The sheep named Dolly, which was cloned using genetic material from a somatic cell.

19

BIOTECHNOLOGY

Biotechnology is broadly defined as the application of technologies that involve the use of living organisms, or products from living organisms, for the development of products that benefit humans. Biotechnology is not a new topic. It began several thousand years ago when humans began to domesticate animals and plants for the production of food. Since that time, many species of microorganisms, animals, and plants have become routinely used by people. More recently, the term biotechnology has become associated with molecular genetics. Since the 1970s, molecular genetic tools have provided novel ways to make use of living organisms for products and services. As discussed in Chapter 18, recombinant DNA techniques can be used to genetically engineer microorganisms. In addition, recombinant methods enable the introduction of genetic material into animals and plants. **Genetically modified organisms (GMOs)** have received genetic material via recombinant DNA technology. If an organism has received genetic material from a different species, it is called a **transgenic organism.** A gene from one species that is introduced into another species is called a transgene.

In the 1980s, court rulings made it possible to patent recombinant organisms such as transgenic animals and plants. This was one factor that contributed to the growth of many biotechnology industries. In this chapter, we will examine how molecular techniques have expanded our knowledge of the genetic characteristics of commercially important species. We will also discuss examples in which recombinant microorganisms and transgenic animals and plants have been given characteristics that are useful in the treatment of disease or in agricultural production. These include recombinant bacteria that make human insulin, transgenic livestock that produce human proteins in their milk, and transgenic tomatoes with a longer shelf life. In addition, the topics of mammalian cloning and stem cell research are examined from a technical point of view. Likewise, the current and potential use of human gene therapy—the introduction of cloned genes into living cells in the treatment of a disease—will be addressed. In the process, we will also touch upon some of the ethical issues associated with these technologies.

19.1 USES OF MICROORGANISMS IN BIOTECHNOLOGY

Microorganisms are used to benefit humans in various ways (**Table 19.1**). In this section, we will examine how molecular genetic tools have become increasingly important for improving our use of microorganisms. Such tools can produce recombinant microorganisms with genes that have been manipulated in vitro. Why are recombinant organisms useful? Recombinant techniques can improve strains of microorganisms and have even yielded strains that make products not normally produced by microorganisms. For example, human genes have been introduced into

TABLE **19.1**	
Common Uses of Microorganisms	
Application	**Examples**
Production of medicines	Antibiotics
	Synthesis of human insulin in recombinant *E. coli*
Food fermentation	Cheese, yogurt, vinegar, wine, and beer
Biological control	Control of plant diseases, insect pests, and weeds
	Symbiotic nitrogen fixation
	Prevention of frost formation
Bioremediation	Cleanup of environmental pollutants such as petroleum hydrocarbons and synthetics that are difficult to degrade

| TABLE **19.2**		
Examples of Medical Agents Produced by Recombinant Microorganisms		
Drug	**Action**	**Treatment**
Insulin	A hormone that promotes glucose uptake	For diabetes
Tissue plasminogen activator (TPA)	Dissolves blood clots	For heart attacks and other arterial occlusions
Superoxide dismutase	Antioxidant	For heart attacks and to minimize tissue damage
Factor VIII	Blood-clotting factor	For certain types of hemophilias
Renin inhibitor	Lowers blood pressure	For hypertension
Erythropoietin	Stimulates the production of red blood cells	For anemia

bacteria to produce medically important products such as insulin and human growth hormone. As discussed in this section, several recombinant strains are in widespread use. However, in some areas of biotechnology and in some parts of the world, the commercialization of recombinant strains has proceeded very slowly. This is particularly true for applications in which recombinant microorganisms may be used to produce food products or where they are released into the environment. In such cases, safety and environmental concerns along with negative public perceptions have slowed or even halted the commercial use of recombinant microorganisms. Nevertheless, molecular genetic research continues, and many biotechnologists expect an expanding use of recombinant microbes in the future.

Many Important Medicines Are Produced by Recombinant Microorganisms

During the 1970s, geneticists became aware of the great potential of recombinant DNA technology to produce therapeutic agents for treating certain human diseases. Healthy individuals possess many different genes that encode short peptide and longer polypeptide hormones. Diseases can result when an individual is unable to produce these hormones.

In 1976, Robert Swanson and Herbert Boyer formed Genentech Inc. The aspiration of this company was to engineer bacteria to synthesize useful products, particularly peptide and polypeptide hormones. Their first contract was with researchers Keiichi Itakura and Arthur Riggs. They were able to engineer a bacterial strain that produced somatostatin, a human hormone that inhibits the secretion of a number of other hormones, including growth hormone, insulin, and glucagon. Somatostatin was not chosen for its commercial potential. Instead, it was chosen because the researchers thought it would be technically less difficult to produce than other hormones. Somatostatin is very small (only 14 amino acids long), which requires a short coding sequence, and it can be detected easily. Since this pioneering work, recombinant DNA technology has been used to develop bacterial strains that synthesize several other medical agents, a few of which are described in **Table 19.2**.

