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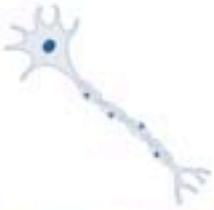
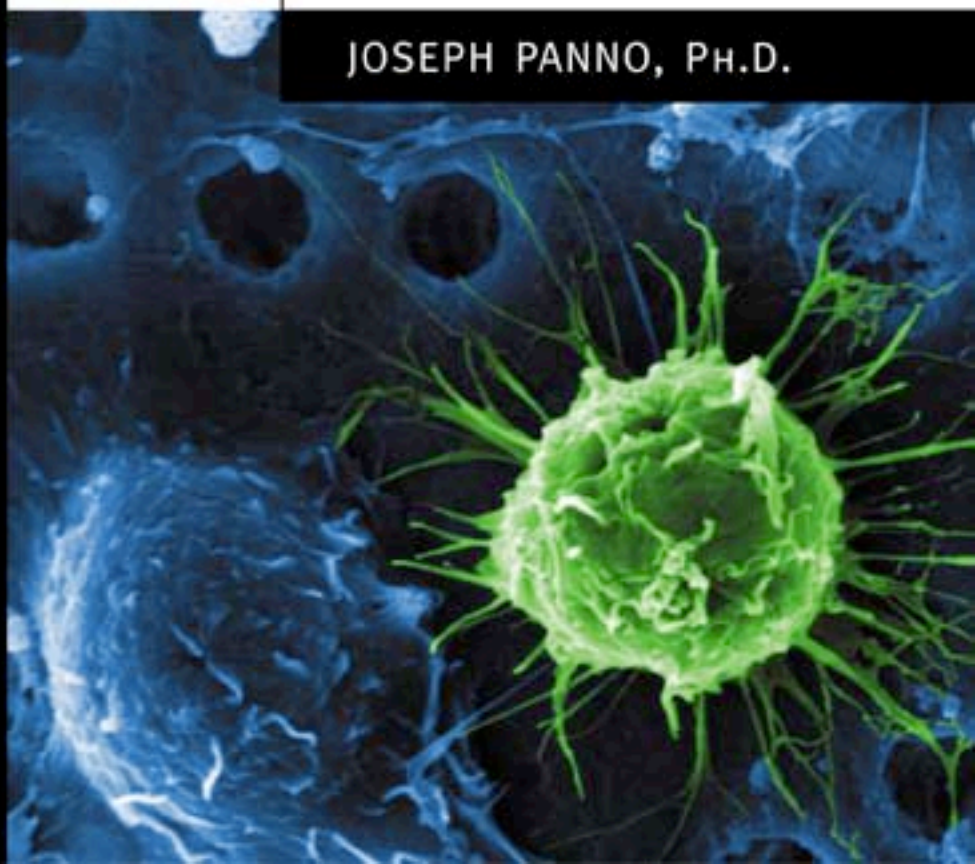


Medical Applications & Ethical Controversy

JOSEPH PANNO, PH.D.



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STEM CELL RESEARCH

Medical Applications and Ethical Controversy

Joseph Panno, Ph.D.

STEM CELL RESEARCH: Medical Applications and Ethical Controversy

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This book is printed on acid-free paper.

For my wife, Diana,
who worked with me in the lab for many years,
and for my daughter Eleanor,
who knew about cells before she could read or write.

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PREFACE

The New Biology set consists of the following six volumes: *The Cell, Animal Cloning, Stem Cell Research, Gene Therapy, Cancer,* and *Aging.* The set is intended primarily for middle and high school students, but it is also appropriate for first-year university students and the general public. In writing this set, I have tried to balance the need for a comprehensive presentation of the material, covering many complex fields, against the danger of burying—and thereby losing—young students under a mountain of detail. Thus the use of lengthy discussions and professional jargon has been kept to a minimum, and every attempt has been made to ensure that this be done without sacrificing the important elements of each topic. A large number of drawings are provided throughout the series to illustrate the subject matter.

The term *new biology* was coined in the 1970s with the introduction of recombinant DNA technology (or biotechnology). At that time, biology was largely a descriptive science in danger of going adrift. Microbiologists at the turn of the century had found cures for a few diseases, and biologists in the 1960s had cracked the genetic code, but there was still no way to study the function of a gene or the cell as a whole. Biotechnology changed all that, and scientists of the period referred to it as the new technique or the new biology. However, since that time it has become clear that the advent of biotechnology was only the first step toward a new biology, a biology that now includes nuclear transfer technology (animal cloning), gene therapy, and stem cell therapy. All these technologies are covered in the six volumes of this set.

The cell is at the very heart of the new biology and thus figures prominently in this book series. Biotechnology was specifically designed for studying cells, and using those techniques, scientists gained insights into cell structure and function that came with unprecedented detail.

As knowledge of the cell grew, the second wave of technologies—animal cloning, stem cell therapy, and gene therapy—began to appear throughout the 1980s and 1990s. The technologies and therapies of the new biology are now being used to treat a wide variety of medical disorders, and someday they may be used to repair a damaged heart, a severed spinal cord, and perhaps even reverse the aging process. These procedures are also being used to enhance food crops and the physical characteristics of dairy cows and to create genetically modified sheep that produce important pharmaceuticals. The last application alone could save millions of lives every year.

While the technologies of the new biology have produced some wonderful results, some of the procedures are very controversial. The ability to clone an animal or genetically engineer a plant raises a host of ethical questions and environmental concerns. Is a cloned animal a freak that we are creating for our entertainment, or is there a valid medical reason for producing such animals? Should we clone ourselves, or use the technology to re-create a loved one? Is the use of human embryonic stem cells to save a patient dying from leukemia a form of high-tech cannibalism? These and many other questions are discussed throughout the series.

The New Biology set is laid out in a specific order, indicated previously, that reflects the natural progression of the discipline. That is, knowledge of the cell came first, followed by animal cloning, stem cell therapy, and gene therapy. These technologies were then used to expand our knowledge of, and develop therapies for, cancer and aging. Although it is recommended that *The Cell* be read first, this is not essential. Volumes 2 through 6 contain extensive background material, located in the final chapter, on the cell and other new biology topics. Consequently, the reader may read the set in the order he or she prefers.

ACKNOWLEDGMENTS

I would first like to thank my friend and mentor, the late Dr. Karun Nair, for helping me understand some of the intricacies of the biological world and for encouraging me to seek that knowledge by looking beyond the narrow confines of any one discipline. The clarity and accuracy of the initial manuscript for this book was greatly improved by reviews and comments from Diana Dowsley and Michael Panno, and later by Frank Darmstadt, Executive Editor; Dorothy Cummings, Project Editor; and Anthony Sacramone, Copy Editor. I am also indebted to Ray Spangenburg, Kit Moser, Sharon O'Brien, and Diana Dowsley for their help in locating photographs for the New Biology set. Finally, I would like to thank my wife and daughter, to whom this book is dedicated, for the support and encouragement that all writers need and are eternally grateful for.

INTRODUCTION

Stem cells are special cells that have the ability to divide for an indefinite period and can give rise to a wide variety of specialized cell types. This ability, known as developmental plasticity, is a common feature of fertilized eggs and early embryonic cells (known as blastomeres). A fertilized egg, being able to give rise to all of the body's cells, has the highest degree of developmental plasticity and, thus, is said to be totipotent. Blastomeres are also totipotent, but this level of plasticity decreases quickly. Consequently, blastomeres from a five-day-old human embryo (consisting of about 200 cells) can only give rise to a limited range of cell types and, accordingly, are said to be pluripotent. As development progresses, individual cells become multipotent (able to give rise to only a few cell types) before assuming their final form as a specialized cell that can only give rise to other cells of its kind. Stem cells may be isolated from embryos, umbilical cords, and adult tissues. When they originate from embryos or umbilical cords, they are equivalent to pluripotent blastomeres. Stem cells isolated from adult tissue possess a wider range of plasticity that varies from pluripotent to multipotent.

When placed in culture, stem cells grow and divide indefinitely, and scientists are now learning how to coax them into producing cell types that may be used to cure many diseases. The extraordinary powers and versatility of these cells have generated an interest level that approaches a fever pitch. Stem cell therapies may be able to treat cardiovascular disease, spinal cord disorders, Parkinson's disease, Alzheimer's disease, and some cancers. Leukemia, a cancer affecting white blood cells (WBCs), is already being treated by replacing the cancerous cells with stem cells programmed to differentiate (transform) into healthy WBCs. Diseases that affect the brain, spinal cord, or heart are ideal candidates for stem cell therapy because these organs have lost the talents that stem cells

retain; in particular, the ability to proliferate (grow and reproduce) and differentiate.

All eukaryote cells at some point in their lives possess the powers of reproduction and differentiation, but those powers become a liability when cells are trying to live as a community. This is particularly true for a community as complex as an animal's body. The human brain, for example, is an intricate assemblage of 10 billion neurons that is constructed during embryonic development; once established, this network would be destroyed if the cells continued dividing. The neurons in our brain can form new associations with other neurons throughout our life, but they become postmitotic (lose the ability to divide) soon after an individual is born. Many other organs, such as the spinal cord, heart, kidneys, and muscles, adhere to the same developmental pattern: active cell division during embryogenesis, loss of cell division in the adult.

The loss of cell division in an animal's body is a trade-off that allows the cell community to produce organs of a predictable size and shape. But if a person suffers a disease or trauma such as a heart attack, the postmitotic cells, in this case myocytes (cardiac muscle cells), are unable to repair the damage. If the damage is extensive, the heart muscle cannot contract properly, and the patient dies or has to have a transplant. However, some of our tissues and organs, such as skin, liver, and bone marrow, retain the power of division throughout the life span of the individual. If we cut our finger, the wound is able to heal because cells in the skin divide to fill the gap. If liver cells die, or a portion of the organ is removed surgically, the cells will divide and grow to repair the damage. Red blood cells, with a life span of only 120 days, have to be replaced on a daily basis. In this case, it is stem cells, located in the bone marrow where blood cells are made, that divide and differentiate into both red and white blood cells, thus replacing them as they wear out.

Scientists believe that if stem cells can replace worn-out red blood cells, it might be possible to train them to repair organs, such as the brain or heart, that are incapable of repairing themselves. If they are successful, we may live to see the day when there is no more cardiovascular disease, no more brains wasting away in an Alzheimer's fog, and quadriplegics will rise up from their chairs and walk again. However, many investigators believe that these results can only be obtained by using embryonic stem cells. But harvesting these cells requires the

destruction of human embryos, a practice that many believe is immoral and unethical. Consequently, stem cell research has become a very contentious area of research that has roused an often-rancorous debate at all levels of society.

This book, another volume in the New Biology set, discusses the different types of stem cells, how they are studied in the laboratory, and the diseases that may be treated with these cells. Two chapters are devoted to the many ethical and legal issues that are associated with stem cell research and therapy. The final chapter provides background material on cell biology, recombinant DNA technology, and other topics that are relevant to stem cell research.

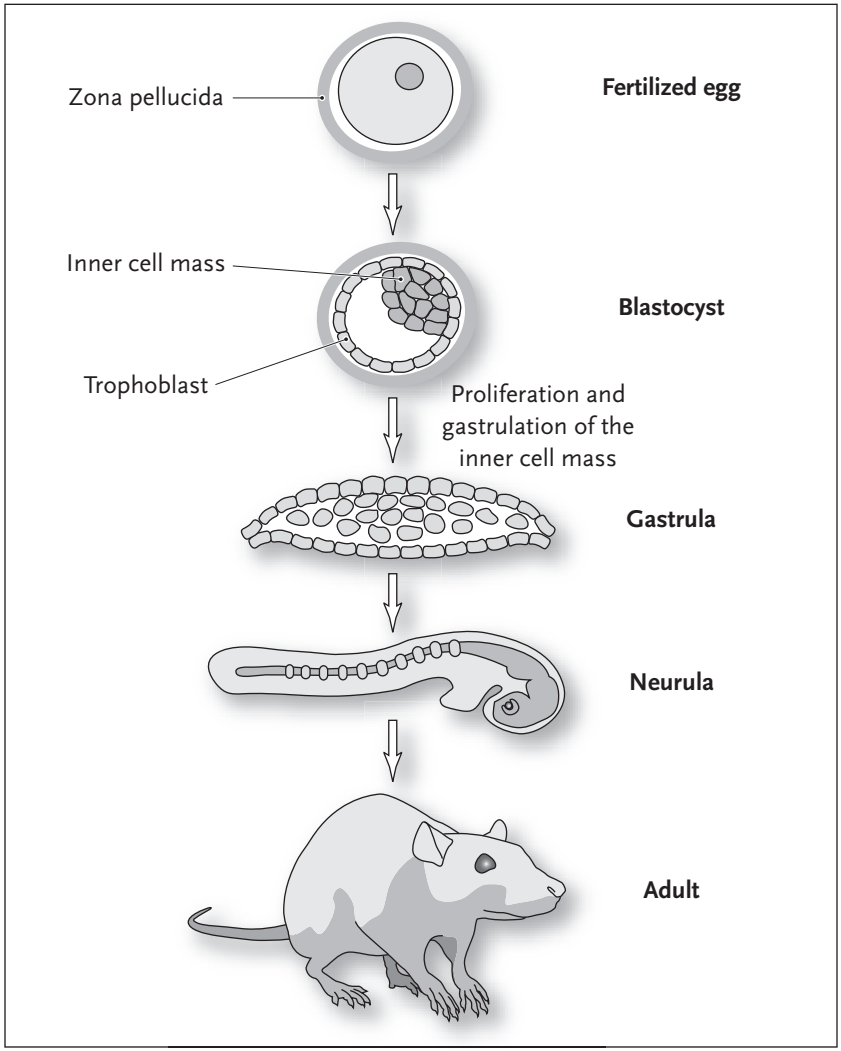
.1.

STEM CELLS ARE NOT ALL EQUAL

Stem cells can differentiate into more than one kind of cell, a characteristic referred to as plasticity. But the plasticity of a stem cell varies depending on whether it originates from an embryo or from an adult organism. In general, stem cells from embryos have greater plasticity than stem cells from adults, although this distinction may change in the near future. Embryonic stem cells have been isolated from mice, monkeys, and humans, but most of what we know about these cells has been learned by studying mouse stem cells.

Throughout the 1990s, when scientists were studying stem cells from rodents, standard protocols were developed for culturing, testing, and manipulating these cells. Other stem cells, from different species or from adult tissues, are now studied by using those protocols, so that one type of stem cell can be easily compared with another. The protocols cover the behavior of stem cells in vivo (within a living organism) and in vitro (in cell culture). The most important in vitro characteristic includes the cell's ability to proliferate (grow and divide) for an indefinite period of time while maintaining an embryonic phenotype. Phenotype, used in this context, refers to all the observable characteristics of the cell: its shape, or morphology (in this case, a simple, rounded shape); its behavior, meaning the way in which it interacts with other cells and the methods it uses to communicate with those cells; and finally, the composition of the glycocalyx, the molecular forest that covers the surface of all cells. The glycocalyx varies depending on the differentiation state of the cell. The types of proteins embedded in the

membrane of an embryonic cell are different from those of a fully differentiated adult cell. In other words, the differentiation of a cell is associated with the restructuring and maturation of the glycocalyx.



Mammalian embryogenesis. Mammalian embryos, like those of all vertebrates, pass through similar developmental stages, marked by the appearance of the blastula, gastrula, and neurula. A mammalian blastula is called a blastocyst.

An important in vitro characteristic of stem cells is their ability to differentiate into many different kinds of cells. Differentiation may occur spontaneously or through a process called directed differentiation, which occurs when the cells are allowed to contact each other, or when certain growth factors are added to the culture medium. The in vivo behavior of candidate stem cells is established by isolating the cells and then injecting them into a mouse to see if they will differentiate. How the stem cells respond to these procedures depends primarily on whether they were isolated from an embryo or an adult.

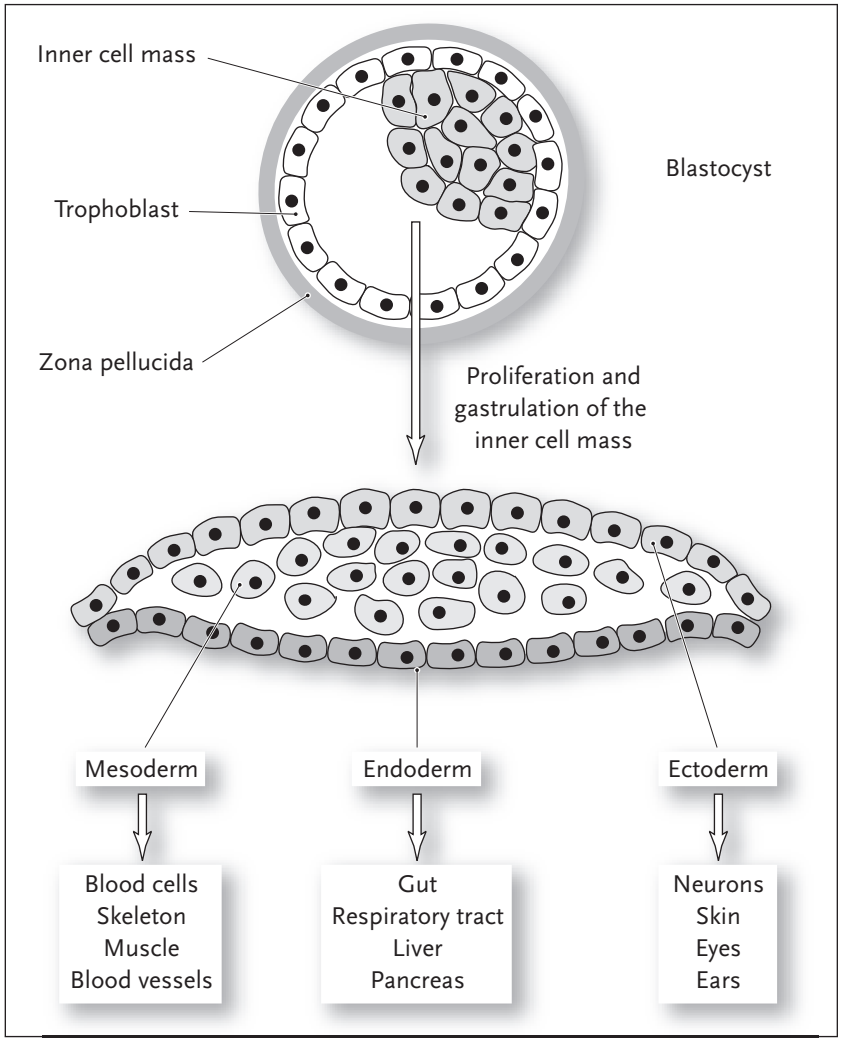
The Mammalian Embryo

Mammalian embryos, like all vertebrate embryos, pass through similar developmental stages that are marked by the appearance of the blastula (blastocyst), gastrula, and neurula. There are, of course, many differences between mammalian and amphibian embryogenesis. Mammals develop inside the mother's womb, where they receive nourishment through the placenta and are surrounded by a chorionic membrane that produces a fluid-filled incubation chamber. Frog embryos, typical of all amphibians, develop in a pond, and the mother places all the nourishment they need in the egg.

Consequently, all the cells in a frog embryo will become part of the adult. But this is not the case with mammalian embryos, since a portion of their cells must be used to produce the placenta and the chorionic membrane. The distinction between embryonic and nonembryonic cells is evident by the blastula stage, when two kinds of cells have developed: the inner cell mass (ICM) and the trophoblast. Embryonic tissue is derived exclusively from the ICM, whereas the trophoblast differentiates into the placenta and the chorionic membrane and performs an essential role in the implantation of the embryo in the mother's uterus.

The mammalian blastula is called a blastocyst because it embeds itself in the lining of the uterus. The mammalian gastrula has a flattened profile, in contrast with the spherical shape of amphibian and many invertebrate embryos. Flattened embryos are a common feature among animals, such as birds and reptiles, that produce very yolky eggs, on top

of which the embryo floats like a tiny raft. Mammals do not develop inside an egg, but being descended from reptilian stock, we have retained their form of embryogenesis.



Formation of the three germ layers in a mammalian embryo. During gastrulation, movement and proliferation of the inner cell mass produces the three primary germ layers: the ectoderm, endoderm, and mesoderm. Eventually, the ectoderm covers the entire embryo. Movements and fate of the trophoblast and zona pellucida are not shown.

An important property of embryonic development is cell-to-cell contact and communication. This intimate and very intricate relationship between the cells is crucial for the induction and coordination of cellular differentiation. By touching one another, or by signaling at a distance, cells can induce other cells to differentiate into a particular type of tissue. By careful regulation of the timing and location of induction, the cells are able to establish the embryo's basic body plan: where's up, where's down, which end is going to be the head, and which will be the tail.

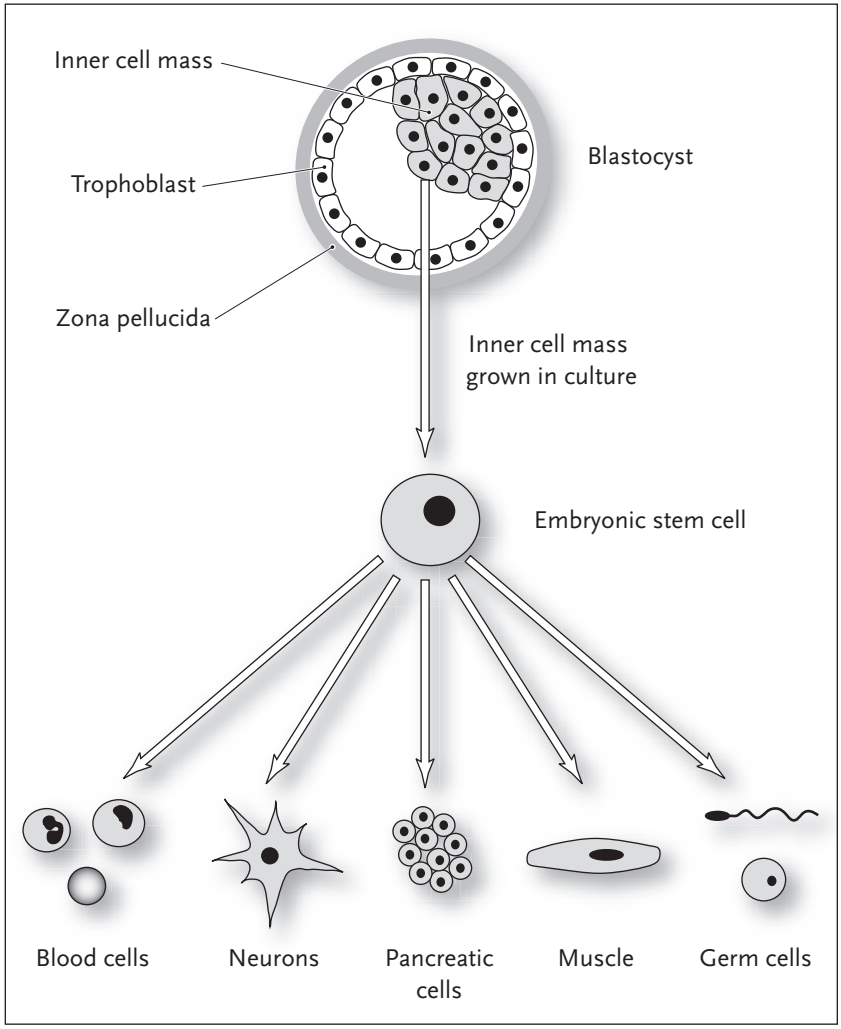
Embryonic induction occurs in three major stages, called primary, secondary, and tertiary induction. Primary induction is associated with gastrulation, a coordinated movement of the cells that leads to the formation of the three germ layers: the ectoderm, mesoderm, and endoderm, which give rise to all the tissues of the adult body. Secondary induction involves a complex interaction between the three germ layers to initiate neurulation, the development of the brain, spinal cord, segmented spinal column, and peripheral nerves. Tertiary induction regulates organogenesis, or development of the body's organs and appendages.

Embryonic Stem Cells

The ultimate stem cell is the fertilized egg, which, being totipotent, can give rise to an entire organism consisting of hundreds of different kinds of cells. Amphibian blastomeres, from two- or four-cell embryos, also retain their totipotency and are a good example of an embryonic stem (ES) cell.

Mammalian ES cells are obtained exclusively from the inner cell mass (ICM) of a blastocyst, and when placed in cell culture, they can differentiate into many kinds of cells, representing all three embryonic germ layers. However, once the association between the ICM and the trophoblast is disrupted (as when ES cells are placed in culture), the ES cells cannot develop into an embryo. For this reason, they are said to be pluripotent rather than totipotent. In culture, ES cells are immortal, proliferating indefinitely while retaining an embryonic phenotype.

Scientists first demonstrated the remarkable plasticity of ES cells in the 1980s, when cells from the ICM of a mouse blastocyst were trans-

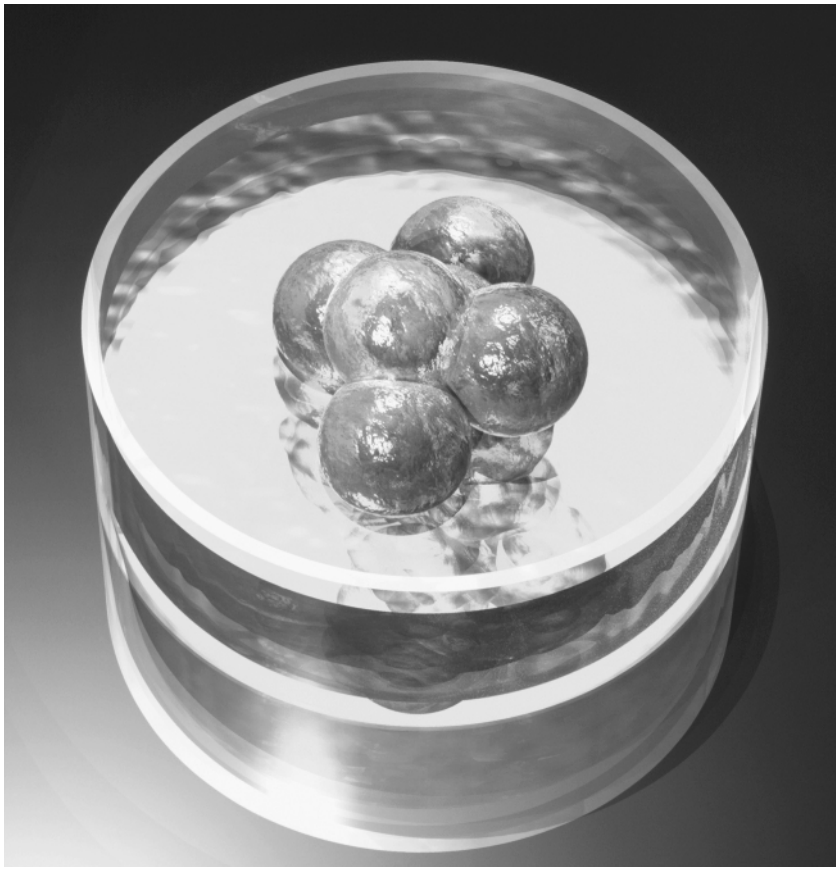


Differentiation of embryonic stem cells. Embryonic stem cells are obtained from the inner cell mass of a blastocyst. When cultured, these cells can differentiate into many different kinds of cells, representing the three germ layers.

ferred to the cavity of a second mouse blastocyst where they differentiated, in vivo, into a variety of tissues. In other experiments, ES cells were placed in culture dishes and allowed to differentiate spontaneously (in

this case, the cells are said to have differentiated in vitro). The first, and crucial, stage of in vitro differentiation involves the aggregation of the cells into small clumps called embryoid bodies. Contact between the cells is necessary for differentiation to occur and echoes the events of normal embryogenesis, in which cell-to-cell contact and interactions between the three germ layers determine the developmental fate of a given group of cells.

In culture, cell-to-cell communication within the embryoid body leads to the formation of neurons, skin cells, contracting muscle tissue, and other cell types. Although embryoid bodies have a loose organization,



Conceptual computer artwork of a bundle of stem cells in culture, showing their typical rounded morphology *(Laguna Design/SPL/Photo Researchers, Inc.)*

CELLS PRODUCED BY EMBRYONIC STEM CELL DIFFERENTIATION

Cell Type	Description
Adipocyte	Cells that make and store fat compounds
Astrocyte	A type of glia (glue) cell that provides structural and metabolic support to the neurons
Cardiomyocyte	Cells that form the heart; also called myocytes
Chondrocyte	Cells that make cartilage
Dendritic cell	Antigen-presenting cell of the immune system
Endothelial cell	Cells that form the inner lining (endothelium) of all blood vessels
Hematopoietic cell	Cells that differentiate into red and white blood cells
Keratinocyte	Cells that form hair and nails
Mast cell	Associated with connective tissue and blood vessels
Neuron	Cells that form the brain, spinal cords, and peripheral nervous system
Oligodendrocyte	Myelin-forming glia cells of the central nervous system
Osteoblast	Give rise to osteocytes, or bone-forming cells
Pancreatic islet cells	Endocrine cells that synthesize insulin
Smooth muscle	Muscle that lines blood vessels and the digestive tract

some of them resemble blastocysts. Something similar happens when ES cells are injected under the skin of adult mice. Again, the cells tend to aggregate into small clumps, which in this case are benign tumors called teratomas. Examination of these tumors has shown that they consist of gutlike structures, neural tissue, cartilage, bone, and sometimes hair.

When cultured ES cells aggregate to form embryoid bodies, or teratomas, they are trying to form a gastrula and the three germ layers, just as they would have done during normal embryonic development.

But without the trophoblast surrounding them and the signals they normally receive after implanting in the mother's womb, these cells are like small children trying to find their way home on a very dark night. They have lost their vision, and have no map to guide them. They can make all of the cells the body will ever need, but they do not know where to put them or how to connect them.

Adult Stem Cells

Not long ago, scientists believed all repairs in the adult body were carried out by the affected tissue: If the skin was cut, other skin cells along the damaged area would divide and migrate to seal the wound; break a leg, and chondrocytes (bone-forming cells) would repair the damage. Other organs, such as the brain and heart, were thought to be incapable of self-repair, because the myocytes and neurons were known to be postmitotic. However, the picture has become much more complicated and, from a clinical point of view, much more interesting in recent years with the discovery of stem cells in many adult tissues and organs (see the table on page 10).

While embryonic stem cells are defined and identified by having been isolated from the ICM of a blastocyst, identifying adult stem cells and determining their source is very difficult. Indeed, no one knows the ultimate source of these cells. Some have suggested that they are embryonic cells, set aside during development of each tissue, whereas others believe they may have been part of a migrating population of embryonic cells that took up residence in various parts of the body during the processes of neurulation and organogenesis. A third possibility is that stem cells were produced after embryonic development was complete by the de-differentiation of a select group of cells within the various tissues of the body. It is not clear why these cells are able to repair some tissue but not others.

The plasticity of adult stem cells appears to be less than that of an ES cell. This difference has been demonstrated by determining the fate of both kinds of cell after being injected into mice. ES cells, being undifferentiated, do not exhibit a tendency to find "home"—that is, to return to the tissue from which they derive. Instead, ES cells associate with each other, forming teratomas in various regions of the body. Adult

ADULT TISSUES AND ORGANS KNOWN TO HAVE STEM CELLS

Source	Description
Brain	Stem cells of the brain can differentiate into the three kinds of nervous tissue—astrocytes, oligodendrocytes, and neurons—and, in some cases, blood-cell precursors.
Bone marrow	These occur as hematopoietic stem cells, which give rise to all blood cells, and as stoma cells, which differentiate into cartilage and bone.
Endothelium	These stem cells are called hemangioblasts and are known to differentiate into blood vessels and cardiomyocytes. They may originate in bone marrow, but this is uncertain.
Skeletal muscle	These stem cells may be isolated from muscle or bone marrow. They mediate muscle growth and may proliferate in response to injury or exercise.
Skin	Stem cells of the skin are associated with the epithelial cells, epidermal cells, hair follicle cells, and the basal layer of the epidermis. These stem cells are involved in repair and replacement of all types of skin cells.
Digestive system	Located in intestinal crypts, or invaginations. These stem cells are responsible for renewing the epithelial lining of the gut.
Pancreas	Many types are believed to exist, but examples have yet to be isolated. Some neural stem cells are known to generate pancreatic β cells.
Liver	The identity of liver stem cells is still unclear. Stem cells from bone marrow may repair some liver damage, but most repairs seem to be carried out by the hepatocytes (liver cells) themselves.

stem cells, on the other hand, have differentiated enough that they know where home is, and that is where they collect: Stems cells derived from bone marrow return to the bone marrow, and those of neural origin migrate to the brain or spinal cord. In culture, ES cells can differentiate into a wide variety of cell types, representing all germ layers, whereas adult stem cells differentiate into a smaller range of cells, representing one or two germ layers.

The first adult stem cell to be characterized was found in the bone marrow and is known to be responsible for replenishing blood cells. Prior to the discovery of stem cells, it was assumed that blood cells were replaced exclusively by precursor cells—that is, cells that could mature into blood cells but could not differentiate into other cell types. Other adult stem cells, found in the skin, may be involved in wound repair. In most cases, division and movement of the skin cells repairs a cut, whereas deeper wounds may activate stem cells to repair the damage.

The existence of adult stem cells is extremely important, since their use to cure diseases removes the ethical problems associated with the use of embryonic stem cells. The limited plasticity of adult stem cells is a major hurdle to overcome before they will be a practical alternative to ES cells, but many believe this feat will be accomplished as study of these cells, grown in tissue culture, improves.

Stem Cells in Culture

All stem cells, whether isolated from adults or embryos, have the ability to proliferate in culture and can differentiate into many different kinds of cells. Differentiation of these cells occurs spontaneously or through directed differentiation. Researchers choose the initial culturing conditions that prevent spontaneous differentiation. This involves growing the cells on a layer of feeder cells that secrete substances into the culture media that help nourish the ES cells. For the most part, the identity of these substances is unknown. The feeder cells in most in vitro experiments are mouse embryonic fibroblasts that have been exposed to γ-radiation, a form of radioactivity that destroys the cell's ability to replicate.

Feeder cells help maintain the stem cells in an undifferentiated state; they also provide a favorable substrate for the ES cells to grow on.

When the stem cells are needed for a directed differentiation experiment, they are transferred to fresh culture plates that lack a feeder cell layer, then given culture media containing one or more growth factors. In some cases, directed differentiation simply involves transferring the cells to fresh plates, lacking a feeder layer, so the cells can form embryoid bodies.

Directed differentiation of adult stem cells has confirmed previous in vivo studies, which concluded that adult stem cells have less plasticity than ES cells. When stem cells from an adult mouse brain are placed in culture, they differentiate into different kinds of neural tissue but not the variety of cell types that are produced by ES cells. Likewise, adult stem cells from the bone marrow differentiate, in vitro, into blood cells, neurons, and fat cells, but not glandular tissue (see table on page 13). Moreover, scientists have less control over the differentiation process of adult stem cells than that of ES cells. Adult bone marrow stem cells differentiate spontaneously when placed in culture, and there is no way to stop them. Consequently, it is very difficult to maintain a continuous culture of these cells. So far, only mouse ES cells are able to proliferate in vitro for an indefinite period of time while retaining an embryonic phenotype.

The gradual loss of plasticity in some stem cells, mouse or human, may not be a serious problem, because some ES cells have been maintained for more than two years in an undifferentiated state. Moreover, even after months of culturing, stem cells will still respond to directed differentiation. This responsiveness is extremely important if these cells are going to be used to treat a disease. For example, to repair a damaged heart, cultured ES cells, kept in stock for years, would be stimulated to differentiate into myocytes, after which they would be injected into the circulatory system of the patient. The trick here is to ensure that the cells are only partially differentiated (that is, they are myocyte precursors); otherwise they would lose the power to proliferate and be unable to effect repairs.

Simply injecting freshly isolated stem cells into the patient would lead to the formation of teratomas, as occurs when stem cells are injected into mice. Injected myocyte precursors, on the other hand, will home to the parent organ, the heart, and, it is hoped, initiate repairs. Of course, this scenario is very hypothetical and may contain a

large dose of wishful thinking. For the present, we simply do not know what a myocyte precursor will do when it reaches the heart, and, perhaps more important, we do not know how the heart itself will respond.

Directed differentiation of cultured mouse or human stem cells has led to the production of many cell types, but in most cases scientists do not understand the details of the process. Manipulating the culture conditions by adding a growth factor or a molecule that influences gene expression may cause the stem cells to differentiate into nervous tissue or bone, but the intermediate steps that lead to the transformation are still obscure.

DIRECTED DIFFERENTIATION OF MOUSE STEM CELLS

Source	Conditions	Resulting Cell Types
Adult bone marrow	Interleukin-3 Interleukin-6	Platelets Red blood cells White blood cells
Adult bone marrow	Epithelial growth factor (EGF) Neurotrophic growth factor Retinoic acid	Astrocyte Neurons
Adult brain	Fibroblast growth factor Oligodendrocyte	Astrocyte Neurons
Embryo	Retinoic acid Insulin	Adipocyte
Embryo	Retinoic acid Oligodendrocyte	Astrocyte Neurons
Embryo	Retinoic acid Dimethylsulfoxide	Cardiac muscle Skeletal muscle Smooth muscle
Embryo	Interleukin-3	Dendritic cells Macrophage

Consequently, directed differentiation requires a good deal of guesswork. Various compounds known to influence gene expression can be added to the culture media to see if they will stimulate differentiation and, if they do, which kind of cell is produced. In this way, scientists are in the process of constructing a catalog of culture additives that will lead to the production of specific cell types from cultured embryonic and adult stem cells. The study of directed differentiation may also help scientists understand the molecular nature of plasticity and the factors that distinguish embryonic and adult stem cells. This information could pave the way to effective therapies based solely on the use of adult stem cells.

Stem Cell Markers

Identifying a stem cell is not always easy. Adult stem cells account for only one out of 100,000 cells of the total cell population, so the odds of finding one, at the best of times, are small indeed. Stem cells have a simple morphology, and one might think this would set them apart from other cells in the body, but there are many differentiated cells that have a similar size and shape. Consequently, it is almost impossible to separate stem cells from differentiated cells by simple visual inspection. The situation with ES cells is a little better, since their source and identity are known without question. However, all stem cells change somewhat when placed in culture, making it necessary to monitor their behavior and to track any changes in their state of differentiation.

To this end, scientists have developed a set of markers that work for both mice and humans, which simplify the identification of stem cells and the evaluation of their phenotype. There are many different stem cell markers, but they all fall within one of three groups: glycoprotein receptors that are embedded in the cell membrane; cell-specific gene expression; and cell-specific molecules such as hormones, enzymes, or structural proteins.

GLYCOPROTEIN RECEPTORS

White blood cells carry cell-surface receptors called CD4 and CD8, which are specific for mature T lymphocytes. The protein that binds to these receptors is called a ligand and can be detected with a procedure

called immunofluorescence. Briefly, this involves placing the cells on a microscope slide and covering them with a solution containing the ligand. During incubation, the ligand binds to the receptors (if present), after which the sample is covered with a solution containing a fluorescent-labeled antibody that will attach specifically to the ligand. After an appropriate incubation period, the slide is examined under a fluorescent microscope. Cells carrying CD4 or CD8 receptors will be colored blue or green, whereas all the other cells will be colorless. A negative reaction—where all cells are colorless—indicates that the stem cells have not differentiated, or that they have not differentiated into T-lymphocytes.

Fluorescent markers are often used in conjunction with a machine called a fluorescence activated cell sorter (FACS). A suspension of thousands of cells is treated with the immunofluorescence procedure to tag any stem cells that may be present with a fluorescent dye, after which the sample is injected into the FACS machine. The cell suspension passes through a very thin tube, which forces the cells to move past a laser beam in single file. The laser beam gives each cell an electric charge: Those that carry the fluorescent marker receive a negative charge; the rest receive a positive charge. An electromagnetic field directs the negatively charged cells into one tube, while the positively charged cells are directed to a separate tube. A FACS machine can isolate a single stem cell from a population of more than 100,000 cells in less than an hour.

