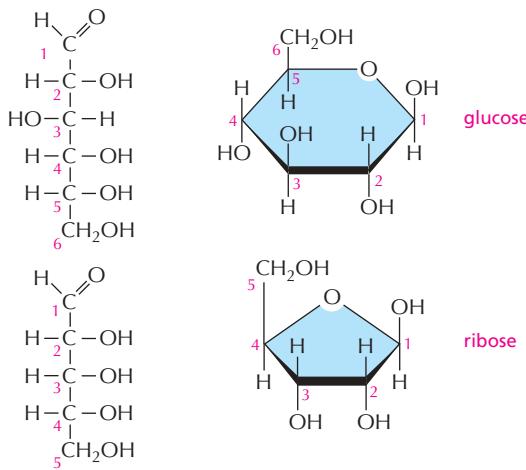
MONOSACCHARIDES

Monosaccharides usually have the general formula $(\text{CH}_2\text{O})_n$, where n can be 3, 4, 5, or 6, and have two or more hydroxyl groups. They either contain an aldehyde group ($-\text{C}=\text{O}$) and are called aldoses, or a ketone group ($\text{C}=\text{O}$) and are called ketoses.



RING FORMATION

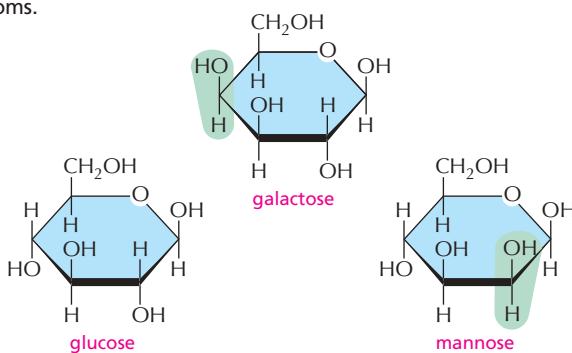
In aqueous solution, the aldehyde or ketone group of a sugar molecule tends to react with a hydroxyl group of the same molecule, thereby closing the molecule into a ring.



Note that each carbon atom has a number.

ISOMERS

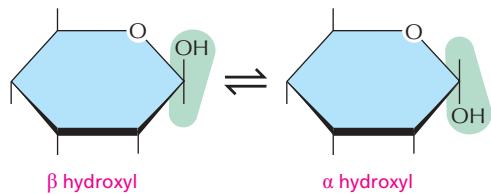
Many monosaccharides differ only in the spatial arrangement of atoms—that is, they are **isomers**. For example, glucose, galactose, and mannose have the same formula ($\text{C}_6\text{H}_{12}\text{O}_6$) but differ in the arrangement of groups around one or two carbon atoms.



These small differences make only minor changes in the chemical properties of the sugars. But the differences are recognized by enzymes and other proteins and therefore can have major biological effects.

α AND β LINKS

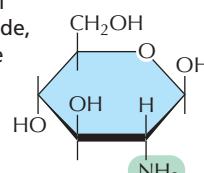
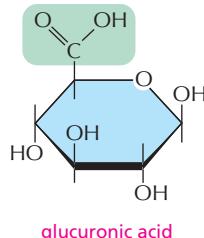
The hydroxyl group on the carbon that carries the aldehyde or ketone can rapidly change from one position to the other. These two positions are called α and β .



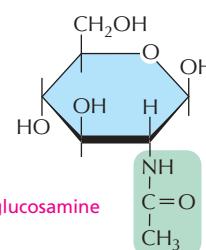
As soon as one sugar is linked to another, the α or β form is frozen.

SUGAR DERIVATIVES

The hydroxyl groups of a simple monosaccharide, such as glucose, can be replaced by other groups.



glucosamine

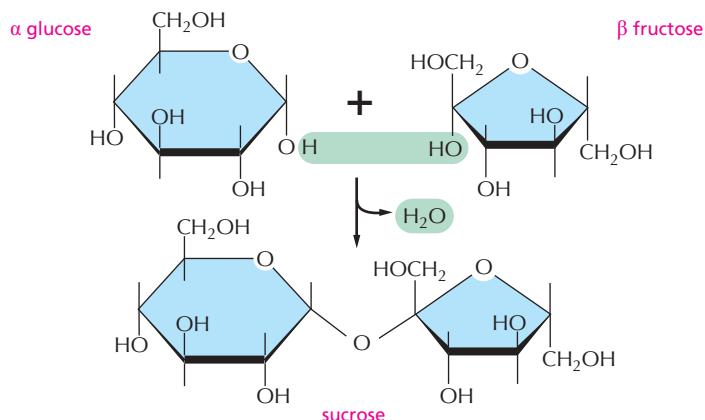


DISACCHARIDES

The carbon that carries the aldehyde or the ketone can react with any hydroxyl group on a second sugar molecule to form a **disaccharide**. Three common disaccharides are

- maltose (glucose + glucose)
- lactose (galactose + glucose)
- sucrose (glucose + fructose)

The reaction forming sucrose is shown here.

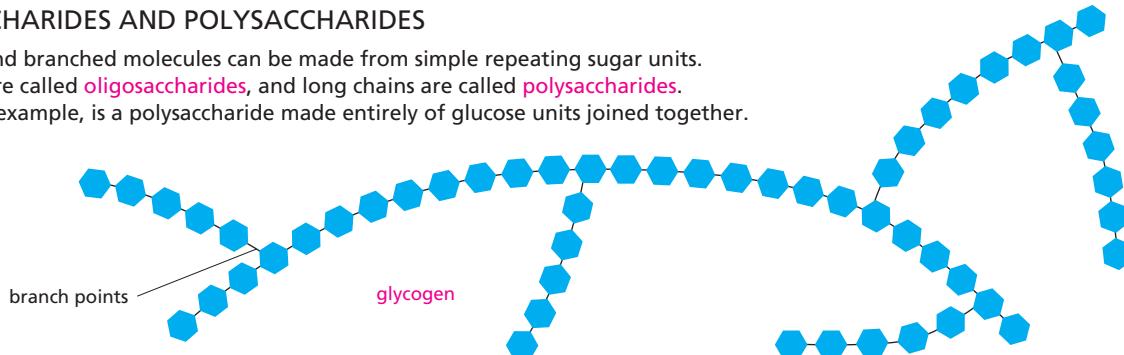


OLIGOSACCHARIDES AND POLYSACCHARIDES

Large linear and branched molecules can be made from simple repeating sugar units.

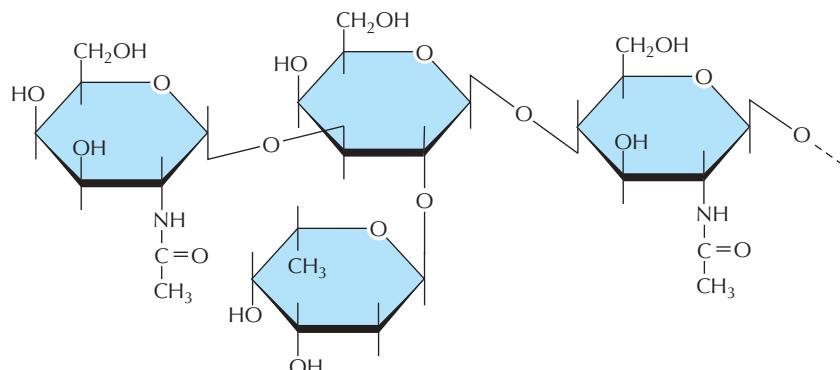
Short chains are called **oligosaccharides**, and long chains are called **polysaccharides**.

Glycogen, for example, is a polysaccharide made entirely of glucose units joined together.



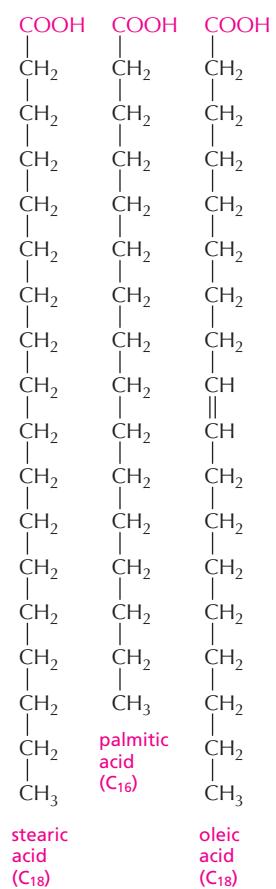
COMPLEX OLIGOSACCHARIDES

In many cases, a sugar sequence is nonrepetitive. Many different molecules are possible. Such complex oligosaccharides are usually linked to proteins or to lipids, as is this oligosaccharide, which is part of a cell-surface molecule that defines a particular blood group.

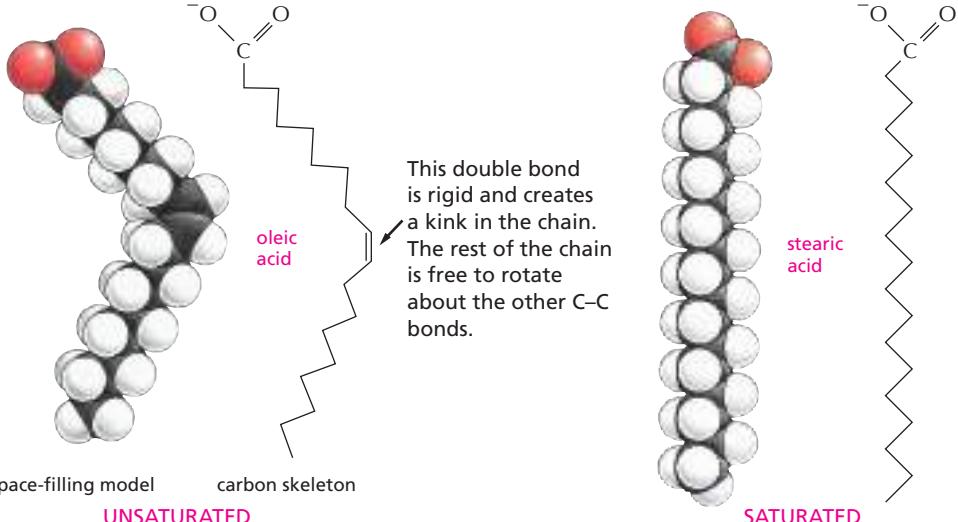


FATTY ACIDS

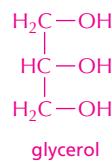
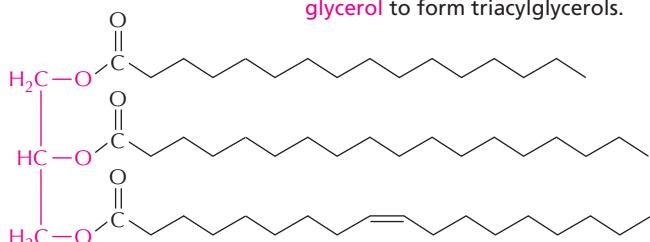
All fatty acids have carboxyl groups at one end and long hydrocarbon tails at the other.



Hundreds of different kinds of fatty acids exist. Some have one or more double bonds in their hydrocarbon tail and are said to be **unsaturated**. Fatty acids with no double bonds are **saturated**.

**TRIACYLGLYCEROLS**

Fatty acids are stored in cells as an energy reserve (fats and oils) through an ester linkage to **glycerol** to form triacylglycerols.

**CARBOXYL GROUP**

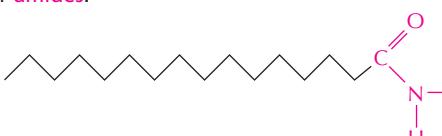
If free, the carboxyl group of a fatty acid will be ionized.



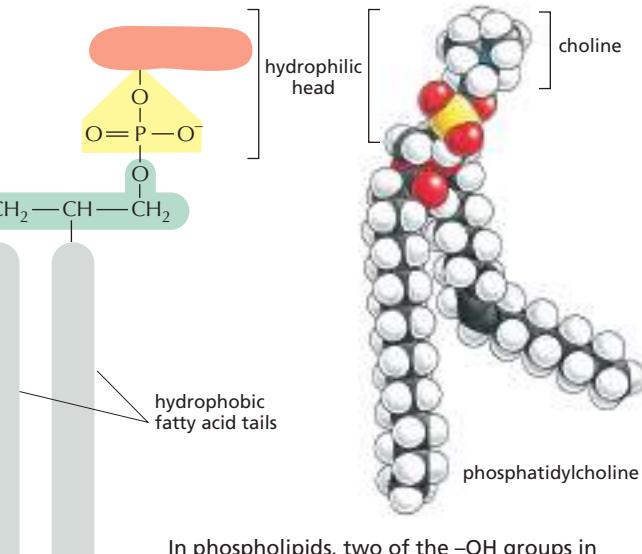
But more often it is linked to other groups to form either **esters**



or **amides**.

**PHOSPHOLIPIDS**

Phospholipids are the major constituents of cell membranes.



general structure of a phospholipid

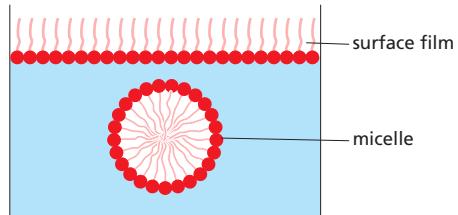
In phospholipids, two of the $-\text{OH}$ groups in glycerol are linked to fatty acids, while the third $-\text{OH}$ group is linked to phosphoric acid. The phosphate is further linked to one of a variety of small polar groups, such as choline.

LIPID AGGREGATES

Fatty acids have a hydrophilic head and a hydrophobic tail.



In water, they can form either a surface film or small, spherical micelles.

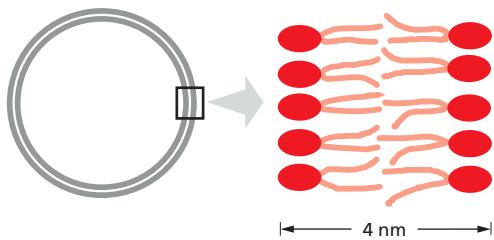


Their derivatives can form larger aggregates held together by hydrophobic forces:

Triacylglycerols form large spherical fat droplets in the cell cytoplasm.

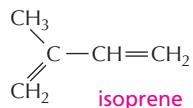


Phospholipids and **glycolipids** form self-sealing *lipid bilayers*, which are the basis for all cell membranes.



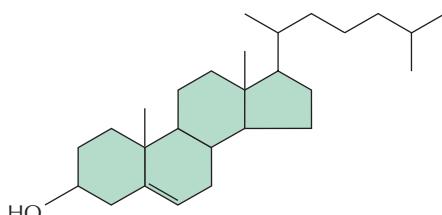
OTHER LIPIDS

Lipids are defined as water-insoluble molecules that are soluble in organic solvents. Two other common types of lipids are steroids and polyisoprenoids. Both are made from isoprene units.

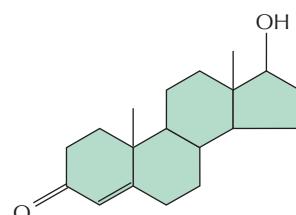


STEROIDS

Steroids have a common multiple-ring structure.



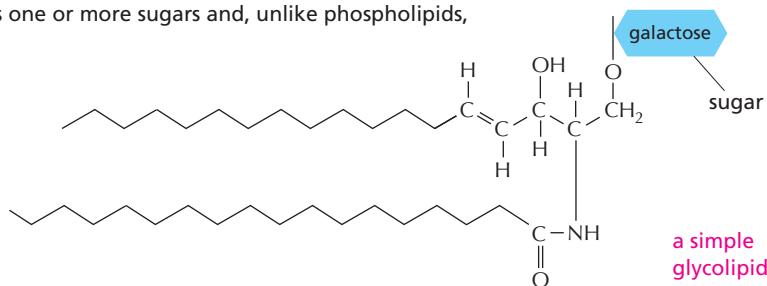
cholesterol—found in many cell membranes



testosterone—male sex hormone

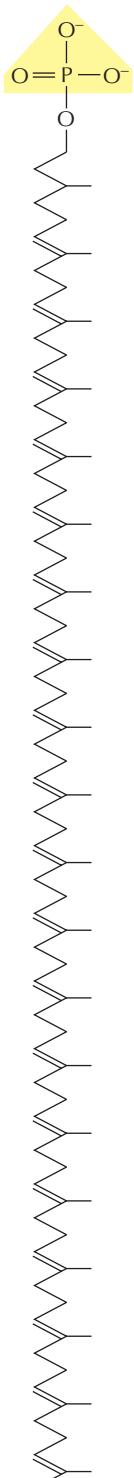
GLYCOLIPIDS

Like phospholipids, these compounds are composed of a hydrophobic region, containing two long hydrocarbon tails, and a polar region, which contains one or more sugars and, unlike phospholipids, no phosphate.



POLYISOPRENOIDS

Long-chain polymers of isoprene



dolichol phosphate—used to carry activated sugars in the membrane-associated synthesis of glycoproteins and some polysaccharides

PANEL 2–5 THE 20 AMINO ACIDS FOUND IN PROTEINS

FAMILIES OF AMINO ACIDS

The common amino acids are grouped according to whether their side chains are

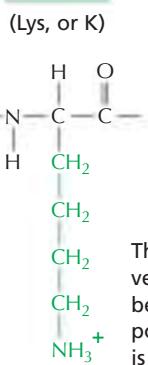
- acidic
- basic
- uncharged polar
- nonpolar

These 20 amino acids are given both three-letter and one-letter abbreviations.

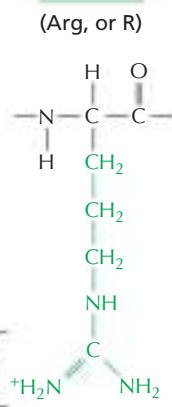
Thus: alanine = Ala = A

BASIC SIDE CHAINS

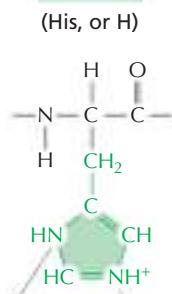
lysine (Lys, or K)



arginine (Arg, or R)



histidine (His, or H)

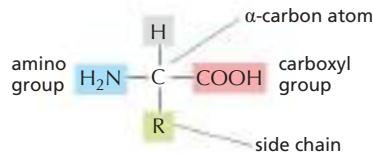


This group is very basic because its positive charge is stabilized by resonance (see Panel 2–1).

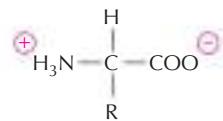
These nitrogens have a relatively weak affinity for an H⁺ and are only partly positive at neutral pH.

THE AMINO ACID

The general formula of an amino acid is

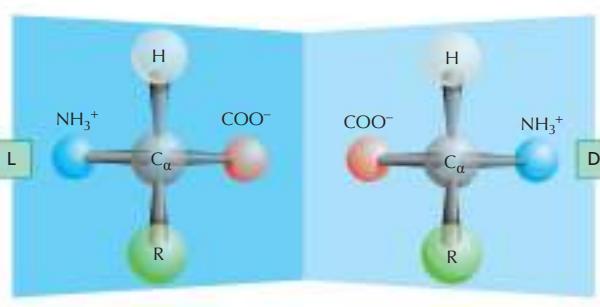


R is commonly one of 20 different side chains. At pH 7, both the amino and carboxyl groups are ionized.



OPTICAL ISOMERS

The α-carbon atom is asymmetric, allowing for two mirror-image (or stereo-) isomers, L and D.



Proteins contain exclusively L-amino acids.

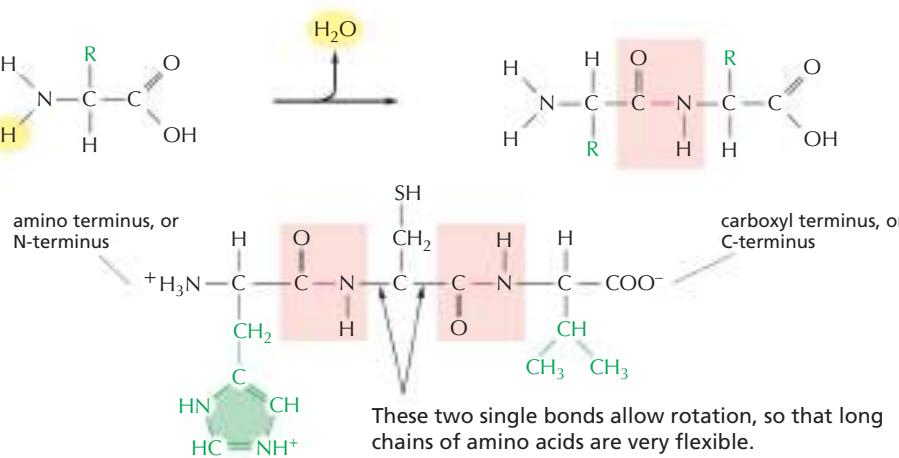
PEPTIDE BONDS

In proteins, amino acids are commonly joined together by an amide linkage, called a peptide bond.



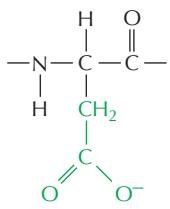
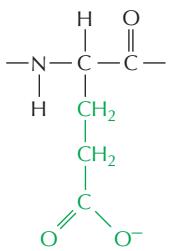
Proteins are long polymers of amino acids linked by peptide bonds, and they are always written with the N-terminus toward the left. **Peptides** are shorter, usually fewer than 50 amino acids long. The sequence of this tripeptide is histidine-cysteine-valine.

The four atoms in each peptide bond (red box) form a rigid planar unit. There is no rotation around the C–N bond.



These two single bonds allow rotation, so that long chains of amino acids are very flexible.

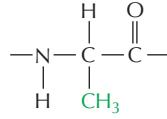
ACIDIC SIDE CHAINS

aspartic acid
(Asp, or D)glutamic acid
(Glu, or E)

NONPOLAR SIDE CHAINS

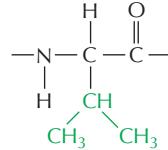
alanine

(Ala, or A)



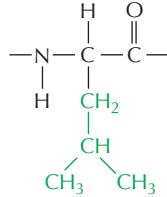
valine

(Val, or V)



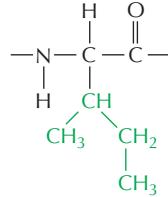
leucine

(Leu, or L)



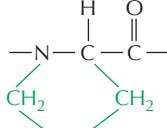
isoleucine

(Ile, or I)



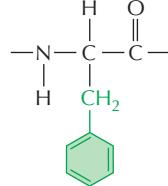
proline

(Pro, or P)



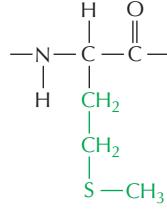
phenylalanine

(Phe, or F)



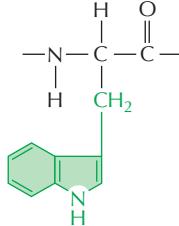
methionine

(Met, or M)



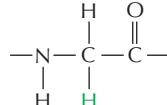
tryptophan

(Trp, or W)



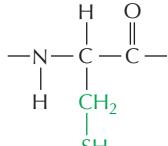
glycine

(Gly, or G)

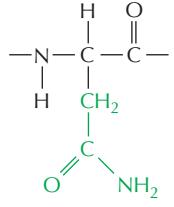
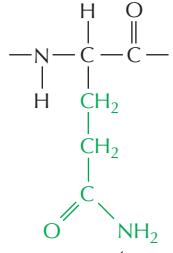


cysteine

(Cys, or C)



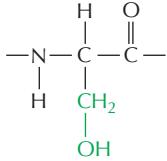
UNCHARGED POLAR SIDE CHAINS

asparagine
(Asn, or N)glutamine
(Gln, or Q)

Although the amide N is not charged at neutral pH, it is polar.

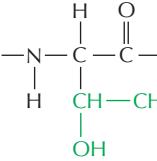
serine

(Ser, or S)



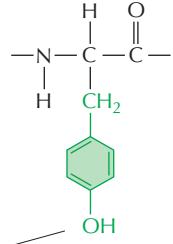
threonine

(Thr, or T)



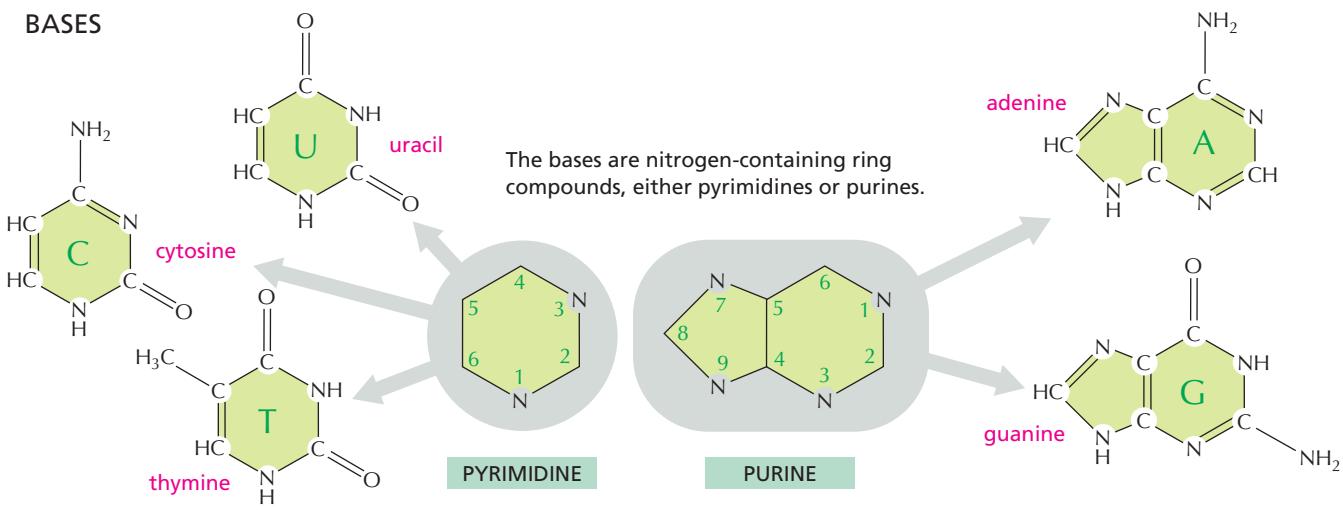
tyrosine

(Tyr, or Y)

The $-\text{OH}$ group is polar.**Disulfide bonds** can form between two cysteine side chains in proteins. $-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-$

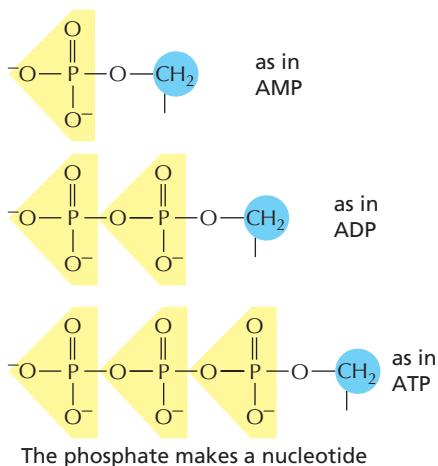
PANEL 2–6 A SURVEY OF THE NUCLEOTIDES

BASES



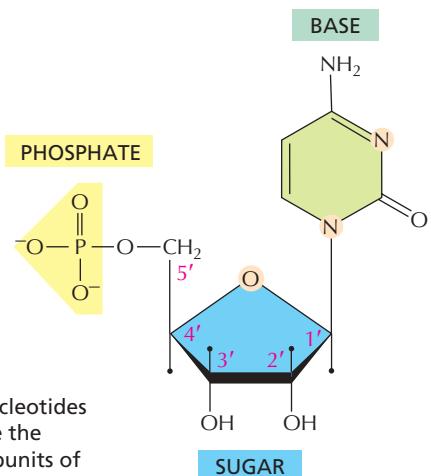
PHOSPHATES

The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.

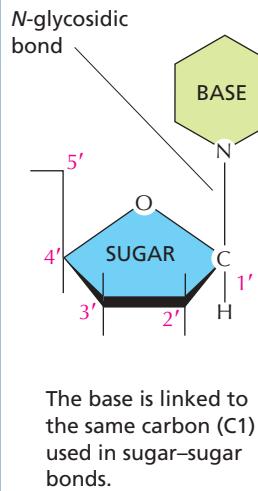


NUCLEOTIDES

A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.

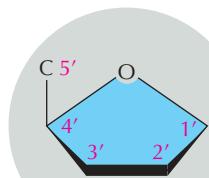


BASE-SUGAR LINKAGE

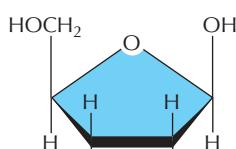


SUGARS

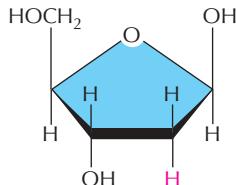
PENTOSE
a five-carbon sugar



two kinds of pentoses are used



Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.



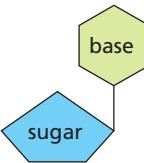
NOMENCLATURE

The names can be confusing, but the abbreviations are clear.

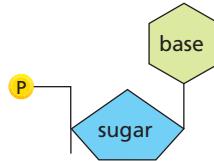
BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

Nucleotides are abbreviated by three capital letters. Some examples follow:

AMP = adenosine monophosphate
DAMP = deoxyadenosine monophosphate
UDP = uridine diphosphate
ATP = adenosine triphosphate



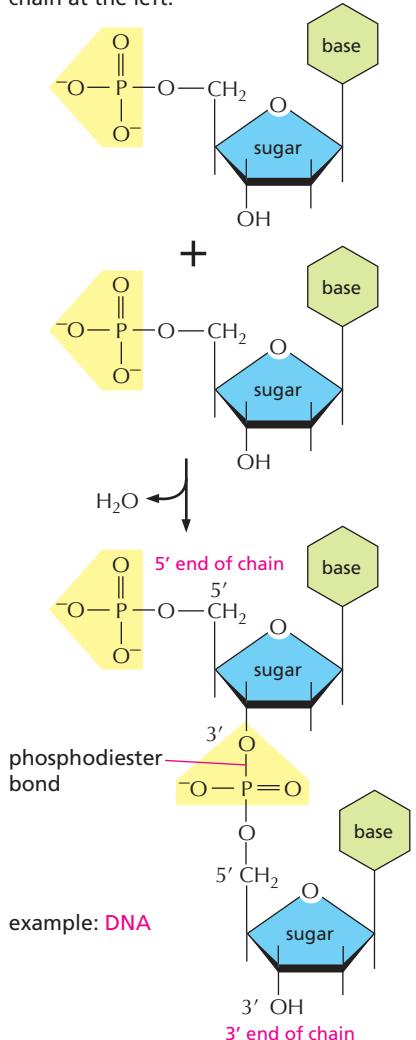
BASE + SUGAR = **NUCLEOSIDE**



BASE + SUGAR + PHOSPHATE = **NUCLEOTIDE**

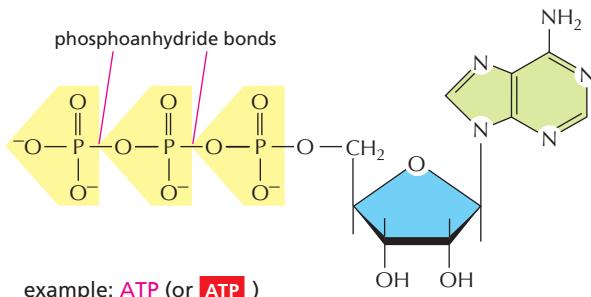
NUCLEIC ACIDS

Nucleotides are joined together by **phosphodiester bonds** between 5' and 3' carbon atoms of the sugar ring, via a phosphate group, to form nucleic acids. The linear sequence of nucleotides in a nucleic acid chain is commonly abbreviated by a one-letter code, such as AGCTTACA, with the 5' end of the chain at the left.

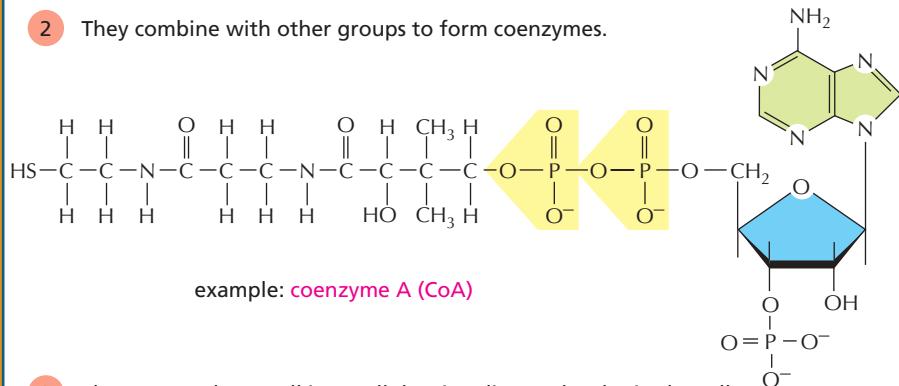


NUCLEOTIDES HAVE MANY OTHER FUNCTIONS

- They carry chemical energy in their easily hydrolyzed phosphoanhydride bonds.

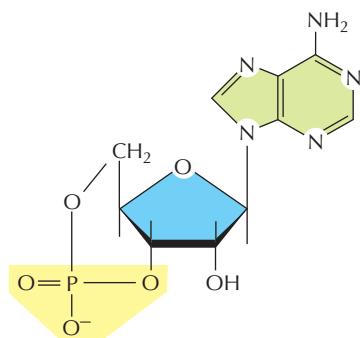


- They combine with other groups to form coenzymes.



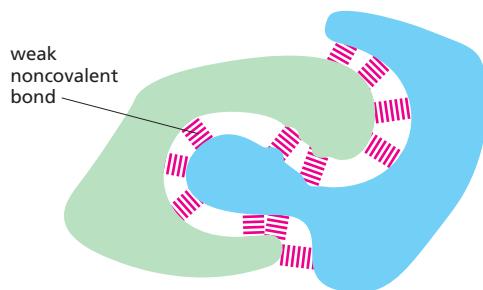
- They are used as small intracellular signaling molecules in the cell.

example: cyclic AMP



WEAK NONCOVALENT CHEMICAL BONDS

Organic molecules can interact with other molecules through three types of short-range attractive forces known as *noncovalent bonds*: van der Waals attractions, electrostatic attractions, and hydrogen bonds. The repulsion of hydrophobic groups from water is also important for these interactions and for the folding of biological macromolecules.



Weak noncovalent bonds have less than 1/20 the strength of a strong covalent bond. They are strong enough to provide tight binding only when many of them are formed simultaneously.

HYDROGEN BONDS

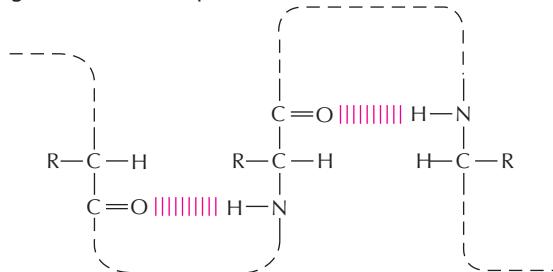
As already described for water (see Panel 2–2, pp. 68–69), **hydrogen bonds** form when a hydrogen atom is “sandwiched” between two electron-attracting atoms (usually oxygen or nitrogen).

Hydrogen bonds are strongest when the three atoms are in a straight line:

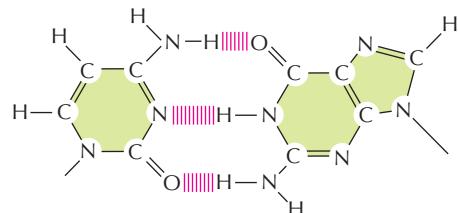


Examples in macromolecules:

Amino acids in a polypeptide chain can be hydrogen-bonded together in a folded protein.

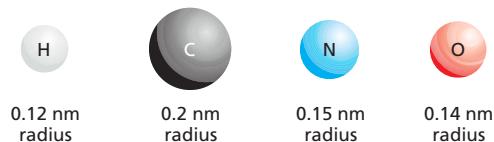


Two bases, G and C, are hydrogen-bonded in a DNA double helix.



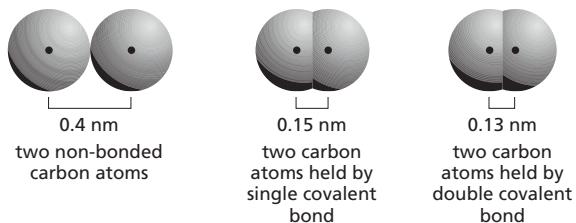
VAN DER WAALS ATTRACTIONS

If two atoms are too close together they repel each other very strongly. For this reason, an atom can often be treated as a sphere with a fixed radius. The characteristic “size” for each atom is specified by a unique **van der Waals radius**. The contact distance between any two noncovalently bonded atoms is the sum of their van der Waals radii.



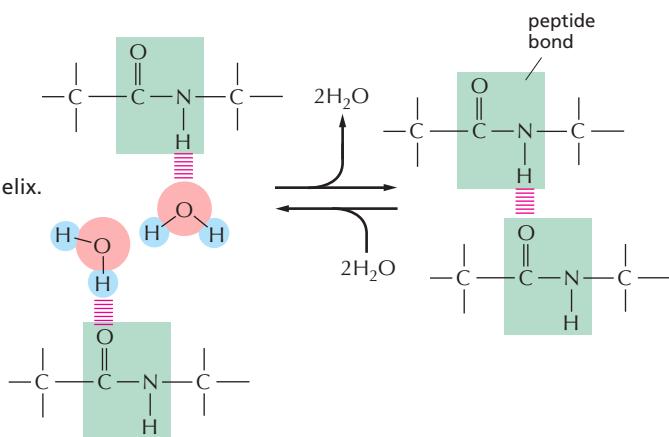
At very short distances, any two atoms show a weak bonding interaction due to their fluctuating electrical charges. The two atoms will be attracted to each other in this way until the distance between their nuclei is approximately equal to the sum of their van der Waals radii. Although they are individually very weak, such **van der Waals attractions** can become important when two macromolecular surfaces fit very close together, because many atoms are involved.

Note that when two atoms form a covalent bond, the centers of the two atoms (the two atomic nuclei) are much closer together than the sum of the two van der Waals radii. Thus,



HYDROGEN BONDS IN WATER

Any two atoms that can form hydrogen bonds to each other can alternatively form hydrogen bonds to water molecules. Because of this competition with water molecules, the hydrogen bonds formed in water between two peptide bonds, for example, are relatively weak.



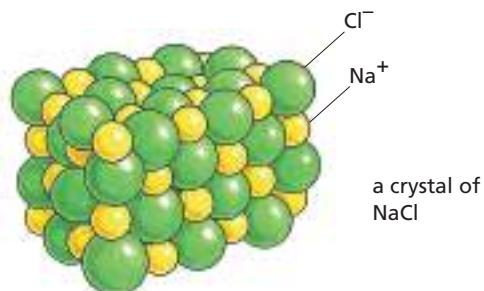
ELECTROSTATIC ATTRACTIONS

Attractive interactions occur both between fully charged groups (ionic bond) and between partially charged groups on polar molecules.



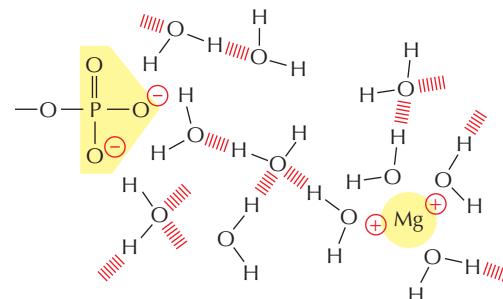
The force of attraction between the two partial charges, δ^+ and δ^- , falls off rapidly as the distance between the charges increases.

In the absence of water, ionic bonds are very strong. They are responsible for the strength of such minerals as marble and agate, and for crystal formation in common table salt, NaCl.

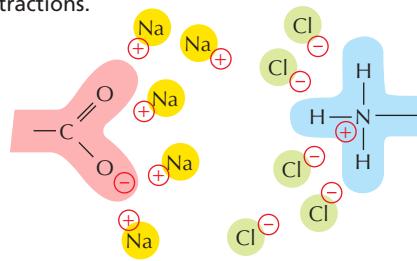


ELECTROSTATIC ATTRACTIONS IN AQUEOUS SOLUTIONS

Charged groups are shielded by their interactions with water molecules. Electrostatic attractions are therefore quite weak in water.

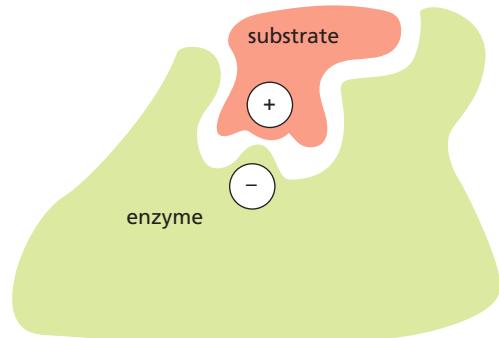
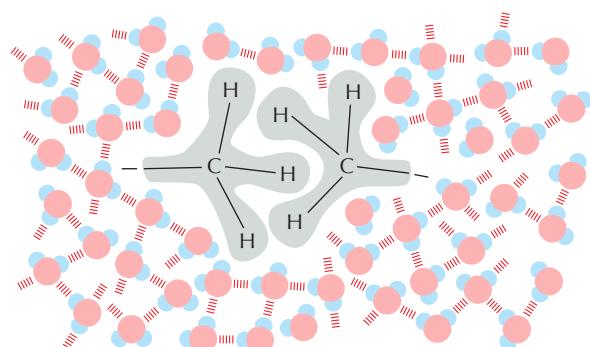
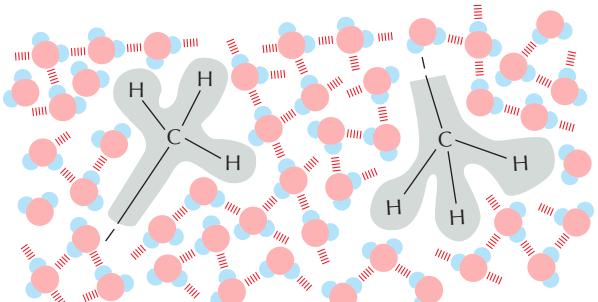


Inorganic ions in solution can also cluster around charged groups and further weaken these electrostatic attractions.



Despite being weakened by water and inorganic ions, electrostatic attractions are very important in biological systems. For example, an enzyme that binds a positively charged substrate will often have a negatively charged amino acid side chain at the appropriate place.

HYDROPHOBIC INTERACTIONS



Water forces hydrophobic groups together in order to minimize their disruptive effects on the water network formed by the H bonds between water molecules. Hydrophobic groups held together in this way are sometimes said to be held together by "hydrophobic bonds," even though the attraction is actually caused by a repulsion from water.

QUESTIONS

QUESTION 2–10

Which of the following statements are correct? Explain your answers.

- An atomic nucleus contains protons and neutrons.
- An atom has more electrons than protons.
- The nucleus is surrounded by a double membrane.
- All atoms of the same element have the same number of neutrons.
- The number of neutrons determines whether the nucleus of an atom is stable or radioactive.
- Both fatty acids and polysaccharides can be important energy stores in the cell.
- Hydrogen bonds are weak and can be broken by thermal energy, yet they contribute significantly to the specificity of interactions between macromolecules.

QUESTION 2–11

To gain a better feeling for atomic dimensions, assume that the page on which this question is printed is made entirely of the polysaccharide cellulose, whose molecules are described by the formula $(C_nH_{2n}O_n)$, where n can be a quite large number and is variable from one molecule to another. The atomic weights of carbon, hydrogen, and oxygen are 12, 1, and 16, respectively, and this page weighs 5 g.

- How many carbon atoms are there in this page?
- In cellulose, how many carbon atoms would be stacked on top of each other to span the thickness of this page (the size of the page is 21.2 cm \times 27.6 cm, and it is 0.07 mm thick)?
- Now consider the problem from a different angle. Assume that the page is composed only of carbon atoms. A carbon atom has a diameter of 2×10^{-10} m (0.2 nm); how many carbon atoms of 0.2 nm diameter would it take to span the thickness of the page?
- Compare your answers from parts B and C and explain any differences.

QUESTION 2–12

- How many electrons can be accommodated in the first, second, and third electron shells of an atom?
- How many electrons would atoms of the elements listed below have to gain or lose to obtain a completely filled outer shell?

helium	gain <u> </u>	lose <u> </u>
oxygen	gain <u> </u>	lose <u> </u>
carbon	gain <u> </u>	lose <u> </u>
sodium	gain <u> </u>	lose <u> </u>
chlorine	gain <u> </u>	lose <u> </u>

- What do the answers tell you about the reactivity of helium and the bonds that can form between sodium and chlorine?

QUESTION 2–13

The elements oxygen and sulfur have similar chemical properties because they both have six electrons in their outermost electron shells. Indeed, both elements form molecules with two hydrogen atoms, water (H_2O) and hydrogen sulfide (H_2S). Surprisingly, at room temperature, water is a liquid, yet H_2S is a gas, despite sulfur being much larger and heavier than oxygen. Explain why this might be the case.

QUESTION 2–14

Write the chemical formula for a condensation reaction of two amino acids to form a peptide bond. Write the formula for its hydrolysis.

QUESTION 2–15

Which of the following statements are correct? Explain your answers.

- Proteins are so remarkably diverse because each is made from a unique mixture of amino acids that are linked in random order.
- Lipid bilayers are macromolecules that are made up mostly of phospholipid subunits.
- Nucleic acids contain sugar groups.
- Many amino acids have hydrophobic side chains.
- The hydrophobic tails of phospholipid molecules are repelled from water.
- DNA contains the four different bases A, G, U, and C.

QUESTION 2–16

- How many different molecules composed of (a) two, (b) three, and (c) four amino acids, linked together by peptide bonds, can be made from the set of 20 naturally occurring amino acids?
- Assume you were given a mixture consisting of one molecule each of all possible sequences of a smallish protein of molecular weight 4800 daltons. If the average molecular weight of an amino acid is, say, 120 daltons, how much would the sample weigh? How big a container would you need to hold it?
- What does this calculation tell you about the fraction of possible proteins that are currently in use by living organisms (the average molecular weight of proteins is about 30,000 daltons)?

QUESTION 2–17

This is a biology textbook. Explain why the chemical principles that are described in this chapter are important in the context of modern cell biology.

QUESTION 2–18

- Describe the similarities and differences between van der Waals attractions and hydrogen bonds.

B. Which of the two bonds would form (a) between two hydrogens bound to carbon atoms, (b) between a nitrogen atom and a hydrogen bound to a carbon atom, and (c) between a nitrogen atom and a hydrogen bound to an oxygen atom?

QUESTION 2-19

What are the forces that determine the folding of a macromolecule into a unique shape?

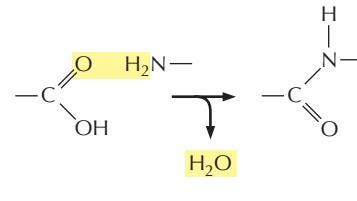
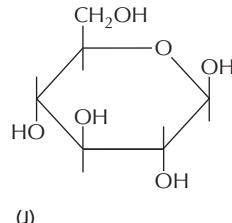
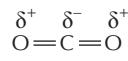
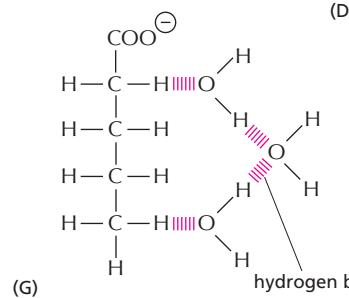
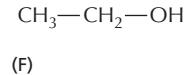
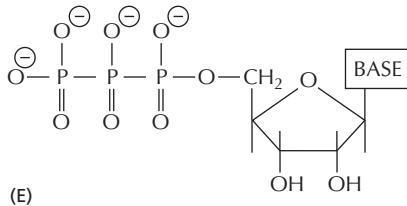
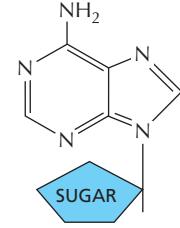
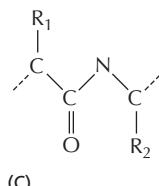
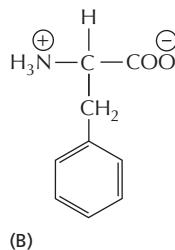
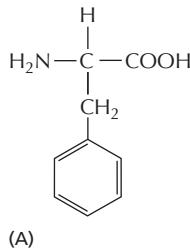


Figure Q2-21

QUESTION 2-20

Fatty acids are said to be "amphipathic." What is meant by this term, and how does an amphipathic molecule behave in water? Draw a diagram to illustrate your answer.

QUESTION 2-21

Are the formulas in **Figure Q2-21** correct or incorrect? Explain your answer in each case.

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CHAPTER THREE

3

Energy, Catalysis, and Biosynthesis

One property above all makes living things seem almost miraculously different from nonliving matter: they create and maintain order in a universe that is tending always toward greater disorder. To accomplish this remarkable feat, the cells in a living organism must carry out a never-ending stream of chemical reactions that produce the molecules the organism requires to meet its metabolic needs. In some of these reactions, small organic molecules—amino acids, sugars, nucleotides, and lipids—are taken apart or modified to supply the many other small molecules that the cell requires. In other reactions, these small molecules are used to construct an enormously diverse range of larger molecules, including the proteins, nucleic acids, and other macromolecules that endow living systems with all of their most distinctive properties. Each cell can be viewed as a tiny chemical factory, performing many millions of these reactions every second.

To carry out the tremendous number of chemical reactions needed to sustain it, a living organism requires both a source of atoms in the form of food molecules and a source of energy. The atoms and the energy must both come, ultimately, from the nonliving environment. In this chapter, we discuss why cells require energy, and how they use energy and atoms from their environment to create the molecular order that makes life possible.

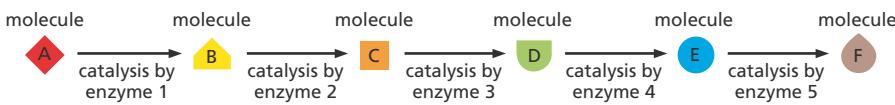
Most of the chemical reactions that cells perform would normally occur only at temperatures that are much higher than those inside a cell. Each reaction therefore requires a major boost in chemical reactivity to enable it to proceed rapidly within the cell. This boost is provided by specialized proteins called *enzymes*, each of which accelerates, or *catalyzes*, just one

THE USE OF ENERGY BY CELLS

FREE ENERGY AND CATALYSIS

ACTIVATED CARRIERS AND BIOSYNTHESIS

Figure 3–1 A series of enzyme-catalyzed reactions forms a metabolic pathway. Each enzyme catalyzes a chemical reaction involving a particular molecule. In this example, a set of enzymes acting in series converts molecule A to molecule F, forming a metabolic pathway.



of the many possible kinds of reactions that a particular molecule might undergo. These enzyme-catalyzed reactions are usually connected in series, so that the product of one reaction becomes the starting material for the next (Figure 3–1). The long linear reaction pathways, or *metabolic pathways*, that result are in turn linked to one another, forming a complex web of interconnected reactions.

Rather than being an inconvenience, the necessity for *catalysis* is a benefit, as it allows the cell to precisely control its **metabolism**—the sum total of all the chemical reactions it needs to carry out to survive, grow, and reproduce. This control is central to the chemistry of life.

Two opposing streams of chemical reactions occur in cells, the *catabolic pathways* and the *anabolic pathways*. The catabolic pathways (**catabolism**) break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks. The anabolic, or *biosynthetic*, pathways (**anabolism**) use the energy harnessed by catabolism to drive the synthesis of the many molecules that form the cell. Together, these two sets of reactions constitute the metabolism of the cell (Figure 3–2).

The details regarding the individual reactions that comprise cell metabolism are part of the subject matter of *biochemistry*, and they need not concern us here. But the general principles by which cells obtain energy from their environment and use it to create order are central to cell biology. We begin this chapter with a discussion of why a constant input of energy is needed to sustain living organisms. We then discuss how enzymes catalyze the reactions that produce biological order. Finally, we describe the molecules that carry the energy that makes life possible.

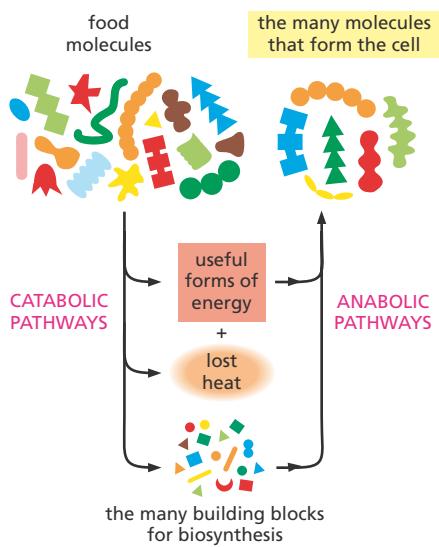


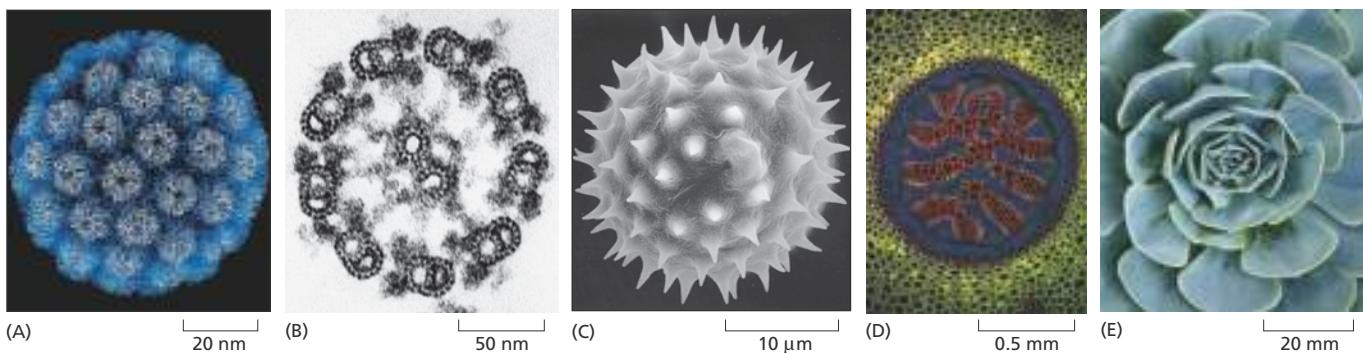
Figure 3–2 Catabolic and anabolic pathways together constitute the cell's metabolism. Note that a major portion of the energy stored in the chemical bonds of food molecules is dissipated as heat. Thus, only some of this energy can be converted to the useful forms of energy needed to drive the synthesis of new molecules.

THE USE OF ENERGY BY CELLS

Nonliving things left to themselves eventually become disordered: buildings crumble and dead organisms decay. Living cells, by contrast, not only maintain, but actually generate order at every level, from the large-scale structure of a butterfly or a flower down to the organization of the molecules that make up these organisms (Figure 3–3). This property of life is made possible by elaborate molecular mechanisms that extract energy from the environment and convert it into the energy stored in chemical bonds. Biological structures are therefore able to maintain their form, even though the materials of which they are made are continually being broken down, replaced, and recycled. Your body has the same basic structure it had 10 years ago, even though you now contain atoms that, for the most part, were not in your body then.

Biological Order Is Made Possible by the Release of Heat Energy from Cells

The universal tendency of things to become disordered is expressed in a fundamental law of physics, the *second law of thermodynamics*. This law states that, in the universe or in any isolated system (a collection of matter that is completely isolated from the rest of the universe), the degree of disorder can only increase. The second law of thermodynamics has such profound implications for living things that it is worth restating in several ways.



We can express the second law in terms of probability by stating that *systems will change spontaneously toward those arrangements that have the greatest probability*. Consider a box of 100 coins all lying heads up. A series of events that disturbs the box will tend to move the arrangement toward a mixture of 50 heads and 50 tails. The reason is simple: there are a huge number of possible arrangements of the individual coins that can achieve the 50–50 result, but only one possible arrangement that keeps them all oriented heads up. Because the 50–50 mixture accommodates a greater number of possibilities and places fewer constraints on the orientation of each individual coin, we say that it is more “disordered.” For the same reason, one’s living space will become increasingly disordered without an intentional effort to keep it organized. Movement toward disorder is a spontaneous process, requiring a periodic input of energy to reverse it (**Figure 3-4**).

The measure of a system’s disorder is called the **entropy** of the system, and the greater the disorder, the greater the entropy. Thus another way to express the second law of thermodynamics is to say that systems will change spontaneously toward arrangements with greater entropy. Living cells—by surviving, growing, and forming complex communities and even whole organisms—generate order and thus might appear to defy the second law of thermodynamics. This is not the case, however,

Figure 3-3 Biological structures are highly ordered. Well-defined, ornate, and beautiful spatial patterns can be found at every level of organization in living organisms. In order of increasing size: (A) protein molecules in the coat of a virus (a parasite that, although not technically alive, contains the same types of molecules as those found in living cells); (B) the regular array of microtubules seen in a cross section of a sperm tail; (C) surface contours of a pollen grain (a single cell); (D) cross section of a fern stem, showing the patterned arrangement of cells; and (E) flower with a spiral array of petals, each made of millions of cells. (A, courtesy of Robert Grant, Stéphane Crainic, and James M. Hogle; B, courtesy of Lewis Tilney; C, courtesy of Colin MacFarlane and Chris Jeffree; D, courtesy of Jim Haseloff.)

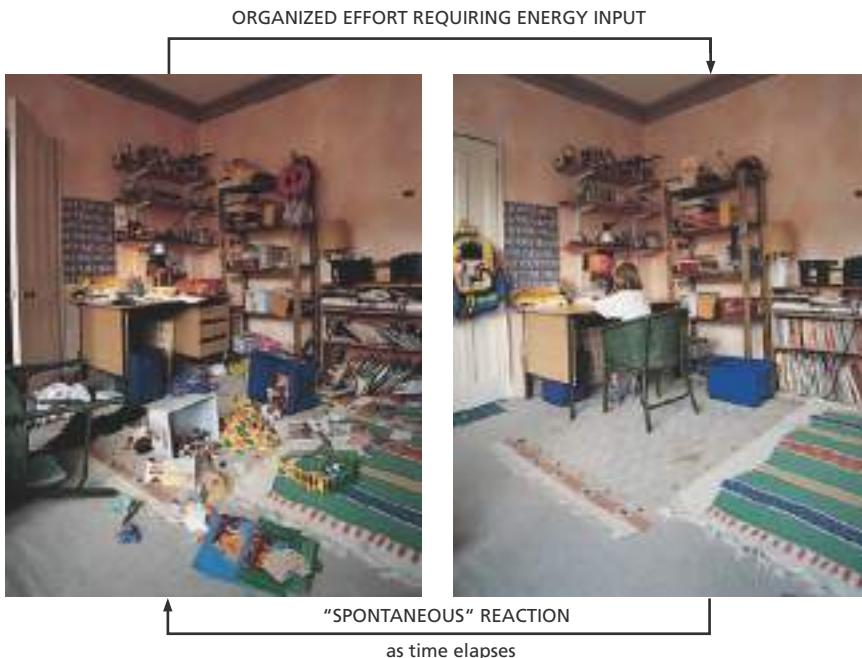
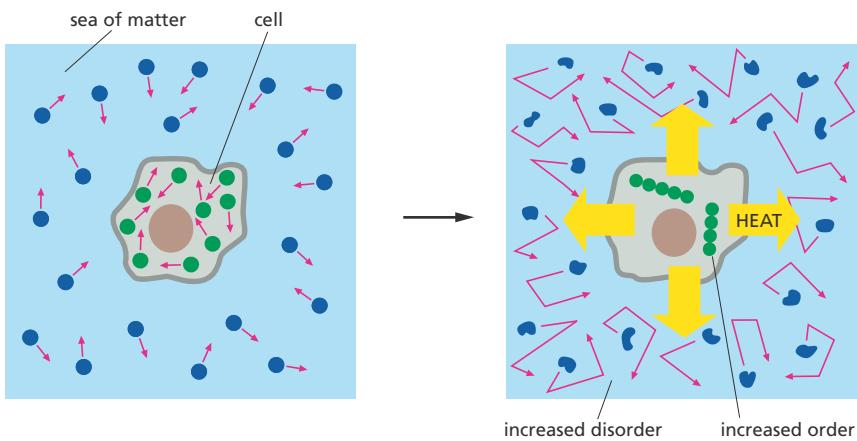


Figure 3-4 The spontaneous tendency toward disorder is an everyday experience. Reversing this natural tendency toward disorder requires an intentional effort and an input of energy. In fact, from the second law of thermodynamics, we can be certain that the human intervention required will release enough heat to the environment to more than compensate for the reestablishment of order in this room.

Figure 3–5 Living cells do not defy the second law of thermodynamics. In the diagram on the left, the molecules of both the cell and the rest of the universe (the environment) are depicted in a relatively disordered state. In the diagram on the right, the cell has taken in energy from food molecules and released heat by carrying out a reaction that orders the molecules that the cell contains. Because the heat increases the disorder in the environment around the cell—as depicted by the longer, jagged red arrows, which represent increased thermal motion, and the distorted molecules, which indicate enhanced molecular vibration and rotation—the second law of thermodynamics is satisfied, even as the cell grows and constructs larger molecules.



because a cell is not an isolated system. Rather, it takes in energy from its environment—in the form of food, inorganic molecules, or photons of light from the sun—and it then uses this energy to generate order within itself, forging new chemical bonds and building large macromolecules. In the course of performing the chemical reactions that generate order, some energy is lost in the form of heat. Heat is energy in its most disordered form—the random jostling of molecules (analogous to the random jostling of the coins in the box). Because the cell is not an isolated system, the heat energy that its reactions generate is quickly dispersed into the cell's surroundings. There, the heat increases the intensity of the thermal motions of nearby molecules, thereby increasing the entropy of the environment (**Figure 3–5**).

The amount of heat released by a cell must be great enough that the increased order generated inside the cell is more than compensated for by the increased disorder generated in the environment. Only in this case is the second law of thermodynamics satisfied, because the total entropy of the system—that of the cell plus its environment—increases as a result of the chemical reactions inside the cell.

Cells Can Convert Energy from One Form to Another

According to the *first law of thermodynamics*, energy cannot be created or destroyed—but it can be converted from one form to another (**Figure 3–6**). Cells take advantage of this law of thermodynamics, for example, when they convert the energy from sunlight into the energy in the chemical bonds of sugars and other small organic molecules during photosynthesis. Although chemical reactions that power such energy conversions can change how much energy is present in one form or another, the first law tells us that the total amount of energy in the universe must always be the same.

When an animal cell breaks down foodstuffs, some of the energy in the chemical bonds in the food molecules (chemical-bond energy) is converted into the thermal motion of molecules (heat energy). This conversion of chemical energy into heat energy causes the universe as a whole to become more disordered—as required by the second law of thermodynamics. But the cell cannot derive any benefit from the heat energy it produces unless the heat-generating reactions are directly linked to processes that maintain molecular order inside the cell. It is the tight coupling of heat production to an increase in order that distinguishes the metabolism of a cell from the wasteful burning of fuel in a fire. Later in this chapter, we illustrate how this coupling occurs. For the moment, it is

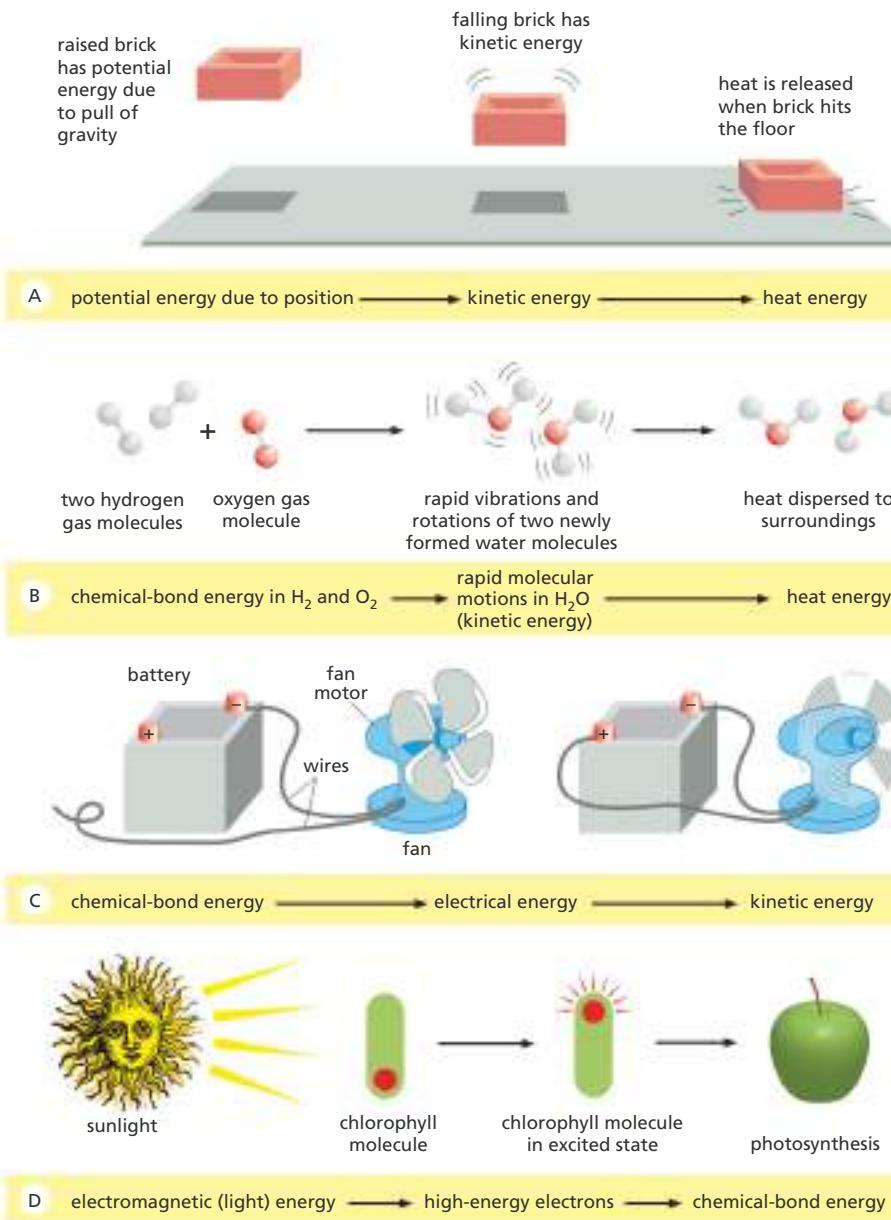


Figure 3–6 Different forms of energy are interconvertible, but the total amount of energy must be conserved. In (A), we can use the height and weight of the brick to predict exactly how much heat will be released when it hits the floor. In (B), the large amount of chemical-bond energy released when water (H₂O) is formed from H₂ and O₂ is initially converted to very rapid thermal motions in the two new H₂O molecules; however, collisions with other H₂O molecules almost instantaneously spread this kinetic energy evenly throughout the surroundings (heat transfer), making the new H₂O molecules indistinguishable from all the rest. (C) Cells can convert chemical-bond energy into kinetic energy to drive, for example, molecular motor proteins; however, this occurs without the intermediate conversion to electrical energy that a man-made appliance such as this fan requires. (D) Some cells can also harvest the energy from sunlight to form chemical bonds via photosynthesis.

sufficient to recognize that—by directly linking the “burning” of food molecules to the generation of biological order—cells are able to create and maintain an island of order in a universe tending toward chaos.

Photosynthetic Organisms Use Sunlight to Synthesize Organic Molecules

All animals live on energy stored in the chemical bonds of organic molecules, which they take in as food. These food molecules also provide the atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals, others by eating plants. Plants, by contrast, obtain their energy directly from sunlight. Thus, the energy animals obtain by eating plants—or by eating animals that have eaten plants—ultimately comes from the sun (**Figure 3–7**).

Solar energy enters the living world through **photosynthesis**, a process that converts the electromagnetic energy in sunlight into chemical-bond energy in cells. Photosynthetic organisms—including plants, algae, and



Figure 3–7 With few exceptions, the radiant energy of sunlight sustains all life. Trapped by plants and some microorganisms through photosynthesis, light from the sun is the ultimate source of all energy for humans and other animals. (*Wheat Field Behind Saint-Paul Hospital with a Reaper* by Vincent van Gogh. Courtesy of Museum Folkwang, Essen.)

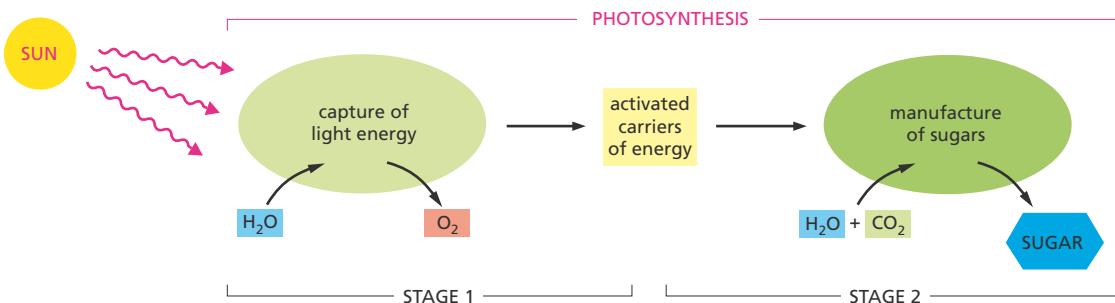


Figure 3–8 Photosynthesis takes place in two stages. The activated carriers generated in the first stage are two molecules that we will discuss shortly: ATP and NADPH.

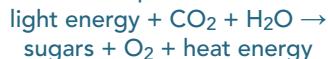
some bacteria—use the energy they derive from sunlight to synthesize small chemical building blocks such as sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the macromolecules—the proteins, nucleic acids, polysaccharides, and lipids—that form the plant.

We describe the elegant mechanisms that underlie photosynthesis in detail in Chapter 14. Generally speaking, the reactions of photosynthesis take place in two stages. In the first stage, energy from sunlight is captured and transiently stored as chemical-bond energy in specialized molecules called *activated carriers*, which we discuss in more detail later in the chapter. All of the oxygen (O_2) in the air we breathe is generated by the splitting of water molecules during this first stage of photosynthesis.

In the second stage, the activated carriers are used to help drive a *carbon-fixation* process, in which sugars are manufactured from carbon dioxide gas (CO_2). In this way, photosynthesis generates an essential source of stored chemical-bond energy and other organic materials—for the plant itself and for any animals that eat it. The two stages of photosynthesis are summarized in **Figure 3–8**.

QUESTION 3–1

Consider the equation



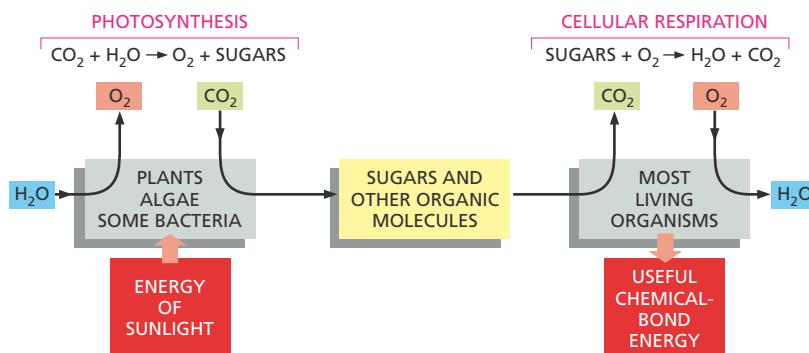
Would you expect this reaction to occur in a single step? Why must heat be generated in the reaction? Explain your answers.

Cells Obtain Energy by the Oxidation of Organic Molecules

All animal and plant cells require the chemical energy stored in the chemical bonds of organic molecules—either the sugars that a plant has produced by photosynthesis as food for itself or the mixture of large and small molecules that an animal has eaten. To use this energy to live, grow, and reproduce, organisms must extract it in a usable form. In both plants and animals, energy is extracted from food molecules by a process of gradual *oxidation*, or controlled burning.

Earth's atmosphere is about 21% oxygen. In the presence of oxygen, the most energetically stable form of carbon is CO_2 and that of hydrogen is H_2O . A cell is therefore able to obtain energy from sugars or other organic molecules by allowing the carbon and hydrogen atoms in these molecules to combine with oxygen—that is, become *oxidized*—to produce CO_2 and H_2O , respectively—a process known as **cellular respiration**.

Photosynthesis and cellular respiration are complementary processes (**Figure 3–9**). This means that the transactions between plants and animals are not all one way. Plants, animals, and microorganisms have existed together on this planet for so long that they have become an essential part of each other's environments. The oxygen released by photosynthesis is consumed by nearly all organisms for the oxidative breakdown of organic molecules. And some of the CO_2 molecules that today are incorporated into organic molecules by photosynthesis in a green leaf were released yesterday into the atmosphere by the respiration of an animal, a fungus, or the plant itself, or by the burning of



fossil fuels. Carbon utilization therefore forms a huge cycle that involves the *biosphere* (all of the living organisms on Earth) as a whole, crossing boundaries between individual organisms (Figure 3–10).

Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism directs the molecules through a large number of reactions, few of which actually involve the direct addition of oxygen. Thus, before we consider some of these reactions, we should explain what is meant by oxidation.

The term **oxidation** literally means the addition of oxygen atoms to a molecule. More generally, though, oxidation is said to occur in any reaction in which electrons are transferred from one atom to another. Oxidation, in this sense, refers to the removal of electrons from an atom. The converse reaction, called **reduction**, involves the addition of electrons to an atom. Thus, Fe^{2+} is oxidized when it loses an electron to become Fe^{3+} , whereas a chlorine atom is reduced when it gains an electron to become Cl^- . Because the number of electrons is conserved in a chemical reaction (there is no net loss or gain), oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule must lose the electron (oxidation). When a sugar molecule is oxidized to CO_2 and H_2O , for example, the O_2 molecules involved in forming H_2O gain electrons and thus are said to have been reduced.

The terms oxidation and reduction apply even when there is only a partial shift of electrons between atoms linked by a covalent bond. When a carbon atom becomes covalently bonded to an atom with a strong affinity for electrons—oxygen, chlorine, or sulfur, for example—it gives up more

Figure 3–9 Photosynthesis and cellular respiration are complementary processes in the living world. The left side of the diagram shows how photosynthesis—carried out by plants and photosynthetic microorganisms—uses the energy of sunlight to produce sugars and other organic molecules from the carbon atoms in CO_2 in the atmosphere. In turn, these molecules serve as food for other organisms. The right side of the diagram shows how cellular respiration in most organisms—including plants and photosynthetic microorganisms—uses O_2 to oxidize food molecules, releasing the same carbon atoms in the form of CO_2 back to the atmosphere. In the process, the organisms obtain the useful chemical-bond energy that they need to survive. The first cells on Earth are thought to have been capable of neither photosynthesis nor cellular respiration (discussed in Chapter 14). However, photosynthesis must have preceded respiration on the Earth, because there is strong evidence that billions of years of photosynthesis were required to release enough O_2 to create an atmosphere that could support respiration.

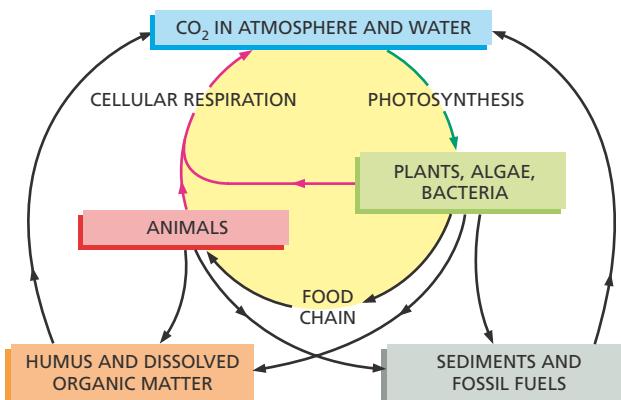
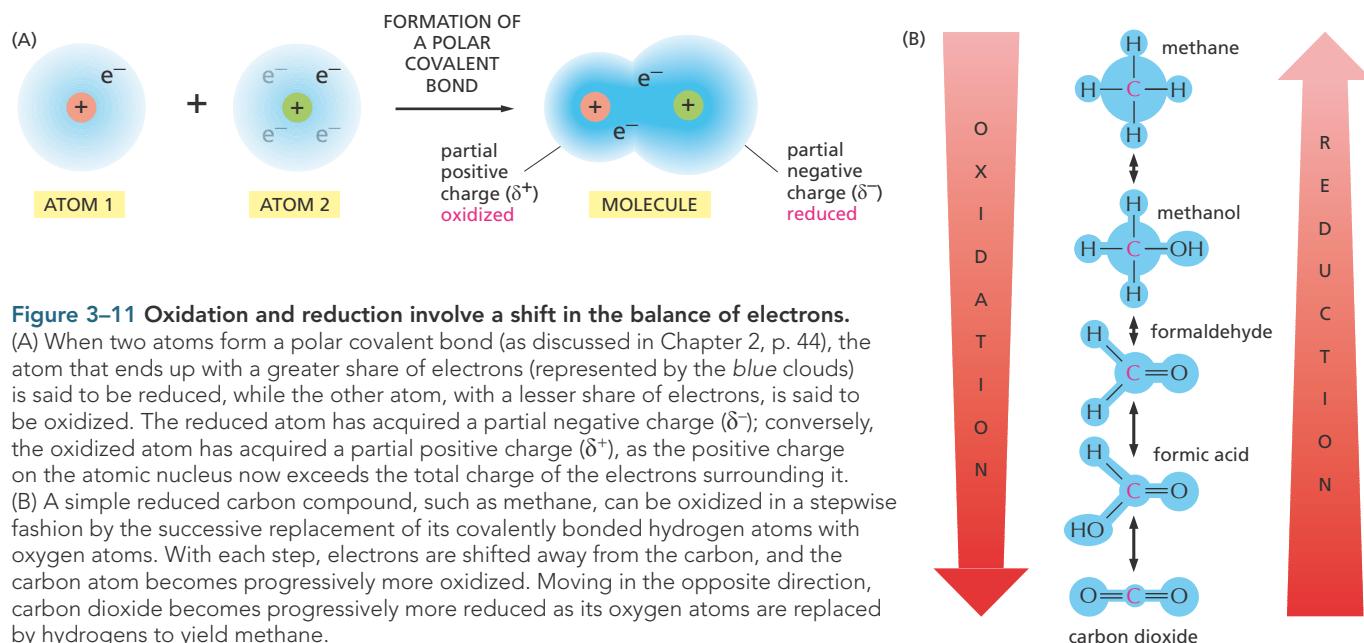


Figure 3–10 Carbon atoms cycle continuously through the biosphere.

Individual carbon atoms are incorporated into organic molecules of the living world by the photosynthetic activity of plants, algae, and bacteria. They then pass to animals and microorganisms—as well as into organic material in soil and oceans—and are ultimately restored to the atmosphere in the form of CO_2 when organic molecules are oxidized by cells during respiration or burned by humans as fossil fuels.



than its equal share of electrons and forms a *polar covalent bond*. The positive charge of the carbon nucleus now slightly exceeds the negative charge of its electrons, so that the carbon atom acquires a partial positive charge (δ^+) and is said to be oxidized. Conversely, the carbon atom in a C-H bond has somewhat more than its share of electrons; it acquires a partial negative charge (δ^-), and so is said to be reduced (Figure 3–11A).

When a molecule in a cell picks up an electron (e^-), it often picks up a proton (H^+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule:



Even though a proton plus an electron is involved (instead of just an electron), such *hydrogenation* reactions are reductions, and the reverse, *dehydrogenation*, reactions are oxidations. An easy way to tell whether an organic molecule is being oxidized or reduced is to count its C-H bonds: reduction occurs when the number of C-H bonds increases, whereas oxidation occurs when the number of C-H bonds decreases (Figure 3–11B).

As we will see later in this chapter—and again in Chapter 13—cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows energy to be harvested in useful forms.

FREE ENERGY AND CATALYSIS

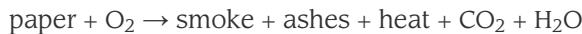
Enzymes, like cells, obey the second law of thermodynamics. Although they can speed up energetically favorable reactions—those that produce disorder in the universe—enzymes cannot by themselves force energetically unfavorable reactions to occur. Cells, however, must do just that in order to grow and divide—or just to survive. They must build highly ordered and energy-rich molecules from small and simple ones—a process that requires an input of energy.

To understand how enzymes promote **catalysis**—the acceleration of the specific chemical reactions needed to sustain life—we first need to examine the energetics involved. In this section, we consider how the free energy of molecules contributes to their chemistry, and we see how

free-energy changes—which reflect how much total disorder is generated in the universe by a reaction— influence whether and how the reaction will proceed. We then discuss how enzymes lower the activation energy needed to initiate reactions in the cell. And we describe how enzymes can exploit differences in the free-energy changes of different reactions to drive the energetically unfavorable reactions that produce biological order. Such enzyme-assisted catalysis is crucial for cells: without it, life could not exist.

Chemical Reactions Proceed in the Direction that Causes a Loss of Free Energy

Paper burns readily, releasing into the atmosphere water and carbon dioxide as gases, while simultaneously releasing energy as heat:



This reaction occurs in only one direction: smoke and ashes never spontaneously gather carbon dioxide and water from the heated atmosphere and reconstitute themselves into paper. When paper burns, much of its chemical energy is dissipated as heat: it is not lost from the universe, since energy can never be created or destroyed; instead, it is irretrievably dispersed in the chaotic random thermal motions of molecules. At the same time, the atoms and molecules of the paper become dispersed and disordered. In the language of thermodynamics, there has been a release of free energy—that is, energy that can be harnessed to do work or drive chemical reactions. This release reflects a loss of orderliness in the way the energy and molecules had been stored in the paper. We will discuss free energy in more detail shortly, but the general principle can be summarized as follows: chemical reactions proceed only in the direction that leads to a loss of free energy. In other words, the spontaneous direction for any reaction is the direction that goes “downhill.” A “downhill” reaction in this sense is said to be energetically favorable.

Enzymes Reduce the Energy Needed to Initiate Spontaneous Reactions

Although the most energetically favorable form of carbon under ordinary conditions is CO_2 , and that of hydrogen is H_2O , a living organism will not disappear in a puff of smoke, and the book in your hands will not burst spontaneously into flames. This is because the molecules in both the living organism and the book are in a relatively stable state, and they cannot be changed to lower-energy states without an initial input of energy. In other words, a molecule requires a boost over an energy barrier before it can undergo a chemical reaction that moves it to a lower-energy (more stable) state (Figure 3–12A). This boost is known as the

QUESTION 3–2

In which of the following reactions does the red atom undergo an oxidation?

- A. $\text{Na} \rightarrow \text{Na}^+$ (Na atom $\rightarrow \text{Na}^+$ ion)
- B. $\text{Cl} \rightarrow \text{Cl}^-$ (Cl atom $\rightarrow \text{Cl}^-$ ion)
- C. $\text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CHO}$
(ethanol \rightarrow acetaldehyde)
- D. $\text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{COO}^-$
(acetaldehyde \rightarrow acetic acid)
- E. $\text{CH}_2=\text{CH}_2 \rightarrow \text{CH}_3\text{CH}_3$
(ethene \rightarrow ethane)

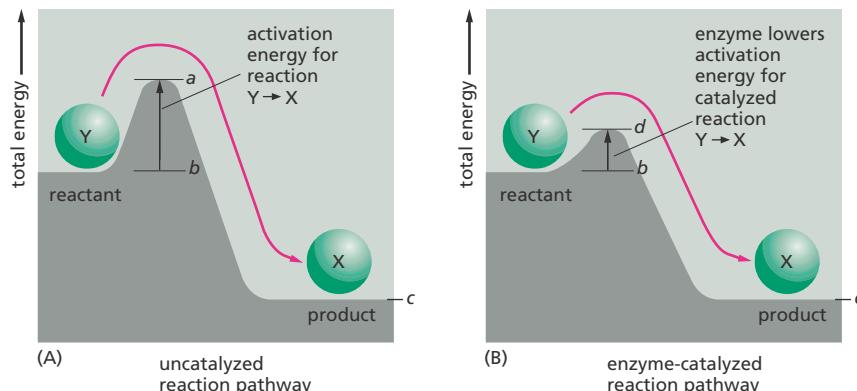


Figure 3–12 Even energetically favorable reactions require activation energy to get them started. (A) Compound Y (a reactant) is in a relatively stable state; thus energy is required to convert it to compound X (a product), even though X is at a lower overall energy level than Y. This conversion will not take place, therefore, unless compound Y can acquire enough activation energy (energy a minus energy b) from its surroundings to undergo the reaction that converts it into compound X. This energy may be provided by means of an unusually energetic collision with other molecules. For the reverse reaction, $\text{X} \rightarrow \text{Y}$, the activation energy required will be much larger (energy a minus energy c); this reaction will therefore occur much more rarely. Activation energies are always positive. The total energy change for the energetically favorable reaction $\text{Y} \rightarrow \text{X}$, is energy c minus energy b, a negative number, which corresponds to a loss of free energy. (B) Energy barriers for specific reactions can be lowered by catalysts, as indicated by the line marked d. Enzymes are particularly effective catalysts because they greatly reduce the activation energy for the reactions they catalyze.

Figure 3–13 Lowering the activation energy greatly increases the probability that a reaction will occur. At any given instant, a population of identical substrate molecules will have a range of energies, distributed as shown on the graph. The varying energies come from collisions with surrounding molecules, which make the substrate molecules jiggle, vibrate, and spin. For a molecule to undergo a chemical reaction, the energy of the molecule must exceed the activation energy barrier for that reaction (dashed lines); for most biological reactions, this almost never happens without enzyme catalysis. Even with enzyme catalysis, only a small fraction of substrate molecules reach an energy state that is high enough for them to undergo a reaction (red shaded area).

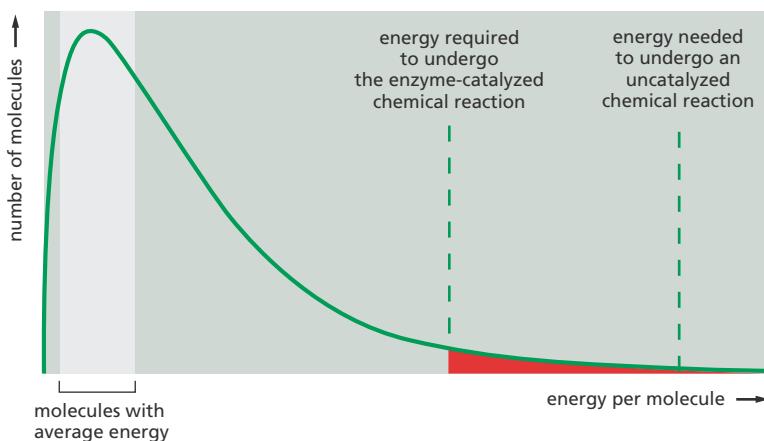
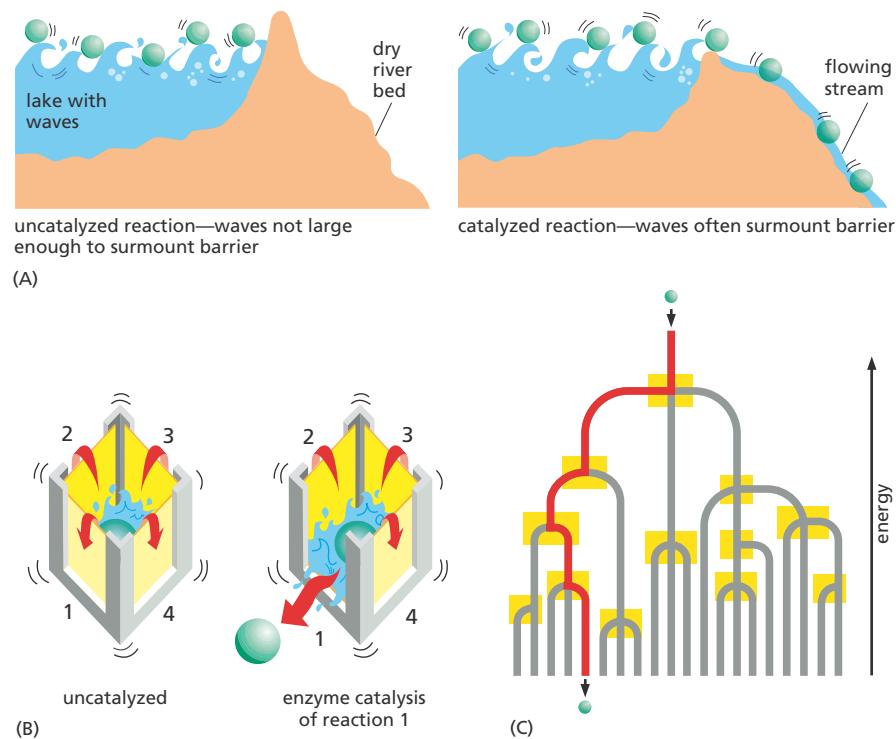
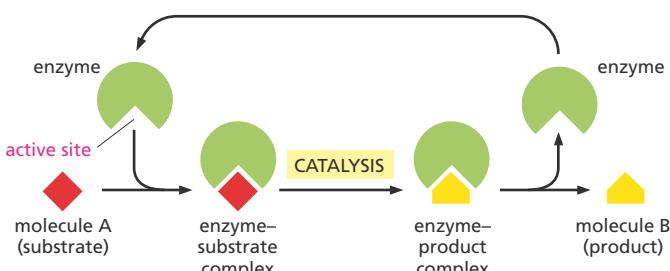


Figure 3–14 Enzymes catalyze reactions by lowering the activation energy barrier. (A) The dam represents the activation energy, which is lowered by enzyme catalysis. Each green ball represents a potential substrate molecule that is bouncing up and down in energy level owing to constant encounters with waves, an analogy for the thermal bombardment of substrate molecules by surrounding water molecules. When the barrier—the activation energy—is lowered significantly, the balls (substrate molecules) with sufficient energy can roll downhill, an energetically favorable movement. (B) The four walls of the box represent the activation energy barriers for four different chemical reactions that are all energetically favorable because the products are at lower energy levels than the substrates. In the left-hand box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the right-hand box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows the substrate molecule to pass over this energy barrier, allowing reaction 1 to proceed (Movie 3.1). (C) A branching river with a set of barrier dams (yellow boxes) serves to illustrate how a series of enzyme-catalyzed reactions determines the exact reaction pathway followed by each molecule inside the cell by controlling specifically which reaction will be allowed at each junction.

activation energy. In the case of a burning book, the activation energy is provided by the heat of a lighted match. But cells can't raise their temperature to drive biological reactions. Inside cells, the push over the energy barrier is aided by specialized proteins called **enzymes**.

Each enzyme binds tightly to one or two molecules, called **substrates**, and holds them in a way that greatly reduces the activation energy needed to facilitate a specific chemical interaction between them (Figure 3–12B). A substance that can lower the activation energy of a reaction is termed a **catalyst**; catalysts increase the rate of chemical reactions because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier, as illustrated in Figure 3–13 and Figure 3–14A. Enzymes are among the most effective catalysts known. They can speed up reactions by a factor of as much as 10^{14} (that is, trillions of times faster than the same reactions would proceed without an enzyme catalyst). Enzymes therefore allow reactions that would not otherwise occur to proceed rapidly at the normal temperature inside cells.





Unlike the effects of temperature, enzymes are highly selective. Each enzyme usually speeds up only one particular reaction out of the several possible reactions that its substrate molecules could undergo. In this way, enzymes direct each of the many different molecules in a cell along specific reaction pathways (Figure 3–14B and C), thereby producing the compounds that the cell actually needs.

Like all catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and therefore can function over and over again (Figure 3–15). In Chapter 4, we will discuss further how enzymes work, after we have looked in detail at the molecular structure of proteins.

The Free-Energy Change for a Reaction Determines Whether It Can Occur

According to the second law of thermodynamics, a chemical reaction can proceed only if it results in a net (overall) increase in the disorder of the universe (see Figure 3–5). Disorder increases when useful energy that could be harnessed to do work is dissipated as heat. The useful energy in a system is known as its **free energy**, or G . And because chemical reactions involve a transition from one molecular state to another, the term that is of most interest to chemists and cell biologists is the **free-energy change**, denoted ΔG ("Delta G ").

Let's consider a collection of molecules. ΔG measures the amount of disorder created in the universe when a reaction involving these molecules takes place. *Energetically favorable* reactions, by definition, are those that create disorder by decreasing the free energy of the system to which they belong; in other words, they have a *negative ΔG* (Figure 3–16).

A reaction can occur spontaneously only if ΔG is negative. On a macroscopic scale, an energetically favorable reaction with a negative ΔG is the relaxation of a compressed spring into an expanded state, releasing its stored elastic energy as heat to its surroundings. On a microscopic scale, an energetically favorable reaction with a negative ΔG occurs when salt (NaCl) dissolves in water. Note that, just because a reaction can occur spontaneously, does not mean it will occur quickly. The decay of diamonds into graphite is a spontaneous process—but it takes millions of years.

Energetically unfavorable reactions, by contrast, create order in the universe; they have a *positive ΔG* . Such reactions—for example, the formation of a peptide bond between two amino acids—cannot occur spontaneously; they take place only when they are coupled to a second reaction with a negative ΔG large enough that the net ΔG of the entire process is negative (Figure 3–17). Life is possible because enzymes can create biological order by coupling energetically unfavorable reactions with energetically favorable ones. These critical concepts are summarized, with examples, in Panel 3–1 (pp. 96–97).

Figure 3–15 Enzymes convert substrates to products while remaining unchanged themselves. Each enzyme has an active site to which one or two substrate molecules bind, forming an enzyme–substrate complex. A reaction occurs at the active site, generating an enzyme–product complex. The product is then released, allowing the enzyme to bind additional substrate molecules and repeat the reaction. An enzyme thus serves as a catalyst, and it usually forms or breaks a single covalent bond in a substrate molecule.

The free energy of Y is greater than the free energy of X. Therefore ΔG is negative (< 0), and the disorder of the universe increases during the reaction $Y \rightarrow X$.

this reaction can occur spontaneously

If the reaction $X \rightarrow Y$ occurred, ΔG would be positive (> 0), and the universe would become more ordered.

this reaction can occur only if it is coupled to a second, energetically favorable reaction

Figure 3–16 Energetically favorable reactions have a negative ΔG , whereas energetically unfavorable reactions have a positive ΔG .

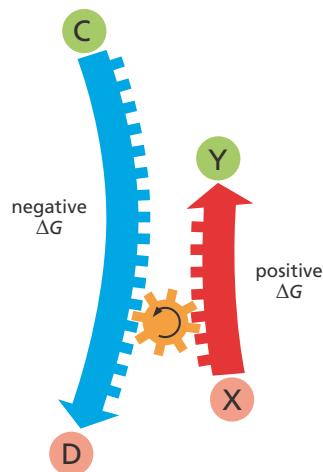


Figure 3-17 Reaction coupling can drive an energetically unfavorable reaction. The energetically unfavorable ($\Delta G > 0$) reaction $X \rightarrow Y$ cannot occur unless it is coupled to an energetically favorable ($\Delta G < 0$) reaction $C \rightarrow D$, such that the net free-energy change for the coupled reactions is negative (less than 0).

ΔG Changes As a Reaction Proceeds Toward Equilibrium

It's easy to see how a tensed spring, when left to itself, will relax and release its stored energy to the environment as heat. But chemical reactions are a bit more complex—and harder to intuit. That's because whether a reaction will proceed depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Recalling our coin analogy, more coins in a jiggling box will flip from a head to a tail orientation when the box contains 90 heads and 10 tails, than when the box contains 10 heads and 90 tails.

The same is true for a chemical reaction. As the energetically favorable reaction $Y \rightarrow X$ proceeds, the concentration of the product X will increase and the concentration of the substrate Y will decrease. This change in relative concentrations of substrate and product will cause the ratio of Y to X to shrink, making the initially favorable ΔG less and less negative. Unless more Y is added, the reaction will slow and eventually stop.

Because ΔG changes as products accumulate and substrates are depleted, chemical reactions will generally proceed until they reach a state of **equilibrium**. At that point, the rates of the forward and reverse reactions are equal, and there is no further net change in the concentrations of substrate or product (Figure 3-18). For reactions at chemical equilibrium, $\Delta G = 0$, so the reaction will not proceed forward or backward, and no work can be done.

Such a state of chemical inactivity would be incompatible with life. Living cells avoid reaching a state of complete chemical equilibrium because they are constantly exchanging materials with their environment: replenishing nutrients and eliminating waste products. Many of the individual reactions in the cell's complex metabolic network also exist in disequilibrium because the products of one reaction are continually being siphoned off to become the substrates in a subsequent reaction. Rarely do products and substrates reach concentrations at which the forward and reverse reaction rates are equal.

The Standard Free-Energy Change, ΔG° , Makes it Possible to Compare the Energetics of Different Reactions

Because ΔG depends on the concentrations of the molecules in the reaction mixture at any given time, it is not a particularly useful value for comparing the relative energies of different types of reactions. But such energetic assessments are necessary, for example, to predict whether an energetically favorable reaction is likely to have a ΔG negative enough to drive an energetically unfavorable reaction. To compare reactions in this way, we need to turn to the *standard free-energy change* of a reaction, ΔG° . The ΔG° is independent of concentration; it depends only on the intrinsic characters of the reacting molecules, based on their behavior under ideal conditions where the concentrations of all the reactants are set to the same fixed value of 1 mole/liter.

A large body of thermodynamic data has been collected from which ΔG° can be calculated for most metabolic reactions. Some common reactions are compared in terms of their ΔG° in Panel 3-1 (pp. 96–97).

The ΔG of a reaction can be calculated from ΔG° if the concentrations of the reactants and products are known. For the simple reaction $Y \rightarrow X$, their relationship follows this equation:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[X]}{[Y]}$$

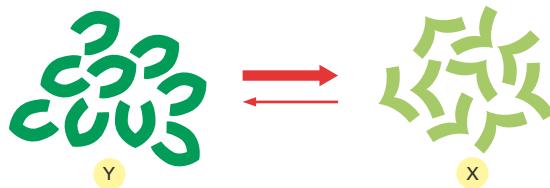
where ΔG is in kilocalories per mole, $[Y]$ and $[X]$ denote the concentrations

QUESTION 3-3

Consider the analogy of the jiggling box containing coins that was described on page 85. The reaction, the flipping of coins that either face heads up (H) or tails up (T), is described by the equation $H \leftrightarrow T$, where the rate of the forward reaction equals the rate of the reverse reaction.

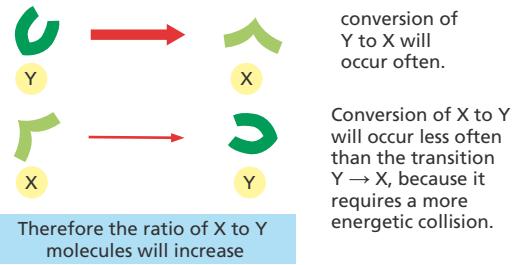
- A. What are ΔG and ΔG° in this analogy?
- B. What corresponds to the temperature at which the reaction proceeds? What corresponds to the activation energy of the reaction? Assume you have an “enzyme,” called *jigglase*, which catalyzes this reaction. What would the effect of *jigglase* be and what, mechanically, might *jigglase* do in this analogy?

FOR THE ENERGETICALLY FAVORABLE REACTION $Y \rightarrow X$,

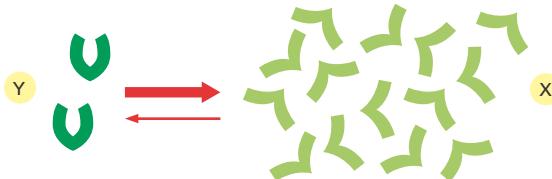


when X and Y are at equal concentrations, $[Y] = [X]$, the formation of X is energetically favored. In other words, the ΔG of $Y \rightarrow X$ is negative and the ΔG of $X \rightarrow Y$ is positive. But because of thermal bombardments, there will always be some X converting to Y.

THUS, FOR EACH INDIVIDUAL MOLECULE,



EVENTUALLY, there will be a large enough excess of X over Y to just compensate for the slow rate of $X \rightarrow Y$, such that the number of Y molecules being converted to X molecules each second is exactly equal to the number of X molecules being converted to Y molecules each second. At this point, the reaction will be at equilibrium.



AT EQUILIBRIUM, there is no net change in the ratio of Y to X, and the ΔG for both forward and backward reactions is zero.

of Y and X in moles/liter, \ln is the natural logarithm, and RT is the product of the gas constant, R , and the absolute temperature, T . At 37°C , $RT = 0.616$. (A mole is 6×10^{23} molecules of a substance.)

From this equation, we can see that when the concentrations of reactants and products are equal, in other words, $[X]/[Y] = 1$, the value of ΔG equals the value of ΔG° (because $\ln 1 = 0$). Thus when the reactants and products are present in equal concentrations, the direction of the reaction depends entirely on the intrinsic properties of the molecules.

The Equilibrium Constant Is Directly Proportional to ΔG°

As mentioned earlier, all chemical reactions tend to proceed toward equilibrium. Knowing where that equilibrium lies for any given reaction will tell you which way the reaction will proceed—and how far it will go. For example, if a reaction is at equilibrium when the concentration of the product is ten times the concentration of the substrate, and we begin with a surplus of substrate and little or no product, the reaction will proceed forward for some time. For the simple reaction $Y \rightarrow X$, that value—the ratio of substrate to product at equilibrium—is called the reaction's **equilibrium constant, K** . Expressed as an equation:

$$K = \frac{[X]}{[Y]}$$

where $[X]$ is the concentration of the product and $[Y]$ is the concentration of the substrate at equilibrium.

Figure 3–18 Reactions will eventually reach a chemical equilibrium. At that point, the forward and the backward fluxes of reacting molecules are equal and opposite. The widths of the arrows indicate the relative rates at which an *individual molecule* converts.

FREE ENERGY

This panel reviews the concept of free energy and offers examples showing how changes in free energy determine whether—and how—biological reactions occur.

The molecules of a living cell possess energy because of their vibrations, rotations, and movement through space, and because of the energy that is stored in the bonds between individual atoms.

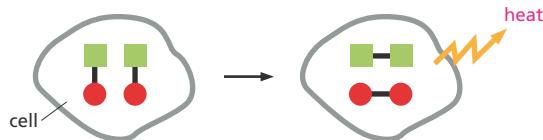


The **free energy**, G (in kcal/mole), measures the energy of a molecule which could in principle be used to do useful work at constant temperature, as in a living cell. Energy can also be expressed in joules (1 cal = 4.184 joules).

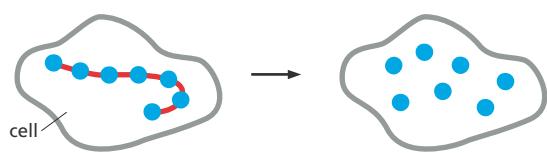
REACTIONS CAUSE DISORDER

Think of a chemical reaction occurring in a cell that has a constant temperature and volume. This reaction can produce disorder in two ways.

- 1 Changes of bond energy of the reacting molecules can cause heat to be released, which disorders the environment around the cell.



- 2 The reaction can decrease the amount of order in the cell—for example, by breaking apart a long chain of molecules, or by disrupting an interaction that prevents bond rotations.



PREDICTING REACTIONS

To predict the outcome of a reaction (Will it proceed to the right or to the left? At what point will it stop?), we must measure its **standard free-energy change** (ΔG°). This quantity represents the gain or loss of free energy as one mole of reactant is converted to one mole of product under “standard conditions” (all molecules present at a concentration of 1 M and pH 7.0).

ΔG° for some reactions

glucose-1-P → glucose-6-P	-1.7 kcal/mole
sucrose → glucose + fructose	-5.5 kcal/mole
ATP → ADP + P _i	-7.3 kcal/mole
glucose + 6O ₂ → 6CO ₂ + 6H ₂ O	-686 kcal/mole

driving force
↓

ΔG (“DELTA G”)

Changes in free energy occurring in a reaction are denoted by ΔG , where “ Δ ” indicates a difference. Thus, for the reaction



$$\Delta G = \text{free energy (C + D)} - \text{free energy (A + B)}$$

ΔG measures the amount of disorder caused by a reaction: the change in order inside the cell, plus the change in order of the surroundings caused by the heat released.

ΔG is useful because it measures how far away from equilibrium a reaction is. Thus the reaction



has a large negative ΔG because cells keep the reaction a long way from equilibrium by continually making fresh ATP. However, if the cell dies, then most of its ATP will be hydrolyzed, until equilibrium is reached; at equilibrium, the forward and backward reactions occur at equal rates and $\Delta G = 0$.

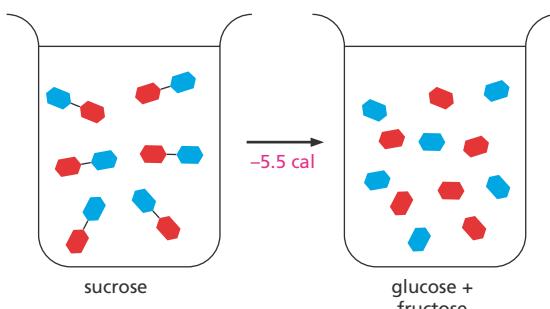
SPONTANEOUS REACTIONS

From the second law of thermodynamics, we know that the disorder of the universe can only increase. ΔG is **negative** if the disorder of the universe (reaction plus surroundings) **increases**.

In other words, a chemical reaction that occurs spontaneously must have a negative ΔG :

$$G_{\text{products}} - G_{\text{reactants}} = \Delta G < 0$$

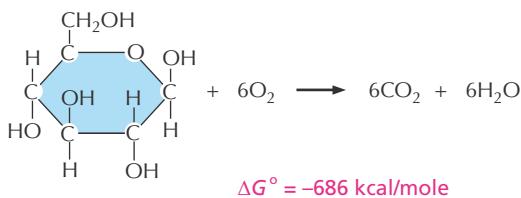
EXAMPLE: The difference in free energy of 100 ml of 10 mM sucrose (common sugar) and 100 ml of 10 mM glucose plus 10 mM fructose is about **-5.5 calories**. Therefore, the hydrolysis reaction that produces two monosaccharides from a disaccharide (sucrose → glucose + fructose) can proceed spontaneously.



In contrast, the reverse reaction (glucose + fructose → sucrose), which has a ΔG of **+5.5 calories**, could not occur without an input of energy from a coupled reaction.

REACTION RATES

A spontaneous reaction is not necessarily an instantaneous reaction: a reaction with a negative free-energy change (ΔG) will not necessarily occur rapidly by itself. Consider, for example, the combustion of glucose in oxygen:



Even this highly favorable reaction may not occur for centuries unless there are enzymes to speed up the process.

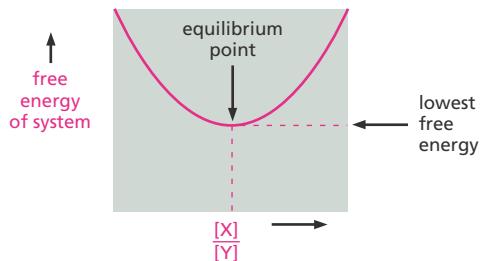
Enzymes are able to catalyze reactions and speed up their rate, but they cannot change the ΔG° of a reaction.

CHEMICAL EQUILIBRIA

A fixed relationship exists between the standard free-energy change of a reaction, ΔG° , and its equilibrium constant K . For example, the reversible reaction



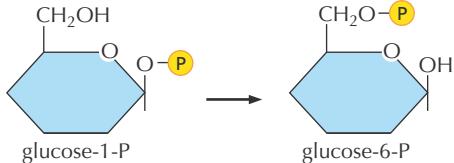
will proceed until the ratio of concentrations $[X]/[Y]$ is equal to K (note: square brackets [] indicate concentration). At this point, the free energy of the system will have its lowest value.



$$\text{At } 37^\circ\text{C}, \quad \Delta G^\circ = -1.42 \log_{10} K \quad (\text{see text, p. 98})$$

$$K = 10^{-\Delta G^\circ/1.42}$$

For example, the reaction



has $\Delta G^\circ = -1.74$ kcal/mole. Therefore, its equilibrium constant

$$K = 10^{(1.74/1.42)} = 10^{(1.23)} = 17$$

So the reaction will reach steady state when

$$[\text{glucose-6-P}] / [\text{glucose-1-P}] = 17$$

COUPLED REACTIONS

Reactions can be “coupled” together if they share one or more intermediates. In this case, the overall free-energy change is simply the sum of the individual ΔG° values. A reaction that is unfavorable (has a positive ΔG°) can for this reason be driven by a second, highly favorable reaction.

SINGLE REACTION

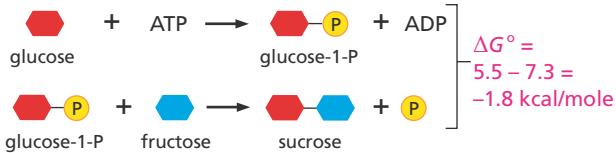


NET RESULT: reaction will not occur



NET RESULT: reaction is highly favorable

COUPLED REACTIONS



NET RESULT: sucrose is made in a reaction driven by the hydrolysis of ATP

HIGH-ENERGY BONDS

One of the most common reactions in the cell is **hydrolysis**, in which a covalent bond is split by adding water.



The ΔG° for this reaction is sometimes loosely termed the “bond energy.” Compounds such as acetyl phosphate and ATP, which have a large negative ΔG° of hydrolysis in an aqueous solution, are said to have “high-energy” bonds.

	ΔG° (kcal/mole)
acetyl P_i \longrightarrow acetate + P_i	-10.3
ATP \longrightarrow ADP + P_i	-7.3
glucose-6-P \longrightarrow glucose + P_i	-3.3

(Note that, for simplicity, H_2O is omitted from the above equations.)

But how do we know at what concentrations of substrate and product a reaction will reach equilibrium? It goes back to the intrinsic properties of the molecules involved, as expressed by ΔG° . Let's see why.

At equilibrium, the rate of the forward reaction is exactly balanced by the rate of the reverse reaction. At that point, $\Delta G = 0$, and there is no net change of free energy to drive the reaction in either direction (see Panel 3–1, pp. 96–97).

Now, if we return to the equation presented on p. 94,

$$\Delta G = \Delta G^\circ + RT \ln \frac{[X]}{[Y]}$$

we can see that, at equilibrium at 37°C, where $\Delta G = 0$ and the constant $RT = 0.616$, this equation becomes:

$$\Delta G^\circ = -0.616 \ln \frac{[X]}{[Y]}$$

In other words, ΔG° is directly proportional to the equilibrium constant, K :

$$\Delta G^\circ = -0.616 \ln K$$

If we convert this equation from natural log (\ln) to the more commonly used base-10 logarithm (\log), we get

$$\Delta G^\circ = -1.42 \log K$$

This equation reveals how the equilibrium ratio of Y to X, expressed as the equilibrium constant K , depends on the intrinsic character of the molecules, as expressed in the value of ΔG° (Table 3–1). It tells us that for every 1.42 kcal/mole difference in free energy at 37°C, the equilibrium constant changes by a factor of 10. Thus, the more energetically favorable the reaction, the more product will accumulate if the reaction proceeds to equilibrium.

In Complex Reactions, the Equilibrium Constant Includes the Concentrations of All Reactants and Products

We have so far discussed the simplest of reactions, $Y \rightarrow X$, in which a single substrate is converted into a single product. But inside cells, it is more common for two reactants to combine to form a single product: $A + B \rightleftharpoons AB$. How can we predict how this reaction will proceed?

The same principles apply, except that in this case the equilibrium constant K includes the concentrations of both of the reactants, in addition to the concentration of the product:

$$K = [AB]/[A][B]$$

As illustrated in Figure 3–19, the concentrations of both reactants are multiplied because the formation of product AB depends on the collision of A and B, and these encounters occur at a rate that is proportional to $[A] \times [B]$. As with single-substrate reactions, $\Delta G^\circ = -1.42 \log K$ at 37°C.

The Equilibrium Constant Indicates the Strength of Molecular Interactions

The concept of free-energy change does not only apply to chemical reactions where covalent bonds are being broken and formed, but also to interactions where one molecule binds to another by means of noncovalent interactions (see Chapter 2, p. 63). Noncovalent interactions are immensely important to cells. They include the binding of substrates to enzymes, the binding of gene regulatory proteins to DNA, and the binding of one protein to another to make the many different structural and functional protein complexes that operate in a living cell.

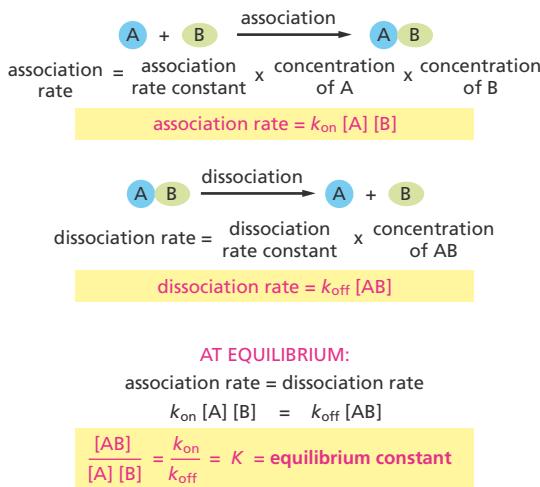
TABLE 3–1 RELATIONSHIP BETWEEN THE STANDARD FREE-ENERGY CHANGE, ΔG° , AND THE EQUILIBRIUM CONSTANT

Equilibrium Constant [X] [Y]	Standard Free Energy (ΔG°) of X minus Free Energy of Y in kcal/mole
10^5	-7.1
10^4	-5.7
10^3	-4.3
10^2	-2.8
10	-1.4
1	0
10^{-1}	1.4
10^{-2}	2.8
10^{-3}	4.3
10^{-4}	5.7
10^{-5}	7.1

Values of the equilibrium constant were calculated for the simple chemical reaction $Y \leftrightarrow X$, using the equation given in the text.

The ΔG° values given here are in kilocalories per mole at 37°C. As explained in the text, ΔG° represents the free-energy difference under standard conditions (where all components are present at a concentration of 1 mole/liter).

From this table, we see that, if there is a favorable free-energy change of -4.3 kcal/mole for the transition $Y \rightarrow X$, there will be 1000 times more molecules of X than of Y at equilibrium.



Two molecules will bind to each other if the free-energy change for the interaction is negative; that is, the free energy of the resulting complex is lower than the sum of the free energies of the two partners when unbound. Because the equilibrium constant of a reaction is related directly to ΔG° , K is commonly employed as a measure of the binding strength of a non-covalent interaction between two molecules. The binding strength is a very useful quantity to know because it also indicates how specific the interaction is between the two molecules.

Consider the reaction that was shown in Figure 3–19, where molecule A interacts with molecule B to form the complex AB. The reaction proceeds until it reaches equilibrium, at which point the number of association events precisely equals the number of dissociation events; at this point, the concentrations of reactants A and B, and of the complex AB, can be used to determine the equilibrium constant K .

K becomes larger as the *binding energy*—that is, the energy released in the binding interaction—increases. In other words, the larger K is, the greater is the drop in free energy between the dissociated and associated states, and the more tightly the two molecules will bind. Even a change of a few noncovalent bonds can have a striking effect on a binding interaction, as illustrated in Figure 3–20. In this example, eliminating a few hydrogen bonds from a binding interaction can be seen to cause a dramatic decrease in the amount of complex that exists at equilibrium.

For Sequential Reactions, the Changes in Free Energy Are Additive

Now we return to our original concern: how can enzymes catalyze reactions that are energetically unfavorable? One way they do so is by directly coupling energetically unfavorable reactions with energetically favorable ones. Consider, for example, two sequential reactions,



where the ΔG° values are +5 and -13 kcal/mole, respectively. (Recall that a mole is 6×10^{23} molecules of a substance.) The unfavorable reaction, $\text{X} \rightarrow \text{Y}$, will not occur spontaneously. However, it can be driven by the favorable reaction $\text{Y} \rightarrow \text{Z}$, provided that the second reaction follows the first. That's because the overall free-energy change for the coupled reaction is equal to the sum of the free-energy changes for each individual reaction. In this case, the ΔG° for the coupled reaction will be -8 kcal/mole, making the overall pathway energetically favorable.

Figure 3–19 The equilibrium constant (K) for the reaction $\text{A} + \text{B} \rightarrow \text{AB}$ depends on both the association and dissociation rate constants. Molecules A and B must collide in order to interact, and the association rate is therefore proportional to the product of their individual concentrations $[\text{A}] \times [\text{B}]$. As shown, the ratio of the rate constants k_{on} and k_{off} for the association and the dissociation reactions, respectively, is equal to the equilibrium constant, K , for the interaction. For two interacting components, K involves the concentrations of both substrates, in addition to that of the product. However, the relationship between K and ΔG° is the same as that shown in Table 3–1. The larger the value of K , the stronger is the binding between A and B.

Consider 1000 molecules of A and 1000 molecules of B in the cytosol of a eukaryotic cell. The concentration of both will be about 10^{-9} M.

If the equilibrium constant (K) for $\text{A} + \text{B} \leftrightarrow \text{AB}$ is 10^{10} liters/mole, then at equilibrium there will be

270	270	730
A molecules	B molecules	AB complexes

If the equilibrium constant is a little weaker, say 10^8 liters/mole—a value that represents a loss of 2.8 kcal/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds—then there will be

915	915	85
A molecules	B molecules	AB complexes

Figure 3–20 Small changes in the number of weak bonds can have drastic effects on a binding interaction. This example illustrates the dramatic effect of the presence or absence of a few weak noncovalent bonds in the interaction between two cytosolic proteins.

Cells can therefore cause the energetically unfavorable transition, $X \rightarrow Y$, to occur if an enzyme catalyzing the $X \rightarrow Y$ reaction is supplemented by a second enzyme that catalyzes the energetically favorable reaction, $Y \rightarrow Z$. In effect, the reaction $Y \rightarrow Z$ acts as a “siphon,” pulling the conversion of all of molecule X to molecule Y, and then to molecule Z (**Figure 3–21**). For example, several of the reactions in the long pathway that converts sugars into CO_2 and H_2O are energetically unfavorable. The pathway nevertheless proceeds rapidly to completion, however, because the total ΔG° for the series of sequential reactions has a large negative value.

Forming a sequential pathway, however, is not the answer for all metabolic needs. Often the desired reaction is simply $X \rightarrow Y$, without further conversion of Y to some other product. Fortunately, there are other, more general ways of using enzymes to couple reactions together, involving the production of activated carriers that can shuttle energy from one reaction site to another. We discuss these systems shortly. Before we do, let’s pause to look at how enzymes find and recognize their substrates and how enzyme-catalyzed reactions proceed. After all, thermodynamic considerations merely establish whether chemical reactions can occur; enzymes actually make them happen.

QUESTION 3–4

For the reactions shown in Figure 3–21, sketch an energy diagram similar to that in Figure 3–12 for the two reactions alone and for the combined reactions. Indicate the standard free-energy changes for the reactions $X \rightarrow Y$, $Y \rightarrow Z$, and $X \rightarrow Z$ in the graph. Indicate how enzymes that catalyze these reactions would change the energy diagram.

Thermal Motion Allows Enzymes to Find Their Substrates

Enzymes and their substrates are both present in relatively small amounts in the cytosol of a cell, yet a typical enzyme can capture and process about a thousand substrate molecules every second. This means that an enzyme can release its product and bind a new substrate in a fraction of a millisecond. How do these molecules find each other so quickly in the crowded cytosol of the cell?

Rapid binding is possible because molecular motions are enormously fast. Because of heat energy, molecules are in constant motion and consequently will explore the cytosolic space very efficiently by wandering

Figure 3–21 An energetically unfavorable reaction can be driven by an energetically favorable follow-on reaction that acts as a chemical siphon. (A) At equilibrium, there are twice as many X molecules as Y molecules. (B) At equilibrium, there are 25 times more Z molecules than Y molecules. (C) If the reactions in (A) and (B) are coupled, nearly all of the X molecules will be converted to Z molecules, as shown. In terms of energetics, the ΔG° of the $Y \rightarrow Z$ reaction is so negative that, when coupled to the $X \rightarrow Y$ reaction, it lowers the ΔG of $X \rightarrow Y$, because the ΔG of $X \rightarrow Y$ decreases as the ratio of Y to X declines. As shown in Figure 3–18, arrow widths reflect the relative rates at which an individual molecule converts; the arrow lengths are the same in both directions here, indicating that there is no net flux.

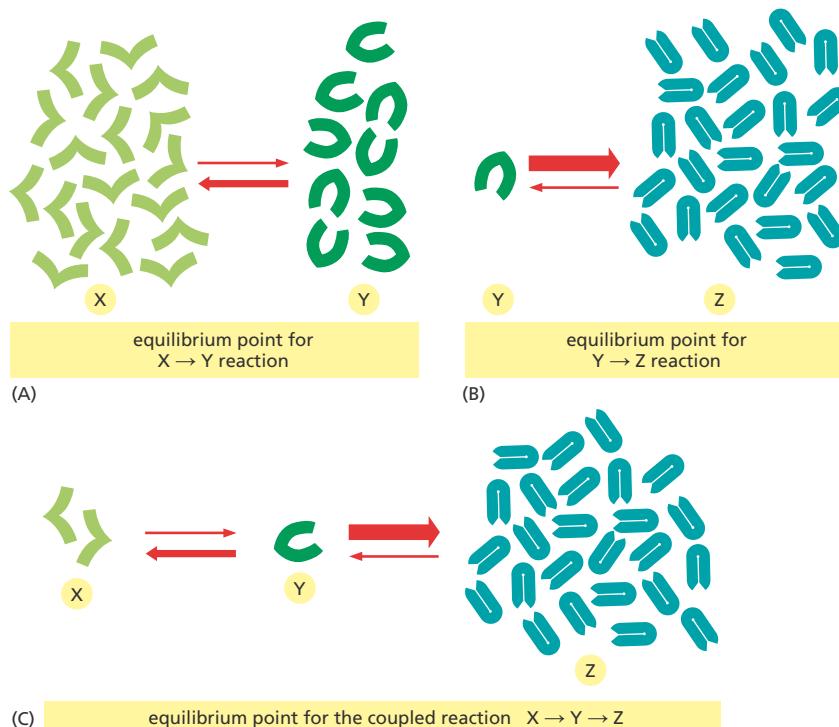


Figure 3–22 A molecule traverses the cytosol by taking a random walk. Molecules in solution move in a random fashion due to the continual buffeting they receive in collisions with other molecules. This movement allows small molecules to diffuse rapidly throughout the cell cytosol ([Movie 3.2](#)).

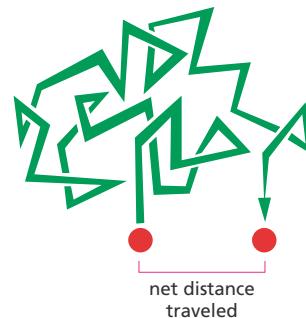
randomly through it—a process called **diffusion**. In this way, every molecule in the cytosol collides with a huge number of other molecules each second. As the molecules in a liquid collide and bounce off one another, an individual molecule moves first one way and then another, its path constituting a *random walk* (Figure 3–22).

Although the cytosol of a cell is densely packed with molecules of various shapes and sizes (Figure 3–23), experiments in which fluorescent dyes and other labeled molecules are injected into the cell cytosol show that small organic molecules diffuse through this aqueous gel nearly as rapidly as they do through water. A small organic molecule, such as a substrate, takes only about one-fifth of a second on average to diffuse a distance of 10 μm . Diffusion is therefore an efficient way for small molecules to move limited distances in the cell.

Because proteins diffuse through the cytosol much more slowly than do small molecules, the rate at which an enzyme will encounter its substrate depends on the concentration of the substrate. The most abundant substrates are present in the cell at a concentration of about 0.5 mM. Because pure water is 55 M, there is only about one such substrate molecule in the cell for every 10^5 water molecules. Nevertheless, the site on an enzyme that binds this substrate will be bombarded by about 500,000 random collisions with the substrate every second. For a substrate concentration tenfold lower (0.05 mM), the number of collisions drops to 50,000 per second, and so on.

The random encounters between an enzyme and its substrate often lead to the formation of an enzyme–substrate complex. This association is stabilized by the formation of multiple, weak bonds between the enzyme and substrate. These weak interactions—which can include hydrogen bonds, van der Waals attractions, and electrostatic attractions (discussed in Chapter 2)—persist until random thermal motion causes the molecules to dissociate again. When two colliding molecules have poorly matching surfaces, few noncovalent bonds are formed, and their total energy is negligible compared with that of thermal motion. In this case, the two molecules dissociate as rapidly as they come together (see Figure 2–33). This is what prevents incorrect and unwanted associations from forming between mismatched molecules, such as those between an enzyme and the wrong substrate. But when the enzyme and substrate are well matched, they form many weak interactions, which keep them held together long enough for a covalent bond in the substrate molecule to be formed or broken. Knowing the speed at which molecules collide and come apart, as well as how fast bonds can be formed and broken, makes the observed rate of enzymatic catalysis seem a little less amazing.

Figure 3–23 The cytosol is crowded with various molecules. Only the macromolecules, which are drawn to scale, are shown. RNAs are blue, ribosomes are green, and proteins are red. Enzymes and other macromolecules diffuse relatively slowly in the cytosol, in part because they interact with so many other macromolecules. Small molecules, by contrast, can diffuse nearly as rapidly as they do in water. (Adapted from D.S. Goodsell, *Trends Biochem. Sci.* 16:203–206, 1991. With permission from Elsevier.)

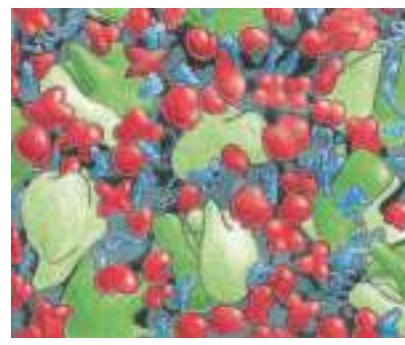


QUESTION 3–5

The enzyme carbonic anhydrase is one of the speediest enzymes known. It catalyzes the rapid conversion of CO_2 gas into the much more soluble bicarbonate ion (HCO_3^-). The reaction:

$\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$

is very important for the efficient transport of CO_2 from tissue, where CO_2 is produced by respiration, to the lungs, where it is exhaled. Carbonic anhydrase accelerates the reaction 10⁷-fold, hydrating 10⁵ CO_2 molecules per second at its maximal speed. What do you suppose limits the speed of the enzyme? Sketch a diagram analogous to the one shown in Figure 3–13 and indicate which portion of your diagram has been designed to display the 10⁷-fold acceleration.



100 nm

V_{\max} and K_M Measure Enzyme Performance

To catalyze a reaction, an enzyme must first bind its substrate. The substrate then undergoes a reaction to form the product, which initially remains bound to the enzyme. Finally, the product is released and diffuses away, leaving the enzyme free to bind another substrate molecule and catalyze another reaction (see Figure 3–15). The rates of the different steps vary widely from one enzyme to another, and they can be measured by mixing purified enzymes and substrates together under carefully defined conditions in a test tube (see **How We Know**, pp. 104–106).

In such experiments, the substrate is introduced in increasing concentrations to a solution containing a fixed concentration of enzyme. At first, the concentration of the enzyme–substrate complex—and therefore the rate at which product is formed—rises in a linear fashion in direct proportion to substrate concentration. However, as more and more enzyme molecules become occupied by substrate, this rate increase tapers off, until at a very high concentration of substrate it reaches a maximum value, termed V_{\max} . At this point, the active sites of all enzyme molecules in the sample are fully occupied by substrate, and the rate of product formation depends only on how rapidly the substrate molecule can undergo a reaction to form the product. For many enzymes, this **turnover number** is of the order of 1000 substrate molecules per second, although turnover numbers between 1 and 100,000 have been measured.

Because there is no clearly defined substrate concentration at which the enzyme can be deemed fully occupied, biochemists instead use a different parameter to gauge the concentration of substrate needed to make the enzyme work efficiently. This value is called the **Michaelis constant**, K_M , named after one of the biochemists who worked out the relationship. The K_M of an enzyme is defined as the concentration of substrate at which the enzyme works at half its maximum speed (**Figure 3–24**). In general, a small K_M indicates that a substrate binds very tightly to the enzyme, and a large K_M indicates weak binding.

Although an enzyme (or any catalyst) functions to lower the activation energy for a reaction such as $Y \rightarrow X$, it is important to note that the enzyme will also lower the activation energy for the reverse reaction $X \rightarrow Y$ to exactly the same degree. The forward and backward reactions will therefore be accelerated by the same factor by an enzyme, and the equilibrium point for the reaction—and thus its ΔG° —remains unchanged (**Figure 3–25**).

QUESTION 3–6

In cells, an enzyme catalyzes the reaction $AB \rightarrow A + B$. It was isolated, however, as an enzyme that carries out the opposite reaction $A + B \rightarrow AB$. Explain the paradox.

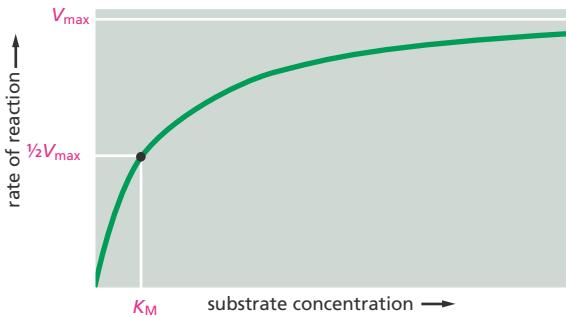


Figure 3–24 An enzyme's performance depends on how rapidly it can process its substrate. The rate of an enzyme reaction (V) increases as the substrate concentration increases, until a maximum value (V_{\max}) is reached. At this point, all substrate-binding sites on the enzyme molecules are fully occupied, and the rate of the reaction is limited by the rate of the catalytic process on the enzyme surface. For most enzymes, the concentration of substrate at which the reaction rate is half-maximal (K_M) is a direct measure of how tightly the substrate is bound, with a large value of K_M (a large amount of substrate needed) corresponding to weak binding.

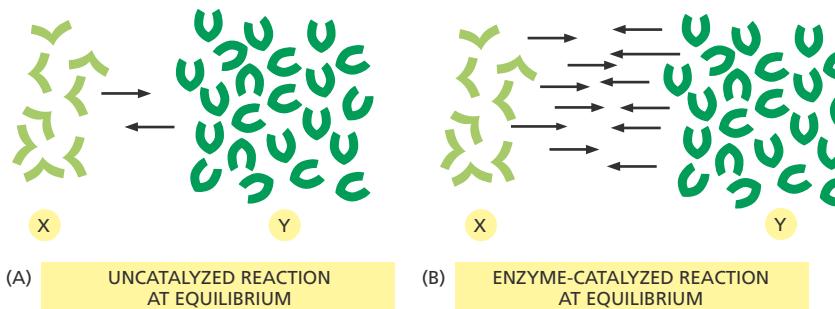


Figure 3–25 Enzymes cannot change the equilibrium point for reactions.

Enzymes, like all catalysts, speed up the forward and reverse rates of a reaction by the same amount. Therefore, for both the (A) uncatalyzed and (B) catalyzed reactions shown here, the number of molecules undergoing the transition $X \rightarrow Y$ is equal to the number of molecules undergoing the transition $Y \rightarrow X$ when the ratio of Y molecules to X molecules is 3.5 to 1, as illustrated. In other words, both the catalyzed and uncatalyzed reactions will eventually reach the same equilibrium point, although the catalyzed reaction will reach equilibrium faster.

ACTIVATED CARRIERS AND BIOSYNTHESIS

The energy released by energetically favorable reactions such as the oxidation of food molecules must be stored temporarily before it can be used by cells to fuel energetically unfavorable reactions, such as the synthesis of all the other molecules needed by the cell. In most cases, the energy is stored as chemical-bond energy in a set of *activated carriers*, small organic molecules that contain one or more energy-rich covalent bonds. These molecules diffuse rapidly and carry their bond energy from the sites of energy generation to the sites where energy is used for **biosynthesis** or for other energy-requiring cell activities (Figure 3–26).

Activated carriers store energy in an easily exchangeable form, either as a readily transferable chemical group or as readily transferable (“high energy”) electrons. They can serve a dual role as a source of both energy and chemical groups for biosynthetic reactions. The most important activated carriers are ATP and two molecules that are closely related to each other, NADH and NADPH. Cells use activated carriers like money to pay for the energetically unfavorable reactions that otherwise would not take place.

The Formation of an Activated Carrier Is Coupled to an Energetically Favorable Reaction

When a fuel molecule such as glucose is oxidized in a cell, enzyme-catalyzed reactions ensure that a large part of the free energy released is captured in a chemically useful form, rather than being released wastefully as heat. (Oxidizing sugar in a cell allows you to power metabolic reactions, whereas burning a chocolate bar in the street will get you nowhere, producing no metabolically useful energy.) In cells, energy capture is achieved by means of a **coupled reaction**, in which an energetically favorable reaction is used to drive an energetically unfavorable one that produces an activated carrier or some other useful molecule.

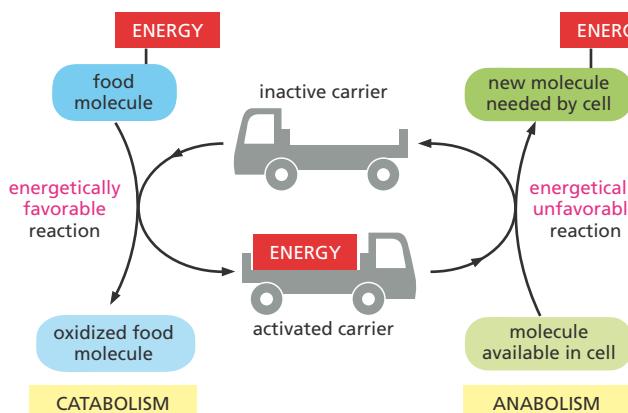


Figure 3–26 Activated carriers can store and transfer energy in a form that cells can use. By serving as intracellular energy shuttles, activated carriers perform their function as go-betweens that link the release of energy from the breakdown of food molecules (catabolism) to the energy-requiring biosynthesis of small and large organic molecules (anabolism).

MEASURING ENZYME PERFORMANCE

At first glance, it seems that a cell's metabolic pathways have been pretty well mapped out, with each reaction proceeding predictably to the next—substrate X is converted to product Y, which is passed along to enzyme Z. So why would anyone need to know exactly how tightly a particular enzyme clutches its substrate or whether it can process 100 or 1000 substrate molecules every second?

In reality, such elaborate metabolic maps merely suggest which pathways a cell might follow as it converts nutrients into small molecules, chemical energy, and the larger building blocks of life. Like a road map, they do not predict the density of traffic under a particular set of conditions: which pathways the cell will use when it is starving, when it is well fed, when oxygen is scarce, when it is stressed, or when it decides to divide. The study of an enzyme's *kinetics*—how fast it operates, how it handles its substrate, how its activity is controlled—makes it possible to predict how an individual catalyst will perform, and how it will interact with other enzymes in a network. Such knowledge leads to a deeper understanding of cell biology, and it opens the door to learning how to harness enzymes to perform desired reactions.

Speed

The first step to understanding how an enzyme performs involves determining the maximal velocity, V_{\max} , for the reaction it catalyzes. This is accomplished by measuring, in a test tube, how rapidly the reaction proceeds

in the presence of different concentrations of substrate (**Figure 3–27A**): the rate should increase as the amount of substrate rises until the reaction reaches its V_{\max} . The velocity of the reaction is measured by monitoring either how quickly the substrate is consumed or how rapidly the product accumulates. In many cases, the appearance of product or the disappearance of substrate can be observed directly with a spectrophotometer. This instrument detects the presence of molecules that absorb light at a particular wavelength; NADH, for example, absorbs light at 340 nm, while its oxidized counterpart, NAD^+ , does not. So, a reaction that generates NADH (by reducing NAD^+) can be monitored by following the formation of NADH at 340 nm in a spectrophotometer.

To determine the V_{\max} of a reaction, you would set up a series of test tubes, where each tube contains a different concentration of substrate. For each tube, add the same amount of enzyme and then measure the velocity of the reaction—the number of micromoles of substrate consumed or product generated per minute. Because these numbers will tend to decrease over time, the rate used is the velocity measured early in the reaction. These initial velocity values (v) are then plotted against the substrate concentration, yielding a curve like the one shown in **Figure 3–27B**.

Looking at this plot, however, it is difficult to determine the exact value of V_{\max} , as it is not clear where the reaction rate will reach its plateau. To get around this problem, the data are converted to their reciprocals

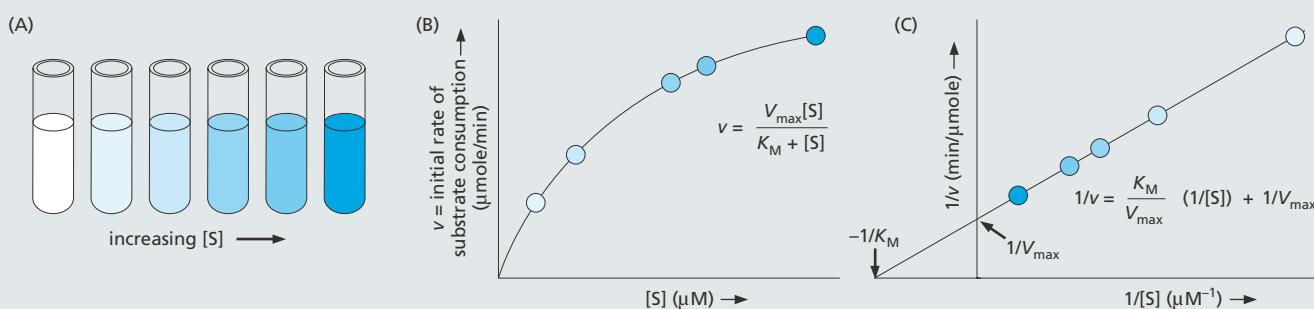


Figure 3–27 Measured reaction rates are plotted to determine V_{\max} and K_M of an enzyme-catalyzed reaction. (A) A series of increasing substrate concentrations is prepared, a fixed amount of enzyme is added, and initial reaction rates (velocities) are determined. (B) The initial velocities (v) plotted against the substrate concentrations $[S]$ give a curve described by the general equation $y = ax/(b + x)$. Substituting our kinetic terms, the equation becomes $v = V_{\max}[S]/(K_M + [S])$, where V_{\max} is the asymptote of the curve (the value of y at an infinite value of x), and K_M is equal to the substrate concentration where v is one-half V_{\max} . This is called the Michaelis-Menten equation, named for the biochemists who provided evidence for this enzymatic relationship. (C) In a double-reciprocal plot, $1/v$ is plotted against $1/[S]$. The equation describing this straight line is $1/v = (K_M/V_{\max})(1/[S]) + 1/V_{\max}$. When $1/[S] = 0$, the y intercept ($1/v$) is $1/V_{\max}$. When $1/v = 0$, the x intercept ($1/[S]$) is $-1/K_M$. Plotting the data this way allows V_{\max} and K_M to be calculated more precisely. By convention, lowercase letters are used for variables (hence v for velocity) and uppercase letters are used for constants (hence V_{\max}).

and graphed in a “double-reciprocal plot,” where the inverse of the velocity ($1/v$) appears on the y axis and the inverse of the substrate concentration ($1/[S]$) on the x axis (Figure 3–27C). This graph yields a straight line whose y intercept (the point where the line crosses the y axis) represents $1/V_{\max}$ and whose x intercept corresponds to $-1/K_M$. These values are then converted to values for V_{\max} and K_M .

Enzymologists use this technique to determine the kinetic parameters of many enzyme-catalyzed reactions (although these days computer programs automatically plot the data and spit out the sought-after values). Some reactions, however, happen too fast to be monitored in this way; the reaction is essentially complete—the substrate entirely consumed—within thousandths of a second. For these reactions, a special piece of equipment must be used to follow what happens during the first few milliseconds after enzyme and substrate meet (Figure 3–28).

Control

Substrates are not the only molecules that can influence how well or how quickly an enzyme works. In many cases, products, substrate lookalikes, inhibitors, and other small molecules can also increase or decrease enzyme activity. Such regulation allows cells to control when and how rapidly various reactions occur, a process we will consider in more detail in Chapter 4.

Determining how an inhibitor decreases an enzyme’s activity can reveal how a metabolic pathway is regulated—and can suggest how those control points can be circumvented by carefully designed mutations in specific genes.

The effect of an inhibitor on an enzyme’s activity is monitored in the same way that we measured the enzyme’s kinetics. A curve is first generated showing the velocity of the uninhibited reaction between enzyme and substrate, as described previously. Additional curves are then produced for reactions in which the inhibitor molecule has been included in the mix.

Comparing these curves, with and without inhibitor, can also reveal how a particular inhibitor impedes enzyme activity. For example, some inhibitors bind to the same site on an enzyme as its substrate. These *competitive inhibitors* block enzyme activity by competing directly with the substrate for the enzyme’s attention. They resemble the substrate enough to tie up the enzyme, but they differ enough in structure to avoid getting converted to product. This blockage can be overcome by adding enough substrate so that enzymes are more likely to encounter a substrate molecule than an inhibitor molecule. From the kinetic data, we can see that competitive inhibitors do not change the V_{\max} of a reaction; in other words, add enough substrate and the enzyme will encounter mostly substrate molecules and will reach its maximum velocity (Figure 3–29).

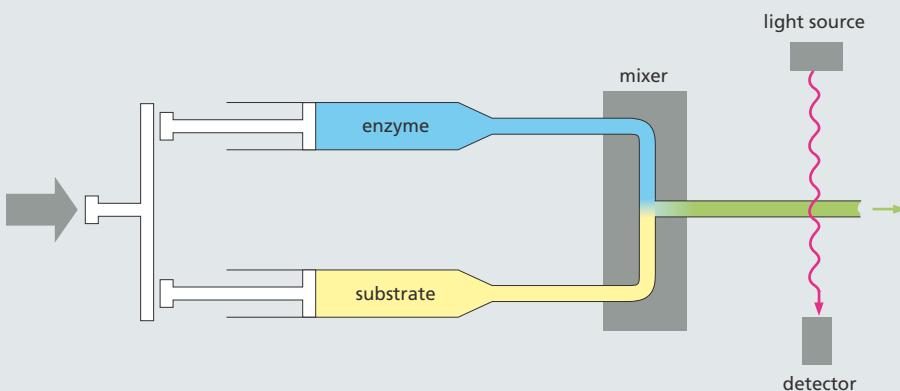


Figure 3–28 A stopped-flow apparatus is used to observe reactions during the first few milliseconds. In this piece of equipment, the enzyme and substrate are rapidly injected into a mixing chamber through two syringes. The enzyme and its substrate meet as they shoot through the mixing tube at flow rates that can easily reach 1000 cm/sec. They then enter another tube and zoom past a detector that monitors, say, the appearance of product. If the detector is located within a centimeter of where the enzyme and substrate meet, it is possible to observe reactions when they are only a few milliseconds old.

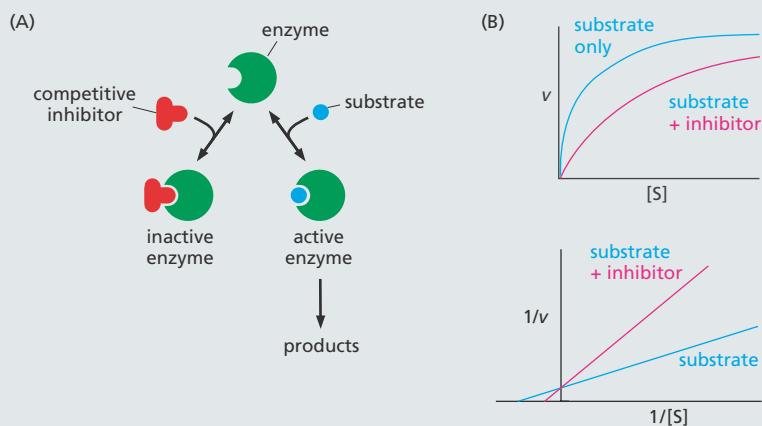


Figure 3–29 A competitive inhibitor directly blocks substrate binding to an enzyme. (A) The active site of the enzyme can bind either the competitive inhibitor or the substrate, but not both together. (B) The upper plot shows that inhibition by a competitive inhibitor can be overcome by increasing the substrate concentration. The double-reciprocal plot below shows that the V_{\max} of the reaction is not changed in the presence of the competitive inhibitor: the y intercept is identical for both the curves.

Competitive inhibitors can be used to treat patients who have been poisoned by ethylene glycol, an ingredient in commercially available antifreeze. Although ethylene glycol is itself not fatally toxic, a by-product of its metabolism—oxalic acid—can be lethal. To prevent oxalic acid from forming, the patient is given a large (though not quite intoxicating) dose of ethanol. Ethanol competes with the ethylene glycol for binding to alcohol dehydrogenase, the first enzyme in the pathway to oxalic acid formation. As a result, the ethylene glycol goes mostly unmetabolized and is safely eliminated from the body.

Other types of inhibitors may interact with sites on the enzyme distant from where the substrate binds. As we discuss in Chapter 4, many biosynthetic enzymes are regulated by feedback inhibition, whereby an enzyme early in a pathway will be shut down by a product generated later in the pathway. Because this type of inhibitor binds to a separate regulatory site on the enzyme, the substrate can still bind, but it might do so more slowly than it would in the absence of inhibitor. Such *noncompetitive inhibition* is not overcome by the addition of more substrate.

Design

With the kinetic data in hand, we can use computer modeling programs to predict how an enzyme will perform, and even how a cell will respond when exposed to different conditions—such as the addition of a particular sugar or amino acid to the culture medium, or the addition of a poison or a pollutant. Seeing how a cell manages its resources—which pathways it favors for dealing with particular biochemical challenges—can also suggest strategies for designing better catalysts for reactions of medical or commercial importance (e.g., for producing drugs or detoxifying industrial waste). Using such tactics, bacteria have even been genetically

engineered to produce large amounts of indigo—the dye, originally extracted from plants, that makes your blue jeans blue.

Computer programs have been developed to facilitate the dissection of complex reaction pathways. They require information about the components in the pathway, including the K_m and V_{\max} of the participating enzymes and the concentrations of enzymes, substrates, products, inhibitors, and other regulatory molecules. The program then predicts how molecules will flow through the pathway, which products will be generated, and where any bottlenecks might be. The process is not unlike balancing an algebraic equation, in which every atom of carbon, nitrogen, oxygen, and so on must be tallied. Such careful accounting makes it possible to rationally design ways to manipulate the pathway, such as re-routing it around a bottleneck, eliminating an important inhibitor, redirecting the reactions to favor the generation of predominantly one product, or extending the pathway to produce a novel molecule. Of course, such computer models must be validated in cells, which may not always behave as predicted.

Producing designer cells that spew out commercial products generally requires using genetic engineering techniques to introduce the gene or genes of choice into a cell, usually a bacterium, that can be manipulated and maintained in the laboratory. We discuss these methods at greater length in Chapter 10. Harnessing the power of cell biology for commercial purposes—even to produce something as simple as the amino acid tryptophan—is currently a multibillion-dollar industry. And, as more genome data come in, presenting us with more enzymes to exploit, it may not be long before vats of custom-made bacteria are churning out drugs and chemicals that represent the biological equivalent of pure gold.

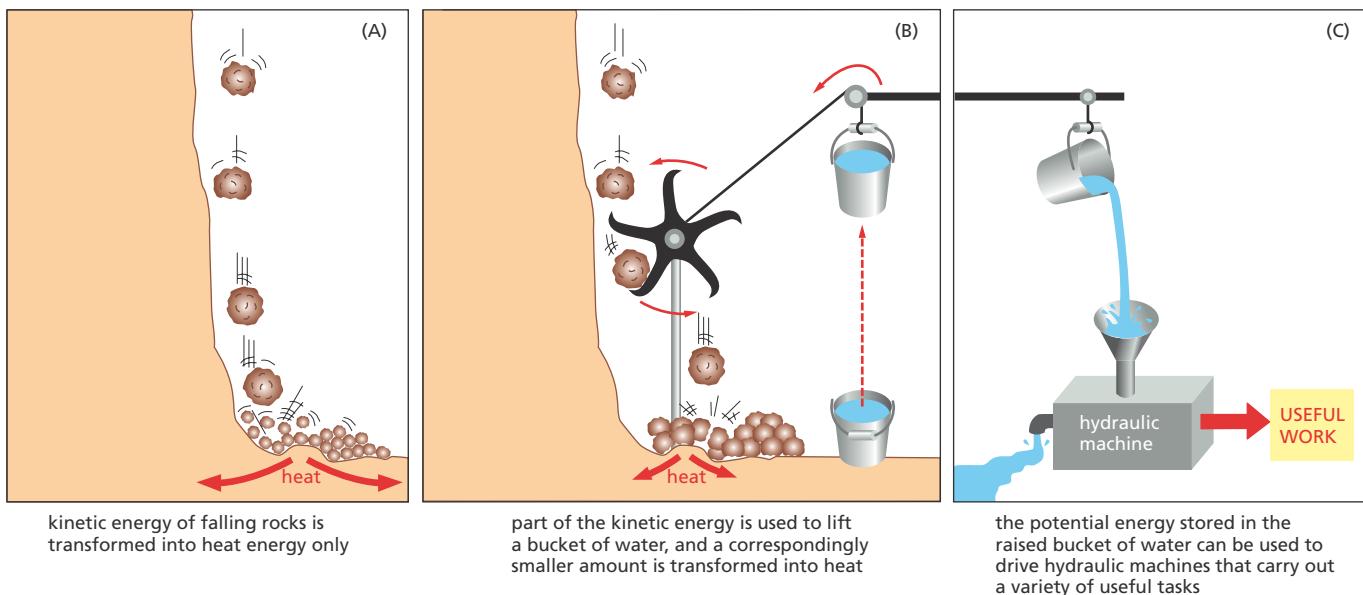


Figure 3–30 A mechanical model illustrates the principle of coupled chemical reactions. The spontaneous reaction shown in (A) could serve as an analogy for the direct oxidation of glucose to CO_2 and H_2O , which produces only heat. In (B), the same reaction is coupled to a second reaction, which could serve as an analogy for the synthesis of activated carriers. The energy produced in (B) is in a more useful form than in (A) and can be used to drive a variety of otherwise energetically unfavorable reactions (C).

Such coupling requires enzymes, which are fundamental to all of the energy transactions in the cell.

The nature of a coupled reaction is illustrated by a mechanical analogy in Figure 3–30, in which an energetically favorable chemical reaction is represented by rocks falling from a cliff. The kinetic energy of falling rocks would normally be entirely wasted in the form of heat generated by friction when the rocks hit the ground (Figure 3–30A). By careful design, however, part of this energy could be used to drive a paddle wheel that lifts a bucket of water (Figure 3–30B). Because the rocks can now reach the ground only after moving the paddle wheel, we say that the energetically favorable reaction of rocks falling has been directly coupled to the energetically unfavorable reaction of lifting the bucket of water. Because part of the energy is used to do work in (B), the rocks hit the ground with less velocity than in (A), and correspondingly less energy is wasted as heat. The energy saved in the elevated bucket of water can then be used to do useful work (Figure 3–30C).

Analogous processes occur in cells, where enzymes play the role of the paddle wheel in Figure 3–30B. By mechanisms that we discuss in Chapter 13, enzymes couple an energetically favorable reaction, such as the oxidation of foodstuffs, to an energetically unfavorable reaction, such as the generation of activated carriers. As a result, the amount of heat released by the oxidation reaction is reduced by exactly the amount of energy that is stored in the energy-rich covalent bonds of the activated carrier. That saved energy can then be used to power a chemical reaction elsewhere in the cell.

ATP Is the Most Widely Used Activated Carrier

The most important and versatile of the activated carriers in cells is **ATP** (adenosine 5'-triphosphate). Just as the energy stored in the raised bucket of water in Figure 3–30B can be used to drive a wide variety of hydraulic machines, ATP serves as a convenient and versatile store, or currency, of energy that can be used to drive a variety of chemical reactions in cells.

QUESTION 3–7

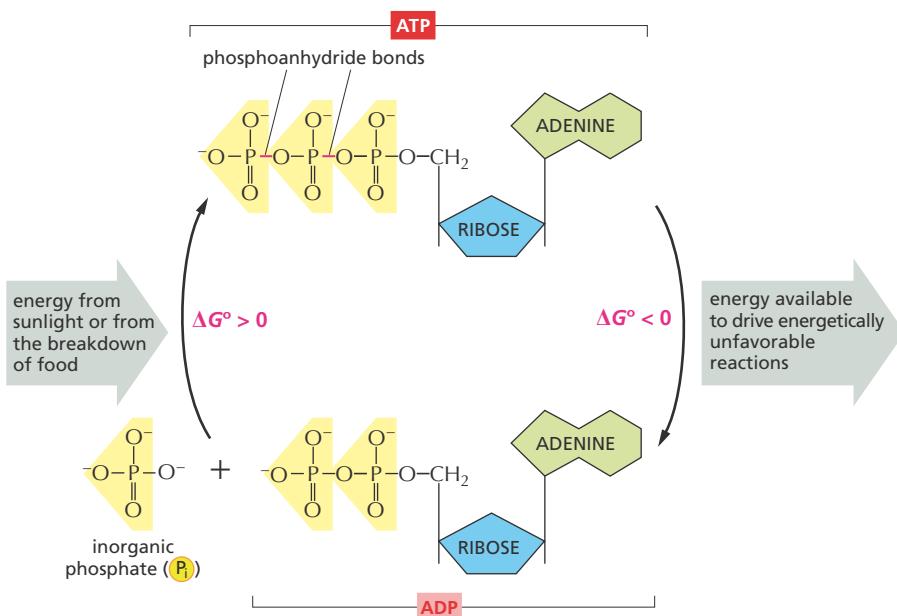
Use Figure 3–30B to illustrate the following reaction driven by the hydrolysis of ATP:



- A. In this case, which molecule or molecules would be analogous to (i) rocks at top of cliff, (ii) broken debris at bottom of cliff, (iii) bucket at its highest point, and (iv) bucket on the ground?
- B. What would be analogous to (i) the rocks hitting the ground in the absence of the paddle wheel in Figure 3–30A and (ii) the hydraulic machine in Figure 3–30C?

Figure 3–31 The interconversion of ATP and ADP occurs in a cycle. The two outermost phosphate groups in ATP are held to the rest of the molecule by high-energy phosphoanhydride bonds and are readily transferred to other organic molecules. Water can be added to ATP to form ADP and inorganic phosphate (P_i). Inside a cell, this hydrolysis of the terminal phosphate of ATP yields between 11 and 13 kcal/mole of usable energy. Although the ΔG° of this reaction is -7.3 kcal/mole, the ΔG is much more negative, because the ratio of ATP to the products ADP and P_i is so high inside the cell.

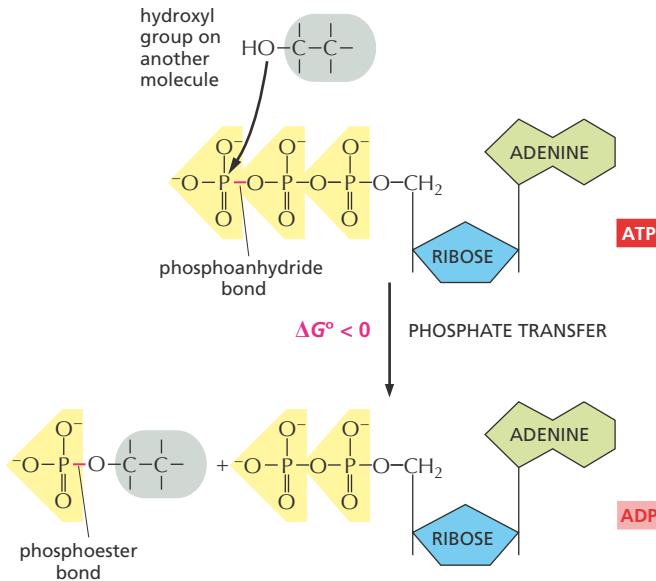
The large negative ΔG° of the reaction arises from a number of factors. Release of the terminal phosphate group removes an unfavorable repulsion between adjacent negative charges; in addition, the inorganic phosphate ion (P_i) released is stabilized by favorable hydrogen-bond formation with water. The formation of ATP from ADP and P_i reverses the hydrolysis reaction; because this condensation reaction is energetically unfavorable, it must be coupled to an energetically more favorable reaction to occur.



As shown in **Figure 3–31**, ATP is synthesized in an energetically unfavorable *phosphorylation* reaction, in which a phosphate group is added to ADP (adenosine 5'-diphosphate). When required, ATP gives up this energy packet in an energetically favorable hydrolysis to ADP and inorganic phosphate (P_i). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP, creating an ATP cycle in the cell.

The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. We will encounter several of these reactions in this chapter, where we will see exactly how this is done. ATP hydrolysis is often coupled to the transfer of the terminal phosphate in ATP to another molecule, as illustrated in **Figure 3–32**. Any reaction that involves the transfer of a phosphate group to a molecule is termed a phosphorylation reaction. Phosphorylation reactions are examples of condensation reactions (see Figure 2–25), and they occur in many important cell processes: they activate substrates, mediate the exchange of chemical energy, and serve as key constituents of intracellular signaling pathways (discussed in Chapter 16).

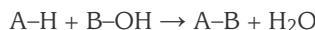
Figure 3–32 The terminal phosphate of ATP can be readily transferred to other molecules. Because an energy-rich phosphoanhydride bond in ATP is converted to a less energy-rich phosphoester bond in the phosphate-accepting molecule, this reaction is energetically favorable, having a large negative ΔG° (see Panel 3–1, pp. 96–97). Phosphorylation reactions of this type are involved in the synthesis of phospholipids and in the initial steps of the breakdown of sugars, as well as in many other metabolic and intracellular signaling pathways.



ATP is the most abundant activated carrier in cells. It is used, for example, to supply energy for many of the pumps that actively transport substances into or out of the cell (discussed in Chapter 12); it also powers the molecular motors that enable muscle cells to contract and nerve cells to transport materials along their lengthy axons (discussed in Chapter 17). Why evolution selected this particular nucleotide over the others as the major carrier of energy, however, remains a mystery. The nucleotide GTP, although similar, has very different functions in the cell, as we discuss in later chapters.

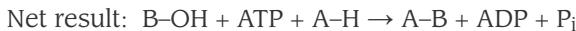
Energy Stored in ATP Is Often Harnessed to Join Two Molecules Together

A common type of reaction that is needed for biosynthesis is one in which two molecules, A and B, are joined together by a covalent bond to produce A–B in the energetically unfavorable condensation reaction:



ATP hydrolysis can be coupled indirectly to this reaction to make it go forward. In this case, energy from ATP hydrolysis is first used to convert B–OH to a higher-energy intermediate compound, which then reacts directly with A–H to give A–B. The simplest mechanism involves the transfer of a phosphate from ATP to B–OH to make B–O–PO₃, in which case the reaction pathway contains only two steps:

1. B–OH + ATP → B–O–PO₃ + ADP
2. A–H + B–O–PO₃ → A–B + P_i



The condensation reaction, which by itself is energetically unfavorable, has been forced to occur by being coupled to ATP hydrolysis in an enzyme-catalyzed reaction pathway (**Figure 3–33A**).

A biosynthetic reaction of exactly this type is employed to synthesize the amino acid glutamine, as illustrated in **Figure 3–33B**. We will see later in the chapter that very similar (but more complex) mechanisms are also used to produce nearly all of the large molecules of the cell.

NADH and NADPH Are Both Activated Carriers of Electrons

Other important activated carriers participate in oxidation-reduction reactions and are commonly part of coupled reactions in cells. These activated carriers are specialized to carry both high-energy electrons and hydrogen atoms. The most important of these *electron carriers* are **NADH** (nicotinamide adenine dinucleotide) and the closely related molecule **NADPH** (nicotinamide adenine dinucleotide phosphate). Both NADH and NADPH carry energy in the form of two high-energy electrons plus a proton (H⁺), which together form a hydride ion (H⁻). When these activated carriers pass their energy (in the form of a hydride ion) to a donor molecule, they become oxidized to form NAD⁺ and NADP⁺, respectively.

Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. NADPH is produced according to the general scheme shown in **Figure 3–34A**. During a special set of energy-yielding catabolic reactions, a hydride ion is removed from the substrate molecule and added to the nicotinamide ring of NADP⁺ to form NADPH. This is a typical oxidation-reduction reaction; the substrate is oxidized and NADP⁺ is reduced.

QUESTION 3–8

The phosphoanhydride bond that links two phosphate groups in ATP in a high-energy linkage has a ΔG° of –7.3 kcal/mole. Hydrolysis of this bond in a cell liberates from 11 to 13 kcal/mole of usable energy. How can this be? Why do you think a range of energies is given, rather than a precise number as for ΔG° ?

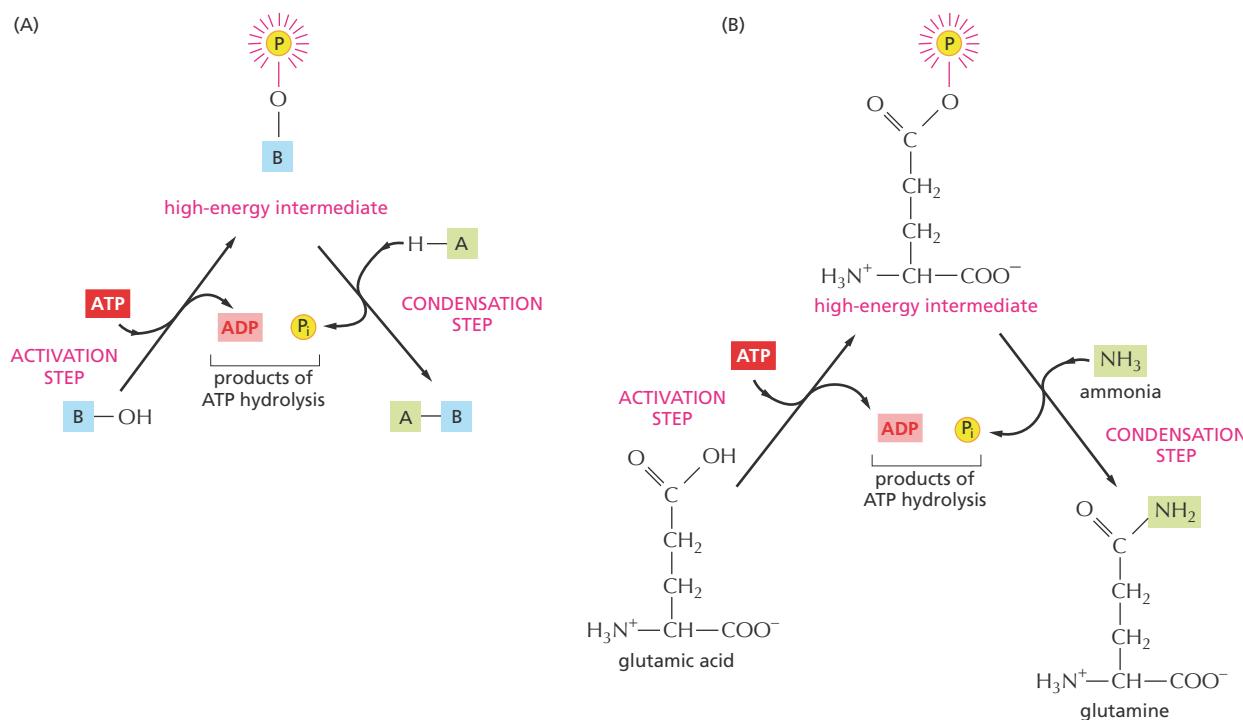


Figure 3–33 An energetically unfavorable biosynthetic reaction can be driven by ATP hydrolysis.

(A) Schematic illustration of the formation of A–B in the condensation reaction described in the text. (B) The biosynthesis of the amino acid glutamine from glutamic acid. Glutamic acid is first converted to a high-energy phosphorylated intermediate (corresponding to the compound B–O–PO₃ described in the text), which then reacts with ammonia (corresponding to A–H) to form glutamine. In this example, both steps occur on the surface of the same enzyme, glutamine synthetase (not shown). For clarity, the glutamic acid side chain is shown in its uncharged form. ATP hydrolysis can drive this energetically unfavorable reaction because it yields more energy (ΔG° of –7.3 kcal/mole) than the energy required for the synthesis of glutamine from glutamic acid plus NH₃ (ΔG° of +3.4 kcal/mole).

