## I. **What are stem cells, and why are they important?**

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells. The functions and characteristics of these cells will be explained in this document. Scientists discovered ways to derive embryonic stem cells from early mouse embryos nearly 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called human embryonic stem cells. The embryos used in these studies were created for reproductive purposes through in vitro fertilization procedures.

When they were no longer needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell, called induced pluripotent stem cells (IPSCs), will be discussed in a later section of this document.

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a blastocyst, the inner cells give rise to the entire body of the organism, including all of the many specialized cell types and organs such as the heart, lung, skin, sperm, eggs and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes and heart disease. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for cell-based therapies to treat disease, which is also referred to as regenerative or reparative medicine.

Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in

the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.

Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

## II. What are the unique properties of all stem cells?

Stem cells differ from other types of cells in the body. All stem cells—regardless of their source—have three general properties: 1) they are capable of dividing and renewing themselves for long periods; 2) they are unspecialized; and 3) they can give rise to specialized cell types.

***Stem cells are capable of dividing and renewing themselves for long periods***. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

Scientists are trying to understand two fundamental properties of stem cells that relate to their long-term self-renewal:

1. Why can embryonic stem cells proliferate for a year or more in the laboratory without differentiating, but most non-embryonic stem cells (adult stem cells) cannot; and

2. What factors in living organisms normally regulate stem cell proliferation and self-renewal?

Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal cell division that leads to cancer. Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow human embryonic stem cells in the laboratory following the development of conditions for growing mouse stem cells. Likewise, scientists must first understand the signals that enable a nonembryonic (adult) stem cell population to proliferate and remain unspecialized before they will be able to grow large numbers of unspecialized adult stem cells in the laboratory.

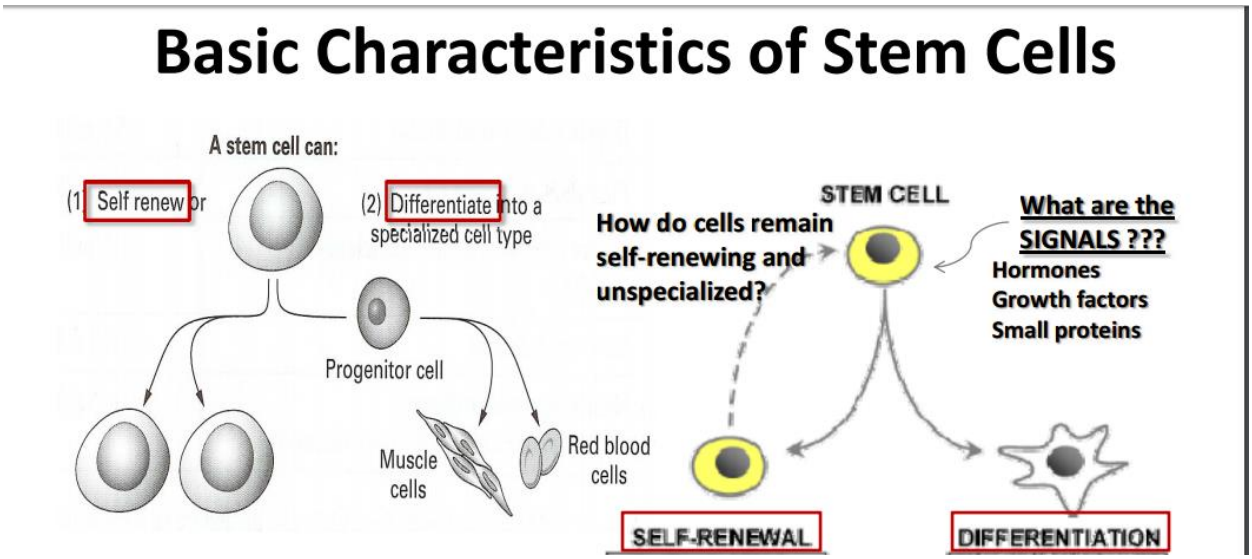
***Stem cells are unspecialized***. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions.

For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

***Stem cells can give rise to specialized cells***. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. Scientists are just beginning to understand the signals inside and outside cells that trigger each step of the differentiation process. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA, and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell's DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division.

Many questions about stem cell differentiation remain. For example, are the internal and external signals for cell differentiation similar for all kinds of stem cells? Can specific sets of signals be identified that promote differentiation into specific cell types? Addressing these questions may lead scientists to find new ways to control stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes such as cell-based therapies or drug screening.

***Adult stem cells typically generate the cell types of the tissue in which they reside***. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the bone marrow—which is called a hematopoietic stem cell—cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue. This remains an area of great debate within the research community. This controversy demonstrates the challenges of studying adult stem cells and suggests that additional research using adult stem cells is necessary to understand their full potential as future therapies.