CELL-SPECIFIC GENE EXPRESSION

The expression of certain genes in specific cell types is another kind of stem cell marker. Some neurons are known to express a gene called *noggin,* which is not expressed in nonneural tissue. Using a procedure called fluorescent in-situ hybridization (FISH), it is possible to detect the cells that express the *noggin* gene. Like the immunofluorescence procedure just described, the final product of the FISH reaction is a field of cells on a microscope slide that are either colored or not. Cells expressing *noggin* will be blue; those that do not express this gene will be colorless. Stem cells that are differentiating into neural tissue will be colored. Stem cells that are not differentiating, or are differentiating into nonneural tissue, will be colorless.

CELL-SPECIFIC MOLECULES

Some cells produce special hormones, macromolecules, and enzymes that may be used as markers because they are not found in all cells. The β cells in the pancreas are the only cells in the body that produce insulin, so this hormone makes an excellent marker for the differentiation of a stem cell into a β cell. All cells make a protein called tubulin, which is an important structural protein, but neurons make their own special neurotubulin, which can serve as a marker of neural differentiation. Embryonic stem cells produce a protein called genesis, which plays an important role in gene transcription. ES cells also have special glycoproteins in their membranes, such as stem cell antigen-number 1 (Sca-1) and embryonic antigen-3 (Ea-3). Thus, genesis, Sca-1, and Ea-3 are markers for the undifferentiated state of a stem cell. The disappearance of these molecules is an early indication the cell is beginning to differentiate, even before an outward change in appearance is evident. Detection of cells carrying these markers is possible using immunofluorescence or FISH.

A special DNA sequence called a telomere may also be used in conjunction with the standard markers to help identify a potential stem cell. A telomere is not a gene but a simple repetitive sequence located at the tip of each chromosome that is needed for the proper duplication of each chromosome during the cell cycle. With each round of cell division, the telomere shrinks in length, but an enzyme called telomerase later restores it. Actively dividing cells have high levels of telomerase, whereas postmitotic cells have none. Consequently, the presence of telomerase is an indication that the cells are actively dividing, as stem cells usually are.

Another approach to monitoring a stem cell's differentiation status employs the technique of gene therapy to introduce a reporter gene, called GFP, into the genome of a stem cell. The GFP gene codes for a fluorescent protein that emits a green light and can be engineered so it is active only when the cell is in an undifferentiated state and is turned off when the cell begins to differentiate. Consequently, differentiation of the cell is associated with a loss of the green color.

An alternative use of the GFP gene is to use it to give cells a permanent green color. Dr. Fred Gage and his colleagues at the Salk Institute

recently obtained stunning results on stem cell differentiation by using the GFP gene. Their intention was to study the ability of astrocytes (a kind of neuron) to stimulate differentiation of adult stem cells into neurons. The in vitro experiments were conducted using cells isolated from the rat. After transfecting stem cells with a GFP reporter gene, Gage's team added them to a culture plate containing astrocytes (a reporter gene is always turned on, so the GFP reporter gene gave the cells a permanent green color). Each time the stem cells divided, they produced green daughter cells, so their fate was easily monitored. This procedure gave clear evidence that the astrocytes could induce stem cells to differentiate into neurons, since green neurons appeared that could only have originated from the green stem cells.

Stem cells are generally characterized through the examination of more than one marker and are referred to accordingly. This produces a very specific, though somewhat awkward, naming convention. A neural stem cell (NSC), isolated from the brain, and evaluated for the expression of CD8, *genesis* and *noggin* would be referred to as NSC ($CD8^{-/low}$, $genesis^{-}$, $noggin^{+}$), meaning the expression of CD8 is absent to low, *genesis* is absent, and *noggin* is present. The use of these markers was established in studies involving research animals, such as the mouse, but they are now an indispensable part of the more recent research effort to identify and characterize human stem cells.

.2.

HUMAN STEM CELLS

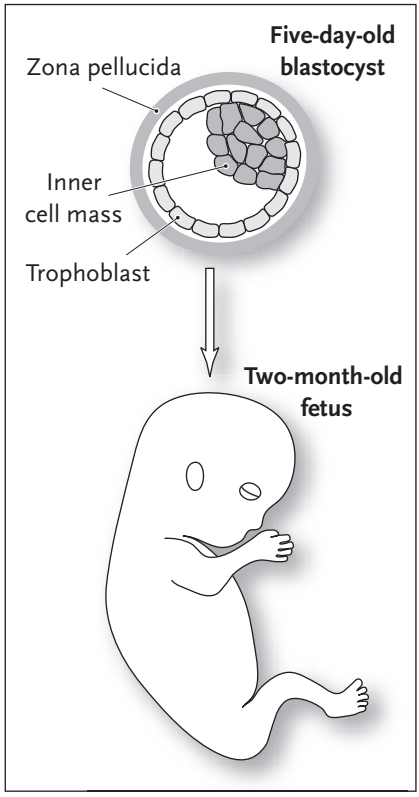
Most of what we know about stem cells has come from studying mice and rats. All the protocols we now use to evaluate stem cells come from work on those animals, and many scientists will continue using rodents to gain a deeper understanding of what it is that makes a stem cell tick. But stem cells from mice cannot be used to cure a human disease, even though stem cell research may make it possible for a human to receive a pig-heart transplant. Repairing a severed spinal cord or curing Parkinson's disease can only be attempted with human stem cells. Consequently, many scientists are eager to study human stem cells and are hoping the cells will show the same properties of plasticity and proliferation demonstrated by rodent stem cells.

Studying human stem cells is a difficult and demanding enterprise, not only because of the science, but because of the greater social issues associated with any experiment in which a human being, no matter at what stage of development, is the guinea pig. Ethical issues in research of this kind set the pace and the scope of the experiments that are allowable. These issues will be dealt with at length in a later chapter. This chapter will focus on the science of human stem cells and the experiments that are being done to study their unique characteristics.

Collecting Human Embryonic Stem Cells

Human embryonic stem cells were collected for the first time in 1998 by two different research teams, one headed by Dr. James Thomson at the University of Wisconsin, and the other by Dr. John Gearhart at Johns

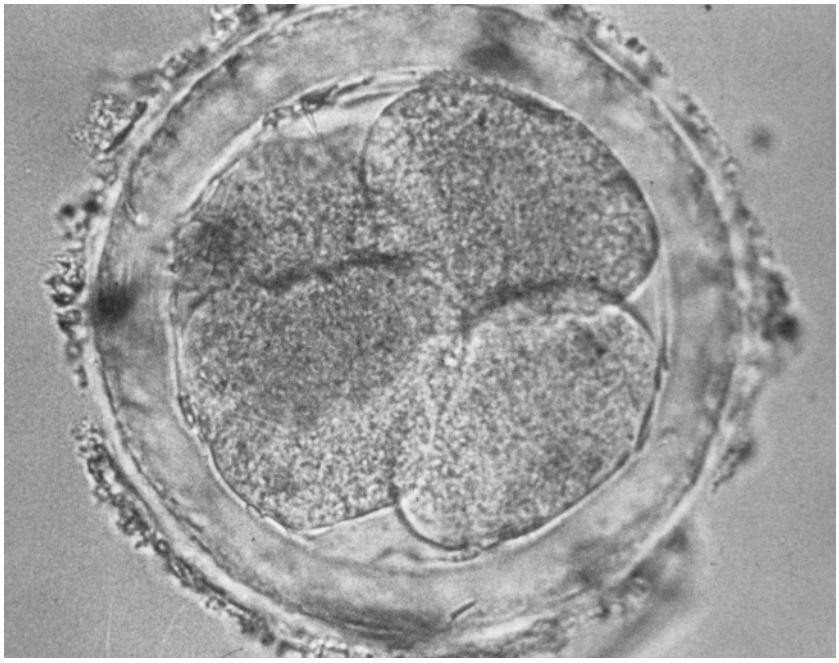
Hopkins University. Thomson's group obtained cells from five-day-old blastocysts that were obtained from in vitro fertilization (IVF) clinics; Gearhart's team isolated cells from two- to four-month-old fetuses that were obtained after elective abortions. The cells obtained from the blastocyst are embryonic stem (ES) cells, whereas the cells obtained from the fetuses are embryonic germ (EG) cells.



Human embryo and fetus used for the collection of stem cells. Embryonic stem cells are collected from the inner cell mass of five-day-old blastocysts. Embryonic germ cells are collected from two- to four-month-old fetuses. At two months of age, the fetus is about 25 mm (or 1 inch) long.

Thomson's team began with 36 embryos, of which only 14 developed to the blastocyst stage. The inner cell mass was isolated from these embryos and used to establish five human ES cell lines: H1, H7, H9, H13, and H14. Culturing of these cells was carried out in a multistep process. After removal of the ICM from the blastocyst, the cells were placed in culture dishes containing a layer of feeder cells. The ES cells were allowed to grow for two weeks, after which they were transferred to plates lacking a feeder layer in order to induce the formation of embryoid bodies. Cells from the edge of the embryoid bodies were collected and transferred to fresh plates.

The five original cell lines continued to divide without differentiating for six months and were able to form teratomas in mice. Cell line H9 went on to proliferate for more than two years and is now being used by many research groups around the world. Karyotyping of the

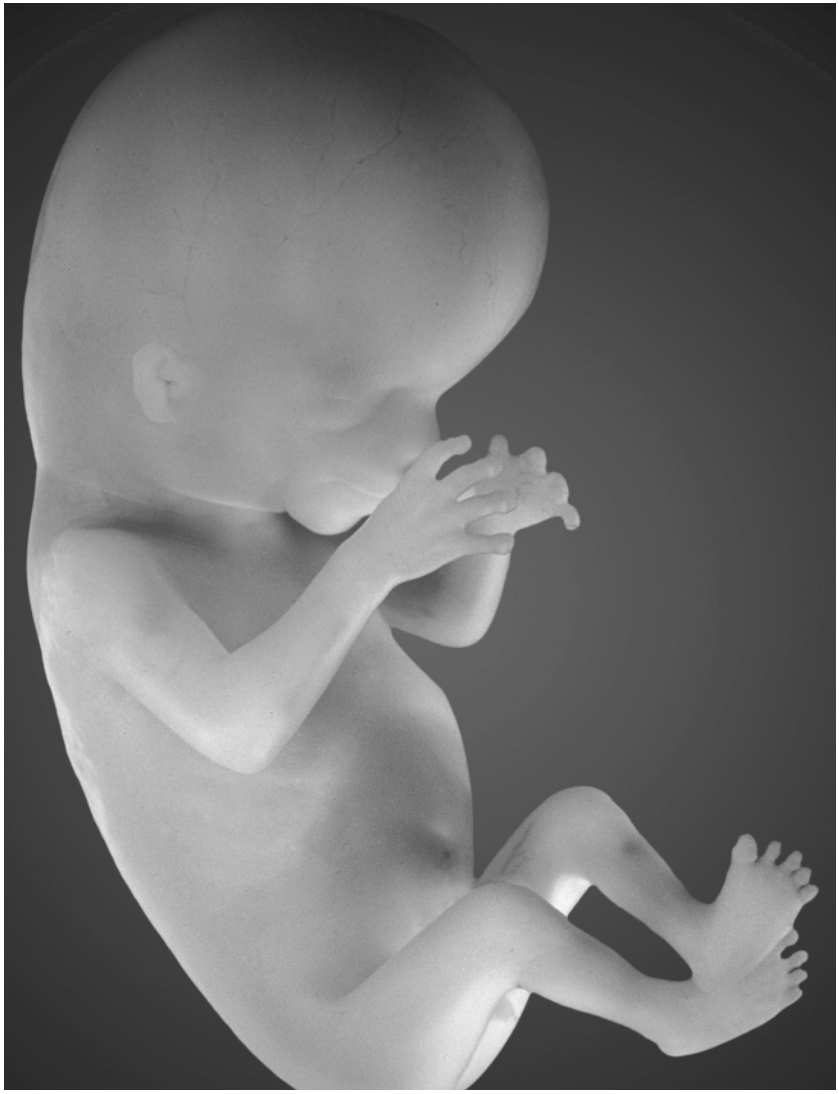


A human embryo, five to 10 days old *(Claude Edelmann/Photo Researchers, Inc.)*

cultures showed that three of the cell lines were male (XY) and two were female (XX). All the cell lines continued to maintain a normal karyotype. This later observation was extremely important, because if the cells grew well in culture but suffered chromosomal damage (i.e., broken chromosomes or daughter cells that received an incorrect number of chromosomes), they would be useless for medical therapies.

Gearhart's team established a different line of stem cell cultures, consisting of EG cells, by collecting cells from the gonadal ridge of nine-week-old fetuses. The cells from each fetus were used to establish a separate culture line. The gonadal ridge is located in the fetuses lower mid-back. Cells in this area form the gonads of the adult, which produce eggs or sperm. The culture conditions were similar to those used by Thomson's team, except for the inclusion of a cytokine called leukemia inhibitory factor (LIF) and a mitogen called fibroblast growth factor (FGF). These compounds were added to the culture plates to enhance cellular proliferation.

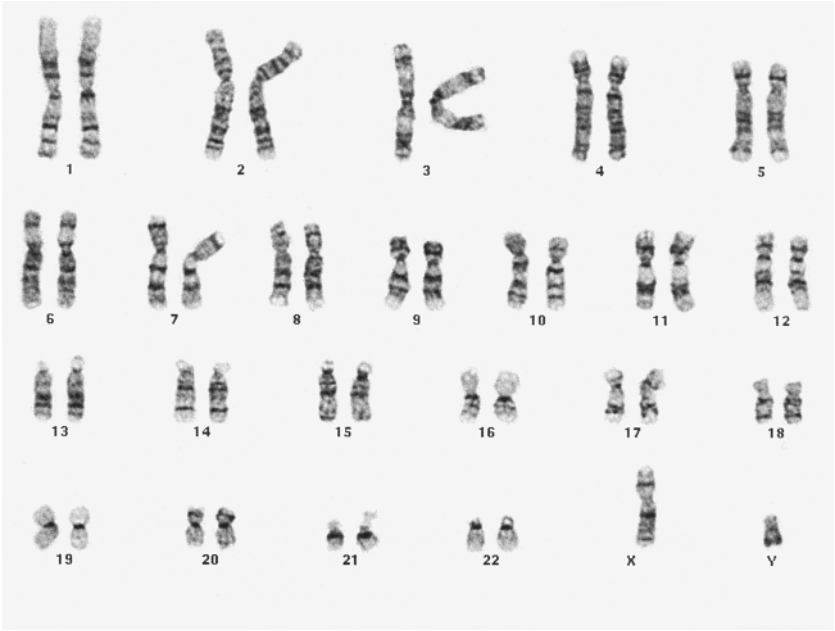
After three weeks of growth, the human EG cells resembled mouse ES cells and expressed several embryonic markers, such as stage-specific embryonic antigen-1 (SSEA-1) and SSEA-3. Both these markers are cell-surface glycoproteins. Some of the cultures were induced to form



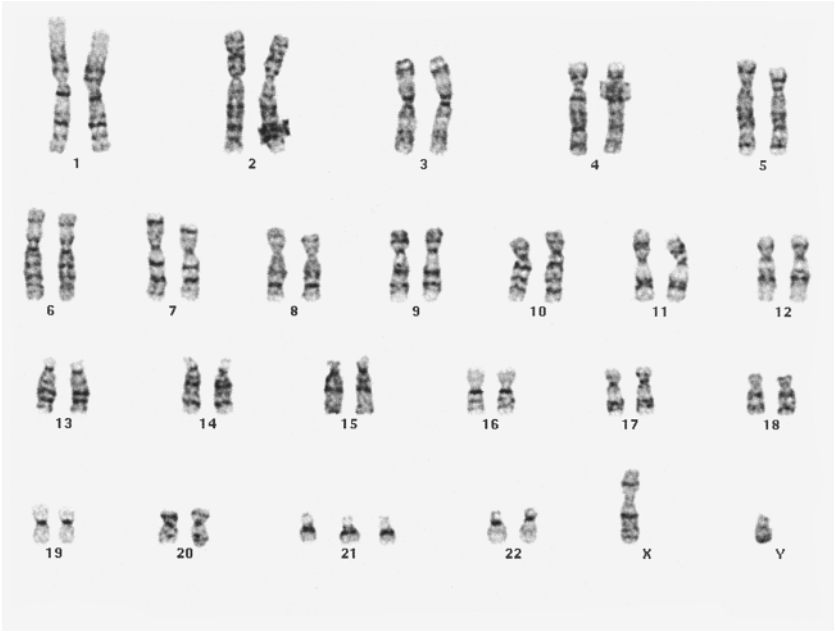
A human fetus after three months of development *(John Watney/Photo Researchers, Inc.)*

embryoid bodies by the removal of LIF, FGF, and the feeder cell layer. Examination of the embryoid bodies showed differentiation of cell types representing all three embryonic germ layers: ectoderm, mesoderm, and endoderm. The identities of the cells were based on physical appearance and the expression of various markers.

Although these results suggest the EG cells are pluripotent, just as ES cells are, they are not pluripotent in vivo, since they failed to produce teratomas in mice. This limited plasticity makes EG cells less desirable candidates for research than ES cells. In addition, everything that can be achieved with EG cells can be accomplished with embryonic or adult stem cells, calling into question the value of this line of research.



A normal human male karyotype. A karyotype represents the full set of chromosomes arranged with respect to size, shape, and number. Human cells contain 23 pairs of chromosomes; 22 autosomes and a pair of sex chromosomes. The sex chromosomes are either X or Y. Females have two X chromosomes, whereas males have an X and a Y chromosome. Karyotypes are used to diagnose genetic illnesses and may also be used to assess the normality of stem cells growing in culture. *(Courtesy of Diana Dowsley)*



An abnormal human male karyotype. This karyotype was produced from an individual suffering from Down's syndrome, a condition that is characterized by an extra chromosome 21. A karyotype such as this may be used to confirm the abnormality of stem cells growing in culture. *(Courtesy of Diana Dowsley)*

Many people are willing to accept the isolation of stem cells from human blastocysts but are opposed to the use of highly developed human fetuses as a source for these cells, particularly if they can be obtained in some other way.

Fate of the Donor Embryo

There is no way to remove cells from the ICM of a blastocyst without killing the embryo. Animal cloning experiments, involving mouse and cow embryos, have been able to separate blastomeres to produce healthy twins or quadruplets, but those experiments were performed on embryos long before they reached the blastocyst stage. An absolute dependence between the ICM and the trophoblast develops by the blastocyst stage, such that neither can survive without the other. Informed

consent from the embryo's parents is required before it can be used for stem cell research.

The situation involving the fetuses used to collect EG cells is somewhat different in that they are killed during the abortion procedure. Nevertheless, informed consent must still be obtained, as is done for embryonic donations. Several methods are available for terminating a pregnancy, and the amount of damage done to the fetal tissue varies depending on which procedure is used. However, the choice of method is up to the attending physician and cannot, by law, be influenced by a researcher who is planning to collect fetal tissue. The use of human fetuses for stem cell research is carefully regulated, and the scientists must obtain special permission to conduct their experiments. Dr. Gearhart's team received permission to carry out their procedure from the Johns Hopkins Joint Committee on Clinical Investigation (JCCI), in accordance with guidelines established by the American Public Health Service Act.

Directed Differentiation

Collecting human stem cells, whether from an adult or an embryo, is just the first part in a long line of procedures that, hopefully, will lead to a treatment for a medical disorder. Once the cells are collected, they are grown in culture and stimulated in various ways to determine the types of cells they may produce. The procedures used are identical to those established in previous experiments with mouse stem cells. Human stem cells, like mouse stem cells, form embryoid bodies when grown on plates lacking a feeder layer. Although the embryoid bodies vary with regard to cellular composition, they usually include cells that look like neurons and myocytes.

Directed differentiation of human stem cells is always carried out on cells that have been isolated from embryoid bodies and replated to form a monolayer of cells. So far, adult stem cells have been stimulated to produce several cell types, either through exposure to various growth factors or by being injected into mice (see table on page 25). Selecting growth factors for these experiments is an example of educated guesswork. If the intention of the experiment is to produce neurons or epithelial cells, the scientists involved will select growth factors such as

epidermal growth factor (EGF) and nerve growth factor (NGF), both of which are known to influence the proliferation of these cells in vivo. Other growth factors, such as transforming growth factor (TGF) or the

DIRECTED DIFFERENTIATION OF HUMAN STEM CELLS

Source	Conditions	Resulting Cell Types
Adult bone marrow	Injection into mice	Hepatocytes Red blood cells White blood cells
Adult bone marrow	Epithelial growth factor (EGF) Neurotrophic growth factor Cultured with fetal rat neurons	Neurons
Adult bone marrow	Transforming growth factor (TGF) Fetal bovine serum Insulin	Adipocyte Chondrocyte Osteocyte
Embryo (H9 cell line)	TGF EGF Cardiomyocyte NGF Fibroblast growth factor (FGF) Bone morphogenic protein (BMP) Hepatocyte growth factor (HGF) Retinoic acid	Neuron Blood cell Precursor Liver Pancreas Muscle
Embryo (H9 cell line)	Injection into mice Leukemia inhibitory factor	Bone Cartilage Gut epithelia Neural epithelia Smooth muscle Striated muscle

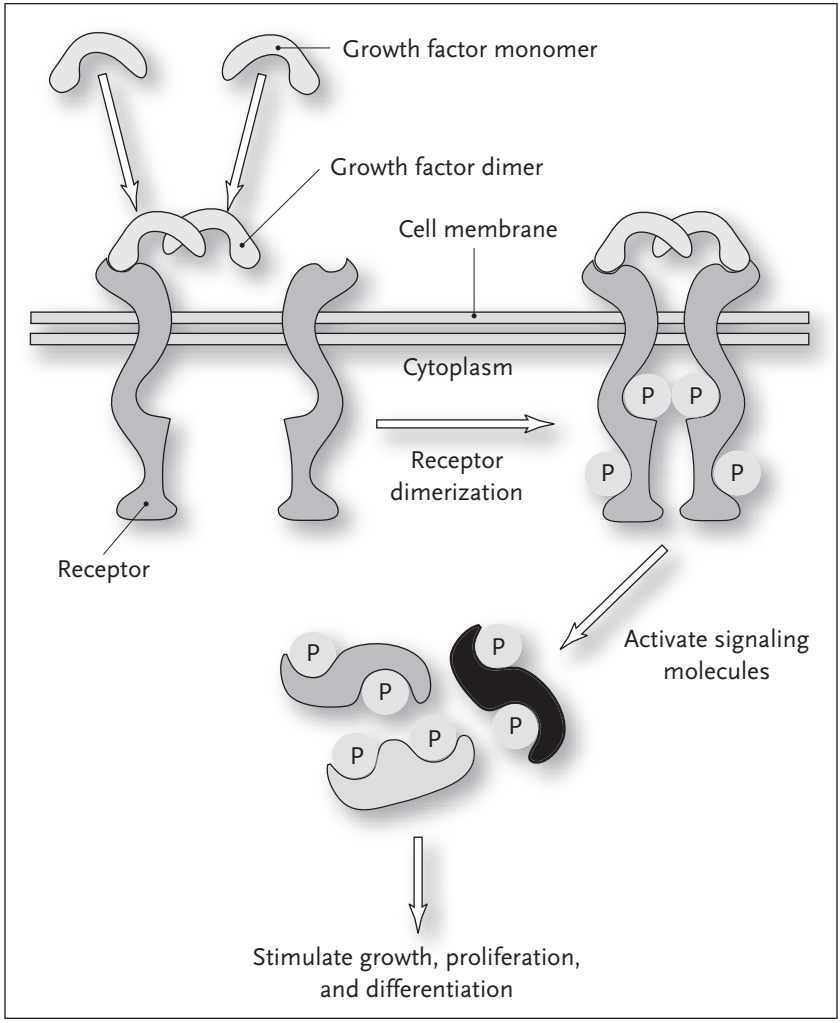
hormone insulin, are known to influence tissues derived from the mesoderm, such as muscle and cartilage. In some cases, the growth factors producing a certain kind of cell are unknown. This occurs when stem cells differentiate in vivo or when they are cultured in the presence of fetal bovine serum (blood serum obtained from a fetal cow), which contains many yet-to-be-identified growth factors.

Directed differentiation of embryonic stem cells is carried out as described for adult stem cells. Retinoic acid, epidermal growth factor (EGF), bone morphogenic protein (BMP), and fibroblast growth factor (FGF) are some of the growth factors that have been used in ES cells. All these factors trigger development of cells that would normally be derived from the ectoderm. Other growth factors, such as activin-A and transforming growth factor (TGF) initiate differentiation of mesoderm-derived cell lines. Hepatocyte growth factor (HGF) and nerve growth factor (NGF) promote differentiation of cells that represent all three germ layers. When all these factors are added individually to cell cultures derived from embryoid bodies, they give rise to 11 different cell types, representing ectoderm, mesoderm, and endoderm (see table on page 25).

Spontaneous differentiation of ES cells in culture will produce several different kinds of cells on a single plate, but stimulating the cultures with any one of the growth factors mentioned above tends to focus the differentiation toward a single cell type. Cultures stimulated with FGF differentiate into epithelial cells that express the marker keratin, a common skin protein. Cultures treated with activin-A produce muscle cells that express a muscle-specific enzyme called enolase. Retinoic acid typically stimulates the production of neurons, but it is also known to initiate development of other cell types.

An important and much sought-after result of directed differentiation is the production of blood cell precursors for the treatment of leukemia. Blood cells are not among the cells produced by human ES cells through spontaneous differentiation, and growth factors so far tested do not initiate the formation of these cells. However, reports indicate some success by growing human ES cells in the presence of γ-irradiated mouse bone marrow cells (γ-irradiated blocks replication of the mouse cells). The mouse cells apparently provide an unknown growth factor that triggers differentiation of the ES cells into blood

cells. The differentiated cells express CD34, a marker for blood cell precursors, and under certain conditions, these cells will form erythroid cells, macrophages, and other blood cells.



Growth factor receptors. The growth factor forms a dimer (two monomers stuck together) and binds to the receptor, stimulating dimerization of the receptor. This stimulates kinase activity of the receptor, which phosphorylates itself and several signaling molecules. The activated signaling molecules stimulate cell growth, proliferation, and differentiation.

Human ES cells have a greater tendency to differentiate spontaneously when placed in culture than do mouse ES cells. Scientists wishing to produce a culture of human myocytes or neurons through directed differentiation must start with a population of undifferentiated cells, otherwise the product of the experiment might be a curious hybrid cell that could yield unpredictable, and perhaps fatal, results if used in a clinical setting. Markers of the embryonic state, such as stage-specific embryonic antigen (SSEA), are being used, in conjunction with a FACS machine, to isolate and segregate the undifferentiated cells from the rest of the population so they can be used for directed differentiation. In addition, the isolation of the undifferentiated cells will make it possible to study the differences between those cells that remain embryonic and those that become partially or wholly differentiated.

Growth Factors

The growth factors used for directed differentiation are themselves a fascinating group of molecules. One of the most famous is nerve growth factor (NGF), isolated in 1981 by Dr. Rita Levi-Montalcini after nearly 20 years of trying to track down factors that influence the behavior of neurons during embryonic development. In 1986, Dr. Levi-Montalcini and her collaborator, Dr. Stanley Cohen, received the Nobel Prize in medicine for discovering NGF and EGF, an epidermal growth factor used extensively in the treatment of severe burns and in stem cell research. Many growth factors (see table on page 25) are proteins that bind to a special kind of cell-surface protein that functions both as a receptor and as an enzyme, called a protein kinase, that can phosphorylate other proteins.

Phosphorylating a protein is analogous to flipping a switch to turn on a light. Phosphorylation of a protein turns it on, converting it from an inactive to an active state. When the activated protein is no longer needed, it is turned off by another enzyme, called a phosphatase, that removes the phosphate group. Protein kinase receptors consist of a single polypeptide chain that spans the cell membrane, with the portion inside the cell containing the enzymatic region. The kinase region of the growth factor receptor adds a phosphate group specifically to the amino acid tyrosine. Other protein kinases specialize in adding phosphate groups to serine or threonine amino acid residues.

Protein growth factors bind to their receptor as dimers. That is, two growth factor molecules join together to form an activated pair that is capable of binding to the receptor. Binding of the dimerized growth factor then stimulates dimerization of the receptor. Dimerization of the receptor activates its own kinase domain, located inside the cell. The receptor, in turn, phosphorylates cytoplasmic signaling proteins that stimulate cell growth, differentiation, and proliferation. Each growth factor binds to its own specific receptor, which activates a unique set of signaling molecules. This is the reason that some growth factors function primarily as mitogens (stimulating cell division, but not necessarily differentiation), while others stimulate differentiation as well as proliferation. Identifying the signaling molecules and the pathways they are on is a very active area of research aimed at improving our understanding of basic cell biology. Stimulating stem cells to grow and differentiate will become a much more precise science when growth factor signaling pathways are understood in greater detail.

Two other growth factors used in directed differentiation are leukemia inhibitory factor (LIF) and retinoic acid. LIF is a cytokine (signal proteins released by many kinds of cells), originally studied for its ability to force the differentiation of certain kinds of cancer cells. Once they differentiate, the cancer cells lose their ability to grow and divide indefinitely, thus inhibiting the spread of the tumor. LIF is also expressed by embryonic trophoblasts and is believed to play an important role in the implantation of the blastocyst in the mother's uterus.

Retinoic acid, closely related to vitamin A, is one of the few growth factors that is not a protein. This compound is a popular ingredient in antiwrinkle creams and is taken by some people to improve their night vision. Retinoic acid has been used in many different kinds of experiments to alter radically the normal gene expression profile of a cell. It is a powerful growth factor but somewhat unpredictable in its behavior and effects. Unlike other growth factor receptors, the receptor for retinoic acid is located inside the nucleus. Being fat-soluble, retinoic acid can diffuse directly into a cell and into the nucleus, where it binds to its receptor. The retinoic acid-receptor complex activates gene expression directly, without relying on the activation of intermediate signaling molecules.

There are many growth factors yet to be discovered. This is demonstrated by the fact that directed differentiation sometimes fails unless the cells are grown in the presence of other types of cells or in the presence of fetal bovine serum (FBS). FBS is obtained by collecting blood from an aborted cow fetus and spinning it in a centrifuge to remove all the blood cells. The amber-colored serum, containing many unidentified growth factors and hormones, is used in a wide variety of cell culture experiments. Some cells simply will not grow well unless FBS is added to the culture media. Efforts to determine the identity of the growth factors in FBS, or those contributed by other cells and in vivo experiments, are in progress in many labs around the world.

Future Prospects for Adult Stem Cells

Adult stem cells can differentiate into several different cell types, but the plasticity of these cells is in question. However, many people, scientists included, are uncomfortable with the current practice of obtaining stem cells from human blastocysts or fetuses and hope that adult stem cells will eventually resolve the issue.

Even if the social objections to the use of human ES cells did not exist, the study of adult stem cells would still be essential because the human ES cells now being isolated and cultured are good only for basic research and not for the treatment of human diseases. This is because our immune system will reject those cells, in a process known as graft-versus-host disease, just as it rejects foreign hearts, livers, or kidneys. Organ transplants are performed today, but they are not very successful, and the patients must be kept on immunosuppressants for the remainder of their lives. In the case of bone marrow transplants, which are used to fight leukemia, physicians try to find a close genetic match by obtaining the tissue from a parent or sibling. But even in those cases, the match, if one is ever found, is imperfect. Organ and tissue transplants, relying on immunosuppressants, are therapies of desperation. They work well enough to keep the patient alive for a few extra years, but they are not particularly healthy or happy years. Three procedures have been suggested that would improve the situation by preventing immune rejection of transplanted stem cells: cloning of the patient, stem cell gene therapy, and directed differentiation of the patient's own stem cells.

CLONING THE PATIENT

This is a theoretical procedure known as therapeutic cloning that mixes animal cloning with stem cell research (therapeutic cloning, as discussed in chapter 7, is illegal in Europe and laws to ban it are expected in the United States by 2004). Nuclei obtained from the patient's skin cells would be injected into enucleated eggs to produce human embryos that would develop up to the blastocyst stage, at which time the ICM would be isolated and cultured. The stem cells so derived would be stimulated with appropriate factors to induce differentiation of the tissue needed to treat the patient. This tissue would carry the patient's unique cell-surface antigens and, consequently, would not be attacked by the immune system. Although this procedure solves the problem of immune rejection, it reintroduces the ethical problems associated with harvesting stem cells from human embryos, since the patient's clone would be sacrificed to provide a cure. A second problem with this approach is that it would be extremely expensive—well beyond the means of anyone living on an average income—and it is very unlikely that medical insurance companies would be able to cover the cost.

STEM CELL GENE THERAPY

Embryonic stem cells obtained from a stock culture can be engineered to carry cell-surface antigens that would be acceptable to the patient's immune system, thus reducing the risk of tissue rejection. However, this procedure does not abolish the threat of tissue rejection. The engineered cell-surface antigens would not account for all the cell-surface antigens present on a typical cell. Consequently, the patient would still require some level of treatment with immunosuppressants. In theory, stem-cell genetic engineering could be tailored for each patient, but this would be even more difficult, and certainly more expensive, than cloning the patient.

DIRECTED DIFFERENTIATION OF THE PATIENT'S OWN STEM CELLS

Adult stem cells can be easily obtained from any patient by a routine bone-marrow tap. After the cells are grown in culture to increase their numbers, they could be directed to differentiate into the tissue needed

to treat the patient. This approach removes the ethical problems associated with harvesting human embryos and it solves the problem of tissue rejection. The cell surface antigens on the tissue so produced would be exactly right, so there would be no fear of rejection and no need for immunosuppressants. The cost of this procedure is not likely to exceed standard bone marrow therapy and would take but a few days to administer. For the treatment of blood disorders such as leukemia, this is the ideal procedure, and it is being tested in clinical trials now.

Brain disorders can also be treated in this way, since adult stem cells isolated from bone marrow can be directed to differentiate into neurons and glia cells (see the tables on pages 13 and 25). However, making this procedure applicable to all forms of disease will require the development of special methods that would give adult stem cells the same developmental plasticity of ES cells. This is especially important with regard to the expense of the procedure. Adult stem cells can be obtained from other organs, such as the pancreas or liver, which would allow production of a wider variety of cell types, but obtaining them would require major surgery. Collecting bone marrow is no more involved than having a tooth filled, so there is a great advantage to obtaining stem cells from this source.

Although the plasticity of adult stem cells is in question, experimental results obtained in 2003 by Dr. Catherine Verfaille at the University of Minnesota suggest that certain stem cells, isolated from adult bone marrow, may have plasticity equivalent to that of ES cells. Another researcher, Dr. Juliet Barker, also at the University of Minnesota, has found that stem cells isolated from umbilical cord blood not only demonstrate a high degree of developmental plasticity but also do not stimulate graft-versus-host disease. A great advantage of the cord blood stem cells is that umbilical cord blood banks could be established to provide a convenient source of stem cells. Verfaille's and Barker's research will be described in greater detail in chapter 5.

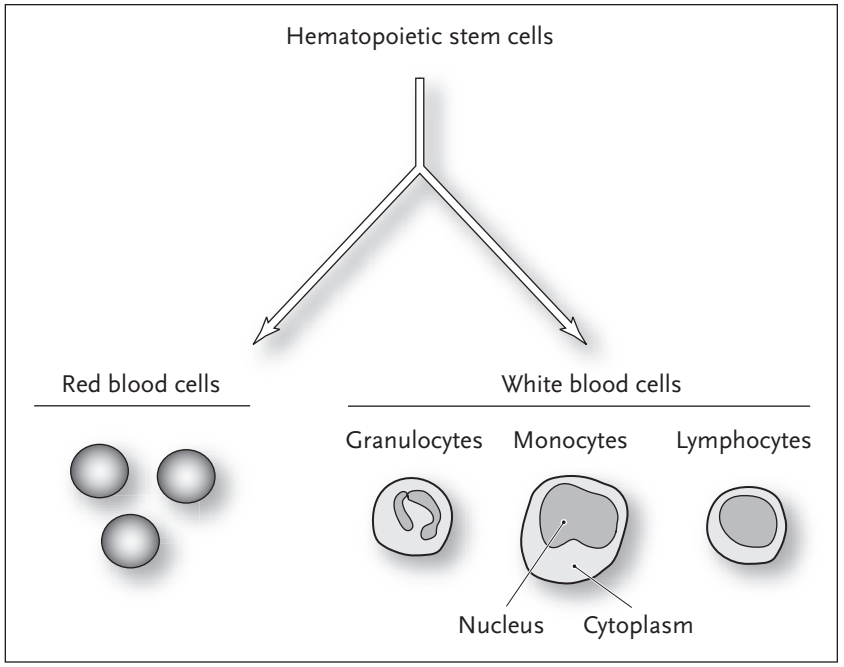
The speed at which stem cell research is progressing is such that we will see, within 10 years, the routine use of adult stem cells to produce any tissue the body needs. This will come about through a better understanding of growth factors and their signaling pathways. Initially, bone marrow stem cells or umbilical cord blood stem cells will be the

starting material for the therapies, but eventually we will be able to take a simple tissue scrape from inside a patient's mouth to obtain skin cells that will be de-differentiated to the level of a stem cell and then directed to differentiate into a different kind of cell, depending on what the patient requires. When we reach that stage, stem cell research will have come of age.

·3·
MEDICAL APPLICATIONS

Scientists were originally interested in stem cells because they provided a way to study totipotency and the plasticity of cells during embryonic development. But it is clear now that these cells can be used to treat, and possibly cure, a wide variety of diseases. Many of the diseases described in this chapter are also being treated with gene therapy, which attempts to correct a genetic abnormality by introducing a normal copy of the affected gene into appropriate cells in the body (for more information on gene therapy, see chapter 8). This an extremely powerful therapy, but the use of viral vectors to deliver the good gene can sometimes lead to deadly consequences. Stem cell therapy, on the other hand, does not depend on viral vectors but attempts to treat a disease by introducing whole human cells into the body, which, it is hoped, will restore the patient's health.

Both therapies are in their early stages of development, so it is not possible to say which is the better therapy. For now, it appears that some diseases may best be treated with gene therapy, while others may respond better to stem cell therapy. Diseases affecting blood cells, such as leukemia or ADA deficiency, or damage to the spinal cord, are best treated with stem cell transplants, whereas cancers of solid organs, such as the brain or lungs, are best treated with gene therapy. Of the two kinds of therapies, treatment with stem cells is more labor intensive. The cells must be isolated, stimulated to differentiate into the desired cell, grown in culture, and then injected into the patient. A major problem associated with stem cell therapy is immune rejection. If the therapy is allogeneic (meaning that stem cells are isolated from an individual other than the patient), the stem cells will be attacked and



Blood cell types. Blood cells, produced from hematopoietic stem cells, located in the bone marrow, are either red or white. Red blood cells (RBC) have no nucleus and are designed to carry oxygen to the tissues. RBCs get their color from a red oxygen-carrying protein called hemoglobin. White blood cells (WBC) are nucleated and part of the immune system. They are not involved in oxygen transport and carry no hemoglobin. WBCs are divided into three categories: granulocytes, monocytes, and lymphocytes. Granulocytes and monocytes are phagocytic, whereas the lymphocytes specialize in producing antibodies and in coordinating the immune response.

destroyed by the patient's immune system long before they have a chance to cure the patient's disease. Immune rejection is a major problem to overcome and is discussed at length in chapter 5. Immune rejection is also a serious problem for gene therapy.

The diseases described here represent those cases where stem cell therapy has a chance of being effective or where clinical trials have already demonstrated the effectiveness of the treatment. Much of the work with stem cells is preclinical, relying on results obtained from mice or rats. In these cases (neurological disorders and cardiovascular

disease), phase I clinical trials are still several years into the future (clinical trial phases are described in chapter 8).