In 1982, the U.S. Food and Drug Administration approved the sale of the first genetically engineered drug, human insulin, which was produced by Genentech and marketed by Eli Lilly. In nondiabetic individuals, insulin is produced by the β cells of the pancreas. Insulin functions to regulate several physiological processes, particularly the uptake of glucose into fat and muscle cells. Persons with insulin-dependent diabetes cannot synthesize an adequate amount of insulin due to a loss of their β cells. Prior to 1982, insulin was isolated from the pancreases removed from cattle and pigs. Unfortunately, in some cases, diabetic individuals became allergic to such insulin and had to use expensive combinations of insulin from other animals and human cadavers. Today, people with diabetes can use genetically engineered human insulin to treat their disease.

Insulin is a hormone composed of two polypeptide chains, called the A and B chains. To make this hormone using bacteria, the coding sequences of the A and B chains are placed next to the coding sequence of a native *E. coli* protein, β-galactosidase (**Figure 19.1**). This creates a fusion protein comprising β-galactosidase and the A or B chain. This step is necessary because the A and B chains are rapidly degraded when expressed in bacterial cells by themselves. The fusion proteins, however, are not. How are the two fusion proteins used to make human insulin? After the fusion proteins are expressed in bacteria, they can be purified and then treated with cyanogen bromide (CNBr), which cleaves after a methionine that is found at the junction between β-galactosidase and the A or B chain. This cleavage step separates β-galactosidase from the A or B chain. The A and B chains are then purified and mixed together under conditions in which they refold and form disulfide bonds with each other to make an active insulin hormone.

Bacterial Species Can Be Used as Biological Control Agents

The term **biological control** refers to the use of living organisms or their products to alleviate plant diseases or damage from
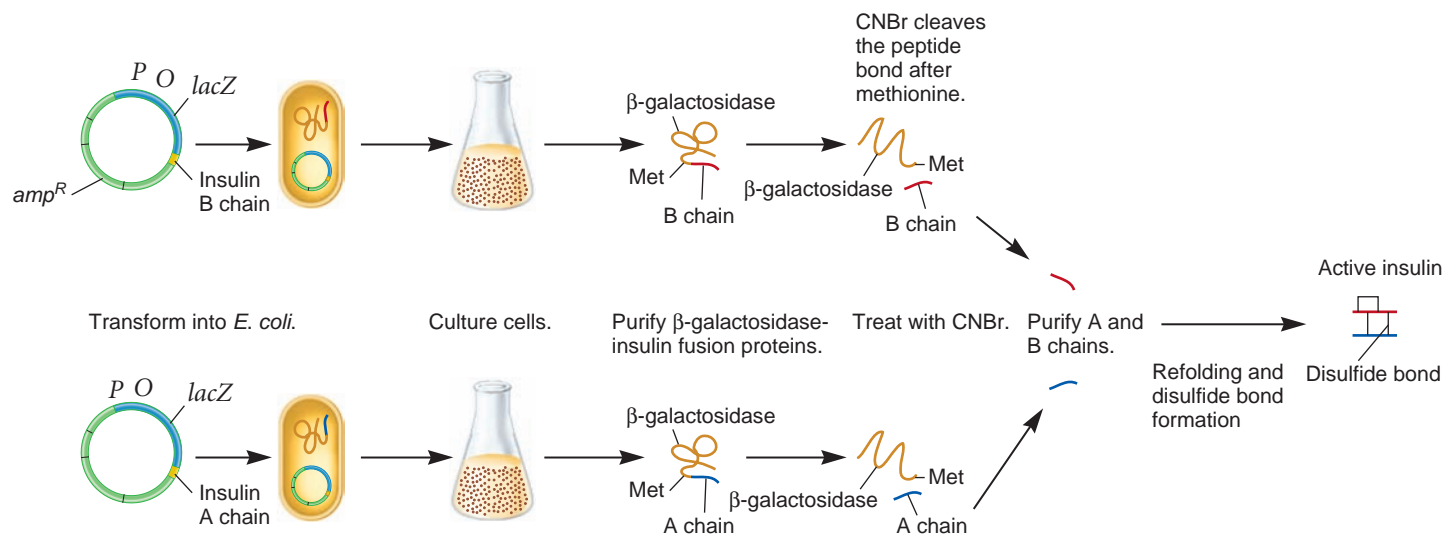
FIGURE 19.1   **The use of bacteria to make human insulin.**

Genes → Traits The synthesis of human insulin is not a trait that bacteria normally possess. However, genetic engineers can introduce the genetic sequences that encode the A and B chains of human insulin via recombinant DNA technology, yielding bacteria that make these polypeptides as fusion proteins with β-galactosidase. Cyanogen bromide (CNBr) treatment releases the A and B polypeptides, which are then purified and mixed together under conditions in which they refold and form functional human insulin.

environmental conditions. During the past 20 years, interest in the biological control of plant diseases and insect pests as an alternative to chemical pesticides has increased. Biological control agents can prevent disease in several ways. In some cases, nonpathogenic microorganisms are used to compete effectively against pathogenic strains for nutrients or space. In other cases, microorganisms may produce a toxin that inhibits other pathogenic microorganisms or insects without harming the plant.