**III. What are embryonic stem cells?**

A. What stages of early embryonic development are important for generating embryonic stem cells?

Embryonic stem cells, as their name suggests, are derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized in vitro—in an in vitro fertilization clinic—and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body.

B. How are embryonic stem cells grown in the laboratory?

Growing cells in the laboratory is known as cell culture. Human embryonic stem cells (hESCs) are generated by transferring cells from a preimplantation-stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium.

The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a feeder layer. The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach.

Also, the feeder cells release nutrients into the culture medium. Researchers have devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time cells from the preimplantation-stage embryo are placed into a culture dish. However, if the plated cells survive, divide, and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or subculturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions

of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

**IV. What are adult stem cells?**

An adult stem cell is thought to be an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Scientists also use the term somatic stem cell instead of adult stem cell, where somatic refers to cells of the body (not the germ cells, sperm or eggs). Unlike embryonic stem cells, which are defined by their origin (cells from the preimplantation-stage embryo), the origin of adult stem cells in some mature tissues is still under investigation.

**V. Where are adult stem cells found, and what do they normally do?**

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche"). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury. Typically, there is a very small number of stem cells in each tissue, and once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult. Scientists in many laboratories are trying to find better ways to grow large quantities of adult stem cells in cell culture and to manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include regenerating bone using cells derived from bone marrow stroma, developing insulin-producing cells for type 1 diabetes, and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

**In conclusion:**

Some stem cells have more potential than others.

**POTENCY** describes this flexibility.

• **Unipotent stem cells** form only one type of specialized cell type.

• **Multipotent stem cells** can form multiple types of cells and tissue types.

• **Pluripotent stem cells** can form most or all cell types in the adult.

• **Totipotent stem cells** can form all adult cell types as well as the specialized tissues to support development of the embryo (e.g., the placenta)

**Properties of Human Embryonic Stem Cells in Culture**

• **Pluripotent** – able to form any of ~200 different types of cells of the body

• Self-renewing in vitro – can propagate or proliferate indefinitely in the undifferentiated state

• Express the enzyme telomerase (required to maintain the ends of chromosomes) and Oct4 (a master regulator of ESC pluripotency)

• Maintain normal chromosome structure and complement even after long periods in culture (unlike many other tissue culture cell lines)

**Applications**

Some Examples of Treatments for Major Diseases

**Type 1 Diabetes in Children**. Type 1 diabetes is an autoimmune disease characterized by destruction of insulin producing cells in the pancreas. Current efforts to treat these patients with human islet transplantation in an effort to restore insulin secretory function (obtained from human pancreas) are limited severely by the small numbers of donated pancreas available each year combined with the toxicity of immunosuppressive drug treatments required to prevent graft rejection. Pluripotent stem cells, instructed to differentiate into a particular pancreatic cell called a beta cell, could overcome the shortage of therapeutically effective material to transplant. They also afford the opportunity to engineer such cells to effectively resist immune attack as well as graft rejection.

**Nervous System Diseases**. Many nervous system diseases result from loss of nerve cells. Mature nerve cells cannot divide to replace those that are lost. Thus, without a "new"source of functioning nerve tissue, no therapeutic possibilities exist. In Parkinson's disease, nerve cells that make the chemical dopamine die. In Alzheimer's disease, cells that are responsible for the production of certain neurotransmitters die. In amyotrophic lateral sclerosis, the motor nerve cells that activate muscles die. In spinal cord injury, brain trauma, and even stroke, many different types of cells are lost or die. In multiple sclerosis, glia, the cells that protect nerve fibers are lost.Perhaps the only hope for treating such individuals comes from the potential to create new nerve tissue restoring function from pluripotent stem cells. Remarkably, human clinical experiments have demonstrated the potential effectiveness of this approach to treatment. Parkinson's patients have been treated by surgical implantation of fetal cells into their brain with some benefit. Although not completely effective, perhaps owing to lack of sufficient numbers of dopamine secreting cells, similar experiments using appropriately differentiated stem cells should overcome those obstacles. More complex experiments have already been successfully conducted in rodent models of Parkinson's.13 Similar approaches could be developed to replace the dead or dysfunctional cells in cortical and hippocampal brain regions that are affected in patients with Alzheimer's. Primary Immunodeficiency Diseases. Pluripotent stem cells could be used in treatment of virtually all

primary immunodeficiency diseases. Presently, there are more than 70 different forms of congenital and inherited deficiencies of the immune system that have been recognized. These are among the most complicated diseases to treat with the worst prognoses. Included here are diseases such as severe combined immunodeficiency disease (the "bubble boy" disease), Wiskott-Aldrich Syndrome, and the autoimmune disease lupus. The immune deficiencies suffered as a result of acquired immune deficiency syndrome (AIDS) following infection with the human immunodeficiency virus are also relevant here. These diseases are characterized by an unusual susceptibility to infection and often associated with anemia, arthritis, diarrhea, and selected malignancies. However, the transplantation of stem cells reconstituted with the normal gene could result in restoration of immune function and effective normalization of life span and quality of life for these people.