Leukemia

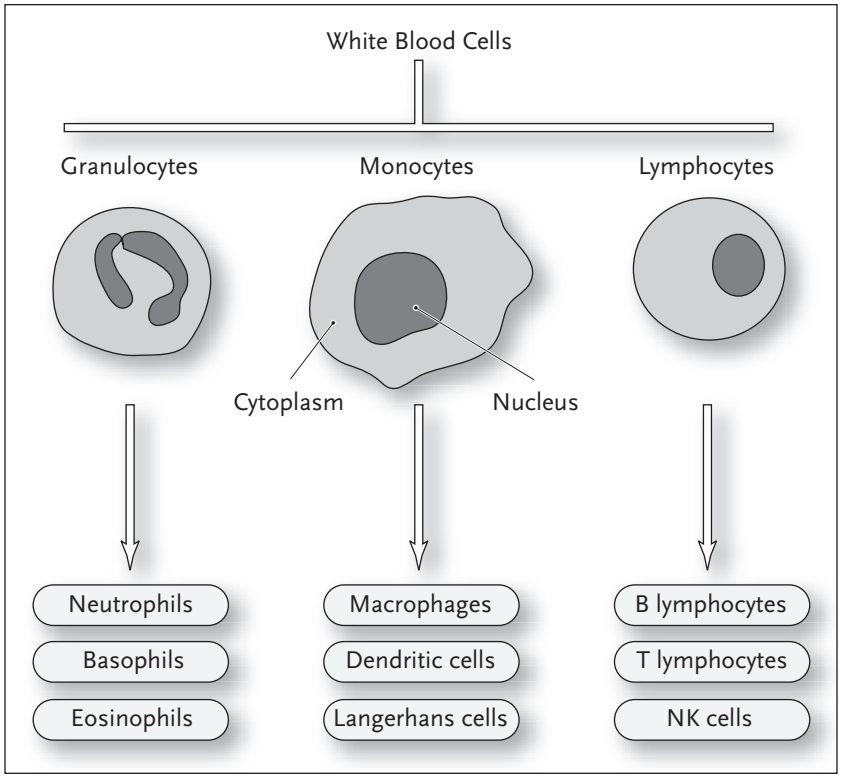
Each year in the United States alone, nearly 32,000 adults and more than 2,000 children develop leukemia, a cancer of the blood cells. There are essentially two kinds of blood cells: red blood cells (RBC) and white blood cells (WBC, or leukocytes). RBCs contain a red protein called hemoglobin and use it to carry oxygen from the lungs to the tissues. White blood cells do not carry oxygen but are part of the body's immune system. Leukemia affects a kind of white blood cell known as lymphocytes, which spend much of their time in the lymphatic system. Another form of lymphocyte, known as myeloid cells, spend their time in the bone marrow or general circulation.

Leukemia affects white blood cells only and can arise in either lymphoid cells (lymphocytic leukemia) or myeloid cells (myelogenous leukemia). The disease has two forms: acute and chronic leukemia. Acute leukemia progresses very quickly, destroying the patient's immune system. Chronic leukemia progresses much more slowly, and even though the leukocytes are transforming, they retain some of their normal functions, so the immune system is not destroyed so quickly or so completely.

The standard treatment for leukemia involves radiation and chemotherapy, which kill the cancerous cells. Extreme forms of this therapy involve the complete destruction of the bone marrow with radiation therapy, after which the patient receives new bone marrow from a suitable donor. The great problem associated with this therapy is the often-impossible task of finding a donor. The best donor is an identical twin, who provides genetically identical, or autologous (or autogeneic), transplant tissue. Nontwin siblings or a parent may also serve as donors, but in these cases, known as allogeneic transplants, rejection of the donated bone marrow is always a threat.

For many patients, there simply are no suitable donors, in which case the outlook is grim. Stem cell therapy has the potential to treat all forms of leukemia with autologous transplants, thus removing the need to find bone marrow donors. Stem cells, isolated from the bone

marrow of the affected patient, can be induced to differentiate into normal white blood cells and then grown in culture to increase their numbers. Once these cells are collected, the patient's cancerous bone marrow is destroyed, and the stem cell–derived blood cells are returned to the patient in order to reconstitute a healthy, cancer-free bone marrow.



White blood cells. These cells are divided into three major categories: granulocytes, monocytes, and lymphocytes. Granulocytes have a distinctive, lobular nucleus and are phagocytic (eat cells, viruses, and debri). Monocytes are large cells with an irregularly shaped nucleus. All monocytes are phagocytic; the largest members, the macrophages, can engulf whole bacteria and damaged or senescent body cells. Lymphocytes have a smooth morphology with a large round nucleus. B lymphocytes are nonphagocytic but produce antibodies. T lymphocytes and natural killer (NK) cells coordinate the immune response and can force infected cells to commit suicide.

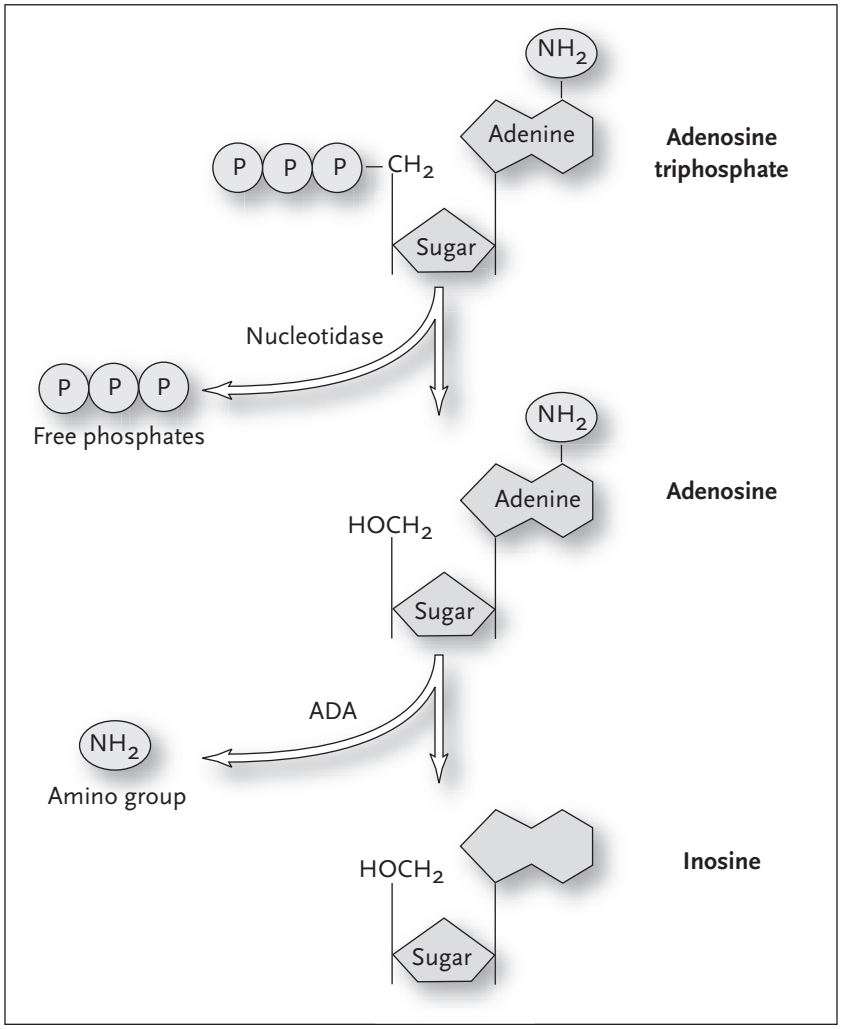
Immune Deficiencies

All animals have an immune system that is designed to combat invading microbes, and without it we face certain death from a multitude of diseases. The immune system consists of an enormous population of white blood cells that appear in many different forms, the most important of which are the B cells, T cells, and macrophages. B and T cells are lymphocytes that develop in bone marrow and the thymus, respectively. Macrophages are phagocytic blood cells that confront invaders head-on by eating them (a process called phagocytosis), whereas B-cells attack them indirectly by producing antibodies. T cells control and coordinate the immune response by releasing signaling molecules called cytokines that recruit macrophages and B cells. T cells also have the remarkable ability to detect invaders that are hiding inside a cell. Even more remarkable, they can force the infected cell to commit suicide in order to control the spread of the infection.

A common form of immune deficiency is called severe combined immunodeficiency-X1 (SCID-X1). This disease represents a group of rare, sometimes fatal, disorders that destroy the immune response. Without special precautions, the patients die during their first year of life. Those who survive are susceptible to repeated bouts of pneumonia, meningitis, and chicken pox.

All forms of SCID are inherited, with as many as half the cases being linked to the X chromosome. The mother passes on this disease, because males born with this disorder, possessing only a single X chromosome, usually die before reaching their reproductive years. SCID-X1 results from a mutation of a gene called *gamma-c* that codes for the interleukin 2 receptor (ILR2). There are many kinds of interleukin receptors, also referred to as cytokine receptors, and all of them are crucial for proper communication between the white blood cells. Defective cytokine receptors and the signaling pathways they activate prevent the normal development of T lymphocytes that play a key role in identifying invading agents as well as activating other members of the immune system.

A second form of SCID is due to a mutation in the adenosine deaminase (ADA) gene, located on chromosome 20. This gene is active in T lymphocytes, and the gene product, ADA, is required for the recycling



The role of adenosine deaminase in recycling nucleotides. Disassembly begins with the removal of the phosphates by a nucleotidase to produce adenosine, followed by the removal of the amino group by adenosine deaminase (ADA) to produce inosine. All three components are recycled to make new nucleotides and amino acids.

of nucleotides. Consequently, a mutation in the ADA gene leads to a toxic buildup of adenosine inside the cell, blocking the normal maturation and activity of this crucial member of the immune system. Some

patients suffering from ADA deficiency can mount a weak immune response, but in most cases, the response is abolished. The conventional treatment, involving a bone marrow transplant, has been successful in saving many lives, but as with leukemia patients, finding a compatible donor is often impossible.

Gene therapy has been used to treat ADA deficiency and SCID-X1 with moderate success. However, a combination of stem cell and gene therapies was used in 2002 by Dr. Alain Fischer of the Necker Hospital in Paris to cure SCID-X1, and by Dr. Claudio Bordignon at the San Raffaele Telethon Institute for Gene Therapy in Milan, Italy, to cure ADA deficiency. Fischer's team extracted bone marrow from affected patients, transfected the hematopoietic stem cells with a healthy copy of the *gamma-c* gene, and then reimplanted the cells into each patient. Of the eight patients so treated, seven developed a functional immune system. Some of the patients developed a normal T cell count within three months of being treated, which has so far been sustained. However, two of the patients subsequently developed a vector-induced leukemia. Bordignon's team, using the same procedure, transfected hematopoietic cells obtained from two patients with a healthy copy of the ADA gene. Both patients showed complete recovery and are now able to lead normal lives.

Diabetes

Diabetes is a chronic metabolic disorder that destroys the body's ability to utilize glucose, a molecule that is critically important to all cells, particularly neurons, as an energy source. The uptake of glucose is regulated by a hormone, called insulin, which is produced by the pancreas, a large gland located just below the liver. The pancreas has two types of cells, called α (alpha) and β (beta). The α cells produce digestive enzymes that are secreted directly into the large intestine. The β cells produce insulin, which stimulates the uptake of glucose by all the cells in the body. When diabetes strikes, β cells lose the ability to manufacture and release insulin, leading to a buildup of glucose in the blood. A chronic elevation of blood glucose levels results in the inappropriate glycosylation (addition of sugar to proteins) of many proteins in the blood, including hemoglobin, as well as many other proteins

associated with the cells and tissues. Systemwide protein glycosylation can lead to blindness, heart disease, kidney failure, and neurological disease. Diabetes is a major health problem in North America, where it causes approximately 500,000 deaths every year. Treatment is very expensive, amounting to about $98 billion annually.

There are two forms of this disease, known as type I and type II diabetes. Type I diabetes is an autoimmune disease in which the white blood cells attack and destroy the β cells of the pancreas. This form of the disease is sometimes called juvenile diabetes because it occurs predominately in teenagers, although it can strike at any age. Type II diabetes affects older people, usually when they are 50 to 60 years of age. In this case, the disease may be due to a genetic predisposition to short-lived β cells, or it may be due to β cell burnout, brought on by a lifelong preference for a diet that is heavy on sweets. This may account for the fact that nearly 80 percent of those suffering from type II diabetes are overweight. At last count, 10 genetic loci were known to be associated with the onset of both types of diabetes.

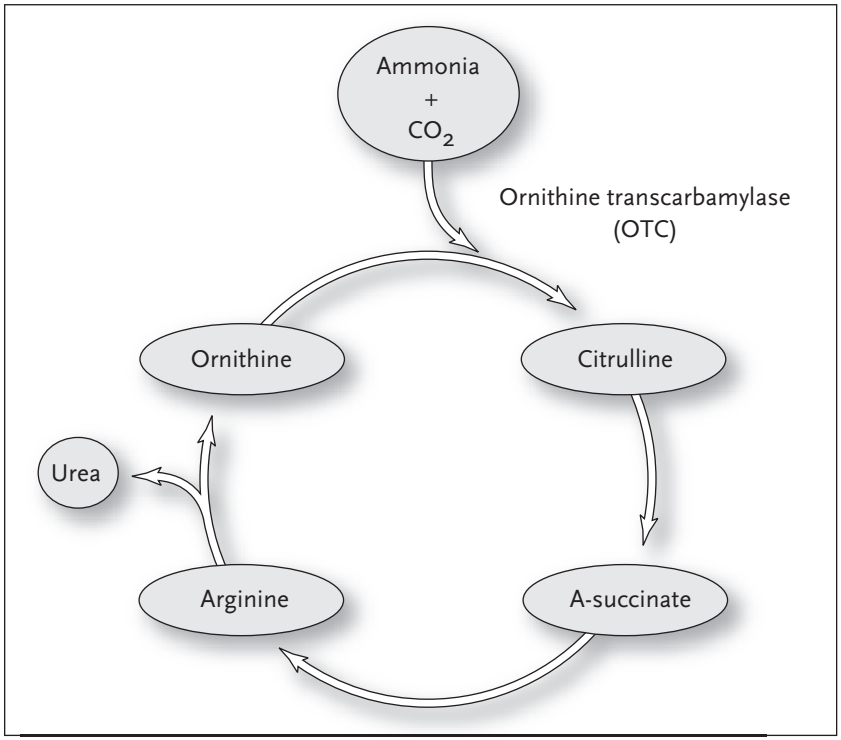
Diabetes is currently treated with daily injections of purified insulin. While this treatment controls the immediate danger of high blood glucose levels, it does not cure diabetes, nor does it remove the long-term threat of kidney failure or the other complications that are associated with this disease. For this reason, scientists have studied the possibility of curing this disease with stem cells. In 2001 research teams in Israel and at the Institutes of Health (NIH) in the United States found a way to direct the differentiation of cultured ES cells into β pancreatic cells that made and secreted insulin. Other investigators are trying to repeat this accomplishment using adult stem cells. If this can be done, stems cells could be harvested from each diabetic patient, stimulated to differentiate into beta cells, and then returned to the patient in the hope they will colonize the pancreas, thus curing the disease.

Liver Disease

Proteins that we eat for food are broken down to amino acids that may be used to generate energy or to construct proteins for our own use. A major by-product in the catabolism of amino acids is ammonia, the

stuff of Earth's ancient atmosphere and a molecule that in high concentrations is toxic. Cells deal with the toxicity by converting the ammonia to urea, a much safer molecule that passes out of our bodies as urine. The production of urea depends on the liver enzyme ornithine transcarbamylase (OTC). If OTC is defective, blood levels of ammonia increase rapidly, resulting in coma, brain damage, and death.

A gene therapy trial in 1999 to treat OTC deficiency was terminated abruptly when one of the patients, Jesse Gelsinger, died. Because this disease affects a solid organ, a viral vector carrying a normal copy of



The urea cycle. Cells in the liver rid the body of toxic ammonia by converting it to urea, which is then excreted by the kidneys as urine. Ammonia and carbon dioxide (CO_2) are added to ornithine to produce citrulline in a reaction that is catalyzed by the enzyme OTC. Other enzymes in the cycle produce argininosuccinate (A-succinate) and arginine; the latter is split into urea and ornithine, thus completing the cycle.

the OTC gene was injected into the liver, but in Gelsinger's case the immune response was so extreme it led to multiorgan failure and death. A safer alternative employs the same strategy used to treat leukemia and SCIDS. Hematopoietic stem cells, isolated from the patient and stimulated to differentiate into liver cells, can be transfected with a vector carrying a good copy of the OTC gene. Reintroduction of these cells into the patient is much safer than injecting naked viruses because transfected stem cells are much less likely to invoke an immune attack. Once injected into the patient, the stem cells, now partially differentiated, will colonize the liver and produce enough OTC to cure the disease.

Cardiovascular Disease

A potentially deadly form of cardiovascular disease affecting the coronary arteries is currently being treated with stem cells in animal models. Coronary arteries carry blood to the cardiomyocytes, or heart muscle cells, and if they become blocked or otherwise damaged, the cardiomyocytes die from lack of oxygen. In serious cases, this can lead to a massive heart attack and death of the patient. In milder cases, damage to the heart is minimal, but coronary circulation is insufficient to allow the patient a normal lifestyle. Stem cells, stimulated to differentiate into cardiomyocytes, could be injected directly into the heart muscle in order to repair the damage. The stem cell transplants may be augmented with gene therapy by introducing a gene directly into the heart that codes for a blood vessel growth factor that stimulates the growth and repair of the coronary arteries, in order to reestablish an adequate blood flow. Stem cell therapy to treat cardiovascular disease is still in a preclinical stage of development.

Neurological Disorders

There are several neurological disorders that may eventually be treatable with stem cells, including Alzheimer's disease (AD), Parkinson's disease (PD), Tay-Sachs disease, Huntington's chorea, and spinal cord trauma. Our understanding of Tay-Sachs disease and Huntington's

chorea is so incomplete that stem cell therapies for these diseases will not be available for many years yet. Although a great deal of progress has been made recently in our understanding of AD, PD, and spinal cord trauma, experimental results obtained in clinical trials in 2004 suggest that stem cell therapies for these diseases may not be available for 10 to 20 years.

ALZHEIMER'S DISEASE (AD)

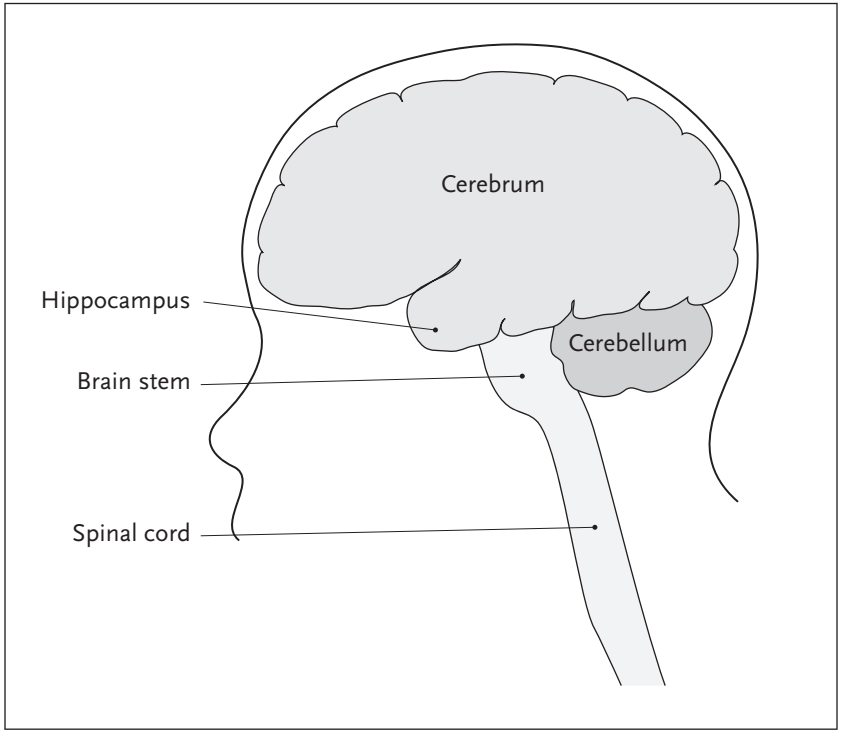
This is a devastating neurological disorder affecting the central nervous system (CNS), which leads to a progressive loss of memory, language, and the ability to recognize friends and family. The average time course of the disease, from early symptoms to complete loss of cognition, is 10 years. Alois Alzheimer first described AD in 1907; the disease has since become the fourth-leading cause of death among the elderly. The incidence of AD increases with age and is twice as common in women as it is in men.

The human CNS is divided into the cerebrum (the main portion of the brain including the cerebral cortex), the cerebellum, and the brain stem. The cerebrum is the home of our intellect and the source of our personality. It also processes and analyzes information from all the sensory nerves of the body. A special area of the cerebrum called the hippocampus is important for coordinating memory functions. The cerebellum regulates fine motor control over our muscles, making it possible for us to learn how to play the piano, manipulate fine objects with precise control, and perform other activities that require intricate coordination. The brain stem is in control of our automatic functions, such as the rate at which the heart beats, muscle contraction of the digestive tract, and our respiratory rate. It also controls our ability to sleep and to stay awake.

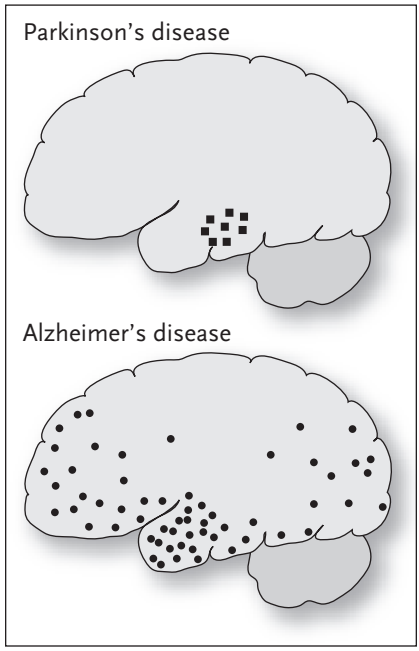
AD begins in the hippocampus. During the early stages, known as preclinical AD, some damage occurs to the brain, but not enough to produce outward signs of the disease. Over a period of years, AD spreads to many areas of the cerebrum, leading to the confusion and loss of memory that accompany this disease. Three genes have been identified that are associated with the onset of AD. The first of these is called *Tau,* which codes for a protein needed for the construction of microtubules. The second gene, *App* (amyloid precursor protein),

codes for a protein that is embedded in the cell membrane. The third gene, *Sen* (senilin, also known as presenilin), codes for an enzyme that may be involved in the processing of *App*. Defects in any or all these genes lead to the extensive death of neurons that is characteristic of AD.

Stem cells, stimulated to differentiate into neurons and glia cells, may be able to repair the damage to the brain that is caused by AD. Experiments with mice have shown that stem cells, injected directly into the brain, can produce functional neurons that make connections with healthy neurons near the lesion. Whether these neurons are making correct connections or not is yet to be determined, and we have no idea



The human central nervous system. The human brain consists of the cerebrum, the cerebellum, and the brain stem, which is continuous with the spinal cord. The brain and spinal cord are called the central nervous system (CNS). The hippocampus, lying beneath the surface, coordinates memory functions.



Neurological disorders. Alzheimer's disease (represented here by black circles) begins in the hippocampus, spreading over a period of years to affect several regions of the cerebrum. In contrast, Parkinson's disease (represented by black squares) is restricted to an area near the top of the brain stem called the substantia nigra.

what effect the growth of these transplanted neurons will have on the psychology of a human patient.

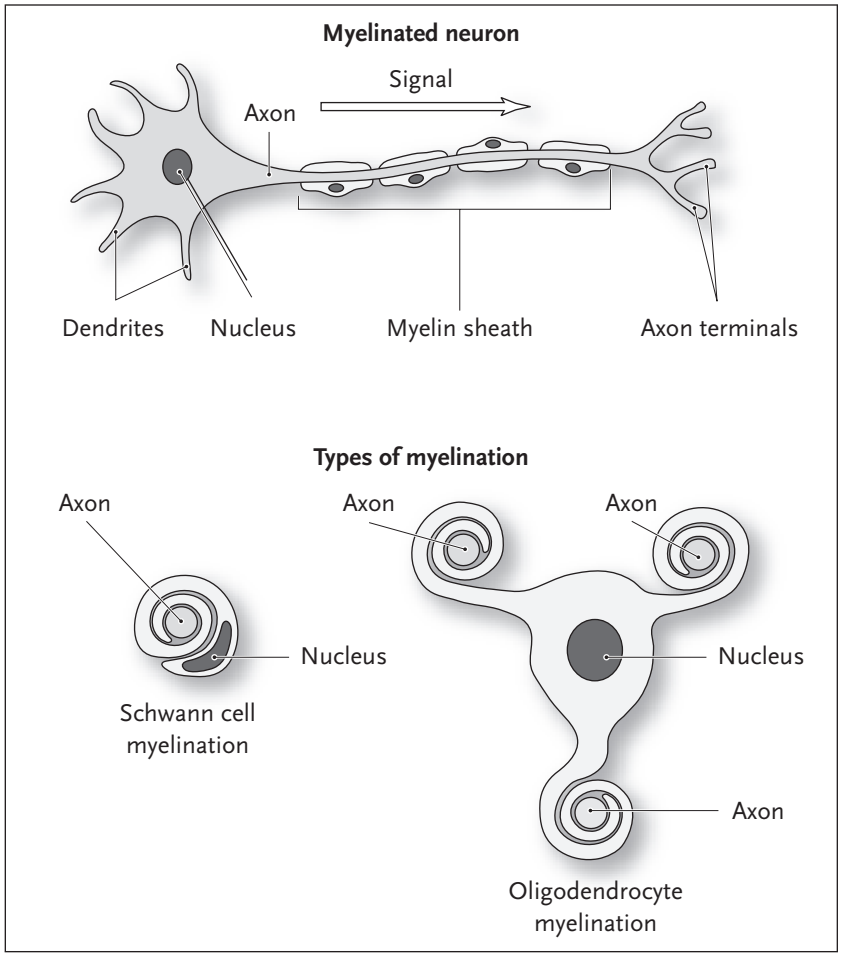
PARKINSON'S DISEASE (PD)

This neurological disorder was first described by James Parkinson in 1817; since then it has become a serious health problem, with more than half a million North Americans affected at any one time. Most people are over 50 years old when the diseasae appears, although it can occur in younger patients. Parkinson's disease (PD) is a neurodegenerative disease, affecting neurons in an area of the brain called the substantia nigra, which results in tremor, muscular stiffness, and difficulty with balance and walking (see the figure above left).

Until recently, Parkinson's disease was not thought to be heritable and research was focused on environmental risk factors such as viral infection or neurotoxins. However, a candidate gene for some cases of Parkinson's disease was mapped to chromosome 4, and mutations in this gene have now been linked to several Parkinson's disease families. The product of this gene is a protein called alpha-synuclein, which may also be involved in the development of Alzheimer's disease.

Since the neurological damage caused by PD is restricted to one region of the brain, stem cell therapy may be successful in treating this disease. Preclinical research has shown that it is possible to isolate

stem cells that can be stimulated to differentiate into dopaminergic neurons (i.e., neurons producing a neurotransmitter called dopamine). This is the type of neuron that populates the substantia



Neural signaling and myelination. A neuron receives signals at its dendrites and passes them on to other neurons through its axon. Circuits are constructed with axon terminals making connections with the dendrites of other neurons. Neural signaling is much more efficient when the axons are insulated with a myelin sheath. Myelin is made by Schwann cells or oligodendrocytes wrapping around the axon. Oligodendrocytes can insulate more than one axon at a time.

nigra and is damaged by PD. Injection of these neuronal stem cells into the brains of Parkinsonian mice relieved some of the disease symptoms, particularly the loss of motor control that is characteristic of this disease.

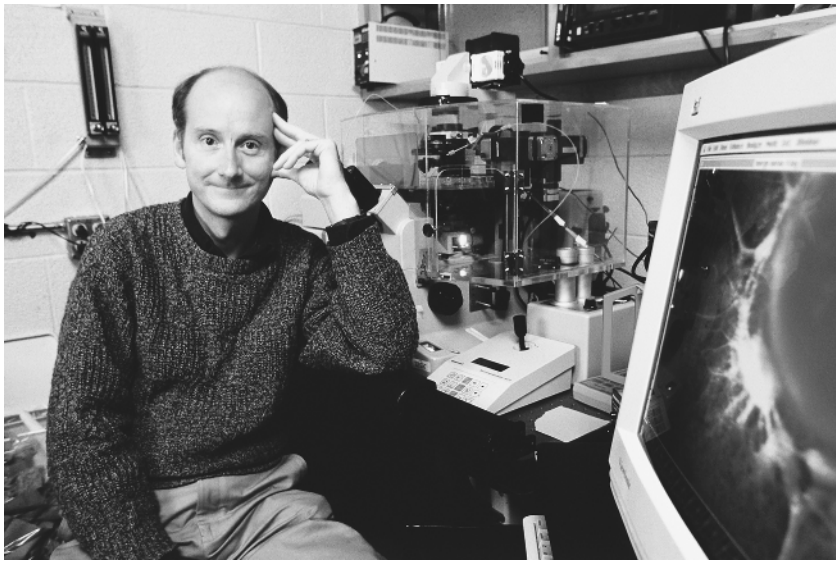
SPINAL CORD TRAUMA

Damage to the spinal cord caused by automobile accidents or falls from high places or from a horse can make it impossible for the brain to control the extremities and internal organs, such as the heart and lungs. The severity of the damage depends on how close to the brain the spinal cord injury is. If the individual's neck is broken, he or she may end up being a quadriplegic (a person who is unable to move the arms and legs) and may not be able to breathe properly. If the damage to the spinal cord is near the middle of the back, the patient will be paraplegic (unable to move the legs) but will retain control over the arms and lungs.

Repairing a damaged spinal cord is extremely difficult for two reasons. First, the neurons that were destroyed must be replaced, and the replacement neurons must make the proper connections to bridge the damaged area. Second, once the new neurons are in place, they must be insulated, much as an electrical wire is insulated, before they can work properly.

Neurons are remarkable cells, specially designed for communication and the construction of elaborate circuits. Signals enter a neuron through its dendrites and leave by its axon (some neurons may have more than one axon). The neural network, or circuitry, is established when the axon from one neuron makes a connection with a dendrite of another neuron. Neural circuitry, particularly in the spinal cord, does not work well unless the axons are insulated with a myelin sheath. The myelin is constructed from cells called oligodendrocytes and Schwann cells that wrap around axons to form a protective multilayered sheath. The oligodendrocytes, located in the central nervous system (CNS, consisting of the brain and spinal cord), can myelinate more than one axon at a time. The Schwann cells, located in the peripheral nervous system (i.e. any nerves outside the CNS, such as those in the toes and fingers), wrap around a single axon.

Stem cell therapists attempting to repair a damaged spinal cord must provide neurons to reestablish the circuit and oligodendrocytes



Dr. James Thomson, a developmental biologist at the University of Wisconsin, Madison. In 1998, his research group reported the first successful culture of human embryonic stem (ES) cells. *(University of Wisconsin, Madison/SPL/ Photo Researchers, Inc.)*

for insulation. Dr. Ronald McKay, a stem researcher at the National Institute of Health, has shown that mouse ES cells can repair some neural damage when injected into rats, but whether stem cells will be able to reestablish normal circuitry and, at the same time, remylenate the axons is yet to be determined. Dr. James Thomson, and other researchers, are confident, however, that this will be accomplished in the near future.

Organ Factories

Direct transplants of stem cells is, for the time being, the most practical application of this type of therapy. However, many scientists are interested in using these cells to grow whole organs in the laboratory that could be used to replace a defective heart, kidney, pancreas, lung, or liver. In 2002 a research team at Harvard Medical School grew a miniature cow's kidney from cultured embryonic stem cells. The ES cells were

obtained by cloning the cow and allowing the fetus to develop up to the stage where kidneys normally begin forming. By isolating cells from the fetus, in the area where the kidneys normally form, the researchers, in effect, isolated an embryonic kidney, which continued growing in cell culture. This cultured kidney was able to produce urine when implanted back into the cow from which the embryo was cloned.

This research demonstrates the feasibility of using stem cells to produce organs, and if successful, its results will go a long way toward relieving the chronic shortness of organ supplies for transplant surgery. Of equal importance is the effect it will have on relieving the ethical and social problems associated with the current practice of obtaining organ donations from family members, who must be subjected to severe surgery—even though they may be perfectly healthy themselves—from which they may not recover. (This aspect of stem cell research will be dealt with more fully in chapter 5.)

.4.

COMMERCIALIZATION OF HUMAN STEM CELLS

Conducting biomedical research is very expensive. Before a drug or therapy can be licensed for use as a standard medical treatment, it has to pass through a review gauntlet consisting of preclinical research using laboratory animals, followed by four stages of clinical trials in which the new treatment is tested on human volunteers. The total cost can range anywhere from $500,000 up to $3 billion. Governmental granting agencies, such as the National Institutes of Health (NIH) in the United States, allocate billions of dollars each year for basic biomedical research, but it is never enough to cover clinical research. That is why clinical trials involving stem cell therapies, and many others, including gene therapies, are backed financially by pharmaceutical companies. Indeed, without the backing of wealthy companies, many drugs and medical therapies would never be brought to market or used to treat the general public. Instead, they would remain within the confines of research laboratories, useful only for curing mice and rats.

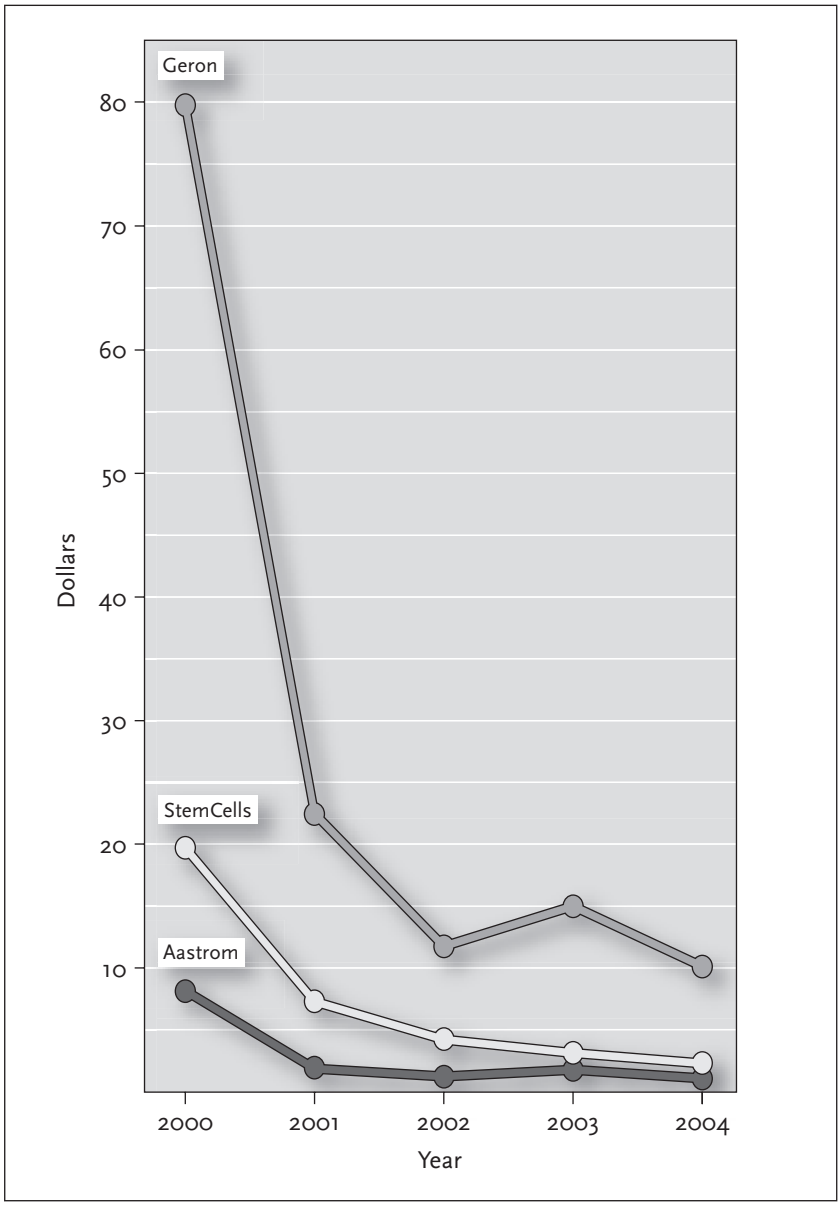
The Stock Market

Where do pharmaceutical companies get the money to fund clinical research? Much of it comes from profits earned by selling products they already have, but a great deal of it comes from selling shares in their company to the general public. Companies that sell shares are said to have "gone public"; the buying and selling of company shares, also referred to as stocks or securities, are handled by a stock exchange like

the New York Stock Exchange (NYSE) or the National Association of Securities Dealers Automated Quotations (NASDAQ), both of which are based in the United States. Most of the pharmaceutical and biotechnology companies are listed on the NASDAQ stock exchange. (NASDAQ gets its rather awkward name from the fact that buying and selling of stocks, including stock price quotations, is completely automated through the use of computers and the Internet. The NASDAQ has no trading floor, unlike the NYSE, where stockbrokers handle the transactions directly.) In 2004 the NASDAQ market listed more than 200 biotech and pharmaceutical companies, of which only five or 10 are actively involved in stem cell research (most of the preclinical work is being undertaken in academic research institutes). The annual value of all biotech and pharmaceutical companies (referred to as market capitalization) is more than $300 billion.

The stock market may seem like a long way from a research laboratory, but the ability of pharmaceutical companies to fund clinical research depends greatly on how successful they are at selling their stock and attracting investors. Not surprisingly, their ability to do so depends on how good their product is, and for biomedical research, the best yardstick is performance of a drug or therapy in a clinical trial. If a company advertises a drug that was proved effective in preclinical trials, many investors will want to buy shares in the company, and the more investors there are who want to buy, the more valuable the stock becomes. Investor confidence can drive the stock price from $1/share to $100/share, practically overnight. If an investor happened to buy 10,000 shares at the $1 price, he or she could suddenly become very rich. This is the hope of most investors in the pharmaceutical sector, that a drug will be proven effective after passing through all the clinical trials and will go on to earn the company, and the shareholders, a great deal of money.

This happy scenario played itself out many times during the early 1990s, when both the economy and investor confidence were high. Many biotech and pharmaceutical companies saw the value of their stocks increase from a few dollars/share to nearly $100/share. Investors gave large sums of money to these companies whether they had a strong product or not. Biotech companies seemed like a sure bet, and if their product succeeded, the companies could earn billions of dollars every year. By the late 1990s this overly optimistic outlook had



Stock prices for companies involved in stem cell research. All three of the companies shown lost large sums of money between 2000 and 2004, as reflected in the five- to 20-fold decrease in the value of their stocks. Declining stock values can reduce a company's operating budget by millions of dollars. Data points shown are the maximum share price for the indicated year.

all but evaporated. Biotech companies that were trading at over $60/share dropped to $10/share in less than a week. In 2002, after the initial sharp decline, many companies were still declining in value.

The financial trouble experienced by the biotech and pharmaceutical companies was caused in part by a general decline in economies all over the world, the ever-present threat of war, and the escalation of terrorists' attacks against the Western world, culminating in the destruction of New York's World Trade Center in 2001. Investor confidence in the stock market as a whole was also badly shaken by a number of accounting frauds that attempted to make certain companies' products look better than they really were. One such case, affecting the biotech sector, involved a company called ImClone Systems Incorporated, which claimed to have developed a very effective drug to fight cancer. Government investigators discovered, however, that the claims were groundless, and the company's chief executive officer (CEO) was tried and convicted of securities fraud. Before the scandal was exposed in early 2002, ImClone's stock sold for $80/share, dropping to less than $10/share by the end of the year. However, the company has since recovered, and as of June 2004 its stock was trading at $81/share.

Honest biotech companies, and there are many of them, have to contend with these problems, but those problems are not solely responsible for drops in their stock value. Many biotech companies have suffered financially simply because they could not bring their product to market or they were not progressing as fast as investors would like (see figure on page 53). The medical therapy or drug a company is working on may have looked good initially but failed in a clinical trial, or the results of a clinical trial may suggest that further preliminary work is required before the therapy can move to the next stage. Investors usually are not scientists, and they do not understand the technical difficulties associated with the biotech company's product. Producing a reliable drug or stem cell therapy rarely happens in a time frame that keeps investors happy.

Aastrom Biosciences Inc.

