Medical Biotechnology

After completing this chapter, you should be able to:

- Explain why model organisms are important for medical biotechnology.
- Describe different molecular techniques for detecting chromosomal abnormalities and for genetic testing.
- Provide examples of how, pharmacogenomics is changing the treatment of genetic conditions.
- Discuss how monoclonal antibodies may be used for treating disease.
- Understand the purpose of gene therapy, compare and contrast different gene therapy strategies, and recognize the limitations of gene therapy.
- Define regenerative medicine and provide examples of how cell and tissue transplantation and tissue engineering can be used.
- Understand what stem cells are, describe how they can be isolated, and provide examples of possible therapies that may be developed from stem cells.
- Compare and contrast therapeutic cloning and reproductive cloning.

Customized medicine is a goal of medical biotechnology.
NatUlrich/Shutterstock.

There is perhaps no topic in biotechnology that provokes greater optimism and debate than **medical biotechnology.** Applications of medical biotechnology have existed for decades. For instance, 100 years ago, leeches were commonly used to treat illness by so-called bloodletting. Some doctors believed that by using leeches to suck blood out of a patient, diseases were being removed from the body. Now, however, much better ways of treating disease have been found thanks to biotechnology. It is an exciting time to learn about medical biotechnology because advances in this field are occurring at a mind-boggling rate. In fact, even the leech is getting attention again—not for bloodletting but for enzymes found in its saliva that can dissolve blood clots and possibly be used to treat strokes and heart attacks.

Medical biotechnology incorporates many topics that we have already discussed. From developing new drugs to the prospects of using stem cells and cloning, the possibilities of medical biotechnology are incredible but also incredibly alarming to many people, including scientists.

In this chapter, we consider a range of different applications of medical biotechnology and discuss many potential impacts of this very exciting area. We begin by providing an overview of how molecular biology techniques can be used to detect and diagnose disease and by considering innovative medical products developed through biotechnology. We then present an introduction to applications and examples of gene therapy before going on to discuss regenerative medicine. The chapter concludes by examining stem cells and their potential applications.

FORECASTING THE FUTURE

Without question medical biotechnology is one of the most rapidly changing disciplines in biotechnology and the possibilities for affecting human life positively are amazing. Because of the range of different technologies being developed and the new applications in medical biotechnology, it is challenging to predict what the next major future discoveries will be. In this "forecasting," we pose several questions that are among the major challenges being addressed by medical biotechnologists.

- To what extent will personalized genomics and sequencing of individual genomes affect disease diagnosis and treatment in the future?
- What new medicines and treatments will result from our understanding of the genome and epigenome?
- What roles will nanotechnologies play for sensing and treating disease?
- What new treatments will emerge from regenerative medicine and the ability to engineer cells, tissues, and organs?
- Will gene therapy techniques advance to become more routine options for treating genetic diseases?
- Will stem cells become reliable, safe, and affordable options for treating disease?

1 The Power of Molecular Biology: Detecting and Diagnosing Human Disease Conditions

The year 2003 marked the 50-year anniversary since Nobel Prize winners James Watson and Francis Crick revealed the structure of DNA. Since then, molecular biology has advanced at an astonishing pace, providing molecular techniques that give scientists and medical doctors very powerful tools for combating human diseases.

Models of Human Disease

Many of the applications you will learn about in this chapter are possible because of important **model organisms.** In particular, mice, rats, worms, and flies have played critical roles in helping scientists study human disease conditions. We think of ourselves as unmatched by other species not only for our ability to communicate through speech and writing but also for walking upright, creating music, making good pizza, and exploring distant planets. But we are not really unique at the genetic level. From yeasts and worms to mice, we share large numbers of genes with other organisms. A number of human genetic diseases also occur in model organisms. Therefore scientists can use model organisms to identify disease genes and test gene therapy and drug-based therapeutic approaches to evaluate their effectiveness and safety in preclinical studies before using them for **clinical trials** in humans.

Model organisms are critically important to scientists because we cannot manipulate human genetics for experimental purposes. It is, of course, unethical and illegal to force humans to breed or to remove their genes to learn how they function. However, these approaches are widely used to study genes in model organisms. Mice, rats, chicks, yeasts, fruit flies, worms, frogs, and even the zebrafish, a common fish in home aquariums, have all played important roles in widening our understanding of human genetics. Many important genes are highly conserved from species to species. If we identify important genes in model organisms, we can form hypotheses and make predictions about how these genes may function in humans.

Many genes identified in different model species have been shown to be related to human genes based

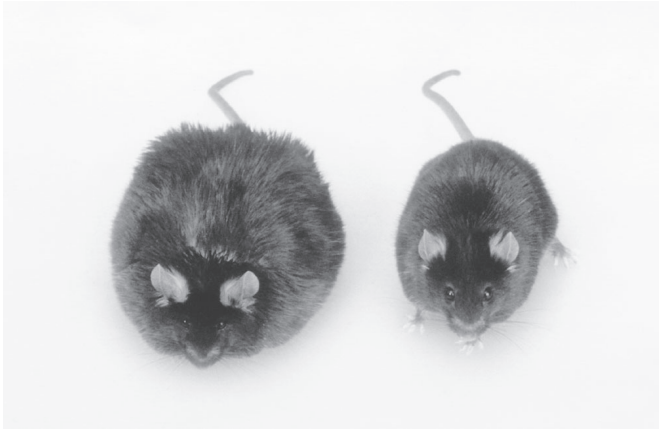


FIGURE 1 **Obese Mice** Model organisms are very valuable for helping scientists learn about human disease genes. The mouse on the left has been genetically engineered by gene knockout to lack the *obese* (*Ob*) gene, which produces a protein hormone called leptin, from the Greek word *leptos* meaning "thin." The *Ob* knockout mouse weighs almost five times as much as its normal sister (right).

John Sholtis/Amgen Inc.

on DNA sequence similarity. Such related genes are called **homologues**. A gene thought to play a role in human illness can be eliminated in model organisms through gene knockout. The effects on the organism can then be studied to learn about the functions of the gene. For instance, several years ago scientists discovered that mice can become obese if they lack a single gene called *Ob* (**Figure 1**). *Ob* codes for a protein hormone called leptin, which is produced by fat cells (adipocytes) and travels through the bloodstream to the brain to regulate hunger, essentially telling the brain when the body is full. The subsequent discovery of a human homologue for leptin has led to a new area of research with great promise for providing insight into fat metabolism in humans and the genetics that may influence weight disorders. Some childhood diseases of obesity are affected by mutation of the *Ob* gene. Extremely obese children in England have been treated with leptin and have responded very well in preliminary studies.

In developing embryos, some cells must die to make room for others. How does the body know where to develop certain organs and determine which cells must die to make room for others? Studies of the nematode *Caenorhabditis elegans*, an unsegmented roundworm, have greatly advanced the quest for answers to these important developmental questions. Maps of *C. elegans*, which has 959 cells, have been created, allowing scientists to determine the fate or lineage of all cells in the embryonic worm that develop to form the mature worm's nervous system, intestine, and other tissues. Of these cells, 131 are destined to die in a form of cell suicide known as programmed cell death, or **apoptosis**. During the development of a human embryo, sheets of skin cells create webs between the fingers and toes; apoptosis is responsible for the degeneration of these webs prior to birth. We know that apoptosis is involved in neurodegenerative diseases such as Alzheimer disease, Huntington disease, amyotrophic lateral sclerosis (Lou Gehrig disease), and Parkinson disease as well as arthritis and some forms of infertility. Model organisms are helping scientists better understand the genes involved and thus to help find ways of slowing or stopping these degenerative processes.

The Human Genome Project and studies in comparative genomics have clearly demonstrated that we share a large number of genes with other organisms. Can you believe that you share approximately 50% of your genes with the pesky fruit flies you bring home on fruit from the grocery store? It may seem hard to believe that it takes only about twice as many genes to make a human as it does a fruit fly. And plants, such as rice, have even more genes than we do. We share nearly 40% of our genes with roundworms and 31% of our genes with yeast—the same yeast we use to help make bread rise and ferment alcoholic beverages. We share even more genes with mice; approximately 90% of our genes are similar in structure and function.

Many genes that determine our body plan, organ development, and eventually our aging and death are virtually identical to genes in fruit flies. Moreover, mutated genes that are known to give rise to disease in humans also cause disease in fruit flies. Approximately 61% of genes mutated in 289 human disease conditions are found in the fruit fly. This group includes the genes involved in prostate cancer, pancreatic cancer, cystic fibrosis, leukemia, and many other human genetic disorders. Heart disease is another example of a condition that scientists are studying in model organisms. Nearly 1 million people die each year in the United States from heart disease. Researchers are using gene knockouts to develop so-called heart attack mice that are deficient in the genes required for cholesterol metabolism. They hope that these mice will show elevated blood cholesterol levels similar to those that occur in atherosclerosis—hardening of the arteries—so that they can test therapies to combat heart disease.

Last, researchers have been hunting for a cure for AIDS for over 20 years. Creating a small animal model for AIDS is a high priority for many AIDS scientists. In addition to humans, HIV and related viruses cause disease in primates such as chimpanzees and rhesus macaque monkeys. But these animals are very expensive and ethical concerns have been raised about experimenting on fellow primates. Each animal can cost over $50,000, and they are available in only limited

numbers. Researchers are making slow but steady progress toward developing rodent models infected with HIV. Even if such animals are created, there is no guarantee their disease will adequately mimic human AIDS. In humans, HIV infects and destroys human T lymphocytes (T cells). A main impediment to a rodent model is that HIV does not recognize and bind to receptor proteins on mouse T cells, the mechanism by which HIV infects human T cells. Nevertheless, scientists might be able to express human T-cell proteins on mouse T cells to trick the virus into infecting mice.

Biomarkers for Disease Detection

In theory, with the right diagnostic tools, it may be possible to detect almost every disease at an early stage. For many diseases, such as cancer, early detection is critical for providing the best treatment and improving the odds of survival. One detection approach is to look for **biomarkers** as indicators of disease. Biomarkers are typically proteins produced by diseased tissue or proteins whose production is increased when a tissue is diseased. Many biomarkers are released into body fluids such as blood and urine as a product of cell damage—released by dead and dying cells such as cells undergoing apoptosis. For example, a protein called **prostate-specific antigen (PSA)** is released into the bloodstream when the prostate gland is inflamed, and elevated levels can be a marker for prostate inflammation and even prostate cancer. Detecting individual genes or gene expression patterns also provides scientists with biomarkers for disease (see Figure 8 later in this chapter), and many biotechnology companies are actively involved in searching for better biomarkers that can be used for early detection and disease diagnosis.

The Human Genome Project Has Revealed Disease Genes on All Human Chromosomes

Many of the disease genes that can currently be tested for were discovered through the Human Genome Project. Prior to the genome project, only about 100 genetic diseases could be tested for; now there are over 2,000 diseases for which genetic tests are available. Through the Human Genome Project, scientists developed complex "maps" showing the locations of normal and diseased genes on all human chromosomes. Chromosome maps are available that pinpoint the locations of normal and disease genes of interest. **Figure 2** shows simplified maps for each chromosome, highlighting one or two prominent genes on each that are known to be involved in human genetic disease.

The Human Genome Project has led to the development of follow-up projects such as **The Cancer Genome Atlas Project (TCGA)**, a comprehensive effort to identify genomic changes involved in a variety of different cancers. Scientists are particularly interested in genetic changes that trigger normal cells to become cancerous cells in the brain, mammary glands, ovaries, pancreas, liver, and lungs because cancers of these organs affect large numbers of Americans. Eventually scientists expect that new information from TCGA will be used to better diagnose and treat cancer.

Detecting Genetic Diseases: Testing for Chromosomal Abnormalities and Defective Genes

Molecular biology techniques have proven to be extremely valuable for detecting many different genetic diseases.

Until relatively recently, most genetic testing occurred on fetuses for the purpose of identifying the sex of a child or to detect a small number of genetic diseases. Most of these procedures involved testing for genetic conditions that occur as a result of alterations in chromosome number or large structural abnormalities of chromosomes. If there are problems with chromosome separation during the formation of sperm or egg cells, a fetus may contain abnormal numbers of chromosomes. One of the best-understood examples of a disorder created by an alteration in chromosome number is **Down syndrome**. Most individuals with this condition have three copies of chromosome 21 (trisomy 21). Affected individuals show a number of symptoms, including cognitive impairments, short stature, and broadened facial features. Scientists have developed a strain of mice with almost a complete copy of human chromosome 21. These mice show characteristics of Down syndrome and may turn out to be a very valuable model for understanding the genetics of this condition.

Fetal testing for Down syndrome is fairly common, particularly in pregnant women older than 40 years, because the incidence of Down syndrome is related to the age of eggs produced by the woman. Trisomy 21 and other abnormalities in chromosome number can be tested in a fetus to provide parents with information that may be used to determine if they want the pregnancy to continue. If a defect is detected, genetic tests also provide information that can be used to treat fetuses during pregnancy and after the child is born.

So how is a developing fetus tested for Down syndrome? Two different techniques can be used: **amniocentesis** and **chorionic villus sampling**. Amniocentesis is performed when the developing fetus is around 16 weeks of age. A needle is inserted through

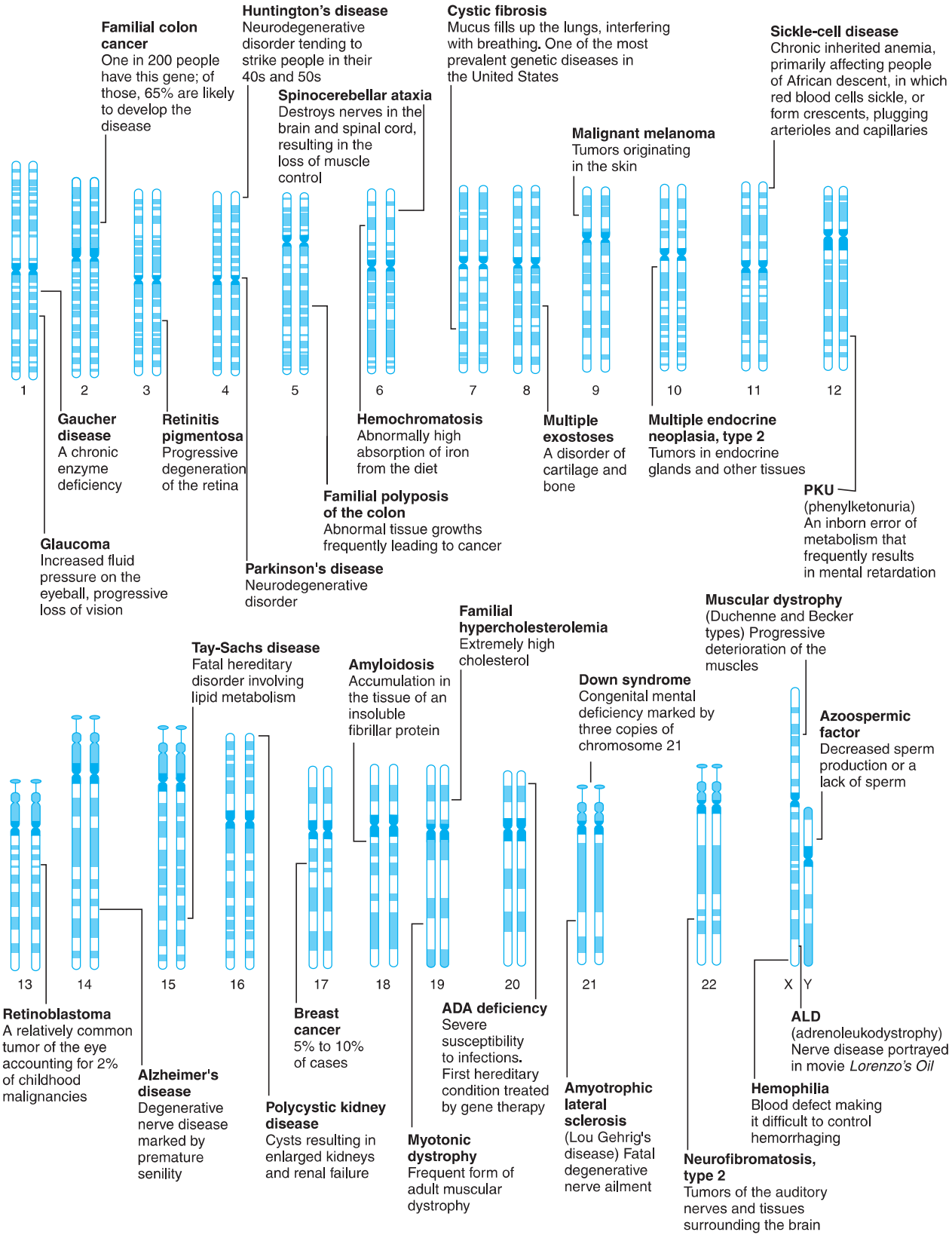


FIGURE 2 **Disease Gene Maps of Human Chromosomes** Maps show one or two representative genes on each human chromosome that are involved in a genetic condition. Many more genes than are shown in this figure are located on each chromosome. Note: Chromosomes are not drawn to scale.

the mother's abdomen into the pocket of amniotic fluid surrounding and cushioning the fetus (**Figure 3**). This fluid contains cells shed from the fetus, such as skin cells. Isolated cells are then cultured for a few days to increase cell numbers, after which the cells are treated to arrest them in mitosis to facilitate the viewing of mitotic chromosomes, which are spread onto a glass slide. The chromosomes are stained with different dyes that bind to proteins attached to the DNA, creating patterns of alternating light and dark bands on each chromosome. Based on the size of each chromosome and its banding pattern, chromosomes can be aligned into pairs. This procedure is called **karyotyping**; it can also be used to determine a child's sex based on the presence of the sex chromosomes (X and Y).

During chorionic villus sampling (CVS) for fetal testing, a suction tube is used to remove a small portion of a layer of cells called the chorionic villus, fetal tissue that helps form the placenta (Figure 2). An advantage of CVS over amniocentesis is that enough cells are obtained so the sample can immediately be used for karyotyping. Another advantage of CVS is that the procedure can be done earlier in the pregnancy, around 8 to 10 weeks. Because the fetus is so small at that stage, CVS carries a higher risk than amniocentesis of disturbing it and causing a miscarriage (overall about 1% of amniocentesis and CVS procedures cause miscarriages).

Several researchers are close to producing noninvasive tests for fetal testing based on identifying and sequencing small amounts of fetal chromosomal fragments present in a pregnant woman's blood. Be on the lookout for further advances in these so-called **noninvasive prenatal genetic diagnosis (NIPD)** tests, which are predicted to be readily available as soon as 2013.

Karyotyping is easily carried out on adults to check for chromosomal abnormalities. Typically, blood is drawn from an adult and the white blood cells are used. A modern technique for karyotyping in both fetuses and adults is **fluorescence in situ hybridization (FISH)**. In FISH, a chromosome spread is prepared on a slide and then fluorescent probes are hybridized to each chromosome. Each probe is specific for certain "marker" sequences on each chromosome. In some cases, FISH can be performed with probes that fluoresce different colors—a procedure called **spectral karyotyping**. FISH is very useful for identifying missing chromosomes and extra chromosomes, but in particular FISH makes it much easier than conventional karyotyping to detect defective chromosomes. A number of human genetic diseases due to chromosomal

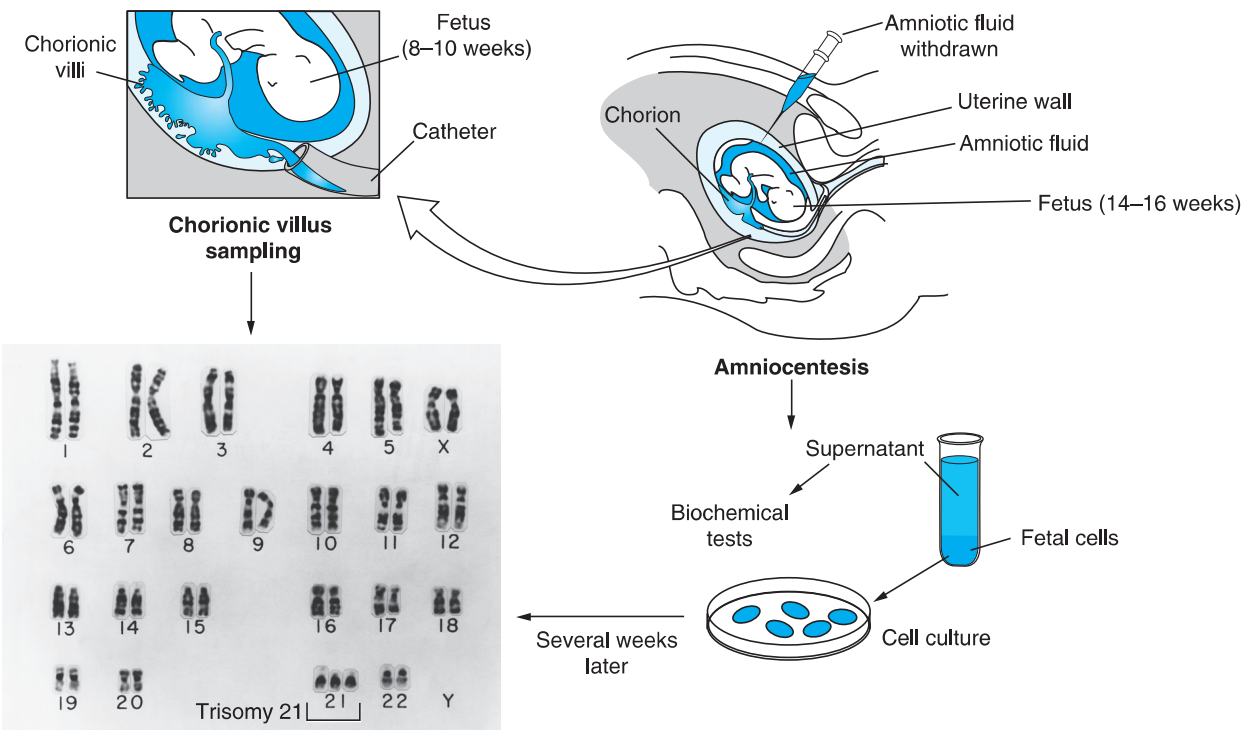


FIGURE 3 Amniocentesis and Chorionic Villus Sampling Fetal testing for chromosomal abnormalities is most commonly achieved through either amniocentesis or chorionic villus sampling. This karyotype from a person with Down syndrome shows three copies of chromosome 21 (trisomy 21).