**Diseases of Bone and Cartilage**. Stem cells, once appropriately differentiated, could correct many diseases and degenerative conditions in which bone or cartilage cells are deficient in numbers or defective in function. This holds promise for treatment of genetic disorders such as osteogenesis imperfecta and chondrodysplasias. Similarly, cells could be cultivated and introduced into damaged areas of joint cartilage in cases of osteoarthritis or into large gaps in bone from fractures or surgery.

**Cancer**. At the present time, bone marrow stem cells, representing a more committed stem cell, are used to rescue patients following high dose chemotherapy. Unfortunately, these recovered cells are limited in their capacity to restore immune function completely in this setting. It is hoped that injections of properly-differentiated stem cells would return the complete repertoire of immune response to patients undergoing bone marrow transplantation. Complete and functional restoration will be required if, for example, immune/vaccine anticancer therapy is to work. More importantly, success would permit use of very toxic (and effective) chemotherapeutic regimens that could not currently be utilized for lack of an ability to restore marrow and immune function.

**Uses in Research**

Much is left to be discovered and understood in all aspects of human biology. What has been frequently lacking are the tools necessary to make the initial discoveries, or to apply the knowledge of discoveries to the understanding of complex systems. These are some of the larger problems in basic and clinical biology where the use of stem cells might be the key to understanding. A new window on human developmental biology. The study of human developmental biology is particularly constrained by practical and ethical limitations. Human ES cells may allow scientists to investigate how early human cells become committed to the major lineages of the body; how these lineages lay down the rudiments of the body's tissues and organs; and how cells within these rudiments differentiate to form the myriad functional cell types which underlie normal function in the adult. The knowledge gained will impact many fields. For example, cancer biology will reap an especially large reward because it is now understood that many cancers arise by perturbations of normal developmental processes. The availability of human ES cells will also greatly accelerate the understanding of the causes of birth defects and thus lead directly to their possible prevention.

Models of human disease that are constrained by current animal and cell culture models. Investigation of a number of human diseases is severely constrained by a lack of in vitro models. A number of pathogenic viruses including human immunodeficiency virus and hepatitis C virus grow only in human or chimpanzee cells. ES cells might provide cell and tissue types that will greatly accelerate investigation into these and other viral diseases. Current animal models of neurodegenerative diseases such as Alzheimer's disease give only a very partial representation of the disease's process.

**Transplantation.** Pluripotent stem cells could be used to create an unlimited supply of cells, tissues, or even organs that could be used to restore function without the requirement for toxic immunosuppression and without regard to tissue matching compatibility. Such cells, when used in transplantation therapies, would in effect be suitable for "universal" donation. Bone marrow transplantation, a difficult and expensive procedure associated with significant hazards, could become safe, cost effective, and be available for treating a wide range of clinical disorders, including aplastic anemia and certain inherited blood disorders. This would be especially important in persons who lost marrow function from toxic exposure, for example to radiation or toxic agents. Growth and transplant of other tissues lost to disease or accident, for example, skin, heart, nervous system components, and other major organs, are foreseeable.

**Gene Therapy**. In gene therapy, genetic material that provides a missing or necessary protein, or causes a clinically-relevant biochemical process, is introduced into an organ for a therapeutic effect. For gene-based therapies (specifically, those using DNA sequences), it is critical that the desired gene be introduced into organ stem cells in order to achieve long-term expression and therapeutic effect. Although techniques for delivering the therapeutic DNA have been greatly improved since the first gene therapy protocol almost 10 years ago, there are as yet no bona fide successes. Besides delivery problems, loss of expression or insufficient expression is an important limiting factor in successful application of gene therapy and could be overcome by transferring genes into stem cells (which presumably will then differentiate and target correctly).

## What is a GMO?

A GMO, or genetically modified organism, is a plant, animal, microorganism or other organism whose genetic makeup has been modified using recombinant DNA methods (also called gene splicing), gene modification or transgenic technology. This relatively new science creates unstable combinations of plant, animal, bacterial and viral genes that do not occur in nature or through traditional crossbreeding methods.