Aastrom is located in Ann Arbor, Michigan, and was formed in 1991, going public in 1997. This company is primarily involved in the isolation

of adult stem cells from bone marrow and umbilical cord blood. It also provides a kit for isolating stem cells, called the AstromReplicell System, which provides standardized methods and reagents for directed differentiation. The company's cells and isolation system are being used in clinical trials to treat breast cancer, leukemia, osteoporosis, and lymphoma.

Aastrom's stock value reached a peak of $8.50/share in 2000 but dropped to $1/share by June 2004 (see the figure on page 53). With nearly 46 million shares originally sold, this company has lost more than $300 million since it began selling shares. Much of its decline has been due to the difficulties involved in obtaining a clear-cut therapeutic effect of its cells in clinical trials.

StemCells Inc.

This company distinguishes itself from Aastrom Biosciences by focusing its attention on cells that may be used to treat injuries to and diseases of the central nervous system (CNS), such as Parkinson's disease and Alzheimer's disease. StemCells Inc. has discovered and characterized several markers for CNS stem cells and ways to prepare them for transplants. It is also developing methods for producing insulin-producing stem cells to treat diabetes, and other cells for treating liver and kidney diseases.

StemCells, located in Palo Alto, California, was formed in 1988 as Cytotherapeutics, changing to its current name in May 2000. A share in this company reached a high of nearly $20 in 2000, dropping to just over a dollar in June 2004 (see the figure on page 53). The company has sold about 3 million shares but has lost nearly $59 million since 1997. The bleak financial picture of this company may improve over the next few years because in 2002 it reported a successful outcome for a preclinical study involving the use of its neural stem cells to treat stroke. The main objective of the study, conducted at Stanford University, was to examine the fate of human stem cells in a rat model for stroke. The results showed that the transplanted stem cells survived in the immunosuppressed rat and migrated toward the stroke site where they began to differentiate into neurons. However, further studies are required to find out if the

transplanted neurons make the appropriate connections to restore normal brain function.

Geron Corporation

In the winter of 2000, stock prices for Geron Corporation, located in Menlo Park, California, were as high as $80/share, but share prices dropped precipitously to $10 by 2002 and fell further to just above $8/share by June 2004 (see figure on page 53). This near-collapse of the company's stock equity (value) amounted to many millions of dollars lost, but despite this, Geron remains the strongest and wealthiest company involved in stem cell research.

In 1996, just months after Ian Wilmut and his team at the Roslin Institute in Scotland announced the cloning of Dolly the sheep, the Scottish government decided to withdraw further funding for the project, possibly because they felt the research was too controversial. The institute managed to obtain alternative funding from two biotech companies: one was PPL therapeutics, based in Scotland, and the other was Geron Corporation. Geron's involvement in the work at Roslin was so complete that in 1999 the corporation bought Roslin Bio-Med, a company formed by the Roslin Institute, and along with it the patent for the nuclear transfer procedure developed by Drs. Wilmut and Keith Campbell to clone Dolly. In addition to the original patent, Geron now has more than 60 patent applications pending for nuclear transfer technology.

Geron's involvement in stem cell research began in 1998, when it funded the work of Dr. James Thomson at the University of Wisconsin (UW), who was among the first to isolate and characterize embryonic stem cells (ES) from humans. In 2002, UW and Geron worked out an agreement in which Geron received exclusive rights to the ES cells when they are used to treat heart disease, diabetes, and neurological disorders, while UW obtained exclusive rights to the cells for treatments involving bone, blood, and liver ailments. Consequently, researchers wishing to use those cells to treat heart disease must pay a licensing fee to Geron, whereas researchers wishing to use the ES cells to treat leukemia must pay the University of Wisconsin a fee.

Owning patents for nuclear transfer technology and for many ES cell lines places Geron in an extremely powerful, and potentially

lucrative, position. The application of stem cell therapy often includes the use of animal cloning procedures. For example, a privately owned biotech company (that is, one that does not sell shares on the stock market) called Advanced Cell Technology is planning to produce ES cells by cloning human eggs, rather than fertilizing them in vitro, as was done for all the current ES cell lines. By doing this, they avoid paying Geron licensing fees for their cells, but they still have to pay a fee for using Geron's nuclear transfer procedures.

Many view the commercialization of stem cell research, especially when it involves human ES cells, as a mixed blessing. Without the money large corporations bring to this area of medical research, many potentially powerful therapies would never see the light of day. But the mixture of science and profit does not always smell so sweet, leading as it sometimes does to exaggerated claims, secretive results, and ethical dilemmas. No one would suggest that corporations should not be involved in this kind of research, but we have a long way to go before the association benefits everyone.

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REALITY CHECK

Stem cells have great potential for forming the basis of medical therapies that could cure cancer, neurological diseases, and spinal cord injuries. But in their enthusiasm for these therapies, many scientists have given the general public an unrealistic impression of what can actually be achieved. In theory, of course, the sky is always the limit, but scientists interested in using stem cells to repair damaged organs fail to make it clear that there are serious obstacles to overcome before these therapies become a reality.

The versatility of stem cells and the promise they hold is without question, but devising a practical method to realize that promise will be extremely difficult. Injecting stem cells into a patient leads to the same problem that transplant surgeons have been trying to overcome since the 1950s: the rejection of those foreign cells by the patient's immune system. Even if scientists can overcome this problem, they are left with an even greater challenge in trying to ensure the cells will go where they are supposed to go and do what they are supposed to do without forming a cancerous tumor, thereby damaging the very organs they are supposed to repair. This is particularly worrisome in the case of a brain disorder, where stem cells are expected to make extremely complex repairs while leaving the patient's original memories and psychology intact. This would be a tall order even if scientists had a clear understanding of memory and personality functions. At the present time, however, they have only an elementary grasp of these processes.

Other researchers have claimed that they will be able to grow vital organs, such as a heart or kidney, by culturing stem cells in special ways.

But acquiring the knowledge to make this possible will be so difficult, and will take so long, that the idea occupies the realm of science fiction. This chapter will explore the many claims that have been made by stem cell researchers and will take a close, critical look at the proposed applications while emphasizing hard realities that are likely to keep most of these therapies on the drawing board for a very long time.

The Problem of Immune Rejection

Stem cell therapies all require that the cells, usually isolated from human embryos, be injected into a patient, where they will effect whatever repairs are necessary. This is equivalent to a tissue or an organ transplant, and like any conventional transplant, the stem cells will have to deal with the patient's immune system.

Transplants are categorized according to the source of the transplant tissue. *Autografts* are transplants of tissue from one part of an individual to some other area of the same individual. A common example involves bypass surgery, in which an artery from the leg is transplanted to the heart in order to repair, or bypass, a blocked coronary artery. Because the artery was obtained from the patient, there is no threat of immune rejection. A second example involves bone marrow transplants, commonly used to treat leukemia. It is sometimes possible to obtain donor bone marrow from an identical twin, in which case, the cells are genetically identical to the eventual host and, again, there is no threat of immune rejection. An autograft is also known as an autogeneic transplant. A syngeneic transplant, involving identical twins, is synonymous with an autograft. *Allografts* involve tissue that is taken from one individual and transplanted into another, unrelated individual. This type of transplant will be attacked by the immune system because it is genetically dissimilar to the host. The great majority of transplants involving organs or tissues are allografts. The proposed transplantation of human embryonic stem (ES) cells into patients suffering from Alzheimer's disease is another example of an allograft. An allograft is also called an allogeneic transplant. *Xenografts* are transplants between different species. Transplanting the heart of a pig into a human is an example of a xenograft; so too is the transplantation of human ES cells into experimental mice or rats.

Autografts do not invoke an immune reaction, but transplant operations involving allografts or xenografts lead to graft-versus-host disease (GVHD), whereby the host's immune system attacks and destroys the transplanted tissue or organ. The current treatment for GVHD is to give the patient drugs that inhibit the immune response. All allograft transplant patients must take these drugs, called immunosuppressants, for the remainder of their lives (this will also have to be done for xenografts if and when they are performed on humans). Immunosuppressants act by blocking the activity of white blood cells, called T lymphocytes, that are responsible for hunting down and destroying invading microbes and infected cells. The immune system cannot distinguish between a deadly microbe and transplanted tissue; both are attacked and, if possible, destroyed.

A considerable amount of survival data, both for the transplanted organ and the patient, has been collected since organ and tissue transplants were first attempted in the 1950s. Prior to the introduction of immunosuppressants in the 1980s, transplant patients rarely survived for more than a few weeks after the operation. After 1980, when two powerful immunosuppressants, called cyclosporine and tacrolimus, were introduced, the one-year survival went up dramatically, but long-term survival is still poor owing to a slow chronic destruction of the transplant by the immune system. Thus, even with a daily dose of immunosuppressants, the transplanted organ is doomed to fail (see the table on page 61).

Several reports have appeared, both in the scientific literature and in the daily news, concerning the use of ES cells to treat neurological disorders. In one such study, mouse ES cells were injected into rats suffering from a surgically induced neurological disorder. The stem cells not only migrated to the appropriate area but, once there, repaired much of the damage. However, what is not generally reported in the daily news is the fact that the animals receiving the stem cells are on powerful immunosuppressants or are so-called knockout rats whose immune systems have been inactivated through genetic engineering. Chemically or genetically suppressing the immune system means, of course, that these animals are especially susceptible to disease and must live out their lives in a sterile environment. These same problems would affect a human patient subjected to this kind of therapy, and yet scientists have not made this point

SURVIVAL OF TRANSPLANT PATIENTS AND TRANSPLANTED ORGANS IN THE UNITED STATES

	1 year		5 year		10 year	
	Patients	**Organs**	**Patients**	**Organs**	**Patients**	**Organs**
Kidney	95.8	91.4	84.8	69.9	69.4	45.9
Heart	85.1	84.4	69.8	68.1	50.0	46.4
Liver	85.8	78.2	79.0	68.2	72.3	43.9
Lung	77.4	87.3	42.5	40.5	22.7	17.5
Pancreas	98.6	81.2	77.8	32.4	68.2	16.2

Table values are percentages. The data was compiled from information provided by the United Network for Organ Sharing for 2003.

clear, leaving the public with the impression that therapies based on stem cells could be available in the very near future. Clearly, the promise of stem cell therapy has been greatly exaggerated. However, hope remains, primarily because there are three alternatives to the use of ES cells: adult stem cells, umbilical cord stem cells, and finally, growth factors to stimulate and mobilize the body's own stem cells.

ADULT STEM CELL THERAPY

The versatility of adult stem (AS) cells has been studied extensively by Dr. Catherine Verfaille and her associates at the University of Minnesota. Verfaille's team has shown that mouse AS cells isolated from bone marrow can be stimulated in vitro to differentiate into a wide variety of cell types, representing mesoderm, neuroectoderm, and endoderm, the three fundamental germ layers. Moreover, when these cells are injected into a mouse embryo at an early stage of development, they contribute to most, if not all, somatic cell types. The use of human AS cells in medical therapies would remove the very substantial problem of immune rejection. AS cells could be isolated from the patients requiring treatment, partially differentiated in vitro into the required cell type, and then returned to the patient to effect repairs. Application of this procedure places stem cell therapy within the realm of possibility. It does not remove the additional problems, mentioned previously, regarding the

Dr. Catherine Verfaille, a professor of medicine at the University of Minnesota, discovered the developmental plasticity of adult stem cells isolated from human bone marrow. *(Courtesy of Tim Rummelhoff)*

repair of neural tissue and the maintenance of normal brain or spinal cord function. But for simpler tissues, such as bone marrow or liver, this approach is extremely promising.

UMBILICAL CORD STEM CELLS

Blood isolated from human umbilical cords has been shown to be an excellent source of stem cells. Moreover, these umbilical cord stem (UCS) cells appear to have a developmental plasticity equal to that of ES cells. Drs. Juliet Barker and John Wagner, also at the University of Minnesota, have successfully treated leukemias in children using UCS cell therapy. These researchers also discovered that UCS cells, although allogeneic, do not stimulate host-versus-graft disease, and thus they offer a great improvement over standard bone marrow transplants, which are eventually rejected even in the presence of immunosuppressants. Many parents are already having umbilical cord blood collected from their newborn infants in case the child should ever need stem cell therapy, and some portion of the blood so collected could be set aside for general use. In time, umbilical cord blood, collected from millions of infants, could become an extremely valuable tissue bank that could be used to treat many diseases without prompting immune rejection and without raising the ethical problems associated with ES cells.

GROWTH FACTORS

Dr. James Fallon and his associates at the University of California, Irvine, have shown that it is possible to activate the body's own stem cells to repair damaged tissue. His team was able to activate adult stem cells by injecting transforming factor α (TF-α) into the damaged area of a rat's brain. The activated stem cells not only repaired the neural damage, but the rats regained some mobility in their limbs, which had been lost as a result of the original brain damage. Fallon's team also found that the response was very specific, in that injections of TF-α into normal rats did not lead to a stimulation of stem cells. Damaged areas of the brain seem to prime the stem cells for repair, making them responsive to the influence of growth factors. In addition to solving the problem of immune rejection, this procedure also removes the technical difficulties associated with in vitro differentiation and with injecting stem cells into the body. The use of growth factors does not, however, resolve the problem of memory retention, nor does it ensure the maintenance of a human patient's psychology. Moreover, growth factors are dangerous drugs to administer because there is a risk they might overstimulate some cells, forcing them to divide inappropriately,

thus setting the stage for cancer development. Nevertheless, the methods of Fallon, Verfaille, and Barker offer a very powerful, and ethically more acceptable, form of stem cell therapy.

Spontaneous In Vitro Differentiation

A major problem associated with the culturing of stem cells is uncontrolled differentiation. Stem cells in culture for six months to a year begin to differentiate into fibroblasts, or cells that look very much like fibroblasts. Once this happens, the researcher has to start a fresh culture. This is easy in the case of adult stem cells because the source is not ethically contentious, but it is becoming increasingly difficult in the case of human ES cells, where starting a new culture means destroying more human embryos.

Spontaneous differentiation is one of the reasons scientists working with human ES cells are interested in having free and ready access to human embryos. It has not been possible to resolve this very difficult problem, nor is it likely to be resolved in the near future. In the meantime, as the available cultures of ES cells differentiate, investigators anxious to produce medical therapies based on ES cells are finding it increasingly difficult to continue their work. Differentiated ES cells cannot be used for therapies. Moreover, funding of stem cell research by agencies such as the National Institutes of Health, is based on merit and the perception that the research will lead to a practical therapy, something that is difficult to achieve when researchers have an insufficient number of cultures to study.

The interest in ES cells is also driven by the race to the patent office. This is an important element in a field where pharmaceutical and biotech companies stand to earn a great deal of money should they succeed in producing a workable therapy. It is also an inevitable outcome when, as described in chapter 4, expensive research is funded by stockholders. It is this arrangement of research and economic priorities that is behind the urgent calls for freer access to human ES cells. In the minds of many scientists, and the heads of biotech companies, ES cells represent the quickest way to medical therapies, international fame, and the front door of the patent office. We need only look at the intense media coverage given to the team that cloned Dolly the sheep to see this

dynamic in action. Embryonic stem cell researchers may achieve the therapies they seek, but the legal environment (described in chapter 7) and the work of Verfaille and Barker may shift the emphasis toward adult or umbilical cord stem cells in the very near future.

Wandering Stem Cells

Conventional stem cell therapy involves injecting the cells into the patient and letting them home in on the target tissue, and there is good evidence available to suggest that these cells, after partial in vitro differentiation, do just that. For example, stem cells that have been stimulated in vitro to become hematopoietic cells will migrate to the bone marrow when injected into the body. However, the number of studies is still too small to guarantee that accurate targeting will always occur. Misdirected stem cells could be a very serious problem for therapies aimed at spinal cord disorders or diseases of the brain. There is currently no method for directing a stem cell to differentiate into a brain-specific neuron, as opposed to a spinal cord–specific neuron or neurons of the peripheral nervous system (such as nerves in the heart, gut, or hands). To improve targeting, the stem cells can be injected directly into the brain or spinal cord, but there is no guarantee that all of them will stay there. They may decide to migrate to other neural tissue. Thus an attempt to treat a brain condition such as Parkinson's or Alzheimer's disease may result in serious complications if therapeutic stem cells colonize the spinal cord or the peripheral nervous system. It is generally considered a wise policy to avoid trying to fix things that are not broken, but stem cells are programmed for making repairs, and that is what they will try to do wherever they land. In the current example, this could lead to severe disruptions in the normal functioning of the spinal cord. Conversely, a therapy to treat spinal cord trauma could result in severe brain damage.

Stem Cell Cancer Induction

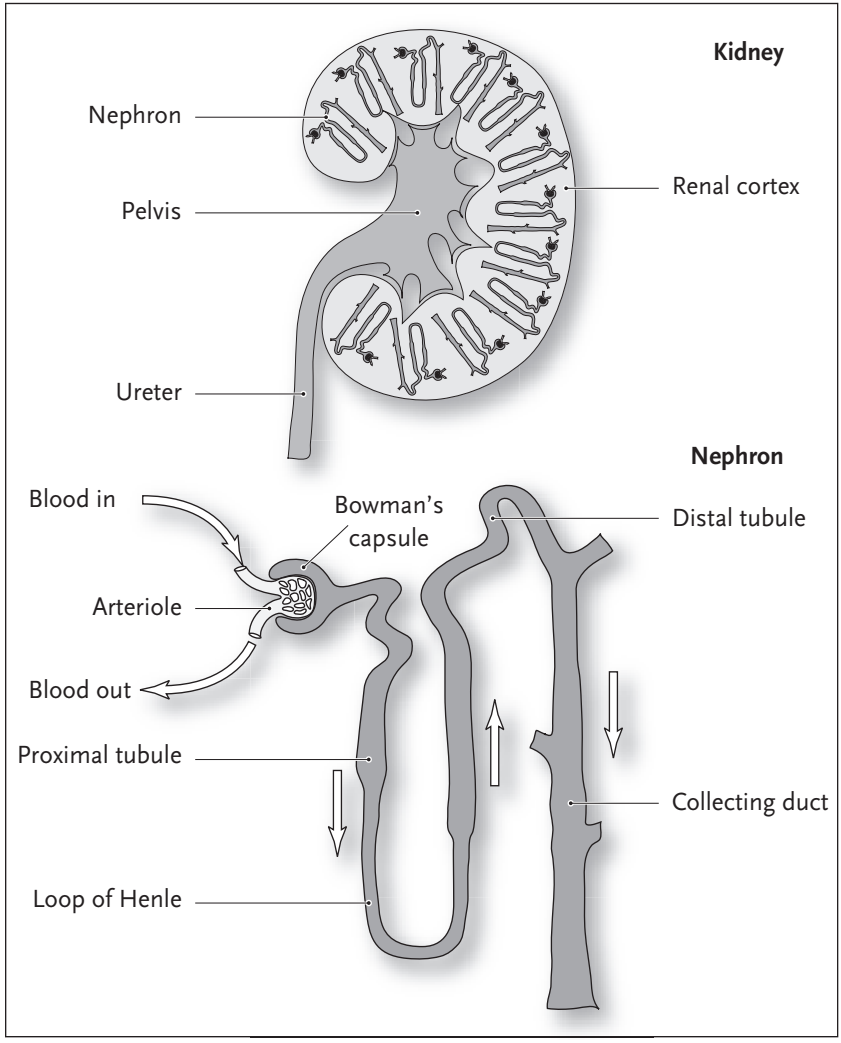
Stem cells have one thing in common with cancer cells: the ability to proliferate indefinitely. This similarity has worried scientists and physicians for some time because it could mean that the use of stem cells to treat a disease may result in the seeding of cancers throughout the body.

With this in mind, researchers at the National Institute of Neurological Disorders in Bethesda, Maryland, began looking for molecular similarities between these two kinds of cell. They found that cancer cells and ES cells both express a protein called nucleostemin. The exact function of this protein is not yet known, but it appears to be a molecular switch that controls cell division. If it turns out that cancer cells escape the normal inhibitions of the cell cycle by activating nucleostemin, this would imply that stem cells have a similar ability, and thus will have to be handled with extreme caution. The use of partially differentiated stem cells may reduce the risk of cancer formation, but additional studies will be needed to reveal more about the body's own stem cells and the controls that keep them in check.

Growing Organs from Cultured Stem Cells

In the summer of 2002, researchers at the biotech company Advanced Cell Technology published a paper in the journal *Nature Biotechnology* in which they described their attempts to grow kidneys from cloned cow embryos. This article, entitled "Generation of Histocompatible Tissues Using Nuclear Transplantation," was also intended to demonstrate the effectiveness of therapeutic cloning, whereby an animal is cloned and the subsequent embryos are used as a source of histocompatible (immune-system compatible) stem cells.

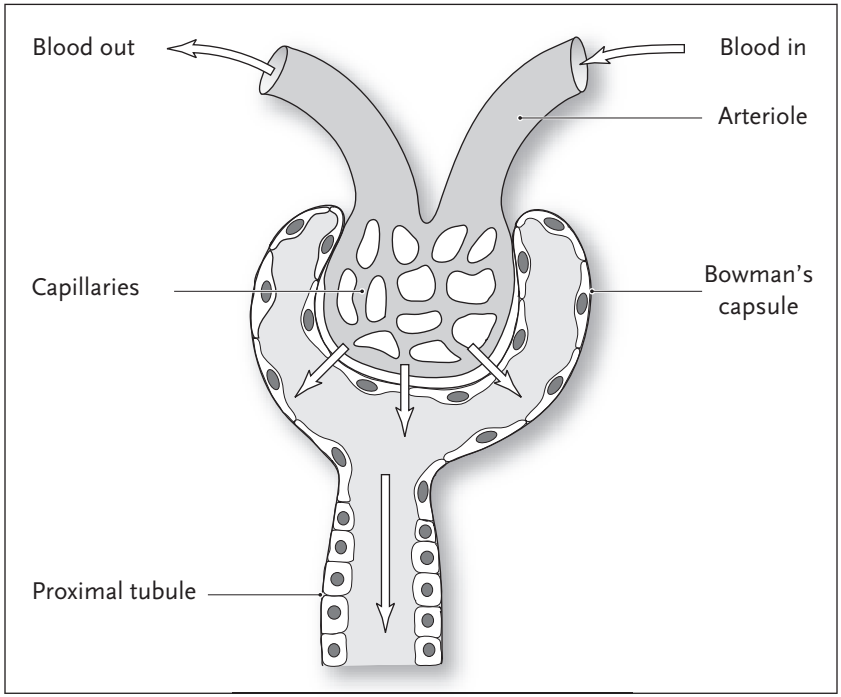
Real kidneys have a very complex structure that poses a huge challenge to anyone trying to grow them in culture. The human kidney, about the size of a child's fist, filters the blood to remove waste products, such as urea, and excess salts and other ions that build up as a normal by-product of metabolism. The functional unit of a kidney is an intricately constructed tubule called the nephron, which consists of several distinct regions: Bowman's capsule, the proximal tubule, the loop of Henle, and the distal tubule. Blood plasma (the fluid portion minus the blood cells) enters the nephron at Bowman's capsule, where the incoming arteriole branches out into a capillary bed (the blood cells are too large to enter the nephron). The combination of Bowman's capsule and the capillary bed is known as the glomerulus. The blood pressure, driven by the heart, forces the plasma out of the capillaries and into the proximal tubule, after which the plasma travels the whole length of the



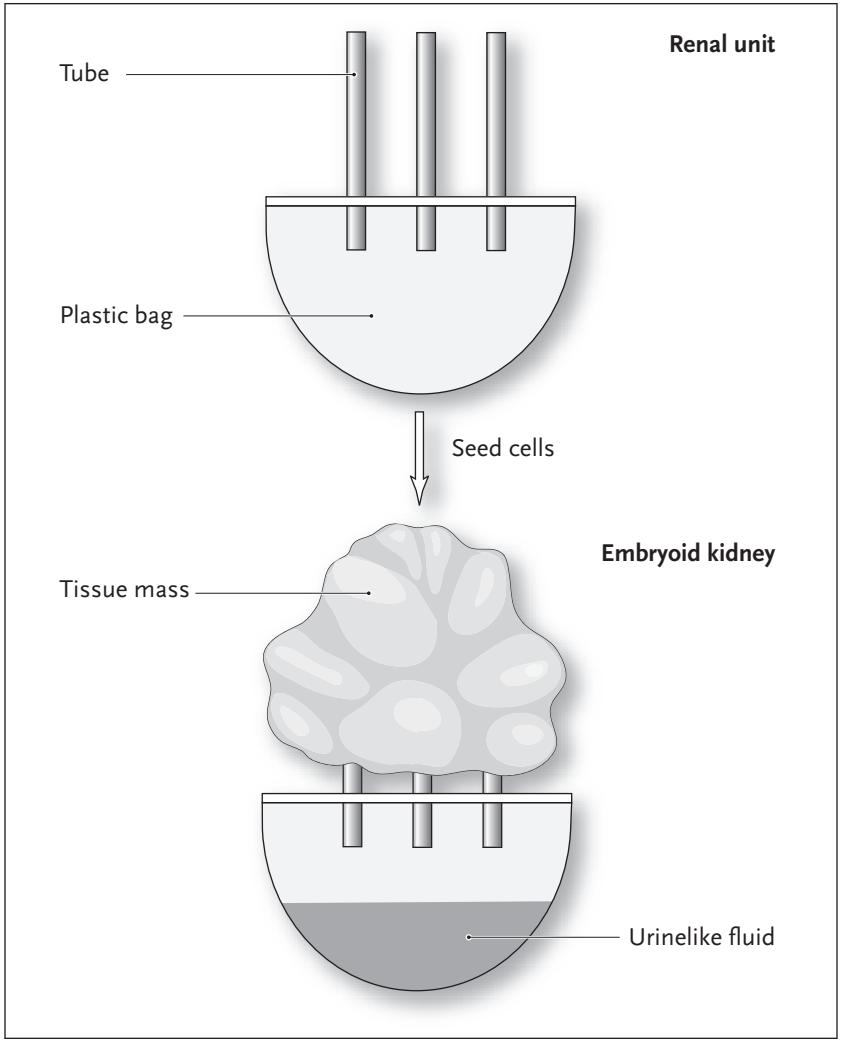
Anatomy of the human kidney. A cross section of a kidney is shown at top, and the functional unit, the nephron, is shown enlarged at the bottom. Each kidney contains millions of nephrons arranged throughout the cortex. (The size of the nephrons is exaggerated for clarity.) The collecting ducts drain into the pelvis, and the urine is carried to the bladder by the ureter. The nephron is a complex tubule that collects blood plasma at Bowman's capsule and then filters it as it passes through to the collecting duct. The main strategy of the nephron is to reclaim essential nutrients while allowing waste materials to pass through as urine. The direction of urine flow through the nephron is indicated by the straight arrows.

tubule and is processed along the way. The basic strategy of the nephron is to reclaim by tubule reabsorption compounds and ions the body needs while allowing the waste material, such as urea, to pass completely through to the collecting ducts and, finally, out of the body by way of the ureter and bladder. Aiding in this remarkable process is a diverse population of cells that are distinct for each region of the tubule.

The attempt to grow a kidney in culture was headed by Dr. Robert Lanza. His team began by cloning cows from ear epithelium and then allowing the embryo to reach an early fetal stage of development (three to four months old), by which time the researchers were able to recognize and isolate embryonic kidney cells. These cells were cultured on a collagen-coated renal unit to give the artificial organs, known as



Bowman's capsule. Blood, under pressure from the beating heart, enters the capillaries. The blood plasma (i.e., fluid minus blood cells) diffuses across the capillary membranes, then enters the nephron by diffusing across the single cell layer of Bowman's capsule (small arrows). The plasma passes through the whole length of the nephron, beginning in the proximal tubule (large arrow).



Embryoid kidney. A renal unit (top) is constructed from collagen-coated tubes and a plastic bag. The unit is seeded with embryonic kidney cells, which form a tissue mass containing loosely organized nephronlike structures. Fluid excreted by the embryoid kidney is collected in the plastic bag.

embryoid kidneys, their final shape and to provide a convenient way to collect the fluid excreted by the cells. The embryoid kidneys were implanted under the skin of the adult cow from which the clones were

derived (to avoid immune rejection), where they continued producing a urinelike substance.

Although the science community greeted it with some enthusiasm, there are many problems associated with this study. First, the investigators used embryonic kidney cells, not stem cells. Thus it is not surprising that these cells, once isolated, tried to construct a kidney, and the study fails to answer the question of whether stem cells can be used to grow artificial organs. Second, despite being cultivated from kidney cells, the final organ bears no resemblance to a mammalian kidney. Although the cells formed nephronlike structures, these structures have a very crude anatomy, lacking regional variations such as the proximal tubule, loop of Henle, and the distal tubule. The embryoid cells failed to construct a kidney because, once they were removed from the body and cultured in vitro, they lost the developmental cues that are crucial for normal organogenesis (as discussed in chapter 2). As a consequence, embryonic cells may form some of the parts, such as a nephron, but they do not know how to organize those parts into a truly functional, anatomically normal organ. Third, a crucial component in this process is the precise interactions between the developing nephron and the arterioles that must form a capillary bed inside every Bowman's capsule. Without the necessary instructions, cultured embryonic cells form an embryoid body, an assemblage of cells that looks more like a tumor than an organ. Lanza's team presented no evidence to document vascularization of the embryoid kidney after implantation in the host cow. Based on the crude appearance of the embryoid nephrons, it is unlikely that capillary beds formed inside the Bowman's capsules to produce a glomerulus. Thus the identity of the fluid collecting in the plastic bags is questionable.

Lanza and his associates set out not only to grow kidneys in culture but also to show the value of therapeutic cloning as applied to organ transplants. For example, a patient suffering from kidney failure currently has two options: kidney dialysis, which is costly and cumbersome, or a kidney transplant. Most kidney transplants are allografts; therefore the patient has to take immunosuppressants for the remainder of his or her life. Moreover (as shown in the table on page 61), even with immunosuppressants, the long-term patient prognosis is not good. Therapeutic cloning offers a third alternative: Clone the patient, allow the

embryo to grow long enough to harvest the stem cells, coax those cells into constructing a kidney, and then implant it in the patient. Since the kidney in this scenario is a clone of the patient, there is no threat of immune rejection. Although in theory, therapeutic cloning will work, it requires the creation of a human embryo that is subsequently killed in order to provide the stem cells needed to grow the body parts. Moreover, Lanza's group used fetal kidney cells, not embryonic stem cells, and even those who may accept the destruction of a two- to three-day-old embryo to harvest stem cells balk at the idea of carrying out the same procedure on a three- or four-month-old fetus.

Society is not likely to accept the idea of using human embryos or fetuses as starter materials for growing organs, but they might accept the procedure if it started with AS or UCS cells, particularly if they can be trained to produce real organs and not just a mass of embryoid tissue. But for this condition to be met, scientists will have to unravel the complex program that guides normal embryonic development and the formation of organs. This is a tall order, even with modern technologies, and is not likely to be achieved in the next 10 to 20 years. By that time, our understanding of basic cell biology may have advanced to a point where it will be possible to repair a defective kidney or heart without the need for organ transplants. Thus the real value in the effort to grow artificial organs may not be the actual production of organs but the knowledge we gain about the control of embryogenesis and the inner workings of the cell.

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THE ETHICS OF STEM CELL RESEARCH

Of all the new biological technologies, none have been more controversial than stem cell therapies. The only other technology to come close is animal cloning, and this is due mainly to its association with stem cells and therapeutic cloning. Ethicists and legislators have been grappling with the implications of stem cell therapy since 1998, when human ES cells were first isolated and cultured. On the face of it, stem cells offer the hope of curing disease and repairing a damaged nervous system. But the strategies that scientists have proposed require the destruction of human embryos and, in its most controversial form (therapeutic cloning), the creation of human embryos for the sole purpose of providing stem cells.

In the minds of many, a stem cell therapy that requires the destruction of human embryos hark back to the cruel experiments that prisoners were forced to endure at the hands of the Nazis during World War II. The association is not as far-fetched as it may at first appear. The problems associated with stem cell research are closely related to the ethical problems affecting biomedical research in general. These issues were analyzed in the Belmont Report (1976) in response to abuses of basic human rights inflicted upon medical research subjects, dating back to World War II. This chapter will discuss the ethics of stem cell research in the context of that report while leaving the legal questions to the next chapter.

Justice and Beneficence

The Belmont Report is a document that provides biomedical researchers with an ethical framework to follow in order to avoid the

kind of abuses that occurred both in Nazi Germany and in an American clinical trial called the Tuskegee study. In 1932 a research institute in Tuskegee, Alabama, enrolled 600 low-income African-American males in a study dealing with the progression of syphilis. These patients, 400 of whom were infected with syphilis, were monitored for 40 years. The infected individuals were never told they had syphilis but instead were told that their medical problems were due to "bad blood." In 1947 penicillin became widely available, and this antibiotic was known throughout the medical world as an effective treatment for syphilis, yet the participants in the Tuskegee study were never told there was an antibiotic available that could cure them. The study was terminated in 1972 by the Department of Health but only after its existence was leaked to the general public and it became a political embarrassment. By the time the study was terminated, 28 of the men had died of syphilis, and 100 others were dead from related complications, 40 of the participants' wives had been infected, and 19 children had contracted the disease at birth. Public revulsion over the details of this study was instrumental in forcing the government to introduce new policies and laws regarding the use of human subjects in medical research. Four years after the termination of the study, the Department of Health published the Belmont Report (see chapter 8 for more details).

At the core of the report is a call for researchers to keep the patient's dignity and well-being in the forefront. The authors of the report used the term "beneficence" to capture this attitude. Beneficence generally refers to acts of kindness or charity and, in the context of the Belmont Report, is a natural extension of the Hippocratic oath that all physicians are expected to abide by: *I will give no deadly medicine to anyone if asked, nor suggest any such counsel.* In other words, doctors must do no harm. For those involved in biomedical research, this means never injuring one person to benefit another. An extension of the beneficence principle is the principle of justice. Researchers must never enlist subjects in an experiment if those subjects do not stand to reap any benefits. The exploitation of prisoners in Nazi concentration camps benefited the Nazis but certainly not the people they experimented on. A second example cited by the report was the Tuskegee study. Aside from committing a gross deviation from the most basic ethical standards, the designers of the Tuskegee study enlisted only black people, even though they are clearly not the only racial group to suffer from this disease. The

principle of justice was clearly not applied to these subjects; instead their treatment was little better than what occurred in Germany's concentration camps.

To address these problems, the Belmont Report introduced for the first time the principle of informed consent. Physicians and biomedical researchers can enroll human subjects in a clinical research trial only if the procedure is carefully explained and the researchers receive written consent from the prospective subjects. This process was modified in 2000 to include a requirement for a patient advocate to be present when physicians are recruiting research subjects. The advocate's job is to make sure the researchers explain the procedure clearly; provide full disclosure of background research, particularly any results that may suggest the subjects will be harmed by the experiment; and, finally, ensure there is no attempt to coerce prospective subjects into joining the trial. The inclusion of an advocate, ordered by the American Food and Drug Administration (FDA), was in response to a failed gene therapy trial in 1999 that led to the death of Jesse Gelsinger, one of the research subjects. The subsequent investigation charged the principal investigators with ignoring the safety and well-being of their subjects and with disregarding the principle of informed consent (see chapter 8 for details).

High-Tech Cannibalism

Opponents of embryonic stem cell research, particularly in the form of therapeutic cloning, believe it is wrong to use human embryos for any kind of research or medical therapy and have characterized the practice as "high-tech cannibalism." The issue of embryonic stem (ES) cell research is extremely complex and involves the very emotional and highly politicized issue of human abortion. Researchers want unlimited access to human embryos, left over from in vitro fertilizations, which they wish to use as a source of stem cells. Scientists at Advanced Cell Technology, a biotech company specializing in stem cell research, would like to push ES cell availability a step further with therapeutic cloning, whereby human embryos are created, using nuclear transfer, for the sole purpose of providing stem cells and, ultimately, culture-grown kidneys and other organs. Embryos do not survive the harvesting of the stem cells; indeed, the stem cells *are* the embryo.

Unfortunately, neither the Belmont Report nor the Gelsinger investigation addresses these new methodologies. Nevertheless, the ethical principles now in place to guide medical research can be used to address the problems associated with ES cell research. The Belmont Report and the Gelsinger investigation established the requirement for informed consent and patient advocates. Both requirements are relevant to fetal and ES cell research. Critics argue that since neither the embryo nor the fetus is recognized as a person, in the legal sense, there is no obligation for ES cell researchers to abide by those reports. However, the absence of legal status does not preclude an ethical policy, as evidenced by the fact that abortion in North America and Europe is only permissible up to the fifth month and not beyond (except in very rare cases where the mother's life is at risk), even though the fetus does not attain the legal status of a person until after birth. With this in mind, opponents of therapeutic cloning and ES cell research maintain that, at the very least, a human embryo or fetus, incapable of giving informed consent, should be afforded the benefit of an advocate. Resistance to this notion centers on the question of when an embryo or a fetus becomes human.

On Becoming Human

The question of when an embryo becomes human is not a new issue but one that has been debated by philosophers, scientists, and politicians for more than 2,000 years. The Greek philosopher Aristotle, always in the thick of things, believed that life arose in three stages, which he characterized as vegetative, animate, and intellectual. Aristotle was likely referring to the emergence of entire populations, but many people took it to mean that human development traveled this course as well, that is, the embryo, immediately after conception, is in a vegetative stage, followed by an animate stage, when muscles differentiate and limbs begin to move, and finally, the individual develops an intellect. People concluded that the first two stages occurred in the womb, while the final stage appeared only after birth. Thus, in the minds of many, an embryo did not become human until after birth, and this idea has influenced public perception and dialogue ever since.

Even modern-day research scientists seem to have soaked up some of Aristotle's reasoning. Dr. John Caplan, a noted science ethicist, has

stated that cloned human embryos, destined for therapeutic research, are not really human. Other scientists, including Dr. Michael West, CEO of Advanced Cell Technology, a company that is involved in therapeutic cloning, have echoed this assessment. But sentiments expressed upon the completion in 2003 of the Human Genome Project run counter to this conclusion. At that time, many scientists declared that the human race, for the first time ever, had access to the information, contained within our genome, that makes us what we are defines our essence, shapes our bodies, and delivers our intellect. Such statements contradict the argument that an embryo, containing a human genome, is not really human. To be sure, Kaplan and West were referring to cloned embryos, which they may think are less than human. But other scientists reject the premise that clones are less than, or even different from, their conventionally conceived genetic counterparts. Specifically Dr. Ian Wilmut, the British scientist who led the team that cloned Dolly the sheep, went to great lengths to assure the general public that Dolly was a sheep like any other sheep of her breed. Wilmut, along with many other scientists, insisted that the fact that Dolly was cloned did not change her sheep-ness; if an embryo has a sheep's genome, it is a sheep. Following the same logic, if an embryo has a human genome, regardless of how it got it, that embryo is human. Moreover, following the logic of genomics (which states that a species identity is defined by its genes), that embryo is human from the day of conception. Many believe that it is wrong to attempt to redefine humanness for, as pointed out by the Belmont Report, such attempts have been used to justify the unethical treatment of those who are the most vulnerable, including early human embryos, which are the primary source of stem cells that researchers wish to use for medical therapies.

The Early Embryo

In such a sensitive area as research on human embryos, terminology is especially important, particularly regarding the developmental stages of the early embryo (up to 14 days old). Debate is often clouded by misconceptions regarding the development of the human embryo. Thus, before beginning a discussion of the ethical issues, let us review the ini-

tial stages of embryonic development. (The basic stages were described in chapter 1.)

Fertilization of the female egg (oocyte) and fusion of the egg and sperm nuclei form the zygote. The zygote undergoes a series of cell divisions beginning about 36 hours after fertilization, at which time it is referred to as an embryo. By the time the embryo is five to six days old, it consists of about 100 cells (still smaller than a period on this page) and is known as a blastocyst. The blastocyst consists of the trophoblast, which eventually forms the placenta and umbilical cord, and the inner cell mass (ICM). The ICM gives rise to the embryo and is the source of the ES cell. The blastocyst, being the source of ES cells, is the focus of much of the debate on the use of embryos in stem cell research and therapy (the rare exception involves EG cells, described in chapter 2). The blastocyst is sometimes referred to as the preimplantation embryo. About a week after fertilization implantation of the blastocyst in the womb takes place. The failure rate of this stage is high, with as many as 75 percent of the blastocysts being naturally lost before implantation. At about 14 days after fertilization, following implantation, the embryo consists of about 200 cells that have begun the process of cellular differentiation. It is at this time that an anatomical feature known as the primitive streak first appears. The appearance of this feature marks the stage at which the central nervous system (CNS) begins to form. For the purpose of this discussion, the term "early embryo" refers to the stages up to the appearance of the primitive streak.