The hydride ion carried by NADPH is given up readily in a subsequent oxidation-reduction reaction, because the ring can achieve a more stable arrangement of electrons without it. In this subsequent reaction, which regenerates NADP⁺, the NADPH becomes oxidized and the substrate becomes reduced—thus completing the NADPH cycle. NADPH is efficient at donating its hydride ion to other molecules for the same reason that ATP readily transfers a phosphate: in both cases, the transfer is accompanied by a large negative free-energy change. One example of the use of NADPH in biosynthesis is shown in **Figure 3–35**.

NADPH and NADH Have Different Roles in Cells

NADPH and NADH differ in a single phosphate group, which is located far from the region involved in electron transfer in NADPH (Figure 3–34B). Although this phosphate group has no effect on the electron-transfer properties of NADPH compared with NADH, it is nonetheless crucial for their distinctive roles, as it gives NADPH a slightly different shape from NADH. This subtle difference in conformation makes it possible for the two carriers to bind as substrates to different sets of enzymes and thereby deliver electrons (in the form of hydride ions) to different target molecules.

Why should there be this division of labor? The answer lies in the need to regulate two sets of electron-transfer reactions independently. NADPH operates chiefly with enzymes that catalyze anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in

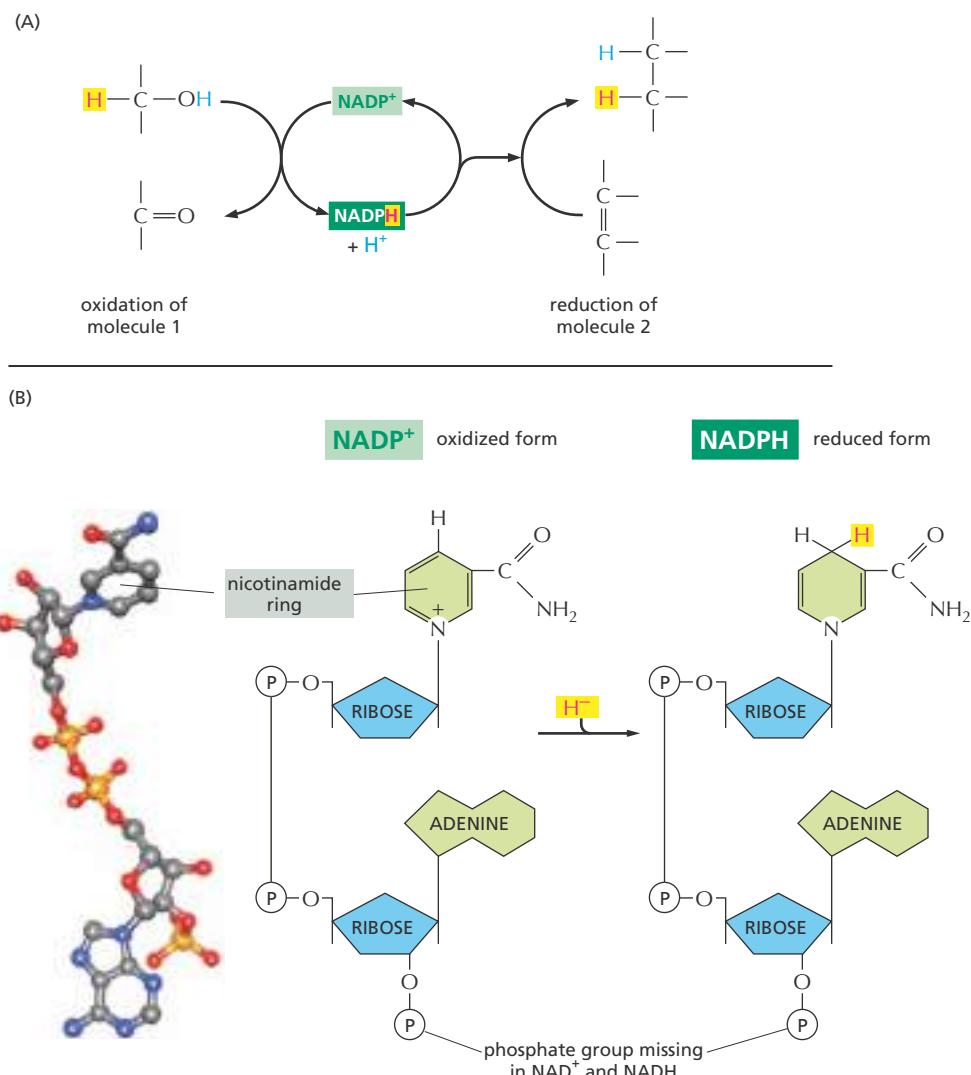


Figure 3–34 NADPH is an activated carrier of electrons. (A) NADPH is produced in reactions of the general type shown on the left, in which two hydrogen atoms are removed from a substrate. The oxidized form of the carrier molecule, NADP⁺, receives one hydrogen atom plus an electron (a hydride ion), while the proton (H⁺) from the other H atom is released into solution. Because NADPH holds its hydride ion in a high-energy linkage, the ion can easily be transferred to other molecules, as shown on the right. (B) The structure of NADP⁺ and NADPH. On the left is a ball-and-stick model of NADP. The part of the NADP⁺ molecule known as the nicotinamide ring accepts two electrons, together with a proton (the equivalent of a hydride ion, H⁻), forming NADPH. NAD⁺ and NADH are identical in structure to NADP⁺ and NADPH, respectively, except that they lack the phosphate group, as indicated.

the catabolic system of reactions that generate ATP through the oxidation of food molecules, as we discuss in Chapter 13. The genesis of NADH from NAD⁺ and that of NADPH from NADP⁺ occurs by different pathways that are independently regulated, so that the cell can adjust the supply of electrons for these two contrasting purposes. Inside the cell, the ratio of NAD⁺ to NADH is kept high, whereas the ratio of NADP⁺ to NADPH is kept low. This arrangement provides plenty of NAD⁺ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

Cells Make Use of Many Other Activated Carriers

In addition to ATP (which transfers a phosphate) and NADPH and NADH (which transfer electrons and hydrogen), cells make use of other activated carriers that pick up and carry a chemical group in an easily transferred, high-energy linkage. *FADH*₂, like NADH and NADPH, carries hydrogen and high-energy electrons (see Figure 13–13B). But other important reactions involve the transfers of acetyl, methyl, carboxyl, and glucose groups from activated carriers for the purpose of biosynthesis (Table 3–2). Coenzyme A, for example, can carry an acetyl group in a readily transferable linkage. This activated carrier, called **acetyl CoA** (acetyl coenzyme A), is shown in Figure 3–36. It is used, for example, to add sequentially two-carbon units in the biosynthesis of the hydrocarbon tails of fatty acids.

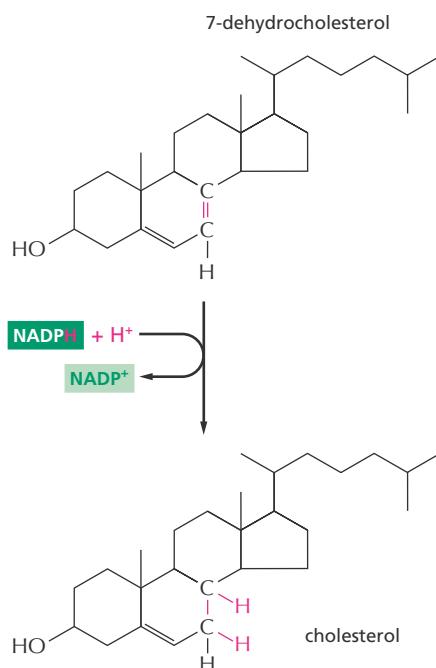


Figure 3–35 NADPH participates in the final stage of one of the biosynthetic routes leading to cholesterol. As in many other biosynthetic reactions, the reduction of the C=C bond is achieved by the transfer of a hydride ion from the activated carrier NADPH, plus a proton (H⁺) from solution.

TABLE 3-2 SOME ACTIVATED CARRIERS WIDELY USED IN METABOLISM

Activated Carrier	Group Carried in High-Energy Linkage
ATP	phosphate
NADH, NADPH, FADH ₂	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

In acetyl CoA and the other activated carriers in Table 3-2, the transferable group makes up only a small part of the molecule. The rest consists of a large organic portion that serves as a convenient “handle,” facilitating the recognition of the carrier molecule by specific enzymes. As with acetyl CoA, this handle portion very often contains a nucleotide. This curious fact may be a relic from an early stage of cell evolution. It is thought that the main catalysts for early life forms on Earth were RNA molecules (or their close relatives) and that proteins were a later evolutionary addition. It is therefore tempting to speculate that many of the activated carriers that we find today originated in an earlier RNA world, where their nucleotide portions would have been useful for binding these carriers to RNA-based catalysts, or *ribozymes* (discussed in Chapter 7).

Activated carriers are usually generated in reactions coupled to ATP hydrolysis, as shown for biotin in Figure 3-37. Therefore, the energy that enables their groups to be used for biosynthesis ultimately comes from the catabolic reactions that generate ATP. Similar processes occur in the synthesis of the very large macromolecules—the nucleic acids, proteins, and polysaccharides—which we discuss next.

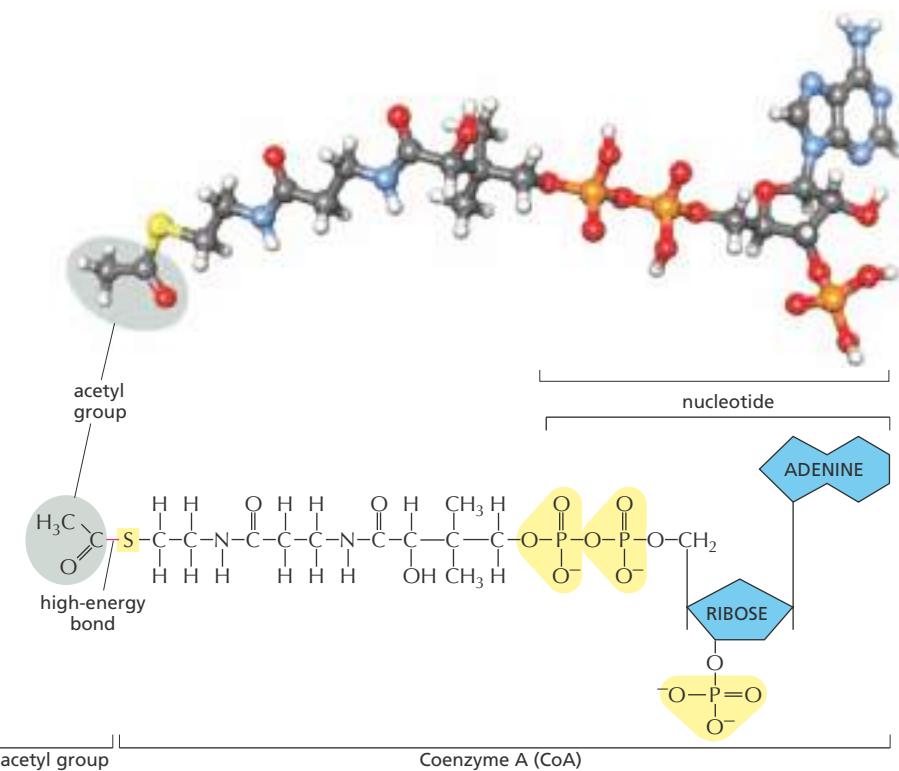


Figure 3-36 Acetyl coenzyme A (CoA) is another important activated carrier.

A ball-and-stick model is shown above the structure of acetyl CoA. The sulfur atom (yellow) forms a thioester bond to acetate. Because the thioester bond is a high-energy linkage, it releases a large amount of free energy when it is hydrolyzed; thus the acetyl group carried by CoA can be readily transferred to other molecules.

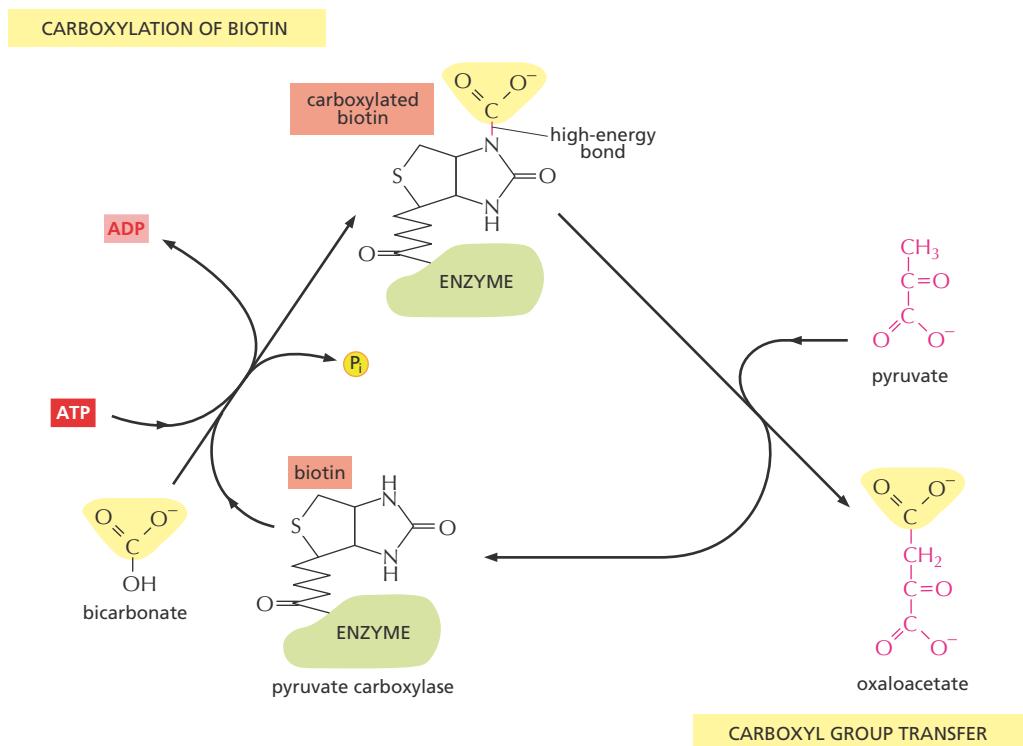


Figure 3–37 An activated carrier transfers a carboxyl group to a substrate. Biotin is a vitamin that is used by a number of enzymes, including pyruvate carboxylase shown here. Once it is carboxylated, biotin can transfer a carboxyl group to another molecule. Here, it transfers a carboxyl group to pyruvate, producing oxaloacetate, a molecule needed in the citric acid cycle (discussed in Chapter 13). Other enzymes use biotin to transfer carboxyl groups to other acceptor molecules. Note that the synthesis of carboxylated biotin requires energy derived from ATP hydrolysis—a general feature of many activated carriers.

The Synthesis of Biological Polymers Requires an Energy Input

The macromolecules of the cell constitute the vast majority of its dry mass—that is, the mass not due to water. These molecules are made from *subunits* (or monomers) that are linked together by bonds formed during an enzyme-catalyzed condensation reaction. The reverse reaction—the breakdown of polymers—occurs through enzyme-catalyzed hydrolysis reactions. These hydrolysis reactions are energetically favorable, whereas the corresponding biosynthetic reactions require an energy input and are more complex (Figure 3–38).

The nucleic acids (DNA and RNA), proteins, and polysaccharides are all polymers that are produced by the repeated addition of a subunit onto one end of a growing chain. The mode of synthesis of each of these macromolecules is outlined in Figure 3–39. As indicated, the condensation step in each case depends on energy provided by the hydrolysis of a nucleoside triphosphate. And yet, except for the nucleic acids, there are no phosphate groups left in the final product molecules. How, then, is the energy of ATP hydrolysis coupled to polymer synthesis?

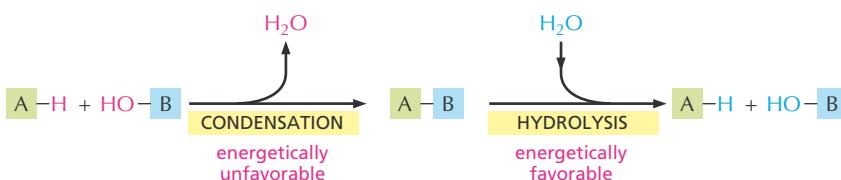
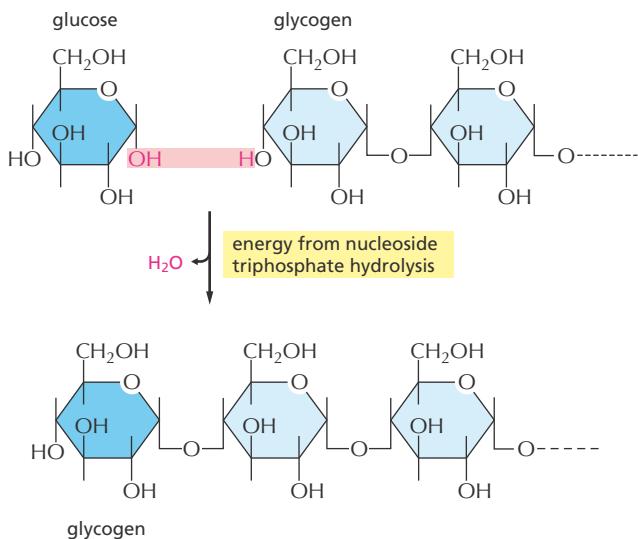
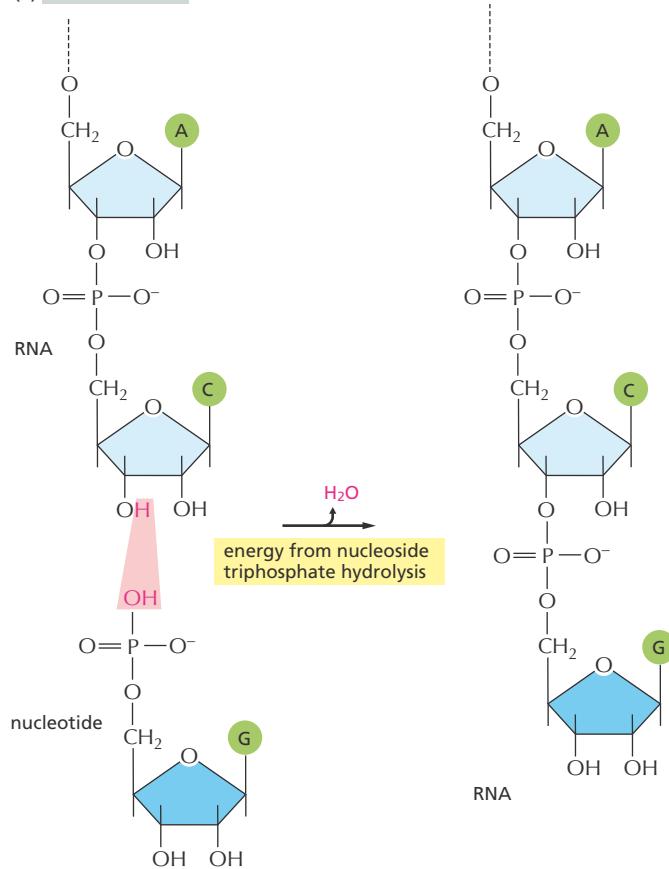


Figure 3–38 In cells, macromolecules are synthesized by condensation reactions and broken down by hydrolysis reactions. Condensation reactions are all energetically unfavorable, whereas hydrolysis reactions are all energetically favorable.

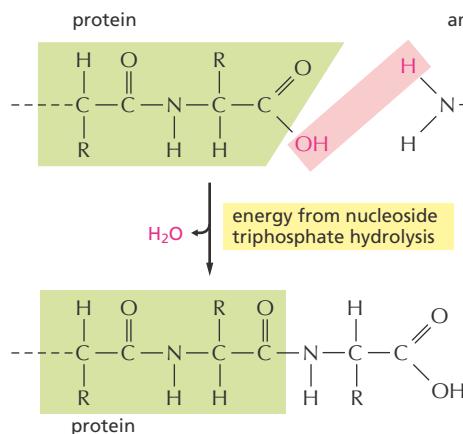
(A) POLYSACCHARIDES



(B) NUCLEIC ACIDS



(C) PROTEINS

**Figure 3–39** The synthesis of macromolecules requires an input of energy.

Synthesis of a portion of (A) a polysaccharide, (B) a nucleic acid, and (C) a protein is shown here. In each case, synthesis involves a condensation reaction in which water is lost; the atoms involved are shaded in pink. Not shown is the consumption of high-energy nucleoside triphosphates that is required to activate each subunit prior to its addition. In contrast, the reverse reaction—the breakdown of all three types of polymers—occurs through the simple addition of water, or hydrolysis (not shown).

QUESTION 3–9

- Which of the following reactions will occur only if coupled to a second, energetically favorable reaction?
- glucose + O₂ → CO₂ + H₂O
 - CO₂ + H₂O → glucose + O₂
 - nucleoside triphosphates → DNA
 - nucleotide bases → nucleoside triphosphates
 - ADP + P_i → ATP

For each type of macromolecule, an enzyme-catalyzed pathway exists, which resembles that discussed previously for the synthesis of the amino acid glutamine (see Figure 3–33). The principle is exactly the same, in that the -OH group that will be removed in the condensation reaction is first activated by forming a high-energy linkage to a second molecule. The mechanisms used to link ATP hydrolysis to the synthesis of proteins and polysaccharides, however, are more complex than that used for glutamine synthesis. In the biosynthetic pathways leading to these macromolecules, a series of high-energy intermediates generates the final high-energy bond that is broken during the condensation step (as discussed in Chapter 7 for protein synthesis).

There are limits to what each activated carrier can do in driving biosynthesis. For example, the ΔG for the hydrolysis of ATP to ADP and inorganic phosphate (P_i) depends on the concentrations of all of the reactants, and under the usual conditions in a cell, is between -11 and -13 kcal/mole. In principle, this hydrolysis reaction can be used to drive an unfavorable reaction with a ΔG of, perhaps, +10 kcal/mole, provided that a suitable reaction path is available. For some biosynthetic reactions, however, even -13 kcal/mole may be insufficient. In these cases, the path of ATP

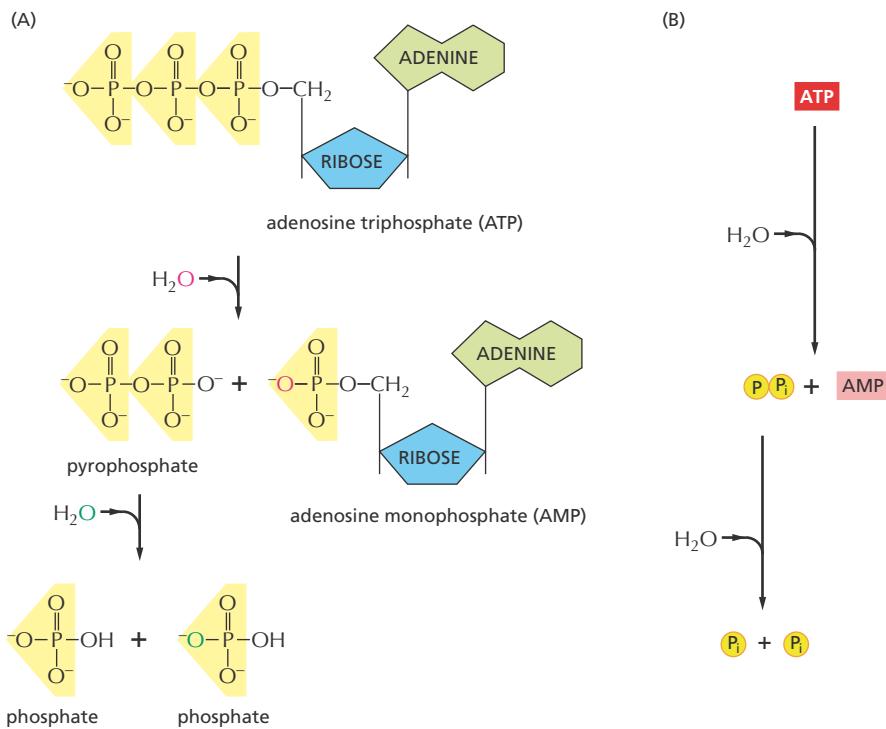


Figure 3–40 In an alternative route for the hydrolysis of ATP, pyrophosphate is first formed and then hydrolyzed in solution. This route releases about twice as much free energy as the reaction shown earlier in Figure 3–31. (A) In each of the two successive hydrolysis reactions, an oxygen atom from the participating water molecule is retained in the products, whereas the hydrogen atoms from water form free hydrogen ions, H^+ . (B) The overall reaction shown in summary form.

hydrolysis can be altered so that it initially produces AMP and pyrophosphate (PP_i), which is itself then hydrolyzed in solution in a subsequent step (Figure 3–40). The whole process makes available a total ΔG of about -26 kcal/mole. The biosynthetic reaction involved in the synthesis of nucleic acids (polynucleotides) is driven in this way (Figure 3–41).

ATP will make many appearances throughout the book as a molecule that powers reactions in the cell. And in Chapters 13 and 14, we discuss how the cell uses the energy from food to generate ATP. In the next chapter, we learn more about the proteins that make such reactions possible.

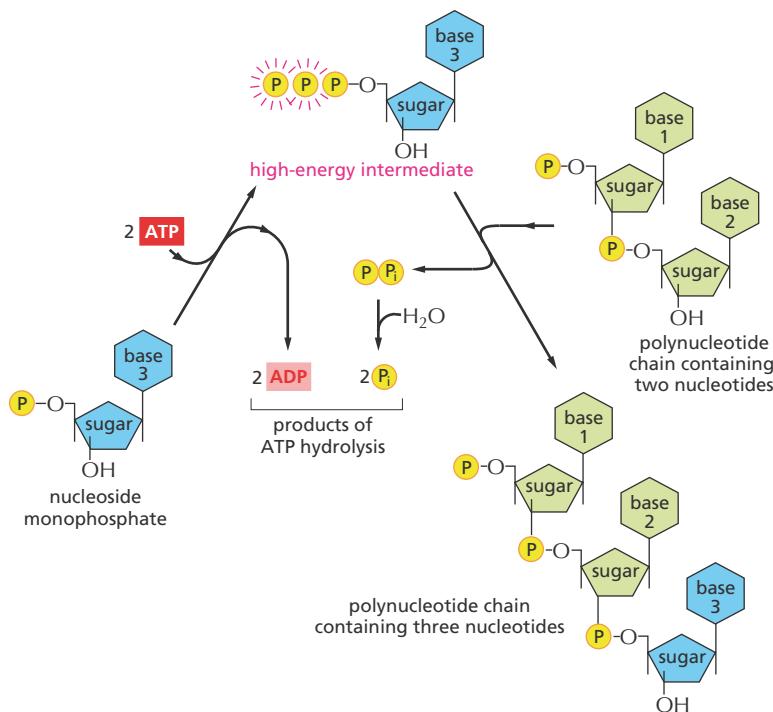


Figure 3–41 Synthesis of a polynucleotide, RNA or DNA, is a multistep process driven by ATP hydrolysis. In the first step, a nucleoside monophosphate is activated by the sequential transfer of the terminal phosphate groups from two ATP molecules. The high-energy intermediate formed—a nucleoside triphosphate—exists free in solution until it reacts with the growing end of an RNA or a DNA chain with release of pyrophosphate. Hydrolysis of the pyrophosphate to inorganic phosphate is highly favorable and helps to drive the overall reaction in the direction of polynucleotide synthesis.

ESSENTIAL CONCEPTS

- Living organisms are able to exist because of a continual input of energy. Part of this energy is used to carry out essential reactions that support cell metabolism, growth, movement, and reproduction; the remainder is lost in the form of heat.
- The ultimate source of energy for most living organisms is the sun. Plants, algae, and photosynthetic bacteria use solar energy to produce organic molecules from carbon dioxide. Animals obtain food by eating plants or by eating animals that feed on plants.
- Each of the many hundreds of chemical reactions that occur in a cell is specifically catalyzed by an enzyme. Large numbers of different enzymes work in sequence to form chains of reactions, called metabolic pathways, each performing a different function in the cell.
- Catabolic reactions release energy by breaking down organic molecules, including foods, through oxidative pathways. Anabolic reactions generate the many complex organic molecules needed by the cell, and they require an energy input. In animal cells, both the building blocks and the energy required for the anabolic reactions are obtained through catabolic reactions.
- Enzymes catalyze reactions by binding to particular substrate molecules in a way that lowers the activation energy required for making and breaking specific covalent bonds.
- The rate at which an enzyme catalyzes a reaction depends on how rapidly it finds its substrates and how quickly the product forms and then diffuses away. These rates vary widely from one enzyme to another.
- The only chemical reactions possible are those that increase the total amount of disorder in the universe. The free-energy change for a reaction, ΔG , measures this disorder, and it must be less than zero for a reaction to proceed spontaneously.
- The ΔG for a chemical reaction depends on the concentrations of the reacting molecules, and it may be calculated from these concentrations if the equilibrium constant (K) of the reaction (or the standard free-energy change, ΔG° , for the reactants) is known.
- Equilibrium constants govern all of the associations (and dissociations) that occur between macromolecules and small molecules in the cell. The larger the binding energy between two molecules, the larger the equilibrium constant and the more likely that these molecules will be found bound to each other.
- By creating a reaction pathway that couples an energetically favorable reaction to an energetically unfavorable one, enzymes can make otherwise impossible chemical transformations occur.
- A small set of activated carriers, particularly ATP, NADH, and NADPH, plays a central part in these coupled reactions in cells. ATP carries high-energy phosphate groups, whereas NADH and NADPH carry high-energy electrons.
- Food molecules provide the carbon skeletons for the formation of macromolecules. The covalent bonds of these larger molecules are produced by condensation reactions that are coupled to energetically favorable bond changes in activated carriers such as ATP and NADPH.

KEY TERMS

acetyl CoA	free energy, G
activated carrier	free-energy change, ΔG
activation energy	hydrolysis
ADP, ATP	K_M
anabolism	metabolism
biosynthesis	Michaelis constant (K_M)
catabolism	NAD ⁺ , NADH
catalysis	NADP ⁺ , NADPH
catalyst	oxidation
condensation reaction	photosynthesis
coupled reaction	reduction
diffusion	respiration
entropy	standard free-energy change, ΔG°
enzyme	substrate
equilibrium	turnover number
equilibrium constant, K	V_{max}

QUESTIONS

QUESTION 3–10

Which of the following statements are correct? Explain your answers.

- A. Some enzyme-catalyzed reactions cease completely if their enzyme is absent.
- B. High-energy electrons (such as those found in the activated carriers NADH and NADPH) move faster around the atomic nucleus.
- C. Hydrolysis of ATP to AMP can provide about twice as much energy as hydrolysis of ATP to ADP.
- D. A partially oxidized carbon atom has a somewhat smaller diameter than a more reduced one.
- E. Some activated carrier molecules can transfer both energy and a chemical group to a second molecule.
- F. The rule that oxidations release energy, whereas reductions require energy input, applies to all chemical reactions, not just those that occur in living cells.
- G. Cold-blooded animals have an energetic disadvantage because they release less heat to the environment than warm-blooded animals do. This slows their ability to make ordered macromolecules.
- H. Linking the reaction $X \rightarrow Y$ to a second, energetically favorable reaction $Y \rightarrow Z$ will shift the equilibrium constant of the first reaction.

QUESTION 3–11

Consider a transition of $X \rightarrow Y$. Assume that the only difference between X and Y is the presence of three hydrogen bonds in Y that are absent in X. What is the ratio of X to Y when the reaction is in equilibrium? Approximate your answer by using Table 3–1 (p. 98), with 1 kcal/mole as the energy of each hydrogen bond. If Y instead has six hydrogen bonds that distinguish it from X, how would that change the ratio?

QUESTION 3–12

Protein A binds to protein B to form a complex, AB. At equilibrium in a cell the concentrations of A, B, and AB are all at 1 μM .

- A. Referring to Figure 3–19, calculate the equilibrium constant for the reaction $A + B \rightleftharpoons AB$.
- B. What would the equilibrium constant be if A, B, and AB were each present in equilibrium at the much lower concentrations of 1 nM each?
- C. How many extra hydrogen bonds would be needed to hold A and B together at this lower concentration so that a similar proportion of the molecules are found in the AB complex? (Remember that each hydrogen bond contributes about 1 kcal/mole.)

QUESTION 3–13

Discuss the following statement: "Whether the ΔG for a reaction is larger, smaller, or the same as ΔG° depends on the concentration of the compounds that participate in the reaction."

QUESTION 3–14

- How many ATP molecules could maximally be generated from one molecule of glucose, if the complete oxidation of 1 mole of glucose to CO_2 and H_2O yields 686 kcal of free energy and the useful chemical energy available in the high-energy phosphate bond of 1 mole of ATP is 12 kcal?
- As we will see in Chapter 14 (Table 14–1), respiration produces 30 moles of ATP from 1 mole of glucose. Compare this number with your answer in part (A). What is the overall efficiency of ATP production from glucose?
- If the cells of your body oxidize 1 mole of glucose, by how much would the temperature of your body (assume that your body consists of 75 kg of water) increase if the heat were not dissipated into the environment? [Recall that a kilocalorie (kcal) is defined as that amount of energy that heats 1 kg of water by 1°C .]
- What would the consequences be if the cells of your body could convert the energy in food substances with only 20% efficiency? Would your body—as it is presently constructed—work just fine, overheat, or freeze?
- A resting human hydrolyzes about 40 kg of ATP every 24 hours. The oxidation of how much glucose would produce this amount of energy? (Hint: Look up the structure of ATP in Figure 2–24 to calculate its molecular weight; the atomic weights of H, C, N, O, and P are 1, 12, 14, 16, and 31, respectively.)

QUESTION 3–15

A prominent scientist claims to have isolated mutant cells that can convert 1 molecule of glucose into 57 molecules of ATP. Should this discovery be celebrated, or do you suppose that something might be wrong with it? Explain your answer.

QUESTION 3–16

In a simple reaction $\text{A} \rightleftharpoons \text{A}^*$, a molecule is interconvertible between two forms that differ in standard free energy G° by 4.3 kcal/mole, with A^* having the higher G° .

- Use Table 3–1 (p. 98) to find how many more molecules will be in state A^* compared with state A at equilibrium.
- If an enzyme lowered the activation energy of the reaction by 2.8 kcal/mole, how would the ratio of A to A^* change?

QUESTION 3–17

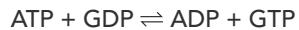
A reaction in a single-step biosynthetic pathway that converts a metabolite into a particularly vicious poison (metabolite \rightleftharpoons poison) in a mushroom is energetically highly unfavorable. The reaction is normally driven by ATP hydrolysis. Assume that a mutation in the enzyme that catalyzes the reaction prevents it from utilizing ATP, but still allows it to catalyze the reaction.

A. Do you suppose it might be safe for you to eat a mushroom that bears this mutation? Base your answer on an estimation of how much less poison the mutant mushroom would produce, assuming the reaction is in equilibrium and most of the energy stored in ATP is used to drive the unfavorable reaction in nonmutant mushrooms.

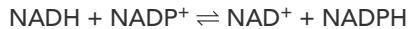
B. Would your answer be different for another mutant mushroom whose enzyme couples the reaction to ATP hydrolysis but works 100 times more slowly?

QUESTION 3–18

Consider the effects of two enzymes, A and B. Enzyme A catalyzes the reaction



and enzyme B catalyzes the reaction



Discuss whether the enzymes would be beneficial or detrimental to cells.

QUESTION 3–19

Discuss the following statement: "Enzymes and heat are alike in that both can speed up reactions that—although thermodynamically feasible—do not occur at an appreciable rate because they require a high activation energy. Diseases that seem to benefit from the careful application of heat—in the form of hot chicken soup, for example—are therefore likely to be due to the insufficient function of an enzyme."

QUESTION 3–20

The curve shown in Figure 3–24 is described by the Michaelis–Menten equation:

$$\text{rate} (v) = V_{\max} [\text{S}] / ([\text{S}] + K_M)$$

Can you convince yourself that the features qualitatively described in the text are accurately represented by this equation? In particular, how can the equation be simplified when the substrate concentration $[\text{S}]$ is in one of the following ranges: (A) $[\text{S}]$ is much smaller than the K_M , (B) $[\text{S}]$ equals the K_M , and (C) $[\text{S}]$ is much larger than the K_M ?

QUESTION 3–21

The rate of a simple enzyme reaction is given by the standard Michaelis–Menten equation:

$$\text{rate} = V_{\max} [\text{S}] / ([\text{S}] + K_M)$$

If the V_{\max} of an enzyme is 100 $\mu\text{mole/sec}$ and the K_M is 1 mM, at what substrate concentration is the rate 50 $\mu\text{mole/sec}$? Plot a graph of rate versus substrate (S) concentration for $[\text{S}] = 0$ to 10 mM. Convert this to a plot of $1/\text{rate}$ versus $1/[\text{S}]$. Why is the latter plot a straight line?

QUESTION 3–22

Select the correct options in the following and explain your choices. If $[\text{S}]$ is much smaller than K_M , the active site of the enzyme is mostly occupied/unoccupied. If $[\text{S}]$ is very much greater than K_M , the reaction rate is limited by the enzyme/substrate concentration.

QUESTION 3–23

A. The reaction rates of the reaction $S \rightarrow P$ catalyzed by enzyme E were determined under conditions such that only very little product was formed. The following data were measured:

Substrate concentration (μM)	Reaction rate ($\mu\text{mole}/\text{min}$)
0.08	0.15
0.12	0.21
0.54	0.7
1.23	1.1
1.82	1.3
2.72	1.5
4.94	1.7
10.00	1.8

Plot the above data as a graph. Use this graph to estimate the K_M and the V_{\max} for this enzyme.

B. Recall from the How We Know essay (pp. 104–106) that to determine these values more precisely, a trick is generally used in which the Michaelis–Menten equation is transformed so that it is possible to plot the data as a straight line.

A simple rearrangement yields

$$1/\text{rate} = (K_M/V_{\max}) (1/[S]) + 1/V_{\max}$$

which is an equation of the form $y = ax + b$. Calculate $1/\text{rate}$ and $1/[S]$ for the data given in part (A) and then plot $1/\text{rate}$ versus $1/[S]$ as a new graph. Determine K_M and V_{\max} from the intercept of the line with the axis, where $1/[S] = 0$, combined with the slope of the line. Do your results agree with the estimates made from the first graph of the raw data?

C. It is stated in part (A) that only very little product was formed under the reaction conditions. Why is this important?

D. Assume the enzyme is regulated such that upon phosphorylation its K_M increases by a factor of 3 without changing its V_{\max} . Is this an activation or inhibition? Plot the data you would expect for the phosphorylated enzyme in both the graph for (A) and the graph for (B).

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CHAPTER FOUR

4

Protein Structure and Function

When we look at a cell in a microscope or analyze its electrical or biochemical activity, we are, in essence, observing the handiwork of proteins. **Proteins** are the main building blocks from which cells are assembled, and they constitute most of the cell's dry mass. In addition to providing the cell with shape and structure, proteins also execute nearly all its myriad functions. *Enzymes* promote intracellular chemical reactions by providing intricate molecular surfaces, contoured with particular bumps and crevices that can cradle or exclude specific molecules. Proteins embedded in the plasma membrane form the channels and pumps that control the passage of nutrients and other small molecules into and out of the cell. Other proteins carry messages from one cell to another, or act as signal integrators that relay information from the plasma membrane to the nucleus of individual cells. Some proteins act as motors that propel organelles through the cytoplasm, and others function as components of tiny molecular machines with precisely calibrated moving parts. Specialized proteins also act as antibodies, toxins, hormones, antifreeze molecules, elastic fibers, or luminescence generators. Before we can hope to understand how genes work, how muscles contract, how nerves conduct electricity, how embryos develop, or how our bodies function, we must understand proteins.

The multiplicity of functions carried out by proteins (**Panel 4–1**, p. 122) arises from the huge number of different shapes they adopt. We therefore begin our description of these remarkable macromolecules by discussing their three-dimensional structures and the properties that these structures confer. We next look at how proteins work: how enzymes catalyze chemical reactions, how some proteins act as molecular switches, and how others generate orderly movement. We then examine how cells

THE SHAPE AND STRUCTURE OF PROTEINS

HOW PROTEINS WORK

HOW PROTEINS ARE CONTROLLED

HOW PROTEINS ARE STUDIED

ENZYMES

function: Catalyze covalent bond breakage or formation.



examples: Living cells contain thousands of different enzymes, each of which catalyzes (speeds up) one particular reaction. Examples include: *tryptophan synthetase*—makes the amino acid tryptophan; *pepsin*—degrades dietary proteins in the stomach; *ribulose bisphosphate carboxylase*—helps convert carbon dioxide into sugars in plants; *DNA polymerase*—copies DNA; *protein kinase*—adds a phosphate group to a protein molecule.

STRUCTURAL PROTEINS

function: Provide mechanical support to cells and tissues.



examples: Outside cells, *collagen* and *elastin* are common constituents of extracellular matrix and form fibers in tendons and ligaments. Inside cells, *tubulin* forms long, stiff microtubules, and *actin* forms filaments that underlie and support the plasma membrane; *keratin* forms fibers that reinforce epithelial cells and is the major protein in hair and horn.

TRANSPORT PROTEINS

function: Carry small molecules or ions.



examples: In the bloodstream, *serum albumin* carries lipids, *hemoglobin* carries oxygen, and *transferrin* carries iron. Many proteins embedded in cell membranes transport ions or small molecules across the membrane. For example, the bacterial protein *bacteriorhodopsin* is a light-activated proton pump that transports H⁺ ions out of the cell; *glucose carriers* shuttle glucose into and out of cells; and a Ca²⁺ pump clears Ca²⁺ from a muscle cell's cytosol after the ions have triggered a contraction.

MOTOR PROTEINS

function: Generate movement in cells and tissues.



examples: *Myosin* in skeletal muscle cells provides the motive force for humans to move; *kinesin* interacts with microtubules to move organelles around the cell; *dynein* enables eukaryotic cilia and flagella to beat.

STORAGE PROTEINS

function: Store amino acids or ions.



examples: Iron is stored in the liver by binding to the small protein *ferritin*; *ovalbumin* in egg white is used as a source of amino acids for the developing bird embryo; *casein* in milk is a source of amino acids for baby mammals.

SIGNAL PROTEINS

function: Carry extracellular signals from cell to cell.



examples: Many of the hormones and growth factors that coordinate physiological functions in animals are proteins; *insulin*, for example, is a small protein that controls glucose levels in the blood; *netrin* attracts growing nerve cell axons to specific locations in the developing spinal cord; *nerve growth factor (NGF)* stimulates some types of nerve cells to grow axons; *epidermal growth factor (EGF)* stimulates the growth and division of epithelial cells.

RECEPTOR PROTEINS

function: Detect signals and transmit them to the cell's response machinery.



examples: *Rhodopsin* in the retina detects light; the *acetylcholine receptor* in the membrane of a muscle cell is activated by acetylcholine released from a nerve ending; the *insulin receptor* allows a cell to respond to the hormone insulin by taking up glucose; the *adrenergic receptor* on heart muscle increases the rate of the heartbeat when it binds to adrenaline.

GENE REGULATORY PROTEINS

function: Bind to DNA to switch genes on or off.



examples: The *lactose repressor* in bacteria silences the genes for the enzymes that degrade the sugar lactose; many different *homeodomain proteins* act as genetic switches to control development in multicellular organisms, including humans.

SPECIAL-PURPOSE PROTEINS

function: Highly variable.



examples: Organisms make many proteins with highly specialized properties. These molecules illustrate the amazing range of functions that proteins can perform. The *antifreeze proteins* of Arctic and Antarctic fishes protect their blood against freezing; *green fluorescent protein* from jellyfish emits a green light; *monellin*, a protein found in an African plant, has an intensely sweet taste; mussels and other marine organisms secrete *glue proteins* that attach them firmly to rocks, even when immersed in seawater.

control the activity and location of the proteins they contain. Finally, we present a brief description of the techniques that biologists use to work with proteins, including methods for purifying them—from tissues or cultured cells—and for determining their structures.

THE SHAPE AND STRUCTURE OF PROTEINS

From a chemical point of view, proteins are by far the most structurally complex and functionally sophisticated molecules known. This is perhaps not surprising, considering that the structure and activity of each protein has been developed and fine-tuned over billions of years of evolution. We start by considering how the position of each amino acid in the long string of amino acids that forms a protein determines its three-dimensional shape, which is stabilized by noncovalent interactions between different parts of the molecule. Understanding the structure of a protein at the atomic level allows us to see how the precise shape of the protein determines its function.

The Shape of a Protein Is Specified by Its Amino Acid Sequence

Proteins, as you may recall from Chapter 2, are assembled mainly from a set of 20 different amino acids, each with different chemical properties. A protein molecule is made from a long chain of these amino acids, held together by covalent peptide bonds (Figure 4–1). Proteins are therefore referred to as **polypeptides**, and their amino acid chains are called **polypeptide chains**. In each type of protein, the amino acids are present in a unique order, called the **amino acid sequence**, which is exactly the same from one molecule of that protein to the next. One molecule of human insulin, for example, has the same amino acid sequence as every other molecule of human insulin. Many thousands of different proteins have been identified, each with its own distinct amino acid sequence.

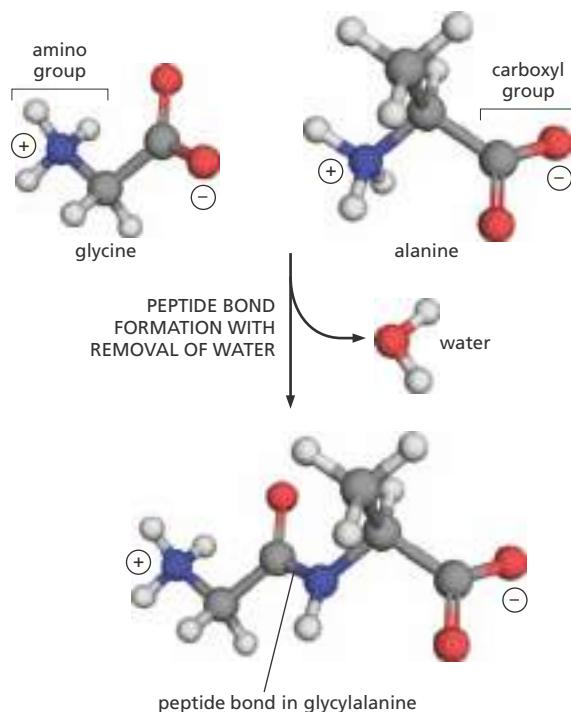
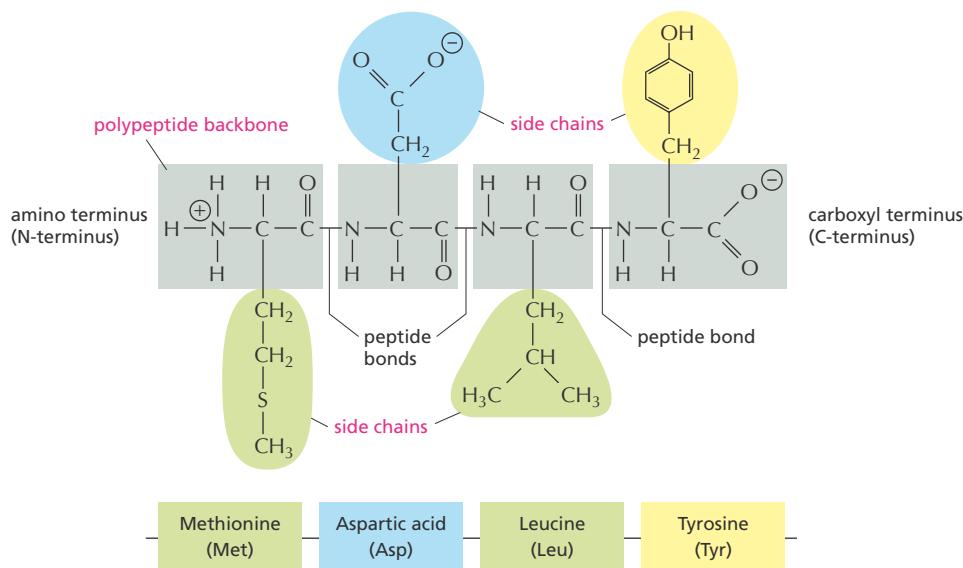


Figure 4–1 Amino acids are linked together by peptide bonds. A covalent peptide bond forms when the carbon atom of the carboxyl group of one amino acid (such as glycine) shares electrons with the nitrogen atom (blue) from the amino group of a second amino acid (such as alanine). Because a molecule of water is eliminated, peptide bond formation is classified as a condensation reaction (see Figure 2–29). In this diagram, carbon atoms are gray, nitrogen blue, oxygen red, and hydrogen white.

Figure 4–2 A protein is made of amino acids linked together into a polypeptide chain. The amino acids are linked by peptide bonds (see Figure 4–1) to form a polypeptide backbone of repeating structure (gray boxes), from which the side chain of each amino acid projects. The character and sequence of the chemically distinct side chains—for example, nonpolar (green), polar uncharged (yellow), and negative (blue) side chains—give each protein its distinct, individual properties. A small polypeptide of just four amino acids is shown here. Proteins are typically made up of chains of several hundred amino acids, whose sequence is always presented starting with the N-terminus reading from left to right.



Each polypeptide chain consists of a backbone that is adorned with a variety of chemical side chains. This **polypeptide backbone** is formed from a repeating sequence of the core atoms ($-\text{N}-\text{C}-\text{C}-$) found in every amino acid (see Figure 4–1). Because the two ends of each amino acid are chemically different—one sports an amino group (NH_3^+ , also written NH_2) and the other a carboxyl group (COO^- , also written COOH)—each polypeptide chain has a directionality: the end carrying the amino group is called the **amino terminus**, or **N-terminus**, and the end carrying the free carboxyl group is the **carboxyl terminus**, or **C-terminus**.

Projecting from the polypeptide backbone are the amino acid **side chains**—the part of the amino acid that is not involved in forming peptide bonds (Figure 4–2). The side chains give each amino acid its unique properties: some are nonpolar and hydrophobic (“water-fearing”), some are negatively or positively charged, some can be chemically reactive, and so on. The atomic formula for each of the 20 amino acids in proteins is presented in Panel 2–5 (pp. 74–75), and a brief list of the 20 common amino acids, with their abbreviations, is provided in Figure 4–3.

AMINO ACID		SIDE CHAIN		AMINO ACID		SIDE CHAIN	
Aspartic acid	Asp	D	negatively charged	Alanine	Ala	A	nonpolar
Glutamic acid	Glu	E	negatively charged	Glycine	Gly	G	nonpolar
Arginine	Arg	R	positively charged	Valine	Val	V	nonpolar
Lysine	Lys	K	positively charged	Leucine	Leu	L	nonpolar
Histidine	His	H	positively charged	Isoleucine	Ile	I	nonpolar
Asparagine	Asn	N	uncharged polar	Proline	Pro	P	nonpolar
Glutamine	Gln	Q	uncharged polar	Phenylalanine	Phe	F	nonpolar
Serine	Ser	S	uncharged polar	Methionine	Met	M	nonpolar
Threonine	Thr	T	uncharged polar	Tryptophan	Trp	W	nonpolar
Tyrosine	Tyr	Y	uncharged polar	Cysteine	Cys	C	nonpolar

Polar amino acids Nonpolar amino acids

Figure 4–3 Twenty different amino acids are commonly found in proteins. Both three-letter and one-letter abbreviations are given, as well as the character of the side chain. There are equal numbers of polar (hydrophilic) and nonpolar (hydrophobic) side chains, and half of the polar side chains carry a positive or negative charge.

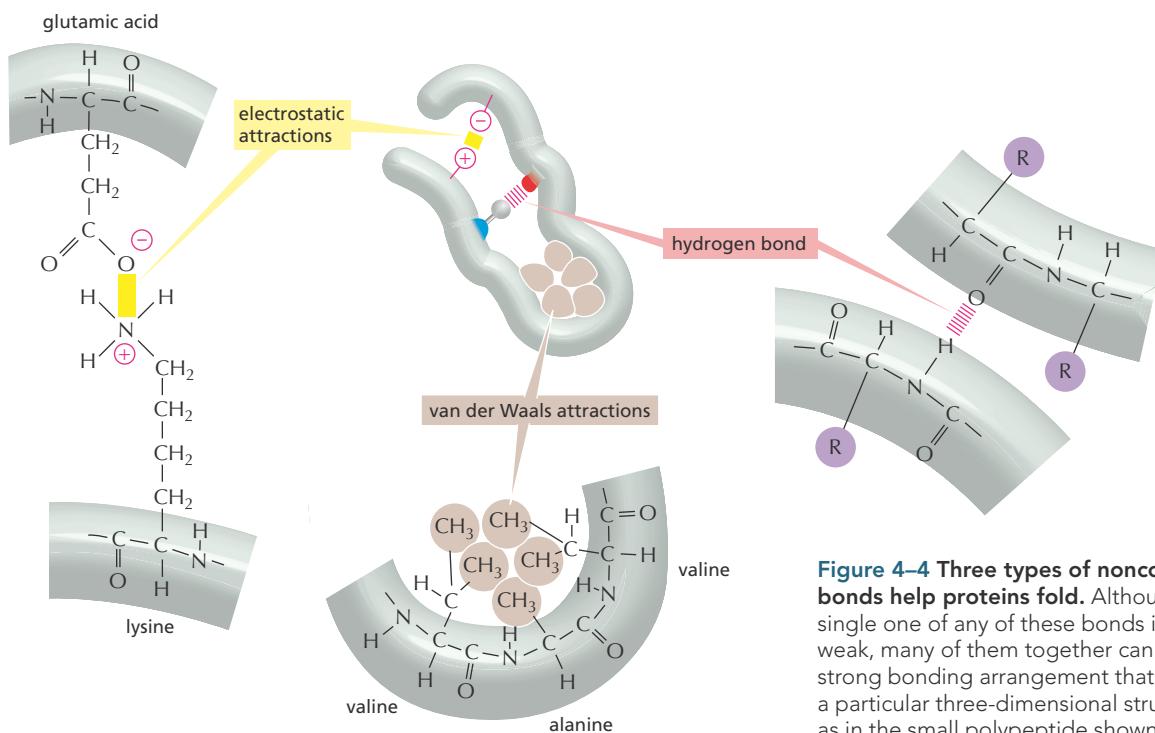


Figure 4-4 Three types of noncovalent

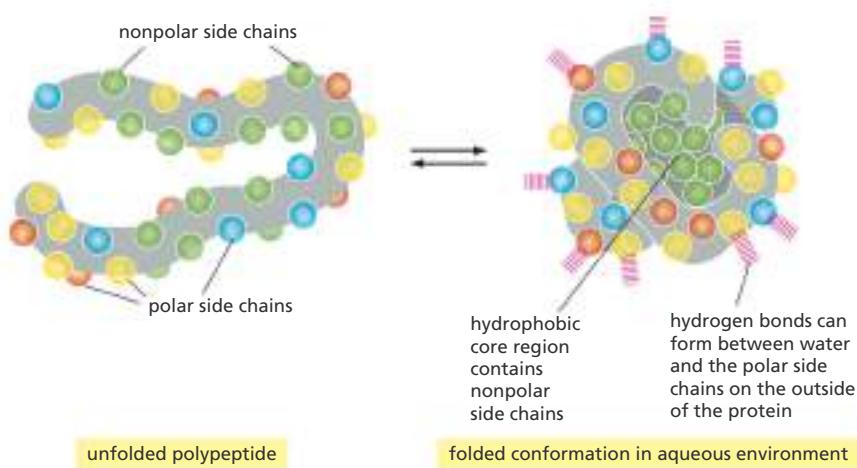
bonds help proteins fold. Although a single one of any of these bonds is quite weak, many of them together can create a strong bonding arrangement that stabilizes a particular three-dimensional structure, as in the small polypeptide shown in the center. R is often used as a general designation for an amino acid side chain. Protein folding is also aided by hydrophobic forces, as shown in Figure 4-5.

Long polypeptide chains are very flexible, as many of the peptide bonds that link the carbon atoms in the polypeptide backbone allow free rotation of the atoms they join. Thus, proteins can in principle fold in an enormous number of ways. The shape of each of these folded chains, however, is constrained by many sets of weak noncovalent bonds that form within proteins. These bonds involve atoms in the polypeptide backbone, as well as atoms in the amino acid side chains. The noncovalent bonds that help proteins fold up and maintain their shape include hydrogen bonds, electrostatic attractions, and van der Waals attractions, which are described in Chapter 2 (see Panel 2-7, pp. 78–79). Because a noncovalent bond is much weaker than a covalent bond, it takes many noncovalent bonds to hold two regions of a polypeptide chain tightly together. The stability of each folded shape is largely influenced by the combined strength of large numbers of noncovalent bonds (Figure 4-4).

A fourth weak force, *hydrophobic interaction*, also has a central role in determining the shape of a protein. In an aqueous environment, hydrophobic molecules, including the nonpolar side chains of particular amino acids, tend to be forced together to minimize their disruptive effect on the hydrogen-bonded network of the surrounding water molecules (see Panel 2-2, pp. 68–69). Therefore, an important factor governing the folding of any protein is the distribution of its polar and nonpolar amino acids. The nonpolar (hydrophobic) side chains—which belong to amino acids such as phenylalanine, leucine, valine, and tryptophan (see Figure 4-3)—tend to cluster in the interior of the folded protein (just as hydrophobic oil droplets coalesce to form one large drop). Tucked away inside the folded protein, hydrophobic side chains can avoid contact with the aqueous cytosol that surrounds them inside a cell. In contrast, polar side chains—such as those belonging to arginine, glutamine, and histidine—tend to arrange themselves near the outside of the folded protein, where they can form hydrogen bonds with water and with other polar molecules (Figure 4-5). When polar amino acids are buried within the protein, they are usually hydrogen-bonded to other polar amino acids or to the polypeptide backbone (Figure 4-6).

Figure 4–5 Hydrophobic forces help proteins fold into compact conformations.

Polar amino acid side chains tend to be displayed on the outside of the folded protein, where they can interact with water; the nonpolar amino acid side chains are buried on the inside to form a highly packed hydrophobic core of atoms that are hidden from water.

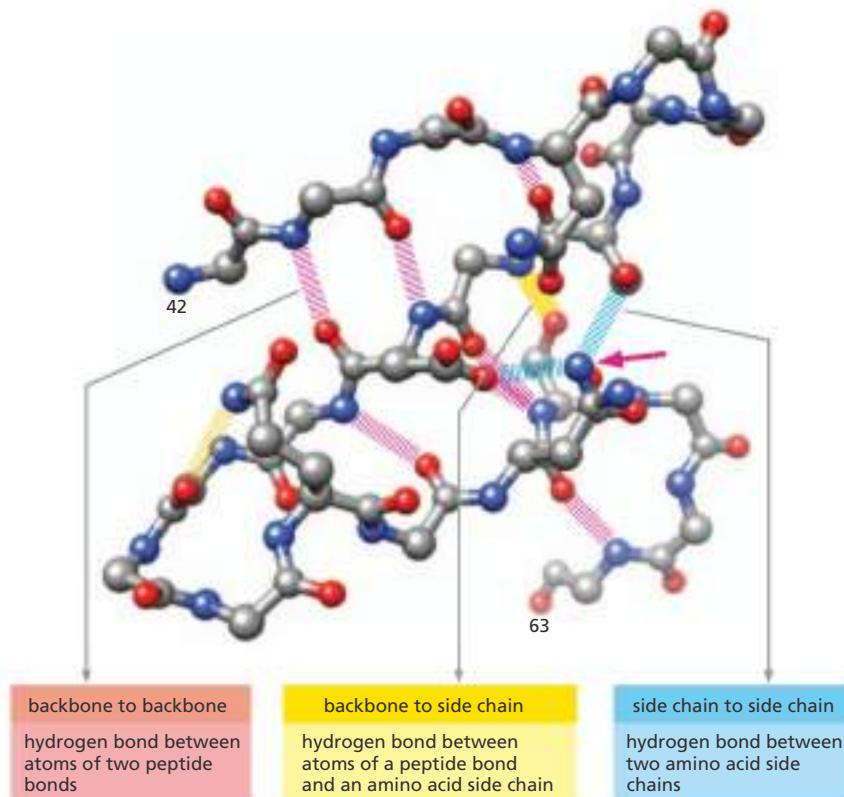


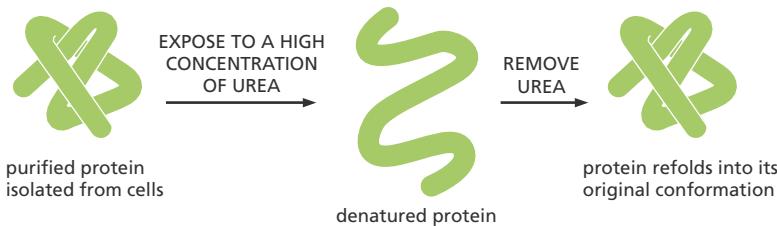
Proteins Fold into a Conformation of Lowest Energy

Each type of protein has a particular three-dimensional structure, which is determined by the order of the amino acids in its polypeptide chain. The final folded structure, or **conformation**, adopted by any polypeptide chain is determined by energetic considerations: a protein generally folds into the shape in which its free energy (G) is minimized. The folding process is thus energetically favorable, as it releases heat and increases the disorder of the universe (see Panel 3–1, pp. 96–97).

Protein folding has been studied in the laboratory using highly purified proteins. A protein can be unfolded, or *denatured*, by treatment with solvents that disrupt the noncovalent interactions holding the folded chain together. This treatment converts the protein into a flexible polypeptide chain that has lost its natural shape. Under the right conditions, when the

Figure 4–6 Hydrogen bonds within a protein molecule help stabilize its folded shape. Large numbers of hydrogen bonds form between adjacent regions of the folded polypeptide chain. The structure shown is a portion of the enzyme lysozyme. Hydrogen bonds between backbone atoms are shown in red; those between the backbone and a side chain are shown in yellow; and those between atoms of two side chains are shown in blue. Note that the same amino acid side chain can make multiple hydrogen bonds (red arrow). The atoms are colored as in Figure 4–1, although the hydrogen atoms are not shown. (After C.K. Mathews, K.E. van Holde, and K.G. Ahern, Biochemistry, 3rd ed. San Francisco: Benjamin Cummings, 2000.)





denaturing solvent is removed, the protein often refolds spontaneously into its original conformation—a process called *renaturation* (Figure 4–7). The fact that a denatured protein can, on its own, refold into the correct conformation indicates that all the information necessary to specify the three-dimensional shape of a protein is contained in its amino acid sequence.

Each protein normally folds into a single stable conformation. This conformation, however, often changes slightly when the protein interacts with other molecules in the cell. This change in shape is crucial to the function of the protein, as we discuss later.

When proteins fold incorrectly, they sometimes form aggregates that can damage cells and even whole tissues. Misfolded proteins are thought to contribute to a number of neurodegenerative disorders, such as Alzheimer's disease and Huntington's disease. Some infectious neurodegenerative diseases—including scrapie in sheep, bovine spongiform encephalopathy (BSE, or “mad cow” disease) in cattle, and Creutzfeldt–Jakob disease (CJD) in humans—are caused by misfolded proteins called prions. The misfolded prion form of a protein can convert the properly folded version of the protein in an infected brain into the abnormal conformation. This allows the misfolded prions, which tend to form aggregates, to spread rapidly from cell to cell, eventually causing the death of the affected animal or human (Figure 4–8). Prions are considered “infectious” because they can also spread from an affected individual to a normal individual via contaminated food, blood, or surgical instruments, for example.

Although a protein chain can fold into its correct conformation without outside help, protein folding in a living cell is generally assisted by special proteins called *chaperone proteins*. Some of these chaperones bind to partly folded chains and help them to fold along the most energetically favorable pathway (Figure 4–9). Others form “isolation chambers” in which single polypeptide chains can fold without the risk of forming aggregates in the crowded conditions of the cytoplasm (Figure 4–10). In either case, the final three-dimensional shape of the protein is still specified by its amino acid sequence; chaperones merely make the folding process more efficient and reliable.

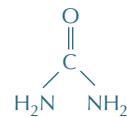
Proteins Come in a Wide Variety of Complicated Shapes

Proteins are the most structurally diverse macromolecules in the cell. Although they range in size from about 30 amino acids to more than

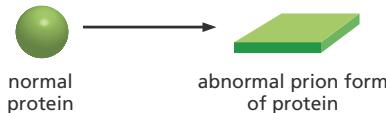
Figure 4–7 Denatured proteins can often recover their natural shapes. This type of experiment demonstrates that the conformation of a protein is determined solely by its amino acid sequence. Renaturation requires the correct conditions and works best for small proteins.

QUESTION 4–1

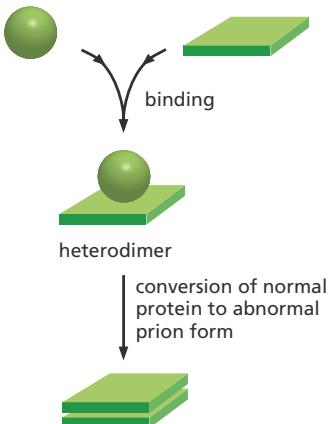
Urea used in the experiment shown in Figure 4–7 is a molecule that disrupts the hydrogen-bonded network of water molecules. Why might high concentrations of urea unfold proteins? The structure of urea is shown here.



(A) normal protein can, on occasion, adopt an abnormal, misfolded prion form



(B) the prion form of the protein can bind to the normal form, inducing conversion to the abnormal conformation

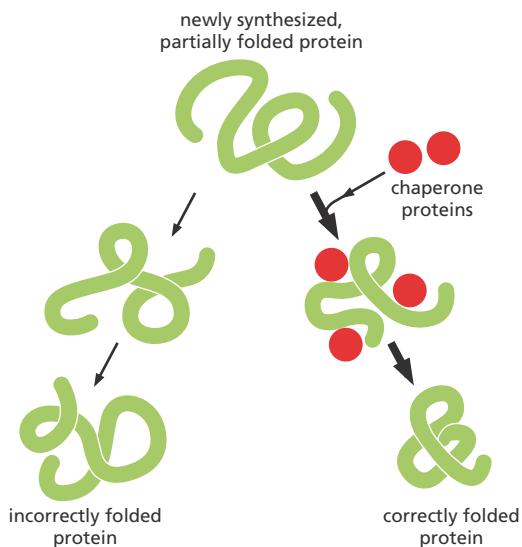


(C) abnormal prion proteins propagate and aggregate to form amyloid fibrils



Figure 4–8 Prion diseases are caused by proteins whose misfolding is infectious. (A) The protein undergoes a rare conformational change to give an abnormally folded prion form. (B) The abnormal form causes the conversion of normal proteins in the host's brain into a misfolded prion form. (C) The prions aggregate into amyloid fibrils, which disrupt brain cell function, causing a neurodegenerative disorder, such as “mad cow” disease (see also Figure 4–18).

Figure 4–9 Chaperone proteins can guide the folding of a newly synthesized polypeptide chain. The chaperones bind to newly synthesized or partially folded chains and help them to fold along the most energetically favorable pathway. Association of these chaperones with the target protein requires an input of energy from ATP hydrolysis.



10,000, the vast majority are between 50 and 2000 amino acids long. Proteins can be globular or fibrous, and they can form filaments, sheets, rings, or spheres (**Figure 4–11**). We will encounter many of these structures later in this chapter and throughout the book.

To date, the structures of about 100,000 different proteins have been determined. We discuss how scientists unravel these structures later in the chapter. Most proteins have a three-dimensional conformation so intricate and irregular that their structure would require an entire chapter to describe in detail. But we can get some sense of the intricacies of polypeptide structure by looking at the conformation of a relatively small protein, such as the bacterial transport protein *HPr*.

This small protein is only 88 amino acids long, and it serves as a carrier protein that facilitates the transport of sugar into bacterial cells. In **Figure 4–12**, we present *HPr*'s three-dimensional structure in four different ways, each of which emphasizes different features of the protein. The backbone model (Figure 4–12A) shows the overall organization of the polypeptide chain and provides a straightforward way to compare the structures of related proteins. The ribbon model (Figure 4–12B) shows the polypeptide backbone in a way that emphasizes its various folds, which we describe in detail shortly. The wire model (Figure 4–12C) includes the positions of all the amino acid side chains; this view is especially useful

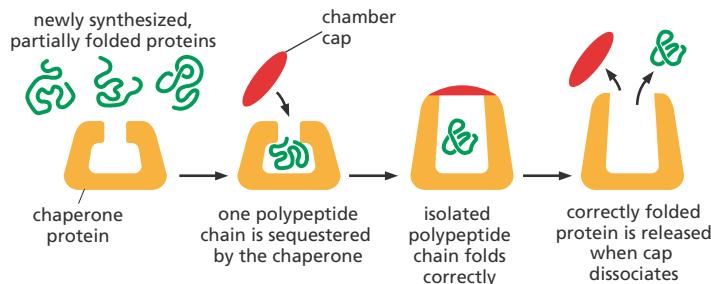


Figure 4–10 Other chaperone proteins act as isolation chambers that help a polypeptide fold. In this case, the barrel of the chaperone provides an enclosed chamber in which a newly synthesized polypeptide chain can fold without the risk of aggregating with other polypeptides in the crowded conditions of the cytoplasm. This system also requires an input of energy from ATP hydrolysis, mainly for the association and subsequent dissociation of the cap that closes off the chamber.

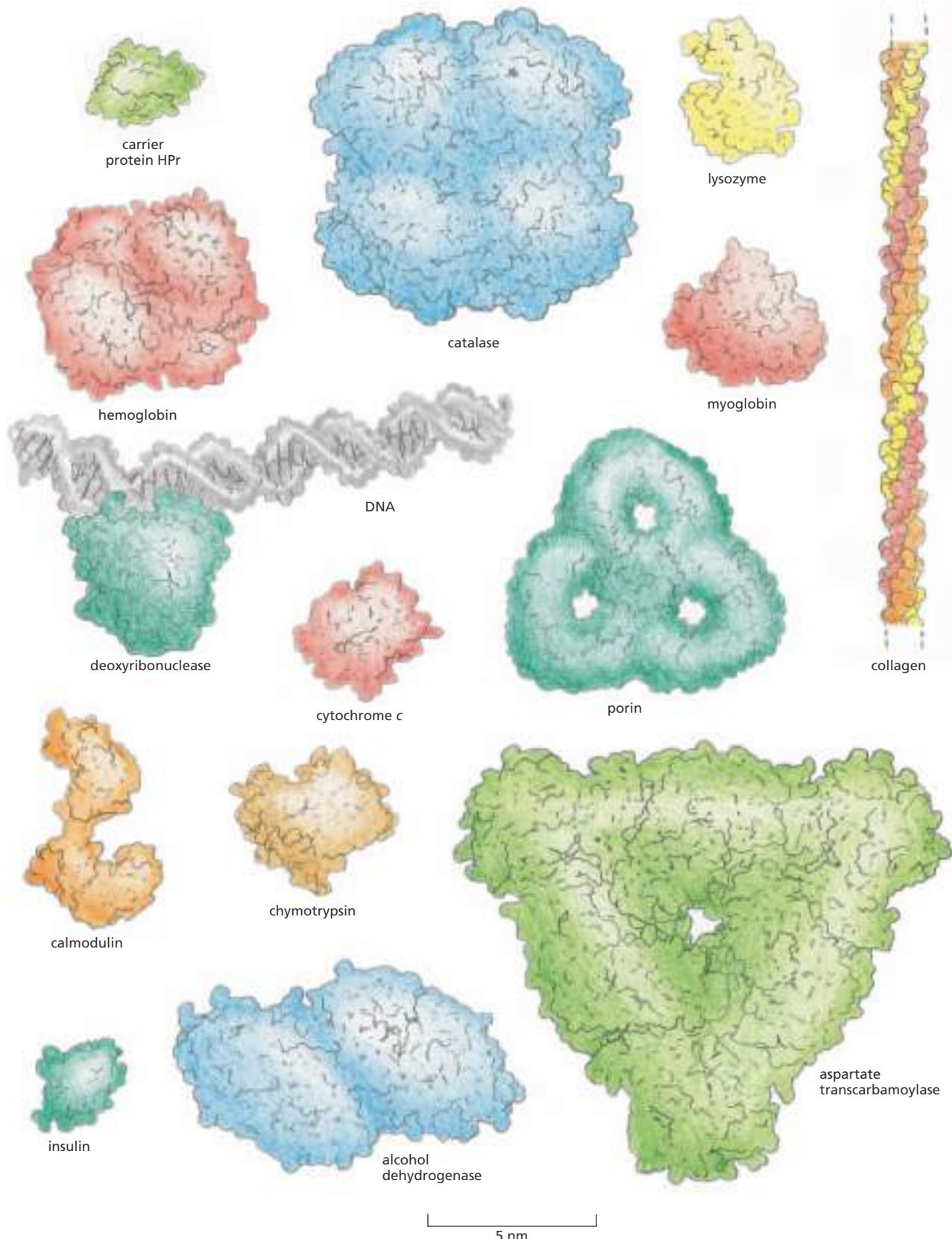
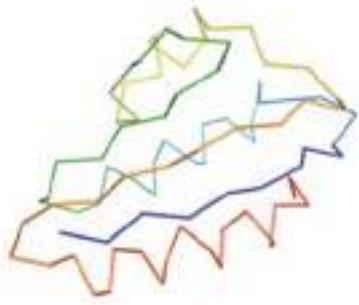


Figure 4–11 Proteins come in a variety of shapes and sizes. Each folded polypeptide is shown as a space-filling model, represented at the same scale. In the top-left corner is HPr, the small protein featured in detail in Figure 4–12. For comparison we also show a portion of a DNA molecule (gray) bound to the protein deoxyribonuclease. (After David S. Goodsell, *Our Molecular Nature*. New York: Springer-Verlag, 1996. With permission from Springer Science and Business Media.)

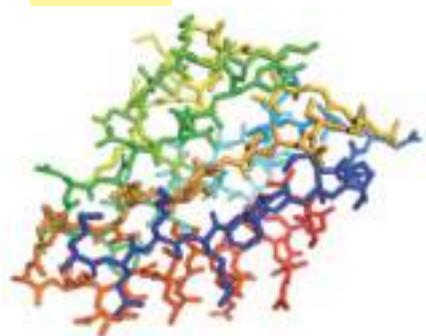
(A) backbone model



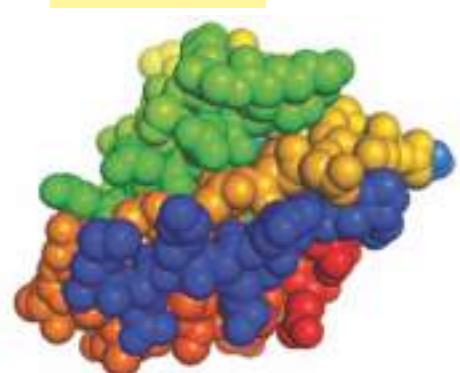
(B) ribbon model



(C) wire model



(D) space-filling model

**Figure 4–12 Protein conformation can be represented in a variety of ways.**

Shown here is the structure of the small bacterial transport protein HPr. The images are colored to make it easier to trace the path of the polypeptide chain. In these models, the region of polypeptide chain carrying the protein's N-terminus is purple and that near its C-terminus is red.

for predicting which amino acids might be involved in the protein's activity. Finally, the space-filling model (Figure 4–12D) provides a contour map of the protein surface, which reveals which amino acids are exposed on the surface and shows how the protein might look to a small molecule such as water or to another macromolecule in the cell.

The structures of larger proteins—or of multiprotein complexes—are even more complex. To visualize such detailed and complicated structures, scientists have developed various graphical and computer-based tools that generate a variety of images of a protein, only some of which are depicted in Figure 4–12. These images can be displayed on a computer screen and readily rotated and magnified to view all aspects of the structure ([Movie 4.1](#)).

When the three-dimensional structures of many different protein molecules are compared, it becomes clear that, although the overall conformation of each protein is unique, some regular folding patterns can be detected, as we discuss next.

The α Helix and the β Sheet Are Common Folding Patterns

More than 60 years ago, scientists studying hair and silk discovered two common folding patterns present in many different proteins. The first to be discovered, called the **α helix**, was found in the protein *α -keratin*, which is abundant in skin and its derivatives—such as hair, nails, and horns. Within a year of the discovery of the α helix, a second folded structure, called a **β sheet**, was found in the protein *fibroin*, the major constituent of silk. (Biologists often use Greek letters to name their discoveries, with the first example receiving the designation α , the second β , and so on.)

These two folding patterns are particularly common because they result from hydrogen bonds that form between the N-H and C=O groups in the polypeptide backbone (see Figure 4–6). Because the amino acid side chains are not involved in forming these hydrogen bonds, α helices and β sheets can be generated by many different amino acid sequences. In each case, the protein chain adopts a regular, repeating form. These structural features, and the shorthand cartoon symbols that are often used to represent them in models of protein structures, are presented in [Figure 4–13](#).

Helices Form Readily in Biological Structures

The abundance of helices in proteins is, in a way, not surprising. A **helix** is a regular structure that resembles a spiral staircase. It is generated simply by placing many similar subunits next to one another, each in the same strictly repeated relationship to the one before. Because it is very rare for subunits to join up in a straight line, this arrangement will generally result in a helix ([Figure 4–14](#)). Depending on the twist of the staircase, a helix is said to be either right-handed or left-handed (Figure 4–14E). Handedness is not affected by turning the helix upside down, but it is reversed if the helix is reflected in a mirror.

An α helix is generated when a single polypeptide chain turns around itself to form a structurally rigid cylinder. A hydrogen bond is made between every fourth amino acid, linking the C=O of one peptide bond to

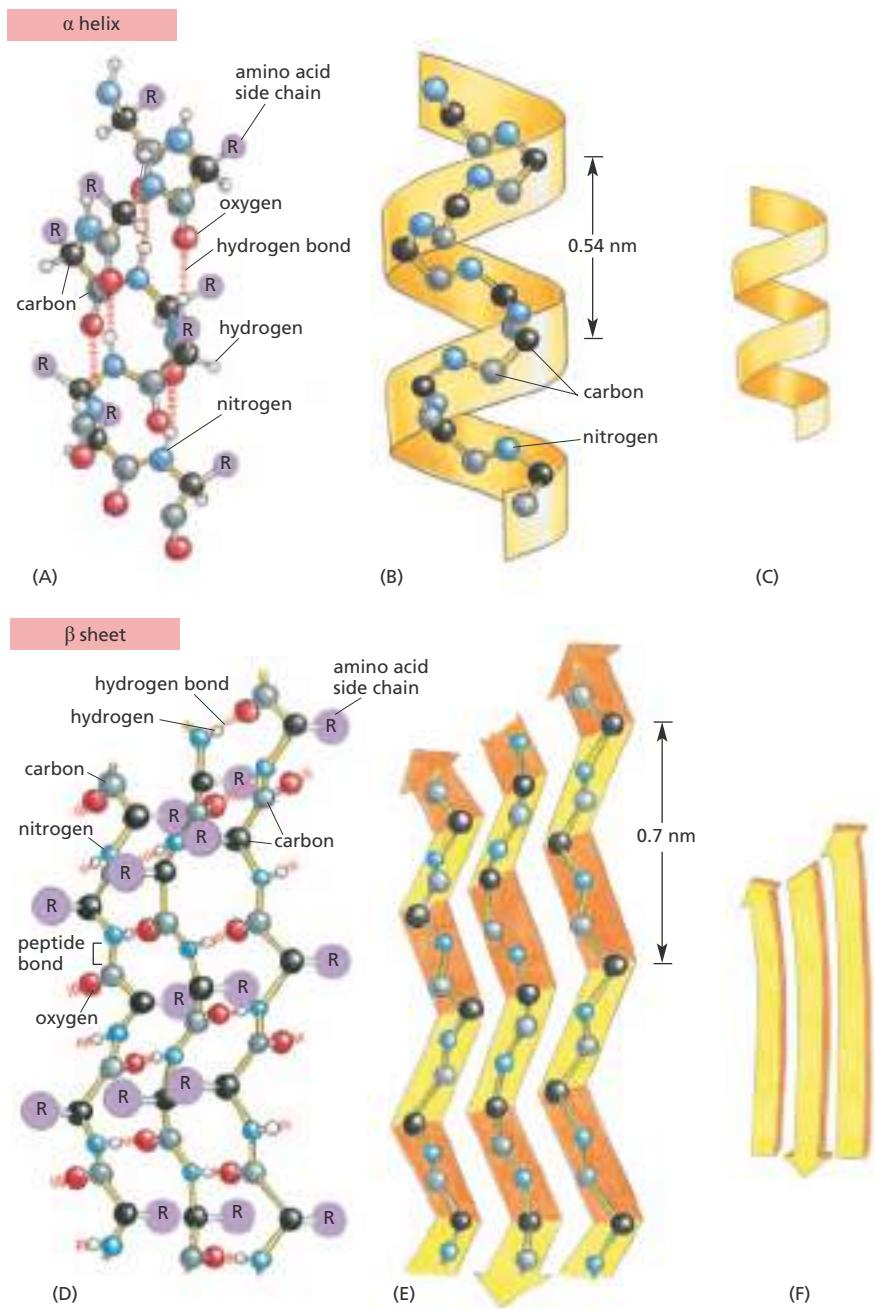


Figure 4–13 Polypeptide chains often fold into one of two orderly repeating forms known as an α helix and a β sheet.
 (A–C) In an α helix, the N–H of every peptide bond is hydrogen-bonded to the C=O of a neighboring peptide bond located four amino acids away in the same chain. (D–F) In a β sheet, several segments (strands) of an individual polypeptide chain are held together by hydrogen-bonding between peptide bonds in adjacent strands. The amino acid side chains in each strand project alternately above and below the plane of the sheet. In the example shown, the adjacent chains run in opposite directions, forming an antiparallel β sheet. (A) and (D) show all of the atoms in the polypeptide backbone, but the amino acid side chains are denoted by R. (B) and (E) show only the carbon (black and gray) and nitrogen (blue) backbone atoms, while (C) and (F) display the cartoon symbols that are used to represent the α helix and the β sheet in ribbon models of proteins (see Figure 4–12B).

the N–H of another (see Figure 4–13A). This gives rise to a regular right-handed helix with a complete turn every 3.6 amino acids (Movie 4.2).

Short regions of α helix are especially abundant in proteins that are embedded in cell membranes, such as transport proteins and receptors. We will see in Chapter 11 that those portions of a transmembrane protein that cross the lipid bilayer usually form an α helix that is composed largely of amino acids with nonpolar side chains. The polypeptide backbone, which is hydrophilic, is hydrogen-bonded to itself in the α helix, and it is shielded from the hydrophobic lipid environment of the membrane by its protruding nonpolar side chains (Figure 4–15).

Sometimes two (or three) α helices will wrap around one another to form a particularly stable structure known as a **coiled-coil**. This structure forms when the α helices have most of their nonpolar (hydrophobic) side chains on one side, so that they can twist around each other with

QUESTION 4–2

Remembering that the amino acid side chains projecting from each polypeptide backbone in a β sheet point alternately above and below the plane of the sheet (see Figure 4–13D), consider the following protein sequence: Leu-Lys-Val-Asp-Ile-Ser-Leu-Arg-Leu-Lys-Ile-Arg-Phe-Glu. Do you find anything remarkable about the arrangement of the amino acids in this sequence when incorporated into a β sheet? Can you make any predictions as to how the β sheet might be arranged in a protein? (Hint: consult the properties of the amino acids listed in Figure 4–3.)

Figure 4–14 The helix is a common, regular, biological structure. A helix will form when a series of similar subunits bind to each other in a regular way. At the bottom, the interaction between two subunits is shown; behind them are the helices that result. These helices have two (A), three (B), or six (C and D) subunits per helical turn. At the top, the arrangement of subunits has been photographed from directly above the helix. Note that the helix in (D) has a wider path than that in (C), but the same number of subunits per turn. (E) A helix can be either right-handed or left-handed. As a reference, it is useful to remember that standard metal screws, which advance when turned clockwise, are right-handed. So to judge the handedness of a helix, imagine screwing it into a wall. Note that a helix preserves the same handedness when it is turned upside down.

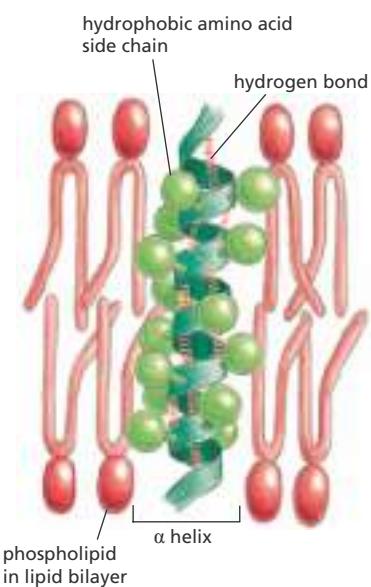
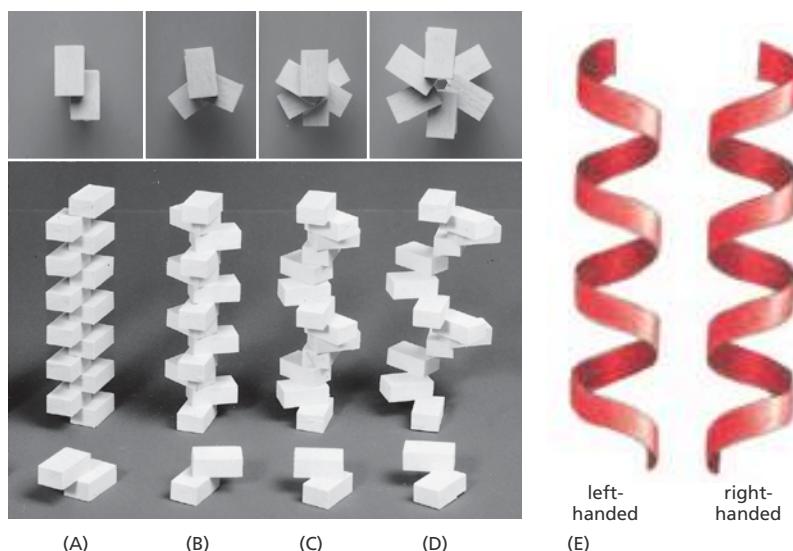


Figure 4–15 Many membrane-bound proteins cross the lipid bilayer as an α helix. The hydrophobic side chains of the amino acids forming the α helix contact the hydrophobic hydrocarbon tails of the phospholipid molecules, while the hydrophilic parts of the polypeptide backbone form hydrogen bonds with one another in the interior of the helix. About 20 amino acids are required to span a membrane in this way. Note that, despite the appearance of a space along the interior of the helix in this schematic diagram, the helix is not a channel: no ions or small molecules can pass through it.

these side chains facing inward—minimizing their contact with the aqueous cytosol (Figure 4–16). Long, rodlike coiled-coils form the structural framework for many elongated proteins. Examples include α -keratin, which forms the intracellular fibers that reinforce the outer layer of the skin, and myosin, the motor protein responsible for muscle contraction (discussed in Chapter 17).

β Sheets Form Rigid Structures at the Core of Many Proteins

A β sheet is made when hydrogen bonds form between segments of a polypeptide chain that lie side by side (see Figure 4–13D). When the neighboring segments run in the same orientation (say, from the N-terminus to the C-terminus), the structure is a *parallel* β sheet; when they run in opposite directions, the structure is an *antiparallel* β sheet (Figure 4–17). Both types of β sheet produce a very rigid, pleated structure, and they form the core of many proteins. Even the small bacterial protein HPr (see Figure 4–12) contains several β sheets.

β sheets have remarkable properties. They give silk fibers their extraordinary tensile strength. They also permit the formation of *amyloid fibers*—insoluble protein aggregates that include those associated with neurodegenerative disorders, such as Alzheimer’s disease and prion diseases (see Figure 4–8). These structures, formed from abnormally folded proteins, are stabilized by β sheets that stack together tightly, with their amino acid side chains interdigitated like the teeth of a zipper (Figure 4–18). Although we tend to associate amyloid fibers with disease, many organisms take advantage of these stable structures to perform novel tasks. Infectious bacteria, for example, can use amyloid fibers to help form the biofilms that allow them to colonize host tissues. Other types of filamentous bacteria use amyloid fibers to extend filaments into the air, enabling the bacteria to disperse their spores far and wide.

Proteins Have Several Levels of Organization

A protein’s structure does not end with α helices and β sheets; there are additional levels of organization. These levels are not independent but are built one upon the next to establish the three-dimensional structure of the entire protein. A protein’s structure begins with its amino acid sequence, which is thus considered its **primary structure**. The next level of organization includes the α helices and β sheets that form within

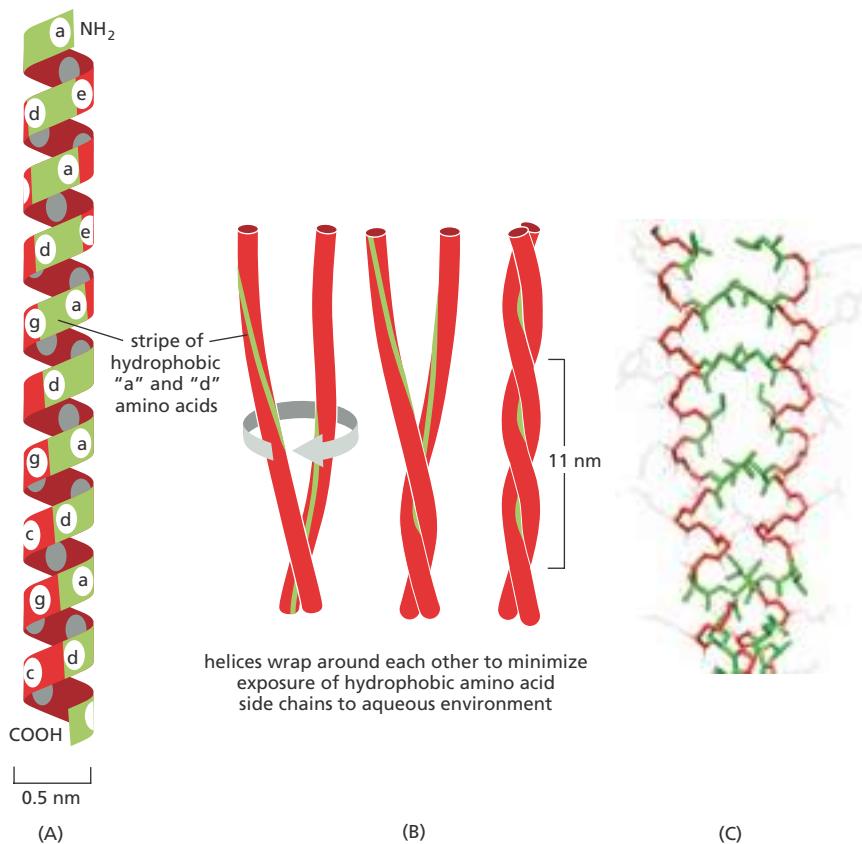
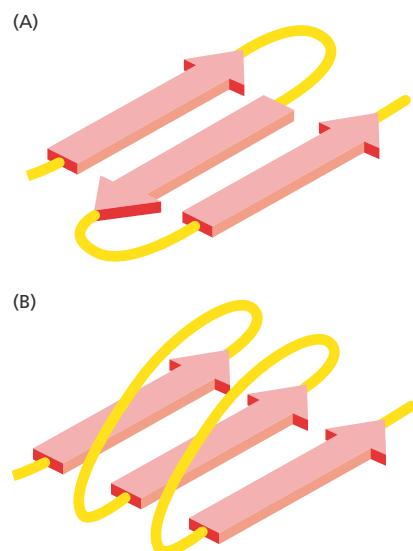


Figure 4–16 Intertwined α helices can form a stiff coiled-coil. In (A), a single α helix is shown, with successive amino acid side chains labeled in a sevenfold repeating sequence "abcdefg." Amino acids "a" and "d" in such a sequence lie close together on the cylinder surface, forming a stripe (shaded in green) that winds slowly around the α helix. Proteins that form coiled-coils typically have nonpolar amino acids at positions "a" and "d." Consequently, as shown in (B), the two α helices can wrap around each other, with the nonpolar side chains of one α helix interacting with the nonpolar side chains of the other, while the more hydrophilic amino acid side chains (shaded in red) are left exposed to the aqueous environment. (C) A portion of the atomic structure of a coiled-coil made of two α helices, as determined by X-ray crystallography. In this structure, atoms that form the backbone of the helices are shown in red; the interacting, nonpolar side chains are green, and the remaining side chains are gray. Coiled-coils can also form from three α helices ([Movie 4.3](#)).

certain segments of the polypeptide chain; these folds are elements of the protein's **secondary structure**. The full, three-dimensional conformation formed by an entire polypeptide chain—including the α helices, β sheets, random coils, and any other loops and folds that form between the N- and C-termini—is sometimes referred to as the **tertiary structure**. Finally, if the protein molecule is formed as a complex of more than one polypeptide chain, then the complete structure is designated its **quaternary structure**.

Studies of the conformation, function, and evolution of proteins have also revealed the importance of a level of organization distinct from the four just described. This organizational unit is the **protein domain**, which is defined as any segment of a polypeptide chain that can fold independently into a compact, stable structure. A protein domain usually contains between 40 and 350 amino acids—folded into α helices and β sheets and other elements of secondary structure—and it is the modular unit from which many larger proteins are constructed ([Figure 4–19](#)). The different domains of a protein are often associated with different functions. For example, the bacterial *catabolite activator protein* (CAP), illustrated in [Figure 4–19](#), has two domains: the small domain binds to DNA, while the large domain binds cyclic AMP, a small intracellular signaling molecule. When the large domain binds cyclic AMP, it causes a conformational change in the protein that enables the small domain to bind to a specific DNA sequence and thereby promote the expression of an adjacent gene. To provide a sense of the many different domain structures observed in proteins, ribbon models of three different domains are shown in [Figure 4–20](#).

Figure 4–17 β sheets come in two varieties. (A) Antiparallel β sheet (see also [Figure 4–13D](#)). (B) Parallel β sheet. Both of these structures are common in proteins. By convention, the arrows point toward the C-terminus of the polypeptide chain ([Movie 4.4](#)).



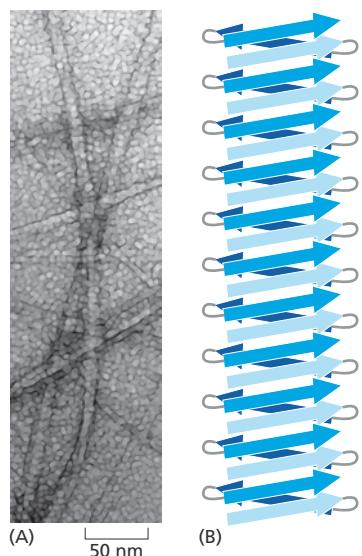


Figure 4–18 Stacking of β sheets allows some misfolded proteins to aggregate into amyloid fibers. (A) Electron micrograph shows an amyloid fiber formed from a segment of a yeast prion protein. (B) Schematic representation shows the stacking of β sheets that stabilize an individual amyloid fiber. (A, courtesy of David Eisenberg.)

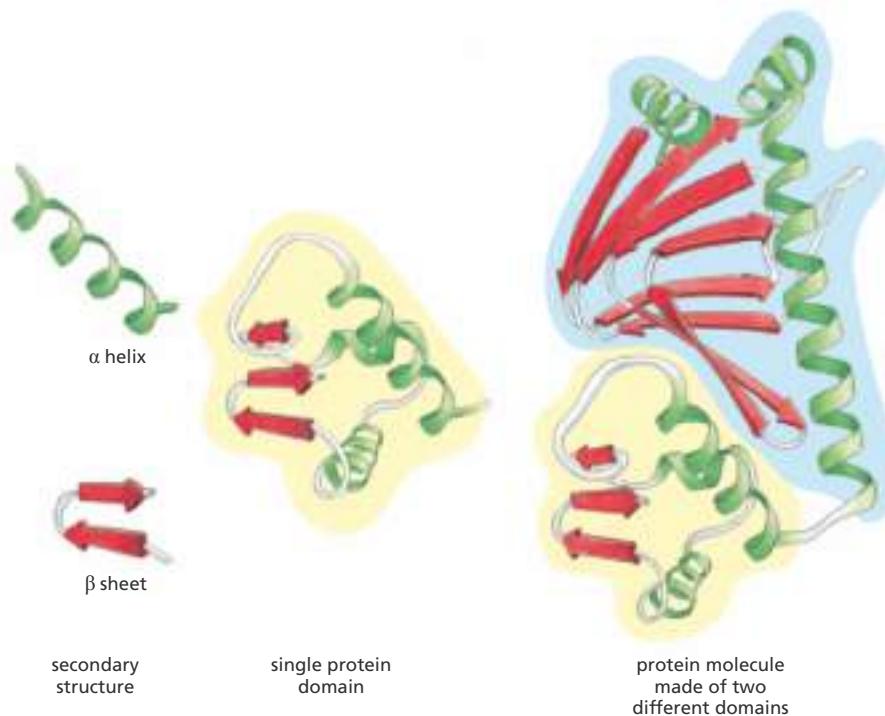
Many Proteins Also Contain Unstructured Regions

Small protein molecules, such as the oxygen-carrying muscle protein myoglobin, contain only a single domain (see Figure 4–11). Larger proteins can contain as many as several dozen domains, which are usually connected by relatively unstructured lengths of polypeptide chain. Such regions of polypeptide chain lacking any definite structure, which continually bend and flex due to thermal buffeting, are abundant in cells. These **intrinsically disordered sequences** are often found as short stretches linking domains in otherwise highly ordered proteins. Other proteins, however, are almost entirely without secondary structure and exist as unfolded polypeptide chains in the cytosol.

Intrinsically disordered sequences remained undetected for many years. Their lack of folded structure makes them prime targets for the proteolytic enzymes that are released when cells are fractionated to isolate their molecular components (see Panel 4–3, pp. 164–165). Unstructured sequences also fail to form protein crystals and for this reason escape the attention of X-ray crystallographers (see How We Know, pp. 162–163). Indeed, the ubiquity of disordered sequences became appreciated only after bioinformatics methods were developed that could recognize them from their amino acid sequences. Present estimates suggest that a third of all eukaryotic proteins have long unstructured regions in their polypeptide chain (greater than 30 amino acids in length), while a substantial number of eukaryotic proteins are mostly disordered under normal conditions.

Unstructured sequences have a variety of important functions in cells. Being able to flex and bend, they can wrap around one or more target proteins like a scarf, binding with both high specificity and low affinity (Figure 4–21). By forming flexible tethers between the compact domains in a protein, they provide flexibility while increasing the frequency of encounters between the domains (Figure 4–21). They can help *scaffold proteins* bring together proteins in an intracellular signaling pathway, facilitating interactions (Figure 4–21). They also give proteins like elastin

Figure 4–19 Many proteins are composed of separate functional domains. Elements of secondary structure such as α helices and β sheets pack together into stable, independently folding, globular elements called protein domains. A typical protein molecule is built from one or more domains, linked by a region of polypeptide chain that is often relatively unstructured. The ribbon diagram on the right represents the bacterial transcription regulator protein CAP, with one large domain (outlined in blue) and one small domain (outlined in yellow).



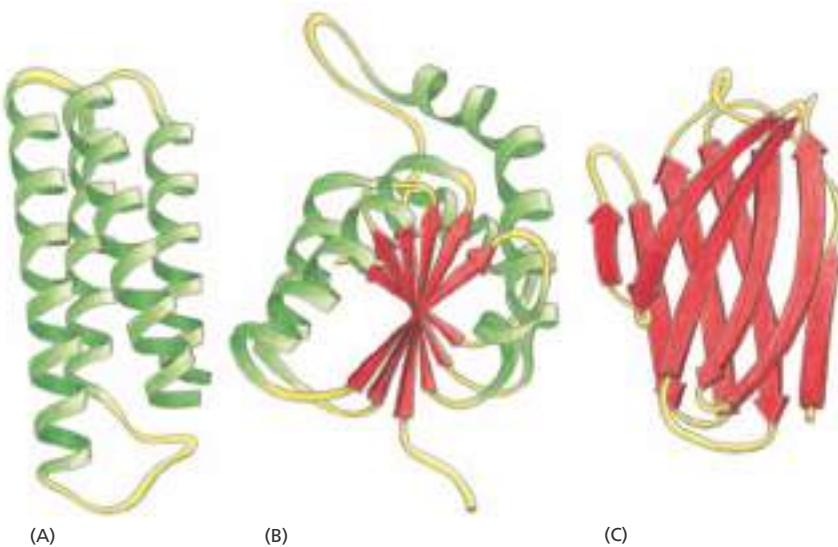


Figure 4–20 Ribbon models show three different protein domains.

(A) Cytochrome b_{562} is a single-domain protein involved in electron transfer in *E. coli*. It is composed almost entirely of α helices. (B) The NAD-binding domain of the enzyme lactic dehydrogenase is composed of a mixture of α helices and β sheets. (C) An immunoglobulin domain of an antibody molecule is composed of a sandwich of two antiparallel β sheets. In these examples, the α helices are shown in green, while strands organized as β sheets are denoted by red arrows. The protruding loop regions (yellow) are often unstructured and can provide binding sites for other molecules. (Redrawn from originals courtesy of Jane Richardson.)

the ability to form rubberlike fibers, allowing our tendons and skin to recoil after being stretched. In addition to providing structural flexibility, unstructured sequences are also ideal substrates for the addition of chemical groups that control the way many proteins behave—a topic we discuss at length later in the chapter.

Few of the Many Possible Polypeptide Chains Will Be Useful

In theory, a vast number of different polypeptide chains could be made from 20 different amino acids. Because each amino acid is chemically distinct and could, in principle, occur at any position, a polypeptide chain four amino acids long has $20 \times 20 \times 20 \times 20 = 160,000$ different possible sequences. In other words, for a polypeptide that is n amino acids long, 20^n different chains are possible. For a typical protein length of 300 amino acids, more than 20^{300} (that's 10^{390}) different polypeptide chains could theoretically be made.

Of the unimaginably large collection of potential polypeptide sequences, only a minuscule fraction is actually present in cells. That's because many biological functions depend on proteins with stable, well-defined three-dimensional conformations. This requirement restricts the list of possible polypeptide sequences. Another constraint is that functional proteins

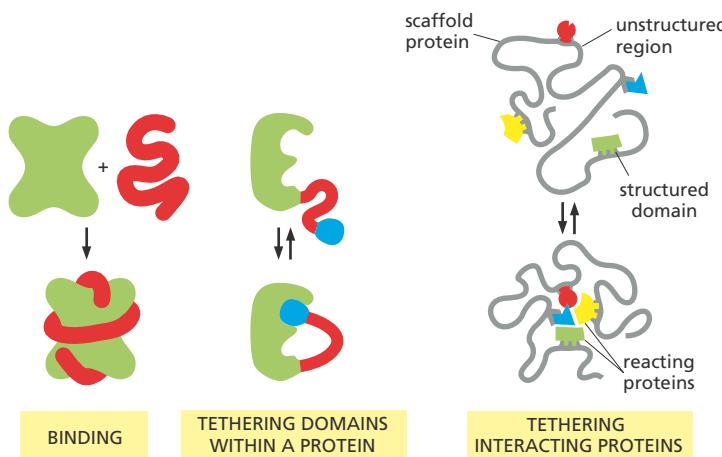


Figure 4–21 Unstructured regions of a polypeptide chain in proteins can perform many functions. A few of these functions are illustrated here.

QUESTION 4–3

Random mutations only very rarely result in changes in a protein that improve its usefulness for the cell, yet useful mutations are selected in evolution. Because these changes are so rare, for each useful mutation there are innumerable mutations that lead to either no improvement or inactive proteins. Why, then, do cells not contain millions of proteins that are of no use?

must be “well-behaved” and not engage in unwanted associations with other proteins in the cell—forming insoluble protein aggregates, for example. Many potential proteins would therefore have been eliminated by natural selection through the long trial-and-error process that underlies evolution (discussed in Chapter 9).

Thanks to this rigorous process of selection, the amino acid sequences of many present-day proteins have evolved to guarantee that the polypeptide will adopt a stable conformation—one that bestows upon the protein the exact chemical properties that will enable it to perform a particular function. Such proteins are so precisely built that a change in even a few atoms in one amino acid can sometimes disrupt the structure of a protein and thereby eliminate its function. In fact, the structures of many proteins—and their constituent domains—are so stable and effective that they have been conserved throughout evolution among many diverse organisms. The three-dimensional structures of the DNA-binding domains from the yeast α 2 protein and the *Drosophila* Engrailed protein, for example, are almost completely superimposable, even though these organisms are separated by more than a billion years of evolution. Other proteins, however, have changed their structure and function over evolutionary time, as we now discuss.

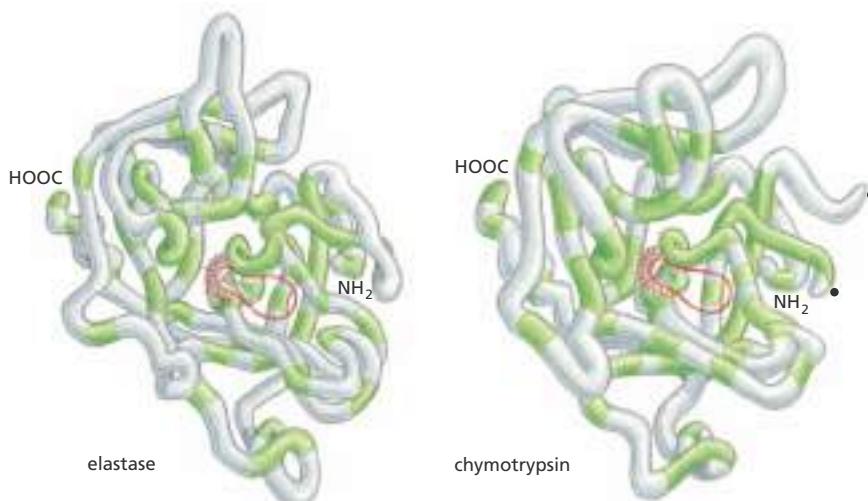
Proteins Can Be Classified into Families

Once a protein had evolved a stable conformation with useful properties, its structure could be modified over time to enable it to perform new functions. We know that this occurred quite often during evolution, because many present-day proteins can be grouped into **protein families**, in which each family member has an amino acid sequence and a three-dimensional conformation that closely resemble those of the other family members.

Consider, for example, the *serine proteases*, a family of protein-cleaving (proteolytic) enzymes that includes the digestive enzymes chymotrypsin, trypsin, and elastase, as well as several proteases involved in blood clotting. When any two of these enzymes are compared, portions of their amino acid sequences are found to be nearly the same. The similarity of their three-dimensional conformations is even more striking: most of the detailed twists and turns in their polypeptide chains, which are several hundred amino acids long, are virtually identical (Figure 4–22). The various serine proteases nevertheless have distinct enzymatic activities, each cleaving different proteins or the peptide bonds between different types of amino acids.

Figure 4–22 Serine proteases constitute a family of proteolytic enzymes. Backbone models of two serine proteases, elastase and chymotrypsin, are illustrated. Although only those amino acid sequences in the polypeptide chain shaded in green are the same in the two proteins, the two conformations are very similar nearly everywhere. Nonetheless, the two proteases prefer different substrates. The active site of each enzyme—where its substrates are bound and cleaved—is circled in red.

Serine proteases derive their name from the amino acid serine, which directly participates in the cleavage reaction. The two black dots on the right side of the chymotrypsin molecule mark the two ends created where the enzyme has cleaved its own backbone.



Large Protein Molecules Often Contain More Than One Polypeptide Chain

The same type of weak noncovalent bonds that enable a polypeptide chain to fold into a specific conformation also allow proteins to bind to each other to produce larger structures in the cell. Any region on a protein's surface that interacts with another molecule through sets of noncovalent bonds is termed a *binding site*. A protein can contain binding sites for a variety of molecules, large and small. If a binding site recognizes the surface of a second protein, the tight binding of two folded polypeptide chains at this site will create a larger protein, whose quaternary structure has a precisely defined geometry. Each polypeptide chain in such a protein is called a **subunit**, and each subunit may contain more than one domain.

In the simplest case, two identical, folded polypeptide chains form a symmetrical complex of two protein subunits (called a *dimer*) that is held together by interactions between two identical binding sites. The CAP protein in bacterial cells is a dimer (**Figure 4–23A**) formed from two identical copies of the protein subunit shown previously in Figure 4–19. Many other symmetrical protein complexes, formed from multiple copies of the same polypeptide chain, are commonly found in cells. The enzyme *neuraminidase*, for example, consists of a ring of four identical protein subunits (**Figure 4–23B**).

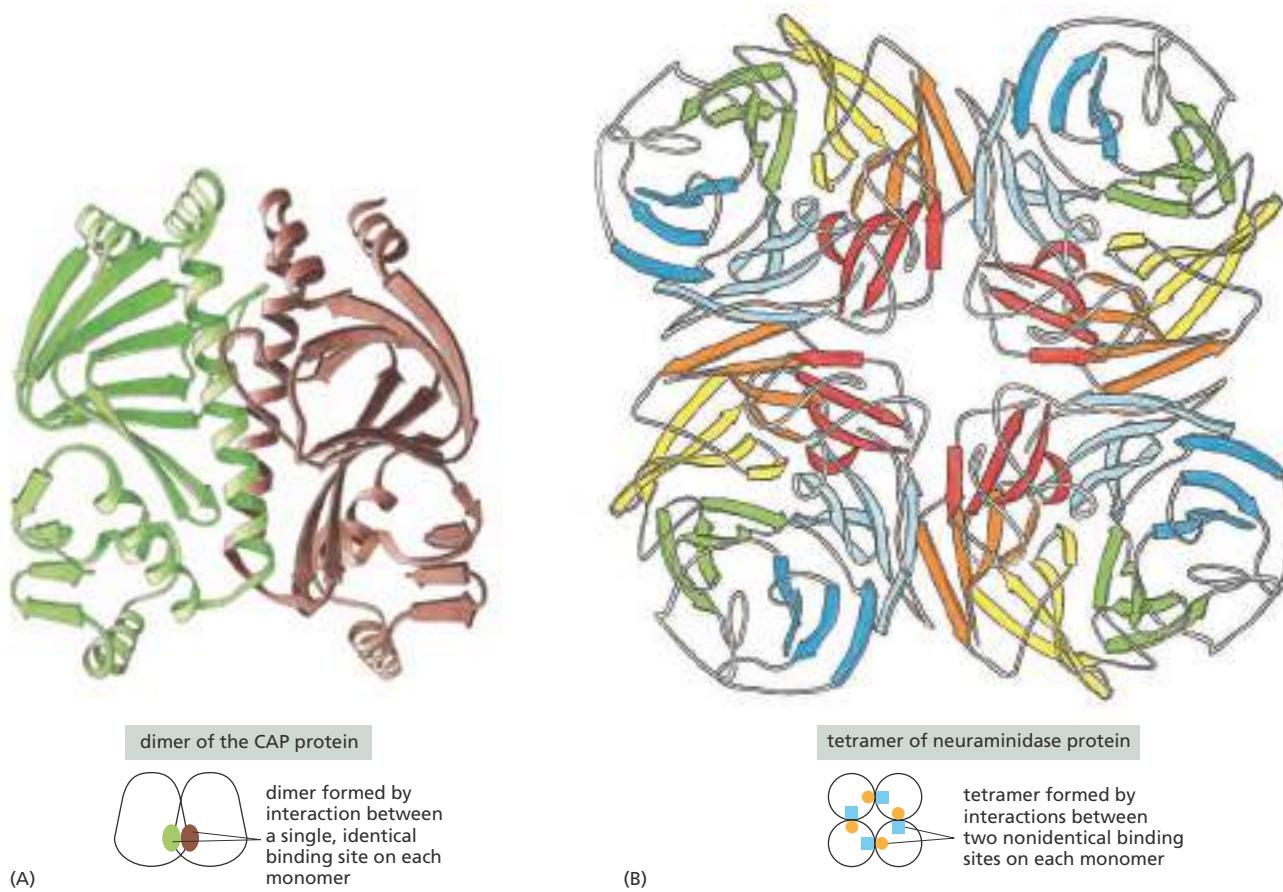
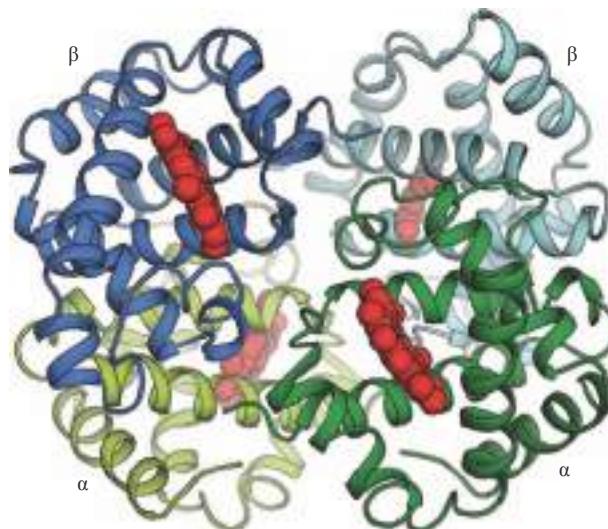


Figure 4–23 Many protein molecules contain multiple copies of the same protein subunit. (A) A symmetrical dimer. The CAP protein is a complex of two identical polypeptide chains (see also Figure 4–19). (B) A symmetrical homotetramer. The enzyme neuraminidase exists as a ring of four identical polypeptide chains. For both (A) and (B), a small schematic below the structure emphasizes how the repeated use of the same binding interaction forms the structure. In (A), the use of the same binding site on each monomer (represented by brown and green ovals) causes the formation of a symmetrical dimer. In (B), a pair of nonidentical binding sites (represented by orange circles and blue squares) causes the formation of a symmetrical tetramer.

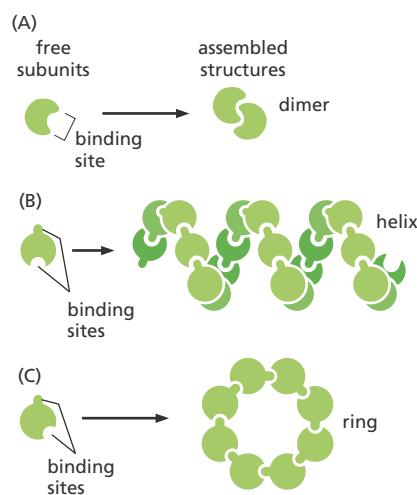
Figure 4–24 Some proteins are formed as a symmetrical assembly of two different subunits. Hemoglobin, an oxygen-carrying protein abundant in red blood cells, contains two copies of α -globin (green) and two copies of β -globin (blue). Each of these four polypeptide chains contains a heme molecule (red), where oxygen (O_2) is bound. Thus, each molecule of hemoglobin in the blood carries four molecules of oxygen.



Other proteins contain two or more different polypeptide chains. *Hemoglobin*, the protein that carries oxygen in red blood cells, is a particularly well-studied example. The protein contains two identical α -globin subunits and two identical β -globin subunits, symmetrically arranged (Figure 4–24). Many proteins contain multiple subunits, and they can be very large (Movie 4.5).

Proteins Can Assemble into Filaments, Sheets, or Spheres

Proteins can form even larger assemblies than those discussed so far. Most simply, a chain of identical protein molecules can be formed if the binding site on one protein molecule is complementary to another region on the surface of another protein molecule of the same type. Because each protein molecule is bound to its neighbor in an identical way (see Figure 4–14), the molecules will often be arranged in a helix that can be extended indefinitely in either direction (Figure 4–25). This type of arrangement can produce an extended protein filament. An actin filament, for example, is a long, helical structure formed from many molecules of the protein actin (Figure 4–26). Actin is extremely abundant in eukaryotic cells, where it forms one of the major filament systems of the cytoskeleton (discussed in Chapter 17). Other sets of identical proteins associate to form tubes, as in the microtubules of the cytoskeleton (Figure 4–27), or cagelike spherical shells, as in the protein coats of virus particles (Figure 4–28).



Many large structures, such as viruses and ribosomes, are built from a mixture of one or more types of protein plus RNA or DNA molecules. These structures can be isolated in pure form and dissociated into their constituent macromolecules. It is often possible to mix the isolated components back together and watch them reassemble spontaneously into the original structure. This demonstrates that all the information needed for assembly of the complicated structure is contained in the macromolecules themselves. Experiments of this type show that much of the

Figure 4–25 Identical protein subunits can assemble into complex structures. (A) A protein with just one binding site can form a dimer with another identical protein. (B) Identical proteins with two different binding sites will often form a long, helical filament. (C) If the two binding sites are disposed appropriately in relation to each other, the protein subunits will form a closed ring instead of a helix (see also Figure 4–23B).

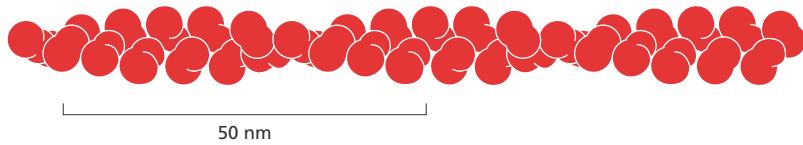


Figure 4–26 An actin filament is composed of identical protein subunits.

The helical array of actin molecules in a filament often contains thousands of molecules and extends for micrometers in the cell.

structure of a cell is self-organizing: if the required proteins are produced in the right amounts, the appropriate structures will form automatically.

Some Types of Proteins Have Elongated Fibrous Shapes

Most of the proteins we have discussed so far are **globular proteins**, in which the polypeptide chain folds up into a compact shape like a ball with an irregular surface. Enzymes, for example, tend to be globular proteins: even though many are large and complicated, with multiple subunits, most have a quaternary structure with an overall rounded shape (see Figure 4–11). In contrast, other proteins have roles in the cell that require them to span a large distance. These proteins generally have a relatively simple, elongated three-dimensional structure and are commonly referred to as **fibrous proteins**.

One large class of intracellular fibrous proteins resembles α -keratin, which we met earlier when we introduced the α -helix. Keratin filaments are extremely stable: long-lived structures such as hair, horns, and nails are composed mainly of this protein. An α -keratin molecule is a dimer of two identical subunits, with the long α helices of each subunit forming a coiled-coil (see Figure 4–16). These coiled-coil regions are capped at either end by globular domains containing binding sites that allow them to assemble into ropelike *intermediate filaments*—a component of the cytoskeleton that gives cells mechanical strength (discussed in Chapter 17).

Fibrous proteins are especially abundant outside the cell, where they form the gel-like *extracellular matrix* that helps bind cells together to form tissues. These proteins are secreted by the cells into their surroundings, where they often assemble into sheets or long fibrils. *Collagen* is the most abundant of these fibrous extracellular proteins in animal tissues. A collagen molecule consists of three long polypeptide chains, each containing the nonpolar amino acid glycine at every third position. This regular structure allows the chains to wind around one another to generate a long, regular, triple helix with glycine at its core (Figure 4–29A). Many such

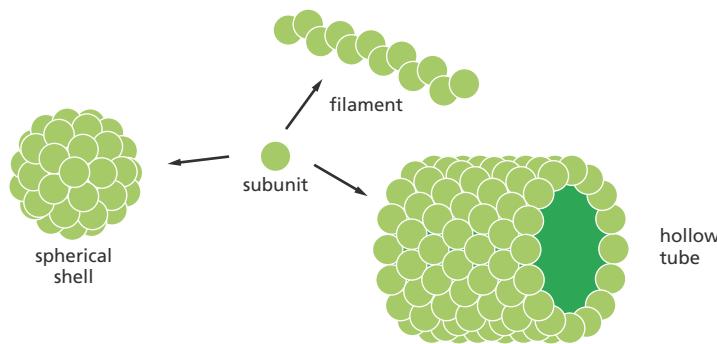


Figure 4–27 A single type of protein subunit can pack together to form a filament, a hollow tube, or a spherical shell. Actin subunits, for example, form actin filaments (see Figure 4–26), whereas tubulin subunits form hollow microtubules, and some virus proteins form a spherical shell (capsid) that encloses the viral genome (see Figure 4–28).

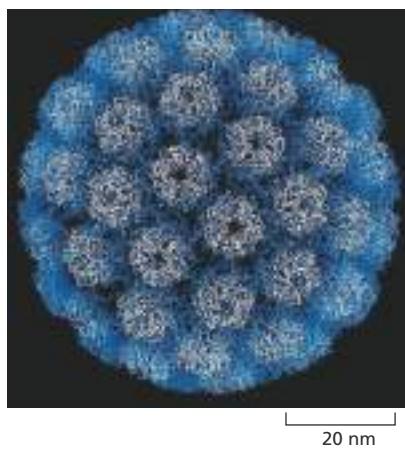


Figure 4–28 Many viral capsids are more or less spherical protein assemblies.

They are formed from many copies of a small set of protein subunits. The nucleic acid of the virus (DNA or RNA) is packaged inside. The structure of the simian virus SV40, shown here, was determined by X-ray crystallography and is known in atomic detail. (Courtesy of Robert Grant, Stephan Crainic, and James M. Hogle.)

collagen molecules bind to one another side-by-side and end-to-end to create long overlapping arrays called *collagen fibrils*, which are extremely strong and help hold tissues together, as described in Chapter 20.

In complete contrast to collagen is another fibrous protein in the extracellular matrix, *elastin*. Elastin molecules are formed from relatively loose and unstructured polypeptide chains that are covalently cross-linked into a rubberlike elastic meshwork. The resulting *elastic fibers* enable skin and other tissues, such as arteries and lungs, to stretch and recoil without tearing. As illustrated in **Figure 4–29B**, the elasticity is due to the ability of the individual protein molecules to uncoil reversibly whenever they are stretched.

Extracellular Proteins Are Often Stabilized by Covalent Cross-Linkages

Many protein molecules are either attached to the outside of a cell's plasma membrane or secreted as part of the extracellular matrix, which exposes them to extracellular conditions. To help maintain their structures, the polypeptide chains in such proteins are often stabilized by covalent cross-linkages. These linkages can either tie together two amino acids in the same polypeptide chain or join together many polypeptide chains in a large protein complex—as for the collagen fibrils and elastic fibers just described.

The most common covalent cross-links in proteins are sulfur–sulfur bonds. These **disulfide bonds** (also called *S–S bonds*) are formed before a protein is secreted by an enzyme in endoplasmic reticulum that links together two –SH groups from cysteine side chains that are adjacent in the folded protein (**Figure 4–30**). Disulfide bonds do not change a protein's conformation, but instead act as a sort of “atomic staple” to reinforce the protein's most favored conformation. For example, lysozyme—an

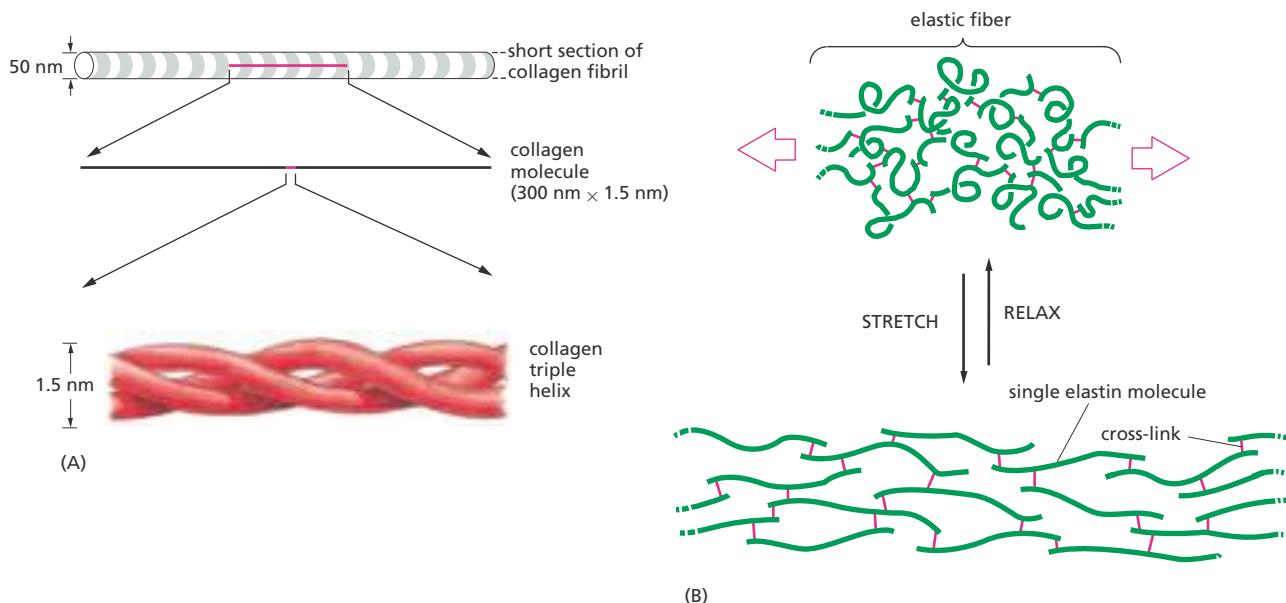


Figure 4–29 Collagen and elastin are abundant extracellular fibrous proteins. (A) A collagen molecule is a triple helix formed by three extended protein chains that wrap around one another. Many rodlike collagen molecules are cross-linked together in the extracellular space to form collagen fibrils (top), which have the tensile strength of steel. The striping on the collagen fibril is caused by the regular repeating arrangement of the collagen molecules within the fibril. (B) Elastin molecules are cross-linked together by covalent bonds (red) to form rubberlike, elastic fibers. Each elastin polypeptide chain uncoils into a more extended conformation when the fiber is stretched, and recoils spontaneously as soon as the stretching force is relaxed.

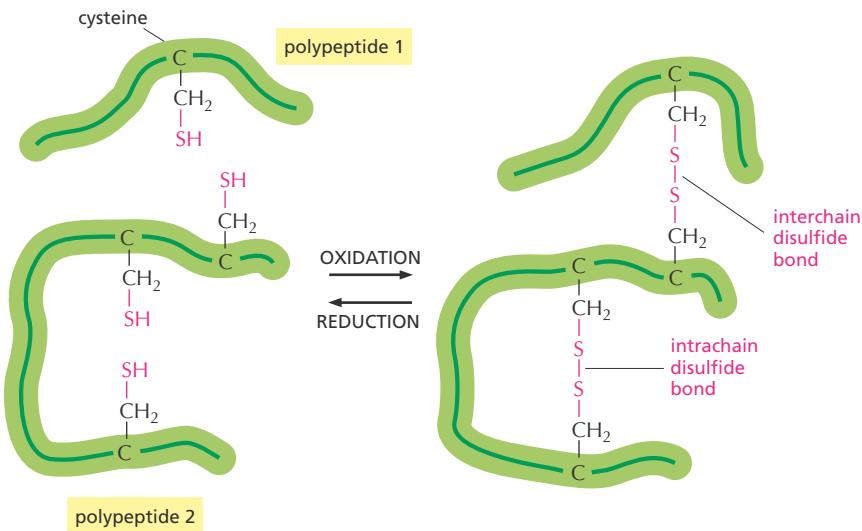


Figure 4–30 Disulfide bonds help stabilize a favored protein conformation. This diagram illustrates how covalent disulfide bonds form between adjacent cysteine side chains by the oxidation of their –SH groups. As indicated, these cross-links can join either two parts of the same polypeptide chain or two different polypeptide chains. Because the energy required to break one covalent bond is much larger than the energy required to break even a whole set of noncovalent bonds (see Table 2–1, p. 48), a disulfide bond can have a major stabilizing effect on a protein’s folded structure (Movie 4.6).

enzyme in tears, saliva, and other secretions that can disrupt bacterial cell walls—retains its antibacterial activity for a long time because it is stabilized by such disulfide cross-links.

Disulfide bonds generally do not form in the cell cytosol, where a high concentration of reducing agents converts such bonds back to cysteine –SH groups. Apparently, proteins do not require this type of structural reinforcement in the relatively mild conditions in the cytosol.

HOW PROTEINS WORK

As we have just seen, proteins are made from an enormous variety of amino acid sequences and can fold into a unique shape. The surface topography of a protein’s side chains endows each protein with a unique function, based on its chemical properties. The union of structure, chemistry, and function gives proteins the extraordinary ability to orchestrate the large number of dynamic processes that occur in cells.

Thus, for proteins, form and function are inextricably linked. But the fundamental question remains: How do proteins actually work? In this section, we will see that the activity of proteins depends on their ability to bind specifically to other molecules, allowing them to act as catalysts, structural supports, tiny motors, and so on. The examples we review here by no means exhaust the vast functional repertoire of proteins. However, the specialized functions of the proteins you will encounter elsewhere in this book are based on the same principles.

All Proteins Bind to Other Molecules

The biological properties of a protein molecule depend on its physical interaction with other molecules. Antibodies attach to viruses or bacteria as part of the body’s defenses; the enzyme hexokinase binds glucose and ATP to catalyze a reaction between them; actin molecules bind to one another to assemble into long filaments; and so on. Indeed, all proteins stick, or bind, to other molecules in a specific manner. In some cases, this binding is very tight; in others, it is weak and short-lived. As we saw in Chapter 3, the affinity of an enzyme for its substrate is reflected in its K_M : the lower the K_M , the tighter the binding.

Regardless of its strength, the binding of a protein to other biological molecules always shows great *specificity*: each protein molecule can bind to just one or a few molecules out of the many thousands of different

QUESTION 4–4

Hair is composed largely of fibers of the protein keratin. Individual keratin fibers are covalently cross-linked to one another by many disulfide (S–S) bonds. If curly hair is treated with mild reducing agents that break a few of the cross-links, pulled straight, and then oxidized again, it remains straight. Draw a diagram that illustrates the three different stages of this chemical and mechanical process at the level of the keratin filaments, focusing on the disulfide bonds. What do you think would happen if hair were treated with strong reducing agents that break all the disulfide bonds?

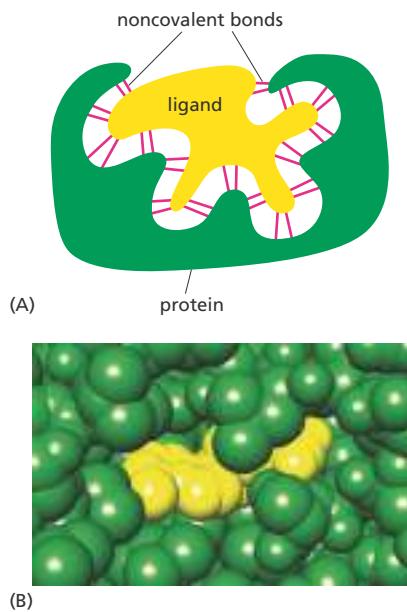


Figure 4–31 The binding of a protein to another molecule is highly selective. Many weak interactions are needed to enable a protein to bind tightly to a second molecule (a ligand). The ligand must therefore fit precisely into the protein's binding site, like a hand into a glove, so that a large number of noncovalent interactions can be formed between the protein and the ligand.

(A) Schematic drawing shows the binding of a hypothetical protein and ligand; (B) space-filling model.

molecules it encounters. Any substance that is bound by a protein—whether it is an ion, a small organic molecule, or a macromolecule—is referred to as a **ligand** for that protein (from the Latin *ligare*, “to bind”).

The ability of a protein to bind selectively and with high affinity to a ligand is due to the formation of a set of weak, noncovalent interactions—hydrogen bonds, electrostatic attractions, and van der Waals attractions—plus favorable hydrophobic forces (see Panel 2–7, pp. 78–79). Each individual noncovalent interaction is weak, so that effective binding requires many such bonds to be formed simultaneously. This is possible only if the surface contours of the ligand molecule fit very closely to the protein, matching it like a hand in a glove (**Figure 4–31**).

When molecules have poorly matching surfaces, few noncovalent interactions occur, and the two molecules dissociate as rapidly as they come together. This is what prevents incorrect and unwanted associations from forming between mismatched molecules. At the other extreme, when many noncovalent interactions are formed, the association can persist for a very long time. Strong binding between molecules occurs in cells whenever a biological function requires that the molecules remain tightly associated for a long time—for example, when a group of macromolecules come together to form a functional subcellular structure such as a ribosome.

The region of a protein that associates with a ligand, known as its **binding site**, usually consists of a cavity in the protein surface formed by a particular arrangement of amino acid side chains. These side chains can belong to amino acids that are widely separated on the linear polypeptide chain, but are brought together when the protein folds (**Figure 4–32**). Other regions on the surface often provide binding sites for different ligands that regulate the protein's activity, as we discuss later. Yet other parts of the protein may be required to attract or attach the protein to a particular location in the cell—for example, the hydrophobic α helix of a

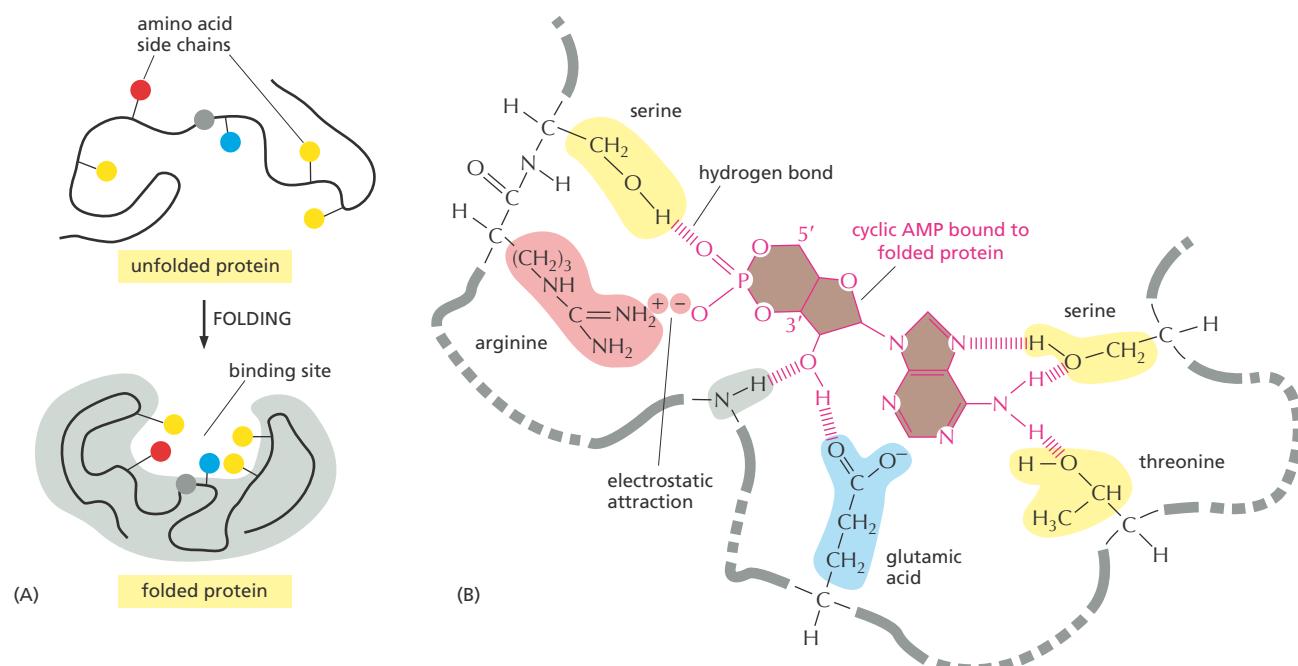


Figure 4–32 Binding sites allow proteins to interact with specific ligands. (A) The folding of the polypeptide chain typically creates a crevice or cavity on the folded protein's surface, where specific amino acid side chains are brought together in such a way that they can form a set of noncovalent bonds only with certain ligands. (B) Close-up view of an actual binding site showing the hydrogen bonds and an electrostatic interaction formed between a protein and its ligand (in this example, the bound ligand is cyclic AMP, shown in dark brown).

membrane-spanning protein allows it to be inserted into the lipid bilayer of a cell membrane (discussed in Chapter 11).

Although the atoms buried in the interior of a protein have no direct contact with the ligand, they provide an essential scaffold that gives the surface its contours and chemical properties. Even tiny changes to the amino acids in the interior of a protein can change the protein's three-dimensional shape and destroy its function.

There Are Billions of Different Antibodies, Each with a Different Binding Site

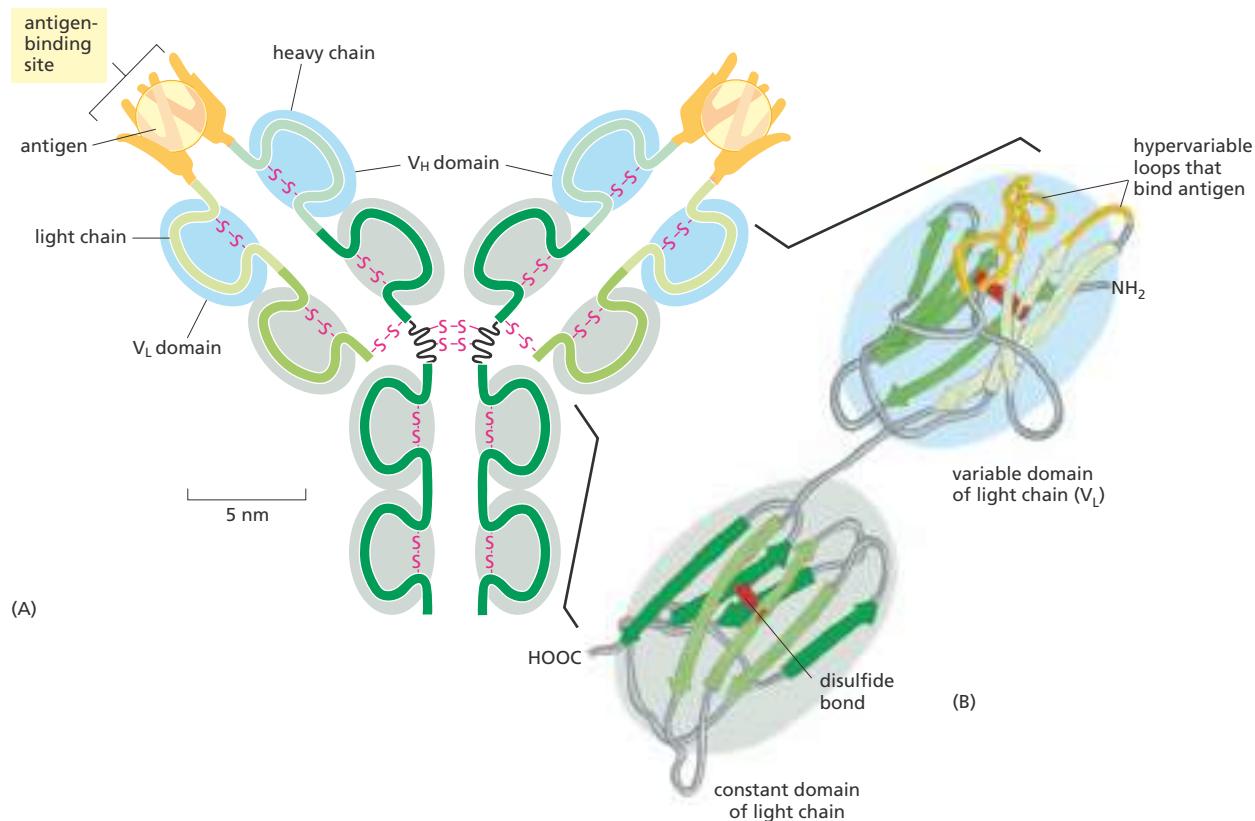
All proteins must bind to particular ligands to carry out their various functions. For antibodies, the universe of possible ligands is limitless. Each of us has the capacity to produce a huge variety of antibodies, among which there will be one that is capable of recognizing and binding tightly to almost any molecule imaginable.

Antibodies are immunoglobulin proteins produced by the immune system in response to foreign molecules, especially those on the surface of an invading microorganism. Each antibody binds to a particular target molecule extremely tightly, either inactivating the target directly or marking it for destruction. An antibody recognizes its target molecule—called an **antigen**—with remarkable specificity, and, because there are potentially billions of different antigens that a person might encounter, we have to be able to produce billions of different antibodies.

Antibodies are Y-shaped molecules with two identical antigen-binding sites, each of which is complementary to a small portion of the surface of the antigen molecule. A detailed examination of the antigen-binding sites of antibodies reveals that they are formed from several loops of polypeptide chain that protrude from the ends of a pair of closely juxtaposed protein domains (Figure 4–33). The amino acid sequence in these

Figure 4–33 An antibody is Y-shaped and has two identical antigen-binding sites, one on each arm of the Y.

(A) Schematic drawing of a typical antibody molecule. The protein is composed of four polypeptide chains (two identical heavy chains and two identical and smaller light chains), held together by disulfide bonds (red). Each chain is made up of several similar domains, here shaded either blue or gray. The antigen-binding site is formed where a heavy-chain variable domain (V_H) and a light-chain variable domain (V_L) come close together. These are the domains that differ most in their amino acid sequence in different antibodies—hence their name. (B) Ribbon drawing of a single light chain showing that the most variable parts of the polypeptide chain (orange) extend as loops at one end of the variable domain (V_L) to form half of one antigen-binding site of the antibody molecule shown in (A). Note that both the constant and variable domains are composed of a sandwich of two antiparallel β sheets (see also Figure 4–20C), connected by a disulfide bond (red).



loops can vary greatly without altering the basic structure of the antibody. An enormous diversity of antigen-binding sites can be generated by changing only the length and amino acid sequence of the loops, which is how the wide variety of different antibodies is formed ([Movie 4.7](#)).

With their unique combination of specificity and diversity, antibodies are not only indispensable for fighting off infections, they are also invaluable in the laboratory, where they can be used to identify, purify, and study other molecules ([Panel 4–2](#), pp. 146–147).

Enzymes Are Powerful and Highly Specific Catalysts

QUESTION 4–5

Use drawings to explain how an enzyme (such as hexokinase, mentioned in the text) can distinguish its normal substrate (here D-glucose) from the optical isomer L-glucose, which is not a substrate. (Hint: remembering that a carbon atom forms four single bonds that are tetrahedrally arranged and that the optical isomers are mirror images of each other around such a bond, draw the substrate as a simple tetrahedron with four different corners and then draw its mirror image. Using this drawing, indicate why only one optical isomer might bind to a schematic active site of an enzyme.)

For many proteins, binding to another molecule is their main function. An actin molecule, for example, need only associate with other actin molecules to form a filament. There are proteins, however, for which ligand binding is simply a necessary first step in their function. This is the case for the large and very important class of proteins called **enzymes**. These remarkable molecules are responsible for nearly all of the chemical transformations that occur in cells. Enzymes bind to one or more ligands, called **substrates**, and convert them into chemically modified products, doing this over and over again with amazing rapidity. As we saw in Chapter 3, they speed up reactions, often by a factor of a million or more, without themselves being changed—that is, enzymes act as **catalysts** that permit cells to make or break covalent bonds at will. This catalysis of organized sets of chemical reactions by enzymes creates and maintains the cell, making life possible.

Enzymes can be grouped into functional classes based on the chemical reactions they catalyze ([Table 4–1](#)). Each type of enzyme is highly specific, catalyzing only a single type of reaction. Thus, *hexokinase* adds a phosphate group to D-glucose but not to its optical isomer L-glucose; the blood-clotting enzyme *thrombin* cuts one type of blood-clotting protein between a particular arginine and its adjacent glycine and nowhere

TABLE 4–1 SOME COMMON FUNCTIONAL CLASSES OF ENZYMES

Enzyme Class	Biochemical Function
Hydrolase	General term for enzymes that catalyze a hydrolytic cleavage reaction
Nuclease	Breaks down nucleic acids by hydrolyzing bonds between nucleotides
Protease	Breaks down proteins by hydrolyzing peptide bonds between amino acids
Ligase	Joins two molecules together; DNA ligase joins two DNA strands together end-to-end
Isomerase	Catalyzes the rearrangement of bonds within a single molecule
Polymerase	Catalyzes polymerization reactions such as the synthesis of DNA and RNA
Kinase	Catalyzes the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins
Phosphatase	Catalyzes the hydrolytic removal of a phosphate group from a molecule
Oxido-reductase	General name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often called oxidases, reductases, or dehydrogenases
ATPase	Hydrolyzes ATP. Many proteins have an energy-harnessing ATPase activity as part of their function, including motor proteins such as myosin (discussed in Chapter 17) and membrane transport proteins such as the sodium pump (discussed in Chapter 12)

Enzyme names typically end in “-ase,” with the exception of some enzymes, such as pepsin, trypsin, thrombin, lysozyme, and so on, which were discovered and named before the convention became generally accepted at the end of the nineteenth century. The name of an enzyme usually indicates the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

else. As discussed in detail in Chapter 3, enzymes often work in tandem, with the product of one enzyme becoming the substrate for the next. The result is an elaborate network of *metabolic pathways* that provides the cell with energy and generates the many large and small molecules that the cell needs.

Lysozyme Illustrates How an Enzyme Works

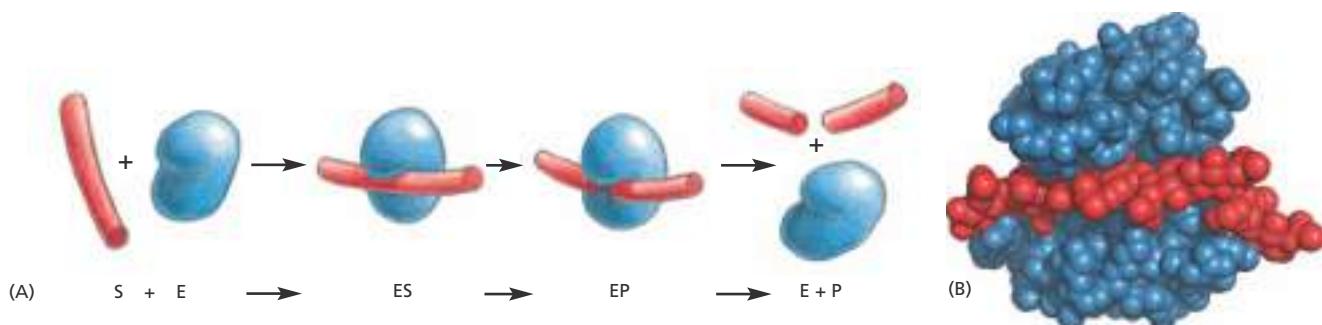
To explain how enzymes catalyze chemical reactions, we will use the example of **lysozyme**—an enzyme that acts as a natural antibiotic in egg white, saliva, tears, and other secretions. Lysozyme severs the polysaccharide chains that form the cell walls of bacteria. Because the bacterial cell is under pressure due to intracellular osmotic forces, cutting even a small number of polysaccharide chains causes the cell wall to rupture and the bacterium to burst, or lyse. Lysozyme is a relatively small and stable protein, which can be isolated easily in large quantities. For these reasons it has been intensively studied, and it was the first enzyme whose structure was worked out in atomic detail by X-ray crystallography.

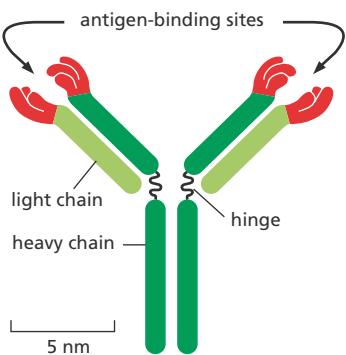
The reaction catalyzed by lysozyme is a hydrolysis: the enzyme adds a molecule of water to a single bond between two adjacent sugar groups in the polysaccharide chain, thereby causing the bond to break. The reaction is energetically favorable because the free energy of the severed polysaccharide chain is lower than the free energy of the intact chain. However, the pure polysaccharide can sit for years in water without being hydrolyzed to any detectable degree. This is because there is an energy barrier to such reactions, called the *activation energy* (discussed in Chapter 3, pp. 91–93). For a colliding water molecule to break a bond linking two sugars, the polysaccharide molecule has to be distorted into a particular shape—the **transition state**—in which the atoms around the bond have an altered geometry and electron distribution. To distort the polysaccharide in this way requires a large input of energy from random molecular collisions. In aqueous solution at room temperature, the energy of such collisions almost never exceeds the activation energy; therefore, hydrolysis occurs extremely slowly, if at all.

This is where the enzyme comes in. Like all enzymes, lysozyme has a binding site on its surface, termed an **active site**, that cradles the contours of its substrate molecule. Here, the catalysis of the chemical reaction occurs. Because its substrate is a polymer, lysozyme's active site is a long groove that holds six linked sugars in the polysaccharide chain at the same time. As soon as the enzyme–substrate complex forms, the enzyme cuts the polysaccharide by catalyzing the addition of a water molecule to one of its sugar-sugar bonds. The severed chain is then quickly released, freeing the enzyme for further cycles of cleavage (**Figure 4–34**).

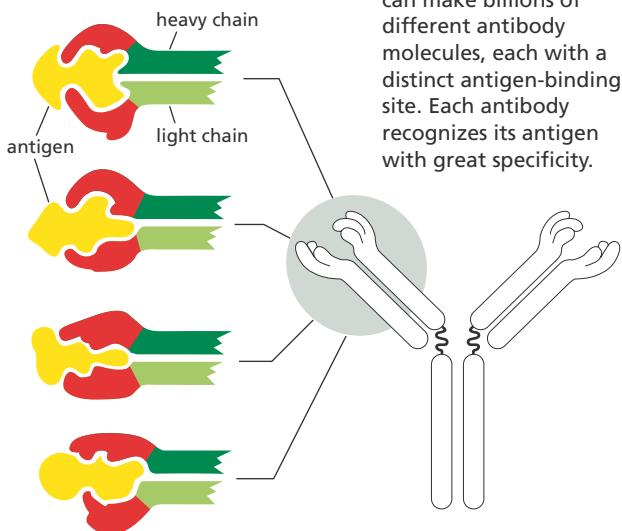
The chemistry that underlies the binding of lysozyme to its substrate is the same as that for antibody binding to its antigen: the formation of

Figure 4–34 Lysozyme cleaves a polysaccharide chain. (A) Schematic view of the enzyme lysozyme (E), which catalyzes the cutting of a polysaccharide substrate molecule (S). The enzyme first binds to the polysaccharide to form an enzyme–substrate complex (ES), then it catalyzes the cleavage of a specific covalent bond in the backbone of the polysaccharide. The resulting enzyme–product complex (EP) rapidly dissociates, releasing the products (P) and leaving the enzyme free to act on another substrate molecule. (B) A space-filling model of lysozyme bound to a short length of polysaccharide chain prior to cleavage. (B, courtesy of Richard J. Feldmann.)



THE ANTIBODY MOLECULE

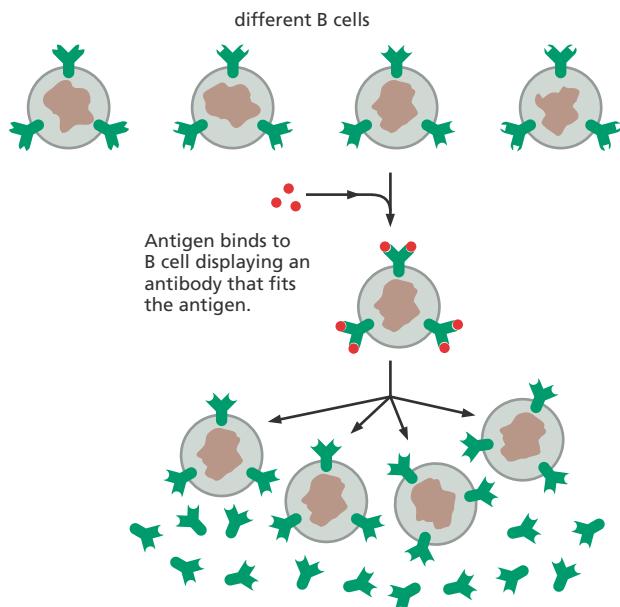
Antibodies are proteins that bind very tightly to their targets (antigens). They are produced in vertebrates as a defense against infection. Each antibody molecule is made of two identical light chains and two identical heavy chains, so the two antigen-binding sites are identical.

ANTIBODY SPECIFICITY

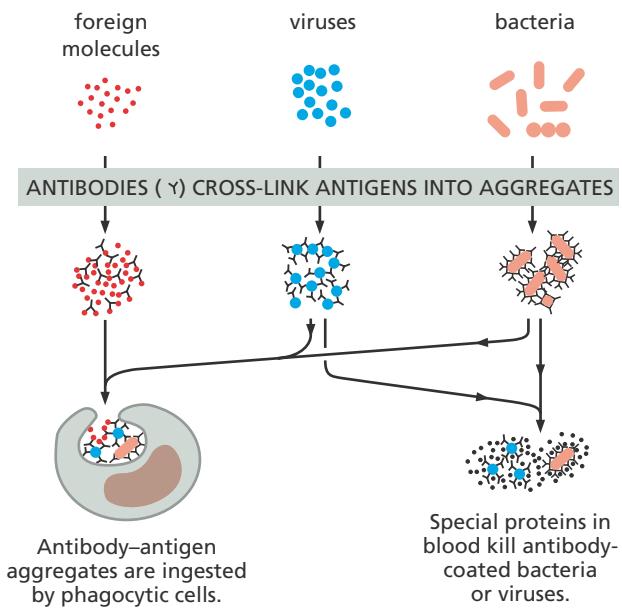
An individual human can make billions of different antibody molecules, each with a distinct antigen-binding site. Each antibody recognizes its antigen with great specificity.

B CELLS PRODUCE ANTIBODIES

Antibodies are made by a class of white blood cells called B lymphocytes, or B cells. Each resting B cell carries a different membrane-bound antibody molecule on its surface that serves as a receptor for recognizing a specific antigen. When antigen binds to this receptor, the B cell is stimulated to divide and to secrete large amounts of the same antibody in a soluble form.



The B cell is stimulated both to proliferate and to make and secrete more of same antibody.

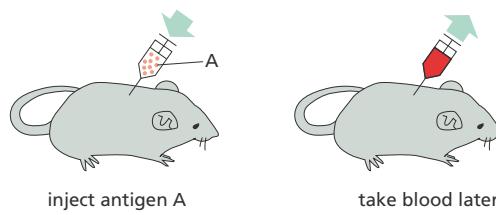
ANTIBODIES DEFEND US AGAINST INFECTION

Antibody–antigen aggregates are ingested by phagocytic cells.

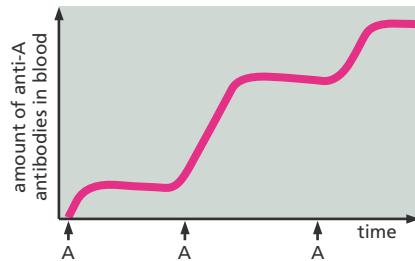
Special proteins in blood kill antibody-coated bacteria or viruses.

RAISING ANTIBODIES IN ANIMALS

Antibodies can be made in the laboratory by injecting an animal (usually a mouse, rabbit, sheep, or goat) with antigen A.



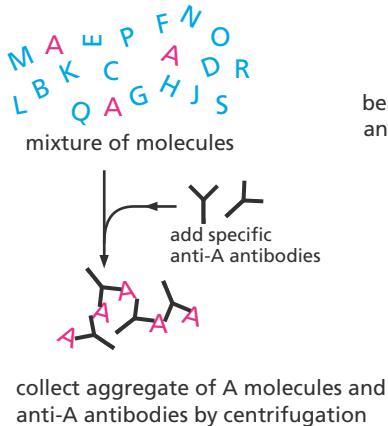
Repeated injections of the same antigen at intervals of several weeks stimulate specific B cells to secrete large amounts of anti-A antibodies into the bloodstream.



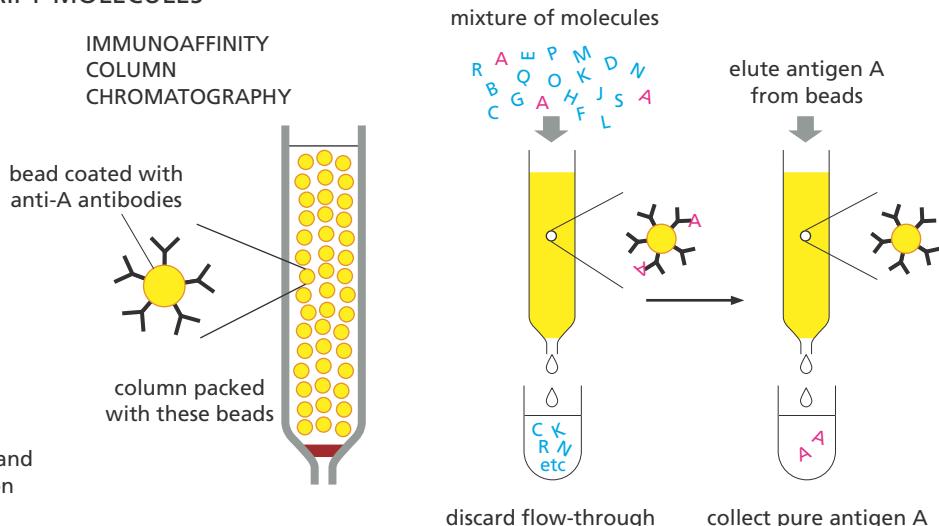
Because many different B cells are stimulated by antigen A, the blood will contain a variety of anti-A antibodies, each of which binds A in a slightly different way.

USING ANTIBODIES TO PURIFY MOLECULES

IMMUNOPRECIPITATION



IMMUNOAFFINITY COLUMN CHROMATOGRAPHY

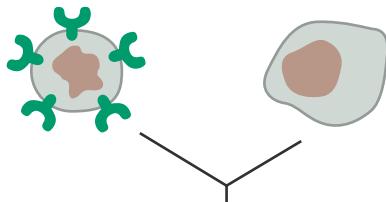


MONOCLONAL ANTIBODIES

Large quantities of a single type of antibody molecule can be obtained by fusing a B cell (taken from an animal injected with antigen A) with a tumor cell. The resulting hybrid cell divides indefinitely and secretes anti-A antibodies of a single (monoclonal) type.

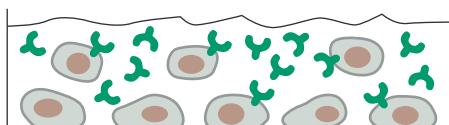
B cell from animal injected with antigen A makes anti-A antibody but does not divide forever.

Tumor cells in culture divide indefinitely but do not make antibody.

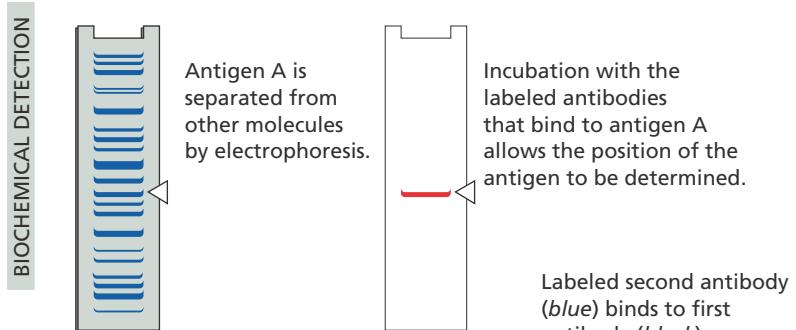
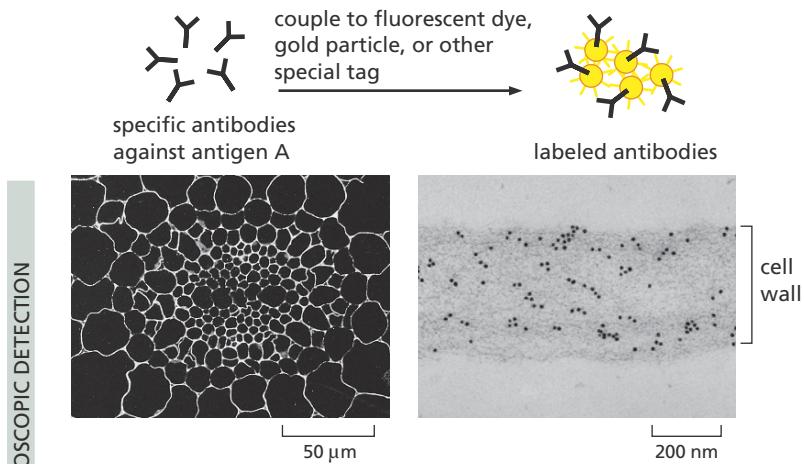


FUSE ANTIBODY-SECRETING B CELL WITH TUMOR CELL

Hybrid cell makes and secretes anti-A antibody and divides indefinitely.

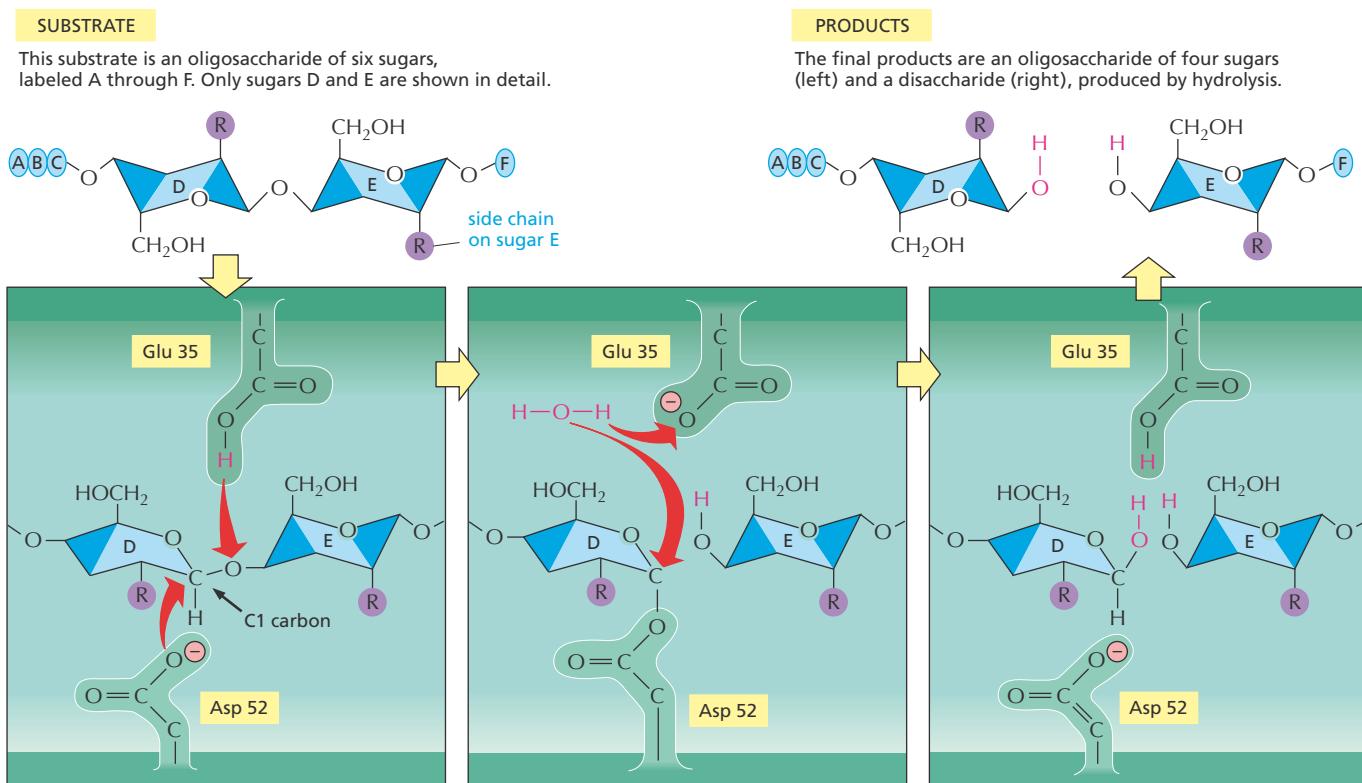


USING ANTIBODIES AS MOLECULAR TAGS



Note: In all cases, the sensitivity can be greatly increased by using multiple layers of antibodies. This "sandwich" method enables smaller numbers of antigen molecules to be detected.



**ES**

In the enzyme–substrate complex (ES), the enzyme forces sugar D into a strained conformation. The Glu 35 in the enzyme is positioned to serve as an acid that attacks the adjacent sugar–sugar bond by donating a proton (H^+) to sugar E; Asp 52 is poised to attack the C1 carbon atom of sugar D.

TRANSITION STATE

The Asp 52 has formed a covalent bond between the enzyme and the C1 carbon atom of sugar D. The Glu 35 then polarizes a water molecule (red), so that its oxygen can readily attack the C1 carbon atom of sugar D and displace Asp 52.

EP

The reaction of the water molecule (red) completes the hydrolysis and returns the enzyme to its initial state, forming the final enzyme–product complex (EP).

Figure 4–35 Enzymes bind to, and chemically alter, substrate molecules.