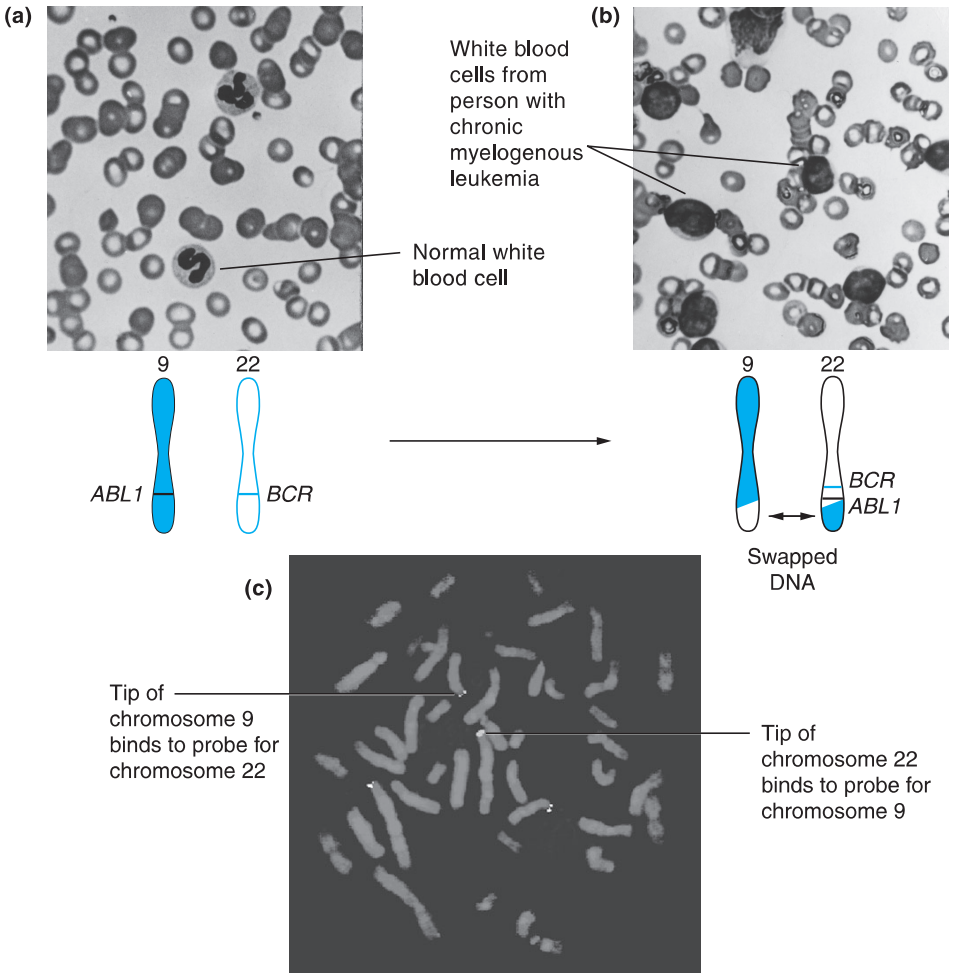


FIGURE 4 FISH Can Be Used to Detect Chromosomal Defects Chronic myelogenous leukemia, a cancer of white blood cells created when genes on chromosome 9 and 22 are swapped, can be detected by FISH.

(a): Centers for Disease Control and Prevention (CDC)., **(c):** Lisa G. Shaffer.

abnormalities occur when a portion of a chromosome is deleted or a piece of chromosome is swapped from one chromosome to another because of problems in chromosomal replication. For instance, in a type of leukemia (a cancer of the white blood cells) called chronic myelogenous leukemia, DNA is exchanged between chromosomes 9 and 22, so that genes from 9 are swapped onto 22 and vice versa (**Figure 4**). This exchange, called a *translocation*, can be detected by FISH using different-colored fluorescent probes for each chromosome.

More genetic diseases result from mutations in specific genes than abnormalities in the numbers or structures of chromosomes. As a result of the Human Genome Project, more sophisticated techniques have been developed to detect *individual* diseased genes in both fetuses and adults. Some genetic diseases can be detected in embryos and adults from either amniotic cells or blood cells, respectively, using **restriction fragment length polymorphism (RFLP) analysis** (pronounced "riff-lips"). The basic idea behind RFLP analysis is that defective gene sequences may be cut differently by restriction enzymes than their normal complements because nucleotide changes in the mutant genes can affect restriction enzyme cutting sites to create more or fewer. Remember that RFLP analysis is used for DNA fingerprinting. As an example, if DNA from a healthy individual and DNA from an individual with sickle-cell disease are both cut with restriction enzymes, they will be of different sizes because of the way restriction enzymes cut each gene. This can be clearly observed when the DNA fragments are subjected to Southern blot analysis with a probe for the ß-globin gene, the gene affected in sickle-cell disease (**Figure 5** on the next page). Hence we have the term ***r***estriction ***f***ragment ***l***ength ***p***olymorphisms—fragments of different lengths or forms ("poly" means many and "morphism" refers to the form or appearance of something) created by restriction enzymes.

Sickle-cell disease occurs when a person has two mutant versions of the ß-globin gene. The mutant copies of ß-globin protein produce an abnormal form of hemoglobin that affects the size and shape of red blood cells, giving them a characteristic "sickled" appearance (see also Figure 20).

The major disadvantage of RFLP analysis is that it

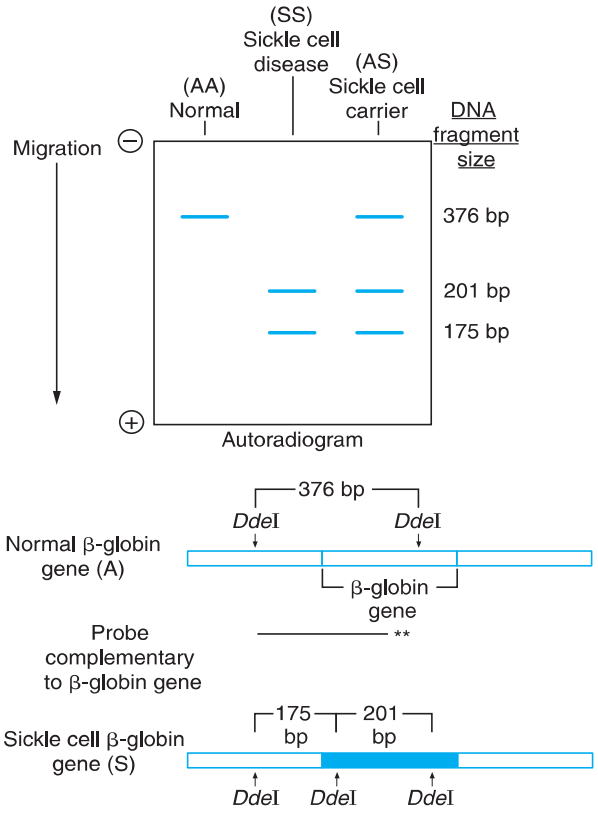


FIGURE 5 Using RFLP Analysis to Detect a Genetic Disease Human DNA cut with a restriction enzyme (*DdeI*) was subjected to agarose gel electrophoresis followed by Southern blotting to transfer the DNA to a nylon membrane and hybridization with a radioactive probe specific for the ß-globin gene. The normal ß-globin gene (A) contains two cutting sites for the enzyme *DdeI*; mutations in the sickle-cell (mutated) ß-globin gene (S) create three cutting sites for *DdeI*. Differences in DNA fragment sizes (polymorphisms) are detected by autoradiography, depending on where the probe binds to complementary sequences in the ß-globin gene. A healthy person with two copies of the normal ß-globin gene (homozygous, AA) shows a single band at 376 bp; a person with sickle-cell disease (homozygous, SS) would have two copies of the mutant ß-globin gene and show bands at 201 and 175 bp. A heterozygous person considered a "carrier" would have one normal and one defective ß-globin gene (AS) but would not have sickle-cell disease because there is one functioning copy of the ß-globin gene; bands show at 376, 201, and 175 bp.

From *Principles of Cell & Molecular Biology*, Second Edition by Lewis J. Kleinsmith and Valerie M. Kish, figure 3-53, p. 110. Copyright © 1995 by HarperCollins College Publishers. Reprinted by permission of Pearson Education, Inc.

can be used only to analyze gene defects in which a mutation changes a restriction site in a gene. **Allele-specific oligonucleotide (ASO) analysis** allows for the detection of a single nucleotide change in a gene even if the mutation does not change a restriction site. In this technique, DNA is isolated from human cells, usually white blood cells, and then amplified by the polymerase chain reaction (PCR) using primers that flank a disease gene of interest. Amplified DNA is then blotted onto nylon filters and hybridized separately to two different ASOs as probes. ASOs are small single-stranded oligonucleotide sequences, usually around 20 nucleotides in length. An ASO that will hybridize to a normal gene and another for the mutant gene are used. Figure 6 shows an example of how ASO analysis can be used to test for the sickle cell gene. PCR-based tests such as this are becoming increasingly valuable for detecting diseased genes. One major advantage of PCR is its high sensitivity for detecting defects in small amounts of DNA. Consequently PCR and ASO analysis as well as FISH are being used to screen for gene defects in single cells from 8- to 32-cell embryos created by in vitro fertilization. Such **preimplantation genetic testing** allows individuals to select a healthy embryo prior to implantation.

Currently, several hundred defective genes can be tested for in adults or fetuses using many of the techniques we have described (Table 1). Refer to "Tools of the Trade" for several excellent websites where you can learn more about human disease genes.

Single nucleotide polymorphisms

If a segment of chromosomal DNA from two different people is compared by DNA sequencing, approximately 99.9% of the DNA sequence will be exactly the same. One of the many intriguing findings of the Human Genome Project was the discovery that **single nucleotide polymorphisms (SNPs**; pronounced "snips") represent one of the most common forms of genetic variation among humans. SNPs are single-nucleotide changes in DNA sequences that vary from individual to individual.

SNPs have been found on all human chromosomes. It has been estimated that SNPs make up about 90% of human genetic variation and occur approximately every 100 to 300 base pairs (bp) in the human genome. Most SNPs are less likely to have an effect on a cell because they occur in non–protein coding regions (introns) of the genome. But when a SNP occurs in a gene sequence, it may cause a change in protein structure that produces disease or influences traits in a variety of ways, including conferring susceptibility for some types of disease conditions.

SNPs represent variations in DNA sequences that may ultimately influence how we respond to stress and disease. The first SNP discovered to be associated with a disease condition was for sickle cell disease. Because SNPs occur frequently throughout the genome, they serve as valuable genetic markers for identifying disease-related genes. Some SNPs are being used to predict susceptibilities to ailments such as stroke, diabetes, cancer, heart disease, behavioral

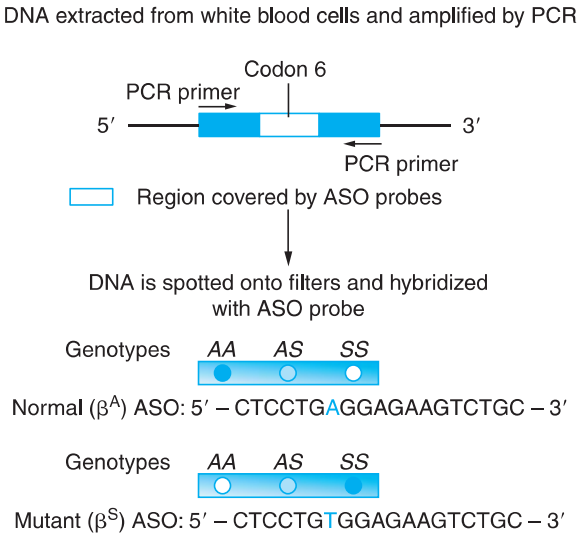


FIGURE 6 Using PCR and ASOs to Test for the Sickle Cell Gene ASO tests are very valuable for detecting single nucleotide mutations such as the one that causes sickle cell disease. In this example, DNA from white blood cells is amplified by PCR, blotted onto nylon membrane, and hybridized to fluorescently labeled ASO probes (probe binding shown as blue spots). The ASO for the normal hemoglobin gene ($ß^A$) will bind to DNA on the nylon only if the normal gene is present; the ASO for the mutant hemoglobin gene ($ß^S$) will bind to the defective gene only if it is present on the nylon. In this figure, probe binding is represented by blue DNA from individuals who are homozygous for the normal gene (AA) and have two copies of the $ß^A$ allele; their DNA will bind only to the normal $ß^A$ ASO. Individuals who are homozygous for the sickle cell hemoglobin gene (SS) have two copies of the $ß^S$ allele and their DNA will bind only to the $ß^S$ ASO and not the normal $ß^A$ ASO. Heterozygous individuals (AS) have one normal copy of the gene and one mutant copy; as a result, their DNA will bind to both probes but produce a lighter hybridization signal than that from homozygous individuals.

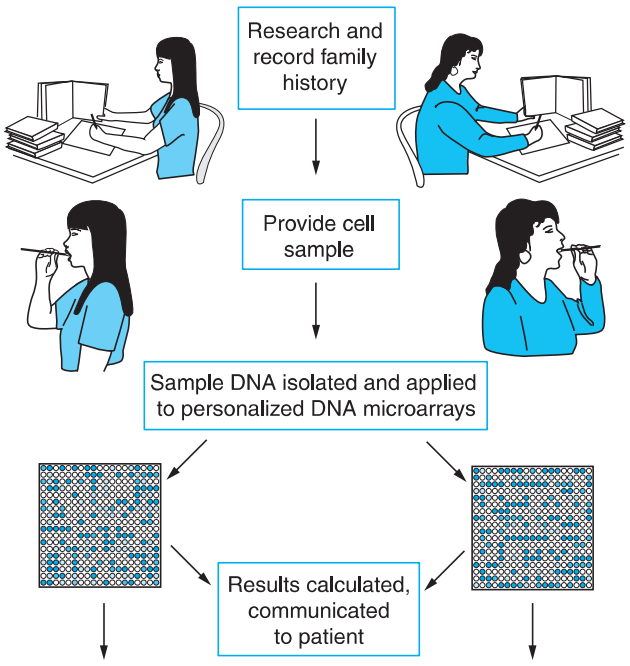
and emotional illnesses, and a host of other disorders that may have a genetic basis.

SNPs are thought to be so promising that pharmaceutical companies have invested millions of dollars in a collaborative partnership called the **HapMap Project**. Many SNPs on the same chromosome are clustered in groups called haplotypes. "Hap" is an abbreviation for haplotype. HapMap is an international effort among companies, academic institutions, and private foundations with an established goal of identifying and cataloguing the chromosomal locations (loci) of the more than 1.4 million SNPs that are present in the 3 billion bp of the human genome and to understand the roles of SNPs in disease diagnosis and treatment. As you just learned, ASO analysis is one way to detect SNPs.

Identifying sets of disease genes by microarray analysis

Another key technique for studying genetic diseases are **DNA microarrays**; also called gene chips. A single microarray can contain probes for thousands of genes. Researchers can use microarrays to screen a patient for a pattern of genes that might be expressed in a particular disease condition. As shown in **Figure 7**, microarray data can then be used to predict the patient's risk of developing disease based on the patient's expressed genes for the disease.

For instance, microarrays created with probes for known disease genes or certain SNPs have become valuable for studying expressed genes in a patient. To do this, DNA or RNA is isolated from a patient's tissue sample—a blood sample or even a scraping of cells lining the cheeks. The patient's DNA is tagged with fluorescent dyes and then hybridized to the chip. Spots on the microarray where the patient's DNA bound are revealed by fluorescence. Binding of a patient's DNA to a gene sequence on the chip indicates that his or her DNA has a particular mutation or SNP. There are even companies



Susan's Genetic Profile

Trait	Risk
Addictive behavior	Greater than general population
Lung cancer	Greater than general population
Colon cancer	Less than general population
Alzheimer's disease	Less than general population

Lisa's Genetic Profile

Trait	Risk
Cystic fibrosis	100% diagnosis
Type II diabetes mellitus	Less than general population
Cardiovascular disease	Greater than general population

FIGURE 7 Using Gene Microarrays to Create a Genetic Profile

TABLE 1 GENETIC DISEASE TESTING

Genetic Disease Condition	Genetic Basis for Disease and Symptoms
Cancers (brain tumors; urinary bladder, prostate, ovarian, breast, brain, lung, and colorectal cancers)	A variety of different mutant genes can serve as markers for genetic testing.
Cystic fibrosis	Large number of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7. Causes lung infections and problems with pancreatic, digestive, and pulmonary functions.
Duchenne muscular dystrophy	Defective gene (dystrophin) on the X chromosome causes muscle weakness and muscle degeneration.
Familial hypercholesterolemia	Mutant gene on chromosome 19 causes extremely high levels of blood cholesterol.
Hemophilia	Defective gene on the X chromosome makes it difficult for blood to clot when there is bleeding.
Huntington disease	Mutation in gene on chromosome 4 causes neurodegenerative disease in adults.
Phenylketonuria (PKU)	Mutation in gene required for converting the amino acid phenylalanine into the amino acid tyrosine. Causes severe neurological damage, including mental retardation.
Severe combined immunodeficiency (SCID)	Immune system disorder caused by mutation of the adenosine deaminase gene.
Sickle cell disease	Mutation in ß-globin gene on chromosome 11 affects hemoglobin structure and shape of red blood cells, which disrupts oxygen transport in blood and causes joint pain.
Tay-Sachs disease	Rare mutation of a gene on chromosome 5 causes certain types of lipids to accumulate in the brain. Causes paralysis, blindness, retardation, and respiratory infections.

working on handheld chip devices that doctors can use to get nearly instant information about a patient's genetics. Microarrays are currently being used to identify genetic differences in patients with various types of cancer. Treatment strategies to combat cancer are being designed based on subtle differences in the expression of cancer-causing genes. But significant questions have been raised about the importance of quality control and consistency measures in using microarrays for genetic testing.

Protein microarrays are another relatively new option for disease diagnosis. They are used in much the same way as DNA chips. For instance, these chips can contain hundreds or thousands of antibodies spotted on a chip. By applying blood proteins from a patient, researchers have been able to detect illness indicated by the presence of proteins from disease-causing organisms.

In the next section we consider how biotechnology is creating new products that can be used to treat human disease.

2 Medical Products and Applications of Biotechnology

Identifying novel drugs and developing new ways to treat disease are major areas of medical biotechnology. We have discussed how different proteins—for example, insulin and human growth hormone—produced by recombinant DNA technology have been used to treat human disease conditions. Here we consider a few examples of important products of medical biotechnology that you will hear more about in the future.

The Search for New Medicines and Drugs

It is estimated that cancer may soon surpass cardiovascular disease as the leading cause of death in the United States. But many promising breakthroughs on the horizon may empower doctors by providing them with new strategies for treating different types of cancer.

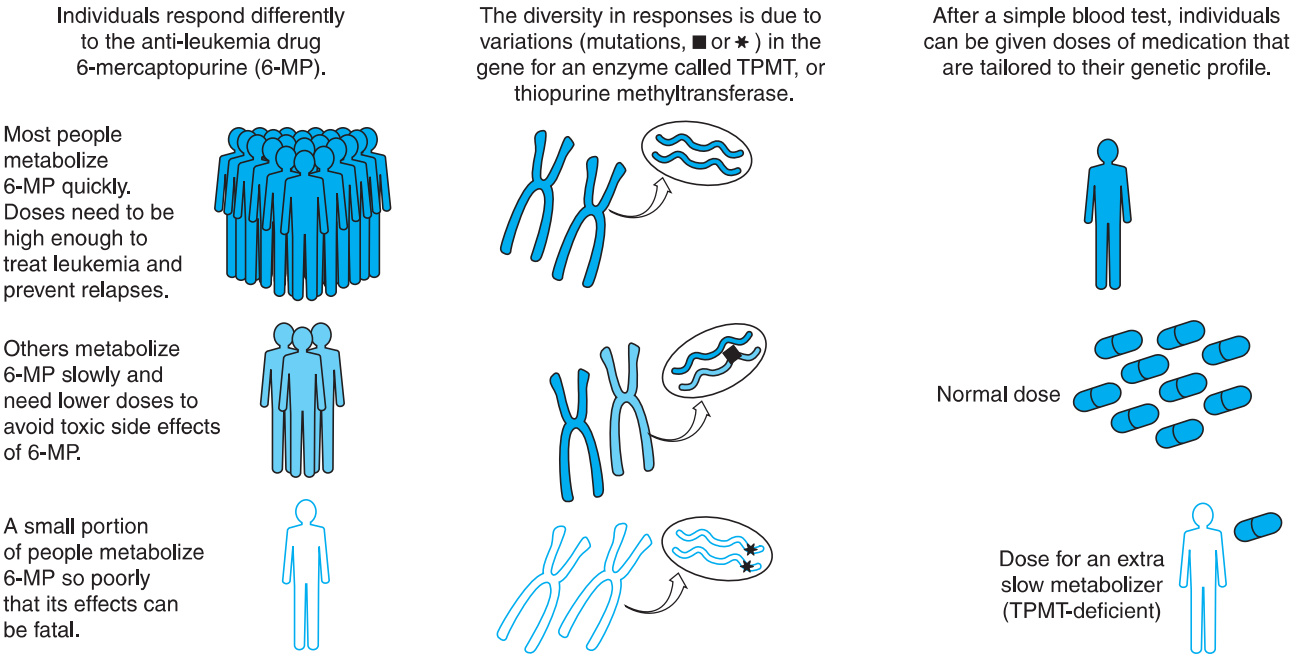


FIGURE 8 **Pharmacogenomics** Different individuals with the same disease often respond differently to a drug treatment because of subtle differences in gene expression. The dose that works for one person may be toxic for another—a basic problem of conventional medicine. This example shows patients' responses to a chemotherapy compound called 6-mercaptopurine (6-MP), which has long been used to treat children with acute lymphocytic anemia (ALL), a form of blood cancer. Since it was found that genetic variations of the gene for the 6-MP-degrading enzyme thiopurine methyltransferase (TPMT) are important for determining how patients respond to 6-MP, physicians now routinely run genetic tests (or blood tests to measure the enzyme levels) on ALL patients before determining the proper dosage of 6-MP.

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Scientists are investigating many of the genes involved in the growth of cancer cells, including genes called **oncogenes**. They produce proteins that may function as transcription factors and receptors for hormones and growth factors; they also serve as enzymes that in many ways help to change the growth properties of cancer-causing cells. Scientists are also actively studying tumor suppressor genes, which produce proteins that can keep cancer formation in check.

Oncogenes and tumor suppressor genes are getting so much attention because researchers are working on ways to make proteins encoded by these genes as targets for *small molecule inhibitors*—drugs that can bind to proteins and block their function. Similarly, researchers are working on drugs that can serve as "activators," binding to and stimulating important proteins that may be used to fight disease. In addition to small molecule drugs, there is a great deal of research designed to personalize medicine and improve drug delivery.

Pharmacogenomics for personalized medicine

The Human Genome Project and discovery of SNPs is partially responsible for a newly emerging field called **pharmacogenomics**: it is customizing medicine by designing effective drug therapy and treatment strategies based on the specific genetic profile of a particular patient. Pharmacogenomics is based on the idea that individuals can react differently to the same drugs, which can have varying degrees of effectiveness and side effects in part because of genetic polymorphisms (**Figure 8**). Each year in the United States alone, over 100,000 deaths occur from the adverse effects of properly prescribed medications. It is unclear whether pharmacogenomics will be a cost-effective approach to medicine; nonetheless, this area of medical biotechnology holds great potential and has already demonstrated success in treating some conditions.