The Parliament of the United Kingdom (U.K.) was among the first to address the ethical and legal status of the early human embryo (the legal issues will be discussed in the next chapter). The initial intention was to regulate the practice of in vitro fertilization (IVF), pioneered by British physicians in the 1970s. The U.K. Parliament established the Committee of Inquiry into Human Fertilization and Embryology, chaired by Baroness Warnock in 1982. After lengthy consultations with a broad segment of the population, the committee submitted its recommendations, known as the Warnock Report, in 1984. The Warnock Report recommended allowing research on early embryos but banning such experiments on embryos that are older than 14 days. With the advent of therapeutic cloning, Parliament set up another committee in 2001 under the chairmanship of Professor Liam Donaldson to

reexamine the ethical issues associated with stem cell research. The Donaldson committee called for presentations from a wide segment of society, including research organizations, church groups, and individuals representing the general public, such as trade unions and the National Federation of Women's Institutes.

The central issue addressed by the Donaldson committee was the status of the early embryo. Positions stated before the committee ranged from those taken by the churches and pro-life groups, that the early embryo is a human being in the fullest sense from the moment of fertilization and should be accorded the same respect as a fetus or baby, to the position that the early embryo is no more than a collection of undifferentiated cells and so deserves no more respect than any other isolated human cell or tissue. (The Warnock committee adopted a position between similar opposing views, concluding that the early embryo has a special status but not one that justifies absolute protection.)

The debate presented to the Donaldson committee focused on the principle of respect for persons. The Roman Catholic Church and pro-life groups argue that an early embryo must be treated as a person, but others, including some non-Catholic church groups, testified that the status of a person is something that develops over time and is not acquired at the point of fertilization. This view is supported by the fact that parents are more likely to grieve the loss of a newborn child than the loss, by miscarriage, of an embryo or a fetus. Moreover, there is no public mourning for the natural loss of a fetus or for surplus embryos that are disposed of at IVF clinics.

The Donaldson committee, upholding the earlier conclusions reached by the Warnock committee, recommended that the 14-day limit on embryo experimentation be maintained and that this should apply both to embryos produced by natural means and by therapeutic cloning. The committee concluded that it would be hypocritical to allow abortion but ban therapeutic cloning and early embryo experimentation. This conclusion, however, was rejected by virtually all other European countries (known as the European Union, or EU, which has its own Parliament). The EU Parliament pointed out that the issues of abortion and therapeutic cloning are separate and that one (abortion) should not be used to justify the other. Abortion is a special case in which the rights of both the mother and the fetus must be taken into

account. Abortion laws in Europe and North America reflect the view that while the human fetus deserves respect, the rights of the mother are paramount. However, in the case of therapeutic cloning, or IVF embryos, the question of the mother's rights is no longer relevant, and thus the fate of the embryo rests solely on the merit of its status as a developing human being.

Moreover, the EU Parliament drew a clear distinction between embryos produced by therapeutic cloning versus surplus IVF embryos. The IVF embryos, as acknowledged by the Donaldson committee, are produced for the purpose of creating life, that is, they are all intended for implantation and subsequent development, whereas therapeutic cloning creates embryos for the sole purpose of harvesting stem cells, thus killing the embryo in the process. Consequently, the EU concluded that therapeutic cloning is inherently unethical and should be banned, whereas experimentation on IVF embryos should be allowed, though tightly regulated.

The debate in the United States is more polarized and more complex than it is in the United Kingdom or the European Union. There is a greater tendency in the United States to view the banning of therapeutic cloning or ES cell research as an encroachment on the availability of abortion and the right of a woman to have one. Indeed, this perception has so far blocked the passage of laws dealing with both reproductive and therapeutic cloning in the United States, while such laws were passed in the U.K. and EU as far back as 2001 (discussed in the next chapter).

Polarization of the debate in the United States is due primarily to a shift in public opinion regarding the eligibility of abortion, as defined by the Supreme Court in 1973 (*Roe v. Wade*). Public opinion polls have shown an even split between adult Americans who are for or against abortion, although many adults who believe abortion should be legal also feel there should be greater restrictions and more concern for the fate of the fetus. This attitude is especially pronounced among American teenagers, the majority of whom are opposed to abortion and believe it should be illegal. Data from the Alan Guttmacher Institute has shown a steady decline in the number of abortions since the early 1990s, due primarily to a drop in teenage abortions. The drop in the abortion rate among teenagers is the result of several factors. First, better education

among high school students, particularly regarding the biology of fetal development. This has been facilitated by the introduction of 3-D ultrasound imagery, which has produced extremely clear images of fetal development. Second, easier access to information about birth control and a family and social climate that encourages its use. Third, greater emphasis is being placed on the option of carrying the fetus full term for eventual adoption. Finally, the reduction in the number of abortions may also be due to reduced funding for abortion clinics and intimidation of abortion providers.

The increased concern for the fate of the embryo and fetus, combined with the ethical principles established by the Belmont Report and the Gelsinger investigation, have led to a call for embryo and fetal advocates. Parents contemplating an abortion or the possibility of donating IVF embryos for research would have access to independent advocates to discuss the issues. In the case of IVF embryos, society should not assume that clinics are acting in the best interest of the embryos any more than they can assume medical researchers are acting in the best interest of the research subjects. There are many who oppose the introduction of advocates because they believe it is an attempt to undermine the legal status of abortion. However, the introduction of patient advocates, called for by the Gelsinger investigation, did not bring an end to clinical trials, nor is the introduction of fetal or embryo advocates likely to overturn abortion laws in the United States. On the contrary, fetal and embryo advocates are a natural extension of the intent of current abortion laws, both in the United States and in Europe. These laws leave the decision to terminate a pregnancy up to the parents and especially up to the mother. The fetal and embryo advocates would simply try to ensure that the parents are making an informed decision.

The United Kingdom and the European Union, though divided on the issue of therapeutic cloning, have decided to allow research on human embryos, specifically those produced in IVF clinics. But it is important to note that these decisions were made without changing their abortion laws. The legislation that has been passed in the U.K. and EU, and now being debated before the United States Congress, will be discussed in the next chapter.

.7.
LEGAL ISSUES

Embryonic stem cell research may provide powerful new methods for treating a variety of medical disorders. It also introduces many ethical problems that require legislation to control the use and spread of this technology. The legal issues deal with stem cells that are harvested from three- to five-day-old human embryos, donated by in vitro fertilization clinics or produced by therapeutic cloning. There are no legal issues associated with the use of adult stem cells, or stem cells isolated from umbilical cord blood. Therapeutic cloning represents a fusion of cloning technology and stem cell research, and its regulation has proved to be an extremely difficult problem to resolve. The legal debate involving stem cells varies from country to country, particularly for therapeutic cloning. We begin by considering the legal issues as they unfolded in the United Kingdom (England, Wales, Scotland, and Northern Ireland), for it was there that laws regulating stem cell research and therapeutic cloning were first discussed and enacted.

The United Kingdom

The regulation of stem cell research in the United Kingdom is governed by the Human Fertilization and Embryology Act of 1990. This legislation, administered by the Human Fertilization and Embryology Authority (HFEA), was enacted to regulate the practice of in vitro fertilization (IVF), which originated in Britain with the birth of the first "test tube" baby, Louise Brown, in 1978. HFEA was debated at length by the British Parliament and by the Committee of Inquiry into Human Fertilization and Embryology. This committee was chaired by Baroness Warnock

and was tabled in 1984. The Act of 1990 largely implemented the recommendations of the Warnock committee.

Under the Act, research on embryos older than 14 days is prohibited. This time period was set to coincide with the appearance of the primitive streak, an anatomical feature of an embryo that indicates the beginning of neurulation and the formation of the central nervous system (CNS). All research dealing with human embryos is licensed by the HFEA, which may be denied if the authority feels the research objectives may be obtained with nonhuman embryos or by some other means. The license is granted only if the research is focused on the following treatments: promoting advances in the treatment for infertility, increasing knowledge about the causes of congenital disease, increasing knowledge about the causes of miscarriages, development of contraceptives, and for developing methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation. There is also a provision for allowing research on embryos for "other purposes" that increase knowledge about the creation and development of embryos, as well as their potential use in developing medical therapies.

With the birth of the first mammalian clone, Dolly the sheep, in 1996, the HFEA and the Human Genetics Advisory Commission undertook a public consultation on human cloning as it pertains to stem cell research. Their report, tabled in 1998, recommended that the HFEA issue licenses for therapeutic cloning and that research involving the embryos so produced would be subject to the 14-day limit imposed by the act for IVF embryos. These recommendations were debated at length by the British government and passed into law as the Human Fertilization and Embryology Regulations (HFER) on January 22, 2001. The passage of this law brought with it the concern that some of the cloned embryos might be implanted into a surrogate mother and brought to full term. To ensure that this did not happen, the government introduced the Human Reproductive Cloning bill, which proposed a ban on reproductive cloning, whereby an embryo is produced by nuclear transfer and then carried to term by a surrogate mother, as was done with Dolly. This bill was passed into law on December 4, 2001.

The British legislation covering therapeutic cloning is highly regarded around the world and has served as a model for all subsequent discussions concerning cloning legislation. The legislation regulating

therapeutic cloning and stem cell research (HFER 2001) was reviewed by a special committee set up by the House of Lords in 2002 and chaired by Professor Liam Donaldson. The Donaldson committee put out a call for evidence from the scientific and research organizations, the churches, medical charities, patients' support groups, pro-life groups, and many organizations representing the general public, such as the trade unions and the National Federation of Women's Institutes. The committee received 52 submissions from various organizations, and they held 12 sessions of oral evidence, at which 42 people representing 17 organizations presented their arguments for or against the proposed legislation. Members of the committee also visited research laboratories to gain a better understanding of the science involved.

The central issue dealt with by the House of Lords committee was the 14-day limit established by HFER 2001. This issue was reexamined because of the great number of people and organizations that questioned the validity of the cut-off period on moral and ethical grounds. Positions range from the view that the early embryo is a human being in the fullest sense from the moment of fertilization and should be accorded the same protection as a human fetus or baby, to the position that an early embryo is an undifferentiated collection of cells that deserves little more attention than any other isolated human cells. The Warnock committee adopted a position between these opposing views, concluding that the early embryo has a special status but not one that justifies its being accorded protection under the law. The House of Lords committee reviewed this position with respect to the principles discussed in chapter 6 (respect for persons and justice and beneficence), and on the current legal and social status of abortion. In this context, the committee focused on three main elements:

1. Legislation allowing abortion has been in place in the U.K. for 30 years. That legislation, known as the Abortion Act, sets an upper limit of 24 weeks for terminating a pregnancy and thus reflects a gradation in the respect accorded to a fetus as it develops from the early embryo to its birth. This does not persuade those opposed to abortion in all circumstances, but the Act reflects majority public opinion and has been tested on several occasions since it was enacted. Thus it would be difficult to justify an absolute prohibition

on the destruction of early embryos while permitting abortion on fetuses long after the formation of the primitive streak.

2. IVF has been practiced in the U.K. for 25 years and has wide public support. As currently practiced, surplus embryos are created, most of which are eventually destroyed. To give the early embryo full protection of a person would be inconsistent with the use of IVF.
3. The 1990 Act, which regulates the use of early embryos for medical research, was enacted after a lengthy period of public and parliamentary debate and still retains a wide measure of public support.

Consequently, the committee concluded that the 14-day limit was valid and should remain the limit for research on early embryos. In assigning this limit, the Warnock committee demonstrated a respect for the early embryo that it did not previously enjoy. The full implication of the embryo's new status was considered further by the House of Lords committee regarding the creation of embryos for research (therapeutic cloning). The committee received many presentations on this issue, some of which took the view that an embryo created for research was clearly being used as a means to an end, with no prospect of implantation, whereas embryos produced in IVF clinics were intended for implantation, even though some would be destroyed. On the weight of this argument, the committee concluded that embryos should not be created specifically for research purposes unless there is an exceptional need that cannot be met by the use of surplus IVF embryos. Thus the committee did not recommend a ban on therapeutic cloning. However, it did call for increased surveillance of the procedure to ensure that every cloned embryo is accounted for and that the experiments to which the embryos are subjected do not extend beyond what is allowed by law.

The European Union

The European Union (EU), which includes Germany, France, Spain, the Netherlands, and 11 other European countries, agrees with the U.K. position on reproductive cloning and has passed laws to ban it. However, the EU strongly disagrees with the U.K. on the issue of therapeutic cloning. Article 18 of the Council of Europe Convention on

Human Rights and Biomedicine states categorically that the creation of human embryos for research purposes is prohibited. The council disagrees with the notion expressed by U.K. legislators that therapeutic cloning should be allowed simply because abortion is allowed. Abortion is a special situation in which the rights of the mother take precedence over the rights of the embryo or fetus. But when this association is broken, or simply does not exist, then the rights of the embryo become paramount. Thus therapeutic cloning, or any kind of research that destroys human embryos, is illegal in Germany, Austria, Portugal, Ireland, Norway, and Poland. Even the Netherlands, a politically liberal country, passed a law in 2003 to ban the cloning of human embryos. In addition, the Council of Europe, which includes not only the 15 EU member states but also more than 40 countries, including Russia and Turkey, adopted a convention on biomedicine that prohibits the creation of human embryos for research purposes. In 2002 the Netherlands and Sweden appeared willing to allow therapeutic cloning provided that laws were enacted to prohibit placing such embryos in surrogate mothers to be carried to full term. But it became clear that enforcing such a law would be nearly impossible, and so a complete ban on all forms of cloning seemed to be the only practical solution.

The United States

A bill to prohibit all forms of cloning (Human Cloning Prohibition Act of 2001, H.R. 2505), which has the support of President George Bush, was passed by the House of Representatives in July 2001 but has not as yet been written into law. The bill, introduced by Representatives David Weldon (R-Florida) and Bart Stupak (D-Michigan), had a broad base of support but met with opposition when submitted to the Senate for debate in 2002. Dissension came from patient advocate groups and members of the biomedical research community who agreed to a ban on reproductive cloning but argued in favor of therapeutic cloning. However, neither side could show that they had at least 60 votes needed to bring the bill to a vote. Consequently, Senate majority leader Tom Daschle put the issue aside.

In 2003 the House of Representatives took a second vote on the bill, and this time it was approved by an overwhelming margin of 241 to

155. Senator Sam Brownback (R-Kansas) introduced the companion bill to the Senate for debate. Both bills call for a maximum penalty of $1 million in civil fines and up to 10-year jail terms for those who attempt reproductive or therapeutic cloning. Competing legislation was also submitted by Senators Arlen Specter (R-Pennsylvania) and Dianne Feinstein (D-California), which calls for a ban only on reproductive cloning.

Legislators debating these bills before the Senate will have to deal with the same moral issues confronting the U.K. and EU Parliaments in 2001: Does a 3-day-old embryo qualify for legal protection? U.K. legislation holds that an embryo less than 14 days old does qualify, whereas a 15-day-old embryo does not. The EU overwhelmingly supports a complete ban on human cloning, and it reiterated this decision in April 2003. Firm support for a total ban on human cloning from the EU, and the perceived difficulties of regulating the use of cloned embryos, will likely influence the outcome of the vote in the Senate. In addition, new research on adult stem cells and umbilical cord blood stem cells indicate that they have the same potential for curing disease as do ES cells. Thus, the argument that therapeutic cloning and ES cells are essential for the development of effective stem cell therapies is no longer convincing.

The issue of therapeutic cloning is especially difficult to resolve in the United States because the abortion issue is much more polarized in that country than it is in the U.K. or the EU. Anti-abortion groups have seized upon the 14-day limit, established by U.K. legislators, as an indication that the issue of abortion needs to be reassessed, rather than using the acceptance of abortion as an argument for allowing research on early embryos. These groups have argued that, if in the context of therapeutic cloning it makes sense to protect a 14-day-old embryo, then it is also wrong to abort an embryo or fetus that is older than 14 days. So far, the American judiciary has refused to hear arguments of this kind, namely that a 14-day-old embryo should have the status of a person, but the various factions that are for or against this position have made it very difficult for Congress to pass legislation even to ban reproductive cloning, a practice that is already banned in all of Europe.

In April 2004, 206 members of the U.S. House of Representatives, in response to public pressure, signed a letter urging President Bush to modify his August 2001 executive order limiting federal funds for ES

cell research to preexisting cell lines. The letter called for a new policy whereby federal funds would be made available for researchers to create new ES cell lines from embryos left over from in vitro fertilization clinics. On May 14, 2004, Dr. Elias Zerhouni, director of the National Institutes of Health, responded to the letter by reiterating President Bush's position that federal funds should not be used to "encourage further destruction of human embryos that have at least the potential for life."

The difficulty of passing legislation to ban or regulate human ES cell research cloning in the United States is simply a reflection of how difficult an issue this is for the American people. Whichever way the vote goes, therapeutic cloning and the use of human embryos for medical research will be debated for a very long time. Therapeutic cloning has forced society to reexamine an issue that was thought to have been resolved with *Roe v. Wade.* But such complex issues, concerning acquisition of humanness and the legal status of an embryo, may never be resolved to the complete satisfaction of the many diverse groups that make up American society.

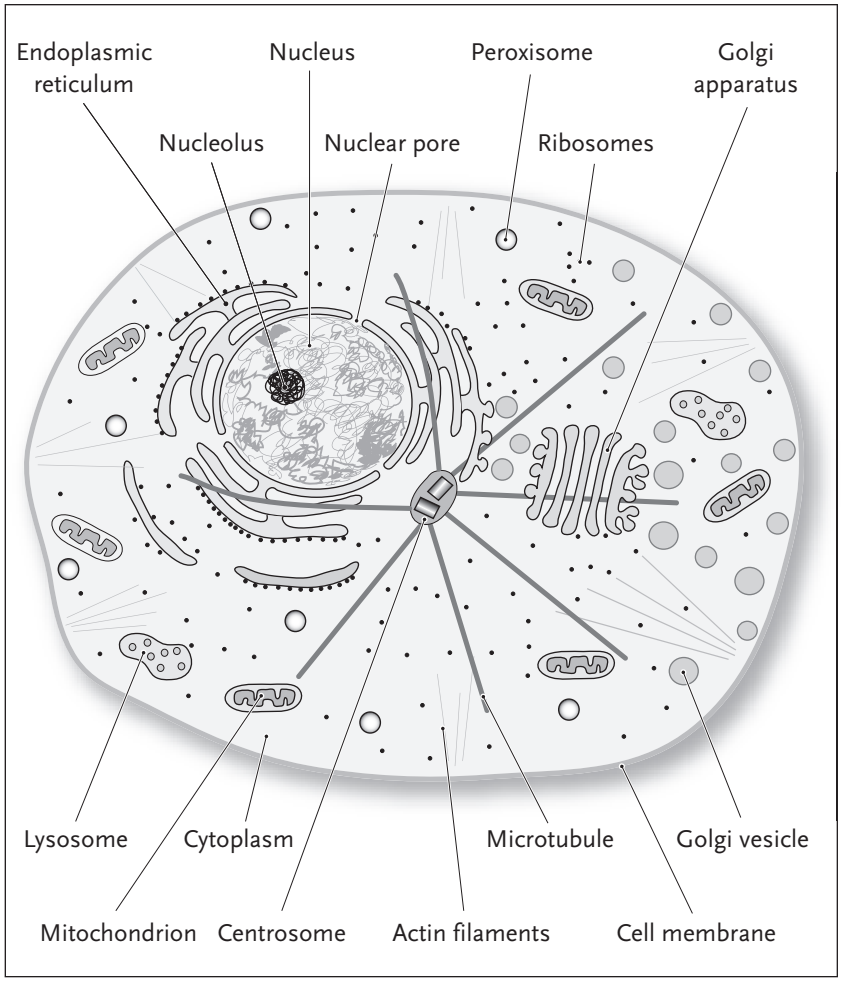
.8.

RESOURCE CENTER

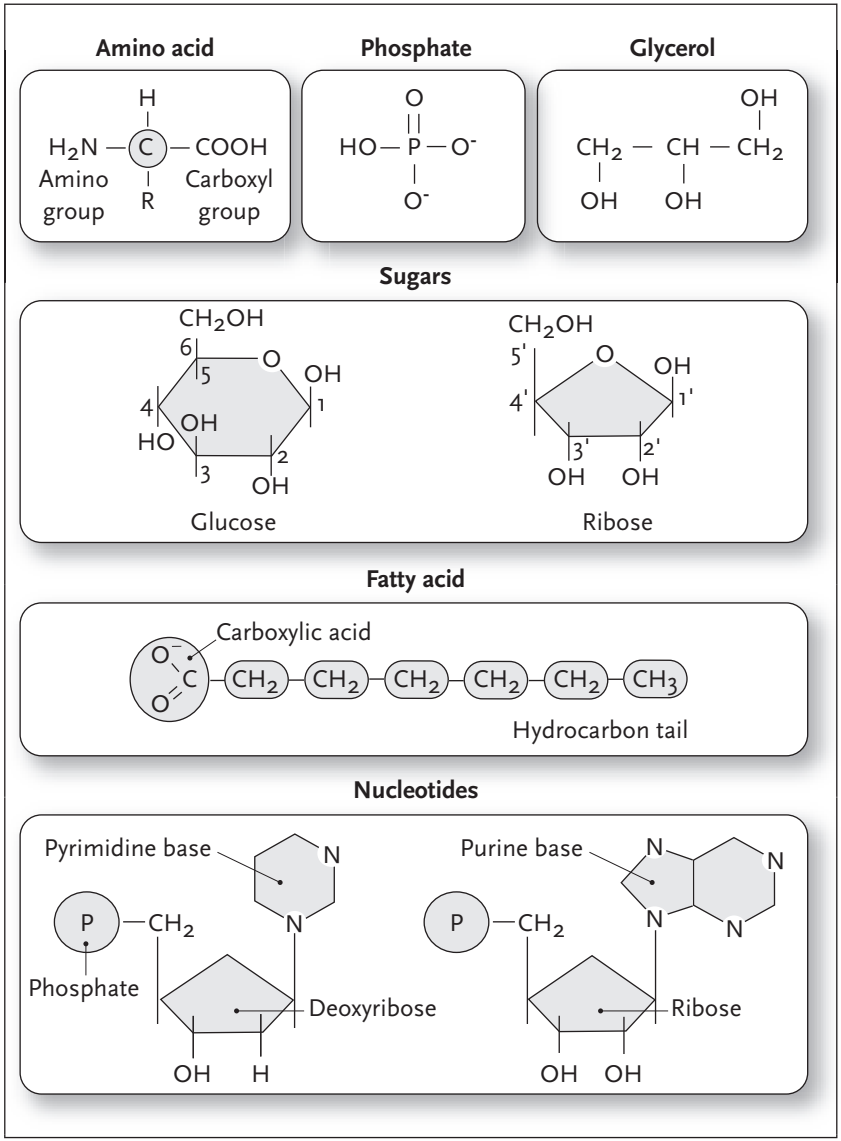
Eukaryote Cell Primer

Life on Earth began 3.5 billion years ago in the form of single cells that appeared in the oceans. These cells evolved into ancestral prokaryotes and about 2 billion years ago gave rise to Archaea, bacteria, and eukaryotes, the three major divisions of life in the world. Eukaryotes, in turn, gave rise to plants, animals, protozoans, and fungi. Each of these groups represents a distinct phylogenetic kingdom. The Archaea and bacteria represent a fifth kingdom, known as the monera or prokaryotes. Archaea and bacteria are very similar anatomically, both lacking a true nucleus and internal organelles. A prokaryote genome is a single, circular piece of naked DNA, called a chromosome, containing fewer than 5,000 genes. Eukaryotes (meaning true nuclei) are much more complex, having many membrane-bounded organelles. These include a nucleus, nucleolus, endoplasmic reticulum (ER), Golgi complex, mitochondria, lysosomes, and peroxisomes.

The eukaryote nucleus, bounded by a double phospholipid membrane, contains a DNA (deoxyribonucleic acid) genome on two or more linear chromosomes, each of which may contain up to 10,000 genes. The nucleus also contains an assembly plant for ribosomal subunits, called the nucleolus. The endoplasmic reticulum (ER) and the Golgi complex work together to glycosylate proteins and lipids (attach sugar molecules to the proteins and lipids producing glycoproteins and glycolipids), most of which are destined for the cell membrane to form a molecular "forest" known as the glycocalyx. The glycoproteins and glycolipids travel from the ER to the Golgi, and from the Golgi to the



The eukaryote cell. The structural components shown here are present in organisms as diverse as protozoans, plants, and animals. The nucleus contains the DNA genome and an assembly plant for ribosomal subunits (the nucleolus). The endoplasmic reticulum (ER) and the Golgi work together to modify proteins, most of which are destined for the cell membrane. These proteins are sent to the membrane in Golgi vesicles. Mitochondria provide the cell with energy in the form of ATP. Ribosomes, some of which are attached to the ER, synthesize proteins. Lysosomes and peroxisomes recycle cellular material and molecules. The microtubules and centrosome form the spindle apparatus for moving chromosomes to the daughter cells during cell division. Actin filaments and a weblike structure consisting of intermediate filaments (not shown) form the cytoskeleton.



Molecules of the cell. Amino acids are the building blocks for proteins. Phosphate is an important component of many other molecules and is added to proteins to modify their behavior. Glycerol is a three-carbon alcohol that is an important ingredient in cell membranes and fat. Sugars, like glucose, are a primary energy source for most cells and also have many structural functions. Fatty acids are involved in the production of cell membranes and storage of fat. Nucleotides are the building blocks for DNA and RNA.

cell surface, in membrane-bounded vesicles that form by budding off the organelle by exocytosis. Thus the cytoplasm contains many transport vesicles that originate from the ER and Golgi. The Golgi vesicles bud off the outer chamber, or the one farthest from the ER. Mitochondria, once free-living prokaryotes and the only other organelle with a double membrane, provide the cell with energy in the form of adenosine triphosphate (ATP). The production of ATP is carried out by an assembly of metal-containing proteins, called the electron transport chain, located in the mitochondrion inner membrane. Ribosomes, some of which are attached to the ER, synthesize proteins. Lysosomes and peroxisomes recycle cellular material and molecules. The microtubules and centrosome form the spindle apparatus for moving chromosomes to the daughter cells during cell division. Actin filaments and a weblike structure consisting of intermediate filaments form the cytoskeleton.

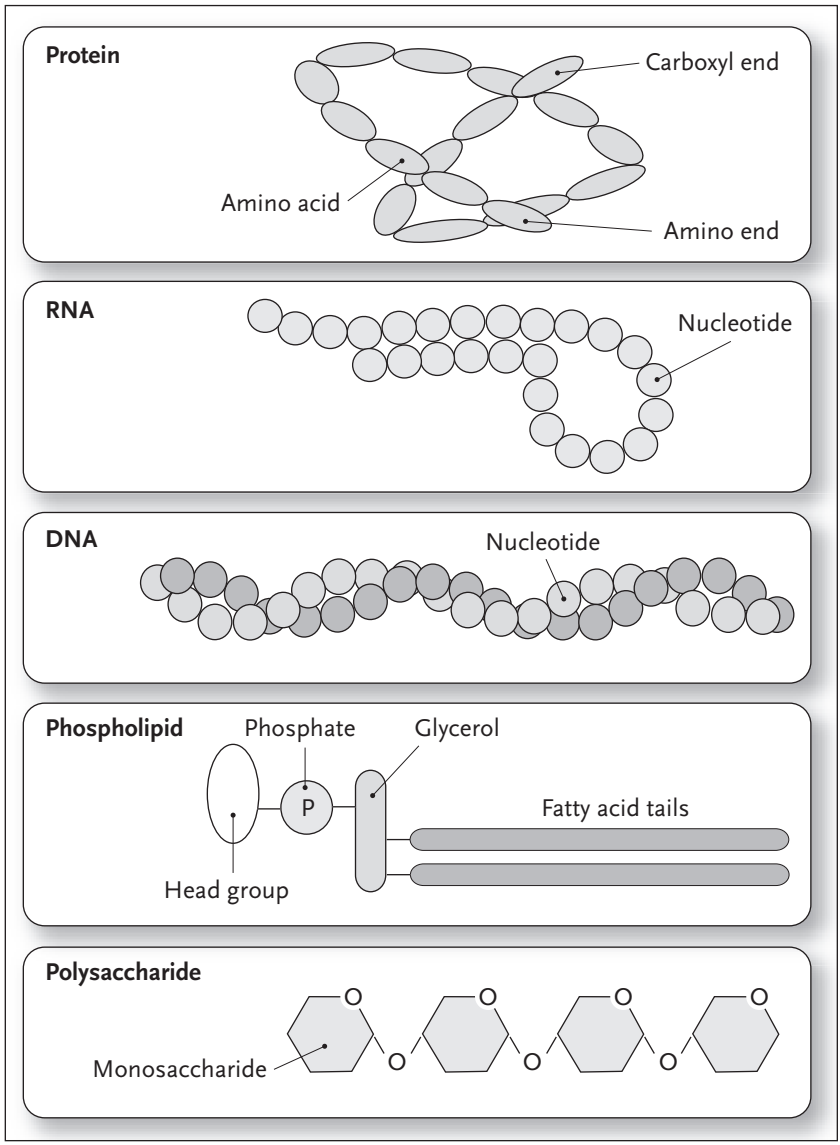
MOLECULES OF THE CELL

Cells are biochemical entities that synthesize many thousands of molecules. Studying these chemicals and the biochemistry of the cell would be a daunting task were it not for the fact that most of the chemical variation is based on six types of molecules assembled into just four types of macromolecules. The six basic molecules are amino acids, phosphate, glycerol, sugars, fatty acids, and nucleotides. Amino acids have a simple core structure consisting of an amino group, a carboxyl group, and a variable R group attached to a carbon atom. There are 20 different kinds of amino acids, each with a unique R group. Phosphates are extremely important molecules that are used in the construction, or modification, of many other molecules. They are also used to store chemical-bond energy. Glycerol is a simple, three-carbon alcohol that is an important component of cell membranes and fat reservoirs. Sugars are extremely versatile molecules that are used as an energy source and for structural purposes. Glucose, a six-carbon sugar, is the primary energy source for most cells and the principle sugar used to glycosylate proteins and lipids for the production of the glycocalyx. Plants have exploited the structural potential of sugars in their production of cellulose, and thus wood, bark, grasses, and reeds are polymers of glucose and other monosaccharides. Ribose, a five-carbon

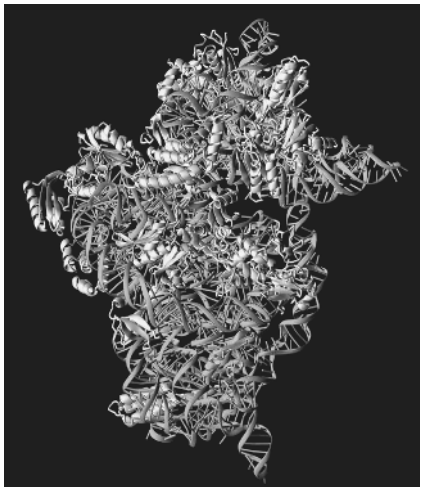
sugar, is a component of nucleic acids as well as of ATP. The numbering convention for sugar carbon atoms is shown in the figure on page 90. Ribose carbons are numbered as 1' (1 prime), 2', and so on. Consequently, references to nucleic acids, which include ribose, often refer to the 3' or 5' carbon. Fatty acids consist of a carboxyl group (when ionized it becomes a carboxylic acid) linked to a hydrophobic hydrocarbon tail. These molecules are used in the construction of cell membranes and fat. Nucleotides are building blocks for DNA and RNA (ribonucleic acid). Nucleotides consist of three components: a phosphate, a ribose sugar, and a nitrogenous (nitrogen containing) ring compound that behaves as a base in solution. Nucleotide bases appear in two forms: a single-ring nitrogenous base, called a pyrimidine, and a double-ringed base, called a purine. There are two kinds of purines (adenine and guanine) and three pyrimidines (uracil, cytosine, and thymine). Uracil is specific to RNA, substituting for thymine. In addition, RNA nucleotides contain ribose, whereas DNA nucleotides contain deoxyribose (hence the origin of their names). Ribose has a hydroxyl (OH) group attached to both the 2' and 3' carbons, whereas deoxyribose is missing the 2' hydroxyl group. ATP, the molecule that is used by all cells as a source of energy, is a ribose nucleotide consisting of the purine base adenine and three phosphates attached to the 5' carbon of the ribose sugar. The phosphates are labeled α (alpha), β (beta), and γ (gamma), and are linked to the carbon in a tandem order, beginning with α. The energy stored by this molecule is carried by the covalent bonds of the β and γ phosphates. Breaking these bonds sequentially releases the energy they contain while converting ATP to adenosine diphosphate (ADP) and then to adenosine monophosphate (AMP). AMP is converted back to ATP by mitochondria.

MACROMOLECULES OF THE CELL

The six basic molecules are used by all cells to construct five essential macromolecules. These include proteins, RNA, DNA, phospholipids, and sugar polymers, known as polysaccharides. Amino acids are linked together by peptide bonds to construct a protein. A peptide bond is formed by linking the carboxyl end of one amino acid to the amino end of second amino acid. Thus, once constructed, every protein has an amino end and a carboxyl end. An average protein may consist of



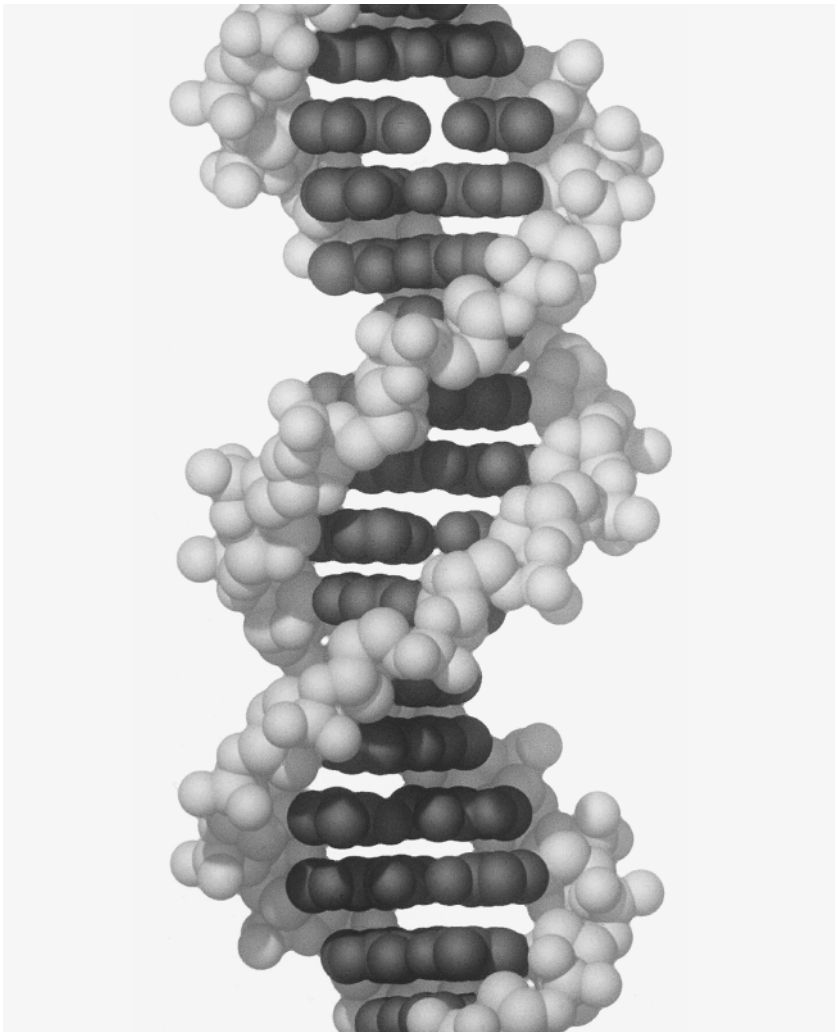
Macromolecules of the cell. Protein is made from amino acids linked together to form a long chain that can fold up into a three-dimensional structure. RNA and DNA are long chains of nucleotides. RNA is generally single stranded, but can form localized double-stranded regions. DNA is a double-stranded helix, with one strand coiling around the other. A phospholipid is composed of a hydrophilic head-group, a phosphate, a glycerol molecule, and two hydrophobic fatty acid tails. Polysaccharides are sugar polymers.



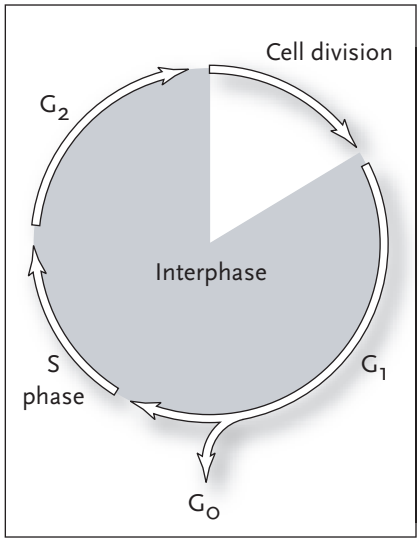
Molecule model of the 30S ribosomal subunit, which consists of protein (light gray corkscrew structures) and RNA (coiled ladders). The RNA that is also responsible for the catalytic function of the ribosome determines the overall shape of the molecule. *(Courtesy of V. Ramakrishnan, MRC Laboratory of Molecular Biology, Cambridge)*

300 to 400 amino acids. Nucleic acids are macromolecules constructed from nucleotides. The 5' phosphate of one nucleotide is linked to the 3' OH of a second nucleotide. Additional nucleotides are always linked to the 3' OH of the last nucleotide in the chain. Consequently, the growth of the chain is said to be in the 5' to 3' direction. RNA nucleotides are adenine, uracil, cytosine, and guanine. A typical RNA molecule consists of 2,000 to 3,000 nucleotides; it is generally single stranded but can form localized double-stranded regions. RNA is involved in the synthesis of proteins and is a structural and enzymatic component of ribosomes. DNA, a double-stranded nucleic acid, encodes cellular genes and is constructed from adenine, thymine, cytosine, and guanine deoxyribonucleotides (dATP, dTTP, dCTP, and dGTP, where "d" indicates deoxyribose). The two DNA strands coil around each other like strands in piece of rope, and for this reason the molecule is known as the double helix. DNA is an extremely large macromolecule, typically consisting of over a million nucleotide pairs (or base pairs). Double-stranded DNA forms when two chains of nucleotides interact through the formation of chemical bonds between complementary base pairs. The chemistry of the bases is such that adenine pairs with thymine, and cytosine pairs with guanine. For stability, the two strands are antiparallel, that is, the orientation of one strand is in the 5' to 3' direction, while the complementary strand runs 3' to 5'. Phospholipids, the main component of cell membranes, are composed of a polar head group (usually an alcohol), a phosphate, glycerol, and

two hydrophobic fatty acid tails. Fat that is stored in the body as an energy reserve has a structure similar to a phospholipid, being composed of three fatty acid chains attached to a molecule of glycerol.



Computer model of DNA. The two strands coil around each other to form a helix that, when looking down on it from above, coils to the right. The spherical structures in this image represent the various atoms in the sugars and bases (dark gray), and phosphates (light gray). *(Kenneth Eward/BioGrafx/Photo Researchers, Inc.)*



The cell cycle. Most cells spend their time cycling between a state of calm (interphase) and cell division. Interphase is further divided into three subphases: Gap 1 (G_1), S phase (DNA synthesis), and Gap 2 (G_2). Cells may exit the cycle by entering a special phase called G_o.

The third fatty acid takes the place of the phosphate and head-group of a phospholipid. Sugars are polymerized to form chains of two or more monosaccharides. Disaccharides (two monosaccharides) and oligosaccharides (three to 12 monosaccharides) are attached to proteins and lipids destined for the glycocalyx. Polysaccharides, such as glycogen and starch, may contain several hundred monosaccharides and are stored in cells as an energy reserve.