In the active site of lysozyme, a covalent bond in a polysaccharide molecule is bent and then broken. The top row shows the free substrate and the free products. The three lower panels depict sequential events at the enzyme active site, during which a sugar–sugar covalent bond is broken. Note the change in the conformation of sugar D in the enzyme–substrate complex compared with the free substrate. This conformation favors the formation of the transition state shown in the middle panel, greatly lowering the activation energy required for the reaction. The reaction, and the structure of lysozyme bound to its product, are shown in **Movie 4.8** and **Movie 4.9**. (Based on D.J. Vocadlo et al., *Nature* 412:835–838, 2001.)

multiple noncovalent bonds. However, lysozyme holds its polysaccharide substrate in such a way that one of the two sugars involved in the bond to be broken is distorted from its normal, most stable conformation. The bond to be broken is held close to two specific amino acids with acidic side chains—a glutamic acid and an aspartic acid—located within the active site of the enzyme. Conditions are thereby created in the microenvironment of the lysozyme active site that greatly reduce the activation energy necessary for the hydrolysis to take place (**Figure 4–35**). The overall chemical reaction, from the initial binding of the polysaccharide on the surface of the enzyme to the final release of the severed chains, occurs many millions of times faster than it would in the absence of enzyme.

Other enzymes use similar mechanisms to lower the activation energies and speed up the reactions they catalyze. In reactions involving two or more substrates, the active site also acts like a template or mold that brings the reactants together in the proper orientation for the reaction to occur (**Figure 4–36A**). As we saw for lysozyme, the active site of an enzyme contains precisely positioned chemical groups that speed up the reaction by altering the distribution of electrons in the substrates (**Figure 4–36B**). Binding to the enzyme also changes the shape of the substrate, bending bonds so as to drive the bound molecule toward a particular transition state (**Figure 4–36C**). Finally, like lysozyme, many enzymes participate intimately in the reaction by briefly forming a covalent bond between the substrate and an amino acid side chain in the active site. Subsequent steps in the reaction restore the side chain to its original state, so the enzyme remains unchanged after the reaction and can go on to catalyze many more reactions.

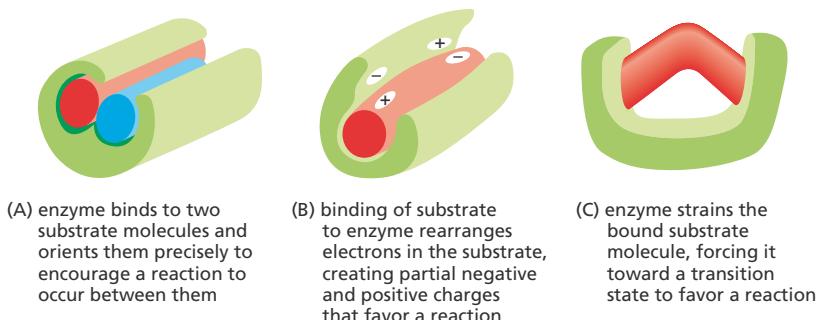


Figure 4-36 Enzymes can encourage a reaction in several ways. (A) Holding reacting substrates together in a precise alignment. (B) Rearranging the distribution of charge in a reaction intermediate. (C) Altering bond angles in the substrate to increase the rate of a particular reaction.

Many Drugs Inhibit Enzymes

Many of the drugs we take to treat or prevent illness work by blocking the activity of a particular enzyme. Cholesterol-lowering *statins* inhibit HMG-CoA reductase, an enzyme involved in the synthesis of cholesterol by the liver. *Methotrexate* kills some types of cancer cells by shutting down dihydrofolate reductase, an enzyme that produces a compound required for DNA synthesis during cell division. Because cancer cells have lost important intracellular control systems, some of them are unusually sensitive to treatments that interrupt chromosome replication, making them susceptible to methotrexate.

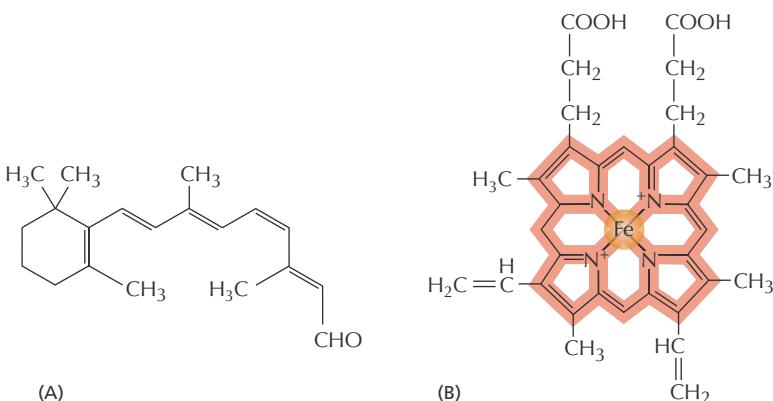
Pharmaceutical companies often develop drugs by first using automated methods to screen massive libraries of compounds to find chemicals that are able to inhibit the activity of an enzyme of interest. They can then chemically modify the most promising compounds to make them even more effective, enhancing their binding affinity and specificity for the target enzyme. As we discuss in Chapter 20, the anticancer drug Gleevec® was designed to specifically inhibit an enzyme whose aberrant behavior is required for the growth of a type of cancer called chronic myeloid leukemia. The drug binds tightly in the substrate-binding pocket of the enzyme, blocking its activity (see Figure 20-56).

Tightly Bound Small Molecules Add Extra Functions to Proteins

Although the order of amino acids in proteins gives these macromolecules their shape and functional versatility, sometimes the amino acids by themselves are not enough for a protein to do its job. Just as we use tools to enhance and extend the capabilities of our hands, so proteins often employ small, nonprotein molecules to perform functions that would be difficult or impossible using amino acids alone. Thus, the photoreceptor protein *rhodopsin*, which is the light-sensitive protein made by the rod cells in the retina, detects light by means of a small molecule, *retinal*, which is attached to the protein by a covalent bond to a lysine side chain (Figure 4-37A). Retinal changes its shape when it absorbs a photon of light, and this change is amplified by rhodopsin to trigger a cascade of reactions that eventually leads to an electrical signal being carried to the brain.

Another example of a protein that contains a nonprotein portion essential for its function is *hemoglobin* (see Figure 4-24). A molecule of hemoglobin carries four noncovalently bound *heme* groups, ring-shaped molecules each with a single central iron atom (Figure 4-37B). Heme gives hemoglobin (and blood) its red color. By binding reversibly to dissolved oxygen gas through its iron atom, heme enables hemoglobin to pick up oxygen in the lungs and release it in tissues that need it.

Figure 4–37 Retinal and heme are required for the function of certain proteins. (A) The structure of retinal, the light-sensitive molecule covalently attached to the rhodopsin protein in our eyes. (B) The structure of a heme group, shown with the carbon-containing heme ring colored red and the iron atom at its center in orange. A heme group is tightly, but noncovalently, bound to each of the four polypeptide chains in hemoglobin, the oxygen-carrying protein whose structure was shown in Figure 4–24.



When these small molecules are attached to their protein, they become an integral part of the protein molecule itself. We discuss in Chapter 11 how proteins can be anchored to cell membranes through covalently attached lipid molecules, and how proteins that are either secreted from the cell or bound to its surface can be modified by the covalent addition of sugars and oligosaccharides.

Enzymes, too, make use of nonprotein molecules: they frequently have a small molecule or metal atom associated with their active site that assists with their catalytic function. *Carboxypeptidase*, an enzyme that cuts polypeptide chains, carries a tightly bound zinc ion in its active site. During the cleavage of a peptide bond by carboxypeptidase, the zinc ion forms a transient bond with one of the substrate atoms, thereby assisting the hydrolysis reaction. In other enzymes, a small organic molecule serves a similar purpose. *Biotin*, for example, is found in enzymes that transfer a carboxyl group ($-COO^-$) from one molecule to another (see Figure 3–37). Biotin participates in these reactions by forming a transient covalent bond to the $-COO^-$ group to be transferred, thereby forming an activated carrier (see Table 3–2, p. 112). This small molecule is better suited for this function than any of the amino acids used to make proteins. Because biotin cannot be synthesized by humans, it must be provided in the diet; thus biotin is classified as a *vitamin*. Other vitamins are similarly needed to make small molecules that are essential components of our proteins; vitamin A, for example, is needed in the diet to make retinal, the light-sensitive part of rhodopsin just discussed.

HOW PROTEINS ARE CONTROLLED

So far, we have examined how proteins do their jobs: how binding to other proteins or small molecules allows them to perform their specific functions. But inside the cell, most proteins and enzymes do not work continuously, or at full speed. Instead, their activities are regulated in a coordinated fashion so the cell can maintain itself in an optimal state, producing only those molecules it requires to thrive under the current conditions. By coordinating when—and how vigorously—proteins function, the cell ensures that it does not deplete its energy reserves by accumulating molecules it does not need or waste its stockpiles of critical substrates. We now consider how cells control the activity of their enzymes and other proteins.

The regulation of protein activity occurs at many levels. At one level, the cell controls the amount of the protein it contains. It can do so by regulating the expression of the gene that encodes that protein (discussed in Chapter 8), and by regulating the rate at which the protein is degraded

(discussed in Chapter 7). At another level, the cell controls enzymatic activities by confining sets of enzymes to particular subcellular compartments, often—but not always—enclosed by distinct membranes (discussed in Chapters 14 and 15). But the most rapid and general mechanism used to adjust the activity of a protein occurs at the level of the protein itself. Although proteins can be switched on or off in various ways, as we see next, all of these mechanisms cause the protein to alter its shape, and therefore its function.

The Catalytic Activities of Enzymes Are Often Regulated by Other Molecules

A living cell contains thousands of different enzymes, many of which are operating at the same time in the same small volume of the cytosol. By their catalytic action, enzymes generate a complex web of metabolic pathways, each composed of chains of chemical reactions in which the product of one enzyme becomes the substrate of the next. In this maze of pathways, there are many branch points where different enzymes compete for the same substrate. The system is so complex that elaborate controls are required to regulate when and how rapidly each reaction occurs.

A common type of control occurs when a molecule other than a substrate specifically binds to an enzyme at a special *regulatory site*, altering the rate at which the enzyme converts its substrate to product. In **feedback inhibition**, for example, an enzyme acting early in a reaction pathway is inhibited by a late product of that pathway. Thus, whenever large quantities of the final product begin to accumulate, the product binds to an earlier enzyme and slows down its catalytic action, limiting further entry of substrates into that reaction pathway (Figure 4–38). Where pathways branch or intersect, there are usually multiple points of control by different final products, each of which works to regulate its own synthesis (Figure 4–39). Feedback inhibition can work almost instantaneously and is rapidly reversed when product levels fall.

Feedback inhibition is a *negative regulation*: it prevents an enzyme from acting. Enzymes can also be subject to *positive regulation*, in which the enzyme's activity is stimulated by a regulatory molecule rather than being suppressed. Positive regulation occurs when a product in one branch of the metabolic maze stimulates the activity of an enzyme in another pathway.

Allosteric Enzymes Have Two or More Binding Sites That Influence One Another

One feature of feedback inhibition was initially puzzling to those who discovered it. Unlike what one expects to see for a competitive inhibitor (see Figure 3–29), the regulatory molecule often has a shape that is totally different from the shape of the enzyme's preferred substrate. Indeed, when this form of regulation was discovered in the 1960s, it was termed *allostery* (from the Greek *allo*, “other,” and *stere*, “solid” or “shape”). As more was learned about feedback inhibition, researchers realized that many enzymes must have at least two different binding sites on their surface: the active site that recognizes the substrates and one or more sites that recognize regulatory molecules. And that these sites must somehow “communicate” to allow the catalytic events at the active site to be influenced by the binding of the regulatory molecule at its separate site.

The interaction between sites that are located in different regions on a protein molecule is now known to depend on *conformational changes* in the protein: binding of a ligand to one of the sites causes a shift in the protein's structure from one folded shape to a slightly different folded shape,

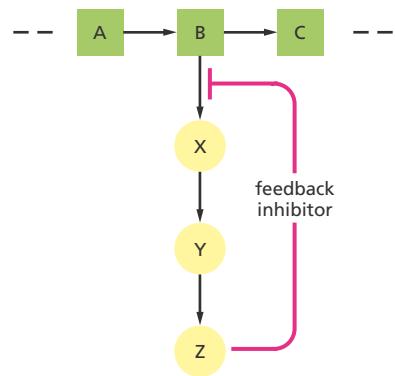


Figure 4–38 Feedback inhibition regulates the flow through biosynthetic pathways.

B is the first metabolite in a pathway that gives the end product Z. Z inhibits the first enzyme that is specific to its own synthesis and thereby limits its own concentration in the cell. This form of negative regulation is called feedback inhibition.

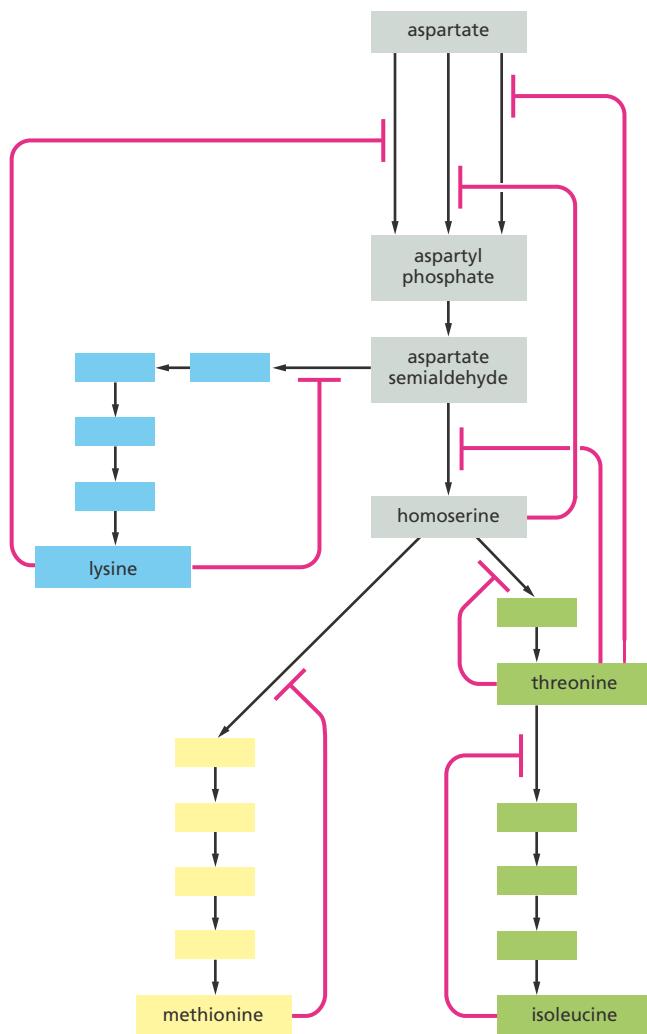
QUESTION 4–6

Consider the drawing in Figure 4–38. What will happen if, instead of the indicated feedback,

- Feedback inhibition from Z affects the step B → C only?
- Feedback inhibition from Z affects the step Y → Z only?
- Z is a positive regulator of the step B → X?
- Z is a positive regulator of the step B → C?

For each case, discuss how useful these regulatory schemes would be for a cell.

Figure 4–39 Feedback inhibition at multiple points regulates connected metabolic pathways. The biosynthetic pathways for four different amino acids in bacteria are shown, starting from the amino acid aspartate. The red lines indicate points at which products feed back to inhibit enzymes and the blank boxes represent intermediates in each pathway. In this example, each amino acid controls the first enzyme specific to its own synthesis, thereby limiting its own concentrations and avoiding a wasteful buildup of intermediates. Some of the products also separately inhibit the initial set of reactions common to all the syntheses. Three different enzymes catalyze the initial reaction from aspartate to aspartyl phosphate, and each of these enzymes is inhibited by a different product.

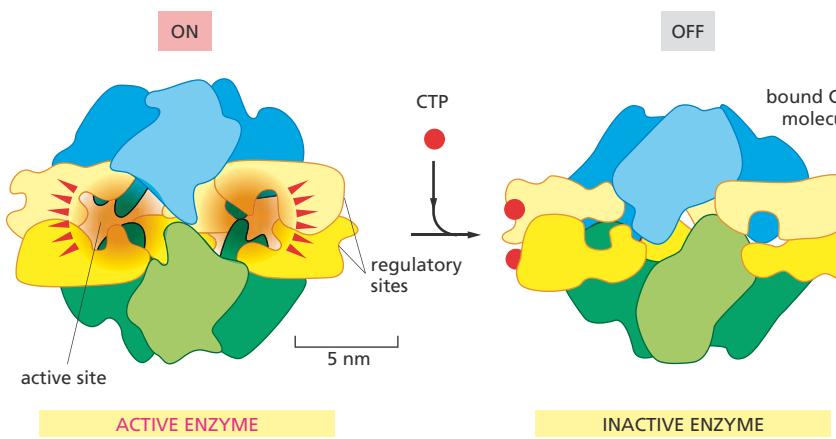


which alters the binding of a ligand to a second site. Many enzymes have two conformations that differ in activity, each stabilized by the binding of different ligands. During feedback inhibition, for example, the binding of an inhibitor at a regulatory site on the protein causes the protein to shift to a conformation in which its active site—located elsewhere in the protein—becomes less accommodating to the substrate molecule (**Figure 4–40**).

Many—if not most—protein molecules are **allosteric**: they can adopt two or more slightly different conformations, and their activity can be regulated by a shift from one to another. This is true not only for enzymes but also for many other proteins as well. The chemistry involved here is extremely simple in concept: because each protein conformation will have somewhat different contours on its surface, the protein’s binding sites for ligands will be altered when the protein changes shape. Each ligand will stabilize the conformation that it binds to most strongly, and at high enough concentrations a ligand will tend to “switch” the population of proteins to the conformation that it favors (**Figure 4–41**).

Phosphorylation Can Control Protein Activity by Causing a Conformational Change

Enzymes are regulated solely by the binding of small molecules. Another method that eukaryotic cells use with great frequency to regulate protein



activity involves attaching a phosphate group covalently to one or more of the protein's amino acid side chains. Because each phosphate group carries two negative charges, the enzyme-catalyzed addition of a phosphate group can cause a major conformational change in a protein by, for example, attracting a cluster of positively charged amino acid side chains from somewhere else in the same protein. This conformational change can, in turn, affect the binding of ligands elsewhere on the protein surface, thereby altering the protein's activity. Removal of the phosphate group by a second enzyme will return the protein to its original conformation and restore its initial activity.

This reversible **protein phosphorylation** controls the activity of many types of proteins in eukaryotic cells; indeed, it is used so extensively that more than one-third of the 10,000 or so proteins in a typical mammalian cell are phosphorylated at any one time. The addition and removal of phosphate groups from specific proteins often occur in response to signals that specify some change in a cell's state. For example, the complicated series of events that takes place as a eukaryotic cell divides is timed largely in this way (discussed in Chapter 18). And many of the intracellular signaling pathways activated by extracellular signals such as hormones depend on a network of protein phosphorylation events (discussed in Chapter 16).

Protein phosphorylation involves the enzyme-catalyzed transfer of the terminal phosphate group of ATP to the hydroxyl group on a serine, threonine, or tyrosine side chain of the protein. This reaction is catalyzed

Figure 4–40 Feedback inhibition triggers a conformational change in an enzyme. The enzyme shown, aspartate transcarbamoylase from *E. coli*, was used in early studies of allosteric regulation. This large multisubunit enzyme (see Figure 4–11) catalyzes an important reaction that begins the synthesis of the pyrimidine ring of C, U, and T nucleotides (see Panel 2–6, p. 76–77). One of the final products of this pathway, cytosine triphosphate (CTP), binds to the enzyme to turn it off whenever CTP is plentiful. This diagram shows the conformational change that occurs when the enzyme is turned off by CTP binding to its four regulatory sites, which are distinct from the active site where the substrate binds. Note that the aspartate transcarbamoylase shown in Figure 4–11 is seen from the top. This figure depicts the enzyme as seen from the side.

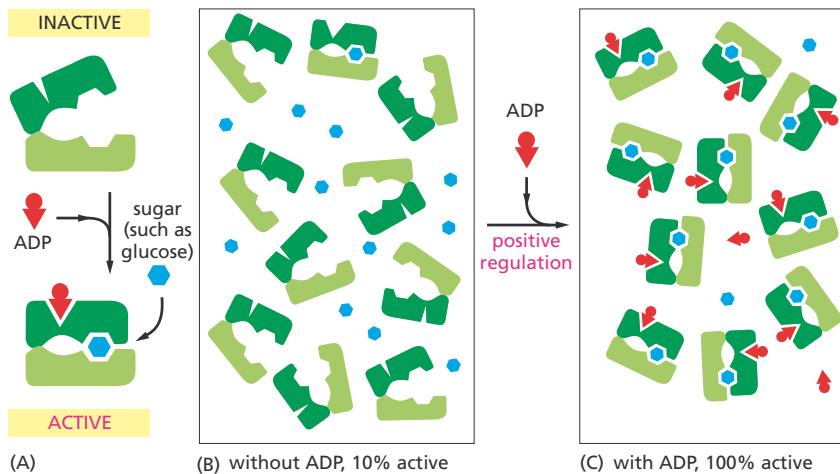


Figure 4–41 The equilibrium between two conformations of a protein is affected by the binding of a regulatory ligand. (A) Schematic diagram of a hypothetical, allosterically regulated enzyme for which a rise in the concentration of ADP molecules (red wedges) increases the rate at which the enzyme catalyzes the oxidation of sugar molecules (blue hexagons). (B) With no ADP present, only a small fraction of the enzyme molecules spontaneously adopt the active (closed) conformation; most are in the inactive (open) conformation. (C) Because ADP can bind to the protein only in its closed, active conformation, an increase in ADP concentration locks nearly all of the enzyme molecules in the active form. Such an enzyme could be used, for example, to sense when ADP is building up in the cell—which is usually a sign that ATP is decreasing. In this way, the increase in ADP would increase the oxidation of sugars to provide more energy for the synthesis of ATP from ADP—an example of positive regulation.

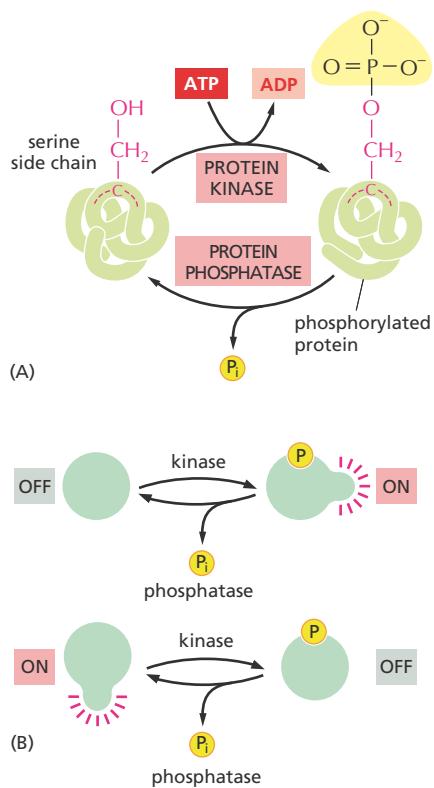


Figure 4–42 Protein phosphorylation is a very common mechanism for regulating protein activity. Many thousands of proteins in a typical eukaryotic cell are modified by the covalent addition of one or more phosphate groups. (A) The general reaction, shown here, entails transfer of a phosphate group from ATP to an amino acid side chain of the target protein by a protein kinase. Removal of the phosphate group is catalyzed by a second enzyme, a protein phosphatase. In this example, the phosphate is added to a serine side chain; in other cases, the phosphate is instead linked to the –OH group of a threonine or tyrosine side chain. (B) Phosphorylation can either increase or decrease the protein’s activity, depending on the site of phosphorylation and the structure of the protein.

by a **protein kinase**. The reverse reaction—removal of the phosphate group, or *dephosphorylation*—is catalyzed by a **protein phosphatase** (**Figure 4–42A**). Phosphorylation can either stimulate protein activity or inhibit it, depending on the protein involved and the site of phosphorylation (**Figure 4–42B**). Cells contain hundreds of different protein kinases, each responsible for phosphorylating a different protein or set of proteins. Cells also contain a smaller set of different protein phosphatases; some of these are highly specific and remove phosphate groups from only one or a few proteins, whereas others act on a broad range of proteins. The state of phosphorylation of a protein at any moment in time, and thus its activity, will depend on the relative activities of the protein kinases and phosphatases that act on it.

For many proteins, a phosphate group is added to a particular side chain and then removed in a continuous cycle. Phosphorylation cycles of this kind allow proteins to switch rapidly from one state to another. The more rapidly the cycle is “turning,” the faster the concentration of a phosphorylated protein can change in response to a sudden stimulus that increases its rate of phosphorylation. However, keeping the cycle turning costs energy, because one molecule of ATP is hydrolyzed with each turn of the cycle.

Covalent Modifications Also Control the Location and Interaction of Proteins

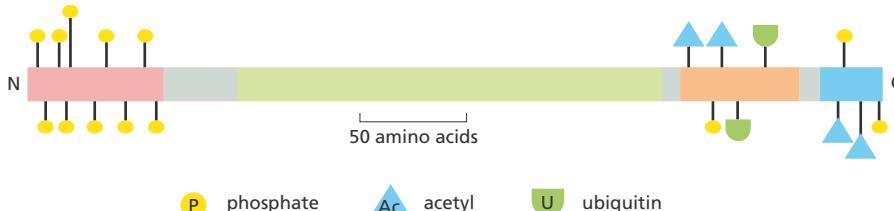
Phosphorylation can do more than control a protein’s activity; it can create docking sites where other proteins can bind, thus promoting the assembly of proteins into larger complexes. For example, when extracellular signals stimulate a class of cell-surface, transmembrane proteins called *receptor tyrosine kinases*, they cause the receptor proteins to phosphorylate themselves on certain tyrosines. The phosphorylated tyrosines then serve as docking sites for the binding and activation of various intracellular signaling proteins, which pass along the message to the cell interior and change the behavior of the cell (see Figure 16–32).

Phosphorylation is not the only form of covalent modification that can affect a protein’s activity or location. More than 100 types of covalent modifications can occur in the cell, each playing its own role in regulating protein function. Many proteins are modified by the addition of an acetyl group to a lysine side chain. And the addition of the fatty acid palmitate to a cysteine side chain drives a protein to associate with cell membranes. Attachment of ubiquitin, a 76-amino-acid polypeptide, can target a protein for degradation, as we discuss in Chapter 7. Each of these modifying groups is enzymatically added or removed depending on the needs of the cell.

A large number of proteins are modified on more than one amino acid side chain. The p53 protein, which plays a central part in controlling how a cell responds to DNA damage and other stresses, can be modified at 20 sites (**Figure 4–43**). Because an enormous number of combinations of these 20 modifications is possible, the protein’s behavior can in principle be altered in a huge number of ways.

The set of covalent modifications that a protein contains at any moment constitutes an important form of regulation. The attachment or removal of these modifying groups controls the behavior of a protein, changing its activity or stability, its binding partners, or its location inside the cell. In some cases, the modification alters the protein’s conformation; in others, it serves as a docking site for other proteins to attach. This layer of control enables the cell to make optimal use of its proteins, and it allows the cell to respond rapidly to changes in its environment.

SOME KNOWN MODIFICATIONS OF PROTEIN p53



GTP-Binding Proteins Are Also Regulated by the Cyclic Gain and Loss of a Phosphate Group

Eukaryotic cells have a second way to regulate protein activity by phosphate addition and removal. In this case, however, the phosphate is not enzymatically transferred from ATP to the protein. Instead, the phosphate is part of a guanine nucleotide—guanosine triphosphate (GTP)—that is bound tightly to various types of **GTP-binding proteins**. These proteins act as molecular switches: they are in their active conformation when GTP is bound, but they can hydrolyze this GTP to GDP, which releases a phosphate and flips the protein to an inactive conformation. As with protein phosphorylation, this process is reversible: the active conformation is regained by dissociation of the GDP, followed by the binding of a fresh molecule of GTP (Figure 4–44).

A large variety of such GTP-binding proteins function as molecular switches in cells. The dissociation of GDP and its replacement by GTP, which turns the switch on, is often stimulated in response to a signal received by the cell. The GTP-binding proteins in turn bind to other proteins to control their activities; their crucial role in intracellular signaling pathways is discussed in detail in Chapter 16.

ATP Hydrolysis Allows Motor Proteins to Produce Directed Movements in Cells

We have seen how conformational changes in proteins play a central part in enzyme regulation and cell signaling. But conformational changes also play another important role in the operation of the eukaryotic cell: they enable certain specialized proteins to drive directed movements of cells and their components. These **motor proteins** generate the forces responsible for muscle contraction and most other eukaryotic cell movements. They also power the intracellular movements of organelles and macromolecules. For example, they help move chromosomes to opposite ends of the cell during mitosis (discussed in Chapter 18), and they move organelles along cytoskeletal tracks (discussed in Chapter 17).

How are shape changes in proteins used to generate such orderly movements? If, for example, a protein is required to walk along a cytoskeletal fiber, it can move by undergoing a series of conformational changes. However, with nothing to drive these changes in an orderly sequence, the shape changes will be perfectly reversible. Thus the protein can only wander randomly back and forth (Figure 4–45).

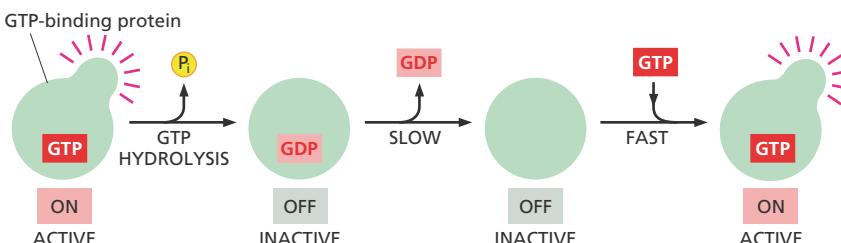


Figure 4–43 The modification of a protein at multiple sites can control the protein's behavior. This diagram shows some of the covalent modifications that control the activity and degradation of the protein p53, an important gene regulatory protein that regulates a cell's response to damage (discussed in Chapter 18). Not all of these modifications will be present at the same time. Colors along the body of the protein represent distinct protein domains, including one that binds to DNA (green) and one that activates gene transcription (pink). All of the modifications shown are located within relatively unstructured regions of the polypeptide chain.

QUESTION 4–7

Explain how phosphorylation and the binding of a nucleotide (such as ATP or GTP) can both be used to regulate protein activity. What do you suppose are the advantages of either form of regulation?

Figure 4–44 GTP-binding proteins function as molecular switches. A GTP-binding protein requires the presence of a tightly bound GTP molecule to be active (switch ON). The active protein can shut itself off by hydrolyzing its bound GTP to GDP and inorganic phosphate (P_i), which converts the protein to an inactive conformation (switch OFF). To reactivate the protein, the tightly bound GDP must dissociate, a slow step that can be greatly accelerated by specific signals; once the GDP dissociates, a molecule of GTP quickly replaces it, returning the protein to its active conformation.



Figure 4–45 Changes in conformation can allow a protein to “walk” along a cytoskeletal filament. This protein’s three different conformations allow it to wander randomly back and forth while bound to a filament. Without an input of energy to drive its movement in a single direction, the protein will only shuffle aimlessly, getting nowhere.

To make the conformational changes unidirectional—and force the entire cycle of movement to proceed in one direction—it is enough to make any one of the steps irreversible. For most proteins that are able to move in a single direction for long distances, this irreversibility is achieved by coupling one of the conformational changes to the hydrolysis of an ATP molecule bound to the protein—which is why motor proteins are also ATPases. A great deal of free energy is released when ATP is hydrolyzed, making it very unlikely that the protein will undergo a reverse shape change—as required for moving backward. (Such a reversal would require that the ATP hydrolysis be reversed, by adding a phosphate molecule to ADP to form ATP.) As a consequence, the protein moves steadily forward (**Figure 4–46**).

Many motor proteins generate directional movement by using the hydrolysis of a tightly bound ATP molecule to drive an orderly series of conformational changes. These movements can be rapid: the muscle motor protein *myosin* walks along actin filaments at about 6 $\mu\text{m/sec}$ during muscle contraction (as discussed in Chapter 17).

Proteins Often Form Large Complexes That Function as Protein Machines

As one progresses from small, single-domain proteins to large proteins formed from many domains, the functions that the proteins can perform become more elaborate. The most complex tasks, however, are carried out by large protein assemblies formed from many protein molecules. Now that it is possible to reconstruct biological processes in cell-free systems in a test tube, it is clear that each central process in a cell—including DNA replication, gene transcription, protein synthesis, vesicle budding, and transmembrane signaling—is catalyzed by a highly coordinated, linked set of many proteins. In most such **protein machines**, the hydrolysis of bound nucleoside triphosphates (ATP or GTP) drives an ordered series of conformational changes in some of the individual protein subunits, enabling the ensemble of proteins to move coordinately. In this way, the appropriate enzymes can be positioned to carry out successive reactions in a series—as during the synthesis of proteins on a ribosome, for example (discussed in Chapter 7). Likewise, a large multiprotein complex moves rapidly along DNA to replicate the DNA double helix during cell division (discussed in Chapter 6). A simple mechanical analogy is illustrated in **Figure 4–47**.

Cells have evolved a large number of different protein machines suited to performing a variety of biological tasks. Cells employ protein machines for the same reason that humans have invented mechanical and electronic machines: for almost any job, manipulations that are spatially and temporally coordinated through linked processes are much more efficient than is the sequential use of individual tools.

Figure 4–46 A schematic model of how a motor protein uses ATP hydrolysis to move in one direction along a cytoskeletal filament. An orderly transition among three conformations is driven by the hydrolysis of a bound ATP molecule and the release of the products: ADP and inorganic phosphate (P_i). Because these transitions are coupled to the hydrolysis of ATP, the entire cycle is essentially irreversible. Through repeated cycles, the protein moves continuously to the right along the filament. The movement of a single molecule of myosin has been captured by atomic force microscopy.

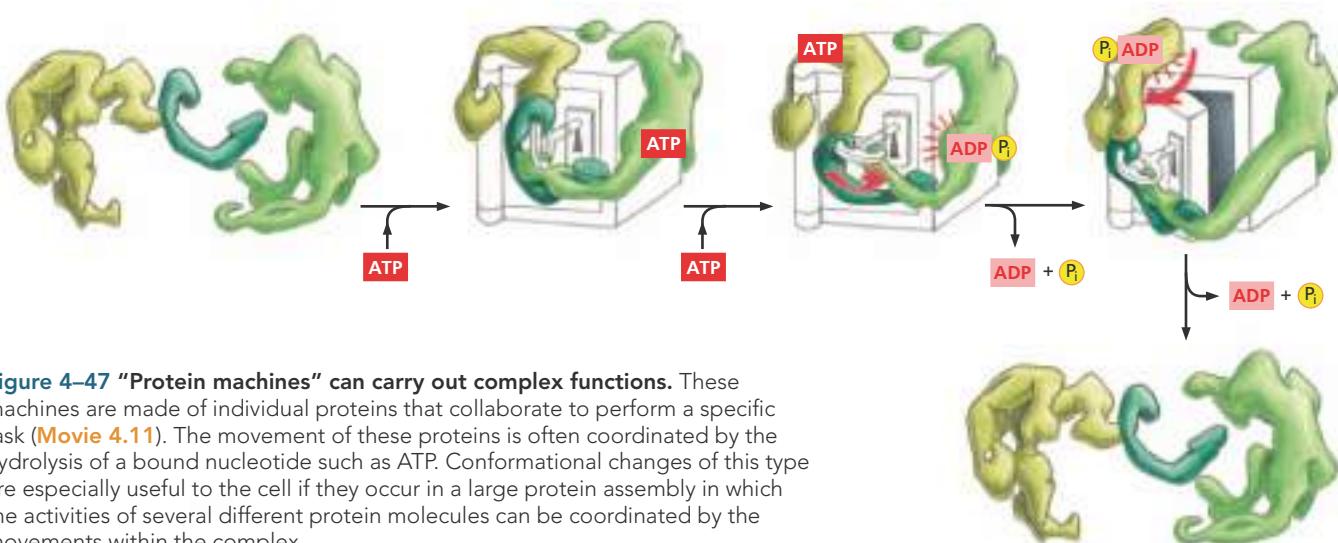


Figure 4–47 “Protein machines” can carry out complex functions. These machines are made of individual proteins that collaborate to perform a specific task ([Movie 4.11](#)). The movement of these proteins is often coordinated by the hydrolysis of a bound nucleotide such as ATP. Conformational changes of this type are especially useful to the cell if they occur in a large protein assembly in which the activities of several different protein molecules can be coordinated by the movements within the complex.

HOW PROTEINS ARE STUDIED

Understanding how a particular protein functions calls for detailed structural and biochemical analyses—both of which require large amounts of pure protein. But isolating a single type of protein from the thousands of other proteins present in a cell is a formidable task. For many years, proteins had to be purified directly from the source—the tissues in which they are most plentiful. That approach was inconvenient, entailing, for example, early-morning trips to the slaughterhouse. More important, the complexity of intact tissues and organs is a major disadvantage when trying to purify particular molecules, because a long series of chromatography steps is generally required. These procedures not only take weeks to perform, but they also yield only a few milligrams of pure protein.

Nowadays, proteins are more often isolated from cells that are grown in a laboratory (see, for example, Figure 1–38). Often these cells have been “tricked” into making large quantities of a given protein using the genetic engineering techniques that we describe in Chapter 10. Such engineered cells frequently allow large amounts of pure protein to be obtained in only a few days.

In this section, we outline how proteins are extracted and purified from cultured cells and other sources. We describe how these proteins are analyzed to determine their amino acid sequence and their three-dimensional structure. Finally, we discuss how technical advances are allowing proteins to be analyzed, cataloged, manipulated, and even designed from scratch.

Proteins Can be Purified from Cells or Tissues

Whether starting with a piece of liver, a dish of cultured cells, or a vat of bacterial, yeast, or animal cells that have been engineered to produce a protein of interest, the first step in any purification procedure is to break open the cells to release their contents. The resulting slurry is called a *cell homogenate* or *extract*. This physical disruption is followed by an initial fractionation procedure to separate out the class of molecules of interest—for example, all the soluble proteins in the cell ([Panel 4–3](#), pp. 164–165).

With this collection of proteins in hand, the job is then to isolate the desired protein. The standard approach involves purifying the protein

QUESTION 4–8

Explain why the hypothetical enzymes in Figure 4–47 have a great advantage in opening the safe if they work together in a protein complex, as opposed to working individually in an unlinked, sequential manner.

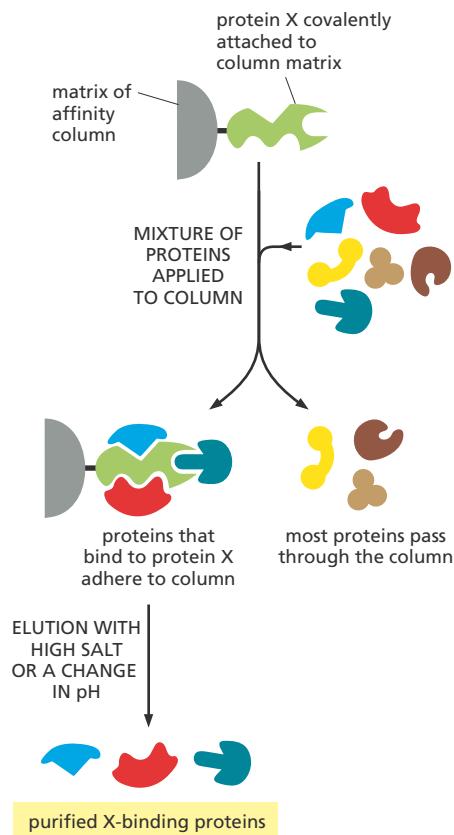


Figure 4–48 Affinity chromatography can be used to isolate the binding partners of a protein of interest. The purified protein of interest (protein X) is covalently attached to the matrix of a chromatography column. An extract containing a mixture of proteins is then loaded onto the column. Those proteins that associate with protein X inside the cell will usually bind to it on the column. Proteins not bound to the column pass right through, and the proteins that are bound tightly to protein X can then be released by changing the pH or ionic composition of the washing solution.

through a series of **chromatography** steps, which use different materials to separate the individual components of a complex mixture into portions, or *fractions*, based on the properties of the protein—such as size, shape, or electrical charge. After each separation step, the fractions are examined to determine which ones contain the protein of interest. These fractions are then pooled and subjected to additional chromatography steps until the desired protein is obtained in pure form.

The most efficient forms of protein chromatography separate polypeptides on the basis of their ability to bind to a particular molecule—a process called *affinity chromatography* (Panel 4–4, p. 166). If large amounts of antibodies that recognize the protein are available, for example, they can be attached to the matrix of a chromatography column and used to help extract the protein from a mixture (see Panel 4–2, pp. 146–147).

Affinity chromatography can also be used to isolate proteins that interact physically with the protein being studied. In this case, a purified protein of interest is attached tightly to the column matrix; the proteins that bind to it will remain in the column and can then be removed by changing the composition of the washing solution (Figure 4–48).

Proteins can also be separated by **electrophoresis**. In this technique, a mixture of proteins is loaded onto a polymer gel and subjected to an electric field; the polypeptides will then migrate through the gel at different speeds depending on their size and net charge (Panel 4–5, p. 167). If too many proteins are present in the sample, or if the proteins are very similar in their migration rate, they can be resolved further using two-dimensional gel electrophoresis (see Panel 4–5). These electrophoretic approaches yield a number of bands or spots that can be visualized by staining; each band or spot contains a different protein. Chromatography and electrophoresis—both developed more than 50 years ago but greatly improved since—have been instrumental in building an understanding of what proteins look like and how they behave (Table 4–2). Both techniques are still frequently used in laboratories.

Once a protein has been obtained in pure form, it can be used in biochemical assays to study the details of its activity. It can also be subjected to techniques that reveal its amino acid sequence and precise three-dimensional structure.

Determining a Protein's Structure Begins with Determining Its Amino Acid Sequence

The task of determining the amino acid sequence of a protein can be accomplished in several ways. For many years, sequencing a protein was done by directly analyzing the amino acids in the purified protein. First, the protein was broken down into smaller pieces using a selective protease; the enzyme trypsin, for example, cleaves polypeptide chains on the carboxyl side of a lysine or an arginine. Then the identities of the amino acids in each fragment were determined chemically. The first protein sequenced in this way was the hormone *insulin*, in 1955.

TABLE 4–2 HISTORICAL LANDMARKS IN OUR UNDERSTANDING OF PROTEINS

1838	The name “protein” (from the Greek <i>proteios</i> , “primary”) was suggested by Berzelius for the complex nitrogen-rich substance found in the cells of all animals and plants.
1819–1904	Most of the 20 common amino acids found in proteins were discovered.
1864	Hoppe-Seyler crystallized, and named, the protein hemoglobin.
1894	Fischer proposed a lock-and-key analogy for enzyme–substrate interactions.
1897	Buchner and Buchner showed that cell-free extracts of yeast can break down sucrose to form carbon dioxide and ethanol, thereby laying the foundations of enzymology.
1926	Sumner crystallized urease in pure form, demonstrating that proteins could possess the catalytic activity of enzymes; Svedberg developed the first analytical ultracentrifuge and used it to estimate the correct molecular weight of hemoglobin.
1933	Tiselius introduced electrophoresis for separating proteins in solution.
1934	Bernal and Crowfoot presented the first detailed X-ray diffraction patterns of a protein, obtained from crystals of the enzyme pepsin.
1942	Martin and Synge developed chromatography, a technique now widely used to separate proteins.
1951	Pauling and Corey proposed the structure of a helical conformation of a chain of amino acids—the α helix—and the structure of the β sheet, both of which were later found in many proteins.
1955	Sanger determined the order of amino acids in insulin, the first protein whose amino acid sequence was determined.
1956	Ingram produced the first protein fingerprints, showing that the difference between sickle-cell hemoglobin and normal hemoglobin is due to a change in a single amino acid (Movie 4.12).
1960	Kendrew described the first detailed three-dimensional structure of a protein (sperm whale myoglobin) to a resolution of 0.2 nm, and Perutz proposed a lower-resolution structure for hemoglobin.
1963	Monod, Jacob, and Changeux recognized that many enzymes are regulated through allosteric changes in their conformation.
1966	Phillips described the three-dimensional structure of lysozyme by X-ray crystallography, the first enzyme to be analyzed in atomic detail.
1973	Nomura reconstituted a functional bacterial ribosome from purified components.
1975	Henderson and Unwin determined the first three-dimensional structure of a transmembrane protein (bacteriorhodopsin), using a computer-based reconstruction from electron micrographs.
1976	Neher and Sakmann developed patch-clamp recording to measure the activity of single ion-channel proteins.
1984	Wüthrich used nuclear magnetic resonance (NMR) spectroscopy to solve the three-dimensional structure of a soluble sperm protein.
1988	Tanaka and Fenn separately developed methods for the analysis of proteins and other biological macromolecules.
1996–2013	Mann, Aebersold, Yates, and others developed efficient methods for using mass spectrometry to identify proteins in complex mixtures, exploiting the availability of complete genome sequences.

A much faster way to determine the amino acid sequence of proteins that have been isolated from organisms for which the full genome sequence is known is a method called *mass spectrometry*. This technique determines the exact mass of every peptide fragment in a purified protein, which then allows the protein to be identified from a database that contains a list of every protein thought to be encoded by the genome of the organism in question. Such lists are computed by taking the genome sequence of the organism and applying the genetic code (discussed in Chapter 7).

To perform **mass spectrometry**, the peptides derived from digestion with trypsin are blasted with a laser. This treatment heats the peptides, causing them to become electrically charged (ionized) and ejected in the form of a gas. Accelerated by a powerful electric field, the peptide ions then fly toward a detector; the time it takes them to arrive is related to their mass and their charge. (The larger the peptide is, the more slowly it moves; the

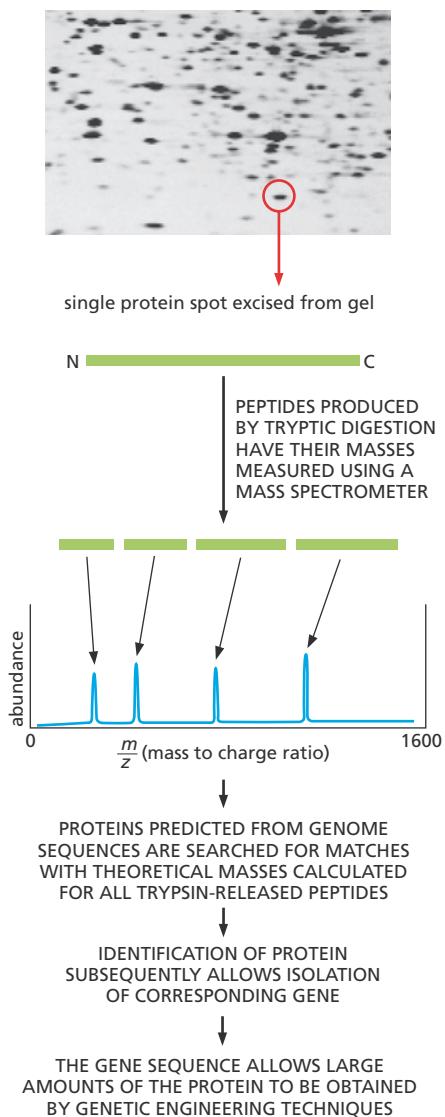


Figure 4–49 Mass spectrometry can be used to identify proteins by determining the precise masses of peptides derived from them. As indicated, this in turn allows the proteins to be produced in the large amounts needed for determining their three-dimensional structure. In this example, the protein of interest is excised from a polyacrylamide gel after two-dimensional electrophoresis (see Panel 4–5, p. 167) and then digested with trypsin. The peptide fragments are loaded into the mass spectrometer, and their exact masses are measured. Genome sequence databases are then searched to find the protein encoded by the organism in question whose profile matches this peptide fingerprint. Mixtures of proteins can also be analyzed in this way. (Image courtesy of Patrick O'Farrell.)

more highly charged it is, the faster it moves.) The set of exact masses of the protein fragments produced by trypsin cleavage then serves as a “fingerprint” that identifies the protein—and its corresponding gene—from publicly accessible databases (Figure 4–49).

This approach can even be applied to complex mixtures of proteins, for example, starting with an extract containing all the proteins made by yeast cells grown under a particular set of conditions. To obtain the increased resolution required to distinguish individual proteins, such mixtures are frequently analyzed using *tandem mass spectrometry*. In this case, after the peptides pass through the first mass spectrometer, they are broken into even smaller fragments and analyzed by a second mass spectrometer.

Although all the information required for a polypeptide chain to fold is contained in its amino acid sequence, we have not yet learned how to reliably predict a protein's detailed three-dimensional conformation—the spatial arrangement of its atoms—from its sequence alone. At present, the only way to discover the precise folding pattern of any protein is by experiment, using either **X-ray crystallography** or **nuclear magnetic resonance (NMR)** spectroscopy (How We Know, pp. 162–163).

Genetic Engineering Techniques Permit the Large-Scale Production, Design, and Analysis of Almost Any Protein

Advances in genetic engineering techniques now permit the production of large quantities of almost any desired protein. In addition to making life much easier for biochemists interested in purifying specific proteins, this ability to churn out huge quantities of a protein has given rise to an entire biotechnology industry (Figure 4–50). Bacteria, yeast, and cultured mammalian cells are now used to mass produce a variety of therapeutic proteins, such as insulin, human growth hormone, and even the fertility-enhancing drugs used to boost egg production in women undergoing *in vitro* fertilization. Preparing these proteins previously required the collection and processing of vast amounts of tissue and other biological products—including, in the case of the fertility drugs, the urine of postmenopausal nuns.

The same sorts of genetic engineering techniques can also be employed to produce new proteins and enzymes that contain novel structures or perform unusual tasks: metabolizing toxic wastes, synthesizing life-saving drugs, or operating under conditions that would destroy most biological catalysts (see Chapter 3 How We Know, pp. 104–106). Most of these synthetic catalysts are nowhere near as effective as naturally occurring enzymes in terms of their ability to speed the rate of selected chemical reactions. But, as we continue to learn more about how proteins and enzymes exploit their unique conformations to carry out their biological functions, our ability to make novel proteins with useful functions can only improve.

Of course, to be able to study—or benefit from—the activity of an engineered protein in a living organism, the DNA encoding that protein must somehow be introduced into cells. Again, thanks to genetic engineering techniques, we are able to do just that. We discuss these methods in great detail in Chapter 10.

The Relatedness of Proteins Aids the Prediction of Protein Structure and Function

Biochemists have made enormous progress in understanding the structure and function of proteins over the past 150 years (see Table 4–2, p. 159). These advances are the fruits of decades of painstaking research on isolated proteins, performed by individual scientists working tirelessly on single proteins or protein families, one by one, sometimes for their entire careers. In the future, however, more and more of these investigations of protein conformation and activity will likely take place on a larger scale.

Improvements in our ability to rapidly sequence whole genomes, and the development of methods such as mass spectrometry, have fueled our ability to determine the amino acid sequences of enormous numbers of proteins. Millions of unique protein sequences from thousands of different species have thereby been deposited into publicly available databases, and the collection is expected to double in size every two years. Comparing the amino acid sequences of all of these proteins reveals that the majority belong to protein families that share specific “sequence patterns”—stretches of amino acids that fold into distinct structural domains. In some of these families, the proteins contain only a single structural domain. In others, the proteins include multiple domains arranged in novel combinations (**Figure 4–51**).

Although the number of multidomain families is growing rapidly, the discovery of novel single domains appears to be leveling off. This plateau suggests that the vast majority of proteins may fold up into a limited number of structural domains—perhaps as few as 10,000 to 20,000. For many single-domain families, the structure of at least one family member is known. And knowing the structure of one family member allows us to say something about the structure of its relatives. By this account, we have some structural information for almost three-quarters of the proteins archived in databases (**Movie 4.13**).

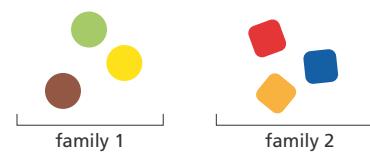
A future goal is to acquire the ability to look at a protein’s amino acid sequence and be able to deduce its structure and gain insight into its function. We are coming closer to being able to predict protein structure based on sequence information, but there is still a long way to go. Predicting how a protein will function, alone, as part of a complex, or as part of a network in the cell, is much more challenging. But, the closer we get to addressing these questions, the closer we should be to understanding the fundamental basis of life.



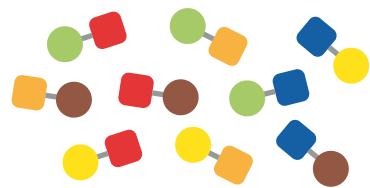
Figure 4–50 Biotechnology companies produce mass quantities of useful proteins. Shown in this photograph are the fermenters used to grow the cells needed for such large-scale protein production. (Courtesy of Bioengineering AG, Switzerland.)

Figure 4–51 Most proteins belong to structurally related families.

(A) More than two-thirds of all well-studied proteins contain a single structural domain. The members of these single-domain families can have different amino acid sequences but fold into a protein with a similar shape. (B) During evolution, structural domains have been combined in different ways to produce families of multidomain proteins. Almost all novelty in protein structure comes from the way these single domains are arranged. The number of multidomain families being added to the public databases is still rapidly increasing, unlike the number of novel single domains.



(A) single-domain protein families



(B) a two-domain protein family

PROBING PROTEIN STRUCTURE

As you've no doubt already concluded in reading this chapter, for many proteins, their three-dimensional shape determines their function. So to learn more about how a protein works, it helps to know exactly what it looks like.

The problem is that most proteins are too small to be seen in any detail, even with a powerful electron microscope. To follow the path of an amino acid chain that is folded into a protein molecule, you need to be able to "see" its individual atoms. Scientists use two main methods to map the locations of atoms in a protein. The first involves the use of X-rays. Like light, X-rays are a form of electromagnetic radiation. But they have a wavelength that's much shorter: 0.1 nanometer (nm) as opposed to the 400–700 nm wavelength of visible light. That tiny wavelength—which is the approximate diameter of a hydrogen atom—allows scientists to probe the structure of very small objects at the atomic level.

A second method, called nuclear magnetic resonance (NMR) spectroscopy, takes advantage of the fact that—in many atoms—the nucleus is intrinsically magnetic. When exposed to a large magnet, these nuclei act like tiny bar magnets and align themselves with the magnetic field. If they are then excited with a blast of radio waves, the nuclei will wobble around their magnetic axes, and, as they relax back into the aligned position, they will give off a signal that can be used to reveal their relative positions in a protein.

Using these techniques, investigators have painstakingly pieced together many thousands of protein structures. With the help of computer graphics programs, they have been able to traverse the surfaces and climb inside these proteins, exploring the nooks where ATP likes to nestle, for example, or examining the loops and helices that proteins use to grab hold of a ligand or wrap around a segment of DNA. If the protein happens to belong to a virus or to a cancer cell, seeing its structure can provide clues to designing drugs that might thwart an infection or eliminate a tumor.

X-rays

To determine a protein's structure using X-ray crystallography, you first need to coax the protein into forming crystals: large, highly ordered arrays of the pure protein in which every molecule has the same conformation and is perfectly aligned with its neighbors. Growing high-quality protein crystals is still something of an art and is largely a matter of trial and error. Although robotic methods increase efficiency, it can still take years to find the right conditions—and some proteins resist crystallization altogether.

If you're lucky enough to get good crystals, you are ready for the X-ray analysis. When a narrow beam of X-rays is directed at a protein crystal, the atoms in the protein molecules scatter the incoming X-rays. These scattered waves either reinforce or cancel one another, producing a complex diffraction pattern that is collected by electronic detectors. The position and intensity of each spot in the diffraction pattern contains information about the position of the atoms in the protein crystal ([Figure 4–52](#)).

Because these patterns are so complex—even a small protein can generate 25,000 discrete spots—computers are used to interpret them and transform them by complex mathematical calculations into maps of the relative spatial positions of the atoms. By combining information obtained from such maps with the amino acid sequence of the protein, you can eventually generate an atomic model of the protein's structure. To determine whether the protein undergoes conformational changes in its structure when it binds a ligand that boosts its activity, you might subsequently try crystallizing it in the presence of its ligand. With crystals of sufficient quality, even small atomic movements can be detected by comparing the structures obtained in the presence and absence of stimulatory or inhibitory ligands.

Magnets

The trouble with X-ray crystallography is that you need crystals. And not all proteins like to form such orderly assemblies. Many have intrinsically disordered regions that wiggle around too much to stack neatly into a crystalline array. Others might not crystallize in the absence of the membranes in which they normally reside.

The other way to solve the structure of a protein does not require protein crystals. If the protein is small—say, 50,000 daltons or less—you can determine its structure by NMR spectroscopy. In this technique, a concentrated solution of pure protein is placed in a strong magnetic field and then bombarded with radio waves of different frequencies. Hydrogen nuclei, in particular, will generate an NMR signal that can be used to determine the distances between these atoms in different parts of the protein. This information is then used to build a model of how the hydrogens are arranged in space. Again, combined with the known amino acid sequence, an NMR spectrum can allow you to compute the three-dimensional structure of the protein ([Figure 4–53](#)). If the protein is larger than 50,000 daltons, you can try to break it up into its constituent functional domains and analyze each domain by NMR.

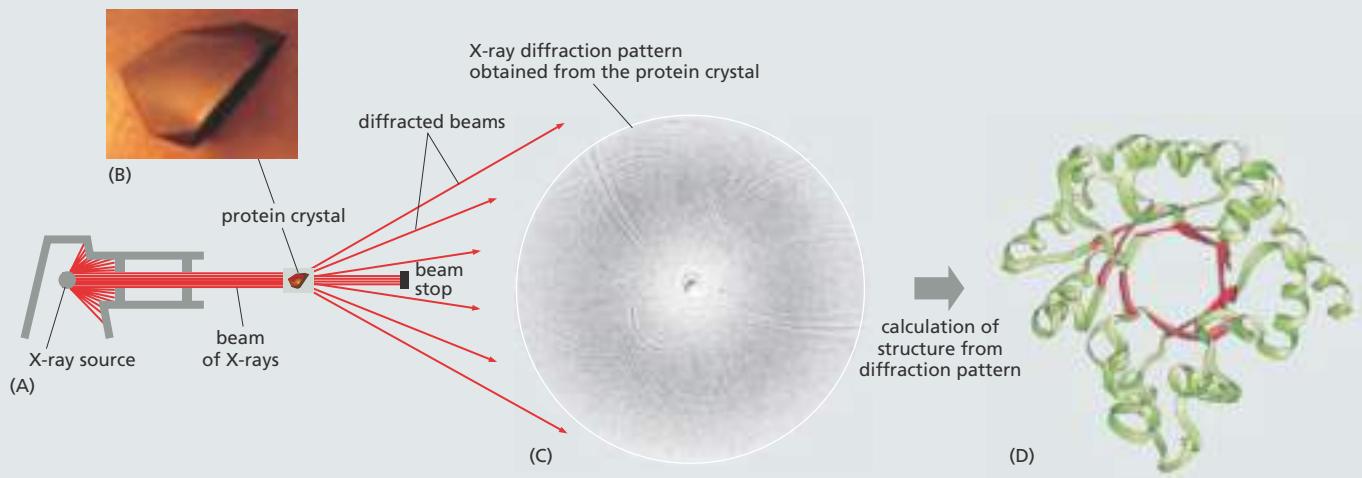


Figure 4–52 The structure of a protein can be determined by X-ray crystallography. Ribulose bisphosphate carboxylase is an enzyme that plays a central role in CO_2 fixation during photosynthesis. (A) X-ray diffraction apparatus; (B) photograph of crystal; (C) diffraction pattern; (D) three-dimensional structure determined from the pattern (α helices are shown in green, and β sheets in red). (B, courtesy of C. Branden; C, courtesy of J. Hajdu and I. Anderson; D, adapted from original provided by B. Furugren.)

Because determining the precise conformation of a protein is so time-consuming and costly—and the resulting insights so valuable—scientists routinely make their structures freely available by submitting the information to a publicly accessible database. Thanks to such databases, anyone interested in viewing the structure of, say, the ribosome—a complex macromolecular machine made of several RNAs and more than 50 proteins—can easily do so. In the future, improvements in

X-ray crystallography and NMR spectroscopy should permit rapid analysis of many more proteins and protein machines. And once enough structures have been determined, it might become possible to generate algorithms for accurately predicting structure solely on the basis of a protein’s amino acid sequence. After all, it is the sequence of the amino acids alone that determines how each protein folds up into its three-dimensional shape.

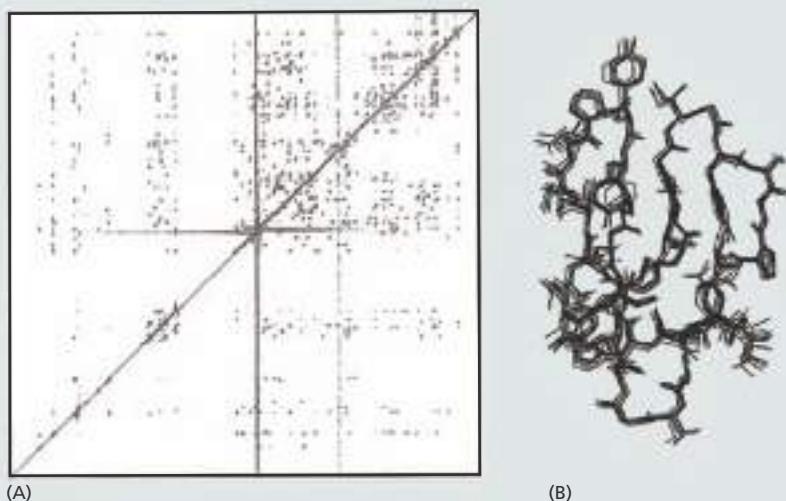


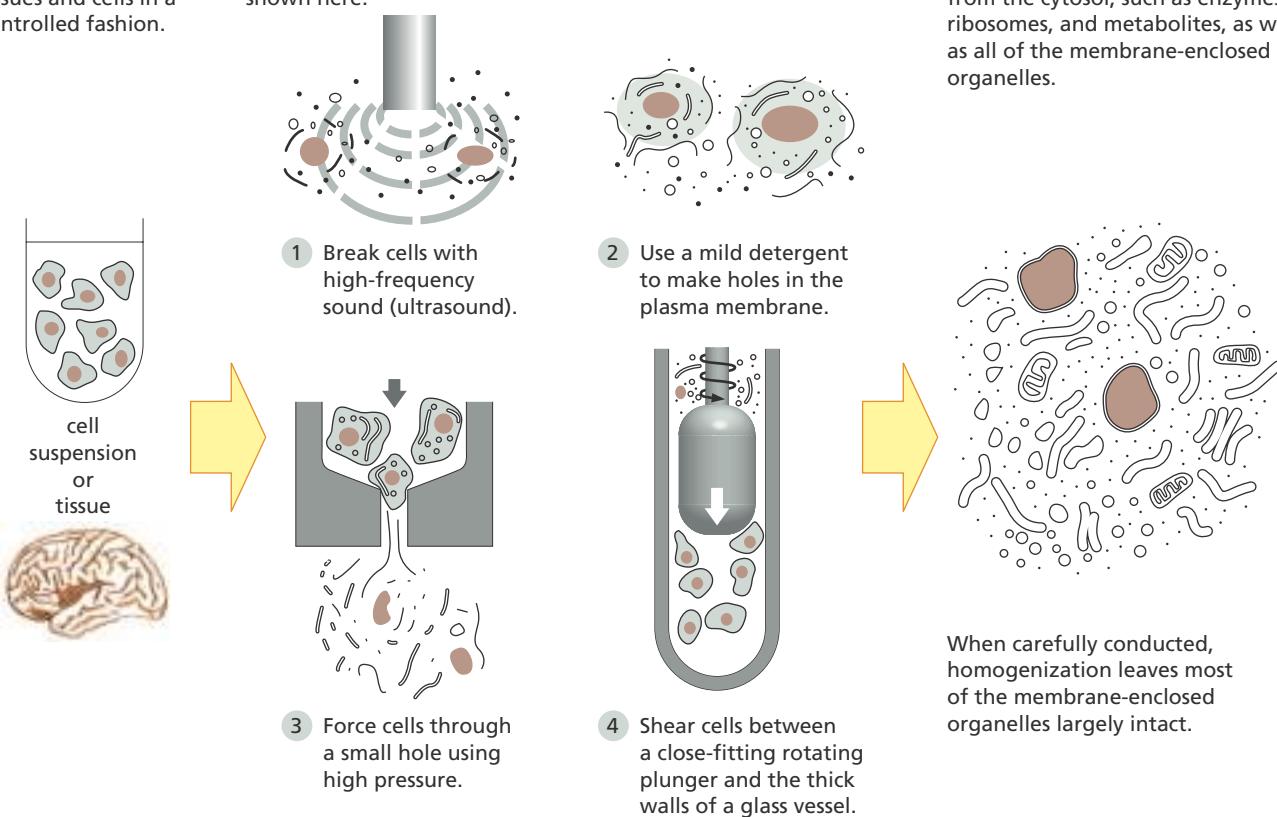
Figure 4–53 NMR spectroscopy can be used to determine the structure of small proteins or protein domains. (A) Two-dimensional NMR spectrum derived from the C-terminal domain of the enzyme cellulase, which breaks down cellulose. The spots represent interactions between neighboring hydrogen atoms. (B) The set of overlapping structures shown all satisfy the distance constraints equally well. (Courtesy of P. Kraulis.)

BREAKING CELLS AND TISSUES

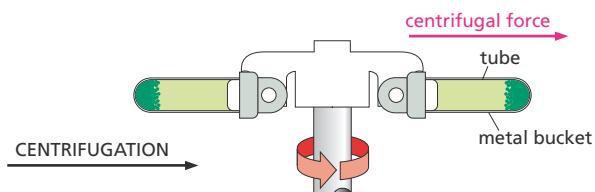
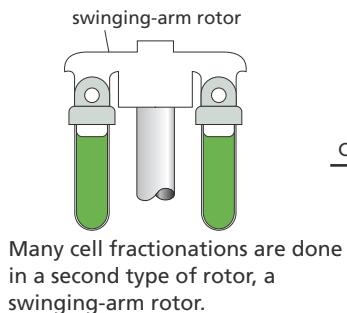
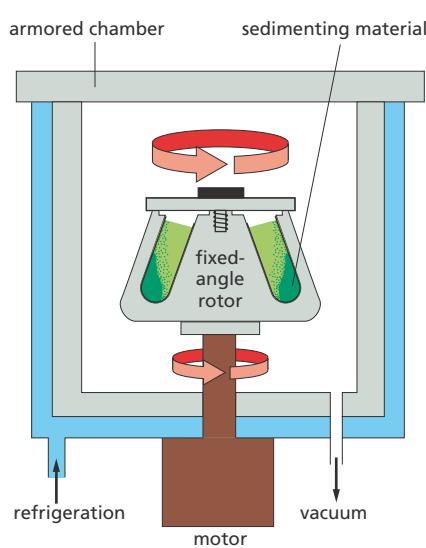
The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called **homogenization**, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

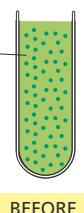
The resulting thick soup (called a **homogenate** or an **extract**) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all of the membrane-enclosed organelles.



THE CENTRIFUGE

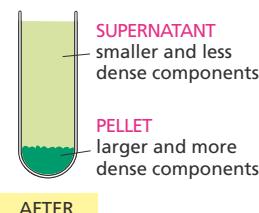


CELL HOMOGENATE
before centrifugation



CENTRIFUGATION

The metal buckets that hold the tubes are free to swing outward as the rotor turns.



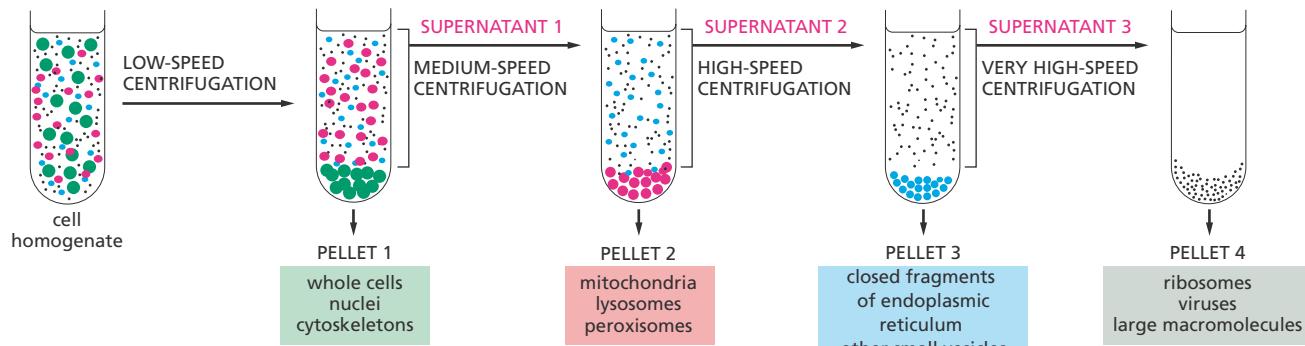
Centrifugation is the most widely used procedure to separate a homogenate into different parts, or fractions. The homogenate is placed in test tubes and rotated at high speed in a centrifuge or ultracentrifuge. Present-day ultracentrifuges rotate at speeds up to 100,000 revolutions per minute and produce enormous forces, as high as 600,000 times gravity.

Such speeds require centrifuge chambers to be refrigerated and have the air evacuated so that friction does not heat up the homogenate. The centrifuge is surrounded by thick armor plating, because an unbalanced rotor can shatter with an explosive release of energy. A fixed-angle rotor can hold larger volumes than a swinging-arm rotor, but the pellet forms less evenly, as shown.

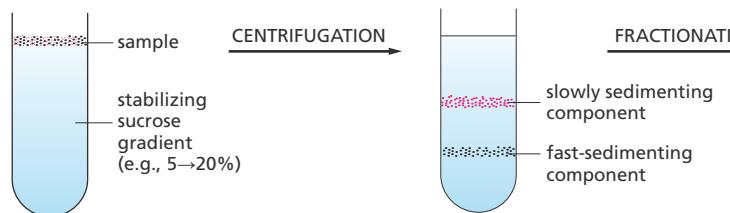
DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

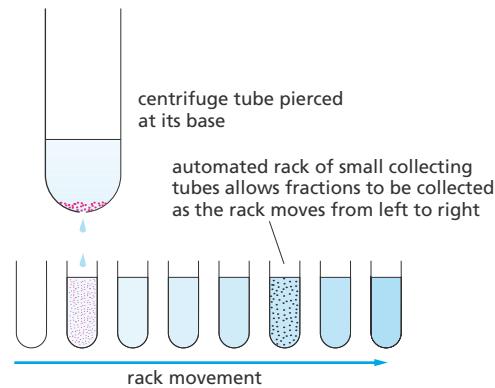
Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, a portion called the supernatant.



VELOCITY SEDIMENTATION



Subcellular components sediment at different rates according to their size after being carefully layered over a dilute salt solution and then centrifuged through it. In order to stabilize the sedimenting components against convective mixing in the tube, the solution contains a continuous shallow gradient of sucrose that increases in concentration toward the bottom of the tube. The gradient is typically 5–20% sucrose. When sedimented through such a dilute sucrose gradient, using a swinging-arm rotor, different cell components separate into distinct bands that can be collected individually.

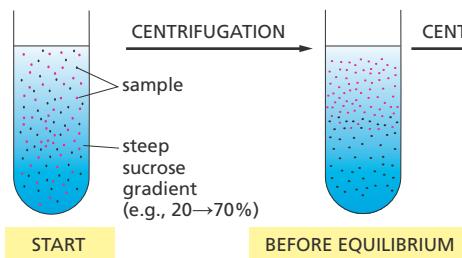


After an appropriate centrifugation time, the bands may be collected, most simply by puncturing the plastic centrifuge tube and collecting drops from the bottom, as shown here.

EQUILIBRIUM SEDIMENTATION

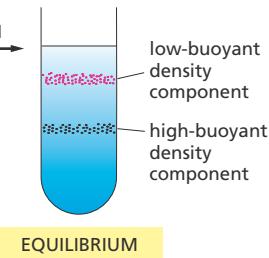
The ultracentrifuge can also be used to separate cell components on the basis of their **buoyant density**, independently of their size or shape. The sample is usually either layered on top of, or dispersed within, a steep density gradient that contains a very high concentration of sucrose or cesium chloride. Each subcellular component will move up or down when centrifuged until it reaches a position where its density matches its surroundings and then will move no further. A series of distinct bands will eventually be produced, with those nearest the bottom of the tube containing the components of highest buoyant density. The method is also called **density gradient centrifugation**.

The sample is distributed throughout the sucrose density gradient.



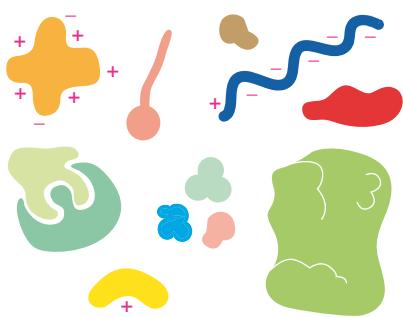
A sucrose gradient is shown here, but denser gradients can be formed with cesium chloride that are particularly useful for separating nucleic acids (DNA and RNA).

At equilibrium, components have migrated to a region in the gradient that matches their own density.



The final bands can be collected from the base of the tube, as shown above for velocity sedimentation.

PROTEIN SEPARATION



Proteins are very diverse. They differ in size, shape, charge, hydrophobicity, and their affinity for other molecules. All of these properties can be exploited to separate them from one another so that they can be studied individually.

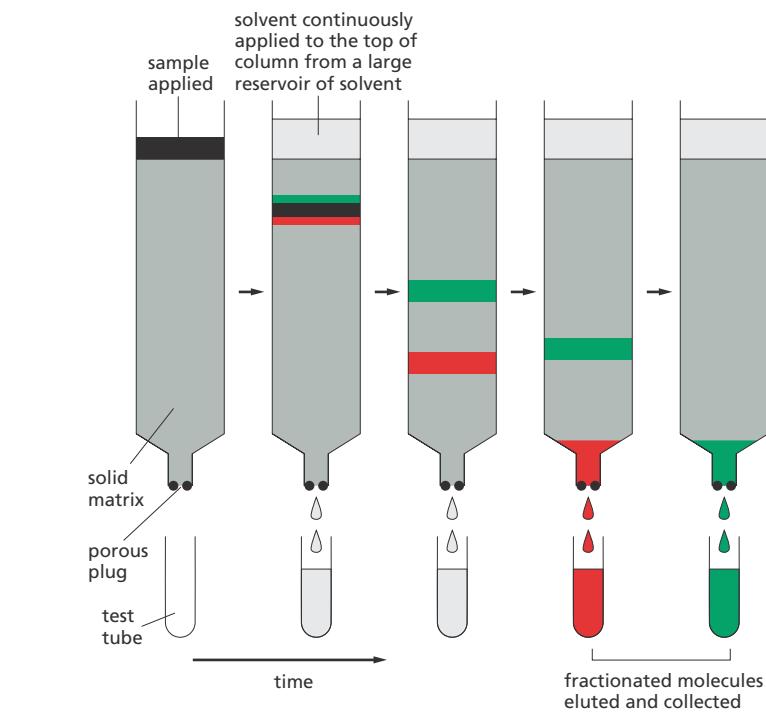
THREE KINDS OF CHROMATOGRAPHY

Although the material used to form the matrix for column chromatography varies, it is usually packed in the column in the form of small beads. A typical protein purification strategy might employ in turn each of the three kinds of matrix described below, with a final protein purification of up to 10,000-fold.

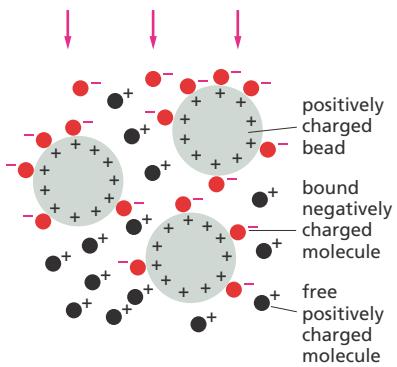
Purity can easily be assessed by gel electrophoresis (Panel 4-5).

COLUMN CHROMATOGRAPHY

Proteins are often fractionated by **column chromatography**. A mixture of proteins in solution is applied to the top of a cylindrical column filled with a permeable solid matrix immersed in solvent. A large amount of solvent is then pumped through the column. Because different proteins are retarded to different extents by their interaction with the matrix, they can be collected separately as they flow out from the bottom. According to the choice of matrix, proteins can be separated according to their charge, hydrophobicity, size, or ability to bind to particular chemical groups (see below).



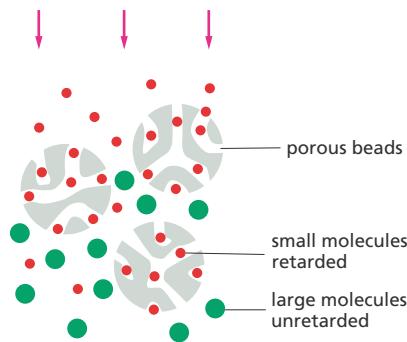
solvent flow



(A) ION-EXCHANGE CHROMATOGRAPHY

Ion-exchange columns are packed with small beads carrying either positive or negative charges that retard proteins of the opposite charge. The association between a protein and the matrix depends on the pH and ionic strength of the solution passing down the column. These can be varied in a controlled way to achieve an effective separation.

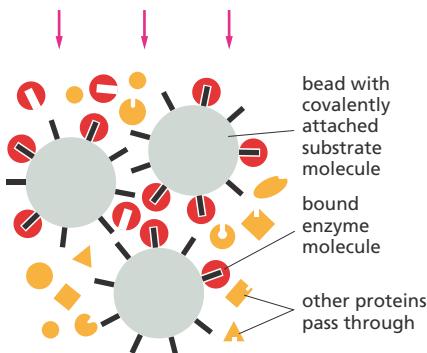
solvent flow



(B) GEL-FILTRATION CHROMATOGRAPHY

Gel-filtration columns separate proteins according to their size. The matrix consists of tiny porous beads. Protein molecules that are small enough to enter the holes in the beads are delayed and travel more slowly through the column. Proteins that cannot enter the beads are washed out of the column first. Such columns also allow an estimate of protein size.

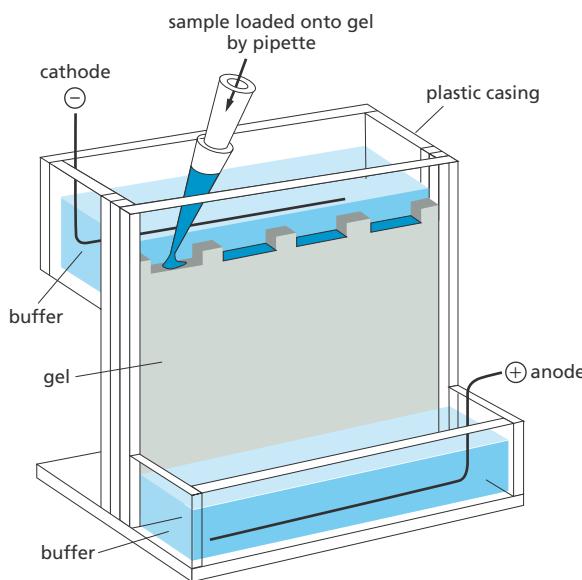
solvent flow



(C) AFFINITY CHROMATOGRAPHY

Affinity columns contain a matrix covalently coupled to a molecule that interacts specifically with the protein of interest (e.g., an antibody, or an enzyme substrate). Proteins that bind specifically to such a column can subsequently be released by a pH change or by concentrated salt solutions, and they emerge highly purified (see also Figure 4-48).

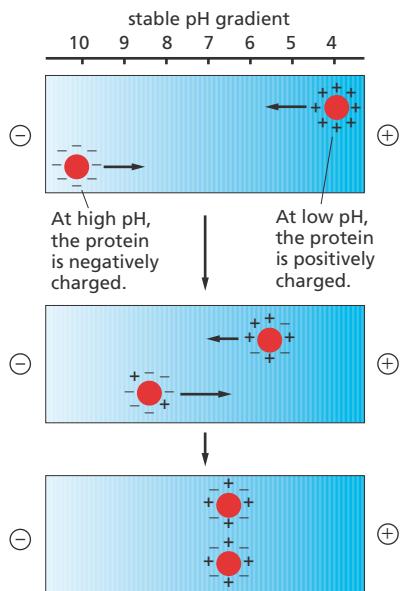
GEL ELECTROPHORESIS



When an electric field is applied to a solution containing protein molecules, the molecules will migrate in a direction and at a speed that reflects their size and net charge. This forms the basis of the technique called **electrophoresis**.

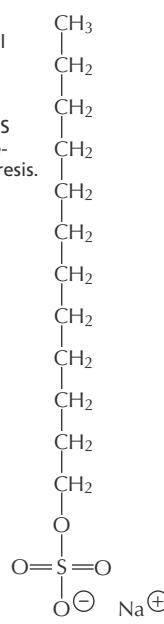
ISOELECTRIC FOCUSING

For any protein there is a characteristic pH, called the **isoelectric point**, at which the protein has no net charge and therefore will not move in an electric field. In **isoelectric focusing**, proteins are electrophoresed in a narrow tube of polyacrylamide gel in which a pH gradient is established by a mixture of special buffers. Each protein moves to a point in the pH gradient that corresponds to its isoelectric point and stays there.

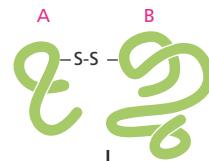


The protein shown here has an isoelectric pH of 6.5.

The detergent sodium dodecyl sulfate (SDS) is used to solubilize proteins for SDS polyacrylamide-gel electrophoresis.



protein with two subunits, A and B, joined by a disulfide (S-S) bond



single-subunit protein

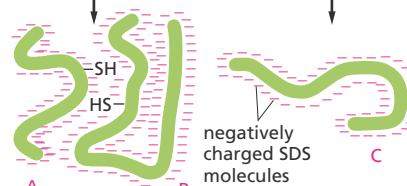


A

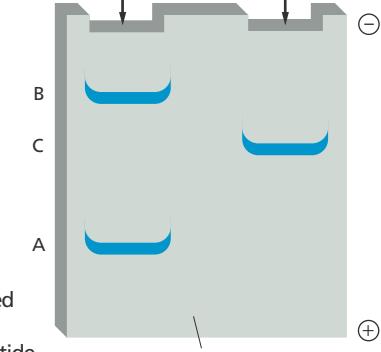
B

C

HEATED WITH SDS AND MERCAPTOETHANOL



POLYACRYLAMIDE-GEL ELECTROPHORESIS



slab of polyacrylamide gel

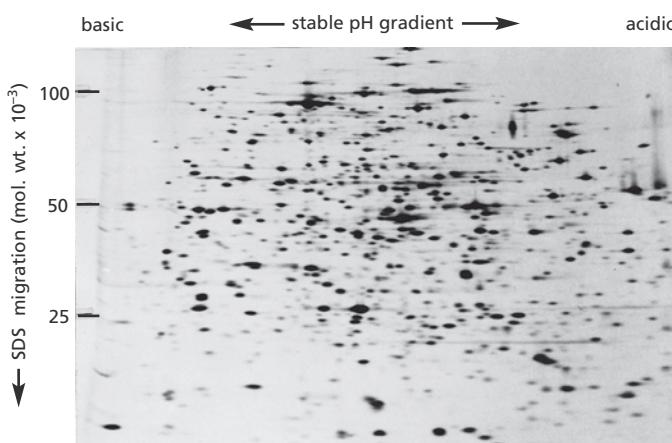
SDS polyacrylamide-gel electrophoresis (SDS-PAGE)

Individual polypeptide chains form a complex with negatively charged molecules of sodium dodecyl sulfate (SDS) and therefore migrate as negatively charged SDS-protein complexes through a slab of porous polyacrylamide gel. The apparatus used for this electrophoresis technique is shown above (left). A reducing agent (mercaptoethanol) is usually added to break any –S–S– linkages within or between proteins. Under these conditions, unfolded polypeptide chains migrate at a rate that reflects their molecular weight.

TWO-DIMENSIONAL POLYACRYLAMIDE-GEL ELECTROPHORESIS

Complex mixtures of proteins cannot be resolved well on one-dimensional gels, but **two-dimensional gel electrophoresis**, combining two different separation methods, can be used to resolve more than 1000 proteins in a two-dimensional protein map. In the first step, native proteins are separated in a narrow gel on the basis of their intrinsic charge using isoelectric focusing (see left). In the second step, this gel is placed on top of a gel slab, and the proteins are subjected to SDS-PAGE (see above) in a direction perpendicular to that used in the first step. Each protein migrates to form a discrete spot.

All the proteins in an *E. coli* bacterial cell are separated in this two-dimensional gel, in which each spot corresponds to a different polypeptide chain. They are separated according to their isoelectric point from left to right and to their molecular weight from top to bottom. (Courtesy of Patrick O'Farrell.)



ESSENTIAL CONCEPTS

- Living cells contain an enormously diverse set of protein molecules, each made as a linear chain of amino acids linked together by covalent peptide bonds.
- Each type of protein has a unique amino acid sequence, which determines both its three-dimensional shape and its biological activity.
- The folded structure of a protein is stabilized by multiple noncovalent interactions between different parts of the polypeptide chain.
- Hydrogen bonds between neighboring regions of the polypeptide backbone often give rise to regular folding patterns, known as α helices and β sheets.
- The structure of many proteins can be subdivided into smaller globular regions of compact three-dimensional structure, known as protein domains.
- The biological function of a protein depends on the detailed chemical properties of its surface and how it binds to other molecules, called ligands.
- When a protein catalyzes the formation or breakage of a specific covalent bond in a ligand, the protein is called an enzyme and the ligand is called a substrate.
- At the active site of an enzyme, the amino acid side chains of the folded protein are precisely positioned so that they favor the formation of the high-energy transition states that the substrates must pass through to be converted to product.
- The three-dimensional structure of many proteins has evolved so that the binding of a small ligand can induce a significant change in protein shape.
- Most enzymes are allosteric proteins that can exist in two conformations that differ in catalytic activity, and the enzyme can be turned on or off by ligands that bind to a distinct regulatory site to stabilize either the active or the inactive conformation.
- The activities of most enzymes within the cell are strictly regulated. One of the most common forms of regulation is feedback inhibition, in which an enzyme early in a metabolic pathway is inhibited by the binding of one of the pathway's end products.
- Many thousands of proteins in a typical eukaryotic cell are regulated by cycles of phosphorylation and dephosphorylation.
- GTP-binding proteins also regulate protein function in eukaryotes; they act as molecular switches that are active when GTP is bound and inactive when GDP is bound; turning themselves off by hydrolyzing their bound GTP to GDP.
- Motor proteins produce directed movement in eukaryotic cells through conformational changes linked to the hydrolysis of ATP to ADP.
- Highly efficient protein machines are formed by assemblies of allosteric proteins in which the various conformational changes are coordinated to perform complex functions.
- Covalent modifications added to a protein's amino acid side chains can control the location and function of the protein and can serve as docking sites for other proteins.
- Starting from crude cell or tissue homogenates, individual proteins can be obtained in pure form by using a series of chromatography steps.
- The function of a purified protein can be discovered by biochemical analyses, and its exact three-dimensional structure can be determined by X-ray crystallography or NMR spectroscopy.

KEY TERMS

active site	mass spectrometry
allosteric	motor protein
α helix	N-terminus
amino acid sequence	nuclear magnetic resonance
antibody	(NMR) spectroscopy
antigen	peptide bond
β sheet	polypeptide, polypeptide chain
binding site	polypeptide backbone
C-terminus	primary structure
chromatography	protein
coiled-coil	protein domain
conformation	protein family
disulfide bond	protein kinase
electrophoresis	protein machine
enzyme	protein phosphatase
feedback inhibition	protein phosphorylation
fibrous protein	quaternary structure
globular protein	secondary structure
GTP-binding protein	side chain
helix	substrate
intrinsically disordered sequence	subunit
ligand	tertiary structure
lysozyme	transition state
	X-ray crystallography

QUESTIONS

QUESTION 4–9

Look at the models of the protein in Figure 4–12. Is the red α helix right- or left-handed? Are the three strands that form the large β sheet parallel or antiparallel? Starting at the N-terminus (the purple end), trace your finger along the peptide backbone. Are there any knots? Why, or why not?

QUESTION 4–10

Which of the following statements are correct? Explain your answers.

- A. The active site of an enzyme usually occupies only a small fraction of the enzyme surface.
- B. Catalysis by some enzymes involves the formation of a covalent bond between an amino acid side chain and a substrate molecule.
- C. A β sheet can contain up to five strands, but no more.
- D. The specificity of an antibody molecule is contained exclusively in loops on the surface of the folded light-chain domain.
- E. The possible linear arrangements of amino acids are so vast that new proteins almost never evolve by alteration of old ones.
- F. Allosteric enzymes have two or more binding sites.
- G. Noncovalent bonds are too weak to influence the three-dimensional structure of macromolecules.

H. Affinity chromatography separates molecules according to their intrinsic charge.

I. Upon centrifugation of a cell homogenate, smaller organelles experience less friction and thereby sediment faster than larger ones.

QUESTION 4–11

What common feature of α helices and β sheets makes them universal building blocks for proteins?

QUESTION 4–12

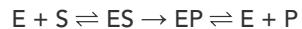
Protein structure is determined solely by a protein's amino acid sequence. Should a genetically engineered protein in which the original order of all amino acids is reversed have the same structure as the original protein?

QUESTION 4–13

Consider the following protein sequence as an α helix: Leu-Lys-Arg-Ile-Val-Asp-Ile-Leu-Ser-Arg-Leu-Phe-Lys-Val. How many turns does this helix make? Do you find anything remarkable about the arrangement of the amino acids in this sequence when folded into an α helix? (Hint: consult the properties of the amino acids in Figure 4–3.)

QUESTION 4–14

Simple enzyme reactions often conform to the equation



where E, S, and P are enzyme, substrate, and product, respectively.

- What does ES represent in this equation?
- Why is the first step shown with bidirectional arrows and the second step as a unidirectional arrow?
- Why does E appear at both ends of the equation?
- One often finds that high concentrations of P inhibit the enzyme. Suggest why this might occur.
- If compound X resembles S and binds to the active site of the enzyme but cannot undergo the reaction catalyzed by it, what effects would you expect the addition of X to the reaction to have? Compare the effects of X and of the accumulation of P.

QUESTION 4–15

Which of the following amino acids would you expect to find more often near the center of a folded globular protein? Which ones would you expect to find more often exposed to the outside? Explain your answers. Ser, Ser-P (a Ser residue that is phosphorylated), Leu, Lys, Gln, His, Phe, Val, Ile, Met, Cys–S–S–Cys (two cysteines that are disulfide-bonded), and Glu. Where would you expect to find the most N-terminal amino acid and the most C-terminal amino acid?

QUESTION 4–16

Assume you want to make and study fragments of a protein. Would you expect that any fragment of the polypeptide chain would fold the same way as it would in the intact protein? Consider the protein shown in Figure 4–19. Which fragments do you suppose are most likely to fold correctly?

QUESTION 4–17

Neurofilament proteins assemble into long, intermediate filaments (discussed in Chapter 17), found in abundance running along the length of nerve cell axons. The C-terminal region of these proteins is an unstructured polypeptide, hundreds of amino acids long and heavily modified by the addition of phosphate groups. The term “polymer brush” has been applied to this part of the neurofilament. Can you suggest why?

QUESTION 4–18

An enzyme isolated from a mutant bacterium grown at 20°C works in a test tube at 20°C but not at 37°C (37°C is the temperature of the gut, where this bacterium normally lives). Furthermore, once the enzyme has been exposed to the higher temperature, it no longer works at the lower one. The same enzyme isolated from the normal bacterium works at both temperatures. Can you suggest what happens (at the molecular level) to the mutant enzyme as the temperature increases?

QUESTION 4–19

A motor protein moves along protein filaments in the cell. Why are the elements shown in the illustration not sufficient to mediate directed movement ([Figure Q4–19](#))? With reference to Figure 4–46, modify the illustration shown here to include other elements that are required to create a unidirectional motor, and justify each modification you make to the illustration.



Figure Q4–19

QUESTION 4–20

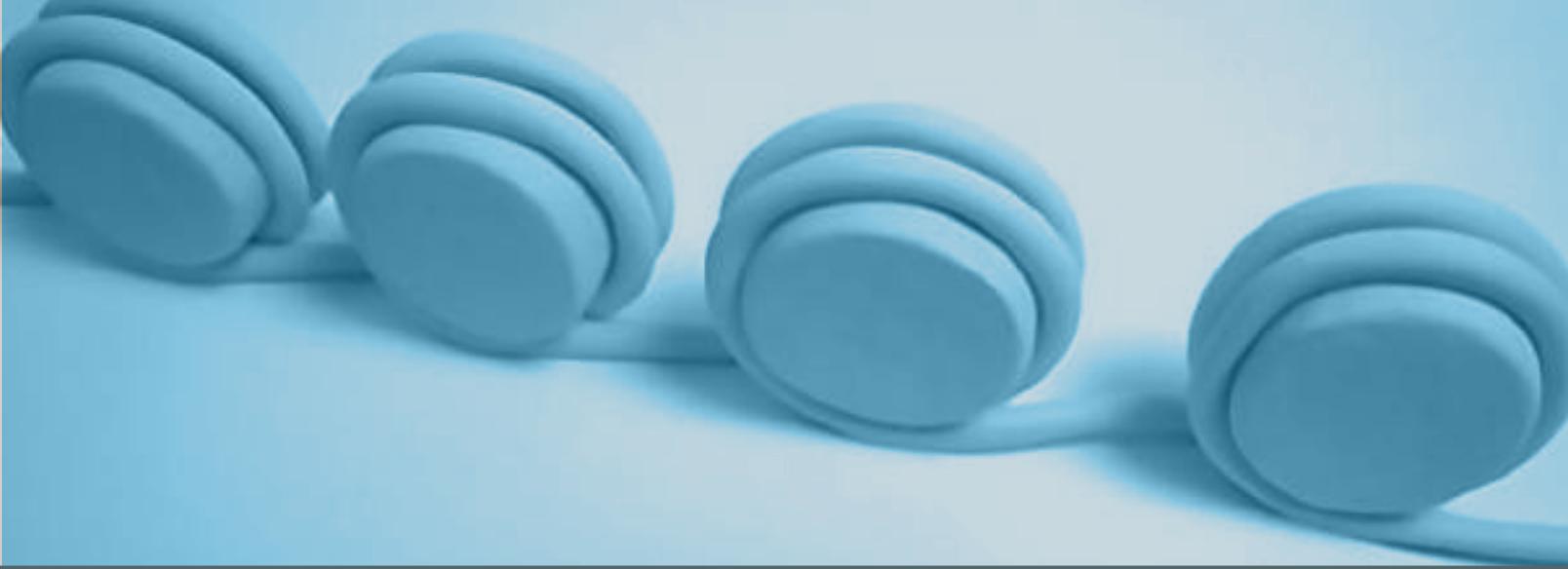
Gel-filtration chromatography separates molecules according to their size (see Panel 4–4, p. 166). Smaller molecules diffuse faster in solution than larger ones, yet smaller molecules migrate more slowly through a gel-filtration column than larger ones. Explain this paradox. What should happen at very rapid flow rates?

QUESTION 4–21

As shown in Figure 4–16, both α helices and the coiled-coil structures that can form from them are helical structures, but do they have the same handedness in the figure? Explain why?

QUESTION 4–22

How is it possible for a change in a single amino acid in a protein of 1000 amino acids to destroy its function, even when that amino acid is far away from any ligand-binding site?



CHAPTER FIVE

5

DNA and Chromosomes

Life depends on the ability of cells to store, retrieve, and translate the genetic instructions required to make and maintain a living organism. This hereditary information is passed on from a cell to its daughter cells at cell division, and from generation to generation in multicellular organisms through the reproductive cells—eggs and sperm. These instructions are stored within every living cell in its *genes*—the information-containing elements that determine the characteristics of a species as a whole and of the individuals within it.

At the beginning of the twentieth century, when genetics emerged as a science, scientists became intrigued by the chemical nature of genes. The information in genes is copied and transmitted from cell to daughter cells millions of times during the life of a multicellular organism, and it survives the process essentially unchanged. What kind of molecule could be capable of such accurate and almost unlimited replication, and also be able to direct the development of an organism and the daily life of a cell? What kind of instructions does the genetic information contain? How are these instructions physically organized so that the enormous amount of information required for the development and maintenance of even the simplest organism can be contained within the tiny space of a cell?

The answers to some of these questions began to emerge in the 1940s, when it was discovered from studies in simple fungi that genetic information consists primarily of instructions for making proteins. Proteins perform most of the cell's functions: they serve as building blocks for cell structures; they form the enzymes that catalyze the cell's chemical reactions; they regulate the activity of genes; and they enable cells to

THE STRUCTURE OF DNA

THE STRUCTURE OF
EUKARYOTIC CHROMOSOMES

THE REGULATION OF
CHROMOSOME STRUCTURE

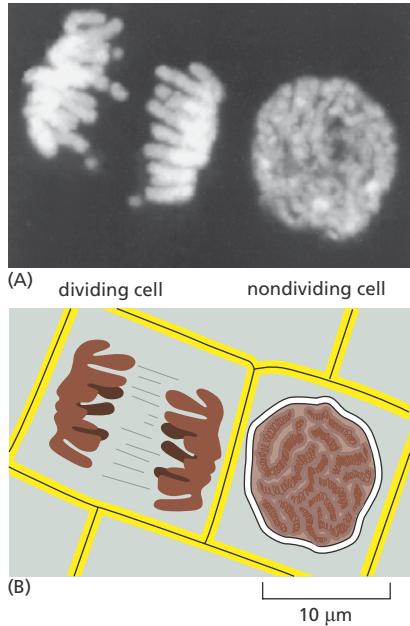


Figure 5-1 Chromosomes become visible as eukaryotic cells prepare to divide.
 (A) Two adjacent plant cells photographed in a fluorescence microscope. The DNA is labeled with a fluorescent dye (DAPI) that binds to it. The DNA is packaged into chromosomes, which become visible as distinct structures only when they condense in preparation for cell division, as shown on the left. The cell on the right, which is not dividing, contains the identical chromosomes, but they cannot be distinguished as individual entities because the DNA is in a much more extended conformation at this phase in the cell's life cycle. (B) Schematic diagram of the outlines of the two cells and their chromosomes.
 (A, courtesy of Peter Shaw.)

move and to communicate with one another. With hindsight, it is hard to imagine what other type of instructions the genetic information could have contained.

The other crucial advance made in the 1940s was the recognition that deoxyribonucleic acid (DNA) was the likely carrier of this genetic information. But the mechanism whereby the hereditary information is copied for transmission from one generation of cells to the next, and how proteins are specified by the instructions in DNA, remained completely mysterious until 1953, when the structure of DNA was determined by James Watson and Francis Crick. The structure immediately revealed how DNA might be copied, or replicated, and it provided the first clues about how a molecule of DNA might encode the instructions for making proteins. Today, the fact that DNA is the genetic material is so fundamental to our understanding of life that it is difficult to appreciate what an enormous intellectual gap this discovery filled.

In this chapter, we begin by describing the structure of DNA. We see how, despite its chemical simplicity, the structure and chemical properties of DNA make it ideally suited for carrying genetic information. The genes of every cell on Earth are made of DNA, and insights into the relationship between DNA and genes have come from experiments in a wide variety of organisms. We then consider how genes and other important segments of DNA are arranged in the single, long DNA molecule that forms the core of each chromosome in the cell. Finally, we discuss how eukaryotic cells fold these long DNA molecules into compact chromosomes inside the nucleus. This packing has to be done in an orderly fashion so that the chromosomes can be duplicated and apportioned correctly between the two daughter cells at each cell division. It must also allow the DNA to be accessed by the proteins that replicate and repair DNA, and regulate the activity of its many genes.

This is the first of five chapters that deal with basic genetic mechanisms—the ways in which the cell maintains and makes use of the genetic information carried in its DNA. In Chapter 6, we discuss the mechanisms by which the cell accurately replicates and repairs its DNA. In Chapter 7, we consider gene expression—how genes are used to produce RNA and protein molecules. In Chapter 8, we describe how a cell controls gene expression to ensure that each of the many thousands of proteins encoded in its DNA is manufactured at the proper time and place. In Chapter 9, we discuss how present-day genes evolved from distant ancestors, and, in Chapter 10, we consider some of the experimental techniques used to study both DNA and its role in fundamental cell processes.

An enormous amount has been learned about these subjects in the past 60 years. Much less obvious, but equally important, is that our knowledge is very incomplete; thus a great deal still remains to be discovered about how DNA provides the instructions to build living things.

THE STRUCTURE OF DNA

Well before biologists understood the structure of DNA, they had recognized that inherited traits and the genes that determine them were associated with the chromosomes. Chromosomes (named from the Greek *chroma*, “color,” because of their staining properties) were discovered in the nineteenth century as threadlike structures in the nucleus of eukaryotic cells that become visible as the cells begin to divide (**Figure 5-1**). As biochemical analysis became possible, researchers learned that chromosomes contain both DNA and protein. But which of these components encoded the organism’s genetic information was not clear.

We now know that the DNA carries the hereditary information of the cell and that the protein components of chromosomes function largely to package and control the enormously long DNA molecules. But biologists in the 1940s had difficulty accepting DNA as the genetic material because of the apparent simplicity of its chemistry (see **How We Know**, pp. 174–176). DNA, after all, is simply a long polymer composed of only four types of nucleotide subunits, which are chemically very similar to one another.

Then, early in the 1950s, DNA was examined by X-ray diffraction analysis, a technique for determining the three-dimensional atomic structure of a molecule (see Figure 4–52). The early results indicated that DNA is composed of two strands wound into a helix. The observation that DNA is double-stranded was of crucial significance. It provided one of the major clues that led, in 1953, to a correct model for the structure of DNA. This structure immediately suggested how DNA could encode the instructions necessary for life, and how these instructions could be copied and passed along when cells divide. In this section, we examine the structure of DNA and explain in general terms how it is able to store hereditary information.

A DNA Molecule Consists of Two Complementary Chains of Nucleotides

A molecule of **deoxyribonucleic acid (DNA)** consists of two long polynucleotide chains. Each *chain*, or *strand*, is composed of four types of nucleotide subunits, and the two strands are held together by hydrogen bonds between the base portions of the nucleotides (**Figure 5–2**).

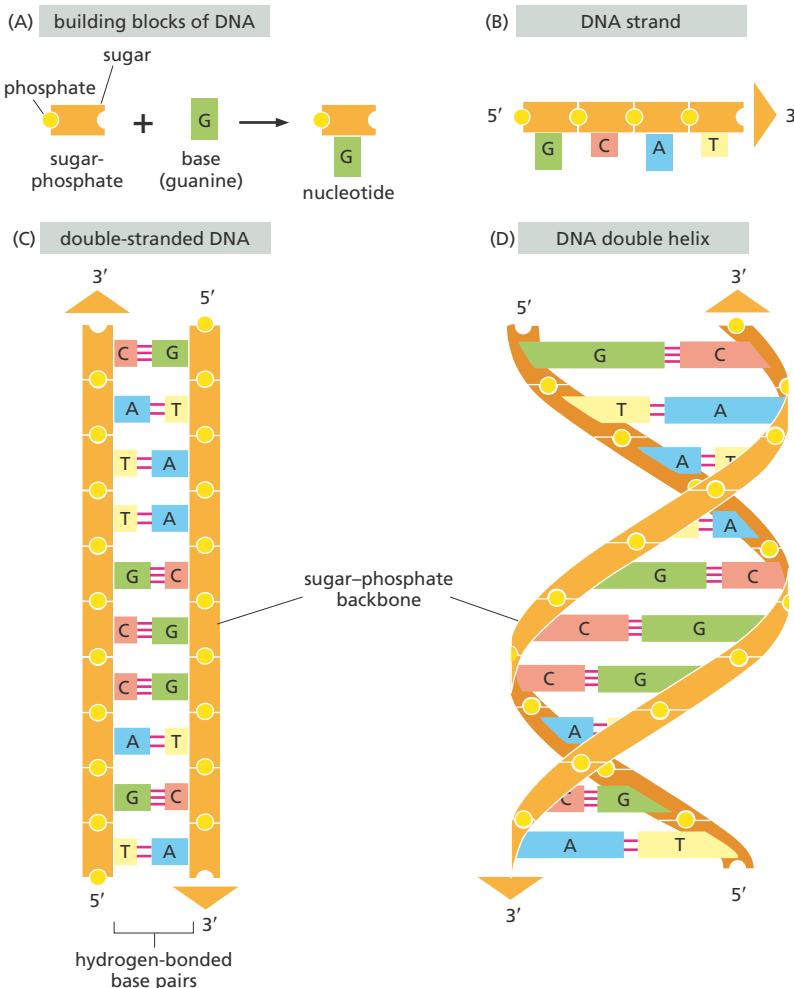


Figure 5–2 DNA is made of four nucleotide building blocks. (A) Each nucleotide is composed of a sugar-phosphate covalently linked to a base—guanine (G) in this figure. (B) The nucleotides are covalently linked together into polynucleotide chains, with a sugar-phosphate backbone from which the bases (A, C, G, and T) extend. (C) A DNA molecule is composed of two polynucleotide chains (DNA strands) held together by hydrogen bonds between the paired bases. The arrows on the DNA strands indicate the polarities of the two strands, which run antiparallel to each other in the DNA molecule. (D) Although the DNA is shown straightened out in (C), in reality, it is wound into a double helix, as shown here.

GENES ARE MADE OF DNA

By the 1920s, scientists generally agreed that genes reside on chromosomes, and they knew that chromosomes are composed of both DNA and proteins. But because DNA is so chemically simple, they naturally assumed that genes had to be made of proteins, which are much more chemically diverse than DNA molecules. Even when the experimental evidence suggested otherwise, this assumption proved hard to shake.

Messages from the dead

The case for DNA began to emerge in the late 1920s, when a British medical officer named Fred Griffith made an astonishing discovery. He was studying *Streptococcus pneumoniae* (pneumococcus), a bacterium that causes pneumonia. As antibiotics had not yet been discovered, infection with this organism was usually fatal. When

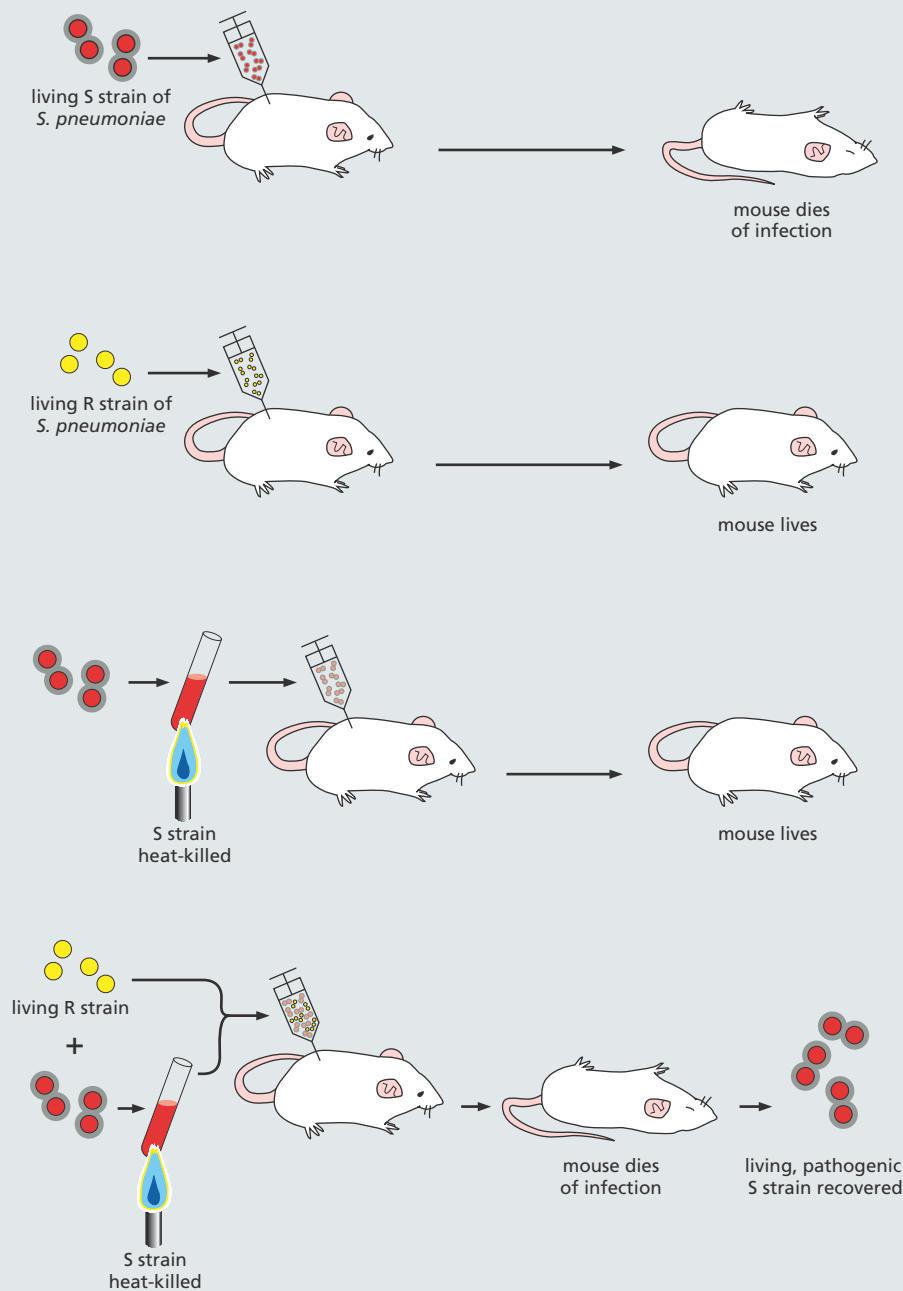


Figure 5–3 Griffith showed that heat-killed, infectious bacteria can transform harmless, living bacteria into pathogenic ones. The bacterium *Streptococcus pneumoniae* comes in two forms that differ from one another in their microscopic appearance and in their ability to cause disease. Cells of the pathogenic strain, which are lethal when injected into mice, are encased in a slimy, glistening polysaccharide capsule. When grown on a plate of nutrients in the laboratory, this disease-causing bacterium forms colonies that look dome-shaped and smooth; hence it is designated the S form. The harmless strain of the pneumococcus, on the other hand, lacks this protective coat; it forms colonies that appear flat and rough—hence, it is referred to as the R form. As illustrated, Griffith found that a substance present in the pathogenic S strain could permanently change, or transform, the nonlethal R strain into the deadly S strain.

grown in the laboratory, pneumococci come in two forms: a pathogenic form that causes a lethal infection when injected into animals, and a harmless form that is easily conquered by the animal's immune system and does not produce an infection.

In the course of his investigations, Griffith injected various preparations of these bacteria into mice. He showed that pathogenic pneumococci that had been killed by heating were no longer able to cause infection. The surprise came when Griffith injected both heat-killed pathogenic bacteria and live harmless bacteria into the same mouse. This combination proved lethal: not only did the animals die of pneumonia, but Griffith found that their blood was teeming with live bacteria of the pathogenic form (Figure 5–3). The heat-killed pneumococci had somehow converted the harmless bacteria into the lethal form. What's more, Griffith found that the change was permanent: he could grow these "transformed" bacteria in culture, and they remained pathogenic. But what was this mysterious material that turned harmless bacteria into killers? And how was this change passed on to progeny bacteria?

Transformation

Griffith's remarkable finding set the stage for the experiments that would provide the first strong evidence that genes are made of DNA. The American bacteriologist Oswald Avery, following up on Griffith's work, discovered that the harmless pneumococcus could be transformed into a pathogenic strain in a culture tube by exposing it to an extract prepared from the pathogenic strain. It would take another 15 years, however, for Avery and his colleagues Colin MacLeod and Maclyn McCarty to successfully purify the "transforming principle" from this soluble extract and to demonstrate that the active ingredient was DNA. Because the transforming principle caused a heritable change in the bacteria that received it, DNA must be the very stuff of which genes are made.

The 15-year delay was in part a reflection of the academic climate—and the widespread supposition that the genetic material was likely to be made of protein. Because of the potential ramifications of their work, the researchers wanted to be absolutely certain that the transforming principle was DNA before they announced their findings. As Avery noted in a letter to his brother, also a bacteriologist, "It's lots of fun to blow bubbles, but it's wiser to prick them yourself before someone else tries to." So the researchers subjected the transforming material to a battery of chemical tests (Figure 5–4). They found that it exhibited all the chemical properties

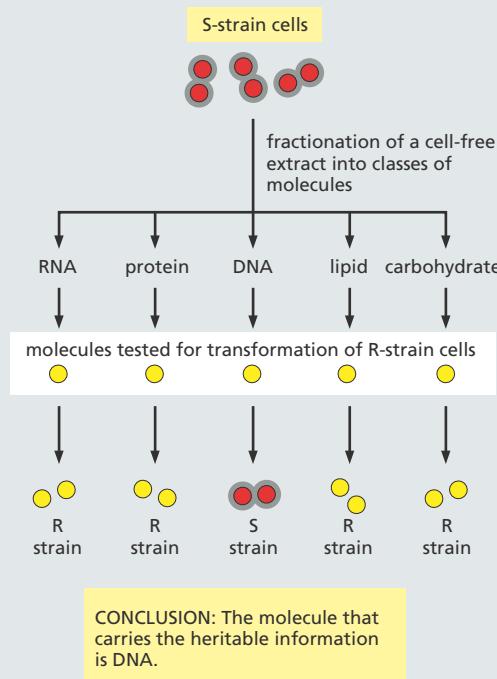


Figure 5–4 Avery, MacLeod, and McCarty demonstrated that DNA is the genetic material. The researchers prepared an extract from the disease-causing S strain of pneumococci and showed that the "transforming principle" that would permanently change the harmless R-strain pneumococci into the pathogenic S strain is DNA. This was the first evidence that DNA could serve as the genetic material.

characteristic of DNA; furthermore, they showed that enzymes that destroy proteins and RNA did not affect the ability of the extract to transform bacteria, while enzymes that destroy DNA inactivated it. And like Griffith before them, the investigators found that their purified preparation changed the bacteria permanently: DNA from the pathogenic species was taken up by the harmless species, and this change was faithfully passed on to subsequent generations of bacteria.

This landmark study offered rigorous proof that purified DNA can act as genetic material. But the resulting paper, published in 1944, drew remarkably little attention. Despite the meticulous care with which these experiments were performed, geneticists were not immediately convinced that DNA is the hereditary material. Many argued that the transformation might have been caused by some trace protein contaminant in the preparations. Or that the extract might contain a mutagen that alters the genetic material of the harmless bacteria—converting them to the pathogenic form—rather than containing the genetic material itself.

Virus cocktails

The debate was not settled definitively until 1952, when Alfred Hershey and Martha Chase fired up their laboratory blender and demonstrated, once and for all, that genes are made of DNA. The researchers were studying T2—a virus that infects and eventually destroys the bacterium *E. coli*. These bacteria-killing viruses behave like little molecular syringes: they inject their genetic material into the bacterial host cell, while the empty virus heads remain attached outside (Figure 5–5A). Once inside the bacterial cell, the viral genes direct the formation of new virus particles. In less than an hour, the infected cells explode, spewing thousands of new viruses into the medium. These then infect neighboring bacteria, and the process begins again.

The beauty of T2 is that these viruses contain only two kinds of molecules: DNA and protein. So the genetic material had to be one or the other. But which? The experiment was fairly straightforward. Because the viral DNA enters the bacterial cell, while the rest of the virus particle remains outside, the researchers decided to radioactively label the protein in one batch of virus and the DNA in another. Then, all they had to do was follow the radioactivity to see whether viral DNA or

viral protein wound up inside the bacteria. To do this, Hershey and Chase incubated their radiolabeled viruses with *E. coli*; after allowing a few minutes for infection to take place, they poured the mix into a Waring blender and hit “puree.” The blender’s spinning blades sheared the empty virus heads from the surfaces of the bacterial cells. The researchers then centrifuged the sample to separate the heavier, infected bacteria, which formed a pellet at the bottom of the centrifuge tube, from the empty viral coats, which remained in suspension (Figure 5–5B).

As you have probably guessed, Hershey and Chase found that the radioactive DNA entered the bacterial cells, while the radioactive proteins remained outside with the empty virus heads. They found that the radioactive DNA was also incorporated into the next generation of virus particles.

This experiment demonstrated conclusively that viral DNA enters bacterial host cells, whereas viral protein does not. Thus, the genetic material in this virus had to be made of DNA. Together with the studies done by Avery, MacLeod, and McCarty, this evidence clinched the case for DNA as the agent of heredity.

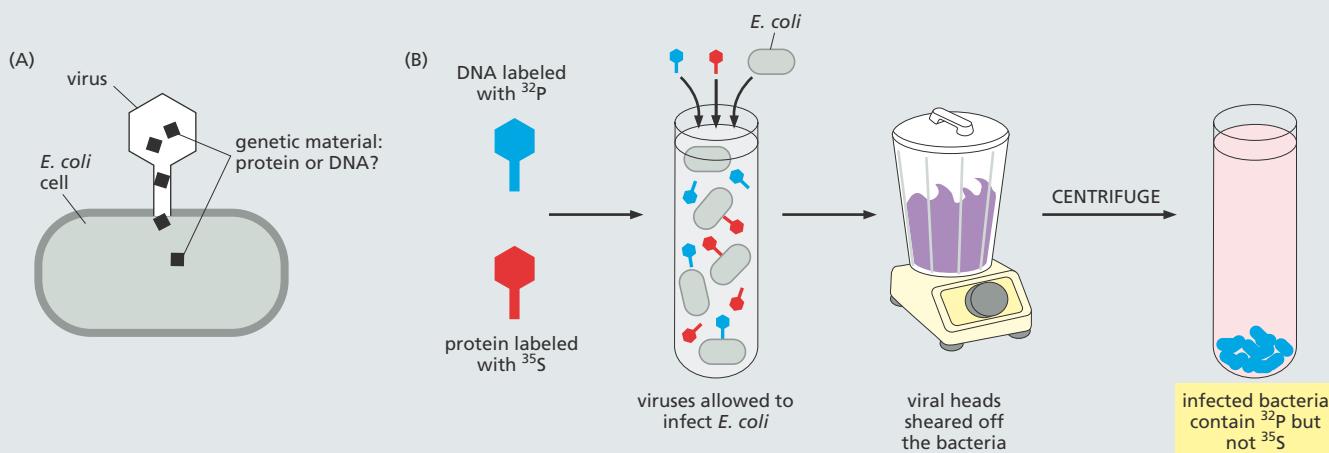


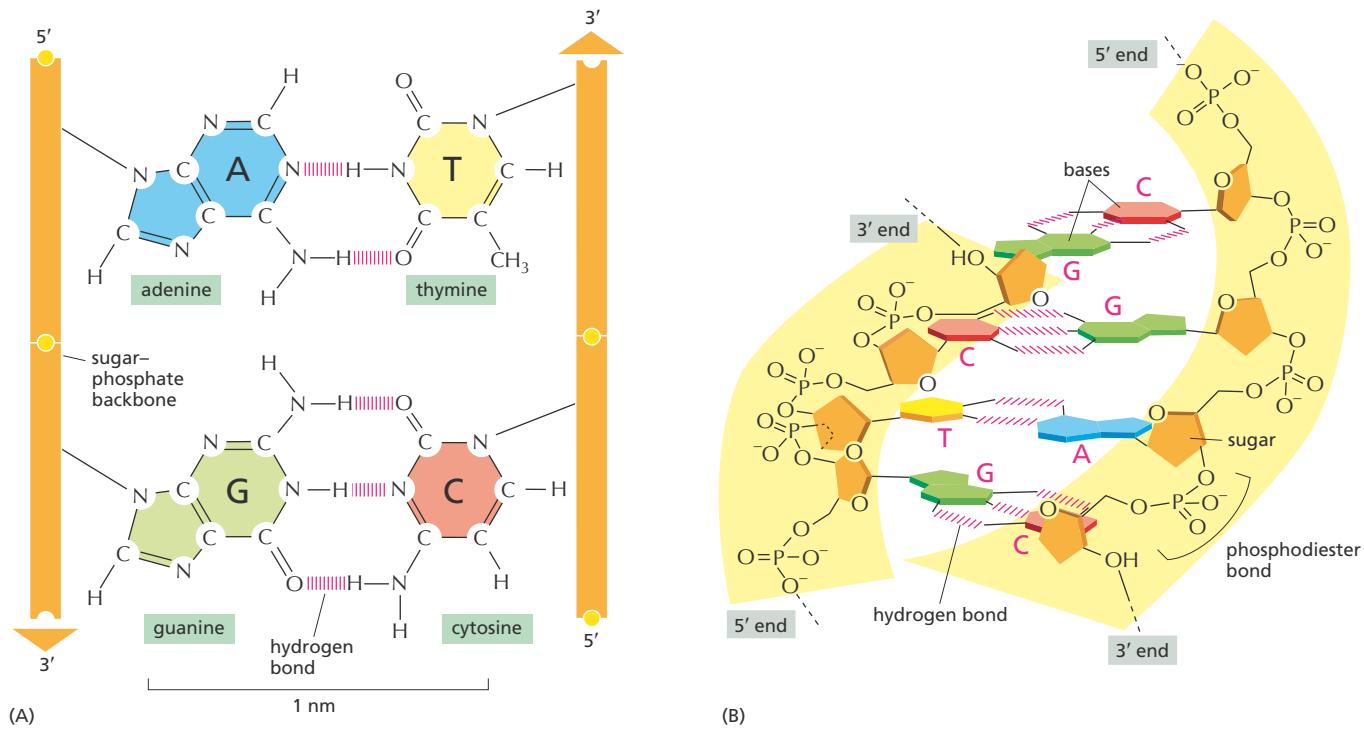
Figure 5–5 Hershey and Chase showed definitively that genes are made of DNA. (A) The researchers worked with T2 viruses, which are made entirely of protein and DNA. Each virus acts as a molecular syringe, injecting its genetic material into a bacterium; the empty viral capsule remains attached to the outside of the cell. (B) To determine whether the genetic material of the virus is protein or DNA, the researchers radioactively labeled the DNA in one batch of viruses with ^{32}P and the proteins in a second batch of viruses with ^{35}S . Because DNA lacks sulfur and the proteins lack phosphorus, these radioactive isotopes provided a handy way for the researchers to distinguish these two types of molecules. These labeled viruses were allowed to infect and replicate inside *E. coli*, and the mixture was then disrupted by brief pulsing in a Waring blender and separated to part the infected bacteria from the empty viral heads. When the researchers measured the radioactivity, they found that much of the ^{32}P -labeled DNA had entered the bacterial cells, while the vast majority of the ^{35}S -labeled proteins remained in solution with the spent viral particles.

As we saw in Chapter 2 (Panel 2–6, pp. 76–77), nucleotides are composed of a nitrogen-containing base and a five-carbon sugar, to which is attached one or more phosphate groups. For the nucleotides in DNA, the sugar is deoxyribose (hence the name deoxyribonucleic acid), and the base can be either *adenine* (A), *cytosine* (C), *guanine* (G), or *thymine* (T). The nucleotides are covalently linked together in a chain through the sugars and phosphates, which thus form a backbone of alternating sugar-phosphate-sugar-phosphate (see Figure 5–2B). Because it is only the base that differs in each of the four types of subunits, each polynucleotide chain in DNA can be thought of as a necklace: a sugar-phosphate backbone strung with four types of beads (the four bases A, C, G, and T). These same symbols (A, C, G, and T) are also commonly used to denote the four different nucleotides—that is, the bases with their attached sugar phosphates.

The way in which the nucleotide subunits are linked together gives a DNA strand a chemical polarity. If we imagine that each nucleotide has a knob (the phosphate) and a hole (see Figure 5–2A), each strand, formed by interlocking knobs with holes, will have all of its subunits lined up in the same orientation. Moreover, the two ends of the strand can be easily distinguished, as one will have a hole (the 3' hydroxyl) and the other a knob (the 5' phosphate). This polarity in a DNA strand is indicated by referring to one end as the 3' end and the other as the 5' end. This convention is based on the details of the chemical linkage between the nucleotide subunits.

The two polynucleotide chains in the DNA **double helix** are held together by hydrogen-bonding between the bases on the different strands. All the bases are therefore on the inside of the double helix, with the sugar-phosphate backbones on the outside (see Figure 5–2D). The bases do not pair at random, however: A always pairs with T, and G always pairs with C (**Figure 5–6**). In each case, a bulkier two-ring base (a purine, see Panel 2–6, pp. 76–77) is paired with a single-ring base (a pyrimidine). Each purine-pyrimidine pair is called a **base pair**, and this *complementary base-pairing* enables the base pairs to be packed in the energetically most favorable

Figure 5–6 The two strands of the DNA double helix are held together by hydrogen bonds between complementary base pairs. (A) The shapes and chemical structure of the bases allow hydrogen bonds to form efficiently only between A and T and between G and C, where atoms that are able to form hydrogen bonds (see Panel 2–2, pp. 68–69) can be brought close together without perturbing the double helix. Two hydrogen bonds form between A and T, whereas three form between G and C. The bases can pair in this way only if the two polynucleotide chains that contain them are antiparallel—that is, oriented in opposite directions. (B) A short section of the double helix viewed from its side. Four base pairs are shown. The nucleotides are linked together covalently by phosphodiester bonds through the 3'-hydroxyl (–OH) group of one sugar and the 5'-phosphate (–PO₃) of the next (see Panel 2–6, pp. 76–77, to review how the carbon atoms in the sugar ring are numbered). This linkage gives each polynucleotide strand a chemical polarity; that is, its two ends are chemically different. The 3' end carries an unlinked –OH group attached to the 3' position on the sugar ring; the 5' end carries a free phosphate group attached to the 5' position on the sugar ring.



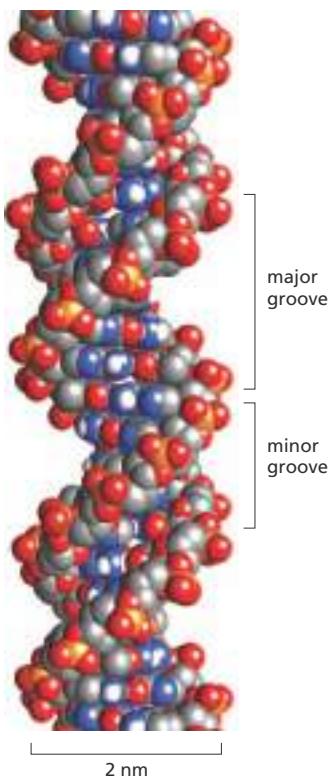


Figure 5–7 A space-filling model shows the conformation of the DNA double helix. The two DNA strands wind around each other to form a right-handed helix (see Figure 4–14) with 10 bases per turn. Shown here are 1.5 turns of the DNA double helix. The coiling of the two strands around each other creates two grooves in the double helix. The wider groove is called the major groove and the smaller one the minor groove. The colors of the atoms are: N, blue; O, red; P, yellow; and H, white.

arrangement in the interior of the double helix. In this arrangement, each base pair has a similar width, thus holding the sugar-phosphate backbones an equal distance apart along the DNA molecule. The members of each base pair can fit together within the double helix because the two strands of the helix run *antiparallel* to each other—that is, they are oriented with opposite polarities (see Figure 5–2C and D). The antiparallel sugar-phosphate strands then twist around each other to form a double helix containing 10 base pairs per helical turn (**Figure 5–7**). This twisting also contributes to the energetically favorable conformation of the DNA double helix.

A consequence of the base-pairing requirements is that each strand of a DNA double helix contains a sequence of nucleotides that is exactly **complementary** to the nucleotide sequence of its partner strand—an A always matches a T on the opposite strand, and a C always matches a G. This complementarity is of crucial importance when it comes to both copying and repairing the DNA, as we discuss in Chapter 6. An animated version of the DNA structure can be seen in **Movie 5.1**.

The Structure of DNA Provides a Mechanism for Heredity

QUESTION 5–1

Which of the following statements are correct? Explain your answers.

- A. A DNA strand has a polarity because its two ends contain different bases.
- B. G-C base pairs are more stable than A-T base pairs.

The need for genes to encode information that must be copied and transmitted accurately when a cell divides raised two fundamental questions: how can the information for specifying an organism be carried in chemical form, and how can the information be accurately copied? The discovery of the structure of the DNA double helix was a landmark in biology because it immediately suggested the answers—and thereby resolved the problem of heredity at the molecular level. In this chapter, we outline the answer to the first question; in the next chapter, we address in detail the answer to the second.

Information is encoded in the order, or sequence, of the nucleotides along each DNA strand. Each base—A, C, T, or G—can be considered a letter in a four-letter alphabet that is used to spell out biological messages (**Figure 5–8**). Organisms differ from one another because their respective DNA molecules have different *nucleotide sequences* and, consequently, carry different biological messages. But how is the nucleotide alphabet used to make up messages, and what do they spell out?

It had already been established some time before the structure of DNA was determined that genes contain the instructions for producing proteins. DNA messages, therefore, must somehow be able to encode proteins. Consideration of the chemical character of proteins makes the problem easier to define. As discussed in Chapter 4, the function of a protein is determined by its three-dimensional structure, and this structure in turn is determined by the sequence of the amino acids in its polypeptide chain. The linear sequence of nucleotides in a gene must therefore be able to spell out the linear sequence of amino acids in a protein.

The exact correspondence between the 4-letter nucleotide alphabet of DNA and the 20-letter amino acid alphabet of proteins—the **genetic code**—is not obvious from the structure of the DNA molecule, and it took more than a decade after the discovery of the double helix to work it

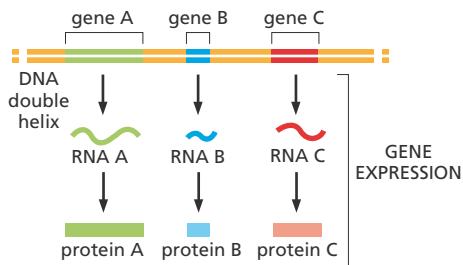
- (A) molecular biology is...
- (B)
- (C) ... - - - - - - - - .
- (D)
- (E) TTGAGCGACCTAACCTATAG

Figure 5–8 Linear messages come in many forms. The languages shown are (A) English, (B) a musical score, (C) Morse code, (D) Chinese, and (E) DNA.

Figure 5–9 Most genes contain information to make proteins. As we discuss in Chapter 7, each protein-coding gene is used to produce RNA molecules, which then direct the production of the specific protein molecules.

out. In Chapter 7, we describe this code in detail when we discuss **gene expression**—the process by which the nucleotide sequence of a gene is *transcribed* into the nucleotide sequence of an RNA molecule, which, in most cases, is then *translated* into the amino acid sequence of a protein (**Figure 5–9**).

The amount of information in an organism's DNA is staggering: written out in the four-letter nucleotide alphabet, the nucleotide sequence of a very small protein-coding gene from humans occupies a quarter of a page of text, while the complete human DNA sequence would fill more than 1000 books the size of this one. Herein lies a problem that affects the architecture of all eukaryotic chromosomes: how can all this information be packed neatly into every cell nucleus? In the remainder of this chapter, we discuss the answer to this question.



THE STRUCTURE OF EUKARYOTIC CHROMOSOMES

Large amounts of DNA are required to encode all the information needed to make even a single-celled bacterium, and far more DNA is needed to encode the information to make a multicellular organism like you. Each human cell contains about 2 meters (m) of DNA; yet the cell nucleus is only 5–8 μm in diameter. Tucking all this material into such a small space is the equivalent of trying to fold 40 km (24 miles) of extremely fine thread into a tennis ball.

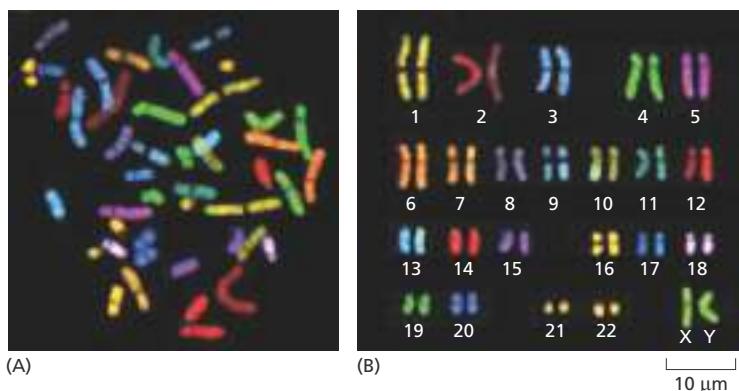
In eukaryotic cells, very long double-stranded DNA molecules are packaged into **chromosomes**. These DNA molecules not only fit readily inside the nucleus, but, after they are replicated, they can be easily apportioned between the two daughter cells at each cell division. The complex task of packaging DNA is accomplished by specialized proteins that bind to and fold the DNA, generating a series of coils and loops that provide increasingly higher levels of organization and prevent the DNA from becoming a tangled, unmanageable mess. Amazingly, the DNA is compacted in a way that allows it to remain accessible to all of the enzymes and other proteins that replicate it, repair it, and control the expression of its genes.

Bacteria typically carry their genes on a single, circular DNA molecule. This molecule is also associated with proteins that condense the DNA, but these proteins differ from the ones that package eukaryotic DNA. Although this prokaryotic DNA is called a bacterial "chromosome," it does not have the same structure as eukaryotic chromosomes, and less is known about how it is packaged. Our discussion of chromosome structure in this chapter will therefore focus entirely on eukaryotic chromosomes.

Eukaryotic DNA Is Packaged into Multiple Chromosomes

In eukaryotes, such as ourselves, the DNA in the nucleus is distributed among a set of different chromosomes. The DNA in a human nucleus, for example, contains approximately 3.2×10^9 nucleotides parceled out into 23 or 24 different types of chromosome (males, with their Y chromosome, have an extra type of chromosome that females do not have). Each chromosome consists of a single, enormously long, linear DNA molecule associated with proteins that fold and pack the fine thread of DNA into a more compact structure. The complex of DNA and protein is called **chromatin**. In addition to the proteins involved in packaging the DNA,

Figure 5–10 Each human chromosome can be “painted” a different color to allow its unambiguous identification. The chromosomes shown here were isolated from a cell undergoing nuclear division (mitosis) and are therefore in a highly compact (condensed) state. Chromosome painting is carried out by exposing the chromosomes to a collection of human DNA molecules that have been coupled to a combination of fluorescent dyes. For example, DNA molecules derived from Chromosome 1 are labeled with one specific dye combination, those from Chromosome 2 with another, and so on. Because the labeled DNA can form base pairs (hybridize) only to its chromosome of origin (discussed in Chapter 10), each chromosome is differently colored. For such experiments, the chromosomes are treated so that the individual strands of the double-helical DNA molecules partly separate to enable base-pairing with the labeled, single-stranded DNA, while keeping the chromosome structure relatively intact. (A) Micrograph shows the array of chromosomes as they originally spilled from the lysed cell. (B) The same chromosomes have been artificially lined up in order. In this so-called karyotype, the homologous chromosomes are numbered and arranged in pairs; the presence of a Y chromosome reveals that these chromosomes came from a male. (From E. Schröck et al., *Science* 273:494–497, 1996. With permission from the AAAS.)



chromosomes are also associated with many other proteins involved in DNA replication, DNA repair, and gene expression.

With the exception of the germ cells (sperm and eggs) and highly specialized cells that lack DNA entirely (such as mature red blood cells), human cells each contain two copies of each chromosome, one inherited from the mother and one from the father. The maternal and paternal chromosomes of a pair are called *homologous chromosomes* (*homologs*). The only nonhomologous chromosome pairs are the sex chromosomes in males, where a *Y chromosome* is inherited from the father and an *X chromosome* from the mother. (Females inherit one X chromosome from each parent and have no Y chromosome.)

In addition to being different sizes, the different human chromosomes can be distinguished from one another by a variety of techniques. Each chromosome can be “painted” a different color using sets of chromosome-specific DNA molecules coupled to different fluorescent dyes (Figure 5–10). This involves a technique called *DNA hybridization*, which takes advantage of complementary base-pairing, as we will describe in detail in Chapter 10. A more traditional way of distinguishing one chromosome from another is to stain the chromosomes with dyes that bind to certain types of DNA sequences. These dyes mainly distinguish between DNA that is rich in A-T nucleotide pairs and DNA that is G-C rich, and they produce a predictable pattern of bands along each type of chromosome. The patterns that result allow each chromosome to be identified and numbered.

An ordered display of the full set of 46 human chromosomes is called the human **karyotype** (see Figure 5–10). If parts of a chromosome are lost, or switched between chromosomes, these changes can be detected. Cytogeneticists analyze karyotypes to detect chromosomal abnormalities that are associated with some inherited defects (Figure 5–11) and with certain types of cancer.

Chromosomes Contain Long Strings of Genes

The most important function of chromosomes is to carry the genes—the functional units of heredity (Figure 5–12). A **gene** is often defined as a

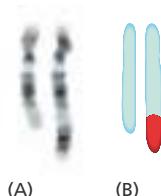


Figure 5–11 Abnormal chromosomes are associated with some inherited genetic defects. (A) A pair of Chromosomes 12 from a patient with inherited ataxia, a genetic disease of the brain characterized by progressive deterioration of motor skills. The patient has one normal Chromosome 12 (left) and one abnormally long Chromosome 12, which contains a piece of Chromosome 4 as identified by its banding pattern. (B) This interpretation was confirmed by chromosome painting, in which Chromosome 12 was painted blue and Chromosome 4 was painted red. (From E. Schröck et al., *Science* 273:494–497, 1996. With permission from the AAAS.)

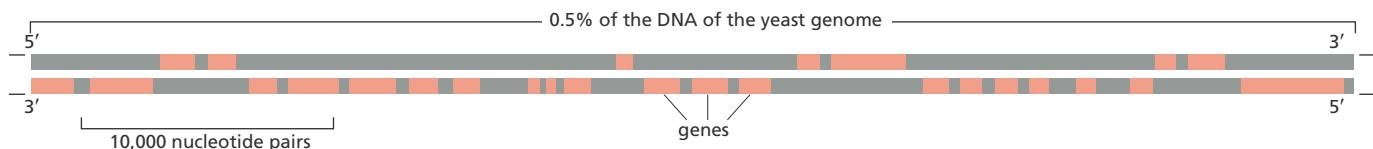


Figure 5–12 Genes are arranged along chromosomes. This figure shows a small region of the DNA double helix in one chromosome from the budding yeast *S. cerevisiae*. The *S. cerevisiae* genome contains about 12 million nucleotide pairs and 6600 genes—spread across 16 chromosomes. Note that, in each gene, only one of the two DNA strands actually encodes the information to make an RNA molecule, and this can be either strand, as indicated by the light red bars. However, a gene is generally denoted to contain both the “coding strand” and its complement, as in Figure 5–9. The high density of genes is characteristic of *S. cerevisiae*.

segment of DNA that contains the instructions for making a particular protein or RNA molecule. Most of the RNA molecules encoded by genes are subsequently used to produce a protein (see Figure 5–9). In some cases, however, the RNA molecule is the final product; like proteins, these RNA molecules have diverse functions in the cell, including structural, catalytic, and gene regulatory roles, as we discuss in later chapters.

Together, the total genetic information carried by all the chromosomes in a cell or organism constitutes its **genome**. Complete genome sequences have been determined for thousands of organisms, from *E. coli* to humans. As might be expected, some correlation exists between the complexity of an organism and the number of genes in its genome. For example, the total number of genes ranges from less than 500 for a simple bacterium to about 30,000 for humans. Bacteria and some single-celled eukaryotes, including *S. cerevisiae*, have especially compact genomes: the DNA molecules that make up their chromosomes are little more than strings of closely packed genes (see Figure 5–12). However, chromosomes from many eukaryotes—including humans—contain, in addition to genes and the specific nucleotide sequences required for normal gene expression, a large excess of interspersed DNA. This extra DNA is sometimes called “junk DNA,” because the usefulness to the cell has not yet been demonstrated. Although the particular nucleotide sequence of most of this DNA might not be important, the DNA itself—acting as spacer material—may be crucial for the long-term evolution of the species and for the proper activity of the genes. In addition, comparisons of the genome sequences from many different species reveal that a portion of this extra DNA is highly conserved among related species, indicating that it serves an important function—although we don’t yet know what that is.

In general, the more complex an organism, the larger is its genome. But this relationship does not always hold true. The human genome, for example, is 200 times larger than that of the yeast *S. cerevisiae*, but 30 times smaller than that of some plants and at least 60 times smaller than some species of amoeba (see Figure 1–40). Furthermore, how the DNA is apportioned over chromosomes also differs from one species to another. Humans have a total of 46 chromosomes (including both maternal and paternal sets), but a species of small deer has only 7, while some carp species have more than 100. Even closely related species with similar genome sizes can have very different numbers and sizes of chromosomes (**Figure 5–13**). Thus, although gene number is roughly correlated with species complexity, there is no simple relationship between gene number, chromosome number, and total genome size. The genomes and chromosomes of modern species have each been shaped by a unique history of seemingly random genetic events, acted on by specific selection pressures, as we discuss in Chapter 9.

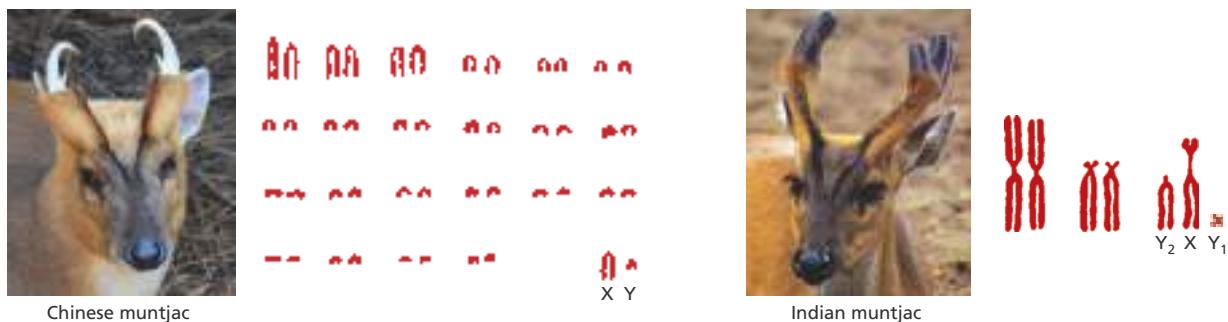


Figure 5-13 Two closely related species can have similar genome sizes but very different chromosome numbers. In the evolution of the Indian muntjac deer, chromosomes that were initially separate, and that remain separate in the Chinese species, fused without having a major effect on the number of genes—or the animal. (Courtesy of Deborah Carreno, Natural Wonders Photography.)

Specialized DNA Sequences Are Required for DNA Replication and Chromosome Segregation

To form a functional chromosome, a DNA molecule must do more than simply carry genes: it must be able to be replicated, and the replicated copies must be separated and partitioned equally and reliably into the two daughter cells at each cell division. These processes occur through an ordered series of events, known collectively as the **cell cycle**. This cycle of cell growth and division is briefly summarized in **Figure 5-14** and will be discussed in detail in Chapter 18. Only two broad stages of the cell cycle need concern us in this chapter: *interphase*, when chromosomes are duplicated, and *mitosis*, when they are distributed, or segregated, to the two daughter nuclei.

During interphase, the chromosomes are extended as long, thin, tangled threads of DNA in the nucleus and cannot be easily distinguished in the light microscope (see Figure 5-1). We refer to chromosomes in this extended state as *interphase chromosomes*. As we discuss in Chapter 6, specialized DNA sequences found in all eukaryotes ensure that DNA replication occurs efficiently during interphase. One type of nucleotide sequence acts as a **replication origin**, where replication of the DNA begins; eukaryotic chromosomes contain many replication origins to ensure that the long DNA molecules are replicated rapidly (**Figure 5-15**). Another DNA sequence forms the **telomeres** at each of the two ends of a chromosome. Telomeres contain repeated nucleotide sequences that are required for the ends of chromosomes to be replicated. They also cap the ends of the DNA molecule, preventing them from being mistaken by the cell as broken DNA in need of repair.

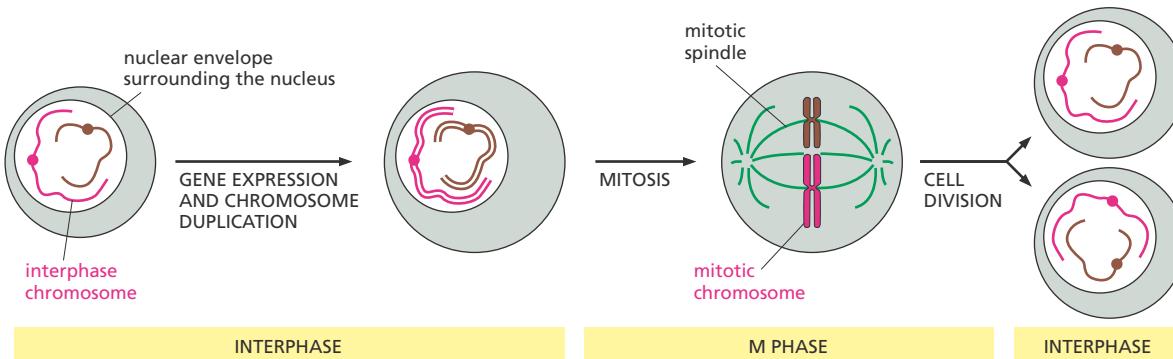


Figure 5-14 The duplication and segregation of chromosomes occurs through an ordered cell cycle in proliferating cells. During interphase, the cell expresses many of its genes, and—during part of this phase—it duplicates chromosomes. Once chromosome duplication is complete, the cell can enter M phase, during which nuclear division, or mitosis, occurs. In mitosis, the duplicated chromosomes condense, gene expression largely ceases, the nuclear envelope breaks down, and the mitotic spindle forms from microtubules and other proteins. The condensed chromosomes are then captured by the mitotic spindle, one complete set is pulled to each end of the cell, and a nuclear envelope forms around each chromosome set. In the final step of M phase, the cell divides to produce two daughter cells. Only two different chromosomes are shown here for simplicity.

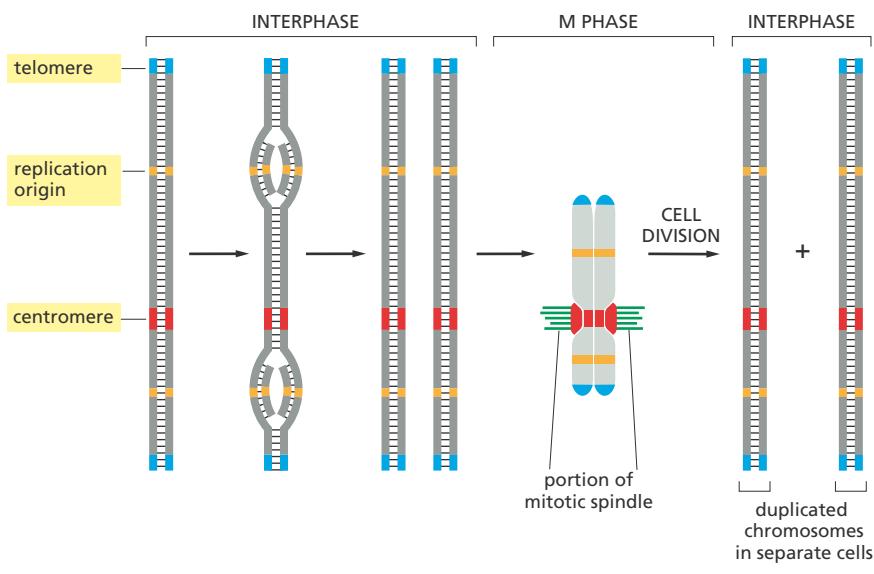


Figure 5–15 Three DNA sequence elements are needed to produce a eukaryotic chromosome that can be replicated and then segregated at **mitosis**. Each chromosome has multiple origins of replication, one centromere, and two telomeres. The sequence of events that a typical chromosome follows during the cell cycle is shown schematically. The DNA replicates in interphase, beginning at the origins of replication and proceeding bidirectionally from the origins across the chromosome. In M phase, the centromere attaches the duplicated chromosomes to the mitotic spindle so that one copy is distributed to each daughter cell when the cell divides. Prior to cell division, the centromere also helps to hold the compact, duplicated chromosomes together until they are ready to be pulled apart. Telomeres, which form special caps at the tips of each chromosome, aid in the replication of chromosome ends.

Eukaryotic chromosomes also contain a third type of specialized DNA sequence, called the **centromere**, that allows duplicated chromosomes to be separated during M phase (see Figure 5–15). During this stage of the cell cycle, the DNA coils up, adopting a more and more compact structure, ultimately forming highly compacted, or condensed, *mitotic chromosomes*. This is the state in which the duplicated chromosomes can be most easily visualized (Figure 5–16) and see Figures 5–1 and 5–14). Once the chromosomes have condensed, the centromere attaches the mitotic spindle to each duplicated chromosome in a way that allows one copy of each chromosome to be segregated to each daughter cell (see Figure 5–15B). We describe the central role that centromeres play in cell division in Chapter 18.

Interphase Chromosomes Are Not Randomly Distributed Within the Nucleus

Inside the nucleus, the interphase chromosomes—although longer and finer than mitotic chromosomes—are nonetheless organized in various

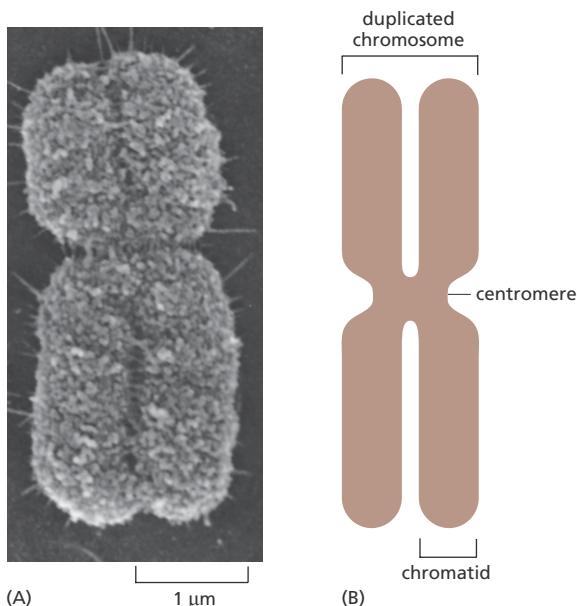
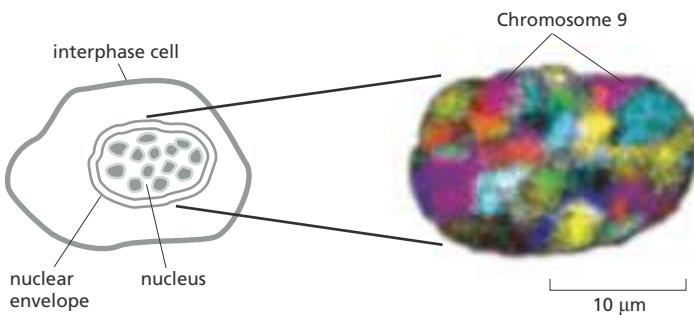


Figure 5–16 A typical duplicated mitotic chromosome is highly compact. Because DNA is replicated during interphase, each duplicated mitotic chromosome contains two identical daughter DNA molecules (see Figure 5–15A). Each of these very long DNA molecules, with its associated proteins, is called a *chromatid*; once the two sister chromatids separate, they are considered individual chromosomes. (A) A scanning electron micrograph of a mitotic chromosome. The two chromatids are tightly joined together. The constricted region reveals the position of the centromere. (B) A cartoon representation of a mitotic chromosome. (A, courtesy of Terry D. Allen.)

Figure 5–17 Interphase chromosomes occupy their own distinct territories within the nucleus. DNA probes coupled with different fluorescent markers were used to paint individual interphase chromosomes in a human cell. Viewed in a fluorescence microscope, each interphase chromosome is seen to occupy its own discrete territory within the nucleus, rather than being mixed with the other chromosomes like spaghetti in a bowl. Note that pairs of homologous chromosomes, such as the two copies of Chromosome 9 indicated, are not generally located in the same position. (From M.R. Speicher and N.P. Carter, *Nat. Rev. Genet.* 6:782–792, 2005. With permission from Macmillan Publishers Ltd.)



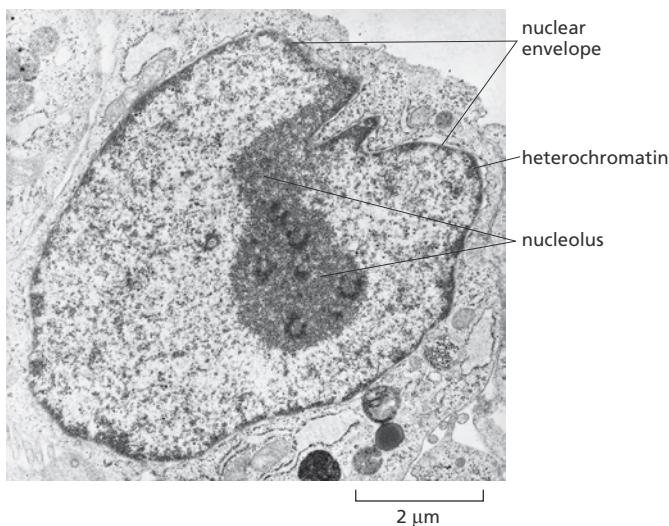
ways. First, each chromosome tends to occupy a particular region of the interphase nucleus, and so different chromosomes do not become extensively entangled with one another (Figure 5–17). In addition, some chromosomes are attached to particular sites on the *nuclear envelope*—the pair of concentric membranes that surround the nucleus—or to the underlying *nuclear lamina*, the protein meshwork that supports the envelope (discussed in Chapter 17).

The most obvious example of chromosome organization in the interphase nucleus is the **nucleolus** (Figure 5–18). The nucleolus is where the parts of the different chromosomes carrying genes that encode *ribosomal RNAs* cluster together. Here, ribosomal RNAs are synthesized and combine with proteins to form ribosomes, the cell's protein-synthesizing machines. As we discuss in Chapter 7, ribosomal RNAs play both structural and catalytic roles in the ribosome.

The DNA in Chromosomes Is Always Highly Condensed

As we have seen, all eukaryotic cells, whether in interphase or mitosis, package their DNA tightly into chromosomes. Human Chromosome 22, for example, contains about 48 million nucleotide pairs; stretched out end-to-end, its DNA would extend about 1.5 cm. Yet, during mitosis, Chromosome 22 measures only about 2 μm in length—that is, nearly 10,000 times more compact than the DNA would be if it were stretched to its full length. This remarkable feat of compression is performed by proteins that coil and fold the DNA into higher and higher levels of organization. The DNA of interphase chromosomes, although about 20 times less condensed than that of mitotic chromosomes (Figure 5–19), is still packed tightly.

Figure 5–18 The nucleolus is the most prominent structure in the interphase nucleus. Electron micrograph of a thin section through the nucleus of a human fibroblast. The nucleus is surrounded by the nuclear envelope. Inside the nucleus, the chromatin appears as a diffuse speckled mass, with regions that are especially dense, called heterochromatin (dark staining). Heterochromatin contains few genes and is located mainly around the periphery of the nucleus, immediately under the nuclear envelope. The large dark region is the nucleolus, which contains the genes for ribosomal RNAs; these genes are located on multiple chromosomes but are clustered together in the nucleolus. (Courtesy of E.G. Jordan and J. McGovern.)



In the next sections, we introduce the specialized proteins that make this compression possible. Bear in mind, though, that chromosome structure is dynamic. Not only do chromosomes condense and decondense during the cell cycle, but chromosome packaging must be flexible enough to allow rapid, on-demand access to different regions of the interphase chromosome, unpacking enough to allow protein complexes access to specific, localized DNA sequences for replication, repair, or gene expression.

Nucleosomes Are the Basic Units of Eukaryotic Chromosome Structure

The proteins that bind to DNA to form eukaryotic chromosomes are traditionally divided into two general classes: the **histones** and the *nonhistone chromosomal proteins*. Histones are present in enormous quantities (more than 60 million molecules of several different types in each cell), and their total mass in chromosomes is about equal to that of the DNA itself. The complex of both classes of protein with nuclear DNA is called **chromatin**.

Histones are responsible for the first and most fundamental level of chromatin packing, the **nucleosome**, which was discovered in 1974. When interphase nuclei are broken open very gently and their contents examined with an electron microscope, much of the chromatin is in the form of *chromatin fibers* with a diameter of about 30 nm (Figure 5–20A). If this chromatin is subjected to treatments that cause it to unfold partially, it can then be seen in the electron microscope as a series of “beads on a string” (Figure 5–20B). The string is DNA, and each bead is a *nucleosome core particle*, which consists of DNA wound around a core of proteins formed from histones.

The structure of the nucleosome core particle was determined after first isolating nucleosomes by treating chromatin in its unfolded, “beads on a string” form with enzymes called nucleases, which break down DNA by cutting the phosphodiester bonds between nucleotides. After digestion for a short period, only the exposed DNA between the core particles—the *linker DNA*—is degraded, allowing the core particles to be isolated. An individual nucleosome core particle consists of a complex of eight histone proteins—two molecules each of histones H2A, H2B, H3, and H4—and a stretch of double-stranded DNA, 147 nucleotide pairs long, that winds around this *histone octamer* (Figure 5–21). The high-resolution structure of the nucleosome core particle was solved in 1997, revealing in atomic detail the disc-shaped histone octamer around which the DNA is tightly wrapped, making 1.7 turns in a left-handed coil (Figure 5–22).

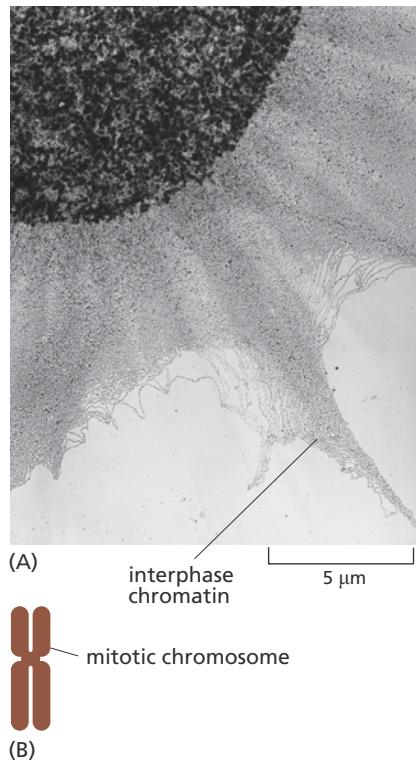


Figure 5–19 DNA in interphase chromosomess is less compact than in mitotic chromosomess. (A) An electron micrograph showing an enormous tangle of chromatin (DNA with its associated proteins) spilling out of a lysed interphase nucleus. (B) Schematic drawing of a human mitotic chromosome drawn to the same scale. (Courtesy of Victoria Foe.)

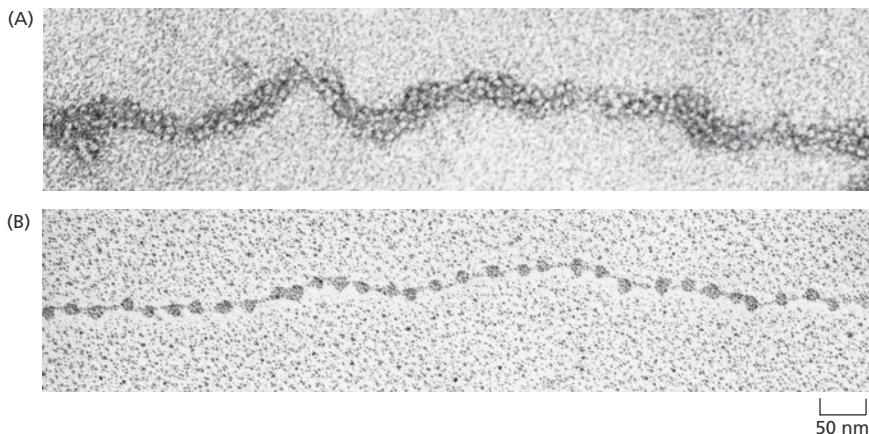


Figure 5–20 Nucleosomes can be seen in the electron microscope. (A) Chromatin isolated directly from an interphase nucleus appears in the electron microscope as a chromatin fiber about 30-nm thick; a part of one such fiber is shown here. (B) This electron micrograph shows a length of a chromatin fiber that has been experimentally unpacked, or decondensed, after isolation to show the “beads-on-a-string” appearance of the nucleosomes. (A, courtesy of Barbara Hamkalo; B, courtesy of Victoria Foe.)

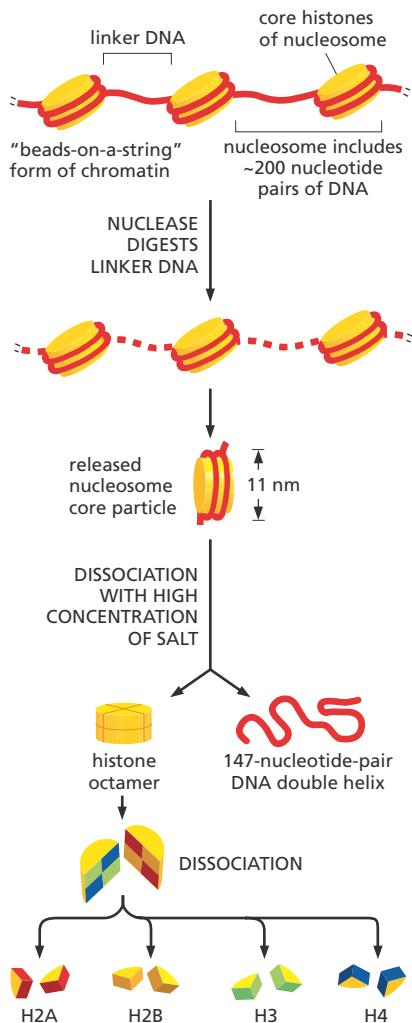


Figure 5–21 Nucleosomes contain DNA wrapped around a protein core of eight histone molecules. In a test tube, the nucleosome core particle can be released from chromatin by digestion of the linker DNA with a nuclease, which degrades the exposed DNA but not the DNA wound tightly around the nucleosome core. The DNA around each isolated nucleosome core particle can then be released and its length determined. With 147 nucleotide pairs in each fragment, the DNA wraps almost twice around each histone octamer.

The linker DNA between each nucleosome core particle can vary in length from a few nucleotide pairs up to about 80. (The term nucleosome technically refers to a nucleosome core particle plus one of its adjacent DNA linkers, as shown in Figure 5–21, but it is often used to refer to the nucleosome core particle itself.) The formation of nucleosomes converts a DNA molecule into a chromatin thread that is approximately one-third the length of the initial piece of DNA, and it provides the first level of DNA packing.

All four of the histones that make up the octamer are relatively small proteins, with a high proportion of positively charged amino acids (lysine and arginine). The positive charges help the histones bind tightly to the negatively charged sugar-phosphate backbone of DNA. These numerous electrostatic interactions explain in part why DNA of virtually any sequence can bind to a histone octamer. Each of the histones in the

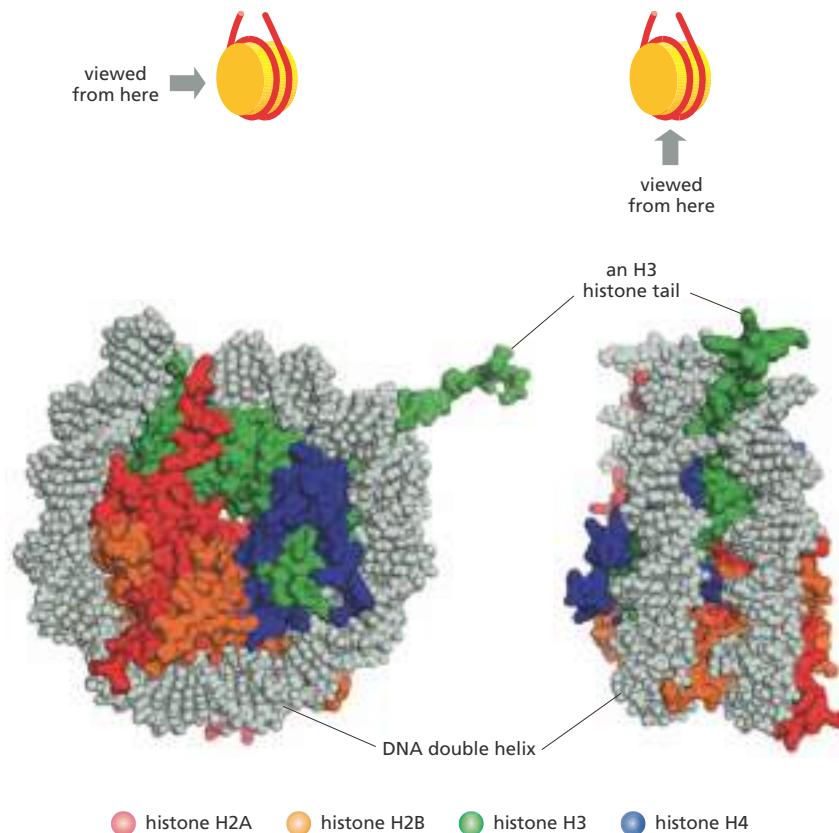


Figure 5–22 The structure of the nucleosome core particle, as determined by X-ray diffraction analysis, reveals how DNA is tightly wrapped around a disc-shaped histone octamer. Two views of a nucleosome core particle are shown here. The two strands of the DNA double helix are shown in gray. A portion of an H3 histone tail (green) can be seen extending from the nucleosome core particle, but the tails of the other histones have been truncated. (Reprinted by permission from K. Luger et al., *Nature* 389:251–260, 1997. With permission from Macmillan Publishers Ltd.)

octamer also has a long, unstructured N-terminal amino acid “tail” that extends out from the nucleosome core particle (see Figure 5–22). These histone tails are subject to several types of reversible, covalent chemical modifications that control many aspects of chromatin structure.