Many drugs currently used in **chemotherapy** can be effective against cancer cells because these drugs target rapidly dividing cells; however, such drugs also affect normal body cells that regularly reproduce, such as hair and skin cells and cells in the bone marrow, the last of which are responsible for making blood cells. As a result, hair loss, dry skin, changes in blood cell counts, and nausea are all related to the ways in which chemotherapy affects normal cells. Researchers have

TOOLS OF THE TRADE

Using the Internet to Learn about Human Chromosomes and Genes

We have discussed the importance of bioinformatics for analyzing genetic information and creating databases as tools that scientists around the world can use to compile, share, and compare DNA sequence information. The wealth of freely available databases that catalog information about human chromosomes and genes resulting in part from the Human Genome Project is a great example. An excellent way to learn about what the Human Genome Project has revealed is to review some of the chromosome maps available on the Web. For instance, if you are interested in the Y chromosome, you could review maps and descriptions of the genes found on it to find out why this chromosome is partially responsible for making male humans.

We encourage you to use the websites mentioned here and available through the Companion Website to follow a chromosome or gene of interest for a few months to see what kinds of information you can uncover. These sites are great resources and among the best sites for learning about human disease genes and chromosome maps.

You can, for example, use them to learn more about a rare disease gene related to a disease condition affecting someone close to you. These sites present up-to-date information that cannot be found in even the most recent books. If you cannot find a gene of interest at these sites, it probably has not been identified yet! The Department of Energy's Human Genome Program Information site provides excellent chromosome maps of identified genes and good historical information about the Human Genome Project. The Online Mendelian Inheritance in Man site (OMIM) is a great database of human genes and genetic disorders. In the keyword box, type in the name of a gene or disease that interests you. For example, type in "breast cancer" and then click the search button. When the next page appears, you will see a list of genes implicated in breast cancer, along with corresponding access numbers highlighted in blue as links. Clicking on one of the links will take you to a wealth of information about that gene, including background information, links to scientific papers about the gene, gene maps, and even nucleotide and protein sequence data (when available). You might also want to search this site to see if a gene has been found for a particular behavioral condition (for example, alcoholism or depression). The National Center for Biotechnology Information (NCBI) sponsors the Genomes and Maps website, which allows you to access detailed maps of chromosomes and disease genes using a feature called Map Viewer.

been looking for "magic bullet" drugs that destroy only cancer cells without harming normal cells. If such drugs were designed, patients might get well faster because the drugs would have little or no effects on normal cells in noncancerous tissues.

Consider the following example. Breast cancer is a disease that shows familial inheritance for some women. Women with defective copies of the genes called *BRCA1* or *BRCA2* have an increased risk of developing breast cancer, but many other cases of breast cancer do not exhibit a clear mode of inheritance. If a woman has a breast tumor thought to be cancerous, a small piece of the tissue could be used to isolate RNA or DNA for SNP and microarray analysis, which could then serve to determine which genes are involved in this particular woman's form of breast cancer. Armed with this genetic information, a physician could design a drug treatment strategy—based on the genes involved—that would be *specific* and *most effective* against this woman's cancer. A second woman with a different genetic profile for her breast cancer might undergo a different treatment.

Scientists at Genentech used this strategy to develop **Herceptin**, a type of monoclonal antibody (discussed in the next section) approved by the FDA in 1998. Herceptin binds to and inhibits HER-2, a protein produced by the human epidermal growth factor receptor 2 gene, which is overexpressed in about 25% to 30% of breast cancer cases. Women with HER-2–positive tumors (HER-2 overexpression) typically develop aggressive breast cancer with a greater likelihood of metastasis (spreading) and poorer prognosis for survival. Herceptin has proven to be effective in some women, but in others tumors become resistant to the antibody. A similar problem has occurred with other pharmacogenomics drugs developed for treating other cancers.

One of the first successful examples of pharmacogenomics involved a drug called **Gleevec,** introduced by Novartis in 2001 and used to treat chronic myelogenous leukemia (CML), the condition discussed in Figure 4. Gleevec targets the BCR-ABL fusion protein, which is created by an exchange of DNA between chromosomes 9 and 22 that occurs in CML; in doing so, Gleevec has proven to be a relatively effective way of treating the

disease. Gleevec and related drugs have increased the survival rate of CML patients from 30% to nearly 90%. Visit the Howard Hughes Medical Institute website listed on the Companion Website and check out the animations link for a good way to observe the action of Gleevec.

Gene expression data from DNA microarrays are being used to diagnose patients based on the genes they express; then patients use pharmacogenomics treatment (if available) based on those genes. **Figure 9** shows an example of microarray data for individuals diagnosed with different forms of leukemia. Notice how the patients can be grouped into different genetic categories of leukemias based on the clusters of genes most actively expressed. Because each group of patients expressed large numbers of different genes and proteins, there is no reason to think that they would all respond well to the same chemotherapy. Knowing this, different chemotherapy approaches can be customized for each category of patients. As a result, patient survival rates have been greatly increased. A similar approach has been used for breast cancer patients, and it is expected that microarray data and pharmacogenomics approaches will increasingly become routine aspects of disease diagnosis and treatment. Several promising clinical trials are under way for pharmacogenomic treatments of melanoma and many other cancers.

It will also be interesting to follow what happens with personalized genomics, the ability to have individual genomes sequenced, and the impact this will have on pharmacogenomics. Similarly, the epigenome is being analyzed for its role in diseases and as a target for new treatment approaches.

Nanotechnology and nanomedicine: Biotechnology at the nanoscale

Nanotechnology is an area of science involved in designing, building, and manipulating structures at the nanometer scale. A nanometer (nm) is one billionth of a meter. For reference, a human hair is approximately 200,000 nm in diameter, DNA is about 2 nm in diameter, and bonds between many atoms are around 0.15 nm long. Nanotechnology is a big business, with applications in materials manufacturing, energy, electronics, and engineering, but **nanomedicine**—applications of nanotechnology for improving human health—is of particular interest for medical biotechnology. Scientists envision tiny devices in the body carrying out a myriad of medical functions, including nanodevice sensors

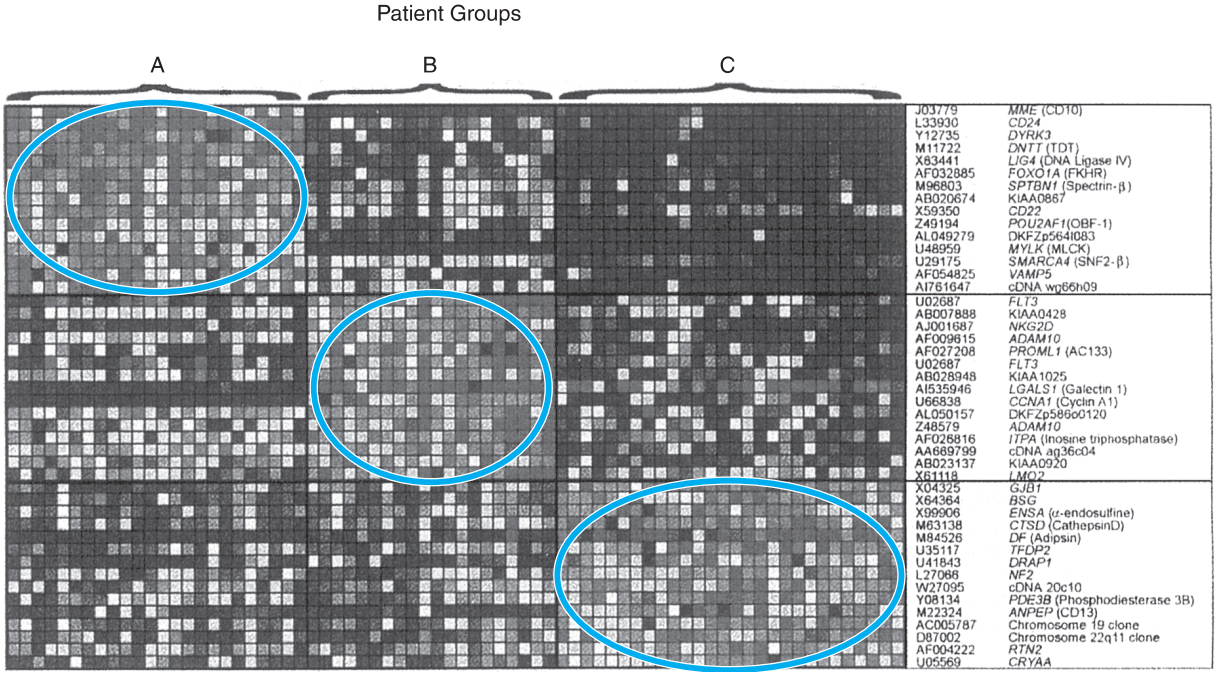

FIGURE 9 Microarrays for Gene Expression Analysis and Pharmacogenomics Shown here are microarray results indicating gene expression profiles in leukemia patients. Each column represents data for one patient, and each row is a different gene (gene symbols and names are on the far right side of the figure). Because this black-and-white image was reproduced from a color image, gray spots shown here represent highly expressed active genes. Red ovals highlight clusters of actively expressed genes (gray and white spots) that can be used to categorize patients into different groupings based on expressed and relatively inactive (dark spots) genes.

DeRisi, J. (1996): Nat. Genet., 14:457–60 adapted for Hollon, T. (2003): The Scientist, Suppl 2, p. 30, 9/22/03 with modification.

YOU DECIDE

Genetic Testing: Issues to Consider

Since genetic testing became available, a number of concerns have been raised about how genetic information could be used and what it may be used for beyond personal and private decisions. Consider some of these issues:

- Should we test unborn children or adults for genetic conditions for which there is currently no treatment or cure?
- What are acceptable consequences if parents learn their unborn child has a genetic defect?
- What are the psychological effects of a false result, which may indicate erroneously that a healthy person has a disease gene or a gene defect that goes undetected in a person with a genetic disorder?
- How do we ensure privacy and confidentiality of genetic information and avoid genetic discrimination? Who should have access to your genetic information? How could your genetic background be used to discriminate against you? How could your health or life insurance company's access to your genetic information affect your premiums? Could your premiums be raised based on "genetic" risk in the same way that premiums are raised based on other risks, such as how old you are and the car you drive?
- What are your obligations to inform others, such as a potential spouse or employer, of your knowledge about a possible genetic disorder?
- If genes are discovered for undesirable human behaviors, how would these genes be perceived in courts of law if accused criminals use genetics as their basis for a plea of not guilty by reason of genetics?
- Would society implement mechanisms to prevent or dissuade individuals with genetic defects from having children?
- Errors in genetic testing can have tragic consequences. Currently there are no federal standards for quality control of genetic testing in the United States. Should mandatory proficiency testing be a requirement to minimize errors?

As you can see, genetic testing is certainly not without its controversies and limitations, and there are few easy answers to these issues. Visit the "Your Genes, Your Choices" website listed on the Companion Website for a thought-provoking series of ethical dilemmas created by genetic testing and genetic technologies. What would you do if you had to face the scenarios presented at this site? You decide.

to monitor blood pressure, blood oxygen levels, and hormone concentrations as well as nanoparticles that can unclog blocked arteries and detect and eliminate cancer cells.

Many companies are working on nanotechnologies to develop innovative ways to improve drug delivery techniques and maximize their effectiveness. Sometimes even a well-designed drug is not as effective as desired because of delivery problems—getting the drug to where it must function. For instance, if a drug to treat knee arthritis is taken as an oral pill, only a small amount of the drug will be absorbed by the body and transported via blood to tissues of the knee joint. Other factors that influence drug effectiveness are a drug's solubility (its ability to dissolve in body fluids), drug breakdown by body organs, and drug elimination by the liver and kidneys.

Microspheres, nanoparticles between 1 to 100 nm in size that can be filled or coated with drugs, may be one way to improve drug delivery and effectiveness. These particles are often made of lipid materials that closely resemble the phospholipids in cell membranes. Delivery of microspheres as a mist sprayed into the airways through the nose and mouth has been used successfully for treating lung cancer and other respiratory illnesses such as asthma, emphysema, tuberculosis, and flu (**Figure 10**). Refer to Section 3 for a discussion of how microspheres called liposomes are used in gene therapy. Researchers are also investigating ways to package anticancer drugs into microspheres for implantation in the body adjacent to growing tumors; they are also working on anesthetics for pain management and adding microspheres to wafers and patches that can be used for drug delivery.

In 2006, the FDA approved Exubera, an inhalable version of insulin produced by Nektar Therapeutics of San Francisco and sold by the pharmaceutical giant Pfizer. Exubera is a recombinant form of insulin delivered as an inhalable powder; it offers diabetic patients the first alternative to needle-based delivery of insulin. But after barely a year, Pfizer stopped selling Exubera because of slow sales. Among the reasons that have been given for Exubera's failure are the unwillingness of physicians and patients to try something new, a bulky inhaler used to deliver the particles, and a poor marketing strategy.

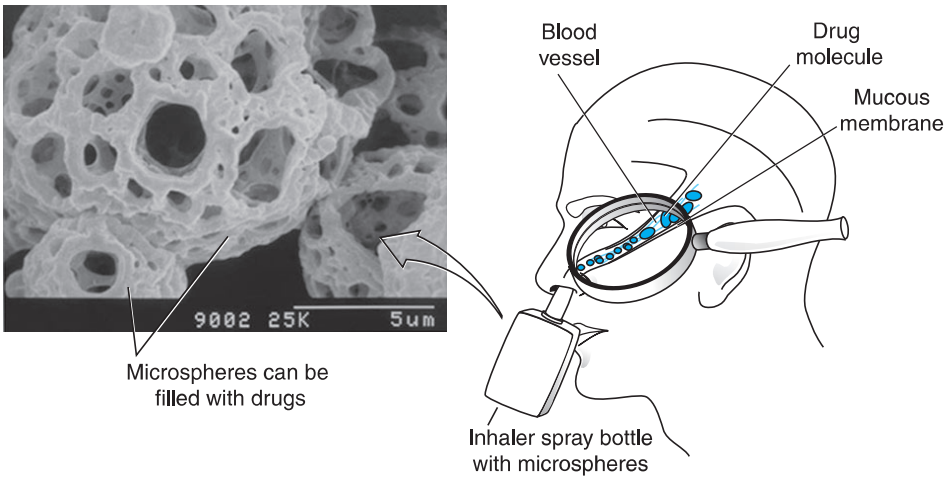

FIGURE 10 Microspheres for Drug Delivery Microspheres can deliver drugs to specific locations in the body or be distributed throughout the body depending on how they are used. In this example, drug-containing microspheres are sprayed into the nose, where they will enter blood vessels and rapidly enter the bloodstream to travel throughout the body.

Robert S. Langer and Kenneth J. Germeshausen.

Currently over a dozen nanoparticle-based drugs are in clinical trials in the United States, and most of these target cancers. Several nanotechnology-enabled drugs are making their way to the market, primarily in areas of cancer treatment, and over 150 nanotechnology cancer therapies are in development. Scientists have developed "smart drugs" using viruses or tiny nanoparticles such as gold particles that are introduced into the body to seek out and target viruses or specific cells, such as cancer cells; they then deliver a cargo intended to treat or destroy those cells rapidly, effectively, and silently, with few side effects (**Figure 11**). Some of these ideas have been tested in patients, with promising results, and nanotechnologists are optimistic about the powers of this technology.

Artificial Blood

Since the 1930s, blood transfusions have been performed routinely and successfully in the United States. Transfusions are often necessary for treating trauma victims, providing blood during surgeries, and treating people with blood-clotting disorders such as hemophilia. In the 1980s, the realization that HIV had contaminated many blood supplies led to new testing techniques that have made blood supplies much safer. Blood donated for transfusions is tested for pathogens such as HIV and hepatitis viruses B and C before it is stored, but donated blood has a shelf life of only a few months and must be refrigerated. Throughout many areas of the world, particularly in developing countries where screening procedures are not very good, there is a serious need for safe blood, free of infectious bacteria and viruses. These and other concerns have prompted scientists to seek ways to develop artificial blood or blood substitutes.

Major advantages of artificial blood could include a disease-free alternative to real blood, a constant supply of blood in the face of blood shortages and emergency situations, and a supply of blood that can be stored for long periods of time. Also, unlike donated blood, synthetic blood would not have to be matched to the recipient's blood type to avoid rejection by the immune system. A major limitation in the development of synthetic blood to date is that artificial bloods have been designed to serve the primary task of normal red blood cells—transporting oxygen to body tissues, a role carried out by the oxygen-carrying protein hemoglobin. Red blood cells are literally hemoglobin factories. But normal red blood cells perform other

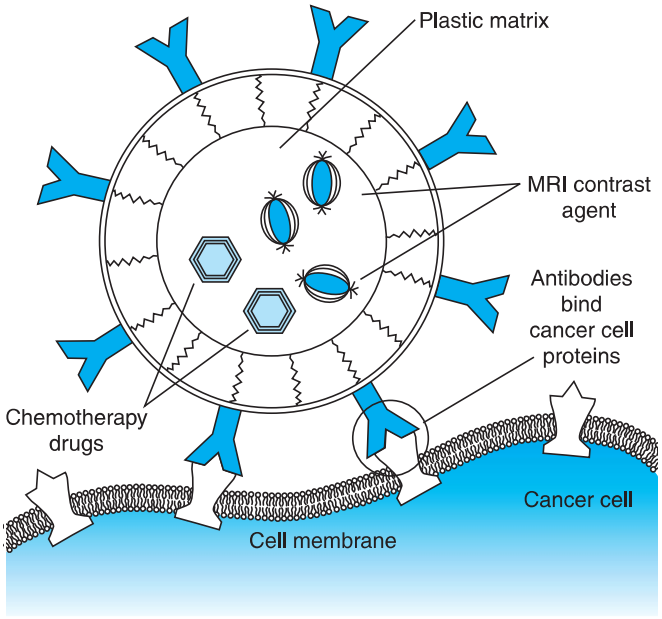

FIGURE 11 Tumor-Seeking and Tumor-Killing Nanoparticles Multifunctional nanoparticles have been developed that have shown promise for seeking out and destroying tumor cells. This example shows a plastic nanoparticle covered with antibodies against cancer cell proteins, which allow the particle to bind to cancer cells. Inside the particle are contrast agents, which can be used for magnetic resonance imaging or x-ray approaches to detect the tumor. The nanoparticle can also contain chemotherapy drugs that can diffuse out of it to kill tumor cells.

From "Nanotechnology takes aim at cancer" by R.F.Service. From *Science* 310: 1132–1134, November 2005. Reprinted with permission from American Association for the Advancement of Science.

YOU DECIDE

Direct-to-Consumer Genetic Tests

The past decade has seen dramatic developments in **direct-to-consumer (DTC) genetic tests**. A simple web search will reveal many companies offering such tests, and there are approximately 1,900 diseases for which such tests are now available (in 1993 there were about 100 such tests). Most DTC tests require that a person mail a saliva sample, hair sample, or cheek cell swab to the company. For a range of pricing options, DTC companies largely use SNP-based tests such as ASO tests to screen for different mutations. For example, in 2007 Myriad Genetics, Inc. began a major DTC marketing campaign of its tests for *BRCA1* and *BRCA2*. Mutations in these genes increase risk of developing breast and ovarian cancer. DTC testing companies report absolute risk, the probability that an individual will develop a disease; but how such risks results are calculated is highly variable and subject to certain assumptions.

Such tests are controversial for many reasons. For example, the test is purchased online by individual consumers and requires no involvement of a physician or other health care professionals, such as a nurse or genetic counselor, to administer or to interpret results. There are significant questions about the quality, effectiveness, and accuracy of such products because the DTC industry is currently largely self-regulated. The U.S. Food and Drug Administration (FDA) does not regulate DTC genetic tests. There is currently no comprehensive way for patients to make comparisons and evaluations about the range of tests available and their relative quality.

Most companies make it clear they are not trying to diagnose or prevent disease, nor are they offering health advice. So what is the purpose of the information that these tests provide? Websites and online programs from DTC companies provide information on what advice a person should pursue if positive results are obtained. But is this enough? If the results are not understood, might negative tests provide a false sense of security? Just because a woman is negative for *BRCA1* and *BRCA2* mutations *does not* mean that she cannot develop breast or ovarian cancer.

In June 2010, the FDA announced that five genetic test manufacturers (Illumina, Pathway Genomics, NaviGenics, 23andMe, deCODE Genetics) would need FDA approval before their tests could be sold to consumers. This action was prompted when Pathway Genomics announced plans to market a DTC kit for "comprehensive genotyping" in the pharmacy chain Walgreens. Pathway Genomics and the other companies have been selling their DTCs through company websites for several years. Pathway and others claim that because their DTC kits are approved by the Clinical Laboratory Improvement Amendments (CLIA), no further regulation is required. CLIA regulates certain laboratory tests but is not part of the FDA. This scenario in particular prompted discussion on how the FDA will oversee DTC genetic tests. However, at the time of publication of this edition, the FDA has not revealed any definitive plans to regulate or oversee DTC genetic tests. There are varying opinions on the regulatory issue. Some believe that the FDA has no business regulating DTC tests and that consumers should be free to purchase products according to their own needs or interests. Others insist that the FDA must regulate DTCs in the overall interest of consumers.

In 2010, the National Institutes of Health announced that it will create a **Genetic Testing Registry (GTR)** designed to increase transparency by publicly sharing information about the utility of their tests and research with the general public, patients, health care workers, genetic counselors, insurance companies, and others. The GTR is intended to allow individuals and families access to key resources to let make them better-informed decisions about their health and genetic tests. But participation in the GTR by DTC companies has not been made mandatory yet. Therefore will companies involved in genetic testing participate? Should DTC genetic tests be more carefully regulated by the FDA? You decide.

functions as well, such as providing the body with a source of iron, and hemoglobin is also important for removing carbon dioxide from the body. Researchers have yet to create blood substitutes that can perform all the functions of normal blood; nevertheless, many promising products are under development.

So how is artificial blood made? Artificial bloods are cell-free solutions containing molecules that can bind to and transport oxygen in much the same way as normal hemoglobin. Some blood substitutes are made from the hemoglobin of cattle; others are made from human hemoglobin. Cow blood is collected from food cattle at slaughterhouses and then processed to purify the hemoglobin. Many other types of artificial blood being tested are produced using fluorocarbons, chemicals that can bind oxygen just as hemoglobin does and then release oxygen to the surrounding tissues. Ultimately, artificial blood products must provide safe alternatives to real blood transfusions. Much work remains to be done, but the potential benefit of these products has many companies investing large amounts of money and time to develop viable blood substitutes.

Vaccines and Therapeutic Antibodies

Vaccines can be used to stimulate the body's immune system to produce antibodies and provide a person with protection against infectious microbes. Certainly vaccination has been very effective for protecting us from pathogens that cause polio, tetanus, typhoid, and dozens of others. Development of vaccines against some of the most deadly pathogens is a very active and important area of research.