People have been altering the genomes of plants and animals for many years using traditional breeding techniques. Artificial selection for specific, desired traits has resulted in a variety of different organisms, ranging from sweet corn to hairless cats. But this artificial selection, in which organisms that exhibit specific traits are chosen to breed subsequent generations, has been limited to naturally occurring variations. In recent decades, however, advances in the field of genetic engineering have allowed for precise control over the genetic changes introduced into an organism.

Today, we can incorporate new genes from one species into a completely unrelated species through genetic engineering, optimizing agricultural performance or facilitating the production of valuable pharmaceutical substances. Crop plants, farm animals, and soil bacteria are some of the more prominent examples of organisms that have been subject to genetic engineering.

**Current Use of Genetically Modified Organisms**

***Table 1: Examples of GMOs Resulting from Agricultural*** Biotechnology

| **Genetically Conferred** Trait | **Example Organism** | **Genetic Change** |
|---|---|---|
| **APPROVED COMMERCIAL PRODUCTS** | | |
| Herbicide tolerance | Soybean | Glyphosate herbicide (Roundup) tolerance conferred by expression of a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) isolated from the soil bacterium *Agrobacterium tumefaciens*, strain CP4 |
| Insect resistance | Corn | Resistance to insect pests, specifically the European corn borer, through expression of the insecticidal protein Cry1Ab from *Bacillus thuringiensis* |
| Altered fatty acid composition | Canola | High laurate levels achieved by inserting the gene for ACP thioesterase from the California bay tree *Umbellularia californica* |
| Virus resistance | Plum | Resistance to plum pox virus conferred by insertion of a coat protein (CP) gene from the virus |
| Vitamin enrichment | Rice | Three genes for the manufacture of beta-carotene, a precursor to vitamin A, in the endosperm of the rice prevent its removal (from husks) during milling |
| Vaccines | Tobacco | Hepatitis B virus surface antigen (HBsAg) produced in transgenic tobacco induces immune response when injected into mice |
| Oral vaccines | Maize | Fusion protein (F) from Newcastle disease virus (NDV) expressed in corn seeds induces an immune response when fed to chickens |
| Faster maturation | Coho salmon | A type 1 growth hormone gene injected into fertilized fish eggs results in 6.2% retention of the vector at one year of age, as well as significantly increased growth rates |

The pharmaceutical industry is another frontier for the use of GMOs. In 1986, human growth hormone was the first protein pharmaceutical made in plants (Barta et al., 1986), and in 1989, the first antibody was produced (Hiatt et al., 1989). Both research groups used tobacco, which has since dominated the industry as the most intensively studied and utilized plant species for the

expression of foreign genes (Ma et al., 2003). As of 2003, several types of antibodies produced in plants had made it to clinical trials. The use of genetically modified animals has also been indispensible in medical research. Transgenic animals are routinely bred to carry human genes, or mutations in specific genes, thus allowing the study of the progression and genetic determinants of various diseases.

**Potential GMO Applications**

Many industries stand to benefit from additional GMO research. For instance, a number of microorganisms are being considered as future clean fuel producers and biodegraders. In addition, genetically modified plants may someday be used to produce recombinant vaccines. In fact, the concept of an oral vaccine expressed in plants (fruits and vegetables) for direct consumption by individuals is being examined as a possible solution to the spread of disease in underdeveloped countries, one that would greatly reduce the costs associated with conducting large-scale vaccination campaigns. Work is currently underway to develop plant-derived vaccine candidates in potatoes and lettuce for hepatitis B virus (HBV), enterotoxigenic Escherichia coli (ETEC), and Norwalk virus. Scientists are also looking into the production of other commercially valuable proteins in plants, such as spider silk protein and polymers that are used in surgery or tissue replacement (Ma et al., 2003). Genetically modified animals have even been used to grow transplant tissues and human transplant organs, a concept called xenotransplantation. The rich variety of uses for GMOs provides a number of valuable benefits to humans, but many people also worry about potential risks.

**Risks and Controversies Surrounding the Use of GMOs**

Despite the fact that the genes being transferred occur naturally in other species, there are unknown consequences to altering the natural state of an organism through foreign gene expression. After all, such alterations can change the organism's metabolism, growth rate, and/or response to external environmental factors. These consequences influence not only the GMO itself, but also the natural environment in which that organism is allowed to proliferate. Potential health risks to humans include the possibility of exposure to new allergens in genetically modified foods, as well as the transfer of antibiotic-resistant genes to gut flora.