The histones that form the nucleosome core are among the most highly conserved of all known eukaryotic proteins: there are only two differences between the amino acid sequences of histone H4 from peas and cows, for example. This extreme evolutionary conservation reflects the vital role of histones in controlling eukaryotic chromosome structure.

Chromosome Packing Occurs on Multiple Levels

Although long strings of nucleosomes form on most chromosomal DNA, chromatin in the living cell rarely adopts the extended beads-on-a-string form seen in Figure 5–20B. Instead, the nucleosomes are further packed on top of one another to generate a more compact structure, such as the chromatin fiber shown in Figure 5–20A and **Movie 5.2**. This additional packing of nucleosomes into a chromatin fiber depends on a fifth histone called histone H1, which is thought to pull adjacent nucleosomes together into a regular repeating array. This “linker” histone changes the path the DNA takes as it exits the nucleosome core, allowing it to form a more condensed chromatin fiber (**Figure 5–23**).

We saw earlier that during mitosis chromatin becomes so highly condensed that individual chromosomes can be seen in the light microscope. How is a chromatin fiber folded to produce mitotic chromosomes? The answer is not yet known in detail, but it is known that the chromatin fiber is folded into a series of loops, and that these loops are further condensed to produce the interphase chromosome; finally, this compact string of loops is thought to undergo at least one more level of packing to form the mitotic chromosome (**Figure 5–24** and **Figure 5–25**).

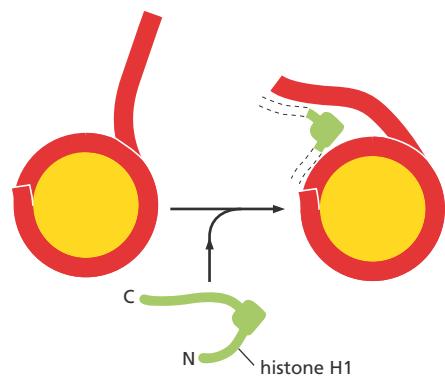
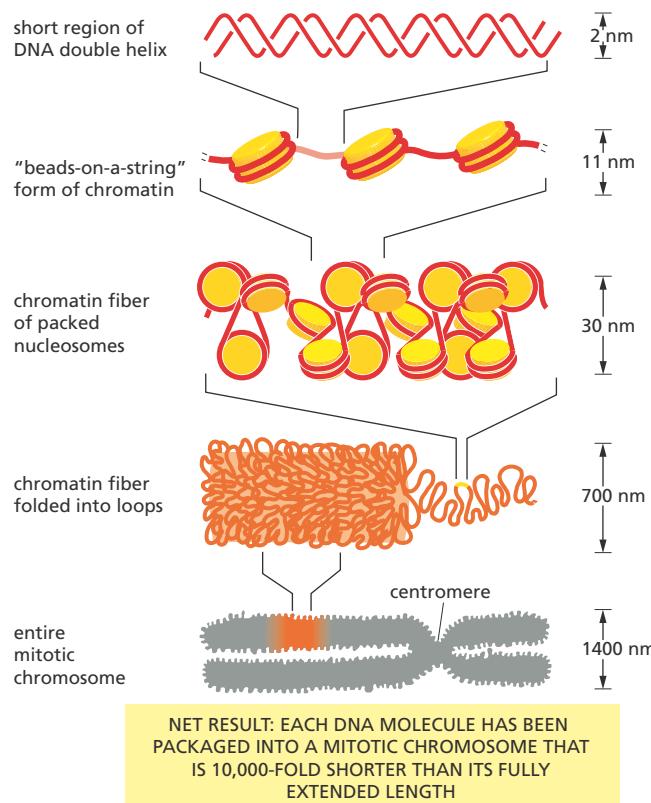


Figure 5–23 A linker histone helps to pull nucleosomes together and pack them into a more compact chromatin fiber.

Histone H1 consists of a globular region plus a pair of long tails at its C-terminal and N-terminal ends. The globular region constrains an additional 20 base pairs of the DNA where it exits from the nucleosome core, an activity that is thought to be important for the formation of the chromatin fiber. The long C-terminal tail is required for H1 to bind to chromatin. The positions of the C-terminal and N-terminal tails in the nucleosome are not known.



QUESTION 5–2

Assuming that the histone octamer (shown in Figure 5–21) forms a cylinder 9 nm in diameter and 5 nm in height and that the human genome forms 32 million nucleosomes, what volume of the nucleus (6 μm in diameter) is occupied by histone octamers? (Volume of a cylinder is $\pi r^2 h$; volume of a sphere is $4/3 \pi r^3$.) What fraction of the total volume of the nucleus do the histone octamers occupy? How does this compare with the volume of the nucleus occupied by human DNA?

Figure 5–24 DNA packing occurs on several levels in chromosomes. This schematic drawing shows some of the levels thought to give rise to the highly condensed mitotic chromosome. The actual structures are still uncertain.

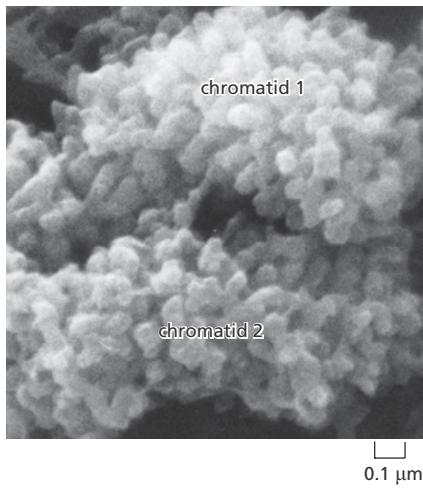


Figure 5–25 The mitotic chromosome contains chromatin that is packed especially tightly. This scanning electron micrograph shows a region near one end of a typical mitotic chromosome. Each knoblike projection is believed to represent the tip of a separate loop of chromatin. The chromosome has duplicated, forming two sister chromatids that are still held close together (see Figure 5–16). The ends of the two chromatids can be distinguished on the right of the photo. (From M.P. Marsden and U.K. Laemmli, *Cell* 17:849–858, 1989. With permission from Elsevier.)

THE REGULATION OF CHROMOSOME STRUCTURE

So far, we have discussed how DNA is packed tightly into chromatin. We now turn to the question of how this packaging can be regulated to allow rapid access to the underlying DNA. The DNA in cells carries enormous amounts of coded information, and cells must be able to get to this information as needed.

In this section, we discuss how a cell can alter its chromatin structure to expose localized regions of DNA and allow access to specific proteins and protein complexes, particularly those involved in gene expression and in DNA replication and repair. We then discuss how chromatin structure is established and maintained—and how a cell can pass on some forms of this structure to its descendants. The regulation and inheritance of chromatin structure play crucial parts in the development of eukaryotic organisms.

QUESTION 5–3

Histone proteins are among the most highly conserved proteins in eukaryotes. Histone H4 proteins from a pea and a cow, for example, differ in only 2 of 102 amino acids. Comparison of the gene sequences shows many more differences, but only two change the amino acid sequence. These observations indicate that mutations that change amino acids must have been selected against during evolution. Why do you suppose that amino-acid-altering mutations in histone genes are deleterious?

Changes in Nucleosome Structure Allow Access to DNA

Eukaryotic cells have several ways to adjust the local structure of their chromatin rapidly. One way takes advantage of **chromatin-remodeling complexes**, protein machines that use the energy of ATP hydrolysis to change the position of the DNA wrapped around nucleosomes (Figure 5–26A). The complexes, which attach to both the histone octamer and the DNA wrapped around it, can locally alter the arrangement of nucleosomes on the DNA, making the DNA either more accessible (Figure 5–26B) or less accessible to other proteins in the cell. During mitosis, many of the chromatin-remodeling complexes are inactivated, which may help mitotic chromosomes maintain their tightly packed structure.

Another way of altering chromatin structure relies on the reversible chemical modification of the histones. The tails of all four of the core histones are particularly subject to these covalent modifications (Figure 5–27A). For example, acetyl, phosphate, or methyl groups can be added to and removed from the tails by enzymes that reside in the nucleus (Figure 5–27B). These and other modifications can have important consequences for the stability of the chromatin fiber. Acetylation of lysines, for instance, can reduce the affinity of the tails for adjacent nucleosomes, thereby loosening chromatin structure and allowing access to particular nuclear proteins.

Most importantly, however, these modifications can serve as docking sites on the histone tails for a variety of regulatory proteins. Different patterns of modifications attract different proteins to particular stretches of chromatin. Some of these proteins promote chromatin condensation, whereas others decondense chromatin and facilitate access to the DNA. Specific combinations of tail modifications and the proteins that bind to them have different meanings for the cell: one pattern, for example, indicates that a particular stretch of chromatin has been newly replicated;

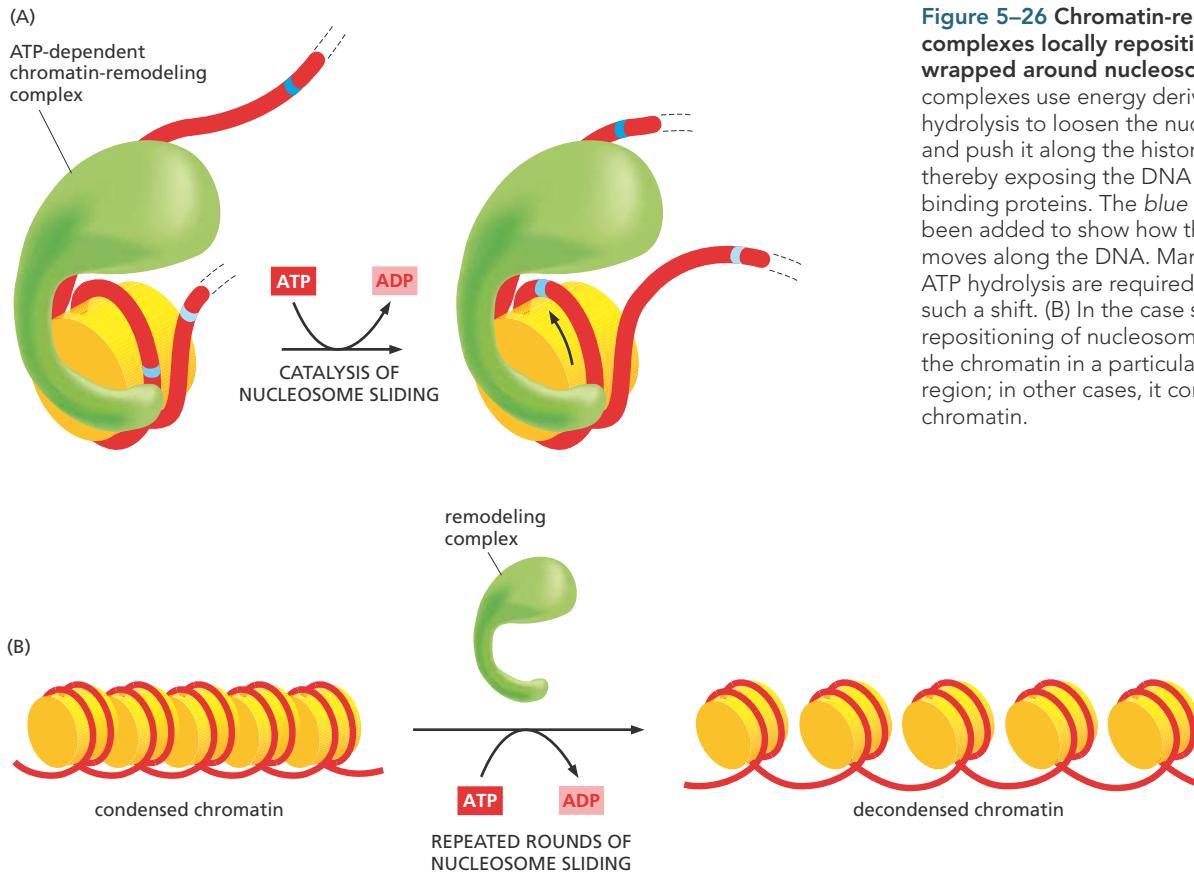


Figure 5-26 Chromatin-remodeling complexes locally reposition the DNA wrapped around nucleosomes. (A) The complexes use energy derived from ATP hydrolysis to loosen the nucleosomal DNA and push it along the histone octamer, thereby exposing the DNA to other DNA-binding proteins. The blue stripes have been added to show how the nucleosome moves along the DNA. Many cycles of ATP hydrolysis are required to produce such a shift. (B) In the case shown, the repositioning of nucleosomes decondenses the chromatin in a particular chromosomal region; in other cases, it condenses the chromatin.

another indicates that the genes in that stretch of chromatin should be expressed; still others indicate that the nearby genes should be silenced (**Figure 5-27C**).

Like the chromatin-remodeling complexes, the enzymes that modify histone tails are tightly regulated. They are brought to particular chromatin regions mainly by interactions with proteins that bind to specific

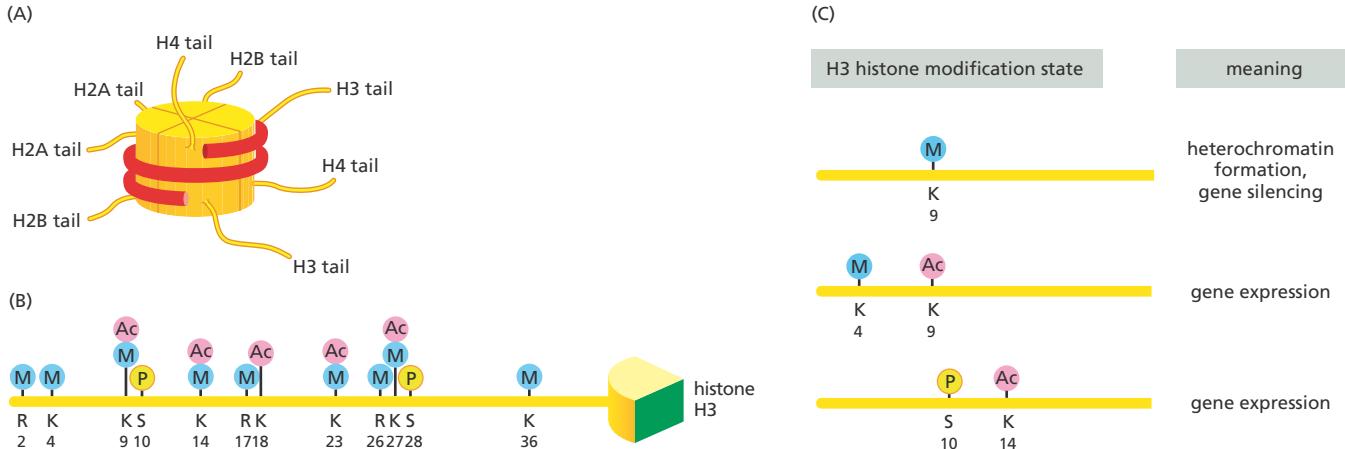


Figure 5-27 The pattern of modification of histone tails can dictate how a stretch of chromatin is treated by the cell.

(A) Schematic drawing showing the positions of the histone tails that extend from each nucleosome. (B) Each histone can be modified by the covalent attachment of a number of different chemical groups, mainly to the tails. Histone H3, for example, can receive an acetyl group (Ac), a methyl group (M), or a phosphate group (P). The numbers denote the positions of the modified amino acids in the protein chain, with each amino acid designated by its one-letter code. Note that some positions, such as lysines (K) 9, 14, 23, and 27, can be modified in more than one way. Moreover, lysines can be modified with either one, two, or three methyl groups (not shown). Note that histone H3 contains 135 amino acids, most of which are in its globular portion (green), and that most modifications are on its N-terminal tail (orange). (C) Different combinations of histone tail modifications can confer a specific meaning on the stretch of chromatin on which they occur, as indicated. Only a few of these “meanings” are known.

sequences in DNA (we discuss these proteins in Chapter 8). The histone-modifying enzymes work in concert with the chromatin-remodeling complexes to condense or decondense stretches of chromatin, allowing local chromatin structure to change rapidly according to the needs of the cell.

Interphase Chromosomes Contain Both Condensed and More Extended Forms of Chromatin

The localized alteration of chromatin packing by remodeling complexes and histone modification has important effects on the large-scale structure of interphase chromosomes. Interphase chromatin is not uniformly packed. Instead, regions of the chromosome that contain genes that are being expressed are generally more extended, while those that contain silent genes are more condensed. Thus, the detailed structure of an interphase chromosome can differ from one cell type to the next, helping to determine which genes are expressed. Most cell types express about 20 to 30 % of the genes they contain.

The most highly condensed form of interphase chromatin is called **heterochromatin** (from the Greek *heteros*, “different,” plus chromatin). It was first observed in the light microscope in the 1930s as discrete, strongly staining regions within the mass of chromatin. Heterochromatin typically makes up about 10% of an interphase chromosome, and in mammalian chromosomes, it is concentrated around the centromere region and in the telomeres at the ends of the chromosomes (see Figure 5–15).

The rest of the interphase chromatin is called **euchromatin** (from the Greek *eu*, “true” or “normal,” plus chromatin). Although we use the term euchromatin to refer to chromatin that exists in a more decondensed state than heterochromatin, it is now clear that both euchromatin and heterochromatin are composed of mixtures of different chromatin structures (Figure 5–28).

Each type of chromatin structure is established and maintained by different sets of histone tail modifications that attract distinct sets of non-histone proteins. The modifications that direct the formation of the most common type of heterochromatin, for example, include the methylation of lysine 9 in histone H3 (see Figure 5–27). Once it has been established, heterochromatin can spread because these histone tail modifications attract a set of heterochromatin-specific proteins, including histone-modifying enzymes, which then create the same histone tail modifications on adjacent nucleosomes. These modifications in turn recruit more of the heterochromatin-specific proteins, causing a wave of condensed chromatin to propagate along the chromosome. This heterochromatin will continue to spread until it encounters a barrier DNA sequence that stops the propagation (Figure 5–29). In this manner, extended regions of heterochromatin can be established along the DNA.

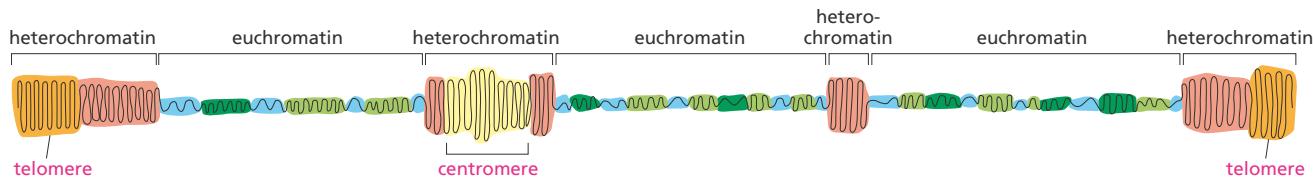


Figure 5–28 The structure of chromatin varies along a single interphase chromosome. As schematically indicated by different colors (and the path of the DNA molecule represented by the central black line), heterochromatin and euchromatin each represent a set of different chromatin structures with different degrees of condensation. Overall, heterochromatin is more condensed than euchromatin.

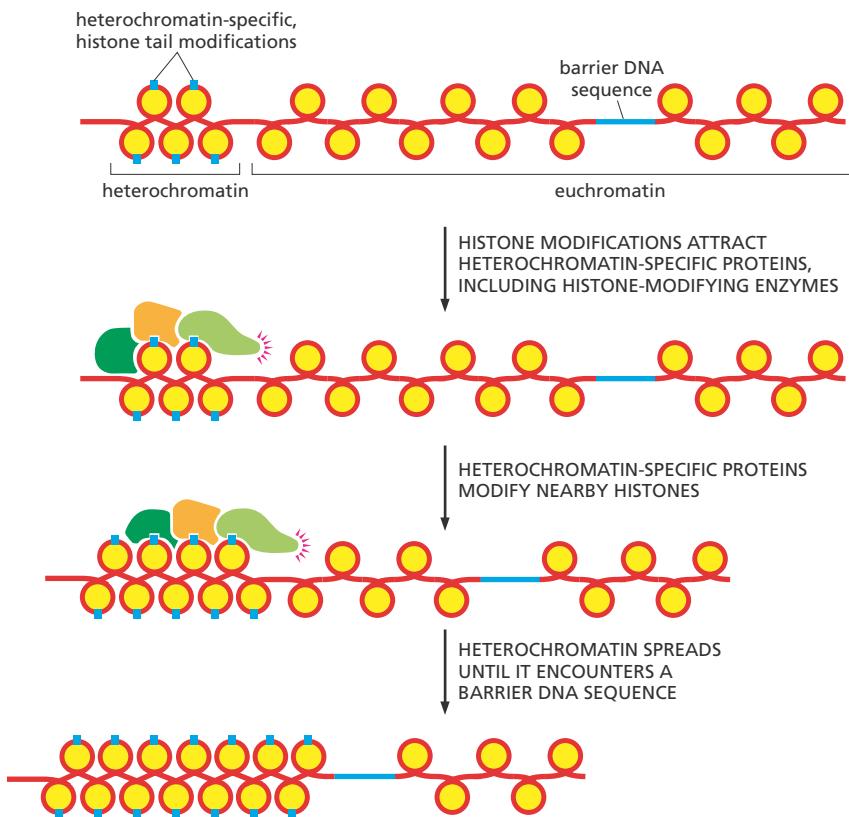


Figure 5–29 Heterochromatin-specific modifications allow heterochromatin to form and to spread. These modifications attract heterochromatin-specific proteins that reproduce the same modifications on neighboring histones. In this manner, heterochromatin can spread until it encounters a barrier DNA sequence that blocks its propagation into regions of euchromatin.

Most DNA that is permanently folded into heterochromatin in the cell does not contain genes. Because heterochromatin is so compact, genes that accidentally become packaged into heterochromatin usually fail to be expressed. Such inappropriate packaging of genes in heterochromatin can cause disease: in humans, the gene that encodes β -globin—which forms part of the oxygen-carrying hemoglobin molecule—is situated next to a region of heterochromatin. If, because of an inherited DNA deletion, that heterochromatin spreads, the β -globin gene is poorly expressed and the person develops a severe form of anemia.

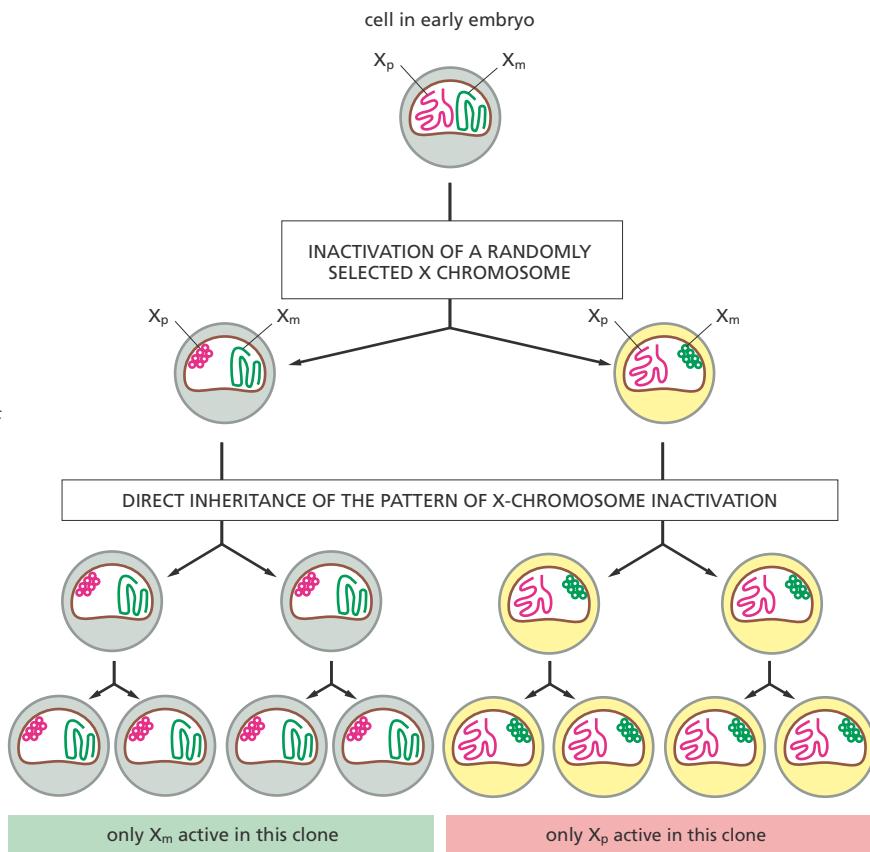
Perhaps the most striking example of the use of heterochromatin to keep genes shut down, or *silenced*, is found in the interphase X chromosomes of female mammals. In mammals, female cells contain two X chromosomes, whereas male cells contain one X and one Y. Because a double dose of X-chromosome products would be lethal, female mammals have evolved a mechanism for permanently inactivating one of the two X chromosomes in each cell. At random, one or other of the two X chromosomes in each cell becomes highly condensed into heterochromatin early in embryonic development. Thereafter, the condensed and inactive state of that X chromosome is inherited in all of the many descendants of those cells (Figure 5–30).

When a cell divides, it generally passes on its histone modifications, chromatin structure, and gene expression patterns to the two daughter cells. Such “cell memory” is critical for the establishment and maintenance of different cell types during the development of a complex multicellular organism. We discuss the mechanisms involved in cell memory in Chapter 8, where we consider the control of gene expression.

QUESTION 5–4

Mutations in a particular gene on the X chromosome result in color blindness in men. By contrast, most women carrying the mutation have proper color vision but see colored objects with reduced resolution, as though functional cone cells (the photoreceptor cells responsible for color vision) are spaced farther apart than normal in the retina. Can you give a plausible explanation for this observation? If a woman is color-blind, what could you say about her father? About her mother? Explain your answers.

Figure 5–30 One of the two X chromosomes is inactivated in the cells of mammalian females by heterochromatin formation. Each female cell contains two X chromosomes, one from the mother (X_m) and the other from the father (X_p). At an early stage in embryonic development, one of these two chromosomes becomes condensed into heterochromatin in each cell, apparently at random. At each cell division, the same X chromosome becomes condensed (and inactivated) in all the descendants of that original cell. Thus, all mammalian females end up as mixtures (mosaics) of cells bearing maternal or paternal inactivated X chromosomes. In most of their tissues and organs, about half the cells will be of one type, and the other half will be of the other.



ESSENTIAL CONCEPTS

- Life depends on the stable storage and inheritance of genetic information.
- Genetic information is carried by very long DNA molecules and is encoded in the linear sequence of four nucleotides: A, T, G, and C.
- Each molecule of DNA is a double helix composed of a pair of antiparallel, complementary DNA strands, which are held together by hydrogen bonds between G-C and A-T base pairs.
- The genetic material of a eukaryotic cell is contained in a set of chromosomes, each formed from a single, enormously long DNA molecule that contains many genes.
- When a gene is expressed, part of its nucleotide sequence is transcribed into RNA molecules, many of which are translated into protein.
- The DNA that forms each eukaryotic chromosome contains, in addition to genes, many replication origins, one centromere, and two telomeres. These special DNA sequences ensure that, before cell division, each chromosome can be duplicated efficiently, and that the resulting daughter chromosomes are parceled out equally to the two daughter cells.
- In eukaryotic chromosomes, the DNA is tightly folded by binding to a set of histone and nonhistone proteins. This complex of DNA and protein is called chromatin.
- Histones pack the DNA into a repeating array of DNA-protein particles called nucleosomes, which further fold up into even more compact chromatin structures.

- A cell can regulate its chromatin structure—temporarily decondensing or condensing particular regions of its chromosomes—using chromatin-remodeling complexes and enzymes that covalently modify histone tails in various ways.
- The loosening of chromatin to a more decondensed state allows proteins involved in gene expression, DNA replication, and DNA repair to gain access to the necessary DNA sequences.
- Some forms of chromatin have a pattern of histone tail modification that causes the DNA to become so highly condensed that its genes cannot be expressed to produce RNA; such condensation occurs on all chromosomes during mitosis and in the heterochromatin of interphase chromosomes.

KEY TERMS

base pair	gene expression
cell cycle	genetic code
centromere	genome
chromatin	heterochromatin
chromatin-remodeling complex	histone
chromosome	karyotype
complementary	nucleolus
deoxyribonucleic acid (DNA)	nucleolus
double helix	replication origin
euchromatin	telomere gene
gene	

QUESTIONS

QUESTION 5–5

A. The nucleotide sequence of one DNA strand of a DNA double helix is

5'-GGATTTCCTGTCACAAATCA-3'.

What is the sequence of the complementary strand?

B. In the DNA of certain bacterial cells, 13% of the nucleotides are adenine. What are the percentages of the other nucleotides?

C. How many possible nucleotide sequences are there for a stretch of DNA that is N nucleotides long, if it is (a) single-stranded or (b) double-stranded?

D. Suppose you had a method of cutting DNA at specific sequences of nucleotides. How many nucleotides long (on average) would such a sequence have to be in order to make just one cut in a bacterial genome of 3×10^6 nucleotide pairs? How would the answer differ for the genome of an animal cell that contains 3×10^9 nucleotide pairs?

QUESTION 5–6

An A-T base pair is stabilized by only two hydrogen bonds. Hydrogen-bonding schemes of very similar strengths can also be drawn between other base combinations that normally do not occur in DNA molecules, such as the A-C and the A-G pairs shown in Figure Q5–6.

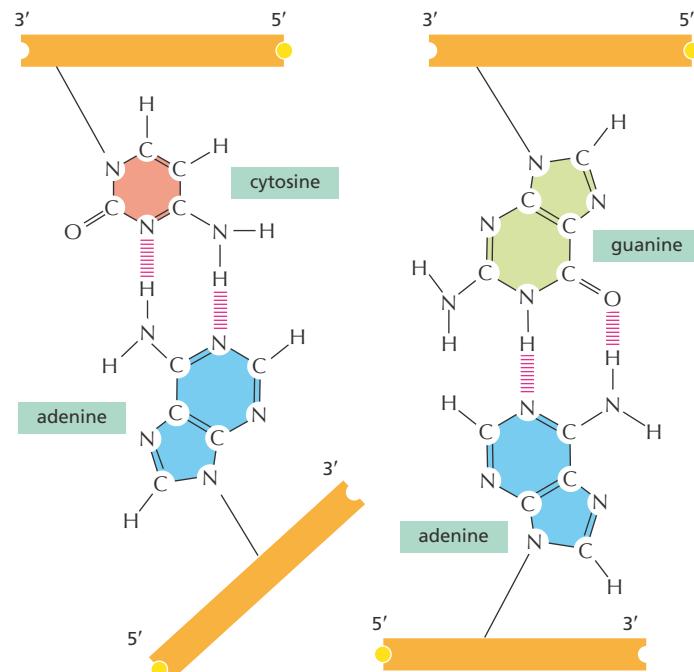


Figure Q5–6

What would happen if these pairs formed during DNA replication and the inappropriate bases were incorporated? Discuss why this does not often happen. (Hint: see Figure 5–6.)

QUESTION 5–7

A. A macromolecule isolated from an extraterrestrial source superficially resembles DNA, but closer analysis reveals that the bases have quite different structures (Figure Q5–7). Bases V, W, X, and Y have replaced bases A, T, G, and C. Look at these structures closely. Could these DNA-like molecules have been derived from a living organism that uses principles of genetic inheritance similar to those used by organisms on Earth?

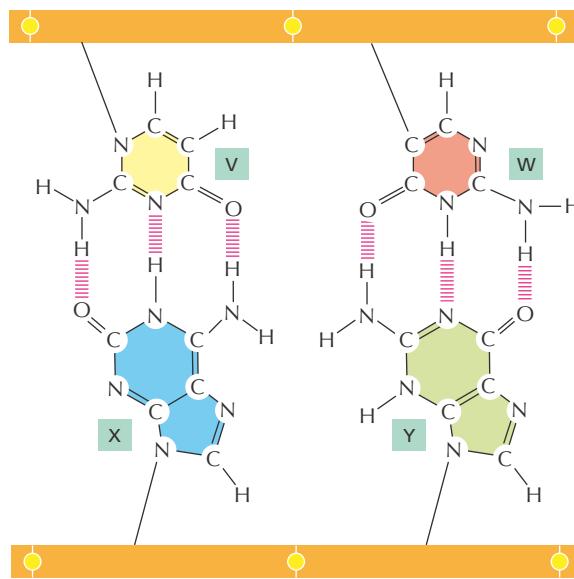


Figure Q5–7

B. Simply judged by their potential for hydrogen-bonding, could any of these extraterrestrial bases replace terrestrial A, T, G, or C in terrestrial DNA? Explain your answer.

QUESTION 5–8

The two strands of a DNA double helix can be separated by heating. If you raised the temperature of a solution containing the following three DNA molecules, in what order do you suppose they would “melt”? Explain your answer.

- A. 5'-GCGGGCCAGCCGAGTTGGTAGGCCAGG-3'
3'-CGCCCGGTCTGGGCTCACCCATCGGGTCC-5'
- B. 5'-ATTATAAAATATTTAGATACTATATTACAA-3'
3'-TAATATTTATAAATCTATGATATAAATGTT-5'
- C. 5'-AGAGCTAGATCGAT-3'
3'-TCTCGATCTAGCTA-5'

QUESTION 5–9

The total length of DNA in the human genome is about 1 m, and the diameter of the double helix is about 2 nm. Nucleotides in a DNA double helix are stacked (see

Figure 5–6B) at an interval of 0.34 nm. If the DNA were enlarged so that its diameter equaled that of an electrical extension cord (5 mm), how long would the extension cord be from one end to the other (assuming that it is completely stretched out)? How close would the bases be to each other? How long would a gene of 1000 nucleotide pairs be?

QUESTION 5–10

A compact disc (CD) stores about 4.8×10^9 bits of information in a 96 cm^2 area. This information is stored as a binary code—that is, every bit is either a 0 or a 1.

- A. How many bits would it take to specify each nucleotide pair in a DNA sequence?
- B. How many CDs would it take to store the information contained in the human genome?

QUESTION 5–11

Which of the following statements are correct? Explain your answers.

- A. Each eukaryotic chromosome must contain the following DNA sequence elements: multiple origins of replication, two telomeres, and one centromere.
- B. Nucleosome core particles are 30 nm in diameter.

QUESTION 5–12

Define the following terms and their relationships to one another:

- A. Interphase chromosome
- B. Mitotic chromosome
- C. Chromatin
- D. Heterochromatin
- E. Histones
- F. Nucleosome

QUESTION 5–13

Carefully consider the result shown in Figure Q5–13. Each of the two colonies shown on the left is a clump of approximately 100,000 yeast cells that has grown up from a single cell, which is now somewhere in the middle of the colony. The two yeast colonies are genetically different, as shown by the chromosomal maps on the right.

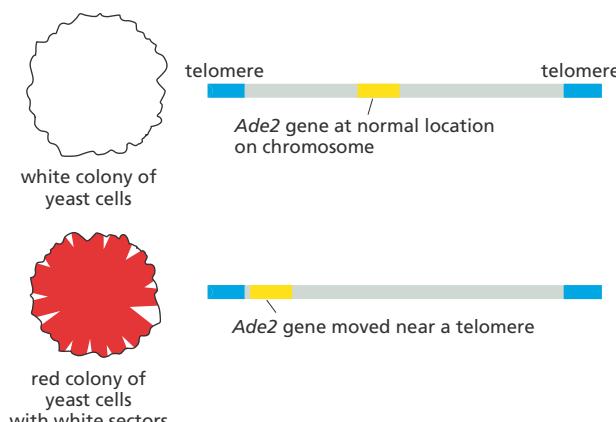
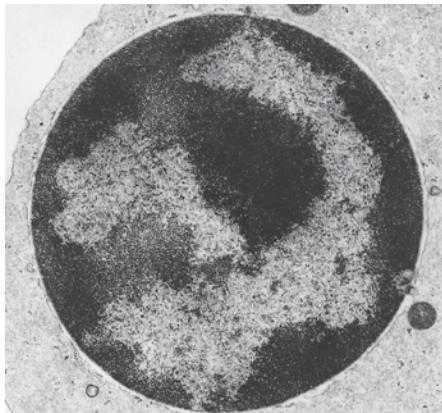


Figure Q5–13

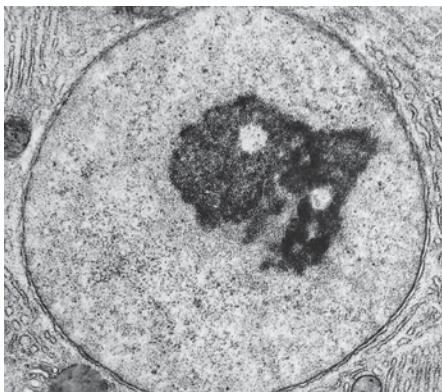
The yeast *Ade2* gene encodes one of the enzymes required for adenine biosynthesis, and the absence of the *Ade2* gene product leads to the accumulation of a red pigment. At its normal chromosome location, *Ade2* is expressed in all cells. When it is positioned near the telomere, which is highly condensed, *Ade2* is no longer expressed. How do you think the white sectors arise? What can you conclude about the propagation of the transcriptional state of the *Ade2* gene from mother to daughter cells?

QUESTION 5–14

The two electron micrographs in **Figure Q5–14** show nuclei of two different cell types. Can you tell from these pictures which of the two cells is transcribing more of its genes? Explain how you arrived at your answer. (Micrographs courtesy of Don W. Fawcett.)



(A)



(B)

Figure Q5–14

QUESTION 5–15

DNA forms a right-handed helix. Pick out the right-handed helix from those shown in **Figure Q5–15**.

**Figure Q5–15**

QUESTION 5–16

A single nucleosome core particle is 11 nm in diameter and contains 147 bp of DNA (the DNA double helix measures 0.34 nm/bp). What packing ratio (ratio of DNA length to nucleosome diameter) has been achieved by wrapping DNA around the histone octamer? Assuming that there are an additional 54 bp of extended DNA in the linker between nucleosomes, how condensed is “beads-on-a-string” DNA relative to fully extended DNA? What fraction of the 10,000-fold condensation that occurs at mitosis does this first level of packing represent?

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CHAPTER SIX

6

DNA Replication, Repair, and Recombination

The ability of a cell to survive and proliferate in a chaotic environment depends on the accurate duplication of the vast quantity of genetic information carried in its DNA. This duplication process, called *DNA replication*, must occur before a cell can divide to produce two genetically identical daughter cells. Maintaining order in a cell also requires the continual surveillance and repair of its genetic information, as DNA is subjected to unavoidable damage by chemicals and radiation in the environment and by reactive molecules that are generated inside the cell. In this chapter, we describe the protein machines that replicate and repair the cell's DNA. These machines catalyze some of the most rapid and accurate processes that take place within cells, and the strategies they have evolved to achieve this feat are marvels of elegance and efficiency.

Despite these systems for protecting a cell's DNA from copying errors and accidental damage, permanent changes—or *mutations*—sometimes do occur. Although most mutations do not affect the organism in any noticeable way, some have profound consequences. Occasionally, these changes can benefit the organism: for example, mutations can make bacteria resistant to antibiotics that are used to kill them. What is more, changes in DNA sequence can produce small variations that underlie the differences between individuals of the same species (**Figure 6–1**); when allowed to accumulate over millions of years, such changes provide the variety in genetic material that makes one species distinct from another, as we discuss in Chapter 9.

But, mutations are much more likely to be detrimental than beneficial: in humans, they are responsible for thousands of genetic diseases, including cancer. The survival of a cell or organism, therefore, depends on keeping

DNA REPLICATION

DNA REPAIR



Figure 6–1 Genetic information is passed from one generation to the next. Differences in DNA can produce the variations that underlie the differences between individuals of the same species—or, over time, the differences between one species and another. In this family photo, the children resemble one another and their parents more closely than they resemble other people because they inherit their genes from their parents. The cat shares many features with humans, but during the millions of years of evolution that have separated humans and cats, both have accumulated many changes in DNA that now make the two species different. The chicken is an even more distant relative.

changes in its DNA to a minimum. Without the protein machines that are continually monitoring and repairing damage to DNA, it is questionable whether life could exist at all.

DNA REPLICATION

At each cell division, a cell must copy its genome with extraordinary accuracy. In this section, we explore how the cell achieves this feat, while duplicating its DNA at rates as high as 1000 nucleotides per second.

Base-Pairing Enables DNA Replication

In the preceding chapter, we saw that each strand of a DNA double helix contains a sequence of nucleotides that is exactly complementary to the nucleotide sequence of its partner strand. Each strand can therefore serve as a **template**, or mold, for the synthesis of a new complementary strand. In other words, if we designate the two DNA strands as S and S', strand S can serve as a template for making a new strand S', while strand S' can serve as a template for making a new strand S (**Figure 6–2**). Thus, the genetic information in DNA can be accurately copied by the beautifully simple process in which strand S separates from strand S', and each separated strand then serves as a template for the production of a new complementary partner strand that is identical to its former partner.

The ability of each strand of a DNA molecule to act as a template for producing a complementary strand enables a cell to copy, or *replicate*, its genes before passing them on to its descendants. But the task is awe-inspiring, as it can involve copying billions of nucleotide pairs every time a cell divides. The copying must be carried out with incredible speed and accuracy: in about 8 hours, a dividing animal cell will copy the equivalent of 1000 books like this one and, on average, get no more than a few letters wrong. This impressive feat is performed by a cluster of proteins that together form a *replication machine*.

Figure 6–2 DNA acts as a template for its own duplication. Because the nucleotide A will successfully pair only with T, and G with C, each strand of a DNA double helix—labeled here as the S strand and its complementary S' strand—can serve as a template to specify the sequence of nucleotides in its complementary strand. In this way, both strands of a DNA double helix can be copied precisely.

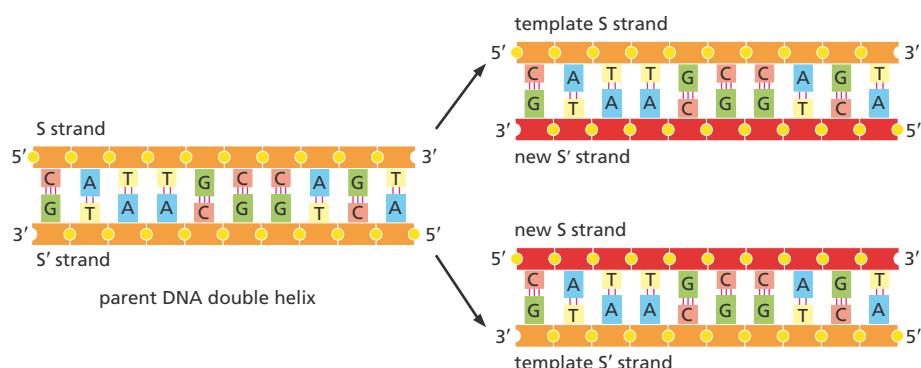


Figure 6–3 In each round of DNA replication, each of the two strands of DNA is used as a template for the formation of a new, complementary strand. DNA replication is “semiconservative” because each daughter DNA double helix is composed of one conserved strand and one newly synthesized strand.

DNA replication produces two complete double helices from the original DNA molecule, with each new DNA helix being identical (except for rare copying errors) in nucleotide sequence to the original DNA double helix (see Figure 6–2). Because each parental strand serves as the template for one new strand, each of the daughter DNA double helices ends up with one of the original (old) strands plus one strand that is completely new; this style of replication is said to be **semiconservative** (Figure 6–3). In **How We Know**, pp. 200–202, we discuss the experiments that first demonstrated that DNA is replicated in this way.

DNA Synthesis Begins at Replication Origins

The DNA double helix is normally very stable: the two DNA strands are locked together firmly by the large numbers of hydrogen bonds between the bases on both strands (see Figure 5–2). As a result, only temperatures approaching those of boiling water provide enough thermal energy to separate the two strands. To be used as a template, however, the double helix must first be opened up and the two strands separated to expose unpaired bases. How does this occur at the temperatures found in living cells?

The process of DNA synthesis is begun by *initiator proteins* that bind to specific DNA sequences called **replication origins**. Here, the initiator proteins pry the two DNA strands apart, breaking the hydrogen bonds between the bases (Figure 6–4). Although the hydrogen bonds collectively make the DNA helix very stable, individually each hydrogen bond is weak (as discussed in Chapter 2). Separating a short length of DNA a few base pairs at a time therefore does not require a large energy input, and the initiator proteins can readily unzip the double helix at normal temperatures.

In simple cells such as bacteria or yeast, replication origins span approximately 100 nucleotide pairs. They are composed of DNA sequences that attract the initiator proteins and are especially easy to open. We saw in Chapter 5 that an A-T base pair is held together by fewer hydrogen bonds than is a G-C base pair. Therefore, DNA rich in A-T base pairs is relatively easy to pull apart, and A-T-rich stretches of DNA are typically found at replication origins.

A bacterial genome, which is typically contained in a circular DNA molecule of several million nucleotide pairs, has a single replication origin. The human genome, which is very much larger, has approximately 10,000 such origins—an average of 220 origins per chromosome. Beginning DNA replication at many places at once greatly shortens the time a cell needs to copy its entire genome.

Once an initiator protein binds to DNA at a replication origin and locally opens up the double helix, it attracts a group of proteins that carry out DNA replication. These proteins form a replication machine, in which each protein carries out a specific function.

Two Replication Forks Form at Each Replication Origin

DNA molecules in the process of being replicated contain Y-shaped junctions called **replication forks**. Two replication forks are formed at

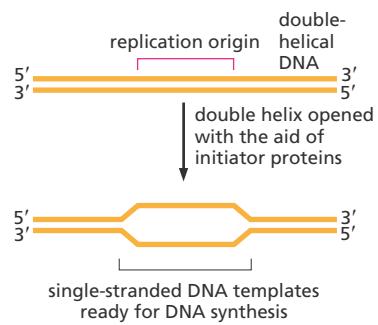
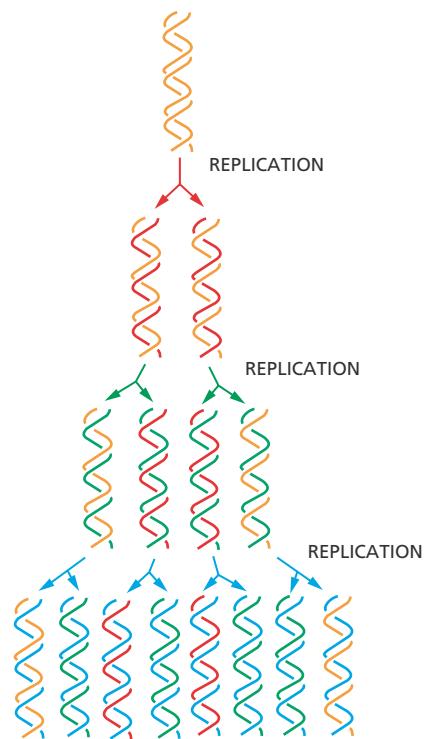


Figure 6–4 A DNA double helix is opened at replication origins. DNA sequences at replication origins are recognized by initiator proteins (not shown), which locally pry apart the two strands of the double helix. The exposed single strands can then serve as templates for copying the DNA.

THE NATURE OF REPLICATION

In 1953, James Watson and Francis Crick published their famous two-page paper describing a model for the structure of DNA (see Figure 5–2). In it, they proposed that complementary bases—adenine and thymine, guanine and cytosine—pair with one another along the center of the double helix, holding together the two strands of DNA. At the very end of this succinct scientific blockbuster, they comment, almost as an aside, “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

Indeed, one month after the classic paper appeared in print in the journal *Nature*, Watson and Crick published a second article, suggesting how DNA might be duplicated. In this paper, they proposed that the two strands of the double helix unwind, and that each strand serves as a template for the synthesis of a complementary daughter strand. In their model, dubbed semiconservative replication, each new DNA molecule consists of one strand derived from the original parent molecule and one newly synthesized strand (Figure 6–5A).

We now know that Watson and Crick’s model for DNA replication was correct—but it was not universally accepted at first. Respected physicist-turned-geneticist Max Delbrück, for one, got hung up on what he termed “the untwiddling problem;” that is: how could the two strands of a double helix, twisted around each other

so many times all along their great length, possibly be unwound without making a big tangled mess? Watson and Crick’s conception of the DNA helix opening up like a zipper seemed, to Delbrück, physically unlikely and simply “too inelegant to be efficient.”

Instead, Delbrück proposed that DNA replication proceeds through a series of breaks and reunions, in which the DNA backbone is broken and the strands are copied in short segments—perhaps only 10 nucleotides at a time—before being rejoined. In this model, which was later dubbed dispersive, the resulting copies would be patchwork collections of old and new DNA, each strand containing a mixture of both (Figure 6–5B). No unwinding was necessary.

Yet a third camp promoted the idea that DNA replication might be *conservative*: that the parent helix would somehow remain entirely intact after copying, and the daughter molecule would contain two entirely new DNA strands (Figure 6–5C). To determine which of these models was correct, an experiment was needed—one that would reveal the composition of the newly synthesized DNA strands. That’s where Matt Meselson and Frank Stahl came in.

As a graduate student working with Linus Pauling, Meselson was toying with a method for telling the difference between old and new proteins. After chatting with Delbrück about Watson and Crick’s replication model, it

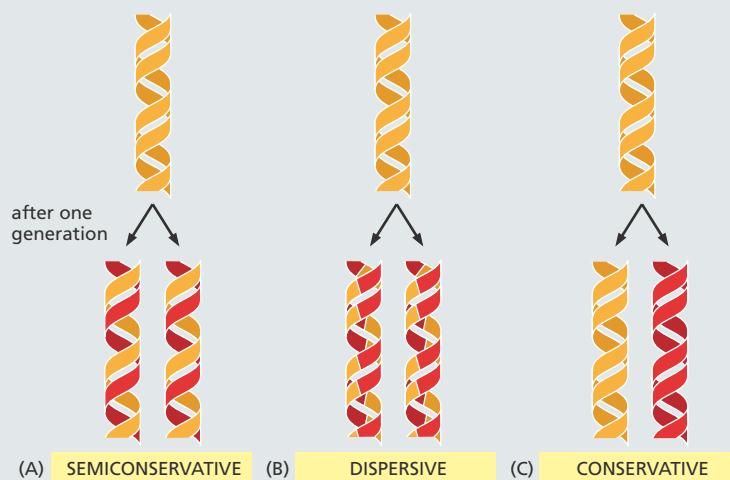


Figure 6–5 Three models for DNA replication make different predictions. (A) In the semiconservative model, each parent strand serves as a template for the synthesis of a new daughter strand. The first round of replication would produce two hybrid molecules, each containing one strand from the original parent in addition to one newly synthesized strand. A subsequent round of replication would yield two hybrid molecules and two molecules that contain none of the original parent DNA (see Figure 6–3). (B) In the dispersive model, each generation of daughter DNA will contain a mixture of DNA from the parent strands and the newly synthesized DNA. (C) In the conservative model, the parent molecule remains intact after being copied. In this case, the first round of replication would yield the original parent double helix and an entirely new double helix. For each model, parent DNA molecules are shown in orange; newly replicated DNA is red. Note that only a very small segment of DNA is shown for each model.

occurred to Meselson that the approach he'd envisaged for exploring protein synthesis might also work for studying DNA. In the summer of 1954, Meselson met Stahl, who was then a graduate student in Rochester, NY, and they agreed to collaborate. It took a few years to get everything working, but the two eventually performed what has come to be known as "the most beautiful experiment in biology."

Their approach, in retrospect, was stunningly straightforward. They started by growing two batches of *E. coli* bacteria, one in a medium containing a heavy isotope of nitrogen, ^{15}N , the other in a medium containing the normal, lighter ^{14}N . The nitrogen in the nutrient medium gets incorporated into the nucleotide bases and, from there, makes its way into the DNA of the organism. After growing bacterial cultures for many generations in either the ^{15}N - or ^{14}N -containing medium, the researchers had two flasks of bacteria, one whose DNA was heavy, the other whose DNA was light. Meselson and Stahl then broke open the bacterial cells and loaded the DNA into tubes containing a high concentration of the salt cesium chloride. When these tubes are centrifuged at high speed, the cesium chloride forms a density gradient, and the DNA molecules float or sink within the solution until they reach the point at which their density equals that of the surrounding salt solution (see Panel 4–3, pp. 164–165). Using this method, called equilibrium

density centrifugation, Meselson and Stahl found that they could distinguish between heavy (^{15}N -containing) DNA and light (^{14}N -containing) DNA by observing the positions of the DNA within the cesium chloride gradient. Because the heavy DNA was denser than the light DNA, it collected at a position nearer to the bottom of the centrifuge tube (Figure 6–6).

Once they had established this method for differentiating between light and heavy DNA, Meselson and Stahl set out to test the various hypotheses proposed for DNA replication. To do this, they took a flask of bacteria that had been grown in heavy nitrogen and transferred the bacteria into a medium containing the light isotope. At the start of the experiment, all the DNA would be heavy. But, as the bacteria divided, the newly synthesized DNA would be light. They could then monitor the accumulation of light DNA and see which model, if any, best fit the data. After one generation of growth, the researchers found that the parental, heavy DNA molecules—those made of two strands containing ^{15}N —had disappeared and were replaced by a new species of DNA that banded at a density halfway between those of ^{15}N -DNA and ^{14}N -DNA (Figure 6–7). These newly synthesized daughter helices, Meselson and Stahl reasoned, must be hybrids—containing both heavy and light isotopes.

Right away, this observation ruled out the conservative model of DNA replication, which predicted that

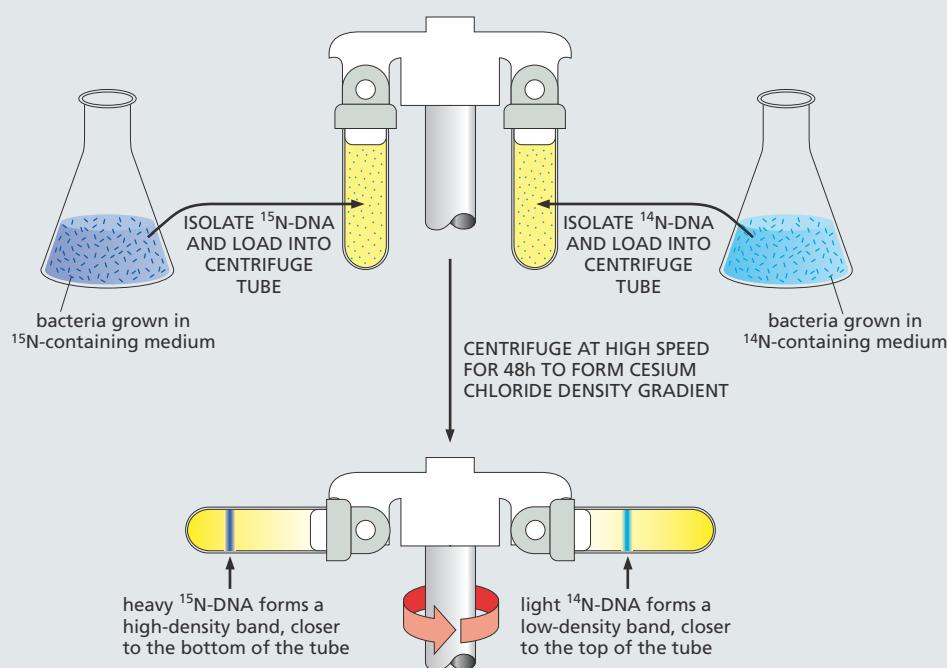


Figure 6–6 Centrifugation in a cesium chloride gradient allows the separation of heavy and light DNA.

Bacteria are grown for several generations in a medium containing either ^{15}N (the heavy isotope) or ^{14}N (the light isotope) to label their DNA. The cells are then broken open, and the DNA is loaded into an ultracentrifuge tube containing a cesium chloride salt solution. These tubes are centrifuged at high speed for two days to allow the DNA to collect in a region where its density matches that of the salt surrounding it. The heavy and light DNA molecules collect in different positions in the tube.

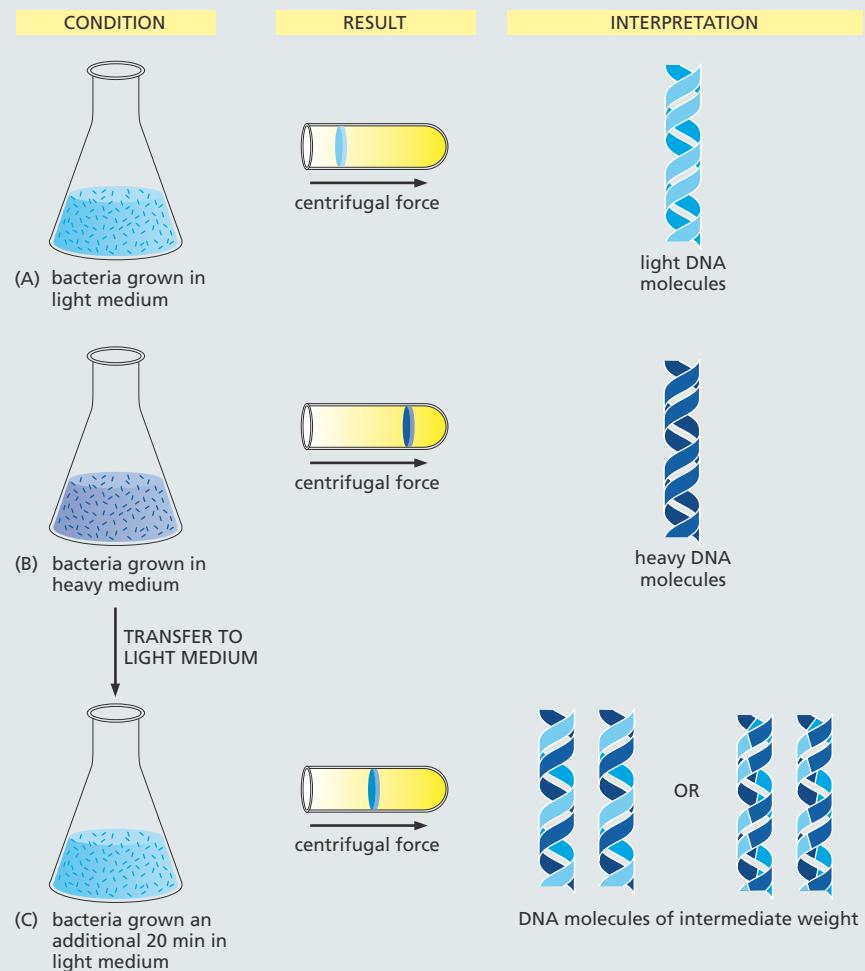


Figure 6–7 The first part of the Meselson–Stahl experiment ruled out the conservative model of DNA replication. (A) Bacteria grown in light medium (containing ^{14}N) yield DNA that forms a band high up in the centrifuge tube, whereas bacteria grown in ^{15}N -containing heavy medium (B) produce DNA that migrates further down the tube. When bacteria grown in a heavy medium are transferred to a light medium and allowed to continue dividing, they produce a band whose position falls somewhere between that of the parent bands (C). These results rule out the conservative model of replication but do not distinguish between the semiconservative and dispersive models, both of which predict the formation of hybrid daughter DNA molecules.

The fact that the results came out looking so clean—with discrete bands forming at the expected positions for newly replicated hybrid DNA molecules—was a happy accident of the experimental protocol. The researchers used a hypodermic syringe to load their DNA samples into the ultracentrifuge tubes (see Figure 6–6). In the process, they unwittingly sheared the large bacterial chromosome into smaller fragments. Had the chromosomes remained whole, the researchers might have isolated DNA molecules that were only partially replicated, because many cells would have been caught in the middle of copying their DNA. Molecules in such an intermediate stage of replication would not have separated into such discrete bands. But because the researchers were instead working with smaller pieces of DNA, the likelihood that any given fragment had been fully replicated—and contained a complete parent and daughter strand—was high, thus yielding nice, clean results.

the parental DNA would remain entirely heavy, while the daughter DNA would be entirely light (see Figure 6–5C). The data matched with the semiconservative model, which predicted the formation of hybrid molecules containing one strand of heavy DNA and one strand of light (see Figure 6–5A). The results, however, were also consistent with the dispersive model, in which hybrid DNA strands would contain a mixture of heavy and light DNA (see Figure 6–5B).

To distinguish between the two models, Meselson and Stahl turned up the heat. When DNA is subjected to high temperature, the hydrogen bonds holding the two strands together break and the helix comes apart, leaving a collection of single-stranded DNAs. When the researchers heated their hybrid molecules before centrifuging, they discovered that one strand of the DNA was heavy, whereas the other was light. This observation supported only the semiconservative model; if the dispersive model were correct, the resulting strands, each containing a mottled assembly of heavy and light DNA, would have all banded together at an intermediate density.

According to historian Frederic Lawrence Holmes, the experiment was so elegant and the results so clean that Stahl—when being interviewed for a position at Yale University—was unable to fill the 50 minutes allotted for his talk. “I was finished in 25 minutes,” said Stahl, “because that is all it takes to tell that experiment. It’s so totally simple and contained.” Stahl did not get the job at Yale, but the experiment convinced biologists that Watson and Crick had been correct. In fact, the results were accepted so widely and rapidly that the experiment was described in a textbook before Meselson and Stahl had even published the data.

each replication origin (**Figure 6–8**). At each fork, a replication machine moves along the DNA, opening up the two strands of the double helix and using each strand as a template to make a new daughter strand. The two forks move away from the origin in opposite directions, unzipping the DNA double helix and replicating the DNA as they go (**Figure 6–9**). DNA replication in bacterial and eukaryotic chromosomes is therefore termed *bidirectional*. The forks move very rapidly—at about 1000 nucleotide pairs per second in bacteria and 100 nucleotide pairs per second in humans. The slower rate of fork movement in humans (indeed, in all eukaryotes) may be due to the difficulties in replicating DNA through the more complex chromatin structure of eukaryotic chromosomes.

DNA Polymerase Synthesizes DNA Using a Parental Strand as Template

The movement of a replication fork is driven by the action of the replication machine, at the heart of which is an enzyme called **DNA polymerase**. This enzyme catalyzes the addition of nucleotides to the 3' end of a growing DNA strand, using one of the original, parental DNA strands as a template. Base pairing between an incoming nucleotide and the template strand determines which of the four nucleotides (A, G, T, or C) will be selected. The final product is a new strand of DNA that is complementary in nucleotide sequence to the template (**Figure 6–10**).

The polymerization reaction involves the formation of a phosphodiester bond between the 3' end of the growing DNA chain and the 5'-phosphate group of the incoming nucleotide, which enters the reaction as a *deoxyribonucleoside triphosphate*. The energy for polymerization is provided

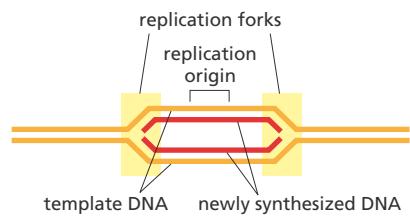


Figure 6–8 DNA synthesis occurs at Y-shaped junctions called **replication forks**. Two replication forks are formed at each replication origin.

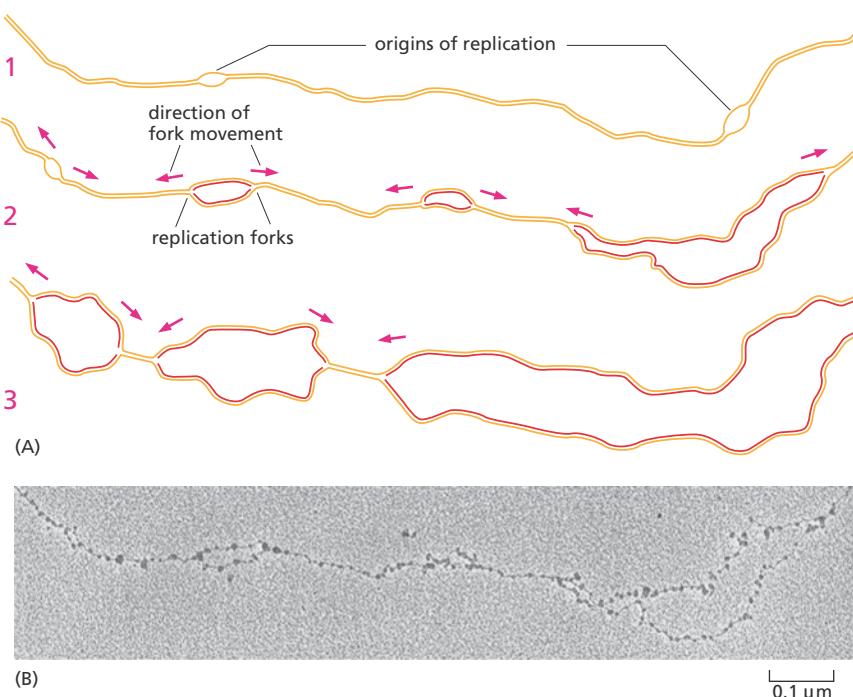


Figure 6–9 The two replication forks move away in opposite directions at each replication origin. (A) These drawings represent the same portion of a DNA molecule as it might appear at different times during replication. The orange lines represent the two parental DNA strands; the red lines represent the newly synthesized DNA strands. (B) An electron micrograph showing DNA replicating in an early fly embryo. The particles visible along the DNA are nucleosomes, structures made of DNA and the protein complexes around which the DNA is wrapped (discussed in Chapter 5). The chromosome in this micrograph is the one that was redrawn in sketch (2) above. (Electron micrograph courtesy of Victoria Foe.)

QUESTION 6–1

Look carefully at the micrograph and drawing 2 in Figure 6–9.

- A. Using the scale bar, estimate the lengths of the DNA strands between the replication forks. Numbering the replication forks sequentially from the left, how long will it take until forks 4 and 5, and forks 7 and 8, respectively, collide with each other? (Recall that the distance between the bases in DNA is 0.34 nm, and eukaryotic replication forks move at about 100 nucleotides per second.) For this question, disregard the nucleosomes seen in the micrograph and assume that the DNA is fully extended.

- B. The fly genome is about 1.8×10^8 nucleotide pairs in size. What fraction of the genome is shown in the micrograph?

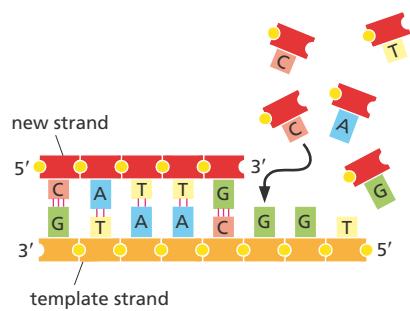


Figure 6–10 A new DNA strand is synthesized in the 5'-to-3' direction.

At each step, the appropriate incoming nucleotide is selected by forming base pairs with the next nucleotide in the template strand: A with T, T with A, C with G, and G with C. Each is added to the 3' end of the growing new strand, as indicated.

by the incoming deoxyribonucleoside triphosphate itself: hydrolysis of one of its high-energy phosphate bonds fuels the reaction that links the nucleotide monomer to the chain, releasing pyrophosphate (**Figure 6–11**). Pyrophosphate is further hydrolyzed to inorganic phosphate (P_i), which makes the polymerization reaction effectively irreversible (see Figure 3–41).

DNA polymerase does not dissociate from the DNA each time it adds a new nucleotide to the growing strand; rather, it stays associated with the DNA and moves along the template strand stepwise for many cycles of the polymerization reaction (**Movie 6.1**). We will see later that a special protein keeps the polymerase attached to the DNA, as it repeatedly adds new nucleotides to the growing strand.

The Replication Fork Is Asymmetrical

The 5'-to-3' direction of the DNA polymerization reaction poses a problem at the replication fork. As illustrated in Figure 5–2, the sugar-phosphate backbone of each strand of a DNA double helix has a unique chemical direction, or polarity, determined by the way each sugar residue is linked to the next, and the two strands in the double helix are antiparallel; that is, they run in opposite directions. As a consequence, at each replication fork, one new DNA strand is being made on a template that runs in one direction (3' to 5'), whereas the other new strand is being made on a template that runs in the opposite direction (5' to 3') (**Figure 6–12**). The replication fork is therefore asymmetrical. Looking at Figure 6–9A, however, it appears that both of the new DNA strands are growing in the same direction; that is, the direction in which the replication fork is moving. That observation suggests that one strand is being synthesized in the 5'-to-3' direction and the other in the 3'-to-5' direction.

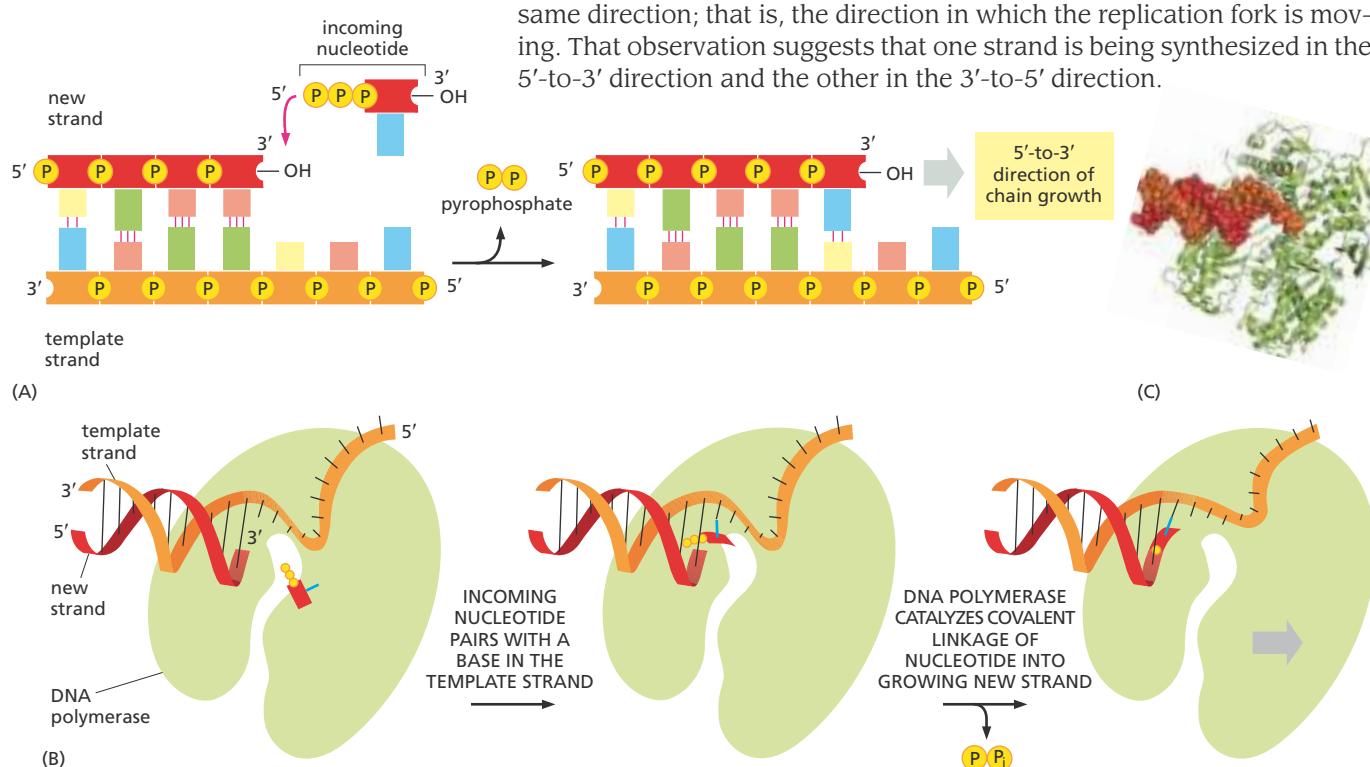


Figure 6–11 DNA polymerase adds a deoxyribonucleotide to the 3' end of a growing DNA chain. (A) Nucleotides enter the reaction as deoxyribonucleoside triphosphates. This incoming nucleotide forms a base pair with its partner in the template strand. It is then linked to the free 3' hydroxyl on the growing DNA strand. The new DNA strand is therefore synthesized in the 5'-to-3' direction. Breakage of a high-energy phosphate bond in the incoming nucleoside triphosphate—accompanied by the release of pyrophosphate—provides the energy for the polymerization reaction. (B) The reaction is catalyzed by the enzyme DNA polymerase (light green). The polymerase guides the incoming nucleotide to the template strand and positions it such that its 5' terminal phosphate will be able to react with the 3'-hydroxyl group on the newly synthesized strand. The gray arrow indicates the direction of polymerase movement. (C) Structure of DNA polymerase, as determined by X-ray crystallography, which shows the positioning of the DNA double helix. The template strand is the longer of the two DNA strands (**Movie 6.1**).

Does the cell have two types of DNA polymerase, one for each direction? The answer is no: all DNA polymerases add new subunits only to the 3' end of a DNA strand (see Figure 6–11A). As a result, a new DNA chain can be synthesized only in a 5'-to-3' direction. This can easily account for the synthesis of one of the two strands of DNA at the replication fork, but what happens on the other? This conundrum is solved by the use of a “backstitching” maneuver. The DNA strand that appears to grow in the incorrect 3'-to-5' direction is actually made *discontinuously*, in successive, separate, small pieces—with the DNA polymerase moving backward with respect to the direction of replication-fork movement so that each new DNA fragment can be polymerized in the 5'-to-3' direction.

The resulting small DNA pieces—called **Okazaki fragments** after the biochemists who discovered them—are later joined together to form a continuous new strand. The DNA strand that is made discontinuously in this way is called the **lagging strand**, because the backstitching imparts a slight delay to its synthesis; the other strand, which is synthesized continuously, is called the **leading strand** (Figure 6–13).

Although they differ in subtle details, the replication forks of all cells, prokaryotic and eukaryotic, have leading and lagging strands. This common feature arises from the fact that all DNA polymerases work only in the 5'-to-3' direction—a restriction that provides cells with an important advantage, as we discuss next.

DNA Polymerase Is Self-correcting

DNA polymerase is so accurate that it makes only about one error in every 10^7 nucleotide pairs it copies. This error rate is much lower than can be explained simply by the accuracy of complementary base-pairing. Although A-T and C-G are by far the most stable base pairs, other, less stable base pairs—for example, G-T and C-A—can also be formed. Such incorrect base pairs are formed much less frequently than correct ones, but, if allowed to remain, they would result in an accumulation of mutations. This disaster is avoided because DNA polymerase has two special qualities that greatly increase the accuracy of DNA replication. First, the enzyme carefully monitors the base-pairing between each incoming nucleotide and the template strand. Only when the match is correct does DNA polymerase catalyze the nucleotide-addition reaction. Second,

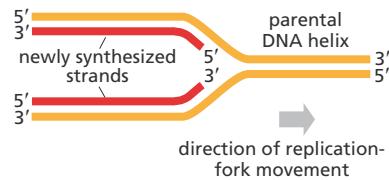


Figure 6–12 At a replication fork, the two newly synthesized DNA strands are of opposite polarities. This is because the two template strands are oriented in opposite directions.

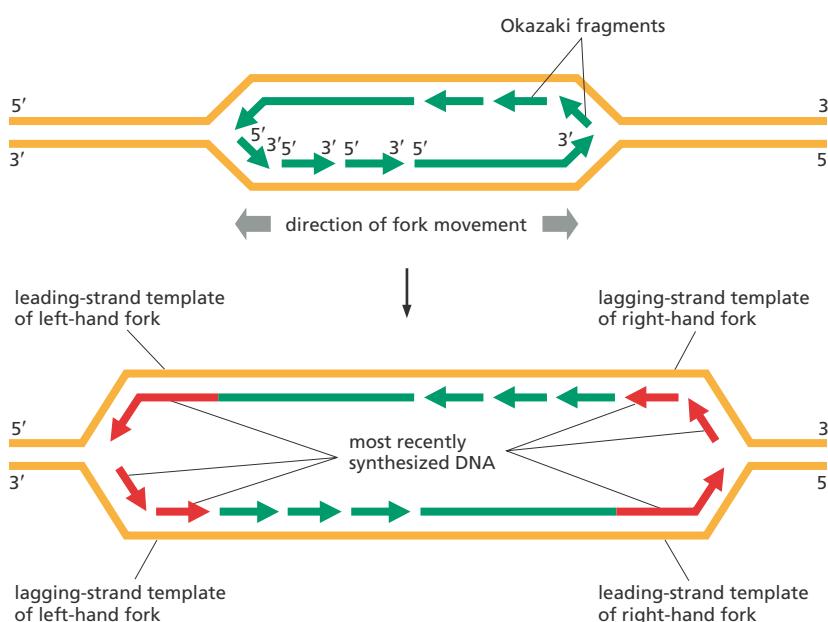


Figure 6–13 At each replication fork, the lagging DNA strand is synthesized in pieces. Because both of the new strands at a replication fork are synthesized in the 5'-to-3' direction, the lagging strand of DNA must be made initially as a series of short DNA strands, which are later joined together. The upper diagram shows two replication forks moving in opposite directions; the lower diagram shows the same forks a short time later. To replicate the lagging strand, DNA polymerase uses a backstitching mechanism: it synthesizes short pieces of DNA (called Okazaki fragments) in the 5'-to-3' direction and then moves back along the template strand (toward the fork) before synthesizing the next fragment.

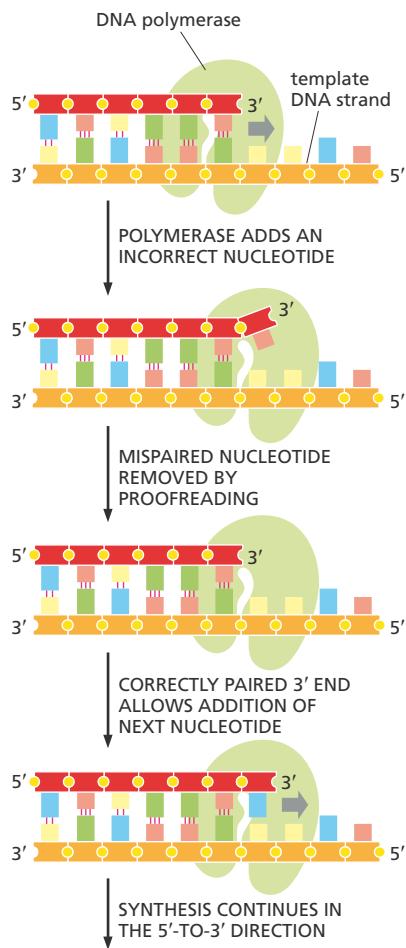


Figure 6-14 During DNA synthesis, DNA polymerase proofreads its own work. If an incorrect nucleotide is added to a growing strand, the DNA polymerase cleaves it from the strand and replaces it with the correct nucleotide before continuing.

Figure 6-15 DNA polymerase contains separate sites for DNA synthesis and proofreading. The diagrams are based on the structure of an *E. coli* DNA polymerase molecule, as determined by X-ray crystallography. DNA polymerase is shown with the replicating DNA molecule and the polymerase in the polymerizing mode (left) and in the proofreading mode (right). The catalytic sites for the polymerization activity (P) and error-correcting proofreading activity (E) are indicated. When the polymerase adds an incorrect nucleotide, the newly synthesized DNA strand (red) transiently unpairs from the template strand (orange), and its growing 3' end moves into the error-correcting catalytic site (E) to be removed.

when DNA polymerase makes a rare mistake and adds the wrong nucleotide, it can correct the error through an activity called **proofreading**.

Proofreading takes place at the same time as DNA synthesis. Before the enzyme adds the next nucleotide to a growing DNA strand, it checks whether the previously added nucleotide is correctly base-paired to the template strand. If so, the polymerase adds the next nucleotide; if not, the polymerase clips off the mispaired nucleotide and tries again (Figure 6-14). This proofreading is carried out by a nuclease that cleaves the phosphodiester backbone. Polymerization and proofreading are tightly coordinated, and the two reactions are carried out by different catalytic domains in the same polymerase molecule (Figure 6-15).

This proofreading mechanism explains why DNA polymerases synthesize DNA only in the 5'-to-3' direction, despite the need that this imposes for a cumbersome backstitching mechanism at the replication fork (see Figure 6-13). A hypothetical DNA polymerase that synthesized in the 3'-to-5' direction (and would thereby circumvent the need for backstitching) would be unable to proofread: if it removed an incorrectly paired nucleotide, the polymerase would create a chemical dead end—a chain that could no longer be elongated. Thus, for a DNA polymerase to function as a self-correcting enzyme that removes its own polymerization errors as it moves along the DNA, it must proceed only in the 5'-to-3' direction.

Short Lengths of RNA Act as Primers for DNA Synthesis

We have seen that the accuracy of DNA replication depends on the requirement of the DNA polymerase for a correctly base-paired 3' end before it can add more nucleotides to a growing DNA strand. How then can the polymerase begin a completely new DNA strand? To get the process started, a different enzyme is needed—one that can begin a new polynucleotide strand simply by joining two nucleotides together without the need for a base-paired end. This enzyme does not, however, synthesize DNA. It makes a short length of a closely related type of nucleic acid—**RNA (ribonucleic acid)**—using the DNA strand as a template. This short length of RNA, about 10 nucleotides long, is base-paired to the template strand and provides a base-paired 3' end as a starting point for DNA polymerase. It thus serves as a *primer* for DNA synthesis, and the enzyme that synthesizes the RNA primer is known as *primase*.

Primase is an example of an *RNA polymerase*, an enzyme that synthesizes RNA using DNA as a template. A strand of RNA is very similar chemically to a single strand of DNA except that it is made of ribonucleotide subunits, in which the sugar is ribose, not deoxyribose; RNA also differs from DNA in that it contains the base uracil (U) instead of thymine (T) (see Panel 2-6, pp. 76–77). However, because U can form a base pair with A, the RNA primer is synthesized on the DNA strand by complementary base-pairing in exactly the same way as is DNA (Figure 6-16).

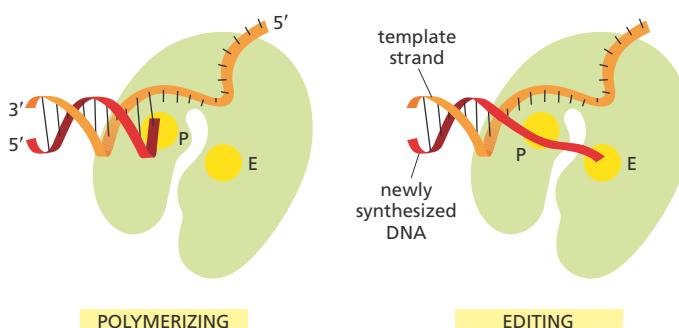


Figure 6–16 RNA primers are synthesized by an RNA polymerase called primase, which uses a DNA strand as a template. Like DNA polymerase, primase works in the 5'-to-3' direction. Unlike DNA polymerase, however, primase can start a new polynucleotide chain by joining together two nucleoside triphosphates without the need for a base-paired 3' end as a starting point. (In this case, ribonucleoside triphosphates, rather than deoxyribonucleoside triphosphates, provide the incoming nucleotides.)

For the leading strand, an RNA primer is needed only to start replication at a replication origin; once a replication fork has been established, the DNA polymerase is continuously presented with a base-paired 3' end as it tracks along the template strand. But on the lagging strand, where DNA synthesis is discontinuous, new primers are needed to keep polymerization going (see Figure 6–13). The movement of the replication fork continually exposes unpaired bases on the lagging strand template, and new RNA primers are laid down at intervals along the newly exposed, single-stranded stretch. DNA polymerase adds a deoxyribonucleotide to the 3' end of each primer to start a new Okazaki fragment, and it will continue to elongate this fragment until it runs into the next RNA primer (Figure 6–17).

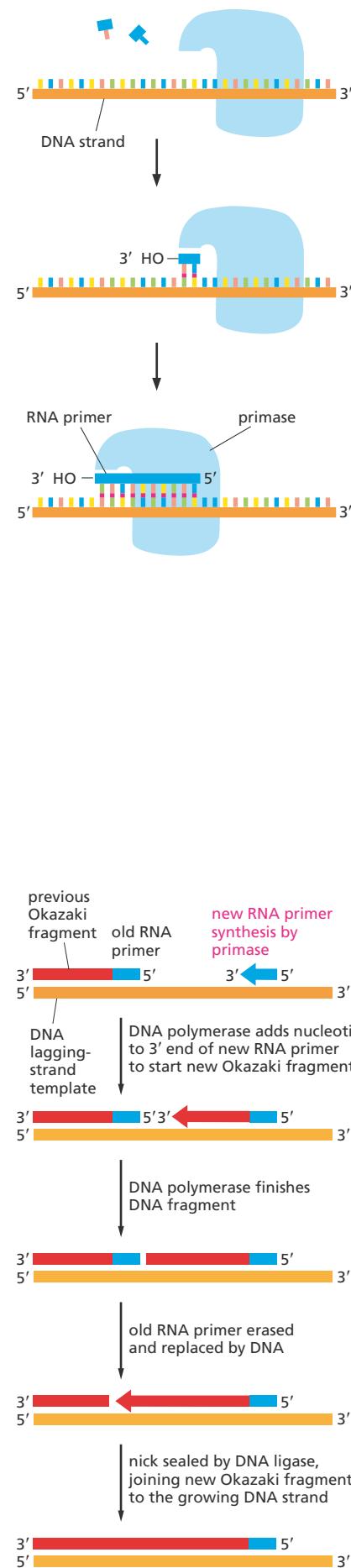
To produce a continuous new DNA strand from the many separate pieces of nucleic acid made on the lagging strand, three additional enzymes are needed. These act quickly to remove the RNA primer, replace it with DNA, and join the DNA fragments together. Thus, a nuclease degrades the RNA primer, a DNA polymerase called a *repair polymerase* then replaces this RNA with DNA (using the end of the adjacent Okazaki fragment as a primer), and the enzyme *DNA ligase* joins the 5'-phosphate end of one DNA fragment to the adjacent 3'-hydroxyl end of the next (Figure 6–18).

Primase can begin new polynucleotide chains, but this activity is possible because the enzyme does not proofread its work. As a result, primers frequently contain mistakes. But because primers are made of RNA instead of DNA, they stand out as “suspect copy” to be automatically removed and replaced by DNA. The repair DNA polymerases that make this DNA, like the replicative polymerases, proofread as they synthesize. In this way, the cell’s replication machinery is able to begin new DNA chains and, at the same time, ensure that all of the DNA is copied faithfully.

Proteins at a Replication Fork Cooperate to Form a Replication Machine

DNA replication requires the cooperation of a large number of proteins that act in concert to open up the double helix and synthesize new DNA. These proteins form part of a remarkably complex replication machine. The first problem faced by the replication machine is accessing the

Figure 6–17 Multiple enzymes are required to synthesize Okazaki fragments on the lagging DNA strand. In eukaryotes, RNA primers are made at intervals of about 200 nucleotides on the lagging strand, and each RNA primer is approximately 10 nucleotides long. Primers are removed by nucleases that recognize an RNA strand in an RNA/DNA helix and degrade it; this leaves gaps that are filled in by a repair DNA polymerase that can proofread as it fills in the gaps. The completed fragments are finally joined together by an enzyme called DNA ligase, which catalyzes the formation of a phosphodiester bond between the 3'-OH end of one fragment and the 5'-phosphate end of the next, thus linking up the sugar-phosphate backbones. This nick-sealing reaction requires an input of energy in the form of ATP (not shown; see Figure 6–18).



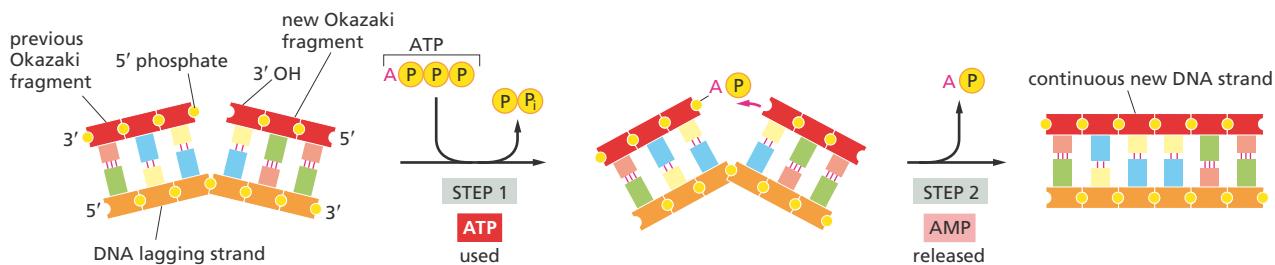


Figure 6–18 DNA ligase joins together Okazaki fragments on the lagging strand during DNA synthesis. The ligase enzyme uses a molecule of ATP to activate the 5' end of one fragment (step 1) before forming a new bond with the 3' end of the other fragment (step 2).

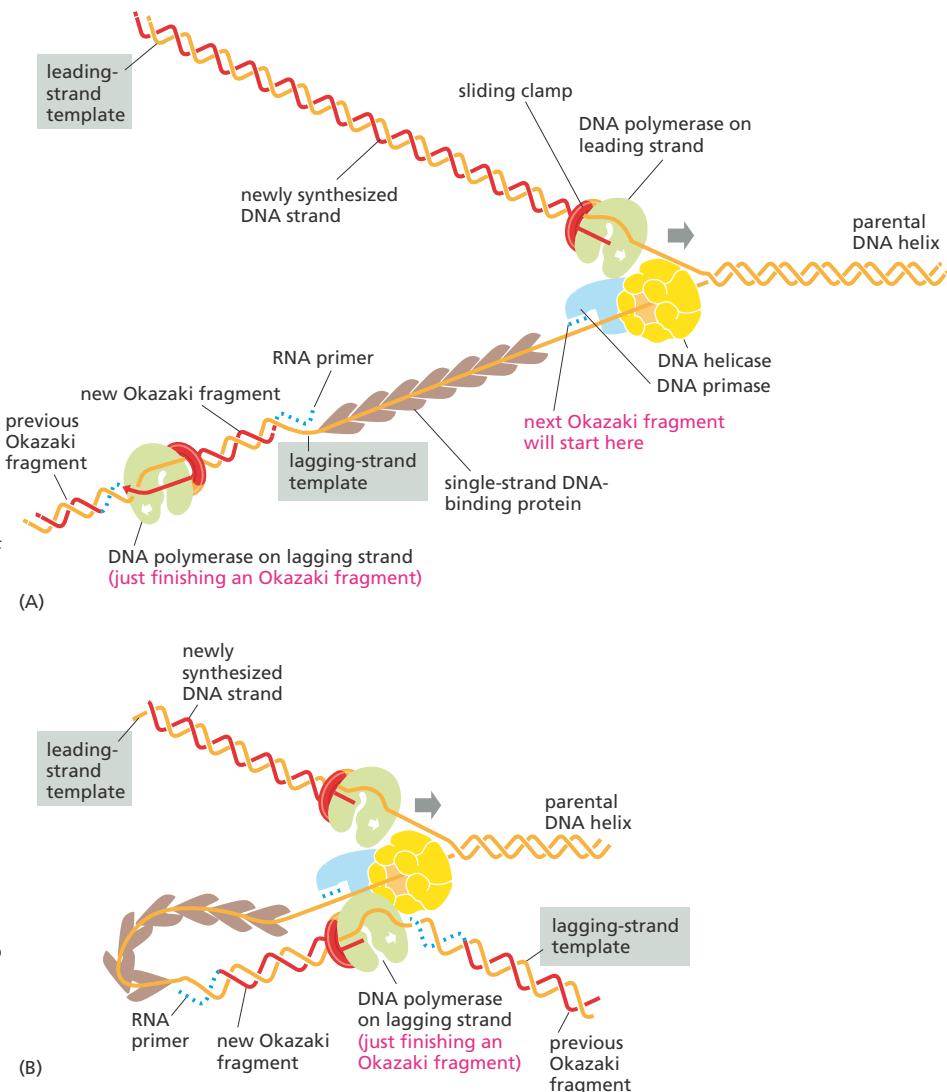
Figure 6–19 DNA synthesis is carried out by a group of proteins that act together as a replication machine.

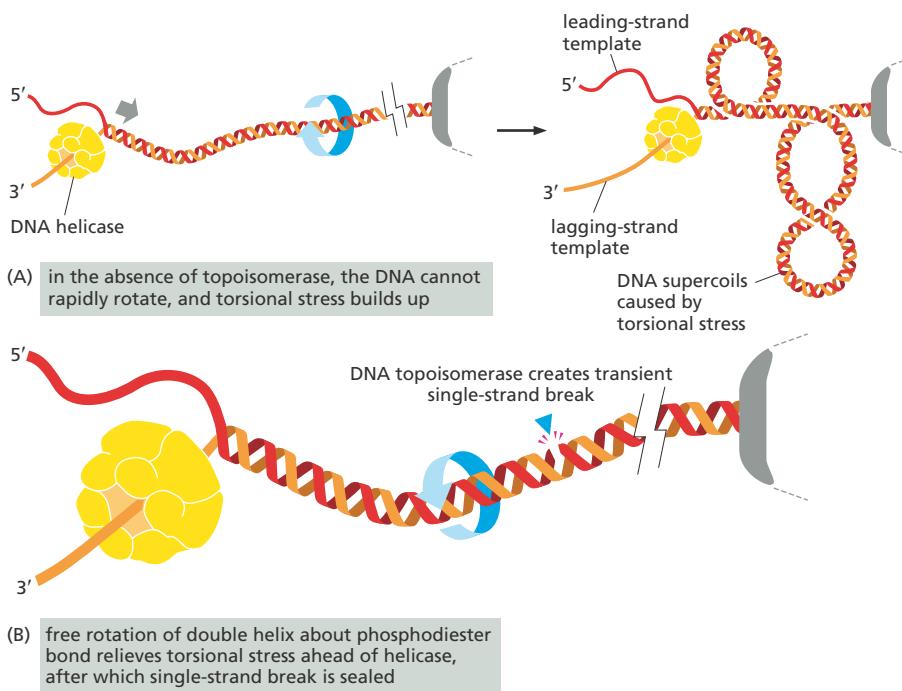
(A) DNA polymerases are held on the leading and lagging strands by circular protein clamps that allow the polymerases to slide. On the lagging-strand template, the clamp detaches each time the polymerase completes an Okazaki fragment. A clamp loader (not shown) is required to attach a sliding clamp each time a new Okazaki fragment is begun. At the head of the fork, a DNA helicase unwinds the strands of the parental DNA double helix. Single-strand DNA-binding proteins keep the DNA strands apart to provide access for the primase and polymerase. For simplicity, this diagram shows the proteins working independently; in the cell, they are held together in a large replication machine, as shown in (B).

(B) This diagram shows a current view of how the replication proteins are arranged when a replication fork is moving. To generate this structure, the lagging strand shown in (A) has been folded to bring its DNA polymerase in contact with the leading-strand DNA polymerase. This folding process also brings the 3' end of each completed Okazaki fragment close to the start site for the next Okazaki fragment. Because the lagging-strand DNA polymerase is bound to the rest of the replication proteins, it can be reused to synthesize successive Okazaki fragments; in this diagram, the lagging-strand DNA polymerase is about to let go of its completed Okazaki fragment and move to the RNA primer that is being synthesized by the nearby primase. To watch the replication complex in action, see **Movies 6.4** and **6.5**.

nucleotides that lie at the center of the helix. For DNA replication to occur, the double helix must be unzipped ahead of the replication fork so that the incoming nucleoside triphosphates can form base pairs with each template strand. Two types of replication proteins—DNA helicases and single-strand DNA-binding proteins—cooperate to carry out this task. The helicase sits at the very front of the replication machine where it uses the energy of ATP hydrolysis to propel itself forward, prying apart the double helix as it speeds along the DNA (**Figure 6–19A** and **Movie 6.2**). Single-strand DNA-binding proteins cling to the single-stranded DNA exposed by the helicase, transiently preventing the strands from re-forming base pairs and keeping them in an elongated form so that they can serve as efficient templates.

This localized unwinding of the DNA double helix itself presents a problem. As the helicase pries open the DNA within the replication fork, the





DNA on the other side of the fork gets wound more tightly. This excess twisting in front of the replication fork creates tension in the DNA that—if allowed to build—makes unwinding the double helix increasingly difficult and impedes the forward movement of the replication machinery (**Figure 6–20A**). Cells use proteins called *DNA topoisomerases* to relieve this tension. These enzymes produce transient nicks in the DNA backbone, which temporarily release the tension; they then reseal the nick before falling off the DNA (**Figure 6–20B**).

An additional replication protein, called a *sliding clamp*, keeps DNA polymerase firmly attached to the template while it is synthesizing new strands of DNA. Left on their own, most DNA polymerase molecules will synthesize only a short string of nucleotides before falling off the DNA template strand. The sliding clamp forms a ring around the newly formed DNA double helix and, by tightly gripping the polymerase, allows the enzyme to move along the template strand without falling off as it synthesizes new DNA (see Figure 6–19A and **Movie 6.3**).

Assembly of the clamp around DNA requires the activity of another replication protein, the *clamp loader*, which hydrolyzes ATP each time it locks a sliding clamp around a newly formed DNA double helix. This loading needs to occur only once per replication cycle on the leading strand; on the lagging strand, however, the clamp is removed and then reattached each time a new Okazaki fragment is made.

Most of the proteins involved in DNA replication are held together in a large multienzyme complex that moves as a unit along the parental DNA double helix, enabling DNA to be synthesized on both strands in a coordinated manner. This complex can be likened to a miniature sewing machine composed of protein parts and powered by nucleoside triphosphate hydrolysis (Figure 6–19B and **Movies 6.4** and **6.5**).

Telomerase Replicates the Ends of Eukaryotic Chromosomes

Having discussed how DNA replication begins at origins and how movement of a replication fork proceeds, we now turn to the special problem

Figure 6–20 DNA topoisomerases relieve the tension that builds up in front of a replication fork. (A) As DNA helicase unwinds the DNA double helix, it generates a section of overwound DNA. Tension builds up because the chromosome is too large to rotate fast enough to relieve the buildup of torsional stress. The broken bars in the left-hand panel represent approximately 20 turns of DNA. (B) DNA topoisomerases relieve this stress by generating temporary nicks in the DNA.

QUESTION 6–2

Discuss the following statement: “Primase is a sloppy enzyme that makes many mistakes. Eventually, the RNA primers it makes are disposed of and replaced with DNA synthesized by a polymerase with higher fidelity. This is wasteful. It would be more energy-efficient if a DNA polymerase made an accurate copy in the first place.”

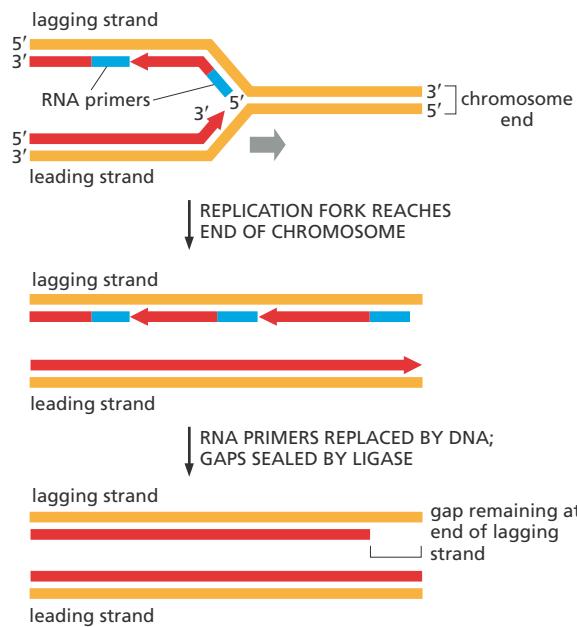


Figure 6–21 Without a special mechanism to replicate the ends of linear chromosomes, DNA would be lost during each round of cell division. DNA synthesis begins at origins of replication and continues until the replication machinery reaches the ends of the chromosome. The leading strand is reproduced in its entirety. But the ends of the lagging strand can't be completed, because once the final RNA primer has been removed there is no way to replace it with DNA. These gaps at the ends of the lagging strand must be filled in by a special mechanism to keep the chromosome ends from shrinking with each cell division.

QUESTION 6–3

A gene encoding one of the proteins involved in DNA replication has been inactivated by a mutation in a cell. In the absence of this protein, the cell attempts to replicate its DNA. What would happen during the DNA replication process if each of the following proteins were missing?

- A. DNA polymerase
- B. DNA ligase
- C. Sliding clamp for DNA polymerase
- D. Nuclease that removes RNA primers
- E. DNA helicase
- F. Primase

of replicating the very ends of chromosomes. As we discussed previously, because DNA replication proceeds only in the 5'-to-3' direction, the lagging strand of the replication fork has to be synthesized in the form of discontinuous DNA fragments, each of which is primed with an RNA primer laid down by a primase (see Figure 6–17). A serious problem arises, however, as the replication fork approaches the end of a chromosome: although the leading strand can be replicated all the way to the chromosome tip, the lagging strand cannot. When the final RNA primer on the lagging strand is removed, there is no way to replace it (Figure 6–21). Without a strategy to deal with this problem, the lagging strand would become shorter with each round of DNA replication; after repeated cell divisions, chromosomes would shrink—and eventually lose valuable genetic information.

Bacteria solve this “end-replication” problem by having circular DNA molecules as chromosomes. Eukaryotes solve it by having long, repetitive nucleotide sequences at the ends of their chromosomes which are incorporated into structures called **telomeres**. These telomeric DNA sequences attract an enzyme called **telomerase** to the chromosome ends. Using an RNA template that is part of the enzyme itself, telomerase extends the ends of the replicating lagging strand by adding multiple copies of the same short DNA sequence to the template strand. This extended template allows replication of the lagging strand to be completed by conventional DNA replication (Figure 6–22).

In addition to allowing replication of chromosome ends, telomeres form structures that mark the true ends of a chromosome. This allows the cell to distinguish unambiguously between the natural ends of chromosomes and the double-strand DNA breaks that sometimes occur accidentally in

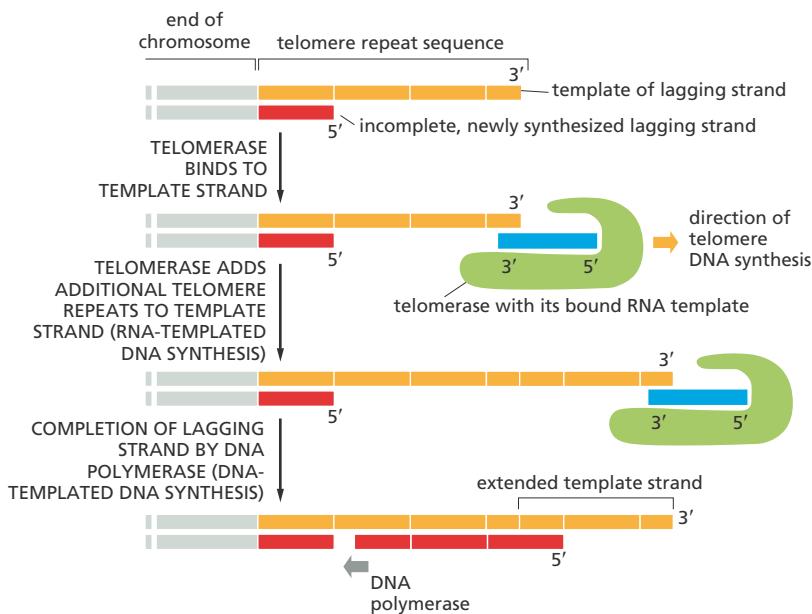


Figure 6–22 Telomeres and telomerase prevent linear eukaryotic chromosomes from shortening with each cell division. For clarity, only the template DNA (orange) and newly synthesized DNA (red) of the lagging strand are shown (see bottom of Figure 6–21). To complete the replication of the lagging strand at the ends of a chromosome, the template strand is first extended beyond the DNA that is to be copied. To achieve this, the enzyme telomerase adds more repeats to the telomere repeat sequences at the 3' end of the template strand, which then allows the lagging strand to be completed by DNA polymerase, as shown. The telomerase enzyme carries a short piece of RNA (blue) with a sequence that is complementary to the DNA repeat sequence; this RNA acts as the template for telomere DNA synthesis. After the lagging-strand replication is complete, a short stretch of single-stranded DNA remains at the ends of the chromosome, as shown. To see telomerase in action, view [Movie 6.6](#).

the middle of chromosomes. These breaks are dangerous and must be immediately repaired, as we see in the next section.

DNA REPAIR

The diversity of living organisms and their success in colonizing almost every part of the Earth's surface depend on genetic changes accumulated gradually over millions of years. Some of these changes allow organisms to adapt to changing conditions and to thrive in new habitats. However, in the short term, and from the perspective of an individual organism, genetic alterations can be detrimental. In a multicellular organism, such permanent changes in the DNA—called mutations—can upset the organism's extremely complex and finely tuned development and physiology.

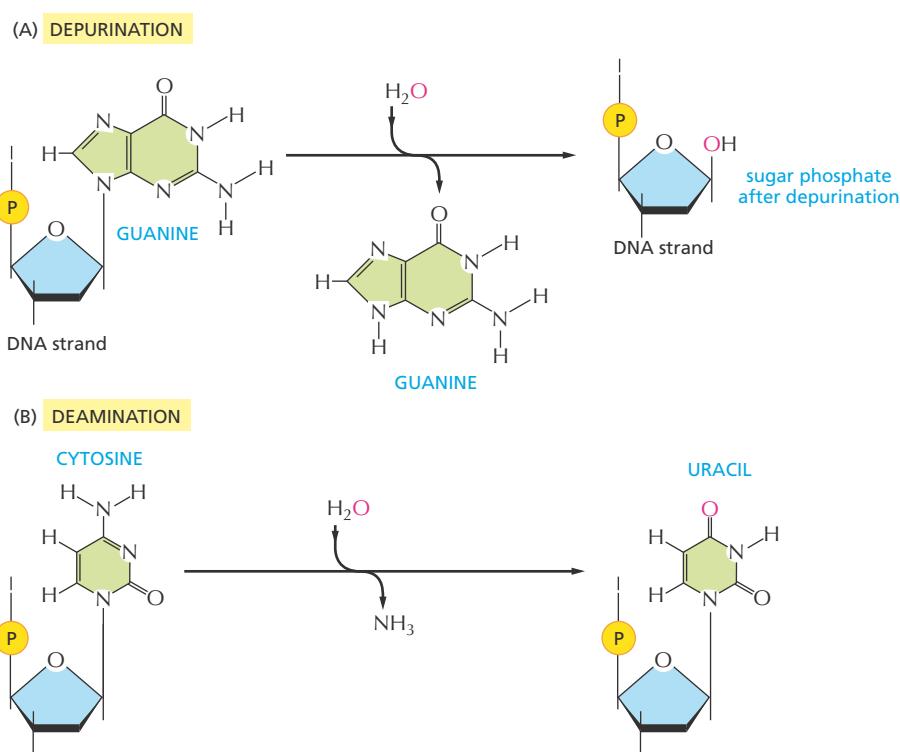
To survive and reproduce, individuals must be genetically stable. This stability is achieved not only through the extremely accurate mechanism for replicating DNA that we have just discussed, but also through the work of a variety of protein machines that continually scan the genome for damage and fix it when it occurs. Although some changes arise from rare mistakes in the replication process, the majority of DNA damage is an unintended consequence of the vast number of chemical reactions that occur inside cells.

Most DNA damage is only temporary, because it is immediately corrected by processes collectively called **DNA repair**. The importance of these DNA repair processes is evident from the consequences of their malfunction. Humans with the genetic disease *xeroderma pigmentosum*, for example, cannot mend the damage done by ultraviolet (UV) radiation because they have inherited a defective gene for one of the proteins involved in this repair process. Such individuals develop severe skin lesions, including skin cancer, because of the accumulation of DNA damage in cells that are exposed to sunlight and the consequent mutations that arise in these cells.

In this section, we describe a few of the specialized mechanisms cells use to repair DNA damage. We then consider examples of what happens when these mechanisms fail—and discuss how the fidelity of DNA replication and repair are reflected in our genome.

Figure 6–23 Depurination and deamination are the most frequent chemical reactions known to create serious DNA damage in cells.

(A) Depurination can remove guanine (or adenine) from DNA. (B) The major type of deamination reaction converts cytosine to an altered DNA base, uracil; however, deamination can also occur on other bases as well. Both depurination and deamination take place on double-helical DNA, and neither break the phosphodiester backbone.



DNA Damage Occurs Continually in Cells

QUESTION 6–4

Discuss the following statement:
"The DNA repair enzymes that fix deamination and depurination damage must preferentially recognize such damage on newly synthesized DNA strands."

Just like any other molecule in the cell, DNA is continually undergoing thermal collisions with other molecules, often resulting in major chemical changes in the DNA. For example, during the time it takes to read this sentence, a total of about a trillion (10^{12}) purine bases (A and G) will be lost from DNA in the cells of your body by a spontaneous reaction called *depurination* (Figure 6–23A). Depurination does not break the DNA phosphodiester backbone but instead removes a purine base from a nucleotide, giving rise to lesions that resemble missing teeth (see Figure 6–25B). Another common reaction is the spontaneous loss of an amino group (*deamination*) from a cytosine in DNA to produce the base uracil (Figure 6–23B). Some chemically reactive by-products of cell metabolism also occasionally react with the bases in DNA, altering them in such a way that their base-pairing properties are changed.

The ultraviolet radiation in sunlight is also damaging to DNA; it promotes covalent linkage between two adjacent pyrimidine bases, forming, for example, the *thymine dimer* shown in Figure 6–24. It is the failure to repair thymine dimers that spells trouble for individuals with the disease xeroderma pigmentosum.

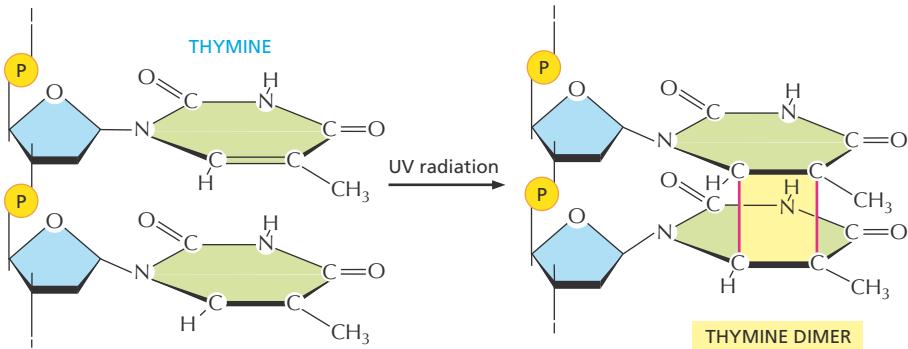
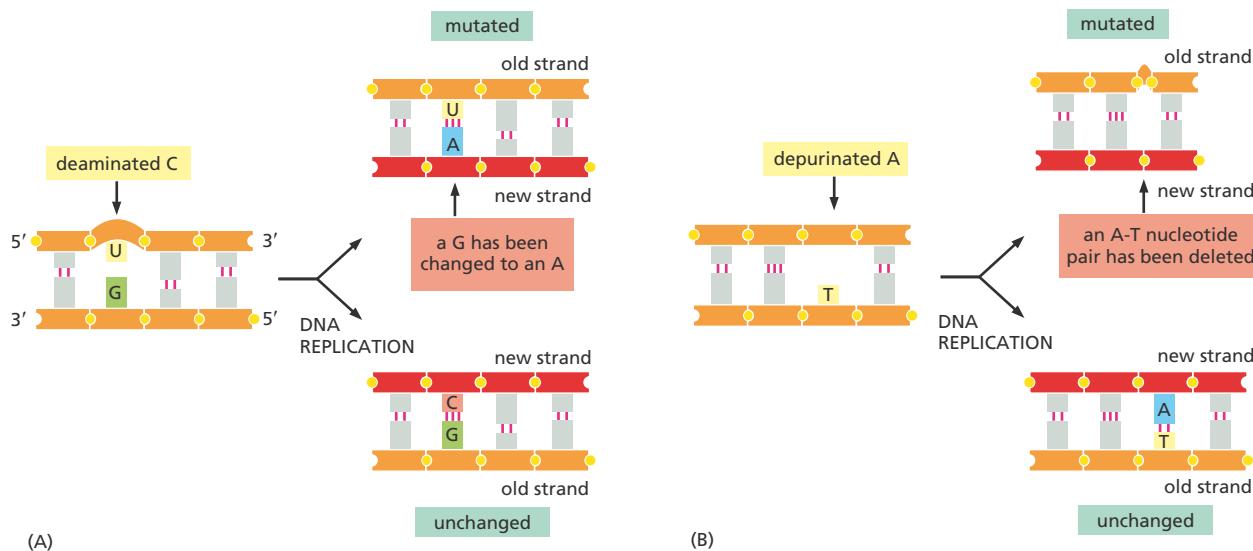


Figure 6–24 The ultraviolet radiation in sunlight can cause the formation of thymine dimers. Two adjacent thymine bases have become covalently attached to each other to form a thymine dimer. Skin cells that are exposed to sunlight are especially susceptible to this type of DNA damage.



These are only a few of many chemical changes that can occur in our DNA. If left unrepaired, many of them would lead either to the substitution of one nucleotide pair for another as a result of incorrect base-pairing during replication (Figure 6–25A) or to deletion of one or more nucleotide pairs in the daughter DNA strand after DNA replication (Figure 6–25B). Some types of DNA damage (thymine dimers, for example) can stall the DNA replication machinery at the site of the damage.

In addition to this chemical damage, DNA can also be altered by replication itself. The replication machinery that copies the DNA can—quite rarely—incorporate an incorrect nucleotide that it fails to correct via proofreading (see Figure 6–14).

For each of these forms of DNA, cells possess a mechanism for repair, as we discuss next.

Cells Possess a Variety of Mechanisms for Repairing DNA

The thousands of random chemical changes that occur every day in the DNA of a human cell—through thermal collisions or exposure to reactive metabolic by-products, DNA-damaging chemicals, or radiation—are repaired by a variety of mechanisms, each catalyzed by a different set of enzymes. Nearly all these repair mechanisms depend on the double-helical structure of DNA, which provides two copies of the genetic information—one in each strand of the double helix. Thus, if the sequence in one strand is accidentally damaged, information is not lost irretrievably, because a backup version of the altered strand remains in the complementary sequence of nucleotides in the other strand. Most DNA damage creates structures that are never encountered in an undamaged DNA strand; thus the good strand is easily distinguished from the bad.

The basic pathway for repairing damage to DNA, illustrated schematically in Figure 6–26, involves three basic steps:

1. The damaged DNA is recognized and removed by one of a variety of mechanisms. These involve nucleases, which cleave the covalent bonds that join the damaged nucleotides to the rest of the DNA strand, leaving a small gap on one strand of the DNA double helix in the region.
2. A *repair DNA polymerase* binds to the 3'-hydroxyl end of the cut DNA strand. It then fills in the gap by making a complementary copy of the information stored in the undamaged strand. Although

Figure 6–25 Chemical modifications of nucleotides, if left unrepaired, produce mutations. (A) Deamination of cytosine, if uncorrected, results in the substitution of one base for another when the DNA is replicated. As shown in Figure 6–23B, deamination of cytosine produces uracil. Uracil differs from cytosine in its base-pairing properties and preferentially base-pairs with adenine. The DNA replication machinery therefore inserts an adenine when it encounters a uracil on the template strand. (B) Depurination, if uncorrected, can lead to the loss of a nucleotide pair. When the replication machinery encounters a missing purine on the template strand, it can skip to the next complete nucleotide, as shown, thus producing a daughter DNA molecule that is missing one nucleotide pair. In other cases (not shown), the replication machinery places an incorrect nucleotide across from the missing base, again resulting in a mutation.

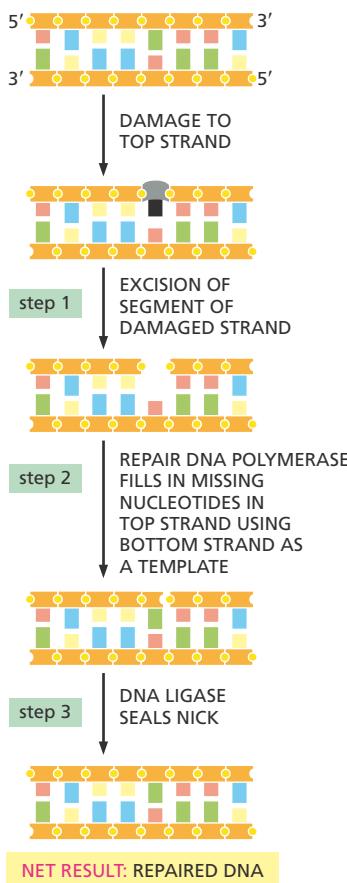


Figure 6–26 The basic mechanism of DNA repair involves three steps. In step 1 (excision), the damage is cut out by one of a series of nucleases, each specialized for a type of DNA damage. In step 2 (resynthesis), the original DNA sequence is restored by a repair DNA polymerase, which fills in the gap created by the excision events. In step 3 (ligation), DNA ligase seals the nick left in the sugar-phosphate backbone of the repaired strand. Nick sealing, which requires energy from ATP hydrolysis, remakes the broken phosphodiester bond between the adjacent nucleotides (see Figure 6–18).

different from the DNA polymerase that replicates DNA, repair DNA polymerases synthesize DNA strands in the same way. For example, they elongate chains in the 5'-to-3' direction and have the same type of proofreading activity to ensure that the template strand is copied accurately. In many cells, this is the same enzyme that fills in the gap left after the RNA primers are removed during the normal DNA replication process (see Figure 6–17).

- When the repair DNA polymerase has filled in the gap, a break remains in the sugar-phosphate backbone of the repaired strand. This nick in the helix is sealed by DNA ligase, the same enzyme that joins the Okazaki fragments during replication of the lagging DNA strand.

Steps 2 and 3 are nearly the same for most types of DNA damage, including the rare errors that arise during DNA replication. However, step 1 uses a series of different enzymes, each specialized for removing different types of DNA damage. Humans produce hundreds of different proteins that function in DNA repair.

A DNA Mismatch Repair System Removes Replication Errors That Escape Proofreading

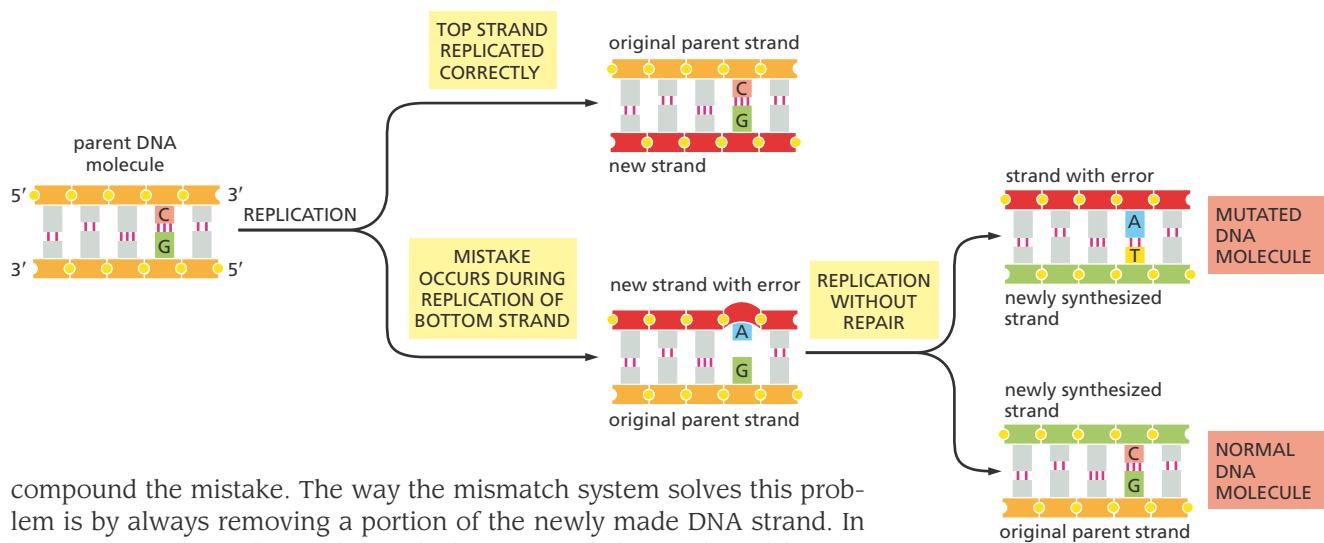
Although the high fidelity and proofreading abilities of the cell's replication machinery generally prevent replication errors from occurring, rare mistakes do happen. Fortunately, the cell has a backup system—called **mismatch repair**—which is dedicated to correcting these errors. The replication machine makes approximately one mistake per 10^7 nucleotides copied; DNA mismatch repair corrects 99% of these replication errors, increasing the overall accuracy to one mistake in 10^9 nucleotides copied. This level of accuracy is much, much higher than that generally encountered in our day-to-day lives (**Table 6–1**).

Whenever the replication machinery makes a copying mistake, it leaves behind a mispaired nucleotide (commonly called a *mismatch*). If left uncorrected, the mismatch will result in a permanent mutation in the next round of DNA replication (**Figure 6–27**). A complex of mismatch repair proteins recognizes such a DNA mismatch, removes a portion of the DNA strand containing the error, and then resynthesizes the missing DNA. This repair mechanism restores the correct sequence (**Figure 6–28**).

To be effective, the mismatch repair system must be able to recognize which of the DNA strands contains the error. Removing a segment from the strand of DNA that contains the correct sequence would only

TABLE 6–1 ERROR RATES

US Postal Service on-time delivery of local first-class mail	13 late deliveries per 100 parcels
Airline luggage system	1 lost bag per 150
A professional typist typing at 120 words per minute	1 mistake per 250 characters
Driving a car in the United States	1 death per 10^4 people per year
DNA replication (without proofreading)	1 mistake per 10^5 nucleotides copied
DNA replication (with proofreading; without mismatch repair)	1 mistake per 10^7 nucleotides copied
DNA replication (with mismatch repair)	1 mistake per 10^9 nucleotides copied



compound the mistake. The way the mismatch system solves this problem is by always removing a portion of the newly made DNA strand. In bacteria, newly synthesized DNA lacks a type of chemical modification that is present on the preexisting parent DNA. Other cells use other strategies for distinguishing their parent DNA from a newly replicated strand.

Mismatch repair plays an important role in preventing cancer. An inherited predisposition to certain cancers (especially some types of colon cancer) is caused by mutations in genes that encode mismatch repair proteins. Humans inherit two copies of these genes (one from each parent), and individuals who inherit one damaged mismatch repair gene are unaffected until the undamaged copy of the same gene is randomly mutated in a somatic cell. This mutant cell—and all of its progeny—are then deficient in mismatch repair; they therefore accumulate mutations more rapidly than do normal cells. Because cancers arise from cells that have accumulated multiple mutations, a cell deficient in mismatch repair has a greatly enhanced chance of becoming cancerous. Thus, inheriting a damaged mismatch repair gene strongly predisposes an individual to cancer.

Figure 6–27 Errors made during DNA replication must be corrected to avoid mutations. If uncorrected, a mismatch will lead to a permanent mutation in one of the two DNA molecules produced by the next round of DNA replication.

Double-Strand DNA Breaks Require a Different Strategy for Repair

The repair mechanisms we have discussed thus far rely on the genetic redundancy built into every DNA double helix. If nucleotides on one strand are damaged, they can be repaired using the information present in the complementary strand.

But what happens when both strands of the double helix are damaged at the same time? Radiation, mishaps at the replication fork, and various chemical assaults can all fracture the backbone of DNA, creating a

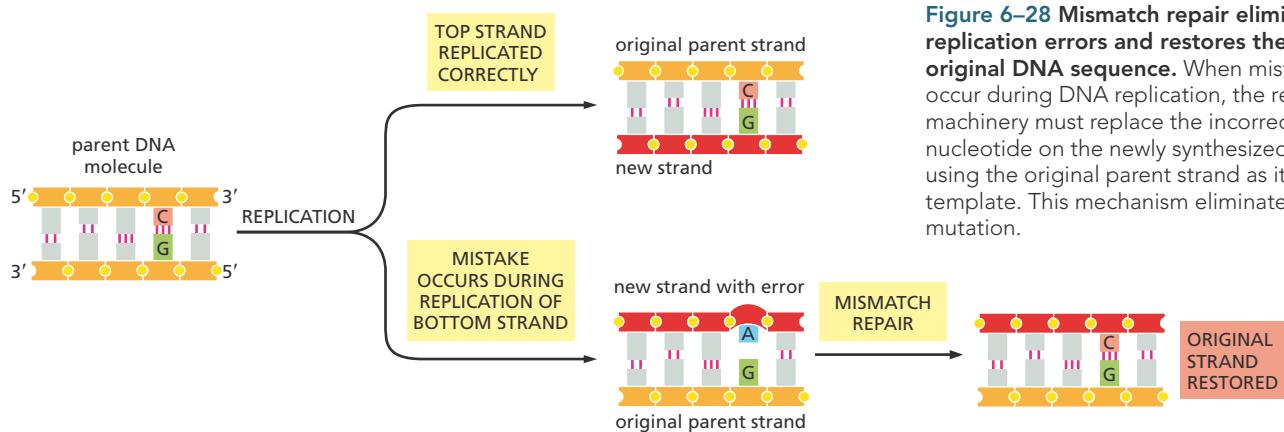
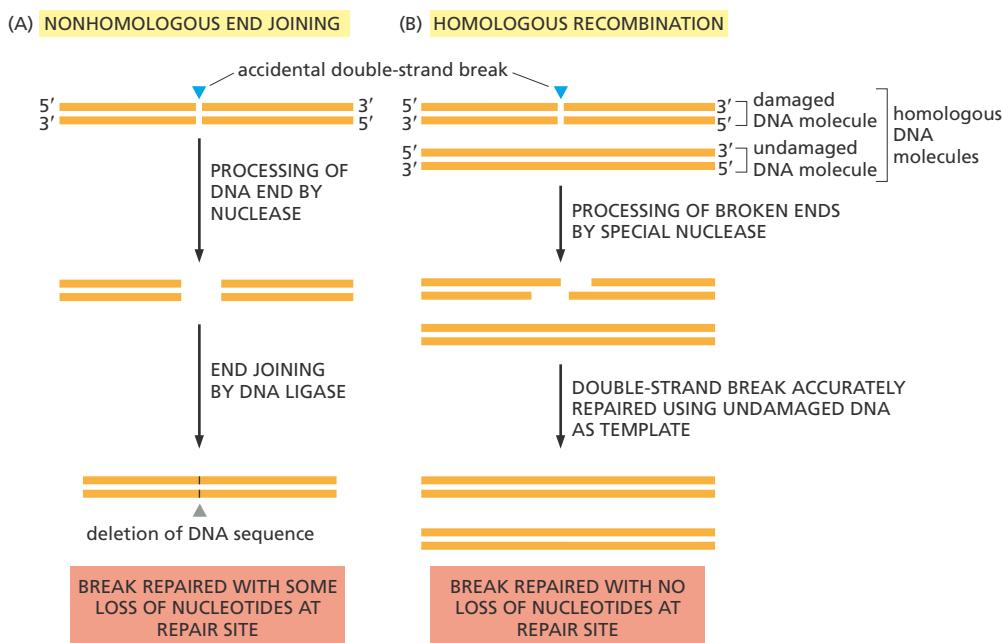


Figure 6–28 Mismatch repair eliminates replication errors and restores the original DNA sequence. When mistakes occur during DNA replication, the repair machinery must replace the incorrect nucleotide on the newly synthesized strand, using the original parent strand as its template. This mechanism eliminates the mutation.

Figure 6–29 Cells can repair double-strand breaks in one of two ways. (A) In nonhomologous end joining, the break is first “cleaned” by a nuclease that chews back the broken ends to produce flush ends. The flush ends are then stitched together by a DNA ligase. Some nucleotides are lost in the repair process, as indicated by the black lines in the repaired DNA. (B) If a double-strand break occurs in one of two daughter DNA double helices after DNA replication has occurred, but before the daughter chromosomes have been separated, the undamaged double helix can be readily used as a template to repair the damaged double helix by homologous recombination. This is a more involved process than non-homologous end joining, but it accurately restores the original DNA sequence at the site of the break. The detailed mechanism is presented in Figure 6–30.



double-strand break. Such lesions are particularly dangerous, because they can lead to the fragmentation of chromosomes and the subsequent loss of genes.

This type of damage is especially difficult to repair. Each chromosome contains unique information; if a chromosome undergoes a double-strand break, and the broken pieces become separated, the cell has no spare copy it can use to reconstruct the information that is now missing.

To handle this potentially disastrous type of DNA damage, cells have evolved two basic strategies. The first involves rapidly sticking the broken ends back together, before the DNA fragments drift apart and get lost. This repair mechanism, called **nonhomologous end joining**, occurs in many cell types and is carried out by a specialized group of enzymes that “clean” the broken ends and rejoin them by DNA ligation. This “quick and dirty” mechanism rapidly repairs the damage, but it comes with a price: in “cleaning” the break to make it ready for ligation, nucleotides are often lost at the site of repair (Figure 6–29A).

In most cases, this emergency repair mechanism mends the damage without creating any additional problems. But if the imperfect repair disrupts the activity of a gene, the cell could suffer serious consequences. Thus, nonhomologous end joining can be a risky strategy for fixing broken chromosomes. So cells have an alternative, error-free strategy for repairing double-strand breaks, called **homologous recombination** (Figure 6–29B), as we discuss next.

Homologous Recombination Can Flawlessly Repair DNA Double-Strand Breaks

The problem with repairing a double-strand break, as we mentioned, is finding an intact template to guide the repair. However, if a double-strand break occurs in one double helix shortly after a stretch of DNA has been replicated, the undamaged double helix can readily serve as a template to guide the repair of the broken DNA: information on the undamaged strand of the intact double helix is used to repair the complementary broken strand in the other. Because the two DNA molecules

are homologous—they have identical nucleotide sequences outside the broken region—this mechanism is known as **homologous recombination**. It results in a flawless repair of the double-strand break, with no loss of genetic information (see Figure 6–29B).

Homologous recombination most often occurs shortly after a cell's DNA has been replicated before cell division, when the duplicated helices are still physically close to each other (Figure 6–30A). To initiate the repair, a nuclease chews back the 5' ends of the two broken strands at the break (Figure 6–30B). Then, with the help of specialized enzymes, one of the broken 3' ends “invades” the unbroken homologous DNA duplex and searches for a complementary sequence through base-pairing (Figure 6–30C). Once an extensive, accurate match is found, the invading strand is elongated by a repair DNA polymerase, using the complementary strand as a template (Figure 6–30D). After the repair polymerase has passed the point where the break occurred, the newly repaired strand rejoins its original partner, forming base pairs that hold the two strands of the broken double helix together (Figure 6–30E). Repair is then completed by additional DNA synthesis at the 3' ends of both strands of the broken double helix (Figure 6–30F), followed by DNA ligation (Figure 6–30G).

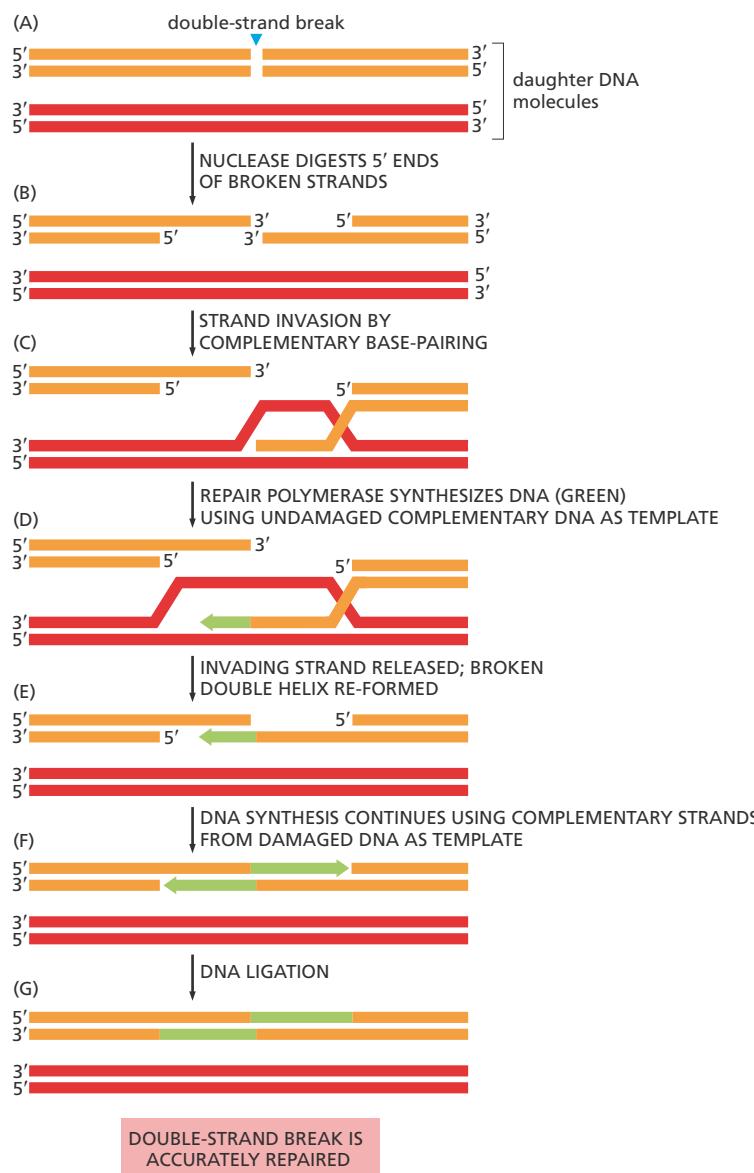


Figure 6–30 Homologous recombination allows the flawless repair of DNA double-strand breaks. This is the preferred method for repairing double-strand breaks that arise shortly after the DNA has been replicated but before the cell has divided. See text for details. (Adapted from M. McVey et al., Proc. Natl. Acad. Sci. USA 101:15694–15699, 2004. With permission from the National Academy of Sciences.)

The net result is two intact DNA helices, where the genetic information from one was used as a template to repair the other.

Homologous recombination can also be used to repair many other types of DNA damage, making it perhaps the most handy DNA repair mechanism available to the cell: all that is needed is an intact homologous chromosome to use as a partner—a situation that occurs transiently each time a chromosome is duplicated. The “all-purpose” nature of homologous recombinational repair probably explains why this mechanism, and the proteins that carry it out, have been conserved in virtually all cells on Earth.

Homologous recombination is versatile, and has a crucial role in the exchange of genetic information during the formation of the germ cells—sperm and eggs. This specialized process, called *meiosis*, enhances the generation of genetic diversity within a species during sexual reproduction. We will discuss it when we talk about sex in Chapter 19.

Failure to Repair DNA Damage Can Have Severe Consequences for a Cell or Organism

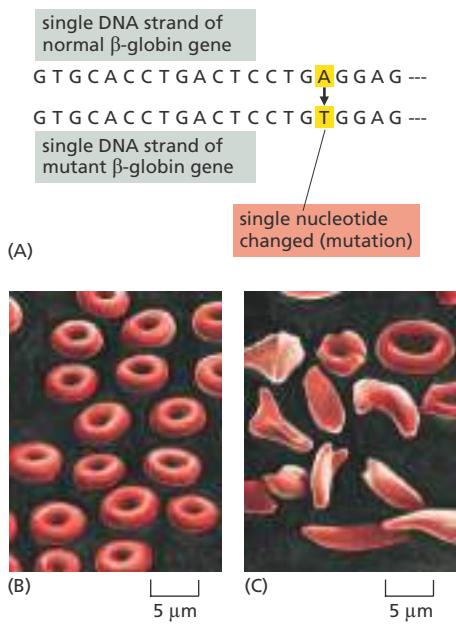


Figure 6–31 A single nucleotide change causes the disease sickle-cell anemia.
 (A) β -globin is one of the two types of protein subunits that form hemoglobin (see Figure 4–24). A single nucleotide change (mutation) in the β -globin gene produces a β -globin subunit that differs from normal β -globin only by a change from glutamic acid to valine at the sixth amino acid position. (Only a small portion of the gene is shown here; the β -globin subunit contains a total of 146 amino acids.) Humans carry two copies of each gene (one inherited from each parent); a sickle-cell mutation in one of the two β -globin genes generally causes no harm to the individual, as it is compensated for by the normal gene. However, an individual who inherits two copies of the mutant β -globin gene will have sickle-cell anemia. Normal red blood cells are shown in (B), and those from an individual suffering from sickle-cell anemia in (C). Although sickle-cell anemia can be a life-threatening disease, the mutation responsible can also be beneficial. People with the disease, or those who carry one normal gene and one sickle-cell gene, are more resistant to malaria than unaffected individuals, because the parasite that causes malaria grows poorly in red blood cells that contain the sickle-cell form of hemoglobin.

On occasion, the cell’s DNA replication and repair processes fail and give rise to a mutation. This permanent change in the DNA sequence can have profound consequences. A mutation that affects just a single nucleotide pair can severely compromise an organism’s fitness if the change occurs in a vital position in the DNA sequence. Because the structure and activity of each protein depend on its amino acid sequence, a protein with an altered sequence may function poorly or not at all. For example, humans use the protein hemoglobin to transport oxygen in the blood (see Figure 4–24). A permanent change in a single nucleotide in a hemoglobin gene can cause cells to make hemoglobin with an incorrect sequence of amino acids. One such mutation causes the disease *sickle-cell anemia*. The sickle-cell hemoglobin is less soluble than normal hemoglobin and forms fibrous intracellular precipitates, which produce the characteristic sickle shape of affected red blood cells (Figure 6–31). Because these cells are more fragile and frequently tear as they travel through the bloodstream, patients with this potentially life-threatening disease have fewer red blood cells than usual—that is, they are anemic. This anemia can cause weakness, dizziness, headaches, and breathlessness. Moreover, the abnormal red blood cells can aggregate and block small vessels, causing pain and organ failure. We know about sickle-cell hemoglobin because individuals with the mutation survive; the mutation even provides a benefit—an increased resistance to malaria. Over the course of evolution, many other mutations in the hemoglobin gene have arisen, but only those that do not completely destroy the protein remain in the population.

The example of sickle-cell anemia, which is an inherited disease, illustrates the importance of protecting reproductive cells (*germ cells*) against mutation. A mutation in a germ cell will be passed on to all the cells in the body of the multicellular organism that develops from it, including the germ cells responsible for the production of the next generation.

The many other cells in a multicellular organism (its *somatic cells*) must also be protected against mutation—in this case, against mutations that arise during the life of an individual. Nucleotide changes that occur in somatic cells can give rise to variant cells, some of which grow and divide in an uncontrolled fashion at the expense of the other cells in the organism. In the extreme case, an unchecked cell proliferation known as **cancer** results. Cancers are responsible for about 30% of the deaths that occur in Europe and North America, and they are caused largely by a gradual accumulation of random mutations in a somatic cell and its

Figure 6–32 Cancer incidence increases dramatically with age. The number of newly diagnosed cases of cancer of the colon in women in England and Wales in one year is plotted as a function of age at diagnosis. Colon cancer, like most human cancers, is caused by the accumulation of multiple mutations. Because cells are continually experiencing accidental changes to their DNA—which accumulate and are passed on to progeny cells when the mutated cells divide—the chance that a cell will become cancerous increases greatly with age. (Data from C. Muir et al., *Cancer Incidence in Five Continents*, Vol. V. Lyon: International Agency for Research on Cancer, 1987.)

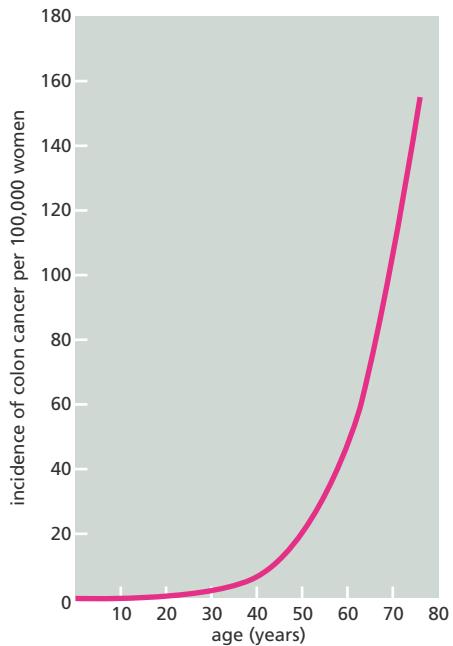
descendants (Figure 6–32). Increasing the mutation frequency even two- or threefold could cause a disastrous increase in the incidence of cancer by accelerating the rate at which such somatic cell variants arise.

Thus, the high fidelity with which DNA sequences are replicated and maintained is important both for reproductive cells, which transmit the genes to the next generation, and for somatic cells, which normally function as carefully regulated members of the complex community of cells in a multicellular organism. We should therefore not be surprised to find that all cells possess a very sophisticated set of mechanisms to reduce the number of mutations that occur in their DNA, devoting hundreds of genes to these repair processes.

A Record of the Fidelity of DNA Replication and Repair Is Preserved in Genome Sequences

Although the majority of mutations do neither harm nor good to an organism, those that have harmful consequences are usually eliminated from the population through natural selection; individuals carrying the altered DNA may die or experience decreased fertility, in which case these changes will be lost. By contrast, favorable changes will tend to persist and spread.

But even where no selection operates—at the many sites in the DNA where a change of nucleotide has no effect on the fitness of the organism—the genetic message has been faithfully preserved over tens of millions of years. Thus humans and chimpanzees, after about 5 million years of divergent evolution, still have DNA sequences that are at least 98% identical. Even humans and whales, after 10 or 20 times this amount of time, have chromosomes that are unmistakably similar in their DNA sequence, and many proteins have amino acid sequences that are almost identical (Figure 6–33). Thus our genome—and those of our relatives—contains a message from the distant past. Thanks to the faithfulness of DNA replication and repair, 100 million years of evolution have scarcely changed its essential content.



whale
human

whale	ATG GGT CGT GAT CAG GCG TAA AGG TGG CTC TGA GAG ATCCC
human	GTG GGT CTG CAG AAG GCG TAA AGT TGG CTC TGA GAG ATCCC

Figure 6–33 The sex-determination genes from humans and whales are unmistakably similar. Although their body plans are strikingly different, humans and whales are built from the same proteins. Despite the many millions of years that have passed since humans and whales diverged, the nucleotide sequences of many of their genes are closely similar. The DNA sequences of a part of the gene that determines maleness in humans and in whales are shown, one above the other; the positions where the two are identical are shaded in green.

ESSENTIAL CONCEPTS

- Before a cell divides, it must accurately replicate the vast quantity of genetic information carried in its DNA.
- Because the two strands of a DNA double helix are complementary, each strand can act as a template for the synthesis of the other. Thus DNA replication produces two identical, double-helical DNA molecules, enabling genetic information to be copied and passed on from a cell to its daughter cells and from a parent to its offspring.
- During replication, the two strands of a DNA double helix are pulled apart at a replication origin to form two Y-shaped replication forks. DNA polymerases at each fork produce a new complementary DNA strand on each parental strand.
- DNA polymerase replicates a DNA template with remarkable fidelity, making only about one error in every 10^7 nucleotides copied. This accuracy is made possible, in part, by a proofreading process in which the enzyme corrects its own mistakes as it moves along the DNA.
- Because DNA polymerase synthesizes new DNA in only one direction, only the leading strand at the replication fork can be synthesized in a continuous fashion. On the lagging strand, DNA is synthesized in a discontinuous backstitching process, producing short fragments of DNA that are later joined together by DNA ligase.
- DNA polymerase is incapable of starting a new DNA chain from scratch. Instead, DNA synthesis is primed by an RNA polymerase called primase, which makes short lengths of RNA primers that are then elongated by DNA polymerase. These primers are subsequently erased and replaced with DNA.
- DNA replication requires the cooperation of many proteins that form a multienzyme replication machine that copies both DNA strands as it moves along the double helix.
- In eukaryotes, a special enzyme called telomerase replicates the DNA at the ends of the chromosomes.
- The rare copying mistakes that escape proofreading are dealt with by mismatch repair proteins, which increase the accuracy of DNA replication to one mistake per 10^9 nucleotides copied.
- Damage to one of the two DNA strands, caused by unavoidable chemical reactions, is repaired by a variety of DNA repair enzymes that recognize damaged DNA and excise a short stretch of the damaged strand. The missing DNA is then resynthesized by a repair DNA polymerase, using the undamaged strand as a template.
- If both DNA strands are broken, the double-strand break can be rapidly repaired by nonhomologous end joining. Nucleotides are lost in the process, altering the DNA sequence at the repair site.
- Homologous recombination can flawlessly repair double-strand breaks using an undamaged homologous double helix as a template.
- Highly accurate DNA replication and DNA repair processes play a key role in protecting us from the uncontrolled growth of somatic cells known as cancer.

KEY TERMS

cancer	nonhomologous end joining
DNA ligase	Okazaki fragment
DNA polymerase	primase
DNA repair	proofreading
DNA replication	replication fork
homologous recombination	replication origin
lagging strand	RNA (ribonucleic acid)
leading strand	telomerase
mismatch repair	telomere
mutation	template

QUESTIONS

QUESTION 6–5

DNA mismatch repair enzymes preferentially repair bases on the newly synthesized DNA strand, using the old DNA strand as a template. If mismatches were simply repaired without regard for which strand served as template, would this reduce replication errors? Explain your answer.

QUESTION 6–6

Suppose a mutation affects an enzyme that is required to repair the damage to DNA caused by the loss of purine bases. The loss of a purine occurs about 5000 times in the DNA of each of your cells per day. As the average difference in DNA sequence between humans and chimpanzees is about 1%, how long will it take you to turn into an ape? What is wrong with this argument?

QUESTION 6–7

Which of the following statements are correct? Explain your answers.

- A. A bacterial replication fork is asymmetrical because it contains two DNA polymerase molecules that are structurally distinct.
- B. Okazaki fragments are removed by a nuclease that degrades RNA.
- C. The error rate of DNA replication is reduced both by proofreading by DNA polymerase and by DNA mismatch repair.
- D. In the absence of DNA repair, genes are unstable.
- E. None of the aberrant bases formed by deamination occur naturally in DNA.
- F. Cancer can result from the accumulation of mutations in somatic cells.

QUESTION 6–8

The speed of DNA replication at a replication fork is about 100 nucleotides per second in human cells. What is the minimum number of origins of replication that a human cell must have if it is to replicate its DNA once every 24 hours? Recall that a human cell contains two copies of the human genome, one inherited from the mother, the other from the father, each consisting of 3×10^9 nucleotide pairs.

QUESTION 6–9

Look carefully at Figure 6–11 and at the structures of the compounds shown in **Figure Q6–9**.

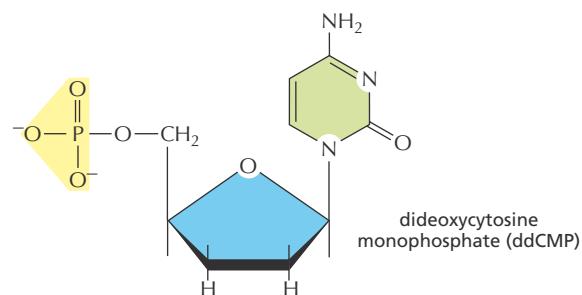
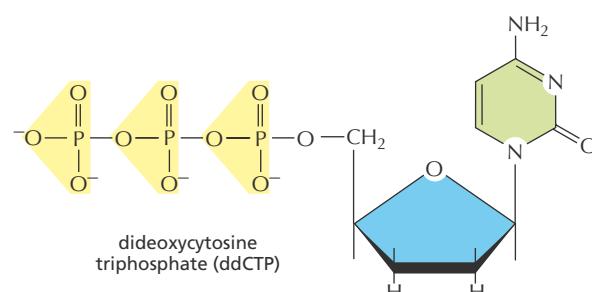
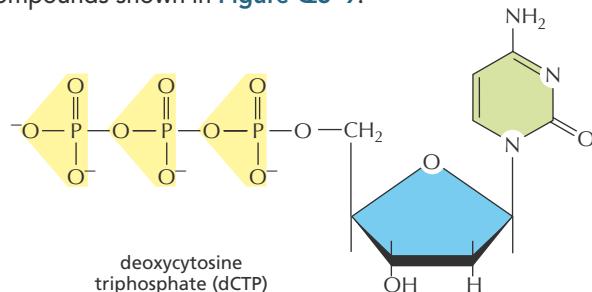
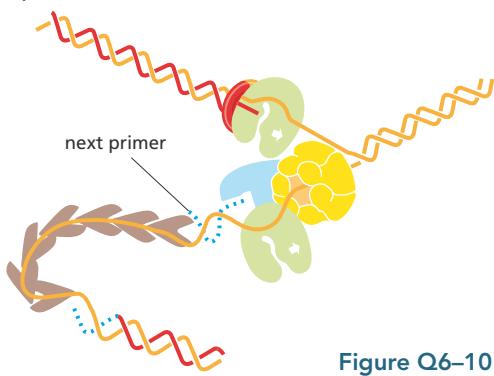


Figure Q6–9

- A. What would you expect if ddCTP were added to a DNA replication reaction in large excess over the concentration of the available deoxycytosine triphosphate (dCTP), the normal deoxycytosine triphosphate?
- B. What would happen if it were added at 10% of the concentration of the available dCTP?
- C. What effects would you expect if ddCMP were added under the same conditions?

QUESTION 6-10

Figure Q6-10 shows a snapshot of a replication fork in which the RNA primer has just been added to the lagging strand. Using this diagram as a guide, sketch the path of the DNA as the next Okazaki fragment is synthesized. Indicate the sliding clamp and the single-strand DNA-binding protein as appropriate.

**Figure Q6-10****QUESTION 6-11**

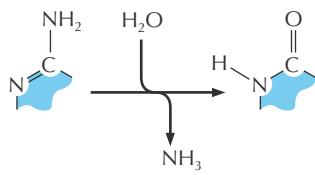
Approximately how many high-energy bonds does DNA polymerase use to replicate a bacterial chromosome (ignoring helicase and other enzymes associated with the replication fork)? Compared with its own dry weight of 10^{-12} g, how much glucose does a single bacterium need to provide enough energy to copy its DNA once? The number of nucleotide pairs in the bacterial chromosome is 3×10^6 . Oxidation of one glucose molecule yields about 30 high-energy phosphate bonds. The molecular weight of glucose is 180 g/mole. (Recall from Figure 2-3 that a mole consists of 6×10^{23} molecules.)

QUESTION 6-12

What, if anything, is wrong with the following statement: "DNA stability in both reproductive cells and somatic cells is essential for the survival of a species." Explain your answer.

QUESTION 6-13

A common type of chemical damage to DNA is produced by a spontaneous reaction termed deamination, in which a nucleotide base loses an amino group (NH_2). The amino group is replaced by a keto group (C=O), by the general reaction shown in **Figure Q6-13**. Write the structures of the bases A, G, C, T, and U and predict the products that will be produced by deamination. By looking at the products of this reaction—and remembering that, in the cell, these will need to be recognized and repaired—can you propose an explanation for why DNA cannot contain uracil?

**Figure Q6-13****QUESTION 6-14**

- Explain why telomeres and telomerase are needed for replication of eukaryotic chromosomes but not for replication of a circular bacterial chromosome. Draw a diagram to illustrate your explanation.
- Would you still need telomeres and telomerase to complete eukaryotic chromosome replication if primase always laid down the RNA primer at the very 3' end of the template for the lagging strand?

QUESTION 6-15

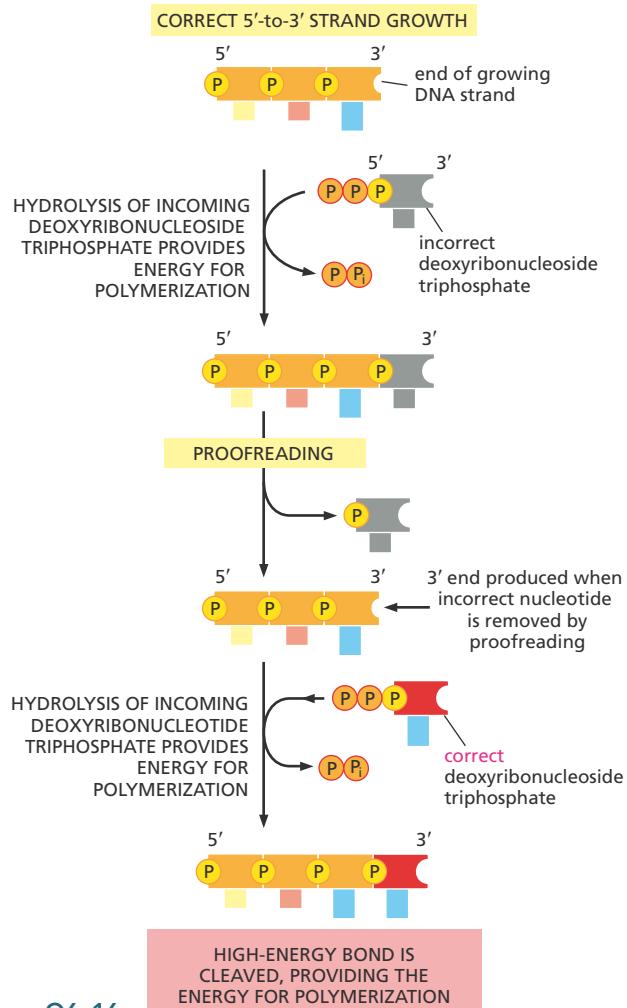
Describe the consequences that would arise if a eukaryotic chromosome

- Contained only one origin of replication:
 - at the exact center of the chromosome
 - at one end of the chromosome
- Lacked one or both telomeres
- Had no centromere

Assume that the chromosome is 150 million nucleotide pairs in length, a typical size for an animal chromosome, and that DNA replication in animal cells proceeds at about 100 nucleotides per second.

QUESTION 6-16

Because DNA polymerase proceeds only in the 5'-to-3' direction, the enzyme is able to correct its own polymerization errors as it moves along the DNA (**Figure Q6-16**). A hypothetical DNA polymerase that synthesized in the 3'-to-5' direction would be unable to proofread. Given what you know about nucleic acid chemistry and DNA synthesis, draw a sketch similar to Figure Q6-16 that shows what would happen if a DNA polymerase operating in the 3'-to-5' direction were to remove an incorrect nucleotide from a growing DNA strand. Why would the edited strand be unable to be elongated?

**Figure Q6-16**



CHAPTER SEVEN

7

From DNA to Protein: How Cells Read the Genome

Once the double-helical structure of DNA (deoxyribonucleic acid) had been determined in the early 1950s, it became clear that the hereditary information in cells is encoded in the linear order—or *sequence*—of the four different nucleotide subunits that make up the DNA. We saw in Chapter 6 how this information can be passed on unchanged from a cell to its descendants through the process of DNA replication. But how does the cell decode and use the information? How do genetic instructions written in an alphabet of just four “letters” direct the formation of a bacterium, a fruit fly, or a human? We still have a lot to learn about how the information stored in an organism’s genes produces even the simplest unicellular bacterium, let alone how it directs the development of complex multicellular organisms like ourselves. But the DNA code itself has been deciphered, and we have come a long way in understanding how cells read it.

Even before the DNA code was broken, it was known that the information contained in genes somehow directed the synthesis of proteins. Proteins are the principal constituents of cells and determine not only cell structure but also cell function. In previous chapters, we encountered some of the thousands of different kinds of proteins that cells can make. We saw in Chapter 4 that the properties and function of a protein molecule are determined by the sequence of the 20 different amino acid subunits in its polypeptide chain: each type of protein has its own unique amino acid sequence, which dictates how the chain will fold to form a molecule with a distinctive shape and chemistry. The genetic instructions carried by DNA must therefore specify the amino acid sequences of proteins. We will see in this chapter exactly how this is done.

FROM DNA TO RNA

FROM RNA TO PROTEIN

RNA AND THE ORIGINS OF LIFE

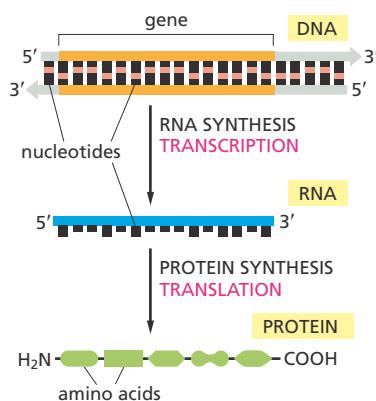


Figure 7–1 Genetic information directs the synthesis of proteins. The flow of genetic information from DNA to RNA (transcription) and from RNA to protein (translation) occurs in all living cells. It was Francis Crick who dubbed this flow of information “the central dogma.” The segments of DNA that are transcribed into RNA are called genes.

DNA does not synthesize proteins itself, but it acts like a manager, delegating the various tasks to a team of workers. When a particular protein is needed by the cell, the nucleotide sequence of the appropriate segment of a DNA molecule is first copied into another type of nucleic acid—RNA (*ribonucleic acid*). That segment of DNA is called a **gene**, and the resulting RNA copies are then used to direct the synthesis of the protein. Many thousands of these conversions from DNA to protein occur every second in each cell in our body. The flow of genetic information in cells is therefore from DNA to RNA to protein (Figure 7–1). All cells, from bacteria to humans, express their genetic information in this way—a principle so fundamental that it has been termed the *central dogma* of molecular biology.

In this chapter, we explain the mechanisms by which cells copy DNA into RNA (a process called *transcription*) and then use the information in RNA to make protein (a process called *translation*). We also discuss a few of the key variations on this basic scheme. Principal among these is *RNA splicing*, a process in eukaryotic cells in which segments of an RNA transcript are removed—and the remaining segments stitched back together—before the RNA is translated into protein. In the final section, we consider how the present scheme of information storage, transcription, and translation might have arisen from much simpler systems in the earliest stages of cell evolution.

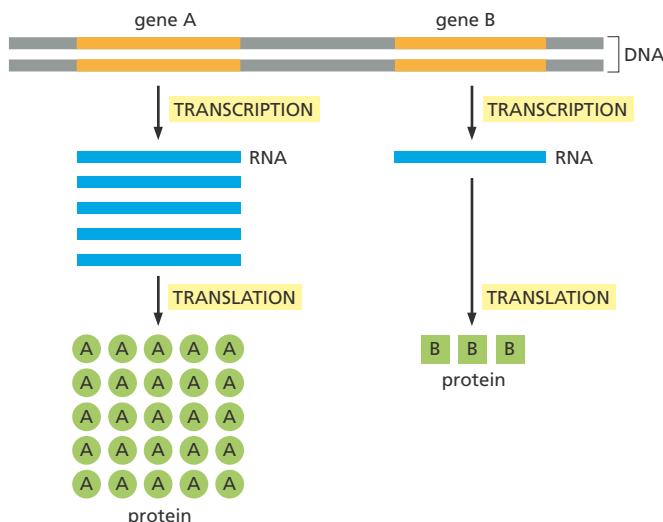
FROM DNA TO RNA

Transcription and translation are the means by which cells read out, or express, the instructions in their genes. Many identical RNA copies can be made from the same gene, and each RNA molecule can direct the synthesis of many identical protein molecules. This successive amplification enables cells to rapidly synthesize large amounts of protein whenever necessary. At the same time, each gene can be transcribed, and its RNA translated, at different rates, providing the cell with a way to make vast quantities of some proteins and tiny quantities of others (Figure 7–2). Moreover, as we discuss in Chapter 8, a cell can change (or regulate) the expression of each of its genes according to the needs of the moment. In this section, we discuss the production of RNA, the first step in *gene expression*.

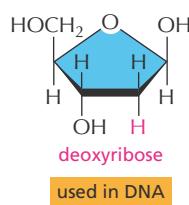
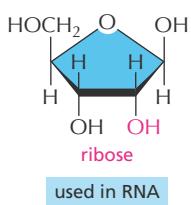
QUESTION 7–1

Consider the expression “central dogma,” which refers to the flow of genetic information from DNA to RNA to protein. Is the word “dogma” appropriate in this context?

Figure 7–2 A cell can express different genes at different rates. In this and later figures, the untranscribed portions of the DNA are shown in gray.



(A) SUGAR DIFFERENCES



(B) BASE DIFFERENCES

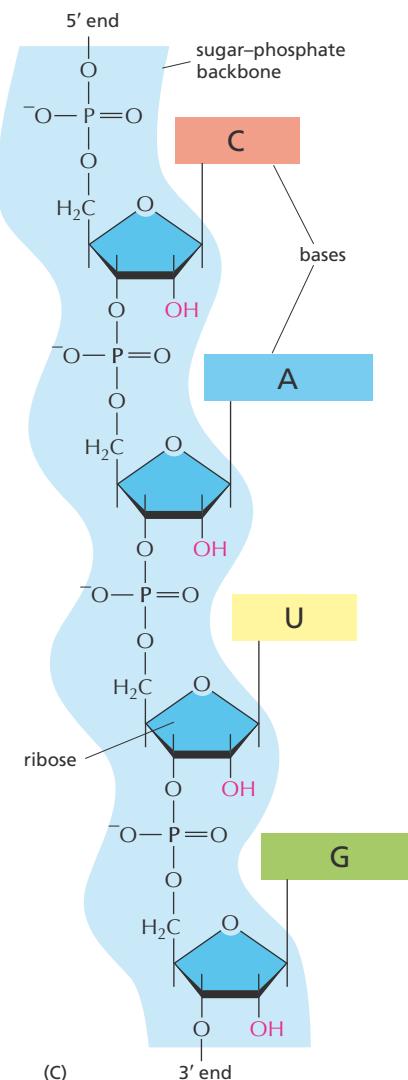
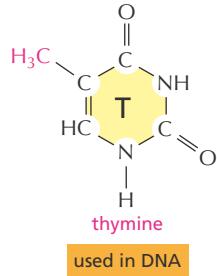
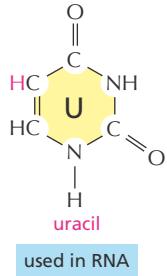


Figure 7–3 The chemical structure of RNA differs slightly from that of DNA. (A) RNA contains the sugar ribose, which differs from deoxyribose, the sugar used in DNA, by the presence of an additional –OH group. (B) RNA contains the base uracil, which differs from thymine, the equivalent base in DNA, by the absence of a –CH₃ group. (C) A short length of RNA. The chemical linkage between nucleotides in RNA—a phosphodiester bond—is the same as that in DNA.

Portions of DNA Sequence Are Transcribed into RNA

The first step a cell takes in expressing one of its many thousands of genes is to copy the nucleotide sequence of that gene into RNA. The process is called **transcription** because the information, though copied into another chemical form, is still written in essentially the same language—the language of nucleotides. Like DNA, RNA is a linear polymer made of four different nucleotide subunits, linked together by phosphodiester bonds. It differs from DNA chemically in two respects: (1) the nucleotides in RNA are **ribonucleotides**—that is, they contain the sugar ribose (hence the name **ribonucleic acid**) rather than deoxyribose; (2) although, like DNA, RNA contains the bases adenine (A), guanine (G), and cytosine (C), it contains uracil (U) instead of the thymine (T) found in DNA (**Figure 7–3**). Because U, like T, can base-pair by hydrogen-bonding with A (**Figure 7–4**), the complementary base-pairing properties described for DNA in Chapter 5 apply also to RNA.