Many scientists hope that vaccination may be useful against conditions such as Alzheimer disease and many different types of cancers, but vaccination for these purposes is still mostly unproven in humans. Cancer vaccines are being experimented with as therapeutic treatments that are not preventative but designed to treat a person who already has cancer. In this approach, a person is injected with cancer cell antigens in an effort to stimulate the patient's immune system to attack existing cancer cells. In fact, there is considerable excitement about new types of "naked DNA" vaccines in which plasmid DNA-encoding genes that produce antigens are injected directly into tissue, where cells take up the plasmid and express the antigens that stimulate antibody production by the body. In other types of DNA vaccines, an immune response is mounted against the DNA itself.

The primary purpose of vaccination is to stimulate antibody production by the immune system and thus to help ward off foreign materials. However, antibodies themselves might be used to treat an existing condition as opposed to preventing infectious microbes from causing disease. Using antibodies in some types of therapy makes good sense because antibodies are very specific for the molecules or pathogens to which they are produced and can find and bind to their target with great affinity. Since their development in 1975, **monoclonal antibodies (MAbs)**, purified antibodies that are very specific for certain molecules, have been considered "magic bullets" for disease treatment. To make a MAb, a mouse or rat is injected with the purified antigen to which researchers are trying to make antibodies. Figure 12 shows production of MAbs specific for proteins from human liver cancer cells. After the mouse makes antibodies to the antigen, a process that usually takes several weeks, the animal's spleen is removed. The spleen is a rich source of antibody-producing B lymphocytes, commonly called B cells. In a culture dish, B cells are mixed with cancerous cells, called myeloma cells, which can grow and divide indefinitely. Under the right conditions, a certain number of B cells and myeloma cells will fuse together to create hybrid cells called **hybridomas**.

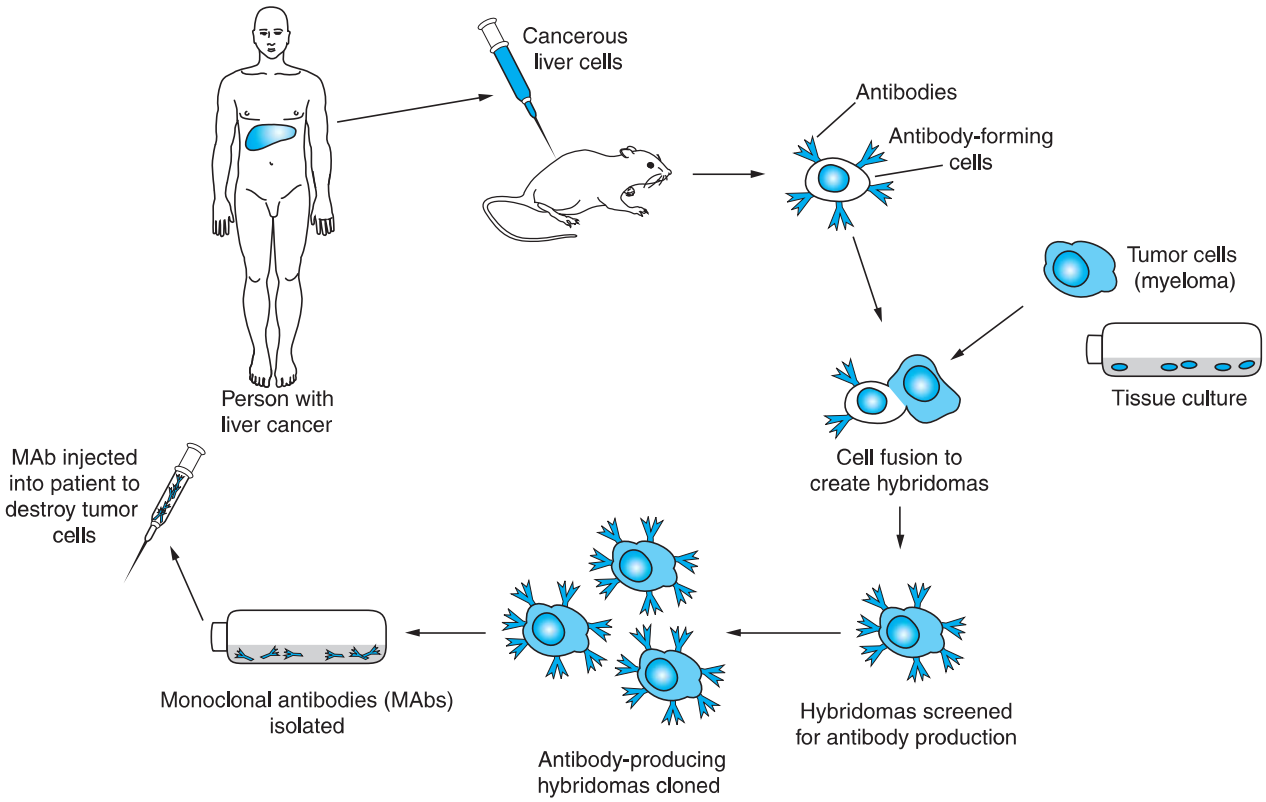

FIGURE 12 **Making Monoclonal Antibodies**

Hybridoma cells grow rapidly in liquid culture because they contain antibody-producing genes from B cells. These cells are literally factories for making antibodies. Hybridoma cells secrete antibodies into the liquid culture medium surrounding the cells. Chemical treatment is used to select for hybridomas and discard unfused mouse and myeloma cells, so that researchers have pure populations of fused, antibody-producing cells. Hybridomas can be transferred to other culture dishes and frozen at ultralow temperatures so that a permanent stock of cells is always available. Antibodies can be isolated from hybridoma cultures in large batches by growing hybridoma cells in batch culture using bioreactors.

Monoclonal antibodies can be injected into patients to seek out and target the antigens to which the MAbs were produced. The MAbs in Figure 12 would bind to liver cancer cells and work on destroying the tumor. In 1986, the FDA approved the first monoclonal antibody, OKT3, which was used to treat organ transplant rejection. In the 1990s, MAbs were developed to treat breast cancer (Herceptin) and lymphoma (Rituxan). There are currently over a dozen MAbs being used worldwide to treat cancer, cardiovascular disease, allergies, and other conditions. Scientists even envision attaching chemicals or radioactive molecules to MAbs in the hope that these might target damaged or cancerous cells and use their payloads to kill these cells. Therapeutic antibody strategies may also be of value for treating people addicted to harmful drugs, such as cocaine and nicotine. In the United States alone, more than 13 million people abuse drugs. Scientists believe that it may be possible to stimulate antibody production to drugs such as cocaine. These antibodies would then bind to the drug as the antigen, trapping and preventing the drug from affecting brain cells. Monoclonal antibodies have also been used for several years in common tests for conditions such as strep throat, and most home pregnancy kits use MAbs to detect hormones produced during pregnancy. Monoclonals for disease treatment have still not lived up to their initial hype, and there have been some setbacks in the field. For example, MAb treatment of Alzheimer patients produced severe inflammation in several people due to a human antimouse antibody response. Humanizing antibodies can alleviate some of the problems with MAbs. Increasingly it appears that MAbs will continue to be valuable tools for medicine in the twenty-first century. In the next section we consider gene therapy, a promising and controversial topic of medical biotechnology.

3 Gene Therapy

Gene therapy involves the delivery of therapeutic genes into the human body to correct disease conditions created by a faulty gene or genes. Think about the awesome power and potential of gene therapy—providing a person with normal genes to supplement defective genes and cure disease or even using normal genes to replace faulty ones. Here we provide an overview of gene therapy strategies for treating and attempting to cure disease.

How Is It Done?

The two primary strategies for gene delivery are **ex vivo gene therapy** and **in vivo gene therapy** (Figure 13). In ex vivo therapy (*ex* means "out of," *vivo* is Latin for "something alive"), cells from a person with a disease condition are removed from that person, treated in the laboratory using techniques similar to bacterial transformation, and then reintroduced into them into him or her. Technically speaking, intro-

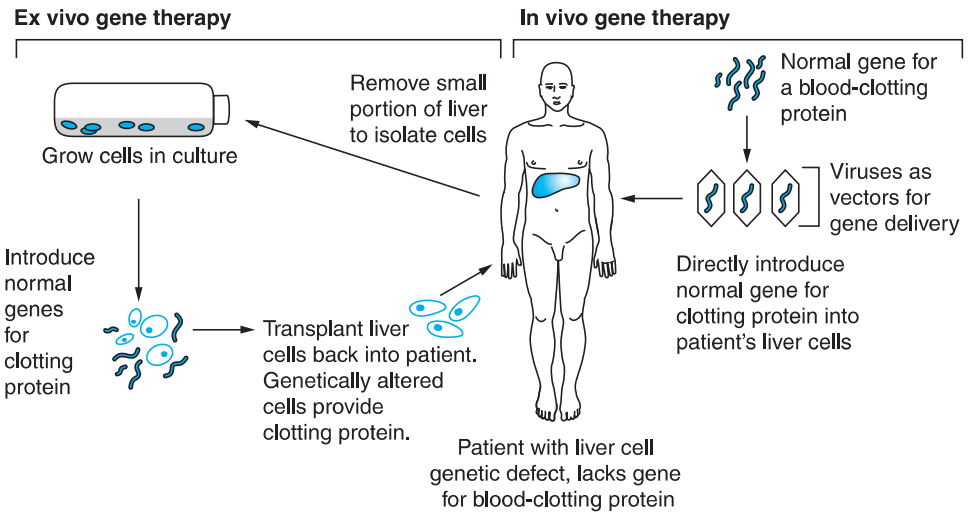

FIGURE 13 Ex Vivo and in Vivo Gene Therapy in a Patient with a Liver Disorder Ex vivo gene therapy involves isolating cells from the patient, introducing normal genes for the clotting protein into these cells, and then transplanting cells back into the body where these cells will produce the required clotting protein. In vivo gene therapy involves introducing DNA directly into cells while they are in the patient. In either mode of gene therapy, genes may be introduced into cells as DNA packaged into viruses as vectors or as naked DNA.

ducing DNA into animal or plant cells is called **transfection**. For instance, liver cells from a patient suffering from a liver disorder would be surgically removed and cultured. Appropriate therapeutic genes would then be delivered into these cells using vectors and other approaches discussed in the next section. These genetically altered liver cells would then be transplanted back into the patient without fear of rejection of the tissue transplant because these cells came from the patient initially (Figure 13).

In vivo gene therapy does not entail removal of a patient's cells; DNA is introduced directly into cells and tissues in the body (Figure 13). One challenge of in vivo gene therapy is delivering genes only to the intended tissues and not to tissues throughout the body. Scientists have primarily relied on using viruses as vectors for gene delivery, but in some cases genes have been directly injected into some tissues. So far, ex vivo strategies have generally proven to be more effective than in vivo approaches.

Delivering the payload: Vectors for gene delivery

A major challenge that must be overcome if gene therapy is to become a reliable tool for treating disease is achieving a safe and effective delivery of therapeutic genes—the payload. Depending on the genetic condition to be treated, some therapeutic strategies may require long-term expression of a corrective gene, whereas others may require rapid expression for shorter periods of time. A majority of gene delivery strategies, both ex vivo and in vivo, rely on viruses as vectors to introduce therapeutic genes into cells.

A viral vector uses a viral genome to carry a therapeutic gene or genes to "infect" human body cells, thereby introducing the therapeutic gene. Scientists have considered various viruses such as **adenovirus**, which causes the common cold, a related virus called **adeno-associated virus (AAV)**; influenza viruses, which cause the flu; and herpes viruses, which can cause cold sores and some cause sexually transmitted diseases, as potential **vectors** for gene delivery. Even human immunodeficiency virus-1 has been considered as a gene therapy vector. For any viral vector to work, scientists must be sure that these vectors have been genetically engineered and inactivated so they neither produce disease nor spread throughout the body and infect other tissues.

Most viruses infect human body cells by binding to and entering cells and then releasing their genetic material into the nucleus or cytoplasm of the human cell. This is usually DNA, but some viruses contain an RNA genome. The infected human cell then serves as a host for reproducing the viral genome and producing viral RNA and proteins. Viral proteins ultimately assemble to create more viral particles that break out of the host cells so they are free to infect other cells and repeat the life cycle.

It may seem strange that viruses would even be considered for carrying genes to cure human diseases. However, since viruses are very effective at introducing their genomes into cells, scientists reasoned that if viruses could be disabled so that they would not cause disease and genetically altered to deliver therapeutic genes safely, we could use viruses for beneficial purposes. In many ways viruses are perfectly designed as gene therapy vehicles or vectors for gene delivery. For instance, adenovirus—which approximately 80% to 90% of the population has been infected with in childhood because it causes the common cold—can infect many types of body cells fairly efficiently. Retroviruses such as **lentivirus,** including even HIV, are of interest as vectors because, on entering a host cell, they copy their RNA genome into DNA and then randomly insert their DNA into the genome of the host cell, where it remains permanently, a process called **integration**. A main reason why retroviruses are used for gene delivery is that they can integrate therapeutic genes into the DNA of human host cells, allowing permanent insertion of genes into the chromosomes of a patient's cells as a way of providing lasting gene therapy.

Viruses have also been widely studied as gene therapy vectors because some viruses infect only certain body cells. This might allow for *targeted* gene therapy—the ability to deliver genes only to the tissues infected by a certain virus. For instance, a strain of herpesvirus (HSV-1) primarily infects cells of the central nervous system. This strain is a candidate for targeted gene delivery to cells of the nervous system, which may be an effective way to treat genetic disorders of the brain such as Alzheimer disease and Parkinson disease. Researchers are also investigating ways that the genetically altered herpes viruses can be used to destroy brain tumors. Preliminary trials of this approach in humans have shown some promise.

Most human cells do not take up DNA easily. If they did, it would then be possible to transfect cells by simply mixing them with DNA in a tube in much the same way that transformation is achieved with bacterial cells. However, some success has been demonstrated for both in vivo and ex vivo strategies using "naked" DNA. Naked DNA is simply DNA by itself, without a viral vector, which is injected directly into body tissues. Small plasmids containing therapeutic genes are often used for this approach. Cells of certain tissues will take in some of the naked DNA and express genes delivered in this way.

Delivery techniques for naked DNA have been somewhat effective in the liver and in skeletal muscle. One of the major problems with transfecting human cells in vivo is that because a relatively small number of cells take up the injected DNA, there may not be enough

cells expressing the therapeutic gene for gene therapy to have any effect on the tissue. Scientists are working on ways to overcome these problems and deliver naked DNA more effectively. For example, electroporation (recall that electrical stimulation is used to move plasmids into bacterial cells) can be used to stimulate movement of DNA into cells.

One approach to deliver DNA without viral vectors involves **liposomes**, small-diameter hollow microspheres made of lipid molecules, similar to the fat molecules in cell membranes. Liposomes are packaged with genes and then injected into tissues or sprayed onto them. A similar technique involves coating tiny gold nanoparticles with DNA and then shooting these into cells using a DNA gun, a pressurized air gun that delivers gold or liposome particles through cell membranes without killing most cells. Biodegradable gelatin particles are also being studied as gene-carrying vectors. Short-term expression of genes through "gene pills" is being explored. In this approach, a pill delivers DNA to the intestines, where it is absorbed by intestinal cells; these then express therapeutic protein encoded by the DNA and secrete these proteins into the bloodstream.

Antisense RNA technology and RNA interference for gene therapy

We have discussed the use of **antisense RNA technology** as a way to block translation of mRNA molecules to silence gene expression. This approach was used to create the Flavr Savr tomato. The basic concept of antisense RNA technology is to design an RNA molecule that will serve as a complementary base pair to the mRNA you want to inhibit, thus blocking it from being translated into a protein (**Figure 14**). This approach for shutting off a gene is frequently called **RNA** or **gene silencing**. Since the development of antisense RNA technologies in the 1970s, scientists have thought that RNA silencing approaches would be promising ways to turn off disease genes as a gene therapy approach.

Antisense RNAs have been effectively used for gene silencing in cultured cells, but this technology has yet to live up to its promise as a treatment for disease. The recent emergence of **RNA interference (RNAi)** as a method to control mRNA stability and protein synthesis has reinvigorated gene therapy approaches by gene silencing. With RNAi, double-stranded RNA molecules are delivered into cells where the enzyme Dicer chops them into 21-nt-long pieces called **small interfering RNAs (siRNAs;** Figure 14). The siRNAs then join with an enzyme complex called the **RNA-induced silencing complex (RISC)**, which shuttles the siRNAs to their target mRNA, where they bind by complementary base pairing. The RISC complex degrades the siRNA-bound mRNAs so they cannot be translated into protein. Using gene therapy to stimulate naturally occurring **microRNAs (miRNAs)** that inhibit gene expression is another area of active investigation. A main challenge to RNAi-based therapeutics so far has been sustained in vivo delivery of the antisense RNA, dsRNA, miRNA, or siRNA to the target tissues. RNAs degrade quickly in the body. It is also hard to get them to penetrate cells and to target the right tissue. Two common delivery approaches are to inject the antisense RNA or siRNA directly or as a plasmid that is taken in by cells to be transcribed to make antisense or RNAi molecules (Figure 14). Liposome, lentivirus delivery mechanisms, and attachment of siRNAs to cholesterol and fatty acids are also used to deliver silencing RNAs. Another problem is that most complex diseases are not caused by just one gene. For example, as mentioned earlier in this chapter, the gene *BCL-2* is overexpressed in about 50% of breast cancer cases, and antisense RNA technology can silence this gene in vitro; but breast cancer is a multigene disease and it is not yet possible to silence all of the genes involved in it.

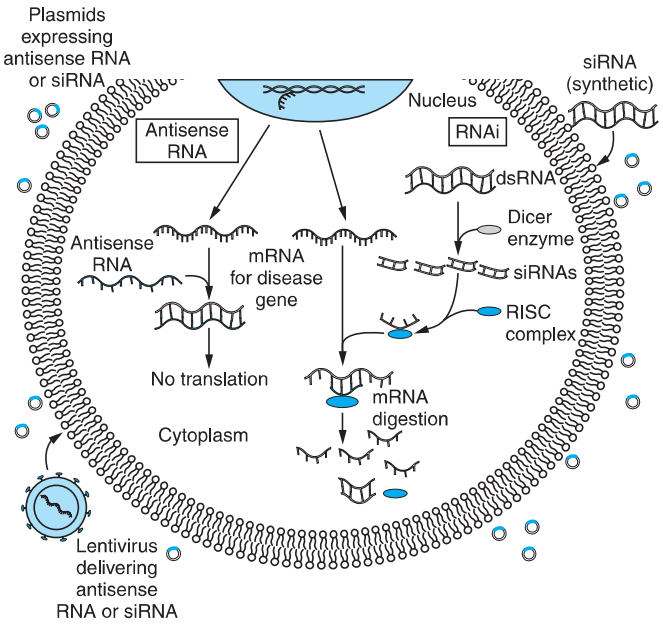

FIGURE 14 Antisense RNA and RNAi Approaches for Gene Therapy by Gene Silencing Antisense technology and RNAi are two ways to silence gene expression and turn off disease genes. In antisense technology (left) an antisense RNA molecule binds to the mRNA for the disease gene and prevents it from being translated into a protein. With RNAi technology, double-stranded RNA (dsRNA) molecules are delivered to the cell. The dsRNAs are then cleaved by Dicer into short interfering RNAs (siRNAs), which are escorted by the RISC protein complex to bind to their target mRNA causing its degradation and thus preventing translation.

More than a dozen clinical trials using RNAi are under way but no RNAi drugs are on the market yet. Several antisense and RNAi clinical trials are under way in the United States for blindness. One study to combat macular degeneration, a form of blindness, attempts to minimize the expression of the *VEGF* gene. The protein encoded by *VEGF* promotes blood vessel growth. Overexpression of this gene leads to excessive production of blood vessels in the retina, which causes impaired vision and eventually blindness. In 2009 a promising RNAi trial for macular degeneration failed in late-stage clinical trials. Others diseases that have been targeted include several different cancers, influenza (scientists have had good success blocking flu infections in the lungs of mice), diabetes, multiple sclerosis, arthritis, and neurodegenerative diseases.

Curing Genetic Diseases: Targets for Gene Therapy

Most gene therapy researchers are focusing on genetic disorders created by single gene mutations or deficiencies—such as sickle cell disease—because in theory these conditions may be easier to cure by gene therapy than genetic diseases involving multiple genes that interact in complex ways. Current estimates indicate there may be more than 3,000 human genetic disease conditions caused by single genes. Table 1 includes some of the diseases that are potential candidates for treatment by gene therapy.

Recent trials for treating deafness, arthritis, melanoma, blindness, AIDS, malignant brain tumors, and other conditions have shown promise. For example, researchers from the University of Pennsylvania used gene therapy to restore retinal cone cell function and day vision in dogs with a condition called congenital **achromatopsia**. This is a rare autosomal recessive condition (1 in 30,000 to 50,000 humans) and affects the cone cells in the retina, which are essential for color vision and some aspects of visual acuity. The therapy cured both young and older canines and appears to be permanent. University of Pennsylvania and Children's Hospital of Philadelphia researchers also reported beneficial results for treatments of **Leber's congenital amaurosis (LCA)**, a degenerative disease of the retina that affects 1 in 50,000 to 100,000 infants each year and causes severe blindness. Young adult patients with defects in the *RPE65* gene were given injections of the normal gene. Complete vision was not restored to these patients, although four of the children who were treated gained enough vision to play sports. Several months after a single treatment with the gene, the patients are still legally blind but they can see more light, some of them can read the lines of an eye chart, and two who had stumbled through an obstacle course were able to navigate it.

Trials using antitumor genes to treat mice with **malignant melanoma**—a cancer of melanin-producing cells called melanocytes, which give the skin its pigmentation—have been successful and similar trials in humans are being planned.

Researchers at the University of Paris and Harvard Medical School have reported that 2 years after gene therapy treatment for ß-thalassemia, a blood disorder that involves a defect in the ß-globin chain of hemoglobin which reduces the production of hemoglobin, a young man no longer needs transfusions and appears to be healthy. A modified, disabled HIV-derived lentivirus vector was used to carry a copy of the normal gene and delivered via blood stem cells. There have also been reports of therapeutic gene integration near a growth factor gene called *HMGA2* that turned this gene on, reminiscent of what occurred in the French X-SCID trials described below. Long-term follow-up will be important to see whether this treatment is effective and safe. Here we consider a few well-studied, successful examples of gene therapy in action.

The first human gene therapy

The first human gene therapy was carried out in 1990 by a group of researchers and physicians at the National Institutes of Health in Bethesda, Maryland, led by W. French Anderson, R. Michael Blaese, and Kenneth Culver. The patient, 4-year-old Ashanti DaSilva, had a genetic disorder called **severe combined immunodeficiency (SCID)**. Patients with SCID lack a functional immune system because of a defect in a gene called adenosine deaminase (*ADA*). *ADA* produces an enzyme involved in metabolism of the nucleotide deoxyadenosine triphosphate (dATP). Mutation of the *ADA* gene results in the accumulation of dATP, which, at high concentration, is toxic to certain types of T cells, resulting in a near-complete loss of these cells in the patient with SCID. This condition is appropriately called "severe combined" immunodeficiency because mutations in the *ADA* gene deliver a knockout blow to the immune system's ability to make antibodies and fight off disease. Without functioning T cells, B cells cannot recognize antigen and make antibodies. Prior to gene therapy, most SCID patients did not live past their teens because their immune systems simply could not fight off infections.