THE CELL CYCLE

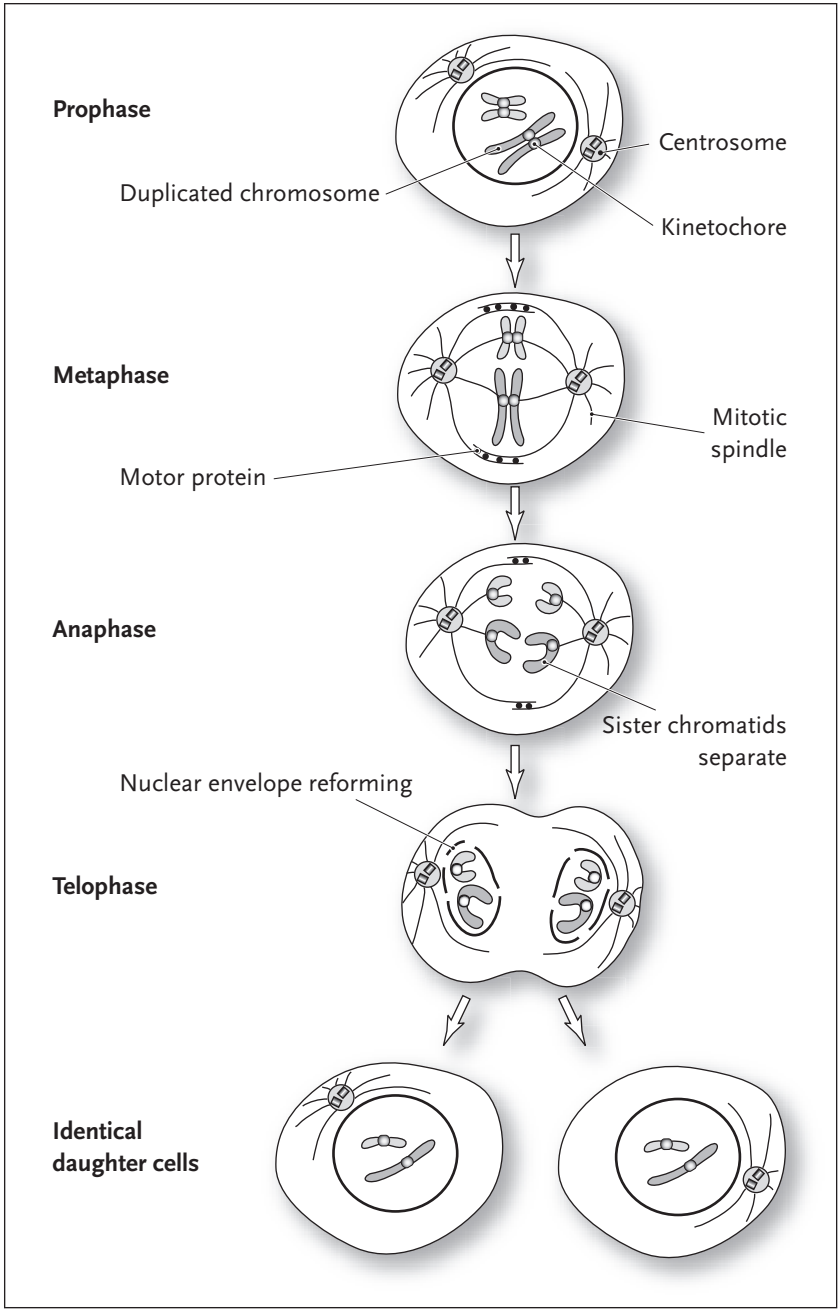
Cells inherited the power of reproduction from prebiotic bubbles that split in half at regular intervals under the influence of the turbulent environment that characterized the Earth more than 3 billion years ago. This pattern of turbulent fragmentation followed by a brief period of calm is now a regular behavior pattern of every cell. Even today, after 3 billion years, many cells still divide every 20 minutes.

The regular alternation between division and calm has come to be known as the cell cycle. In studying this cycle, scientists have recognized different states of calm and different ways in which a cell can divide. The calm state of the cell cycle, referred to as interphase, is divided into three subphases called Gap (G_1), S phase (a period of DNA synthesis), and Gap 2 (G_2). The conclusion of interphase, and with it the termination of G_2, occurs with division of the cell and a return to G_1. Cells may leave the cycle by entering a special phase called G_0. Some cells, such as postmitotic neurons in an animal's brain, remain in G_0 for the life of the organism.

Although interphase is a period of relative calm, the cell grows continuously during this period, working hard to prepare for the next round of division. Two notable events are the duplication of the spindle (the centrosome and associated microtubules), a structure that is crucial for the movement of the chromosomes during cell division, and the appearance of an enzyme called maturation promoting factor (MPF) at the end of G_2. MPF phosphorylates histones. The histones are proteins that bind to the DNA, which, when phosphorylated, compact (or condense) the chromosomes in preparation for cell division. MPF is also responsible for the breakdown of the nuclear membrane. When cell division is complete, MPF disappears, allowing the chromosomes to decondense and the nuclear envelope to reform. Completion of a normal cell cycle always involves the division of a cell into two daughter cells. This can occur by a process known as mitosis, which is intended for cell multiplication, and by second process known as meiosis, which is intended for sexual reproduction.

MITOSIS

Mitosis is used by all free-living eukaryotes (protozoans) as a means of asexual reproduction. The growth of a plant or an animal is also accomplished with this form of cell division. Mitosis is divided into four stages: prophase, metaphase, anaphase, and telophase. All these stages are marked out in accordance with the behavior of the nucleus and the chromosomes. Prophase marks the period during which the duplicated chromosomes begin condensation and the two centrosomes begin moving to opposite poles of the cell. Under the microscope, the chromosomes become visible as X-shaped structures, which are the two duplicated chromosomes, often called sister chromatids. A special region of each chromosome, called a centromere, holds the chromatids together. Proteins bind to the centromere to form a structure called the kinetochore. Metaphase is a period during which the chromosomes are sorted out and aligned between the two centrosomes. By this time, the nuclear membrane has completely broken down. The two centrosomes and the microtubules fanning out between them form the mitotic spindle. The area in between the spindles, where the chromosomes are aligned, is often referred to as the metaphase plate. Some of the microtubules make contact with the kinetochores, while others overlap, with

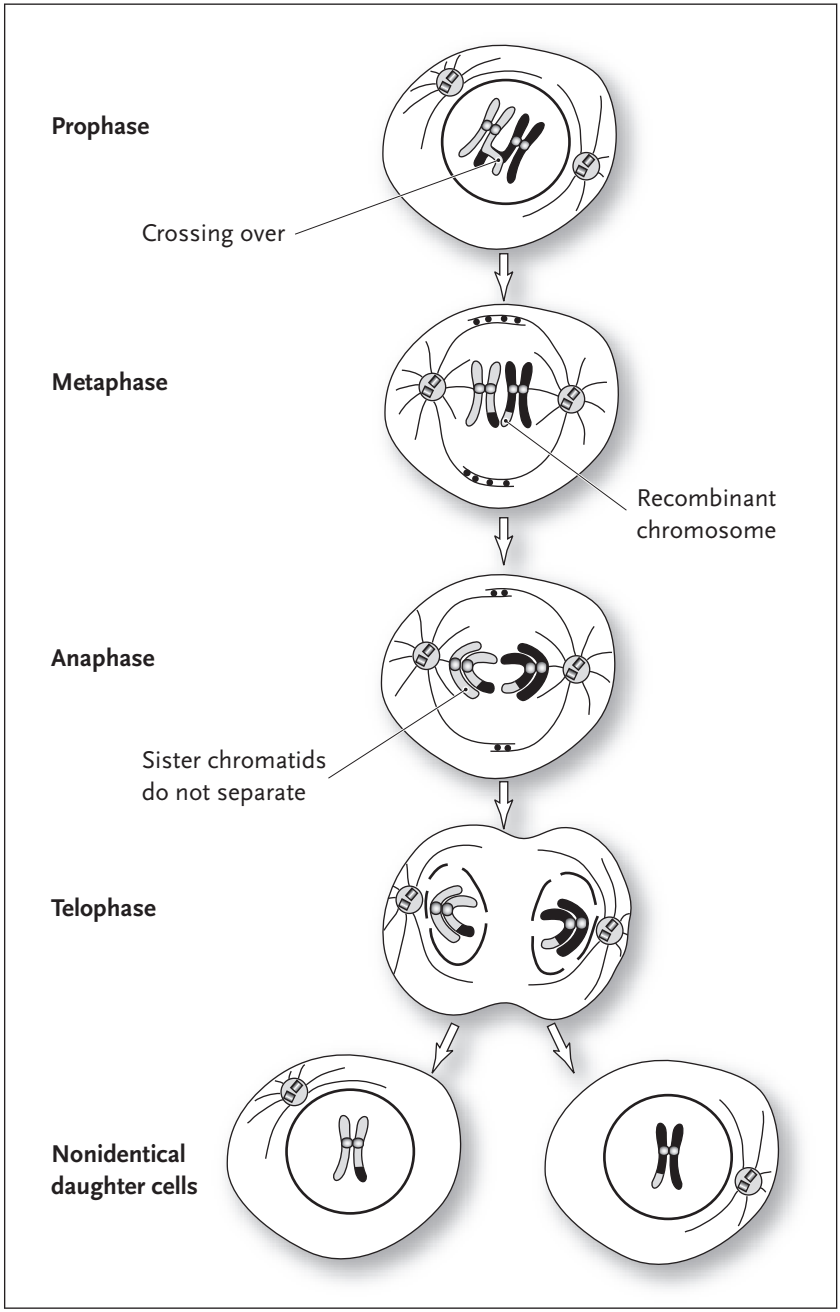


Mitosis. Principal stages dealing with the movement and partitioning of the chromosomes between the future daughter cells. For clarity, only two chromosomes are shown.

motor proteins situated in between. Eukaryotes are normally diploid, so a cell would have two copies of each chromosome, one from the mother and one from the father. Anaphase is characterized by the movement of the duplicated chromosomes to opposite poles of the cell. The first step is the release of an enzyme that breaks the bonds holding the kinetochores together, thus allowing the sister chromatids to separate from each other while remaining bound to their respective microtubules. Motor proteins then move along the microtubule dragging the chromosomes to opposite ends of the cell. Using energy supplied by ATP, the motor proteins break the microtubule down as it drags the chromosome along, so that the microtubule is gone by the time the chromosome reaches the spindle pole. Throughout this process, the motor proteins and the chromosome manage to stay one step ahead of the disintegrating microtubule. The overlapping microtubules aid movement of the chromosomes toward the poles as another type of motor protein pushes the microtubules in opposite directions effectively forcing the centrosomes toward the poles. This accounts for the greater overlap of microtubules in metaphase as compared with anaphase. During telophase, the daughter chromosomes arrive at the spindle poles and decondense to form the relaxed chromatin characteristic of interphase nuclei. The nuclear envelope begins forming around the chromosomes, marking the end of mitosis. During the same period, a contractile ring, made of the proteins myosin and actin, begins pinching the parental cell in two. This stage, separate from mitosis, is called cytokinesis and leads to the formation of two daughter cells, each with one nucleus.

MEIOSIS

Unlike mitosis, which leads to the growth of an organism, meiosis is intended for sexual reproduction and occurs exclusively in ovaries and testes. Eukaryotes, being diploid, receive chromosomes from both parents; if gametes were produced using mitosis, a catastrophic growth in the number of chromosomes would occur each time a sperm fertilized an egg. Meiosis is a special form of cell division that produces haploid gametes (eggs and sperm), each possessing half as many chromosomes as the diploid cell. When haploid gametes fuse, they produce an embryo with the correct number of chromosomes.



Meiosis I. The most notable features include genetic recombination (crossing over) between the homologous chromosomes during prophase, comigration of the sister chromatids during anaphase, and the production of nonidentical daughter cells. Only one homologous pair is shown.

The existence of meiosis was first suggested 100 years ago when microbiologists counted the number of chromosomes in somatic and germ cells. The roundworm, for example, was found to have four chromosomes in its somatic cells but only two in its gametes. Many other studies also compared the amount of DNA in nuclei from somatic cells and gonads, always with the same result: The amount of DNA in somatic cells is exactly double the amount in fully mature gametes. To understand how this could be, scientists studied cell division in the gonads and were able to show that meiosis occurs as two rounds of cell division with only one round of DNA synthesis. The two rounds of division were called meiosis I and meiosis II, and scientists observed that both could be divided into the same four stages known to occur in mitosis. Indeed, meiosis II is virtually identical to a mitotic division. Meiosis I resembles mitosis, but close examination shows three important differences: Gene swapping occurs between homologous chromosomes in prophase; homologs (i.e., two homologous chromosomes) remain paired at metaphase, instead of lining up at the plate as is done in mitosis; and the kinetochores do not separate at anaphase.

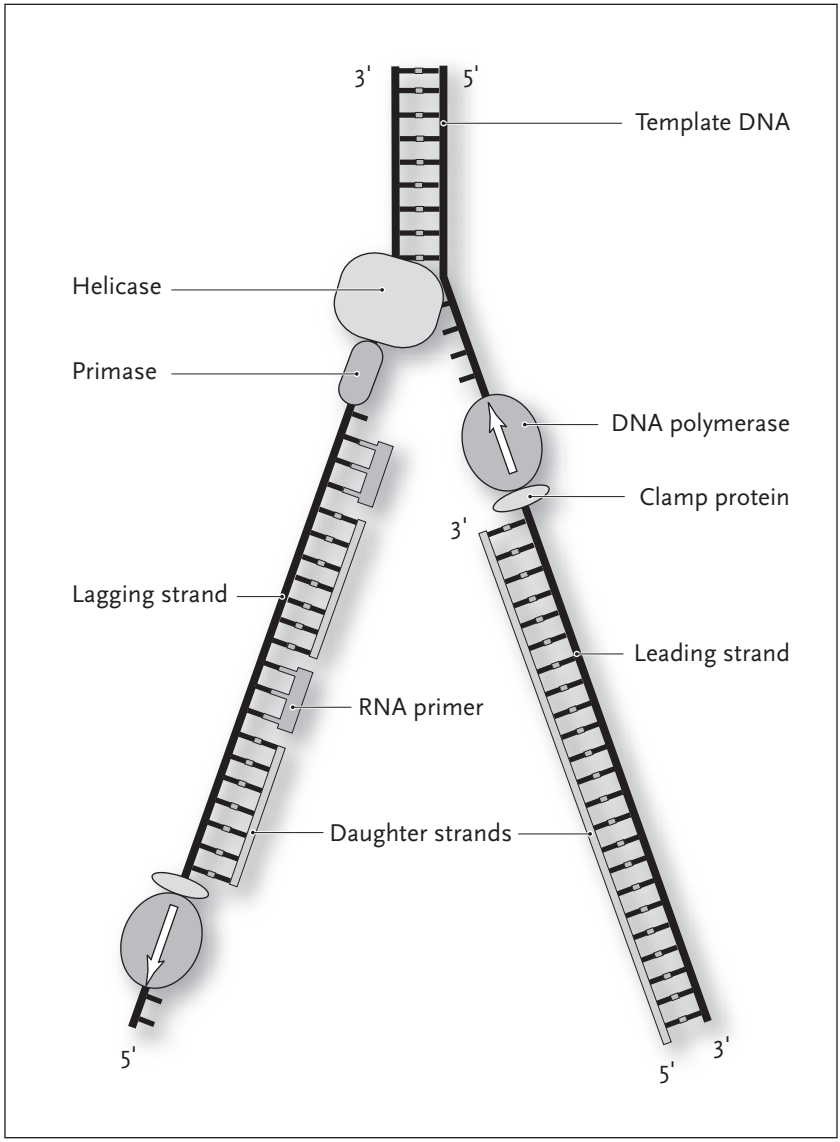
Homologous chromosomes are two identical chromosomes that come from different parents. For example, humans have 23 chromosomes from the father and the same 23 from the mother. We each have a maternal chromosome 1 and a paternal chromosome 1; they carry the same genes but specify slightly different traits. Chromosome 1 may carry the gene for eye color, but the maternal version, or allele, may specify blue eyes, whereas the paternal allele specifies brown. During prophase, homologous pairs exchange large numbers of genes by swapping whole pieces of chromosome. Thus one of the maternal chromatids (gray in the figure) ends up with a piece of paternal chromosome, and a paternal chromatid receives the corresponding piece of maternal chromosome. Mixing genetic material in this way is unique to meiosis, and it is one of the reasons sexual reproduction has been such a powerful evolutionary force.

During anaphase of meiosis I, the kinetochores do not separate as they do in mitosis. The effect of this is to separate the maternal and paternal chromosomes by sending them to different daughter cells, although the segregation is random. That is, the daughter cells receive a random assortment of maternal and paternal chromosomes, rather

than one daughter cell receiving all paternal chromosomes and the other all-maternal chromosomes. Random segregation, along with genetic recombination, accounts for the fact that while children resemble their parents, they do not look or act exactly like them. These two mechanisms are responsible for the remarkable adaptability of all eukaryotes. Meiosis II begins immediately after the completion of meiosis I, which produces two daughter cells, each containing a duplicated parent chromosome and a recombinant chromosome consisting of both paternal and maternal DNA. These two cells divide mitotically to produce four haploid cells, each of which is genetically unique, containing unaltered or recombinant maternal and paternal chromosomes. Meiosis produces haploid cells by passing through two rounds of cell division with only one round of DNA synthesis. However, as we have seen, the process is not just concerned with reducing the number of chromosomes but is also involved in stirring up the genetic pot in order to produce unique gametes that may someday give rise to an equally unique individual.

DNA REPLICATION

DNA replication, which occurs during the S phase of the cell cycle, requires the coordinated effort of a team of enzymes, led by DNA helicase and primase. The helicase is a remarkable enzyme that is responsible for separating the two DNA strands, a feat that it accomplishes at an astonishing rate of 1,000 nucleotides every second. This enzyme gets its name from the fact that it unwinds the DNA helix as it separates the two strands. The enzyme that is responsible for reading the template strand, and for synthesizing the new daughter strand, is called DNA polymerase. This enzyme reads the parental DNA in the 3' to 5' direction and creates a daughter strand that grows 5' to 3'. DNA polymerase also has an editorial function, in that it checks the preceding nucleotide to make sure it is correct before it adds a nucleotide to the growing chain. The editor function of this enzyme introduces an interesting problem: How can the polymerase add the very first nucleotide when it has to check a preceding nucleotide before adding a new one? A special enzyme, called primase, attached to the helicase solves this problem. Primase synthesizes short pieces of RNA that form a DNA-RNA double-stranded region. The RNA becomes a temporary part of



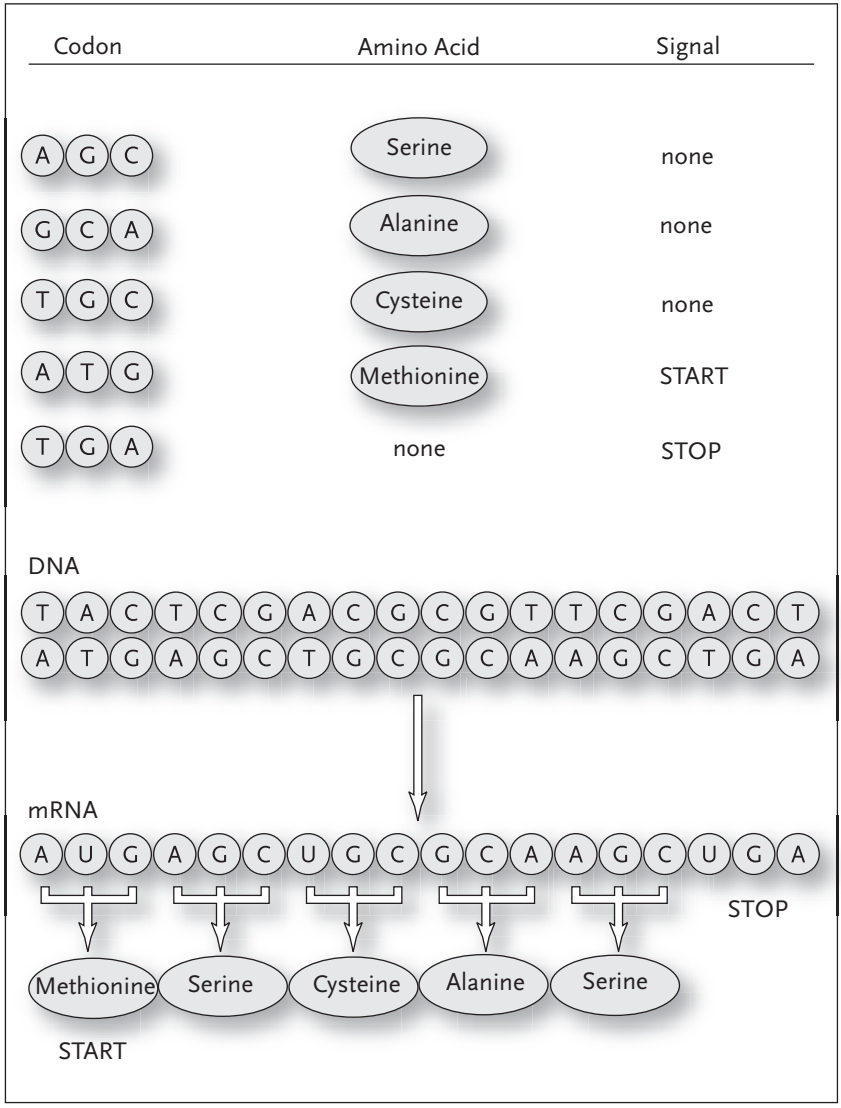
DNA replication. The helicase separates the two strands so the DNA polymerase can synthesize new strands. The primase provides replication signals for the polymerase in the form of RNA primers, and the clamp protein keeps the polymerase from falling off the DNA. The leading strand requires only a single primer (not shown). The lagging strand requires many primers, and the daughter strand is synthesized as a series of DNA fragments that are later joined into one continuous strand.

the daughter strand, thus priming the DNA polymerase by providing the crucial first nucleotide in the new strand. Once the chromosome is duplicated, DNA repair enzymes remove the RNA primers and replace them with DNA nucleotides.

TRANSCRIPTION, TRANSLATION, AND THE GENETIC CODE

Genes encode proteins and several kinds of RNA. Extracting the information from DNA requires the processes of transcription and translation. Transcription, catalyzed by the enzyme RNA polymerase, copies one strand of the DNA into a complementary strand of messenger RNA (mRNA) or ribosomal RNA (rRNA) that is used in the construction of ribosomes. Messenger RNA translocates to the cytoplasm, where it is translated into a protein by ribosomes. Newly transcribed rRNA is sent to the nucleolus for ribosome assembly and is never translated. Ribosomes are complex structures consisting of about 50 proteins and four kinds of rRNA, known as 5S, 5.8S, 18S, and 28S rRNA (the "S" refers to a sedimentation coefficient that is proportional to size). These RNAs range in size from about 500 bases up to 2,000 bases for the 28S. The ribosome is assembled in the nucleolus as two nonfunctional subunits before being sent out to the cytoplasm, where they combine, along with an mRNA, to form a fully functional unit. The production of ribosomes in this way ensures that translation never occurs in the nucleus.

The genetic code provides a way for the translation machinery to interpret the sequence information stored in the DNA molecule and represented by mRNA. DNA is a linear sequence of four different kinds of nucleotides, so the simplest code could be one in which each nucleotide specifies a different amino acid. That is, adenine coding for the amino acid glycine, cytosine for lysine, and so on. The first cells may have used this coding system, but it is limited to the construction of proteins consisting of only four different kinds of amino acids. Eventually, a more elaborate code evolved in which a combination of three out of the four possible DNA nucleotides, called codons, specifies a single amino acid. With this scheme, it is possible to have a unique code for each of the 20 naturally occurring amino acids. For example, the codon AGC specifies the amino acid serine, whereas TGC specifies the amino acid cysteine. Thus, a gene may be viewed as a long continuous



Transcription, translation, and the genetic code. Five codons are shown, four specifying amino acids (protein subunits) and two of the five serving as start and stop signals. The codons, including the start and stop signals, are linked together to form a gene on the bottom, or coding, DNA strand. The coding strand is copied into messenger RNA (mRNA), which is used to synthesize the protein. Nucleotides appear as round beads: adenine (A), thymine (T), cytosine (C), and guanine (G). Amino acids appear as labeled elliptical beads. Note that in mRNA, uracil (U) replaces the thymine (T) found in DNA.

sequence of codons. However, not all codons specify an amino acid. The sequence TGA signals the end of the gene, and a special codon, ATG, signals the start site, in addition to specifying the amino acid methionine. Consequently, all proteins begin with this amino acid, although it is sometimes removed once construction of the protein is complete. As mentioned above, an average protein may consist of 300 to 400 amino acids; since the codon consists of three nucleotides for each amino acid, a typical gene may be 900 to 1,200 nucleotides long.

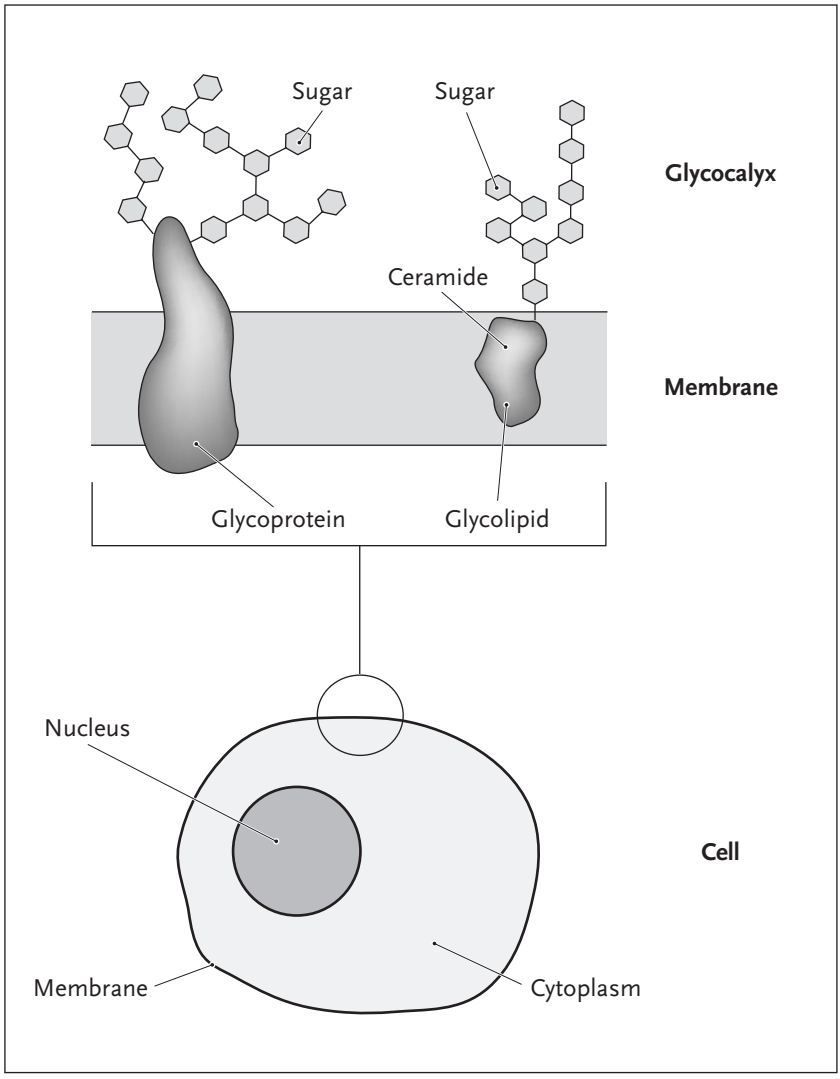
POWER GENERATION

ATP is produced in mitochondria from AMP, or ADP, and phosphate (PO_4). This process involves a number of metal-binding proteins, called the respiratory chain (also known as the electron transport chain), and a special ion channel-enzyme, called ATP synthetase. The respiratory chain consists of three major components: NADH dehydrogenase, cytochrome b, and cytochrome oxidase. All these components are protein complexes that have an iron (NADH dehydrogenase, cytochrome b) or a copper core (cytochrome oxidase), and together with the ATP synthetase are located in the inner membrane of the mitochondria.

The respiratory chain is analogous to an electric cable that transports electricity from a hydroelectric dam to our homes, where it is used to turn on lights or run stereos. The human body, like that of all animals, generates electricity by processing food molecules through a metabolic pathway, called the Krebs cycle. The electricity, or electrons so generated, travel through the respiratory chain, and as they do, they power the synthesis of ATP. All electric circuits must have a ground, that is, the electrons need some place to go once they have completed the circuit. In the case of the respiratory chain, the ground is oxygen. After passing through the chain, the electrons are picked up by oxygen, which combines with hydrogen ions to form water.

THE GLYCOCALYX

This structure is an enormously diverse collection of glycoproteins and glycolipids that covers the surface of every cell, like trees on the surface of the Earth, and has many important functions. All eukaryotes originated from free-living cells that hunted bacteria for food. The glycocalyx evolved to meet the demands of this kind of lifestyle, providing a



The eukaryote glycocalyx. The eukaryote's molecular forest consists of glycoproteins and glycolipids. Two examples are shown at the top, a glycoprotein on the left and a glycolipid on the right. The glycoprotein trees have "trunks" made of protein and "leaves" made of sugar molecules. Glycolipids also have "leaves" made of sugar molecules, but the "trunks" are a fatty compound called ceramide that is completely submerged within the plane of the membrane. The glycocalyx has many jobs, including cell-to-cell communication, and the transport and detection of food molecules. It also provides recognition markers so the immune system can detect foreign cells.

way for the cell to locate, capture, and ingest food molecules or prey organisms. Cell-surface glycoproteins also form transporters and ion channels that serve as gateways into the cell. Neurons have refined ion channels for the purpose of cell-to-cell communication, giving rise to the nervous systems found in most animal species. In higher vertebrates, certain members of the glycocalyx are used by cells of the immune system as recognition markers to detect invading microbes or foreign cells introduced as an organ or tissue transplant.

Recombinant DNA Primer

Recombinant technology is a collection of procedures that make it possible to isolate a gene and produce enough of it for a detailed study of its structure and function. Central to this technology is the ability to construct libraries of DNA fragments that represent the genetic repertoire of an entire organism or of a specific cell type. Constructing these libraries involves splicing different pieces of DNA together to form a novel or recombinant genetic entity, from which the procedure derives its name. DNA cloning and library construction were made possible by the discovery of DNA-modifying enzymes that can seal two pieces of DNA together or can cut DNA at sequence-specific sites. Many of the procedures that are part of recombinant technology, such as DNA sequencing or filter hybridization, were developed in order to characterize DNA fragments that were isolated from cells or gene libraries. Obtaining the sequence of a gene has made it possible to study the organization of the genome, but more important, it has provided a simple way of determining the protein sequence and the expression profile for any gene.

DNA-MODIFYING ENZYMES

Two of the most important enzymes used in recombinant technology are those that can modify DNA by sealing two fragments together and others that can cut DNA at specific sites. The first modifying enzyme to be discovered was DNA ligase, an enzyme that can join two pieces of DNA together and is an important component of the cell's DNA replication and repair machinery. Other DNA-modifying enzymes, called restriction enzymes, cut DNA at sequence-specific sites, with different

members of the family cutting at different sites. Restriction enzymes are isolated from bacteria, and since their discovery in 1970, more than 90 such enzymes have been isolated from more than 230 bacterial strains.

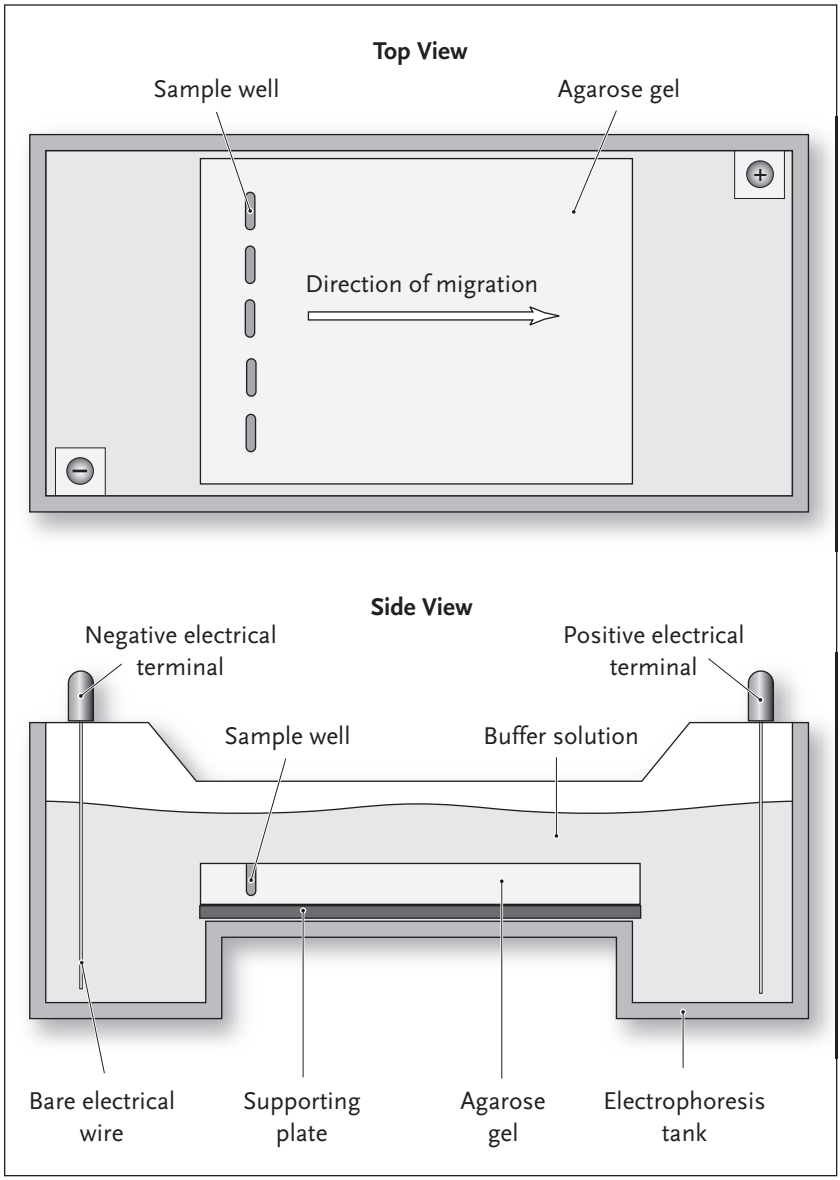
The name *restriction enzyme* is cryptic and calls for an explanation. During the period when prokaryotes began to appear on Earth, their environment contained a wide assortment of molecules that were released into the soil or water by other cells, either deliberately or when the cells died. DNA of varying lengths were among these molecules and were readily taken up by living cells. If the foreign DNA contained complete genes from a competing bacterial species, there was the real possibility that those genes could have been transcribed and translated by the host cell with potentially fatal results. As a precaution, prokaryotes evolved a set of enzymes that would *restrict* the foreign DNA population by cutting it up into smaller pieces before being broken down completely to individual nucleotides.

GEL ELECTROPHORESIS

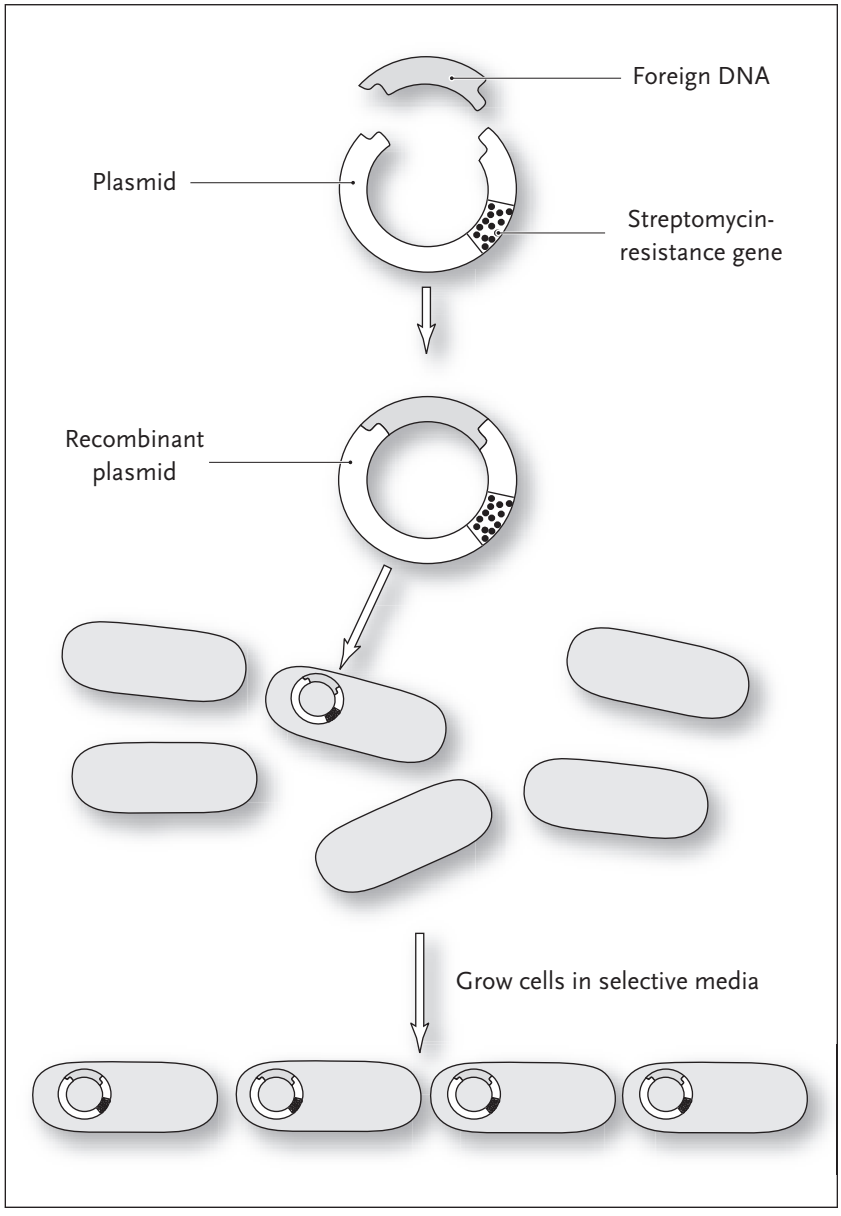
This procedure is used to separate different DNA and RNA fragments in a slab of agar or polyacrylamide subjected to an electric field. Nucleic acids carry a negative charge and thus will migrate toward a positively charged electrode. The gel acts as a sieving medium that impedes the movement of the molecules. Thus the rate at which the fragments migrate is a function of their size; small fragments migrate more rapidly than large fragments. The gel, containing the samples, is run submerged in a special pH-regulated solution, or buffer, containing a nucleic acid–specific dye, ethidium bromide. This dye produces a strong reddish-yellow fluorescence when exposed to ultraviolet (UV) radiation. Consequently, after electrophoresis, the nucleic acid can be detected by photographing the gel under UV illumination.

DNA CLONING

In 1973 scientists discovered that restriction enzymes, DNA ligase, and bacterial plasmids could be used to clone DNA molecules. Plasmids are small (about 4,000 base pairs, also expressed as 4.0 kilo base pairs or 4 Kbp) circular minichromosomes that occur naturally in bacteria and are often exchanged between cells by passive diffusion. When a bacterium acquires a new plasmid, it is said to have been transfected. For



Agarose gel electrophoresis. An agarose gel is placed in an electrophoresis tank and submerged in a buffer solution. The electrical terminals are connected to a power source, with the sample wells positioned near the negative terminal. When the current is turned on, the negatively charged nucleic acids migrate toward the positive terminal. The migration rate is an inverse function of molecular size. (Large molecules travel slower than small ones.)