Although their chemical differences are small, DNA and RNA differ quite dramatically in overall structure. Whereas DNA always occurs in cells as a double-stranded helix, RNA is single-stranded. This difference has important functional consequences. Because an RNA chain is single-stranded, it can fold up into a variety of shapes, just as a polypeptide chain folds up to form the final shape of a protein (**Figure 7–5**); double-stranded DNA cannot fold in this fashion. As we discuss later, the ability to fold into a complex three-dimensional shape allows RNA to carry out various functions in cells, in addition to conveying information between DNA and protein. Whereas DNA functions solely as an information store, some RNAs have structural, regulatory, or catalytic roles.

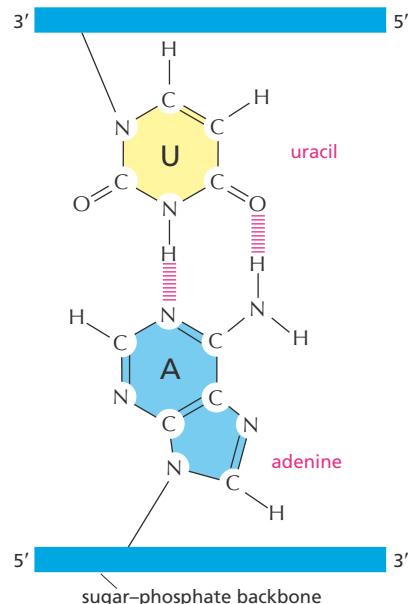


Figure 7–4 Uracil forms a base pair with adenine. The hydrogen bonds that hold the base pair together are shown in red. Uracil has the same base-pairing properties as thymine. Thus U-A base pairs in RNA closely resemble T-A base pairs in DNA (see Figure 5–6A).

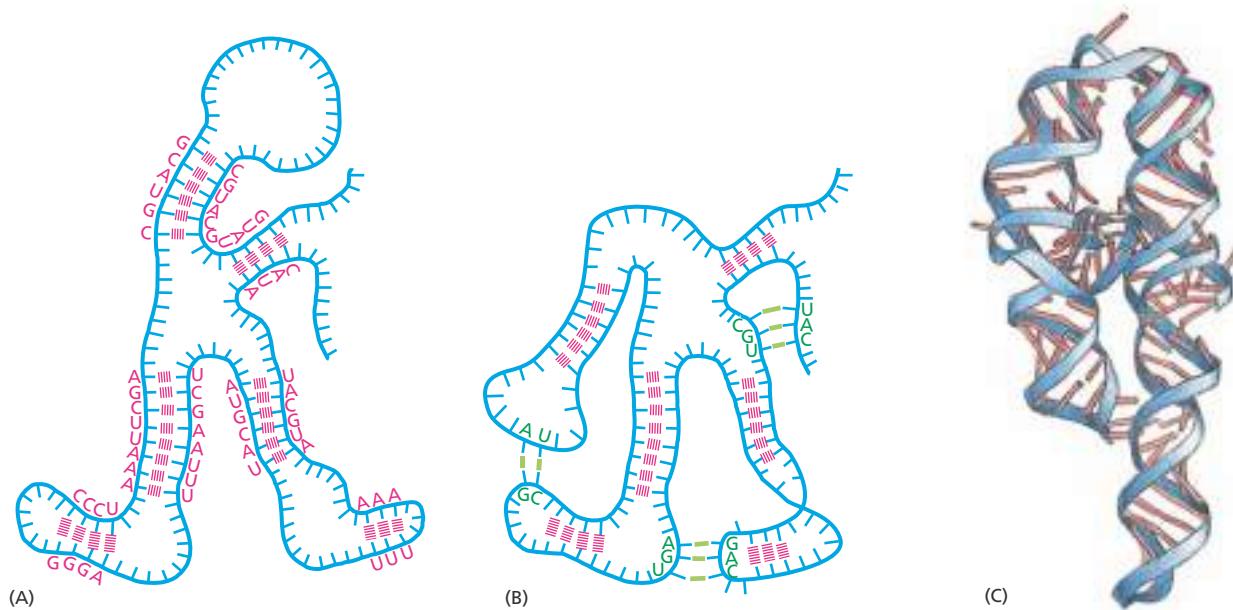


Figure 7–5 RNA molecules can form intramolecular base pairs and fold into specific structures. RNA is single-stranded, but it often contains short stretches of nucleotides that can base-pair with complementary sequences found elsewhere on the same molecule. These interactions—along with some “nonconventional base-pair” interactions (e.g., A-G)—allow an RNA molecule to fold into a three-dimensional structure that is determined by its sequence of nucleotides. (A) A diagram of a hypothetical, folded RNA structure showing only conventional (G-C and A-U) base-pair interactions. (B) Incorporating nonconventional base-pair interactions (green) changes the structure of the hypothetical RNA shown in (A). (C) Structure of an actual RNA molecule that is involved in RNA splicing. This RNA contains a considerable amount of double-helical structure. The sugar-phosphate backbone is blue and the bases are red; the conventional base-pair interactions are indicated by red “rungs” that are continuous, and nonconventional base pairs are indicated by broken red rungs. For an additional view of RNA structure, see [Movie 7.1](#).

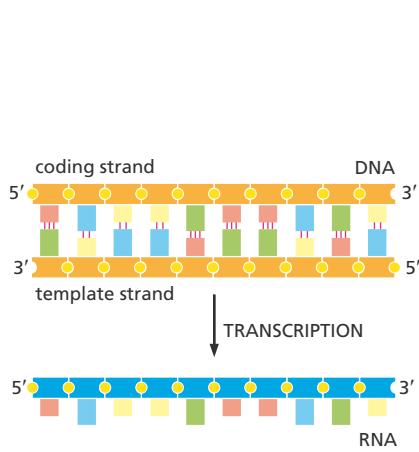
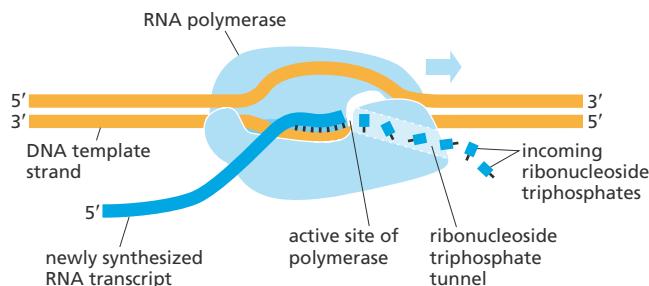


Figure 7–6 Transcription of a gene produces an RNA complementary to one strand of DNA. The transcribed strand of the gene, the bottom strand in this example, is called the template strand. The nontemplate strand of the gene (here, shown at the top) is sometimes called the *coding strand* because its sequence is equivalent to the RNA product, as shown. Which DNA strand serves as the template varies, depending on the gene, as we discuss later. By convention, an RNA molecule is always depicted with its 5' end—the first part to be synthesized—to the left.

Transcription Produces RNA That Is Complementary to One Strand of DNA

All the RNA in a cell is made by transcription, a process that has certain similarities to DNA replication (discussed in Chapter 6). Transcription begins with the opening and unwinding of a small portion of the DNA double helix to expose the bases on each DNA strand. One of the two strands of the DNA double helix then acts as a template for the synthesis of RNA. Ribonucleotides are added, one by one, to the growing RNA chain; as in DNA replication, the nucleotide sequence of the RNA chain is determined by complementary base-pairing with the DNA template. When a good match is made, the incoming ribonucleotide is covalently linked to the growing RNA chain by the enzyme *RNA polymerase*. The RNA chain produced by transcription—the **RNA transcript**—is therefore elongated one nucleotide at a time and has a nucleotide sequence exactly complementary to the strand of DNA used as the template ([Figure 7–6](#)).

Transcription differs from DNA replication in several crucial respects. Unlike a newly formed DNA strand, the RNA strand does not remain hydrogen-bonded to the DNA template strand. Instead, just behind the region where the ribonucleotides are being added, the RNA chain is displaced and the DNA helix re-forms. For this reason—and because only one strand of the DNA molecule is transcribed—RNA molecules are single-stranded. Further, because RNAs are copied from only a limited region of DNA, RNA molecules are much shorter than DNA molecules; DNA molecules in a human chromosome can be up to 250 million nucleotide pairs long, whereas most mature RNAs are no more than a few thousand nucleotides long, and many are much shorter than that.



Like the DNA polymerase that carries out DNA replication (discussed in Chapter 6), **RNA polymerases** catalyze the formation of the phosphodiester bonds that link the nucleotides together and form the sugar-phosphate backbone of the RNA chain (see Figure 7–3). The RNA polymerase moves stepwise along the DNA, unwinding the DNA helix just ahead to expose a new region of the template strand for complementary base-pairing. In this way, the growing RNA chain is extended by one nucleotide at a time in the 5'-to-3' direction (Figure 7–7). The incoming ribonucleoside triphosphates (ATP, CTP, UTP, and GTP) provide the energy needed to drive the reaction forward (see Figure 6–11).

The almost immediate release of the RNA strand from the DNA as it is synthesized means that many RNA copies can be made from the same gene in a relatively short time; the synthesis of the next RNA is usually started before the first RNA has been completed (Figure 7–8). A medium-sized gene—say, 1500 nucleotide pairs—requires approximately 50 seconds for a molecule of RNA polymerase to transcribe it (Movie 7.2). At any given time, there could be dozens of polymerases speeding along this single stretch of DNA, hard on one another's heels, allowing more than 1000 transcripts to be synthesized in an hour. For most genes, however, the amount of transcription is much less than this.

Although RNA polymerase catalyzes essentially the same chemical reaction as DNA polymerase, there are some important differences between the two enzymes. First, and most obviously, RNA polymerase uses ribonucleoside for phosphates as substrates, so it catalyzes the linkage of ribonucleotides, not deoxyribonucleotides. Second, unlike the DNA polymerase involved in DNA replication, RNA polymerases can start an RNA chain without a primer. This difference likely evolved because transcription need not be as accurate as DNA replication; unlike DNA, RNA is not used as the permanent storage form of genetic information in cells, so mistakes in RNA transcripts have relatively minor consequences for a cell. RNA polymerases make about one mistake for every 10^4 nucleotides copied into RNA, whereas DNA polymerase makes only one mistake for every 10^7 nucleotides copied.

Cells Produce Various Types of RNA

The vast majority of genes carried in a cell's DNA specify the amino acid sequences of proteins. The RNA molecules encoded by these genes—which

Figure 7–7 DNA is transcribed into RNA by the enzyme RNA polymerase. RNA polymerase (pale blue) moves stepwise along the DNA, unwinding the DNA helix in front of it. As it progresses, the polymerase adds ribonucleotides one by one to the RNA chain, using an exposed DNA strand as a template. The resulting RNA transcript is thus single-stranded and complementary to this template strand (see Figure 7–6). As the polymerase moves along the DNA template (in the 3'-to-5' direction), it displaces the newly formed RNA, allowing the two strands of DNA behind the polymerase to rewind. A short region of hybrid DNA/RNA helix (approximately nine nucleotides in length) therefore forms only transiently, causing a "window" of DNA/RNA helix to move along the DNA with the polymerase (Movie 7.2).

QUESTION 7–2

In the electron micrograph in Figure 7–8, are the RNA polymerase molecules moving from right to left or from left to right? Why are the RNA transcripts so much shorter than the DNA segments (genes) that encode them?

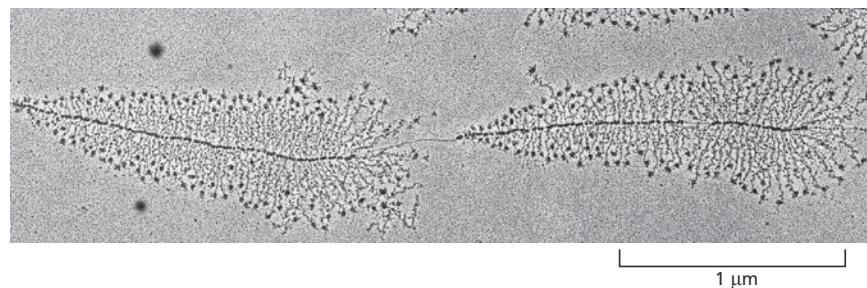


Figure 7–8 Transcription can be visualized in the electron microscope. The micrograph shows many molecules of RNA polymerase simultaneously transcribing two adjacent ribosomal genes on a single DNA molecule. Molecules of RNA polymerase are barely visible as a series of tiny dots along the spine of the DNA molecule; each polymerase has an RNA transcript (a short, fine thread) radiating from it. The RNA molecules being transcribed from the two ribosomal genes—ribosomal RNAs (rRNAs)—are not translated into protein, but are instead used directly as components of ribosomes, macromolecular machines made of RNA and protein. The large particles that can be seen at the free, 5' end of each rRNA transcript are believed to be ribosomal proteins that have assembled on the ends of the growing transcripts. (Courtesy of Ulrich Scheer.)

ultimately direct the synthesis of proteins—are called **messenger RNAs (mRNAs)**. In eukaryotes, each mRNA typically carries information transcribed from just one gene, which codes for a single protein; in bacteria, a set of adjacent genes is often transcribed as a single mRNA, which therefore carries the information for several different proteins.

The final product of other genes, however, is the RNA itself. As we see later, these nonmessenger RNAs, like proteins, have various roles, serving as regulatory, structural, and catalytic components of cells. They play key parts, for example, in translating the genetic message into protein: *ribosomal RNAs (rRNAs)* form the structural and catalytic core of the ribosomes, which translate mRNAs into protein, and *transfer RNAs (tRNAs)* act as adaptors that select specific amino acids and hold them in place on a ribosome for their incorporation into protein. Other small RNAs, called *microRNAs (miRNAs)*, serve as key regulators of eukaryotic gene expression, as we discuss in Chapter 8. The most common types of RNA are summarized in **Table 7–1**.

In the broadest sense, the term **gene expression** refers to the process by which the information encoded in a DNA sequence is translated into a product that has some effect on a cell or organism. In cases where the final product of the gene is a protein, gene expression includes both transcription and translation. When an RNA molecule is the gene's final product, however, gene expression does not require translation.

Signals in DNA Tell RNA Polymerase Where to Start and Finish Transcription

The initiation of transcription is an especially critical process because it is the main point at which the cell selects which proteins or RNAs are to be produced. To begin transcription, RNA polymerase must be able to recognize the start of a gene and bind firmly to the DNA at this site. The way in which RNA polymerases recognize the *transcription start site* of a gene differs somewhat between bacteria and eukaryotes. Because the situation in bacteria is simpler, we describe it first.

When an RNA polymerase collides randomly with a DNA molecule, the enzyme sticks weakly to the double helix and then slides rapidly along its length. RNA polymerase latches on tightly only after it has encountered a gene region called a **promoter**, which contains a specific sequence of nucleotides that lies immediately upstream of the starting point for RNA synthesis. Once bound tightly to this sequence, the RNA polymerase opens up the double helix immediately in front of the promoter to expose the nucleotides on each strand of a short stretch of DNA. One of the two exposed DNA strands then acts as a template for complementary base-pairing with incoming ribonucleoside triphosphates, two of which are

TABLE 7–1 TYPES OF RNA PRODUCED IN CELLS

Type of RNA	Function
messenger RNAs (mRNAs)	code for proteins
ribosomal RNAs (rRNAs)	form the core of the ribosome's structure and catalyze protein synthesis
microRNAs (miRNAs)	regulate gene expression
transfer RNAs (tRNAs)	serve as adaptors between mRNA and amino acids during protein synthesis
other noncoding RNAs	used in RNA splicing, gene regulation, telomere maintenance, and many other processes

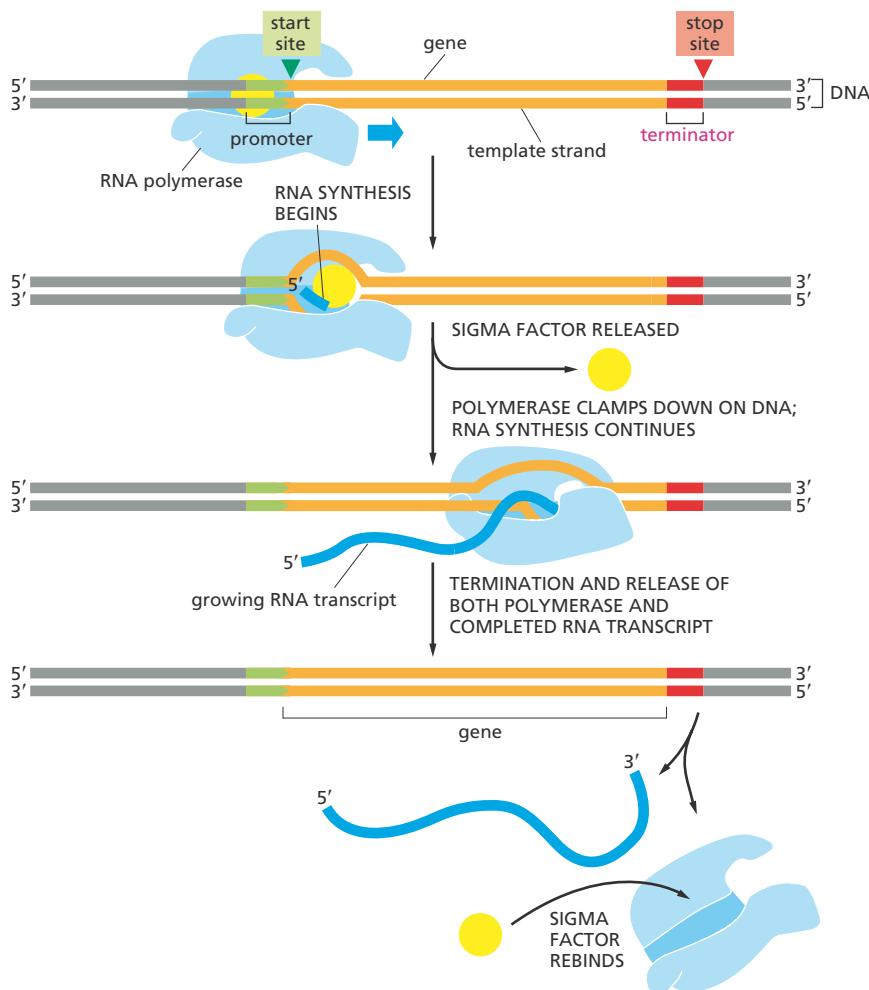


Figure 7–9 Signals in the nucleotide sequence of a gene tell bacterial RNA polymerase where to start and stop transcription. Bacterial RNA polymerase (light blue) contains a subunit called sigma factor (yellow) that recognizes the promoter of a gene (green). Once transcription has begun, sigma factor is released, and the polymerase moves forward and continues synthesizing the RNA. Chain elongation continues until the polymerase encounters a sequence in the gene called the terminator (red). There the enzyme halts and releases both the DNA template and the newly made RNA transcript. The polymerase then reassociates with a free sigma factor and searches for another promoter to begin the process again.

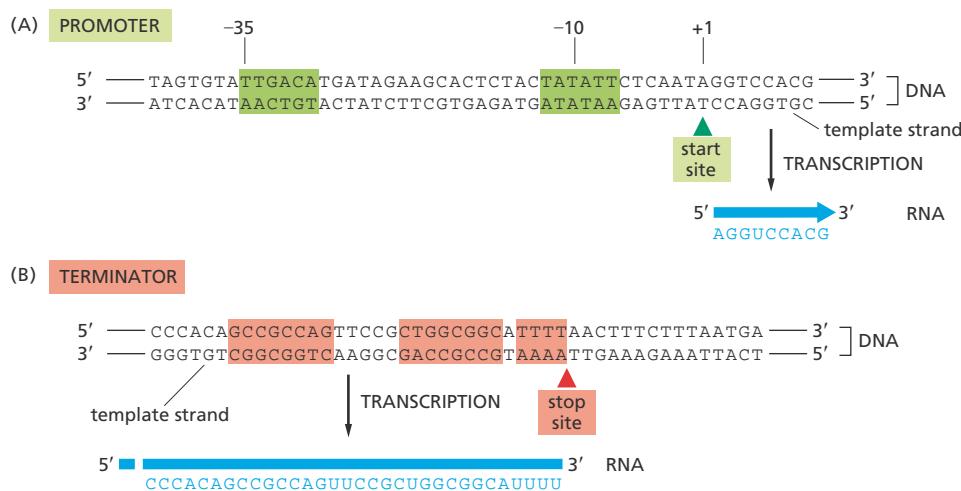
joined together by the polymerase to begin synthesis of the RNA chain. Chain elongation then continues until the enzyme encounters a second signal in the DNA, the *terminator* (or stop site), where the polymerase halts and releases both the DNA template and the newly made RNA transcript (Figure 7–9). This terminator sequence is contained within the gene and is transcribed into the 3' end of the newly made RNA.

Because the polymerase must bind tightly before transcription can begin, a segment of DNA will be transcribed only if it is preceded by a promoter. This ensures that those portions of a DNA molecule that contain a gene will be transcribed into RNA. The nucleotide sequences of a typical promoter—and a typical terminator—are presented in Figure 7–10.

In bacteria, it is a subunit of RNA polymerase, the *sigma* (σ) factor (see Figure 7–9), that is primarily responsible for recognizing the promoter sequence on the DNA. But how can this factor “see” the promoter, given that the base-pairs in question are situated in the interior of the DNA double helix? It turns out that each base presents unique features to the outside of the double helix, allowing the sigma factor to find the promoter sequence without having to separate the entwined DNA strands.

The next problem an RNA polymerase faces is determining which of the two DNA strands to use as a template for transcription: each strand has a different nucleotide sequence and would produce a different RNA transcript. The secret lies in the structure of the promoter itself. Every promoter has a certain polarity: it contains two different nucleotide sequences upstream of the transcriptional start site that position the RNA polymerase, ensuring that it binds to the promoter in only one orientation

Figure 7–10 Bacterial promoters and terminators have specific nucleotide sequences that are recognized by RNA polymerase. (A) The green-shaded regions represent the nucleotide sequences that specify a promoter. The numbers above the DNA indicate the positions of nucleotides counting from the first nucleotide transcribed, which is designated +1. The polarity of the promoter orients the polymerase and determines which DNA strand is transcribed. All bacterial promoters contain DNA sequences at –10 and –35 that closely resemble those shown here. (B) The red-shaded regions represent sequences in the gene that signal the RNA polymerase to terminate transcription. Note that the regions transcribed into RNA contain the terminator but not the promoter nucleotide sequences. By convention, the sequence of a gene is that of the non-template strand, as this strand has the same sequence as the transcribed RNA (with T substituting for U).



(see Figure 7–10A). Because the polymerase can only synthesize RNA in the 5'-to-3' direction once the enzyme is bound it must use the DNA strand oriented in the 3'-to-5' direction as its template.

This selection of a template strand does not mean that on a given chromosome, transcription always proceeds in the same direction. With respect to the chromosome as a whole, the direction of transcription varies from gene to gene. But because each gene typically has only one promoter, the orientation of its promoter determines in which direction that gene is transcribed and therefore which strand is the template strand (**Figure 7–11**).

Initiation of Eukaryotic Gene Transcription Is a Complex Process

Many of the principles we just outlined for bacterial transcription also apply to eukaryotes. However, transcription initiation in eukaryotes differs in several important ways from that in bacteria:

- The first difference lies in the RNA polymerases themselves. While bacteria contain a single type of RNA polymerase, eukaryotic cells have three—RNA polymerase I, RNA polymerase II, and RNA polymerase III. These polymerases are responsible for transcribing different types of genes. RNA polymerases I and III transcribe the genes encoding transfer RNA, ribosomal RNA, and various other RNAs that play structural and catalytic roles in the cell (**Table 7–2**). RNA polymerase II transcribes the vast majority of eukaryotic genes, including all those that encode proteins and miRNAs (**Movie 7.3**). Our subsequent discussion will therefore focus on RNA polymerase II.
- A second difference is that, whereas the bacterial RNA polymerase (along with its sigma subunit) is able to initiate transcription on its own, eukaryotic RNA polymerases require the assistance of a large set of accessory proteins. Principal among these are the *general transcription factors*, which must assemble at each promoter, along with the polymerase, before the polymerase can begin transcription.

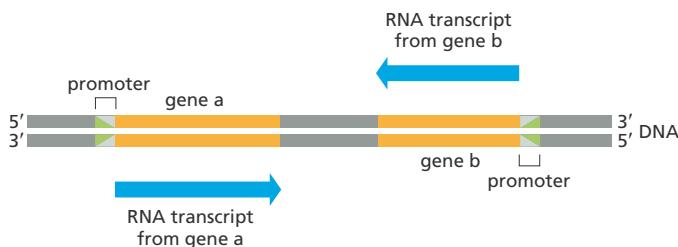


Figure 7–11 On an individual chromosome, some genes are transcribed using one DNA strand as a template, and others are transcribed from the other DNA strand. RNA polymerase always moves in the 3'-to-5' direction and the selection of the template strand is determined by the orientation of the promoter (green arrowheads) at the beginning of each gene. Thus the genes transcribed from left to right use the bottom DNA strand as the template (see Figure 7–10); those transcribed from right to left use the top strand as the template.

TABLE 7–2 THE THREE RNA POLYMERASES IN EUKARYOTIC CELLS

Type of Polymerase	Genes Transcribed
RNA polymerase I	most rRNA genes
RNA polymerase II	all protein-coding genes, miRNA genes, plus genes for other noncoding RNAs (e.g., those in spliceosomes)
RNA polymerase III	tRNA genes 5S rRNA gene genes for many other small RNAs

- A third distinctive feature of transcription in eukaryotes is that the mechanisms that control its initiation are much more elaborate than those in prokaryotes—a point we discuss in detail in Chapter 8. In bacteria, genes tend to lie very close to one another in the DNA, with only very short lengths of nontranscribed DNA between them. But in plants and animals, including humans, individual genes are spread out along the DNA, with stretches of up to 100,000 nucleotide pairs between one gene and the next. This architecture allows a single gene to be controlled by a large variety of *regulatory DNA sequences* scattered along the DNA, and it enables eukaryotes to engage in more complex forms of transcriptional regulation than do bacteria.
- Last but not least, eukaryotic transcription initiation must take into account the packing of DNA into *nucleosomes* and more compact forms of chromatin structure, as we describe in Chapter 8.

We now turn to the general transcription factors and discuss how they help eukaryotic RNA polymerase II initiate transcription.

Eukaryotic RNA Polymerase Requires General Transcription Factors

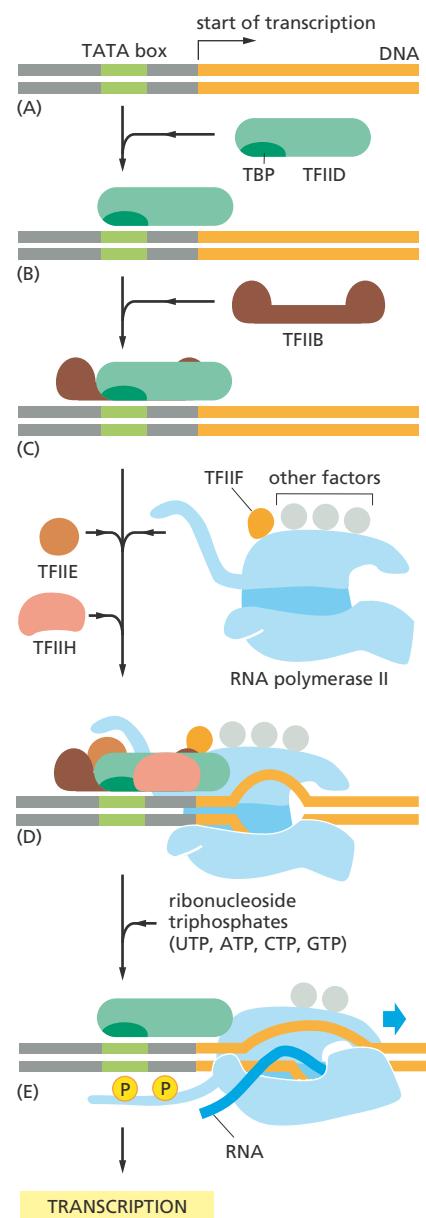
The initial finding that, unlike bacterial RNA polymerase, purified eukaryotic RNA polymerase II could not initiate transcription on its own in a test tube led to the discovery and purification of the **general transcription factors**. These accessory proteins assemble on the promoter, where they position the RNA polymerase and pull apart the DNA double helix to expose the template strand, allowing the polymerase to begin transcription. Thus the general transcription factors have a similar role in eukaryotic transcription as sigma factor has in bacterial transcription.

Figure 7–12 shows how the general transcription factors assemble at a promoter used by RNA polymerase II. The assembly process typically begins with the binding of the general transcription factor TFIID to a short

Figure 7–12 To begin transcription, eukaryotic RNA polymerase II requires a set of general transcription factors. These transcription factors are called TFIIB, TFIID, and so on. (A) Many eukaryotic promoters contain a DNA sequence called the TATA box. (B) The TATA box is recognized by a subunit of the general transcription factor TFIID, called the TATA-binding protein (TBP). For simplicity, the DNA distortion produced by the binding of the TBP (see Figure 7–13) is not shown. (C) The binding of TFIID enables the adjacent binding of TFIIB. (D) The rest of the general transcription factors, as well as the RNA polymerase itself, assemble at the promoter. (E) TFIIH then pries apart the double helix at the transcription start point, using the energy of ATP hydrolysis, which exposes the template strand of the gene (not shown). TFIIH also phosphorylates RNA polymerase II, releasing the polymerase from most of the general transcription factors, so it can begin transcription. The site of phosphorylation is a long polypeptide “tail” that extends from the polymerase.

QUESTION 7–3

Could the RNA polymerase used for transcription be used as the polymerase that makes the RNA primer required for DNA replication (discussed in Chapter 6)?



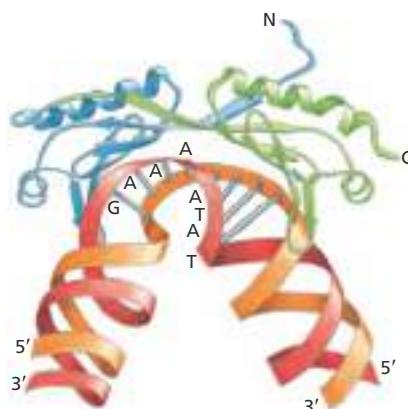


Figure 7–13 TATA-binding protein (TBP) binds to the TATA box (indicated by letters) and bends the DNA double helix. The unique distortion of DNA caused by TBP, which is a subunit of TFIID (see Figure 7–12), helps attract the other general transcription factors. TBP is a single polypeptide chain that is folded into two very similar domains (blue and green). The protein sits atop the DNA double helix like a saddle on a bucking horse (Movie 7.4). (Adapted from J.L. Kim et al., Nature 365:520–527, 1993. With permission from Macmillan Publishers Ltd.)

segment of DNA double helix composed primarily of T and A nucleotides; because of its composition, this part of the promoter is known as the *TATA box*. Upon binding to DNA, TFIID causes a dramatic local distortion in the DNA double helix (Figure 7–13), which helps to serve as a landmark for the subsequent assembly of other proteins at the promoter. The TATA box is a key component of many promoters used by RNA polymerase II, and it is typically located 25 nucleotides upstream from the transcription start site. Once TFIID has bound to the TATA box, the other factors assemble, along with RNA polymerase II, to form a complete *transcription initiation complex*. Although Figure 7–12 shows the general transcription factors piling onto the promoter in a certain order, the actual order of assembly probably differs from one promoter to the next.

After RNA polymerase II has been positioned on the promoter, it must be released from the complex of general transcription factors to begin its task of making an RNA molecule. A key step in liberating the RNA polymerase is the addition of phosphate groups to its “tail” (see Figure 7–12E). This liberation is initiated by the general transcription factor TFIIH, which contains a protein kinase as one of its subunits. Once transcription has begun, most of the general transcription factors dissociate from the DNA and then are available to initiate another round of transcription with a new RNA polymerase molecule. When RNA polymerase II finishes transcribing a gene, it too is released from the DNA; the phosphates on its tail are stripped off by protein phosphatases, and the polymerase is then ready to find a new promoter. Only the dephosphorylated form of RNA polymerase II can initiate RNA synthesis.

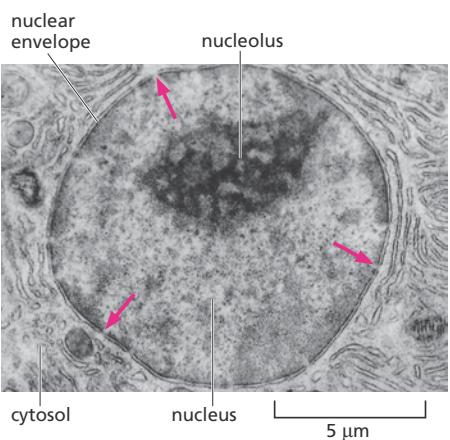


Figure 7–14 Before they can be translated, mRNA molecules made in the nucleus must be exported to the cytosol via pores in the nuclear envelope (red arrows). Shown here is a section of a liver cell nucleus. The nucleolus is where ribosomal RNAs are synthesized and combined with proteins to form ribosomes, which are then exported to the cytoplasm. (From D.W. Fawcett, A Textbook of Histology, 11th ed. Philadelphia: Saunders, 1986. With permission from Elsevier.)

Eukaryotic mRNAs Are Processed in the Nucleus

Although the templating principle by which DNA is transcribed into RNA is the same in all organisms, the way in which the RNA transcripts are handled before they can be used by the cell to make protein differs greatly between bacteria and eukaryotes. Bacterial DNA lies directly exposed to the cytoplasm, which contains the *ribosomes* on which protein synthesis takes place. As an mRNA molecule in a bacterium starts to be synthesized, ribosomes immediately attach to the free 5' end of the RNA transcript and begin translating it into protein.

In eukaryotic cells, by contrast, DNA is enclosed within the *nucleus*. Transcription takes place in the nucleus, but protein synthesis takes place on ribosomes in the cytoplasm. So, before a eukaryotic mRNA can be translated into protein, it must be transported out of the nucleus through small pores in the nuclear envelope (Figure 7–14). Before it can be exported to the cytosol, however, a eukaryotic RNA must go through several **RNA processing** steps, which include *capping*, *splicing*, and *polyadenylation*, as we discuss shortly. These steps take place as the RNA is being synthesized. The enzymes responsible for RNA processing ride on the phosphorylated tail of eukaryotic RNA polymerase II as it synthesizes an RNA molecule (see Figure 7–12), and they process the transcript as it emerges from the polymerase (Figure 7–15).

Different types of RNA are processed in different ways before leaving the nucleus. Two processing steps, capping and polyadenylation, occur only on RNA transcripts destined to become mRNA molecules (called *precursor mRNAs*, or *pre-mRNAs*).

- RNA capping** modifies the 5' end of the RNA transcript, the end that is synthesized first. The RNA is capped by the addition of an atypical nucleotide—a guanine (G) nucleotide bearing a methyl group, which is attached to the 5' end of the RNA in an unusual way (**Figure 7–16**). This capping occurs after RNA polymerase II has produced about 25 nucleotides of RNA, long before it has completed transcribing the whole gene.
- Polyadenylation** provides a newly transcribed mRNA with a special structure at its 3' end. In contrast with bacteria, where the 3' end of an mRNA is simply the end of the chain synthesized by the RNA polymerase, the 3' end of a forming eukaryotic mRNA is first trimmed by an enzyme that cuts the RNA chain at a particular sequence of nucleotides. The transcript is then finished off by a second enzyme that adds a series of repeated adenine (A) nucleotides to the cut end. This *poly-A tail* is generally a few hundred nucleotides long (see Figure 7–16A).

These two modifications—capping and polyadenylation—increase the stability of a eukaryotic mRNA molecule, facilitate its export from the nucleus to the cytoplasm, and generally mark the RNA molecule as an mRNA. They are also used by the protein-synthesis machinery to make sure that both ends of the mRNA are present and that the message is therefore complete before protein synthesis begins.

In Eukaryotes, Protein-Coding Genes Are Interrupted by Noncoding Sequences Called Introns

Most eukaryotic pre-mRNAs have to undergo an additional processing step before they are functional mRNAs. This step involves a far more radical modification of the pre-mRNA transcript than capping or polyadenylation, and it is the consequence of a surprising feature of most eukaryotic genes. In bacteria, most proteins are encoded by an uninterrupted stretch of DNA sequence that is transcribed into an mRNA that, without any further processing, can be translated into protein. Most protein-coding eukaryotic genes, in contrast, have their coding sequences interrupted by long, noncoding, *intervening sequences* called **introns**. The scattered pieces of coding sequence—called *expressed sequences* or

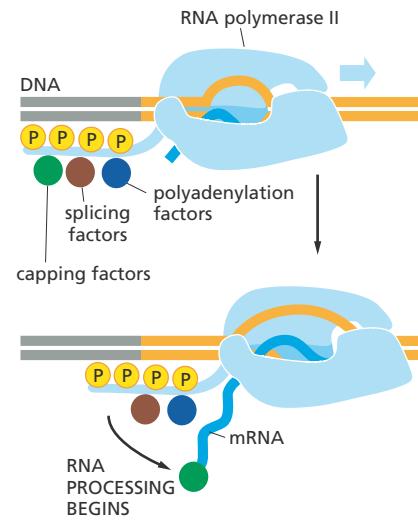


Figure 7–15 Phosphorylation of the tail of RNA polymerase II allows RNA-processing proteins to assemble there. Note that the phosphates shown here are in addition to the ones required for transcription initiation (see Figure 7–12). Capping, polyadenylation, and splicing are all modifications that occur during RNA processing in the nucleus.

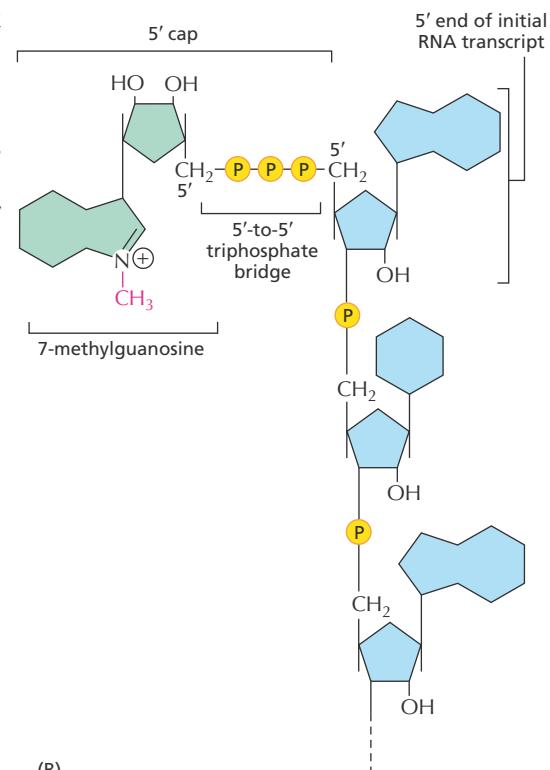
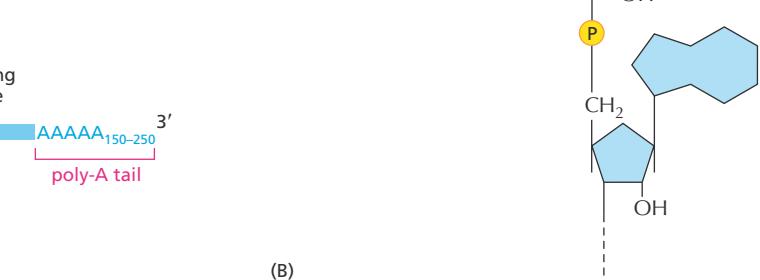
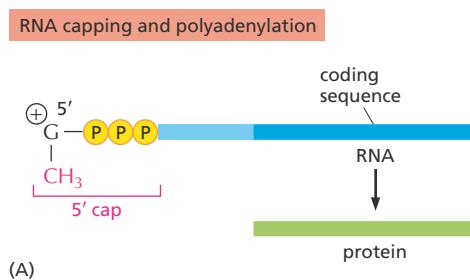


Figure 7–16 Eukaryotic pre-mRNA molecules are modified by capping and polyadenylation. (A) A eukaryotic mRNA has a cap at the 5' end and a poly-A tail at the 3' end. Note that not all of the RNA transcript shown codes for protein. (B) The structure of the 5' cap. Many eukaryotic mRNA caps carry an additional modification: the 2'-hydroxyl group on the second ribose sugar in the mRNA is methylated (not shown).



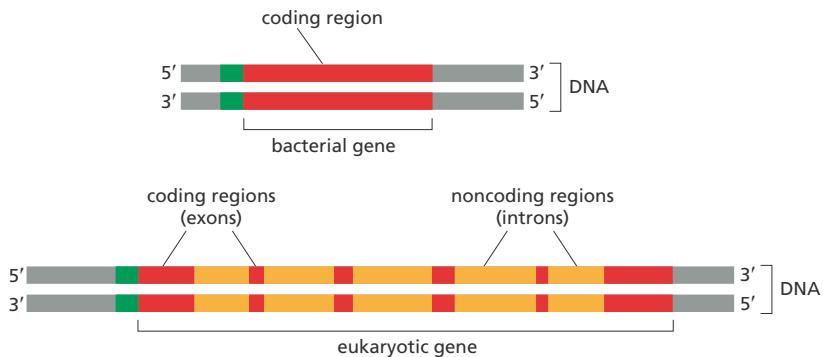


Figure 7–17 Eukaryotic and bacterial genes are organized differently. A bacterial gene consists of a single stretch of uninterrupted nucleotide sequence that encodes the amino acid sequence of a protein (or more than one protein). In contrast, the protein-coding sequences of most eukaryotic genes (exons) are interrupted by noncoding sequences (introns). Promoters for transcription are indicated in green.

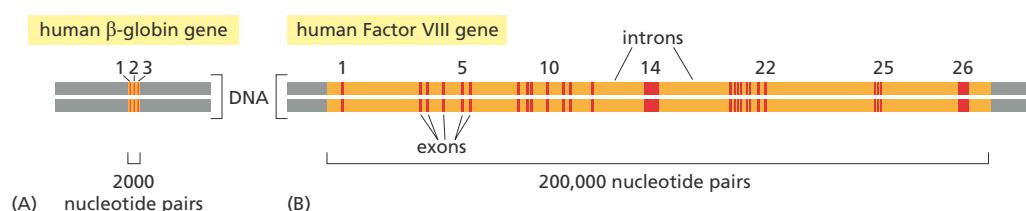
exons—are usually shorter than the introns, and they often represent only a small fraction of the total length of the gene (Figure 7–17). Introns range in length from a single nucleotide to more than 10,000 nucleotides. Some protein-coding eukaryotic genes lack introns altogether, and some have only a few; but most have many (Figure 7–18). Note that the terms “exon” and “intron” apply to both the DNA and the corresponding RNA sequences.

Introns Are Removed From Pre-mRNAs by RNA Splicing

To produce an mRNA in a eukaryotic cell, the entire length of the gene, introns as well as exons, is transcribed into RNA. After capping, and as RNA polymerase II continues to transcribe the gene, the process of **RNA splicing** begins, in which the introns are removed from the newly synthesized RNA and the exons are stitched together. Each transcript ultimately receives a poly-A tail; in some cases, this happens after splicing, whereas in other cases, it occurs before the final splicing reactions have been completed. Once a transcript has been spliced and its 5' and 3' ends have been modified, the RNA is now a functional mRNA molecule that can leave the nucleus and be translated into protein.

How does the cell determine which parts of the RNA transcript to remove during splicing? Unlike the coding sequence of an exon, most of the nucleotide sequence of an intron is unimportant. Although there is little overall resemblance between the nucleotide sequences of different introns, each intron contains a few short nucleotide sequences that act as cues for its removal from the pre-mRNA. These special sequences are found at or near each end of the intron and are the same or very similar in all introns (Figure 7–19). Guided by these sequences, an elaborate splicing machine cuts out the intron in the form of a “lariat” structure (Figure 7–20), formed by the reaction of the “A” nucleotide highlighted in red in Figures 7–19 and 7–20.

Figure 7–18 Most protein-coding human genes are broken into multiple exons and introns. (A) The β -globin gene, which encodes one of the subunits of the oxygen-carrying protein hemoglobin, contains 3 exons. (B) The Factor VIII gene, which encodes a protein (Factor VIII) that functions in the blood-clotting pathway, contains 26 exons. Mutations in this large gene are responsible for the most prevalent form of the blood disorder hemophilia.



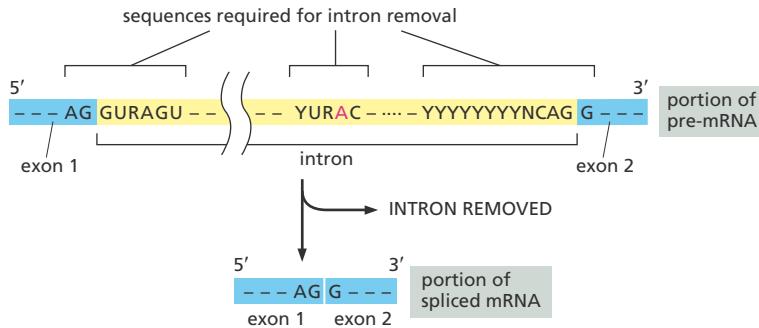


Figure 7–19 Special nucleotide sequences in a pre-mRNA transcript signal the beginning and the end of an intron. Only the nucleotide sequences shown are required to remove an intron; the other positions in an intron can be occupied by any nucleotide. The special sequences are recognized primarily by small nuclear ribonucleoproteins (snRNPs), which direct the cleavage of the RNA at the intron-exon borders and catalyze the covalent linkage of the exon sequences. Here, in addition to the standard symbols for nucleotides (A, C, G, U), R stands for either A or G; Y stands for either C or U; N stands for any nucleotide. The A shown in red forms the branch point of the lariat produced in the splicing reaction shown in Figure 7–20. The distances along the RNA between the three splicing sequences are highly variable; however, the distance between the branch point and the 5' splice junction is typically much longer than that between the 3' splice junction and the branch point (see Figure 7–20). The splicing sequences shown are from humans; similar sequences direct RNA splicing in other eukaryotes.

We will not describe the splicing machinery in detail, but it is worthwhile to note that, unlike the other steps of mRNA production we have discussed, RNA splicing is carried out largely by RNA molecules rather than proteins. These RNA molecules, called **small nuclear RNAs (snRNAs)**, are packaged with additional proteins to form *small nuclear ribonucleoproteins* (*snRNPs*, pronounced “snurps”). The snRNPs recognize splice-site sequences through complementary base-pairing between their RNA components and the sequences in the pre-mRNA, and they also participate intimately in the chemistry of splicing (Figure 7–21). Together, these snRNPs form the core of the **spliceosome**, the large assembly of RNA and protein molecules that carries out RNA splicing in the nucleus. To watch the spliceosome in action, see [Movie 7.5](#).

The intron-exon type of gene arrangement in eukaryotes may, at first, seem wasteful. It does, however, have a number of important benefits. First, the transcripts of many eukaryotic genes can be spliced in different ways, each of which can produce a distinct protein. Such **alternative splicing** thereby allows many different proteins to be produced from the same gene (Figure 7–22). About 95% of human genes are thought to undergo alternative splicing. Thus RNA splicing enables eukaryotes to increase the already enormous coding potential of their genomes.

RNA splicing also provides another advantage to eukaryotes, one that is likely to have been profoundly important in the early evolutionary history of genes. As we discuss in detail in Chapter 9, the intron-exon structure of genes is thought to have sped up the emergence of new and useful proteins: novel proteins appear to have arisen by the mixing and matching of different exons of preexisting genes, much like the assembly of a new type of machine from a kit of preexisting functional components. Indeed, many proteins in present-day cells resemble patchworks composed from a common set of protein pieces, called protein *domains* (see Figure 4–51).

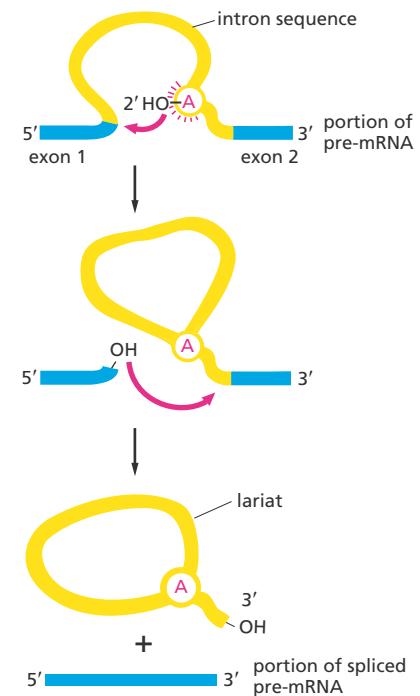
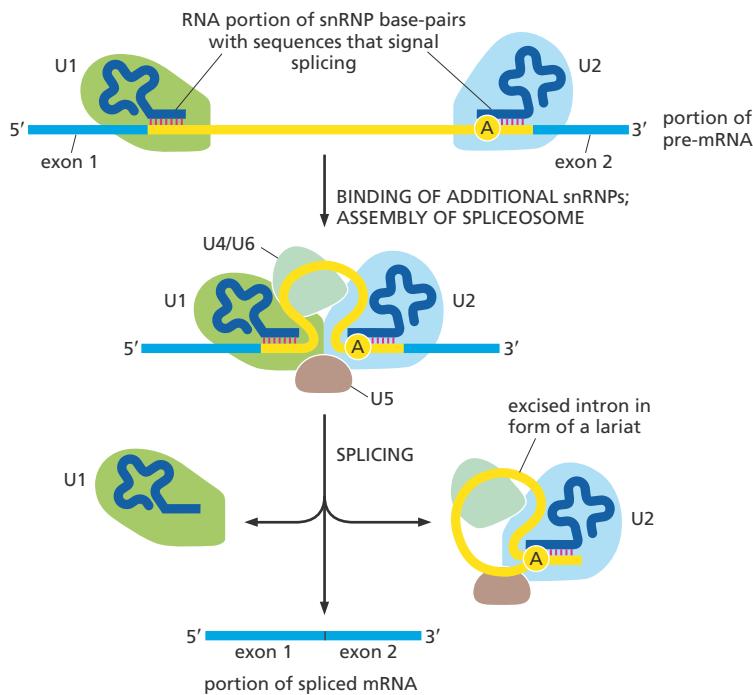


Figure 7–20 An intron in a pre-mRNA molecule forms a branched structure during RNA splicing. In the first step, the branch point adenine (red A) in the intron sequence attacks the 5' splice site and cuts the sugar-phosphate backbone of the RNA at this point (this is the same A highlighted in red in Figure 7–19). In this process, the cut 5' end of the intron becomes covalently linked to the 2'-OH group of the ribose of the A nucleotide to form a branched structure. The free 3'-OH end of the exon sequence then reacts with the start of the next exon sequence, joining the two exons together into a continuous coding sequence and releasing the intron in the form of a lariat structure, which is eventually degraded in the nucleus.

Figure 7–21 Splicing is carried out by a collection of RNA–protein complexes called snRNPs. There are five snRNPs, called U1, U2, U4, U5, and U6. As shown here, U1 and U2 bind to the 5' splice site (U1) and the lariat branch point (U2) through complementary base-pairing. Additional snRNPs are attracted to the splice site, and interactions between their protein components drive the assembly of the complete spliceosome. Rearrangements in the base pairs that hold together the snRNPs and the RNA transcript then reorganize the spliceosome to form the active site that excises the intron, leaving the spliced mRNA behind (see also Figure 7–20).



Mature Eukaryotic mRNAs Are Exported from the Nucleus

We have seen how eukaryotic pre-mRNA synthesis and processing take place in an orderly fashion within the cell nucleus. However, these events create a special problem for eukaryotic cells: of the total number of pre-mRNA transcripts that are synthesized, only a small fraction—the mature mRNAs—will be useful to the cell. The remaining RNA fragments—excised introns, broken RNAs, and aberrantly spliced transcripts—are not only useless, but they could be dangerous to the cell if allowed to leave the nucleus. How, then, does the cell distinguish between the relatively rare mature mRNA molecules it needs to export to the cytosol and the overwhelming amount of debris generated by RNA processing?

The answer is that the transport of mRNA from the nucleus to the cytosol, where mRNAs are translated into protein, is highly selective: only correctly processed mRNAs are exported. This selective transport is mediated by *nuclear pore complexes*, which connect the nucleoplasm with the cytosol and act as gates that control which macromolecules can enter or leave the nucleus (discussed in Chapter 15). To be “export ready,” an mRNA molecule must be bound to an appropriate set of proteins, each of which recognizes different parts of a mature mRNA molecule. These proteins include poly-A-binding proteins, a cap-binding complex, and

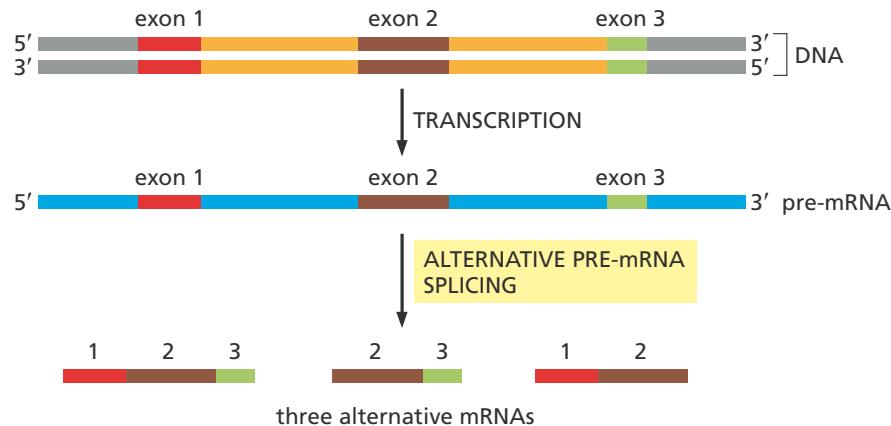
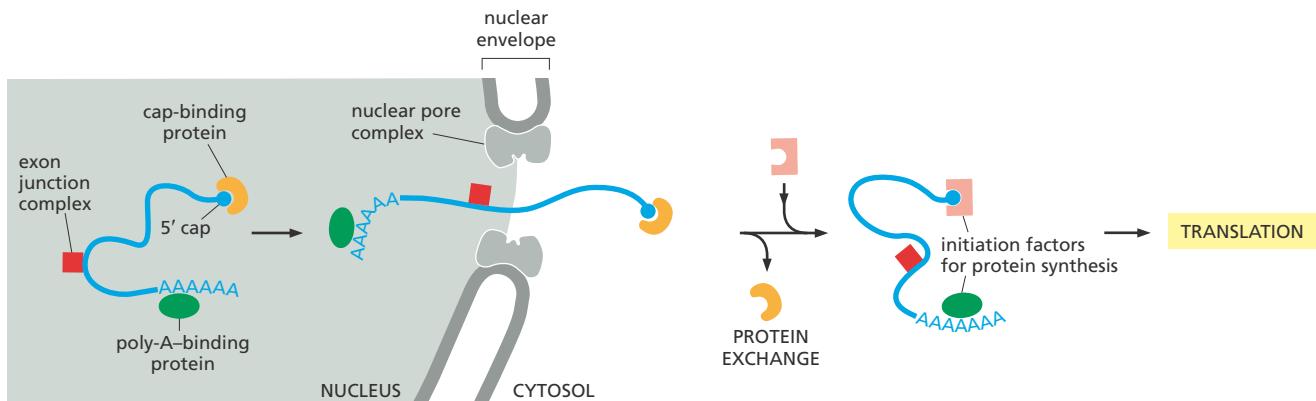


Figure 7–22 Some pre-mRNAs undergo alternative RNA splicing to produce various mRNAs and proteins from the same gene. Whereas all exons are present in a pre-mRNA, some exons can be excluded from the final mRNA molecule. In this example, three of four possible mRNAs are produced. The 5' caps and poly-A tails on the mRNAs are not shown.



proteins that bind to mRNAs that have been appropriately spliced (Figure 7–23). The entire set of bound proteins, rather than any single protein, ultimately determines whether an mRNA molecule will leave the nucleus. The “waste RNAs” that remain behind in the nucleus are degraded there, and their nucleotide building blocks are reused for transcription.

mRNA Molecules Are Eventually Degraded in the Cytosol

Because a single mRNA molecule can be translated into protein many times (see Figure 7–2), the length of time that a mature mRNA molecule persists in the cell affects the amount of protein it produces. Each mRNA molecule is eventually degraded into nucleotides by ribonucleases (RNases) present in the cytosol, but the lifetimes of mRNA molecules differ considerably—depending on the nucleotide sequence of the mRNA and the type of cell. In bacteria, most mRNAs are degraded rapidly, having a typical lifetime of about 3 minutes. The mRNAs in eukaryotic cells usually persist longer: some, such as those encoding β -globin, have lifetimes of more than 10 hours, whereas others have lifetimes of less than 30 minutes.

These different lifetimes are in part controlled by nucleotide sequences in the mRNA itself, most often in the portion of RNA called the *3' untranslated region*, which lies between the 3' end of the coding sequence and the poly-A tail. The different lifetimes of mRNAs help the cell control the amount of each protein that it synthesizes. In general, proteins made in large amounts, such as β -globin, are translated from mRNAs that have long lifetimes, whereas proteins made in smaller amounts, or whose levels must change rapidly in response to signals, are typically synthesized from short-lived mRNAs.

The Earliest Cells May Have Had Introns in Their Genes

The process of transcription is universal: all cells use RNA polymerase and complementary base-pairing to synthesize RNA from DNA. Indeed, bacterial and eukaryotic RNA polymerases are almost identical in overall structure and clearly evolved from a shared ancestral polymerase. It may therefore seem puzzling that the resulting RNA transcripts are handled so differently in eukaryotes and in prokaryotes (Figure 7–24). In particular, RNA splicing seems to mark a fundamental difference between those two types of cells. But how did this dramatic difference arise?

As we have seen, RNA splicing provides eukaryotes with the ability to produce a variety of proteins from a single gene. It also allows them to evolve new genes by mixing-and-matching exons from preexisting genes, as we discuss in Chapter 9. However, these advantages come with a cost: the cell has to maintain a larger genome and has to discard a

Figure 7–23 A specialized set of RNA-binding proteins signals that a mature mRNA is ready for export to the cytosol. As indicated on the left, the cap and poly-A tail of a mature mRNA molecule are “marked” by proteins that recognize these modifications. In addition, a group of proteins called the exon junction complex is deposited on the pre-mRNA after each successful splice has occurred. Once the mRNA is deemed “export ready,” a nuclear transport receptor (discussed in Chapter 15) associates with the mRNA and guides it through the nuclear pore. In the cytosol, the mRNA can shed some of these proteins and bind new ones, which, along with poly-A-binding protein, act as initiation factors for protein synthesis, as we discuss later.

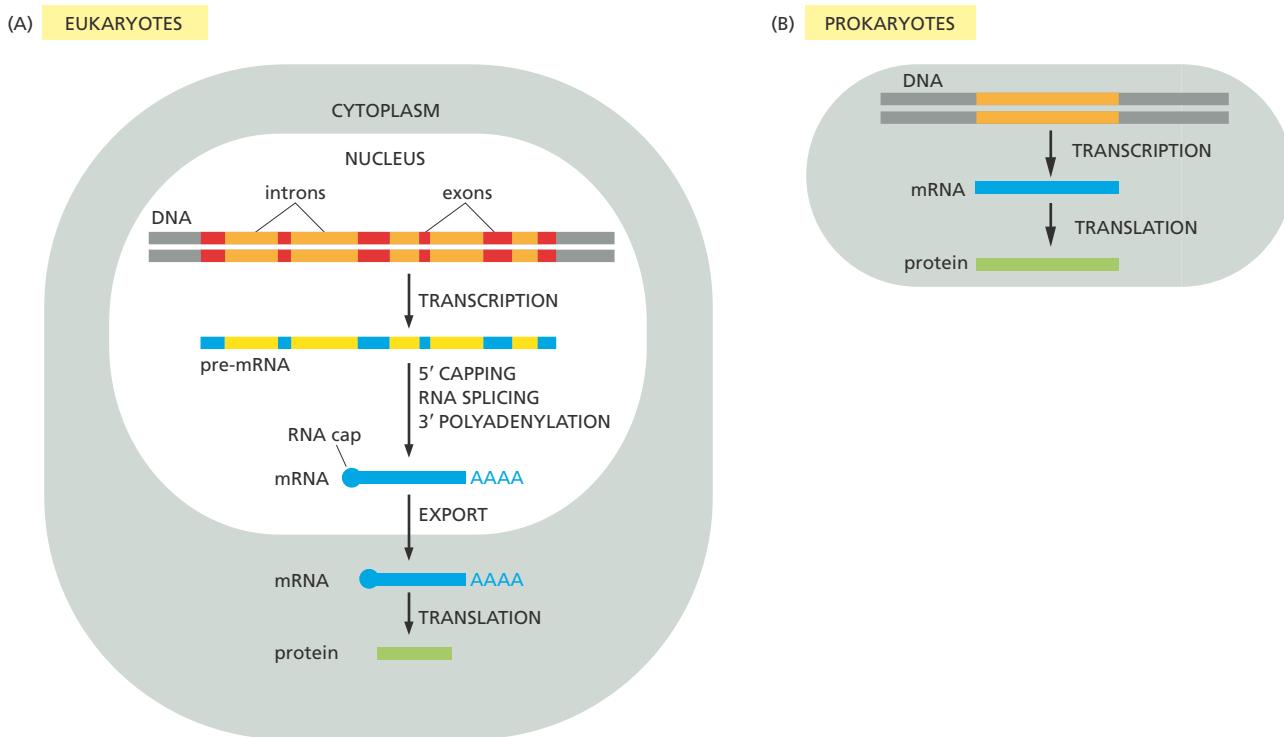


Figure 7-24 Prokaryotes and eukaryotes handle their RNA transcripts differently. (A) In eukaryotic cells, the pre-mRNA molecule produced by transcription contains both intron and exon sequences. Its two ends are modified, and the introns are removed by RNA splicing. The resulting mRNA is then transported from the nucleus to the cytoplasm, where it is translated into protein. Although these steps are depicted as occurring in sequence, one at a time, in reality they occur simultaneously. For example, the RNA cap is usually added and splicing usually begins before transcription has been completed. Because of this overlap, transcripts of the entire gene (including all introns and exons) do not typically exist in the cell. (B) In prokaryotes, the production of mRNA molecules is simpler. The 5' end of an mRNA molecule is produced by the initiation of transcription by RNA polymerase, and the 3' end is produced by the termination of transcription. Because prokaryotic cells lack a nucleus, transcription and translation take place in a common compartment. Translation of a bacterial mRNA can therefore begin before its synthesis has been completed. In both eukaryotes and prokaryotes, the amount of a protein in a cell depends on the rates of each of these steps, as well as on the rates of degradation of the mRNA and protein molecules.

large fraction of the RNA it synthesizes without ever using it. According to one school of thought, early cells—the common ancestors of prokaryotes and eukaryotes—contained introns that were lost in prokaryotes during subsequent evolution. By shedding their introns and adopting a smaller, more streamlined genome, prokaryotes would have been able to reproduce more rapidly and efficiently. Consistent with this idea, simple eukaryotes that reproduce rapidly (some yeasts, for example) have relatively few introns, and these introns are usually much shorter than those found in higher eukaryotes.

On the other hand, some argue that introns were originally parasitic mobile genetic elements (discussed in Chapter 9) that happened to invade an early eukaryotic ancestor, colonizing its genome. These host cells then unwittingly replicated the “stowaway” nucleotide sequences along with their own DNA; modern eukaryotes simply never bothered to sweep away the genetic clutter left from that ancient infection. The issue, however, is far from settled; whether introns evolved early—and were lost in prokaryotes—or evolved later in eukaryotes is still a topic of scientific debate, and we return to it in Chapter 9.

FROM RNA TO PROTEIN

By the end of the 1950s, biologists had demonstrated that the information encoded in DNA is copied first into RNA and then into protein. The debate then shifted to the “coding problem”: How is the information in a linear sequence of nucleotides in an RNA molecule translated into the linear sequence of a chemically quite different set of subunits—the amino acids in a protein? This fascinating question intrigued scientists at the time. Here was a cryptogram set up by nature that, after more than 3 billion years of evolution, could finally be solved by one of the products of evolution—human beings! Indeed, scientists have not only cracked the code but have revealed, in atomic detail, the precise workings of the machinery by which cells read this code.

codons	AGA		UUA		AGC																
	AGG		UUG		AGU																
	GCA	CGA		CUA		ACA															
	GCC	CGC	GGA	CUC	ACC	GUA															
	GCG	CGG	GAC	AAC	GAA	GGG	AUA	CCC	UCA	ACA	GUC	UAA									
	GCU	CGU	GAU	AAU	UGC	GAG	CAG	CAU	AUC	CUG	AAA	UUC	CCG	UCG	ACG	UAC	GUU	UAG	UGA		
amino acids	Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
	A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 7–25 The nucleotide sequence of an mRNA is translated into the amino acid sequence of a protein via the genetic code.

All the three-nucleotide codons in mRNAs that specify a given amino acid are listed above that amino acid, which is given in both its three-letter and one-letter abbreviations (see Panel 2–5, pp. 74–75, for the full name of each amino acid and its structure). Like RNA molecules, codons are always written with the 5'-terminal nucleotide to the left. Note that most amino acids are represented by more than one codon, and there are some regularities in the set of codons that specify each amino acid. Codons for the same amino acid tend to contain the same nucleotides at the first and second positions and to vary at the third position. There are three codons that do not specify any amino acid but act as termination sites (**stop codons**), signaling the end of the protein-coding sequence in an mRNA. One codon—AUG—acts both as an initiation codon, signaling the start of a protein-coding message, and as the codon that specifies the amino acid methionine.

An mRNA Sequence Is Decoded in Sets of Three Nucleotides

Transcription as a means of information transfer is simple to understand: DNA and RNA are chemically and structurally similar, and DNA can act as a direct template for the synthesis of RNA through complementary base-pairing. As the term transcription signifies, it is as if a message written out by hand were being converted, say, into a typewritten text. The language itself and the form of the message do not change, and the symbols used are closely related.

In contrast, the conversion of the information in RNA into protein represents a **translation** of the information into another language that uses different symbols. Because there are only 4 different nucleotides in mRNA but 20 different types of amino acids in a protein, this translation cannot be accounted for by a direct one-to-one correspondence between a nucleotide in RNA and an amino acid in protein. The rules by which the nucleotide sequence of a gene, through an intermediary mRNA molecule, is translated into the amino acid sequence of a protein are known as the **genetic code**.

In 1961, it was discovered that the sequence of nucleotides in an mRNA molecule is read consecutively in groups of three. And because RNA is made of 4 different nucleotides, there are $4 \times 4 \times 4 = 64$ possible combinations of three nucleotides: AAA, AUA, AUG, and so on. However, only 20 different amino acids are commonly found in proteins. Either some nucleotide triplets are never used, or the code is redundant, with some amino acids being specified by more than one triplet. The second possibility turned out to be correct, as shown by the completely deciphered genetic code shown in **Figure 7–25**. Each group of three consecutive nucleotides in RNA is called a **codon**, and each codon specifies one amino acid. The strategy by which this code was cracked is described in **How We Know**, pp. 240–241.

The same genetic code is used in nearly all present-day organisms. Although a few slight differences have been found, these occur chiefly in the mRNA of mitochondria and of some fungi and protozoa. Mitochondria have their own DNA replication, transcription, and protein-synthesis machinery, which operates independently from the corresponding machinery in the rest of the cell (discussed in Chapter 14), and they have been able to accommodate minor changes to the otherwise universal genetic code. Even in fungi and protozoa, the similarities in the code far outweigh the differences.

In principle, an mRNA sequence can be translated in any one of three different **reading frames**, depending on where the decoding process begins (**Figure 7–26**). However, only one of the three possible reading frames

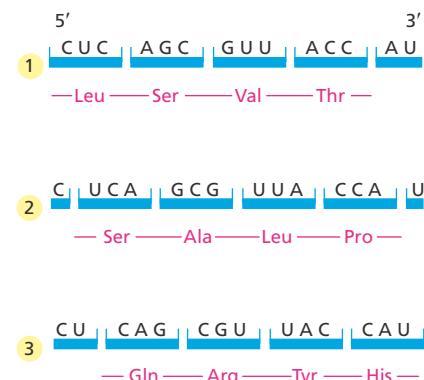


Figure 7–26 In principle, an mRNA molecule can be translated in three possible reading frames. In the process of translating a nucleotide sequence (blue) into an amino acid sequence (red), the sequence of nucleotides in an mRNA molecule is read from the 5' to the 3' end in sequential sets of three nucleotides. In principle, therefore, the same mRNA sequence can specify three completely different amino acid sequences, depending on where translation begins—that is, on the reading frame used. In reality, however, only one of these reading frames encodes the actual message and is therefore used in translation, as we discuss later.

CRACKING THE GENETIC CODE

By the beginning of the 1960s, the *central dogma* had been accepted as the pathway along which information flows from gene to protein. It was clear that genes encode proteins, that genes are made of DNA, and that mRNA serves as an intermediary, carrying the information from DNA to the ribosome, where the RNA is translated into protein.

Even the general format of the genetic code had been worked out: each of the 20 amino acids found in proteins is represented by a triplet codon in an mRNA molecule. But an even greater challenge remained: biologists, chemists, and even physicists set their sights on breaking the genetic code—attempting to figure out which amino acid each of the 64 possible nucleotide triplets designates. The most straightforward path to the solution would have been to compare the sequence of a segment of DNA or of mRNA with its corresponding polypeptide product. Techniques for sequencing nucleic acids, however, would not be devised for another 10 years.

So researchers decided that, to crack the genetic code, they would have to synthesize their own simple RNA molecules. If they could feed these RNA molecules to ribosomes—the machines that make proteins—and then analyze the resulting polypeptide product, they would be on their way to deciphering which triplets encode which amino acids.

Losing the cells

Before researchers could test their synthetic mRNAs, they needed to perfect a cell-free system for protein synthesis. This would allow them to translate their messages into polypeptides in a test tube. (Generally speaking, when working in the laboratory, the simpler the system, the easier it is to interpret the results.) To isolate the molecular machinery they needed for such a cell-free translation system, researchers broke open *E. coli* cells and loaded their contents into a centrifuge tube. Spinning these samples at high speed caused the membranes and other large chunks of cellular debris to be dragged to the bottom of the tube; the lighter cellular components required for protein synthesis—including mRNA, the tRNA adaptors, ribosomes, enzymes, and other small molecules—were left floating in the supernatant. Researchers found that simply adding radioactive amino acids to this cell “soup” would trigger the production of radiolabeled polypeptides. By centrifuging this supernatant again, at a higher speed, the researchers could force the ribosomes, and any newly synthesized peptides attached to them, to the bottom of the tube; the labeled polypeptides could then be detected by measuring the radioactivity in the sediment remaining in the tube after the top layer had been discarded.

The trouble with this particular system was that it produced proteins encoded by the cell's own mRNAs already present in the extract. But researchers wanted to use their own synthetic messages to direct protein synthesis. This problem was solved when Marshall Nirenberg discovered that he could destroy the cells' mRNA in the extract by adding a small amount of ribonuclease—an enzyme that degrades RNA—to the mix. Now all he needed to do was prepare large quantities of synthetic mRNA, add it to the cell-free system, and see what peptides came out.

Faking the message

Producing a synthetic polynucleotide with a defined sequence was not as simple as it sounds. Again, it would be years before chemists and bioengineers developed machines that could synthesize any given string of nucleic acids quickly and cheaply. Nirenberg decided to use polynucleotide phosphorylase, an enzyme that would join ribonucleotides together in the absence of a template. The sequence of the resulting RNA would then depend entirely on which nucleotides were presented to the enzyme. A mixture of nucleotides would be sewn into a random sequence; but a single type of nucleotide would yield a homogeneous polymer containing only that one nucleotide. Thus Nirenberg, working with his collaborator Heinrich Matthaei, first produced synthetic mRNAs made entirely of uracil—poly U.

Together, the researchers fed this poly U to their cell-free translation system. They then added a single type of radioactively labeled amino acid to the mix. After testing each amino acid—one at a time, in 20 different experiments—they determined that poly U directs the synthesis of a polypeptide containing only phenylalanine (**Figure 7–27**). With this electrifying result, the first word in the genetic code had been deciphered (see Figure 7–25).

Nirenberg and Matthaei then repeated the experiment with poly A and poly C and determined that AAA codes for lysine and CCC for proline. The meaning of poly G could not be ascertained by this method because this polynucleotide forms an odd triple-stranded helix that did not serve as a template in the cell-free system.

Feeding ribosomes with synthetic RNA seemed a fruitful technique. But with the single-nucleotide possibilities exhausted, researchers had nailed down only three codons; they had 61 still to go. The other codons, however, were harder to decipher, and a new synthetic approach was needed. In the 1950s, the organic chemist Gobind Khorana had been developing methods for preparing mixed polynucleotides of defined sequence—but his techniques worked only for DNA. When he

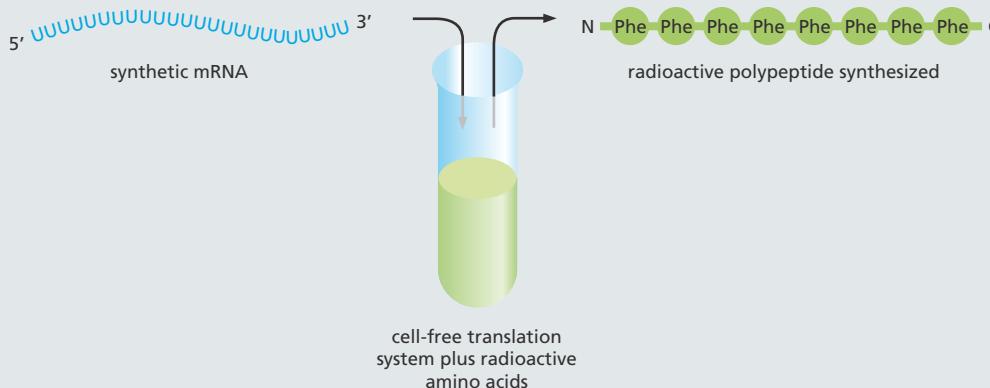


Figure 7–27 UUU codes for phenylalanine. Synthetic mRNAs are fed into a cell-free translation system containing bacterial ribosomes, tRNAs, enzymes, and other small molecules. Radioactive amino acids are added to this mix and the resulting polypeptides analyzed. In this case, poly U is shown to encode a polypeptide containing only phenylalanine.

learned of Nirenberg's work with synthetic RNAs, Khorana directed his energies and skills to producing polyribonucleotides. He found that if he started out by making DNAs of a defined sequence, he could then use RNA polymerase to produce RNAs from those. In this way, Khorana prepared a collection of different RNAs of defined repeating sequence: he generated sequences of repeating dinucleotides (such as poly UC), trinucleotides (such as poly UUC), or tetranucleotides (such as poly UAUC).

These mixed polynucleotides, however, yielded results that were much more difficult to decode than the mononucleotide messages that Nirenberg had used. Take poly UG, for example. When this repeating dinucleotide is added to the translation system, researchers discovered that it codes for a polypeptide of alternating cysteines and valines. This RNA, of course, contains two different alternating codons: UGU and GUG. So researchers could say that UGU and GUG code for cysteine and valine, although they could not tell which went with which. Thus these mixed messages provided useful information, but they did not definitively reveal which codons specified which amino acids (Figure 7–28).

Trapping the triplets

These final ambiguities in the code were resolved when Nirenberg and a young medical graduate named Phil Leder discovered that RNA fragments that were only three nucleotides in length—the size of a single codon—could bind to a ribosome and attract the appropriate amino-acid-containing tRNA molecule to the protein-making machinery. These complexes—containing one ribosome, one mRNA codon, and one radiolabeled aminoacyl-tRNA—could then be captured on a piece of filter paper and the attached amino acid identified.

Their trial run with UUU—the first word—worked splendidly. Leder and Nirenberg primed the usual cell-free translation system with snippets of UUU. These

trinucleotides bound to the ribosomes, and Phe-tRNAs bound to the UUU. The new system was up and running, and the researchers had confirmed that UUU codes for phenylalanine.

All that remained was for researchers to produce all 64 possible codons—a task that was quickly accomplished in both Nirenberg's and Khorana's laboratories. Because these small trinucleotides were much simpler to synthesize chemically, and the triplet-trapping tests were easier to perform and analyze than the previous decoding experiments, the researchers were able to work out the complete genetic code within the next year.

MESSAGE	PEPTIDES PRODUCED	CODON ASSIGNMENTS
poly UG	...Cys–Val–Cys–Val...	UGU GUG] Cys, Val*
poly AG	...Arg–Glu–Arg–Glu...	AGA GAG] Arg, Glu
poly UUC	...Phe–Phe–Phe... + ...Ser–Ser–Ser... + ...Leu–Leu–Leu...	UUC UCU CUU] Phe, Ser, Leu
poly UAUC	...Tyr–Leu–Ser–Ile...	UAU CUA UCU AUC] Tyr, Leu, Ser, Ile

* One codon specifies Cys, the other Val, but which is which? The same ambiguity exists for the other codon assignments shown here.

Figure 7–28 Using synthetic RNAs of mixed, repeating ribonucleotide sequences, scientists further narrowed the coding possibilities. Although these mixed messages produced mixed polypeptides, they did not permit the unambiguous assignment of a single codon to a specific amino acid. For example, the results of the poly-UG experiment cannot distinguish whether UGU or GUG encodes cysteine. As indicated, the same type of ambiguity confounded the interpretation of all the experiments using di-, tri-, and tetranucleotides.

in an mRNA specifies the correct protein. We discuss later how a special punctuation signal at the beginning of each mRNA molecule sets the correct reading frame.

tRNA Molecules Match Amino Acids to Codons in mRNA

The codons in an mRNA molecule do not directly recognize the amino acids they specify: the group of three nucleotides does not, for example, bind directly to the amino acid. Rather, the translation of mRNA into protein depends on adaptor molecules that can recognize and bind to a codon at one site on their surface and to an amino acid at another site. These adaptors consist of a set of small RNA molecules known as **transfer RNAs** (**tRNAs**), each about 80 nucleotides in length.

We saw earlier that an RNA molecule generally folds into a three-dimensional structure by forming base pairs between different regions of the molecule. If the base-paired regions are sufficiently extensive, they will fold back on themselves to form a double-helical structure, like that of double-stranded DNA. The tRNA molecule provides a striking example of this. Four short segments of the folded tRNA are double-helical, producing a molecule that looks like a cloverleaf when drawn schematically (**Figure 7–29A**). For example, a 5'-GCUC-3' sequence in one part of a polynucleotide chain can base-pair with a 5'-GAGC-3' sequence in another region of the same molecule. The cloverleaf undergoes further folding to form a compact, L-shaped structure that is held together by additional hydrogen bonds between different regions of the molecule (**Figure 7–29B and C**).

Two regions of unpaired nucleotides situated at either end of the L-shaped tRNA molecule are crucial to the function of tRNAs in protein synthesis. One of these regions forms the **anticodon**, a set of three consecutive nucleotides that bind, through base-pairing, to the complementary codon

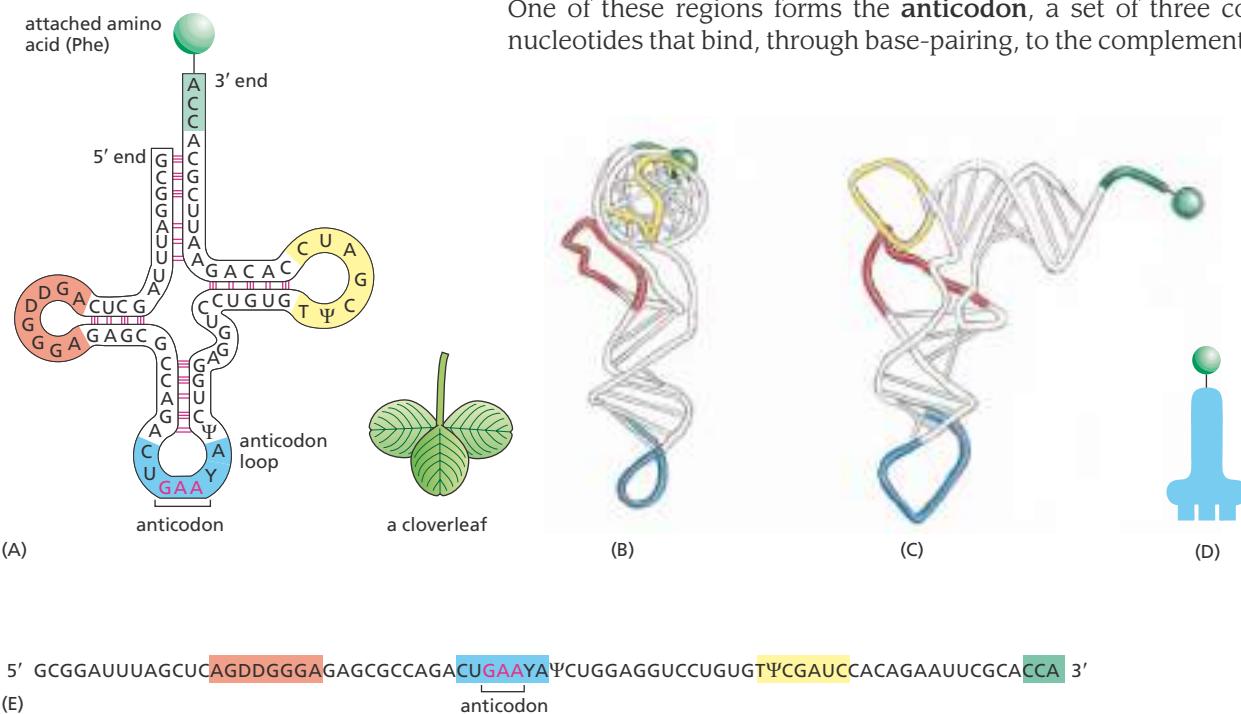


Figure 7–29 tRNA molecules are molecular adaptors, linking amino acids to codons. In this series of diagrams, the same tRNA molecule—in this case, a tRNA specific for the amino acid phenylalanine (Phe)—is depicted in various ways. (A) The conventional “cloverleaf” structure shows the complementary base-pairing (red lines) that creates the double-helical regions of the molecule. The anticodon loop (blue) contains the sequence of three nucleotides (red letters) that base-pairs with a codon in mRNA. The amino acid matching the codon–anticodon pair is attached at the 3' end of the tRNA. tRNAs contain some unusual bases, which are produced by chemical modification after the tRNA has been synthesized. The bases denoted Ψ (for pseudouridine) and D (for dihydrouridine) are derived from uracil. (B and C) Views of the actual L-shaped molecule, based on X-ray diffraction analysis. These two images are rotated 90° with respect to each other. (D) Schematic representation of tRNA, emphasizing the anticodon, that will be used in subsequent figures. (E) The linear nucleotide sequence of the tRNA molecule, color-coded to match A, B, and C.

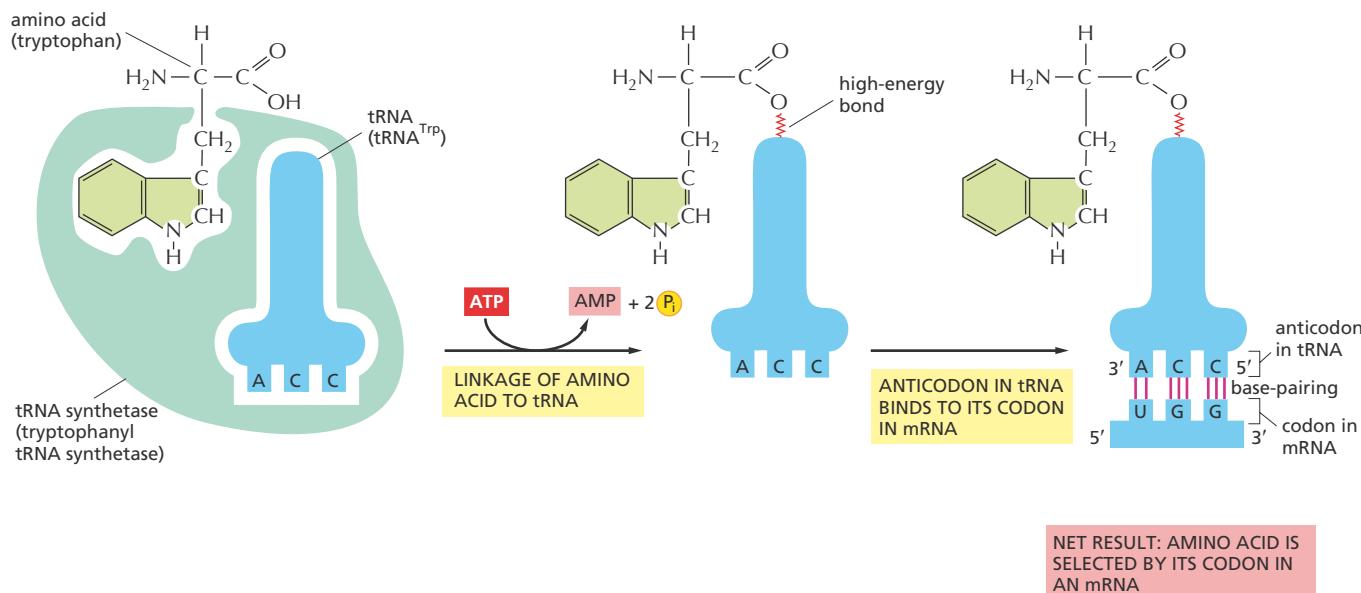
in an mRNA molecule. The other is a short single-stranded region at the 3' end of the molecule; this is the site where the amino acid that matches the codon is covalently attached to the tRNA.

We saw in the previous section that the genetic code is redundant; that is, several different codons can specify a single amino acid (see Figure 7–25). This redundancy implies either that there is more than one tRNA for many of the amino acids or that some tRNA molecules can base-pair with more than one codon. In fact, both situations occur. Some amino acids have more than one tRNA, and some tRNAs are constructed so that they require accurate base-pairing only at the first two positions of the codon and can tolerate a mismatch (or *wobble*) at the third position. This wobble base-pairing explains why so many of the alternative codons for an amino acid differ only in their third nucleotide (see Figure 7–25). Wobble base-pairings make it possible to fit the 20 amino acids to their 61 codons with as few as 31 kinds of tRNA molecules. The exact number of different kinds of tRNAs, however, differs from one species to the next. For example, humans have nearly 500 different tRNA genes, but only 48 anticodons are represented among them.

Specific Enzymes Couple tRNAs to the Correct Amino Acid

For a tRNA molecule to carry out its role as an adaptor, it must be linked—or charged—with the correct amino acid. How does each tRNA molecule recognize the one amino acid in 20 that is its right partner? Recognition and attachment of the correct amino acid depend on enzymes called **aminoacyl-tRNA synthetases**, which covalently couple each amino acid to its appropriate set of tRNA molecules. In most organisms, there is a different synthetase enzyme for each amino acid. That means that there are 20 synthetases in all: one attaches glycine to all tRNAs that recognize codons for glycine, another attaches phenylalanine to all tRNAs that recognize codons for phenylalanine, and so on. Each synthetase enzyme recognizes specific nucleotides in both the anticodon and the amino-acid-accepting arm of the correct tRNA (Movie 7.6). The synthetases are thus equal in importance to the tRNAs in the decoding process, because it is the combined action of the synthetases and tRNAs that allows each codon in the mRNA molecule to specify its proper amino acid (Figure 7–30).

Figure 7–30 The genetic code is translated by the cooperation of two adaptors: aminoacyl-tRNA synthetases and tRNAs. Each synthetase couples a particular amino acid to its corresponding tRNAs, a process called charging. The anticodon on the charged tRNA molecule then forms base pairs with the appropriate codon on the mRNA. An error in either the charging step or the binding of the charged tRNA to its codon will cause the wrong amino acid to be incorporated into a protein chain. In the sequence of events shown, the amino acid tryptophan (Trp) is selected by the codon UGG on the mRNA.



The synthetase-catalyzed reaction that attaches the amino acid to the 3' end of the tRNA is one of many reactions in cells coupled to the energy-releasing hydrolysis of ATP (see Figure 3–33). The reaction produces a high-energy bond between the charged tRNA and the amino acid. The energy of this bond is later used to link the amino acid covalently to the growing polypeptide chain.

The mRNA Message Is Decoded by Ribosomes

The recognition of a codon by the anticodon on a tRNA molecule depends on the same type of complementary base-pairing used in DNA replication and transcription. However, accurate and rapid translation of mRNA into protein requires a molecular machine that can move along the mRNA, capture complementary tRNA molecules, hold the tRNAs in position, and then covalently link the amino acids that they carry to form a polypeptide chain. In both prokaryotes and eukaryotes, the machine that gets the job done is the **ribosome**—a large complex made from dozens of small proteins (the *ribosomal proteins*) and several crucial RNA molecules called **ribosomal RNAs (rRNAs)**. A typical eukaryotic cell contains millions of ribosomes in its cytoplasm (Figure 7–31).

Eukaryotic and prokaryotic ribosomes are very similar in structure and function. Both are composed of one large subunit and one small subunit, which fit together to form a complete ribosome with a mass of several million daltons (Figure 7–32); for comparison, an average-sized protein has a mass of 30,000 daltons. The small ribosomal subunit matches the tRNAs to the codons of the mRNA, while the large subunit catalyzes the formation of the peptide bonds that covalently link the amino acids together into a polypeptide chain. These two subunits come together on an mRNA molecule near its 5' end to start the synthesis of a protein. The mRNA is then pulled through the ribosome like a long piece of tape. As the mRNA inches forward in a 5'-to-3' direction, the ribosome translates its nucleotide sequence into an amino acid sequence, one codon at a time, using the tRNAs as adaptors. Each amino acid is thereby added in the correct sequence to the end of the growing polypeptide chain (Movie 7.7). When synthesis of the protein is finished, the two subunits of the ribosome separate. Ribosomes operate with remarkable efficiency: a eukaryotic ribosome adds about 2 amino acids to a polypeptide chain each second; a bacterial ribosome operates even faster, adding about 20 amino acids per second.

QUESTION 7–4

In a clever experiment performed in 1962, a cysteine already attached to its tRNA was chemically converted to an alanine. These “hybrid” tRNA molecules were then added to a cell-free translation system from which the normal cysteine-tRNAs had been removed. When the resulting protein was analyzed, it was found that alanine had been inserted at every point in the polypeptide chain where cysteine was supposed to be. Discuss what this experiment tells you about the role of aminoacyl-tRNA synthetases during the normal translation of the genetic code.

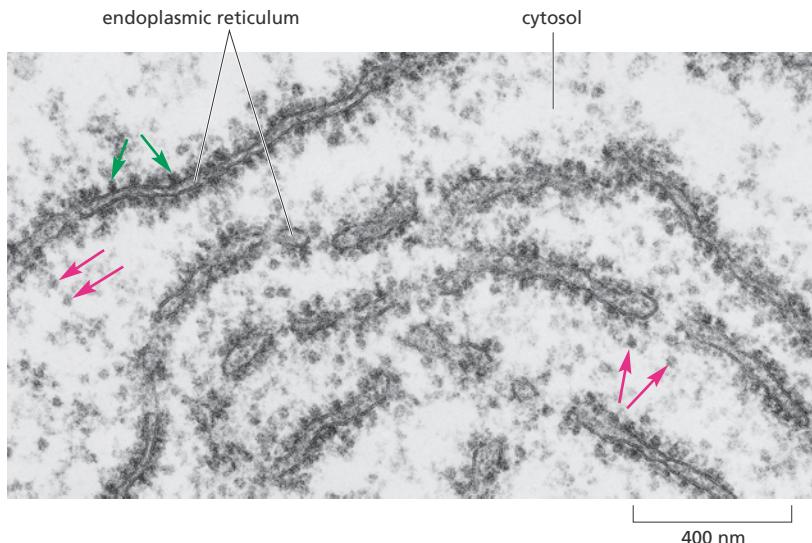


Figure 7–31 Ribosomes are located in the cytoplasm of eukaryotic cells. This electron micrograph shows a thin section of a small region of cytoplasm. The ribosomes appear as small gray blobs. Some are free in the cytosol (red arrows); others are attached to membranes of the endoplasmic reticulum (green arrows). (Courtesy of George Palade.)

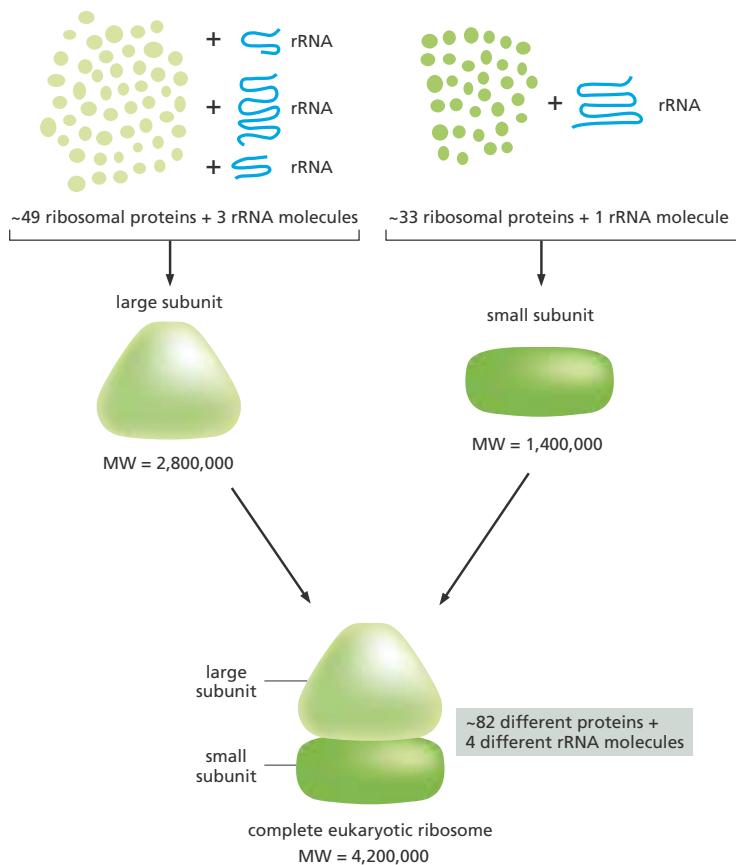


Figure 7–32 The eukaryotic ribosome is a large complex of four rRNAs and more than 80 small proteins. Prokaryotic ribosomes are very similar: both are formed from a large and small subunit, which only come together after the small subunit has bound an mRNA. Although ribosomal proteins greatly outnumber rRNAs, the RNAs account for most of the mass of the ribosome and give it its overall shape and structure.

How does the ribosome choreograph all the movements required for translation? In addition to a binding site for an mRNA molecule, each ribosome contains three binding sites for tRNA molecules, called the A site, the P site, and the E site (Figure 7–33). To add an amino acid to a growing peptide chain, the appropriate charged tRNA enters the A site by base-pairing with the complementary codon on the mRNA molecule. Its amino acid is then linked to the peptide chain held by the tRNA in the neighboring P site. Next, the large ribosomal subunit shifts forward, moving the spent tRNA to the E site before ejecting it (Figure 7–34). This cycle of reactions is repeated each time an amino acid is added to the polypeptide chain, with the new protein growing from its amino to its carboxyl end until a stop codon in the mRNA is encountered.

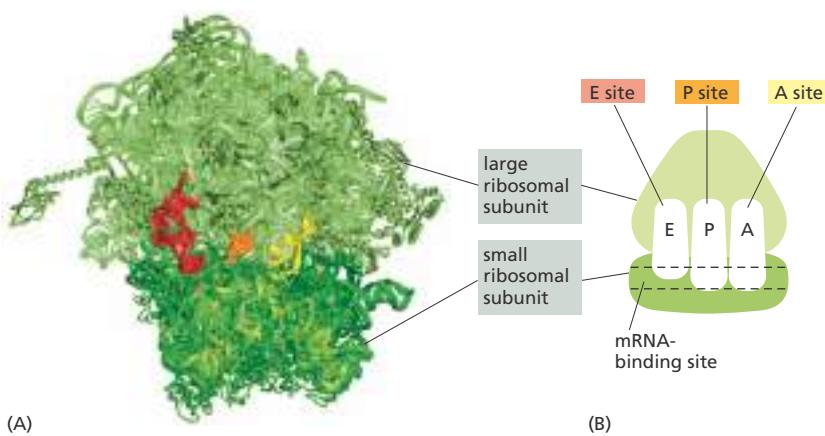


Figure 7–33 Each ribosome has a binding site for mRNA and three binding sites for tRNA. The tRNA sites are designated the A, P, and E sites (short for aminoacyl-tRNA, peptidyl-tRNA, and exit, respectively). (A) Three-dimensional structure of a bacterial ribosome, as determined by X-ray crystallography, with the small subunit in dark green and the large subunit in light green. Both the rRNAs and the ribosomal proteins are shown in green. tRNAs are shown bound in the E site (red), the P site (orange), and the A site (yellow). Although all three tRNA sites are shown occupied here, during the process of protein synthesis only two of these sites are occupied at any one time (see Figure 7–34). (B) Highly schematized representation of a ribosome (in the same orientation as A), which will be used in subsequent figures. Note that both the large and small subunits are involved in forming the A, P, and E sites, while only the small subunit forms the binding site for an mRNA. (B, adapted from M.M. Yusupov et al., Science 292:883–896, 2001, with permission from AAAS. Courtesy of Albion Baucom and Harry Noller.)

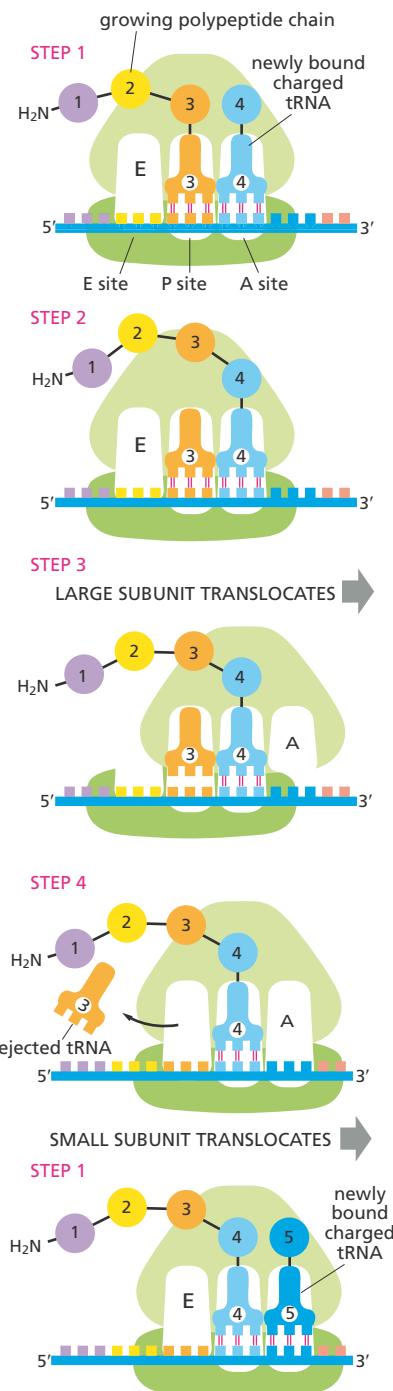


Figure 7-34 Translation takes place in a four-step cycle. This cycle is repeated over and over during the synthesis of a protein. In step 1, a charged tRNA carrying the next amino acid to be added to the polypeptide chain binds to the vacant A site on the ribosome by forming base pairs with the mRNA codon that is exposed there. Because only the appropriate tRNA molecules can base-pair with each codon, this codon determines the specific amino acid added. The A and P sites are sufficiently close together that their two tRNA molecules are forced to form base pairs with codons that are contiguous, with no stray bases in between. This positioning of the tRNAs ensures that the correct reading frame will be preserved throughout the synthesis of the protein. In step 2, the carboxyl end of the polypeptide chain (amino acid 3 in step 1) is uncoupled from the tRNA at the P site and joined by a peptide bond to the free amino group of the amino acid linked to the tRNA at the A site. This reaction is catalyzed by an enzymatic site in the large subunit. In step 3, a shift of the large subunit relative to the small subunit moves the two tRNAs into the E and P sites of the large subunit. In step 4, the small subunit moves exactly three nucleotides along the mRNA molecule, bringing it back to its original position relative to the large subunit. This movement ejects the spent tRNA and resets the ribosome with an empty A site so that the next charged tRNA molecule can bind ([Movie 7.8](#)).

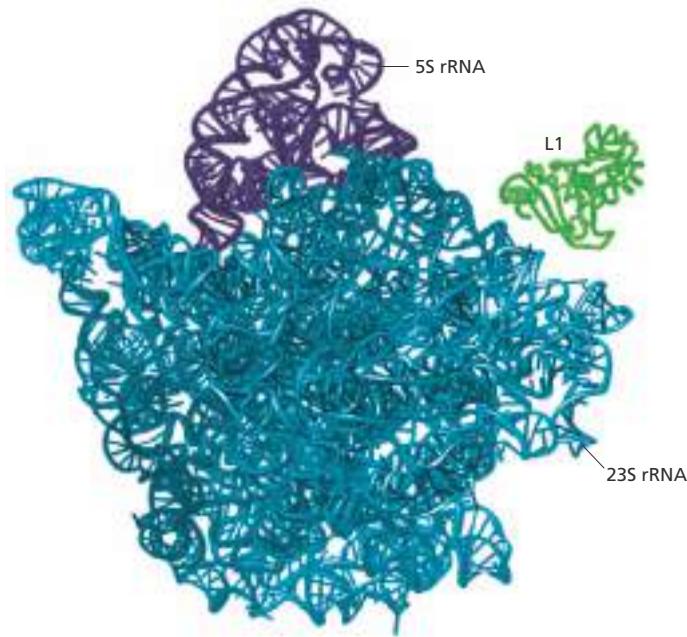
As indicated, the mRNA is translated in the 5'-to-3' direction, and the N-terminal end of a protein is made first, with each cycle adding one amino acid to the C-terminus of the polypeptide chain. To watch the translation cycle in atomic detail, see [Movie 7.9](#).

The Ribosome Is a Ribozyme

The ribosome is one of the largest and most complex structures in the cell, composed of two-thirds RNA and one-third protein by weight. The determination of the entire three-dimensional structure of its large and small subunits in 2000 was a major triumph of modern biology. The structure confirmed earlier evidence that the rRNAs—not the proteins—are responsible for the ribosome's overall structure and its ability to choreograph and catalyze protein synthesis.

The rRNAs are folded into highly compact, precise three-dimensional structures that form the core of the ribosome ([Figure 7-35](#)). In marked contrast to the central positioning of the rRNAs, the ribosomal proteins are generally located on the surface, where they fill the gaps and crevices of the folded RNA. The main role of the ribosomal proteins seems to be

Figure 7-35 Ribosomal RNAs give the ribosome its overall shape. Shown here are the detailed structures of the two rRNAs that form the core of the large subunit of a bacterial ribosome—the 23S rRNA (blue) and the 5S rRNA (purple). One of the protein subunits of the ribosome (L1) is included as a reference point, as this protein forms a characteristic protrusion on the ribosome surface. Ribosomal components are commonly designated by their "S values," which refer to their rate of sedimentation in an ultracentrifuge. (Adapted from N. Ban et al., *Science* 289:905–920, 2000. With permission from AAAS.)



to help fold and stabilize the RNA core, while permitting the changes in rRNA conformation that are necessary for this RNA to catalyze efficient protein synthesis.

Not only are the three tRNA-binding sites (the A, P, and E sites) on the ribosome formed primarily by the rRNAs, but the catalytic site for peptide bond formation is formed by the 23S rRNA of the large subunit; the nearest ribosomal protein is located too far away to make contact with the incoming charged tRNA or with the growing polypeptide chain. The catalytic site in this rRNA—a peptidyl transferase—is similar in many respects to that found in some protein enzymes: it is a highly structured pocket that precisely orients the two reactants—the elongating polypeptide and the charged tRNA—thereby greatly increasing the probability of a productive reaction.

RNA molecules that possess catalytic activity are called **ribozymes**. Later, in the final section of this chapter, we will consider other ribozymes and discuss what the existence of RNA-based catalysis might mean for the early evolution of life on Earth. Here we need only note that there is good reason to suspect that RNA rather than protein molecules served as the first catalysts for living cells. If so, the ribosome, with its catalytic RNA core, could be viewed as a relic of an earlier time in life's history, when cells were run almost entirely by ribozymes.

Specific Codons in mRNA Signal the Ribosome Where to Start and to Stop Protein Synthesis

In the test tube, ribosomes can be forced to translate any RNA molecule (see How We Know, pp. 240–241). In a cell, however, a specific start signal is required to initiate translation. The site at which protein synthesis begins on an mRNA is crucial, because it sets the reading frame for the whole length of the message. An error of one nucleotide either way at this stage will cause every subsequent codon in the mRNA to be misread, resulting in a nonfunctional protein with a garbled sequence of amino acids (see Figure 7–26). And the rate of initiation determines the rate at which the protein is synthesized from the mRNA.

The translation of an mRNA begins with the codon AUG, and a special charged tRNA is required to initiate translation. This **initiator tRNA** always carries the amino acid methionine (or a modified form of methionine, formyl-methionine, in bacteria). Thus newly made proteins all have methionine as the first amino acid at their N-terminal end, the end of a protein that is synthesized first. This methionine is usually removed later by a specific protease.

In eukaryotes, an initiator tRNA, charged with methionine, is first loaded into the P site of the small ribosomal subunit, along with additional proteins called **translation initiation factors** (Figure 7–36). The initiator tRNA is distinct from the tRNA that normally carries methionine. Of all the tRNAs in the cell, only a charged initiator tRNA molecule is capable of binding tightly to the P site in the absence of the large ribosomal subunit. Next, the small ribosomal subunit loaded with the initiator tRNA binds to

QUESTION 7–5

A sequence of nucleotides in a DNA strand—5'-TTAACGGCTTTTTC-3'—was used as a template to synthesize an mRNA that was then translated into protein. Predict the C-terminal amino acid and the N-terminal amino acid of the resulting polypeptide. Assume that the mRNA is translated without the need for a start codon.

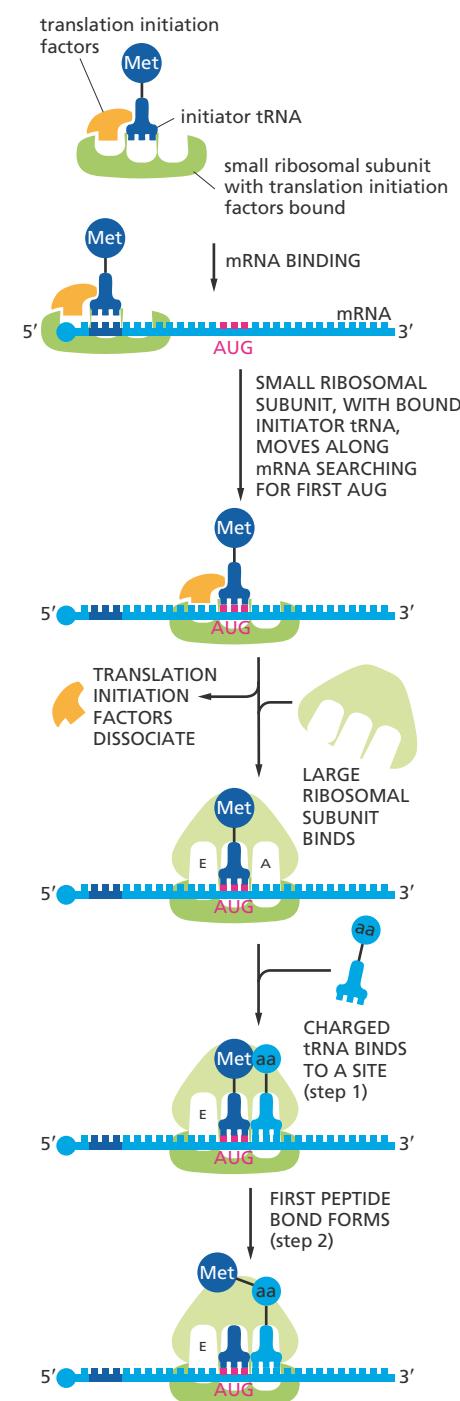


Figure 7–36 Initiation of protein synthesis in eukaryotes requires translation initiation factors and a special initiator tRNA. Although not shown here, efficient translation initiation also requires additional proteins that are bound at the 5' cap and poly-A tail of the mRNA (see Figure 7–23). In this way, the translation apparatus can ascertain that both ends of the mRNA are intact before initiating translation. Following initiation, the protein is elongated by the reactions outlined in Figure 7–34.

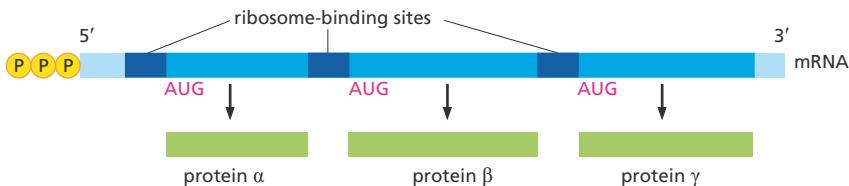
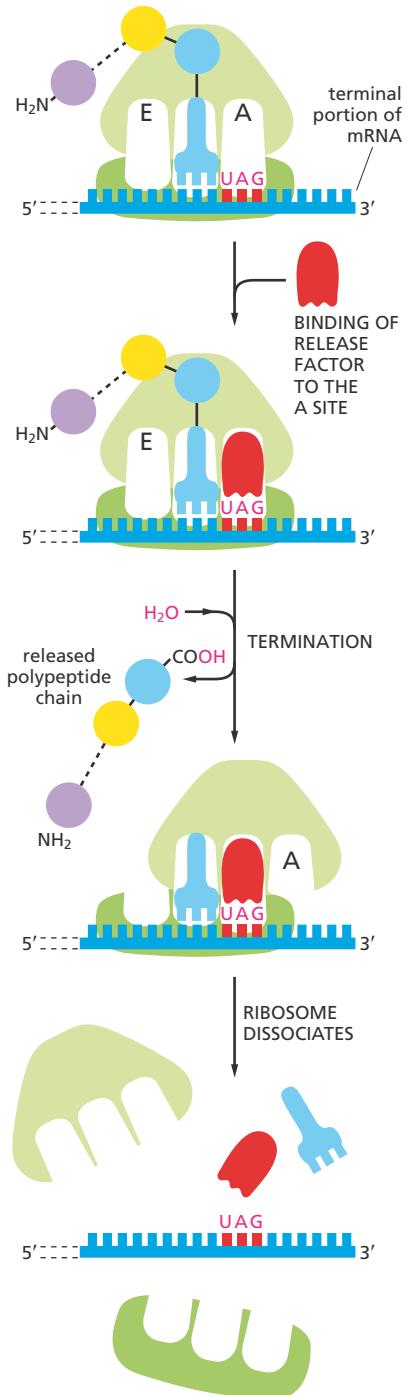


Figure 7–37 A single prokaryotic mRNA molecule can encode several different proteins. In prokaryotes, genes directing the different steps in a process are often organized into clusters (operons) that are transcribed together into a single mRNA. A prokaryotic mRNA does not have the same sort of 5' cap as a eukaryotic mRNA, but instead has a triphosphate at its 5' end. Prokaryotic ribosomes initiate translation at ribosome-binding sites (dark blue), which can be located in the interior of an mRNA molecule. This feature enables prokaryotes to synthesize different proteins from a single mRNA molecule, with each protein made by a different ribosome.



the 5' end of an mRNA molecule, which is marked by the 5' cap that is present on all eukaryotic mRNAs (see Figure 7–16). The small ribosomal subunit then moves forward (5' to 3') along the mRNA searching for the first AUG. When this AUG is encountered and recognized by the initiator tRNA, several initiation factors dissociate from the small ribosomal subunit to make way for the large ribosomal subunit to bind and complete ribosomal assembly. Because the initiator tRNA is bound to the P site, protein synthesis is ready to begin with the addition of the next charged tRNA to the A site (see Figure 7–34).

The mechanism for selecting a start codon is different in bacteria. Bacterial mRNAs have no 5' caps to tell the ribosome where to begin searching for the start of translation. Instead, they contain specific ribosome-binding sequences, up to six nucleotides long, that are located a few nucleotides upstream of the AUGs at which translation is to begin. Unlike a eukaryotic ribosome, a prokaryotic ribosome can readily bind directly to a start codon that lies in the interior of an mRNA, as long as a ribosome-binding site precedes it by several nucleotides. Such ribosome-binding sequences are necessary in bacteria, as prokaryotic mRNAs are often *polycistronic*—that is, they encode several different proteins, each of which is translated from the same mRNA molecule (Figure 7–37). In contrast, a eukaryotic mRNA usually carries the information for a single protein.

The end of translation in both prokaryotes and eukaryotes is signaled by the presence of one of several codons, called *stop codons*, in the mRNA (see Figure 7–25). The stop codons—UAA, UAG, and UGA—are not recognized by a tRNA and do not specify an amino acid, but instead signal to the ribosome to stop translation. Proteins known as *release factors* bind to any stop codon that reaches the A site on the ribosome; this binding alters the activity of the peptidyl transferase in the ribosome, causing it to catalyze the addition of a water molecule instead of an amino acid to the peptidyl-tRNA (Figure 7–38). This reaction frees the carboxyl end of the polypeptide chain from its attachment to a tRNA molecule; because this is the only attachment that holds the growing polypeptide to the ribosome, the completed protein chain is immediately released. At this point, the ribosome also releases the mRNA and dissociates into its two separate subunits, which can then assemble on another mRNA molecule to begin a new round of protein synthesis.

Figure 7–38 Translation halts at a stop codon. In the final phase of protein synthesis, the binding of release factor to an A site bearing a stop codon terminates translation of an mRNA molecule. The completed polypeptide is released, and the ribosome dissociates into its two separate subunits. Note that only the 3' end of the mRNA molecule is shown here.

We saw in Chapter 4 that many proteins can fold into their three-dimensional shape spontaneously, and some do so as they are spun out of the ribosome. Most proteins, however, require *chaperone proteins* to help them fold correctly in the cell. Chaperones can “steer” proteins along productive folding pathways and prevent them from aggregating inside the cell (see Figures 4–9 and 4–10). Newly synthesized proteins are typically met by their chaperones as they emerge from the ribosome.

Proteins Are Made on Polyribosomes

The synthesis of most protein molecules takes between 20 seconds and several minutes. But even during this short period, multiple ribosomes usually bind to each mRNA molecule being translated. If the mRNA is being translated efficiently, a new ribosome hops onto the 5' end of the mRNA molecule almost as soon as the preceding ribosome has translated enough of the nucleotide sequence to move out of the way. The mRNA molecules being translated are therefore usually found in the form of *polyribosomes*, also known as *polysomes*. These large cytoplasmic assemblies are made up of many ribosomes spaced as close as 80 nucleotides apart along a single mRNA molecule (Figure 7–39). With multiple ribosomes working simultaneously on a single mRNA, many more protein molecules can be made in a given time than would be possible if each polypeptide had to be completed before the next could be started.

Polysomes operate in both bacteria and eukaryotes, but bacteria can speed up the rate of protein synthesis even further. Because bacterial mRNA does not need to be processed and is also physically accessible to ribosomes while it is being made, ribosomes will typically attach to the free end of a bacterial mRNA molecule and start translating it even before the transcription of that RNA is complete; these ribosomes follow closely behind the RNA polymerase as it moves along DNA.

Inhibitors of Prokaryotic Protein Synthesis Are Used as Antibiotics

The ability to translate mRNAs accurately into proteins is a fundamental feature of all life on Earth. Although the ribosome and other molecules that carry out this complex task are very similar among organisms, we

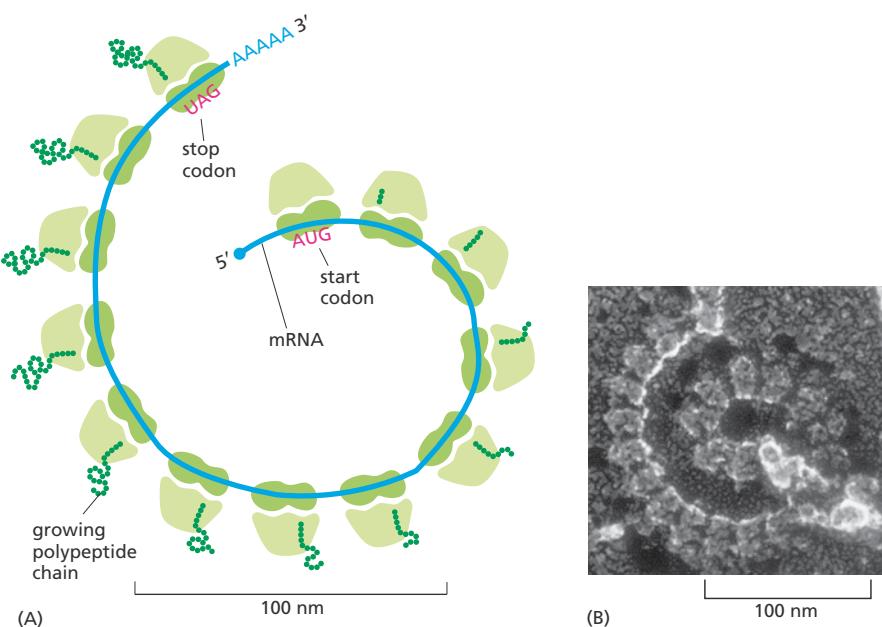


Figure 7–39 Proteins are synthesized on polyribosomes. (A) Schematic drawing showing how a series of ribosomes can simultaneously translate the same mRNA molecule (Movie 7.10). (B) Electron micrograph of a polyribosome in the cytosol of a eukaryotic cell. (B, courtesy of John Heuser.)

TABLE 7–3 ANTIBIOTICS THAT INHIBIT BACTERIAL PROTEIN OR RNA SYNTHESIS

Antibiotic	Specific Effect
Tetracycline	blocks binding of aminoacyl-tRNA to A site of ribosome (step 1 in Figure 7–34)
Streptomycin	prevents the transition from initiation complex to chain elongation (see Figure 7–36); also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 7–34)
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 7–34)
Rifamycin	blocks initiation of transcription by binding to RNA polymerase

have seen that there are some subtle differences in the way that bacteria and eukaryotes synthesize RNA and proteins. Through a quirk of evolution, these differences form the basis of one of the most important advances in modern medicine.

Many of our most effective antibiotics are compounds that act by inhibiting bacterial, but not eukaryotic, RNA and protein synthesis. Some of these drugs exploit the small structural and functional differences between bacterial and eukaryotic ribosomes, so that they interfere preferentially with bacterial protein synthesis. These compounds can thus be taken in doses high enough to kill bacteria without being toxic to humans. Because different antibiotics bind to different regions of the bacterial ribosome, these drugs often inhibit different steps in protein synthesis. A few of the antibiotics that inhibit bacterial RNA and protein synthesis are listed in **Table 7–3**.

Many common antibiotics were first isolated from fungi. Fungi and bacteria often occupy the same ecological niches; to gain a competitive edge, fungi have evolved, over time, potent toxins that kill bacteria but are harmless to themselves. Because fungi and humans are both eukaryotes, and are thus more closely related to each other than either is to bacteria (see Figure 1–28), we have been able to borrow these weapons to combat our own bacterial foes.

Controlled Protein Breakdown Helps Regulate the Amount of Each Protein in a Cell

After a protein is released from the ribosome, a cell can control its activity and longevity in various ways. The number of copies of a protein in a cell depends, like the human population, not only on how quickly new individuals are made but also on how long they survive. So controlling the breakdown of proteins into their constituent amino acids helps cells regulate the amount of each particular protein. Proteins vary enormously in their life-span. Structural proteins that become part of a relatively stable tissue such as bone or muscle may last for months or even years, whereas other proteins, such as metabolic enzymes and those that regulate cell growth and division (discussed in Chapter 18), last only for days, hours, or even seconds. How does the cell control these lifetimes?

Cells possess specialized pathways that enzymatically break proteins down into their constituent amino acids (a process termed *proteolysis*). The enzymes that degrade proteins, first to short peptides and finally to individual amino acids, are known collectively as **proteases**. Proteases

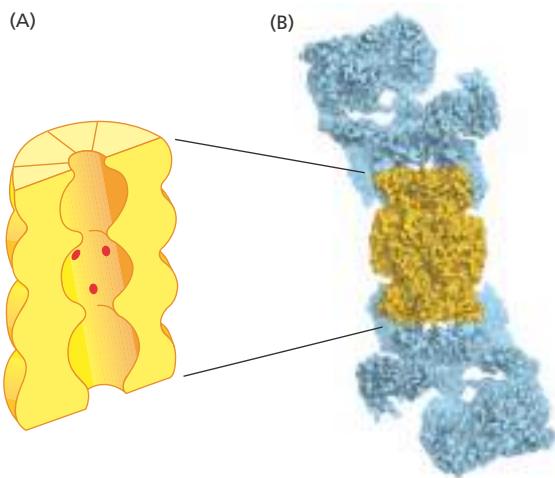


Figure 7–40 A proteasome degrades short-lived and misfolded proteins. The structures shown were determined by X-ray crystallography. (A) A cut-away view of the central cylinder of the proteasome, with the active sites of the proteases indicated by red dots. (B) The structure of the entire proteasome, in which access to the central cylinder (yellow) is regulated by a stopper (blue) at each end. (B, adapted from P.C.A. da Fonseca et al., Mol. Cell 46:54–66, 2012.)

act by cutting (hydrolyzing) the peptide bonds between amino acids (see Panel 2–5, pp. 74–75). One function of proteolytic pathways is to rapidly degrade those proteins whose lifetimes must be kept short. Another is to recognize and remove proteins that are damaged or misfolded. Eliminating improperly folded proteins is critical for an organism, as misfolded proteins tend to aggregate, and protein aggregates can damage cells and even trigger cell death. Eventually, all proteins—even long-lived ones—accumulate damage and are degraded by proteolysis.

In eukaryotic cells, proteins are broken down by large protein machines called **proteasomes**, present in both the cytosol and the nucleus. A proteasome contains a central cylinder formed from proteases whose active sites face into an inner chamber. Each end of the cylinder is stoppered by a large protein complex formed from at least 10 types of protein subunits (Figure 7–40). These protein stoppers bind the proteins destined for degradation and then—using ATP hydrolysis to fuel this activity—unfold the doomed proteins and thread them into the inner chamber of the cylinder. Once the proteins are inside, proteases chop them into short peptides, which are then jettisoned from either end of the proteasome. Housing proteases inside these molecular destruction chambers makes sense, as it prevents the enzymes from running rampant in the cell.

How do proteasomes select which proteins in the cell should be degraded? In eukaryotes, proteasomes act primarily on proteins that have been marked for destruction by the covalent attachment of a small protein called *ubiquitin*. Specialized enzymes tag selected proteins with a short chain of ubiquitin molecules; these ubiquitylated proteins are then recognized, unfolded, and fed into proteasomes by proteins in the stopper (Figure 7–41).

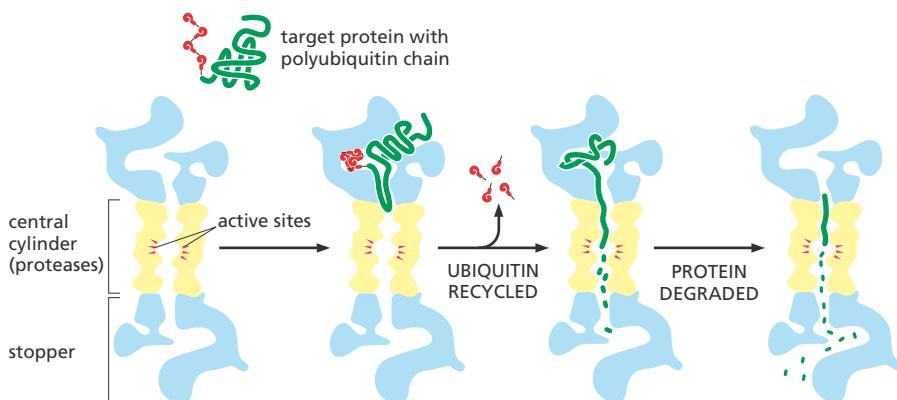


Figure 7–41 Proteins marked by a polyubiquitin chain are degraded by the proteasome. Proteins in the stopper of a proteasome (blue) recognize target proteins marked by a specific type of polyubiquitin chain. The stopper then unfolds the target protein and threads it into the proteasome's central cylinder (yellow), which is lined with proteases that chop the protein to pieces.

Proteins that are meant to be short-lived often contain a short amino acid sequence that identifies the protein as one to be ubiquitylated and degraded in proteasomes. Damaged or misfolded proteins, as well as proteins containing oxidized or otherwise abnormal amino acids, are also recognized and degraded by this ubiquitin-dependent proteolytic system. The enzymes that add a polyubiquitin chain to such proteins recognize signals that become exposed on these proteins as a result of the misfolding or chemical damage—for example, amino acid sequences or conformational motifs that remain buried and inaccessible in the normal “healthy” protein.

There Are Many Steps Between DNA and Protein

We have seen that many types of chemical reactions are required to produce a protein from the information contained in a gene. The final concentration of a protein in a cell therefore depends on the rate at which each of the many steps is carried out (Figure 7–42). In addition, many proteins—once they leave the ribosome—require further attention before they are useful to the cell. Examples of such *post-translational modifications* include covalent modification (such as phosphorylation), the binding of small-molecule cofactors, or association with other protein subunits, which are often needed for a newly synthesized protein to become fully functional (Figure 7–43).

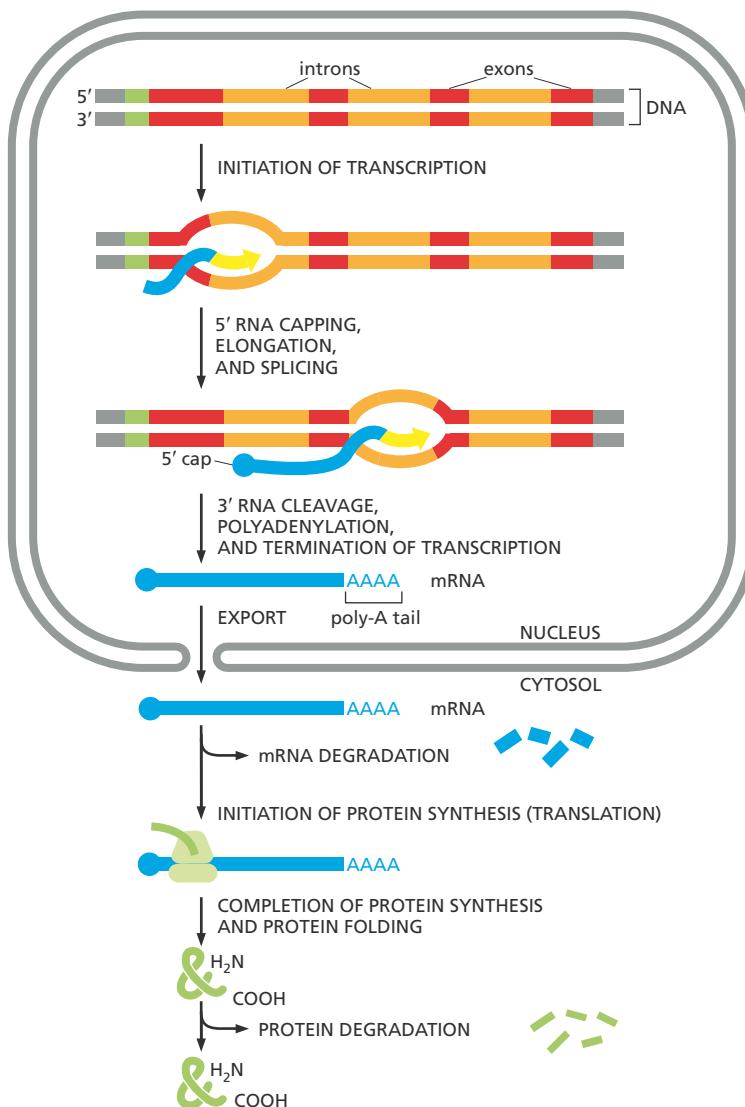


Figure 7–42 Protein production in a eukaryotic cell requires many steps. The final concentration of each protein depends on the rate of each step depicted. Even after an mRNA and its corresponding protein have been produced, their concentrations can be regulated by degradation. Although not shown here, the activity of the protein can also be regulated by other post-translational modifications or the binding of small molecules (see Figure 7–43).

We will see in the next chapter that cells have the ability to change the concentrations of most of their proteins according to their needs. In principle, all of the steps in Figure 7–42 can be regulated by the cell—and many of them, in fact, are. However, as we will see in the next chapter, the initiation of transcription is the most common point for a cell to regulate the expression of its genes.

Transcription and translation are universal processes that lie at the heart of life. However, when scientists came to consider how the flow of information from DNA to protein might have originated, they came to some unexpected conclusions.

RNA AND THE ORIGINS OF LIFE

The central dogma—that DNA makes RNA that makes protein—presented evolutionary biologists with a knotty puzzle: if nucleic acids are required to direct the synthesis of proteins, and proteins are required to synthesize nucleic acids, how could this system of interdependent components have arisen? One view is that an **RNA world** existed on Earth before cells containing DNA and proteins appeared. According to this hypothesis, RNA—which today serves largely as an intermediate between genes and proteins—both stored genetic information and catalyzed chemical reactions in primitive cells. Only later in evolutionary time did DNA take over as the genetic material and proteins become the major catalysts and structural components of cells (Figure 7–44). If this idea is correct, then the transition out of the RNA world was never completed; as we have seen, RNA still catalyzes several fundamental reactions in modern cells. These RNA catalysts—or ribozymes—including those that operate in the ribosome and in the RNA-splicing machinery, can thus be viewed as molecular fossils of an earlier world.

Life Requires Autocatalysis

The origin of life requires molecules that possess, if only to a small extent, one crucial property: the ability to catalyze reactions that lead—directly or indirectly—to the production of more molecules like themselves. Catalysts with this self-producing property, once they had arisen by chance, would divert raw materials from the production of other substances to make more of themselves. In this way, one can envisage the gradual development of an increasingly complex chemical system of organic monomers and polymers that function together to generate more molecules of the same types, fueled by a supply of simple raw materials in the primitive environment on Earth. Such an *autocatalytic* system would have many of the properties we think of as characteristic of living matter: the system would contain a far-from-random selection of interacting molecules; it would tend to reproduce itself; it would compete with other systems dependent on the same raw materials; and, if deprived of its raw materials or maintained at a temperature that upset the balance of reaction rates, it would decay toward chemical equilibrium and “die.”

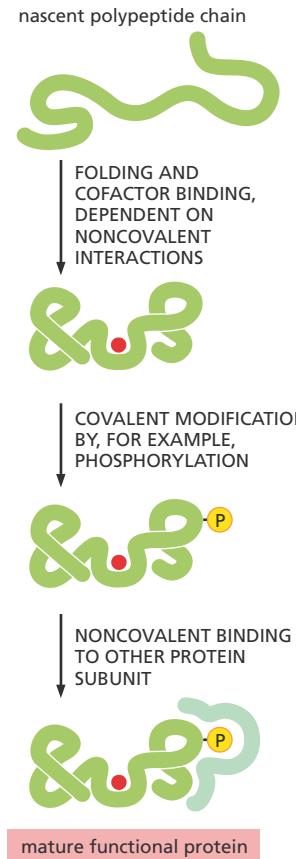


Figure 7–43 Many proteins require various modifications to become fully functional. To be useful to the cell, a completed polypeptide must fold correctly into its three-dimensional conformation and then bind any required cofactors (red) and protein partners—all via noncovalent bonding. Many proteins also require one or more covalent modifications to become active—or to be recruited to specific membranes or organelles (not shown). Although phosphorylation and glycosylation are the most common, more than 100 types of covalent modifications of proteins are known.

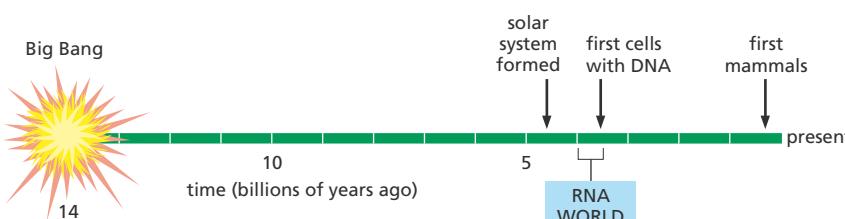


Figure 7–44 An RNA world may have existed before modern cells with DNA and proteins evolved.

But what molecules could have had such autocatalytic properties? In present-day living cells, the most versatile catalysts are proteins, which are able to adopt diverse three-dimensional forms that bristle with chemically reactive sites on their surface. However, there is no known way in which a protein can reproduce itself directly. RNA molecules, by contrast, could—at least, in principle—catalyze their own synthesis.

RNA Can Both Store Information and Catalyze Chemical Reactions

We have seen that complementary base-pairing enables one nucleic acid to act as a template for the formation of another. Thus a single strand of RNA or DNA can specify the sequence of a complementary polynucleotide, which, in turn, can specify the sequence of the original molecule, allowing the original nucleic acid to be replicated (Figure 7–45). Such complementary templating mechanisms lie at the heart of both DNA replication and transcription in modern-day cells.

But the efficient synthesis of polynucleotides by such complementary templating mechanisms also requires catalysts to promote the polymerization reaction: without catalysts, polymer formation is slow, error-prone, and inefficient. Today, nucleotide polymerization is catalyzed by protein enzymes—such as DNA and RNA polymerases. But how could this reaction be catalyzed before proteins with the appropriate catalytic ability existed? The beginnings of an answer were obtained in 1982, when it was discovered that RNA molecules themselves can act as catalysts. The unique potential of RNA molecules to act both as information carriers and as catalysts is thought to have enabled them to have a central role in the origin of life.

In present-day cells, RNA is synthesized as a single-stranded molecule, and we have seen that complementary base-pairing can occur between nucleotides in the same chain. This base-pairing, along with nonconventional hydrogen bonds, can cause each RNA molecule to fold up in a unique way that is determined by its nucleotide sequence (see Figure 7–5). Such associations produce complex three-dimensional shapes.

As we discuss in Chapter 4, protein enzymes are able to catalyze biochemical reactions because they have surfaces with unique contours and chemical properties. In the same way, RNA molecules, with their unique folded shapes, can serve as catalysts (Figure 7–46). RNAs do not have the same structural and functional diversity as do protein enzymes; they are, after all, built from only four different subunits. Nonetheless, ribozymes can catalyze many types of chemical reactions. Most of the ribozymes that have been studied were constructed in the laboratory and selected for their catalytic activity in a test tube (Table 7–4), as relatively few catalytic RNAs exist in present-day cells. But the processes in which catalytic RNAs still seem to have major roles include some of the most

Figure 7–45 An RNA molecule can in principle guide the formation of an exact copy of itself. In the first step, the original RNA molecule acts as a template to form an RNA molecule of complementary sequence. In the second step, this complementary RNA molecule itself acts as a template to form an RNA molecule of the original sequence. Since each template molecule can produce many copies of the complementary strand, these reactions can result in the amplification of the original sequence.

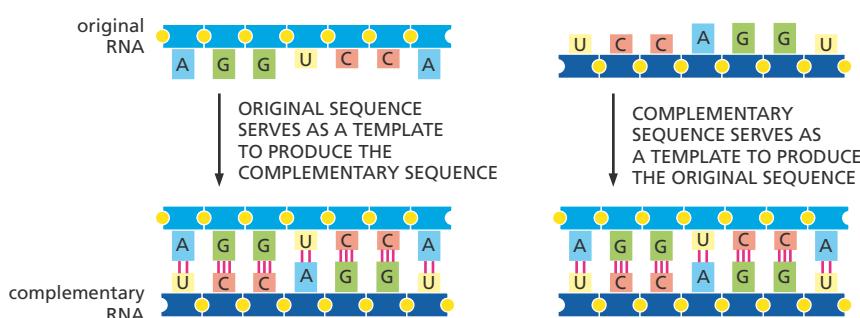


TABLE 7–4 BIOCHEMICAL REACTIONS THAT CAN BE CATALYZED BY RIBOZYMES

Activity	Ribozymes
Peptide bond formation in protein synthesis	ribosomal RNA
DNA ligation	<i>in vitro</i> selected RNA
RNA splicing	self-splicing RNAs, small nuclear RNAs
RNA polymerization	<i>in vitro</i> selected RNA
RNA phosphorylation	<i>in vitro</i> selected RNA
RNA aminoacylation	<i>in vitro</i> selected RNA
RNA alkylation	<i>in vitro</i> selected RNA
C–C bond rotation (isomerization)	<i>in vitro</i> selected RNA

fundamental steps in the expression of genetic information—especially those steps where RNA molecules themselves are spliced or translated into protein.

RNA, therefore, has all the properties required of a molecule that could catalyze its own synthesis (Figure 7–47). Although self-replicating systems of RNA molecules have not been found in nature, scientists appear to be well on the way to constructing them in the laboratory. Although this demonstration would not prove that self-replicating RNA molecules were essential to the origin of life on Earth, it would establish that such a scenario is possible.

RNA Is Thought to Predate DNA in Evolution

The first cells on Earth would presumably have been much less complex and less efficient in reproducing themselves than even the simplest present-day cells. They would have consisted of little more than a simple membrane enclosing a set of self-replicating molecules and a few other components required to provide the materials and energy for this autocatalytic replication. If the evolutionary role for RNA proposed above is correct, these earliest cells would also have differed fundamentally from the cells we know today in having their hereditary information stored in RNA rather than DNA.

Evidence that RNA arose before DNA in evolution can be found in the chemical differences between them. Ribose (see Figure 7–3A), like

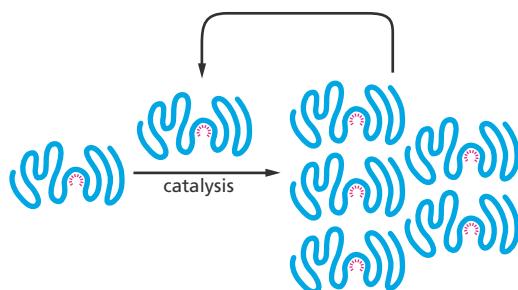


Figure 7–47 Could an RNA molecule catalyze its own synthesis? This hypothetical process would require that the RNA catalyze both steps shown in Figure 7–45. The red rays represent the active site of this ribozyme.

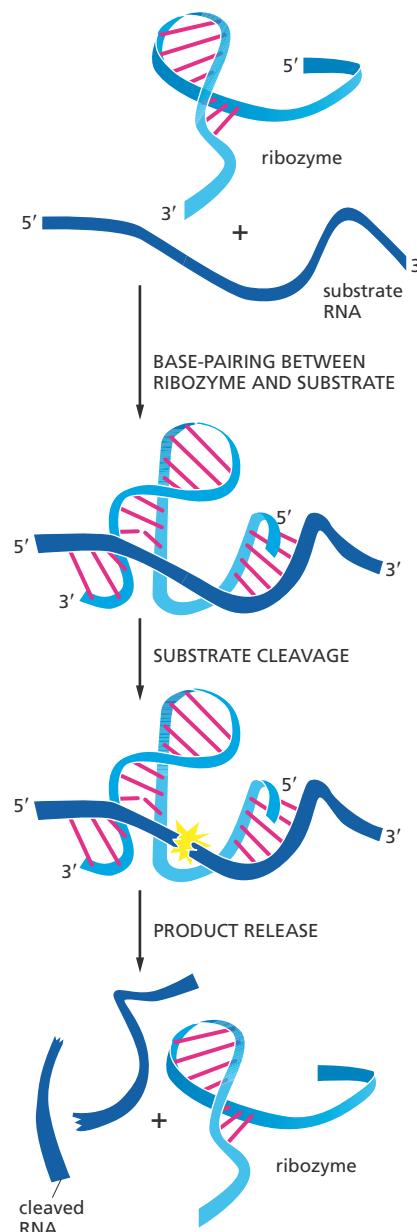


Figure 7–46 A ribozyme is an RNA molecule that possesses catalytic activity. The RNA molecule shown catalyzes the cleavage of a second RNA at a specific site. Similar ribozymes are found embedded in large RNA genomes—called viroids—that infect plants, where the cleavage reaction is one step in the replication of the viroid. (Adapted from T.R. Cech and O.C. Uhlenbeck, *Nature* 372:39–40, 1994. With permission from Macmillan Publishers Ltd.)

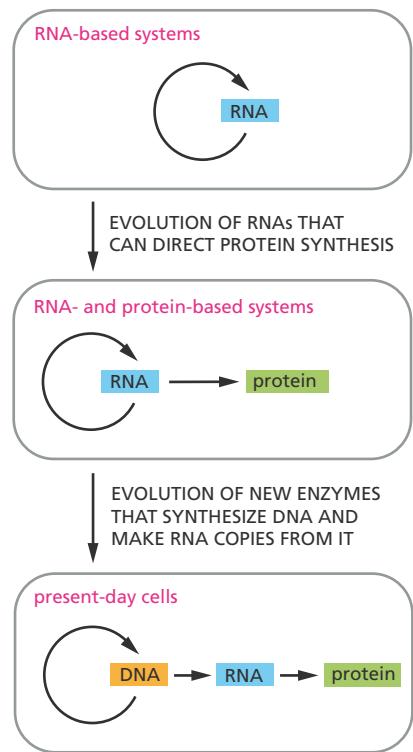


Figure 7–48 RNA may have preceded DNA and proteins in evolution. According to this hypothesis, RNA molecules provided genetic, structural, and catalytic functions in the earliest cells. DNA is now the repository of genetic information, and proteins carry out almost all catalysis in cells. RNA now functions mainly as a go-between in protein synthesis, while remaining a catalyst for a few crucial reactions (including protein synthesis).

QUESTION 7–6

Discuss the following: “During the evolution of life on Earth, RNA lost its glorious position as the first self-replicating catalyst. Its role now is as a mere messenger in the information flow from DNA to protein.”

glucose and other simple carbohydrates, is readily formed from formaldehyde (HCHO), which is one of the principal products of experiments simulating conditions on the primitive Earth. The sugar deoxyribose is harder to make, and in present-day cells it is produced from ribose in a reaction catalyzed by a protein enzyme, suggesting that ribose predates deoxyribose in cells. Presumably, DNA appeared on the scene after RNA, and then proved more suited than RNA as a permanent repository of genetic information. In particular, the deoxyribose in its sugar-phosphate backbone makes chains of DNA chemically much more stable than chains of RNA, so that greater lengths of DNA can be maintained without breakage.

The other differences between RNA and DNA—the double-helical structure of DNA and the use of thymine rather than uracil—further enhance DNA stability by making the molecule easier to repair. We saw in Chapter 6 that a damaged nucleotide on one strand of the double helix can be repaired by using the other strand as a template. Furthermore, deamination, one of the most common unwanted chemical changes occurring in polynucleotides, is easier to detect and repair in DNA than in RNA (see Figure 6–23). This is because the product of the deamination of cytosine is, by chance, uracil, which already exists in RNA, so that such damage would be impossible for repair enzymes to detect in an RNA molecule. However, in DNA, which has thymine rather than uracil, any uracil produced by the accidental deamination of cytosine is easily detected and repaired.

Taken together, the evidence we have discussed supports the idea that RNA—with its ability to provide genetic, structural, and catalytic functions—preceded DNA in evolution. As cells more closely resembling present-day cells appeared, it is believed that many of the functions originally performed by RNA were taken over by DNA and proteins: DNA took over the primary genetic function, and proteins became the major catalysts, while RNA remained primarily as the intermediary connecting the two (Figure 7–48). With the advent of DNA, cells were able to become more complex, for they could then carry and transmit more genetic information than could be stably maintained by RNA alone. Because of the greater chemical complexity of proteins and the variety of chemical reactions they can catalyze, the shift (albeit incomplete) from RNA to proteins also provided a much richer source of structural components and enzymes. This enabled cells to evolve the great diversity of structure and function that we see in life today.

ESSENTIAL CONCEPTS

- The flow of genetic information in all living cells is $\text{DNA} \rightarrow \text{RNA} \rightarrow \text{protein}$. The conversion of the genetic instructions in DNA into RNAs and proteins is termed gene expression.
- To express the genetic information carried in DNA, the nucleotide sequence of a gene is first transcribed into RNA. Transcription is catalyzed by the enzyme RNA polymerase, which uses nucleotide sequences in the DNA molecule to determine which strand to use as a template, and where to start and stop transcribing.
- RNA differs in several respects from DNA. It contains the sugar ribose instead of deoxyribose and the base uracil (U) instead of thymine (T). RNAs in cells are synthesized as single-stranded molecules, which often fold up into complex three-dimensional shapes.
- Cells make several functional types of RNAs, including messenger RNAs (mRNAs), which carry the instructions for making proteins; ribosomal RNAs (rRNAs), which are the crucial components of

ribosomes; and transfer RNAs (tRNAs), which act as adaptor molecules in protein synthesis.

- To begin transcription, RNA polymerase binds to specific DNA sites called promoters that lie immediately upstream of genes. To initiate transcription, eukaryotic RNA polymerases require the assembly of a complex of general transcription factors at the promoter, whereas bacterial RNA polymerase requires only an additional subunit, called sigma factor.
- Most protein-coding genes in eukaryotic cells are composed of a number of coding regions, called exons, interspersed with larger noncoding regions, called introns. When a eukaryotic gene is transcribed from DNA into RNA, both the exons and introns are copied.
- Introns are removed from the RNA transcripts in the nucleus by RNA splicing, a reaction catalyzed by small ribonucleoprotein complexes known as snRNPs. Splicing removes the introns from the RNA and joins together the exons—often in a variety of combinations, allowing multiple proteins to be produced from the same gene.
- Eukaryotic pre-mRNAs go through several additional RNA processing steps before they leave the nucleus as mRNAs, including 5' RNA capping and 3' polyadenylation. These reactions, along with splicing, take place as the pre-mRNA is being transcribed.
- Translation of the nucleotide sequence of an mRNA into a protein takes place in the cytoplasm on large ribonucleoprotein assemblies called ribosomes. As the mRNA moves through the ribosome, its message is translated into protein.
- The nucleotide sequence in mRNA is read in sets of three nucleotides called codons; each codon corresponds to one amino acid.
- The correspondence between amino acids and codons is specified by the genetic code. The possible combinations of the 4 different nucleotides in RNA give 64 different codons in the genetic code. Most amino acids are specified by more than one codon.
- tRNAs act as adaptor molecules in protein synthesis. Enzymes called aminoacyl-tRNA synthetases covalently link amino acids to their appropriate tRNAs. Each tRNA contains a sequence of three nucleotides, the anticodon, which recognizes a codon in an mRNA through complementary base-pairing.
- Protein synthesis begins when a ribosome assembles at an initiation codon (AUG) in an mRNA molecule, a process that depends on proteins called translation initiation factors. The completed protein chain is released from the ribosome when a stop codon (UAA, UAG, or UGA) in the mRNA is reached.
- The stepwise linking of amino acids into a polypeptide chain is catalyzed by an rRNA molecule in the large ribosomal subunit, which thus acts as a ribozyme.
- The concentration of a protein in a cell depends on the rate at which the mRNA and protein are synthesized and degraded. Protein degradation in the cytosol and nucleus occurs inside large protein complexes called proteasomes.
- From our knowledge of present-day organisms and the molecules they contain, it seems likely that life on Earth began with the evolution of RNA molecules that could catalyze their own replication.
- It has been proposed that RNA served as both the genome and the catalysts in the first cells, before DNA replaced RNA as a more stable molecule for storing genetic information, and proteins replaced RNAs as the major catalytic and structural components. RNA catalysts in modern cells are thought to provide a glimpse into an ancient, RNA-based world.

KEY TERMS

alternative splicing	messenger RNA (mRNA)	RNA polymerase
aminoacyl-tRNA synthetase	polyadenylation	RNA processing
anticodon	promoter	RNA splicing
codon	protease	RNA transcript
exon	proteasome	RNA world
gene	reading frame	small nuclear RNA (snRNA)
gene expression	ribosomal RNA (rRNA)	spliceosome
general transcription factors	ribosome	transcription
genetic code	ribozyme	transfer RNA (tRNA)
initiator tRNA	RNA	translation
intron	RNA capping	translation initiation factor

QUESTIONS

QUESTION 7–7

Which of the following statements are correct? Explain your answers.

- A. An individual ribosome can make only one type of protein.
- B. All mRNAs fold into particular three-dimensional structures that are required for their translation.
- C. The large and small subunits of an individual ribosome always stay together and never exchange partners.
- D. Ribosomes are cytoplasmic organelles that are encapsulated by a single membrane.
- E. Because the two strands of DNA are complementary, the mRNA of a given gene can be synthesized using either strand as a template.
- F. An mRNA may contain the sequence
ATTGACCCGGTCAA.
- G. The amount of a protein present in a cell depends on its rate of synthesis, its catalytic activity, and its rate of degradation.

QUESTION 7–8

The *Lacheinmal* protein is a hypothetical protein that causes people to smile more often. It is inactive in many chronically unhappy people. The mRNA isolated from a number of different unhappy individuals in the same family was found to lack an internal stretch of 173 nucleotides that is present in the *Lacheinmal* mRNA isolated from happy members of the same family. The DNA sequences of the *Lacheinmal* genes from the happy and unhappy family members were determined and compared. They differed by a single nucleotide substitution, which lay in an intron. What can you say about the molecular basis of unhappiness in this family?

(Hints: [1] Can you hypothesize a molecular mechanism by which a single nucleotide substitution in a gene could cause the observed deletion in the mRNA? Note that the deletion is *internal* to the mRNA. [2] Assuming the 173-base-pair deletion removes coding sequences from the *Lacheinmal* mRNA, how would the *Lacheinmal* protein differ between the happy and unhappy people?)

QUESTION 7–9

Use the genetic code shown in Figure 7–25 to identify which of the following nucleotide sequences would code for the polypeptide sequence arginine-glycine-aspartate:

1. 5'-AGA-GGA-GAU-3'
2. 5'-ACA-CCC-ACU-3'
3. 5'-GGG-AAA-UUU-3'
4. 5'-CGG-GGU-GAC-3'

QUESTION 7–10

"The bonds that form between the anticodon of a tRNA molecule and the three nucleotides of a codon in mRNA are _____. Complete this sentence with each of the following options and explain why each of the resulting statements is correct or incorrect.

- A. Covalent bonds formed by GTP hydrolysis
- B. Hydrogen bonds that form when the tRNA is at the A site
- C. Broken by the translocation of the ribosome along the mRNA

QUESTION 7–11

List the ordinary, dictionary definitions of the terms *replication*, *transcription*, and *translation*. By their side, list the special meaning each term has when applied to the living cell.

QUESTION 7–12

In an alien world, the genetic code is written in pairs of nucleotides. How many amino acids could such a code specify? In a different world, a triplet code is used, but the sequence of nucleotides is not important; it only matters which nucleotides are present. How many amino acids could this code specify? Would you expect to encounter any problems translating these codes?

QUESTION 7–13

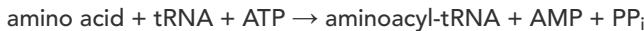
One remarkable feature of the genetic code is that amino acids with similar chemical properties often have similar codons. Thus codons with U or C as the second nucleotide tend to specify hydrophobic amino acids. Can you suggest a possible explanation for this phenomenon in terms of the early evolution of the protein-synthesis machinery?

QUESTION 7–14

A mutation in DNA generates a UGA stop codon in the middle of the mRNA coding for a particular protein. A second mutation in the cell's DNA leads to a single nucleotide change in a tRNA that allows the correct translation of the protein; that is, the second mutation "suppresses" the defect caused by the first. The altered tRNA translates the UGA as tryptophan. What nucleotide change has probably occurred in the mutant tRNA molecule? What consequences would the presence of such a mutant tRNA have for the translation of the normal genes in this cell?

QUESTION 7–15

The charging of a tRNA with an amino acid can be represented by the following equation:



where PP_i is pyrophosphate (see Figure 3–40). In the aminoacyl-tRNA, the amino acid and tRNA are linked with a high-energy covalent bond; a large portion of the energy derived from the hydrolysis of ATP is thus stored in this bond and is available to drive peptide bond formation at the later stages of protein synthesis. The free-energy change of the charging reaction shown in the equation is close to zero and therefore would not be expected to favor attachment of the amino acid to tRNA. Can you suggest a further step that could drive the reaction to completion?

QUESTION 7–16

A. The average molecular weight of a protein in the cell is about 30,000 daltons. A few proteins, however, are much larger. The largest known polypeptide chain made by any cell is a protein called titin (made by mammalian muscle cells), and it has a molecular weight of 3,000,000 daltons. Estimate how long it will take a muscle cell to translate an mRNA coding for titin (assume the average molecular weight of an amino acid to be 120, and a translation rate of two amino acids per second for eukaryotic cells).

B. Protein synthesis is very accurate: for every 10,000 amino acids joined together, only one mistake is made. What is the fraction of average-sized protein molecules and of titin molecules that are synthesized without any errors? (Hint: the probability P of obtaining an error-free protein is given by $P = (1 - E)^n$, where E is the error frequency and n the number of amino acids.)

C. The molecular weight of all eukaryotic ribosomal proteins combined is about 2.5×10^6 daltons. Would it be advantageous to synthesize them as a single protein?

D. Transcription occurs at a rate of about 30 nucleotides per second. Is it possible to calculate the time required to synthesize a titin mRNA from the information given here?

QUESTION 7–17

Which of the following types of mutations would be predicted to harm an organism? Explain your answers.

- A. Insertion of a single nucleotide near the end of the coding sequence.
- B. Removal of a single nucleotide near the beginning of the coding sequence.
- C. Deletion of three consecutive nucleotides in the middle of the coding sequence.
- D. Deletion of four consecutive nucleotides in the middle of the coding sequence.
- E. Substitution of one nucleotide for another in the middle of the coding sequence.

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CHAPTER EIGHT

8

Control of Gene Expression

An organism's DNA encodes all of the RNA and protein molecules that are needed to make its cells. Yet a complete description of the DNA sequence of an organism—be it the few million nucleotides of a bacterium or the few billion nucleotides in each human cell—does not enable us to reconstruct that organism any more than a list of all the English words in a dictionary enables us to reconstruct a play by Shakespeare. We need to know how the elements in the DNA sequence or the words on a list work together to make the masterpiece.

For cells, the question involves *gene expression*. Even the simplest single-celled bacterium can use its genes selectively—for example, switching genes on and off to make the enzymes needed to digest whatever food sources are available. In multicellular plants and animals, however, gene expression is under much more elaborate control. Over the course of embryonic development, a fertilized egg cell gives rise to many cell types that differ dramatically in both structure and function. The differences between an information-processing nerve cell and an infection-fighting white blood cell, for example, are so extreme that it is difficult to imagine that the two cells contain the same DNA (**Figure 8–1**). For this reason, and because cells in an adult organism rarely lose their distinctive characteristics, biologists originally suspected that certain genes might be selectively lost when a cell becomes specialized. We now know, however, that nearly all the cells of a multicellular organism contain the same genome. Cell *differentiation* is instead achieved by changes in gene expression.

In mammals, hundreds of different cell types carry out a range of specialized functions that depend upon genes that are switched on in that

AN OVERVIEW OF GENE EXPRESSION

HOW TRANSCRIPTIONAL SWITCHES WORK

THE MOLECULAR MECHANISMS THAT CREATE SPECIALIZED CELL TYPES

POST-TRANSCRIPTIONAL CONTROLS

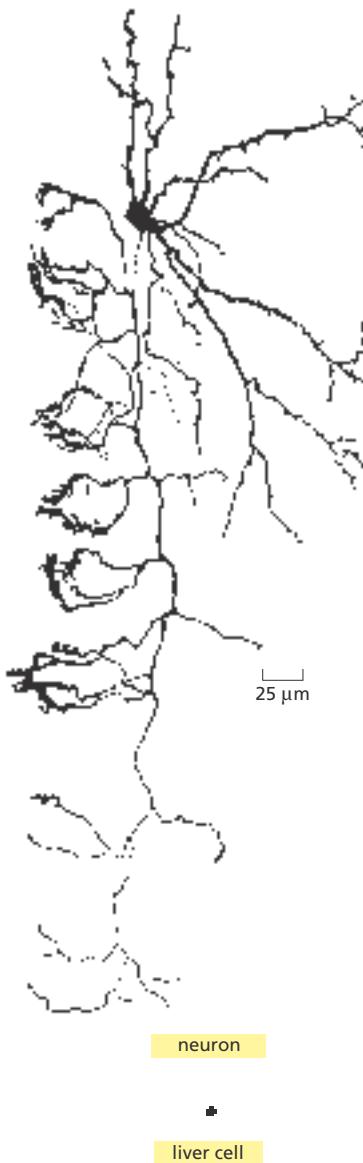


Figure 8–1 A neuron and a liver cell share the same genome.

The long branches of this neuron from the retina enable it to receive electrical signals from many other neurons and carry them to many neighboring neurons. The liver cell, which is drawn to the same scale, is involved in many metabolic processes, including digestion and the detoxification of alcohol and other drugs. Both of these mammalian cells contain the same genome, but they express many different RNAs and proteins. (Neuron adapted from S. Ramón y Cajal, *Histoologie du Système Nerveux de l'Homme et de Vertébrés*, 1909–1911. Paris: Maloine; reprinted, Madrid: C.S.I.C., 1972.)

cell type but not in most others: for example, the β cells of the pancreas make the protein hormone insulin, while the α cells of the pancreas make the hormone glucagon; the B lymphocytes of the immune system make antibodies, while developing red blood cells make the oxygen-transport protein hemoglobin. The differences between a neuron, a white blood cell, a pancreatic β cell, and a red blood cell depend upon the precise control of gene expression. A typical differentiated cell expresses only about half the genes in its total repertoire.

In this chapter, we discuss the main ways in which gene expression is regulated, with a focus on those genes that encode proteins as their final product. Although some of these control mechanisms apply to both eukaryotes and prokaryotes, eukaryotic cells—with their more complex chromosomal structure—have some ways of controlling gene expression that are not available to bacteria.

AN OVERVIEW OF GENE EXPRESSION

Gene expression is a complex process by which cells selectively direct the synthesis of the many thousands of proteins and RNAs encoded in their genome. But how do cells coordinate and control such an intricate process—and how does an individual cell specify which of its genes to express? This decision is an especially important problem for animals because, as they develop, their cells become highly specialized, ultimately producing an array of muscle, nerve, and blood cells, along with the hundreds of other cell types seen in the adult. Such cell **differentiation** arises because cells make and accumulate different sets of RNA and protein molecules: that is, they express different genes.

The Different Cell Types of a Multicellular Organism Contain the Same DNA

The evidence that cells have the ability to change which genes they express without altering the nucleotide sequence of their DNA comes from experiments in which the genome from a differentiated cell is made to direct the development of a complete organism. If the chromosomes of the differentiated cell were altered irreversibly during development, they would not be able to accomplish this feat.

Consider, for example, an experiment in which the nucleus is taken from a skin cell in an adult frog and injected into a frog egg from which the nucleus has been removed. In at least some cases, that doctored egg will develop into a normal tadpole (Figure 8–2). Thus, the transplanted skin-cell nucleus cannot have lost any critical DNA sequences. Nuclear transplantation experiments carried out with differentiated cells taken from adult mammals—including sheep, cows, pigs, goats, and mice—have shown similar results. And in plants, individual cells removed from a carrot, for example, can regenerate an entire adult carrot plant. These experiments all show that the DNA in specialized cell types of multicellular organisms still contains the entire set of instructions needed to form

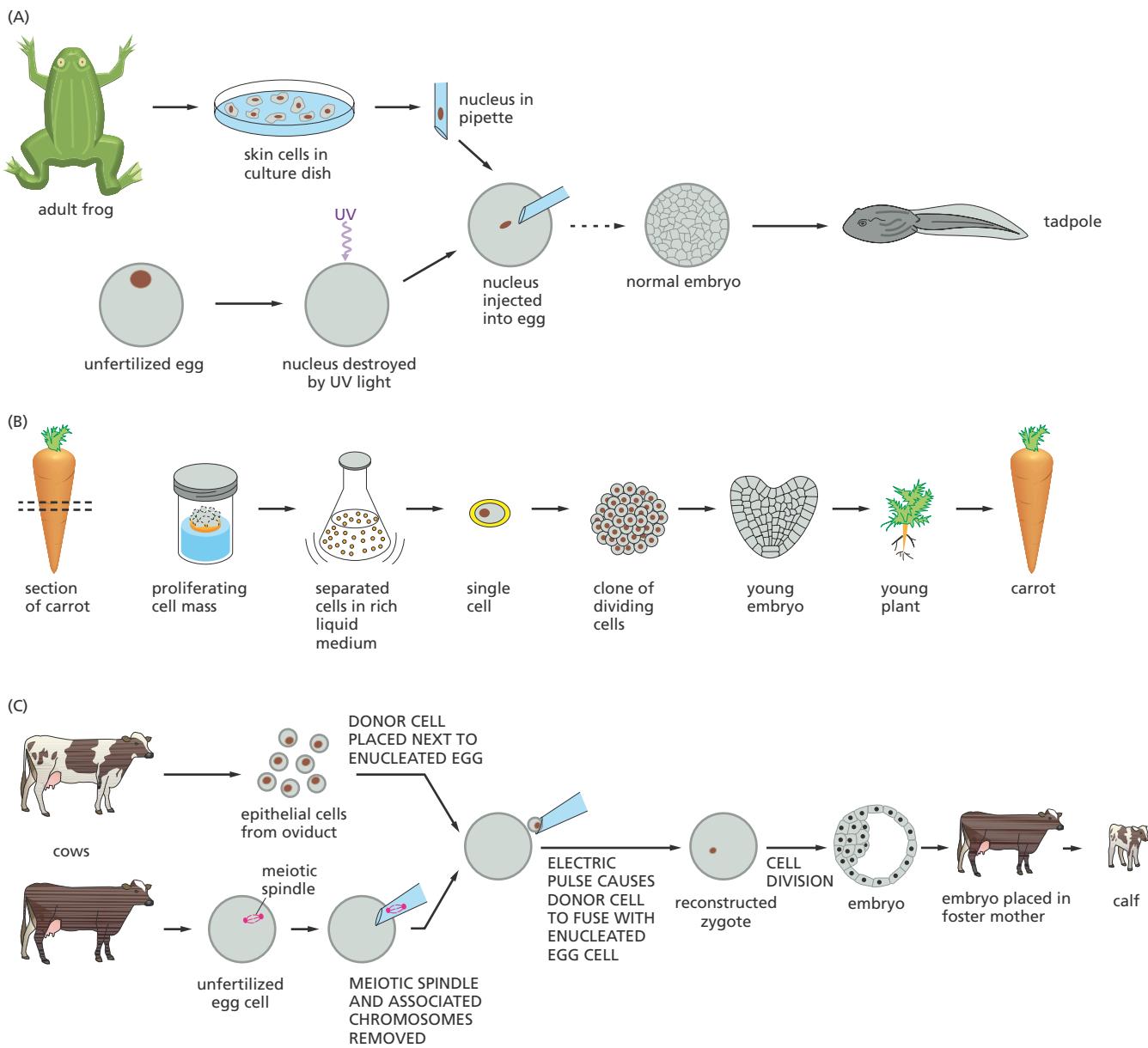


Figure 8–2 Differentiated cells contain all the genetic instructions necessary to direct the formation of a complete organism. (A) The nucleus of a skin cell from an adult frog transplanted into an egg whose nucleus has been destroyed can give rise to an entire tadpole. The broken arrow indicates that to give the transplanted genome time to adjust to an embryonic environment, a further transfer step is required in which one of the nuclei is taken from the early embryo that begins to develop and is put back into a second enucleated egg. (B) In many types of plants, differentiated cells retain the ability to “de-differentiate,” so that a single cell can proliferate to form a clone of progeny cells that later give rise to an entire plant. (C) A nucleus removed from a differentiated cell from an adult cow can be introduced into an enucleated egg from a different cow to give rise to a calf. Different calves produced from the same differentiated cell donor are all clones of the donor and are therefore genetically identical. (A, modified from J.B. Gurdon, *Sci. Am.* 219:24–35, 1968, with permission from the Estate of Bunji Tagawa.)

a whole organism. The various cell types of an organism therefore differ not because they contain different genes, but because they express them differently.

Different Cell Types Produce Different Sets of Proteins

The extent of the differences in gene expression between different cell types may be roughly gauged by comparing the protein composition of cells in liver, heart, brain, and so on. In the past, such analysis was performed by two-dimensional gel electrophoresis (see Panel 4–5, p. 167). Nowadays, the total protein content of a cell can be rapidly analyzed by

a method called mass spectrometry (see Figure 4–49). This technique is much more sensitive than electrophoresis and it enables the detection of even proteins that are produced in minor quantities.