To treat Ashanti, the normal gene for *ADA* was cloned into a vector that was then introduced into a retrovirus. An ex vivo gene therapy approach was used in which a small number of T cells were isolated from Ashanti's blood and cultured in the lab. Her T cells were then infected with the ADA-containing retrovirus, and the infected T cells were further cultured. Because retroviruses integrate their genome into the genome of host cells, the retrovirus was integrating

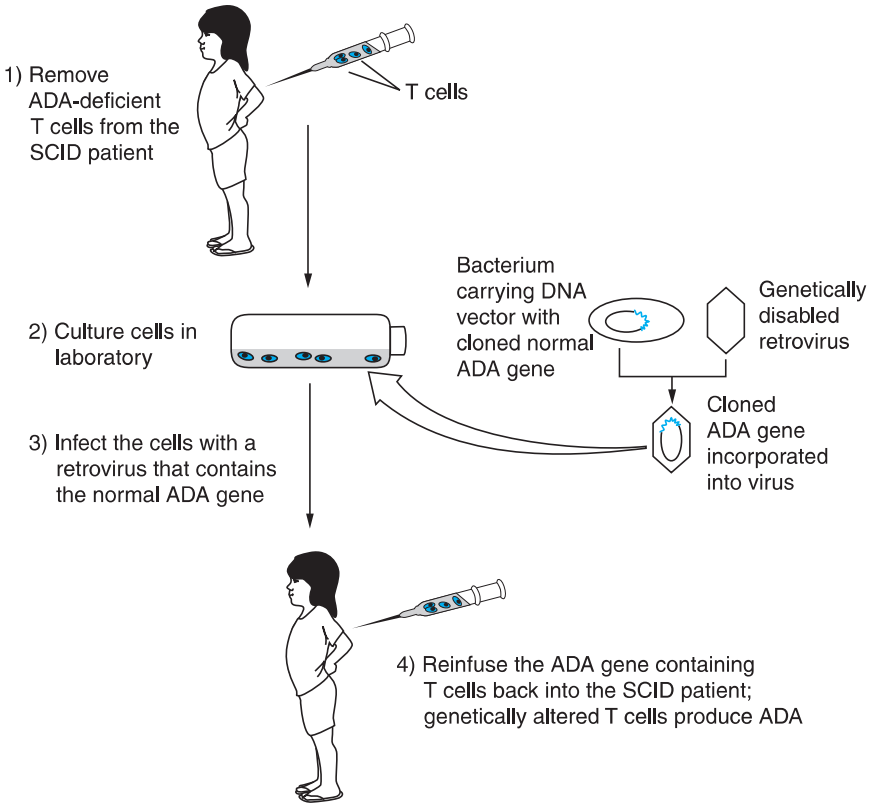

FIGURE 15 The First Human Gene Therapy An ex vivo gene therapy strategy was used in a 4-year-old SCID patient with a defective ADA gene.

the normal *ADA* gene into the chromosomes of Ashanti's T cells during this culturing. After a short period of culturing, these ADA-containing T cells were reintroduced back into Ashanti (Figure 15).

Ashanti received multiple treatments. Within a few months after gene therapy, the T-cell numbers in Ashanti began to increase. After 2 years, Ashanti's ADA enzyme activity was relatively high, and she was showing near-normal T-cell counts with about 20% to 25% of her T cells showing the added ADA gene. Ashanti is currently enjoying a healthy life. Since Ashanti's treatment, gene therapy has successfully restored the immune systems of over two dozen children with SCID.

Treating cystic fibrosis

Cystic fibrosis (CF) is one of the most common genetic diseases. Approximately 1,000 children with CF are born in the United States each year, and currently more than 30,000 people in the United States have been diagnosed with CF. This disease occurs when a person has two defective copies of a gene encoding a protein called the **cystic fibrosis transmembrane conductance regulator (CFTR)**. The normal CFTR protein serves as a pump at the cell membrane to move electrically charged chloride atoms (ions) out of cells. Chloride ions enter cells in a number of ways and are required for many cellular reactions. The CFTR is important for maintaining the proper balance of chloride inside cells. Mutations in the *CFTR* gene, which may cause the total absence of the protein or result in defective protein, are responsible for CF.

The CFTR protein is made by cells in many areas of the body, including the skin, pancreas, liver, digestive tract, male reproductive tract, and respiratory tract (trachea and bronchi). An abnormally functioning or absent CFTR causes an imbalance in chloride ions inside the cells because the defective CFTR does not pump out these ions (Figure 6). In organs such as the trachea, an accumulation of chloride ions in these cells leads to the production of an extremely thick sticky mucus that clogs the airways. This occurs because water moves into chloride-rich cells in an effort to balance chloride concentrations inside the cells. Normally, mucus in the trachea helps sweep dust and particles out of the airways to keep these materials from reaching the lungs. But when water enters tracheal cells, the mucus becomes extremely thick. In addition to clogging the airways, the thick mucus provides an ideal environment for microbes to grow; as a result, patients with CF experience infections from bacteria such as *Pseudomonas*.

Infections of the airways and lungs can lead to pneumonia and respiratory failure, the leading cause of death among patients with CF. There are similar effects

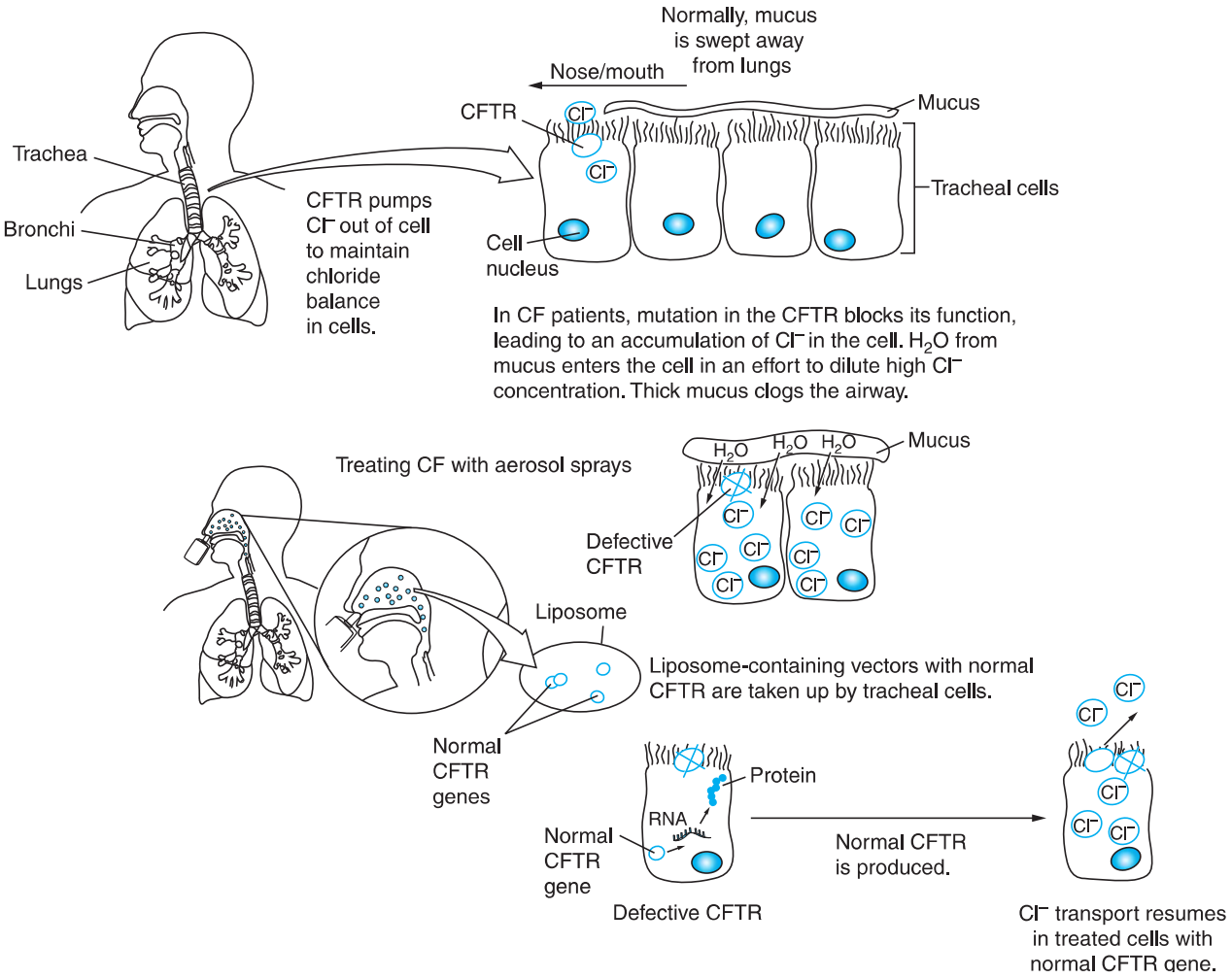

FIGURE 11.16 Treating Cystic Fibrosis by Gene Therapy

in many other organs of the body. Males experience infertility owing to problems related to ion transport in male reproductive organs, and both males and females have extremely salty sweat owing to abnormalities in ion transport in sweat glands. In fact, prior to the discovery of the *CFTR* gene, a sweat test was the standard for diagnosing CF and is still used today.

Treatments for patients with CF vary from back clapping—holding the patient almost upside down and slapping the back—and moving body positions to drain lung mucus, to using drugs to thin the mucus and antibiotic treatment to fight infections. However, there is no cure for CF. This disease often causes death due to lung malfunctions and respiratory infections early in life. Many new treatment strategies have enabled these patients to live to well into adulthood; as a result, the average life span of CF patients has increased from 19 years in the 1980s to over 37 years currently. In 1989, the *CFTR* gene was discovered, and by 1993 scientists had begun gene therapy trials by introducing the normal *CFTR* gene into viral vectors. Recent studies are using liposomes and spraying these into the noses and mouths of CF patients as an aerosol or administering into the airways through a hose (Figure 16). Liposomes fuse with lipids in cell membranes of tracheal cells, releasing the normal *CFTR* gene into the cytoplasm of cells. The normal *CFTR* gene is copied into mRNA and the normal protein translated. The normal CFTR protein enters cell membranes and starts to transport chloride ions out of cells, thereby thinning the mucus and alleviating CF symptoms.

Gene therapy for CF is not yet a reliable cure. It is expensive, requiring multiple reapplications, because DNA delivered via liposomes does not integrate into chromosomes. Each time tracheal cells divide, and they divide rapidly, delivered genes are lost and more spraying is required. Also, *CFTR*-containing liposomes are taken up by a small percentage of tracheal cells. Even cells containing the delivered gene may not produce enough CFTR protein to allow for adequate

transport of chloride ions. Furthermore, there have been problems with the expressed CFTR protein being toxic to cells. In addition, there are now over 1,500 known mutations of the *CFTR* gene, potentially resulting in variations of CF and complicating the administration of gene therapy. Although a gene therapy cure for CF is not yet available, scientists are aggressively moving forward on strategies that may eventually lead to the permanent introduction of the normal gene or correction of the defective *CFTR* in an effort to improve the lives of people with CF.

Challenges Facing Gene Therapy

Scientists have always been concerned about the potential risks associated with gene therapy and the safety of these procedures. Discussions about the safety of gene therapy greatly intensified after 18-year-old Jesse Gelsinger died during a gene therapy clinical trial at the University of Pennsylvania in 1999. Jesse's death was directly attributed to complications related to the adenovirus vector used to deliver therapeutic genes to treat him for a liver disorder (ornithine transcarbamylase deficiency) that affected his ability to break down dietary amino acids. Jesse's death was triggered by a massive inflammatory response to a modified adenovirus vector bearing the *ornithine transcarbamylase* (*OTC*) gene that had been injected into his hepatic artery. The vectors were intended to enter liver cells and result in the production of OTC protein in the hope that this treatment would cure him of his liver disease. Within hours of his first treatment, a massive immune reaction surged through Jesse's body. He developed a high fever, his lungs filled with fluid, multiple organs shut down, and he died 4 days later of acute respiratory failure.

During inquiries into this tragedy, it was learned that clinical trial scientists had not reported other adverse reactions to gene therapy and that some of the scientists involved in this trial were affiliated with private companies that could benefit financially from the trials. It was found that serious side effects seen in animal studies were not explained to patients during informed-consent discussions. The FDA then scrutinized gene therapy trials across the country, halted a number of them, and shut down several. Other research groups voluntarily suspended their gene therapy studies. Over 500 gene therapy clinical trials have been carried out around the world, a majority of these in the United States, and more than 600 trials are ongoing in 20 countries. Jesse Gelsinger was the first person in a gene therapy clinical trial to die as a result of his treatment. His death raised more questions about using viral vectors, placed greater emphasis on the development of nonviral vectors, and called for greater scrutiny of gene therapy and tighter restrictions on gene therapy trials.

In 2002, concerns about gene therapy involving retroviruses were further elevated as a result of trials in France for treating X-linked severe combined immunodeficiency syndrome (SCID-X). In this trial, 3 of 11 children treated developed leukemia because of the therapy, and one of them died in 2004. They had received injections of bone marrow cells that had been treated (ex vivo) with a retrovirus-delivered therapeutic gene. The 2 surviving leukemic children were 1 and 3 months old at the time of treatment and had returned home to a normal life, seemingly cured, until they developed a leukemia-like cancer about 2½ years later. Their cancer was caused by retrovirus vectors that randomly integrated the therapeutic gene into a critical location of the genome containing the promoter region for a gene called *LM02*, which encodes a transcription factor required for the normal formation of white blood cells. This integration led to aberrant transcription of the *LM02* gene and overexpression of *LM02*, which triggered the uncontrolled division of mature T cells.

This tragedy resulted in the temporary cessation of a large number of gene therapy trials, and the FDA completely stopped most retroviral studies. Trials eventually resumed, but with greater patient monitoring.

Currently there are more barriers to gene therapy than solutions to medical problems. These include the following:

- How can expression of the therapeutic gene be controlled in the patient? What happens if therapeutic genes are overexpressed or if a gene shuts off shortly after it has been introduced?
- How can scientists safely and efficiently target only the cells and tissues that require the therapeutic gene without affecting other cells in the body where the gene is not needed?
- How can gene therapy be targeted to specific regions of the genome to prevent the random integration problems encountered in the French trials or instability and movement of the inserted DNA? One active area of research showing promise involves targeted genome editing using proteins called **zinc finger nucleases (ZFNs)**. These nucleases act as DNA-cleaving genome scissors to cut out specific areas of DNA, such as a defective gene, and ZFNs can be used to cut out and replace a sequence. Plasmids encoding ZFNs to "edit" out and replace defective genes is an area of intense research in gene therapy. A ZFN-based strategy is being used in the first gene therapy trial under way for patients with HIV in an attempt to disrupt the *CCR5* gene, which encodes a protein that HIV

uses to enter cells, in the hope that this will halt the spread of the virus.

- How can gene therapy provide lasting, permanent treatment without frequent administration of the therapeutic gene?
- How can rejection of the therapeutic gene be avoided? Whenever gene therapy is used, it is not always known if the recipient's immune system will reject the protein produced by therapeutic genes or reject genetically altered cells containing therapeutic genes.
- How many cells must express the therapeutic gene to treat the condition effectively? This will vary depending on the disease condition, but it remains to be determined if a majority of diseased cells must be affected by the therapeutic gene or if a disease can be treated by correcting only a small number of cells.

These and other barriers must be overcome before gene therapy becomes a safe and reliable treatment approach, but scientists are making excellent progress in this field. They are also making incredibly rapid advances in another hot area of medical biotechnology called regenerative medicine, the topic of the next section of this chapter.

4 The Potential of Regenerative Medicine

Currently, physicians treating most human illness are primarily limited to approaches such as surgical techniques, radiation treatment, and drug therapy. Although these approaches all have a place in medicine to treat certain human conditions, they do not offer the ability to regenerate tissue or restore the functions of damaged organs. For example, when a person has a heart attack or stroke, tissue damage often results. When an organ is damaged, the only way to restore its functions fully is to replace the damaged tissue with new ones—something that often does not occur naturally in heart or brain tissue.

When organ development occurs in the embryo, changes in the expression of many genes must occur in an ordered sequence. Unwanted changes that even drugs cannot fix may occur in cells, and no one drug can stimulate the growth and repair of new tissue when an organ is severely damaged. Even with new knowledge gained from the Human Genome Project, it is highly unlikely that any one drug or even a few drugs could be used to stimulate hundreds of changes in gene expression with the proper timing required for tissue regeneration and the restoration of organ function.

Regenerative medicine, growing cells and tissues that can be used to replace or repair defective tissues and organs, is an exciting field of biotechnology that holds the promise and potential for radically changing medicine and the delivery of health care as we know it. Most researchers in the field agree that the goal of regenerative medicine is not to extend the human life span and achieve immortality but to improve the quality of life by making it healthier.

Cell and Tissue Transplantation

Organ transplantation is not a new idea, but applications that involve transplanting specific cells and tissues to replace or repair damaged tissues are relatively new aspects of medical biotechnology research.

Fetal tissue grafts

Neurodegenerative diseases occur gradually, leading to progressive loss of brain functions over time. Alzheimer disease and Parkinson disease are perhaps the two best-known examples. These diseases rank first and second, respectively, as the most common neurodegenerative disorders. For Parkinson disease alone, approximately 50,000 cases are diagnosed yearly, and an estimated 500,000 Americans currently have the disease. Parkinson disease is due to the loss of cells in an area deep inside the brain called the *substantia nigra*. Neurons in this region produce a chemical called *dopamine*, a neurotransmitter or chemical used by neurons (nerve cells) to signal one another. Loss of these dopamine-producing cells causes tremors, weakness, poor balance, loss of dexterity, muscle rigidity, a reduced sense of smell, inability to swallow, and speech problems, among other effects. Most treatments involve drugs that increase the production or accumulation of dopamine in the brain; however, after about 4 to 10 years of drug treatment, the disease progresses and the effectiveness of these drugs diminishes, leading to a poor quality of life for the patient, who typically dies of complications related to the disease.

Unlike fetal neurons, which can divide, most adult neurons will not repair themselves when damaged, and most neurons do not undergo cell division. Scientists have long been interested in using fetal neuron transplants as a way of treating Parkinson disease and other neurological conditions. The basic idea is to introduce fetal neurons in the hope that these cells can establish connections with other neurons, replace the damaged brain cells, and restore brain function. After demonstrated success in rodents, fetal tissue transplants have been used since the late 1980s, and well over 100 patients have received such transplants. Most human fetal tissue comes from embryos or fetuses obtained from accident victims and legally aborted

embryos. Patients receiving fetal transplants have shown varying degrees of improvement, including relief of parkinsonian symptoms in over 40% of patients and in some cases the almost complete elimination of most symptoms even several years later, but fetal transplants have not provided full recovery.

Over 250,000 individuals have been paralyzed by trauma to the spinal cord, and nearly 2 million people worldwide are living with spinal cord injuries. Each year approximately 85,000 people suffer spinal cord injuries, including roughly 10,000 in the United States. Damage can occur when the cord is crushed or the nerve fibers are severed. Incomplete or complete severing of the spinal cord may result in paraplegia, paralysis of the lower body, or quadriplegia, paralysis of the body from the neck down, depending on where the injury occurred. Many strategies have been used in attempts to repair spinal cord injuries. One approach has been to graft nerve fibers from fetal or adult neurons into the damaged area of the spinal cord so as to bridge the parts of the cord that were severed (**Figure 17**). Such bridge implants have shown promise in dogs and rats. As scientists learn more about the inflammatory chemicals that hinder nerve growth and the factors that stimulate it, it may be possible to use such molecules to minimize scar tissue formation, reduce damage caused by scar tissue to supporting cells called glial cells, block growth inhibitor molecules, and stimulate neuron regeneration at the same time.

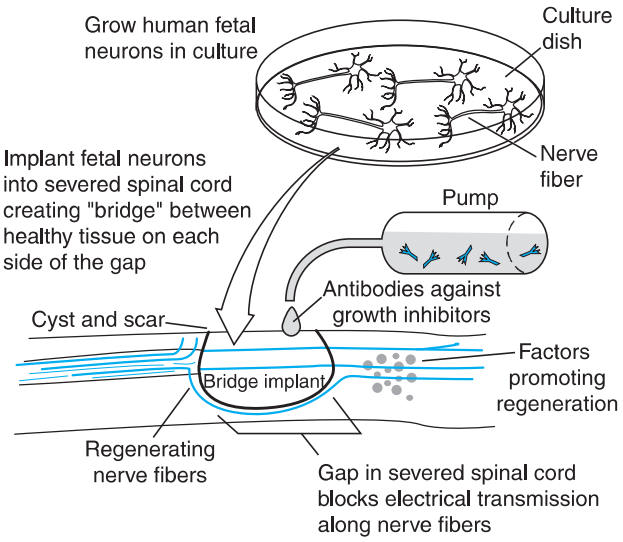

FIGURE 17 Bridging the Gap In many types of spinal cord injuries, a gap occurs when the spinal cord is severed. This blocks electrical transmission of nerve impulses and can result in paralysis and the loss of body sensations such as temperature, pain, and touch. One experimental strategy for repairing spinal cord injuries involves implanting fetal neurons into the severed area of the spinal cord to create a bridge between normal neurons. Scientists are using this strategy in combination with antibodies that may block chemicals that inhibit spinal cord repair and growth factors that can stimulate neuron regeneration in an effort to bridge the gap in spinal cord injuries.

Organ transplantation

Organ transplantation can and does save lives. Approximately 8 million surgeries related to tissue damage and organ failure are performed in the United States each year, but about 4,000 people also die each year while waiting for an organ transplant. At least 100,000 people die each year without ever qualifying to be on a waiting list. Well over $400 billion is spent on organ failure and tissue-related health care costs in the United States. This number represents nearly half of the nation's health care bill.

Autografting—that is, the transplantation of a patient's own tissue from one region of the body to another—can alleviate some transplantation problems. For example, coronary artery bypass operations involve removing segments of a vein from the patient's leg and connecting it surgically to arteries in the heart as a bypass around obstructed vessels. But if a patient needs a heart or a liver transplant, another person who can donate an organ for the recipient must be found. Even when a human donor who appears to be a match is found, organ rejection is a major problem. Rejection typically occurs when the recipient's immune system recognizes that the donor organ is foreign. Matching organs for transplantation involves tissue typing to check if a donor organ is compatible for a recipient.