Cloning DNA in a plasmid. The foreign DNA and the plasmid are cut with the same restriction enzyme, allowed to fuse, and then sealed with DNA ligase. The recombinant plasmid is mixed with bacterial cells, some of which pick up the plasmid, allowing them to grow in a culture medium containing the antibiotic streptomycin. The bacteria's main chromosome is not shown.

bacteria, the main advantage to swapping plasmids is that they often carry antibiotic-resistant genes, so that a cell sensitive to ampicillin can become resistant simply by acquiring the right plasmid.

The first cloning experiment used a plasmid from *Escherichia coli* that was cut with the restriction enzyme *Eco*RI. The plasmid had a single *Eco*RI site, so the restriction enzyme simply opened the circular molecule, rather than cutting it up into many useless pieces. Foreign DNA, cut with the same restriction enzyme, was incubated with the plasmid. Because the plasmid and foreign DNA were both cut with *Eco*RI, the DNA could insert itself into the plasmid to form a hybrid, or recombinant plasmid, after which DNA ligase sealed the two together. The reaction mixture was added to a small volume of *E. coli* so that some of the cells could take up the recombinant plasmid before being transferred to a nutrient broth containing streptomycin. Only those cells carrying the recombinant plasmid, which contained an antistreptomycin gene, could grow in the presence of this antibiotic. Each time the cells divided, the plasmid DNA was duplicated along with the main chromosome. After the cells had grown overnight, the foreign DNA had been amplified, or cloned, billions of times and was easily isolated for sequencing or expression studies.

GENOMIC AND cDNA LIBRARIES

The basic cloning procedure described above not only provides a way to amplify a specific piece of DNA, but it can also be used to construct gene libraries. In this case, however, the cloning vector is a bacteriophage called lambda. The lambda genome is double-stranded linear DNA of about 40 Kbp, much of which can be replaced by foreign DNA without sacrificing the ability of the virus to infect bacteria. This is the great advantage of lambda over a plasmid. Lambda can accommodate very long pieces of DNA, often long enough to contain an entire gene, whereas a plasmid cannot accommodate foreign DNA that is larger than 4 Kbp. Moreover, bacteriophage has the natural ability to infect bacteria so that the efficiency of transfection is 100 times greater than it is for plasmids.

The construction of a gene library begins by isolating genomic DNA and digesting it with a restriction enzyme to produce fragments of 1,000 to 10,000 base pairs. These fragments are ligated into lambda

genomes, which are subjected to a packaging reaction to produce mature viral particles, most of which carry a different piece of the genomic DNA. This collection of viruses is called a genomic library and is used to study the structure and organization of specific genes. Clones from a library such as this contains the coding sequences, in addition to introns, intervening sequences, promoters, and enhancers. An alternative form of a gene library can be constructed by isolating mRNA from a specific cell type. This RNA is converted to the complementary DNA (cDNA), using an RNA-dependent DNA polymerase called reverse transcriptase. The cDNA is ligated to lambda genomes and packaged as for the genomic library. This collection of recombinant viruses is a cDNA library and only contains genes that were being expressed by the cells when the RNA was extracted. It does not include introns or controlling elements, as these are lost during transcription and the processing that occurs in the cell to make mature mRNA. Thus a cDNA library is intended for the purpose of studying gene expression and the structure of the coding region only.

LABELING CLONED DNA

Many of the procedures used in the area of recombinant technology were inspired by the events that occur during DNA replication. This includes the labeling of cloned DNA for use as probes in expression studies, DNA sequencing, and polymerase chain reaction (PCR, described in a following section). DNA replication involes duplicating one of the strands (the parent, or template strand) by linking nucleotides in an order specified by the template and depends on a large number of enzymes, the most important of which is DNA polymerase. This enzyme, guided by the template strand, constructs a daughter strand by linking nucleotides together. One such nucleotide is deoxyadenine triphosphate (dATP). Deoxyribonucleotides have a single hydroxyl group located at the 3' carbon of the sugar group while the triphosphate is attached to the 5' carbon. The procedure for labeling DNA probes, developed in 1983, introduces radioactive nucleotides into a DNA molecule. This method supplies DNA polymerase with a single stranded DNA template, a primer, and the four nucleotides, in a buffered solution to induce in vitro replication. The daughter strand, which becomes the probe, is labeled by including a nucleotide in the

reaction mix that is linked to a radioactive isotope. The radioactive nucleotide is usually deoxycytosine triphosphate (dCTP) or dATP.

Single-stranded DNA hexamers (six bases long) are used as primers, and these are produced in such a way that they contain all possible permutations of four bases taken six at a time. Randomizing the base sequence for the primers ensures that there will be at least one primer site in a template that is only 50 bp long. Templates used in labeling reactions such as this are generally 100 to 800 bp long. This strategy of labeling DNA, known as random primer or oligo labeling, is widely used in cloning and in DNA and RNA filter hybridizations (described in following sections).

DNA SEQUENCING

A sequencing reaction developed by the British biochemist Dr. Fred Sanger in 1976 is another technique that takes its inspiration from the natural process of DNA replication. DNA polymerase requires a primer with a free 3' hydroxyl group. The polymerase adds the first nucleotide to this group, and all subsequent bases are added to the 3' hydroxyl of the previous base. Sequencing by the Sanger method is usually performed with the DNA cloned into a plasmid. This simplifies the choice of the initial primers because their sequence can be derived from the known plasmid sequence. An engineered plasmid primer site adjacent to a cloned DNA fragment is shown in the accompanying figure. Once the primer binds to the primer site, the cloned DNA may be

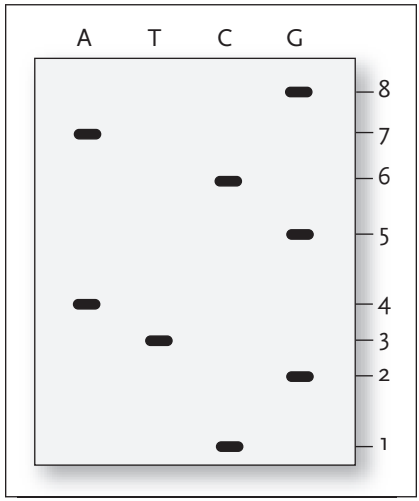
Plasmid primer site for DNA sequencing. The cloned DNA is inserted into the plasmid near an engineered primer site. Once the primer binds to the primer site, the cloned DNA may be replicated, as part of a sequencing reaction, in the direction indicated by the arrow. Only one strand of the double-stranded plasmid and cloned DNA is shown.

EXAMPLE OF A SEQUENCING REACTION

Tube	Reaction Products	
A	G-C-A-T-C-G-T-C C-G-T-**A**	G-C-A-T-C-G-T-C C-G-T-A-G-C-**A**
T	G-C-A-T-C-G-T-C C-G-**T**	
C	G-C-A-T-C-G-T-C **C** G-C-A-T-C-G-T-C	G-C-A-T-C-G-T-C C-G-T-A-G-**C** G-C-A-T-C-G-T-C
G	C-**G** G-C-A-T-C-G-T-C C-G-T-A-G-C-A-**G**	C-G-T-A-**G**

The Sanger sequencing reaction is set up in four separate tubes, each containing a different dideoxynucleotide (ddATP, ddTTP, ddCTP, and ddGTP). The reaction products are shown for each of the tubes: A (ddATP), T (ddTTP), C (ddCTP), and G (ddGTP). The template strand is GCATCGTC. Replication of the template begins after the primer binds to the primer site on the sequencing plasmid. The dideoxynucleotide terminating the reaction is shown in bold. The daughter strands, all of different lengths, are fractionated on a polyacrylamide gel.

replicated. Sanger's innovation involved the synthesis of artificial nucleotides lacking the 3' hydroxyl group, thus producing dideoxynucleotides (ddATP, ddCTP, ddGTP, and ddTTP). Incorporation of a dideoxynucleotide terminates the growth of the daughter strand at that point, and this can be used to determine the size of each daughter strand. The shortest daughter strand represents the complementary nucleotide at the beginning of the template; whereas the longest strand represents the complementary nucleotide at the end of the template (see the table above). The reaction products, consisting of all the daughter strands, are fractionated on a polyactylamide gel. Polyacrylamide serves the same function as agarose. It has the advantage of being a tougher material, essential for the large size of a typical sequencing gel. Some of the nucleotides included in the Sanger reaction are labeled with a radioactive isotope so the fractionated daughter strands can be visualized by drying the gel and then exposing it to X-ray film. Thus the



A representation of a sequencing gel. The reaction products (shown in the table on p. 115) run from the top to the bottom, with the smallest fragment migrating at the highest rate. The sequence is read beginning with the smallest fragment on the gel (band # 1, in the "C" lane) and ending with the largest fragment at the top (band # 8, in the "G" lane). The sequence is CGTAGCAG. The complementary sequence is GCATCGTC. This is the template strand indicated in the table.

Sanger method uses the natural process of replication to mark the position of each nucleotide in the DNA fragment so the sequence of the fragment can be determined.

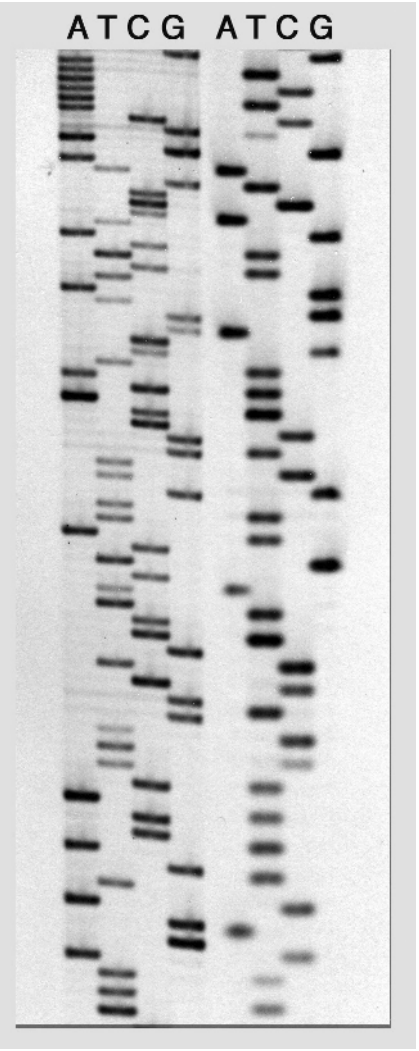
A representation of a sequencing gel is shown in the accompanying figure. The sequence of the daughter strand is read beginning with the smallest fragment at the bottom of the gel and ending with the largest fragment at the top. The sequence of the template strand (see table on page 115) is obtained simply by taking the complement of the sequence obtained from the gel (the daughter strand).

SOUTHERN AND NORTHERN BLOTTING

One of the most important techniques to be developed as part of recombinant technology is the transfer of nucleic acids from an agarose gel to nylon filter paper that can be hybridized to a labeled probe to detect specific genes. This procedure was introduced by the Scottish scientist E. M. Southern in 1975 for transferring DNA and is now known as Southern blotting. Since the DNA is transferred to filter paper, the detection stage is known as filter hybridization. In 1980 the procedure was modified to transfer RNA to nylon membranes for the study of gene expression and, in reference to the original, is called northern blotting.

Northern blotting is used to study the expression of specific genes and is usually performed on messenger RNA (mRNA). Typical experiments may wish to determine the expression of specific genes in

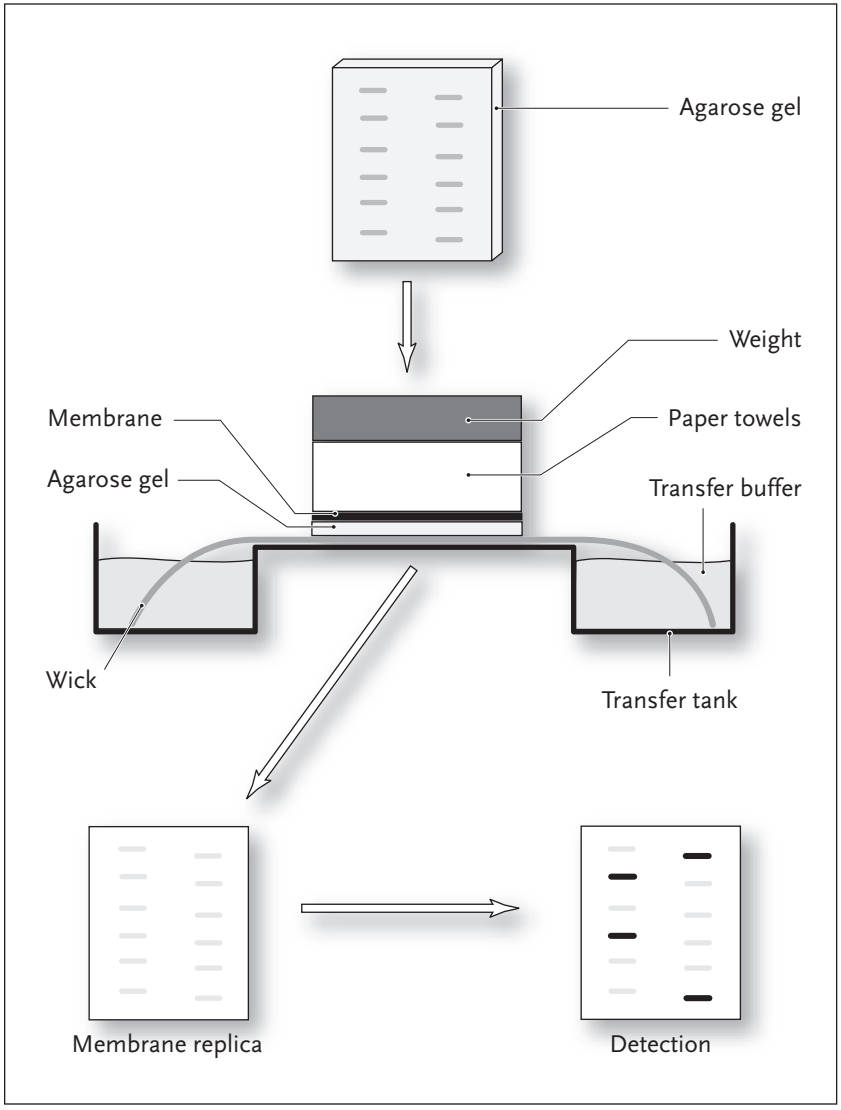
normal, as opposed to cancerous, tissue or tissues obtained from groups of different ages. The RNA is fractionated on an agarose gel and then transferred to a nylon membrane. The paper towels placed on top of the assembly pull the transfer buffer through the gel, eluting the RNA from the gel and trapping it on the membrane. The location of specific mRNA can be determined by hybridizing the membrane to a radiolabeled cDNA or genomic clone. The hybridization procedure involves placing the filter in a buffer solution containing a labeled probe. During a long incubation period, the probe binds to the target sequence immobilized on the membrane. A-T and G-C base pairing mediate the binding between the probe and target. The double-stranded molecule that is formed is a hybrid, being formed between the RNA target, on the membrane, and the DNA probe.



An autoradiogram of a portion of a DNA sequencing gel. A partial sequence (the first 20 bases) of the left set, beginning at the bottom of the "T" lane, is TTTAGGATGACCACTTTGGC. *(Dr. Joseph P. Panno)*

FLUORESCENT IN SITU HYBRIDIZATION (FISH)

Studying gene expression does not always depend on northern blots and filter hybridization. In the 1980s, scientists found that cDNA probes could be hybridized to DNA or mRNA in situ, that is, while located within cells or tissue sections fixed on a



Northern transfer and membrane hybridization. RNA is fractionated on an agarose gel and then placed face down on a paper wick in a transfer tank. The gel is overlaid with a piece of nylon membrane, paper towels, and weight. The paper towels draw the buffer through the gel and the membrane. The flow of buffer elutes the RNA from the gel, transferring it to the membrane. A radiolabeled cDNA probe is hybridized to the membrane to detect specific mRNA transcripts. Note that the thickness of the membrane is exaggerated for clarity.

microscope slide. In this case, the probe is labeled with a fluorescent dye molecule, rather than a radioactive isotope. The samples are then examined and photographed under a fluorescent microscope. FISH is an extremely powerful variation on Southern and northern blots. This procedure gives precise information regarding the identity of a cell that expresses a specific gene, information that usually cannot be obtained with filter hybridization. Organs and tissues are generally composed of many different kinds of cells, which cannot be separated from each other using standard biochemical extraction procedures. Histological sections, however, show clearly the various cell types and, when subjected to FISH analysis, provide clear results as to which cells express specific genes. FISH is also used in clinical laboratories for the diagnosis of genetic abnormalities.

POLYMERASE CHAIN REACTION (PCR)

PCR is simply repetitive DNA replication over a limited, primer-defined region of a suitable template. The region defined by the primers is amplified to such an extent that it can be easily isolated for further study. The reaction exploits the fact that a DNA duplex, in a low-salt buffer, will melt (i.e., separate into two single strands) at 75°C but will reanneal (rehybridize) at 37°C. The reaction is initiated by melting the template in the presence of primers and polymerase in a suitable buffer, cooling quickly to 37°C and allowing sufficient time for the polymerase to replicate both strands of the template. The temperature is then increased to 75°C to melt the newly formed duplexes and then cooled to 37°C. At the lower temperature, more primer will anneal to initiate another round of replication. The heating-cooling cycle is repeated 20 to 30 times, after which the reaction products are fractionated on an agarose gel and photographed. The band containing the amplified fragment may be cut out of the gel and purified for further study. The DNA polymerase used in these reactions is isolated from thermophilic bacteria that can withstand temperatures of 70°C to 80°C. PCR applications are nearly limitless. It is used to amplify DNA from samples containing, at times, no more than a few cells. It can be used to screen libraries and to identify genes that are turned on or off during embryonic development or during cellular transformation.

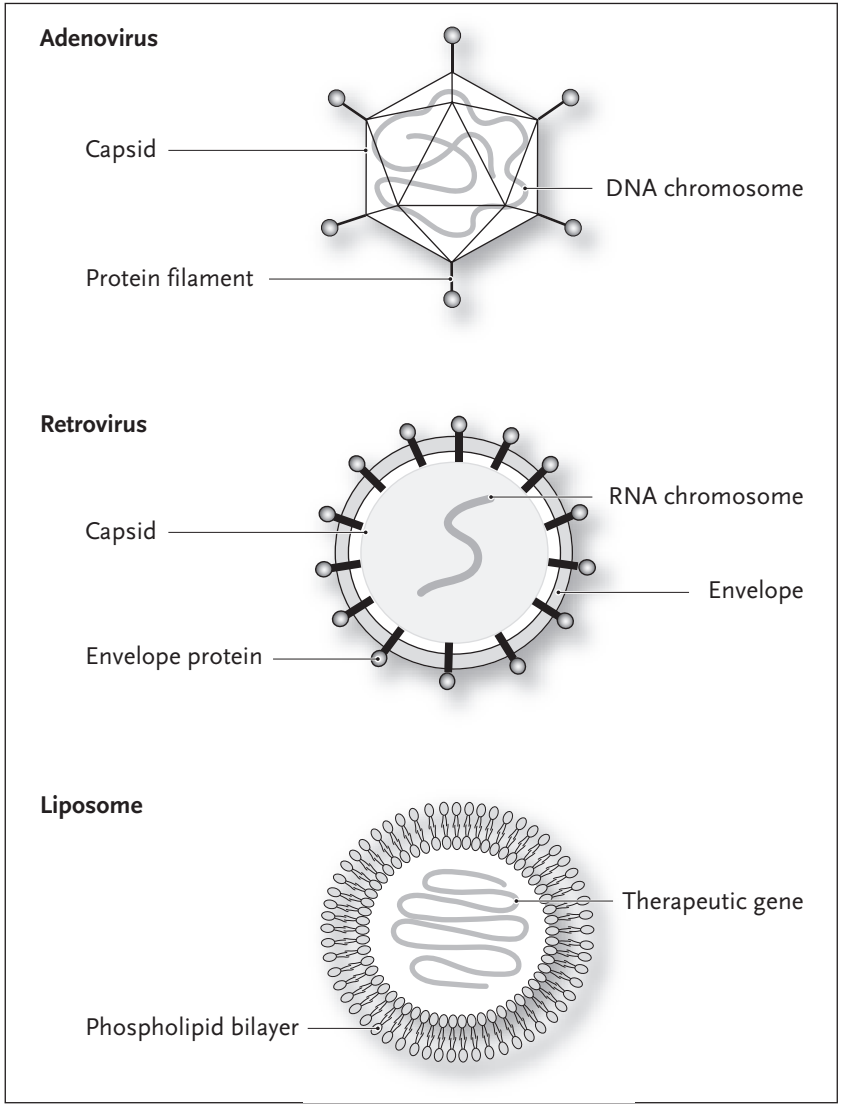
Gene Therapy Primer

When we get sick it often is due to invading microbes that destroy or damage cells and organs in our body. Cholera, smallpox, measles, diphtheria, AIDS, and the common cold are all examples of what we call an infectious disease. If we catch any of these diseases, our doctor may prescribe a drug that will, in some cases, remove the microbe from our bodies, thus curing the disease. Unfortunately, most of the diseases that we fall prey to are not of the infectious kind. In such cases, there are no microbes to fight, no drugs to apply. Instead, we are faced with a far more difficult problem, for this type of disease is an ailment that damages a gene. Gene therapy attempts to cure these diseases by replacing, or supplementing, the damaged gene.

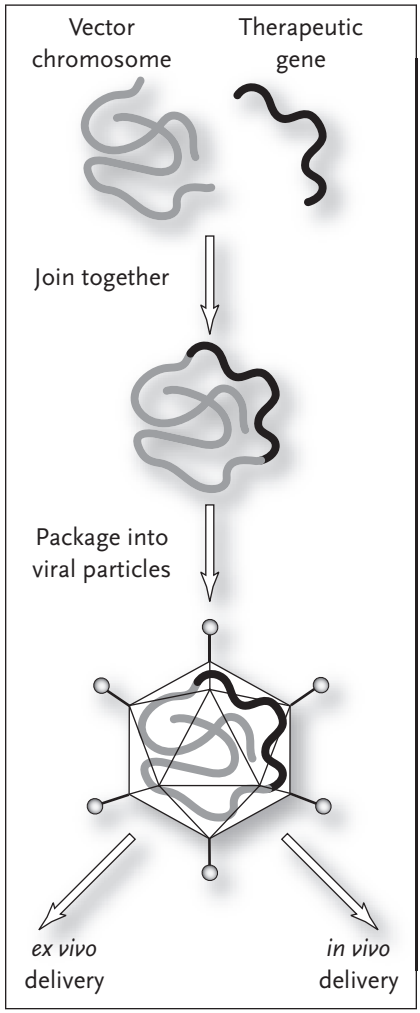
When a gene is damaged, it usually is caused by a point mutation, a change that affects a single nucleotide. Sickle-cell anemia, a disease affecting red blood cells, was the first genetic disorder of this kind to be described. The mutation occurs in a gene that codes for the β (beta) chain of hemoglobin, converting the codon GAG to GTG, which substitutes the amino acid valine at position 6 for glutamic acid. This single amino-acid substitution is enough to cripple the hemoglobin molecule, making it impossible for it to carry enough oxygen to meet the demands of a normal adult. Scientists have identified several thousand genetic disorders known to be responsible for diseases such as breast cancer, colon cancer, hemophilia, and two neurological disorders, Alzheimer's disease and Parkinson's disease.

Gene therapy is made possible by recombinant DNA technology (biotechnology). Central to this technology is the use of viruses to clone specific pieces of DNA. That is, the DNA is inserted into a viral chromosome and is amplified as the virus multiplies. Viruses are parasites that specialize in infecting bacterial and animal cells. Consequently, scientists realized that a therapeutic gene could be inserted into a patient's cells by first introducing it into a virus and then letting the virus carry it into the affected cells. In this context, the virus is referred to as a *gene therapy delivery vehicle* or *vector* (in recombinant technology it is referred to as a *cloning vector*).

The most commonly used viruses are the retrovirus and the adenovirus. A retrovirus gets its name from the fact that it has an RNA



Vectors used in gene therapy. Adenoviruses have a DNA genome, contained in a crystalline protein capsid, and normally infect cells of the upper respiratory tract, causing colds and flulike symptoms. The protein filaments are used to infect cells. Retroviruses have an RNA genome that is converted to DNA when a cell is infected. The capsid is enclosed in a phospholipid envelope studded with proteins used to infect cells. The HIV (AIDS) virus is a common example of a retrovirus. Artificial vectors have also been used, consisting of a phospholipid bilayer enclosing the therapeutic gene.



Vector preparation and delivery. A viral chromosome and a therapeutic gene are cut with the same restriction enzyme, and the two are joined together, after which the recombinant chromosome is packaged into viral particles to form the vector. The vector may be introduced into cultured cells and then returned to the patient from whom they were derived (*ex vivo* delivery), or the vector may be injected directly into the patient's circulatory system (*in vivo* delivery).

genome that is copied into DNA after it infects a cell. Corona viruses (cause the common cold) and the AIDS virus are common examples of retroviruses. The adenovirus (from "adenoid," a gland from which the virus was first isolated) normally infects the upper respiratory tract, causing colds and flulike symptoms. This virus, unlike the retrovirus, has a DNA genome. Artificial vectors, called liposomes, have also been used that consist of a phospholipid vesicle (bubble), containing the therapeutic gene.

Gene therapy vectors are prepared by cutting the viral chromosome and the therapeutic gene with the same restriction enzyme, after which the two are joined together with a DNA ligase. This recombinant chromosome is packaged into viral particles to form the final vector. The vector may be introduced into cultured cells, suffering from a genetic defect, and then returned to the patient from whom they were derived (*ex vivo* delivery). Alternatively, the vector may be injected directly into

the patient's circulatory system (in vivo delivery). The ex vivo procedure is used when the genetic defect appears in white blood cells or stem cells that may be harvested from the patient and grown in culture. The in vivo procedure is used when the genetic defect appears in an organ, such as the liver, brain, or pancreas. This is the most common form of gene therapy, but it is also potentially hazardous because the vector, being free in the circulatory system, may infect a wide range of cells, thus activating an immune response that could lead to widespread tissue and organ damage.

The first gene therapy trial, conducted in 1990, used ex vivo delivery. This trial cured a young patient named Ashi DeSilva of an immune deficiency (adenosine deaminase deficiency) that affects white blood cells. Other trials since then have either been ineffective or were devastating failures. Such a case occurred in 1999, when Jesse Gelsinger, an 18-year-old patient suffering from a liver disease, died while participating in a gene therapy trial. His death was caused by multiorgan failure brought on by the viral vector. In 2002 two children being treated for another form of immune deficiency developed vector-induced leukemia (cancer of the white blood cells). Despite these setbacks, gene therapy holds great promise as a medical therapy, and there are currently more than 600 trials in progress, in the United States alone, to treat a variety of genetic disorders.

The Belmont Report

On July 12, 1975, the American National Research Act was signed into law, thereby creating a national commission to protect human research subjects. This commission was charged with the task of identifying basic ethical principles that should govern the conduct of any research involving human subjects, and in February 1976 the commission produced the Belmont Report (so named because the report was finalized at the Smithsonian Institution's Belmont Conference Center). The report began by defining three basic ethical principles that should be applied to research involving human subjects: respect for persons, beneficence, and justice.

RESPECT FOR PERSONS

Respect for persons demands that subjects enter into research voluntarily and with adequate information. This assumes the individuals are

autonomous agents, that is, are competent to make up their own minds. However, there are many instances where potential research subjects are not really autonomous; prisoners, patients in a mental institution, children, the elderly, and the infirm. All these people require special protection to ensure they are not being coerced or fooled into volunteering as research subjects. The subjects in the Tuskegee study were all poor, uneducated farm workers who were especially vulnerable to coercion.

BENEFICENCE

It is not enough to respect a potential subject's decisions and to protect them from harm; in addition, it is necessary to do everything possible to ensure their well-being. Beneficence is generally regarded as acts of kindness or charity, but the report insisted that in the case of research subjects, it be made an obligation. In this sense, it is the natural extension of the Hippocratic oath that all physicians are expected to adhere by: *I will give no deadly medicine to anyone if asked, nor suggest any such counsel.* In other words, physicians should do no harm, and those involved in biomedical research should never injure one person to benefit another.

JUSTICE

Those volunteering to be research subjects should, if at all possible, reap some of the benefits. This is a question of justice, in the sense of fairness of distribution. The exploitation of prisoners in Nazi concentration camps may have produced results that benefited the Nazis but certainly not the people they experimented on. The Tuskegee study used disadvantaged rural black men to study the untreated course of a disease that is by no means restricted to that population.

Guided by these three ethical principles, the report introduced the following requirements that all human research trials must adhere to: informed consent, risk/benefit assessment, and fair selection of research subjects.

INFORMED CONSENT

All participants must provide informed consent, in writing. Moreover, steps must be taken to ensure the consent is, in fact, informed. This might involve an independent assessment of the individual's ability to

understand the language on the consent form and any instructions or explanations the investigators have given. Since the Gelsinger investigation, this process was amended to include a patient advocate, to be present at any meeting between the physicians and the prospective volunteers. This has the added advantage of ensuring that in a case where the patient is fully competent, the scientists do not give them misleading or inaccurate information or try to coerce them in any way.

RISK/BENEFIT ASSESSMENT

There is no point in having an ethical standard based on doing no harm if there is no formalized method available for assessing the risk to patient. It is the risk that is paramount in a patient's mind. No matter how grand the possible benefits, few would volunteer if they thought they would die as a consequence. The only exception to this might be terminally ill patients who volunteer for a clinical trial, even though they know they are not likely to survive it. Independent committees based on information supplied by the investigators monitor risk assessment. In general, risks should be reduced to those necessary to achieve the research objective. If there is significant risk, review committees are expected to demand a justification for it.

SELECTION OF SUBJECTS

The selection process must be fair. Low-risk, potentially beneficial research should not be offered to one segment of our society, while high-risk research is conducted on prisoners, low-income groups, or anyone in a disadvantaged social position.

CONCLUSIONS

The Belmont Report introduced, for the first time, the principle of informed consent. Backing this up is the recommendation for independent review committees that ensure the ethical guidelines are being followed. In the United States, the FDA and NIH are responsible for enforcing the guidelines laid out by the Belmont Report. There are, in addition, local review committees, called institutional review boards, that must approve any experimentation using human subjects. The Belmont Report was inspired by the general public's anger over the Tuskegee study, and thus it is fitting that on May 16, 1997, the surviving

members of the Tuskegee study were invited to the White House where then-president Bill Clinton issued a formal apology and reaffirmed the nation's commitment to rigorous ethical standards in biomedical research to ensure that such flagrant abuses of basic human rights would never happen again. No one would have believed at the time that further trouble was just around the corner.

The Gelsinger Investigation

In the fall of 1998, a gene therapy trial to treat a liver disease was begun at the University of Pennsylvania. The investigators recruited 18 patients, and the 18th patient, who happened to be 18 years of age, was Jesse Gelsinger. Gelsinger joined the trial on September 13, 1999. On the second day of his treatment, he lapsed into a coma and was pronounced dead 24 hours later. Within days of Gelsinger's death, the National Institutes of Health (NIH) ordered a halt to all American gene therapy trials that were using a similar research protocol. The ban was to last a full year and was accompanied by an investigation that was not concluded until the fall of 2001.

The team leader of the clinical trial, Dr. James Wilson, reported Gelsinger's death immediately. A preliminary review was conducted from November 30, 1999, to January 19, 2000. The full review was to last for more than a year and covered every aspect of Dr. Wilson's protocol and the criteria used to admit patients to the trial. In January 2000, NIH released preliminary results of their investigation, which cited the principal investigators for failure to adhere to the clinical protocol and an apparent disregard for the safety of the study subjects. The report focused on four main points: Failure to adhere to the stopping rules, failure to adhere to the principle of informed consent, failure to keep adequate records regarding vector lineage and titer, and changing the protocol without approval.

FAILURE TO ADHERE TO THE STOPPING RULES

The study was designed around several cohorts that were treated in tandem so that in the event of toxic reactions in one cohort treatment the study could be terminated before other cohorts were treated. However, toxic reactions observed in five of the cohorts did not lead to termination

of the trial before Gelsinger was treated. Many of the patients suffered harsher reactions to the treatment than was expected, and this should have been sufficient reason to stop the trial. In addition, most of the toxic reactions experienced by the patients in this study were never reported to FDA or NIH. In the months following the conclusion of the Gelsinger preliminary investigation, other investigations showed that failure to report toxic reactions was a common failure in many gene therapy trials. In one study, the patients experienced 691 serious side effects, and of these, only 39 were reported as required by the federal agencies.

FAILURE TO ADHERE TO THE PRINCIPLE OF INFORMED CONSENT

When a toxic response occurred in cohort 1, cohort 2 should have been informed of this response to give those patients the option of withdrawing from the study. This was not done. Moreover, the investigators discovered that none of the subjects were told about adverse affects on monkeys in the preclinical trial. One of the monkeys received the same virus used in the clinical trial, though at a higher dose, and within a week of being treated, it was euthanized because it developed the same clotting disorder that killed Gelsinger. Since the subjects were not told about this, the consent forms were ruled invalid. It was this charge that led to the call for a patient advocate in all future biomedical research trials, regardless of their nature.

FAILURE TO KEEP ADEQUATE RECORDS REGARDING VECTOR LINEAGE AND TITER

This was an especially damaging finding because it implied that the researchers gave Gelsinger more virus than they thought they had. The term *titer* refers to the number of vector particles in a given solution. Determining the titer is not straightforward, and if errors are made, the concentration may be out in increments of 10, rather than double or triple the amount expected. The possibility that Gelsinger was accidentally given a higher-than-stated dose is suggested by the fact that a woman in his cohort received a nearly identical dose (3.0×10^{13}) without signs of liver damage or multiorgan failure. As mentioned above, a monkey in a preclinical trial received a higher dose ($17 \times$ greater) of the same virus and subsequently died of multiorgan failure. If there was an

error made in calculating the dose for Gelsinger, it is possible he received an equivalent, fatal amount.

CHANGING THE PROTOCOL WITHOUT APPROVAL

The most serious infraction here had to do with the ammonia levels in the blood of prospective volunteers. As laid out in the original protocol, patients having more that 50 micromoles of ammonia per milliliter of blood were barred from volunteering because such a test result indicates severe liver damage. This was increased, sometime after the trial began, to 70 micromoles, without formal approval from the FDA. Gelsinger's ammonia level on the day he was treated was about 60 micromoles. If the original cutoff had been adhered to, he would have been excluded from the study. This is another indication of how important it is to adhere to the principle of informed consent and to the inclusion of an independent patient advocate.

Clinical Trials

Clinical trials are conducted in four phases and are always preceded by research conducted on experimental animals such as mice, rats, or monkeys. The format for preclinical research is informal; it is conducted in a variety of research labs around the world, with the results being published in scientific journals. Formal approval from a governmental regulatory body is not required.

PHASE I CLINICAL TRIAL

Pending the outcome of the preclinical research, investigators may apply for permission to try the experiments on human subjects. Applications in the United States are made to the Food and Drug Administration (FDA), the National Institutes of Health (NIH), and the Recombinant DNA Advisory Committee (RAC). RAC was set up by NIH to monitor any research, including clinical trials, dealing with cloning, recombinant DNA, or gene therapy. Phase I trials are conducted on a small number of adult volunteers, usually between two and 20, who have given informed consent. That is, the investigators explain the procedure, the possible outcomes, and especially the dangers associated with the procedure before the subjects sign a consent form. The

purpose of the Phase I trial is to determine the overall effect the treatment has on humans. A treatment that works well in monkeys or mice may not work at all on humans. Similarly, a treatment that appears safe in lab animals may be toxic, even deadly, when given to humans. Since most clinical trials are testing a new drug of some kind, the first priority is to determine a safe dosage for humans. Consequently, subjects in the Phase I trial are given a range of doses, all of which, even the high dose, are less than the highest dose given to experimental animals. If the results from the Phase I trial are promising, the investigators may apply for permission to proceed to Phase II.

PHASE II CLINICAL TRIAL

Having established the general protocol, or procedure, the investigators now try to replicate the encouraging results from Phase I, but with a much larger number of subjects (100 to 300). Only with a large number of subjects is it possible to prove the treatment has an effect. In addition, dangerous side effects may have been missed in Phase I because of a small sample size. The results from Phase II will determine how safe the procedure is and whether it works or not. If the statistics show the treatment is effective, and toxicity is low, the investigators may apply for permission to proceed to Phase III.

PHASE III CLINICAL TRIAL

Based on Phase II results, the procedure may look very promising, but before it can be used as a routine treatment, it must be tested on thousands of patients at a variety of research centers. This is the expensive part of bringing a new drug or therapy to market, costing millions, sometimes billions, of dollars. It is for this reason that Phase III clinical trials invariably have the financial backing of large pharmaceutical or biotechnology companies. If the results of the Phase II trial are confirmed in Phase III, the FDA will approve the use of the drug for routine treatment. The use of the drug or treatment now passes into an informal Phase IV trial.

PHASE IV CLINICAL TRIAL

Even though the treatment has gained formal approval, its performance is monitored for very long-term effects, sometimes stretching on for 10

to 20 years. In this way, the FDA retains the power to recall the drug long after it has become a part of standard medical procedure. It can happen that in the long term, the drug costs more than an alternative, in which case, health insurance providers may refuse to cover the cost of the treatment.

GLOSSARY

acetyl A chemical group derived from acetic acid. Important in energy metabolism and for the modification of proteins.

acetylcholine A neurotransmitter released at axonal terminals by cholinergic neurons. Found in the central and peripheral nervous system and released at the vertebrate neuromuscular junction.

acetyl-CoA A water-soluble molecule, coenzyme A (CoA), that carries acetyl groups in cells.

acid A substance that releases protons when dissolved in water. Carries a net negative charge.

actin filament A protein filament formed by the polymerization of globular actin molecules. Forms the cytoskeleton of all eukaryotes and part of the contractile apparatus of skeletal muscle.

action potential A self-propagating electrical impulse that occurs in the membranes of neurons, muscles, photoreceptors, and hair cells of the inner ear.

active transport Movement of molecules across the cell membrane, utilizing the energy stored in ATP.

adenylate cyclase A membrane-bound enzyme that catalyzes the conversion of ATP to cyclic AMP. An important component of cell-signaling pathways.

adherens junction A cell junction in which the cytoplasmic face of the membrane is attached to actin filaments.

adipocyte A fat cell.

adrenaline (epinephrine) A hormone released by chromaffin cells in the adrenal gland. Prepares an animal for extreme activity, increases the heart rate and blood-sugar levels.

adult stem cells Stem cells isolated from adult tissues, such as bone marrow or epithelium.

aerobic Refers to a process that either requires oxygen or occurs in its presence.

allele An alternate form of a gene. Diploid organisms have two alleles for each gene, located at the same locus (position) on homologous chromosomes.

allogeneic transplant A patient receives a tissue or organ transplant from an unrelated individual.

alpha helix A common folding pattern of proteins in which a linear sequence of amino acids twists into a right-handed helix stabilized by hydrogen bonds.

amino acid An organic molecule containing amino and carboxyl groups that is a building block of protein.

aminoacyl-tRNA An amino acid linked by its carboxyl group to a hydroxyl group on tRNA.

aminoacyl-tRNA synthetase An enzyme that attaches the correct amino acid to a tRNA.

amino terminus The end of a protein or polypeptide chain that carries a free amino group.

amphipathic Having both hydrophilic and hydrophobic regions, as in a phospholipid.

anabolism A collection of metabolic reactions in a cell whereby large molecules are made from smaller ones.

anaerobic A cellular metabolism that does not depend on molecular oxygen.

anaphase A mitotic stage in which the two sets of chromosomes move away from each other toward opposite and spindle poles.

anchoring junction A cell junction that attaches cells to each other.

angiogenesis Sprouting of new blood vessels from preexisting ones.

angstrom A unit of length, equal to 10^{-10} meter or 0.1 nanometer (nm), that is used to measure molecules and atoms.

anterior A position close to or at the head end of the body.

antibiotic A substance made by bacteria, fungi, and plants that is toxic to microorganisms. Common examples are penicillin and streptomycin.

antibody A protein made by B cells of the immune system in response to invading microbes.

anticodon A sequence of three nucleotides in tRNA that is complementary to a messenger RNA codon.

antigen A molecule that stimulates an immune response, leading to the formation of antibodies.

antigen-presenting cell A cell of the immune system, such as a monocyte, that presents pieces of an invading microbe (the antigen) to lymphocytes.

antiparallel The relative orientation of the two strands in a DNA double helix; the polarity of one strand is oriented in the opposite direction to the other.

antiporter A membrane carrier protein that transports two different molecules across a membrane in opposite directions.

apoptosis Regulated or programmed form of cell death that may be activated by the cell itself or by the immune system to force cells to commit suicide when they become infected with a virus.

asexual reproduction The process of forming new individuals without gametes or the fertilization of an egg by a sperm. Individuals produced this way are identical to the parent and referred to as a clone.

aster The star-shaped arrangement of microtubules that is characteristic of a mitotic or meiotic spindle.