Both techniques reveal that many proteins are common to all the cells of a multicellular organism. These *housekeeping* proteins include, for example, the structural proteins of chromosomes, RNA polymerases, DNA repair enzymes, ribosomal proteins, enzymes involved in glycolysis and other basic metabolic processes, and many of the proteins that form the cytoskeleton. In addition, each different cell type also produces specialized proteins that are responsible for the cell's distinctive properties. In mammals, for example, hemoglobin is made almost exclusively in developing red blood cells.

Gene expression can also be studied by cataloging a cell's RNAs, including the mRNAs that encode protein. The most comprehensive methods for such analyses involve determining the nucleotide sequence of every RNA molecule made by the cell, an approach that can also reveal their relative abundance. Estimates of the number of different mRNA sequences in human cells suggest that, at any one time, a typical differentiated human cell expresses perhaps 5000–15,000 protein-coding genes from a total of about 21,000. It is the expression of a different collection of genes in each cell type that causes the large variations seen in the size, shape, behavior, and function of differentiated cells.

A Cell Can Change the Expression of Its Genes in Response to External Signals

The specialized cells in a multicellular organism are capable of altering their patterns of gene expression in response to extracellular cues. For example, if a liver cell is exposed to the steroid hormone cortisol, the production of several proteins is dramatically increased. Released by the adrenal gland during periods of starvation, intense exercise, or prolonged stress, cortisol signals liver cells to boost the production of glucose from amino acids and other small molecules. The set of proteins whose production is induced by cortisol includes enzymes such as tyrosine aminotransferase, which helps convert tyrosine to glucose. When the hormone is no longer present, the production of these proteins returns to its resting level.

Other cell types respond to cortisol differently. In fat cells, for example, the production of tyrosine aminotransferase is reduced, while some other cell types do not respond to cortisol at all. The fact that different cell types often respond in different ways to the same extracellular signal contributes to the specialization that gives each cell type its distinctive character.

Gene Expression Can Be Regulated at Various Steps from DNA to RNA to Protein

If differences among the various cell types of an organism depend on the particular genes that the cells express, at what level is the control of gene expression exercised? As we saw in the last chapter, there are many steps in the pathway leading from DNA to protein, and all of them can in principle be regulated. Thus a cell can control the proteins it contains by (1) controlling when and how often a given gene is transcribed, (2) controlling how an RNA transcript is spliced or otherwise processed, (3) selecting which mRNAs are exported from the nucleus to the cytosol, (4) regulating how quickly certain mRNA molecules are degraded, (5) selecting which mRNAs are translated into protein by ribosomes, or

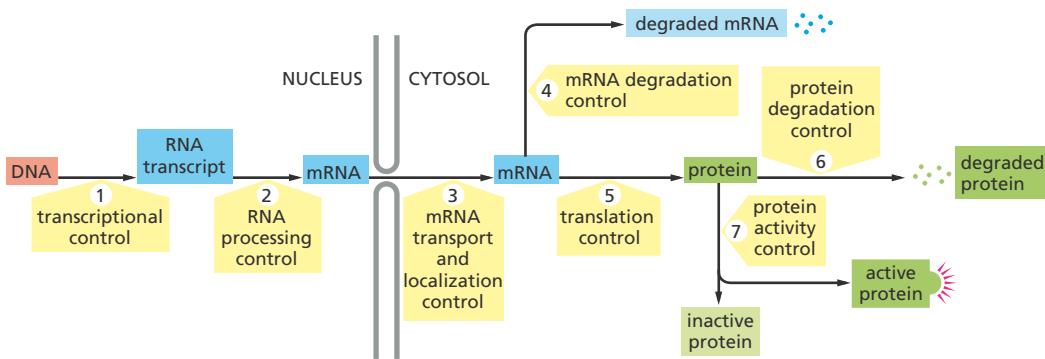


Figure 8–3 Gene expression in eukaryotic cells can be controlled at various steps. Examples of regulation at each of these steps are known, although for most genes the main site of control is step 1—transcription of a DNA sequence into RNA.

(6) regulating how rapidly specific proteins are destroyed after they have been made; in addition, the activity of individual proteins can be further regulated in a variety of ways. These steps are illustrated in **Figure 8–3**.

Gene expression can be regulated at each of these steps. For most genes, however, the control of transcription (step number 1 in Figure 8–3) is paramount. This makes sense because only transcriptional control can ensure that no unnecessary intermediates are synthesized. So it is the regulation of transcription—and the DNA and protein components that determine which genes a cell transcribes into RNA—that we address first.

HOW TRANSCRIPTIONAL SWITCHES WORK

Until 50 years ago, the idea that genes could be switched on and off was revolutionary. This concept was a major advance, and it came originally from studies of how *E. coli* bacteria adapt to changes in the composition of their growth medium. Many of the same principles apply to eukaryotic cells. However, the enormous complexity of gene regulation in higher organisms, combined with the packaging of their DNA into chromatin, creates special challenges and some novel opportunities for control—as we will see. We begin with a discussion of the *transcription regulators*, proteins that bind to DNA and control gene transcription.

Transcription Regulators Bind to Regulatory DNA Sequences

Control of transcription is usually exerted at the step at which the process is initiated. In Chapter 7, we saw that the **promoter** region of a gene binds the enzyme *RNA polymerase* and correctly orients the enzyme to begin its task of making an RNA copy of the gene. The promoters of both bacterial and eukaryotic genes include a *transcription initiation site*, where RNA synthesis begins, plus a sequence of approximately 50 nucleotide pairs that extends upstream from the initiation site (if one likens the direction of transcription to the flow of a river). This upstream region contains sites that are required for the RNA polymerase to recognize the *promoter*, although they do not bind to RNA polymerase directly. Instead, these sequences contain recognition sites for proteins that associate with the active polymerase—sigma factor in bacteria (see Figure 7–9) or the general transcription factors in eukaryotes (see Figure 7–12).

In addition to the promoter, nearly all genes, whether bacterial or eukaryotic, have **regulatory DNA sequences** that are used to switch the gene on or off. Some regulatory DNA sequences are as short as 10 nucleotide pairs and act as simple switches that respond to a single signal; such simple regulatory switches predominate in bacteria. Other regulatory DNA sequences, especially those in eukaryotes, are very long (sometimes spanning more than 10,000 nucleotide pairs) and act as molecular

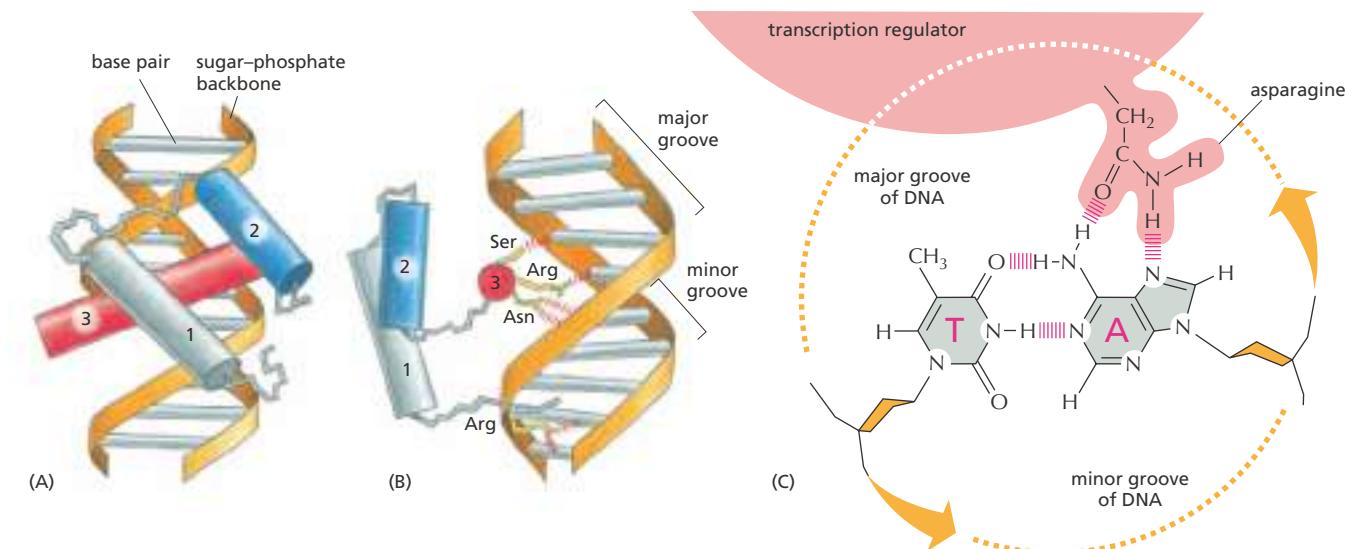


Figure 8–4 A transcription regulator interacts with the major groove of a DNA double helix. (A) This regulator recognizes DNA via three α helices, shown as numbered cylinders, which allow the protein to fit into the major groove and form tight associations with the base pairs in a short stretch of DNA. This particular structural motif, called a **homeodomain**, is found in many eukaryotic DNA-binding proteins (Movie 8.1). (B) Most of the contacts with the DNA bases are made by helix 3 (red), which is shown here end-on. The protein interacts with the edges of the nucleotides without disrupting the hydrogen bonds that hold the base pairs together. (C) An asparagine residue from helix 3 forms two hydrogen bonds with the adenine in an A-T base pair. The view is end-on looking down the DNA double helix, and the protein contacts the base pair from the major groove side. For simplicity, only one amino acid-base contact is shown; in reality, transcription regulators form hydrogen bonds (as shown here), ionic bonds, and hydrophobic interactions with individual bases in the major groove. Typically, the protein–DNA interface would consist of 10–20 such contacts, each involving a different amino acid and each contributing to the overall strength of the protein–DNA interaction.

microprocessors, integrating information from a variety of signals into a command that dictates how often transcription of the gene is initiated.

Regulatory DNA sequences do not work by themselves. To have any effect, these sequences must be recognized by proteins called **transcription regulators**. It is the binding of a transcription regulator to a regulatory DNA sequence that acts as the switch to control transcription. The simplest bacterium produces several hundred different transcription regulators, each of which recognizes a different DNA sequence and thereby regulates a distinct set of genes. Humans make many more—several thousand—indicating the importance and complexity of this form of gene regulation in the development and function of a complex organism.

Proteins that recognize a specific nucleotide sequence do so because the surface of the protein fits tightly against the surface features of the DNA double helix in that region. Because these surface features will vary depending on the nucleotide sequence, different DNA-binding proteins will recognize different nucleotide sequences. In most cases, the protein inserts into the major groove of the DNA helix and makes a series of intimate molecular contacts with the nucleotide pairs within the groove (Figure 8–4). Although each individual contact is weak, the 10 to 20 contacts that are typically formed at the protein–DNA interface combine to ensure that the interaction is both highly specific and very strong; indeed, protein–DNA interactions are among the tightest and most specific molecular interactions known in biology.

Many transcription regulators bind to the DNA helix as dimers (Figure 8–5). Such dimerization roughly doubles the area of contact with the DNA, thereby greatly increasing the strength and specificity of the protein–DNA interaction.

Transcriptional Switches Allow Cells to Respond to Changes in Their Environment

The simplest and best understood examples of gene regulation occur in bacteria and in the viruses that infect them. The genome of the bacterium *E. coli* consists of a single circular DNA molecule of about 4.6×10^6 nucleotide pairs. This DNA encodes approximately 4300 proteins, although only a fraction of these are made at any one time. Bacteria regulate the expression of many of their genes according to the food sources that are available in the environment. For example, in *E. coli*, five genes code for enzymes that manufacture the amino acid tryptophan. These genes are arranged in a cluster on the chromosome and are transcribed from a single promoter as one long mRNA molecule; such coordinately transcribed clusters are called *operons* (Figure 8–6). Although operons are common in bacteria, they are rare in eukaryotes, where genes are transcribed and regulated individually (see Figure 7–2).

When tryptophan concentrations are low, the operon is transcribed; the resulting mRNA is translated to produce a full set of biosynthetic enzymes, which work in tandem to synthesize tryptophan. When tryptophan is abundant, however—for example, when the bacterium is in the gut of a mammal that has just eaten a protein-rich meal—the amino acid is imported into the cell and shuts down production of the enzymes, which are no longer needed.

We now understand in considerable detail how this repression of the tryptophan operon comes about. Within the operon's promoter is a short DNA sequence, called the operator (see Figure 8–6), that is recognized by a transcription regulator. When this regulator binds to the operator, it blocks access of RNA polymerase to the promoter, preventing transcription of the operon and production of the tryptophan-producing enzymes. The transcription regulator is known as the *tryptophan repressor*, and it is controlled in an ingenious way: the repressor can bind to DNA only if it has also bound several molecules of tryptophan (Figure 8–7).

The tryptophan repressor is an allosteric protein (see Figure 4–41): the binding of tryptophan causes a subtle change in its three-dimensional structure so that the protein can bind to the operator sequence. When the concentration of free tryptophan in the bacterium drops, the repressor no longer binds to DNA, and the tryptophan operon is transcribed. The repressor is thus a simple device that switches production of a set of biosynthetic enzymes on and off according to the availability of the end product of the pathway that the enzymes catalyze.

The tryptophan repressor protein itself is always present in the cell. The gene that encodes it is continuously transcribed at a low level, so that a small amount of the repressor protein is always being made. Thus the bacterium can respond very rapidly to a rise in tryptophan concentration.

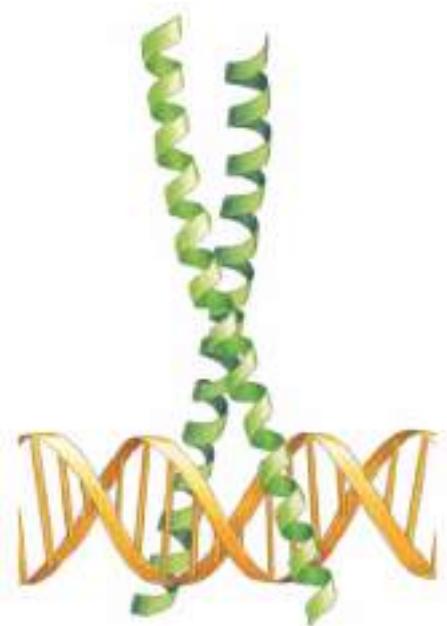
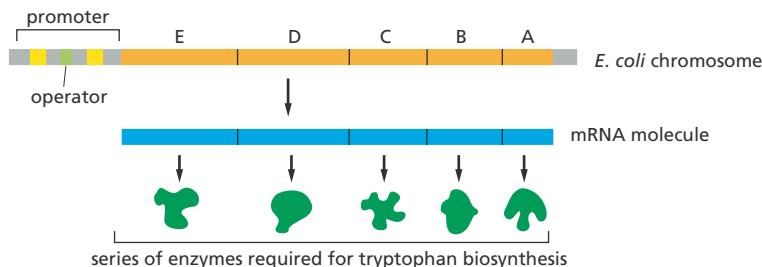


Figure 8–5 Many transcription regulators bind to DNA as dimers. This transcription regulator contains a leucine zipper motif, which is formed by two α helices, each contributed by a different protein subunit. Leucine zipper proteins thus bind to DNA as dimers, gripping the double helix like a clothespin on a clothesline (Movie 8.2).

Figure 8–6 A cluster of bacterial genes can be transcribed from a single promoter. Each of these five genes encodes a different enzyme; all of the enzymes are needed to synthesize the amino acid tryptophan. The genes are transcribed as a single mRNA molecule, a feature that allows their expression to be coordinated. Clusters of genes transcribed as a single mRNA molecule are common in bacteria. Each of these clusters is called an operon because its expression is controlled by a regulatory DNA sequence called the operator (green), situated within the promoter. The yellow blocks in the promoter represent DNA sequences that bind RNA polymerase.

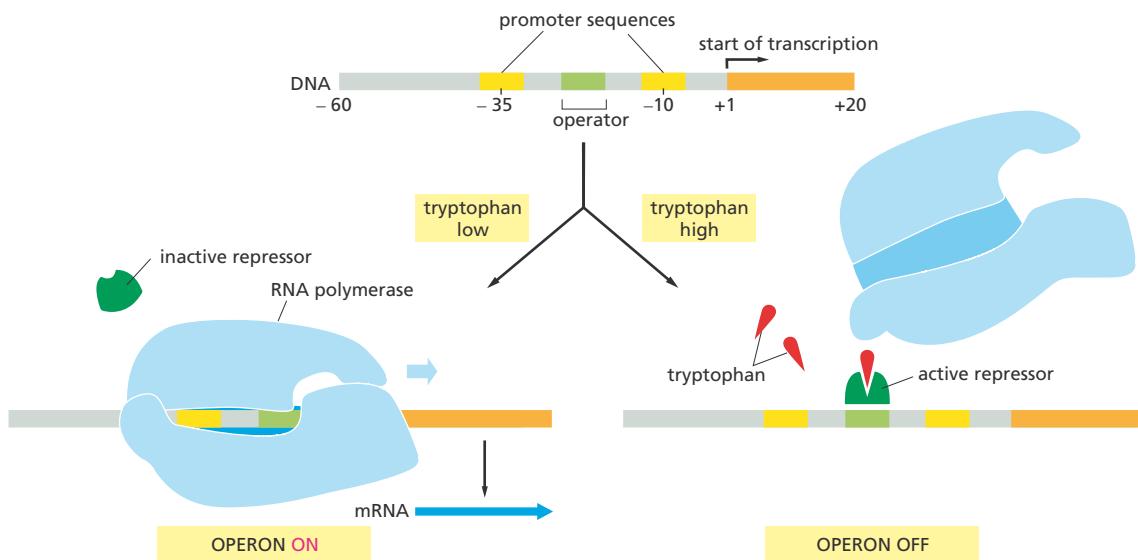


Figure 8–7 Genes can be switched off by repressor proteins. If the concentration of tryptophan inside a bacterium is low (left), RNA polymerase (blue) binds to the promoter and transcribes the five genes of the tryptophan operon. However, if the concentration of tryptophan is high (right), the repressor protein (dark green) becomes active and binds to the operator (light green), where it blocks the binding of RNA polymerase to the promoter. Whenever the concentration of intracellular tryptophan drops, the repressor falls off the DNA, allowing the polymerase to again transcribe the operon. The promoter contains two key blocks of DNA sequence information, the -35 and -10 regions, highlighted in yellow, which are recognized by RNA polymerase (see Figure 7–10). The complete operon is shown in Figure 8–6.

Repressors Turn Genes Off and Activators Turn Them On

The tryptophan repressor, as its name suggests, is a **transcriptional repressor** protein: in its active form, it switches genes off, or *represses* them. Some bacterial transcription regulators do the opposite: they switch genes on, or *activate* them. These **transcriptional activator** proteins work on promoters that—in contrast to the promoter for the tryptophan operon—are only marginally able to bind and position RNA polymerase on their own. However, these poorly functioning promoters can be made fully functional by activator proteins that bind nearby and contact the RNA polymerase to help it initiate transcription (**Figure 8–8**).

Like the tryptophan repressor, activator proteins often have to interact with a second molecule to be able to bind DNA. For example, the bacterial activator protein CAP has to bind cyclic AMP (cAMP) before it can bind to DNA (see Figure 4–19). Genes activated by CAP are switched on in response to an increase in intracellular cAMP concentration, which rises when glucose, the bacterium's preferred carbon source, is no longer available; as a result, CAP drives the production of enzymes that allow the bacterium to digest other sugars.

An Activator and a Repressor Control the Lac Operon

In many instances, the activity of a single promoter is controlled by two different transcription regulators. The *Lac operon* in *E. coli*, for example,

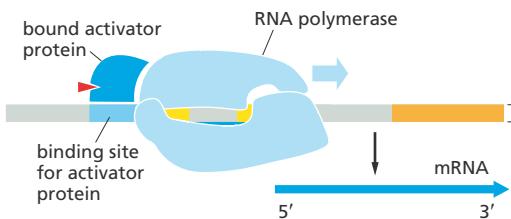


Figure 8–8 Genes can be switched on by activator proteins. An activator protein binds to a regulatory sequence on the DNA and then interacts with the RNA polymerase to help it initiate transcription. Without the activator, the promoter fails to initiate transcription efficiently. In bacteria, the binding of the activator to DNA is often controlled by the interaction of a metabolite or other small molecule (red triangle) with the activator protein. The *Lac operon* works in this manner, as we discuss shortly.

is controlled by both the *Lac* repressor and the CAP activator that we just discussed. The *Lac* operon encodes proteins required to import and digest the disaccharide lactose. In the absence of glucose, the bacterium makes cAMP, which activates CAP to switch on genes that allow the cell to utilize alternative sources of carbon—including lactose. It would be wasteful, however, for CAP to induce expression of the *Lac* operon if lactose itself were not present. Thus the Lac repressor shuts off the operon in the absence of lactose. This arrangement enables the control region of the *Lac* operon to integrate two different signals, so that the operon is highly expressed only when two conditions are met: glucose must be absent and lactose must be present (Figure 8–9). This genetic circuit thus behaves much like a switch that carries out a logic operation in a computer. When lactose is present AND glucose is absent, the cell executes the appropriate program—in this case, transcription of the genes that permit the uptake and utilization of lactose.

The elegant logic of the *Lac* operon first attracted the attention of biologists more than 50 years ago. The molecular basis of the switch in *E. coli* was uncovered by a combination of genetics and biochemistry, providing the first insight into how transcription is controlled. In a eukaryotic cell, similar transcription regulatory devices are combined to generate increasingly complex circuits, including those that enable a fertilized egg to form the tissues and organs of a multicellular organism.

QUESTION 8–1

Bacterial cells can take up the amino acid tryptophan (Trp) from their surroundings, or if there is an insufficient external supply they can synthesize tryptophan from other small molecules. The Trp repressor is a transcription regulator that shuts off the transcription of genes that code for the enzymes required for the synthesis of tryptophan (see Figure 8–7).

A. What would happen to the regulation of the tryptophan operon in cells that express a mutant form of the tryptophan repressor that (1) cannot bind to DNA, (2) cannot bind tryptophan, or (3) binds to DNA even in the absence of tryptophan?

B. What would happen in scenarios (1), (2), and (3) if the cells, in addition, produced normal tryptophan repressor protein from a second, normal gene?

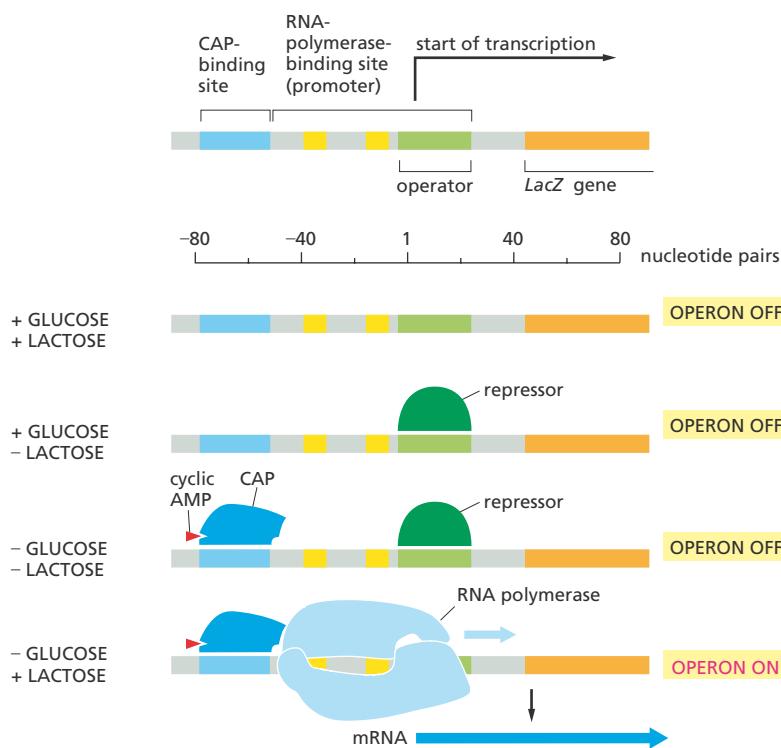


Figure 8–9 The *Lac* operon is controlled by two transcription regulators, the *Lac* repressor and CAP. When lactose is absent, the *Lac* repressor binds to the *Lac* operator and shuts off expression of the operon. Addition of lactose increases the intracellular concentration of a related compound, allolactose; allolactose binds to the *Lac* repressor, causing it to undergo a conformational change that releases its grip on the operator DNA (not shown). When glucose is absent, cyclic AMP (red triangle) is produced by the cell, and CAP binds to DNA. *LacZ*, the first gene of the operon, encodes the enzyme β -galactosidase, which breaks down lactose to galactose and glucose.

QUESTION 8–2

Explain how DNA-binding proteins can make sequence-specific contacts to a double-stranded DNA molecule without breaking the hydrogen bonds that hold the bases together. Indicate how, through such contacts, a protein can distinguish a T-A from a C-G pair. Indicate the parts of the nucleotide base pairs that could form noncovalent interactions—hydrogen bonds, electrostatic attractions, or hydrophobic interactions (see Panel 2–7, pp. 78–79)—with a DNA-binding protein. The structures of all the base pairs in DNA are given in Figure 5–6.

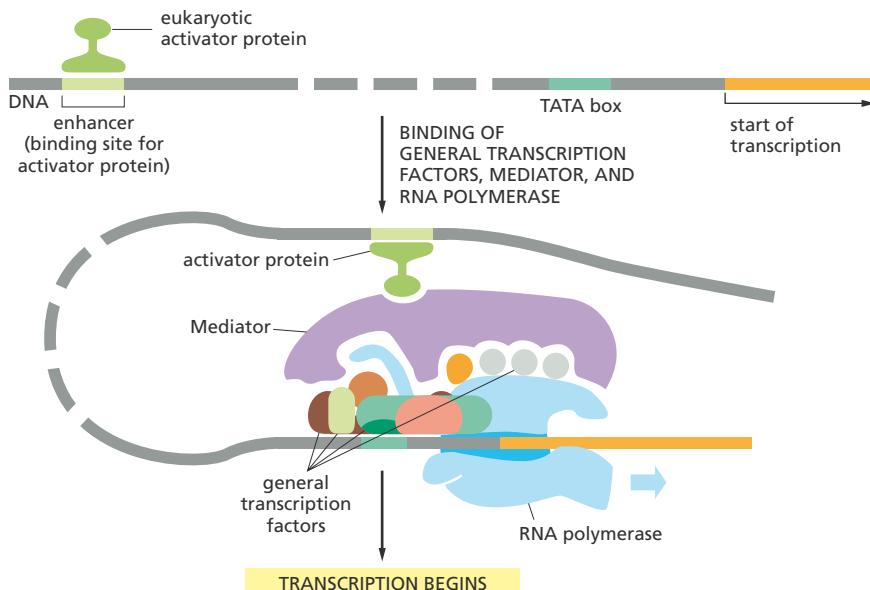
Eukaryotic Transcription Regulators Control Gene Expression from a Distance

Eukaryotes, too, use transcription regulators—both activators and repressors—to regulate the expression of their genes. The DNA sites to which eukaryotic gene activators bind are termed *enhancers*, because their presence dramatically enhances the rate of transcription. It was surprising to biologists when, in 1979, it was discovered that these activator proteins could enhance transcription even when they are bound thousands of nucleotide pairs away from a gene's promoter. They also work when bound either upstream or downstream from the gene. These observations raised several questions. How do enhancer sequences and the proteins bound to them function over such long distances? How do they communicate with the promoter?

Many models for this “action at a distance” have been proposed, but the simplest of these seems to apply in most cases. The DNA between the enhancer and the promoter loops out to allow eukaryotic activator proteins to influence directly events that take place at the promoter (**Figure 8–10**). The DNA thus acts as a tether, allowing a protein that is bound to an enhancer—even one that is thousands of nucleotide pairs away—to interact with the proteins in the vicinity of the promoter—including RNA polymerase and the general transcription factors (see Figure 7–12). Often, additional proteins serve to link the distantly bound transcription regulators to these proteins at the promoter; the most important of these regulators is a large complex of proteins known as *Mediator* (see Figure 8–10). One of the ways in which these proteins function is by aiding the assembly of the general transcription factors and RNA polymerase to form a large *transcription complex* at the promoter. Eukaryotic repressor proteins do the opposite: they decrease transcription by preventing the assembly of the same protein complex.

In addition to promoting—or repressing—the assembly of a transcription initiation complex directly, eukaryotic transcription regulators have an additional mechanism of action: they attract proteins that modify chromatin structure and thereby affect the accessibility of the promoter to the general transcription factors and RNA polymerase, as we discuss next.

Figure 8–10 In eukaryotes, gene activation can occur at a distance. An activator protein bound to a distant enhancer attracts RNA polymerase and general transcription factors to the promoter. Looping of the intervening DNA permits contact between the activator and the transcription initiation complex bound to the promoter. In the case shown here, a large protein complex called Mediator serves as a go-between. The broken stretch of DNA signifies that the length of DNA between the enhancer and the start of transcription varies, sometimes reaching tens of thousands of nucleotide pairs in length. The TATA box is a DNA recognition sequence for the first general transcription factor that binds to the promoter (see Figure 7–12).



Eukaryotic Transcription Regulators Help Initiate Transcription by Recruiting Chromatin-Modifying Proteins

Initiation of transcription in eukaryotic cells must also take into account the packaging of DNA into chromosomes. As discussed in Chapter 5, eukaryotic DNA is packed into nucleosomes, which, in turn, are folded into higher-order structures. How do transcription regulators, general transcription factors, and RNA polymerase gain access to such DNA? Nucleosomes can inhibit the initiation of transcription if they are positioned over a promoter, because they physically block the assembly of the general transcription factors or RNA polymerase on the promoter. Such chromatin packaging may have evolved in part to prevent leaky gene expression by blocking the initiation of transcription in the absence of the proper activator proteins.

In eukaryotic cells, activator and repressor proteins exploit chromatin structure to help turn genes on and off. As we saw in Chapter 5, chromatin structure can be altered by *chromatin-remodeling complexes* and by enzymes that covalently modify the histone proteins that form the core of the nucleosome (see Figures 5–26 and 5–27). Many gene activators take advantage of these mechanisms by recruiting such chromatin-modifying proteins to promoters. For example, the recruitment of *histone acetyltransferases* promotes the attachment of acetyl groups to selected lysines in the tail of histone proteins. This modification alters chromatin structure, allowing greater accessibility to the underlying DNA; moreover, the acetyl groups themselves attract proteins that promote transcription, including some of the general transcription factors (Figure 8–11).

Likewise, gene repressor proteins can modify chromatin in ways that reduce the efficiency of transcription initiation. For example, many repressors attract *histone deacetylases*—enzymes that remove the acetyl groups from histone tails, thereby reversing the positive effects that acetylation has on transcription initiation. Although some eukaryotic repressor proteins work on a gene-by-gene basis, others can orchestrate the formation of large swathes of transcriptionally inactive chromatin containing many

QUESTION 8–3

Some transcription regulators bind to DNA and cause the double helix to bend at a sharp angle. Such “bending proteins” can stimulate the initiation of transcription without contacting either the RNA polymerase, any of the general transcription factors, or any other transcription regulators. Can you devise a plausible explanation for how these proteins might work to modulate transcription? Draw a diagram that illustrates your explanation.

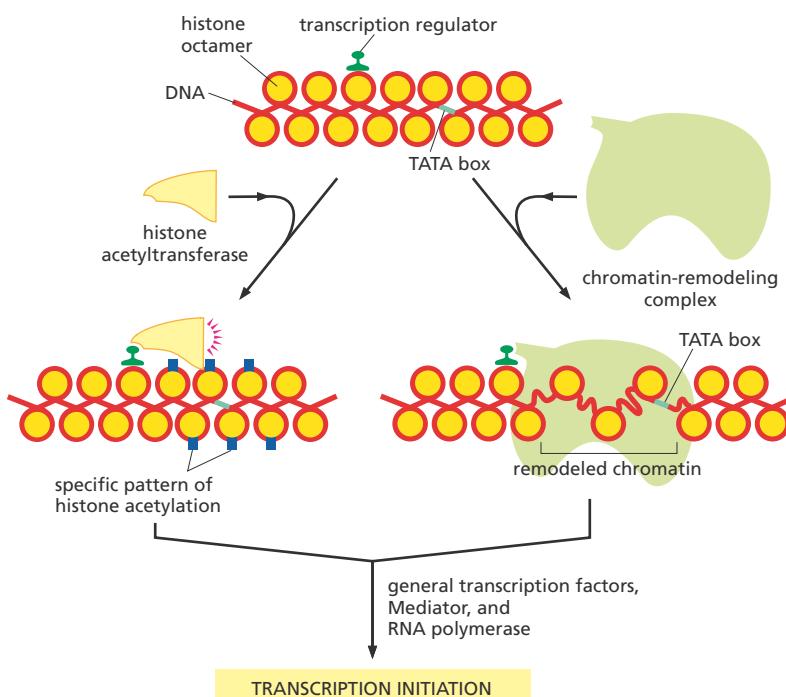


Figure 8–11 Eukaryotic transcriptional activators can recruit chromatin-modifying proteins to help initiate gene transcription. On the right, chromatin-remodeling complexes render the DNA packaged in chromatin more accessible to other proteins in the cell, including those required for transcription initiation; notice, for example, the increased exposure of the TATA box. On the left, the recruitment of histone-modifying enzymes such as histone acetyltransferases adds acetyl groups to specific histones, which can then serve as binding sites for proteins that stimulate transcription initiation (not shown).

genes. As discussed in Chapter 5, these transcription-resistant regions of DNA include the heterochromatin found in interphase chromosomes and the inactive X chromosome in the cells of female mammals.

THE MOLECULAR MECHANISMS THAT CREATE SPECIALIZED CELL TYPES

All cells must be able to switch genes on and off in response to signals in their environment. But the cells of multicellular organisms have evolved this capacity to an extreme degree and in highly specialized ways to form organized arrays of differentiated cell types. In particular, once a cell in a multicellular organism becomes committed to differentiate into a specific cell type, the choice of fate is generally maintained through subsequent cell divisions. This means that the changes in gene expression, which are often triggered by a transient signal, must be remembered by the cell. This phenomenon of *cell memory* is a prerequisite for the creation of organized tissues and for the maintenance of stably differentiated cell types. In contrast, the simplest changes in gene expression in both eukaryotes and bacteria are often only transient; the tryptophan repressor, for example, switches off the tryptophan operon in bacteria only in the presence of tryptophan; as soon as the amino acid is removed from the medium, the genes switch back on, and the descendants of the cell will have no memory that their ancestors had been exposed to tryptophan.

In this section, we discuss some of the special features of transcriptional regulation that are found in multicellular organisms. Our focus will be on how these mechanisms create and maintain the specialized cell types that give a worm, a fly, or a human its distinctive characteristics.

Eukaryotic Genes Are Controlled by Combinations of Transcription Regulators

Because eukaryotic transcription regulators can control transcription initiation when bound to DNA many base pairs away from the promoter, the nucleotide sequences that control the expression of a gene can be spread over long stretches of DNA. In animals and plants, it is not unusual to find the regulatory DNA sequences of a gene dotted over tens of thousands of nucleotide pairs, although much of the intervening DNA serves as “spacer” sequence and is not directly recognized by the transcription regulators.

So far in this chapter, we have treated transcription regulators as though each functions individually to turn a gene on or off. While this idea holds true for many simple bacterial activators and repressors, most eukaryotic transcription regulators work as part of a “committee” of regulatory proteins, all of which are necessary to express the gene in the right place, in the right cell type, in response to the right conditions, at the right time, and in the required amount.

The term **combinatorial control** refers to the way that groups of transcription regulators work together to determine the expression of a single gene. We saw a simple example of such regulation by multiple regulators when we discussed the bacterial *Lac* operon (see Figure 8–9). In eukaryotes, the regulatory inputs have been amplified, and a typical gene is controlled by dozens of transcription regulators. These help assemble chromatin-remodeling complexes, histone-modifying enzymes, RNA polymerase, and general transcription factors via the multiprotein Mediator complex (Figure 8–12). In many cases, both repressors and activators will be present in the same complex; how the cell integrates the effects of all of these proteins to determine the final level of gene

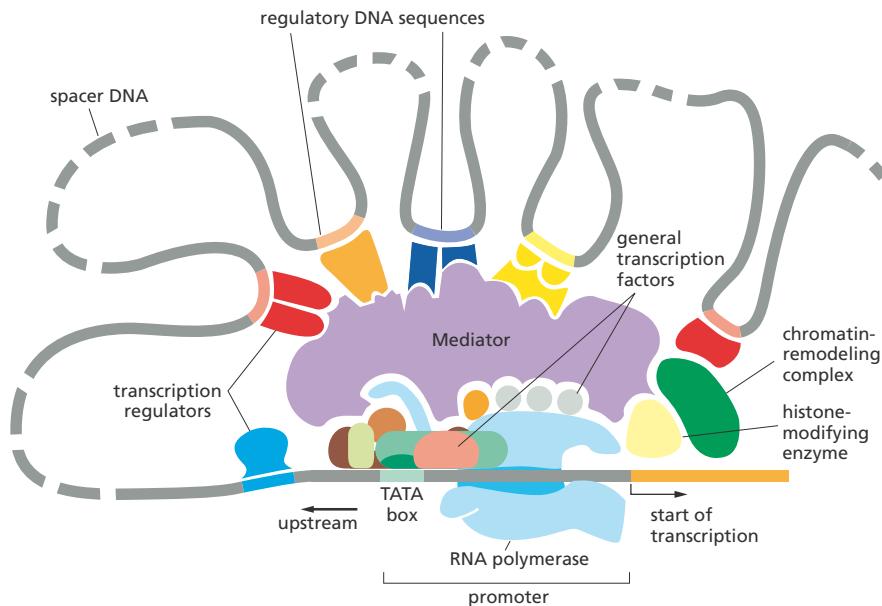


Figure 8–12 Transcription regulators work together as a “committee” to control the expression of a eukaryotic gene. Whereas the general transcription factors that assemble at the promoter are the same for all genes transcribed by RNA polymerase (see Figure 7–12), the transcription regulators and the locations of their DNA binding sites relative to the promoters are different for different genes. These regulators, along with chromatin-modifying proteins, are assembled at the promoter by the Mediator. The effects of multiple transcription regulators combine to determine the final rate of transcription initiation.

expression is only now beginning to be understood. An example of such a complex regulatory system—one that participates in the development of a fruit fly from a fertilized egg—is described in **How We Know**, pp. 274–275.

The Expression of Different Genes Can Be Coordinated by a Single Protein

In addition to being able to switch individual genes on and off, all cells—whether prokaryote or eukaryote—need to coordinate the expression of different genes. When a eukaryotic cell receives a signal to divide, for example, a number of hitherto unexpressed genes are turned on together to set in motion the events that lead eventually to cell division (discussed in Chapter 18). As discussed earlier, one way in which bacteria coordinate the expression of a set of genes is by having them clustered together in an operon under the control of a single promoter (see Figure 8–6). Such clustering is not seen in eukaryotic cells, where each gene is transcribed and regulated individually. So how do these cells coordinate gene expression? In particular, given that a eukaryotic cell uses a committee of transcription regulators to control each of its genes, how can it rapidly and decisively switch whole groups of genes on or off?

The answer is that even though control of gene expression is combinatorial, the effect of a single transcription regulator can still be decisive in switching any particular gene on or off, simply by completing the combination needed to activate or repress that gene. This is like dialing in the final number of a combination lock: the lock will spring open if the other numbers have been previously entered. Just as the same number can complete the combination for different locks, the same protein can complete the combination for several different genes. As long as different genes contain regulatory DNA sequences that are recognized by the same transcription regulator, they can be switched on or off together, as a coordinated unit.

An example of such coordinated regulation in humans is seen with the *cortisol receptor protein*. In order to bind to regulatory sites in DNA, this

GENE REGULATION—THE STORY OF EVE

The ability to regulate gene expression is crucial to the proper development of a multicellular organism from a fertilized egg to a fertile adult. Beginning at the earliest moments in development, a succession of transcriptional programs guides the differential expression of genes that allows an animal to form a proper body plan—helping to distinguish its back from its belly, and its head from its tail. These programs ultimately direct the correct placement of a wing or a leg, a mouth or an anus, a neuron or a sex cell.

A central challenge in development, then, is to understand how an organism generates these patterns of gene expression, which are laid down within hours of fertilization. Among the most important genes involved in these early stages of development are those that encode transcription regulators. By interacting with different regulatory DNA sequences, these proteins instruct every cell in the embryo to switch on the genes that are appropriate for that cell at each time point during development. How can a protein binding to a piece of DNA help direct the development of a complex multicellular organism? To see how we can address that large question, we review the story of *Eve*.

Seeing Eve

Even-skipped—*Eve*, for short—is a gene whose expression plays an important part in the development of the *Drosophila* embryo. If this gene is inactivated by mutation, many parts of the embryo fail to form and the fly larva dies early in development. But *Eve* is not expressed uniformly throughout the embryo. Instead, the *Eve* protein is produced in a striking series of seven neat stripes, each of which occupies a very precise position along the length of the embryo. These seven stripes correspond to seven of the fourteen segments that define the body plan of the fly—three for the head, three for the thorax, and eight for the abdomen.

This pattern never varies: *Eve* can be found in the very same places in every *Drosophila* embryo (see Figure 8–13B). How can the expression of a gene be regulated with such spatial precision—such that one cell will produce a protein while a neighboring cell does not? To find out, researchers took a trip upstream.

Dissecting the DNA

As we have seen in this chapter, regulatory DNA sequences control which cells in an organism will express a particular gene, and at what point during development that gene will be turned on. In eukaryotes,

these regulatory sequences are frequently located upstream of the gene itself. One way to locate a regulatory DNA sequence—and study how it operates—is to remove a piece of DNA from the region upstream of a gene of interest and insert that DNA upstream of a **reporter gene**—one that encodes a protein with an activity that is easy to monitor experimentally. If the piece of DNA contains a regulatory sequence, it will drive the expression of the reporter gene. When this patchwork piece of DNA is subsequently introduced into a cell or organism, the reporter gene will be expressed in the same cells and tissues that normally express the gene from which the regulatory sequence was derived (see Figure 10–31).

By excising various segments of the DNA sequences upstream of *Eve*, and coupling them to a reporter gene, researchers found that the expression of the gene is controlled by a series of seven regulatory modules—each of which specifies a single stripe of *Eve* expression. In this way, researchers identified, for example, a single segment of regulatory DNA that specifies stripe 2. They could excise this regulatory segment, link it to a reporter gene, and introduce the resulting DNA segment into the fly. When they examined embryos that carried this engineered DNA, they found that the reporter gene is expressed in the precise position of stripe 2 (Figure 8–13). Similar experiments revealed the existence of six other regulatory modules, one for each of the other *Eve* stripes.

The next question is: How does each of these seven regulatory segments direct the formation of a single stripe in a specific position? The answer, researchers found, is that each segment contains a unique combination of regulatory sequences that bind different combinations of transcription regulators. These regulators, like *Eve* itself, are distributed in unique patterns within the embryo—some toward the head, some toward the rear, some in the middle.

The regulatory segment that defines stripe 2, for example, contains regulatory DNA sequences for four transcription regulators: two that activate *Eve* transcription and two that repress it (Figure 8–14). In the narrow band of tissue that constitutes stripe 2, it just so happens the repressor proteins are not present—so the *Eve* gene is expressed; in the bands of tissue on either side of the stripe, the repressors keep *Eve* quiet. And so a stripe is formed.

The regulatory segments controlling the other stripes are thought to function along similar lines; each regulatory segment reads “positional information” provided

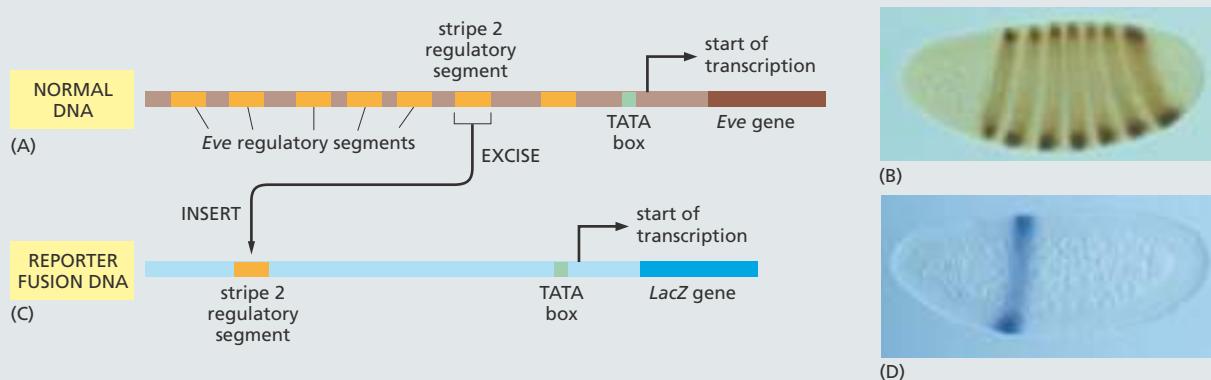


Figure 8–13 An experimental approach that involves the use of a reporter gene reveals the modular construction of the *Eve* gene regulatory region. (A) Expression of the *Eve* gene is controlled by a series of regulatory segments (orange) that direct the production of *Eve* protein in stripes along the embryo. (B) Embryos stained with antibodies to the *Eve* protein show the seven characteristic stripes of *Eve* expression. (C) In the laboratory, the regulatory segment that directs the formation of stripe 2 can be excised from the DNA shown in part A and inserted upstream of the *E. coli* *LacZ* gene, which encodes the enzyme β-galactosidase (see Figure 8–9). (D) When the engineered DNA containing the stripe 2 regulatory segment is introduced into the genome of a fly, the resulting embryo expresses β-galactosidase precisely in the position of the second *Eve* stripe. Enzyme activity is assayed by the addition of X-gal, a modified sugar that when cleaved by β-galactosidase generates an insoluble blue product. (B and D, courtesy of Stephen Small and Michael Levine.)

by some unique combination of transcription regulators in the embryo and expresses *Eve* on the basis of this information. The entire regulatory region is strung out over 20,000 nucleotide pairs of DNA and, altogether, binds more than 20 transcription regulators. This large regulatory region is built from a series of smaller regulatory segments, each of which consists of a unique arrangement of regulatory DNA sequences recognized by specific transcription regulators. In this way, the

Eve gene can respond to an enormous combination of inputs.

The *Eve* protein is itself a transcription regulator, and it—in combination with many other regulatory proteins—controls key events in the development of the fly. This complex organization of a discrete number of regulatory elements begins to explain how the development of an entire organism can be orchestrated by repeated applications of a few basic principles.

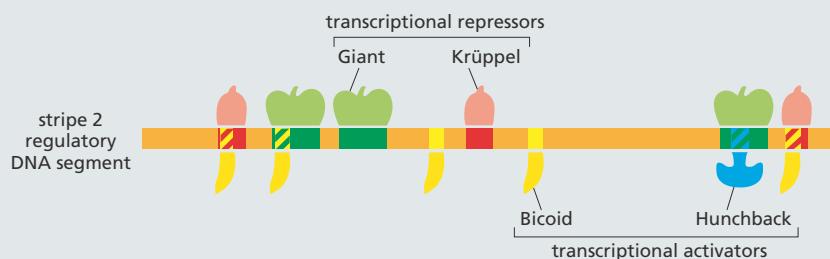
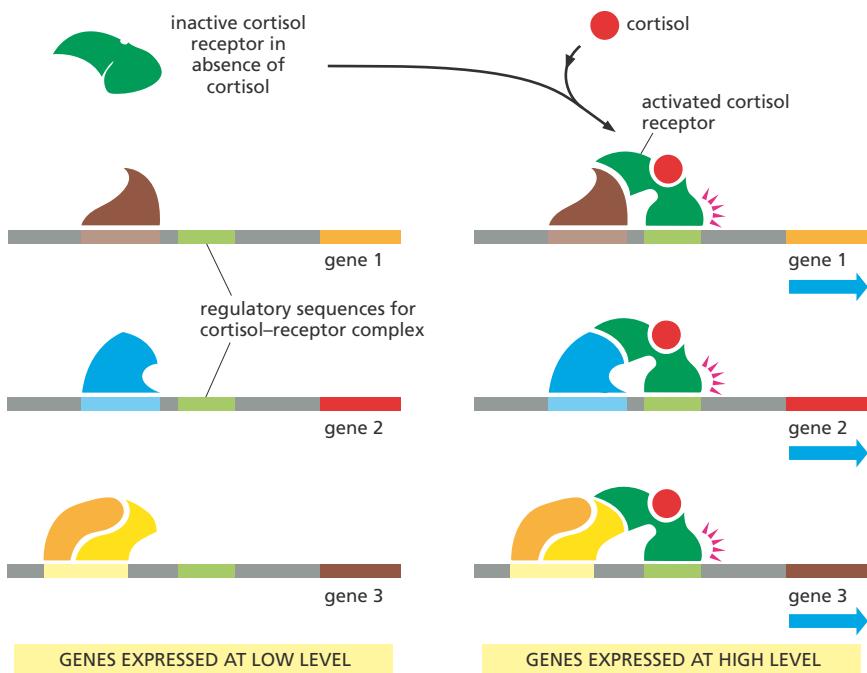


Figure 8–14 The regulatory segment that specifies *Eve* stripe 2 contains binding sites for four different transcription regulators. All four regulators are responsible for the proper expression of *Eve* in stripe 2. Flies that are deficient in the two activators, called Bicoid and Hunchback, fail to form stripe 2 efficiently; in flies deficient in either of the two repressors, called Giant and Krüppel, stripe 2 expands and covers an abnormally broad region of the embryo. As indicated in the diagram, in some cases the binding sites for the transcription regulators overlap, and the proteins compete for binding to the DNA. For example, the binding of Bicoid and Krüppel to the site at the far right is thought to be mutually exclusive. The regulatory segment is 480 base pairs in length.

Figure 8–15 A single transcription regulator can coordinate the expression of many different genes. The action of the cortisol receptor is illustrated. On the left is a series of genes, each of which has a different gene activator protein bound to its respective regulatory DNA sequences. However, these bound proteins are not sufficient on their own to activate transcription efficiently. On the right is shown the effect of adding an additional transcription regulator—the cortisol-receptor complex—that can bind to the same regulatory DNA sequence in each gene. The activated cortisol receptor completes the combination of transcription regulators required for efficient initiation of transcription, and the genes are now switched on as a set.



transcription regulator must first form a complex with a molecule of cortisol (see Table 16–1, p. 529). In response to cortisol, liver cells increase the expression of many genes, one of which encodes the enzyme tyrosine aminotransferase, as discussed earlier. All these genes are regulated by the binding of the cortisol-receptor complex to a regulatory sequence in the DNA of each gene. When the cortisol concentration decreases again, the expression of all of these genes drops to its normal level. In this way, a single transcription regulator can coordinate the expression of many different genes (Figure 8–15).

Combinatorial Control Can Also Generate Different Cell Types

The ability to switch many different genes on or off using a limited number of transcription regulators is not only useful in the day-to-day regulation of cell function. It is also one of the means by which eukaryotic cells diversify into particular types of cells during embryonic development. A striking example is the development of muscle cells. A mammalian skeletal muscle cell is distinguished from other cells by the production of a large number of characteristic proteins, such as the muscle-specific forms of actin and myosin that make up the contractile apparatus (discussed in Chapter 17), as well as the receptor proteins and ion channel proteins in the plasma membrane that make the muscle cell sensitive to nerve stimulation. The genes encoding these muscle-specific proteins are all switched on coordinately as the muscle cell differentiates. Studies of developing muscle cells in culture have identified a small number of key transcription regulators, expressed only in potential muscle cells, that coordinate muscle-specific gene expression and are thus crucial for muscle-cell differentiation. This set of regulators activates the transcription of the genes that code for muscle-specific proteins by binding to specific DNA sequences present in their regulatory regions.

Some transcription regulators can even convert one specialized cell type to another. For example, when the gene encoding the transcription regulator MyoD is artificially introduced into fibroblasts cultured from skin

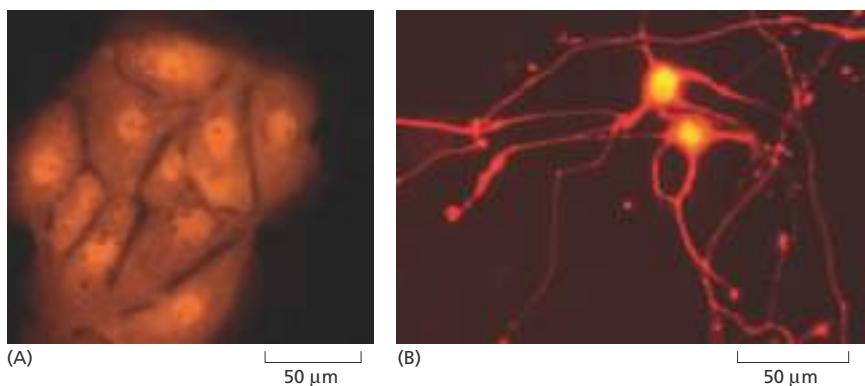


Figure 8–16 A small number of transcription regulators can convert one differentiated cell type directly into another. In this experiment, liver cells grown in culture (A) were converted into neuronal cells (B) via the artificial introduction of three nerve-specific transcription regulators. The cells are labeled with a fluorescent dye. (From S. Marro et al., *Cell Stem Cell* 9:374–378, 2011. With permission from Elsevier.)

connective tissue, the fibroblasts form musclelike cells. It appears that the fibroblasts, which are derived from the same broad class of embryonic cells as muscle cells, have already accumulated many of the other necessary transcription regulators required for the combinatorial control of the muscle-specific genes, and that addition of MyoD completes the unique combination required to direct the cells to become muscle.

This type of reprogramming can produce even more dramatic effects. For example, a set of nerve-specific transcription regulators, when artificially expressed in cultured liver cells, can convert them into functional neurons (Figure 8–16). Such dramatic results suggest that it may someday be possible to produce in the laboratory any cell type for which the correct combination of transcription regulators can be identified. How these transcription regulators can then lead to the generation of different cell types is illustrated schematically in Figure 8–17.

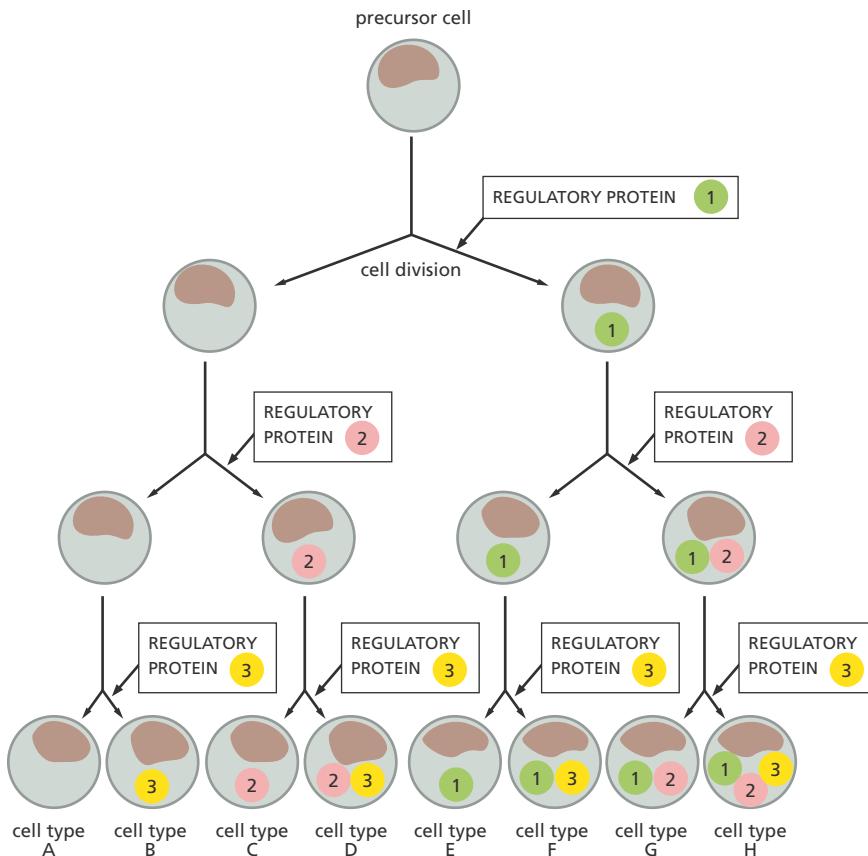
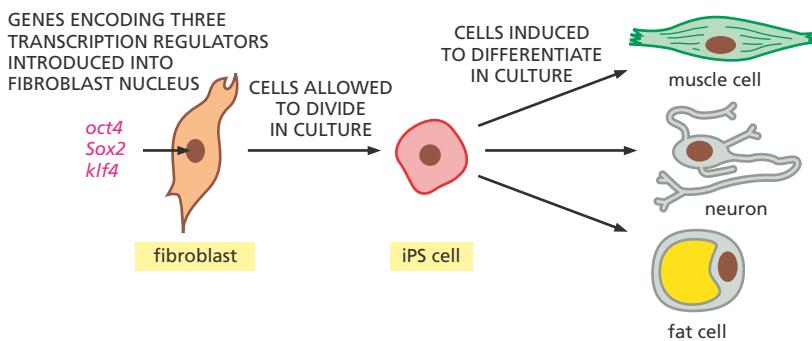


Figure 8–17 Combinations of a few transcription regulators can generate many cell types during development. In this simple scheme, a “decision” to make a new transcription regulator (shown as a numbered circle) is made after each cell division. Repetition of this simple rule can generate eight cell types (A through H), using only three transcription regulators. Each of these hypothetical cell types would then express many different genes, as dictated by the combination of transcription regulators that each cell type produces.

Figure 8–18 A combination of transcription regulators can induce a differentiated cell to de-differentiate into a pluripotent cell. The artificial expression of a set of four genes, each of which encodes a transcription regulator, can reprogram a fibroblast into a pluripotent cell with ES cell-like properties. Like ES cells, such iPS cells can proliferate indefinitely in culture and can be stimulated by appropriate extracellular signal molecules to differentiate into almost any cell type in the body.



Specialized Cell Types Can Be Experimentally Reprogrammed to Become Pluripotent Stem Cells

We have seen that, in some cases, one type of differentiated cell can be experimentally converted into another type by the artificial expression of specific transcription regulators (see Figure 8–16). Even more surprising, transcription regulators can coax various differentiated cells to *de-differentiate* into **pluripotent stem cells** that are capable of giving rise to all the specialized cell types in the body, much like the embryonic stem (ES) cells discussed in Chapter 20 (see pp. 708–711).

Using a defined set of transcription regulators, cultured mouse fibroblasts have been reprogrammed to become **induced pluripotent stem (iPS) cells**—cells that look and behave like the pluripotent ES cells that are derived from embryos (Figure 8–18). The approach was quickly adapted to produce iPS cells from a variety of specialized cell types, including cells taken from humans. Such human iPS cells can then be directed to generate a population of differentiated cells for use in the study or treatment of disease, as we discuss in Chapter 20.

The Formation of an Entire Organ Can Be Triggered by a Single Transcription Regulator

We have seen that a small number of transcription regulators can control the expression of whole sets of genes and can even convert one cell type into another. But an even more stunning example of the power of transcriptional control comes from studies of eye development in *Drosophila*. In this case, a single “master” transcription regulator called *Ey* could be used to trigger the formation of not just a single cell type but a whole organ. In the laboratory, the *Ey* gene can be artificially expressed in fruit fly embryos in cells that would normally give rise to a leg. When these modified embryos develop into adult flies, some have an eye in the middle of a leg (Figure 8–19).



Figure 8–19 Artificially induced expression of the *Drosophila* *Ey* gene in the precursor cells of the leg triggers the misplaced development of an eye on a fly's leg. (Courtesy of Walter Gehring.)

How the *Ey* protein coordinates the specification of each type of cell found in the eye—and directs their proper organization in three-dimensional space—is an actively studied topic in developmental biology. In essence, however, *Ey* functions like any other transcription regulator, controlling the expression of multiple genes by binding to DNA sequences in their regulatory regions. Some of the genes controlled by *Ey* encode additional transcription regulators that, in turn, control the expression of other genes. In this way, the action of a single transcription regulator can produce a cascade of regulators that, working in combination, lead to the formation of an organized group of many different types of cells. One can begin to imagine how, by repeated applications of this principle, a complex organism self-assembles, piece by piece.

Epigenetic Mechanisms Allow Differentiated Cells to Maintain Their Identity

Once a cell has become differentiated into a particular cell type, it will generally remain differentiated, and all its progeny cells will remain that same cell type. Some highly specialized cells, including skeletal muscle cells and neurons, never divide again once they have differentiated—that is, they are *terminally differentiated* (as discussed in Chapter 18). But many other differentiated cells—such as fibroblasts, smooth muscle cells, and liver cells—will divide many times in the life of an individual. When they do, these specialized cell types give rise only to cells like themselves: smooth muscle cells do not give rise to liver cells, nor liver cells to fibroblasts.

For a proliferating cell to maintain its identity—a property called **cell memory**—the patterns of gene expression responsible for that identity must be remembered and passed on to its daughter cells through all subsequent cell divisions. Thus, in the model illustrated in Figure 8–17, the production of each transcription regulator, once begun, has to be continued in the daughter cells of each cell division. How is such perpetuation accomplished?

Cells have several ways of ensuring that their daughters “remember” what kind of cells they are. One of the simplest and most important is through a **positive feedback loop**, where a master transcription regulator activates transcription of its own gene, in addition to that of other cell-type-specific genes. Each time a cell divides the regulator is distributed to both daughter cells, where it continues to stimulate the positive feedback loop. The continued stimulation ensures that the regulator will continue to be produced in subsequent cell generations. The Ey protein discussed earlier functions in such a positive feedback loop. Positive feedback is crucial for establishing the “self-sustaining” circuits of gene expression that allow a cell to commit to a particular fate—and then to transmit that information to its progeny (Figure 8–20).

Although positive feedback loops are probably the most prevalent way of ensuring that daughter cells remember what kind of cells they are meant to be, there are other ways of reinforcing cell identity. One involves the methylation of DNA. In vertebrate cells, **DNA methylation** occurs on certain cytosine bases (Figure 8–21). This covalent modification generally

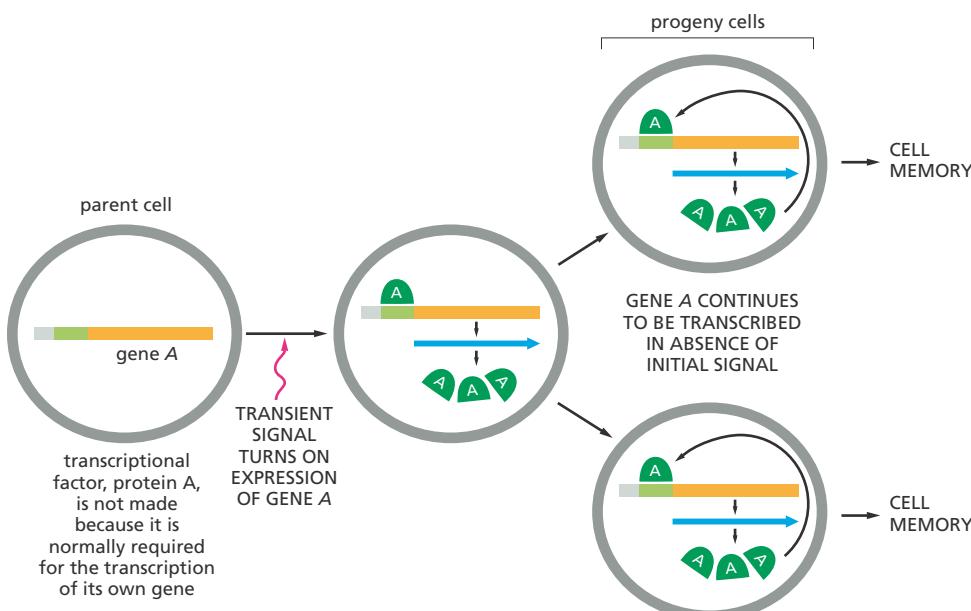


Figure 8–20 A positive feedback loop can create cell memory.

Protein A is a master transcription regulator that activates the transcription of its own gene—as well as other cell-type-specific genes (not shown). All of the descendants of the original cell will therefore “remember” that the progenitor cell had experienced a transient signal that initiated the production of protein A.

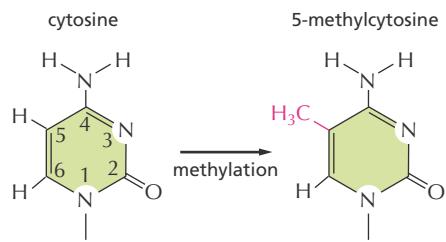


Figure 8-21 Formation of 5-methylcytosine occurs by methylation of a cytosine base in the DNA double helix. In vertebrates, this modification is confined to selected cytosine (C) nucleotides that fall next to a guanine (G) in the sequence CG.

turns off genes by attracting proteins that bind to methylated cytosines and block gene transcription. DNA methylation patterns are passed on to progeny cells by the action of an enzyme that copies the methylation pattern on the parent DNA strand to the daughter DNA strand as it is synthesized (**Figure 8-22**).

Another mechanism for inheriting gene expression patterns involves the modification of histones. When a cell replicates its DNA, each daughter double helix receives half of its parent's histone proteins, which contain the covalent modifications of the parent chromosome. Enzymes responsible for these modifications may bind to the parental histones and confer the same modifications to the new histones nearby. This cycle of modification reestablishes the pattern of chromatin structure found in the parent chromosome (**Figure 8-23**).

Because all of these cell-memory mechanisms transmit patterns of gene expression from parent to daughter cell without altering the actual nucleotide sequence of the DNA, they are considered to be forms of **epigenetic inheritance**. Such epigenetic changes play an important part in controlling patterns of gene expression, allowing transient signals from the environment to be permanently recorded by our cells—a fact that has important implications for understanding how cells operate and how they malfunction in disease.

POST-TRANSCRIPTIONAL CONTROLS

We have seen that transcription regulators control gene expression by promoting or hindering the transcription of specific genes. The vast majority of genes in all organisms are regulated in this way. But many additional points of control can come into play later in the pathway from DNA to protein, giving cells a further opportunity to regulate the amount or activity of the gene products that they make (see Figure 8-3). These **post-transcriptional controls**, which operate after transcription has begun, play a crucial part in regulating the expression of almost all genes.

We have already encountered a few examples of such post-transcriptional control. We have seen how alternative RNA splicing allows different

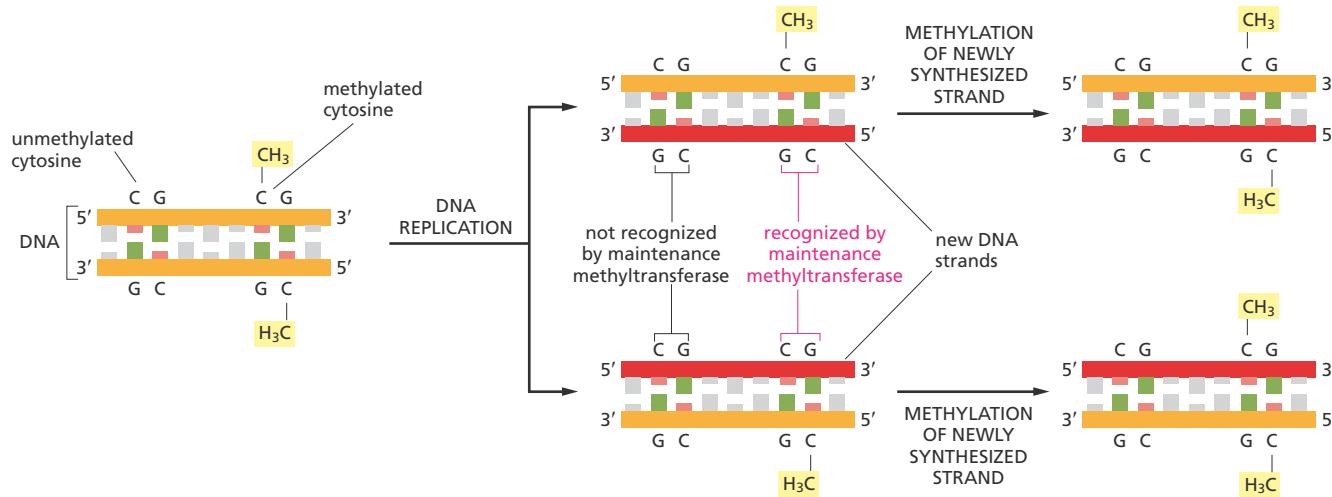


Figure 8-22 DNA methylation patterns can be faithfully inherited when a cell divides. An enzyme called a maintenance methyltransferase guarantees that once a pattern of DNA methylation has been established, it is inherited by newly made DNA. Immediately after DNA replication, each daughter double helix will contain one methylated DNA strand—Inherited from the parent double helix—and one unmethylated, newly synthesized strand. The maintenance methyltransferase interacts with these hybrid double helices and methylates only those CG sequences that are base-paired with a CG sequence that is already methylated.

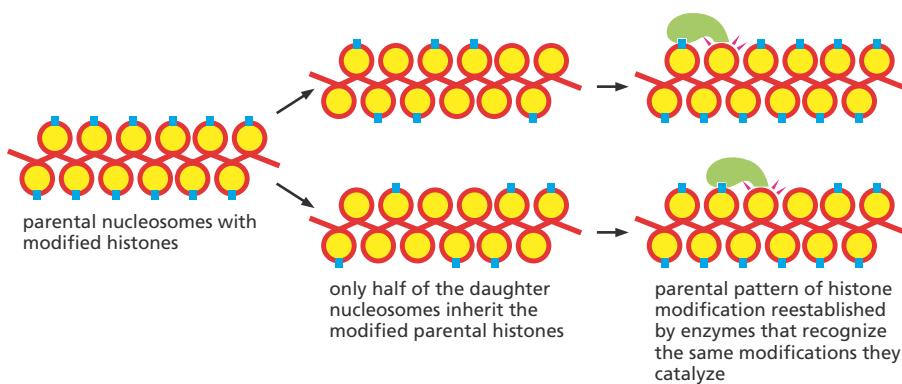


Figure 8–23 Histone modifications may be inherited by daughter chromosomes. When a chromosome is replicated, its resident histones are distributed more or less randomly to each of the two daughter DNA double helices. Thus, each daughter chromosome will inherit about half of its parent's collection of modified histones. The remaining stretches of DNA receive newly synthesized, not-yet-modified histones. If the enzymes responsible for each type of modification bind to the specific modification they create, they can catalyze the spread of this modification on the new histones. This cycle of modification and recognition can restore the parental histone modification pattern and, ultimately, allow the inheritance of the parental chromatin structure. This mechanism may apply to some but not all types of histone modifications.

forms of a protein, encoded by the same gene, to be made in different tissues (Figure 7–22). And we have discussed how various post-translational modifications of a protein can regulate its concentration and activity (see Figure 4–43). In the remainder of this chapter, we consider several other examples—some only recently discovered—of the many ways in which cells can manipulate the expression of a gene after transcription has commenced.

Each mRNA Controls Its Own Degradation and Translation

The more time an mRNA persists in the cell before it is degraded, the more protein it will produce. In bacteria, most mRNAs last only a few minutes before being destroyed. This instability allows a bacterium to adapt quickly to environmental changes. Eukaryotic mRNAs are generally more stable. The mRNA that encodes β -globin, for example, has a half-life of more than 10 hours. Most eukaryotic mRNAs, however, have half-lives of less than 30 minutes, and the most short-lived are those that encode proteins whose concentrations need to change rapidly based on the cell's needs, such as transcription regulators. Whether bacterial or eukaryotic, an mRNA's lifetime is dictated by specific nucleotide sequences within the untranslated regions that lie both upstream and downstream of the protein-coding sequence. These sequences often harbor binding sites for proteins that are involved in RNA degradation.

In addition to the nucleotide sequences that regulate its half-life, each mRNA possesses sequences that help control how often or how efficiently it will be translated into protein. These sequences control translation initiation. Although the details differ between eukaryotes and bacteria, the general strategy is similar for both.

Bacterial mRNAs contain a short ribosome-binding sequence located a few nucleotide pairs upstream of the AUG codon where translation begins (see Figure 7–37). This binding sequence forms base pairs with the RNA in the small ribosomal subunit, correctly positioning the initiating AUG codon within the ribosome. Because this interaction is needed for efficient translation initiation, it provides an ideal target for translational control. By blocking—or exposing—the ribosome-binding sequence, the bacterium can either inhibit—or promote—the translation of an mRNA (Figure 8–24).

Eukaryotic mRNAs possess a 5' cap that helps guide the ribosome to the first AUG, the codon where translation will start (see Figure 7–36). Eukaryotic repressor proteins can inhibit translation initiation by binding to specific nucleotide sequences in the 5' untranslated region of the mRNA, thereby preventing the ribosome from finding the first AUG—a mechanism similar to that in bacteria. When conditions change, the cell can inactivate the repressor to initiate translation of the mRNA.

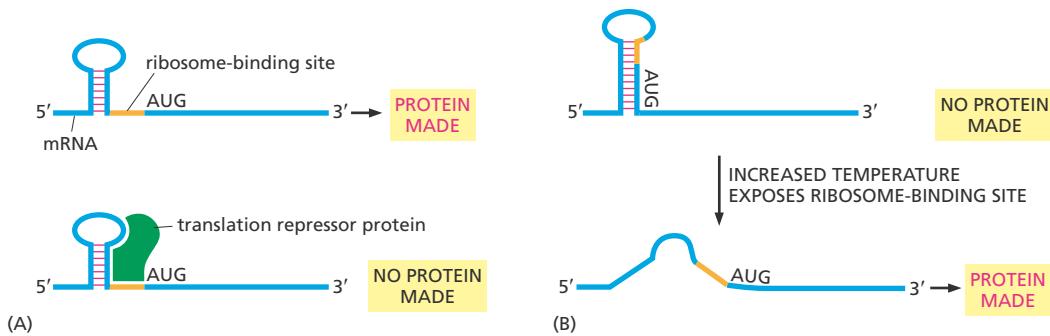


Figure 8–24 A bacterial gene’s expression can be controlled by regulating translation of its mRNA. (A) Sequence-specific RNA-binding proteins can repress the translation of specific mRNAs by keeping the ribosome from binding to the ribosome-binding sequence (orange) in the mRNA. Some ribosomal proteins exploit this mechanism to inhibit the translation of their own mRNA. In this way, “extra” ribosomal proteins—those not incorporated into ribosomes—serve as a signal to halt their synthesis. (B) An mRNA from the pathogen *Listeria monocytogenes* contains a “thermosensor” RNA sequence that controls the translation of a set of mRNAs produced by virulence genes. At the warmer temperature that the bacterium encounters inside its human host, the thermosensor sequence denatures, exposing the ribosome-binding sequence, so the virulence proteins are made.

Regulatory RNAs Control the Expression of Thousands of Genes

As we saw in Chapter 7, RNAs perform many critical tasks in cells. In addition to the mRNAs, which code for proteins, *noncoding RNAs* have various functions. It has long been known that some have key structural and catalytic roles, particularly in protein synthesis by ribosomes (see pp. 246–247). But a recent series of surprising discoveries has revealed several new classes of noncoding RNAs and shown that these RNAs are far more prevalent than previously suspected.

What, then, are all these newly discovered noncoding RNAs doing? Many have unanticipated but important roles in regulating gene expression and are therefore referred to as **regulatory RNAs**. There are at least three major types of regulatory RNAs—*microRNAs*, *small interfering RNAs*, and *long noncoding RNAs*. We discuss each one in turn.

MicroRNAs Direct the Destruction of Target mRNAs

MicroRNAs, or **miRNAs**, are tiny RNA molecules that control gene expression by base-pairing with specific mRNAs and reducing both their stability and their translation into protein. In humans, miRNAs are thought to regulate the expression of at least one-third of all protein-coding genes.

Like other noncoding RNAs, such as tRNA and rRNA, a precursor miRNA transcript undergoes a special type of processing to yield the mature, functional miRNA molecule, which is only about 22 nucleotides in length. This small but mature miRNA is packaged with specialized proteins to form an *RNA-induced silencing complex* (*RISC*), which patrols the cytoplasm in search of mRNAs that are complementary to the bound miRNA molecule (Figure 8–25). Once a target mRNA forms base pairs with an miRNA, it is either destroyed immediately by a nuclease present within the RISC or its translation is blocked. In the latter case, the bound mRNA molecule is delivered to a region of the cytoplasm where other nucleases eventually degrade it. Destruction of the mRNA releases the RISC and allows it to seek out additional mRNA targets. Thus, a single miRNA—as part of a RISC—can eliminate one mRNA molecule after another, thereby efficiently blocking production of the protein that the mRNAs encode.

Two features of miRNAs make them especially useful regulators of gene expression. First, a single miRNA can inhibit the transcription of a whole set of different mRNAs so long as all the mRNAs carry a common sequence, usually located in either their 5' or 3' untranslated regions. In humans, some individual miRNAs influence the transcription of hundreds of different mRNAs in this manner. Second, a gene that encodes an miRNA occupies relatively little space in the genome compared with one that encodes a transcription regulator. Indeed, their very small size is one reason that miRNAs were discovered only recently. There are thought

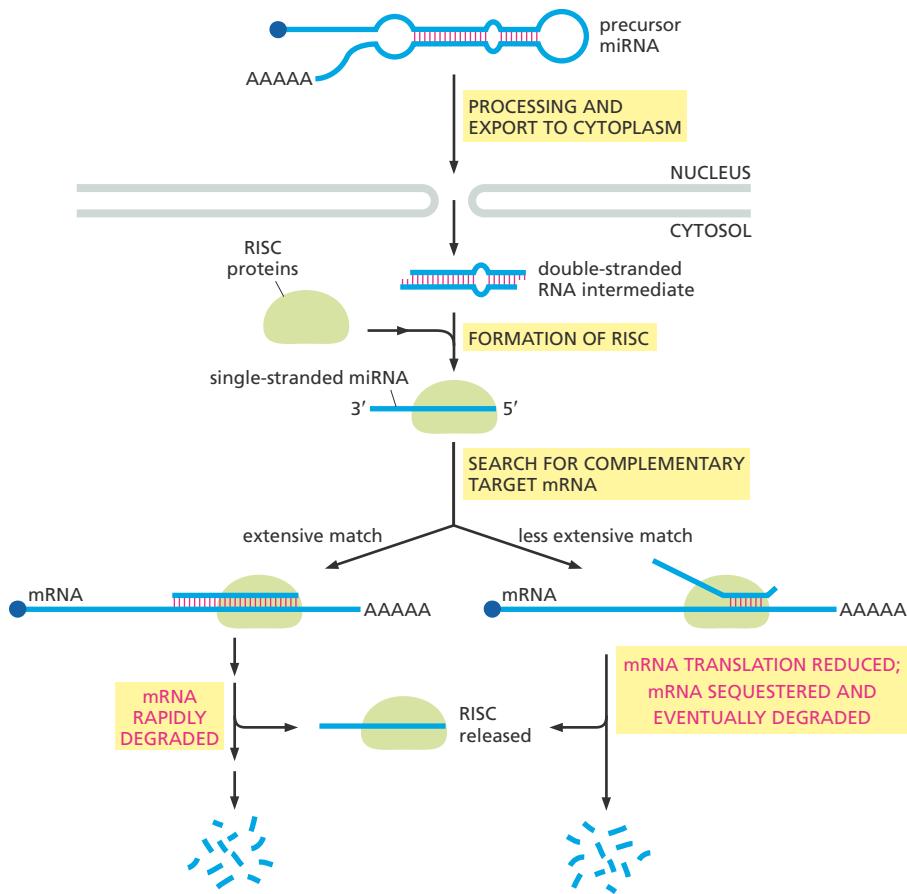


Figure 8–25 An miRNA targets a complementary mRNA molecule for destruction. Each precursor miRNA transcript is processed to form a double-stranded intermediate, which is further processed to form a mature, single-stranded miRNA. This miRNA assembles with a set of proteins into a complex called RISC, which then searches for mRNAs that have a nucleotide sequence complementary to its bound miRNA. Depending on how extensive the region of complementarity is, the target mRNA is either rapidly degraded by a nuclease within the RISC or transferred to an area of the cytoplasm where other cellular nucleases destroy it.

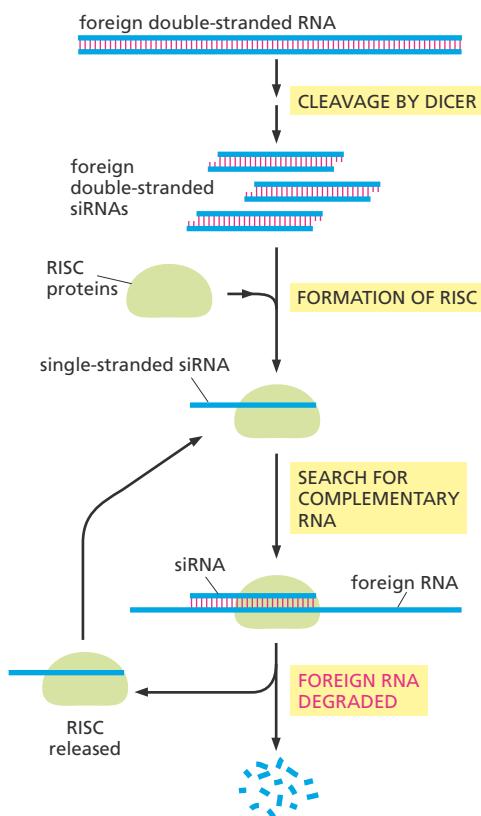


Figure 8–26 siRNAs are produced from double-stranded, foreign RNAs in the process of RNA interference. Double-stranded RNAs from a virus or transposable genetic element are first cleaved by a nuclease called Dicer. The resulting double-stranded fragments are incorporated into RISCs, which discard one strand of the foreign RNA duplex and use the other strand to locate and destroy foreign RNAs with a complementary sequence.

to be roughly 500 different miRNAs encoded by the human genome. Although we are only beginning to understand the full impact of these miRNAs, it is clear that they play a critical part in regulating gene expression and thereby influence many cell functions.

Small Interfering RNAs Are Produced From Double-Stranded, Foreign RNAs to Protect Cells From Infections

Some of the same components that process and package miRNAs also play another crucial part in the life of a cell: they serve as a powerful cell defense mechanism. In this case, the system is used to eliminate “foreign” RNA molecules—in particular, the double-stranded RNAs produced by many viruses and transposable genetic elements (discussed in Chapter 9). The process is called **RNA interference (RNAi)**.

In the first step of RNAi, the double-stranded, foreign RNAs are cut into short fragments (approximately 22 nucleotide pairs in length) by a protein called Dicer—the same protein used to generate the double-stranded RNA intermediate in miRNA production (see Figure 8–25). The resulting double-stranded RNA fragments, called **small interfering RNAs (siRNAs)**, are then taken up by the same RISCs that carry miRNAs. The RISC discards one strand of the siRNA duplex and uses the remaining single-stranded RNA to seek and destroy complementary foreign RNA molecules (Figure 8–26). In this way, the infected cell turns the foreign RNA back on itself.

RNAi operates in a wide variety of organisms, including single-celled fungi, plants, and worms, indicating that it is an evolutionarily ancient defense mechanism. In some organisms, including plants, the RNAi defense response can spread from tissue to tissue, allowing the entire organism to become resistant to a virus after only a few of its cells

have been infected. In this sense, RNAi resembles certain aspects of the adaptive immune responses of vertebrates; in both cases, an invading pathogen elicits the production of molecules—either siRNAs or antibodies—that are custom-made to inactivate the specific invader and thereby protect the host.

Thousands of Long Noncoding RNAs May Also Regulate Mammalian Gene Activity

At the other end of the size spectrum are the **long noncoding RNAs**, a class of RNA molecules that are more than 200 nucleotides in length. There are thought to be upwards of 8000 of these RNAs encoded in the human and mouse genomes. Yet, with few exceptions, their roles in the biology of the organism are not entirely clear.

One of the best understood of the long noncoding RNAs is *Xist*. This enormous RNA molecule, some 17,000 nucleotides long, is a key player in X inactivation—the process by which one of the two X chromosomes in the cells of female mammals is permanently silenced (see Figure 5–30). Early in development, *Xist* is produced by only one of the X chromosomes in each female nucleus. The transcript then “sticks around,” coating the chromosome and presumably attracting the enzymes and chromatin-remodeling complexes that promote the formation of highly condensed heterochromatin. Other long noncoding RNAs may promote the silencing of specific genes in a similar manner.

Some long noncoding RNAs arise from protein-coding regions of the genome, but are transcribed from the “wrong” DNA strand. Some of these *antisense* transcripts are known to bind to the mRNAs produced from that DNA segment, regulating their translation and stability—in some cases by producing siRNAs (see Figure 8–26).

Regardless of how the various long noncoding RNAs operate—or what exactly they do—the discovery of this large class of RNAs reinforces the idea that a eukaryotic genome is densely packed with information that provides not only an inventory of the molecules and structures every cell must make, but a set of instructions for how and when to assemble these parts to guide the growth and development of a complete organism.

ESSENTIAL CONCEPTS

- A typical eukaryotic cell expresses only a fraction of its genes, and the distinct types of cells in multicellular organisms arise because different sets of genes are expressed as cells differentiate.
- In principle, gene expression can be controlled at any of the steps between a gene and its ultimate functional product. For the majority of genes, however, the initiation of transcription is the most important point of control.
- The transcription of individual genes is switched on and off in cells by transcription regulator proteins, which bind to short stretches of DNA called regulatory DNA sequences.
- In bacteria, transcription regulators usually bind to regulatory DNA sequences close to where RNA polymerase binds. This binding can either activate or repress transcription of the gene. In eukaryotes, regulatory DNA sequences are often separated from the promoter by many thousands of nucleotide pairs.
- Eukaryotic transcription regulators act in two main ways: (1) they can directly affect the assembly process that requires RNA polymerase

and the general transcription factors at the promoter, and (2) they can locally modify the chromatin structure of promoter regions.

- In eukaryotes, the expression of a gene is generally controlled by a combination of different transcription regulator proteins.
- In multicellular plants and animals, the production of different transcription regulators in different cell types ensures the expression of only those genes appropriate to the particular type of cell.
- One differentiated cell type can be converted to another by artificially expressing an appropriate set of transcription regulators. A differentiated cell can also be reprogrammed into a stem cell by artificially expressing a particular set of such regulators.
- Cells in multicellular organisms have mechanisms that enable their progeny to “remember” what type of cell they should be. A prominent mechanism for propagating cell memory relies on transcription regulators that perpetuate transcription of their own gene—a form of positive feedback.
- A master transcription regulator, if expressed in the appropriate precursor cell, can trigger the formation of a specialized cell type or even an entire organ.
- The pattern of DNA methylation can be transmitted from one cell generation to the next, producing a form of epigenetic inheritance that helps a cell remember the state of gene expression in its parent cell. There is also evidence for a form of epigenetic inheritance based on transmitted chromatin structures.
- Cells can regulate gene expression by controlling events that occur after transcription has begun. Many of these post-transcriptional mechanisms rely on RNA molecules that can influence their own stability or translation.
- MicroRNAs (miRNAs) control gene expression by base-pairing with specific mRNAs and inhibiting their stability and translation.
- Cells have a defense mechanism for destroying “foreign” double-stranded RNAs, many of which are produced by viruses. It makes use of small interfering RNAs (siRNAs) that are produced from the foreign RNAs in a process called RNA interference (RNAi).
- Scientists can take advantage of RNAi to inactivate specific genes of interest.
- The recent discovery of thousands of long noncoding RNAs in mammals has opened a new window to the roles of RNAs in gene regulation.

KEY TERMS

combinatorial control	promoter
differentiation	regulatory DNA sequence
DNA methylation	regulatory RNA
epigenetic inheritance	reporter gene
gene expression	RNA interference (RNAi)
long noncoding RNA	small interfering RNA (siRNA)
microRNA (miRNA)	transcription regulator
positive feedback loop	transcriptional activator
post-transcriptional control	transcriptional repressor

QUESTIONS

QUESTION 8–4

A virus that grows in bacteria (bacterial viruses are called bacteriophages) can replicate in one of two ways. In the prophage state, the viral DNA is inserted into the bacterial chromosome and is copied along with the bacterial genome each time the cell divides. In the lytic state, the viral DNA is released from the bacterial chromosome and replicates many times in the cell. This viral DNA then produces viral coat proteins that together with the replicated viral DNA form many new virus particles that burst out of the bacterial cell. These two forms of growth are controlled by two transcription regulators, called *c1* ("c one") and *Cro*, that are encoded by the virus. In the prophage state, *c1* is expressed; in the lytic state, *Cro* is expressed. In addition to regulating the expression of other genes, *c1* represses the *Cro* gene, and *Cro* represses the *c1* gene (**Figure Q8–4**). When bacteria containing a phage in the prophage state are briefly irradiated with UV light, *c1* protein is degraded.

- What will happen next?
- Will the change in (A) be reversed when the UV light is switched off?
- Why might this response to UV light have evolved?

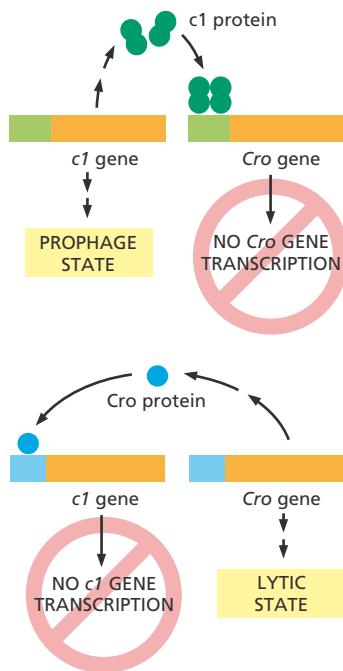


Figure Q8–4

QUESTION 8–5

Which of the following statements are correct? Explain your answers.

- In bacteria, but not in eukaryotes, many mRNAs contain the coding region for more than one gene.
- Most DNA-binding proteins bind to the major groove of the DNA double helix.
- Of the major control points in gene expression (transcription, RNA processing, RNA transport, translation, and control of a protein's activity), transcription initiation is one of the most common.

QUESTION 8–6

Your task in the laboratory of Professor Quasimodo is to determine how far an enhancer (a binding site for an activator protein) could be moved from the promoter of the *straightspine* gene and still activate transcription. You systematically vary the number of nucleotide pairs between these two sites and then determine the amount of transcription by measuring the production of *Straightspine* mRNA. At first glance, your data look confusing (**Figure Q8–6**). What would you have expected for the results of this experiment? Can you save your reputation and explain these results to Professor Quasimodo?

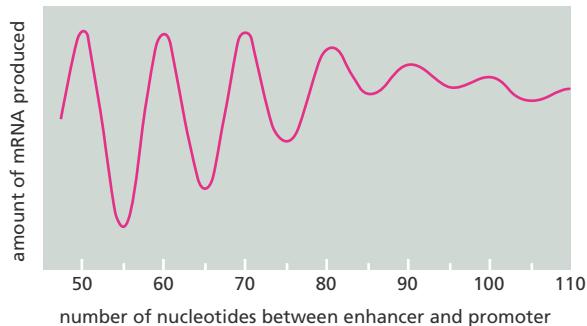


Figure Q8–6

QUESTION 8–7

The λ repressor binds as a dimer to critical sites on the λ genome to repress the virus's lytic genes. This is necessary to maintain the prophage (integrated) state. Each molecule of the repressor consists of an N-terminal DNA-binding domain and a C-terminal dimerization domain (**Figure Q8–7**). Upon induction (for example, by irradiation with UV light), the genes for lytic growth are expressed, λ progeny are produced, and the bacterial cell is lysed (see Question 8–4). Induction is initiated by cleavage of the λ repressor at a site between the DNA-binding domain and the dimerization domain, which causes the repressor to dissociate from the DNA. In the absence of bound repressor, RNA polymerase binds and initiates lytic growth. Given that the number (concentration) of DNA-binding domains is unchanged by cleavage of the repressor, why do you suppose its cleavage results in its dissociation from the DNA?

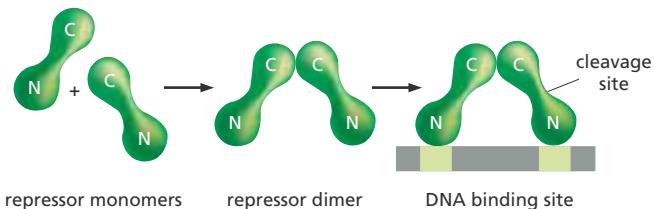


Figure Q8–7

QUESTION 8–8

The genes that encode the enzymes for arginine biosynthesis are located at several positions around the genome of *E. coli*, and they are regulated coordinately by a transcription regulator encoded by the *ArgR* gene.

The activity of the ArgR protein is modulated by arginine. Upon binding arginine, ArgR alters its conformation, dramatically changing its affinity for the DNA sequences in the promoters of the genes for the arginine biosynthetic enzymes. Given that ArgR is a repressor protein, would you expect that ArgR would bind more tightly or less tightly to the DNA sequences when arginine is abundant? If ArgR functioned instead as an activator protein, would you expect the binding of arginine to increase or to decrease its affinity for its regulatory DNA sequences? Explain your answers.

QUESTION 8–9

When enhancers were initially found to influence transcription many thousands of nucleotide pairs from the promoters they control, two principal models were invoked to explain this action at a distance. In the “DNA looping” model, direct interactions between proteins bound at enhancers and promoters were proposed to stimulate transcription initiation. In the “scanning” or “entry-site” model, RNA polymerase (or another component of the transcription machinery) was proposed to bind at the enhancer and then scan along the DNA until it reached the promoter. These two models were tested using an enhancer on one piece of DNA and a β -globin gene and promoter on a separate piece of DNA (Figure Q8–9). The β -globin gene was not expressed from the mixture of pieces. However, when the two segments of DNA were joined via a linker (made of a protein that binds to a small molecule called biotin), the β -globin gene was expressed.

Does this experiment distinguish between the DNA looping model and the scanning model? Explain your answer.

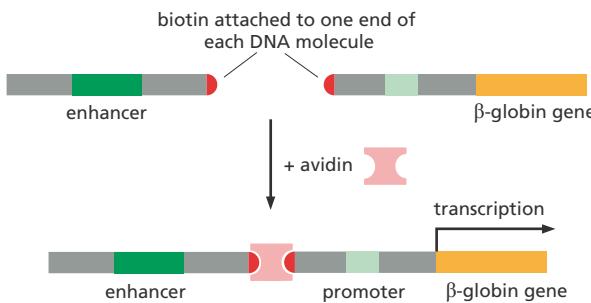


Figure Q8–9

QUESTION 8–10

Differentiated cells of an organism contain the same genes. (Among the few exceptions to this rule are the cells of the mammalian immune system, in which the formation of specialized cells is based on limited rearrangements of the genome.) Describe an experiment that substantiates the first sentence of this question, and explain why it does.

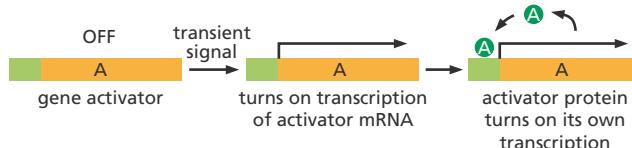
QUESTION 8–11

Figure 8–17 shows a simple scheme by which three transcription regulators are used during development to create eight different cell types. How many cell types could you create, using the same rules, with four different transcription regulators? As described in the text, MyoD is a transcription regulator that by itself is sufficient to induce muscle-specific gene expression in fibroblasts. How does this observation fit the scheme in Figure 8–17?

QUESTION 8–12

Imagine the two situations shown in Figure Q8–12. In cell I, a transient signal induces the synthesis of protein A, which is a transcriptional activator that turns on many genes including its own. In cell II, a transient signal induces the synthesis of protein R, which is a transcriptional repressor that turns off many genes including its own. In which, if either, of these situations will the descendants of the original cell “remember” that the progenitor cell had experienced the transient signal? Explain your reasoning.

(A) CELL I



(B) CELL II

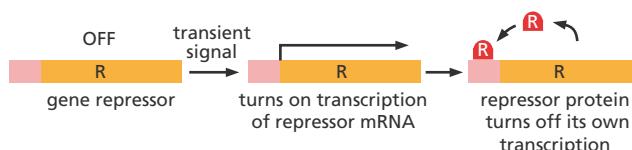


Figure Q8–12

QUESTION 8–13

Discuss the following argument: “If the expression of every gene depends on a set of transcription regulators, then the expression of these regulators must also depend on the expression of other regulators, and their expression must depend on the expression of still other regulators, and so on. Cells would therefore need an infinite number of genes, most of which would code for transcription regulators.” How does the cell get by without having to achieve the impossible?

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CHAPTER NINE

9

How Genes and Genomes Evolve

For a given individual, the nucleotide sequence of the genome in virtually every one of its cells is the same. But compare the DNA of two individuals—even parent and child—and that is no longer the case: the genomes of individuals within a species contain slightly different information. And between members of different species, the deviations are even more extensive.

Such differences in DNA sequence are responsible for the diversity of life on Earth, from the subtle variations in hair color, eye color, and skin color that characterize members our own species (**Figure 9–1**) to the dramatic differences in phenotype that distinguish a fish from a fungus or a robin from a rose. But if all life emerged from a common ancestor—a single-celled organism that existed some 3.5 billion years ago—where did these genetic improvisations come from? How did they arise, why were they preserved, and how do they contribute to the breathtaking biological diversity that surrounds us?

Improvements in the methods used to sequence and analyze whole genomes—from pufferfish and the plague bacterium to people from around the world—are now allowing us to address some of these questions. In Chapter 10, we describe these revolutionary technologies, which continue to transform the modern era of genomics. In this chapter, we present some of the fruits of these technological innovations. Our ability to compare the genomes of a wide-ranging collection of organisms has provided striking confirmation of Darwin’s explanations for the diversity of life on Earth—revealing how processes of mutation and natural selection have been sculpting DNA sequences for billions of years, giving rise to the spectacular menagerie of present-day life-forms that crowd every corner of the planet.

GENERATING GENETIC VARIATION

RECONSTRUCTING LIFE’S FAMILY TREE

TRANSPOSONS AND VIRUSES

EXAMINING THE HUMAN GENOME



Figure 9–1 Small differences in DNA sequence account for differences in appearance between one individual and the next. A group of English schoolchildren displays a sampling of the characteristics that define the unity and diversity of our own species. (Courtesy of Fiona Pragoff, Wellcome Images.)

In this chapter, we discuss how genes and genomes change over time. We examine the molecular mechanisms that generate genetic diversity, and we consider how the information in present-day genomes can be deciphered to yield a historical record of the evolutionary processes that have shaped these DNA sequences. We take a brief look at mobile genetic elements and consider how these elements, along with modern-day viruses, can carry genetic information from place to place and from organism to organism. Finally, we end the chapter by taking a closer look at the human genome to see what our own DNA sequences tell us about who we are and where we come from.

GENERATING GENETIC VARIATION

Evolution is more a tinkerer than an inventor: it uses as its raw materials the DNA sequences that each organism inherits from its ancestors. There is no natural mechanism for making long stretches of entirely novel nucleotide sequences. In this sense, no gene or genome is ever entirely new. Instead, the astonishing diversity in form and function in the living world is all the result of variations on preexisting themes. As genetic variations pile up over millions of generations, they can produce radical change.

Several basic types of genetic change are especially crucial in evolution (**Figure 9–2**):

- *Mutation within a gene:* An existing gene can be modified by a mutation that changes a single nucleotide or deletes or duplicates one or more nucleotides. These mutations can alter the splicing of a gene's transcript or change the stability, activity, location, or interactions of its encoded protein or RNA product.
- *Mutation within regulatory DNA:* When and where a gene is expressed can be affected by a mutation in the stretches of DNA sequence that regulate the gene's activity (described in Chapter 8). For example, humans and fish have a surprisingly large number of genes in common, but changes in the regulation of those shared genes underlie many of the most dramatic differences between those species.
- *Gene duplication:* An existing gene, a larger segment of DNA, or even a whole genome can be duplicated, creating a set of closely related genes within a single cell. As this cell and its progeny divide, the original DNA sequence and its duplicate can acquire additional mutations and thereby assume new functions and patterns of expression.
- *Exon shuffling:* Two or more existing genes can be broken and rejoined to make a hybrid gene containing DNA segments that originally belonged to separate genes. In eukaryotes, the breaking and rejoining often occurs within the long intron sequences, which do not encode protein. Because intron sequences are removed by RNA splicing, the breaking and joining do not have to be precise to result in a functional gene.
- *Mobile genetic elements:* Specialized DNA sequences that can move from one chromosomal location to another can alter the activity or regulation of a gene; they can also promote gene duplication, exon shuffling, and other genome rearrangements.
- *Horizontal gene transfer:* A piece of DNA can be transferred from the genome of one cell to that of another—even to that of another species. This process, which is rare among eukaryotes but common among bacteria, differs from the usual “vertical” transfer of genetic information from parent to progeny.



Figure 9–2 Genes and genomes can be altered by several different mechanisms.

Small mutations, duplications, deletions, rearrangements, and even the infusion of fresh genetic material all contribute to genome evolution. Although the mobile genetic element here is shown interrupting a gene regulatory sequence, the movement of these parasitic elements can promote a variety of genetic variations, including gene duplication, exon shuffling, and other regulatory and structural alterations.

Each of these forms of genetic variation—from the simple mutations that occur within a gene to the more extensive duplications, deletions, rearrangements, and additions that occur within a genome—has played an important part in the evolution of modern organisms. And they still play that part today, as organisms continue to evolve. In this section, we discuss these basic mechanisms of genetic change, and we consider their consequences for genome evolution. But first, we pause to consider the contribution of sex—the mechanism that many organisms use to pass genetic information on to future generations.

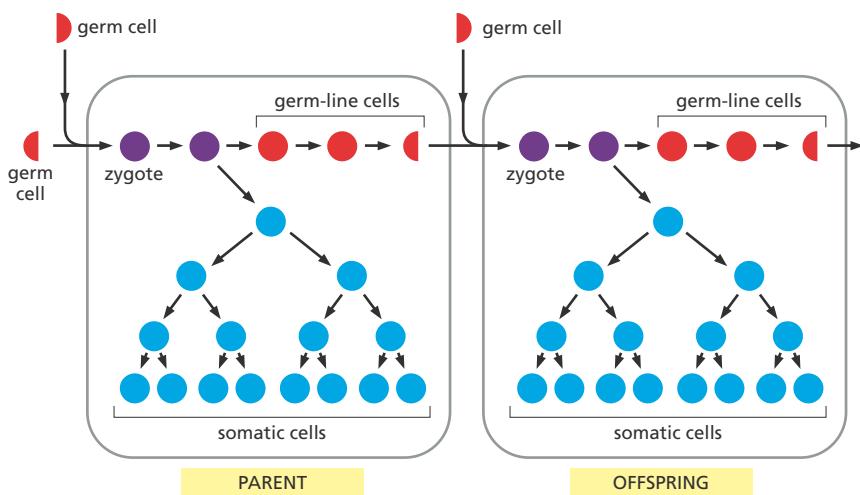
In Sexually Reproducing Organisms, Only Changes to the Germ Line Are Passed On To Progeny

For bacteria and unicellular organisms that reproduce mainly asexually, the inheritance of genetic information is fairly straightforward. Each individual duplicates its genome and donates one copy to each daughter cell when the individual divides in two. The family tree of such unicellular organisms is simply a branching diagram of cell divisions that directly links each individual to its progeny and to its ancestors.

QUESTION 9–1

In this chapter, it is argued that genetic variability is beneficial for a species because it enhances that species' ability to adapt to changing conditions. Why, then, do you think that cells go to such great lengths to ensure the fidelity of DNA replication?

Figure 9–3 Germ-line cells and somatic cells have fundamentally different functions. In sexually reproducing organisms, genetic information is propagated into the next generation exclusively by germ-line cells (red). This cell lineage includes the specialized reproductive cells—the germ cells (eggs and sperm, red half circles)—which contain only half the number of chromosomes than do the other cells in the body (full circles). When two germ cells come together during fertilization, they form a fertilized egg or zygote (purple), which once again contains a full set of chromosomes (discussed in Chapter 19). The zygote gives rise to both germ-line cells and to somatic cells (blue). Somatic cells form the body of the organism but do not contribute their DNA to the next generation.

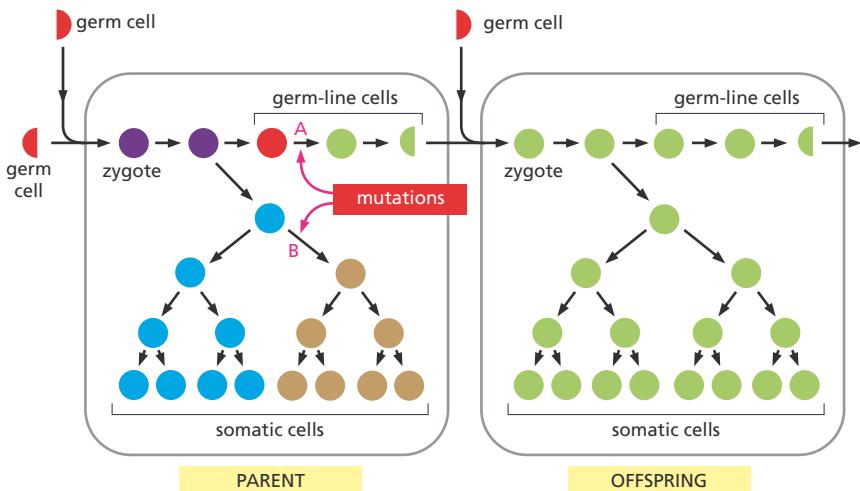


For a multicellular organism that reproduces sexually, however, the family connections are considerably more complex. Although individual cells within that organism divide, only the specialized reproductive cells—the **germ cells**—carry a copy of its genome to the next generation of organisms (discussed in Chapter 19). All the other cells of the body—the **somatic cells**—are doomed to die without leaving evolutionary descendants of their own (Figure 9–3). In a sense, somatic cells exist only to help the germ cells survive and propagate.

A mutation that occurs in a somatic cell—although it might have unfortunate consequences for the individual in which it occurs (causing cancer, for example)—will not be transmitted to the organism’s offspring. For a mutation to be passed on to the next generation, it must alter the **germ line**—the cell lineage that gives rise to the germ cells (Figure 9–4). Thus, when we track the genetic changes that accumulate during the evolution of sexually reproducing organisms, we are looking at events that took place in a germ-line cell. It is through a series of germ-line cell divisions that sexually reproducing organisms trace their descent back to their ancestors and, ultimately, back to the ancestors of us all—the first cells that existed, at the origin of life more than 3.5 billion years ago.

In addition to perpetuating a species, sex also introduces its own form of genetic change: when germ cells from a male and female unite during fertilization, they generate offspring that are genetically distinct from either parent. We discuss this form of genetic diversification in detail in Chapter 19. In the meantime, aside from this mating-based genome

Figure 9–4 Mutations in germ-line cells and somatic cells have different consequences. A mutation that occurs in a germ-line cell (A) can be passed on to the cell’s progeny and, ultimately, to the progeny of the organism (green). By contrast, a mutation that arises in a somatic cell (B) affects only the progeny of that cell (orange) and will not be passed on to the organism’s progeny. As we discuss in Chapter 20, somatic mutations are responsible for most human cancers (see pp. 714–717).



reshuffling, which influences how mutations are inherited in organisms that reproduce sexually, most of the mechanisms that generate genetic change are the same for all living things, as we now discuss.

Point Mutations Are Caused by Failures of the Normal Mechanisms for Copying and Repairing DNA

Despite the elaborate mechanisms that exist to faithfully copy and repair DNA sequences, each nucleotide pair in an organism's genome runs a small risk of changing each time a cell divides. Changes that affect a single nucleotide pair are called **point mutations**. These typically arise from rare errors in DNA replication or repair (discussed in Chapter 6).

The point mutation rate has been determined directly in experiments with bacteria such as *E. coli*. Under laboratory conditions, *E. coli* divides about once every 20–25 minutes; in less than a day, a single *E. coli* can produce more descendants than there are humans on Earth—enough to provide a good chance for almost any conceivable point mutation to occur. A culture containing 10^9 *E. coli* cells thus harbors millions of mutant cells whose genomes differ subtly from the ancestor cell. Some of these mutations may confer a selective advantage on individual cells: resistance to a poison, for example, or the ability to survive when deprived of a standard nutrient. By exposing the culture to a selective condition—adding an antibiotic or removing an essential nutrient, for example—one can find these needles in the haystack; that is, the cells that have undergone a specific mutation enabling them to survive in conditions where the original cells cannot (Figure 9–5). Such experiments have revealed that the overall point mutation frequency in *E. coli* is about 3 changes per 10^{10} nucleotide pairs each cell generation. The mutation rate in humans, as determined by comparing the DNA sequences of children and their parents (and estimating how many times the parental germ cells divided), is

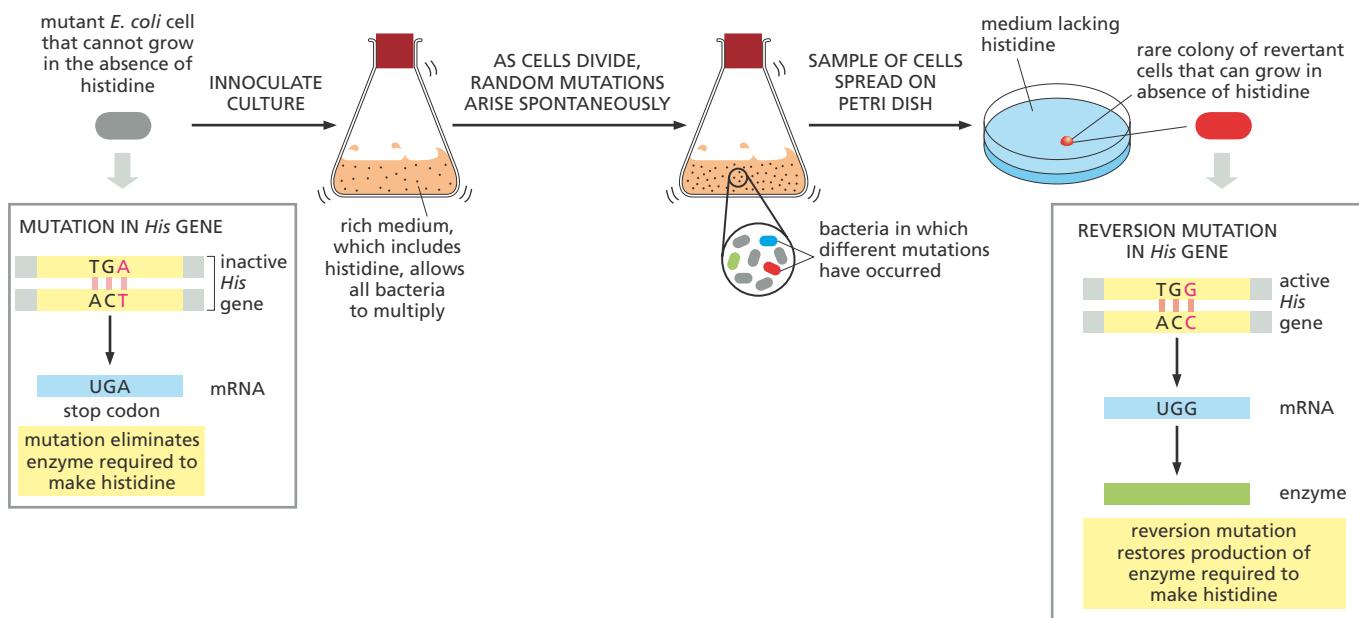


Figure 9–5 Mutation rates can be measured in the laboratory. In this experiment, an *E. coli* strain that carries a deleterious point mutation in the *His* gene—which is needed to manufacture the amino acid histidine—is used. The mutation converts a G-C nucleotide pair to an A-T, resulting in a premature stop signal in the mRNA produced from the mutant gene (left box). As long as histidine is supplied in the growth medium, this strain can grow and divide normally. If a large number of mutant cells (say 10^{10}) is spread on an agar plate that lacks histidine, the great majority will die. The rare survivors will contain a “reversion” mutation (in which the A-T is changed back to a G-C). This reversion corrects the original defect and now allows the bacterium to make the enzyme it needs to survive in the absence of histidine. Such mutations happen by chance and only rarely, but the ability to work with very large numbers of *E. coli* cells makes it possible to detect this change and to accurately measure its frequency.

about one-third that of *E. coli*—which suggests that the mechanisms that evolved to maintain genome integrity operate with an efficiency that does not differ significantly in even distantly related species.

Point mutations can destroy a gene's activity or—very rarely—improve it (as shown in Figure 9–5). More often, however, they do neither of these things. At many sites in the genome, a point mutation has absolutely no effect on the organism's appearance, viability, or ability to reproduce. Such *neutral mutations* often fall in regions of the gene where the DNA sequence is unimportant, including most of an intron's sequence. In cases where they occur within an exon, neutral mutations can change the third position of a codon such that the amino acid it specifies is unchanged—or is so similar that the protein's function is unaffected.

Point Mutations Can Change the Regulation of a Gene

Mutations in the coding sequences of genes are fairly easy to spot because they change the amino acid sequence of the encoded protein in predictable ways. But mutations in regulatory DNA are more difficult to recognize, because they don't affect protein sequence and can be located some distance from the coding sequence of the gene.

Despite these difficulties, many examples have been discovered where point mutations in regulatory DNA have a profound effect on the protein's production and thereby on the organism. For example, a small number of people are resistant to malaria because of a point mutation that affects the expression of a cell-surface receptor to which the malaria parasite *Plasmodium vivax* binds. The mutation prevents the receptor from being produced in red blood cells, rendering the individuals who carry this mutation immune to malarial infection.

Point mutations in regulatory DNA also have a role in our ability to digest lactose, the main sugar in milk. Our earliest ancestors were lactose intolerant, because the enzyme that breaks down lactose—called lactase—was made only during infancy. Adults, who were no longer exposed to breast milk, did not need the enzyme. When humans began to get milk from domestic animals some 10,000 years ago, variant genes—produced by random mutation—enabled those who carried the variation to continue to express lactase as adults. We now know that people who retain the ability to digest milk as adults contain a point mutation in the regulatory DNA of the lactase gene, allowing it to be efficiently transcribed throughout life. In a sense, these milk-drinking adults are “mutants” with respect to their ability to digest lactose. It is remarkable how quickly this trait spread through the human population, especially in societies that depended heavily on milk for nutrition ([Figure 9–6](#)).

These evolutionary changes in the regulatory sequence of the lactase gene occurred relatively recently (10,000 years ago), well after humans became a distinct species. However, much more ancient changes in regulatory sequences have occurred in other genes, and some of these are thought to underlie many of the profound differences among species ([Figure 9–7](#)).

DNA Duplications Give Rise to Families of Related Genes

Point mutations can influence the activity of an existing gene, but how do new genes with new functions come into being? Gene duplication is perhaps the most important mechanism for generating new genes from old ones. Once a gene has been duplicated, each of the two copies is free to accumulate mutations that might allow it to perform a slightly different function—as long as the original activity of the gene is not lost. This specialization of duplicated genes occurs gradually, as mutations



Figure 9–6 The ability of adult humans to digest milk followed the domestication of cattle. Approximately 10,000 years ago, humans in northern Europe and central Africa began to raise cattle. The subsequent availability of cow's milk—particularly during periods of starvation—gave a selective advantage to those humans able to digest lactose as adults. Two independent point mutations that allow the expression of lactase in adults arose in human populations—one in northern Europe and another in central Africa. These mutations have since spread through different regions of the world. For example, the migration of Northern Europeans to North America and Australia explains why most people living on these continents can digest lactose as adults; the native populations of North America and Australia, however, remain lactose intolerant.

accumulate in the descendants of the original cell in which gene duplication occurred. By repeated rounds of this process of **gene duplication and divergence** over many millions of years, one gene can give rise to a whole family of genes, each with a specialized function, within a single genome. Analysis of genome sequences reveals many examples of such **gene families**: in *Bacillus subtilis*, for example, nearly half of the genes have one or more obvious relatives elsewhere in the genome. And in vertebrates, the globin family of genes, which encode oxygen-carrying proteins, clearly arose from a single primordial gene, as we see shortly. But how does gene duplication occur in the first place?

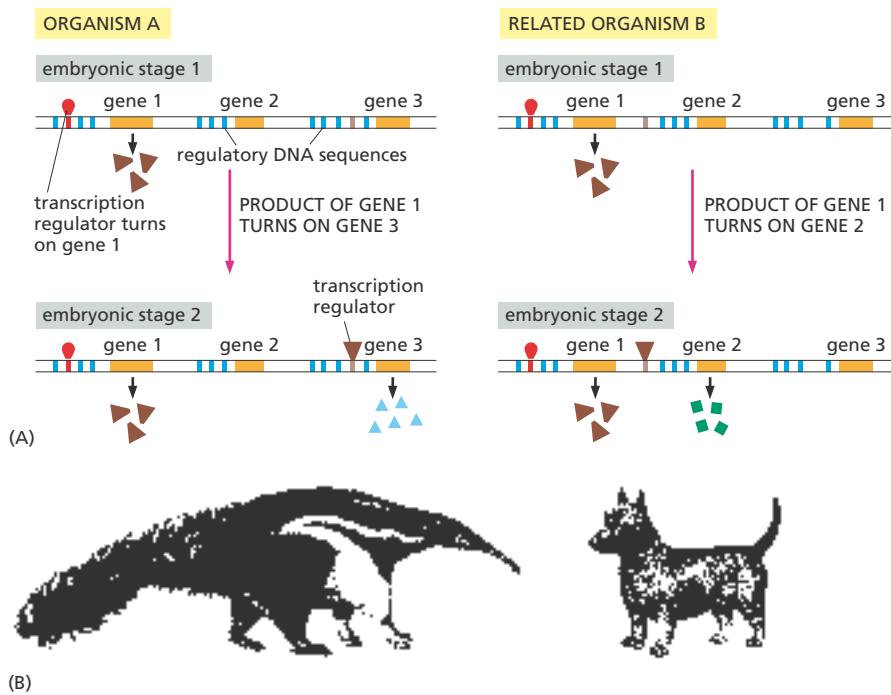
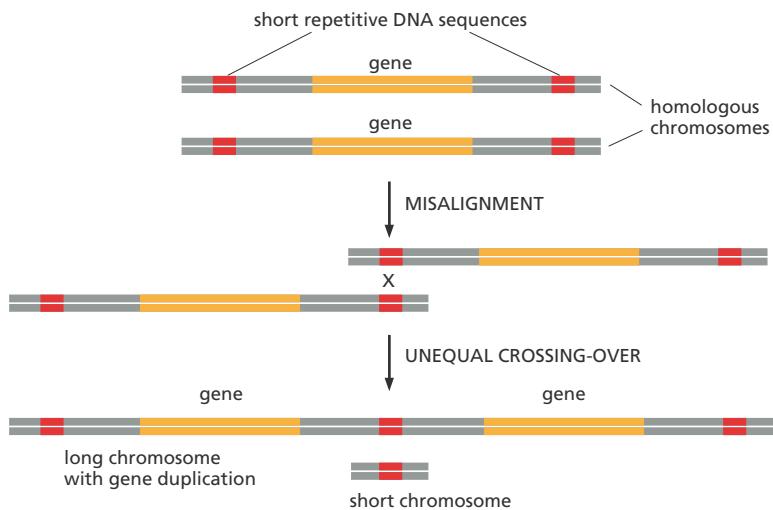


Figure 9–7 Changes in regulatory DNA sequences can have dramatic consequences for the development of an organism. (A) In this hypothetical example, the genomes of organisms A and B contain the same three genes (1, 2, and 3) and encode the same two transcription regulators (red oval, brown triangle). However, the regulatory DNA controlling expression of genes 2 and 3 is different in the two organisms. Although both express the same gene—gene 1—during embryonic stage 1, the differences in their regulatory DNA cause them to express different genes in stage 2. (B) In principle, a collection of such regulatory changes can have profound effects on an organism's developmental program—and, ultimately, on the appearance of the adult.

Figure 9–8 Gene duplication can be caused by crossovers between short, repeated DNA sequences in adjacent homologous chromosomes. The two chromosomes shown here undergo homologous recombination at short repeated sequences (red), that bracket a gene (orange). These repeated sequences can be remnants of mobile genetic elements, which are present in many copies in the human genome, as we discuss shortly. When crossing-over occurs unequally, as shown, one chromosome will get two copies of the gene, while the other will get none. The type of homologous recombination that produces gene duplications is called *unequal crossing-over* because the resulting products are unequal in size. If this process occurs in the germ line, some progeny will inherit the long chromosome, while others will inherit the short one.



Many gene duplications are believed to be generated by *homologous recombination*. As discussed in Chapter 6, homologous recombination provides an important mechanism for mending a broken double helix; it allows an intact chromosome to be used as a template to repair a damaged sequence on its homolog. Homologous recombination normally takes place only after two long stretches of nearly identical DNA become paired, so that the information in the intact piece of DNA can be used to "restore" the sequence in the broken DNA. On rare occasions, however, a recombination event can occur between a pair of shorter DNA sequences—identical or very similar—that fall on either side of a gene. If these short sequences are not aligned properly during recombination, a lopsided exchange of genetic information can occur. Such *unequal crossovers* can generate one chromosome that has an extra copy of the gene and another with no copy (Figure 9–8). Once a gene has been duplicated in this way, subsequent unequal crossovers can readily add extra copies to the duplicated set by the same mechanism. As a result, entire sets of closely related genes, arranged in series, are commonly found in genomes.

The Evolution of the Globin Gene Family Shows How Gene Duplication and Divergence Can Produce New Proteins

The evolutionary history of the globin gene family provides a striking example of how gene duplication and divergence has generated new proteins. The unmistakable similarities in amino acid sequence and structure among the present-day globin proteins indicate that all the globin genes must derive from a single ancestral gene.

The simplest globin protein has a polypeptide chain of about 150 amino acids, which is found in many marine worms, insects, and primitive fish. Like our hemoglobin, this protein transports oxygen molecules throughout the animal's body. The oxygen-carrying protein in the blood of adult mammals and most other vertebrates, however, is more complex; it is composed of four globin chains of two distinct types— α globin and β globin (Figure 9–9). The four oxygen-binding sites in the $\alpha_2\beta_2$ molecule interact, allowing an allosteric change in the molecule as it binds and releases oxygen. This structural shift enables the four-chain hemoglobin molecule to efficiently take up and release four oxygen molecules in an all-or-none fashion, a feat not possible for the single-chain version. This efficiency is particularly important for large multicellular animals, which

Figure 9–9 An ancestral globin gene encoding a single-chain globin molecule is thought to have given rise to the pair of genes that produce four-chain hemoglobin proteins of modern humans and other mammals. The mammalian hemoglobin molecule is a complex of two α - and two β -globin chains. Each chain has a bound heme group (red) that is responsible for binding oxygen.

cannot rely on the simple diffusion of oxygen through the body to oxygenate their tissues adequately.

The α - and β -globin genes are the result of gene duplications that occurred early in vertebrate evolution. Genome analyses suggest that one of our ancient ancestors had a single globin gene. But about 500 million years ago, gene duplications followed by mutation are thought to have given rise to two slightly different globin genes, one encoding α globin, the other encoding β globin. Still later, as the different mammals began diverging from their common ancestor, the β -globin gene underwent its own duplication and divergence to give rise to a second β -like globin gene that is expressed specifically in the fetus (Figure 9–10). The resulting fetal hemoglobin molecule has a higher affinity for oxygen compared with adult hemoglobin, a property that helps transfer oxygen from mother to fetus.

Subsequent rounds of duplication in both the α - and β -globin genes gave rise to additional members of these families. Each of these duplicated genes has been modified by point mutations that affect the properties of the final hemoglobin molecule, and by changes in regulatory DNA that determine when—and how strongly—each gene is expressed. As a result, each globin differs slightly in its ability to bind and release oxygen and in the stage of development during which it is expressed.

In addition to these specialized globin genes, there are several duplicated DNA sequences in the α - and β -globin gene clusters that are not functional genes. They are similar in DNA sequence to the functional globin genes, but they have been disabled by the accumulation of many mutations that inactivate them. The existence of such *pseudogenes* makes it clear that, as might be expected, not every DNA duplication leads to a new functional gene. Most gene duplication events are unsuccessful in that one copy is gradually inactivated by mutation. Although we have focused here on the evolution of the globin genes, similar rounds of gene duplication and divergence have clearly taken place in many other gene families present in the human genome.

Figure 9–10 Repeated rounds of duplication and mutation are thought to have generated the globin gene family in humans.

About 500 million years ago, an ancestral globin gene duplicated and gave rise to the β -globin gene family (including the five genes shown) and the related α -globin gene family. In most vertebrates, a molecule of hemoglobin (see Figure 9–9) is formed from two chains of α globin and two chains of β globin—which can be any one of the five subtypes of the β family listed here.

The evolutionary scheme shown was worked out by comparing globin genes from many different organisms. The nucleotide sequences of the γ^G and γ^A genes—which produce the β -globin-like chains that form fetal hemoglobin—are much more similar to each other than either of them is to the adult β gene. And the δ -globin gene that arose during primate evolution encodes a minor β -globin form that's only made in adult primates. In humans, the β -globin genes are located in a cluster on Chromosome 11. A subsequent chromosome breakage event, which occurred about 300 million years ago, is believed to have separated the α - and β -globin genes; the α -globin genes now reside on human Chromosome 16 (not shown).

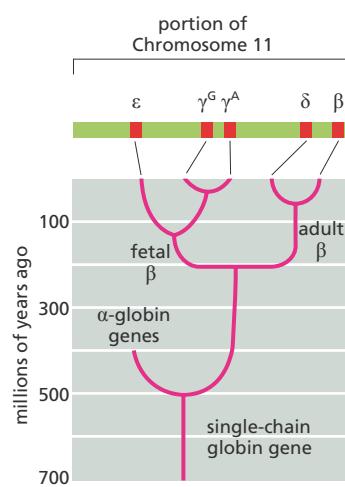
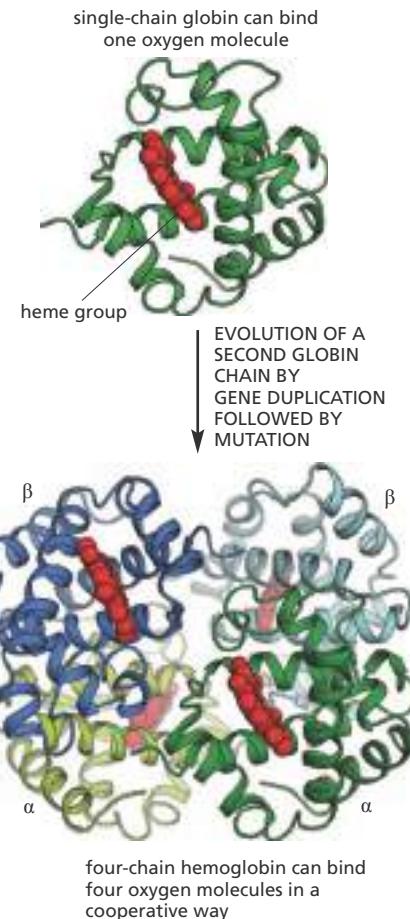




Figure 9-11 Different species of the frog *Xenopus* have different DNA contents.

DNA contents. *X. tropicalis* (above) has an ordinary diploid genome with two sets of chromosomes in every somatic cell; the tetraploid *X. laevis* (below) has a duplicated genome containing twice as much DNA per cell. (Courtesy of Enrique Amaya.)

Whole-Genome Duplications Have Shaped the Evolutionary History of Many Species

Almost every gene in the genomes of vertebrates exists in multiple versions, suggesting that, rather than single genes being duplicated in a piecemeal fashion, the whole vertebrate genome was long ago duplicated in one fell swoop. Early in vertebrate evolution, it appears that the entire genome actually underwent duplication twice in succession, giving rise to four copies of every gene. In some groups of vertebrates, such as the salmon and carp families (including the zebrafish; see Figure 1-37), there may have been yet another duplication, creating an eightfold multiplicity of genes.

The precise history of whole-genome duplications in vertebrate evolution is difficult to chart because many other changes have occurred since these ancient evolutionary events. In some organisms, however, full genome duplications are especially obvious, as they have occurred relatively recently—evolutionarily speaking. The frog genus *Xenopus*, for example, comprises a set of closely similar species related to one another by repeated duplications or triplications of the whole genome (Figure 9-11). Such large-scale duplications can happen if cell division fails to occur following a round of genome replication in the germ line of a particular individual. Once an accidental doubling of the genome occurs in a germ-line cell, it will be faithfully passed on to germ-line progeny cells in that individual and, ultimately, to any offspring these cells might produce.

Novel Genes Can Be Created by Exon Shuffling

As we discussed in Chapter 4, many proteins are composed of a set of smaller functional *domains*. In eukaryotes, each of these protein domains is usually encoded by a separate exon, which is surrounded by long stretches of noncoding introns (see Figures 7-17 and 7-18). This organization of eukaryotic genes can facilitate the evolution of new proteins by allowing exons from one gene to be added to another—a process called **exon shuffling**.

This duplication and movement of exons is promoted by the same type of recombination that gives rise to gene duplications (see Figure 9-8). In this case, recombination occurs within the introns that surround the exons. If the introns in question are from two different genes, this recombination can generate a hybrid gene that includes complete exons from both. The presumed results of such exon shuffling are seen in many present-day proteins, which contain a patchwork of many different protein domains (Figure 9-12).

It has been proposed that all the proteins encoded by the human genome (approximately 21,000) arose from the duplication and shuffling of a few thousand distinct exons, each encoding a protein domain of approximately 30–50 amino acids. This remarkable idea suggests that the great

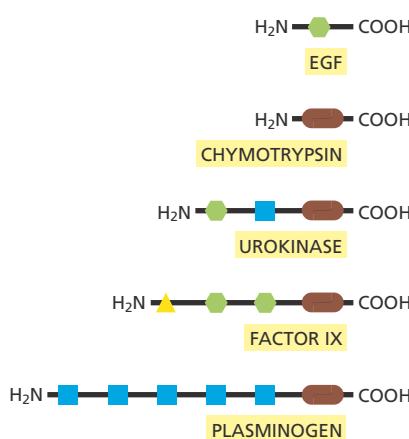


Figure 9-12 Exon shuffling during evolution can generate proteins with new combinations of protein domains. Each type of colored symbol represents a different protein domain. These different domains are thought to have been joined together by exon shuffling during evolution to create the modern-day human proteins shown here.

diversity of protein structures is generated from a quite small universal “list of parts,” pieced together in different combinations.

The Evolution of Genomes Has Been Profoundly Influenced by the Movement of Mobile Genetic Elements

Mobile genetic elements—DNA sequences that can move from one chromosomal location to another—are an important source of genomic change and have profoundly affected the structure of modern genomes. These parasitic DNA sequences can colonize a genome and then spread within it. In the process, they often disrupt the function or alter the regulation of existing genes; sometimes they even create novel genes through fusions between mobile sequences and segments of existing genes.

The insertion of a mobile genetic element into the coding sequence of a gene or into its regulatory region can cause the “spontaneous” mutations that are observed in many of today’s organisms. Mobile genetic elements can severely disrupt a gene’s activity if they land directly within its coding sequence. Such an *insertion mutation* destroys the gene’s capacity to encode a useful protein—as is the case for a number of mutations that cause hemophilia in humans, for example.

The activity of mobile genetic elements can also change the way existing genes are regulated. An insertion of an element into a regulatory DNA region, for instance, will often have a striking effect on where and when genes are expressed (Figure 9–13). Many mobile genetic elements carry DNA sequences that are recognized by specific transcription regulators; if these elements insert themselves near a gene, that gene can be brought under the control of these transcription regulators, thereby changing the gene’s expression pattern. Thus, mobile genetic elements can be a major source of developmental changes: They are thought to have been particularly important in the evolution of the body plans of multicellular plants and animals.

Finally, mobile genetic elements provide opportunities for genome rearrangements by serving as targets of homologous recombination (see Figure 9–8). For example, the duplications that gave rise to the β -globin gene cluster are thought to have occurred by crossovers between the abundant mobile genetic elements sprinkled throughout the human genome. Later in the chapter, we describe these elements in more detail and discuss the mechanisms that have allowed them to establish a stronghold within our genome.

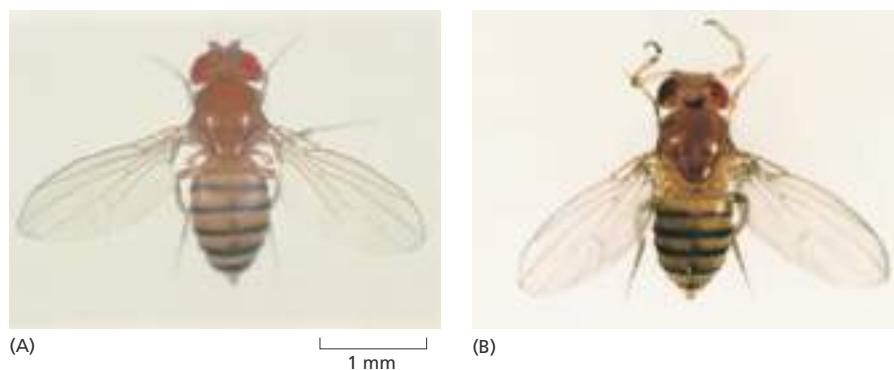


Figure 9–13 Mutation due to a mobile genetic element can induce dramatic alterations in the body plan of an organism. (A) A normal fruit fly (*Drosophila melanogaster*). (B) A mutant fly in which the antennae have been transformed into legs because of a mutation in a regulatory DNA sequence that causes genes for leg formation to be activated in the positions normally reserved for antennae. Although this particular change is not advantageous to the fly, it illustrates how the movement of a transposable element can produce a major change in the appearance of an organism. (A, courtesy of E.B. Lewis; B, courtesy of Matthew Scott.)

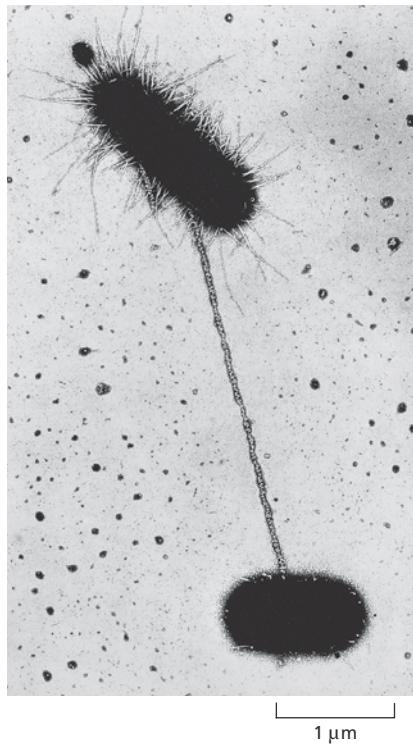


Figure 9–14 Bacterial cells can exchange DNA through

conjugation. Conjugation begins when a donor cell (top) attaches to a recipient cell (bottom) by a fine appendage, called a sex pilus. DNA from the donor cell then moves through the pilus into the recipient cell. In this electron micrograph, the sex pilus has been labeled along its length by viruses that specifically bind to it and make the structure more visible. Conjugation is one of several ways in which bacteria carry out horizontal gene transfer. (Courtesy of Charles C. Brinton Jr. and Judith Carnahan.)

Genes Can Be Exchanged Between Organisms by Horizontal Gene Transfer

So far we have considered genetic changes that take place within the genome of an individual organism. However, genes and other portions of genomes can also be exchanged between individuals of different species. This mechanism of **horizontal gene transfer** is rare among eukaryotes but common among bacteria, which can exchange DNA by the process of conjugation (Figure 9–14 and Movie 9.1).

E. coli, for example, has acquired about one-fifth of its genome from other bacterial species within the past 100 million years. And such genetic exchanges are currently responsible for the rise of new and potentially dangerous strains of drug-resistant bacteria. Genes that confer resistance to antibiotics are readily transferred from species to species, providing the recipient bacterium with an enormous selective advantage in evading the antimicrobial compounds that constitute modern medicine's frontline attack against bacterial infection. As a result, many antibiotics are no longer effective against the common bacterial infections for which they were originally used; as an example, most strains of *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea, are now resistant to penicillin, which is therefore no longer the primary drug used to treat this disease.

QUESTION 9–2

Why do you suppose that horizontal gene transfer is more prevalent in single-celled organisms than in multicellular organisms?

RECONSTRUCTING LIFE'S FAMILY TREE

We have seen how genomes can change over evolutionary time. The nucleotide sequences of present-day genomes provide a record of those changes that conferred biological success. By comparing the genomes of a variety of living organisms, we can thus begin to decipher our evolutionary history, seeing how our ancestors veered off in adventurous new directions that led us to where we are today.

The most astonishing revelation of such genome comparisons has been that **homologous genes**—those that are similar in nucleotide sequence because of their common ancestry—can be recognized across vast evolutionary distances. Unmistakable homologs of many human genes are easy to detect in organisms such as worms, fruit flies, yeasts, and even bacteria. Although the lineage that led to the evolution of vertebrates is thought to have diverged from the one that led to nematode worms and insects more than 600 million years ago, when we compare the genomes of the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and *Homo sapiens*, we find that about 50% of the genes in each of these species have clear homologs in one or both of the other two species. In other words, clearly recognizable versions of at least half of all human genes were already present in the common ancestor of worms, flies, and humans.

By tracing such relationships among genes, we can begin to define the evolutionary relationships among different species, placing each bacterium, animal, plant, or fungus in a single vast family tree of life. In this

section, we discuss how these relationships are determined and what they tell us about our genetic heritage.

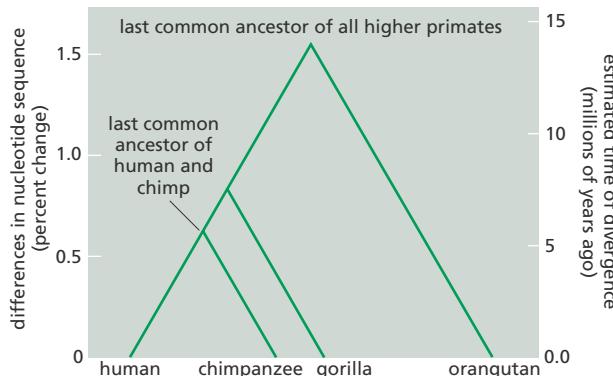
Genetic Changes That Provide a Selective Advantage Are Likely to Be Preserved

Evolution is commonly thought of as progressive, but at the molecular level the process is random. Consider the fate of a point mutation that occurs in a germ-line cell. On rare occasions, the mutation might cause a change for the better. But most often it will either have no consequence or cause serious damage. Mutations of the first type will tend to be perpetuated, because the organism that inherits them will have an increased likelihood of reproducing itself. Mutations that are *selectively neutral* may or may not be passed on. And mutations that are deleterious will be lost. Through endless repetition of such cycles of error and trial—of mutation and natural selection—organisms gradually evolve. Their genomes change and they develop new ways to exploit the environment—to out-compete others and to reproduce successfully.

Clearly, some parts of the genome can accumulate mutations more easily than others in the course of evolution. A segment of DNA that does not code for protein or RNA and has no significant regulatory role is free to change at a rate limited only by the frequency of random mutation. In contrast, deleterious alterations in a gene that codes for an essential protein or RNA molecule cannot be accommodated so easily: when mutations occur, the faulty organism will almost always be eliminated or fail to reproduce. Genes of this latter sort are therefore *highly conserved*; that is, the proteins they encode are very similar from organism to organism. Throughout the 3.5 billion years or more of evolutionary history, the most highly conserved genes remain perfectly recognizable in all living species. They encode crucial proteins such as DNA and RNA polymerases, and they are the ones we turn to when we wish to trace family relationships among the most distantly related organisms in the tree of life.

Closely Related Organisms Have Genomes That Are Similar in Organization As Well As Sequence

For species that are closely related, it is often most informative to focus on selectively neutral mutations. Because they accumulate steadily at a rate that is unconstrained by selection pressures, these mutations provide a metric for gauging how much modern species have diverged from their common ancestor. Such comparisons of nucleotide changes allow the construction of a **phylogenetic tree**, a diagram that depicts the evolutionary relationships among a group of organisms. **Figure 9–15** presents a phylogenetic tree that lays out the relationships among higher primates.



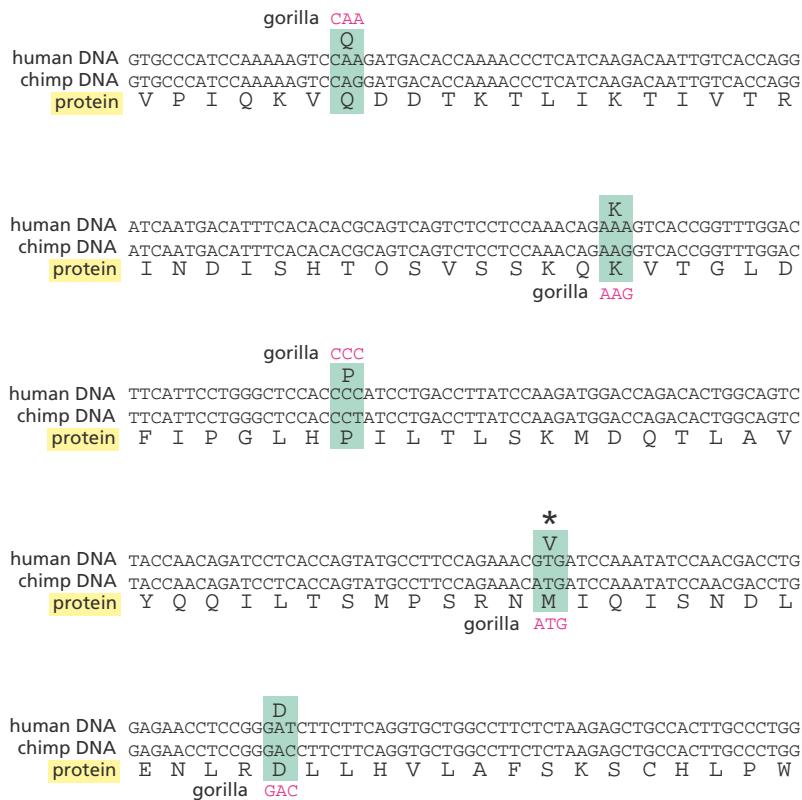
QUESTION 9–3

Highly conserved genes such as those for ribosomal RNA are present as clearly recognizable relatives in all organisms on Earth; thus, they have evolved very slowly over time. Were such genes “born” perfect?

Figure 9–15 Phylogenetic trees display the relationships among modern life-forms. In this family tree of higher primates, humans fall closer to chimpanzees than to gorillas or orangutans, as there are fewer differences between human and chimp DNA sequences than there are between those of humans and gorillas, or of humans and orangutans. As indicated, the genome sequences of each of these four species are estimated to differ from the sequence of the last common ancestor of higher primates by about 1.5%. Because changes occur independently in each lineage, the divergence between any two species will be twice as much as the amount of change that takes place between each of the species and their last common ancestor. For example, although humans and orangutans differ from their common ancestor by about 1.5% in terms of nucleotide sequence, they typically differ from one another by slightly more than 3%; human and chimp genomes differ by about 1.2%. Although this phylogenetic tree is based solely on nucleotide sequences, the estimated dates of divergence, shown on the right side of the graph, derive from data obtained from the fossil record. (Modified from F.C. Chen and W.H. Li, Am. J. Hum. Genet. 68:444–456, 2001. With permission from Elsevier.)

Figure 9–16 Ancestral gene sequences can be reconstructed by comparing closely related present-day species.

Shown here, in five contiguous segments of DNA, are nucleotide sequences from the protein-coding region of the leptin gene from humans and chimpanzees. Leptin is a hormone that regulates food intake and energy utilization. As indicated by the codons boxed in green, only 5 out of a total 441 nucleotides differ between the chimp and human sequences. Only one of these changes (marked with an asterisk) results in a change in the amino acid sequence. The nucleotide sequence of the last common ancestor was probably the same as the human and chimp sequences where they agree; in the few places where they disagree, the gorilla sequence (red) can be used as a “tiebreaker.” This strategy is based on the relationship shown in Figure 9–15: differences between humans and chimpanzees reflect relatively recent events in evolutionary history, and the gorilla sequence reveals the most likely precursor sequence. For convenience, only the first 300 nucleotides of the leptin-coding sequences are shown. The last 141 nucleotides are identical between humans and chimpanzees.

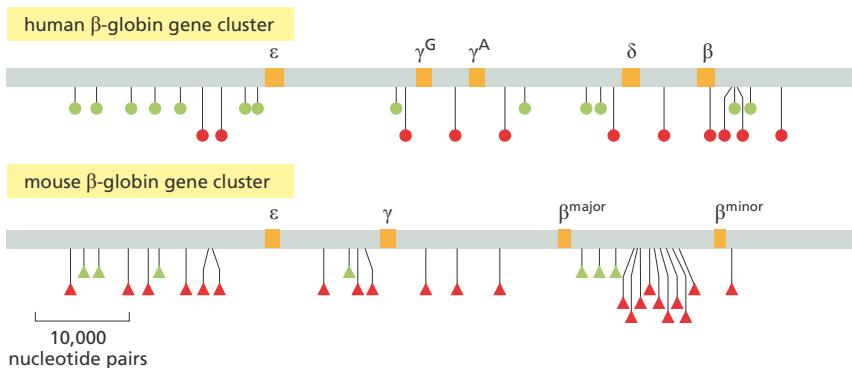


It is clear from this figure that chimpanzees are our closest living relative among the higher primates. Not only do chimpanzees seem to have essentially the same set of genes as we do, but their genes are arranged in nearly the same way. The only substantial exception is human Chromosome 2, which arose from a fusion of two chromosomes that remain separate in the chimpanzee, gorilla, and orangutan. Humans and chimpanzees are so closely related that it is possible to use DNA sequence comparisons to reconstruct the sequence of genes that must have been present in the now-extinct, common ancestor of the two species (**Figure 9–16**).

Even the rearrangement of genomes by recombination, which we described earlier, has produced only minor differences between the human and chimp genomes. For example, both the chimp and human genomes contain a million copies of a type of mobile genetic element called an *Alu* sequence. More than 99% of these elements are in corresponding positions in both genomes, indicating that most of the *Alu* sequences in our genome were in place before humans and chimpanzees diverged.

Functionally Important Genome Regions Show Up As Islands of Conserved DNA Sequence

As we delve back further into our evolutionary history and compare our genomes with those of more distant relatives, the picture begins to change. The lineages of humans and mice, for example, diverged about 75 million years ago. These genomes are about the same size, contain practically the same genes, and are both riddled with mobile genetic elements. However, the mobile genetic elements found in mouse and human DNA, although similar in sequence, are distributed differently, as they have had more time to proliferate and move around the two genomes since these species diverged (**Figure 9–17**).



In addition to the movement of mobile genetic elements, the large-scale organization of the human and mouse genomes has been scrambled by many episodes of chromosome breakage and recombination in the past 75 million years: it is estimated that about 180 such “break-and-join” events have dramatically altered chromosome structure. For example, in humans most centromeres lie near the middle of the chromosome, whereas those of mouse are located at the chromosome ends.

In spite of this significant degree of genetic shuffling, one can nevertheless still recognize many blocks of **conserved synteny**, regions where corresponding genes are strung together in the same order in both species. These genes were neighbors in the ancestral species and, despite all the chromosomal upheavals, they remain neighbors in the two present-day species. More than 90% of the mouse and human genomes can be partitioned into such corresponding regions of conserved synteny. Within these regions, we can align the DNA of mouse with that of humans so that we can compare the nucleotide sequences in detail. Such genome-wide sequence comparisons reveal that, in the roughly 75 million years since humans and mice diverged from their common ancestor, about 50% of the nucleotides have changed. Against this background of dissimilarity, however, one can now begin to see very clearly the regions where changes are not tolerated, so that the human and mouse sequences have remained nearly the same (**Figure 9–18**). Here, the sequences have been conserved by **purifying selection**—that is, by the elimination of individuals carrying mutations that interfere with important functions.

The power of *comparative genomics* can be increased by stacking our genome up against the genomes of additional animals, including the rat, chicken, and dog. Such comparisons take advantage of the results of the “natural experiment” that has lasted for hundreds of millions of years, and they highlight some of the most important regions of these genomes. These comparisons reveal that roughly 4.5% of the human genome consists of DNA sequences that are highly conserved in many other mammals (**Figure 9–19**). Surprisingly, only about one-third of these sequences code for proteins. Some of the conserved noncoding sequences correspond

Figure 9–17 The positions of mobile genetic elements in the human and mouse genomes reflect the long evolutionary time separating the two species. This stretch of human Chromosome 11 (introduced in Figure 9–10) contains five functional β-globin-like genes (orange); the comparable region from the mouse genome contains only four. The positions of two types of mobile genetic element—Alu sequences (green) and L1 sequences (red)—are shown in each genome. Although the mobile genetic elements in human (circles) and mouse (triangles) are not identical, they are closely related. The absence of these elements within the globin genes can be attributed to natural selection, which most likely eliminated any insertion that compromised gene function. (The mobile genetic element that falls inside the human β-globin gene (far right) is actually located within an intron.) (Courtesy of Ross Hardison and Webb Miller.)

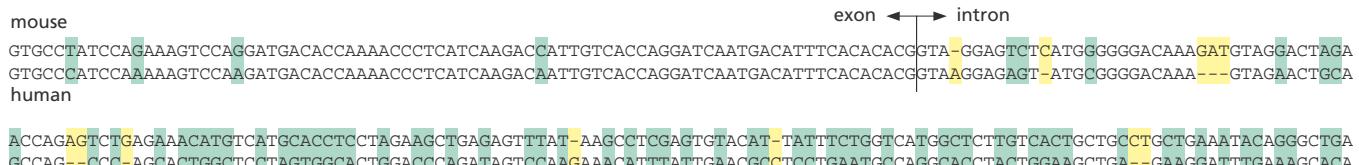


Figure 9–18 Accumulated mutations have resulted in considerable divergence in the nucleotide sequences of the human and the mouse genomes. Shown here in two contiguous segments of DNA are portions of the human and mouse leptin gene sequences. Positions where the sequences differ by a single nucleotide substitution are boxed in green, and positions where they differ by the addition or deletion of nucleotides are boxed in yellow. Note that the coding sequence of the exon is much more conserved than the adjacent intron sequence.

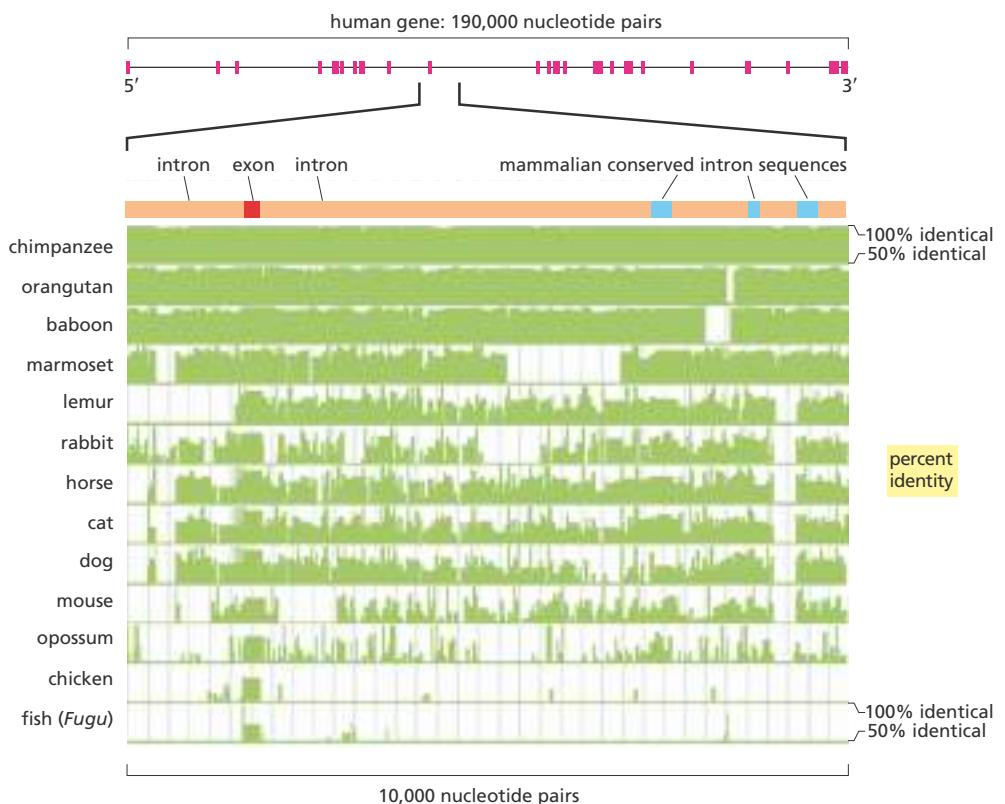


Figure 9–19 Comparison of nucleotide sequences from many different vertebrates reveals regions of high conservation. The nucleotide sequence examined in this diagram is a small segment of the human gene for a plasma membrane transporter protein. Exons in the complete gene (top) and in the expanded region of the gene are indicated in red. Three blocks of intron sequence that are conserved in mammals are shown in blue. In the lower part of the figure, the expanded human DNA sequence is aligned with the corresponding sequences of different vertebrates; the percent identity with the human sequences for successive stretches of 100 nucleotide pairs is plotted in green, with only identities above 50% shown. Note that the sequence of the exon is highly conserved in all the species, including chicken and fish, but the three intron sequences that are conserved in mammals are not conserved in chickens or fish. The functions of most conserved intron sequences in the human genome (including these three) are not known. (Courtesy of Eric D. Green.)

to regulatory DNA, whereas others are transcribed to produce RNA molecules that are not translated into protein but serve regulatory functions (discussed in Chapter 8). The functions of the majority of these conserved noncoding sequences, however, remain unknown. The unexpected discovery of these mysterious conserved DNA sequences suggests that we understand much less about the cell biology of mammals than we had previously imagined. With the plummeting cost and accelerating speed of whole-genome sequencing, we can expect many more surprises that will lead to an increased understanding in the years ahead.

Genome Comparisons Show That Vertebrate Genomes Gain and Lose DNA Rapidly

Going back even further in evolution, we can compare our genome with those of more distantly related vertebrates. The lineages of fish and mammals diverged about 400 million years ago. This is long enough for random sequence changes and differing selection pressures to have obliterated almost every trace of similarity in nucleotide sequence—except where purifying selection has operated to prevent change. Regions of the genome conserved between humans and fishes thus stand out even more strikingly than those conserved between different mammals. In fishes, one can still recognize most of the same genes as in humans and even many of the same segments of regulatory DNA. On the other hand, the extent of duplication of any given gene is often different, resulting in different numbers of members of gene families in the two species.

But even more striking is the finding that although all vertebrate genomes contain roughly the same number of genes, their overall size varies considerably. Whereas human, dog, and mouse are all in the same size range (around 3×10^9 nucleotide pairs), the chicken genome is only one-third this size. An extreme example of genome compression is the pufferfish *Fugu rubripes* (Figure 9–20), whose tiny genome is one-tenth the size of mammalian genomes, largely because of the small size of

Figure 9–20 The pufferfish, *Fugu rubripes*, has a remarkably compact genome. At 400 million nucleotide pairs, the *Fugu* genome is only one-quarter the size of the zebrafish genome, even though the two species have nearly the same genes. (From a woodcut by Hiroshige, courtesy of Arts and Designs of Japan.)



its introns. *Fugu* introns, as well as other noncoding segments in the animal's genome, lack the repetitive DNA that makes up a large portion of most mammalian genomes. Nonetheless, the positions of most *Fugu* introns are perfectly conserved when compared with their positions in mammalian genomes (Figure 9–21). Clearly, the intron structure of most vertebrate genes was already in place in the common ancestor of fish and mammals.

What factors could be responsible for the size differences among modern vertebrate genomes? Detailed comparisons of many genomes have led to the unexpected finding that small blocks of sequence are being lost from and added to genomes at a surprisingly rapid rate. It seems likely, for example, that the *Fugu* genome is so tiny because it lost DNA sequences faster than it gained them. Over long periods, this imbalance apparently cleared out those DNA sequences whose loss could be tolerated. This "cleansing" process has been enormously helpful to biologists: by "trimming the fat" from the *Fugu* genome, evolution has provided a conveniently slimmed-down version of a vertebrate genome in which the only DNA sequences that remain are those that are very likely to have important functions.

Sequence Conservation Allows Us to Trace Even the Most Distant Evolutionary Relationships

As we go back further still to the genomes of our even more distant relatives—beyond apes, mice, fish, flies, worms, plants, and yeasts, all the way to bacteria—we find fewer and fewer resemblances to our own genome. Yet even across this enormous evolutionary divide, purifying selection has maintained a few hundred fundamentally important genes. By comparing the sequences of these genes in different organisms and seeing how far they have diverged, we can attempt to construct a phylogenetic tree that goes all the way back to the ultimate ancestors—the cells at the very origins of life, from which we all derive.

To construct such a tree, biologists have focused on one particular gene that is conserved in all living species: the gene that codes for the ribosomal RNA (rRNA) of the small ribosomal subunit (see Figure 7–32). Because the process of translation is fundamental to all living cells, this

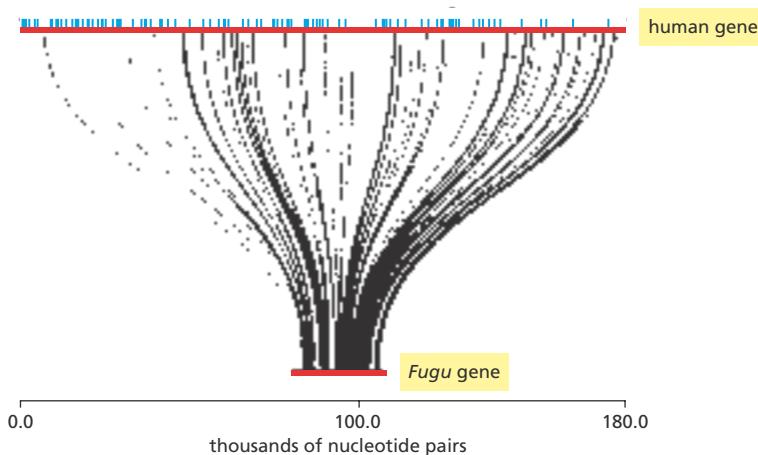


Figure 9–21 The positions of introns and exons are conserved between *Fugu* and humans. Comparison of the nucleotide sequences of the genes that encode the huntingtin protein in human and in *Fugu*. Both genes (red) contain 67 short exons, which align in 1:1 correspondence with one another; the corresponding exons are connected by the curved black lines. The human gene is 7.5 times larger than the *Fugu* gene (180,000 versus 24,000 nucleotide pairs), due entirely to the larger introns in the human sequence. The larger size of the human introns is due in part to mobile genetic elements, whose positions are represented by the blue vertical lines. These elements are absent in *Fugu*. In humans, mutation of this gene causes Huntington's disease, an inherited neurodegenerative disorder of the brain. (Adapted from S. Baxendale et al., Nat. Genet. 10:67–76, 1995. With permission from Macmillan Publishers Ltd.)

GTTCCGGGGGGAGTATGGTTCGAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAAGGAGTGGAGGCCCGCGCTTAATTGACTCAACACGGGAAACCTCACCC	human
GCCCCCTGGGAGTACGGTCCAAGACTGAAACTTAAAGGAATTGGGGGGAGCACTACAACGGTGGAGGCCCGCGCTTAATTGGATTCAACGCCGGCATCTTACCA	<i>Methanococcus</i>
ACCGCCTGGGAGTACGGCCGAAGGTAAAACCTCAAATGAATTGACGGGGGCCGC•ACAAGGGTGGAGCATGTGGTTAACCTGATGCAACGCCAAGAACCTTACCT	<i>E. coli</i>
GTTCCGGGGGGAGTATGGTTCGAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAAGGAGTGGAGGCCCGCGCTTAATTGACTCAACACGGGAAACCTCACCC	human

Figure 9–22 Some genetic information has been conserved since the beginnings of life. A part of the gene for the small subunit rRNA (see Figure 7–32) is shown. Corresponding segments of nucleotide sequence from this gene in three distantly related species (*Methanococcus jannaschii*, an archaeon; *Escherichia coli*, a bacterium; and *Homo sapiens*, a eukaryote) are aligned in parallel. Sites where the nucleotides are identical between species are indicated by green shading; the human sequence is repeated at the bottom of the alignment so that all three two-way comparisons can be seen. The red dot halfway along the *E. coli* sequence denotes a site where a nucleotide has been either deleted from the bacterial lineage in the course of evolution or inserted in the other two lineages. Note that the three sequences have all diverged from one another to a roughly similar extent, while still retaining unmistakable similarities.

component of the ribosome has been highly conserved since early in the history of life on Earth (Figure 9–22).

By applying the same principles used to construct the primate family tree (see Figure 9–15), the small subunit rRNA nucleotide sequences have been used to create a single, all-encompassing tree of life. Although many aspects of this phylogenetic tree were anticipated by classical taxonomy (which is based on the outward appearance of organisms), there were also many surprises. Perhaps the most important was the realization that some of the organisms that were traditionally classed as “bacteria” are as widely divergent in their evolutionary origins as is any prokaryote from any eukaryote. As discussed in Chapter 1, it is now apparent that the prokaryotes comprise two distinct groups—the *bacteria* and the *archaea*—that diverged early in the history of life on Earth. The living world therefore has three major divisions or *domains*: bacteria, archaea, and eukaryotes (Figure 9–23).

Although we humans have been classifying the visible world since antiquity, we now realize that most of life’s genetic diversity lies in the world of microscopic organisms. These microbes have tended to go unnoticed, unless they cause disease or rot the timbers of our houses. Yet they make up most of the total mass of living matter on our planet. Many of these organisms cannot be grown under laboratory conditions. Thus it is only through the analysis of DNA sequences, obtained from around the globe, that we are beginning to obtain a more detailed understanding of all life on Earth—knowledge that is less distorted by our biased perspective as large animals living on dry land.

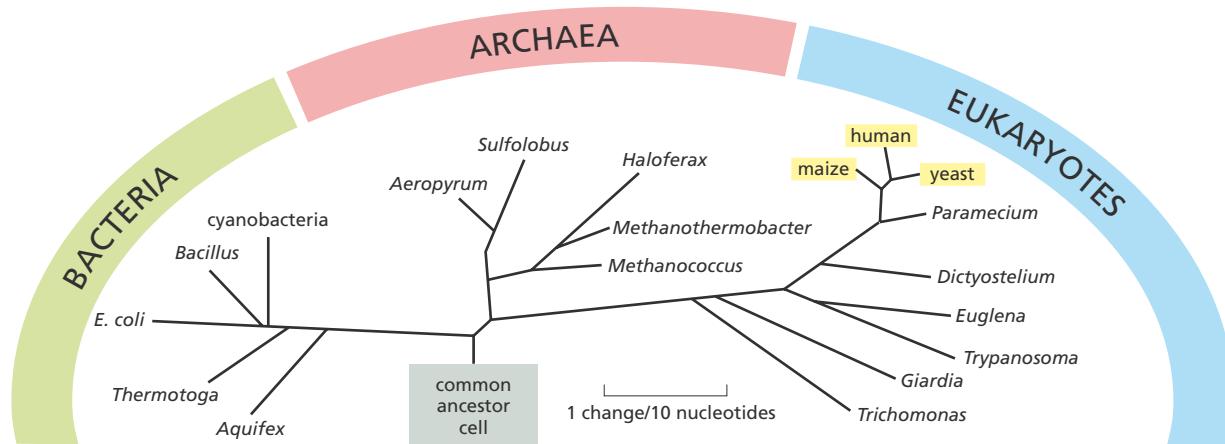


Figure 9–23 The tree of life has three major divisions. Each branch on the tree is labeled with the name of a representative member of that group, and the length of each branch corresponds to the degree of difference in the DNA sequences that encode their small subunit rRNAs (see Figure 9–22). Note that all the organisms we can see with the unaided eye—animals, plants, and some fungi (highlighted in yellow)—represent only a small subset of the diversity of life.

TRANSPOSONS AND VIRUSES

The tree of life depicted in Figure 9–23 includes representatives from life's most distant branches, from the cyanobacteria that release oxygen into the atmosphere to the animals, like us, that use that oxygen to boost their metabolism. What the diagram does not encompass, however, are the parasitic genetic elements that operate on the outskirts of life. Although these elements are built from the same nucleic acids contained in all life-forms and can multiply and move from place to place, they do not cross the threshold of actually being alive. Yet because of their prevalence and behavior, these diminutive genetic parasites have major implications for the evolution of species and for human health.