Tissue typing is based on marker proteins found on the cell surface (membrane) of every cell in the body. Tissue typing proteins are part of a large group of over 70 genes called the **major histocompatibility complex (MHC)**, aptly named because *histo* means "tissues," and MHC molecules must be matched between donor and recipient to have a compatible organ for transplantation. There are many different types of MHC proteins. One common group, called the human leukocyte antigens (HLAs, named because they were first discovered on white blood cells, or leukocytes), are found on virtually all body cells. Immune system cells such as B and T cells recognize HLAs on all body cells present since birth as "self" (belonging to the same individual), whereas any other cells are "nonself," or foreign cells that may be attacked by the immune system and destroyed. Some common HLAs are found on most human tissues, and others are unique to a given individual. To have a successful transplantation of an organ from one human to another requires a close match of several types of HLAs between the donor organ and the recipient's cells; otherwise the recipient will reject the transplanted organ.

Since the first human liver transplant in 1963, transplant surgeons have been using immunosuppres-

sive drugs to weaken the recipient's immune system and minimize organ rejection. Most transplant recipients must use immunosuppressive drugs for the rest of their lives. One obvious problem with this approach is that patients on immunosuppressive drugs can and do develop infections, which, because of their weakened immune systems, can be life-threatening. The lack of sufficient human organ donors and the problem of organ rejection are major reasons why scientists are looking at other ways to provide donor organs.

Xenotransplantation—the transfer of organs from different species—may one day become a viable alternative to human-to-human organ donation, thus helping relieve the tremendous need for human donor organs. Baboons were once considered the animal of choice for providing organs to human recipients. The first animal-to-human organ transplant in a child, carried out in 1984 by doctors at Loma Linda University Medical Center in California, involved transplanting a baboon heart into Baby Fae, a 12-day-old girl. Baby Fae lived with the baboon heart for 3 weeks before she died of complications related to organ rejection. Similar transplants have been performed without great success. Although baboons and other primates may still be candidates for providing organs, many groups are choosing to investigate the potential of using pigs as organ sources. Pigs may be a good choice because they are plentiful, easy to breed, and relatively inexpensive. Many pig organs are also similar in function and size to human organs. Progress on using pig organs for transplantation in humans has been slowed by concerns that viruses may be transmitted from pigs to humans, causing the transplanted organs to be rejected and creating other health problems.

Transplantation scientists have combined molecular techniques and transplantation technologies to produce cloned pigs that may help overcome current fears of organ rejection and viral disease transmission. Researchers at the University of Missouri have created cloned piglets that lack a key gene called *GGTA1* (ß-1, 3-galactosyltransferase). *GGTA1* produces a sugar on the surface of pig tissues, which, when transplanted into a human, would be recognized as a foreign antigen, leading to antibody production and rejection of the organ. The *GGTA1* knockout pigs were cloned using nuclear transfer cloning techniques. Creating *GGTA1* knockout pigs may be a way to generate pigs that could produce organs for transplantation that the human immune system may not recognize as foreign (**Figure 18**).

Xenotransplantation does not always have to involve the transfer of a whole organ, as you will learn in the next section. Scientists are working hard to develop ways to deliver small clusters of cells as a technique for cell and tissue transplantation.

Cellular therapeutics

Cellular therapeutics involves using cells, instead of whole tissues or organs, to replace defective tissues or to deliver important biological molecules. One alternative for avoiding organ rejection of transplants is to use living cells that have been encapsulated into tiny plastic beads or tubes called **biocapsules** or microcapsules. Biocapsules may also contain genetically engineered cells designed to produce therapeutic molecules such as recombinant proteins.

Biocapsules have tiny holes in their walls, making them permeable to nutrient exchange and allowing molecules produced by the encapsulated cells to escape from the capsule and enter the bloodstream or surrounding tissues (**Figure 19** on the next page). For instance, capsules containing insulin-producing cells (beta cells) from the pancreas, implanted in patients with type I diabetes, would produce insulin that could travel out of the capsule into the bloodstream of the patient to all body organs requiring insulin. Another important feature of biocapsules is that they protect cells from being attacked by the recipient's immune system by hiding them within capsules, where immune cells and antibodies cannot reach and destroy them. Although not a permanent cure, biocapsules can provide lasting release of molecules into the body. This approach would likely require that biocapsules be changed every few months; however, in the case of diabetes, this might be a better alternative than daily injections of insulin.

FIGURE 18 Pigs Could Potentially Save the Lives of Patients Waiting for a Transplant These piglets have been engineered to lack a sugar-producing gene that causes human bodies to reject pig organs, potentially providing a source of rejection-free pig organs.

Bill Ling/Dorling Kindersley Media Library.

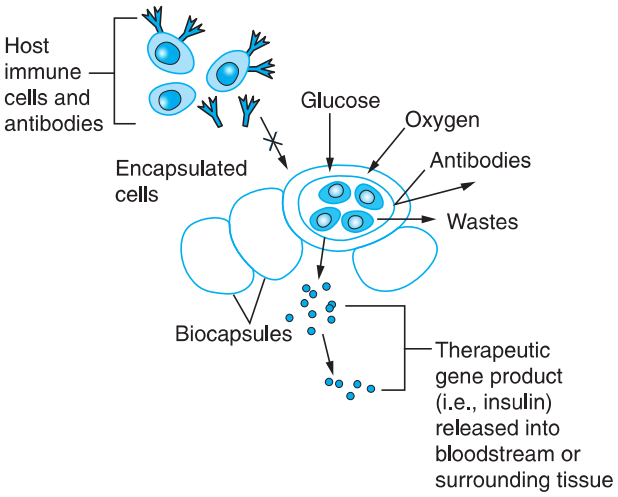

FIGURE 19 **Biocapsules** Encapsulated cells may provide valuable ways to deliver therapeutic molecules. Cells in the biocapsule are protected from attack by immune cells and antibodies of the host. At the same time, biocapsules allow molecules produced by the cells to leave the capsule and provide therapeutic benefits for the host. This figure illustrates how insulin-producing cells could be used to provide a patient with diabetes with a source of insulin.

Tissue Engineering

Whether you pay $30,000 or $300 for your first car, one thing is certain: over time parts will wear out and break. A trip to the local mechanic can repair and replace some car parts, but eventually the car wears out to the point of no return, and it is time to buy another car. Wear and tear on human body parts also take their toll. Over time, organs do not work as well as they should; in some cases, an organ may stop functioning altogether. Even if a person lives a relatively healthy life, the wear and tear of aging or a sudden event such as a stroke or heart attack will lead to a decline in organ function and perhaps organ failure. But our bodies are not like cars: we cannot go to a warehouse of body parts for replacements.

In the future, however, the emerging science of **tissue engineering** may provide tissues and organs that can be used to replace damaged or diseased tissues. This small but growing industry—there are over 60 biotechnology companies involved in tissue engineering in the United States alone and experts predict that the field will grow by 50% in the next decade—is actively involved in research to engineer human tissues and organs as replacements for worn out and damaged tissues. As we age, we outlive the functional abilities of our organs. It has been estimated that roughly 20% of people over the age of 65 in developing nations will benefit from some type of tissue engineering application in their lifetimes. Tissue engineering and regenerative medicine approaches are also predicted to lead to substantial cost savings for care of chronic diseases such as congestive heart failure and diabetes. In the case of congestive heart failure, if regenerated heart tissues restored cardiac function for even a small percentage of individuals, cost savings could be several billion dollars annually worldwide.

Tissue engineering scientists often begin by designing and constructing a biomaterial framework or scaffold made of biological substances such as calcium, collagen, a polysaccharide called alginate, or biodegradable materials (**Figure 20a**). The scaffold is shaped as a mold of the tissue or organ to be made, and its purpose is to create a three-dimensional framework onto which cells are placed. Growing human cells on the scaffolding is called *seeding* because the cells literally act as "seeds" to create more cells that will grow over the scaffold. Scaffolds seeded with cells are bathed in a nutrient-rich medium and, over time, cell layers build up over the scaffolding material to assume the shape of the scaffold. Engineered sheets of human skin have already proven useful for treating severe burn victims who have lost substantial portions of their skin, a very painful and life-threatening condition. Sheets of skin grafts have proven to be successful organs grown by tissue engineering. Engineered bone structures for healing bone fractures have also worked fairly well. Scaffolding to engineer teeth and blood vessels has shown promise, and many products of tissue engineering are FDA-approved and being used to treat patients or in clinical trials.

In the 1990s, tissue engineering pioneer Charles Vacanti and colleagues made headlines around the world when they revealed a mouse with an engineered ear growing on its back. In this example, a biodegradable scaffold in the shape of an outer ear was attached to a mouse and then seeded with cartilage cells from cows. The cartilage cells infiltrated the scaffolding and produced cartilage as they grew; then, as the scaffolding degraded, the cartilage developed enough strength to support itself. The human ear–looking tissue was never transplanted onto a human. Because it was made of cow cells, it would have been rejected by the human immune system. Also, this tissue was just the outer ear without the inner ear structures that actually detect sound, but it provided strong evidence that tissue engineering could work.

Creating large and complex organs such as the liver, heart, and kidney has proven to be much more difficult, although fetal tissue has been used to grow a rudimentary kidney in rats that was able to produce a urine-like fluid. At least two phase II clinical trials are under way in which human bladders were created using tissue

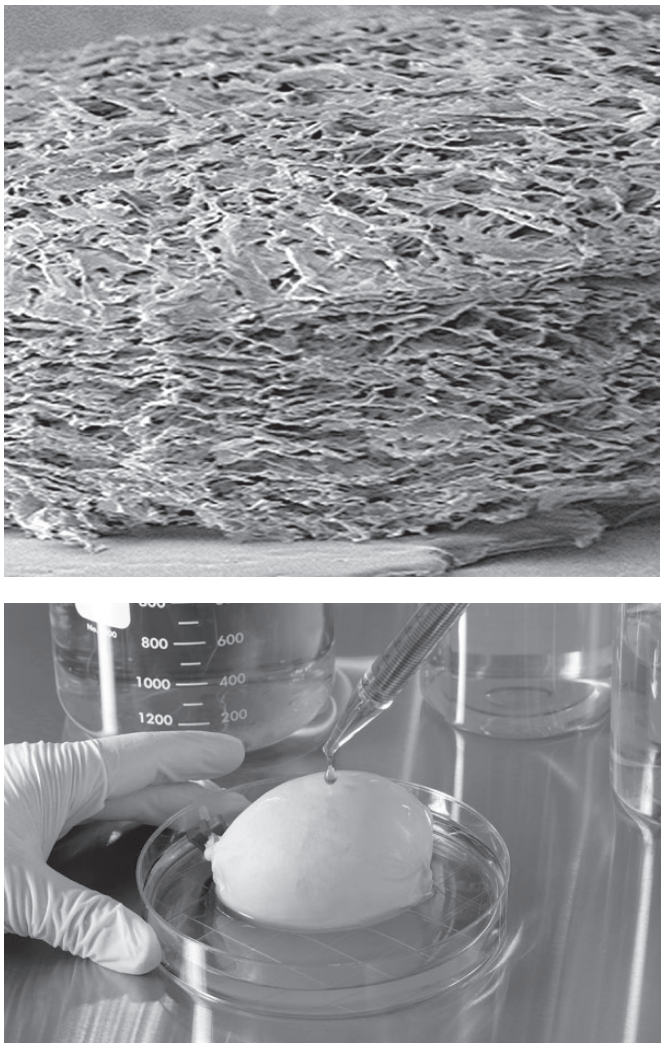

FIGURE 20 **Tissue Engineering** (a) An example of collagen scaffolding used to engineer tissues. (b) A urinary bladder created from stem cells.

(a): Becton Dickinson and Company.

biopsies from patients' bladders to seed scaffolding and produce new bladders (Figure 20b). The field of tissue engineering is in its infancy. But progress is advancing at an incredibly rapid rate and, as discussed in the next section, applications involving stem cells are adding to this progress, and there is every reason to expect that engineered organs will become a reality.

The telomere story

In normal human cells, the ends of chromosomes contain sequences of DNA nucleotides called **telomeres**. Telomeres are usually 8,000 to 12,000 bp units of the repeating sequence 5′-TTAGGG-3′. Think of these as the plastic tabs at the ends of shoelaces that prevent the laces from unraveling, a sort of chromosome "cap." Normal cells have a limited ability to proliferate. Most human body cells can divide a maximum of 50 to 90 times before they show signs of aging—a process called **senescence**—which eventually leads to cell death. A cell's life span is affected in part by telomeres.

Each time a cell divides, telomeres shorten slightly. This occurs because of a basic flaw that prevents DNA polymerase from completely copying the ends of both strands of a DNA molecule. In many ways, telomeres serve as a biological clock for counting down cell divisions leading to senescence and cell death. Telomeres shorten, and senescence occurs until the cell can no longer divide. If there are multiple copies of the TTAGGG repeats at the ends of chromosomes, cells can lose this DNA without the loss of precious gene sequences. Eventually loss of repeat sequences produces a critical loss of DNA, so cells no longer divide (**Figure 21** on the next page).

Scientists have long known that many cancer cells can divide indefinitely—a property called *immortality*. One way in which cancer cells achieve immortality is through the actions of an enzyme called **telomerase**. It repairs telomere length at the ends of chromosomes by adding DNA nucleotides to cap the telomere after each round of cell division. Telomerase is not active in normal cells but is active in over 90% of human cancers. By preventing telomere shortening, telomerase activity is a major reason why cancer cells can divide indefinitely. In fact, biologists call cancer cells immortal because of their ability to avoid senescence indefinitely.

Telomere shortening is involved in the aging process—in the aches and pains, wrinkles, arthritis, gray hair, and other symptoms humans experience as we age. Telomerase is not a "fountain of youth" cure for the effects of old age. Aging and cancer are far more complex processes that involve many proteins, not just telomerase. Although high levels of telomerase are found in almost every human cancer cell, telomerase itself does not *cause* cancer. Telomerase in combination with genetic mutations in genes that control cell division can create immortal cells that avoid senescence. Overproduction of telomerase often correlates with the aggressive growth of tumors. Researchers are working on cancer treatment strategies such as telomerase peptide vaccines to inhibit telomerase and stop cancer cells from dividing. Also, two companies have recently produced tests to measure the length of one's telomeres as an indicator of health status, although the diagnostic and predictive values of such tests are being debated.

From a tissue engineering perspective, scientists are investigating how introducing telomerase genes into cultured human cells can allow them to produce normal human cells that display immortality. If these efforts are successful, immortal human cells could be valuable in treating individuals with age-related disorders ranging from arthritis to neurodegenerative diseases. Such cells could also be used in many other ways, as for providing skin cells for healing bedsores and ulcers and

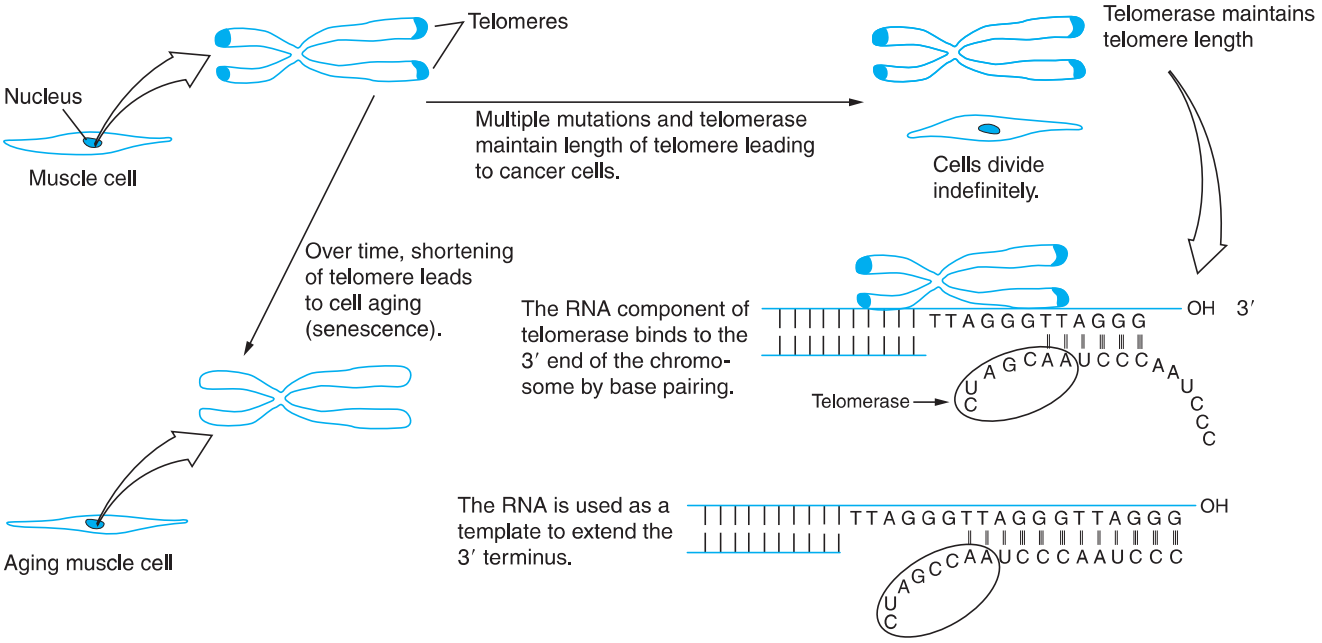

FIGURE 21 **Telomere Shortening Leads to Cell Aging** Telomeres shorten with each round of DNA replication. Eventually telomere shortening leads to cell senescence and death. Through mutations that affect the functions of different genes and expression of the enzyme telomerase, cancer cells can divide indefinitely and avoid senescence. Telomerase plays a role in this process by continually filling in telomere sequences to prevent them from shortening during cell division.

(b): Wake Forest University School of Medicine.

treating patients with late-onset blindness, and muscular dystrophy. In the future, the technology might even be available to remove a patient's aging cells, introduce telomerase genes to these cells to extend the patient's life, and then return the cells to the patient. Some of this work could be done in combination with stem cells, which are perhaps the hottest and most controversial topic in medical biotechnology today.

Stem Cells

The tremendous promise and controversy surrounding stem cells has made stem cell research and related topics regular themes of front-page news items and TV headlines. Stem cells evoke emotional and controversial responses from scientists, clergy, politicians, and the general public. Among some people, the isolation and use of these cells engenders excitement, fear, anger, and a range of other emotions.

What are stem cells?

As you will soon learn, there are many different types of **stem cells**; but in general all stem cells have two basic characteristics that set them apart from other cell types: **self-renewal** and **differentiation** into specialized cells:

- Self-renewal: Stem cells grow and divide (proliferate) indefinitely by mitosis to create populations of identical stem cells.
- Differentiation: This is a complex process involving many genes that must be activated and silenced, and differentiating cells rely on chemical signals such as growth factors and hormones from other cells to help them change. Stem cells are special because they can eventually differentiate to form all of the more than 200 cell types that make up the human body. Stem cells are called **pluripotent** because they have the potential to develop into a variety of different types.

To understand what stem cells are, we must look briefly at the development of the human embryo. We do this by considering how **in vitro fertilization (IVF)** is carried out. IVF first gained public attention in 1978 when Louise Brown, the first test tube baby, was born. To create a child by IVF, sperm and egg from donor parents are mixed together in a culture dish to produce an embryo. After several days of division, the embryo is surgically implanted in the uterus of a woman, usually the egg donor, who has been treated with hormones to prepare her uterus for implantation. When a couple agrees to undergo IVF, several embryos are usually created, but often only one is implanted during each procedure. The remaining embryos are frozen for future use as needed. Potentially, the leftover embryos can be a source of **human embryonic stem cells**

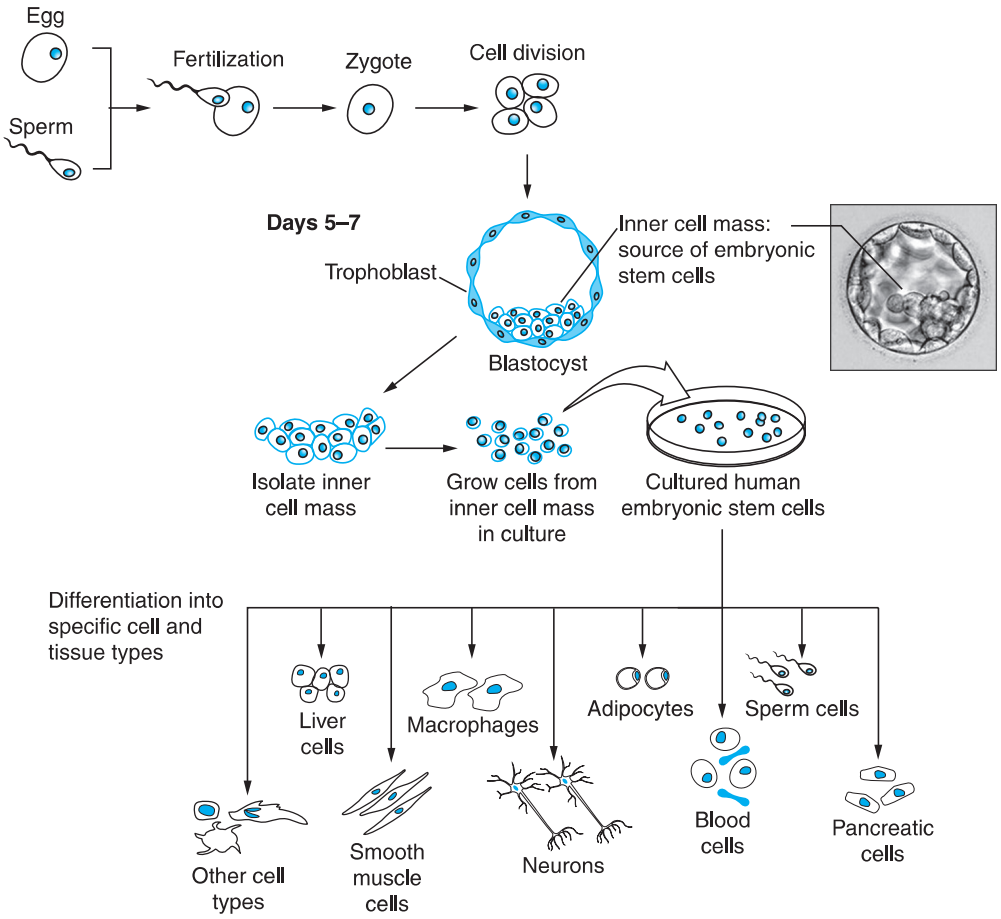

FIGURE 22 Isolating and Culturing Human Embryonic Stem Cells (hESCs) Cells isolated from the inner cell mass of human embryos can be grown in culture as a source of hESCs. Under the proper growth conditions, hESCs can be stimulated to differentiate into virtually any cell type in the body.

(hESCs), but they are also the source of a great deal of controversy.