Horizontal gene transfer of pesticide, herbicide, or antibiotic resistance to other organisms would not only put humans at risk, but it would also cause ecological imbalances, allowing previously innocuous plants to grow uncontrolled, thus promoting the spread of disease among both plants and animals. Although the possibility of horizontal gene transfer between GMOs and other organisms cannot be denied, in reality, this risk is considered to be quite low. Horizontal gene transfer occurs naturally at a very low rate and, in most cases, cannot be simulated in an optimized laboratory environment without active modification of the target genome to increase susceptibility (Ma et al., 2003).

In contrast, the alarming consequences of vertical gene transfer between GMOs and their wild-type counterparts have been highlighted by studying transgenic fish released into wild populations of the same species (Muir & Howard, 1999). The enhanced mating advantages of the genetically modified fish led to a reduction in the viability of their offspring. Thus, when a new transgene is introduced into a wild fish population, it propagates and may eventually threaten the viability of both the wild-type and the genetically modified organisms.

**Unintended Impacts on Other Species: The Bt Corn Controversy**

One example of public debate over the use of a genetically modified plant involves the case of Bt corn. Bt corn expresses a protein from the bacterium Bacillus thuringiensis. Prior to construction of the recombinant corn, the protein had long been known to be toxic to a number of pestiferous insects, including the monarch caterpillar, and it had been successfully used as an environmentally friendly insecticide for several years. The benefit of the expression of this protein by corn plants is a reduction in the amount of insecticide that farmers must apply to their crops. Unfortunately, seeds containing genes for recombinant proteins can cause unintentional spread of recombinant genes or exposure of non-target organisms to new toxic compounds in the environment.

The now-famous Bt corn controversy started with a laboratory study by in which the mortality of monarch larvae was reportedly higher when fed with milkweed (their natural food supply) covered in pollen from transgenic corn than when fed milkweed covered with pollen from regular corn. The report by Losey et al. was followed by another publication suggesting that natural levels of Bt corn pollen in the field were harmful to monarchs.

Debate ensued when scientists from other laboratories disputed the study, citing the extremely high concentration of pollen used in the laboratory study as unrealistic, and concluding that migratory patterns of monarchs do not place them in the vicinity of corn during the time it sheds pollen. For the next two years, six teams of researchers from government, academia, and industry investigated the issue and concluded that the risk of Bt corn to monarchs was "very low", providing the basis for the U.S. Environmental Protection Agency to approve Bt corn for an additional seven years.

**Unintended Economic Consequences**

Another concern associated with GMOs is that private companies will claim ownership of the organisms they create and not share them at a reasonable cost with the public. If these claims are correct, it is argued that use of genetically modified crops will hurt the economy and environment, because monoculture practices by large-scale farm production centers (who can afford the costly seeds) will dominate over the diversity contributed by small farmers who can't afford the technology. However, a recent meta-analysis of 15 studies reveals that, on average, two-thirds of the benefits of first-generation genetically modified crops are shared downstream, whereas only one-third accrues upstream. These benefit shares are exhibited in both industrial and developing countries. Therefore, the argument that private companies will not share ownership of GMOs is not supported by evidence from first-generation genetically modified crops.

**GMOs and the General Public: Philosophical and Religious Concerns**

In a 2007 survey of 1,000 American adults conducted by the International Food Information Council (IFIC), 33% of respondents believed that biotech food products would benefit them or their families, but 23% of respondents did not know biotech foods had already reached the market. In addition, only 5% of those polled said they would take action by altering their purchasing habits as a result of concerns associated with using biotech products.

According to the Food and Agriculture Organization of the United Nations, public acceptance trends in Europe and Asia are mixed depending on the country and current mood at the time of the survey (Hoban, 2004). Attitudes toward cloning, biotechnology, and genetically modified products

differ depending upon people's level of education and interpretations of what each of these terms mean. Support varies for different types of biotechnology; however, it is consistently lower when animals are mentioned.

Furthermore, even if the technologies are shared fairly, there are people who would still resist consumable GMOs, even with thorough testing for safety, because of personal or religious beliefs. The ethical issues surrounding GMOs include debate over our right to "play God," as well as the introduction of foreign material into foods that are abstained from for religious reasons. Some people believe that tampering with nature is intrinsically wrong, and others maintain that inserting plant genes in animals, or vice versa, is immoral. When it comes to genetically modified foods, those who feel strongly that the development of GMOs is against nature or religion have called for clear labeling rules so they can make informed selections when choosing which items to purchase. Respect for consumer choice and assumed risk is as important as having safeguards to prevent mixing of genetically modified products with non-genetically modified foods. In order to determine the requirements for such safeguards, there must be a definitive assessment of what constitutes a GMO and universal agreement on how products should be labeled.