Mobile genetic elements, known informally as jumping genes, are found in virtually all cells. Their DNA sequences make up almost half of the human genome. Although they can insert themselves into virtually any DNA sequence, most mobile genetic elements lack the ability to leave the cell in which they reside. This is not the case for their relatives, the *viruses*. Not much more than strings of genes wrapped in a protective coat, viruses can escape from one cell and infect another.

In this section, we briefly discuss mobile genetic elements as well as viruses. We review their structure and outline how they operate—and we consider the effects they have on gene expression, genome evolution, and the transmission of disease.

Mobile Genetic Elements Encode the Components They Need for Movement

Mobile genetic elements, also called **transposons**, are typically classified according to the mechanism by which they move or *transpose*. In bacteria, the most common mobile genetic elements are the *DNA-only transposons*. The name is derived from the fact that the element moves from one place to another as a piece of DNA, as opposed to being converted into an RNA intermediate—which is the case for another type of mobile element we discuss below. Bacteria contain many different DNA-only transposons. Some move to the target site using a simple cut-and-paste mechanism, whereby the element is simply excised from the genome and inserted into a different site; other DNA-only transposons replicate their DNA before inserting into the new chromosomal site, leaving the original copy intact at its previous location (Figure 9–24).

Each mobile genetic element typically encodes a specialized enzyme, called a *transposase*, that mediates its movement. These enzymes recognize and act on unique DNA sequences that are present on each mobile genetic element. Many mobile genetic elements also carry additional genes: some

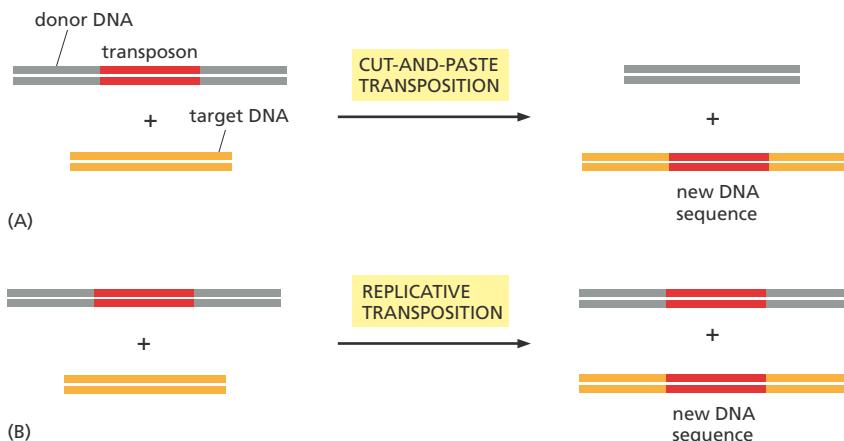
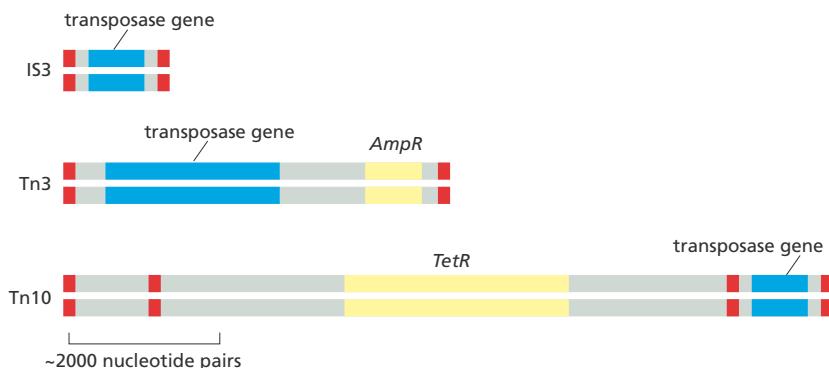


Figure 9–24 The most common mobile genetic elements in bacteria, DNA-only transposons, move by two types of mechanism. (A) In cut-and-paste transposition, the element is cut out of the donor DNA and inserted into the target DNA, leaving behind a broken donor DNA molecule, which is subsequently repaired. (B) In replicative transposition, the mobile genetic element is copied by DNA replication. The donor molecule remains unchanged, and the target molecule receives a copy of the mobile genetic element. In general, a particular type of transposon moves by only one of these mechanisms. However, the two mechanisms have many enzymatic similarities, and a few transposons can move by either mechanism. The donor and target DNAs can be part of the same DNA molecule or reside on different DNA molecules.

Figure 9–25 Transposons contain the components they need for transposition.

Shown here are three types of bacterial DNA-only transposons. Each carries a gene that encodes a transposase (blue)—the enzyme that catalyzes the element's movement—as well as DNA sequences (red) that are recognized by that transposase.

Some transposons carry additional genes (yellow) that encode enzymes that inactivate antibiotics such as ampicillin (*AmpR*) and tetracycline (*TetR*). The spread of these transposons is a serious problem in medicine, as it has allowed many disease-causing bacteria to become resistant to antibiotics developed during the twentieth century.



mobile genetic elements, for example, carry antibiotic-resistance genes, which have contributed greatly to the widespread dissemination of antibiotic resistance in bacterial populations (Figure 9–25).

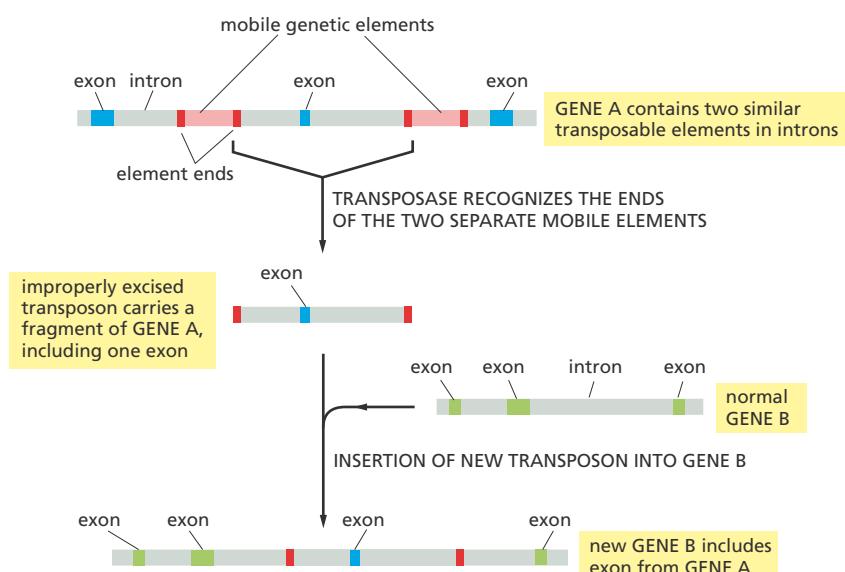
In addition to relocating themselves, mobile genetic elements occasionally rearrange the DNA sequences of the genome in which they are embedded. For example, if two mobile genetic elements that are recognized by the same transposase integrate into neighboring regions of the same chromosome, the DNA between them can be accidentally excised and inserted into a different gene or chromosome (Figure 9–26). In eukaryotic genomes, such accidental transposition provides a pathway for generating novel genes, both by altering gene expression and by duplicating existing genes.

QUESTION 9–4

Many transposons move within a genome by replicative mechanisms (such as those shown in Figure 9–24B). They therefore increase in copy number each time they transpose. Although individual transposition events are rare, many transposons are found in multiple copies in genomes. What do you suppose keeps the transposons from completely overrunning their hosts' genomes?

The Human Genome Contains Two Major Families of Transposable Sequences

The sequencing of human genomes has revealed many surprises, as we describe in detail in the next section. But one of the most stunning was the finding that a large part of our DNA is not entirely our own. Nearly half of the human genome is made up of mobile genetic elements, which number in the millions. Some of these elements have moved from place to place within the human genome using the cut-and-paste mechanism discussed earlier (see Figure 9–24A). However, most have moved not as DNA, but via an RNA intermediate. These **retrotransposons** appear to be unique to eukaryotes.

**Figure 9–26 Mobile genetic elements can move exons from one gene to another.**

When two mobile genetic elements of the same type (red) happen to insert near each other in a chromosome, the transposition mechanism occasionally recognizes the ends of two different elements (instead of the two ends of the same element). As a result, the chromosomal DNA that lies between the mobile genetic elements gets excised and moved to a new site. Such inadvertent transposition of chromosomal DNA can either generate novel genes, as shown, or alter gene regulation (not shown).

One abundant human retrotransposon, the ***L1* element** (sometimes referred to as *LINE-1*, a long interspersed nuclear element), is transcribed into RNA by a host cell's RNA polymerase. A double-stranded DNA copy of this RNA is then made using an enzyme called **reverse transcriptase**, an unusual DNA polymerase that can use RNA as a template. The reverse transcriptase is encoded by the *L1* element itself. The DNA copy of the element is then free to reintegrate into another site in the genome (Figure 9–27).

L1 elements constitute about 15% of the human genome. Although most copies have been immobilized by the accumulation of deleterious mutations, a few still retain the ability to transpose. Their movement can sometimes precipitate disease: for example, about 40 years ago, movement of an *L1* element into the gene that encodes Factor VIII—a protein essential for proper blood clotting—caused hemophilia in an individual with no family history of the disease.

Another type of retrotransposon, the ***Alu* sequence**, is present in about 1 million copies, making up about 10% of our genome. *Alu* elements do not encode their own reverse transcriptase and thus depend on enzymes already present in the cell to help them move.

Comparisons of the sequence and locations of the *L1* and *Alu* elements in different mammals suggest that these sequences have proliferated in primates relatively recently in evolutionary history (see Figure 9–17). Given that the placement of mobile genetic elements can have profound effects on gene expression, it is humbling to contemplate how many of our uniquely human qualities we might owe to these prolific genetic parasites.

Viruses Can Move Between Cells and Organisms

Viruses are also mobile, but unlike the transposons we have discussed so far, they can actually escape from cells and move to other cells and organisms. Viruses were first categorized as disease-causing agents that, by virtue of their tiny size, passed through ultrafine filters that can hold back even the smallest bacterial cell. We now know that viruses are essentially genomes enclosed by a protective protein coat, and that they must enter a cell and coopt its molecular machinery to express their genes, make their proteins, and reproduce. Although the first viruses that were discovered attack mammalian cells, it is now recognized that many types of viruses exist, and virtually all organisms—including plants, animals, and bacteria—can serve as viral hosts.

Viral reproduction is often lethal to the host cells; in many cases, the infected cell breaks open (lyses), releasing progeny viruses, which can then infect neighboring cells. Many of the symptoms of viral infections reflect this lytic effect of the virus. The cold sores formed by herpes simplex virus and the blisters caused by the chickenpox virus, for example, reflect the localized killing of human skin cells.

Most viruses that cause human disease have genomes made of either double-stranded DNA or single-stranded RNA (Table 9–1). However, viral genomes composed of single-stranded DNA and of double-stranded RNA are also known. The simplest viruses found in nature have a small genome, composed of as few as three genes, enclosed by a protein coat built from many copies of a single polypeptide chain. More complex viruses have larger genomes of up to several hundred genes, surrounded by an elaborate shell composed of many different proteins (Figure 9–28). The amount of genetic material that can be packaged inside a viral protein shell is limited. Because these shells are too small to encode the

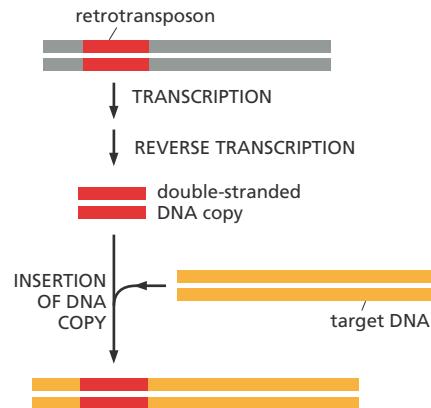


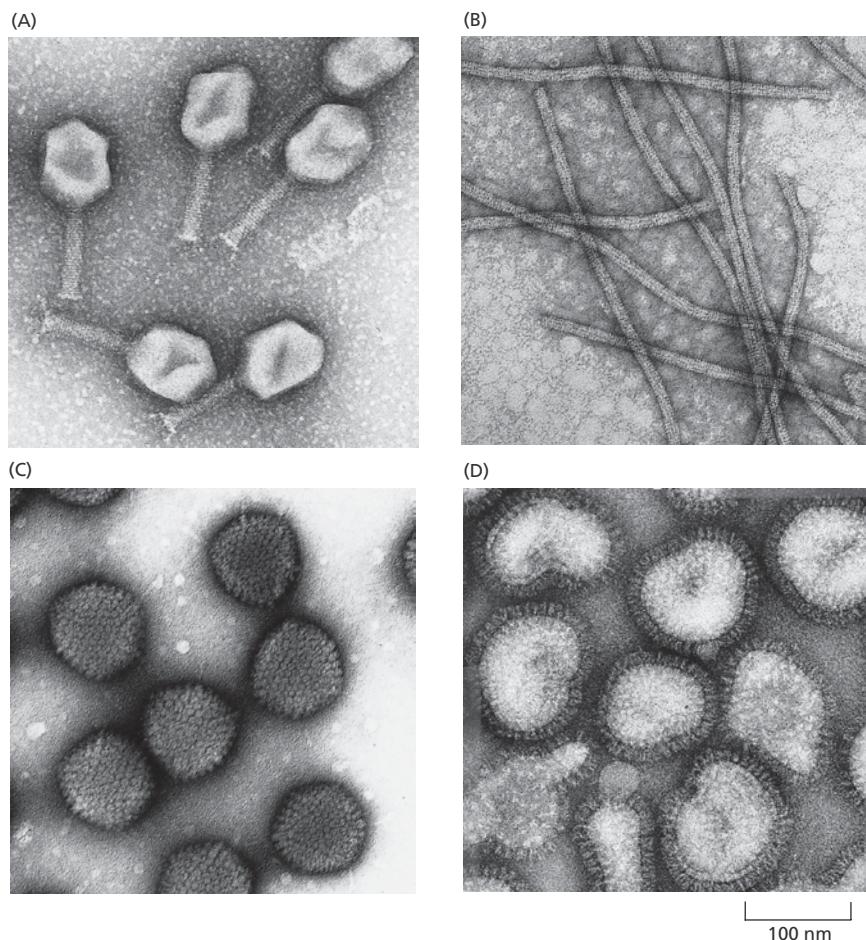
Figure 9–27 Retrotransposons move via an RNA intermediate. These transposable elements are first transcribed into an RNA intermediate. Next, a double-stranded DNA copy of this RNA is synthesized by the enzyme reverse transcriptase. This DNA copy is then inserted into the target location, which can be on either the same or a different DNA molecule. The donor retrotransposon remains at its original location, so each time it transposes, it duplicates itself. These mobile genetic elements are called retrotransposons because at one stage in their transposition their genetic information flows backward, from RNA to DNA.

QUESTION 9–5

Discuss the following statement:
"Viruses exist in the twilight zone of life: outside cells they are simply dead assemblies of molecules; inside cells, however, they are alive."

Figure 9–28 Viruses come in different shapes and sizes.

shapes and sizes. These electron micrographs of virus particles are all shown at the same scale. (A) T4 bacteriophage, a large DNA-containing virus that infects *E. coli* cells. The DNA is stored in the viral head and is injected into the bacterium through the cylindrical tail. (B) Potato virus X, a tubelike plant virus that contains an RNA genome. (C) Adenovirus, a DNA-containing animal virus that can infect human cells. (D) Influenza virus, a large RNA-containing animal virus whose protein coat is further enclosed in a lipid-bilayer-based envelope. The spikes protruding from the envelope are viral coat proteins embedded in the lipid bilayer. (A, courtesy of James R. Paulson; B, courtesy of Graham Hills; C, courtesy of Mei Lie Wong; D, courtesy of R.C. Williams and H.W. Fisher.)



100 nm

many enzymes and other proteins that are required to replicate even the simplest virus, viruses must hijack their host's biochemical machinery to reproduce themselves (**Figure 9–29**). The viral genome will typically encode both viral coat proteins and proteins that help them to coopt the host enzymes needed to replicate their genetic material.

Retroviruses Reverse the Normal Flow of Genetic Information

Although there are many similarities between bacterial and eukaryotic viruses, one important class of viruses—the **retroviruses**—is found only in eukaryotic cells. In many respects, retroviruses resemble the retrotransposons we just discussed. A key feature of the life cycle of both is a step in which DNA is synthesized using RNA as a template—hence the prefix *retro*, which refers to the reversal of the usual flow of DNA information to RNA. Retroviruses are thought to have derived from a retrotransposon that long ago acquired additional genes encoding the coat proteins and other proteins required to make a virus particle. The RNA stage of its replicative cycle could then be packaged into a viral particle that could leave the cell. The complete life cycle of a retrovirus is shown in **Figure 9–30**.

Like retrotransposons, retroviruses use the enzyme reverse transcriptase to convert RNA into DNA. The enzyme is encoded by the retroviral genome, and a few molecules of the enzyme are packaged along with the RNA genome in each virus particle. When the single-stranded RNA genome of the retrovirus enters a cell, the reverse transcriptase brought in with it makes a complementary DNA strand to form a DNA/RNA hybrid double helix. The RNA strand is removed, and the reverse transcriptase

TABLE 9–1 VIRUSES THAT CAUSE HUMAN DISEASE

Virus	Genome Type	Disease
Herpes simplex virus	double-stranded DNA	recurrent cold sores
Epstein–Barr virus (EBV)	double-stranded DNA	infectious mononucleosis
Varicella-zoster virus	double-stranded DNA	chickenpox and shingles
Smallpox virus	double-stranded DNA	smallpox
Hepatitis B virus	part single-, part double-stranded DNA	serum hepatitis
Human immunodeficiency virus (HIV)	single-stranded RNA	acquired immune deficiency syndrome (AIDS)
Influenza virus type A	single-stranded RNA	respiratory disease (flu)
Poliovirus	single-stranded RNA	poliomyelitis
Rhinovirus	single-stranded RNA	common cold
Hepatitis A virus	single-stranded RNA	infectious hepatitis
Hepatitis C virus	single-stranded RNA	non-A, non-B type hepatitis
Yellow fever virus	single-stranded RNA	yellow fever
Rabies virus	single-stranded RNA	rabies encephalitis
Mumps virus	single-stranded RNA	mumps
Measles virus	single-stranded RNA	measles

(which can use either DNA or RNA as a template) now synthesizes a complementary DNA strand to produce a DNA double helix. This DNA is then inserted, or integrated, into a randomly selected site in the host genome by a virally encoded *integrase* enzyme. In this integrated state, the virus is *latent*: each time the host cell divides, it passes on a copy of the integrated viral genome, which is known as a *provirus*, to its progeny cells.

The next step in the replication of a retrovirus—which can take place long after its integration into the host genome—is the copying of the integrated viral DNA into RNA by a host-cell RNA polymerase, which produces large numbers of single-stranded RNAs identical to the original infecting genome. These viral RNAs are then translated by the host-cell ribosomes to produce the viral shell proteins, the envelope proteins, and reverse transcriptase—all of which are assembled with the RNA genome into new virus particles.

The human immunodeficiency virus (HIV), which is the cause of AIDS, is a retrovirus. As with other retroviruses, the HIV genome can persist in a latent state as a provirus embedded in the chromosomes of an infected cell. This ability to hide in host cells complicates attempts to treat the infection with antiviral drugs. But because the HIV reverse transcriptase is not used by cells for any purpose of their own, it is one of the prime targets of drugs currently used to treat AIDS.

EXAMINING THE HUMAN GENOME

The human genome contains an enormous amount of information about who we are and where we came from (Figure 9–31). Its 3.2×10^9 nucleotide pairs, spread out over 23 sets of chromosomes—22 autosomes and

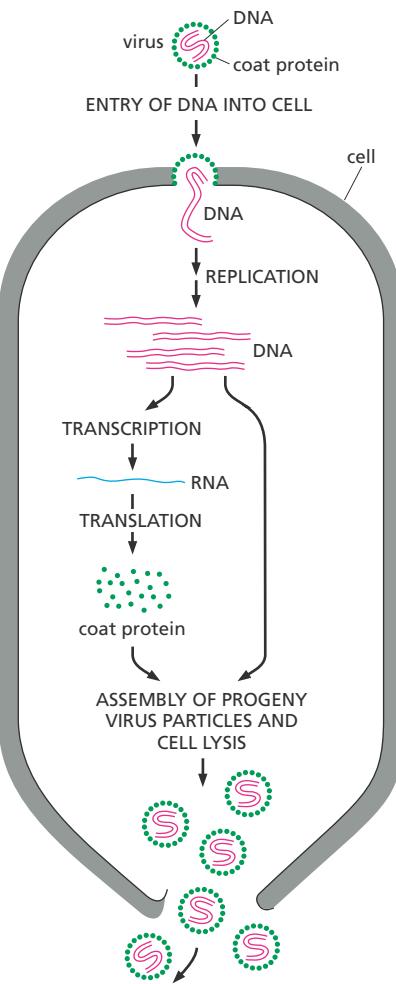


Figure 9–29 Viruses commandeer the host cell's molecular machinery to reproduce. The hypothetical simple virus illustrated here consists of a small double-stranded DNA molecule that encodes just a single type of viral coat protein. To reproduce, the viral genome must first enter a host cell, where it is replicated to produce multiple copies, which are transcribed and translated to produce the viral coat protein. The viral genomes can then assemble spontaneously with the coat protein to form new virus particles, which escape from the cell by lysing it.

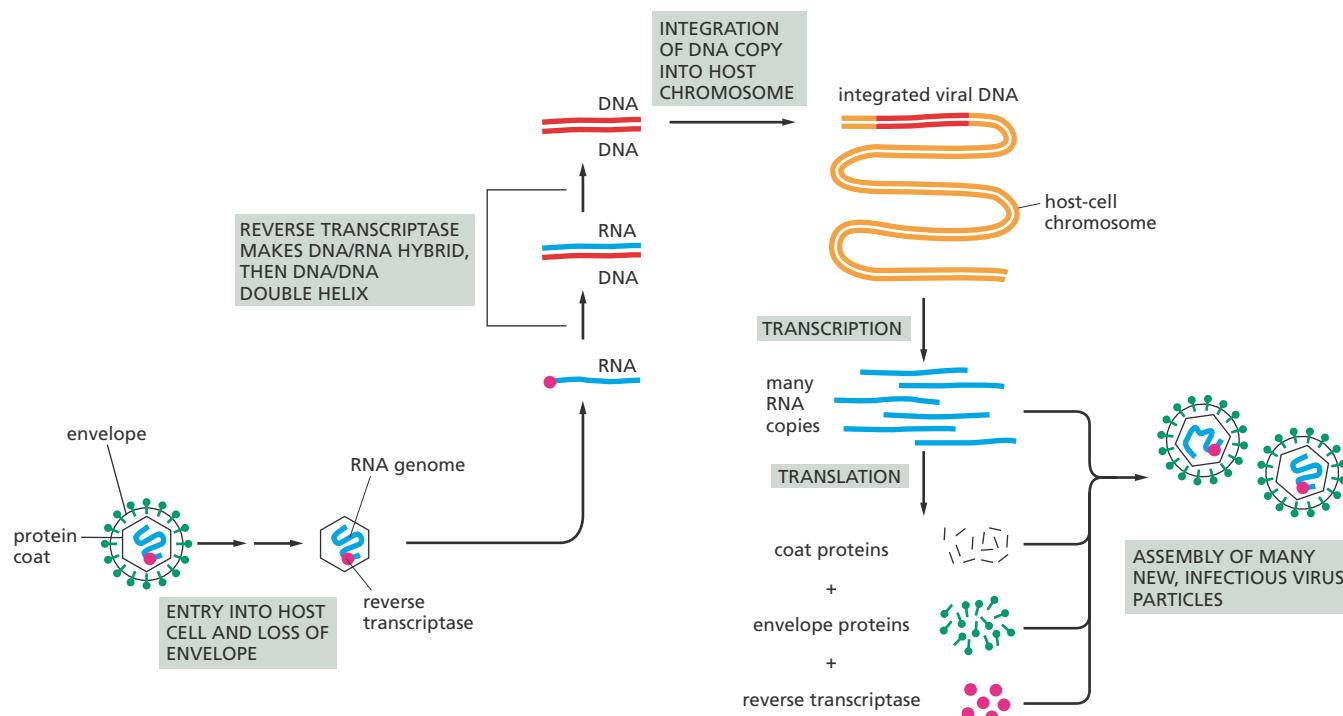


Figure 9–30 The life cycle of a retrovirus includes reverse transcription and integration of the viral genome into the host cell’s DNA. The retrovirus genome consists of an RNA molecule (blue) that is typically between 7000 and 12,000 nucleotides in size. It is packaged inside a protein coat, which is surrounded by a lipid-based envelope that contains virus-encoded envelope proteins (green). The enzyme reverse transcriptase (red circle), encoded by the viral genome and packaged with its RNA, first makes a single-stranded DNA copy of the viral RNA molecule and then a second DNA strand, generating a double-stranded DNA copy of the RNA genome. This DNA double helix is then integrated into a host chromosome, a step required for the synthesis of new viral RNA molecules by a host-cell RNA polymerase.

a pair of sex chromosomes (X and Y)—provide the instructions needed to build a human being. Yet, 25 years ago, biologists actively debated the value of determining the *human genome sequence*—the complete list of nucleotides contained in our DNA.

The task was not simple. An international consortium of investigators labored tirelessly for the better part of a decade—and spent nearly \$3 billion—to give us our first glimpse of this genetic blueprint. But the effort turned out to be well worth the cost, as the data continue to shape our thinking about how our genome functions and how it has evolved.

The first human genome sequence was just the beginning. Spectacular improvements in sequencing technologies, coupled with powerful new tools for handling massive amounts of data, are taking genomics to a whole new level. The cost of DNA sequencing has dropped about 100,000-fold since the human genome project was launched in 1990, such that a whole human genome can now be sequenced in a few days for about \$1000. Investigators around the world are collaborating to collect and compare the nucleotide sequences of thousands of human genomes. This resulting deluge of data promises to tell us what makes us human, and what makes each of us unique.

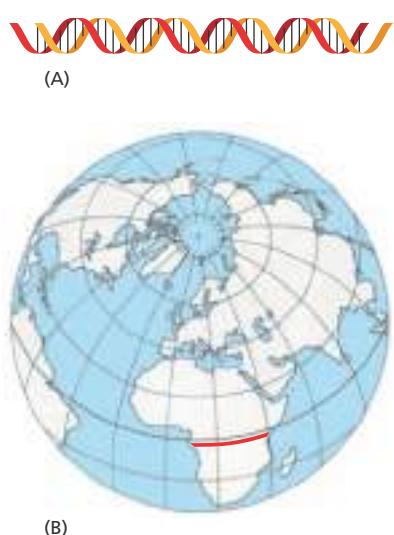


Figure 9–31 The 3 billion nucleotide pairs of the human genome contain a vast amount of information, including clues about our origins. If each nucleotide pair is drawn to span 1 mm, as shown in (A), the human genome would extend 3200 km (approximately 2000 miles)—far enough to stretch across central Africa, where humans first arose (red line in B). At this scale, there would be, on average, a protein-coding gene every 150 m. An average gene would extend for 30 m, but the coding sequences (exons) in this gene would add up to only just over a meter; the rest would be introns.

Although it will take decades to analyze the rapidly accumulating genome data, the recent findings have already influenced the content of every chapter in this book. In this section, we describe some of the most striking features of the human genome—many of which were entirely unexpected. We review what genome comparisons can tell us about how we evolved, and we discuss some of the mysteries that still remain.

The Nucleotide Sequences of Human Genomes Show How Our Genes Are Arranged

When the DNA sequence of human Chromosome 22, one of the smallest human chromosomes, was completed in 1999, it became possible for the first time to see exactly how genes are arranged along an entire vertebrate chromosome (Figure 9–32). The subsequent publication of the whole human genome sequence—a first draft in 2001 and a finished draft in 2004—provided a more panoramic view of the complete genetic landscape, including how many genes we have, what those genes look like, and how they are distributed across the genome (Table 9–2).

The first striking feature of the human genome is how little of it—less than 2%—codes for proteins (Figure 9–33). In addition, almost half of our DNA is made up of mobile genetic elements that have colonized our genome over evolutionary time. Because these elements have accumulated mutations, most can no longer move; rather, they are relics from an earlier evolutionary era when mobile genetic elements ran rampant through our genome.

It was a surprise to discover how few protein-coding genes our genome actually contains. Earlier estimates had been in the neighborhood of 100,000 (see **How We Know**, pp. 316–317). Although the exact count is still being refined, current estimates place the number of human

QUESTION 9–6

Mobile genetic elements, such as the *Alu* sequences, are found in many copies in human DNA. In what ways could the presence of an *Alu* sequence affect a nearby gene?

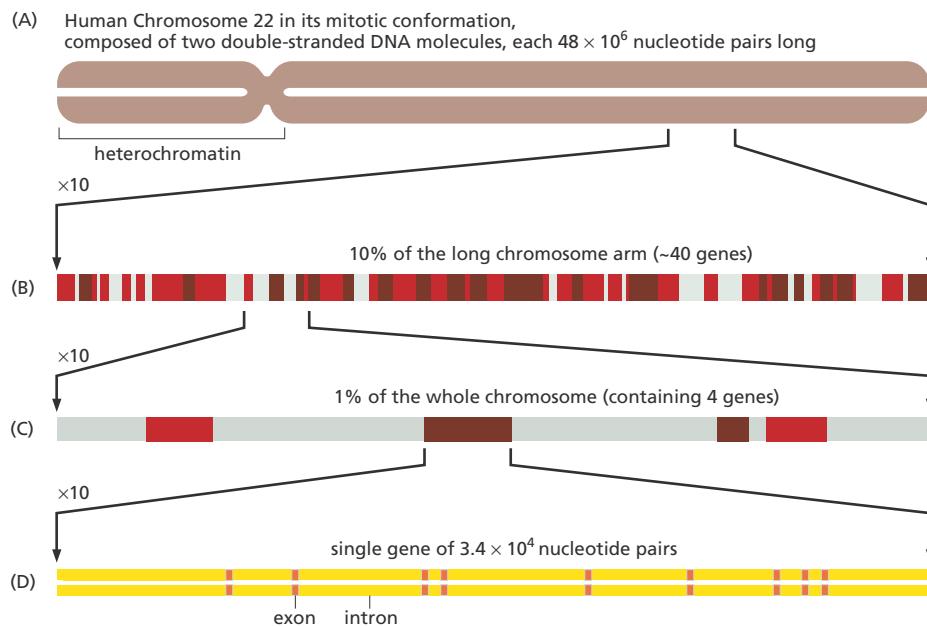


Figure 9–32 The sequence of Chromosome 22 shows how human chromosomes are organized. (A) Chromosome 22, one of the smallest human chromosomes, contains 48×10^6 nucleotide pairs and makes up approximately 1.5% of the entire human genome. Most of the left arm of Chromosome 22 consists of short repeated sequences of DNA that are packaged in a particularly compact form of chromatin (heterochromatin), as discussed in Chapter 5. (B) A tenfold expansion of a portion of Chromosome 22 shows about 40 genes. Those in dark brown are known genes, and those in red are predicted genes. (C) An expanded portion of (B) shows the entire length of several genes. (D) The intron–exon arrangement of a typical gene is shown after a further tenfold expansion. Each exon (orange) codes for a portion of the protein, while the DNA sequence of the introns (yellow) is relatively unimportant. (Adapted from The International Human Genome Sequencing Consortium, *Nature* 409:860–921, 2001. With permission from Macmillan Publishers Ltd.)

TABLE 9–2 SOME VITAL STATISTICS FOR THE HUMAN GENOME

DNA length	3.2×10^9 nucleotide pairs*
Number of protein-coding genes	approximately 21,000
Number of non-protein-coding genes**	approximately 9000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes***	approximately 11,000
Percentage of DNA sequence in exons (protein-coding sequences)	1.5%
Percentage of DNA conserved with other mammals that does not encode protein****	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%

*The sequence of 2.85 billion nucleotide pairs is known precisely (error rate of only about one in 100,000 nucleotides). The remaining DNA consists primarily of short, highly repeated sequences that are tandemly repeated, with repeat numbers differing from one individual to the next.

**These include genes that encode structural, catalytic, and regulatory RNAs.

***A pseudogene is a DNA sequence that closely resembles that of a functional gene but contains numerous mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene, followed by the accumulation of damaging mutations in one copy.

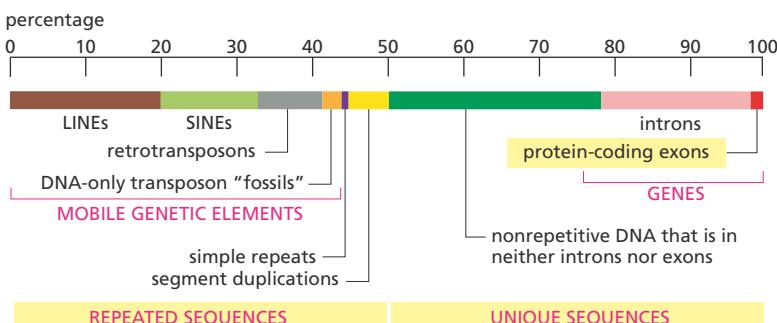
****This includes DNA encoding 5' and 3' UTRs (untranslated regions of mRNAs), regulatory DNA, and conserved regions of unknown function.

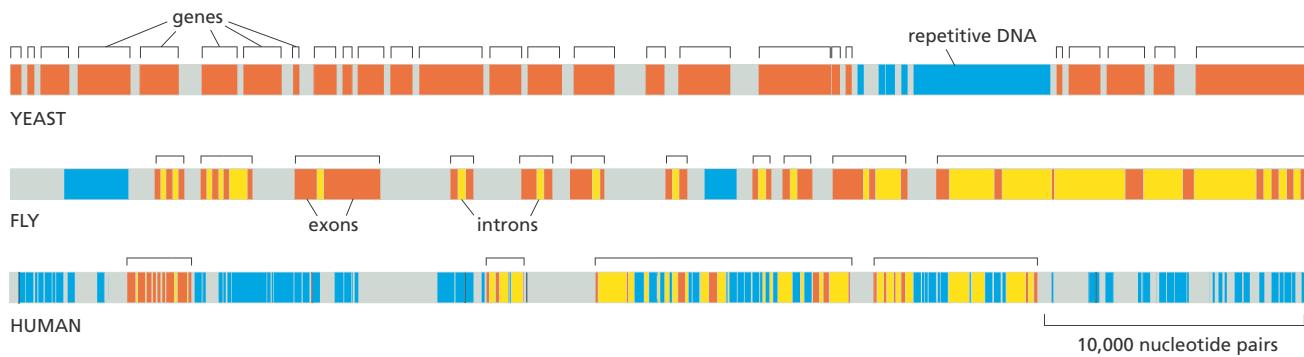
Figure 9–33 The bulk of the human genome is made of repetitive nucleotide sequences and other noncoding DNA.

The LINEs (which include *L1*), SINEs (short interspersed nuclear element, which include *Alu*), retrotransposons, and DNA-only transposons are mobile genetic elements that have multiplied in our genome by replicating themselves and inserting the new copies in different positions. Simple repeats are short nucleotide sequences (less than 14 nucleotide pairs) that are repeated again and again for long stretches. Segment duplications are large blocks of the genome (1000–200,000 nucleotide pairs) that are present at two or more locations in the genome. The unique sequences that are not part of any introns or exons (dark green) include gene regulatory sequences, sequences that code for functional RNA, and sequences whose functions are not known. The most highly repeated blocks of DNA in heterochromatin have not yet been completely sequenced; therefore about 10% of human DNA sequences are not represented in this diagram. (Data courtesy of E.H. Margulies.)

protein-coding genes at about 21,000. Perhaps another 9000 genes encode functional RNAs that are not translated into proteins. The estimate of 30,000 total genes brings us much closer to the gene numbers for simpler multicellular animals—for example, 13,000 for *Drosophila*, 21,000 for *C. elegans*, and 28,000 for the small weed *Arabidopsis* (see Table 1–2).

The number of protein-coding genes we have may be unexpectedly small, but their relative size is unusually large. Only about 1300 nucleotide pairs are needed to encode an average-sized human protein of about 430 amino acids. Yet the average length of a human gene is 27,000 nucleotide





pairs. Most of this DNA is in noncoding introns. In addition to the voluminous introns (see Figure 9–32D), each gene is associated with regulatory DNA sequences that ensure that the gene is expressed at the proper level, time, and place. In humans, these regulatory DNA sequences are typically interspersed along tens of thousands of nucleotide pairs, much of which seems to be “spacer” DNA. Indeed, compared to many other eukaryotic genomes, the human genome is much less densely packed (Figure 9–34).

Although exons and their associated gene regulatory sequences comprise less than 2% of the human genome, comparative studies indicate that about 5% of the human genome is highly conserved when compared with other mammalian genomes (see Figure 9–19). An additional 4% of the genome shows reduced variation in the human population, as determined by comparing the DNA sequence of thousands of individuals. Taken together, this conservation suggests that about 9% of the human genome contains sequences that are likely to be functionally important—but we do not yet know the function of much of this DNA.

Accelerated Changes in Conserved Genome Sequences Help Reveal What Makes Us Human

When the chimpanzee genome sequence became available in 2005, scientists began searching for DNA sequence changes that might account for the striking differences between us and them (Figure 9–35). With about 3 billion nucleotide pairs to compare between the two species, the task is daunting. But the search is made much easier by confining the comparison to those sequences that are highly conserved across multiple mammalian species (see Figure 9–19). These conserved sequences represent parts of the genome that are most likely to be functionally important—and are thus areas of particular interest when we search for genetic changes that make humans different from our mammalian cousins.

Although these sequences are conserved, they are not identical: when the version from one mammal is compared with that of another, they are typically found to have drifted apart by a small amount, which corresponds to the time elapsed since the species diverged during evolution. In a small proportion of cases, however, the sequences show signs of a sudden evolutionary spurt. For example, some DNA sequences that have been highly conserved in most mammalian species are found to have changed exceptionally fast during the last six million years of human evolution. Such *human accelerated regions* are thought to reflect functions that have been especially important in making us the unique animal that we are.

One study identified about 50 such sites—one-quarter of which were located near genes associated with brain development. The sequence

Figure 9–34 Genes are sparsely distributed in the human genome.

Compared to these other eukaryotic genomes, the human genome is less gene-dense. Shown here are DNA segments about 50,000 nucleotide pairs in length from yeast, *Drosophila*, and human. The human segment contains only 4 genes, compared to 26 in the yeast and 11 in the fly. Exons are shown in orange, introns in yellow, repetitive elements in blue, and “spacer” DNA in gray. The genes of yeast and flies are generally more compact, with fewer introns, than the genes of humans.



Figure 9–35 DNA sequences that have changed rapidly in the past six million years may account for the differences between chimps and humans. Many of these changes may have affected the way human brains develop. Shown here is anthropologist Jane Goodall with one of her chimpanzee subjects. (Courtesy of the Jane Goodall Institute of Canada.)

COUNTING GENES

How many genes does it take to make a human? It seems a natural thing to wonder. If 6000 genes can produce a yeast and 13,000 a fly, how many are needed to make a human being—a creature curious and clever enough to study its own genome? Until researchers completed the first draft of the human genome sequence, the most frequently cited estimate was 100,000. But where did that figure come from? And how was the revised estimate of only 21,000 protein-coding genes derived?

Walter Gilbert, a physicist-turned-biologist who won a Nobel Prize for developing techniques for sequencing DNA, was one of the first to throw out a ballpark estimate of the number of human genes. In the mid-1980s, Gilbert suggested that humans could have 100,000 genes, an estimate based on the average size of the few human genes known at the time (about 3×10^4 nucleotide pairs) and the size of our genome (about 3×10^9 nucleotide pairs). This back-of-the-envelope calculation yielded a number with such a pleasing roundness that it wound up being quoted widely in articles and textbooks.

The calculation provides an estimate of the number of genes a human could have in principle, but it does not address the question of how many genes we actually have. As it turns out, that question is not so easy to answer, even with the complete human genome sequence in hand. The problem is, how does one identify a gene? Consider protein-coding genes, which comprise only 1.5% of the human genome. Looking at a given piece of raw DNA sequence—an apparently random string of As, Ts, Gs, and Cs—how can one tell which parts represent protein-coding segments? Being able to accurately

and reliably distinguish the rare coding sequences from the more plentiful noncoding sequences in a genome is necessary before one can hope to locate and count its genes.

Signals and chunks

As always, the situation is simplest in bacteria and simple eukaryotes such as yeasts. In these genomes, genes that encode proteins are identified by searching through the entire DNA sequence looking for **open reading frames (ORFs)**. These are long sequences—say, 100 codons or more—that lack stop codons. A random sequence of nucleotides will by chance encode a stop codon about once every 20 codons (as there are three stop codons in the set of 64 possible codons—see Figure 7–25). So finding an ORF—a continuous nucleotide sequence that encodes more than 100 amino acids—is the first step in identifying a good candidate for a protein-coding gene. Today, computer programs are used to search for such ORFs, which begin with an initiation codon, usually ATG, and end with a termination codon, TAA, TAG, or TGA (Figure 9–36).

In animals and plants, the process of identifying ORFs is complicated by the presence of large intron sequences, which interrupt the protein-coding portions of genes. As we have seen, these introns are generally much larger than the exons, which might represent only a few percent of the gene. In human DNA, exons sometimes contain as few as 50 codons (150 nucleotide pairs), while introns may exceed 10,000 nucleotide pairs in length. Fifty codons is too short to generate a statistically significant

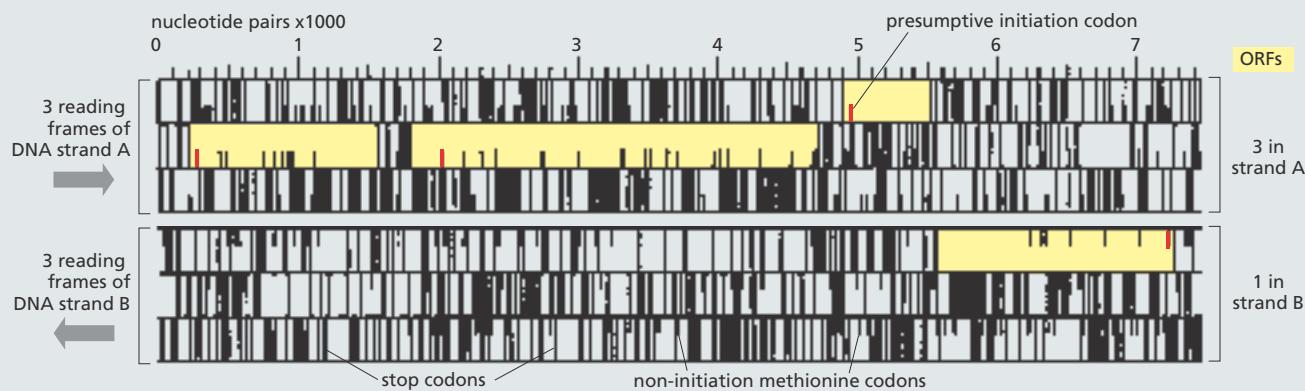


Figure 9–36 Computer programs are used to identify protein-coding genes. In this example, a DNA sequence of 7500 nucleotide pairs from the pathogenic yeast *Candida albicans* was fed into a computer, which then calculated the proteins that could, in theory, be produced from each of its six possible reading frames—three on each of the two strands (see Figure 7–26). The output shows the location of start and stop codons for each reading frame. The reading frames are laid out in horizontal columns. Stop, or termination, codons (TGA, TAA, and TAG) are represented by tall, vertical black lines, and methionine codons (ATG) are represented by shorter black lines. Four open-reading frames, or ORFs (shaded yellow), can be clearly identified by the statistically significant absence of stop codons. For each ORF, the presumptive initiation codon (ATG) is indicated in red. The additional ATG codons in the ORFs code for methionine in the protein.

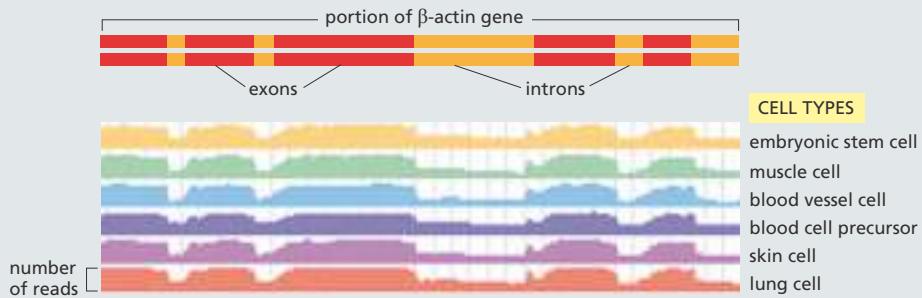


Figure 9–37 RNA sequencing can be used to identify protein-coding genes. Presented here is a set of data corresponding to RNAs produced from a segment of the gene for β -actin, which is depicted schematically at the top. Millions of RNA “sequence reads,” each approximately 200 nucleotides long, were collected from a variety of cell types (right) and matched to DNA sequences within the β -actin gene. The height of each trace is proportional to how often each sequence appears in a read. Exon sequences are present at high levels, reflecting their presence in mature β -actin mRNAs. Intron sequences are present at low levels, most likely reflecting their presence in pre-mRNA molecules that have not yet been spliced or spliced introns that have not yet been degraded.

“ORF signal,” as it is not all that unusual for 50 random codons to lack a stop signal. Moreover, introns are so long that they are likely to contain by chance quite a bit of “ORF noise,” numerous stretches of sequence lacking stop signals. Finding the true ORFs in this sea of information in which the noise often outweighs the signal can be difficult. To make the task more manageable, computers are used to search for other distinctive features that mark the presence of a protein-coding gene. These include the splicing sequences that signal an intron-exon boundary (see Figure 7–19), gene regulatory sequences, or conservation with coding sequences from other organisms.

In 1992, researchers used a computer program to predict protein-coding regions in a preliminary human sequence. They found two genes in a 58,000-nucleotide-pair segment of Chromosome 4, and five genes in a 106,000-nucleotide-pair segment of Chromosome 19. That works out to an average of 1 gene every 23,000 nucleotide pairs. Extrapolating from that density to the whole genome would give humans nearly 130,000 genes. It turned out, however, that the chromosomes the researchers analyzed had been chosen for sequencing precisely because they appeared to be gene-rich. When the estimate was adjusted to take into account the gene-poor regions of the human genome—guessing that half of the human genome had maybe one-tenth of that gene-rich density—the estimated number dropped to 71,000.

Matching RNAs

Of course, these estimates are based on what we think genes look like; to get around this bias, we must employ

more direct, experiment-based methods for locating genes. Because genes are transcribed into RNA, the preferred strategy for finding genes involves isolating all of the RNAs produced by a particular cell type and determining their nucleotide sequence—a technique called RNA Seq. These sequences are then mapped back to the genome to locate their genes. For protein-coding genes, exon segments are more highly represented among the sequenced transcripts, as intron sequences tend to be spliced out and destroyed. Because different cell types express different genes, and splice their RNA transcripts differently, a variety of cell types are used in the analysis (Figure 9–37).

RNA Seq also offers a few additional benefits. First, the relative abundance of each sequence can be used to assess how highly its gene is expressed. Furthermore, the approach also locates genes that do not code for proteins, but instead encode functional or regulatory RNAs. Many noncoding RNAs were first identified through RNA Seq.

Human gene countdown

Based on a combination of all of these computational and experimental techniques, current estimates of the number of human genes are now converging around 30,000. It could be many years, however, before we have the final answer to how many genes it takes to make a human. In the end, having an exact count will not be nearly as important as understanding the functions of each gene and how they interact to build the living organism.

exhibiting the most rapid change (18 changes between human and chimp, compared with only two changes between chimp and chicken) was examined further and found to encode a short, non-protein-coding RNA that is produced in the human cerebral cortex at a critical time during brain development. Although the function of this RNA is not yet known, this exciting finding is stimulating further studies that might help shed light on features of the human brain that distinguish us from chimps.

Similar studies have identified genes that may have played a role in even more recent human evolution. In 2010, investigators completed their analysis of the first Neanderthal genome. Our closest evolutionary relative, Neanderthals lived side by side with the ancestors of modern humans in Europe and Western Asia. By comparing the Neanderthal genome sequence—obtained from DNA that was extracted from a fossilized bone fragment found in a cave in Croatia—with those of five people from different parts of the world, the researchers identified a handful of genomic regions that have undergone a sudden spurt of changes in modern humans. These regions include genes involved in metabolism, brain development, and the shape of the skeleton, particularly the rib cage and head—all features thought to differ between modern humans and our extinct cousins.

Remarkably, these studies also revealed that some modern humans—those that hail from Europe and Asia—share from 1 to 4 percent of their genomes with Neanderthals. This genetic overlap suggests that our ancestors may have mated with Neanderthals—before outcompeting or actively exterminating them—on the way out of Africa, a relationship that left a permanent mark in the human genome.

Genome Variation Contributes to Our Individuality—But How?

With the possible exception of some identical twins, no two people have exactly the same genome sequence. When the same region of the genome from two different humans is compared, the nucleotide sequences typically differ by about 0.1%. That might seem an insignificant degree of variation, but considering the size of the human genome, it amounts to some 3 million genetic differences per genome between one person and the next. Detailed analyses of human genetic variation suggest that the bulk of this variation was already present early in our evolution, perhaps 100,000 years ago, when the human population was still small. This means that a great deal of the genetic variation in present-day humans was inherited from our early human ancestors.

Most of the genetic variation in the human genome takes the form of single base changes called **single-nucleotide polymorphisms (SNPs)**, pronounced snips). These polymorphisms are simply points in the genome that differ in nucleotide sequence between one portion of the population and another—positions where more than 1% of the population has a G-C nucleotide pair, for example, while another has an A-T (**Figure 9–38**). Two human genomes chosen at random from the world's population will differ by approximately 2.5×10^6 SNPs that are scattered throughout the genome.

Another important source of variation inherited from our ancestors involves the duplication and deletion of large segments of DNA. When the genome of any person is compared with a standard reference genome, one observes roughly 100 instances in which a relatively long stretch of DNA has been gained or lost. Some of these **copy-number variations (CNVs)** are very common, whereas others are present in only a small minority of people. From an initial sampling, nearly half of

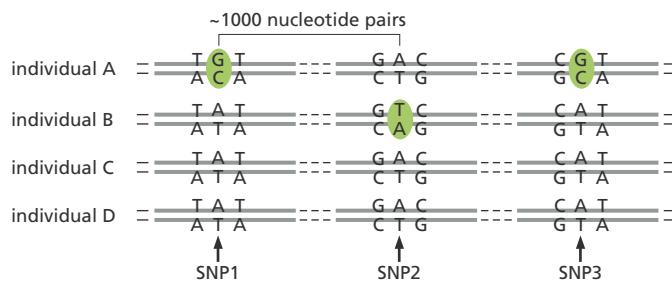


Figure 9–38 Single-nucleotide polymorphisms (SNPs) are points in the genome that differ by a single nucleotide pair between one portion of the population and another. By convention, to count as a polymorphism, a genetic difference must be present in at least 1% of the total population of the species. Most, but not all, SNPs in the human genome occur in regions where they do not affect the function of a gene. As indicated, when comparing any two humans one finds, on average, about one SNP per every 1000 nucleotide pairs.

these segments contain known genes and can affect one's susceptibility to certain diseases. In retrospect, this type of structural variation is not surprising, given the extensive history of DNA addition and DNA loss in vertebrate genomes discussed earlier. Exactly how it contributes to our individuality, however, remains to be determined.

In addition to the SNPs and the CNVs that we inherited from our ancestors, humans also possess repetitive nucleotide sequences that are particularly prone to new mutations. CA repeats, for example, are ubiquitous in the human genome. Nucleotide sequences containing large numbers of CA repeats are often replicated inaccurately (imagine trying to copy a word that is nothing more than a string of CACACACAC...); hence, the precise length of such repeats can vary widely between individuals and can increase from one generation to the next. Because they show such exceptional variability, and because this variability has arisen so recently in human history, CA repeats, and others like them, make ideal markers for distinguishing the DNA of individual humans. For this reason, differences in the numbers of *short tandem repeats* at different positions in the genome are used to identify individuals by *DNA fingerprinting* in crime investigations, paternity suits, and other forensic applications (see Figure 10–18).

Most of the variations in the human genome sequence are genetically silent, as they fall within noncritical regions of the genome. Such variations have no effect on how we look or how our cells function. This means that only a small subset of the variation we observe in our DNA is responsible for the heritable differences from one human to the next. It remains a major challenge to identify those genetic variations that are functionally important—a problem we return to in Chapter 19.

Differences in Gene Regulation May Help Explain How Animals With Similar Genomes Can Be So Different

The finding that humans, chimps, and mice contain essentially the same protein-coding genes has raised a fundamental question: What makes these creatures so different from one another?

To a large extent, the instructions needed to produce a multicellular animal from a fertilized egg are provided by the regulatory DNA associated with each gene. These noncoding DNA sequences contain, scattered within them, dozens of separate regulatory elements, including short DNA segments that serve as binding sites for specific transcription regulators (discussed in Chapter 8). Regulatory DNA ultimately dictates each organism's developmental program—the rules its cells follow as they proliferate, assess their positions in the embryo, and specialize by switching on and off specific genes at the right time and place. The evolution of species is likely to have more to do with innovations in gene regulatory sequences than in the proteins or functional RNAs those genes encode.

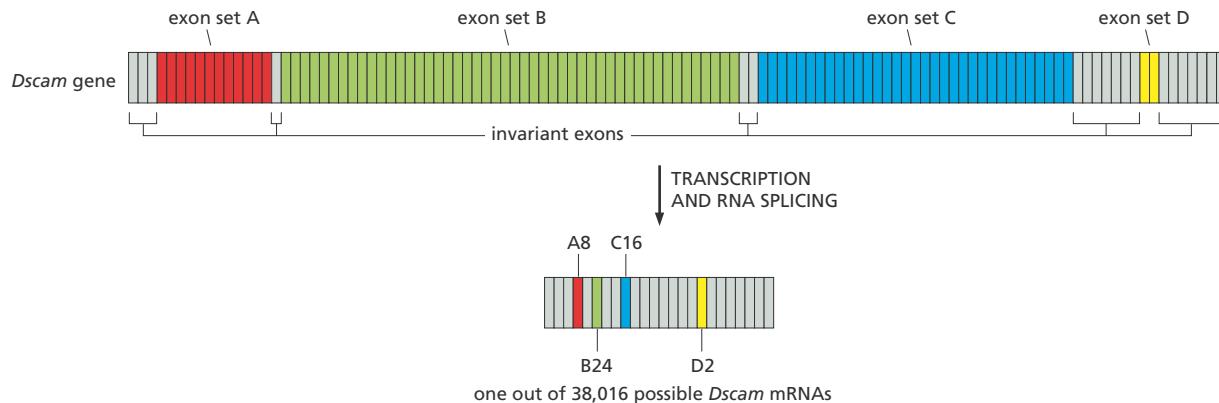


Figure 9–39 Alternative splicing of RNA transcripts can produce many distinct proteins. The *Drosophila* *Dscam* proteins are receptors that help nerve cells make their appropriate connections. The final mRNA transcript contains 24 exons, four of which (denoted A, B, C, and D) are present in the *Dscam* gene as arrays of alternative exons. Each mature mRNA contains 1 of 12 alternatives for exon A (red), 1 of 48 alternatives for exon B (green), 1 of 33 alternatives for exon C (blue), 1 of 2 alternatives for exon D (yellow), and all of the 19 invariant exons (gray). If all possible splicing combinations were used, 38,016 different proteins could in principle be produced from the *Dscam* gene. Only one of the many possible splicing patterns and the mature mRNA it produces is shown. (Adapted from D.L. Black, *Cell* 103:367–370, 2000. With permission from Elsevier.)

Although we have made great strides in recognizing many of these regulatory sequences amidst the excess of noncritical “spacer” DNA, we still do not know how to “read” these sequences so that we can predict exactly how they operate in cells to control development. For example, the same short stretch of regulatory DNA may be recognized by several different transcription regulators, so simply knowing its nucleotide sequence will not reveal which transcription regulator—or regulators—might bind to the sequence in a particular cell at a particular time or place. In addition, gene expression is controlled by complex combinations of proteins (see Figure 8–12), which further complicates our attempts to decipher when in development and in which type of cell any given gene will be expressed.

Even if we could predict when a particular protein-coding gene would be expressed, we would not necessarily be able to predict what protein that gene would produce. Recent studies suggest that more than 90% of human genes undergo alternative RNA splicing, which allows cells to produce a range of related but distinct proteins from a single gene (see Figure 7–22). RNA splicing is often regulated, so that one form of a protein is produced in one type of cell, while other forms are produced preferentially in other cell types. In one extreme example, from *Drosophila*, a single gene can produce thousands of different protein variants through alternative RNA splicing (Figure 9–39). Thus an organism can produce far more proteins than it has genes. We do not yet know enough about alternative splicing to predict exactly which human genes are subject to this process—and when, where, and how during development such regulation occurs. Nonetheless, it seems likely that these differences in alternative RNA splicing could help explain how animals with very similar protein-coding genes develop so differently.

Another part of the explanation may involve regulatory RNAs, such as the microRNAs and long noncoding RNAs discussed in Chapter 8. Thus for example, microRNAs have diverse roles in controlling gene expression, especially during development. They regulate as many as one-third of all human genes, for example, yet few of them have been studied in any detail—and new ones are still being found. And even less is known about the long noncoding RNAs.

The information that guides the countless decisions made by developing cells as they divide and specialize is all contained within the genome sequence of an organism. But we are only just beginning to learn the grammar and rules by which this genetic information orchestrates development. Deciphering this code—which has been shaped by evolution and refined by individual variation—is one of the great challenges facing the next generation of cell biologists.

ESSENTIAL CONCEPTS

- By comparing the DNA and protein sequences of contemporary organisms, we are beginning to reconstruct how genomes have evolved in the billions of years that have elapsed since the appearance of the first cells.
- Genetic variation—the raw material for evolutionary change—arises through a variety of mechanisms that alter the nucleotide sequence of genomes. These changes in sequence range from simple point mutations to larger-scale deletions, duplications, and rearrangements.
- Genetic changes that give an organism a selective advantage are the most likely to be perpetuated. Changes that compromise an organism's fitness or ability to reproduce are eliminated through natural selection.
- Gene duplication is one of the most important sources of genetic diversity. Once duplicated, the two genes can accumulate different mutations and thereby diversify to perform different roles.
- Repeated rounds of gene duplication and divergence during evolution have produced many large gene families.
- The evolution of new proteins is thought to have been greatly facilitated by the swapping of exons between genes to create hybrid proteins with new functions.
- The human genome contains 3.2×10^9 nucleotide pairs distributed among 23 pairs of chromosomes—22 autosomes and a pair of sex chromosomes. Less than a tenth of this DNA is transcribed to produce protein-coding or otherwise functional RNAs.
- Individual humans differ from one another by an average of 1 nucleotide pair in every 1000; this and other genetic variation underlies most of our individuality and provides the basis for identifying individuals by DNA analysis.
- Nearly half of the human genome consists of mobile genetic elements that can move from one site to another within a genome. Two classes of these elements have multiplied to especially high copy numbers.
- Viruses are genes packaged in protective coats that can move from cell to cell and organism to organism, but they require host cells to reproduce themselves.
- Some viruses have RNA instead of DNA as their genetic material. Retroviruses copy their RNA genomes into DNA before integrating into the host-cell genome.
- Comparing genome sequences of different species provides a powerful way to identify conserved, functionally important DNA sequences.
- Related species, such as human and mouse, have many genes in common; evolutionary changes in the regulatory DNA sequences that affect how these genes are expressed are especially important in determining the differences between species.

KEY TERMS

<i>Alu sequence</i>	<i>germ line</i>	<i>purifying selection</i>
<i>conserved synteny</i>	<i>homologous gene</i>	<i>retrotransposon</i>
<i>copy-number variation</i>	<i>horizontal gene transfer</i>	<i>retrovirus</i>
<i>divergence</i>	<i>L1 element</i>	<i>reverse transcriptase</i>
<i>exon shuffling</i>	<i>mobile genetic element</i>	<i>single-nucleotide polymorphism (SNP)</i>
<i>gene duplication and divergence</i>	<i>open reading frame (ORF)</i>	<i>somatic cell</i>
<i>gene family</i>	<i>phylogenetic tree</i>	<i>transposon</i>
<i>germ cell</i>	<i>point mutation</i>	<i>virus</i>

QUESTIONS

QUESTION 9–7

Discuss the following statement: "Mobile genetic elements are parasites. They are harmful to the host organism and therefore place it at an evolutionary disadvantage."

QUESTION 9–8

Human Chromosome 22 (48×10^6 nucleotide pairs in length) has about 700 protein-coding genes, which average 19,000 nucleotide pairs in length and contain an average of 5.4 exons, each of which averages 266 nucleotide pairs. What fraction of the average protein-coding gene is converted into mRNA? What fraction of the chromosome do these genes occupy?

QUESTION 9–9

(True/False) The majority of human DNA is unimportant junk. Explain your answer.

QUESTION 9–10

Mobile genetic elements make up nearly half of the human genome and are inserted more or less randomly throughout it. However, in some spots these elements are rare, as illustrated for a cluster of genes called HoxD, which lies on Chromosome 2 (Figure Q9–10). This cluster is about 100 kb in length and contains nine genes whose differential expression along the length of the developing embryo helps establish the basic body plan for humans (and for other animals). Why do you suppose that mobile genetic elements are so rare in this cluster? In Figure Q9–10, lines that project upward indicate exons of known genes. Lines that project downward indicate mobile genetic elements; they are so numerous they merge into nearly a solid block outside the HoxD cluster. For comparison, an equivalent region of Chromosome 22 is shown.

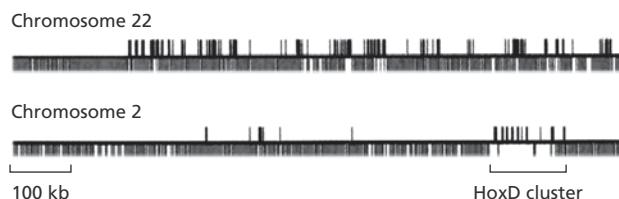


Figure Q9–10

QUESTION 9–11

An early graphical method for comparing nucleotide sequences—the so-called diagon plot—still yields one

of the best visual comparisons of sequence relatedness. An example is illustrated in Figure Q9–11, in which the human β -globin gene is compared with the human cDNA for β globin (which contains only the coding portion of the gene; Figure Q9–11A) and to the mouse β -globin gene (Figure Q9–11B). Diagon plots are generated by comparing blocks of sequence, in this case blocks of 11 nucleotides at a time. If 9 or more of the nucleotides match, a dot is placed on the diagram at the coordinates corresponding to the blocks being compared. A comparison of all possible blocks generates diagrams such as the ones shown in Figure Q9–11, in which sequence similarities show up as diagonal lines.

- From the comparison of the human β -globin gene with the human β -globin cDNA (Figure Q9–11A), can you deduce the positions of exons and introns in the β -globin gene?
- Are the exons of the human β -globin gene (indicated by shading in Figure Q9–11B) similar to those of the mouse β -globin gene? Identify and explain any key differences.
- Is there any sequence similarity between the human and mouse β -globin genes that lies outside the exons? If so, identify its location and offer an explanation for its preservation during evolution.
- Did the mouse or human gene undergo a change of intron length during their evolutionary divergence? How can you tell?

QUESTION 9–12

Your advisor, a brilliant bioinformatician, has high regard for your intellect and industry. She suggests that you write a computer program that will identify the exons of protein-coding genes directly from the sequence of the human genome. In preparation for that task, you decide to write down a list of the features that might distinguish protein-coding sequences from intronic DNA and from other sequences in the genome. What features would you list? (You may wish to review basic aspects of gene expression in Chapter 7.)

QUESTION 9–13

You are interested in finding out the function of a particular gene in the mouse genome. You have determined the nucleotide sequence of the gene, defined the portion that

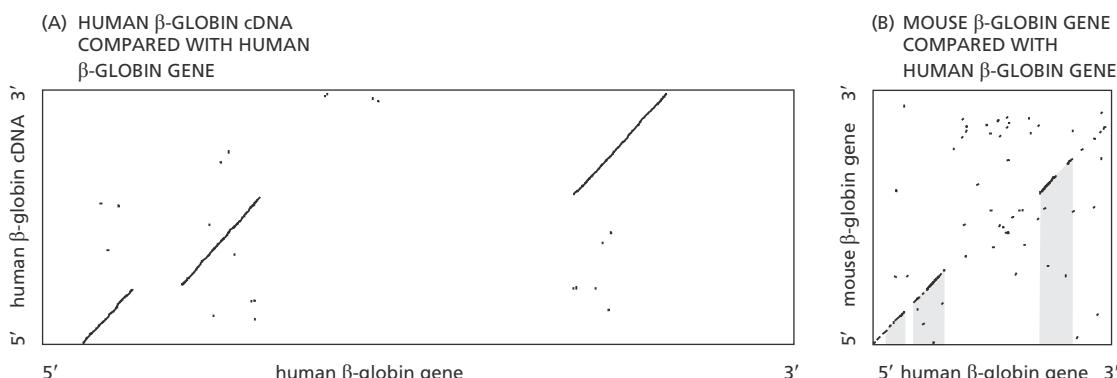


Figure Q9–11

codes for its protein product, and searched the relevant database for similar sequences; however, neither the gene nor the encoded protein resembles anything previously described. What types of additional information about the gene and the encoded protein would you like to know in order to narrow down its function, and why? Focus on the information you would want, rather than on the techniques you might use to get that information.

QUESTION 9–14

Why do you expect to encounter a stop codon about every 20 codons or so in a random sequence of DNA?

QUESTION 9–15

The genetic code (see Figure 7–25) relates the nucleotide sequence of mRNA to the amino acid sequence of encoded proteins. Ever since the code was deciphered, some have claimed it must be a frozen accident—that is, the system randomly fell into place in some ancestral organism and was then perpetuated unchanged throughout evolution; others have argued that the code has been shaped by natural selection.

A striking feature of the genetic code is its inherent resistance to the effects of mutation. For example, a change in the third position of a codon often specifies the same amino acid or one with similar chemical properties. But is the natural code more resistant to mutation than other possible versions? The answer is an emphatic “Yes,” as illustrated in **Figure Q9–15**. Only one in a million computer-generated “random” codes is more error-resistant than the natural genetic code.

Does the resistance to mutation of the actual genetic code argue in favor of its origin as a frozen accident or as a result of natural selection? Explain your reasoning.

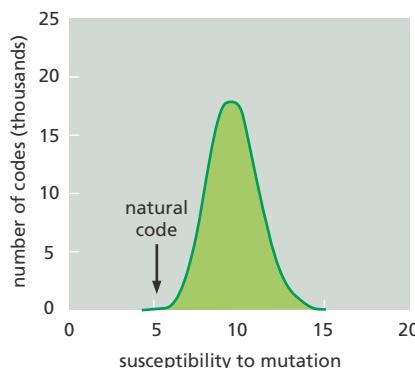


Figure Q9–15

QUESTION 9–16

Which of the processes listed below contribute significantly to the evolution of new protein-coding genes?

- Duplication of genes to create extra copies that can acquire new functions.
- Formation of new genes *de novo* from noncoding DNA in the genome.
- Horizontal transfer of DNA between cells of different species.
- Mutation of existing genes to create new functions.
- Shuffling of protein domains by gene rearrangement.

QUESTION 9–17

Some genes evolve more rapidly than others. But how can this be demonstrated? One approach is to compare several genes from the same two species, as shown for rat and human in the table above. Two measures of rates of nucleotide substitution are indicated in the table. Nonsynonymous changes refer to single-nucleotide changes in the DNA sequence that alter the encoded amino acid ($ATC \rightarrow TTC$, which gives isoleucine \rightarrow phenylalanine, for example). Synonymous changes refer to those that do not alter the encoded amino acid ($ATC \rightarrow ATT$, which gives isoleucine \rightarrow isoleucine, for example). (As is apparent in the genetic code, Figure 7–25, there are many cases where several codons correspond to the same amino acid.)

Gene	Amino Acids	Rates of Change	
		Nonsynonymous	Synonymous
Histone H3	135	0.0	4.5
Hemoglobin α	141	0.6	4.4
Interferon γ	136	3.1	5.5

Rates were determined by comparing rat and human sequences and are expressed as nucleotide changes per site per 10^9 years. The average rate of nonsynonymous changes for several dozen rat and human genes is about 0.8.

- Why are there such large differences between the synonymous and nonsynonymous rates of nucleotide substitution?
- Considering that the rates of synonymous changes are about the same for all three genes, how is it possible for the histone H3 gene to resist so effectively those nucleotide changes that alter its amino acid sequence?
- In principle, a protein might be highly conserved because its gene exists in a “privileged” site in the genome that is subject to very low mutation rates. What feature of the data in the table argues against this possibility for the histone H3 protein?

QUESTION 9–18

Plant hemoglobins were found initially in legumes, where they function in root nodules to lower the oxygen concentration, allowing the resident bacteria to fix nitrogen. These hemoglobins impart a characteristic pink color to the root nodules. The discovery of hemoglobin in plants was initially surprising because scientists regarded hemoglobin as a distinctive feature of animal blood. It was hypothesized that the plant hemoglobin gene was acquired by horizontal transfer from an animal. Many more hemoglobin genes have now been sequenced from a variety of organisms, and a phylogenetic tree of hemoglobins is shown in **Figure Q9–18**.

- Does the evidence in the tree support or refute the hypothesis that the plant hemoglobins arose by horizontal gene transfer?
- Supposing that the plant hemoglobin genes were originally derived by horizontal transfer (from a parasitic nematode, for example), what would you expect the phylogenetic tree to look like?

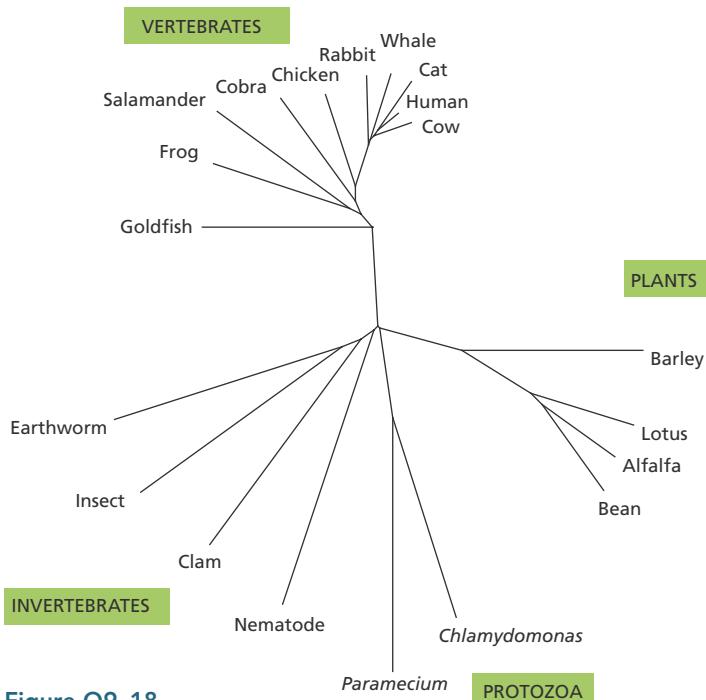


Figure Q9-18

QUESTION 9-19

The accuracy of DNA replication in the human germ-cell line is such that on average only about 0.6 out of the 6 billion nucleotides is altered at each cell division. Because most of our DNA is not subject to any precise constraint on its sequence, most of these changes are selectively neutral. Any two modern humans picked at random will show about 1 difference of nucleotide sequence in every 1000 nucleotides. Suppose we are all descended from a single pair of ancestors—Adam and Eve—who were genetically identical and homozygous (each chromosome was identical to its homolog). Assuming that all germ-line mutations that arise are preserved in descendants, how many cell generations must have elapsed since the days of Adam and Eve for 1 difference per 1000 nucleotides to have accumulated in modern humans? Assuming that each human generation corresponds on average to 200 cell-division cycles in the germ-cell lineage and allowing 30 years per human generation, how many years ago would this ancestral couple have lived?

QUESTION 9-20

Reverse transcriptases do not proofread as they synthesize DNA using an RNA template. What do you think the consequences of this are for the treatment of AIDS?



CHAPTER TEN

10

Modern Recombinant DNA Technology

Since the turn of the century, biologists have amassed an unprecedented wealth of information on the genes that direct the development and behavior of living things. Thanks to advances in our ability to rapidly determine the nucleotide sequence of entire genomes, we now have access to the complete molecular blueprints for thousands of different organisms, from the platypus to the plague bacterium, and for thousands of different people from all over the world.

This information explosion would not have been possible without the technological revolution that enabled us to manipulate DNA molecules. In the early 1970s, it became possible, for the first time, to isolate a selected piece of DNA from the many millions of nucleotide pairs in a typical chromosome—and to replicate, sequence, and modify this DNA. These modified DNA molecules can then be introduced into another organism's genome, where they become a functional and heritable part of that organism's genetic instructions.

These technical breakthroughs—dubbed **recombinant DNA technology**, or *genetic engineering*—have had a dramatic impact on all aspects of cell biology. They have advanced our understanding of the organization and evolutionary history of complex eukaryotic genomes (as discussed in Chapter 9) and have led to the discovery of whole new classes of genes, RNAs, and proteins. They continue to generate new ways of determining the functions of genes and proteins in living organisms, and they provide an important set of tools for unraveling the mechanisms—still poorly understood—by which a complex organism can develop from a single fertilized egg.

Recombinant DNA technology has also had a profound influence on our understanding and treatment of disease: it is used, for example, to detect

MANIPULATING AND
ANALYZING DNA MOLECULES

DNA CLONING IN BACTERIA

DNA CLONING BY PCR

EXPLORING AND EXPLOITING
GENE FUNCTION

QUESTION 10–1

DNA sequencing of your own two β -globin genes (one from each of your two Chromosome 11s) reveals a mutation in one of the genes. Given this information alone, should you worry about being a carrier of an inherited disease that could be passed on to your children? What other information would you like to have to assess your risk?

the mutations in human genes that are responsible for inherited disorders or that predispose us to a variety of common diseases, including cancer; it is used to produce an increasing number of pharmaceuticals, such as insulin for diabetics and blood-clotting proteins for hemophiliacs. But recombinant DNA technology also has applications outside the clinic. It allows, for example, forensic science to identify or acquit suspects in a crime. Even our laundry detergents contain heat-stable, stain-removing proteases, courtesy of DNA technology. Of all the discoveries described in this book, those that led to the development of recombinant DNA technology have the greatest impact on our everyday lives.

In this chapter, we present a brief overview of how we learned to manipulate DNA, identify genes, and produce many copies of any given nucleotide sequence in the laboratory. We discuss several approaches to exploring gene function, including new ways to monitor gene expression and to inactivate or modify genes in cells, animals, and plants. These methods—which are continuously being improved and made ever-more powerful—are not only revolutionizing the way we do science, they are transforming our understanding of cell biology and human disease. Indeed, they are responsible for a substantial portion of the information we present in this book.

MANIPULATING AND ANALYZING DNA MOLECULES

Humans have been experimenting with DNA, albeit without realizing it, for millennia. The roses in our gardens, the corn on our plate, and the dogs in our yards are all the product of selective breeding that has taken place over many, many generations (Figure 10–1). But it wasn't until the development of recombinant DNA techniques in the 1970s that we could begin to engineer organisms with desired properties by directly tinkering with their genes.

Isolating and manipulating individual genes is not a trivial matter. Unlike a protein, a gene does not exist as a discrete entity in cells; it is a small part of a much larger DNA molecule. Even bacterial genomes, which are much less complex than the chromosomes of eukaryotes, are enormously long. The *E. coli* genome, for example, contains 4.6 million nucleotide pairs.

How, then, can a single gene be separated from a eukaryotic genome—which is considerably larger—so that it can be handled in the laboratory? The solution to this problem emerged, in large part, with the discovery of a class of bacterial enzymes known as *restriction nucleases*. These

Figure 10–1 By breeding plants and animals, humans have been unwittingly experimenting with DNA for millennia.

(A) The oldest known depiction of a rose in Western art, from the palace of Knossos in Crete, around 2000 BC. Modern roses are the result of centuries of breeding between such wild roses. (B) Dogs have been bred to exhibit a wide variety of characteristics, including different head shapes, coat colors, and of course size. All dogs, regardless of breed, belong to a single species that was domesticated from the gray wolf some 10,000 to 15,000 years ago. (B, from A.L. Shearin & E.A. Ostrander, *PLoS Biol.* 8:e1000310, 2010.)



enzymes cut double-stranded DNA at particular sequences. They can therefore be used to produce a reproducible set of specific DNA fragments from any genome. In this section, we describe how these enzymes work and how the DNA fragments they produce can be separated and visualized. We then discuss how these fragments can be probed to identify the ones that contain the DNA sequence of interest.

Restriction Nucleases Cut DNA Molecules at Specific Sites

Like many of the tools of recombinant DNA technology, restriction nucleases were discovered by researchers trying to understand an intriguing biological phenomenon. It had been observed that certain bacteria always degraded “foreign” DNA that was introduced into them experimentally. A search for the mechanism responsible revealed a novel class of bacterial nucleases that cleave DNA at specific nucleotide sequences. The bacteria’s own DNA is protected from cleavage by chemical modification of these specific sequences. Because these enzymes function to restrict the transfer of DNA between strains of bacteria, they were called **restriction nucleases**. The pursuit of this seemingly arcane biological puzzle set off the development of technologies that have forever changed the way cell and molecular biologists study living things.

Different bacterial species produce different restriction nucleases, each cutting at a different, specific nucleotide sequence (Figure 10–2). Because these target sequences are short—generally four to eight nucleotide pairs—many sites of cleavage will occur, purely by chance, in any long DNA molecule. The reason restriction nucleases are so useful in the laboratory is that each enzyme will cut a particular DNA molecule, at the same sites. Thus for a given sample of DNA, a particular restriction nuclease will reliably generate the same set of DNA fragments.

The size of the resulting fragments depends on the target sequences of the restriction nucleases. As shown in Figure 10–2, the enzyme HaeIII cuts at a sequence of four nucleotide pairs; a sequence this long would be expected to occur purely by chance approximately once every 256 nucleotide pairs (1 in 4^4). In comparison, a restriction nuclease with a target sequence that is eight nucleotides long would be expected to cleave DNA on average once every 65,536 nucleotide pairs (1 in 4^8). This difference in sequence selectivity makes it possible to cleave a long DNA molecule into the fragment sizes that are most suitable for a given application.

Gel Electrophoresis Separates DNA Fragments of Different Sizes

After a large DNA molecule is cleaved into smaller pieces with a restriction nuclease, the DNA fragments can be separated from one another on

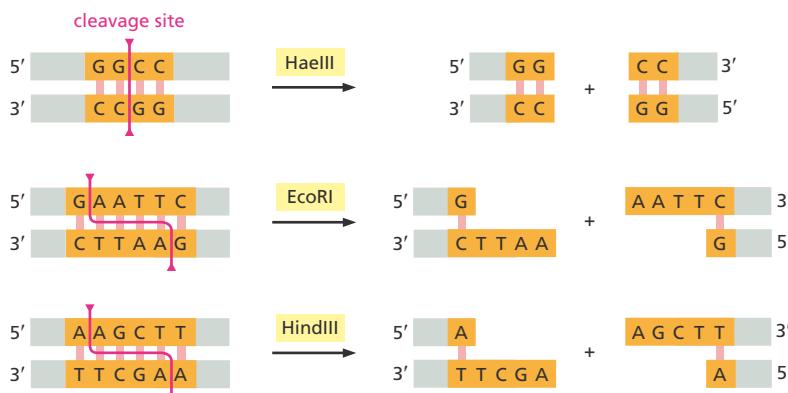
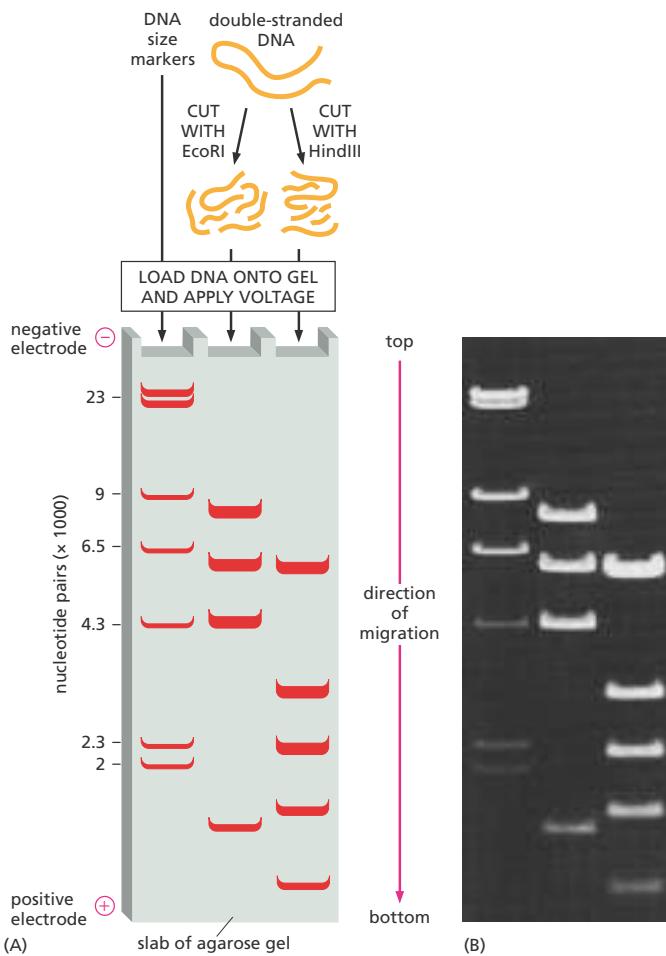


Figure 10–2 Restriction nucleases cleave DNA at specific nucleotide sequences.

Target sequences are often palindromic (that is, the nucleotide sequence is symmetrical around a central point). Here, both strands of the DNA double helix are cut at specific points within the target sequence (orange). Some enzymes, such as HaeIII, cut straight across the double helix and leave two blunt-ended DNA molecules; with others, such as EcoRI and HindIII, the cuts on each strand are staggered. These staggered cuts generate “sticky ends”—short, single-stranded overhangs that help the cut DNA molecules join back together through complementary base-pairing. This rejoining of DNA molecules becomes important for DNA cloning, as we discuss later. Restriction nucleases are usually obtained from bacteria, and their names reflect their origins: for example, the enzyme EcoRI comes from *Escherichia coli*.

Figure 10–3 DNA molecules can be separated by size using gel electrophoresis. (A) Schematic illustration compares the results of cutting the same DNA molecule (in this case, the genome of a virus that infects parasitic wasps) with two different restriction nucleases, EcoRI (middle) and HindIII (right). The fragments are then separated by gel electrophoresis. Because larger fragments migrate more slowly than smaller ones, the lowermost bands on the gel contain the smallest DNA fragments. The sizes of the fragments can be estimated by comparing them to a set of DNA fragments of known sizes (left). (B) Photograph of an actual gel shows the positions of DNA bands that have been labeled with a fluorescent dye. (B, from U. Albrecht et al., *J. Gen. Virol.* 75:3353–3363, 1994.)



the basis of their length by gel electrophoresis—the same method used to separate mixtures of proteins (see Panel 4–5, p. 167). A mixture of DNA fragments is loaded at one end of a slab of agarose or polyacrylamide gel, which contains a microscopic network of pores. When a voltage is applied across the gel, the negatively charged DNA fragments migrate toward the positive electrode; larger fragments will migrate more slowly because their progress is impeded to a greater extent by the gel matrix. Over several hours, the DNA fragments become spread out across the gel according to size, forming a ladder of discrete bands, each composed of a collection of DNA molecules of identical length (Figure 10–3). To isolate a desired DNA fragment, the small section of the gel that contains the band is excised with a scalpel or a razor blade, and the DNA is then extracted.

QUESTION 10–2

Which products result when the double-stranded DNA molecule below is digested with (A) EcoRI, (B) HaeIII, (C) HindIII, or (D) all three of these enzymes together? (See Figure 10–2 for the target sequences of these enzymes.)

5'-AAGAATTGCGGAATTGGGCCCTAACGCGCCGCGTCGAGGCCTTAAA-3'
3'-TTCTTAACGCCCTAACGCCCGAATTGCGGGCGAGCTCCGGAATTT-5'

Bands of DNA in a Gel Can Be Visualized Using Fluorescent Dyes or Radioisotopes

The separated DNA bands on an agarose or polyacrylamide gel are not, by themselves, visible. To see these bands, the DNA must be labeled or stained in some way. One sensitive method involves exposing the gel to a dye that fluoresces under ultraviolet (UV) light when it is bound to DNA. When the gel is placed on a UV light box, the individual bands glow bright orange—or bright white when the gel is photographed in black and white (see Figure 10–3B).

An even more sensitive detection method involves incorporating a radioisotope into the DNA molecules before they are separated by electrophoresis; ^{32}P is often used, as it can be incorporated into the phosphates of DNA. Because the β particles emitted from ^{32}P can activate the radiation-sensitive particles in photographic film, a sheet of film placed flat on top of the agarose gel will, when developed, show the position of all the DNA bands.

Exposing a gel to a fluorescent dye that binds to DNA—or starting with DNA that has been pre-labeled with ^{32}P —will allow every band on the gel to be seen. But it does not reveal which of those bands contains a DNA sequence of interest. To do that, a probe is designed to bind specifically to the desired nucleotide sequence by complementary base-pairing, as we see next.

Hybridization Provides a Sensitive Way to Detect Specific Nucleotide Sequences

Under normal conditions, the two strands of a DNA double helix are held together by hydrogen bonds between the complementary base pairs (see Figure 5–6). But these relatively weak, noncovalent bonds can be fairly easily broken. Such *DNA denaturation* will release the two strands from each other, but does not break the covalent bonds that link together the nucleotides within each strand. Perhaps the simplest way to achieve this separation involves heating the DNA to around 90°C. When the conditions are reversed—by slowly lowering the temperature—the complementary strands will readily come back together to re-form a double helix. This **hybridization**, or *DNA renaturation*, is driven by the re-formation of the hydrogen bonds between complementary base pairs (Figure 10–4).

This fundamental capacity of a single-stranded nucleic acid molecule, either DNA or RNA, to form a double helix with a single-stranded molecule of a complementary sequence provides a very powerful and sensitive technique for detecting specific nucleotide sequences in both DNA and RNA. Today, one simply designs a short, single-stranded *DNA probe* that is complementary to the nucleotide sequence of interest. Because the nucleotide sequences of so many genomes are known—and are stored in publicly accessible databases—designing such a probe is straightforward. The desired probe can then be synthesized in the laboratory—usually by a

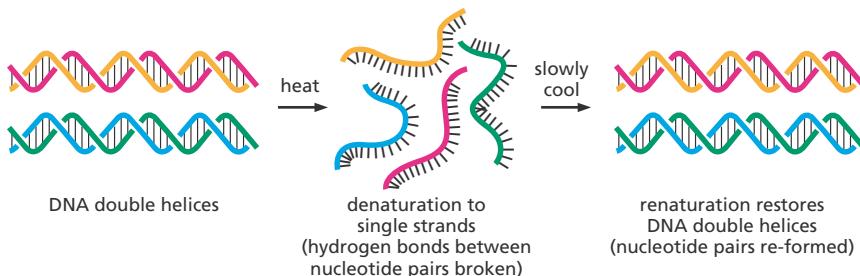


Figure 10–4 A molecule of DNA can undergo denaturation and renaturation (**hybridization**). For two single-stranded molecules to hybridize, they must have complementary nucleotide sequences that allow base-pairing. In this example, the red and orange strands are complementary to each other, and the blue and green strands are complementary to each other. Although denaturation by heating is shown, DNA can also be renatured after being denatured by alkali treatment. The 1961 discovery that single strands of DNA could readily re-form a double helix in this way was a big surprise to scientists.

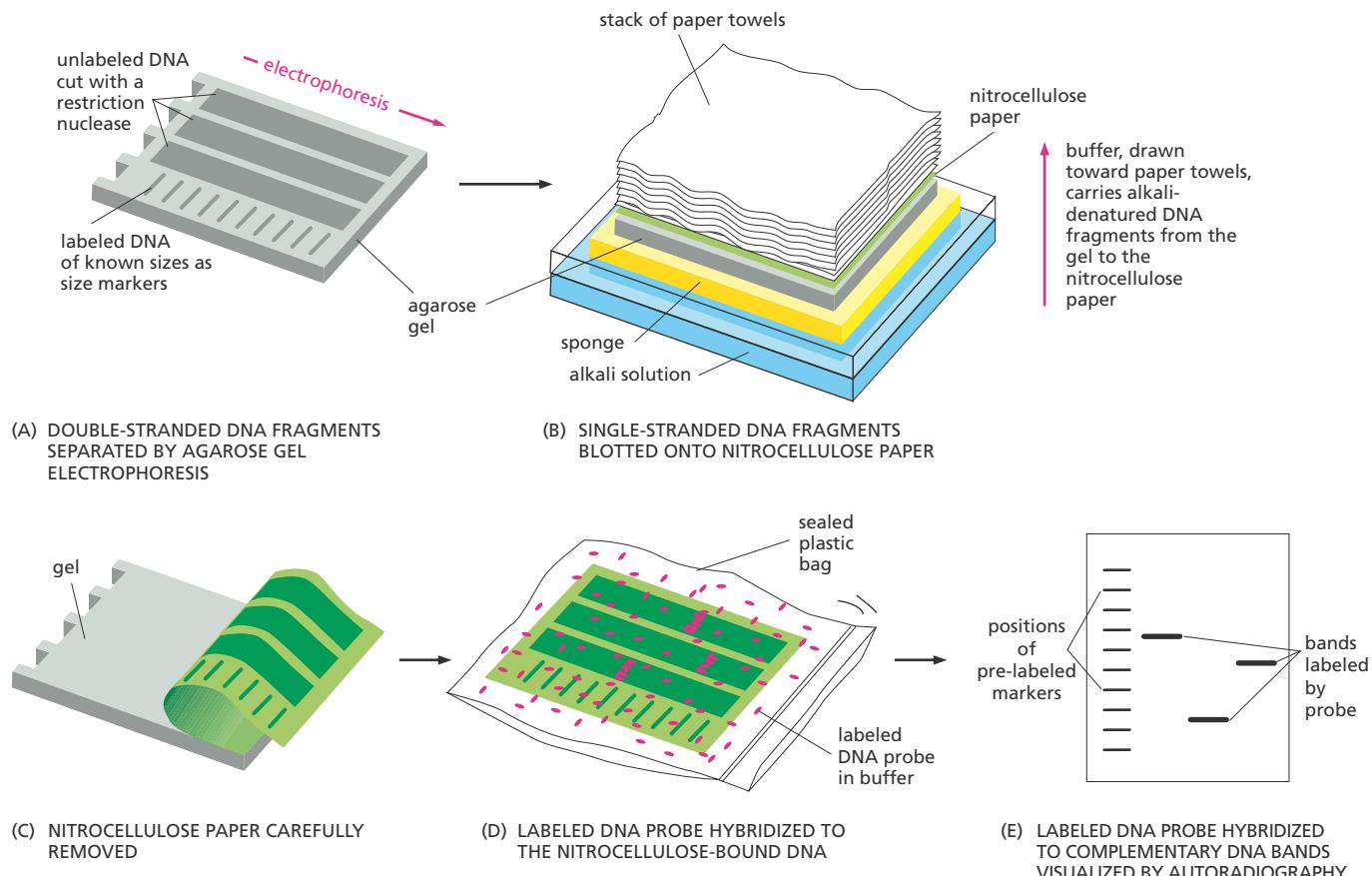


Figure 10-5 Gel-transfer hybridization, or Southern blotting, is used to detect specific DNA fragments. (A) The mixture of double-stranded DNA fragments generated by restriction nuclease treatment of DNA is separated according to length by gel electrophoresis. (B) A sheet of nitrocellulose paper is laid over the gel, and the separated DNA fragments are denatured with alkali and transferred to the sheet by blotting. In this process, a stack of absorbent paper towels is used to suck buffer up through the gel, transferring the single-stranded DNA fragments from the gel to the nitrocellulose paper. (C) The nitrocellulose sheet is carefully peeled off the gel. (D) The sheet containing the bound single-stranded DNA fragments is exposed to a radioactive, single-stranded DNA probe specific for the DNA sequence of interest under conditions that favor hybridization. (E) The sheet is washed thoroughly, so that only probe molecules that have hybridized to the DNA on the paper remain attached. After autoradiography, the DNA that has hybridized to the labeled probe will show up as a band on the autoradiograph. An adaptation of this technique, used to detect specific RNA sequences, is called Northern blotting. In this case, RNA molecules are electrophoresed through the gel, and the probe is usually a single-stranded DNA molecule. The same procedures can be carried out with non-radioactive probes using an appropriate method of detection.

commercial organization or a centralized academic facility. Such probes carry a fluorescent or radioactive label to facilitate detection of the nucleotide sequence to which they bind.

Once a suitable probe has been obtained, it can be used in a variety of situations to search for nucleic acids with a complementary sequence—for example, finding a sequence of interest among DNA fragments that have been separated on an agarose gel. In this case, the fragments are first transferred to a special sheet of paper, which is then exposed to the labeled probe. This common technique, called *Southern blotting*, was named after the scientist who invented it (**Figure 10–5**).

DNA probes are widely used in cell biology. Later in the chapter, we discuss how they can be used to determine in which tissues and at what stages of development a gene is transcribed. But first, we consider how hybridization facilitates the process of DNA cloning.

DNA CLONING IN BACTERIA

The term **DNA cloning** refers to the production of many identical copies of a DNA sequence. It is this amplification that makes it possible to separate a defined segment of DNA—often a gene of interest—from the rest of a cell’s genome. DNA cloning is one of the most important feats of recombinant DNA technology, as it is the starting point for understanding the function of any stretch of DNA within the genome.

In this section, we describe the classical approach to DNA cloning, in which one copies all of the DNA from a cell or tissue and then finds and isolates the specific DNA of interest. Later, we discuss how the development of the *polymerase chain reaction* (PCR) has facilitated a more direct

approach to cloning, allowing one to copy, in a test tube, only the DNA fragment of interest.

DNA Cloning Begins with Genome Fragmentation and Production of Recombinant DNAs

Whole genomes, even small ones, are too large and unwieldy to be handled easily in the laboratory. Thus the first step in cloning any gene is to break the genome into smaller, more manageable pieces. These fragments can then be joined together, or recombined, to produce the DNA molecules that will be amplified. Our ability to generate such **recombinant DNA molecules** is made possible by the use of molecular tools that are provided by cells themselves.

As we discussed earlier, bacterial restriction nucleases can be used to cut long DNA molecules into conveniently sized fragments (see Figure 10–2). These fragments can then be joined to one another—or to any piece of DNA—using **DNA ligase**, an enzyme that reseals the nicks that arise in the DNA backbone during DNA replication and DNA repair in cells (see Figure 6–18). DNA ligase allows investigators to join together any two pieces of DNA in a test tube, producing recombinant DNA molecules that are not found in nature (Figure 10–6).

The production of recombinant DNA molecules in this way is a key step in the classical approach to DNA cloning. It allows the DNA fragments generated by treatment with a restriction nuclease to be inserted into another, special DNA molecule that serves as a carrier, or *vector*, which can be copied—and thereby amplified—inside a cell, as we discuss next.

Recombinant DNA Can Be Inserted Into Plasmid Vectors

The vectors typically used for gene cloning are relatively small, circular DNA molecules called **plasmids**. (Figure 10–7). Each plasmid contains a replication origin, which enables it to replicate in a bacterial cell independently of the bacterial chromosome. It also has cleavage sites for common restriction nucleases, so that the plasmid can be conveniently opened and a foreign DNA fragment inserted.

The plasmids used for cloning are basically streamlined versions of plasmids that occur naturally in many bacteria. Bacterial plasmids were first recognized by physicians and scientists because they often carry

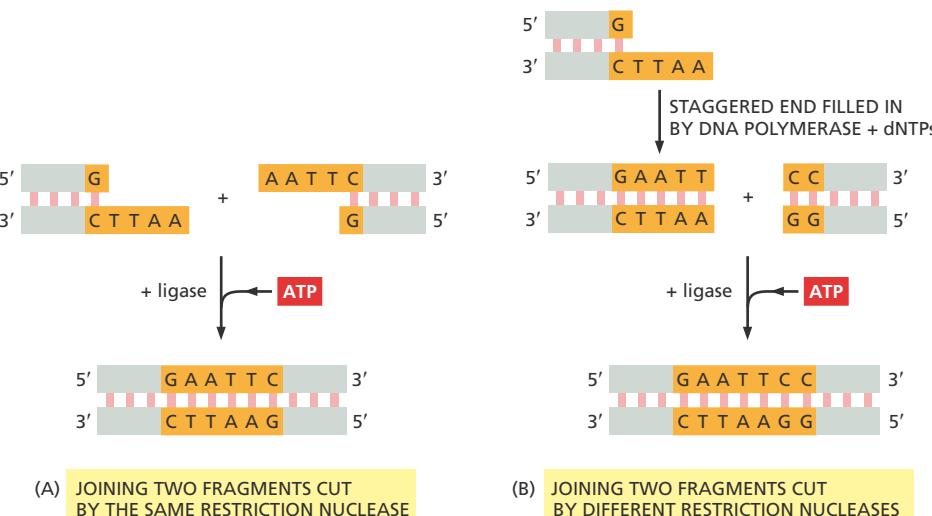


Figure 10–6 DNA ligase can join together any two DNA fragments *in vitro* to produce recombinant DNA molecules. ATP provides the energy necessary for the ligase to reseal the sugar-phosphate backbone of DNA. (A) DNA ligase can readily join two DNA fragments produced by the same restriction nuclease, in this case EcoRI. Note that the staggered ends produced by this enzyme enable the ends of the two fragments to base-pair correctly with each other, greatly facilitating their rejoicing. (B) DNA ligase can also be used to join DNA fragments produced by different restriction nucleases—for example, EcoRI and HaeIII. In this case, before the fragments undergo ligation, DNA polymerase plus a mixture of deoxyribonucleoside triphosphates (dNTPs) are used to fill in the staggered cut produced by EcoRI. Each DNA fragment shown in the figure is oriented so that its 5' ends are the left end of the upper strand and the right end of the lower strand, as indicated.

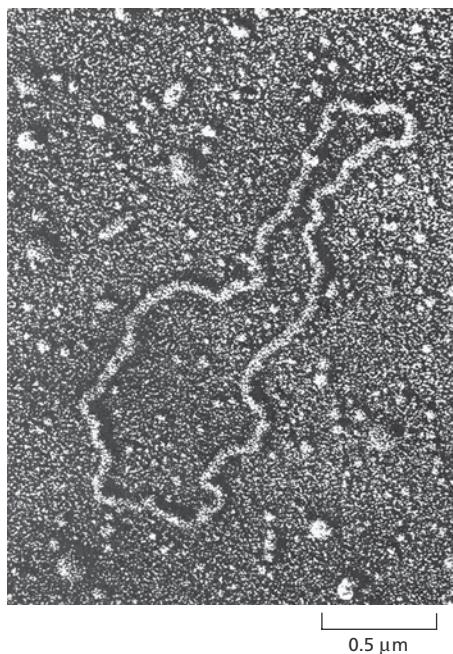


Figure 10–7 Bacterial plasmids are commonly used as cloning vectors. This circular, double-stranded DNA molecule was the first plasmid for DNA cloning; it contains about nine thousand nucleotide pairs. The staining procedure used to make the DNA visible in this electron micrograph causes the DNA to appear much thicker than it actually is. (Courtesy of Stanley N. Cohen, Stanford University.)

genes that render their microbial host resistant to one or more antibiotics. Indeed, historically potent antibiotics—penicillin, for example—are no longer effective against many of today's bacterial infections because plasmids that confer resistance to the antibiotic have spread among bacterial species by horizontal gene transfer (see Figure 9–14).

To insert a piece of DNA into a plasmid vector, the purified plasmid DNA is opened up by a restriction nuclease that cleaves it at a single site, and the DNA fragment to be cloned is then spliced into that site using DNA ligase (Figure 10–8). This recombinant DNA molecule is now ready to be introduced into a bacterium, where it will be copied and amplified, as we see next.

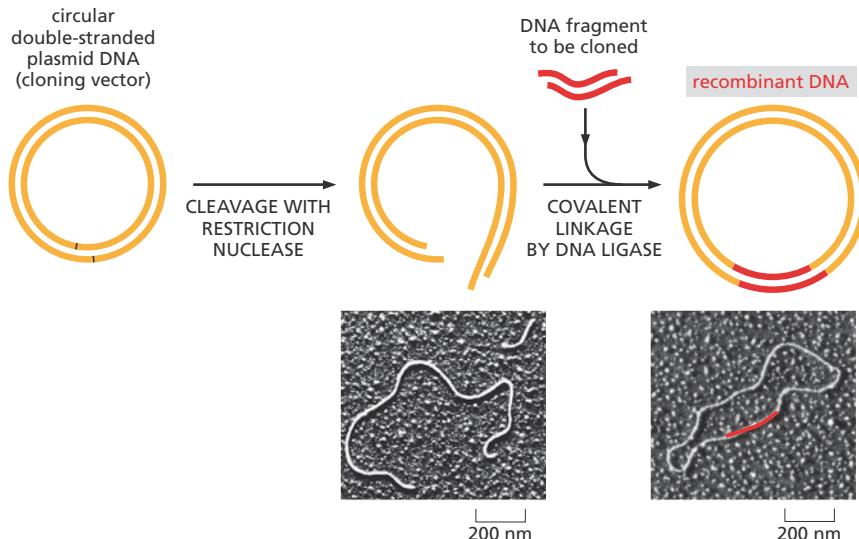
Recombinant DNA Can Be Copied Inside Bacterial Cells

To introduce recombinant DNA into a bacterial cell, investigators take advantage of the fact that some bacteria naturally take up DNA molecules present in their surroundings. The mechanism that controls this uptake is called **transformation**, because early observations suggested it could “transform” one bacterial strain into another. Indeed, the first proof that genes are made of DNA came from an experiment in which DNA purified from a pathogenic strain of pneumococcus was used to transform a harmless bacterium into a deadly one (see How We Know, pp. 174–176).

In a natural bacterial population, a source of DNA for transformation is provided by bacteria that have died and released their contents, including DNA, into the environment. In a test tube, however, bacteria such as *E. coli* can be coaxed to take up recombinant DNA that has been created in the laboratory. These bacteria are then suspended in a nutrient-rich broth and allowed to proliferate.

Each time the bacterial population doubles—every 30 minutes or so—the number of copies of the recombinant DNA molecule also doubles. Thus, in 24 hours, the engineered cells will produce hundreds of millions of copies of the plasmid, along with the DNA fragment it contains. The bacteria can then be split open (lysed) and the plasmid DNA purified from

Figure 10–8 A DNA fragment is inserted into a bacterial plasmid by using the enzyme DNA ligase. The plasmid is first cut open at a single site with a restriction nuclease (in this case, one that produces staggered ends). It is then mixed with the DNA fragment to be cloned, which has been cut with the same restriction nuclease. DNA ligase and ATP are also added to the mix. The staggered ends base-pair, and the nicks in the DNA backbone are sealed by the DNA ligase to produce a complete recombinant DNA molecule. In the accompanying micrographs, we have colored the DNA fragment red to make it easier to see. (Micrographs courtesy of Huntington Potter and David Dressler.)



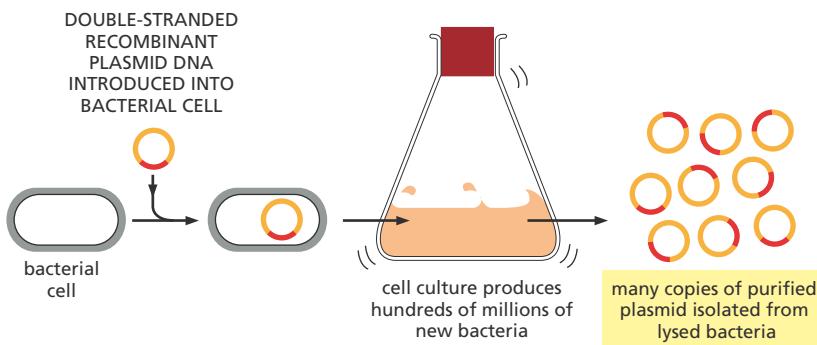


Figure 10–9 A DNA fragment can be replicated inside a bacterial cell. To clone a particular fragment of DNA, it is first inserted into a plasmid vector, as shown in Figure 10–8. The resulting recombinant plasmid DNA is then introduced into a bacterium, where it is replicated many millions of times as the bacterium multiplies. For simplicity, the genome of the bacterial cell is not shown.

the rest of the cell contents, including the large bacterial chromosome (**Figure 10–9**).

The DNA fragment can be readily recovered by cutting it out of the plasmid DNA with the same restriction nuclease that was used to insert it, and then separating it from the plasmid DNA by gel electrophoresis (see Figure 10–3). Together, these steps allow the amplification and purification of any segment of DNA from the genome of any organism.

Genes Can Be Isolated from a DNA Library

Thus far, we have described the amplification of a single DNA fragment. In reality, when a genome is cut by a restriction nuclease, millions of different DNA fragments are generated. How can the single fragment that contains the DNA of interest be isolated from this collection? The solution involves introducing all of the fragments into bacteria and then selecting those bacterial cells that have amplified the desired DNA molecule.

The entire collection of DNA fragments can be ligated into plasmid vectors, using conditions that favor the insertion of a single DNA fragment into each plasmid molecule. These recombinant plasmids are then introduced into *E. coli* at a concentration that ensures that no more than one plasmid molecule is taken up by each bacterium. The collection of cloned DNA fragments in this bacterial culture is known as a **DNA library**. Because the DNA fragments were derived directly from the chromosomal DNA of the organism of interest, the resulting collection—called a **genomic library**—should represent the entire genome of that organism (**Figure 10–10**).

To find a particular gene within this library, one can use a labeled DNA probe designed to bind specifically to part of the gene's DNA sequence. Using such a probe, the rare bacterial clones in the DNA library that contain the gene—or a portion of it—can be identified by hybridization (**Figure 10–11**).

But before a gene has been cloned, how can one design a probe to detect it? In the early days of cloning, investigators wishing to study a protein-coding gene would first determine at least part of the protein's amino acid sequence. By applying the genetic code in reverse, they could use this amino acid sequence to deduce the corresponding gene sequence, which allowed them to generate an appropriate DNA probe.

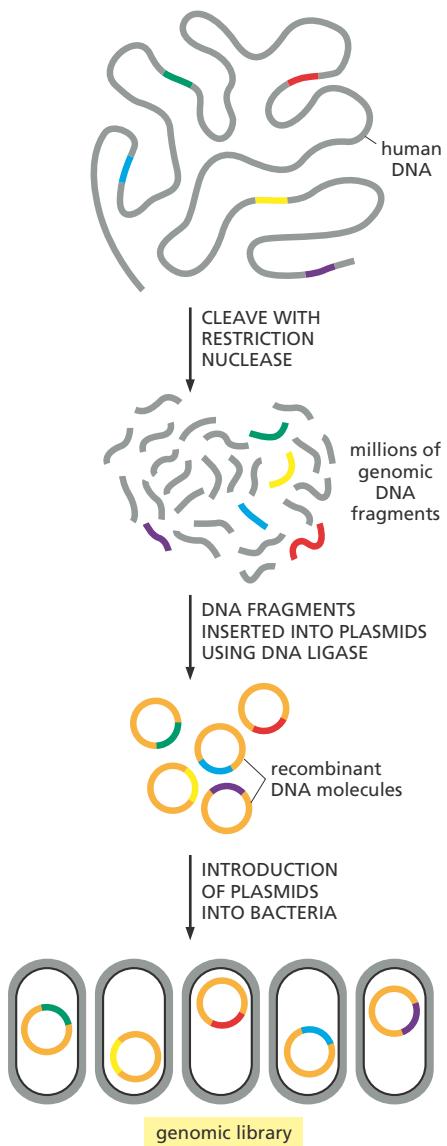


Figure 10–10 Human genomic libraries containing DNA fragments representing the whole human genome can be constructed using restriction nucleases and DNA ligase. Such a genomic library consists of a set of bacteria, each carrying a different small fragment of human DNA. For simplicity, only the colored DNA fragments are shown in the library; in reality, all of the different gray fragments will also be represented.

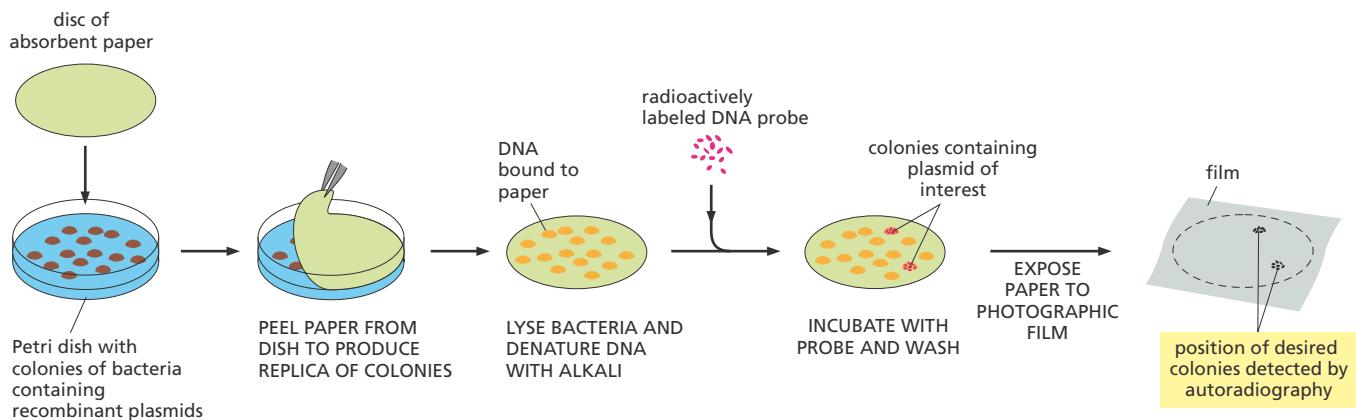


Figure 10–11 A bacterial colony carrying a particular DNA clone can be identified by hybridization. A replica of the arrangement of the bacterial colonies (clones) on the Petri dish is made by pressing a piece of absorbent paper against the surface of the dish. This replica is treated with alkali (to lyse the cells and dissociate the plasmid DNA into single strands), and the paper is then hybridized to a highly radioactive DNA probe. Those bacterial colonies that have bound the probe are identified by autoradiography. Living bacterial cells containing the plasmid can then be isolated from the original Petri dish.

Many genes were originally identified and cloned using variations on this basic approach. Now that the complete genome sequences of many organisms, including humans, are known, however, cloning genes is very much easier, faster, and cheaper. The sequence of any gene in an organism can be looked up in an electronic database, making it a simple matter to design a probe that can be synthesized to order. As we discuss shortly, gene cloning today is typically done directly on the original DNA sample, bypassing the use of a DNA library entirely.

cDNA Libraries Represent the mRNAs Produced by Particular Cells

For many applications—for example, when attempting to clone a protein-coding gene, it is advantageous to obtain the gene in a form that contains only the coding sequence; that is, a form that lacks the intron DNA. For some genes, the complete genomic clone—including introns and exons—is too large and unwieldy to handle conveniently in the laboratory (see, for example, Figure 7–18B). What's more, the bacterial or yeast cells typically used to amplify cloned DNA are unable to remove introns from mammalian RNA transcripts. So if the goal is to use a cloned mammalian gene to produce a large amount of the protein it encodes, it is essential to use only the coding sequence of the gene. Fortunately, it is relatively simple to isolate a gene free of all its introns, by using a different type of DNA library, called a **cDNA library**.

A cDNA library is similar to a genomic library in that it also contains numerous clones containing many different DNA sequences. But it differs in one important respect. The DNA that goes into a cDNA library is not genomic DNA; it is DNA copied from the mRNAs present in a particular type of cell. To prepare a **cDNA** library, all of the mRNAs are extracted, and double-stranded DNA copies of these mRNAs are produced by the enzymes *reverse transcriptase* and DNA polymerase (Figure 10–12). These **complementary DNA**—or **cDNA**—molecules are then introduced into bacteria and amplified, as described for genomic DNA fragments (see Figure 10–10). The gene of interest—in this case, without its introns—can then be isolated by using a probe that hybridizes to the DNA sequence (see Figure 10–11). We discuss later how such cDNAs can be used to produce purified proteins on a commercial scale.

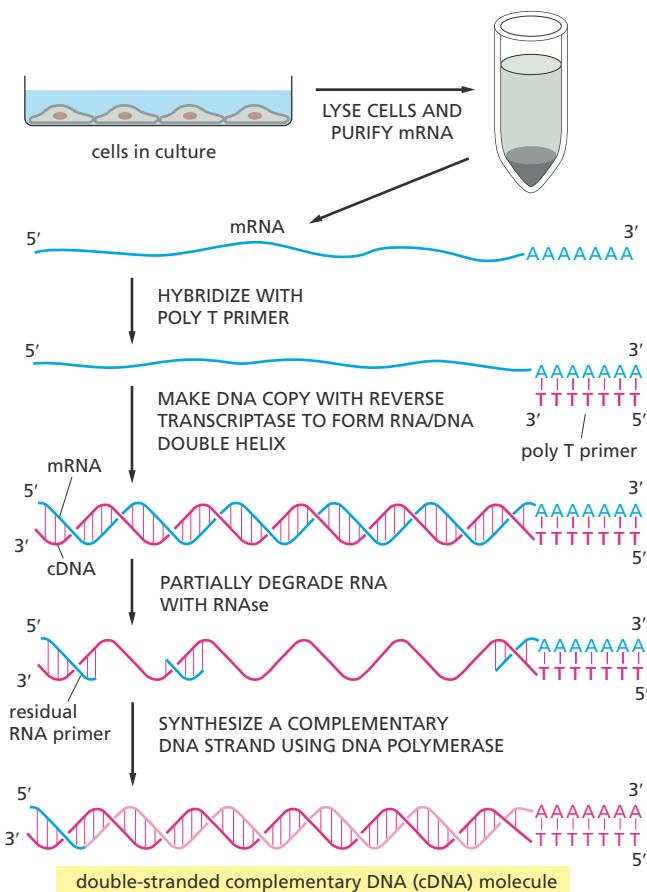


Figure 10–12 Complementary DNA (cDNA) is prepared from mRNA. Total mRNA is extracted from a selected type of cell, and double-stranded, complementary DNA (cDNA) is produced using reverse transcriptase (see Figure 9–30) and DNA polymerase. For simplicity, the copying of just one of these mRNAs into cDNA is illustrated here. Note that an RNA fragment that remains hybridized to the first cDNA strand after partial RNase digestion serves as the primer needed for DNA polymerase to begin synthesis of the complementary DNA strand.

There are several important differences between genomic DNA clones and cDNA clones, as illustrated in **Figure 10–13**. Genomic clones represent a random sample of all of the DNA sequences found in an organism's genome and, with very rare exceptions, will contain the same sequences regardless of the cell type from which the DNA came. Also, genomic clones from eukaryotes contain large amounts of noncoding DNA, repetitive DNA sequences, introns, regulatory DNA, and spacer DNA; sequences that code for proteins will make up only a few percent of the library (see Figure 9–33). By contrast, cDNA clones contain predominantly protein-coding sequences, and only those for genes that have been transcribed into mRNA in the cells from which the cDNA was made. As different types of cells produce distinct sets of mRNA molecules, each yields a different cDNA library. Furthermore, patterns of gene expression change during development, so cells at different stages in their development will also yield different cDNA libraries.

As we discuss later, cDNAs are used to assess which genes are expressed in specific cells, at particular times in development, or under a particular set of conditions. In contrast, genomic clones—which include introns and exons, as well as regulatory DNA sequences—provide the starting material for determining the complete nucleotide sequence of an organism's genome.

QUESTION 10–3

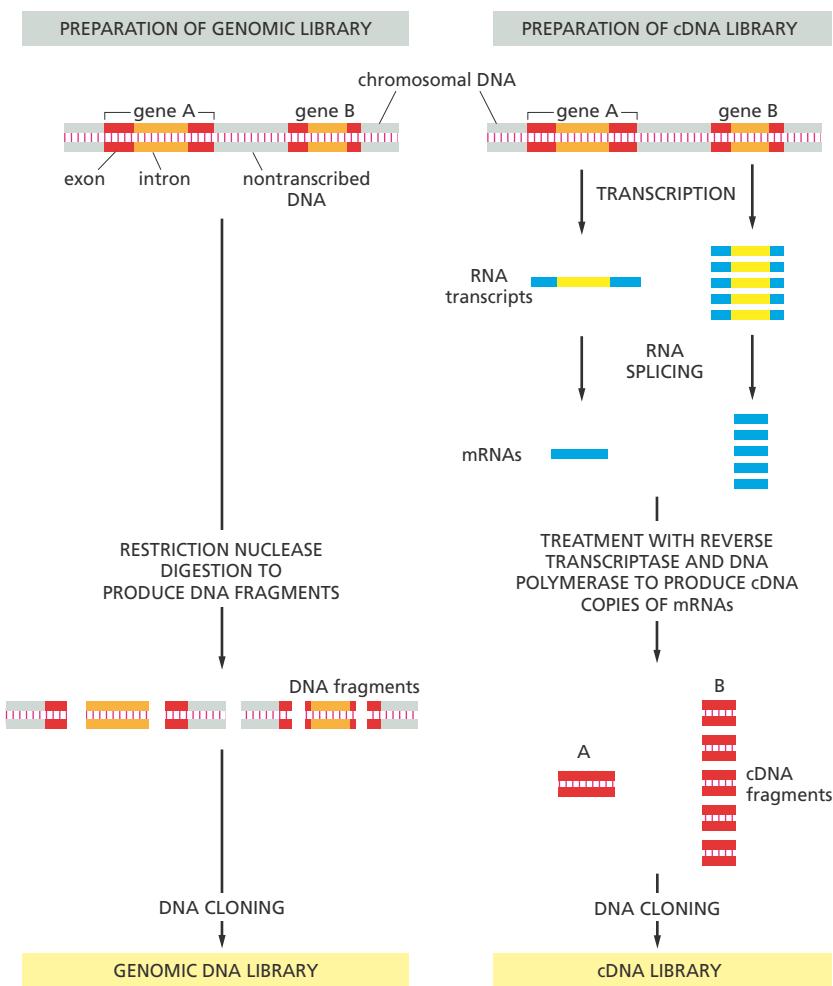
Discuss the following statement:
"From the nucleotide sequence of a cDNA clone, the complete amino acid sequence of a protein can be deduced by applying the genetic code. Thus, protein biochemistry has become superfluous because there is nothing more that can be learned by studying the protein."

DNA CLONING BY PCR

Genomic and cDNA libraries were once the only route to gene cloning, and they are still used for cloning very large genes and for sequencing whole genomes. However, a powerful and versatile method for amplifying DNA, known as the **polymerase chain reaction (PCR)**, provides a

Figure 10–13 Genomic DNA clones and cDNA clones derived from the same region of the genome are different.

In this example, gene A is infrequently transcribed, whereas gene B is frequently transcribed, and both genes contain introns (orange). In the genomic DNA library, both introns and nontranscribed DNA (gray) are included in the clones, and most clones will contain either no coding sequence or only part of the coding sequence of a gene (red); the DNA sequences that regulate the expression of each gene are also included (not indicated). In the cDNA clones, the intron sequences have been removed by RNA splicing during the formation of the mRNA (blue), and a continuous coding sequence is therefore present in each clone. Because gene B is transcribed more frequently than gene A in the cells from which the cDNA library was made, it will be represented much more often than A in the cDNA library. In contrast, genes A and B should be represented equally in the genomic library.



more rapid and straightforward approach to DNA cloning, particularly in organisms whose complete genome sequence is known. Today, most genes are cloned via PCR.

Invented in the 1980s, PCR revolutionized the way that DNA and RNA are analyzed. The technique can amplify any nucleotide sequence rapidly and selectively. Unlike the traditional approach of cloning using vectors—which relies on bacteria to make copies of the desired DNA sequences—PCR is performed entirely in a test tube. Eliminating the need for bacteria makes PCR convenient and incredibly quick—billions of copies of a nucleotide sequence can be generated in a matter of hours. At the same time, PCR is remarkably sensitive: the method can be used to detect the trace amounts of DNA in a drop of blood left at a crime scene or in a few copies of a viral genome in a patient's blood sample. Because of its sensitivity, speed, and ease of use, PCR has many applications in addition to DNA cloning, including forensics and diagnostics.

In this section, we provide a brief overview of how PCR works and how it is used for a range of purposes that require the amplification of specific DNA sequences.

PCR Uses a DNA Polymerase to Amplify Selected DNA Sequences in a Test Tube

The success of PCR depends on the exquisite selectivity of DNA hybridization, along with the ability of DNA polymerase to copy a DNA template

reliably, through repeated rounds of replication *in vitro*. The enzyme works by adding nucleotides to the 3' end of a growing strand of DNA (see Figure 6–11). To initiate the reaction, the polymerase requires a primer—a short nucleotide sequence that provides a 3' end from which synthesis can begin. The beauty of PCR is that the primers that are added to the reaction mixture not only serve as starting points, they also direct the polymerase to the specific DNA sequence to be amplified. These primers, like the DNA probes used to identify specific nucleotide sequences as discussed earlier, are designed by the experimenter based on the DNA sequence of interest and then synthesized chemically. Thus, PCR can only be used to clone a DNA segment for which the sequence is known in advance. With the large and growing number of genome sequences available in public databases, this requirement is rarely a drawback.

Multiple Cycles of Amplification *In Vitro* Generate Billions of Copies of the Desired Nucleotide Sequence

PCR is an iterative process in which the cycle of amplification is repeated dozens of times. At the start of each cycle, the two strands of the double-stranded DNA template are separated and a unique primer is annealed to each. DNA polymerase is then allowed to replicate each strand independently (Figure 10–14). In subsequent cycles, all the newly synthesized DNA molecules produced by the polymerase serve as templates for the next round of replication (Figure 10–15). Through this iterative amplification process, many copies of the original sequence can be made—billions after about 20 to 30 cycles.

PCR is now the method of choice for cloning relatively short DNA fragments (say, under 10,000 nucleotide pairs). Each cycle takes only about five minutes, and automation of the whole procedure enables cell-free cloning of a DNA fragment in a few hours, compared with the several days required for cloning in bacteria. The original template for PCR can be either DNA or RNA, so this method can be used to obtain either a full genomic clone (complete with introns and exons) or a cDNA copy of an mRNA (Figure 10–16). A major benefit of PCR is that genes can be cloned directly from any piece of DNA or RNA without the time and effort needed to first construct a DNA library.

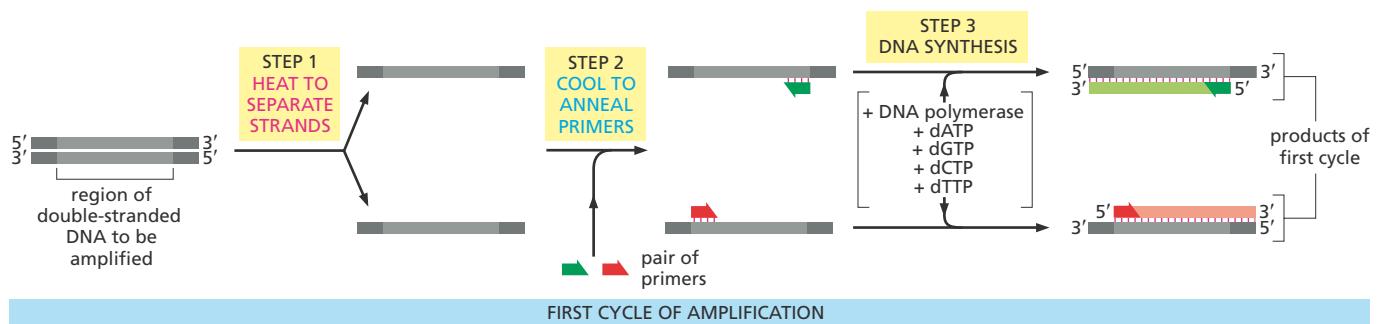


Figure 10–14 A pair of PCR primers directs the amplification of a desired segment of DNA in a test tube. Each cycle of PCR includes three steps: (1) The double-stranded DNA is heated briefly to separate the two strands. (2) The DNA is exposed to a large excess of a pair of specific primers—designed to bracket the region of DNA to be amplified—and the sample is cooled to allow the primers to hybridize to complementary sequences in the two DNA strands. (3) This mixture is incubated with DNA polymerase and the four deoxyribonucleoside triphosphates so that DNA can be synthesized, starting from the two primers. The cycle can then be repeated by reheating the sample to separate the newly synthesized DNA strands (see Figure 10–15).

The technique depends on the use of a special DNA polymerase isolated from a thermophilic bacterium; this polymerase is stable at much higher temperatures than eukaryotic DNA polymerases, so it is not denatured by the heat treatment shown in step 1. The enzyme therefore does not have to be added again after each cycle.

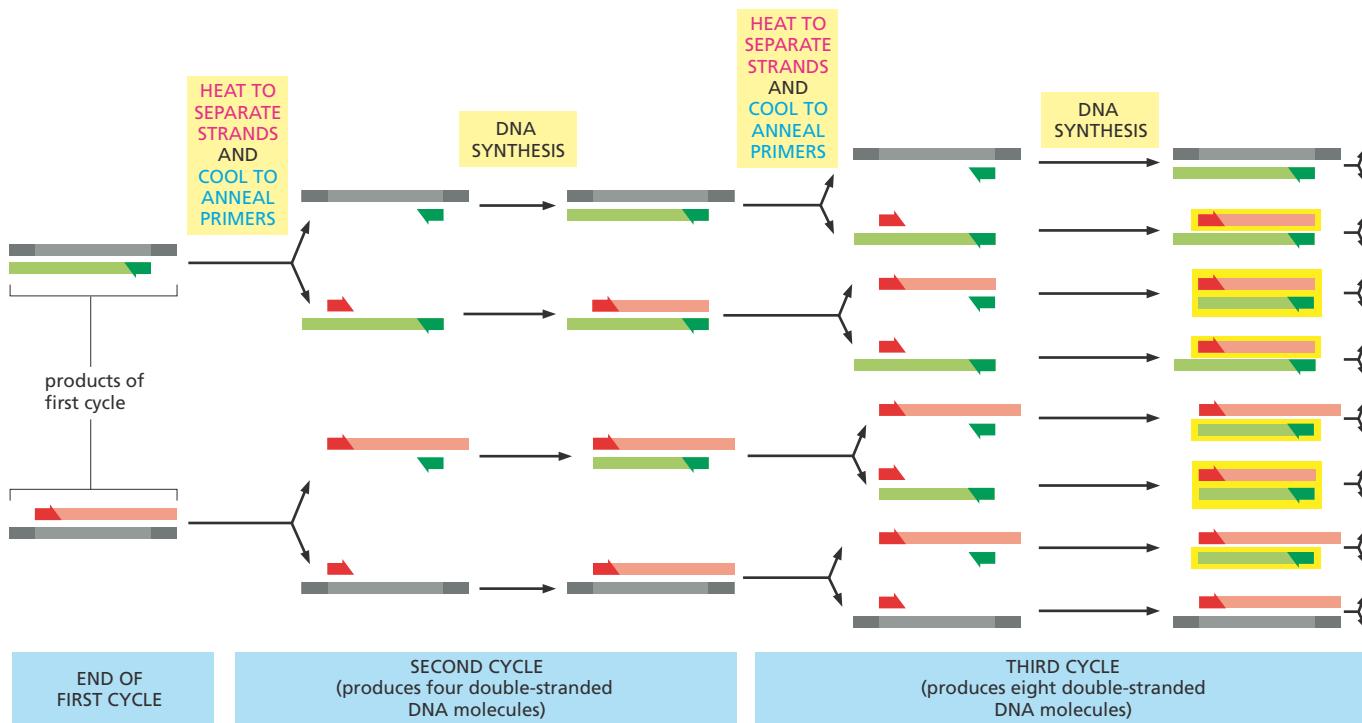


Figure 10–15 PCR uses repeated rounds of strand separation, hybridization, and synthesis to amplify DNA. As the procedure outlined in Figure 10–14 is repeated, all the newly synthesized fragments serve as templates in their turn. Because the polymerase and the primers remain in the sample after the first cycle, PCR involves simply heating and then cooling the same sample, in the same test tube, again and again. Each cycle doubles the amount of DNA synthesized in the previous cycle, so that within a few cycles, the predominant DNA is identical to the sequence bracketed by and including the two primers in the original template. In the example illustrated here, three cycles of reaction produce 16 DNA chains, 8 of which (boxed in yellow) correspond exactly to one or the other strand of the original bracketed sequence. After four more cycles, 240 of the 256 DNA chains will correspond exactly to the original sequence, and after several more cycles, essentially all of the DNA strands will be this length. The whole procedure is shown in [Movie 10.1](#).

PCR is Also Used for Diagnostic and Forensic Applications

In addition to its use in gene cloning, PCR is frequently employed to amplify DNA for other, more practical purposes. Because of its extraordinary sensitivity, PCR can be used to detect invading microorganisms at very early stages of infection. In this case, short sequences complementary to a segment of the infectious agent's genome are used as primers, and following many cycles of amplification, even a few copies of an invading bacterial or viral genome in a patient sample can be detected ([Figure 10–17](#)). For many infections, PCR has replaced the use of antibodies against microbial molecules to detect the presence of pathogens. PCR can also be used to track epidemics, detect bioterrorist attacks, and test food products for the presence of potentially harmful microbes. It is also used to verify the authenticity of a food source—for example, whether a sample of beef actually came from a cow.

Finally, PCR is now widely used in forensic medicine. The method's extreme sensitivity allows forensic investigators to isolate DNA from minute traces of human blood or other tissue to obtain a *DNA fingerprint* of the person who left the sample behind. With the possible exception of identical twins, the genome of each human differs in DNA sequence from that of every other person on Earth. Using primer pairs targeted at genome sequences that are known to be highly variable in the human population, PCR makes it possible to generate a distinctive DNA fingerprint for any individual ([Figure 10–18](#)). Such forensic analyses can be used not only to point the finger at those who have done wrong, but—equally important—to help exonerate those who have been wrongfully convicted.

QUESTION 10–4

- A. If the PCR shown in Figure 10–15 is carried through an additional two rounds of amplification, how many of the DNA fragments labeled in gray, green, or red or outlined in yellow are produced? If many additional cycles are carried out, which fragments will predominate?

- B. Assume you start with one double-stranded DNA molecule and amplify a 500-nucleotide-pair sequence contained within it.

Approximately how many cycles of PCR amplification will you need to produce 100 ng of this DNA? 100 ng is an amount that can be easily detected after staining with a fluorescent dye. (Hint: for this calculation, you need to know that each nucleotide has an average molecular mass of 330 g/mole.)

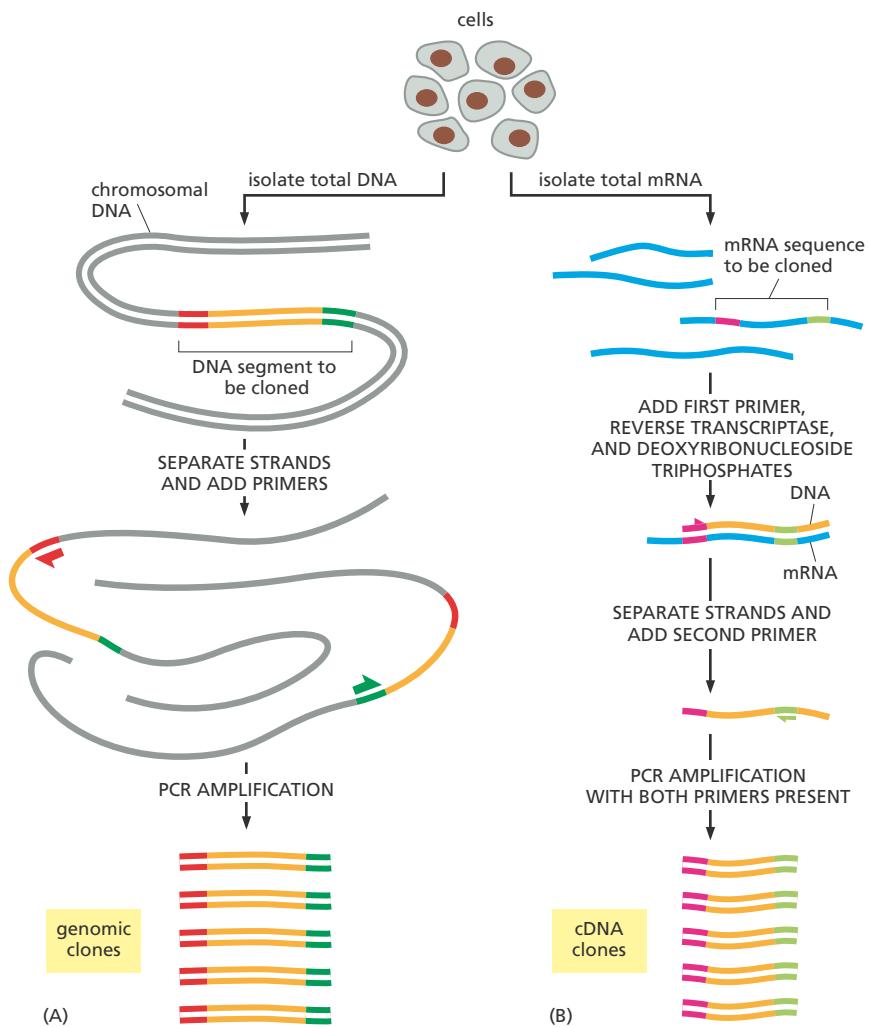


Figure 10–16 PCR can be used to obtain either genomic or cDNA clones. (A) To use PCR to clone a segment of chromosomal DNA, total DNA is first purified from cells. PCR primers that flank the stretch of DNA to be cloned are added, and many cycles of PCR are completed (see Figure 10–15). Because only the DNA between (and including) the primers is amplified, PCR provides a way to obtain selectively any short stretch of chromosomal DNA in an effectively pure form. (B) To use PCR to obtain a cDNA clone of a gene, total mRNA is first purified from cells. The first primer is added to the population of mRNAs, and reverse transcriptase is used to make a DNA strand complementary to the specific RNA sequence of interest. The second primer is then added, and the DNA molecule is amplified through many cycles of PCR.

EXPLORING AND EXPLOITING GENE FUNCTION

The procedures we have described thus far enable biologists to obtain large amounts of DNA in a form that is easy to work with in the laboratory. Whether present as fragments stored in a DNA library in bacteria or as a collection of PCR products nestled in the bottom of a test tube, this DNA also provides the raw material for experiments designed to unravel how individual genes—and the RNA molecules and proteins they encode—function in cells and organisms.

This is where creativity comes in. There are as many ways to study gene function as there are scientists interested in studying it. The techniques

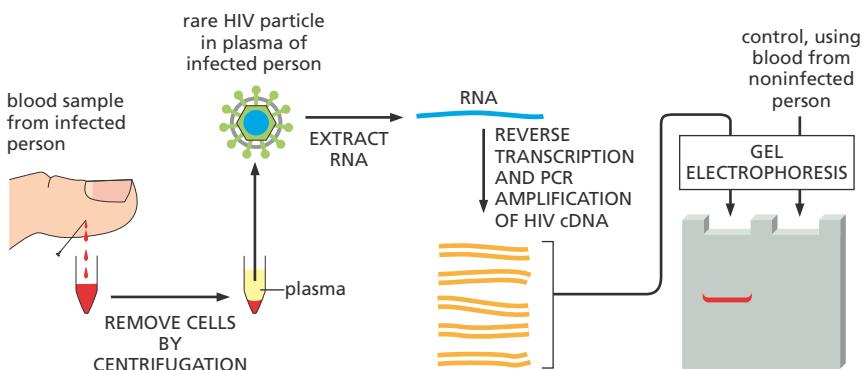


Figure 10–17 PCR can be used to detect the presence of a viral genome in a sample of blood. Because of its ability to amplify enormously the signal from every single molecule of nucleic acid, PCR is an extraordinarily sensitive method for detecting trace amounts of virus in a sample of blood or tissue without the need to purify the virus. For HIV, the virus that causes AIDS, the genome is a single-stranded molecule of RNA, as illustrated here. In addition to HIV, many other viruses that infect humans are now detected in this way.

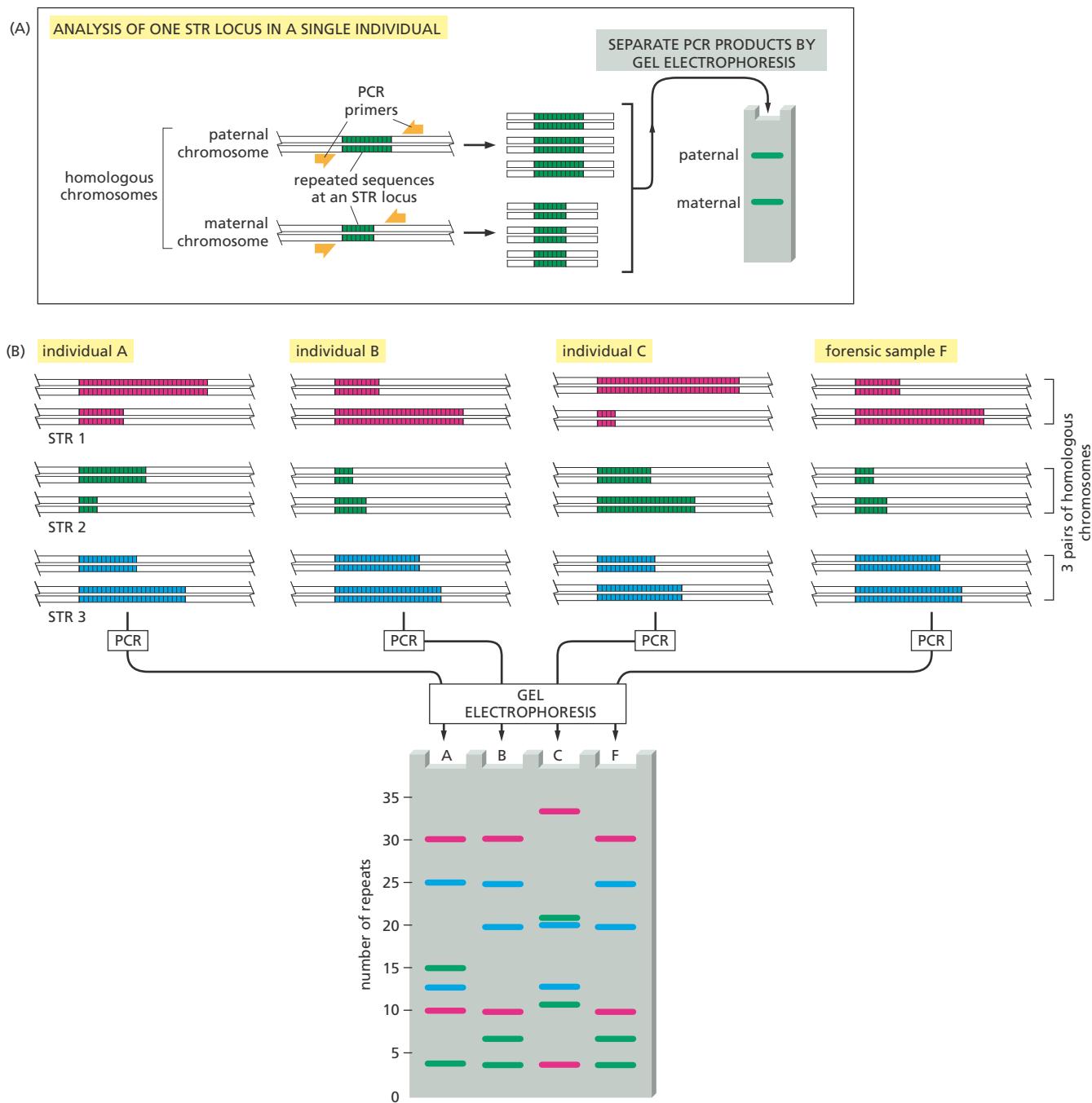


Figure 10–18 PCR is used in forensic science to distinguish one individual from another. The DNA sequences analyzed are short tandem repeats (STRs) composed of sequences such as CACACA... or GTGTGT.... STRs are found in various positions (loci) in the human genome. The number of repeats in each STR locus is highly variable in the population, ranging from 4 to 40 in different individuals. Because of the variability in these sequences, individuals will usually inherit a different number of repeats at each STR locus from their mother and from their father; two unrelated individuals, therefore, rarely contain the same pair of sequences at a given STR locus. (A) PCR using primers that recognize unique sequences on either side of one particular STR locus produces a pair of bands of amplified DNA from each individual, one band representing the maternal STR variant and the other representing the paternal STR variant. The length of the amplified DNA, and thus its position after gel electrophoresis, will depend on the exact number of repeats at the locus. (B) In the schematic example shown here, the same three STR loci are analyzed in samples from three suspects (individuals A, B, and C), producing six bands for each individual. Although different people can have several bands in common, the overall pattern is quite distinctive for each person. The band pattern can therefore serve as a *DNA fingerprint* to identify an individual nearly uniquely. The fourth lane (F) contains the products of the same PCR amplifications carried out on a hypothetical forensic DNA sample, which could have been obtained from a single hair or a tiny spot of blood left at a crime scene.

The more loci that are examined, the more confident one can be about the results. When examining the variability at 5–10 different STR loci, the odds that two random individuals would share the same fingerprint by chance are approximately one in 10 billion. In the case shown here, individuals A and C can be eliminated from inquiries, while B is a clear suspect. A similar approach is now used routinely in paternity testing.

an investigator chooses often depend on his or her background and training: a geneticist might, for example, engineer mutant organisms in which the activity of the gene has been disrupted, whereas a biochemist might take the same gene and produce large amounts of its protein to determine its three-dimensional structure.

In this section, we present a few of the methods that investigators currently use to study the function of a gene—all of which depend on recombinant DNA technology. Because a gene's activity is specified by its nucleotide sequence, we begin by outlining the techniques used to determine—and begin to interpret—the nucleotide sequence of a stretch of DNA. We then explore a variety of approaches for investigating when and where a gene is expressed. We describe how disrupting the activity of a gene in a cell, tissue, or whole plant or animal can provide insights into what that gene normally does. Finally, we explain how recombinant DNA technology can be harnessed to produce large amounts of any protein. Together, the methods we discuss have revolutionized all aspects of cell biology.

Whole Genomes Can Be Sequenced Rapidly

In the late 1970s, researchers developed several schemes for determining, simply and quickly, the nucleotide sequence of any purified DNA fragment. The one that became the most widely used is called **dideoxy sequencing** or **Sanger sequencing** (after the scientist who invented it). The technique uses DNA polymerase, along with special chain-terminating nucleotides called dideoxynucleoside triphosphates (Figure 10–19), to make partial copies of the DNA fragment to be sequenced. It ultimately produces a collection of different DNA copies that terminate at every position in the original DNA sequence.

Until recently, these DNA copies, which differ in length by a single nucleotide, would then be separated by gel electrophoresis, and the nucleotide sequence of the original DNA would be determined manually from the order of labeled DNA fragments in the gel (Figure 10–20). These days, however, Sanger sequencing is fully automated: robotic devices mix the reagents—including the four different chain-terminating dideoxynucleotides, each tagged with a different-colored fluorescent dye—and load the reaction samples onto long, thin capillary gels, which have replaced the flat gel slabs used since the 1970s. A detector then records the color of each band in the gel, and a computer translates the information into a nucleotide sequence (Figure 10–21). How such sequence information is then analyzed to assemble a complete genome sequence—for example, the first draft of the human genome—is described in **How We Know**, pp. 344–345.

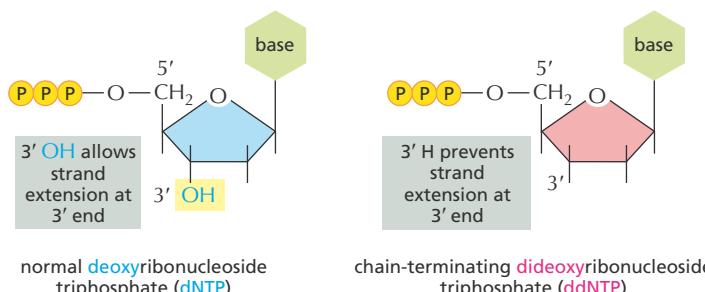


Figure 10–19 The dideoxy, or Sanger, method of sequencing DNA relies on chain-terminating dideoxynucleoside triphosphates (ddNTPs). These ddNTPs are derivatives of the normal deoxyribonucleoside triphosphates that lack the 3' hydroxyl group. When incorporated into a growing DNA strand, they block further elongation of that strand.

Figure 10–20 The Sanger method produces four sets of labeled DNA molecules. To determine the complete sequence of a single-stranded fragment of DNA (gray), the DNA is first hybridized with a short DNA primer (orange) that is labeled with a fluorescent dye or radioisotope. DNA polymerase and an excess of all four normal deoxyribonucleoside triphosphates (blue A, C, G, and T) are added to the primed DNA, which is then divided into four reaction tubes. Each of these tubes receives a small amount of a single chain-terminating dideoxyribonucleoside triphosphate (red A, C, G, or T). Because the chain-terminating ddNTPs will be incorporated only occasionally, each reaction produces a set of DNA copies that terminate at different points in the sequence. The products of these four reactions are separated by electrophoresis in four parallel lanes of a polyacrylamide gel (labeled here A, T, C, and G). In each lane, the bands represent fragments that have terminated at a given nucleotide (e.g., A in the leftmost lane) but at different positions in the DNA. By reading off the bands in order, starting at the bottom of the gel and reading across all lanes, the DNA sequence of the newly synthesized strand can be determined. The sequence, which is given in the green arrow to the right of the gel, is complementary to the sequence of the original gray single-stranded DNA, as shown on the bottom.

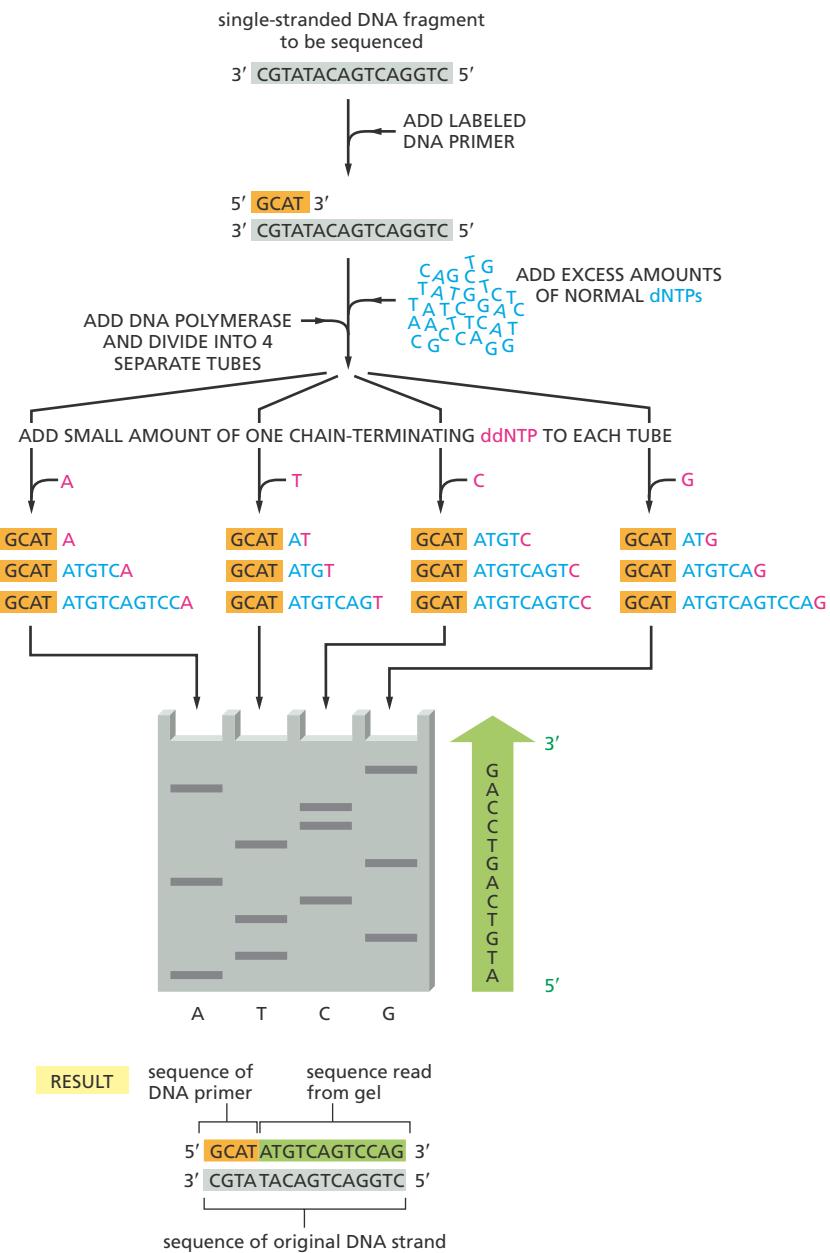
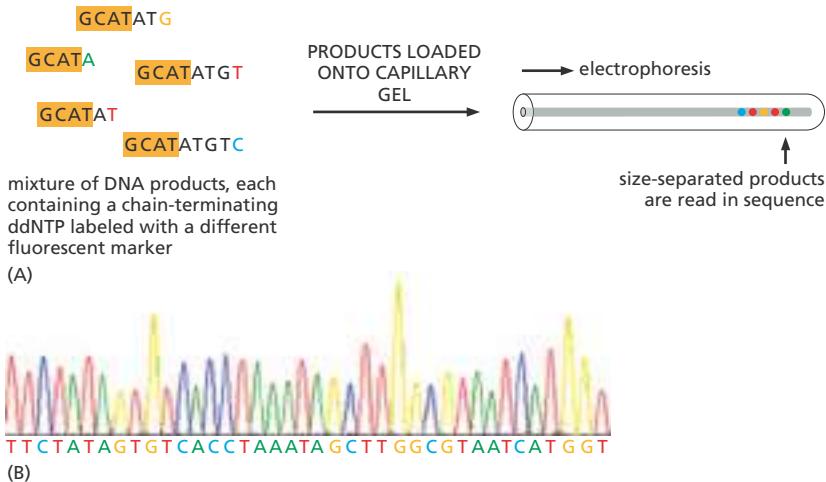


Figure 10–21 Fully automated machines can set up and run Sanger sequencing reactions. (A) The automated method uses an excess amount of normal dNTPs plus a mixture of four different chain-terminating ddNTPs, each of which is labeled with a fluorescent tag of a different color. The reaction products are loaded onto a long, thin capillary gel and separated by electrophoresis. A camera reads the color of each band on the gel and feeds the data to a computer that assembles the sequence (not shown). (B) A tiny part of the data from such an automated sequencing run. Each colored peak represents a nucleotide in the DNA sequence.



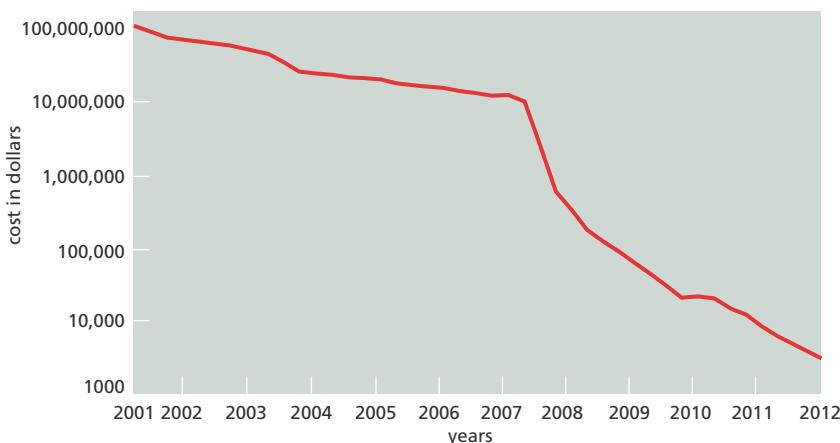


Figure 10–22 The cost of DNA sequencing has dropped precipitously since the advent of next-generation sequencing technologies. Shown here are the costs of sequencing a human genome which was \$100 million in 2001 and not much more than a thousand dollars by the end of 2012. (Data from the National Human Genome Research Initiative.)

Next-Generation Sequencing Techniques Make Genome Sequencing Faster and Cheaper

The Sanger method has made it possible to sequence the genomes of humans and of many other organisms including most of those discussed in this book. But newer methods, developed since 2005, have made genome sequencing even more rapid—and very much cheaper. With these so-called *second-generation sequencing methods*, the cost of sequencing DNA has plummeted (Figure 10–22). At the same time, the number of genomes that have been sequenced has skyrocketed. These rapid methods allow multiple genomes to be sequenced in parallel in a matter of weeks, enabling investigators to examine thousands of human genomes, catalog the variation in nucleotide sequences from people around the world, and uncover the mutations that increase the risk of various diseases—from cancer to autism—as we discuss in Chapter 19.

Although each method differs in detail, most rely on PCR amplification of a random collection of DNA fragments attached to a solid support, such as a glass slide or a microwell plate. For each fragment, the amplification generates a “cluster” that contains about 1000 copies of an individual DNA fragment. These clusters—tens of millions of which can fit on a single slide or plate—are then sequenced at the same time (Figure 10–23).

Even more remarkable are the newest, *third-generation sequencing methods*, which permit the sequencing of just a single molecule of DNA. In one of these techniques, for example, each DNA molecule is slowly pulled through a very tiny channel, like thread through the eye of a needle. Because each of the four nucleotides has a different, characteristic shape, the way a nucleotide obstructs the pore as it passes through reveals

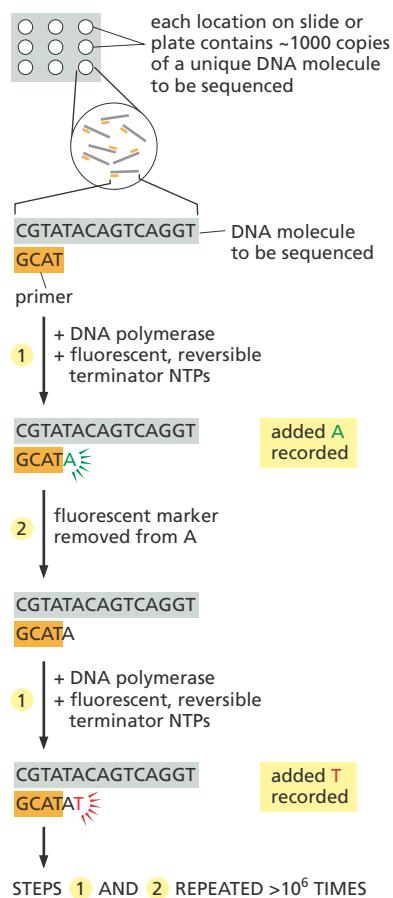


Figure 10–23 Second-generation sequencing methods rely on massively parallel sequencing reactions carried out on clusters of PCR-amplified DNA. Each spot on a slide or plate contains about a thousand copies of a single DNA fragment. In the first step, the plate is incubated with DNA polymerase and a special set of four nucleoside triphosphates (NTPs) that terminate DNA synthesis in a reversible manner, each of which carries a fluorescent marker of a different color; no normal dNTPs are present. A camera then images and records the fluorescence at each position on the plate. In the second step, the DNA is chemically treated to remove the fluorescent markers and chemical blockers from each nucleoside; strand synthesis then continues after a new batch of fluorescent NTPs is added. These steps are repeated until the sequence is complete. The snapshots of each round of synthesis are compiled by computer to yield the sequence of the cluster of fragments located at each of the potentially millions of positions on the plate.

SEQUENCING THE HUMAN GENOME

When DNA sequencing techniques became fully automated, determining the order of the nucleotides in a piece of DNA went from being an elaborate Ph.D. thesis project to a routine laboratory chore. Feed DNA into the sequencing machine, add the necessary reagents, and out comes the sought-after result: the order of As, Ts, Gs, and Cs. Nothing could be simpler.

So why was sequencing the human genome such a formidable task? Largely because of its size. The DNA sequencing methods employed at the time were limited by the physical size of the gel used to separate the labeled fragments (see Figure 10–20). At most, only a few hundred nucleotides could be read from a single gel. How, then, do you handle a genome that contains billions of nucleotide pairs?

The solution is to break the genome into fragments and sequence these smaller pieces. The main challenge then comes in piecing the short fragments together in the correct order to yield a comprehensive sequence of a whole chromosome, and ultimately a whole genome. There are two main strategies for accomplishing this genomic breakage and reassembly: the shotgun method and the clone-by-clone approach.

Shotgun sequencing

The most straightforward approach to sequencing a genome is to break it into random fragments, separate and sequence each of the single-stranded fragments, and then use a powerful computer to order these pieces using sequence overlaps to guide the assembly (Figure 10–24). This approach is called the shotgun sequencing strategy. As an analogy, imagine shredding several

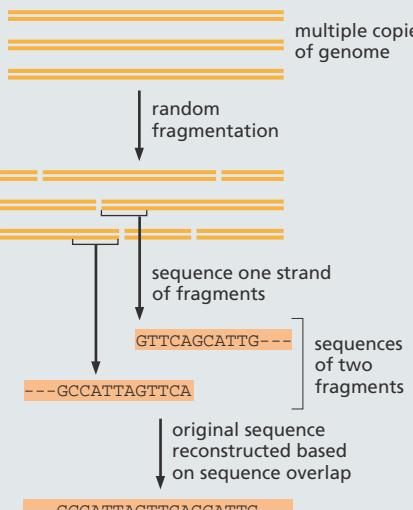


Figure 10–24 Shotgun sequencing is the method of choice for small genomes. The genome is first broken into much smaller, overlapping fragments. Each fragment is then sequenced, and the genome is assembled based on overlapping sequences.

copies of *Essential Cell Biology* (*ECB*), mixing up the pieces, and then trying to put one whole copy of the book back together again by matching up the words or phrases or sentences that appear on each piece. (Several copies would be needed to generate enough overlap for reassembly.) It could be done, but it would be much easier if the book were, say, only two pages long.

For this reason, a straight-out shotgun approach is the strategy of choice only for sequencing small genomes. The method proved its worth in 1995, when it was used to sequence the genome of the infectious bacterium *Haemophilus influenzae*, the first organism to have its complete genome sequence determined. The trouble with shotgun sequencing is that the reassembly process can be derailed by repetitive nucleotide sequences. Although rare in bacteria, these sequences make up a large fraction of vertebrate genomes (see Figure 9–33). Highly repetitive DNA segments make it difficult to piece DNA sequences back together accurately (Figure 10–25). Returning to the *ECB* analogy, this chapter alone contains more than a few instances of the phrase “the human genome.” Imagine that one slip of paper from the shredded *ECBs* contains the information: “So why was sequencing the human genome” (which appears at the start of this section); another contains the information: “the human genome sequence consortium combined shotgun sequencing with a clone-by-clone approach” (which appears below). You might be tempted to join these two segments together based on the overlapping phrase “the human genome.” But you would wind up with the nonsensical statement: “So why was sequencing the human genome sequence consortium combined shotgun sequencing with a clone-by-clone approach.” You would also lose the several paragraphs of important text that originally appeared between these two instances of “the human genome.”

And that’s just in this section. The phrase “the human genome” appears in many chapters of this book. Such repetition compounds the problem of placing each fragment in its correct context. To circumvent these assembly problems, researchers in the human genome sequence consortium combined shotgun sequencing with a clone-by-clone approach.

Clone-by-clone

In this approach, researchers started by preparing a genomic DNA library. They broke the human genome into overlapping fragments, 100–200 kilobase pairs in size. They then plugged these segments into bacterial artificial chromosomes (BACs) and inserted them into *E. coli*. (BACs are similar to the bacterial plasmids discussed earlier, except they can carry much larger pieces of DNA.) As the bacteria divided, they copied the BACs,

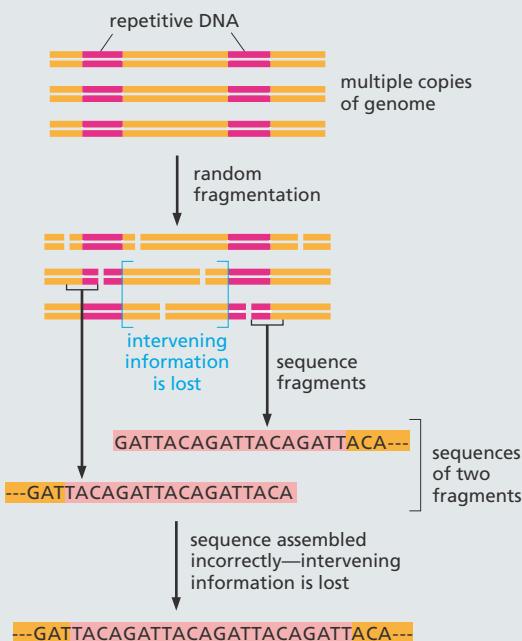


Figure 10–25 Repetitive DNA sequences in a genome make it difficult to accurately assemble its fragments. In this example, the DNA contains two segments of repetitive DNA, each made of many copies of the sequence GATTACA. When the resulting sequences are examined, two fragments from different parts of the DNA appear to overlap. Assembling these sequences incorrectly would result in a loss of the information (in brackets) that lies between the original repeats.

thus producing a collection of overlapping cloned fragments (see Figure 10–10).

The researchers then determined where each of these DNA fragments fit into the existing map of the human genome. To do this, different restriction nucleases were used to cut each clone to generate a unique restriction-site “signature.” The locations of the restriction sites in each fragment allowed researchers to map each BAC clone onto a restriction map of a whole human genome that had been generated previously using the same set of restriction nucleases (Figure 10–26).

Knowing the relative positions of the cloned fragments, the researchers then selected some 30,000 BACs, sheared each into smaller fragments, and determined the

nucleotide sequence of each BAC separately using the shotgun method. They could then assemble the whole genome sequence by stitching together the sequences of thousands of individual BACs that span the length of the genome.

The beauty of this approach was that it was relatively easy to accurately determine where the BAC fragments belong in the genome. This mapping step reduces the likelihood that regions containing repetitive sequences will be assembled incorrectly, and it virtually eliminates the possibility that sequences from different chromosomes will be mistakenly joined together. Returning to the textbook analogy, the BAC-based approach is akin to first separating your copies of *ECB* into individual pages and then shredding each page into its own separate pile. It should be much easier to put the book back together when one pile of fragments contains words from page 1, a second pile from page 2, and so on. And there's virtually no chance of mistakenly sticking a sentence from page 40 into the middle of a paragraph on page 412.

All together now

The clone-by-clone approach produced the first draft of the human genome sequence in 2000 and the completed sequence in 2004. As the set of instructions that specify all of the RNA and protein molecules needed to build a human being, this string of genetic bits holds the secrets to human development and physiology. But the sequence was also of great value to researchers interested in comparative genomics or in the physiology of other organisms: it eased the assembly of nucleotide sequences from other mammalian genomes—mice, rats, dogs, and other primates. It also made it much easier to determine the nucleotide sequences of the genomes of individual humans by providing a framework on which the new sequences could be simply superimposed.

The first human sequence was the only mammalian genome completed in this methodical way. But the human genome project was an unqualified success in that it provided the techniques, confidence, and momentum that drove the development of the next generation of DNA sequencing methods, which are now rapidly transforming all areas of biology.

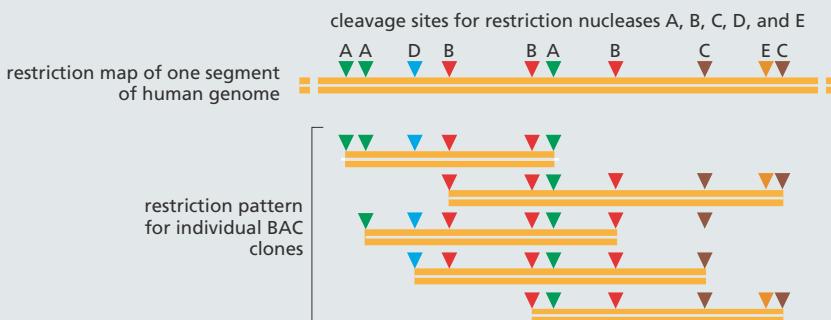


Figure 10–26 Individual BAC clones are positioned on the physical map of the human genome sequence on the basis of their restriction site “signatures.” Clones are digested with five different restriction nucleases, and the sites at which the different enzymes cut each clone are recorded. The distinctive pattern of restriction sites allows investigators to order the fragments and place them on a restriction map of a human genome that had been previously generated using the same nucleases.