An embryo goes through a predictable series of developmental stages (Figure 22). After an egg cell is fertilized, it is called a **zygote**. The zygote divides rapidly and, after 3 to 5 days, first forms a compact ball of about 12 cells called a **morula**, meaning "little mulberry." Around 5 to 7 days after fertilization, the embryo consists of a small hollow cluster of approximately 100 cells called a **blastocyst**. The blastocyst is approximately one-seventh of a millimeter in diameter. It contains an outer row of single cells called the trophoblast; this layer develops to form part of the placenta, which nourishes the developing embryo. The area of cells of primary interest to stem cell biologists is a small cluster of around 30 cells tucked inside the blastocyst, which form a structure known as the **inner cell mass**, the source of hESCs (Figure 22).

Cells of the inner cell mass develop to form the embryo itself. Stem cells in the inner cell mass have the ability to undergo differentiation. Successful isolation and culture of the first hESCs from a human blastocyst was reported in 1998 by James Thomson of the University of Wisconsin at Madison who had cultured hESCs from rhesus monkeys 2 years earlier. Also in 1998, John Gearhart and colleagues at Johns Hopkins University isolated embryonic germ cells, primitive cells that form the gametes—sperm and egg cells—from human fetal tissue and demonstrated that these cells could develop into different cell types. These discoveries followed the work of other scientists who had isolated stem cells in species such as mice, pigs, cows, rabbits, and sheep. In fact, stem cell researchers credit much of what is now known about isolating hESCs from pioneering work initiated in mice in the 1980s—another outstanding example of how model organisms contribute greatly to the advancement of science.

Human ESCs avoid senescence and show no signs of aging, in part because they express high levels of telomerase. Several groups have maintained stem cells for over 3 years and over 600 rounds of division without apparent problems. Cultured cells such as these, which can be maintained and grown successively, are called **cell lines**. Stem cells also grow rapidly and can be frozen for long periods of time and still retain their properties. Under the right conditions, when they are stimulated with different molecules including hormones and substances called **growth factors**, stem cell lines

can be coaxed to differentiate into different types of cells. This *directed differentiation* of stem cells into specific differentiated cells of interest is key to creating tissues for regenerative medicine applications. A major focus of stem cell research is experimentation to determine what controls the pluripotency of stem cells and to identify the factors that stimulate their differentiation into discrete cell types. These signals include substances called growth factors, hormones, and peptides, which stimulate differentiation in tissue-specific ways.

For example, signaling systems that involve transforming growth factor-ß (TGF-ß), bone morphogenetic proteins (BMPs), and other growth differentiation factors act on a gene for a transcription factor called *Nanog*. NANOG is one key protein that maintains hESCs in an undifferentiated pluripotent state. Once stem cells are produced and isolated, scientists use a number of tests to determine their pluripotency. In the laboratory under the proper culturing conditions, ESCs from humans, mice, rats, primates, and other species have been shown to differentiate into a myriad of cells including skin cells; brain cells (both neurons and glial cells, which support, nourish, and protect neurons); cartilage (chondrocytes); spermatozoa; osteoblasts (bone-forming cells); liver cells (hepatocytes); insulin-secreting pancreatic beta cells; muscle cells including smooth muscle, which forms the walls of blood vessels; skeletal muscle cells, which form the muscles that attach to and move the skeleton; and cardiac muscle cells (myocytes), which form the muscular walls of the heart. How do researchers obtain hESCs? hESCs for research purposes are derived from the blastocysts of embryos that are no longer needed for IVF or from human embryos created by IVF from sperm and egg cells donated in order to provide embryos for research purposes. Typically, leftover blastocysts would either be destroyed or frozen indefinitely, but with the consent of the couple who provided the sperm and egg, they can be used to derive hESCs, as described in Figure 22. In U.S. fertility clinics alone, an estimated 400,000 frozen unused embryos and several thousand eggs are discarded annually.

Other Sources of Stem Cells

Research on hESCs is very controversial because of their source—an early embryo. Scientists have discovered **adult-derived stem cells (ASCs)**, cells that reside in mature adult tissue and could be cultured and differentiated to produce other cell types. ASCs appear in very small numbers, and although they have been isolated from the heart, brain, intestine, hair, skin, pancreas, bone marrow, fat, mammary glands, teeth, muscle, and blood, they have not yet been discovered in all adult tissues.

Opponents of hESC research often claim that ASCs are a more acceptable alternative than using hESCs because isolating ASCs does not require the destruction of an embryo. ASCs can be harvested from people by fine-needle biopsy, through a thin diameter needle inserted into muscle or bone tissue. It may even be possible to isolate ASCs from cadavers. We also know that ASCs are present in fat (adipose) tissue, which could potentially be an outstanding source of stem cells, especially if you consider that over 500,000 L of fat tissue collected by liposuction and other cosmetic surgery techniques are discarded in the United States each year.

Experiments have shown that ASCs from one tissue can differentiate into another different specialized cell type. For instance, an ASC isolated from muscle tissue could be used to develop into a blood cell. But other studies have demonstrated that ASCs may not be as pluripotent as hESCs. Much more research is required to determine whether ASCs can be as valuable as hESCs might be.

Stem cells can be isolated from human amniotic fluid, the protective fluid that surrounds a developing fetus. In the lab, these **amniotic fluid–derived stem cells (AFSs)** have been coaxed to become neurons, muscle cells, adipocytes, bone, blood vessels, and liver cells. It is not entirely clear whether these cells are truly different from hESCs or ASCs; but if so, they may be a key breakthrough in stem cell technologies.

Cancer stem cells (CSCs), also called tumor-initiating cells, have been identified and implicated in the development of cancers, tumor progression, tumor metastasis, and the recurrence of cancers. Like normal stem cells, CSCs can self-renew and differentiate to form the tissues from which they were derived. Certain CSCs grow slowly in clusters or *niches* within a tissue. It is not clear what properties CSCs may have besides the ability to form a tumor. Researchers are also not sure whether CSCs are derived from normal cells or if they are involved in cancer tumor resistance to chemotherapies, but these cells are a focus of intense research and potential therapeutic treatments for the treatment of cancers.

Creating stem cells by nuclear reprogramming of somatic cells

Research on stem cell biology is an extremely active field. A primary focus continues to be alternative approaches for producing pluripotent stem cells without destroying an embryo. One of the most promising new approaches for doing this involves a technique called **nuclear reprogramming of somatic cells**. The basic concept of this approach is to use genes involved in cell development to push a somatic cell back to an earlier stage of development and affect gene expression and thus to reprogram the somatic cell

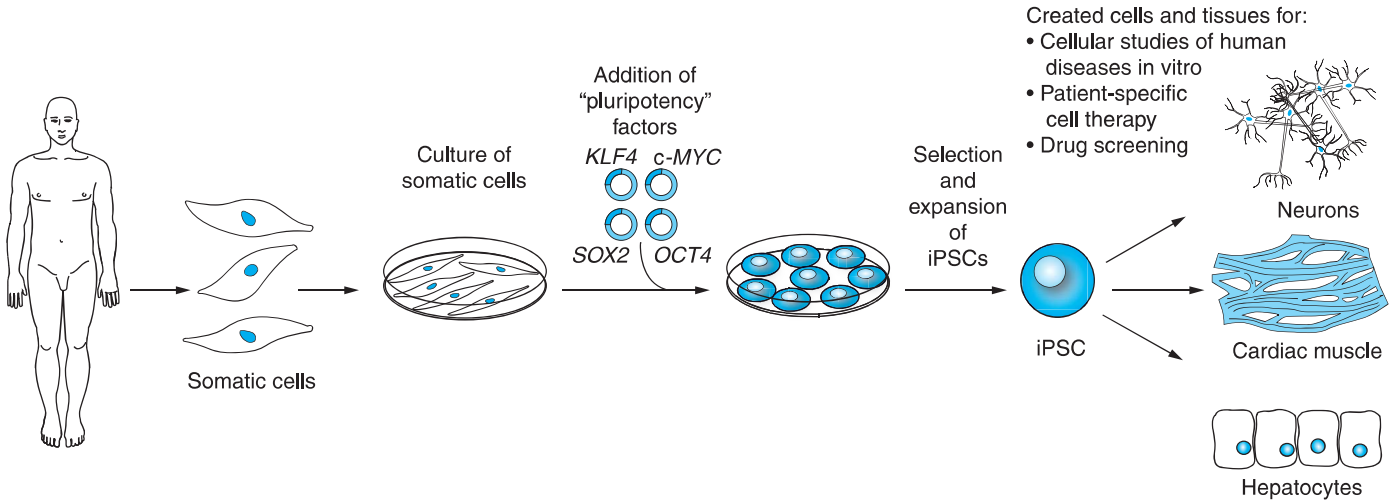

FIGURE 23 Nuclear Reprogramming of Somatic Cells to Produce Induced Pluripotent Stem Cells The introduction of four transcription factor genes (*Oct3/4*, *Sox2*, *c-myc*, *Klf4*) into mouse fibroblasts results in the formation of induced pluripotent stem cells (iPSCs). Invariably a number of these cells are defective in reprogramming and must be selected out during culture. They must also be selected for the expression of endogenous marker genes (such as *Nanog* and *Oct*) known to be expressed in pluripotent stem cells.

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genetically to return to a pluripotent state characteristic of the stem cells from which it was derived. It was previously thought that once cells differentiated to become specific, specialized cell types, for example a skin cell, that their differentiation fate was irreversible. But we now know that this is not the case. One of the first successful reprogramming techniques involved fusing hESCs with skin cells called *fibroblasts*. The hybrid cells generated in this way displayed several properties of hESCs both in vitro and in vivo.

There are several different approaches to nuclear reprogramming, and these are being heralded as a revolution in stem cell research. One approach has involved using retroviruses to deliver four transgenes—*OCT3/4*, *SOX2*, *c-MYC*, and *KLF4*—into fibroblasts (**Figure 23**). Expression of these four genes, which encode transcription factors involved in cell development, "reprograms" the fibroblasts back to an earlier stage of differentiation. Such reprogrammed cells are called **induced pluripotent stem cells (iPSCs)**. iPSCs demonstrate many properties of hESCs, such as self-renewal and pluripotency, and appear to be indistinguishable from hESCs. iPSCs have been produced from human, mouse, rat, pig and monkey cells. Subsequently a cocktail of RNA molecules for the four genes described above has also been used to create iPSCs from somatic cells. Also, human neural stem cells have been reprogrammed to iPSCs by introducing only *OCT4*.

These iPSCs express genes such as *Nanog* and *Oct*, which are characteristic markers known to be expressed in undifferentiated hESCs. Other experiments have also demonstrated that iPSCs can differentiate into other cell types including neural cells and cardiac muscle cells. Nuclear reprogramming may be a way to generate patient-specific iPSCs without the need for an embryo and hESCs. iPSCs have been successfully derived by reprogramming human skin cells from patients. Reprogrammed skin cells taken from the face of a 36-year-old woman, connective tissue cells from the joints of a 69-year-old man, and skin cells from the foreskin of a newborn boy are examples. iPSCs have also been produced without using the *c-myc* gene. This is a potentially important advance because *c-myc* is a known oncogene.

Stem cells derived in this way could be used for patient-specific cell therapies. In addition, with iPSC technologies, it is theoretically possible to create disease-specific stem cells from individual patients. For example, one could take a tissue biopsy, such as skin, from a person with a particular disease and reprogram those cells into stem cells that could then be used to create cell types for combating the disease. Patient-specific iPSCs could be used for cell-based therapies without the risk of immune rejection. But even the most optimistic iPSC researchers believe that such cell therapies will not be ready for at least another decade or more.

Scientists are also very excited about how reprogrammed cells from patients can be used to study "diseases in a dish." Cultured reprogrammed cells from diseased patients are being studied to help scientists better understand human disease progression and disease processes. They are also being utilized for drug screening tests in order to determine the effectiveness of potential

drug treatments for diseased cells. Labs around the world are making iPSCs from patients with a disease and differentiating these cells to become the tissues affected by a particular disease so that these diseases can be modeled in vitro and cells can be used for drug treatments.

On the surface, iPSCs circumvent some of the legal and ethical controversies associated with hESCs. But as promising as iPSCs are, there are challenges associated with them that will have to be addressed. For example, scientists still do not fully understand how pluripotent iPSCs may be and how to best control the potency of these cells. In addition, iPSCs:

- Are relatively inefficient to produce (only about one in 1000 somatic cells exposed to most reprogramming approaches becomes an iPSC)
- Require constant feeding to maintain viable cells lines
- Show low viability compared with other cell types once they have been stored frozen
- Can be prone to forming tumors
- Occasionally show spontaneous differentiation into mature cell types when in culture
- Can sometimes be difficult to use for directing differentiation into particular cell types

Research with iPSCs is progressing at an astonishing and exciting pace as scientists work to better understand the properties and capabilities of these cells. Promising results demonstrate that nuclear reprogramming may be a potentially viable way to generate person-specific stem cells without the need for an embryo. Watch for exciting new developments in the next few years involving nuclear reprogramming and iPSCs.

Potential applications of stem cells

The CDC's National Center for Human Statistics indicates that approximately 3,000 Americans die every day from diseases that may one day be treated by stem cell technologies. In the future, stem cell research may affect the lives of millions of people throughout the world.

There are many potential applications for stem cells—from growing healthy tissues, to studying them to understand and treat birth defects, to genetic manipulation for delivering genes in gene therapy approaches, to creating whole tissues in the laboratory using tissue engineering. Many scientists believe that stem cell technologies will play key roles in developing treatments for diseases such as stroke, heart disease, Parkinson disease, Alzheimer disease, Lou Gehrig disease, diabetes, and other conditions (refer to Table 2).

Potential and *promise* are frequently used words when stem cell applications are being discussed, but use of these cells for treating disease is still largely unproven. Here we consider some of the most promising examples of stem cell applications to date. Patients with leukemia frequently require chemotherapy or radiation treatment to destroy defective white blood cells. As a result, the patient's immune system is greatly weakened. Leukemia treatments may also involve blood transfusions to replace white blood cells and red blood cells damaged by chemotherapy. The use of stem cells to make white blood cells has already become an effective way to treat leukemia. Stem cells from umbilical cord blood have also been used to provide red blood cells for patients with sickle cell disease and those with other blood deficiencies. The isolation of stem cells from cord blood is becoming so popular that, in many U.S. states, parents can opt to pay to have cord blood stem cells frozen indefinitely should their child need them at some time in the future.

So far there have been a number of promising results in animal models as well as in human clinical trials using stem cells for tissue repair. For example, stem cells from fat have been used to form bone tissue in the human skull. The repair of heart tissue has shown strong potential. Stem cells might be used to replace dead and dying cells following trauma, such as a heart

TABLE 2 STEM CELL–BASED THERAPIES MAY POTENTIALLY BENEFIT MILLIONS OF PEOPLE

Disease Condition	Number of Patients in the United States
Cardiovascular disease	58 million
Autoimmune diseases	30 million
Diabetes	16 million
Osteoporosis	10 million
Cancers (urinary bladder, prostate, ovarian, breast, brain, lung, and colorectal cancers; brain tumors)	8.2 million
Degenerative retinal disease	5.5 million
Phenylketonuria (PKU)	5.5 million
Severe combined immunodeficiency (SCID)	0.3 million
Sickle cell disease	0.25 million
Neurodegenerative diseases (Alzheimer and Parkinson diseases)	0.15 million

Source: Adapted from Stem Cells and the Future of Regenerative Medicine, www.nap.edu/catalog/10195.html.

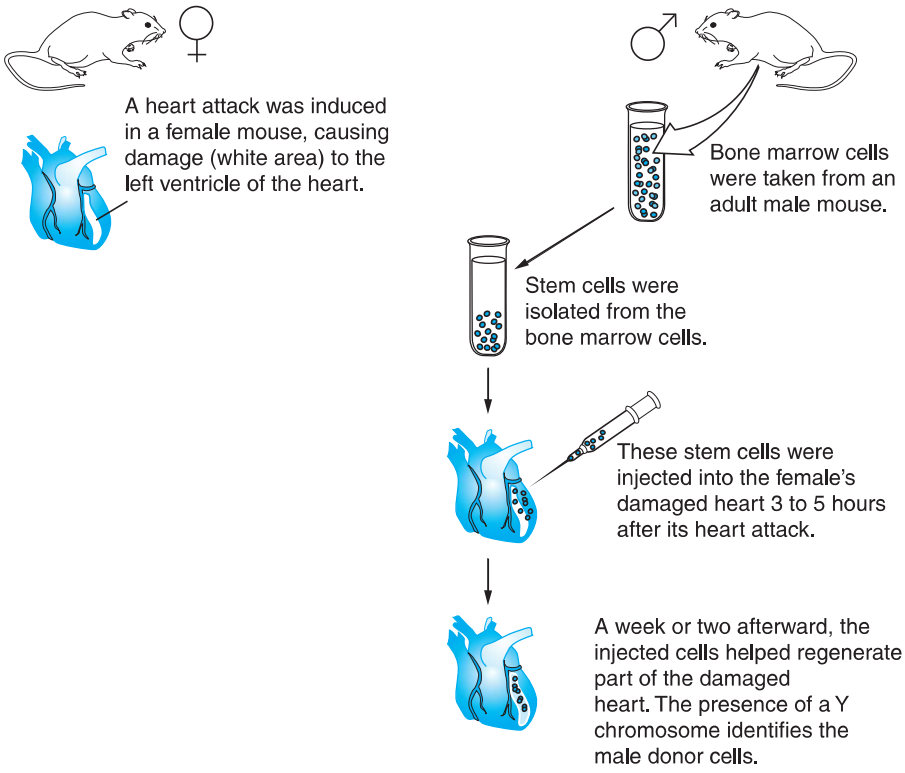

FIGURE 24 Repairing a Damaged Heart with Adult Bone Marrow Stem Cells Mouse adult bone marrow stem cells can be used to repair areas of the mouse heart damaged by a heart attack. Courtesy of Dr. Donald Orlic.

attack. Heart attacks are a leading cause of death in the United States, killing nearly half a million people each year. The death of cardiac muscle cells weakens the heart and can prevent it from beating with the proper strength to maintain normal blood flow. Adult cardiac muscle cells do not repair themselves well. Several groups of researchers have injected adult stem cells from different sources into damaged areas of the mouse heart (**Figure 24**). These stem cells can develop into cardiac muscle cells, form electrical connections with healthy muscle cells, and improve heart function by over 35%. Researchers have reported improved pumping efficiency in mice at least a year after treatment. A similar approach was used to transplant hESCs into the ventricular walls of damaged hearts in pigs and in humans involved in clinical trials. In both examples transplanted stem cells differentiated to form cardiac muscle cells, which restored a significant percentage of electrical activity and contractility to the damaged areas. One study used stem cells from human umbilical cord blood to improve cardiac function in rats.

Scientists are optimistic that this approach may someday work in humans. Consider this: in the future, a surgeon may order a few grams of cardiac muscle cells from a regenerative medicine lab to transplant into a heart attack patient in much the same way that surgeons routinely order blood from a blood bank for a transfusion during a surgical procedure.

A potentially promising and innovative treatment using iPSCs to correct sickle cell anemia in mice has been demonstrated (**Figure 25** on the next page). In this work, iPSCs were produced from skin cells of transgenic mice that express a mutated version of the human sickle cell hemoglobin gene and display sickle cell disease. These iPSCs were genetically engineered to correct the hemoglobin gene mutation. The corrected iPSCs were then induced to form blood stem cells and transferred into donor sickle cell mice, which produced functional red blood cells that, in turn, corrected the disease condition. This is an incredibly exciting result, combining aspects of both stem cell technologies and gene therapy.

Earlier in this chapter, we discussed problems faced by patients who suffer from spinal cord injuries. In the last few years, researchers have disproved a long-standing belief that the human brain and spinal cord cannot grow new neurons. Adult stem cells have been isolated from the human brain and used to make neurons in culture, and scientists have already demonstrated that ESCs can be differentiated to form neurons that can be injected into mice and rats to improve neural function in those with spinal cord injuries. Researchers at Johns Hopkins University have demonstrated that human stem cell transplants can enable mice with paralyzed hind limbs to walk. These studies were carried out on mice that were paralyzed after

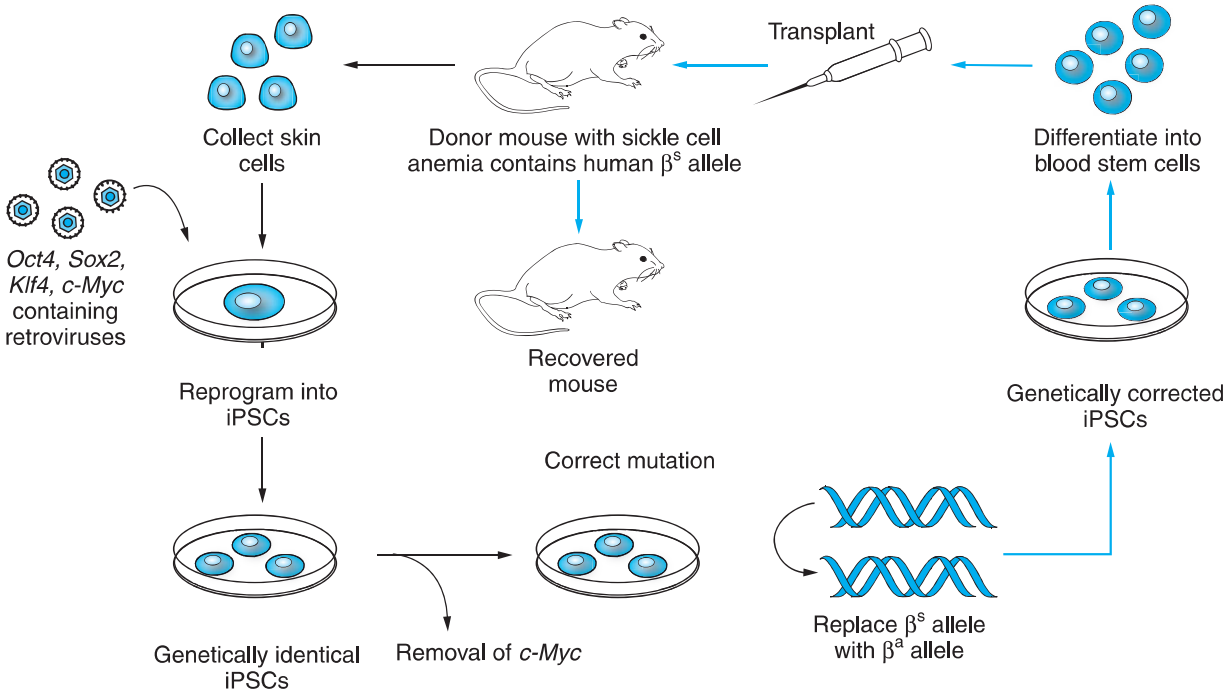

FIGURE 25 A Mouse Model for Correcting Sickle Cell Anemia Using iPSCs

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they were infected with a virus similar to the poliomyelitis virus that causes polio. Recently, the California-based biotechnology company Geron Corporation received approval from the FDA for the first U.S. clinical trial using hESCs to treat individuals with spinal cord injuries. There is still much work to be done in the field of spinal cord repair and regeneration, but researchers are optimistic that adult neural stem cell transplants may be ready for human clinical trials in the next 3 to 5 years, offering hope to the many individuals who are affected by spinal cord injuries.