These issues are increasingly important to consider as the number of GMOs continues to increase due to improved laboratory techniques and tools for sequencing whole genomes, better processes for cloning and transferring genes, and improved understanding of gene expression systems. Thus, legislative practices that regulate this research have to keep pace. Prior to permitting commercial use of GMOs, governments perform risk assessments to determine the possible consequences of their use, but difficulties in estimating the impact of commercial GMO use makes regulation of these organisms a challenge.

History of International Regulations for GMO Research and Development

In 1971, the first debate over the risks to humans of exposure to GMOs began when a common intestinal microorganism, E. coli, was infected with DNA from a tumor-inducing virus (Devos et al., 2007). Initially, safety issues were a concern to individuals working in laboratories with GMOs, as well as nearby residents. However, later debate arose over concerns that recombinant organisms might be used as weapons. The growing debate, initially restricted to scientists, eventually spread to the public, and in 1974, the National Institutes of Health (NIH) established the Recombinant DNA Advisory Committee to begin to address some of these issues.

In the 1980s, when deliberate releases of GMOs to the environment were beginning to occur, the U.S. had very few regulations in place. Adherence to the guidelines provided by the NIH was voluntary for industry. Also during the 1980s, the use of transgenic plants was becoming a valuable endeavor for production of new pharmaceuticals, and individual companies, institutions, and whole countries were beginning to view biotechnology as a lucrative means of making money (Devos et al., 2007). Worldwide commercialization of biotech products sparked new debate over the patentability of living organisms, the adverse effects of exposure to recombinant proteins, confidentiality issues, the morality and credibility of scientists, the role of government in regulating science, and other issues. In the U.S., the Congressional Office of Technology Assessment initiatives were developed, and they were eventually adopted worldwide as a top-down approach to advising policymakers by forecasting the societal impacts of GMOs.

Then, in 1986, a publication by the Organization for Economic Cooperation and Development (OECD), called "Recombinant DNA Safety Considerations," became the first intergovernmental document to address issues surrounding the use of GMOs. This document recommended that risk assessments be performed on a case-by-case basis. Since then, the case-by-case approach to risk assessment for genetically modified products has been widely accepted; however, the U.S. has generally taken a product-based approach to assessment, whereas the European approach is more process based (Devos et al., 2007). Although in the past, thorough regulation was lacking in many countries, governments worldwide are now meeting the demands of the public and implementing stricter testing and labeling requirements for genetically modified crops.

Increased Research and Improved Safety Go Hand in Hand

Proponents of the use of GMOs believe that, with adequate research, these organisms can be safely commercialized. There are many experimental variations for expression and control of engineered genes that can be applied to minimize potential risks. Some of these practices are already necessary as a result of new legislation, such as avoiding superfluous DNA transfer (vector sequences) and replacing selectable marker genes commonly used in the lab (antibiotic resistance) with innocuous plant-derived markers (Ma et al., 2003). Issues such as the risk of vaccine-expressing plants being mixed in with normal foodstuffs might be overcome by having built-in identification factors, such as pigmentation, that facilitate monitoring and separation of genetically modified products from non-GMOs. Other built-in control techniques include having inducible promoters (e.g., induced by stress, chemicals, etc.), geographic isolation, using male-sterile plants, and separate growing seasons.

GMOs benefit mankind when used for purposes such as increasing the availability and quality of food and medical care, and contributing to a cleaner environment. If used wisely, they could result in an improved economy without doing more harm than good, and they could also make the most of their potential to alleviate hunger and disease worldwide. However, the full potential of GMOs cannot be realized without due diligence and thorough attention to the risks associated with each new GMO on a case-by-case basis.

**Gene therapy** can be defined as the introduction of nucleic acids into cells for the purpose of altering the course of a medical condition or disease. In general, with some exceptions, the nucleic acids are DNA molecules encoding gene products or proteins. The original ideas were directed toward treating monogenic (single-gene) disorders, but it has become clear that the gene can be considered a new pharmaceutical agent for treating many types of diseases. Over the last 20 years, the initial thoughts of gene therapy have been transformed into reality with more than 175 clinical trials and 2,000 patients already treated.

The two main types of gene therapy are somatic cell gene therapy and reproductive or germ-line gene therapy.

**Germ-line cell therapy** involves the introduction of corrective genes into reproductive cells (sperm and eggs) or zygotes, with the objective of creating a beneficial genetic change that is transmitted to the offspring. When genes are introduced in a reproductive cell, descendant cells can inherit the genes. Gene therapy of somatic cells, those not directly related to reproduction, results in changes that are not transmitted to offspring. An example of gene therapy in somatic cells is the introduction of genes in an organ or tissue to induce the production of an enzyme. This alteration does not affect the individual's genetic makeup as a whole and it is not transmitted to its descendants. With somatic cell gene therapy, a disabled organ is better able to function normally. This technology has many applications to human health. One variant of somatic cell gene therapy is DNA vaccines, which allow cells of the immune system to fight certain diseases in a method similar to conventional vaccines.