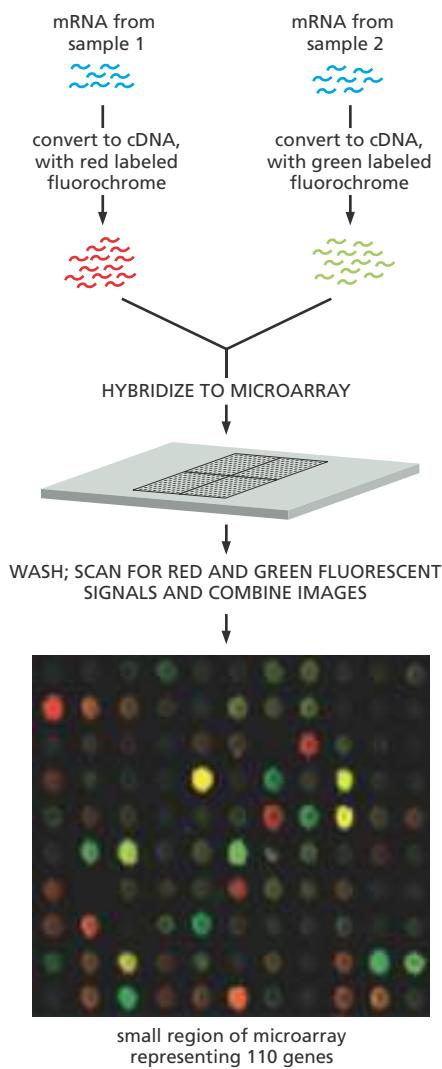


Figure 10–27 DNA microarrays are used to analyze the production of thousands of different mRNAs in a single experiment.

In this example, mRNA is collected from two different cell samples—for example, cells treated with a hormone and untreated cells of the same type—to allow for a direct comparison of the specific genes expressed under both conditions. The mRNAs are converted to cDNAs that are labeled with a red fluorescent dye for one sample, and a green fluorescent dye for the other. The labeled samples are mixed and then allowed to hybridize to the microarray. After incubation, the array is washed and the fluorescence scanned. Only a small proportion of the microarray, representing 110 genes, is shown. Red spots indicate that the gene in sample 1 is expressed at a higher level than the corresponding gene in sample 2, and green spots indicate the opposite. Yellow spots reveal genes that are expressed at about equal levels in both cell samples. The intensity of the fluorescence provides an estimate of how much RNA is present from a gene. Dark spots indicate little or no expression of the gene whose fragment is located at that position in the array.

its identity—information that is then used to compile the sequence of the DNA molecule. Such methods require no amplification or chemical labeling, and thereby reduce the cost and time of sequencing even further, making it possible to obtain a complete human genome sequence for under \$1000 in hours.

Comparative Genome Analyses Can Identify Genes and Predict Their Function

Strings of nucleotides, at first glance, reveal nothing about how that genetic information directs the development of a living organism—or even what type of organism it might encode. One way to learn something about the function of a particular nucleotide sequence is to compare it with the multitude of sequences available in public databases. Using a computer program to search for sequence similarity, one can determine whether a nucleotide sequence contains a gene and what that gene is likely to do—based on the gene’s known activity in other organisms.

Comparative analyses have revealed that the coding regions of genes from a wide variety of organisms show a large degree of sequence conservation (see Figure 9–19). The sequences of noncoding regions, however, tend to diverge over evolutionary time (see Figure 9–18). Thus, a search for sequence similarity can often indicate from which organism a particular piece of DNA was derived, and which species are most closely related. Such information is particularly useful when the origin of a DNA sample is unknown—because it was extracted, for example, from a sample of soil or seawater or the blood of a patient with an undiagnosed infection.

But knowing where a nucleotide sequence comes from—or even what activity it might have—is only the first step toward determining what role it has in the development or physiology of the organism. The knowledge that a particular DNA sequence encodes a transcription regulator, for example, does not reveal when and where that protein is produced, or which genes it might regulate. To learn that, investigators must head back to the laboratory.

Analysis of mRNAs By Microarray or RNA-Seq Provides a Snapshot of Gene Expression

As we discussed in Chapter 8, a cell expresses only a subset of the thousands of genes available in its genome. This subset differs from one cell type to another. One way to determine which genes are being expressed in a population of cells or in a tissue is to analyze which mRNAs are being produced.

The first tool that allowed investigators to analyze simultaneously the thousands of different RNAs produced by cells or tissues was the **DNA microarray**. Developed in the 1990s, DNA microarrays are glass microscope slides that contain hundreds of thousands of DNA fragments, each of which serves as a probe for the mRNA produced by a specific gene. Such microarrays allow investigators to monitor the expression of every gene in an entire genome in a single experiment. To do the analysis, mRNAs are extracted from cells or tissues and converted to cDNAs (see Figure 10–12). The cDNAs are fluorescently labeled and allowed to hybridize to the fragments on the microarray. An automated fluorescence microscope then determines which mRNAs were present in the original sample based on the array positions to which the cDNAs are bound (**Figure 10–27**).

Although microarrays are relatively inexpensive and easy to use, they suffer from one obvious drawback: the sequences of the mRNA samples to be analyzed must be known in advance and represented by a corresponding probe on the array. With the development of next-generation

sequencing technologies, investigators increasingly use a more direct approach for cataloging the RNAs produced by a cell. The RNAs are converted to cDNAs, which are then sequenced using second-generation sequencing methods. The approach, called **RNA-Seq**, provides a more quantitative analysis of the *transcriptome*—the complete collection of RNAs produced by a cell under a certain set of conditions. It also determines the number of times a particular sequence appears in a sample and detects rare mRNAs, RNA transcripts that are alternatively spliced, mRNAs that harbor sequence variations, and noncoding RNAs. For these reasons, RNA-Seq is replacing microarrays as the method of choice for analyzing the transcriptome.

In Situ Hybridization Can Reveal When and Where a Gene Is Expressed

Although microarrays and RNA-Seq provide a list of genes that are being expressed by a cell or tissue, they do not reveal exactly where in the cell or tissue those mRNAs are produced. To see where a particular RNA is made, investigators use a technique called ***in situ* hybridization** (from the Latin *in situ*, “in place”), which allows a specific nucleic acid sequence—either DNA or RNA—to be visualized in its normal location.

In situ hybridization uses single-stranded DNA or RNA probes, labeled with either fluorescent dyes or radioactive isotopes, to detect complementary nucleic acid sequences within a tissue, a cell (Figure 10–28), or even an isolated chromosome (Figure 10–29). The latter application is used in the clinic to determine, for example, whether fetuses carry abnormal chromosomes.

In situ hybridization is frequently used to study the expression patterns of a particular gene or collection of genes in an adult or developing tissue. In one particularly ambitious project, neuroscientists are using the method to assemble a three-dimensional map of all the genes expressed in both the mouse and human brain (Figure 10–30). Knowing where and when a gene is expressed can provide important clues about its function.

Reporter Genes Allow Specific Proteins to be Tracked in Living Cells

For a gene that encodes a protein, the location of the protein within the cell, tissue, or organism yields clues to the gene’s function. Traditionally, the most effective way to visualize a protein within a cell or tissue involved using a labeled antibody. That approach requires the generation of an antibody that specifically recognizes the protein of interest—a process that can be time-consuming and has no guarantee of success.

An alternative approach is to use the regulatory DNA sequences of the protein-coding gene to drive the expression of some type of

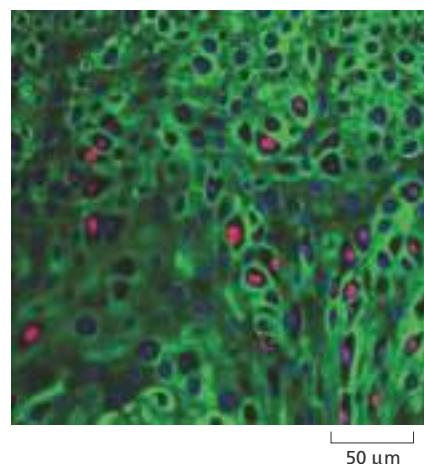


Figure 10–28 *In situ* hybridization can be used to detect the presence of a virus in cells. In this micrograph, the nuclei of cultured epithelial cells infected with the human papillomavirus (HPV) are stained pink by a fluorescent probe that recognizes a viral DNA sequence. The cytoplasm of all cells is stained green. (Courtesy of Høgne Røed Nilsen.)

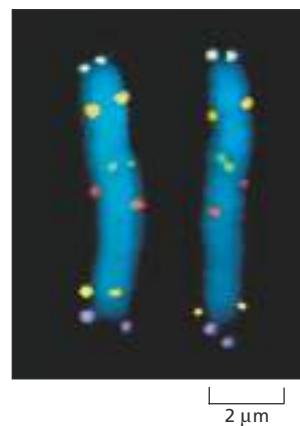


Figure 10–29 *In situ* hybridization can be used to locate genes on isolated chromosomes. Here, six different DNA probes have been used to mark the locations of their respective nucleotide sequences on human Chromosome 5 isolated from a mitotic cell in metaphase (see Figure 5–16 and Panel 18–1, pp. 622–623). The DNA probes have been labeled with different chemical groups and are detected using fluorescent antibodies specific for those groups. Both the maternal and paternal copies of Chromosome 5 are shown, aligned side by side. Each probe produces two dots on each chromosome because chromosomes undergoing mitosis have already replicated their DNA; therefore, each chromosome contains two identical DNA helices. The technique employed here is nicknamed FISH, for fluorescence *in situ* hybridization. (Courtesy of David C. Ward.)

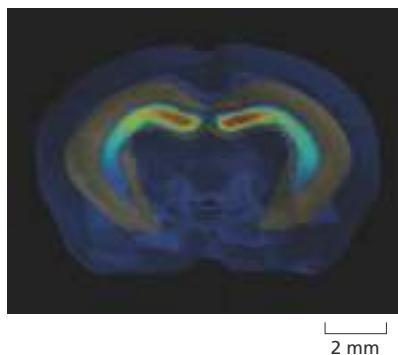


Figure 10-30 *In situ* hybridization has been used to generate an atlas of gene expression in the mouse brain. This computer-generated image shows the expression of genes specific to an area of the brain associated with learning and memory. Similar maps of expression patterns of all known genes in the mouse brain are compiled in the brain atlas project, which is available for free online. (From M. Hawrylycz et al., *PLoS Comput. Biol.* 7:e1001065, 2011.)

reporter gene, one that encodes a protein that can be easily monitored by its fluorescence or enzymatic activity. A recombinant gene of this type usually mimics the expression of the gene of interest, producing the reporter protein when, where, and in the same amounts as the normal protein would be made (**Figure 10-31A**). The same approach can be used to study the regulatory DNA sequences that control the gene's expression (**Figure 10-31B**).

One of the most popular reporter proteins used today is **green fluorescent protein (GFP)**, the molecule that gives luminescent jellyfish their greenish glow. In many cases, the gene that encodes GFP is simply attached to one end of the gene of interest. The resulting *GFP fusion protein* often behaves in the same way as the normal protein produced by the gene of interest, and its location can be monitored by fluorescence microscopy (**Figure 10-32**). GFP fusion has become a standard strategy for tracking not only the location but also the movement of specific proteins in living cells. In addition, the use of multiple GFP variants that fluoresce at different wavelengths can provide insights into how different cells interact in a living tissue (**Figure 10-33**).

The Study of Mutants Can Help Reveal the Function of a Gene

Although it may seem counterintuitive, one of the best ways to determine a gene's function is to see what happens to an organism when the gene is inactivated by a mutation. Before the advent of gene cloning, geneticists

Figure 10-31 Reporter genes can be used to determine the pattern of a gene's expression. (A) Suppose the goal is to find out which cell types (A–F) express protein X, but it is difficult to detect the protein directly—with antibodies, for example. Using recombinant DNA techniques, the coding sequence for protein X can be replaced with the coding sequence for reporter protein Y, which can be easily monitored visually; two commonly used reporter proteins are the enzyme β -galactosidase (see Figure 8–13C) and green fluorescent protein (GFP, see Figure 10–32). The expression of the reporter protein Y will now be controlled by the regulatory sequences (here labeled 1, 2, and 3) that control the expression of the normal protein X. (B) To determine which regulatory sequences normally control expression of gene X in particular cell types, reporters with various combinations of the regulatory regions associated with gene X can be constructed. These recombinant DNA molecules are then tested for expression after their introduction into the different cell types.

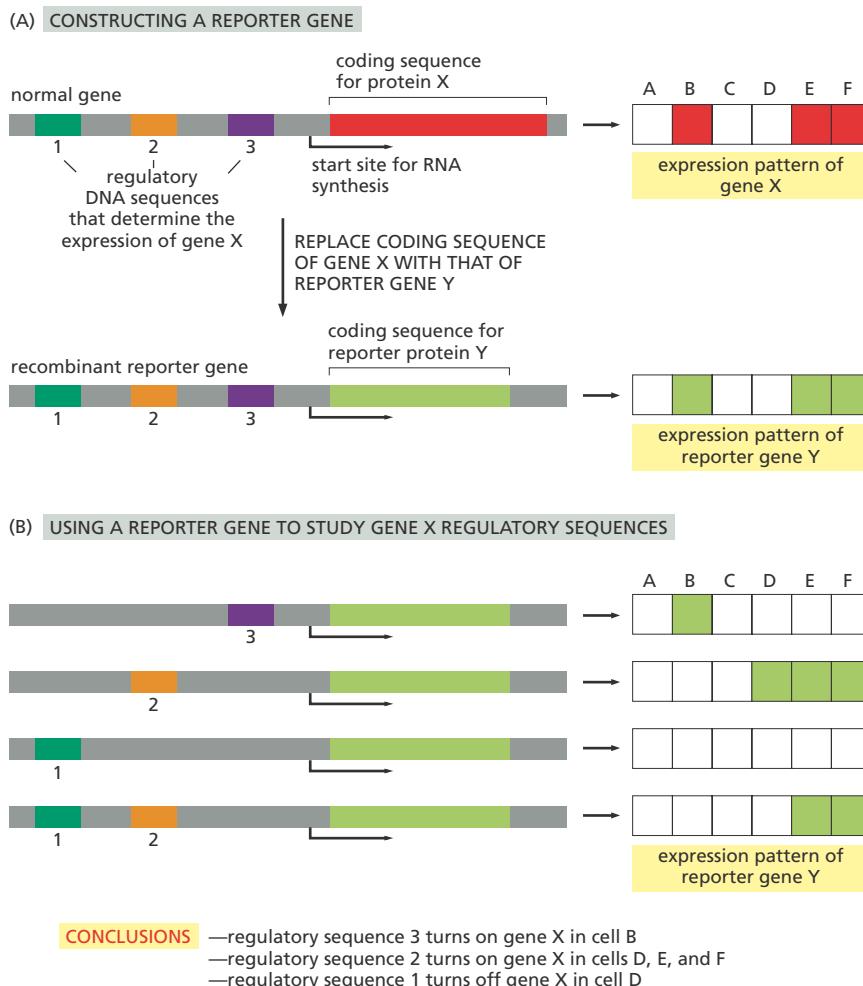
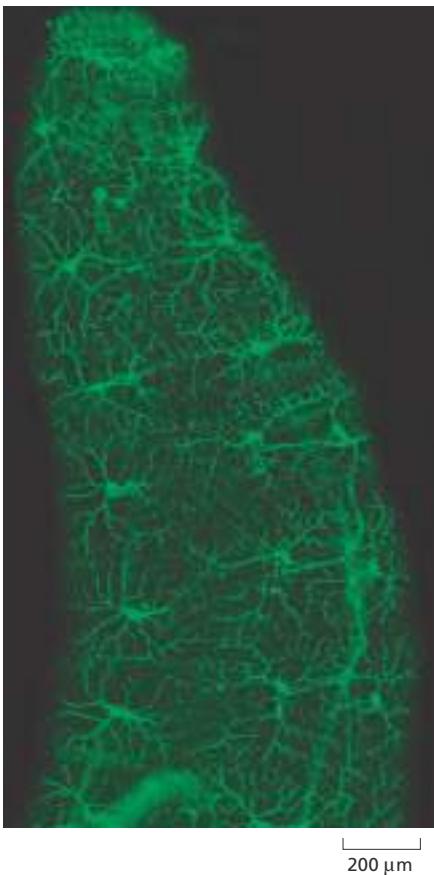


Figure 10–32 Green fluorescent protein (GFP) can be used to identify specific cells in a living animal. For this experiment, carried out in the fruit fly, recombinant DNA techniques were used to join the gene encoding GFP to the regulatory DNA sequences that direct the production of a particular *Drosophila* protein. Both the GFP and the normal fly protein are made only in a specialized set of neurons. This image of a live fly embryo was captured by a fluorescence microscope and shows approximately 20 neurons, each with long projections (axons and dendrites) that communicate with other (nonfluorescent) cells. These neurons, located just under the embryo's surface, allow the organism to sense its immediate environment. (From W.B. Gruener et al., *Curr. Biol.* 13:618–626, 2003. With permission from Elsevier.)



studied the mutant organisms that arise spontaneously in a population. The mutants of most interest were often selected because of their unusual *phenotype*—fruit flies with white eyes or curly wings, for example. The gene responsible for the mutant phenotype could then be studied by breeding experiments, as Gregor Mendel did with peas in the nineteenth century (discussed in Chapter 19).

Although mutant organisms can arise spontaneously, they do so infrequently. The process can be accelerated by treating organisms with either radiation or chemical mutagens, which randomly disrupt gene activity. Such random mutagenesis generates large numbers of mutant organisms, each of which can then be studied individually. This “classical genetic approach,” which we discuss in detail in Chapter 19, is most applicable to organisms that reproduce rapidly and can be analyzed genetically in the laboratory—such as bacteria, yeasts, nematode worms, and fruit flies—although it has also been used in zebrafish and mice.

RNA Interference (RNAi) Inhibits the Activity of Specific Genes

Recombinant DNA technology has made possible a more targeted genetic approach to studying gene function. Instead of beginning with a randomly generated mutant and then identifying the responsible gene, a gene of known sequence can be inactivated deliberately and the effects on the cell or organism’s phenotype can be observed. Because this strategy is essentially the reverse of that used in classical genetics—which goes from mutants to genes—it is often referred to as *reverse genetics*.

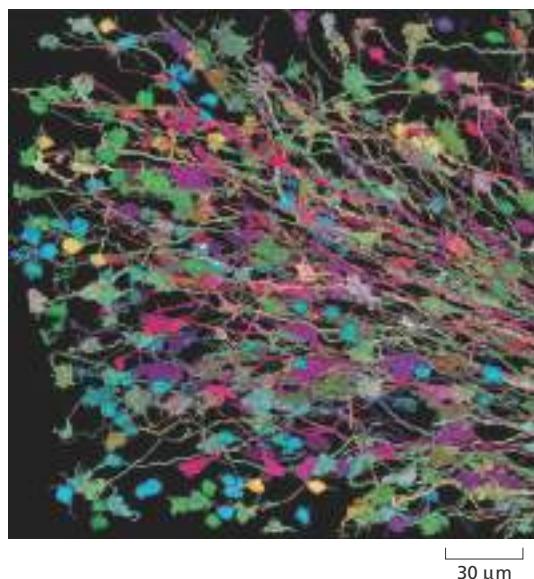


Figure 10–33 GFPs that fluoresce at different wavelengths help reveal the connections that individual neurons make within the brain. This image shows differently colored neurons in one region of a mouse brain. The neurons randomly express different combinations of differently colored GFPs, making it possible to distinguish and trace many individual neurons within a population. The stunning appearance of these labeled neurons have earned these animals the colorful nickname “rainbow mice.” (From J. Livet et al., *Nature* 450:56–62, 2007. With permission from Macmillan Publishers Ltd.)

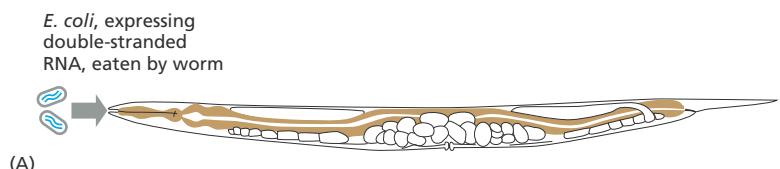
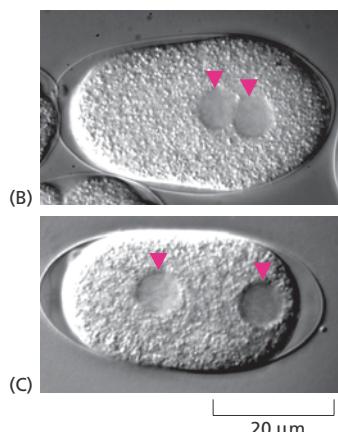


Figure 10–34 Gene function can be tested by RNA interference.

(A) Double-stranded RNA (dsRNA) can be introduced into *C. elegans* by (1) feeding the worms *E. coli* that express the dsRNA or (2) injecting the dsRNA directly into the animal's gut. (B) In a wild-type worm embryo, the egg and sperm pronuclei (red arrowheads) come together in the posterior half of the embryo shortly after fertilization. (C) In an embryo in which a particular gene has been silenced by RNAi, the pronuclei fail to migrate. This experiment revealed an important but previously unknown function of this gene in embryonic development. (B and C, from P. Gönczy et al., *Nature* 408:331–336, 2000. With permission from Macmillan Publishers Ltd.)



One of the fastest and easiest ways to silence genes in cells and organisms is via **RNA interference (RNAi)**. Discovered in 1998, RNAi exploits a natural mechanism used in a wide variety of plants and animals to protect themselves against certain viruses and the proliferation of mobile genetic elements (discussed in Chapter 9). The technique involves introducing into a cell or organism double-stranded RNA molecules with a nucleotide sequence that matches the gene to be inactivated. The double-stranded RNA is cleaved and processed by special RNAi machinery to produce shorter, double-stranded fragments called small interfering RNAs (siRNAs). These siRNAs are unwound to form single-stranded RNA fragments that hybridize with the target gene's mRNAs and direct their degradation (see Figure 8–26). In some organisms, the same fragments can direct the production of more siRNAs allowing continued inactivation of the target mRNAs.

RNAi is frequently used to inactivate genes in cultured mammalian cell lines, *Drosophila*, and the nematode *C. elegans*. Introducing double-stranded RNAs into *C. elegans* is particularly easy: the worm can be fed with *E. coli* that have been genetically engineered to produce the double-stranded RNAs that trigger RNAi (Figure 10–34). These RNAs get converted into siRNAs, which get distributed throughout the animal's body to inhibit expression of the target gene in various tissues. For the many organisms whose genomes have been completely sequenced, RNAi can, in principle, be used to explore the function of any gene, and large collections of DNA vectors that produce these double-stranded RNAs are available for several species.

A Known Gene Can Be Deleted or Replaced With an Altered Version

Despite its usefulness, RNAi has some limitations. Non-target genes are sometimes inhibited along with the gene of interest, and certain cell types are resistant to RNAi entirely. Even for cell types in which the mechanism functions effectively, gene inactivation by RNAi is often temporary, earning the description “gene knockdown.”

Fortunately, there are other, more specific and effective means of eliminating gene activity in cells and organisms. Using recombinant DNA techniques, the coding sequence of a cloned gene can be mutated *in vitro* to change the functional properties of its protein product. Alternatively, the coding region can be left intact and the regulatory region of the gene changed, so that the amount of protein made will be altered or the gene will be expressed in a different type of cell or at a different time during development. By re-introducing this altered gene back into the organism from which it originally came, one can produce a mutant organism

that can be studied to determine the gene's function. Often the altered gene is inserted into the genome of reproductive cells so that it can be stably inherited by subsequent generations. Organisms whose genomes have been altered in this way are known as **transgenic organisms**, or *genetically modified organisms (GMOs)*; the introduced gene is called a **transgene**.

To study the function of a gene that has been altered *in vitro*, ideally one would like to generate an organism in which the normal gene has been replaced by the altered one. In this way, the function of the mutant protein can be analyzed in the absence of the normal protein. A common way of doing this in mice makes use of cultured mouse embryonic stem (ES) cells (discussed in Chapter 20). These cells are first subjected to targeted gene replacement before being transplanted into a developing embryo to produce a mutant mouse, as illustrated in **Figure 10–35**.

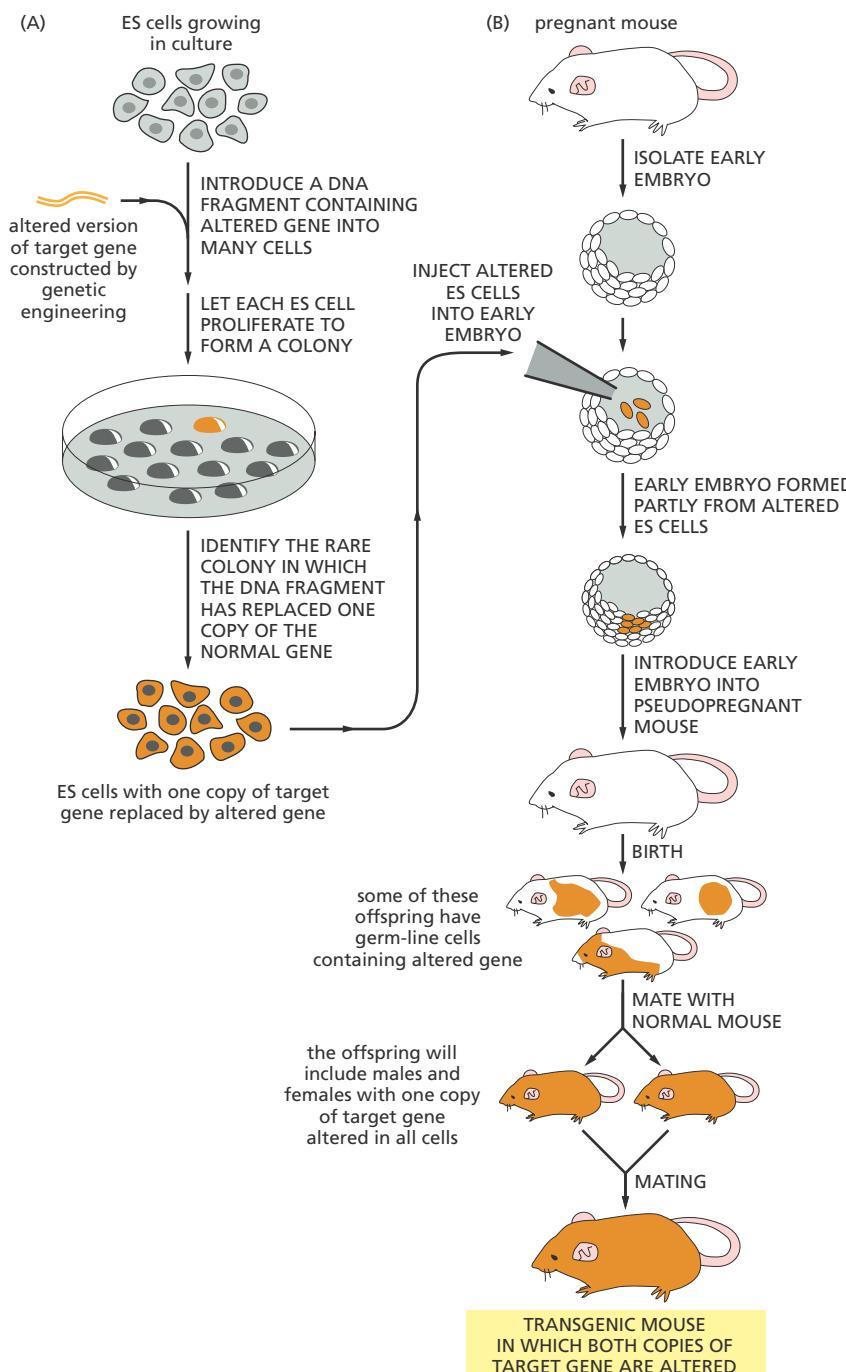
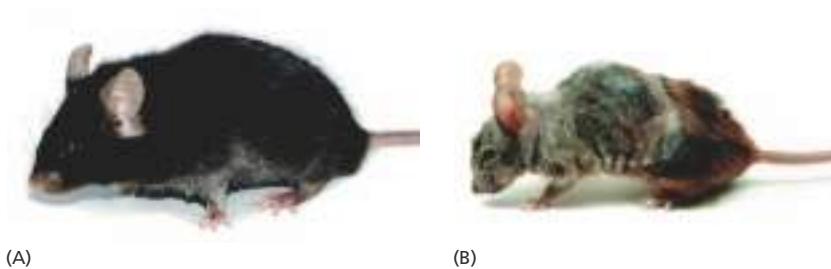


Figure 10–35 Targeted gene replacement in mice utilizes embryonic stem (ES) cells. (A) First, an altered version of the gene is introduced into cultured ES cells. In a few rare ES cells, the altered gene will replace the corresponding normal gene through homologous recombination. Although the procedure is often laborious, these rare cells can be identified and cultured to produce many descendants, each of which carries an altered gene in place of one of its two normal corresponding genes. (B) Next, the altered ES cells are injected into a very early mouse embryo; the cells are incorporated into the growing embryo, which then develops into a mouse that contains some somatic cells (colored orange) that carry the altered gene. Some of these mice may also have germ-line cells that contain the altered gene; when bred with a normal mouse, some of the progeny of these mice will contain the altered gene in all of their cells. Such a mouse is called a "knock-in" mouse. If two such mice are bred, one can obtain progeny that contain two copies of the altered gene—one on each chromosome—in all of their cells.

Figure 10–36 Transgenic mice with a mutant DNA helicase show premature aging. The helicase, encoded by the *Xpd* gene, is involved in both transcription and DNA repair. Compared with a wild-type mouse (A), a transgenic mouse that expresses a defective version of *Xpd* (B) exhibits many of the symptoms of premature aging, including osteoporosis, emaciation, early graying, infertility, and reduced life-span. The mutation in *Xpd* used here impairs the activity of the helicase and mimics a human mutation that causes trichothiodystrophy, a disorder characterized by brittle hair, skeletal abnormalities, and a greatly reduced life expectancy. These results support the hypothesis that an accumulation of DNA damage contributes to the aging process in both humans and mice. (From J. de Boer et al., *Science* 296:1276–1279, 2002. With permission from the AAAS.)



Using a similar strategy, the activity of both copies of a gene can also be eliminated entirely, creating a “**gene knockout**.” To do this, one can either introduce an inactive, mutant version of the gene into cultured ES cells or delete the gene altogether. The ability to use ES cells to produce such “knockout mice” revolutionized the study of gene function, and the technique is now being used to systematically determine the function of every mouse gene (Figure 10–36). A variation of this technique is used to produce *conditional knockout mice*, in which a known gene can be disrupted more selectively—only in a particular cell type or at a certain time in development. Such conditional knockouts are useful for studying genes with a critical function during development, because mice missing these crucial genes often die before birth.

Mutant Organisms Provide Useful Models of Human Disease

Technically speaking, transgenic approaches could be used to alter genes in the human germ line. For ethical reasons, such manipulations are unlawful. But transgenic technologies are widely used to generate animal models of human diseases in which mutant genes play a major part.

With the explosion of DNA sequencing technologies, investigators can rapidly search the genomes of patients for mutations that cause or greatly increase the risk of their disease (discussed in Chapter 19). These mutations can then be introduced into animals, such as mice, that can be studied in the laboratory. The resulting transgenic animals, which often mimic some of the phenotypic abnormalities associated with the condition in patients, can be used to explore the cellular and molecular basis of the disease and to screen for drugs that could potentially be used therapeutically in humans.

An encouraging example is provided by *fragile X syndrome*, a neuropsychiatric disorder associated with intellectual impairment, neurological abnormalities, and often autism. The disease is caused by a mutation in the *fragile X mental retardation gene (FMR1)*, which encodes a protein that inhibits the translation of mRNAs into proteins at synapses—the junctions where nerve cells communicate with one another (see Figure 12–38). Transgenic mice in which the *FMR1* gene has been disabled show many of the same neurological and behavioral abnormalities seen in patients with the disorder, and drugs that return synaptic protein synthesis to near-normal levels also reverse many of the problems seen in these mutant mice. Preliminary studies suggest that at least one of these drugs may benefit patients with the disease.

Transgenic Plants Are Important for Both Cell Biology and Agriculture

Although we tend to think of recombinant DNA research in terms of animal biology, these techniques have also had a profound impact on the

study of plants. In fact, certain features of plants make them especially amenable to recombinant DNA methods.

When a piece of plant tissue is cultured in a sterile medium containing nutrients and appropriate growth regulators, some of the cells are stimulated to proliferate indefinitely in a disorganized manner, producing a mass of relatively undifferentiated cells called a *callus*. If the nutrients and growth regulators are carefully manipulated, one can induce the formation of a shoot within the callus, and in many species a whole new plant can be regenerated from such shoots. In a number of plants—including tobacco, petunia, carrot, potato, and *Arabidopsis*—a single cell from such a callus can be grown into a small clump of cells from which a whole plant can be regenerated (see Figure 8–2B). Just as mutant mice can be derived by the genetic manipulation of embryonic stem cells in culture, so transgenic plants can be created from plant cells transfected with DNA in culture (Figure 10–37).

The ability to produce transgenic plants has greatly accelerated progress in many areas of plant cell biology. It has played an important part, for example, in isolating receptors for growth regulators and in analyzing the mechanisms of morphogenesis and of gene expression in plants. These techniques have also opened up many new possibilities in agriculture that could benefit both the farmer and the consumer. They have made it possible, for example, to modify the ratio of lipid, starch, and protein in seeds, to impart pest and virus resistance to plants, and to create modified plants that tolerate extreme habitats such as salt marshes or water-stressed soil. One variety of rice has been genetically engineered to produce β-carotene, the precursor of vitamin A. If it replaced conventional rice, this “golden rice”—so called because of its faint yellow color—could help to alleviate severe vitamin A deficiency, which causes blindness in hundreds of thousands of children in the developing world each year.

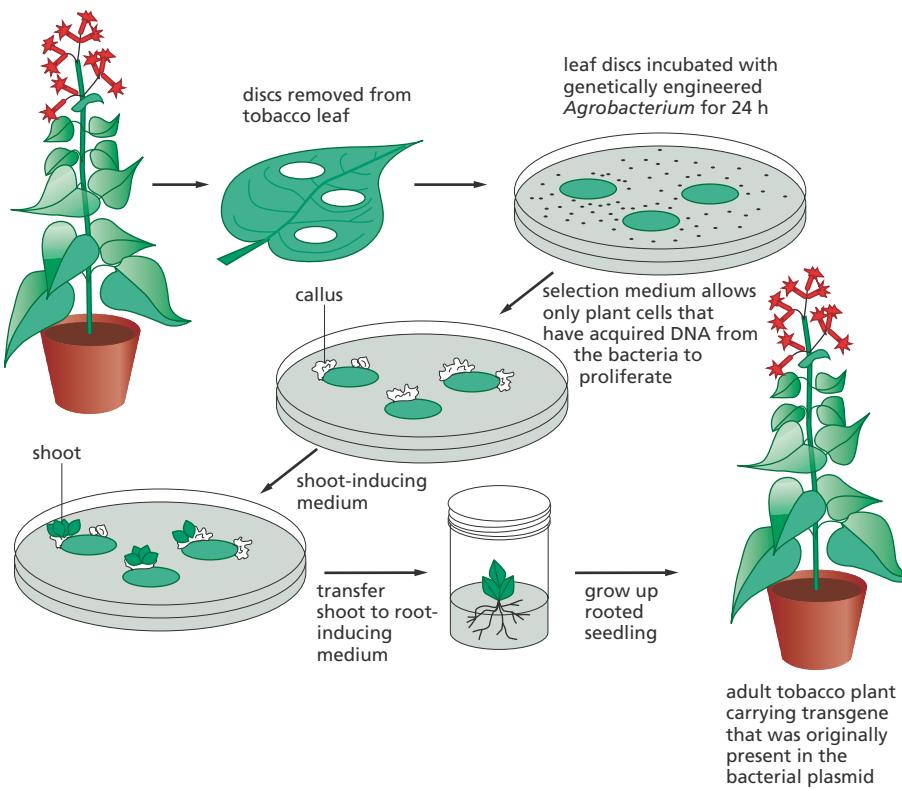


Figure 10–37 Transgenic plants can be made using recombinant DNA techniques optimized for plants. A disc is cut out of a leaf and incubated in a culture of *Agrobacterium* that carries a recombinant plasmid with both a selectable marker and a desired genetically engineered gene. The wounded plant cells at the edge of the disc release substances that attract the bacteria, which inject their DNA into the plant cells. Only those plant cells that take up the appropriate DNA and express the selectable marker gene survive and proliferate and form a callus. The manipulation of growth factors supplied to the callus induces it to form shoots, which subsequently root and grow into adult plants carrying the engineered gene.

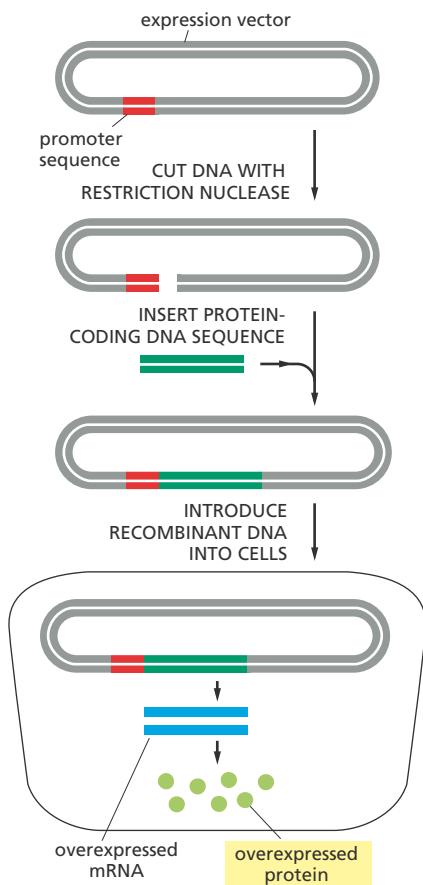


Figure 10–38 Large amounts of a protein can be produced from a protein-coding DNA sequence inserted into an expression vector and introduced into cells. Here, a plasmid vector has been engineered to contain a highly active promoter, which causes unusually large amounts of mRNA to be produced from the inserted protein-coding gene. Depending on the characteristics of the cloning vector, the plasmid is introduced into bacterial, yeast, insect, or mammalian cells, where the inserted gene is efficiently transcribed and translated into protein.

Even Rare Proteins Can Be Made in Large Amounts Using Cloned DNA

One of the most important contributions of DNA cloning and genetic engineering to cell biology is that they make it possible to produce any protein, including the rare ones, in nearly unlimited amounts. Such high-level production is usually accomplished by using specially designed vectors known as *expression vectors*. These vectors include transcription and translation signals that direct an inserted gene to be expressed at very high levels. Different expression vectors are designed for use in bacterial, yeast, insect, or mammalian cells, each containing the appropriate regulatory sequences for transcription and translation in these cells (Figure 10–38). The expression vector is replicated at each round of cell division, so that the transfected cells in the culture are able to synthesize very large amounts of the protein of interest—often comprising 1–10% of the total cell protein. It is usually a simple matter to purify this protein away from the other proteins made by the host cell.

This technology is now used to make large amounts of many medically useful proteins, including hormones (such as insulin), growth factors, and viral coat proteins for use in vaccines. Expression vectors also allow scientists to produce many proteins of biological interest in large enough amounts for detailed structural and functional studies that were once impossible—especially for proteins that are normally present in very small amounts, such as some receptors and transcription regulators. Recombinant DNA techniques thus allow scientists to move with ease from protein to gene, and vice versa, so that the functions of both can be explored on multiple fronts (Figure 10–39).

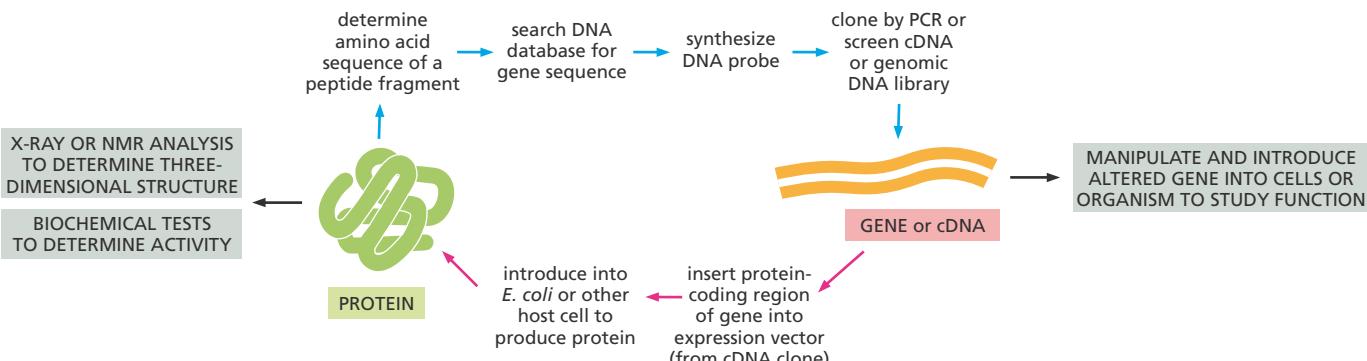


Figure 10–39 Recombinant DNA techniques make it possible to move experimentally from gene to protein and from protein to gene. A small quantity of a purified protein or peptide fragment is used to obtain a partial amino acid sequence, which is used to search a DNA database for the corresponding nucleotide sequence. This sequence is used to synthesize a DNA probe, which can be used either to pick out the corresponding gene from a DNA library by DNA hybridization (see Figure 10–11) or to clone the gene by PCR from a sequenced genome (see Figure 10–16). Once the gene has been isolated and sequenced, its protein-coding sequence can be inserted into an expression vector to produce large quantities of the protein (see Figure 10–38), which can then be studied biochemically or structurally. In addition to producing protein, the gene or DNA can also be manipulated and introduced into cells or organisms to study its function. (NMR stands for nuclear magnetic resonance; see How We Know, pp. 162–163.)

ESSENTIAL CONCEPTS

- Recombinant DNA technology has revolutionized the study of cells, making it possible to pick out any gene at will from the thousands of genes in a cell and to determine its nucleotide sequence.
- A crucial element in this technology is the ability to cut a large DNA molecule into a specific and reproducible set of DNA fragments using restriction nucleases, each of which cuts the DNA double helix only at a particular nucleotide sequence.
- DNA fragments can be separated from one another on the basis of size by gel electrophoresis.
- Nucleic acid hybridization can detect any given DNA or RNA sequence in a mixture of nucleic acid fragments. This technique depends on highly specific base-pairing between a labeled, single-stranded DNA or RNA probe and another nucleic acid with a complementary sequence.
- DNA cloning techniques enable any DNA sequence to be selected from millions of other sequences and produced in unlimited amounts in pure form.
- DNA fragments can be joined together *in vitro* by using DNA ligase to form recombinant DNA molecules that are not found in nature.
- DNA fragments can be maintained and amplified by inserting them into a larger DNA molecule capable of replication, such as a plasmid. This recombinant DNA molecule is then introduced into a rapidly dividing host cell, usually a bacterium, so that the DNA is replicated at each cell division.
- A collection of cloned fragments of chromosomal DNA representing the complete genome of an organism is known as a genomic library. The library is often maintained as millions of clones of bacteria, each different clone carrying a different fragment of the organism's genome.
- cDNA libraries contain cloned DNA copies of the total mRNA of a particular type of cell or tissue. Unlike genomic DNA clones, cDNA clones contain predominantly protein-coding sequences; they lack introns, regulatory DNA sequences, and promoters. Thus they are useful when the cloned gene is needed to make a protein.
- The polymerase chain reaction (PCR) is a powerful form of DNA amplification that is carried out *in vitro* using a purified DNA polymerase. PCR requires prior knowledge of the sequence to be amplified, because two synthetic oligonucleotide primers must be synthesized that bracket the portion of DNA to be replicated.
- Historically, genes were cloned using hybridization techniques to identify the bacteria carrying the desired sequence in a DNA library. Today, a gene is usually cloned using PCR to specifically amplify it from a sample of DNA or mRNA.
- DNA sequencing techniques have become increasingly fast and cheap, so that the entire genomes of thousands of different organisms are now known, including thousands of individual humans.
- Using recombinant DNA techniques, a protein can be joined to a molecular tag, such as green fluorescent protein (GFP), which allows its movement to be tracked inside a cell and, in some cases, inside a living organism.
- *In situ* nucleic acid hybridization can be used to detect the precise location of genes on chromosomes and of RNAs in cells and tissues.
- DNA microarrays and RNA-Seq can be used to monitor the expression of tens of thousands of genes at once.
- Cloned genes can be altered *in vitro* and stably inserted into the genome of a cell or an organism to study their function. Such mutants are called transgenic organisms.

- The expression of particular genes can be inhibited in cells or organisms by the technique of RNA interference (RNAi), which prevents an mRNA from being translated into protein.
- Bacteria, yeasts, and mammalian cells can be engineered to synthesize large quantities of any protein whose gene has been cloned, making it possible to study proteins that are otherwise rare or difficult to isolate.

KEY TERMS

cDNA	hybridization
cDNA library	<i>in situ</i> hybridization
dideoxy (Sanger) DNA sequencing	plasmid
DNA cloning	polymerase chain reaction (PCR)
DNA library	recombinant DNA
DNA ligase	recombinant DNA technology
DNA microarray	reporter gene
gene knockout	restriction nuclease
gene replacement	RNA interference (RNAi)
genomic DNA library	RNA-Seq
green fluorescent protein (GFP)	transformation
	transgenic organism

QUESTIONS

QUESTION 10–5

What are the consequences for a DNA sequencing reaction if the ratio of dideoxynucleoside triphosphates to deoxyribonucleoside triphosphates is increased? What happens if this ratio is decreased?

QUESTION 10–6

Almost all the cells in an individual animal contain identical genomes. In an experiment, a tissue composed of several different cell types is fixed and subjected to *in situ* hybridization with a DNA probe to a particular gene. To your surprise, the hybridization signal is much stronger in some cells than in others. How might you explain this result?

QUESTION 10–7

After decades of work, Dr. Ricky M. isolated a small amount of attractase—an enzyme that produces a powerful human pheromone—from hair samples of Hollywood celebrities. To take advantage of attractase for his personal use, he obtained a complete genomic clone of the attractase gene, connected it to a strong bacterial promoter on an expression plasmid, and introduced the plasmid into *E. coli* cells. He was devastated to find that no attractase was produced in the cells. What is a likely explanation for his failure?

QUESTION 10–8

Which of the following statements are correct? Explain your answers.

- Restriction nucleases cut DNA at specific sites that are always located between genes.
- DNA migrates toward the positive electrode during electrophoresis.
- Clones isolated from cDNA libraries contain promoter sequences.
- PCR utilizes a heat-stable DNA polymerase because for each amplification step, double-stranded DNA must be heat-denatured.
- Digestion of genomic DNA with AluI, a restriction enzyme that recognizes a four-nucleotide sequence, produces fragments that are all exactly 256 nucleotides in length.
- To make a cDNA library, both a DNA polymerase and a reverse transcriptase must be used.
- DNA fingerprinting by PCR relies on the fact that different individuals have different numbers of repeats in STR regions in their genome.
- It is possible for a coding region of a gene to be present

in a genomic library prepared from a particular tissue but to be absent from a cDNA library prepared from the same tissue.

QUESTION 10–9

- A. What is the sequence of the DNA that was used in the sequencing reaction shown in [Figure Q10–9](#)?

The four lanes show the products of sequencing reactions that contained ddG (lane 1), ddA (lane 2), ddt (lane 3), and ddC (lane 4). The numbers to the right of the autoradiograph represent the positions of marker DNA fragments of 50 and 116 nucleotides.

- B. This DNA was derived from the middle of a cDNA clone of a mammalian protein. Using the genetic code table (see [Figure 7–25](#)), can you determine the amino acid sequence of this portion of the protein?

QUESTION 10–10

- A. How many different DNA fragments would you expect to obtain if you cleaved human genomic DNA with HaeIII? (Recall that there are 3×10^9 nucleotide pairs per haploid genome.) How many fragments would you expect with EcoRI?

- B. Human genomic libraries used for DNA sequencing are often made from fragments obtained by cleaving human DNA with HaeIII in such a way that the DNA is only partially digested; that is, not all the possible HaeIII sites have been cleaved. What is a possible reason for doing this?

QUESTION 10–11

A molecule of double-stranded DNA was cleaved with restriction nucleases, and the resulting products were separated by gel electrophoresis ([Figure Q10–11](#)). DNA fragments of known sizes were electrophoresed on the same gel for use as size markers (left lane). The size of the

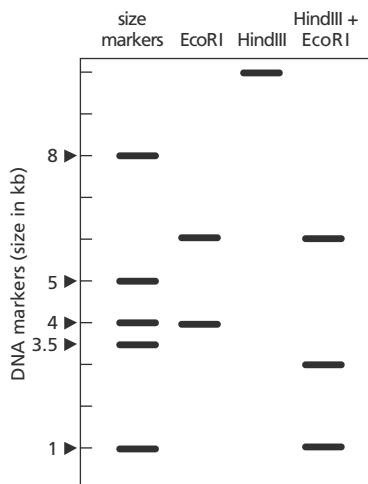


Figure Q10–11

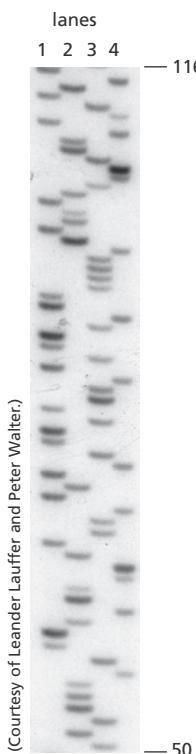


Figure Q10–9

DNA markers is given in kilobase pairs (kb), where 1 kb = 1000 nucleotide pairs. Using the size markers as a guide, estimate the length of each restriction fragment obtained. From this information, construct a map of the original DNA molecule indicating the relative positions of all the restriction enzyme cleavage sites.

QUESTION 10–12

You have isolated a small amount of a rare protein. You cleaved the protein into fragments using proteases, separated some of the fragments by chromatography, and determined their amino acid sequence. Unfortunately, as is often the case when only small amounts of protein are available, you obtained only three short stretches of amino acid sequence from the protein:

1. Trp-Met-His-His-Lys
2. Leu-Ser-Arg-Leu-Arg
3. Tyr-Phe-Gly-Met-Gln

A. Using the genetic code (see [Figure 7–25](#)), design a collection of DNA probes specific for each peptide that could be used to detect the gene in a cDNA library by hybridization. Which of the three collections of oligonucleotide probes would it be preferable to use first? Explain your answer. (Hint: the genetic code is redundant, so each peptide has multiple potential coding sequences.)

B. You have also been able to determine that the Gln of your peptide #3 is the C-terminal (i.e., the final) amino acid of your protein. How would you go about designing oligonucleotide primers that could be used to amplify a portion of the gene from a cDNA library using PCR?

C. Suppose the PCR amplification in (B) yields a DNA that is precisely 300 nucleotides long. Upon determining the nucleotide sequence of this DNA, you find the sequence CTATCACGCCTAGG approximately in its middle. What would you conclude from these observations?

QUESTION 10–13

Assume that a DNA sequencing reaction is carried out as shown in [Figure 10–20](#), except that the four different dideoxyribonucleoside triphosphates are modified so that each contains a covalently attached dye of a different color (which does not interfere with its incorporation into the DNA chain). What would the products be if you added a mixture of all four of these labeled dideoxyribonucleoside triphosphates along with the four unlabeled deoxyribonucleoside triphosphates into a single sequencing reaction? What would the results look like if you electrophoresed these products in a single lane of a gel?

QUESTION 10–14

Genomic DNA clones are often used to “walk” along a chromosome. In this approach, one cloned DNA is used to isolate other clones that contain overlapping DNA sequences ([Figure Q10–14](#)). Using this method, it is possible to build up a long stretch of DNA and thus identify new genes in near proximity to a previously cloned gene.

- A. Would it be faster to use cDNA clones in this method, because they do not contain any intron sequences?

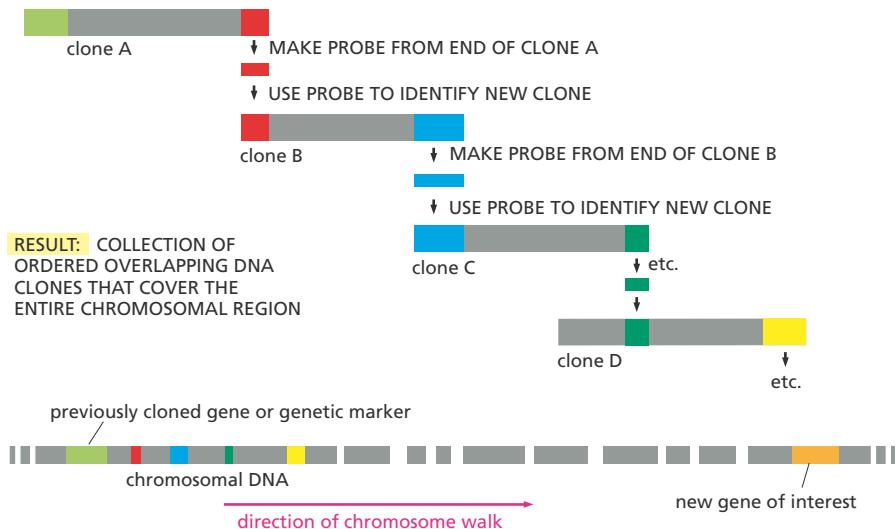


Figure Q10-14

- B. What would happen if you encountered a repetitive DNA sequence, like the *L1* transposon (see Figure 9-17), which is found in many copies and in many different places in the genome?

QUESTION 10-15

There has been a colossal snafu in the maternity ward of your local hospital. Four sets of male twins, born within an hour of each other, were inadvertently shuffled in the excitement occasioned by that unlikely event. You have been called in to set things straight. As a first step, you would like to match each baby with his twin. (Many newborns look alike so you don't want to rely on appearance alone.) To that end you analyze a small blood sample from each infant using a hybridization probe that detects short tandem repeats (STRs) located in widely scattered regions of the genome. The results are shown in Figure Q10-15.

- Which infants are twins? Which are identical twins?
- How could you match a pair of twins to the correct parents?



Figure Q10-15



Figure Q10-16

(Courtesy of John Bedbrook and DNA Plant Technology Corporation)

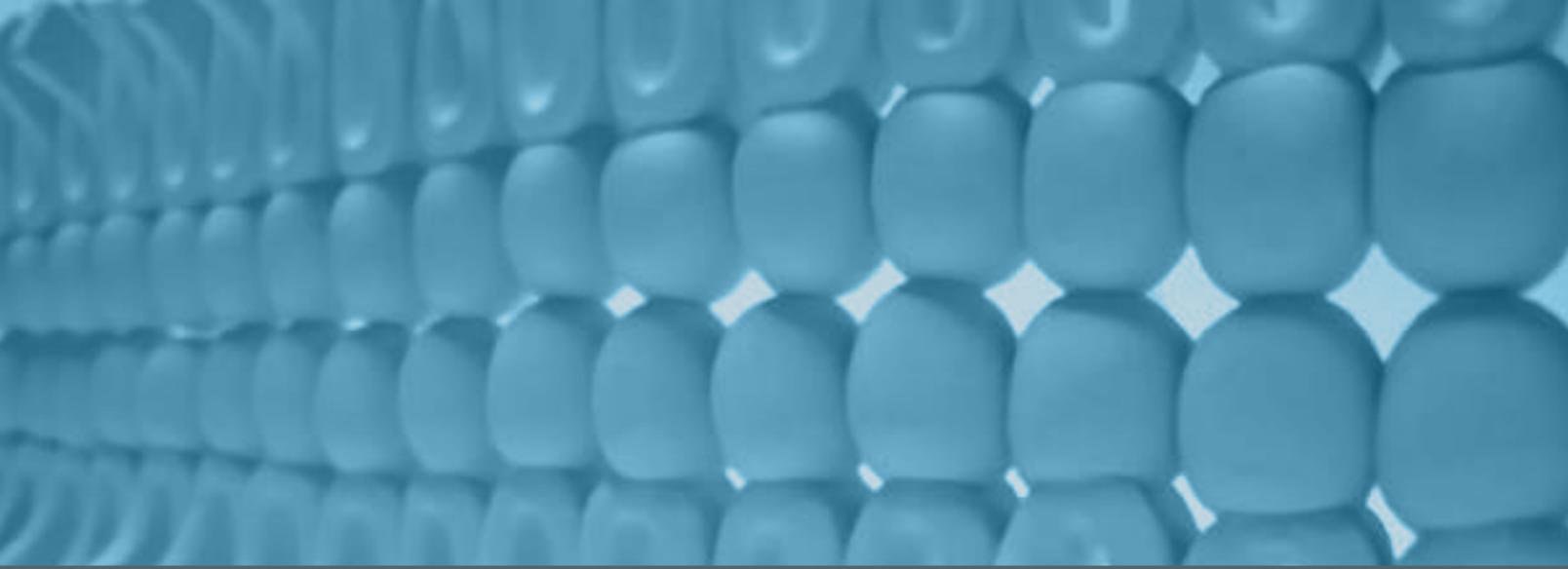
QUESTION 10-16

One of the first organisms that was genetically modified using recombinant DNA technology was a bacterium that normally lives on the surface of strawberry plants. This bacterium makes a protein, called ice-protein, that causes the efficient formation of ice crystals around it when the temperature drops to just below freezing. Thus, strawberries harboring this bacterium are particularly susceptible to frost damage because their cells are destroyed by the ice crystals. Consequently, strawberry farmers have a considerable interest in preventing ice crystallization.

A genetically engineered version of this bacterium was constructed in which the ice-protein gene was knocked out. The mutant bacteria were then introduced in large numbers into strawberry fields, where they displaced the normal bacteria by competition for their ecological niche. This approach has been successful: strawberries bearing the mutant bacteria show a much reduced susceptibility to frost damage.

At the time they were first carried out, the initial open-field trials triggered an intense debate because they represented the first release into the environment of an organism that had been genetically engineered using recombinant DNA technology. Indeed, all preliminary experiments were carried out with extreme caution and in strict containment (Figure Q10-16).

Do you think that bacteria lacking the ice-protein could be isolated without the use of modern DNA technology? Is it likely that such mutations have already occurred in nature? Would the use of a mutant bacterial strain isolated from nature be of lesser concern? Should we be concerned about the risks posed by the application of recombinant DNA techniques in agriculture and medicine? Explain your answers.



CHAPTER ELEVEN

11

Membrane Structure

A living cell is a self-reproducing system of molecules held inside a container. That container is the **plasma membrane**—a protein-studded, fatty film so thin that it cannot be seen directly in the light microscope. Every cell on Earth uses such a membrane to separate and protect its chemical components from the outside environment. Without membranes, there would be no cells, and thus no life.

The structure of the plasma membrane is simple: it consists of a two-ply sheet of lipid molecules about 5 nm—or 50 atoms—thick, into which proteins have been inserted. Its properties, however, are unlike those of any sheet of material we are familiar with in the everyday world. Although it serves as a barrier to prevent the contents of the cell from escaping and mixing with the surrounding medium (**Figure 11–1**), the plasma membrane does much more than that. If a cell is to survive and grow, nutrients must pass inward across the plasma membrane, and waste products must pass out. To facilitate this exchange, the membrane is penetrated by highly selective channels and transporters—proteins that allow specific,

THE LIPID BILAYER

MEMBRANE PROTEINS

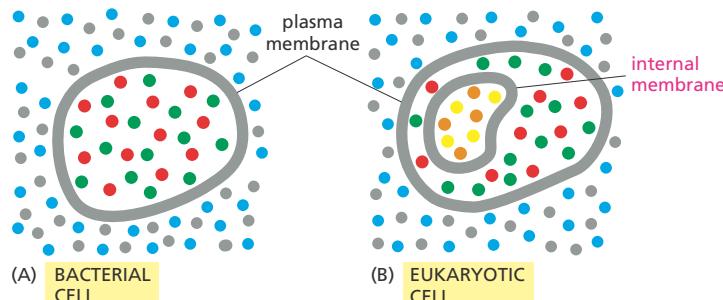
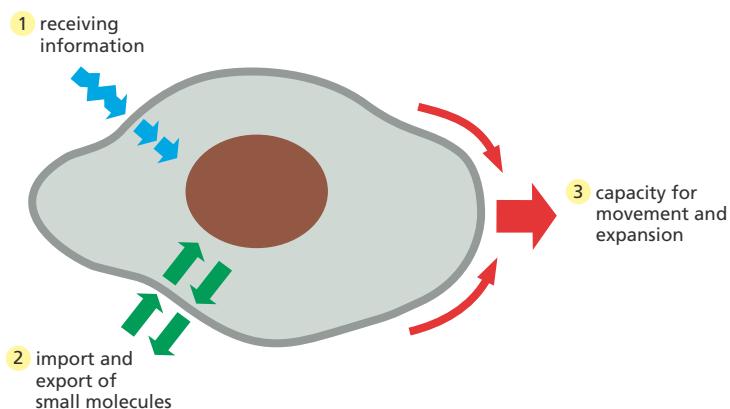


Figure 11–1 Cell membranes act as **selective barriers**. The plasma membrane separates a cell from its surroundings, enabling the molecular composition of a cell to differ from that of its environment. (A) In some bacteria, the plasma membrane is the only membrane. (B) Eukaryotic cells also have internal membranes that enclose individual organelles. All cell membranes prevent molecules on one side from freely mixing with those on the other, as schematically indicated by the colored dots.

Figure 11–2 The plasma membrane is involved in cell communication, import and export of molecules, and cell growth and motility. (1) Receptor proteins in the plasma membrane enable the cell to receive signals from the environment; (2) transport proteins in the membrane enable the import and export of small molecules; (3) the flexibility of the membrane and its capacity for expansion allow the cell to grow, change shape, and move.



small molecules and ions to be imported and exported. Other proteins in the membrane act as sensors, or receptors, that enable the cell to receive information about changes in its environment and respond to them in appropriate ways. The mechanical properties of the plasma membrane are equally remarkable. When a cell grows or changes shape, so does its membrane: it enlarges in area by adding new membrane without ever losing its continuity, and it can deform without tearing ([Figure 11–2](#)). If the membrane is pierced, it neither collapses like a balloon nor remains torn; instead, it quickly reseals.

As shown in [Figure 11–1](#), the simplest bacteria have only a single membrane—the plasma membrane—whereas eukaryotic cells also contain internal membranes that enclose intracellular compartments. The internal membranes form various organelles, including the endoplasmic reticulum, Golgi apparatus, and mitochondria ([Figure 11–3](#)). Although these internal membranes are constructed on the same principles as the plasma membrane, there are subtle differences in their composition, especially in their resident proteins.

Regardless of their location, all cell membranes are composed of lipids and proteins and share a common general structure ([Figure 11–4](#)). The lipids are arranged in two closely apposed sheets, forming a *lipid bilayer* (see [Figure 11–4B](#) and C). This lipid bilayer serves as a permeability barrier to most water-soluble molecules. The proteins carry out the other functions of the membrane and give different membranes their individual characteristics.

In this chapter, we consider the structure of biological membranes and the organization of their two main constituents: lipids and proteins. Although we focus mainly on the plasma membrane, most of the concepts we discuss also apply to internal membranes. The functions of cell membranes, including their role in cell communication, the transport of small molecules, and energy generation, are considered in later chapters.

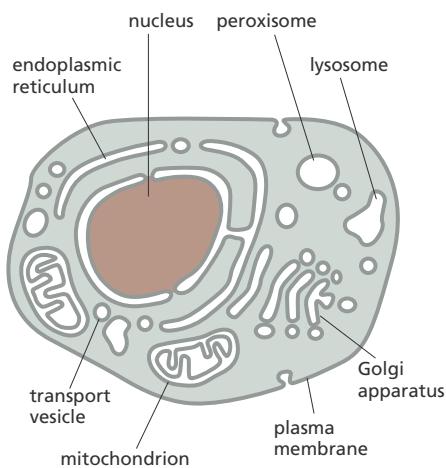


Figure 11–3 Internal membranes form many different compartments in a eukaryotic cell. Some of the main membrane-enclosed organelles in a typical animal cell are shown here. Note that the nucleus and mitochondria are each enclosed by two membranes.

THE LIPID BILAYER

Because cells are filled with—and surrounded by—water, the structure of cell membranes is determined by the way membrane lipids behave in a watery (aqueous) environment. In this section, we take a closer look at the **lipid bilayer**, which constitutes the fundamental structure of all cell membranes. We consider how lipid bilayers form, how they are maintained, and how their properties establish the general properties of all cell membranes.

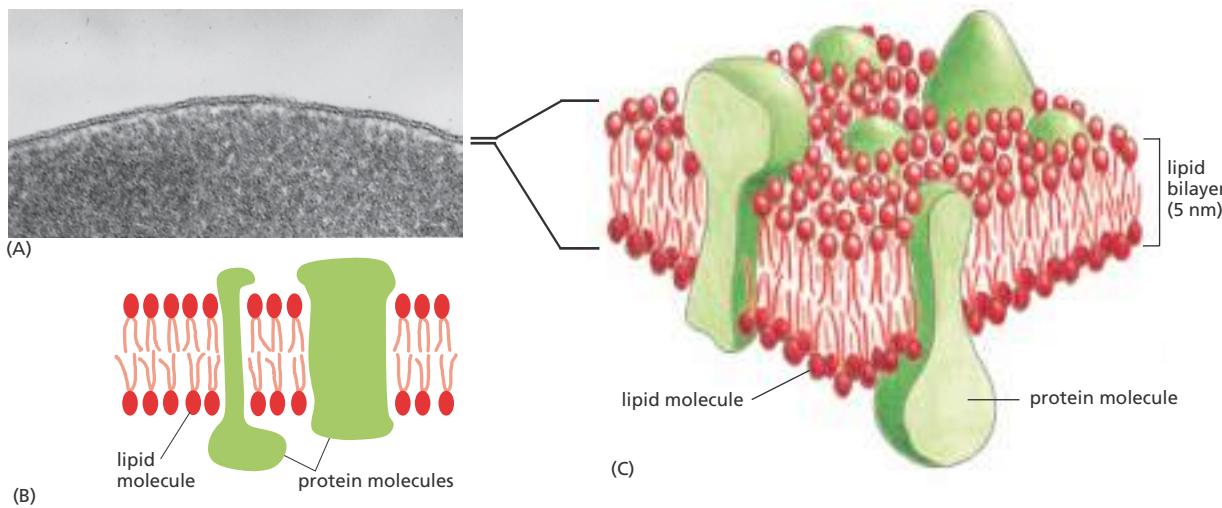


Figure 11-4 A cell membrane can be viewed in a number of ways. (A) An electron micrograph of a plasma membrane of a human red blood cell seen in cross section. (B and C) Schematic drawings showing two-dimensional and three-dimensional views of a cell membrane. (A, courtesy of Daniel S. Friend.)

Membrane Lipids Form Bilayers in Water

The lipids in cell membranes combine two very different properties in a single molecule: each lipid has a hydrophilic ("water-loving") head and a hydrophobic ("water-fearing") tail. The most abundant lipids in cell membranes are the **phospholipids**, which have a phosphate-containing, hydrophilic head linked to a pair of hydrophobic tails (Figure 11–5). **Phosphatidylcholine**, for example, has the small molecule choline attached to a phosphate group as its hydrophilic head (Figure 11–6).

Molecules with both hydrophilic and hydrophobic parts are termed **amphiphatic**, a property shared by other types of membrane lipids, including the cholesterol, which is found in animal cell membranes and the glycolipids, which have sugars as part of their hydrophilic head (Figure 11–7). Having both hydrophilic and hydrophobic parts plays a crucial part in driving these lipid molecules to assemble into bilayers in an aqueous environment.

As discussed in Chapter 2 (see Panel 2–2, pp. 68–69), hydrophilic molecules dissolve readily in water because they contain either charged groups or uncharged polar groups that can form either electrostatic attractions or hydrogen bonds with water molecules (Figure 11–8). Hydrophobic molecules, by contrast, are insoluble in water because all—or almost all—of their atoms are uncharged and nonpolar; they therefore cannot form favorable interactions with water molecules. Instead, they force adjacent water molecules to reorganize into a cagelike structure around them (Figure 11–9). Because this cagelike structure is more highly ordered than the rest of the water, its formation requires free energy. This energy cost is minimized when the hydrophobic molecules cluster together, limiting their contacts with the surrounding water molecules. Thus purely hydrophobic molecules, like the fats found in animal fat cells and the oils found in plant seeds (Figure 11–10A), coalesce into a single large drop when dispersed in water.

Amphiphatic molecules, such as phospholipids (Figure 11–10B), are subject to two conflicting forces: the hydrophilic head is attracted to water, while the hydrophobic tails shun water and seek to aggregate with other hydrophobic molecules. This conflict is beautifully resolved by the

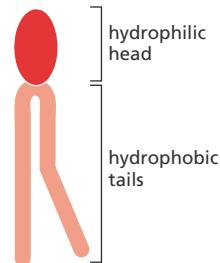


Figure 11-5 A typical membrane lipid molecule has a hydrophilic head and two hydrophobic tails.

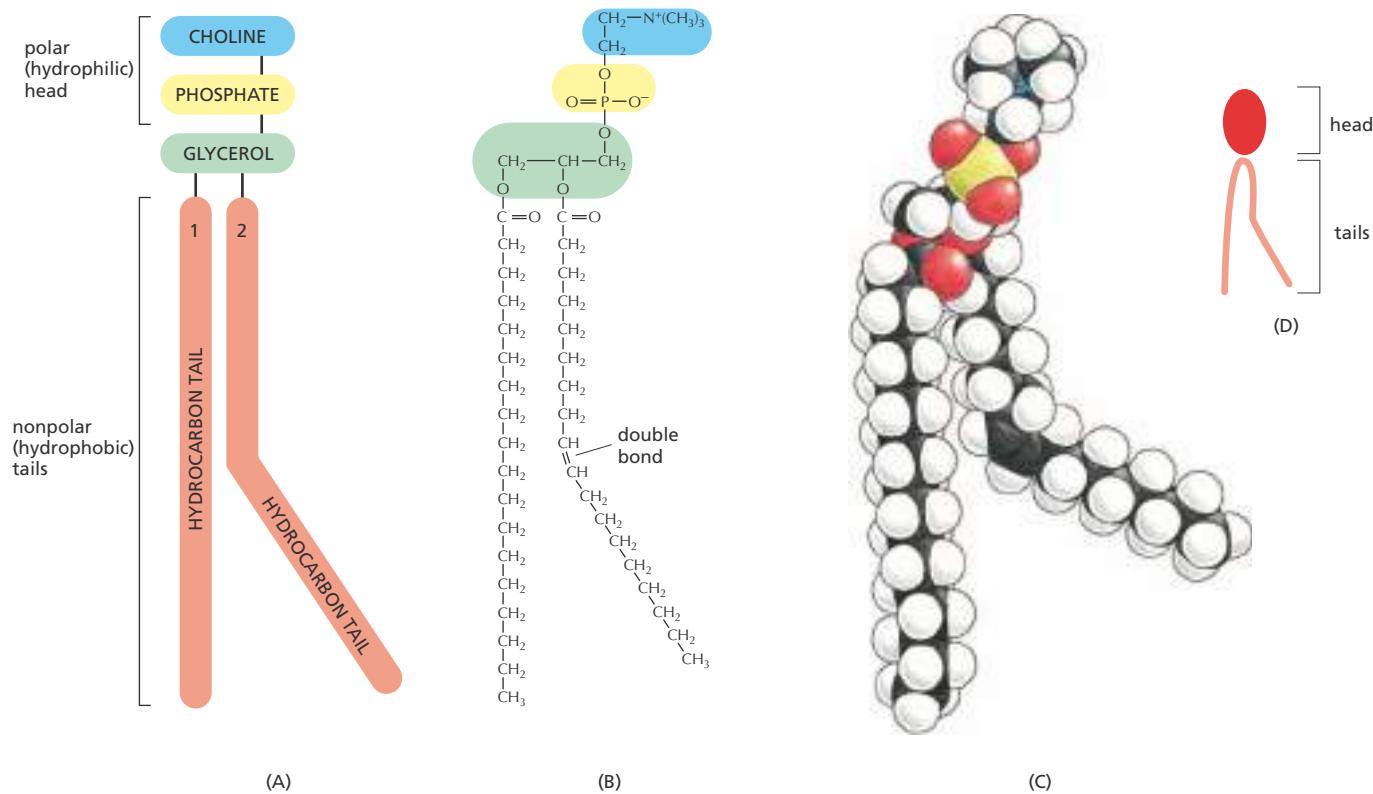


Figure 11–6 Phosphatidylcholine is the most common phospholipid in cell membranes. It is represented schematically in (A), as a chemical formula in (B), as a space-filling model in (C), and as a symbol in (D). This particular phospholipid is built from five parts: the hydrophilic head, which consists of choline linked to a phosphate group; two hydrocarbon chains, which form the hydrophobic tails; and a molecule of glycerol, which links the head to the tails. Each of the hydrophobic tails is a fatty acid—a hydrocarbon chain with a $-COOH$ group at one end—which has been attached to glycerol via this group. A kink in one of the hydrocarbon chains occurs where there is a double bond between two carbon atoms. (The “phosphatidyl” part of the name of a phospholipid refers to the phosphate-glycerol-fatty acid portion of the molecule.)

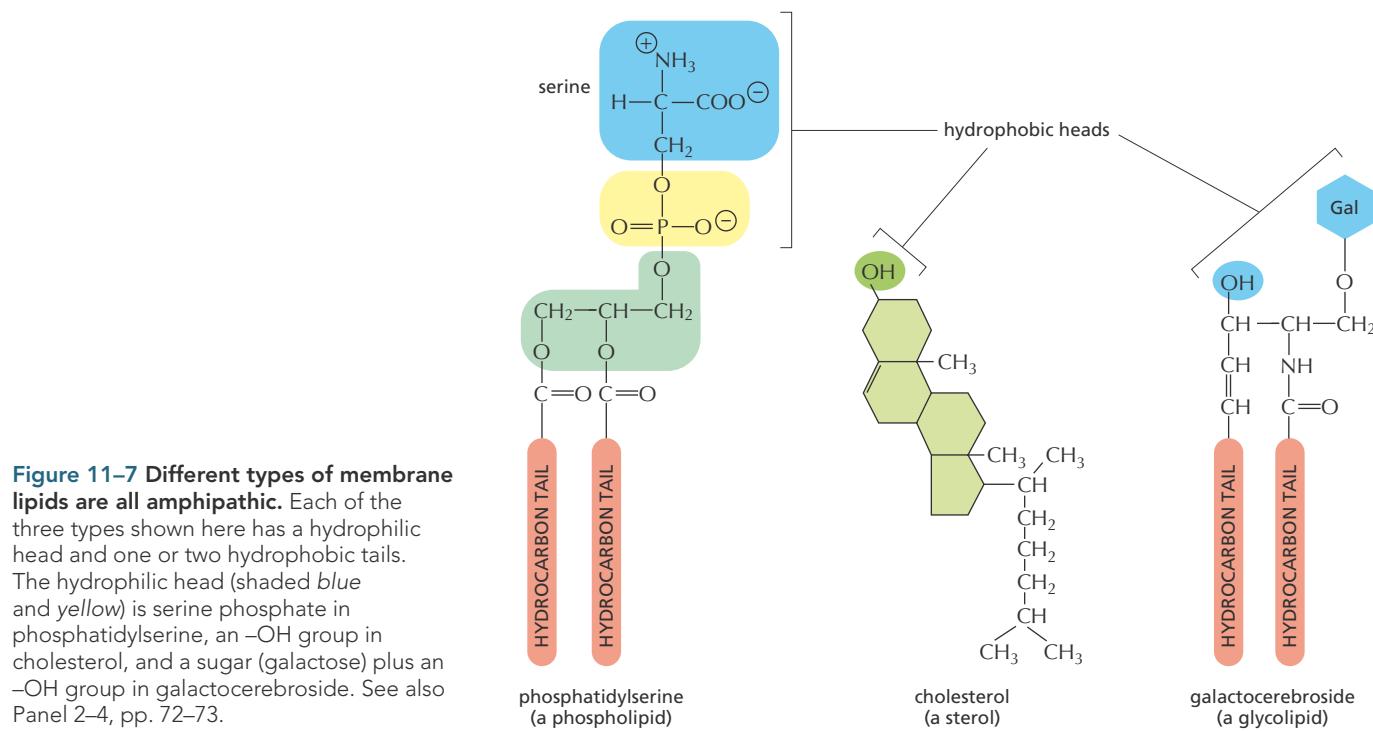


Figure 11–7 Different types of membrane lipids are all amphipathic. Each of the three types shown here has a hydrophilic head and one or two hydrophobic tails. The hydrophilic head (shaded blue and yellow) is serine phosphate in phosphatidylserine, an $-OH$ group in cholesterol, and a sugar (galactose) plus an $-OH$ group in galactocerebroside. See also Panel 2–4, pp. 72–73.

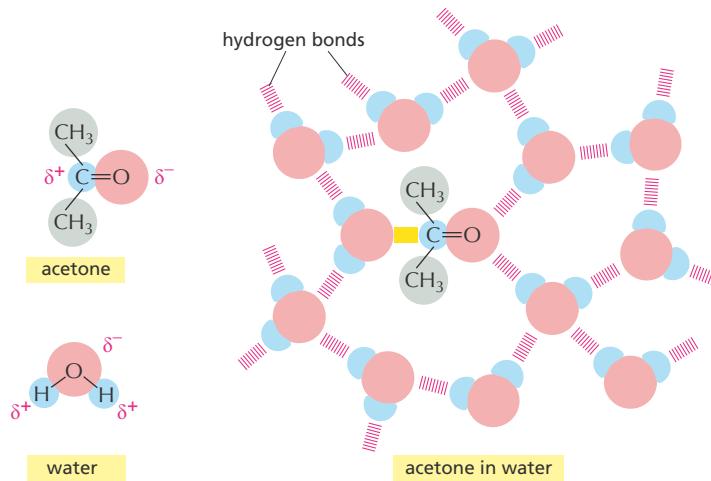


Figure 11–8 A hydrophilic molecule attracts water molecules. Both acetone and water are polar molecules; thus acetone readily dissolves in water. Polar atoms are shown in red and blue, with δ^- indicating a partial negative charge, and δ^+ indicating a partial positive charge. Hydrogen bonds (red) and an electrostatic attraction (yellow) form between acetone and the surrounding water molecules. Nonpolar groups are shown in gray.

formation of a lipid bilayer—an arrangement that satisfies all parties and is energetically most favorable. The hydrophilic heads face water on both surfaces of the bilayer; but the hydrophobic tails are all shielded from the water, as they lie next to one another in the interior, like the filling in a sandwich (Figure 11–11).

The same forces that drive the amphipathic molecules to form a bilayer help to make the bilayer self-sealing. Any tear in the sheet will create a free edge that is exposed to water. Because this situation is energetically unfavorable, the molecules of the bilayer will spontaneously rearrange to eliminate the free edge. If the tear is small, this spontaneous rearrangement will exclude the water molecules and lead to repair of the bilayer, restoring a single continuous sheet. If the tear is large, the sheet may begin to fold in on itself and break up into separate closed vesicles. In either case, the overriding principle is that free edges are quickly eliminated.

The prohibition on free edges has a profound consequence: the only way a finite amphipathic sheet can avoid having free edges is to bend and seal,

QUESTION 11–1

Water molecules are said “to reorganize into a cagelike structure” around hydrophobic compounds (e.g., see Figure 11–9). This seems paradoxical because water molecules do not interact with the hydrophobic compound. So how could they “know” about its presence and change their behavior to interact differently with one another? Discuss this argument and, in doing so, develop a clear concept of what is meant by a “cagelike” structure. How does it compare to ice? Why would this cagelike structure be energetically unfavorable?

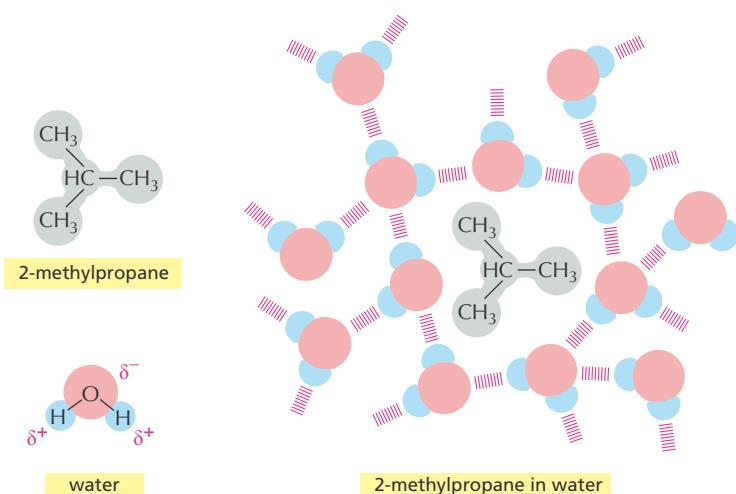


Figure 11–9 A hydrophobic molecule tends to avoid water. Because the 2-methylpropane molecule is entirely hydrophobic, it cannot form favorable interactions with water. This causes the adjacent water molecules to reorganize into a cagelike structure around it, in order to maximize their hydrogen bonds with each other.

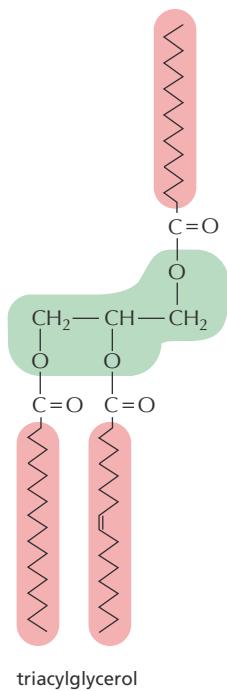


Figure 11–10 Fat molecules are hydrophobic, unlike phospholipids.

Triacylglycerols, which are the main constituents of animal fats and plant oils, are entirely hydrophobic. Here, the third hydrophobic tail of the triacylglycerol molecule in (A) is drawn facing upward for comparison with the phospholipid (see Figure 11–6), although normally it is depicted facing down (see Panel 2–4, pp. 72–73).

forming a boundary around a closed space (**Figure 11–12**). Therefore, amphipathic molecules such as phospholipids necessarily assemble into self-sealing containers that define closed compartments. This remarkable behavior, fundamental to the creation of a living cell, is simply a result of the property that each molecule is hydrophilic at one end and hydrophobic at the other.

The Lipid Bilayer Is a Flexible Two-dimensional Fluid

The aqueous environment inside and outside a cell prevents membrane lipids from escaping from the bilayer, but nothing stops these molecules from moving about and changing places with one another within the plane of the bilayer. The membrane therefore behaves as a two-dimensional fluid, a fact that is crucial for membrane function and integrity (**Movie 11.1**).

The lipid bilayer is also flexible—that is, it is able to bend. Like fluidity, flexibility is important for membrane function, and it sets a lower limit of about 25 nm to the size of vesicle that cell membranes can form.

The fluidity of lipid bilayers can be studied using synthetic lipid bilayers, which are easily produced by the spontaneous aggregation of amphipathic lipid molecules in water. Pure phospholipids, for example, will form closed spherical vesicles, called liposomes, when added to water; they vary in size from about 25 nm to 1 mm in diameter (**Figure 11–13**).

Such simple synthetic bilayers allow the movements of the lipid molecules to be measured. These measurements reveal that some types of movement are rare, while others are frequent and rapid. Thus, in synthetic lipid bilayers, phospholipid molecules very rarely tumble from one half of the bilayer, or monolayer, to the other. Without proteins to facilitate the process, it is estimated that this event, called “flip-flop,” occurs less than once a month for any individual lipid molecule under conditions

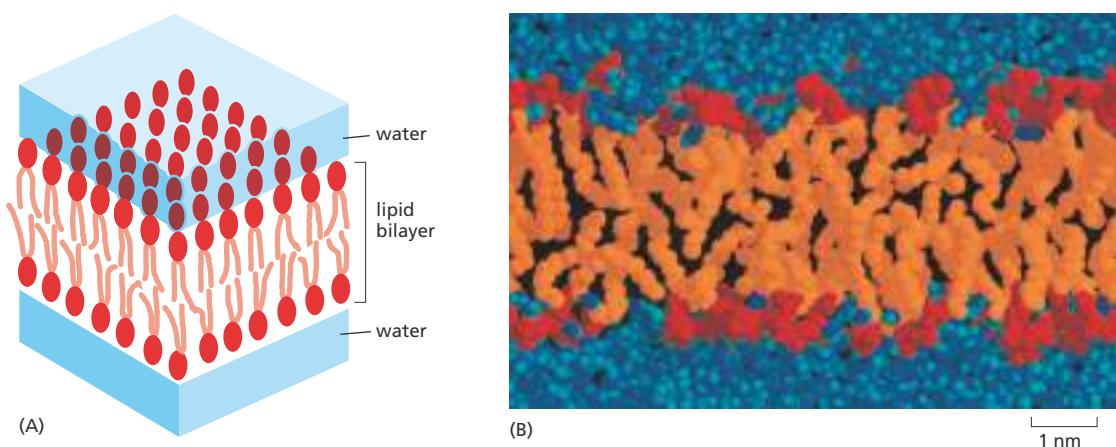


Figure 11–11 Amphipathic phospholipids form a bilayer in water. (A) Schematic drawing of a phospholipid bilayer in water. (B) Computer simulation showing the phospholipid molecules (red heads and orange tails) and the surrounding water molecules (blue) in a cross section of a lipid bilayer. (B, adapted from *Science* 262:223–228, 1993, with permission from the AAAS; courtesy of R. Venable and R. Pastor.)

Figure 11–12 Phospholipid bilayers spontaneously close in on themselves to form sealed compartments. The closed structure is stable because it avoids the exposure of the hydrophobic hydrocarbon tails to water, which would be energetically unfavorable.

similar to those in a cell. On the other hand, as the result of random thermal motions, lipid molecules continuously exchange places with their neighbors in the same monolayer. This exchange leads to rapid lateral diffusion of lipid molecules within the plane of each monolayer, so that, for example, a lipid in an artificial bilayer may diffuse a length equal to that of an entire bacterial cell (~2 μm) in about one second.

Similar studies show that individual lipid molecules not only flex their hydrocarbon tails, but they also rotate rapidly about their long axis—some reaching speeds of 500 revolutions per second. Studies of whole cells—and isolated cell membranes—indicate that lipid molecules in cell membranes undergo the same movements as they do in synthetic bilayers. The movements of membrane phospholipid molecules are summarized in **Figure 11–14**.

The Fluidity of a Lipid Bilayer Depends on Its Composition

The fluidity of a cell membrane—the ease with which its lipid molecules move within the plane of the bilayer—is important for membrane function and has to be maintained within certain limits. Just how fluid a lipid bilayer is at a given temperature depends on its phospholipid composition and, in particular, on the nature of the hydrocarbon tails: the closer and more regular the packing of the tails, the more viscous and less fluid the bilayer will be. Two major properties of hydrocarbon tails affect how tightly they pack in the bilayer: their length and the number of double bonds they contain.

A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another and therefore increases the fluidity of the bilayer. The hydrocarbon tails of membrane phospholipids vary in length between 14 and 24 carbon atoms, with 18–20 atoms being most usual. Most phospholipids contain one hydrocarbon tail that has one or more double bonds between adjacent carbon atoms, and a second tail with single bonds only (see Figure 11–6). The chain that harbors a double bond does not contain the maximum number of hydrogen atoms that could, in principle, be attached to its carbon backbone; it is thus said to be **unsaturated** with respect to hydrogen. The hydrocarbon tail with no double bonds has a full complement of hydrogen atoms and is said to be **saturated**. Each double bond in an unsaturated tail creates a small kink in the tail (see Figure 11–6), which makes it more difficult for the tails to pack against one another. For this reason, lipid bilayers that contain a large proportion of unsaturated hydrocarbon tails are more fluid than those with lower proportions.

In bacterial and yeast cells, which have to adapt to varying temperatures, both the lengths and the unsaturation of the hydrocarbon tails in the bilayer are constantly adjusted to maintain the membrane at a relatively constant fluidity: at higher temperatures, for example, the cell makes

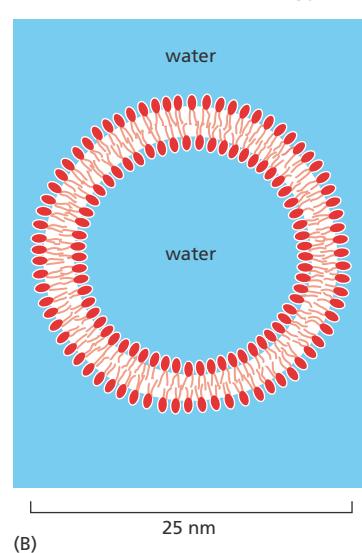
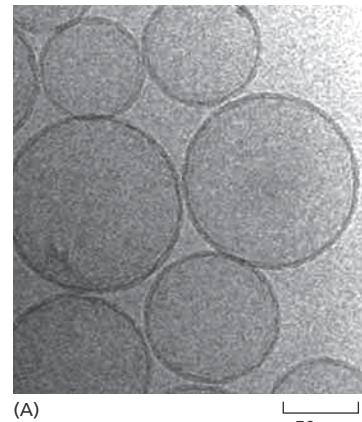
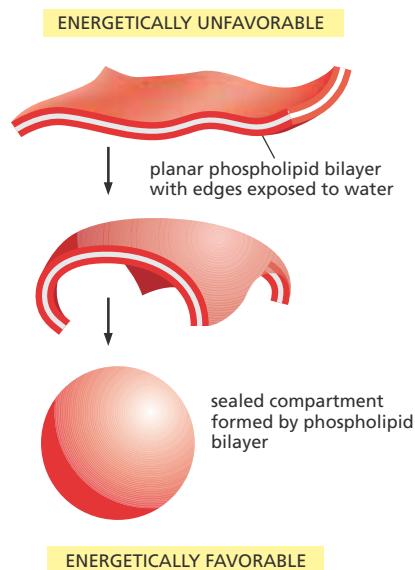


Figure 11–13 Pure phospholipids can form closed, spherical liposomes. (A) An electron micrograph of phospholipid vesicles (liposomes) showing the bilayer structure of the membrane. (B) A drawing of a small, spherical liposome seen in cross section. (A, courtesy of Jean Lepault.)

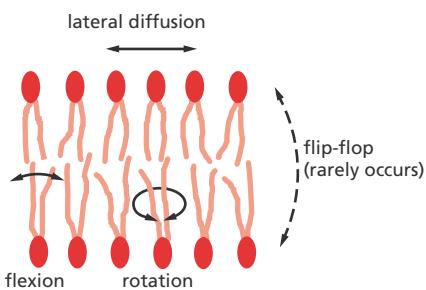


Figure 11-14 Membrane phospholipids are motile. The drawing shows the types of movement that phospholipid molecules undergo in a lipid bilayer. Because of these movements, the bilayer behaves as a two-dimensional fluid, in which the individual lipid molecules are able to move in their own monolayer. Note that lipid molecules do not move spontaneously from one monolayer to the other.

QUESTION 11-2

Five students in your class always sit together in the front row. This could be because (A) they really like each other or (B) nobody else in your class wants to sit next to them. Which explanation holds for the assembly of a lipid bilayer? Explain. Suppose, instead, that the other explanation held for lipid molecules. How would the properties of the lipid bilayer be different?

membrane lipids with tails that are longer and that contain fewer double bonds. A similar trick is used in the manufacture of margarine from vegetable oils. The fats produced by plants are generally unsaturated and therefore liquid at room temperature, unlike animal fats such as butter or lard, which are generally saturated and therefore solid at room temperature. Margarine is made of hydrogenated vegetable oils; their double bonds have been removed by the addition of hydrogen, so that they are more solid and butterlike at room temperature.

In animal cells, membrane fluidity is modulated by the inclusion of the sterol **cholesterol**. This molecule is present in especially large amounts in the plasma membrane, where it constitutes approximately 20% of the lipids in the membrane by weight. Because cholesterol molecules are short and rigid, they fill the spaces between neighboring phospholipid molecules left by the kinks in their unsaturated hydrocarbon tails (Figure 11-15). In this way, cholesterol tends to stiffen the bilayer, making it less flexible, as well as less permeable. The chemical properties of membrane lipids—and how they affect membrane fluidity—are reviewed in Movie 11.2.

For all cells, membrane fluidity is important for many reasons. It enables many membrane proteins to diffuse rapidly in the plane of the bilayer and to interact with one another, as is crucial, for example, in cell signaling (discussed in Chapter 16). It permits membrane lipids and proteins to diffuse from sites where they are inserted into the bilayer after their synthesis to other regions of the cell. It ensures that membrane molecules are distributed evenly between daughter cells when a cell divides. And, under appropriate conditions, it allows membranes to fuse with one another and mix their molecules (discussed in Chapter 15). If biological membranes were not fluid, it is hard to imagine how cells could live, grow, and reproduce.

Membrane Assembly Begins in the ER

In eukaryotic cells, new phospholipids are manufactured by enzymes bound to the cytosolic surface of the *endoplasmic reticulum* (ER; see Figure 11-3). Using free fatty acids as substrates (see Panel 2-4, pp. 72–73), the enzymes deposit the newly made phospholipids exclusively in the cytosolic half of the bilayer.

Despite this preferential treatment, cell membranes manage to grow evenly. So how do new phospholipids make it to the opposite monolayer?

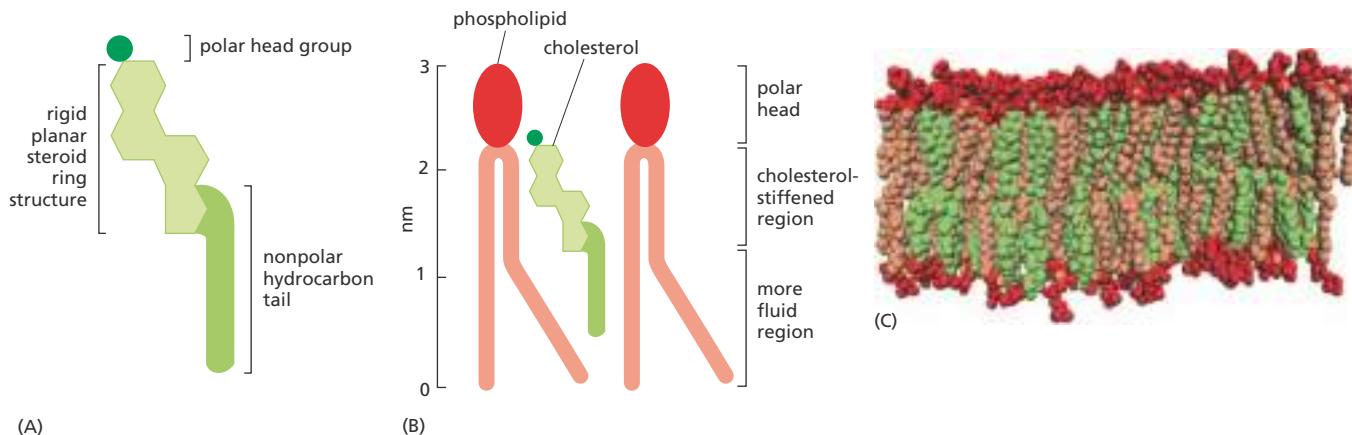


Figure 11-15 Cholesterol tends to stiffen cell membranes. (A) The shape of a cholesterol molecule. (B) How cholesterol fits into the gaps between phospholipid molecules in a lipid bilayer. (C) Space-filling model of the bilayer, with cholesterol molecules in green. The chemical formula of cholesterol is shown in Figure 11-7. (C, from H.L. Scott, *Curr. Opin. Struct. Biol.* 12: 499, 2002.)

As we saw in Figure 11–14, the transfer of lipids from one monolayer to the other rarely occur spontaneously. Instead, they are catalyzed by enzymes called *scramblases*, which remove randomly selected phospholipids from one half of the lipid bilayer and insert them in the other. As a result of this scrambling, newly made phospholipids are redistributed equally between each monolayer of the ER membrane (Figure 11–16A).

Some of this newly assembled membrane will remain in the ER; the rest will be used to supply fresh membrane to other compartments in the cell. Bits of membrane are continually pinching off the ER to form small, spherical vesicles that then fuse with other membranes, such as those of the Golgi apparatus. Additional vesicles bubble from the Golgi to become incorporated into the plasma membrane. We discuss this dynamic process of membrane transport in detail in Chapter 15.

Certain Phospholipids Are Confined to One Side of the Membrane

Most cell membranes are asymmetrical: the two halves of the bilayer often include strikingly different sets of phospholipids. But if membranes emerge from the ER with an evenly scrambled set of phospholipids, where does this asymmetry arise? It begins in the Golgi apparatus. The Golgi membrane contains another family of phospholipid-handling enzyme, called *flippases*. These enzymes remove specific phospholipids from the side of the bilayer facing the exterior space and flip them into the monolayer that faces the cytosol (Figure 11–16B).

The action of these flippases—and similar enzymes in the plasma membrane—initiates and maintains the asymmetric arrangement of phospholipids that is characteristic of the membranes of animal cells. This asymmetry is preserved as membranes bud from one organelle and fuse with another—or with the plasma membrane. This means that all

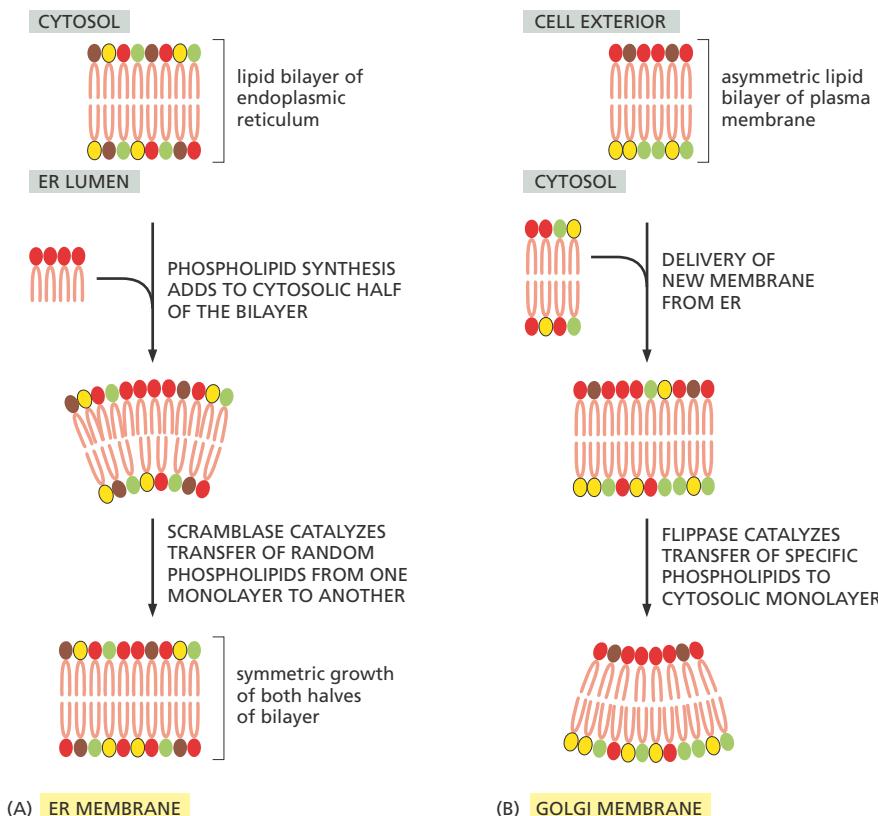


Figure 11–16 Newly synthesized phospholipids are added to the cytosolic side of the ER membrane and then redistributed by enzymes that transfer them from one half of the lipid bilayer to the other. (A) Biosynthetic enzymes bound to the cytosolic monolayer of the ER membrane (not shown) produce new phospholipids from free fatty acids and insert them into the cytosolic monolayer. Enzymes called *scramblases* then randomly transfer phospholipid molecules from one monolayer to the other, allowing the membrane to grow as a bilayer. (B) When membranes leave the ER and are incorporated in the Golgi, they encounter enzymes called *flippases*, which selectively remove phosphatidylserine (*light green*) and phosphatidylethanolamine (*yellow*) from the noncytosolic monolayer and flip them to the cytosolic side. This transfer leaves phosphatidylcholine (*red*) and sphingomyelin (*brown*) concentrated in the noncytosolic monolayer. The resulting curvature of the membrane may actually help drive subsequent vesicle budding.

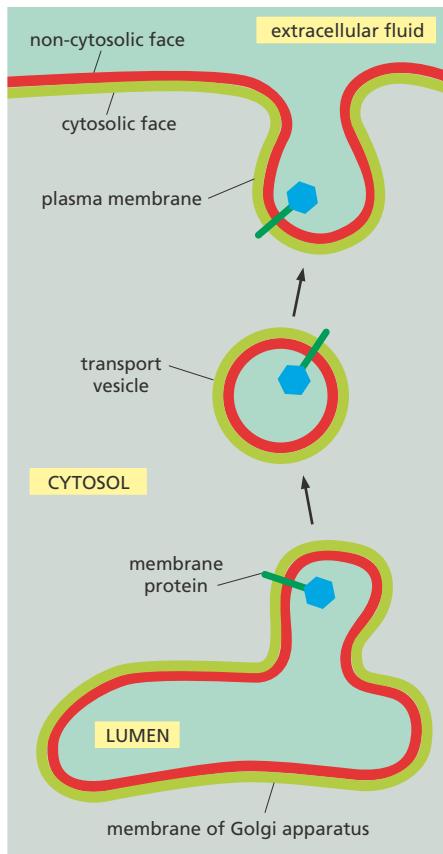


Figure 11–17 Membranes retain their orientation during transfer between cell compartments. Membranes are transported by a process of vesicle budding and fusing. Here, a vesicle is shown budding from the Golgi apparatus and fusing with the plasma membrane. Note that the orientations of both the membrane lipids and proteins are preserved during the process: the original cytosolic surface of the lipid bilayer (green) remains facing the cytosol, and the noncytosolic surface (red) continues to face away from the cytosol, toward the lumen of the Golgi or transport vesicle—or toward the extracellular compartment. Similarly, the glycoprotein shown here remains in the same orientation, with its attached sugar facing the noncytosolic side.

cell membranes have distinct “inside” and “outside” faces: the cytosolic monolayer always faces the cytosol, while the noncytosolic monolayer is exposed to either the cell exterior—in the case of the plasma membrane—or to the interior space (*lumen*) of an organelle. This conservation of orientation applies not only to the phospholipids that make up the membrane, but to any proteins that might be inserted in the membrane (Figure 11–17). For membrane proteins, this positioning is very important, as their orientation within the lipid bilayer is often crucial for their function (see Figure 11–19).

Among lipids, those that show the most dramatically lopsided distribution in cell membranes are the glycolipids, which are located mainly in the plasma membrane, and only in the noncytosolic half of the bilayer (Figure 11–18). Their sugar groups face the cell exterior, where they form part of a continuous coat of carbohydrate that surrounds and protects animal cells. Glycolipid molecules acquire their sugar groups in the Golgi apparatus, where the enzymes that engineer this chemical modification are confined. These enzymes are oriented such that sugars are added only to lipid molecules in the noncytosolic half of the bilayer. Once a glycolipid molecule has been created in this way, it remains trapped in this monolayer, as there are no flippases that transfer glycolipids to the cytosolic side. Thus, when a glycolipid molecule is finally delivered to the plasma membrane, it displays its sugars to the exterior of the cell.

Other lipid molecules show different types of asymmetric distributions, which relate to their specific functions. For example, the inositol phospholipids—a minor component of the plasma membrane—have a special

QUESTION 11–3

It seems paradoxical that a lipid bilayer can be fluid yet asymmetrical. Explain.

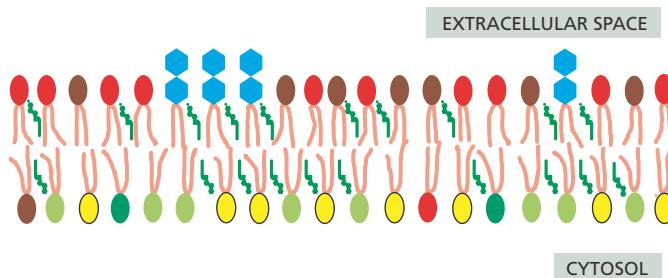


Figure 11–18 Phospholipids and glycolipids are distributed asymmetrically in the lipid bilayer of a eukaryotic plasma membrane. Phosphatidylcholine (red) and sphingomyelin (brown) are concentrated in the noncytosolic monolayer, whereas phosphatidylserine (light green), and phosphatidylethanolamine (yellow) are found mainly on the cytosolic side. In addition to these phospholipids, phosphatidylinositols (dark green), a minor constituent of the plasma membrane, are shown in the cytosolic monolayer, where they participate in cell signaling. Glycolipids are drawn with hexagonal blue head groups to represent sugars; these are found exclusively in the noncytosolic monolayer of the membrane. Within the bilayer, cholesterol (green) is distributed almost equally in both monolayers.

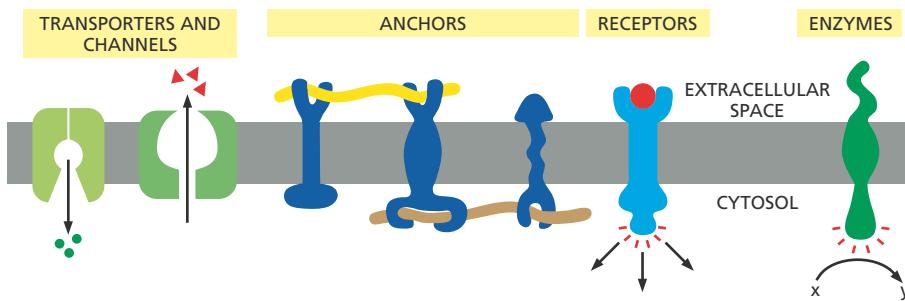


Figure 11–19 Plasma membrane proteins have a variety of functions.

role in relaying signals from the cell surface to the cell interior (discussed in Chapter 16); thus they are concentrated in the cytosolic half of the lipid bilayer.

MEMBRANE PROTEINS

Although the lipid bilayer provides the basic structure of all cell membranes and serves as a permeability barrier to the hydrophilic molecules on either side of it, most membrane functions are carried out by **membrane proteins**. In animals, proteins constitute about 50% of the mass of most plasma membranes, the remainder being lipid plus the relatively small amounts of carbohydrate found on some of the lipids (glycolipids) and many of the proteins (glycoproteins). Because lipid molecules are much smaller than proteins, however, a cell membrane typically contains about 50 times more lipid molecules than protein molecules (see Figure 11–4C).

Membrane proteins serve many functions. Some transport particular nutrients, metabolites, and ions across the lipid bilayer. Others anchor the membrane to macromolecules on either side. Still others function as receptors that detect chemical signals in the cell's environment and relay them into the cell interior, or work as enzymes to catalyze specific reactions at the membrane (Figure 11–19 and Table 11–1). Each type of cell membrane contains a different set of proteins, reflecting the specialized functions of the particular membrane. In this section, we discuss the structure of membrane proteins and how they associate with the lipid bilayer.

TABLE 11–1 SOME EXAMPLES OF PLASMA MEMBRANE PROTEINS AND THEIR FUNCTIONS

Functional Class	Protein Example	Specific Function
Transporters	Na ⁺ pump	actively pumps Na ⁺ out of cells and K ⁺ in (discussed in Chapter 12)
Ion channels	K ⁺ leak channel	allows K ⁺ ions to leave cells, thereby having a major influence on cell excitability (discussed in Chapter 12)
Anchors	integrins	link intracellular actin filaments to extracellular matrix proteins (discussed in Chapter 20)
Receptors	platelet-derived growth factor (PDGF) receptor	binds extracellular PDGF and, as a consequence, generates intracellular signals that cause the cell to grow and divide (discussed in Chapters 16 and 18)
Enzymes	adenylyl cyclase	catalyzes the production of the small intracellular signaling molecule cyclic AMP in response to extracellular signals (discussed in Chapter 16)

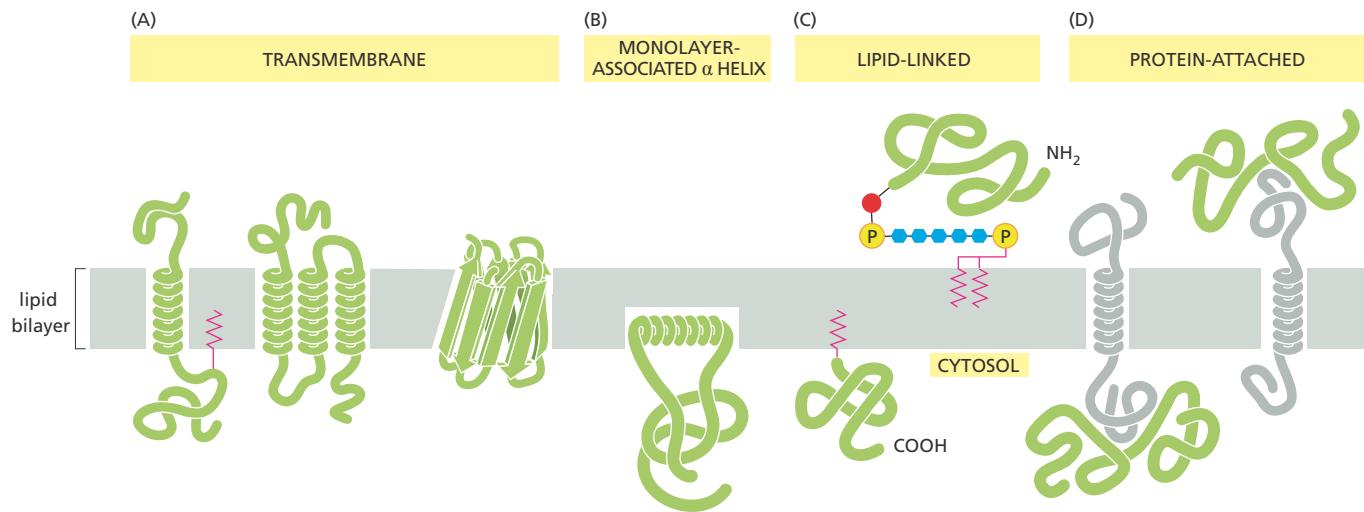


Figure 11–20 Membrane proteins can associate with the lipid bilayer in different ways. (A) Transmembrane proteins extend across the bilayer as a single α helix, as multiple α helices, or as a rolled-up β sheet (called a β barrel). (B) Some membrane proteins are anchored to the cytosolic half of the lipid bilayer by an amphipathic α helix. (C) Others are linked to either side of the bilayer solely by a covalently attached lipid molecule (red zigzag lines). (D) Many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins. All except (D) are *integral membrane proteins*.

Membrane Proteins Associate with the Lipid Bilayer in Different Ways

Proteins can be associated with the lipid bilayer of a cell membrane in any one of the ways illustrated in **Figure 11–20**.

1. Many membrane proteins extend through the bilayer, with part of their mass on either side (Figure 11–20A). Like their lipid neighbors, these *transmembrane proteins* are amphipathic, having both hydrophobic and hydrophilic regions. Their hydrophobic regions lie in the interior of the bilayer, nestled against the hydrophobic tails of the lipid molecules. Their hydrophilic regions are exposed to the aqueous environment on either side of the membrane.
2. Other membrane proteins are located almost entirely in the cytosol and are associated with the cytosolic half of the lipid bilayer by an amphipathic α helix exposed on the surface of the protein (Figure 11–20B).
3. Some proteins lie entirely outside the bilayer, on one side or the other, attached to the membrane only by one or more covalently attached lipid groups (Figure 11–20C).
4. Yet other proteins are bound indirectly to one or the other face of the membrane, held in place only by their interactions with other membrane proteins (Figure 11–20D).

Proteins that are directly attached to the lipid bilayer—whether they are transmembrane, associated with the lipid monolayer, or lipid-linked—can be removed only by disrupting the bilayer with detergents, as discussed shortly. Such proteins are known as *integral membrane proteins*. The remaining membrane proteins are known as *peripheral membrane proteins*; they can be released from the membrane by more gentle extraction procedures that interfere with protein–protein interactions but leave the lipid bilayer intact.

A Polypeptide Chain Usually Crosses the Lipid Bilayer as an α Helix

All membrane proteins have a unique orientation in the lipid bilayer, which is essential for their function. For a transmembrane receptor protein, for example, the part of the protein that receives a signal from the environment must be on the outside of the cell, whereas the part that passes along the signal must be in the cytosol (see Figure 11–19). This orientation is a consequence of the way in which membrane proteins are synthesized (discussed in Chapter 15). The portions of a transmembrane protein located on either side of the lipid bilayer are connected by specialized membrane-spanning segments of the polypeptide chain (see Figure 11–20A). These segments, which run through the hydrophobic environment of the interior of the lipid bilayer, are composed largely of amino acids with hydrophobic side chains. Because these side chains cannot form favorable interactions with water molecules, they prefer to interact with the hydrophobic tails of the lipid molecules, where no water is present.

In contrast to the hydrophobic side chains, however, the peptide bonds that join the successive amino acids in a protein are normally polar, making the polypeptide backbone hydrophilic (Figure 11–21). Because water is absent from the interior of the bilayer, atoms forming the backbone are driven to form hydrogen bonds with one another. Hydrogen-bonding is maximized if the polypeptide chain forms a regular α helix, and so the great majority of the membrane-spanning segments of polypeptide chains traverse the bilayer as α helices (see Figure 4–13). In these membrane-spanning α helices, the hydrophobic side chains are exposed on the outside of the helix, where they contact the hydrophobic lipid tails, while atoms in the polypeptide backbone form hydrogen bonds with one another on the inside of the helix (Figure 11–22).

In many transmembrane proteins, the polypeptide chain crosses the membrane only once (see Figure 11–20A). Many of these *single-pass* transmembrane proteins are receptors for extracellular signals. Other transmembrane proteins function as channels, forming aqueous pores across the lipid bilayer to allow small, water-soluble molecules to cross the membrane. Such channels cannot be formed by proteins with a single transmembrane α helix. Instead, they usually consist of a series of α helices that cross the bilayer a number of times (see Figure 11–20A). In many of these *multipass* transmembrane proteins, one or more of the membrane-spanning regions are amphipathic—formed from α helices that contain both hydrophobic and hydrophilic amino acid side chains. These amino acids tend to be arranged so that the hydrophobic side chains fall on one side of the helix, while the hydrophilic side chains are concentrated on the other side. In the hydrophobic environment of the lipid bilayer, α helices of this sort pack side by side in a ring, with the hydrophobic side chains exposed to the lipids of the membrane and the hydrophilic side chains forming the lining of a hydrophilic pore through the lipid bilayer (Figure 11–23). How such channels function in the selective transport of small, water-soluble molecules, especially inorganic ions, is discussed in Chapter 12.

Figure 11–22 A transmembrane polypeptide chain usually crosses the lipid bilayer as an α helix. In this segment of a transmembrane protein, the hydrophobic side chains (light green) of the amino acids forming the α helix contact the hydrophobic hydrocarbon tails of the phospholipid molecules, while the hydrophilic parts of the polypeptide backbone form hydrogen bonds with one another in the interior of the helix. An α helix containing about 20 amino acids is required to completely traverse a cell membrane.

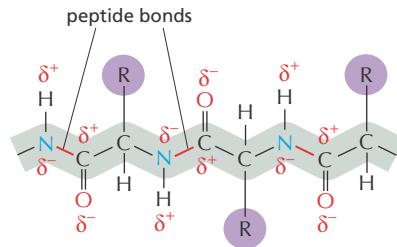
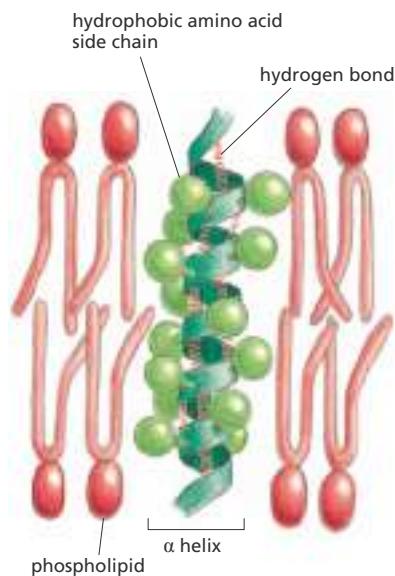


Figure 11–21 The backbone of a polypeptide chain is hydrophilic. The atoms on either side of a peptide bond (red line) are polar and carry partial positive or negative charges (δ^+ or δ^-). These charges allow these atoms to hydrogen-bond with one another when the polypeptide folds into an α helix that spans the lipid bilayer (see Figure 11–22).



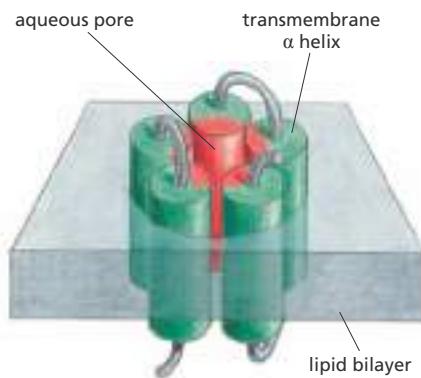


Figure 11–23 A transmembrane hydrophilic pore can be formed by multiple amphipathic α helices. In this example, five such transmembrane α helices form a water-filled channel across the lipid bilayer. The hydrophobic amino acid side chains (green) on one side of each helix contact the hydrophobic lipid tails, while the hydrophilic side chains (red) on the opposite side of the helices form a water-filled pore.

QUESTION 11–4

Explain why the polypeptide chain of most transmembrane proteins crosses the lipid bilayer as an α helix or a β barrel.

Although the α helix is by far the most common form in which a polypeptide chain crosses a lipid bilayer, the polypeptide chain of some transmembrane proteins crosses the lipid bilayer as a β sheet that is rolled into a cylinder, forming a keglike structure called a β barrel (see Figure 11–20A). As expected, the amino acid side chains that face the inside of the barrel, and therefore line the aqueous channel, are mostly hydrophilic, while those on the outside of the barrel, which contact the hydrophobic core of the lipid bilayer, are exclusively hydrophobic. The most striking example of a β -barrel structure is found in the *porin* proteins, which form large, water-filled pores in mitochondrial and bacterial outer membranes (Figure 11–24). Mitochondria and some bacteria are surrounded by a double membrane, and porins allow the passage of small nutrients, metabolites, and inorganic ions across their outer membranes, while preventing unwanted larger molecules from crossing.

Membrane Proteins Can Be Solubilized in Detergents

To understand a protein fully, one needs to know its structure in detail. For membrane proteins, this presents special problems. Most biochemical procedures are designed for studying molecules in aqueous solution. Membrane proteins, however, are built to operate in an environment that is partly aqueous and partly fatty, and taking them out of this environment and purifying them while preserving their essential structure is no easy task.

Before an individual protein can be studied in detail, it must be separated from all the other cell proteins. For most membrane proteins, the first step in this separation process involves solubilizing the membrane with agents that destroy the lipid bilayer by disrupting hydrophobic associations. The most widely used disruptive agents are **detergents** (Movie 11.3). These small, amphipathic, lipidlike molecules differ from membrane phospholipids in that they have only a single hydrophobic tail (Figure 11–25). Because they have one tail, detergent molecules are shaped like cones; in water, they thus tend to aggregate into small clusters called *micelles*, rather than forming a bilayer as do the phospholipids, which—with their two tails—are more cylindrical in shape.

When mixed in great excess with membranes, the hydrophobic ends of detergent molecules interact with the membrane-spanning hydrophobic regions of the transmembrane proteins, as well as with the hydrophobic

Figure 11–24 Porin proteins form water-filled channels in the outer membrane of a bacterium. The protein illustrated is from *E. coli*, and it consists of a 16-stranded β sheet curved around on itself to form a transmembrane water-filled channel. The three-dimensional structure was determined by X-ray crystallography. Although not shown in the drawing, three porin proteins associate to form a trimer, which has three separate channels.

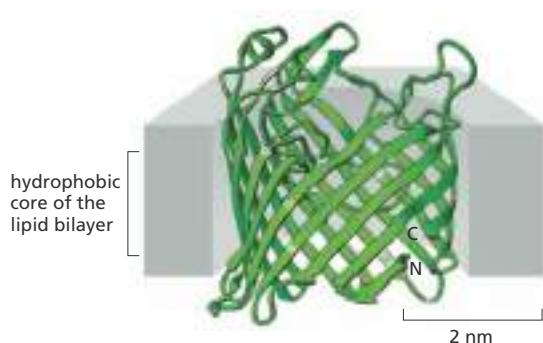


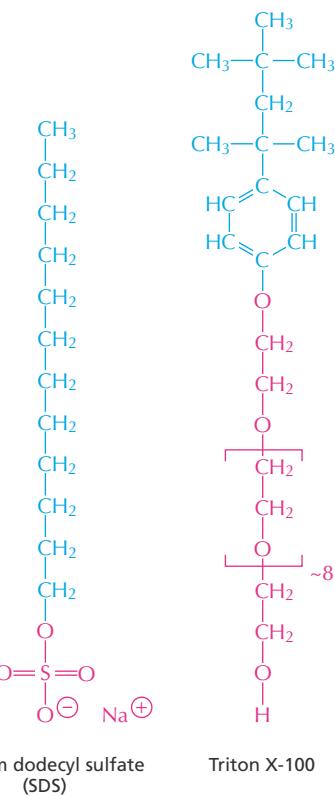
Figure 11–25 SDS and Triton X-100 are two commonly used detergents. Sodium dodecyl sulfate (SDS) is a strong ionic detergent—that is, it has an ionized (charged) group at its hydrophilic end. Triton X-100 is a mild nonionic detergent—that is, it has a nonionized but polar structure at its hydrophilic end. The hydrophobic portion of each detergent is shown in blue, and the hydrophilic portion in red. The bracketed portion of Triton X-100 is repeated about eight times. Strong ionic detergents like SDS not only displace lipid molecules from proteins but also unfold the proteins (see Panel 4–5, p. 167).

tails of the phospholipid molecules, thereby disrupting the lipid bilayer and separating the proteins from most of the phospholipids. Because the other end of the detergent molecule is hydrophilic, these interactions bring the membrane proteins into solution as protein–detergent complexes; at the same time, the detergent solubilizes the phospholipids (**Figure 11–26**). The protein–detergent complexes can then be separated from one another and from the lipid–detergent complexes for further analysis.

We Know the Complete Structure of Relatively Few Membrane Proteins

For many years, much of what we knew about the structure of membrane proteins was learned by indirect means. The standard method for determining a protein's three-dimensional structure directly is X-ray crystallography (see Figure 4–52), but this requires ordered crystalline arrays of the molecule. Because membrane proteins have to be purified in detergent micelles that are often heterogeneous in size, they are harder to crystallize than the soluble proteins that inhabit the cell cytosol or extracellular fluids. Nevertheless, with recent advances in protein preparation and X-ray crystallography, the structures of an increasing number of membrane proteins have now been determined to high resolution.

One example is bacteriorhodopsin, the structure of which first revealed exactly how α helices cross the lipid bilayer. **Bacteriorhodopsin** is a small protein (about 250 amino acids) found in large amounts in the plasma membrane of an archaean, called *Halobacterium halobium*, that lives in salt marshes. Bacteriorhodopsin acts as a membrane transport protein that pumps H⁺ (protons) out of the cell. Pumping requires energy, and bacteriorhodopsin gets its energy directly from sunlight. Each bacteriorhodopsin molecule contains a single light-absorbing nonprotein



sodium dodecyl sulfate (SDS) Triton X-100

QUESTION 11–5

For the two detergents shown in Figure 11–25, explain why the red portions of the molecules are hydrophilic and the blue portions hydrophobic. Draw a short stretch of a polypeptide chain made up of three amino acids with hydrophobic side chains (see Panel 2–5, pp. 74–75) and apply a similar color scheme.

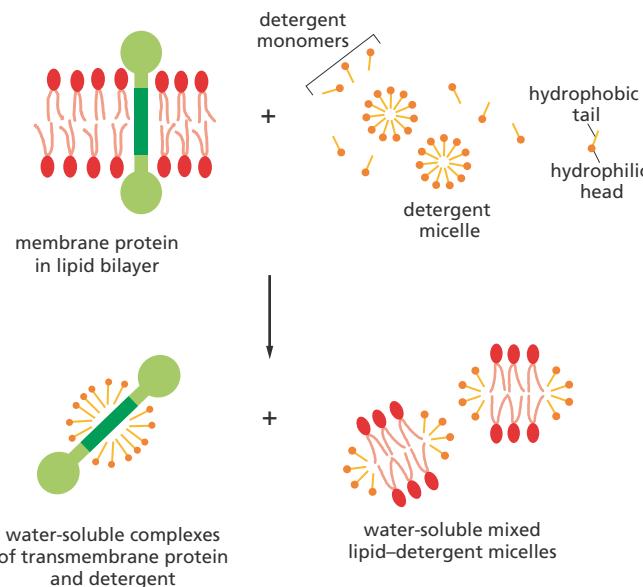
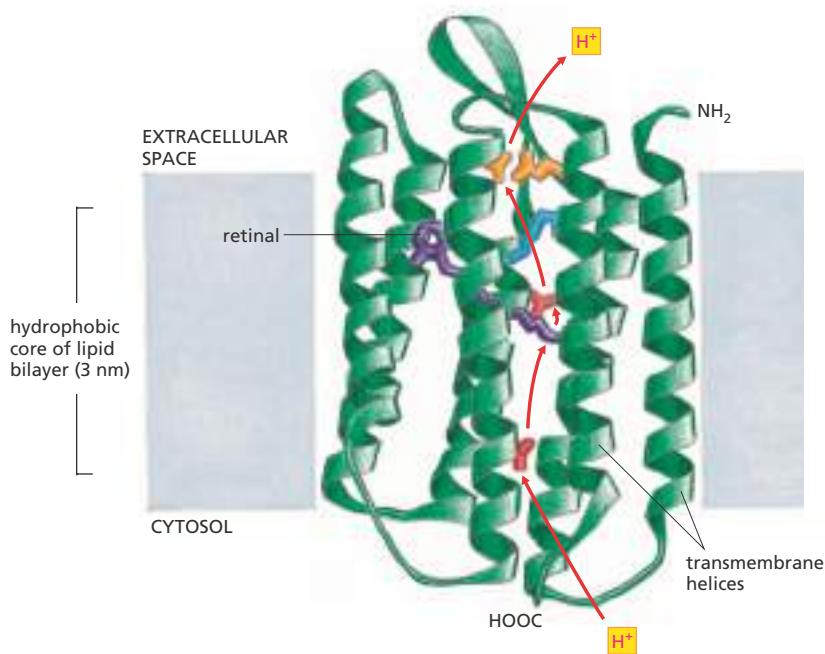


Figure 11–26 Membrane proteins can be solubilized by a mild detergent such as Triton X-100. The detergent molecules (gold) are shown as both monomers and micelles, the form in which detergent molecules tend to aggregate in water. The detergent disrupts the lipid bilayer and brings the proteins into solution as protein–detergent complexes. As illustrated, the phospholipids in the membrane are also solubilized by the detergents, forming lipid–detergent micelles.

Figure 11–27 Bacteriorhodopsin acts as a proton pump. The polypeptide chain crosses the lipid bilayer as seven α helices. The location of the retinal (purple) and the probable pathway taken by protons during the light-activated pumping cycle (red arrows) are highlighted. Strategically placed polar amino acid side chains, shown in red, yellow, and blue, guide the movement of the proton across the bilayer, allowing the proton to avoid contact with the lipid environment. The proton-transfer steps are shown in Movie 11.4. Retinal is also used to detect light in our own eyes, where it is attached to a protein with a structure very similar to bacteriorhodopsin. (Adapted from H. Luecke et al., *Science* 286:255–260, 1999. With permission from the AAAS.)



molecule, called *retinal*, that gives the protein—and the bacterium—a deep purple color. This small hydrophobic molecule is covalently attached to one of bacteriorhodopsin's seven transmembrane α helices (Figure 11–27). When retinal absorbs a photon of light, it changes shape, and in doing so, it causes the protein embedded in the lipid bilayer to undergo a series of small conformational changes. These changes result in the transfer of one H^+ from the retinal to the outside of the bacterium (see Figure 11–27). The retinal is then regenerated by taking up a H^+ from the cytosol, returning the protein to its original conformation so that it can repeat the cycle. The overall outcome is the movement of one H^+ from inside to outside the cell.

In the presence of sunlight, thousands of bacteriorhodopsin molecules pump H^+ out of the cell, generating a concentration gradient of H^+ across the plasma membrane. The cell uses this proton gradient to store energy and convert it into ATP, as we discuss in detail in Chapter 14. Bacteriorhodopsin is a pump protein, a class of transmembrane protein that actively moves small organic molecules and inorganic ions into and out of cells (see Figure 11–19). We will meet other pump proteins in Chapter 12.

The Plasma Membrane Is Reinforced by the Underlying Cell Cortex

A cell membrane by itself is extremely thin and fragile. It would require nearly 10,000 cell membranes laid on top of one another to achieve the thickness of this paper. Most cell membranes are therefore strengthened and supported by a framework of proteins, attached to the membrane via transmembrane proteins. For plants, yeasts, and bacteria, the cell's shape and mechanical properties are conferred by a rigid cell wall—a meshwork of proteins, sugars, and other macromolecules that encases the plasma membrane. By contrast, the plasma membrane of animal cells is stabilized by a meshwork of fibrous proteins, called the cell cortex, that is attached to the underside of the membrane.

The cortex of human red blood cells is a relatively simple and regular structure and has been especially well studied. These cells are small and have a distinctive flattened shape (Figure 11–28). The main component of their cortex is the dimeric protein spectrin, a long, thin, flexible rod



Figure 11–28 Human red blood cells have a characteristic flattened biconcave shape, as seen in this scanning electron micrograph. These cells lack a nucleus and other intracellular organelles. (Courtesy of Bernadette Chailley.)

QUESTION 11–6

Look carefully at the transmembrane proteins shown in Figure 11–29. What can you say about their mobility in the membrane?

about 100 nm in length. It forms a meshwork that provides support for the plasma membrane and maintains the cell's biconcave shape. The spectrin meshwork is connected to the membrane through intracellular attachment proteins that link the spectrin to specific transmembrane proteins (Figure 11–29 and Movie 11.5). The importance of this meshwork is seen in mice and humans that have genetic abnormalities in spectrin structure. These individuals are anemic: they have fewer red blood cells than normal. The red cells they do have are spherical instead of flattened and are abnormally fragile.

Proteins similar to spectrin and to its associated attachment proteins are present in the cortex of most animal cells. But the cortex in these cells is especially rich in actin and the motor protein *myosin*, and it is much more complex than that of red blood cells. While red blood cells need their cortex mainly to provide mechanical strength as they are pumped through blood vessels, other cells also need their cortex to allow them to selectively take up materials from their environment, to change their shape actively, and to move, as we discuss in Chapter 17. In addition, cells use their cortex to restrain the diffusion of proteins within the plasma membrane, as we see next.

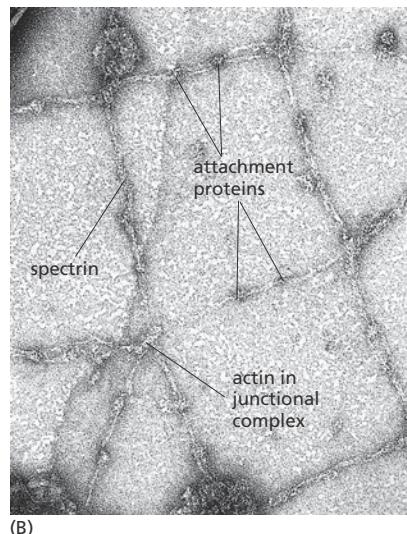
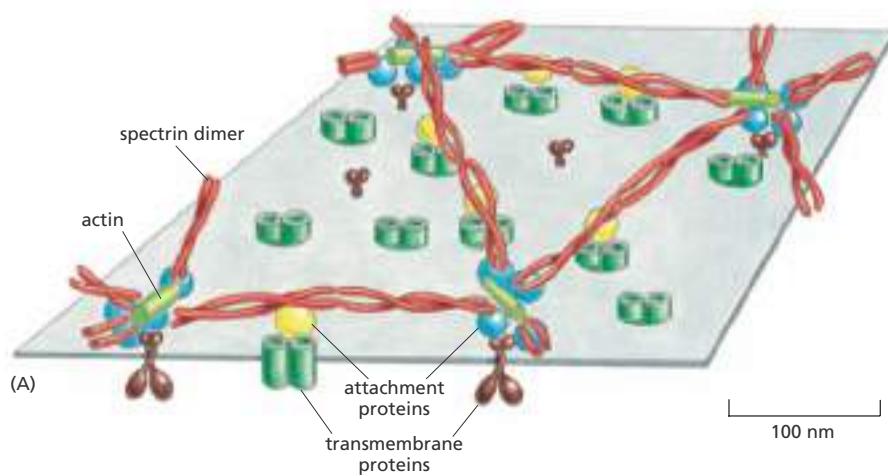
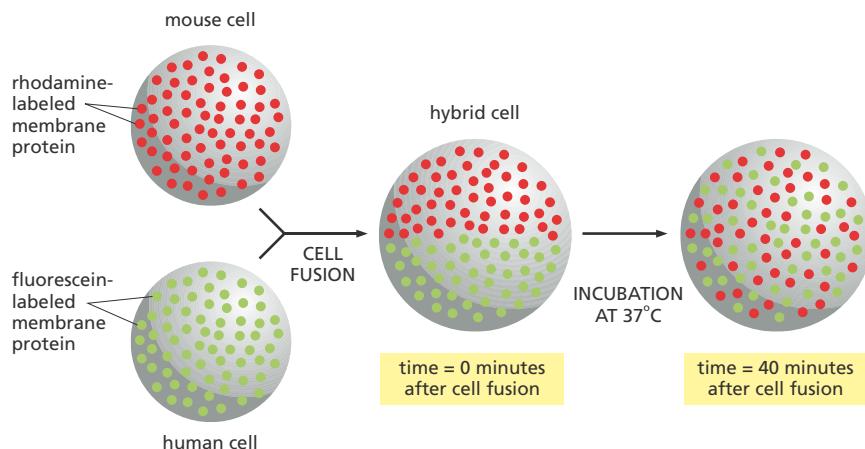


Figure 11–29 A spectrin meshwork forms the cell cortex in human red blood cells. (A) Spectrin dimers are linked end-to-end to form longer tetramers. The spectrin tetramers, together with a smaller number of actin molecules, are linked together into a mesh. This network is attached to the plasma membrane by the binding of at least two types of attachment proteins (shown here in yellow and blue) to two kinds of transmembrane proteins (shown here in green and brown). (B) Electron micrograph showing the spectrin meshwork on the cytoplasmic side of a red blood cell membrane. The meshwork has been stretched out to show the details of its structure; in the normal cell, the meshwork shown would be much more crowded and would occupy only about one-tenth of this area. (B, courtesy of T. Byers and D. Branton, *Proc. Natl. Acad. Sci. USA* 82:6153–6157, 1985. With permission from the National Academy of Sciences.)

Figure 11–30 Formation of mouse–human hybrid cells shows that some plasma membrane proteins can move laterally in the lipid bilayer. When the mouse and human cells are first fused, their proteins are confined to their own halves of the newly formed hybrid-cell plasma membrane. Within a short time, however, they completely intermix. To monitor the movement of a selected sampling of these proteins, the cells are labeled with antibodies that bind to either human or mouse proteins; the antibodies are coupled to two different fluorescent tags—rhodamine (red) or fluorescein (green)—so they can be distinguished in a fluorescence microscope (see Panel 4–2, pp. 146–147). (Based on observations of L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319–335, 1970. With permission from The Company of Biologists Ltd.)



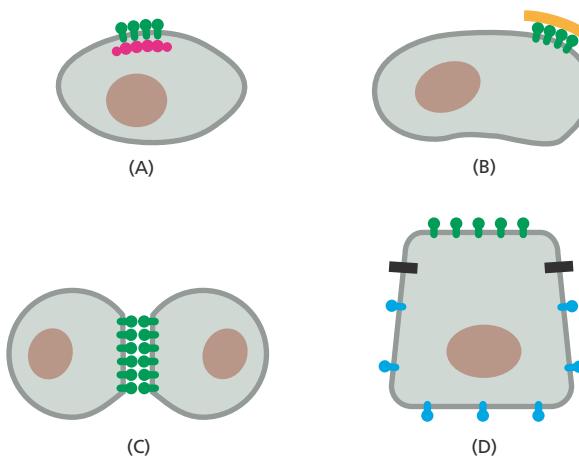
A Cell Can Restrict the Movement of Its Membrane Proteins

Because a membrane is a two-dimensional fluid, many of its proteins, like its lipids, can move freely within the plane of the lipid bilayer. This lateral diffusion was initially demonstrated by experimentally fusing a mouse cell with a human cell to form a double-sized hybrid cell and then monitoring the distribution of certain mouse and human plasma membrane proteins. At first, the mouse and human proteins are confined to their own halves of the newly formed hybrid cell, but within half an hour or so the two sets of proteins become evenly mixed over the entire cell surface (Figure 11–30). We describe some other techniques for studying the movement of membrane proteins in **How We Know**, pp. 378–379.

The picture of a cell membrane as a sea of lipid in which all proteins float freely is too simple, however. Cells have ways of confining particular proteins to localized areas within the bilayer membrane, thereby creating functionally specialized regions, or **membrane domains**, on the cell or organelle surface.

As illustrated in Figure 11–31, plasma membrane proteins can be tethered to structures outside the cell—for example, to molecules in the extracellular matrix or on an adjacent cell (discussed in Chapter 20)—or to relatively immobile structures inside the cell, especially to the cell cortex (see Figure 11–29). Additionally, cells can create barriers that restrict particular membrane components to one membrane domain. In epithelial cells that line the gut, for example, it is important that transport proteins involved in the uptake of nutrients from the gut be confined to

Figure 11–31 The lateral mobility of plasma membrane proteins can be restricted in several ways. Proteins can be tethered to the cell cortex inside the cell (A), to extracellular matrix molecules outside the cell (B), or to proteins on the surface of another cell (C). Diffusion barriers (shown as black bars) can restrict proteins to a particular membrane domain (D).



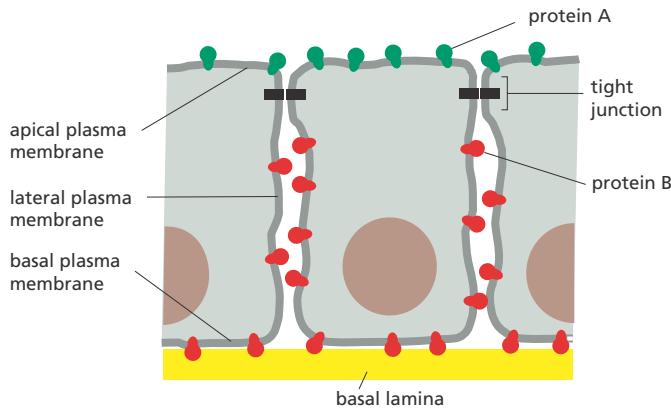


Figure 11–32 Membrane proteins are restricted to particular domains of the plasma membrane of epithelial cells in the gut. Protein A (in the apical membrane) and protein B (in the basal and lateral membranes) can diffuse laterally in their own membrane domains but are prevented from entering the other domain by a specialized cell junction called a tight junction. The basal lamina is a mat of extracellular matrix that supports all epithelial sheets (discussed in Chapter 20).

the *apical* surface of the cells (the surface that faces the gut contents) and that other transport proteins involved in the export of solutes out of the epithelial cell into the tissues and bloodstream be confined to the *basal* and *lateral* surfaces (see Figure 12–17). This asymmetric distribution of membrane proteins is maintained by a barrier formed along the line where the cell is sealed to adjacent epithelial cells by a so-called *tight junction* (Figure 11–32). At this site, specialized junctional proteins form a continuous belt around the cell where the cell contacts its neighbors, creating a seal between adjacent plasma membranes (see Figure 20–23). Membrane proteins cannot diffuse past the junction.

The Cell Surface Is Coated with Carbohydrate

We saw earlier that some of the lipids in the outer layer of the plasma membrane have sugars covalently attached to them. The same is true for most of the proteins in the plasma membrane. The great majority of these proteins have short chains of sugars, called oligosaccharides, linked to them; they are called *glycoproteins*. Other membrane proteins, the *proteoglycans*, contain one or more long polysaccharide chains. All of the carbohydrate on the glycoproteins, proteoglycans, and glycolipids is located on the outside of the plasma membrane, where it forms a sugar coating called the *carbohydrate layer* or *glycocalyx* (Figure 11–33).

This layer of carbohydrate helps protect the cell surface from mechanical damage. As the oligosaccharides and polysaccharides adsorb water, they also give the cell a slimy surface, which helps motile cells such as white blood cells squeeze through narrow spaces and prevents blood cells from sticking to one another or to the walls of blood vessels.

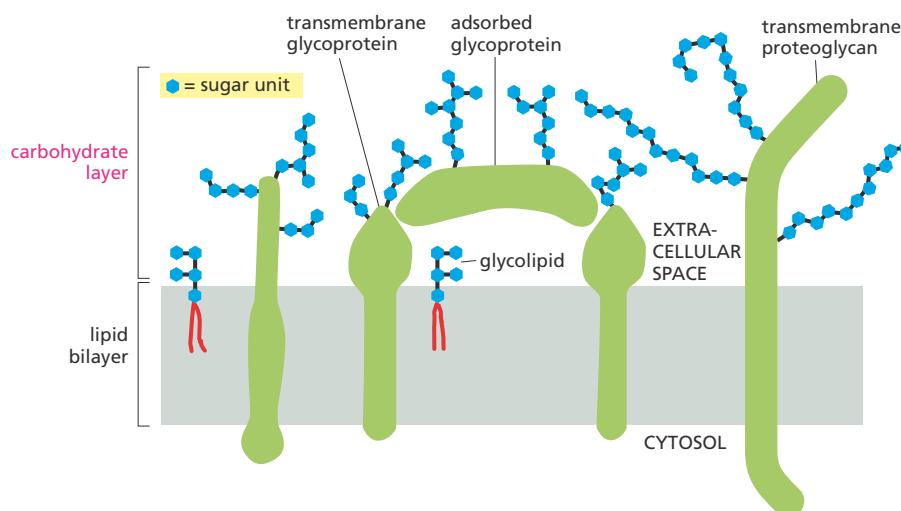


Figure 11–33 Eukaryotic cells are coated with sugars. The carbohydrate layer is made of the oligosaccharide side chains attached to membrane glycolipids and glycoproteins, and of the polysaccharide chains on membrane proteoglycans. As shown, glycoproteins that have been secreted by the cell and then adsorbed back onto its surface can also contribute. Note that all the carbohydrate is on the external (noncytosolic) surface of the plasma membrane.

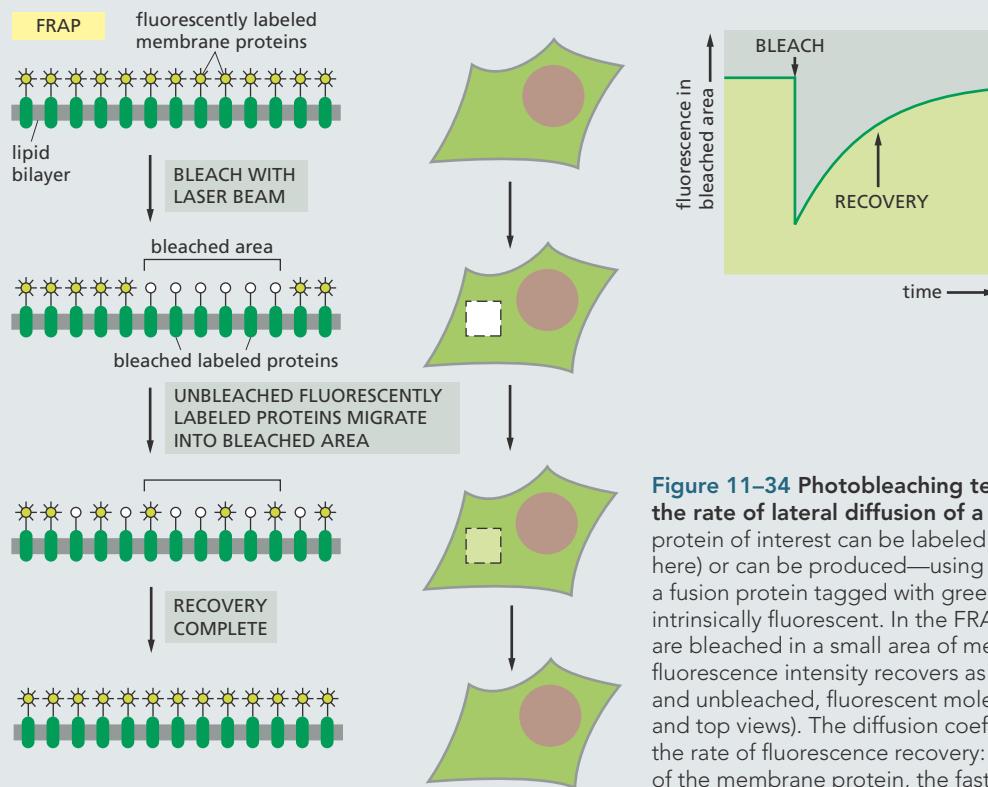
MEASURING MEMBRANE FLOW

An essential feature of the lipid bilayer is its fluidity, which is crucial for cell membrane integrity and function. This property allows many membrane-embedded proteins to move laterally in the plane of the bilayer, so that they can engage in the various protein–protein interactions on which cells depend. The fluid nature of cell membranes is so central to their proper function that it may seem surprising that this property was not recognized until the early 1970s.

Given its importance for membrane structure and function, how do we measure and study the fluidity of cell membranes? The most common methods are visual: simply label some of the molecules native to the membrane and then watch them move. Such an approach first demonstrated the lateral movement of membrane proteins that had been tagged with labeled antibodies (see Figure 11–30). This experiment seemed to suggest that membrane proteins diffuse freely, without restriction, in an open sea of lipids. We now know that this image is not entirely accurate. To probe membrane fluidity more thoroughly, researchers had to invent more precise methods for tracking the movement of proteins within a membrane such as the plasma membrane of a living cell.

The FRAP attack

One such technique, called fluorescence recovery after photobleaching (FRAP), involves uniformly labeling the



components of the cell membrane—its lipids or, more often, its proteins—with some sort of fluorescent marker. Labeling membrane proteins can be accomplished by incubating living cells with a fluorescent antibody or by covalently attaching a fluorescent protein such as green fluorescent protein (GFP) to a membrane protein of interest using recombinant DNA techniques (discussed in Chapter 10).

Once a protein has been labeled, a small patch of membrane is irradiated with an intense pulse of light from a sharply focused laser beam. This treatment irreversibly “bleaches” the fluorescence from the labeled proteins in that small patch of membrane, typically an area about 1 μm square. The fluorescence of this irradiated membrane is monitored in a fluorescence microscope, and the amount of time it takes for the neighboring, unbleached fluorescent proteins to migrate into the bleached region of the membrane is measured (Figure 11–34). The rate of this “fluorescence recovery” is a direct measure of the rate at which the protein molecules can diffuse within the membrane (Movie 11.6). Such experiments have revealed that, generally speaking, a cell membrane is about as viscous as olive oil.

One-by-one

One drawback to the FRAP approach is that the technique monitors the movement of fairly large populations of proteins—hundreds or thousands—across a relatively

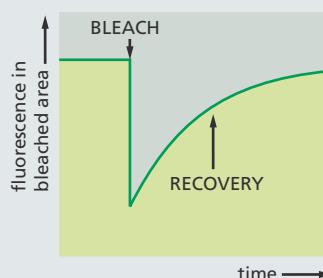


Figure 11–34 Photobleaching techniques can be used to measure the rate of lateral diffusion of a membrane protein. A specific protein of interest can be labeled with a fluorescent antibody (as shown here) or can be produced—using genetic engineering techniques—as a fusion protein tagged with green fluorescent protein (GFP), which is intrinsically fluorescent. In the FRAP technique, fluorescent molecules are bleached in a small area of membrane using a laser beam. The fluorescence intensity recovers as the bleached molecules diffuse away and unbleached, fluorescent molecules diffuse in (shown here in side and top views). The diffusion coefficient is calculated from a graph of the rate of fluorescence recovery: the greater the diffusion coefficient of the membrane protein, the faster the recovery.

large area of the membrane. With this technique it is impossible to track the motion of individual molecules. If the labeled proteins fail to migrate into the bleached zone over the course of a FRAP study, for example, is it because they are immobile, essentially anchored in one place in the membrane? Or, alternatively, are they restricted to movement within a very small region—fenced in by cytoskeletal proteins—and thus only appear motionless?

To get around this problem, researchers have developed methods for labeling and observing the movement of individual molecules or small clusters of molecules. One such technique, dubbed single-particle tracking (SPT) microscopy, relies on tagging protein molecules with antibody-coated gold nanoparticles. The gold particles look like tiny black dots when seen with a light microscope, and their movement, and thus the movement of individually tagged protein molecules, can be followed using video microscopy.

From the studies carried out to date, it appears that membrane proteins can display a variety of patterns of movement, from random diffusion to complete immobility (Figure 11–35). Some proteins rapidly switch between these different kinds of motion.

Freed from cells

In many cases, researchers wish to study the behavior of a particular type of membrane protein in a synthetic lipid bilayer, in the absence of other proteins that might restrain its movement or alter its activity. For such studies, membrane proteins can be isolated from cells and the protein of interest purified and reconstituted in artificial phospholipid vesicles (Figure 11–36). The lipids

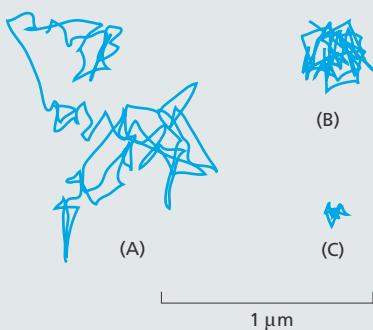


Figure 11–35 Proteins show different patterns of diffusion. Single-particle tracking studies reveal some of the pathways that real proteins follow on the surface of a living cell. Shown here are some trajectories representative of different kinds of proteins in the plasma membrane. (A) Tracks made by a protein that is free to diffuse randomly in the lipid bilayer. (B) Tracks made by a protein that is corralled within a small membrane domain by other proteins. (C) Tracks made by a protein that is tethered to the cytoskeleton and hence is essentially immobile. The movement of the proteins is monitored over a period of seconds.

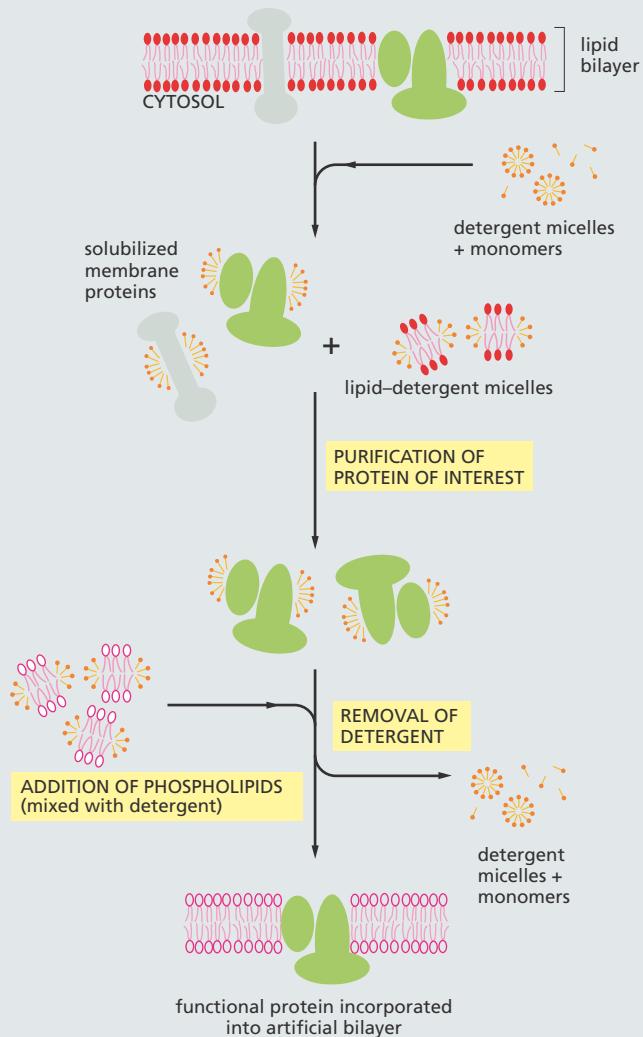


Figure 11–36 Mild detergents can be used to solubilize and reconstitute functional membrane proteins.

allow the purified protein to maintain its proper structure and function, so that its activity and behavior can be analyzed in detail.

It is apparent from such studies that membrane proteins diffuse more freely and rapidly in artificial lipid bilayers than in cell membranes. The fact that most proteins show reduced mobility in a cell membrane makes sense, as these membranes are crowded with many types of proteins and contain a greater variety of lipids than an artificial lipid bilayer. Furthermore, many membrane proteins in a cell are tethered to proteins in the extracellular matrix, or anchored to the cell cortex just under the plasma membrane, or both (as illustrated in Figure 11–31).

Taken together, such studies have revolutionized our understanding of membrane proteins and of the architecture and organization of cell membranes.

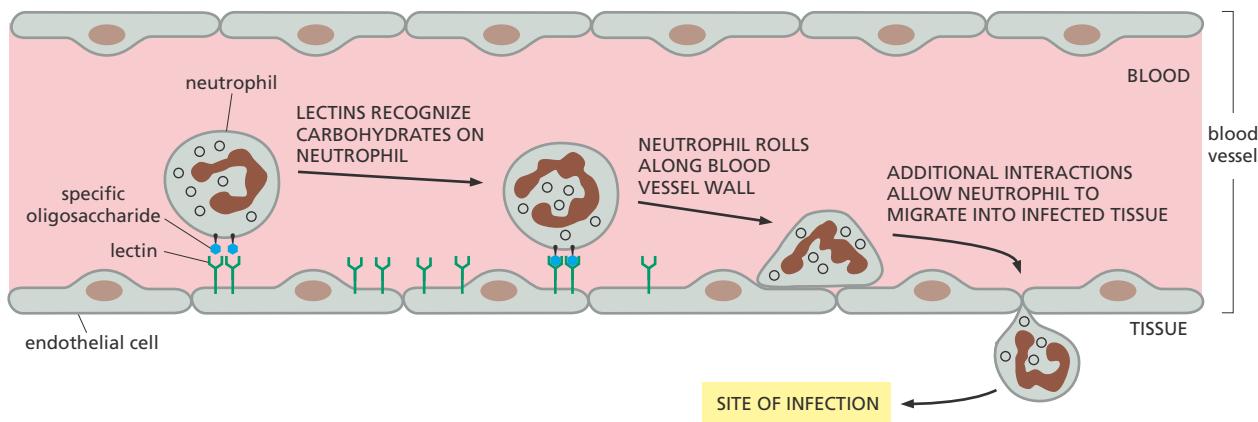


Figure 11–37 The recognition of the cell-surface carbohydrate on neutrophils is the first stage of their migration out of the blood at sites of infection. Specialized transmembrane proteins (called lectins) are made by the endothelial cells lining the blood vessel in response to chemical signals emanating from a site of infection. These proteins recognize particular sugar groups carried by glycolipids and glycoproteins on the surface of neutrophils (a type of white blood cell) circulating in the blood. The neutrophils consequently stick to the endothelial cells that line the blood vessel wall. This association is not very strong, but it leads to another, much stronger protein–protein interaction (not shown) that helps the neutrophil slip between the endothelial cells, so it can migrate out of the bloodstream and into the tissue at the site of infection ([Movie 11.7](#)).

Cell-surface carbohydrates do more than just protect and lubricate the cell, however. They have an important role in cell-cell recognition and adhesion. Just as many proteins will recognize a particular site on another protein, proteins called *lectins* are specialized to bind to particular oligosaccharide side chains. The oligosaccharide side chains of glycoproteins and glycolipids, although short (typically fewer than 15 sugar units), are enormously diverse. Unlike proteins, in which the amino acids are all joined together in a linear chain by identical peptide bonds, sugars can be joined together in many different arrangements, often forming elaborate branched structures (see Panel 2–3, pp. 70–71). Using a variety of covalent linkages, even three different sugars can form hundreds of different trisaccharides.

The carbohydrate layer on the surface of cells in a multicellular organism serves as a kind of distinctive clothing, like a police officer's uniform. It is characteristic of each cell type and is recognized by other cell types that interact with it. Specific oligosaccharides in the carbohydrate layer are involved, for example, in the recognition of an egg by a sperm (discussed in Chapter 19). Similarly, in the early stages of a bacterial infection, the carbohydrate on the surface of white blood cells called neutrophils is recognized by a lectin on the cells lining the blood vessels at the site of infection; this recognition causes the neutrophils to adhere to the blood vessel wall and then migrate from the bloodstream into the infected tissue, where they help destroy the invading bacteria (**Figure 11–37**).

ESSENTIAL CONCEPTS

- Cell membranes enable cells to create barriers that confine particular molecules to specific compartments. They consist of a continuous double layer—a bilayer—of lipid molecules in which proteins are embedded.
- The lipid bilayer provides the basic structure and barrier function of all cell membranes.
- Membrane lipid molecules are amphipathic, having both hydrophobic and hydrophilic regions. This property promotes their spontaneous assembly into bilayers when placed in water, forming closed compartments that reseal if torn.
- There are three major classes of membrane lipid molecules: phospholipids, sterols, and glycolipids.
- The lipid bilayer is fluid, and individual lipid molecules are able to diffuse within their own monolayer; they do not, however, spontaneously flip from one monolayer to the other.

- The two lipid monolayers of a cell membrane have different lipid compositions, reflecting the different functions of the two faces of the membrane.
- Cells that live at different temperatures maintain their membrane fluidity by modifying the lipid composition of their membranes.
- Membrane proteins are responsible for most of the functions of cell membranes, including the transport of small, water-soluble molecules across the lipid bilayer.
- Transmembrane proteins extend across the lipid bilayer, usually as one or more α helices but sometimes as a β sheet rolled into the form of a barrel.
- Other membrane proteins do not extend across the lipid bilayer but are attached to one or the other side of the membrane, either by noncovalent association with other membrane proteins, by covalent attachment of lipids, or by association of an exposed amphipathic α helix with a single lipid monolayer.
- Most cell membranes are supported by an attached framework of proteins. An especially important example is the meshwork of fibrous proteins that forms the cell cortex underneath the plasma membrane.
- Although many membrane proteins can diffuse rapidly in the plane of the membrane, cells have ways of confining proteins to specific membrane domains. They can also immobilize particular membrane proteins by attaching them to intracellular or extracellular macromolecules.
- Many of the proteins and some of the lipids exposed on the surface of cells have attached sugar chains, which form a carbohydrate layer that helps protect and lubricate the cell surface, while also being involved in specific cell-cell recognition.

KEY TERMS

amphipathic
bacteriorhodopsin
cholesterol
detergent
glycocalyx
lipid bilayer
membrane domain

membrane protein
phosphatidylcholine
phospholipid
plasma membrane
saturated
unsaturated

QUESTIONS

QUESTION 11–7

Describe the different methods that cells use to restrict proteins to specific regions of the plasma membrane. Is a membrane with many of its proteins restricted still fluid?

QUESTION 11–8

Which of the following statements are correct? Explain your answers.

- A. Lipids in a lipid bilayer spin rapidly around their long axis.
- B. Lipids in a lipid bilayer rapidly exchange positions with one another in their own monolayer.

- C. Lipids in a lipid bilayer do not flip-flop readily from one lipid monolayer to the other.
- D. Hydrogen bonds that form between lipid head groups and water molecules are continually broken and re-formed.
- E. Glycolipids move between different membrane-enclosed compartments during their synthesis but remain restricted to one side of the lipid bilayer.
- F. Margarine contains more saturated lipids than the vegetable oil from which it is made.
- G. Some membrane proteins are enzymes.
- H. The sugar layer that surrounds all cells makes cells more slippery.

QUESTION 11–9

What is meant by the term “two-dimensional fluid”?

QUESTION 11–10

The structure of a lipid bilayer is determined by the particular properties of its lipid molecules. What would happen if

- A. Phospholipids had only one hydrocarbon tail instead of two?
- B. The hydrocarbon tails were shorter than normal, say, about 10 carbon atoms long?
- C. All of the hydrocarbon tails were saturated?
- D. All of the hydrocarbon tails were unsaturated?
- E. The bilayer contained a mixture of two kinds of phospholipid molecules, one with two saturated hydrocarbon tails and the other with two unsaturated hydrocarbon tails?
- F. Each phospholipid molecule were covalently linked through the end carbon atom of one of its hydrocarbon tails to a phospholipid tail in the opposite monolayer?

QUESTION 11–11

What are the differences between a phospholipid molecule and a detergent molecule? How would the structure of a phospholipid molecule need to change to make it a detergent?

QUESTION 11–12

A. Membrane lipid molecules exchange places with their lipid neighbors every 10^{-7} second. A lipid molecule diffuses from one end of a 2- μm -long bacterial cell to the other in about 1 second. Are these two numbers in agreement (assume that the diameter of a lipid head group is about 0.5 nm)? If not, can you think of a reason for the difference?

B. To get an appreciation for the great speed of molecular diffusion, assume that a lipid head group is about the size of a ping-pong ball (4 cm in diameter) and that the floor of your living room ($6 \text{ m} \times 6 \text{ m}$) is covered wall-to-wall with these balls. If two neighboring balls exchanged positions once every 10^{-7} second, what would their speed be in kilometers per hour? How long would it take for a ball to move from one side of the room to the opposite side?

QUESTION 11–13

Why does a red blood cell plasma membrane need transmembrane proteins?

QUESTION 11–14

Consider a transmembrane protein that forms a hydrophilic pore across the plasma membrane of a eukaryotic cell, allowing Na^+ to enter the cell when it is activated upon binding a specific ligand on its extracellular side. It is made of five similar transmembrane subunits, each containing a membrane-spanning α helix with hydrophilic amino acid side chains on one surface of the helix and hydrophobic amino acid side chains on the opposite surface. Considering the function of the protein as a channel for Na^+ ions to enter the cell, propose a possible arrangement of the five membrane-spanning α helices in the membrane.

QUESTION 11–15

In the membrane of a human red blood cell, the ratio of the mass of protein (average molecular weight 50,000) to phospholipid (molecular weight 800) to cholesterol (molecular weight 386) is about 2:1:1. How many lipid molecules are there for every protein molecule?

QUESTION 11–16

Draw a schematic diagram that shows a close-up view of two plasma membranes as they come together during cell fusion, as shown in Figure 11–30. Show membrane proteins in both cells that were labeled from the outside by the binding of differently colored fluorescent antibody molecules. Indicate in your drawing the fates of these color tags as the cells fuse. Will they remain on the outside of the hybrid cell after cell fusion and still be there after the mixing of membrane proteins that occurs during the incubation at 37°C? How would the experimental outcome be different if the incubation were done at 0°C?

QUESTION 11–17

Compare the hydrophobic forces that hold a membrane protein in the lipid bilayer with those that help proteins fold into a unique three-dimensional structure.

QUESTION 11–18

Predict which one of the following organisms will have the highest percentage of unsaturated phospholipids in its membranes. Explain your answer.

- A. Antarctic fish
- B. Desert snake
- C. Human being
- D. Polar bear
- E. Thermophilic bacterium that lives in hot springs at 100°C.

QUESTION 11–19

Which of the three 20-amino-acid sequences listed below in the single-letter amino acid code is the most likely candidate to form a transmembrane region (α helix) of a transmembrane protein? Explain your answer.

- A. I T L I Y F G N M S S V T Q T I L L I S
- B. L L L I F F G V M A L V I V V I L L I A
- C. L L K K F F R D M A A V H E T I L E E S