Many fundamental questions about stem cells, however, must be answered before stem cell technologies can become viable treatment strategies. Currently studies that have used pluripotent stem cells of any source and introduced those into animals or patients have largely been plagued by problems. What do you think is the main issue here? The main problem is how to control the differentiation of pluripotent stem cells into desired tissues of interest. For example, if the stem cells are injected into a tissue, say skeletal muscle, it is difficult to control how they will respond to differentiation cues in vivo. As a result, such cells often develop into many different and undesirable cell and tissue types other than the types that were intended.

When stem cells instead of differentiated adult tissues are injected, scientists cannot fully control the spread of the cells to other places in the body, nor can they control the differentiation of stem cells into tissues other than those that were intended. Injected hESCs have formed tumors, including types of tumors called *teratomas,* which contain mixtures of differentiated tissues such as teeth, bone, and hair, all in one tumor. Another problem is avoiding chromosomal abnormalities that are known to occur when stem cells differentiate. For example, alterations in chromosome number (trisomy 12, trisomy 17, and others) frequently occur when stem cells differentiate. Patients in unregulated stem cell clinics in China, Thailand, Korea, Romania, and other countries have died as a direct result of complications after having received injections of stem cells.

It is important to recognize that the most effective and safe stem cell treatments in the future are likely to involve differentiating stem cells in vitro into desired cell and tissue types and then introducing those cells into a patient as appropriate instead of injecting them directly.

Other important questions to be answered are these:

- Why do stem cells self-renew and maintain an undifferentiated state?
- What factors trigger the division of stem cells?
- What are the growth signals (chemical, genetic, environmental) that influence the differentiation of stem cells?

- What factors affect the integration of new tissues and cells into existing organs?
- Can nuclear reprogramming of somatic cells or other approaches that do not require an embryo become reliable techniques for producing pluripotent stem cells with the properties of hESCs?
- Which diseases can be most effectively treated by stem cell technologies?
- What strategies will be most effective for delivering stem cell treatments?

Answers to these and many other questions will help scientists and physicians in their quest to develop stem cell–based applications for treating human disease.

Cloning

We have discussed many types of cloning in this book. Remember that *cloning* refers to making a copy of something—a gene, a cell, or an entire organism. Recombinant DNA technology is used for gene cloning. When bacteria or cultured cells divide in a petri dish or bioreactor, clones of cells are being produced. But clearly no aspect of cloning is as controversial as animal cloning. With the announcement of the cloning of Dolly the sheep in 1997, the world was immediately faced with the prospect that advances in biotechnology could lead to human cloning. Dolly's creation generated a great increase in public awareness and additional discussion about cloning. In this section, we consider the scientific implications of human cloning applications.

Therapeutic Cloning and Reproductive Cloning

There are two main approaches to cloning: **reproductive cloning** and **therapeutic cloning** (Table 3). The intent of reproductive cloning is to create a baby. Dolly was the first of many mammals to be produced by reproductive cloning. Unlike reproductive cloning, therapeutic cloning provides stem cells that are a genetic match to a patient who requires a transplant. In therapeutic cloning, the chromosomes from a patient's cell (for instance, skin cells) are injected into an enucleated egg—an egg that has had its nucleus removed—that is then stimulated to divide in culture to create an embryo (**Figure 26** on the next page). The embryo produced will not be used to produce a child; instead, it will be grown for several days until it reaches the blastocyst stage so it can be used to harvest stem cells. Stem cells isolated from this embryo can be grown in culture and then introduced into the donor patient (Figure 26).

Prior to the development of iPSCs, therapeutic cloning was seen as a potential way to provide patient-specific stem cells that could be used to treat disease without fear of immune rejection by the recipient because he or she was the original source of the cells. In theory, stem cells from a patient can also be used to create cell lines from humans with genetic diseases to provide scientists with unprecedented potential to study and learn more about human disease conditions. We say "in theory" because it has not been proven that therapeutic cloning in humans can work.

Many scientists do not like the term *therapeutic cloning* because it implies creating a human clone. Creating stem cells to treat human diseases is not the same as cloning a human being.

Most stem cell researchers prefer the term **somatic cell nuclear transfer (SCNT)** because *nuclear transfer* truly describes the biological processes taking place. For therapeutic cloning, the blastocyst would be used as a source of stem cells and then destroyed. For reproductive cloning, the blastocyst would be implanted in a woman's uterus to allow it to grow and develop to form a baby, which would take the normal 9 months. Reproductive cloning by nuclear transfer is a very inefficient process at best. In the case of Dolly the sheep, it took 277 nuclear fusion attempts to produce only one successfully implanted embryo that developed completely and formed Dolly. We will briefly discuss controversies surrounding the work of Dr. Woo Suk Hwang of Seoul National University. Recall that Dr. Hwang had claimed to have cloned a human embryo by SCNT, but this work was proven to be fraudulent.

Many scientists think that reproductive cloning of humans is unethical, immoral, and scientifically unsafe; others think that it may be inevitable at least somewhere in the world. There have been several well-publicized announcements from a few private groups about their ongoing plans to produce humans by reproductive cloning. These claims have generated much skepticism and have been widely disapproved and strongly denounced by most of the scientific community. At the time this text was printed, no definitive proof existed that a human has ever been cloned.

Regulations Governing Embryonic Stem Cell and Therapeutic Cloning in the United States

Although there are international guidelines on the ethical use of stem cells, there is currently no international policy governing stem cell use. In the United States, the FDA has rules regarding the purity of stem

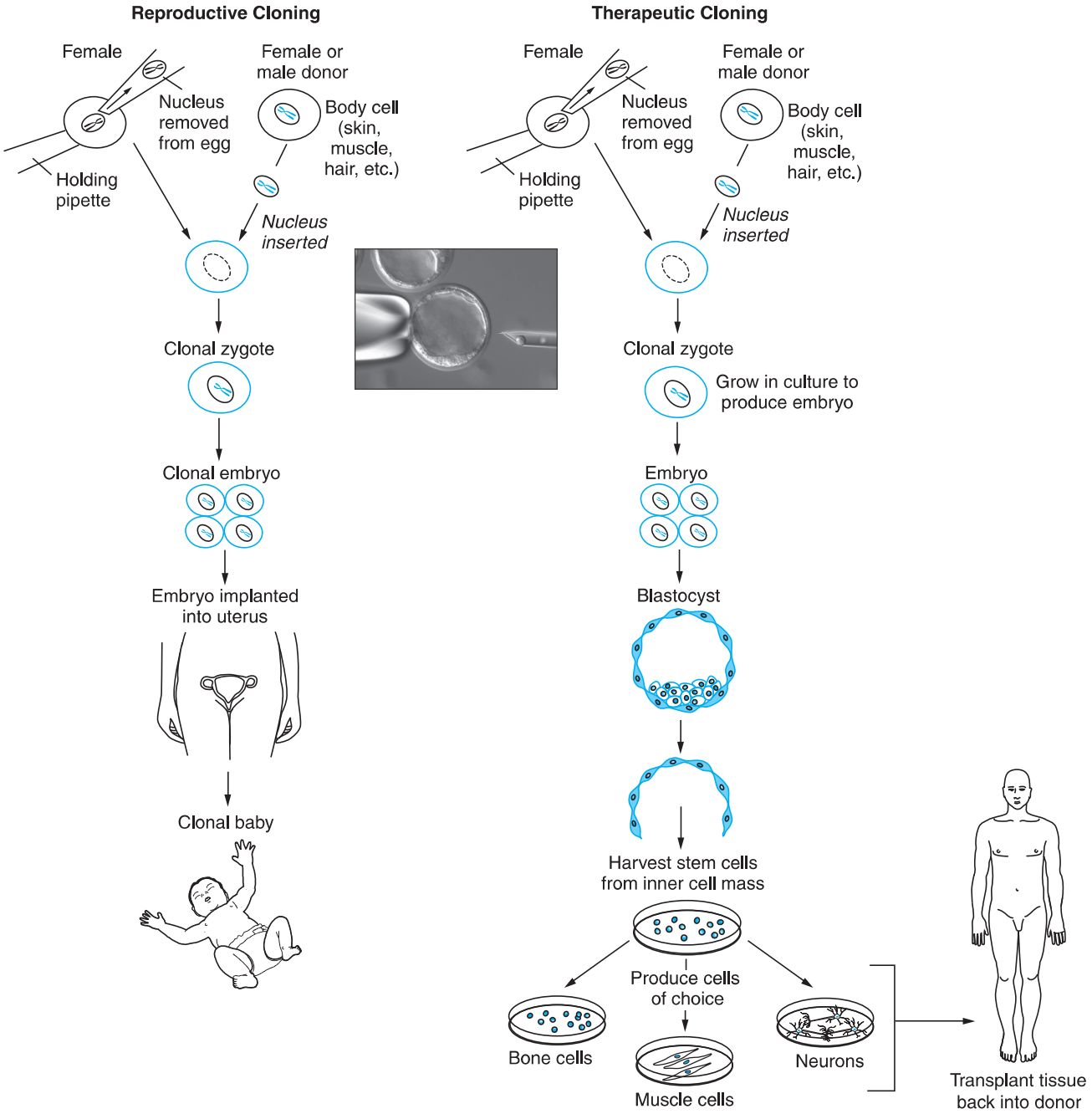

FIGURE 26 Reproductive Cloning and Therapeutic Cloning In reproductive cloning, the goal is to produce a cloned baby. In therapeutic cloning, stem cells that are genetically identical to the cells taken from a patient are produced to provide patient-specific stem cell therapy. This photo shows a holding pipette (left side of egg) holding an egg while the nucleus is being removed with a glass micropipette (right).

Anne Bower.

cells and their applications in clinical trials and medical products. Shortly after hESCs were first isolated in the United States, the National Bioethics Advisory Committee and the National Institutes of Health (NIH) began working on guidelines for hESCs research. On August 9, 2001, President George W. Bush announced a ban on the use of federal funds to create an embryo for the purpose of isolating ESCs. This ban did provide for the use of federal funds for research on 78 cell lines that had already been established and were available through the NIH prior to this date.

However, only 21 of these lines became available for research, and many of the lines turned out to be far less valuable than initially believed. Some failed to

TABLE 3 COMPARISON OF STEM CELLS, THERAPEUTIC CLONING, AND REPRODUCTIVE CLONING

	Embryonic Stem Cells (ESCs)	Adult Stem Cells (ASCs)	Induced Pluripotent Stem Cells (iPSCs)	Therapeutic Cloning (Somatic Cell Nuclear Transfer)	Reproductive Cloning
Final or "end" product	Undifferentiated stem cells (isolated from fetal or embryonic tissue such as an embryo at the blastocyst stage) growing in culture	Undifferentiated stem cells (isolated from adult tissue such as bone marrow cells) growing in a culture dish	Undifferentiated stem cells created from somatic cells; may be patient-specific	Undifferentiated stem cells growing in a culture dish (obtained from the person who will also serve as the recipient of these cells)	"Cloned" human
Purpose/ application	Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue	Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue	Potential source of patient-specific stem cells for studying disease and for treating human disease conditions	Source of stem cells that are genetically matched to recipient for treating human disease conditions such as replacing diseased or injured tissue	Create, duplicate, or replace a human by producing an embryo for implantation, leading to the birth of a child
Embryo Required	Yes	No	No	Yes	Yes
Surrogate mother required	No	No	No	No	Yes
Human created	Depends on how one defines and embryo	No	No	Depends on how one defines and embryo	Yes
Time frame	A few weeks of growth in culture	A few weeks of growth in culture	Weeks to months	A few weeks of growth in culture	9 months, the duration of a normal biological pregnancy (after growth of the embryo in culture)

grow without differentiating. Others showed genetic instability with abnormalities in chromosome number. Some lines were contaminated by mouse feeder cells (which were commonly used to provide key nutrients to growing stem cells in the early days of ESC work). Many scientists and stem cell advocacy groups fought hard to lift the ban on federal funding for the creation of new hESC lines.

In 2006 a bill to lift the ban on federal funding passed the Senate but was subsequently vetoed by President Bush. Congress tried again with a bill in 2007 which was also quickly vetoed by President Bush. In March 2009 President Barack Obama issued an executive order to have the ban lifted and charged the NIH to develop guidelines for governing federal funding of ESC work within 4 months. By April 2009 the NIH had released draft guidelines to allow federal funding for research on hESCs (but not for the use of federal funds to derive hESCs).

In late 2010, a District of Columbia federal judge ruling temporarily blocked President Obama's 2009 order to expand ESC funding based on a lawsuit ruling that the Obama order violated a previous law banning federal funding for research to destroy an embryo. This ruling created major turmoil for stem cell researchers relying on federal funding for their work and resulted in an injunction prohibiting the use of federal funds for hESC work, although it did allow work from funds previously awarded to continue. To demonstrate the impact of this ruling on U.S.-based companies in stem cell research, stock share prices for such companies dropped over

YOU DECIDE

Stem Cell and Cloning Debates

Frequently, when science and medicine produce innovative discoveries, society is not prepared for the consequences of new technology. In 1850, the development of anesthesia was considered very controversial. Many worried about unanticipated adverse reactions from anesthesia, and religious groups protested over "painless" childbirth based on scripture, suggesting that Eve was to go forth from the Garden of Eden to deliver children in pain. Over 150 years later, few people dispute anesthesia as an important tool for complex surgeries and even routine procedures such as having a wisdom tooth extracted.

When recombinant DNA technology was first developed, there was great fear and speculation about what would result from such experiments. Recombinant DNA technology has resulted in many innovative and safe products that have been used to treat more than 250 million people worldwide. Not unlike the anesthesia and recombinant DNA controversies, clergy, politicians, researchers, and the general public currently debate the merits of stem cells and cloning. At the root of these debates is the source of human stem cells, in particular hESCs and their potential uses and abuses. In large part, hESCs are controversial because of their source—the early human embryo. Knowing that hESCs may have enormous potential for treating and curing many devastating diseases and providing people with an opportunity for healthier, longer lives, what do you think about their use? ESCs are perhaps the most controversial scientific issue ever debated by the public and by politicians. The range of questions surrounding stem cells and cloning is seemingly endless.

- Is it acceptable to produce a human embryo for the sole purpose of destroying it for other uses?
- Some fear that stem cells and cloning technologies will cause a great need for human eggs to support research. Is it acceptable to pay women to collect their eggs surgically?
- What is the moral status of early embryos created by therapeutic cloning?
- What rights does a cell donor have to stem cell lines or technologies created from cells they have donated? Should tissue donors share in the commercial potential and monetary awards of stem cell line created from their cells?

Some people believe that a person is formed at the moment an egg is fertilized, so they consider therapeutic cloning the equivalent of killing a child deliberately for the benefit of another person. Others believe that the early embryo is a cluster of living cells with the *potential* for forming a person but the early embryo itself is not a human being.

- Should we justify destroying embryos that are developed through in vitro fertilization approaches?
- Why not use these embryos in an attempt to reduce pain and suffering in other humans?

Scientists define life in many ways. Biologists agree that the cluster of cells called the blastocyst is alive at the cellular level. Although all life forms deserve respect, the blastocyst is not a person because it does not have limbs, a nervous system, organs, or other physical features of a human individual. So a major source of debate continues about whether we should assign moral status to human embryos and if so, at what stage.

- Does the moral value of an embryo increase as it develops? Or is its moral value equal to that of a baby or adult? If an early embryo is deemed a living person, then it has all the rights of other living persons. Consequently to destroy an embryo intentionally is immoral. Taxpaying citizens must decide how their money will be spent and what they believe is ethical, responsible, and safe research. The scientific establishment must share its knowledge to ensure that citizens make well-informed decisions on such topics as ESCs and cloning.

Human cloning is banned in the United States, but many countries have less restrictive policies. There is concern that severe restrictions on stem cell and cloning research in the United States could create a "brain drain" in which top scientists in these fields will move to countries where cloning is legal. The biotechnology industry in the United States could suffer as a result. Many private companies took the lead in stem cell research, using private funding, and in many cases these companies moved research operations to states and countries that were most supportive of stem cell research.

Even if therapeutic cloning is eventually approved in the United States, will its acceptance make it more likely that people will accept reproductive cloning? Probably not. Therapeutic cloning, which is intended to be used to treat illness, is a very different issue than creating a new human. If creating iPSCs turns out to be a viable

Continued

way to produce stem cells, scientific and ethical debates about therapeutic cloning and the use of hESCs may become irrelevant because stem cells could be generated without an egg or embryo.

Various public opinion polls have reported mixed feedback from Americans about the use of hESCs. Recent polls indicate that the percentage of Americans who consider the use of hESCs morally acceptable has gradually increased from about 50% to 65% over the past 8 years. Generally, Americans expect the highest level of health care in the world. If scientists in another country use therapeutic cloning to produce treatments for Parkinson disease, Alzheimer disease, and others, how will the American public feel about not having access to such technologies? Some have even argued that reproductive cloning is a fundamental right of people living in the United States. How would you feel if you were part of an infertile couple who could not have a biologically related child any way other than through reproductive cloning? Are there proper and ethically acceptable applications of using early embryos and their stem cells? Can the same be said for cloning?

You decide.

8% when the ruling was first announced. The NIH was forced to order an immediate shutdown of hESC research by its investigators. By spring 2011, the U.S. Court of Appeals lifted the 2010 injunction, but it is likely this case will eventually go to the U.S. Supreme Court. Check the NIH website on stem cells, which is listed in the Companion Website, for updates on current regulations and other resources.

Many private foundations are providing hundreds of millions of dollars for stem cell researchers, and several states have enacted legislation to create stem cell research institutes and provide state-funded support for ESC research. Some of the more active states include California (which in 2004 passed Proposition 71 approving a budget of nearly $300 million in bonds and over $3 billion overall), New Jersey, Connecticut, Illinois, Maryland, and Wisconsin. Around the world, regulations vary on the production of new hESC lines and policies on therapeutic cloning. For instance, production of new lines and therapeutic cloning is legal in the United Kingdom, Israel, South Korea, China, and Singapore. Therapeutic cloning is banned in Brazil, Australia, and the European Union (although in member nations that allow it, hESCs can be derived from unused embryos from in vitro fertilization procedures).

Legitimate stem cell research centers have been established around the world in countries considered major powers as well as in many relatively small countries (Belgium, Sweden, Turkey, Israel, Switzerland, etc.). But as mentioned earlier, many stem cell treatment clinics have also sprouted up around the world and patients desperate for cures have taken to traveling to other countries seeking stem cell–based cures, which are often overhyped and promoted as successful despite baseless information and adverse side effects. Often called "stem cell tourists," patients desperate for a cure have been known to travel around the world for unproven stem cell treatments, and the number of individuals involved in this "tourism" continues to grow at an alarming rate. The European Union has laws similar to FDA regulations in the United States that govern stem cell usage. But it is clear that international regulations must be established to govern safe applications of stem cells and avoid the fraudulent and tragic cases of their inappropriate and unethical use. Such abuses and the resulting tragedies will only impede progress and erode confidence in the legitimate treatments being developed.

MAKING A DIFFERENCE

In 1968, about 40 years before hESCs were isolated, physicians performed the first successful bone marrow transplant. Bone marrow contains ASCs, specifically hematopoietic stem cells, which can produce all of the different cell types present in blood. Since this first transplantation, bone marrow transplantation has become routine; it is commonly used to treat a variety of blood and bone marrow diseases, blood cancers such as leukemia, blood clotting disorders, and immune disorders of the bloodstream. Many of these diseases were once thought to be incurable and, in the early days of bone marrow transplants, there were many skeptics and many challenges to be overcome. In some ways research with hESCs and iPSCs is facing some of these same challenges and the future success of these technologies remains to be seen. In the United States alone, some 10,000 patients are treated by bone marrow transplants each year, and the success of such treatments is "making a difference" for many patients and their families.

CAREER PROFILE

Career Options in Medical Biotechnology

Medical biotechnology offers an exciting range of potential career choices, primarily in biotechnology and pharmaceutical companies. Courses in chemistry, cell biology, molecular biology, biochemistry, and bioinformatics are essential, but industry experts recommend that students interested in a career in medical biotechnology identify an area of interest and then gain life and work experience that will helps them fit in with a company's needs. In particular, an internship or summer research experience at a local chemical, pharmaceutical, or biotechnology company is highly recommended.

If possible, get involved in a research project with a professor while you are an undergraduate student. Undergraduate research can provide invaluable opportunities for learning how to plan research projects, execute and troubleshoot experiments, and interpret data. Such research may even lead to a presentation at regional or national meetings—a great way to network and interact with other scientists—or even a publication. Your research professor can also provide an essential reference to help you land your first job.

Hiring experts strongly recommend that students study companies of different sizes to get a feel for the type of work and size of company that appeals to them. Consider what you want to do, and then identify companies that intrigue you and whose needs match your interests and skills. Are you interested in drug discovery and development, regenerative medicine and stem cell technologies, gene therapy, cancer research, aging research, surgical materials, genomics, or curing childhood diseases? Would you prefer a large company to a smaller, more personalized company or even a biotechnology start-up company?

With the wealth of information available online, this is easier than ever. Most biotechnology companies have websites. On an interview, it can help to demonstrate a little background knowledge and a true interest in the company with which you would like to land a job.

There are many entry-level job opportunities in medical biotechnology for people with associate's or bachelor's degrees. Many start as laboratory technicians. In some companies, this position involves routine procedures such as preparing solutions and setting up materials for experiments, but individuals who show initiative are often given latitude to do more and to assume decision-making responsibilities in research projects. Application scientists develop new products and procedures, often working directly at the lab bench conducting research. In some companies, however, application scientists may give on-site product demonstrations and present technical seminars to potential customers. Application scientists are also frequently involved in customer relations issues, teaching customers how to troubleshoot a product, interpret data, and the like. Clinical scientists work together with physicians and research scientists to help carry out clinical trials for testing a new drug or medical product. Medical biotechnology companies also employ people who want to combine an interest in science with business skills through marketing and product sales positions.

The interview process at most biotechnology companies is very rigorous. Companies want people who are highly organized, systematic, and attentive to detail. Medical biotechnology research is a team effort. Good writing and communication skills are essential because you will be working with other people routinely and interacting in verbal and written form. In addition, virtually every biotechnology and pharmaceutical company today seeks people with computer skills who can work together with teams of software engineers and information technology professionals to create new software with which to manage and store data, test hypotheses, and model molecular structures.

Industry insiders consistently point out that enthusiasm, an ability to take novel approaches to problem solving, and a commitment to professional growth are valuable tools that companies look for in potential employees. People who are eager and excited about the challenge of applying their skills in teamwork approaches to problem solving are highly desired. Even if a person's educational background is not an exact match for a particular position, as is often the case, enthusiasm and a willingness to learn can determine whether the applicant is hired. Biotechnology companies seek people who want to make a difference—if you have a burning desire to use science to help improve human health, a career in medical biotechnology might be for you.