**Stem cell therapy** involves the use of pluripotent cells, or cells that can differentiate into any other cell type. Stem cells are found in developing embryos and in some tissues of adult individuals. This therapy is similar to a conventional transplant, with the objective of regenerating or repairing a damaged organ or tissue. The procedure 88 7 • Gene Therapy has a reduced probability of rejection because it uses the individual's own cells. For instance, stem cells differentiated into nerve cells could be used by patients suffering from paralysis, with the goal of helping them recovering movement; or in cases of heart stroke, muscle cells might be used to rejuvenate the cardiac muscles. Furthermore, the future may bring the growth of stem cells from an individual's body to produce certain tissues or organs in vitro. Stem cell research could eventually blend gene therapy with genetic engineering to create healthy stem cells that can be used to generate healthy organs and tissue. A fundamental requirement for gene therapy is the correct identification of genes coding for diseases. This can be accomplished at a spectacular speed with the information from the Human Genome Project. Scientific magazines have been announcing, with great frequency, the discovery of genes responsible for several medical conditions, from Alzheimer's disease to baldness. The knowledge of the genes involved in these traits allows unequivocal diagnosis of the disease in the patient, an essential step before treatment can be initiated for the genetic disease. Biotechnology is contributing to the development of the needed genetic tests for detection of defective genes. The most complex phase in gene therapy is the development of mechanisms to deliver the therapeutic genes to the target organ in an accurate, controlled, and effective way. That step has been developing more slowly and is currently the most limiting factor for gene therapy.

GERM-LINE CELL THERAPY . . . . . .

The **main advantages of germ-line cell gene therapy** are the following:

1. It offers the possibility for a true cure of several diseases and it is not only a temporary solution.

2. It might be the only way to treat some genetic diseases.

3. The benefits would be extended for several generations, because genetic defects are eliminated in the individual's genome and, consequently, the benefits would be passed to his or her offspring.

Some of the arguments presented against germ-line cell gene therapy are the following:

1. It involves many steps that are poorly understood, and the long-term results cannot be estimated.

2. It would open the door for genetic modifications in human traits with profound social and ethical implications.

3. It is very expensive and it would not benefit the common citizen.

4. The extension of the cure to a person's offspring would be possible only if the defective gene was directly modified, but probably not if a new gene was added to another part of the genome.

STEM CELL THERAPY . . . . . .

Stem cell therapy or therapeutic cloning does not involve gene therapy itself. However, in the future it might be used in conjunction with gene therapy for regeneration of tissue and organs after they have been treated with corrective genes. Visually, stem cells are not distinguishable from any other cells of the human body. Under a common microscope (magnification 20 to 40 times), those cells can only be observed using special dyes. Visually there is no significant difference in such cells. The real differences exist at the DNA level, where gene expression is amendable to signals influencing protein expression. The cells can differentiate into any of the 220 cell types of the human body (e.g., kidneys, heart, liver, skin, or retina), a phenomenon called pluripotency. At birth, stem cells can be harvested from an individual's bone marrow, fat tissue, and the umbilical cord. Embryonic stem cells are harvested from embryos up to a few days after fertilization. Stem Cell Therapy 95 Another characteristic of stem cells is their capability to grow indefinitely. Whereas the remaining body cells have a biological programming that limits the number of cell divisions they can go through before dying, stem cells can be maintained indefinitely in a petri dish with nutritive media. Stem cell therapy provides hope for a cure for patients of incurable afflictions such as Parkinson's disease and Alzheimer's disease, and also for people suffering from paralysis resulting from spinal cord injuries. At first, some opponents speculated that stem cells would be used in nurseries to produce organs such as livers, hearts, and virtually any other body part. However, most organs possess complex structures with ducts and valves, making it impossible to produce them outside of the organism. Stem cells have opened a new avenue for disease treatment. For example, the injection of stem cells into the liver of a patient with cirrhosis or hepatitis could result in new tissue capable of performing its role. Stem cell therapy also has great potential to cure rheumatoid arthritis and some heart diseases. Recent research has found that spine-injured mice suffering from paralysis were able to move their legs following an injection of stem cells. Some people believe that if human stem cells are as versatile as those of mice, they might be the long sought after fountain of youth. The combination of stem cells with gene therapy might allow rebuilding of new body parts to substitute for old and defective ones. Right now, different procedures are being tested for curing ADA deficiency. Somatic cell gene therapies have the limitation of lasting for only a few months, which in turn requires repeated applications. With the use of stem cells to

regenerate healthy bone marrow cells, a permanent cure is expected, as healthy cells have the capability to grow and divide continuously. Embryonic stem cells, from embryos about four days old, have been at the center of a heated debate due to ethical issues. The main disagreement is whether or not a four-day-old embryo is already a human life.