ATP (adenosine triphosphate) A nucleoside consisting of adenine, ribose, and three phosphate groups that is the main carrier of chemical energy in the cell.

ATPase Any enzyme that catalyzes a biochemical reaction by extracting the necessary energy from ATP.

ATP synthase A protein located in the inner membrane of the mitochondrion that catalyzes the formation of ATP from ADP and inorganic phosphate using the energy supplied by the electron transport chain.

autogeneic transplant A patient receives a transplant of his or her own tissue.

autosome Any chromosome other than a sex chromosome.

axon A long extension of a neuron's cell body that transmits an electrical signal to other neurons.

axonal transport The transport of organelles, such as Golgi vesicles, along an axon to the axonal terminus. Transport also flows from the terminus to the cell body.

bacteria One of the most ancient forms of cellular life (the other is the Archaea). Bacteria are prokaryotes and some are known to cause disease.

bacterial artificial chromosome (BAC) A cloning vector that accommodates DNA inserts of up to 1 million base pairs.

bacteriophage A virus that infects bacteria. Bacteriophages were used to prove that DNA is the cell's genetic material and are now used as cloning vectors.

base A substance that can accept a proton in solution. The purines and pyrimidines in DNA and RNA are organic bases and are often referred to simply as bases.

base pair Two nucleotides in RNA or DNA that are held together by hydrogen bonds. Adenine bound to thymine or guanine bound to cytosine are examples of base pairs.

B cell (B lymphocyte) A white blood cell that makes antibodies and is part of the adaptive immune response.

benign Tumors that grow to a limited size and do not spread to other parts of the body.

beta sheet Common structural motif in proteins in which different strands of the protein run alongside each other and are held together by hydrogen bonds.

biopsy The removal of cells or tissues for examination under a microscope. When only a sample of tissue is removed, the procedure is called an incisional biopsy or core biopsy. When an entire lump or suspicious area is removed, the procedure is called an excisional biopsy. When a sample of tissue or fluid is removed with a needle, the procedure is called a needle biopsy or fine-needle aspiration.

biosphere The world of living organisms.

bivalent A duplicated chromosome paired with its homologous duplicated chromosome at the beginning of meiosis.

blastomere A cell formed by the cleavage of a fertilized egg. Blastomeres are the totipotent cells of the early embryo.

blotting A technique for transferring DNA (Southern blotting), RNA (northern blotting), or proteins (western blotting) from an agarose or polyacrylamide gel to a nylon membrane.

BRCA1 (breast cancer gene 1) A gene on chromosome 17 that may be involved in regulating the cell cycle. A person who inherits an

altered version of the BRCA1 gene has a higher risk of getting breast, ovarian, or prostate cancer.

BRCA2 (breast cancer gene 2) A gene on chromosome 13 that, when mutated, increases the risk of getting breast, ovarian, or prostate cancer.

budding yeast The common name for the baker's yeast *Saccharomyces cerevisiae,* a popular experimental organism that reproduces by budding off a parental cell.

cadherin Belongs to a family of proteins that mediates cell-to-cell adhesion in animal tissues.

calorie A unit of heat. One calorie is the amount of heat needed to raise the temperature of one gram of water by 1°C. Kilocalories (1,000 calories) are used to describe the energy content of foods.

capsid The protein coat of a virus, formed by auto-assembly of one or more proteins into a geometrically symmetrical structure.

carbohydrate A general class of compounds that includes sugars, containing carbon, hydrogen, and oxygen.

carboxyl group A carbon atom attached to an oxygen and a hydroxyl group.

carboxyl terminus The end of a protein containing a carboxyl group.

carcinogen A compound or form of radiation that can cause cancer.

carcinogenesis The formation of a cancer.

carcinoma Cancer of the epithelium, representing the majority of human cancers.

cardiac muscle Muscle of the heart. Composed of myocytes that are linked together in a communication network based on free passage of small molecules through gap junctions.

caspase A protease involved in the initiation of apoptosis.

catabolism Enzyme-regulated breakdown of large molecules for the extraction of chemical-bond energy. Intermediate products are called catabolites.

catalyst A substance that lowers the activation energy of a reaction.

CD28 Cell-surface protein located in T cell membranes, necessary for the activation of T cells by foreign antigens.

cDNA (complementary DNA) DNA that is synthesized from mRNA, thus containing the complementary sequence. cDNA contains coding sequence but not the regulatory sequences that are present in the

genome. Labeled probes are made from cDNA for the study of gene expression.

cell adhesion molecule (CAM) A cell surface protein that is used to connect cells to each other.

cell body The main part of a cell containing the nucleus, Golgi complex, and endoplasmic reticulum. Used in reference to neurons that have long processes (dendrites and axons) extending some distance from the nucleus and cytoplasmic machinery.

cell coat See **glycocalyx**.

cell-cycle control system A team of regulatory proteins that governs progression through the cell cycle.

cell-division-cycle gene (*cdc* gene) A gene that controls a specific step in the cell cycle.

cell fate The final differentiated state that a pluripotent embryonic cell is expected to attain.

cell-medicated immune response Activation of specific cells to launch an immune response against an invading microbe.

cell nuclear replacement Animal-cloning technique whereby a somatic cell nucleus is transferred to an enucleated oocyte. Synonomous with somatic-cell nuclear transfer.

central nervous system (CNS) That part of a nervous system that analyzes signals from the body and the environment. In animals, the CNS includes the brain and spinal cord.

centriole A cylindrical array of microtubules that is found at the center of a centrosome in animal cells.

centromere A region of a mitotic chromosome that holds sister chromatids together. Microtubules of the spindle fiber connect to an area of the centromere called the kinetochore.

centrosome Organizes the mitotic spindle and the spindle poles. In most animal cells it contains a pair of centrioles.

chiasma (plural: chiasmata) An X-shaped connection between homologous chromosomes that occurs during meiosis I, representing a site of crossing-over, or genetic exchange between the two chromosomes.

chromatid A duplicate chromosome that is still connected to the original at the centromere. The identical pair are called sister chromatids.

chromatin A complex of DNA and proteins (histones and nonhistones) that forms each chromosome and is found in the nucleus of all eukaryotes. Decondensed and threadlike during interphase.

chromatin condensation Compaction of different regions of interphase chromosomes that is mediated by the histones.

chromosome One long molecule of DNA that contains the organism's genes. In prokaryotes, the chromosome is circular and naked; in eukaryotes, it is linear and complexed with histone and nonhistone proteins.

chromosome condensation Compaction of entire chromosomes in preparation for cell division.

clinical breast exam An exam of the breast performed by a physician to check for lumps or other changes.

cyclic adenosine monophosphate (cAMP) A second messenger in a cell-signaling pathway that is produced from ATP by the enzyme adenylate cyclase.

cyclin A protein that activates protein kinases (cyclin-dependent protein kinases, or Cdk) that control progression from one state of the cell cycle to another.

cytochemistry The study of the intracellular distribution of chemicals.

cytochrome Colored, iron-containing protein that is part of the electron transport chain.

cytotoxic T cell A T lymphocyte that kills infected body cells.

dendrite An extension of a nerve cell that receives signals from other neurons.

dexrazoxane A drug used to protect the heart from the toxic effects of anthracycline drugs such as doxorubicin. It belongs to the family of drugs called chemoprotective agents.

dideoxy sequencing A method for sequencing DNA that employs dideoxyribose nucleotides.

diploid A genetic term meaning two sets of homologous chromosomes, one set from the mother and the other from the father. Thus diploid organisms have two versions (alleles) of each gene in the genome.

DNA (deoxyribonucleic acid) A long polymer formed by linking four different kinds of nucleotides together like beads on a string. The sequence of nucleotides is used to encode an organism's genes.

DNA helicase An enzyme that separates and unwinds the two DNA strands in preparation for replication or transcription.

DNA library A collection of DNA fragments that are cloned into plasmids or viral genomes.

DNA ligase An enzyme that joins two DNA strands together to make a continuous DNA molecule.

DNA microarray A technique for studying the simultaneous expression of a very large number of genes.

DNA polymerase An enzyme that synthesizes DNA using one strand as a template.

DNA primase An enzyme that synthesizes a short strand of RNA that serves as a primer for DNA replication.

dorsal The backside of an animal. Also refers to the upper surface of anatomical structures, such as arms or wings.

dorsonventral The body axis running from the backside to the frontside or the upperside to the underside of a structure.

double helix The three-dimensional structure of DNA in which the two strands twist around each other to form a spiral.

doxorubicin An anticancer drug that belongs to a family of antitumor antibiotics.

Drosophila melanogaster Small species of fly, commonly called a fruit fly, that is used as an experimental organism in genetics, embryology, and gerontology.

ductal carcinoma in situ (DCIS) Abnormal cells that involve only the lining of a breast duct. The cells have not spread outside the duct to other tissues in the breast. Also called intraductal carcinoma.

dynein A motor protein that is involved in chromosome movements during cell division.

dysplasia Disordered growth of cells in a tissue or organ, often leading to the development of cancer.

ectoderm An embryonic tissue that is the precursor of the epidermis and the nervous system.

electrochemical gradient A differential concentration of an ion or molecule across the cell membrane that serves as a source of potential energy and may polarize the cell electrically.

electron microscope A microscope that uses electrons to produce a high-resolution image of the cell.

embryogensis The development of an embryo from a fertilized egg.

embryonic stem cell (ES cell) A pluripotent cell derived from the inner cell mass (the cells that give rise to the embryo instead of the placenta) of a mammalian embryo.

endocrine cell A cell that is specialized for the production and release of hormones. Such cells make up hormone-producing tissue such as the pituitary gland or gonads.

endocytosis Cellular uptake of material from the environment by invagination of the cell membrane to form a vesicle called an endosome. The endosome's contents are made available to the cell after it fuses with a lysosome.

endoderm An embryonic tissue layer that gives rise to the gut.

endoplasmic reticulum (ER) Membrane-bounded chambers that are used to modify newly synthesized proteins with the addition of sugar molecules (glycosylation). When finished, the glycosylated proteins are sent to the Golgi apparatus in exocytotic vesicles.

endothelial cell A cell that forms the endothelium, a thin sheet of cells lining the inner surface of all blood vessels.

enhancer A DNA regulatory sequence that provides a binding site for transcription factors capable of increasing the rate of transcription for a specific gene. Often located thousands of base pairs away from the gene it regulates.

enveloped virus A virus containing a capsid that is surrounded by a lipid bilayer originally obtained from the membrane of a previously infected cell.

enzyme A protein or RNA that catalyzes a specific chemical reaction.

epidermis The epithelial layer, or skin, that covers the outer surface of the body.

ER signal sequence The amino terminal sequence that directs proteins to enter the endoplasmic reticulum (ER). This sequence is removed once the protein enters the ER.

erythrocyte A red blood cell that contains the oxygen-carrying pigment hemoglobin used to deliver oxygen to cells in the body.

***Escherichia coli* (*E. coli*)** Rod shape, gram negative bacterium that inhabits the intestinal tract of most animals and is used as an experimental organism by geneticists and biomedical researchers.

euchromatin Lightly staining portion of interphase chromatin, in contrast to the darkly staining heterochromatin (condensed chromatin). Euchromatin contains most, if not all, of the active genes.

eukaryote (eucaryote) A cell containing a nucleus and many membrane-bounded organelles. All life-forms, except bacteria and viruses, are composed of eukaryote cells.

exocytosis The process by which molecules are secreted from a cell. Molecules to be secreted are located in Golgi-derived vesicles that fuse with the inner surface of the cell membrane, depositing the contents into the intercellular space.

exon Coding region of a eukaryote gene that is represented in messenger RNA, and thus directs the synthesis of a specific protein.

expression studies Examination of the type and quantity of mRNA or protein that is produced by cells, tissues, or organs.

fat A lipid material, consisting of triglycerides (fatty acids bound to glycerol), that is stored adipocytes as an energy reserve.

fatty acid A compound that has a carboxylic acid attached to a long hydrocarbon chain. A major source of cellular energy and a component of phospholipids.

filter hybridization The detection of specific DNA or RNA molecules, fixed on a nylon filter, by incubating the filter with a labeled probe that hybridizes to the target sequence.

fertilization The fusion of haploid male and female gametes to form a diploid zygote.

fibroblast The cell type that, by secreting an extracellular matrix, gives rise to the connective tissue of the body.

fixative A chemical that is used to preserve cells and tissues. Common examples are formaldehyde, methanol, and acetic acid.

flagellum (plural: flagella) Whiplike structure found in prokaryotes and eukaryotes that are used to propel cells through water.

fluorescein Fluorescent dye that produces a green light when illuminated with ultraviolet or blue light.

fluorescent dye A dye that absorbs UV or blue light and emits light of a longer wavelength, usually as green or red light.

fluorescent microscope A microscope that is equipped with special filters and a beam splitter for the examination of tissues and cells stained with a fluorescent dye.

follicle cell Cells that surround and help feed a developing oocyte.

G_0 G "zero" refers to a phase of the cell cycle. State of withdrawal from the cycle as the cell enters a resting or quiescent stage. Occurs in differentiated body cells as well as developing oocytes.

G_1 Gap 1 refers to the phase of the cell cycle that occurs just after mitosis and before the next round of DNA synthesis.

G_2 Gap 2 refers to the phase of the cell cycle that follows DNA replication and precedes mitosis.

gap junction A communication channel in the membranes of adjacent cells that allows free passage of ions and small molecules.

gastrulation An embryological event in which a spherical embryo is converted into an elongated structure with a head end, a tail end, and a gut (gastrula).

gene A region of the DNA that specifies a specific protein or RNA molecule that is handed down from one generation to the next. This region includes both the coding, noncoding, and regulatory sequences.

gene regulatory protein Any protein that binds to DNA and thereby affects the expression of a specific gene.

gene repressor protein A protein that binds to DNA and blocks transcription of a specific gene.

gene therapy A method for treating disease whereby a defective gene, causing the disease, is either repaired, replaced, or supplemented with a functional copy.

genetic code A set of rules that assigns a specific DNA or RNA triplet, consisting of a three-base sequence, to a specific amino acid.

genome All of the genes that belong to a cell or an organism.

genomic library A collection of DNA fragments, obtained by digesting genomic DNA with a restriction enzyme, that are cloned into plasmid or viral vectors.

genomics The study of DNA sequences and their role in the function and structure of an organism.

genotype The genetic composition of a cell or organism.

germ cell Cells that develop into gametes, either sperm or oocytes.

glucose Six-carbon monosaccharide (sugar) that is the principal source of energy for many cells and organisms. Stored as glycogen in animal cells and as starch in plants. Wood is an elaborate polymer of glucose and other sugars.

glycerol A three-carbon alcohol that is an important component of phospholipids.

glycocalyx A molecular "forest," consisting of glycosylated proteins and lipids, that covers the surface of every cell. The glycoproteins and glycolipids, carried to the cell membrane by Golgi-derived vesicles, have many functions, including the formation of ion channels, cell-signaling receptors and transporters.

glycogen A polymer of glucose used to store energy in an animal cell.

glycolysis The degradation of glucose with production of ATP.

glycoprotein Any protein that has a chain of glucose molecules (oligosaccharide) attached to some of the amino acid residues.

glycosylation The process of adding one or more sugar molecules to proteins or lipids.

glycosyl transferase An enzyme in the Golgi complex that adds glucose to proteins.

Golgi complex (Golgi apparatus) Membrane-bounded organelle in eukaryote cells that receives glycoproteins from the ER, which are modified and sorted before being sent to their final destination. The Golgi complex is also the source of glycolipids that are destined for the cell membrane. The glycoproteins and glycolipids leave the Golgi by exocytosis. This organelle is named after the Italian histologist Camillo Golgi, who discovered it in 1898.

granulocyte A type of white blood cell that includes the neutrophils, basophils, and eosinophils.

growth factor A small protein (polypeptide) that can stimulate cells to grow and proliferate.

haploid Having only one set of chromosomes. A condition that is typical in gametes, such as sperm and eggs.

HeLa cell A tumor-derived cell line, originally isolated from a cancer patient in 1951. Currently used by many laboratories to study the cell biology of cancer and carcinogenesis.

helix-loop-helix A structural motif common to a group of gene regulatory proteins.

helper T cell A type of T lymphocyte that helps stimulate B cells to make antibodies directed against a specific microbe or antigen.

hemoglobin An iron-containing protein complex, located in red blood cells that picks up oxygen in the lungs and carries it to other tissues and cells of the body.

hemopoiesis Production of blood cells, occurring primarily in the bone marrow.

hepatocyte A liver cell.

heterochromatin A region of a chromosome that is highly condensed and transcriptionally inactive.

histochemistry The study of chemical differentiation of tissues.

histology The study of tissues.

histone Small nuclear proteins, rich in the amino acids arginine and lysine, that form the nucleosome in eukaryote nuclei, a beadlike structure that is a major component of chromatin.

HIV The human immunodeficiency virus that is responsible for AIDS.

homolog One of two or more genes that have a similar sequence and are descended from a common ancestor gene.

homologous Organs or molecules that are similar in structure because they have descended from a common ancestor. Used primarily in reference to DNA and protein sequences.

homologous chromosomes Two copies of the same chromosome, one inherited from the mother and the other from the father.

hormone A signaling molecule, produced and secreted by endocrine glands. Usually released into general circulation for coordination of an animal's physiology.

housekeeping gene A gene that codes for a protein that is needed by all cells, regardless of the cell's specialization. Genes encoding enzymes involved in glycolysis and the Krebs cycle are common examples.

hybridization A term used in molecular biology (recombinant DNA technology) meaning the formation of a double-stranded nucleic acid through complementary base-pairing. A property that is exploited in filter hybridization, a procedure that is used to screen gene libraries and to study gene structure and expression.

hydrophilic A polar compound that mixes readily with water.

hydrophobic A nonpolar molecule that dissolves in fat and lipid solutions but not in water.

hydroxyl group (-OH) Chemical group consisting of oxygen and hydrogen that is a prominent part of alcohol.

image analysis A computerized method for extracting information from digitized microscopic images of cells or cell organelles.

immunofluorescence Detection of a specific cellular protein with the aid of a fluorescent dye that is coupled to an antibody.

immunoglobulin (Ig) An antibody made by B cells as part of the adaptive immune response.

incontinence Inability to control the flow of urine from the bladder (urinary incontinence) or the escape of stool from the rectum (fecal incontinence).

in situ hybridization A method for studying gene expression, whereby a labeled cDNA or RNA probe hybridizes to a specific mRNA in intact cells or tissues. The procedure is usually carried out on tissue sections or smears of individual cells.

insulin Polypeptide hormone secreted by β (beta) cells in the vertebrate pancreas. Production of this hormone is regulated directly by the amount of glucose that is in the blood.

interleukin A small protein hormone, secreted by lymphocytes, to activate and coordinate the adaptive immune response.

interphase The period between each cell division, which includes the G_1, S, and G_2 phases of the cell cycle.

intron A section of a eukaryotic gene that is non-coding. It is transcribed, but does not appear in the mature mRNA.

in vitro Refers to cells growing in culture, or a biochemical reaction occurring in a test tube (Latin for "in glass").

in vivo A biochemical reaction, or a process, occurring in living cells or a living organism (Latin for "in life").

ion An atom that has gained or lost electrons, thus acquiring a charge. Common examples are Na^+ and Ca^{++} ions.

ion channel A transmembrane channel that allows ions to diffuse across the membrane and down their electrochemical gradient.

Jak-STAT signaling pathway One of several cell-signaling pathways that activates gene expression. The pathway is activated through cell-surface receptors and cytoplasmic Janus kinases (Jaks), and signal transducers and activators of transcription (STATs).

karyotype A pictorial catalog of a cell's chromosomes, showing their number, size, shape, and overall banding pattern.

keratin Proteins produced by specialized epithelial cells called keratinocytes. Keratin is found in hair, fingernails, and feathers.

kinesin A motor protein that uses energy obtained from the hydrolysis of ATP to move along a microtubule.

kinetochore A complex of proteins that forms around the centromere of mitotic or meiotic chromosomes, providing an attachment site for microtubules. The other end of each microtubule is attached to a chromosome.

Krebs cycle (citric acid cycle) The central metabolic pathway in all eukaryotes and aerobic prokaryotes, discovered by the German chemist Hans Krebs in 1937. The cycle oxidizes acetyl groups derived from food molecules. The end products are CO_2, H_2O, and high-energy electrons, which pass via NADH and FADH2 to the respiratory chain. In eukaryotes, the Krebs cycle is located in the mitochondria.

labeling reaction The addition of a radioactive atom or fluorescent dye to DNA or RNA for use as a probe in filter hybridization.

lagging strand One of the two newly synthesized DNA strands at a replication fork. The lagging strand is synthesized discontinuously, and therefore, its completion lags behind the second, or leading, strand.

lambda bacteriophage A viral parasite that infects bacteria. Widely used as a DNA cloning vector.

leading strand One of the two newly synthesized DNA strands at a replication fork. The leading strand is made by continuous synthesis in the 5' to 3' direction.

leucine zipper A structural motif of DNA binding proteins, in which two identical proteins are joined together at regularly spaced leucine residues, much like a zipper, to form a dimer.

leukemia Cancer of white blood cells.

lipid bilayer Two closely aligned sheets of phospholipids that form the core structure of all cell membranes. The two layers are aligned such that the hydrophobic tails are interior, while the hydrophilic head groups are exterior on both surfaces.

liposome An artificial lipid bilayer vesicle used in membrane studies and as an artificial gene therapy vector.

locus A term from genetics that refers to the position of a gene along a chromosome. Different alleles of the same gene occupy the same locus.

long-term potentiation (LTP) A physical remodeling of synaptic junctions that receive continuous stimulation.

lymphocyte A type of white blood cell that is involved in the adaptive immune response. There are two kinds of lymphocytes: T lymphocytes and B lymphocytes. T lymphocytes (T cells) mature in the thymus and attack invading microbes directly. B lymphocytes (B cells) mature in the bone marrow and make antibodies that are designed to immobilize or destroy specific microbes or antigens.

lysis The rupture of the cell membrane followed by death of the cell.

lysosome Membrane-bounded organelle of eukaryotes that contains powerful digestive enzymes.

macromolecule A very large molecule that is built from smaller molecular subunits. Common examples are DNA, proteins, and polysaccharides.

magnetic resonance imaging (MRI) A procedure in which radio waves and a powerful magnet linked to a computer are used to create detailed pictures of areas inside the body. These pictures can show the difference between normal and diseased tissue. MRI makes better images of organs and soft tissue than other scanning techniques, such as CT or X-ray. MRI is especially useful for imaging the brain, spine, the soft tissue of joints, and the inside of bones. Also called nuclear magnetic resonance imaging.

major histocompatibility complex Vertebrate genes that code for a large family of cell-surface glycoproteins that bind foreign antigens and present them to T cells to induce an immune response.

malignant Refers to the functional status of a cancer cell that grows aggressively and is able to metastasize, or colonize, other areas of the body.

mammography The use of X-rays to create a picture of the breast.

MAP-kinase (mitogen-activated protein kinase) A protein kinase that is part of a cell-proliferation-inducing signaling pathway.

M-cyclin A eukaryote enzyme that regulates mitosis.

meiosis A special form of cell division by which haploid gametes are produced. This is accomplished with two rounds of cell division but only one round of DNA replication.

melanocyte A skin cell that produces the pigment melanin.

membrane The lipid bilayer, and the associated glycocalyx, that surrounds and encloses all cells.

membrane channel A protein complex that forms a pore or channel through the membrane for the free passage of ions and small molecules.

membrane potential A buildup of charged ions on one side of the cell membrane establishes an electrochemical gradient that is measured in millivolts (mV). An important characteristic of neurons as it provides the electric current, when ion channels open, that enable these cells to communicate with each other.

mesoderm An embryonic germ layer that gives rise to muscle, connective tissue, bones, and many internal organs.

messenger RNA (mRNA) An RNA transcribed from a gene that is used as the gene template by the ribosomes, and other components of the translation machinery, to synthesize a protein.

metabolism The sum total of the chemical processes that occur in living cells.

metaphase The stage of mitosis at which the chromosomes are attached to the spindle but have not begun to move apart.

metaphase plate Refers to the imaginary plane established by the chromosomes as they line up at right angles to the spindle poles.

metaplasia A change in the pattern of cellular behavior that often precedes the development of cancer.

metastasis Spread of cancer cells from the site of the original tumor to other parts of the body.

methyl group (-CH_3) Hydrophobic chemical group derived from methane. Occurs at the end of a fatty acid.

micrograph Photograph taken through a light, or electron, microscope.

micrometer (μm or micron) Equal to 10^{-6} meters.

microtubule A fine cylindrical tube made of the protein tubulin, forming a major component of the eukaryote cytoskeleton.

millimeter (mm) Equal to 10^{-3} meters.

mitochondrion (plural: mitochondria) Eukaryote organelle, formerly free-living, that produces most of the cell's ATP.

mitogen A hormone or signaling molecule that stimulates cells to grow and divide.

mitosis Division of a eucaryotic nucleus. From the Greek *mitos,* meaning "a thread," in reference to the threadlike appearance of interphase chromosomes.

mitotic chromosome Highly condensed duplicated chromosomes held together by the centromere. Each member of the pair is referred to as a sister chromatid.

mitotic spindle Array of microtubules, fanning out from the polar centrioles and connecting to each of the chromosomes.

molecule Two or more atoms linked together by covalent bonds.

monoclonal antibody An antibody produced from a B cell–derived clonal line. Since all of the cells are clones of the original B cell, the antibodies produced are identical.

monocyte A type of white blood cell that is involved in the immune response.

motif An element of structure or pattern that may be a recurring domain in a variety of proteins.

M phase The period of the cell cycle (mitosis or meiosis) when the chromosomes separate and migrate to the opposite poles of the spindle.

multipass transmembrane protein A membrane protein that passes back and forth across the lipid bilayer.

mutant A genetic variation within a population.

mutation A heritable change in the nucleotide sequence of a chromosome.

myelin sheath Insulation applied to the axons of neurons. The sheath is produced by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system.

myeloid cell White blood cells other than lymphocytes.

myoblast Muscle precursor cell. Many myoblasts fuse into a syncytium, containing many nuclei, to form a single muscle cell.

myocyte A muscle cell.

NAD (nicotine adenine dinucleotide) Accepts a hydride ion (H^-), produced by the Krebs cycle, forming NADH, the main carrier of electrons for oxidative phosphorylation.

NADH dehydrogenase Removes electrons from NADH and passes them down the electron transport chain.

nanometer (nm) Equal to 10^{-9} meters or 10^{-3} microns.

natural killer cell (NK cell) A lymphocyte that kills virus-infected cells in the body. It also kills foreign cells associated with a tissue or organ transplant.

neuromuscular junction A special form of synapse between a motor neuron and a skeletal muscle cell.

neuron A cell specially adapted for communication that forms the nervous system of all animals.

neurotransmitter A chemical released by neurons at a synapse that transmits a signal to another neuron.

non-small-cell lung cancer A group of lung cancers that includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The small cells are endocrine cells.

northern blotting A technique for the study of gene expression. Messenger RNA (mRNA) is fractionated on an agarose gel and then transferred to a piece of nylon filter paper (or membrane). A specific mRNA is detected by hybridization with a labeled DNA or RNA probe. The original blotting technique invented by E. M. Southern inspired the name.

nuclear envelope The double membrane (two lipid bilayers) enclosing the cell nucleus.

nuclear localization signal (NLS) A short amino acid sequence located on proteins that are destined for the cell nucleus after they are translated in the cytoplasm.

nucleic acid DNA or RNA, a macromolecule consisting of a chain of nucleotides.

nucleolar organizer Region of a chromosome containing a cluster of ribosomal RNA genes that gives rise to the nucleolus.

nucleolus A structure in the nucleus where ribosomal RNA is transcribed and ribosomal subunits are assembled.

nucleoside A purine or pyrimidine linked to a ribose or deoxyribose sugar.

nucleosome A beadlike structure, consisting of histone proteins.

nucleotide A nucleoside containing one or more phosphate groups linked to the 5' carbon of the ribose sugar. DNA and RNA are nucleotide polymers.

nucleus Eukaryote cell organelle that contains the DNA genome on one or more chromosomes.

oligodendrocyte A myelinating glia cell of the vertebrate central nervous system.

oligo labeling A method for incorporating labeled nucleotides into a short piece of DNA or RNA. Also known as the random-primer labeling method.

oligomer A short polymer, usually consisting of amino acids (oligopeptides), sugars (oligosaccharides), or nucleotides (oligonucleotides). Taken from the Greek word *oligos,* meaning "few" or "little."

oncogene A mutant form of a normal cellular gene, known as a proto-oncogene, that can transform a cell to a cancerous phenotype.

oocyte A female gamete or egg cell.

operator A region of a prokaryote chromosome that controls the expression of adjacent genes.

operon Two or more prokaryote genes that are transcribed into a single mRNA.

organelle A membrane-bounded structure, occurring in eukaryote cells, that has a specialized function. Examples are the nucleus, Golgi complex, and endoplasmic reticulum.

osmosis The movement of solvent across a semipermeable membrane that separates a solution with a high concentration of solutes from one with a low concentration of solutes. The membrane must be permeable to the solvent but not to the solutes. In the context of cellular osmosis, the solvent is always water, the solutes are ions and molecules, and the membrane is the cell membrane.

osteoblast Cells that form bones.

ovulation Rupture of a mature follicle with subsequent release of a mature oocyte from the ovary.

oxidative phosphorylation Generation of high-energy electrons from food molecules that are used to power the synthesis of ATP from ADP and inorganic phosphate. The electrons are eventually transferred to oxygen to complete the process. Occurs in bacteria and mitochondria.

p53 A tumor-suppressor gene that is mutated in about half of all human cancers. The normal function of the *p53* protein is to block passage through the cell cycle when DNA damage is detected.

parthenogenesis A natural form of animal cloning whereby an individual is produced without the formation of haploid gametes and the fertilization of an egg.

pathogen An organism that causes disease.

PCR (polymerase chain reaction) A method for amplifying specific regions of DNA by temperature cycling a reaction mixture containing the template, a heat-stable DNA polymerase, and replication primers.

peptide bond The chemical bond that links amino acids together to form a protein.

pH Measures the acidity of a solution as a negative logarithmic function (p) of H^+ concentration (H). Thus a pH of 2.0 (10^{-2} molar H^+) is acidic, whereas a pH of 8.0 (10^{-8} molar H^+) is basic.

phagocyte A cell that engulfs other cells or debris by phagocytosis.

phagocytosis A process whereby cells engulf other cells or organic material by endocytosis. A common practice among protozoans and cells of the vertebrate immune system. (Derived from the Greek word *phagein,* "to eat.")

phenotype Physical characteristics of a cell or organism.

phospholipid The kind of lipid molecule used to construct cell membranes. Composed of a hydrophilic head-group, phosphate, glycerol, and two hydrophobic fatty acid tails.

phosphorylation A chemical reaction in which a phosphate is covalently bonded to another molecule.

photoreceptor A molecule or cell that responds to light.

photosynthesis A biochemical process in which plants, algae, and certain bacteria use energy obtained from sunlight to synthesize macromolecules from CO_2 and H_2O.

phylogeny The evolutionary history of an organism, or group of organisms, often represented diagrammatically as a phylogenetic tree.

pinocytosis A form of endocytosis whereby fluid is brought into the cell from the environment.

placebo An inactive substance that looks the same, and is administered in the same way, as a drug in a clinical trial.

plasmid A minichromosome, often carrying antibiotic-resistant genes, that occurs naturally among prokaryotes. Used extensively as a DNA cloning vector.

platelet A cell fragment, derived from megakaryocytes and lacking a nucleus, that is present in the bloodstream and is involved in blood coagulation.

ploidy The total number of chromosomes (n) that a cell has. Ploidy is also measured as the amount of DNA (C) in a given cell relative to a haploid nucleus of the same organism. Most organisms are diploid, having two sets of chromosomes, one from each parent, but there is great variation among plants and animals. The silk gland of the moth *Bombyx mori,* for example, has cells that are extremely polyploid, reaching values of 100,000C. Flowers are often highly polyploid, and vertebrate hepatocytes may be 16C.

point mutation A change in DNA, particularly in a region containing a gene, that alters a single nucleotide.

polyploid Possessing more than two sets of homologous chromosomes.

portal system A system of liver vessels that carries liver enzymes directly to the digestive tract.

probe Usually a fragment of a cloned DNA molecule that is labeled with a radioisotope or fluorescent dye and used to detect specific DNA or RNA molecules on Southern or northern blots.

promoter A DNA sequence to which RNA polymerase binds to initiate gene transcription.

prophase The first stage of mitosis. The chromosomes are duplicated and beginning to condense but are attached to the spindle.

protein A major constituent of cells and organisms. Proteins, made by linking amino acids together, are used for structural purposes and regulate many biochemical reactions in their alternative role as enzymes. Proteins range in size from just a few amino acids to more than 200.

protein glycosylation The addition of sugar molecules to a protein.

proto-oncogene A normal gene that can be converted to a cancer-causing gene (oncogene) by a point mutation or through inappropriate expression.

protozoa Free-living, single-cell eukaryotes that feed on bacteria and other microorganisms. Common examples are *Paramecium* and *Amoeba.* Parasitic forms are also known that inhabit the digestive and urogenital tract of many animals, including humans.

purine A nitrogen-containing compound that is found in RNA and DNA. Two examples are adenine and guanine.

pyrimidine A nitrogen-containing compound found in RNA and DNA. Examples are cytosine, thymine, and uracil (RNA only).

radioactive isotope An atom with an unstable nucleus that emits radiation as it decays.

randomized clinical trial A study in which the participants are assigned by chance to separate groups that compare different treatments; neither the researchers nor the participants can choose which group. Using chance to assign people to groups means that the groups will be similar and that the treatments they receive can be compared objectively. At the time of the trial, it is not known which treatment is best.

reagent A chemical solution designed for a specific biochemical or histochemical procedure.

recombinant DNA A DNA molecule that has been formed by joining two or more fragments from different sources.

regulatory sequence A DNA sequence to which proteins bind that regulate the assembly of the transcriptional machinery.

replication bubble Local dissociation of the DNA double helix in preparation for replication. Each bubble contains two replication forks.

replication fork The Y-shaped region of a replicating chromosome. Associated with replication bubbles.

replication origin (origin of replication, ORI) The location at which DNA replication begins.

respiratory chain (electron transport chain) A collection of iron- and copper-containing proteins, located in the inner mitochondrion membrane, that utilize the energy of electrons traveling down the chain to synthesize ATP.

restriction enzyme An enzyme that cuts DNA at specific sites.

restriction map The size and number of DNA fragments obtained after digesting with one or more restriction enzymes.

retrovirus A virus that converts its RNA genome to DNA once it has infected a cell.

reverse transcriptase An RNA-dependent DNA polymerase. This enzyme synthesizes DNA by using RNA as a template, the reverse of the usual flow of genetic information from DNA to RNA.

ribosomal RNA (rRNA) RNA that is part of the ribosome and serves both a structural and functional role, possibly by catalyzing some of the steps involved in protein synthesis.

ribosome A complex of protein and RNA that catalyzes the synthesis of proteins.

rough endoplasmic reticulum (rough ER) Endoplasmic reticulum that has ribosomes bound to its outer surface.

Saccharomyces Genus of budding yeast that are frequently used in the study of eukaryote cell biology.

sarcoma Cancer of connective tissue.

Schwann cell Glia cell that produces myelin in the peripheral nervous system.

screening Checking for disease when there are no symptoms.

senescence Physical and biochemical changes that occur in cells and organisms with age.

signal transduction A process by which a signal is relayed to the interior of a cell where it elicits a response at the cytoplasmic or nuclear level.

smooth muscle cell Muscles lining the intestinal tract and arteries. Lacks the striations typical of cardiac and skeletal muscle, giving it a smooth appearance when viewed under a microscope.

somatic cell Any cell in a plant or animal except those that produce gametes (germ cells or germ cell precursors).

somatic cell nuclear transfer Animal cloning technique whereby a somatic cell nucleus is transferred to an enucleated oocyte. Synonomous with cell nuclear replacement.

Southern blotting The transfer of DNA fragments from an agarose gel to a piece of nylon filter paper. Specific fragments are identified by hybridizing the filter to a labeled probe. Invented by the Scottish scientist E. M. Southern in 1975.

stem cell Pluripotent progenitor cell, found in embryos and various parts of the body, that can differentiate into a wide variety of cell types.

steroid A hydrophobic molecule with a characteristic four-ringed structure. Sex hormones, such as estrogen and testosterone, are steroids.

structural gene A gene that codes for a protein or an RNA. Distinguished from regions of the DNA that are involved in regulating gene expression but are non-coding.

synapse A neural communication junction between an axon and a dendrite. Signal transmission occurs when neurotransmitters, released into the junction by the axon of one neuron, stimulate receptors on the dendrite of a second neuron.

syncytium A large multinucleated cell. Skeletal muscle cells are syncytiums produced by the fusion of many myoblasts.

syngeneic transplants A patient receives tissue or an organ from an identical twin.

tamoxifen A drug that is used to treat breast cancer. Tamoxifen blocks the effects of the hormone estrogen in the body. It belongs to the family of drugs called antiestrogens.

T cell (T lymphocyte) A white blood cell involved in activating and coordinating the immune response.

telomere The end of a chromosome. Replaced by the enzyme telomerase with each round of cell division to prevent shortening of the chromosomes.

telophase The final stage of mitosis in which the chromosomes decondense and the nuclear envelope reforms.

template A single strand of DNA or RNA whose sequence serves as a guide for the synthesis of a complementary, or daughter, strand.

therapeutic cloning The cloning of a human embryo for the purpose of harvesting the inner cell mass (ES cells).

topoisomerase An enzyme that makes reversible cuts in DNA to relieve strain or to undo knots.

transcription The copying of a DNA sequence into RNA, catalyzed by RNA polymerase.

transcriptional factor A general term referring to a wide assortment of proteins needed to initiate or regulate transcription.

transfection Introduction of a foreign gene into a eukaryote cell.

transfer RNA (tRNA) A collection of small RNA molecules that transfer an amino acid to a growing polypeptide chain on a ribosome. There is a separate tRNA for amino acid.

transgenic organism A plant or animal that has been transfected with a foreign gene.

trans-Golgi network The membrane surfaces where glycoproteins and glycolipids exit the Golgi complex in transport vesicles.

translation A ribosome-catalyzed process whereby the nucleotide sequence of a mRNA is used as a template to direct the synthesis of a protein.

transposable element (transposon) A segment of DNA that can move from one region of a genome to another.

ultrasound (ultrasonography) A procedure in which high-energy sound waves (ultrasound) are bounced off internal tissues or organs producing echoes that are used to form a picture of body tissues (a sonogram).

umbilical cord blood stem cells Stem cells, produced by a human fetus and the placenta, that are found in the blood that passes from the placenta to the fetus.

vector A virus or plasmid used to carry a DNA fragment into a bacterial cell (for cloning) or into a eukaryote to produce a transgenic organism.

vesicle A membrane-bounded bubble found in eukaryote cells. Vesicles carry material from the ER to the Golgi and from the Golgi to the cell membrane.

virus A particle containing an RNA or DNA genome surrounded by a protein coat. Viruses are cellular parasites that cause many diseases.

western blotting The transfer of protein from a polyacrylamide gel to a piece of nylon filter paper. Specific proteins are detected with labeled antibodies. The name was inspired by the original blotting technique invented by E. M. Southern.

yeast Common term for unicellular eukaryotes that are used to brew beer and make bread. Bakers yeast, *Saccharomyces cerevisiae,* is also widely used in studies on cell biology.

zygote A diploid cell produced by the fusion of a sperm and egg.

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