• Gene Therapy life begins at conception (i.e., at the moment of the fertilization of the egg by the sperm). For many, the destruction of embryos for the purpose of treating another human being is wrong. Recently, in the United States, the Bush administration broadened the definition of a child eligible for coverage under the Children's Health Insurance Program by classifying a developing fetus as an "unborn child." Many activists are arguing that the Bush administration's proposal demonstrates its commitment to the strategy of undermining a woman's right to choose abortion by ascribing legal rights to embryos.

# What are biosensors?

A biosensor is an analytical device which converts a biological response into an electrical signal (Figure 6.1). The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly. This very broad definition is used by some scientific journals (e.g. Biosensors, Elsevier Applied Science) but will not be applied to the coverage here. The emphasis of this Chapter concerns enzymes as the biologically responsive material, but it should be recognised that other biological systems may be utilised by biosensors, for example, whole cell metabolism, ligand binding and the antibody-antigen reaction. Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry (e.g. 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. The estimated world analytical market is about 12,000,000,000 $year^{-1}$ of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors. Research and development in this field is wide and multidisciplinary, spanning biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering. Most of this current endeavour concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips. However, all the main transducer types are likely to be thoroughly examined, for use in biosensors, over the next few years.

A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks (see later), show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme.

3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case, the biosensor should not be prone to fouling or proteolysis.
5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
6. There should be a market for the biosensor. There is clearly little purpose developing a biosensor if other factors (e.g. government subsidies, the continued employment of skilled analysts, or poor customer perception) encourage the use of traditional methods and discourage the decentralisation of laboratory testing.

The biological response of the biosensor is determined by the biocatalytic membrane which accomplishes the conversion of reactant to product. Immobilised enzymes possess a number of advantageous features which makes them particularly applicable for use in such systems. They may be re-used, which ensures that the same catalytic activity is present for a series of analyses. This is an important factor in securing reproducible results and avoids the pitfalls associated with the replicate pipetting of free enzyme otherwise necessary in analytical protocols. Many enzymes are intrinsically stabilised by the immobilisation process, but even where this does not occur there is usually considerable apparent stabilisation. It is normal to use an excess of the enzyme within the immobilised sensor system. This gives a catalytic redundancy (i.e. $\eta << 1$) which is sufficient to ensure an increase in the apparent stabilisation of the immobilised enzyme. Even where there is some inactivation of the immobilised enzyme over a period of time, this inactivation is usually steady and predictable. Any activity decay is easily incorporated into an analytical scheme by regularly interpolating standards between the analyses of unknown samples. For these reasons, many such immobilised enzyme systems are re-usable up to 10,000 times over a period of several months. Clearly, this results in a considerable saving in terms of the enzymes' cost relative to the analytical usage of free soluble enzymes.

When the reaction, occurring at the immobilised enzyme membrane of a biosensor, is limited by the rate of external diffusion, the reaction process will possess a number of valuable analytical assets. In particular, it will obey the

relationship shown in equation. It follows that the biocatalyst gives a proportional change in reaction rate in response to the reactant (substrate) concentration over a substantial linear range, several times the intrinsic $K_m$. This is very useful as analyte concentrations are often approximately equal to the $K_m$s of their appropriate enzymes which is roughly 10 times more concentrated than can be normally determined, without dilution, by use of the free enzyme in solution. Also following from equation is the independence of the reaction rate with respect to pH, ionic strength, temperature and inhibitors. This simply avoids the tricky problems often encountered due to the variability of real analytical samples (e.g, fermentation broth, blood and urine) and external conditions. Control of biosensor response by the external diffusion of the analyte can be encouraged by the use of permeable membranes between the enzyme and the bulk solution. The thickness of these can be varied with associated effects on the proportionality constant between the substrate concentration and the rate of reaction (i.e. increasing membrane thickness increases the unstirred layer ($\delta$) which, in turn, decreases the proportionality constant, $k_L$, in equation. Even if total dependence on the external diffusional rate is not achieved (or achievable), any increase in the dependence of the reaction rate on external or internal diffusion will cause a reduction in the dependence on the pH, ionic strength, temperature and inhibitor concentrations.

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