Biological control can also involve the use of microorganisms living in the field. A successful example is the use of *Agrobacterium radiobacter* to prevent crown gall disease caused by *Agrobacterium tumefaciens*. The disease gets its name from the large swellings (galls) produced by the plant in response to the bacteria. *A. radiobacter* produces agrocin 84, an antibiotic that kills *A. tumefaciens*. Molecular geneticists have determined that *A. radiobacter* contains a plasmid with genes responsible for agrocin 84 synthesis and resistance. Unfortunately, this plasmid is occasionally transferred from *A. radiobacter* to *A. tumefaciens* during interspecies conjugation. When this occurs, *A. tumefaciens* can gain resistance to agrocin 84. Researchers have identified *A. radiobacter* strains in which this plasmid has been altered genetically to prevent its transfer during conjugation. This conjugation-deficient strain is now used commercially worldwide to prevent crown gall disease.

Another biological control agent is *Bacillus thuringiensis*, usually referred to as Bt (pronounced "bee-tee"). This naturally occurring bacterium produces toxins that are lethal to many caterpillars and beetles that feed on a wide variety of food crops and ornamental plants. Bt is generally harmless to plants and other animals, such as humans, and does not usually harm beneficial insects that act as pollinators. Therefore, it is viewed as an environmentally friendly pesticide. Commercially, Bt is sold in a powder form that is used as a dust or mixed with water as a foliage spray. Bt is then dusted or sprayed on plants that are under

attack by caterpillars or beetles so the pests will ingest the bacteria as they eat the leaves, flowers, or fruits. The toxins produced by Bt bring about paralysis of the insect's digestive tract, causing it to stop feeding within hours and die within a few days. Geneticists have cloned the genes that encode Bt toxins, which are proteins. As discussed later, such genes have been introduced into crops, such as corn, to produce transgenic plants resistant to insect attack.

The Release of Recombinant Microorganisms into the Environment Is Sometimes Controversial

As we have seen, genetically altered strains can have commercial applications in the field. Whether or not a microorganism is recombinant has become an important issue in the use of biological control agents that are released into the environment. Alterations in the genetic characteristics of a microorganism, such as the acquisition of naturally occurring plasmids or mutagenesis by chemical agents and radiation, do not produce bacteria that are classified as recombinant strains. The *A. radiobacter* strain and Bt bacterium fall into this category. This distinction is important from the perspective of both governmental regulation and public perception, even though the organisms produced by nonrecombinant and recombinant approaches may be genetically identical or nearly identical.

Knowledge from molecular genetics research is used to develop both nonrecombinant and recombinant strains with desirable characteristics. Each year, many new strains of nonrecombinant microorganisms are analyzed in field tests for the biological control of plant diseases and insect pests. By comparison, the use of recombinant microorganisms in field tests has proceeded much more slowly. This slow progress is related to increased levels of governmental regulation and, in some cases, to negative public perception of recombinant microorganisms.

As an example of the controversial nature of this topic, let's consider the first field test of a recombinant bacterium, which involved the use of a genetically engineered strain of *Pseudomonas syringae* to control frost damage. Experiments by Steven Lindow and colleagues showed that the formation of ice on the surface of plants is enhanced by the presence of certain bacterial species. These *Ice*$^+$ species synthesize cellular proteins that promote ice nucleation—the initiation of ice crystals. Using recombinant DNA technology, Lindow constructed an *Ice*$^-$ strain of *P. syringae* in the early 1980s, which lacks the gene responsible for the production of ice-nucleation proteins. When applied to the surface of plants, an *Ice*$^-$ strain can compete with and thereby reduce the proliferation of *Ice*$^+$ bacteria, thus inhibiting the formation of frost.

Lindow sought approval for field tests of an *Ice*$^-$ recombinant strain in Tulelake, California. For several years, these tests were delayed because of a lawsuit from the Foundation on Economic Trends. During that time, Lindow made great efforts to ensure the safety of this project by studying the local environment. He also consulted with local townspeople where the field test was to take place. Initially, the idea was well received by the local residents. However, another company tested similar bacteria on the roof of an Oakland facility without Environmental Protection Agency (EPA) approval. The media reported this incident, and it caused many Tulelake townspeople to become apprehensive about the release of recombinant bacteria. Nevertheless, in 1987, approval was finally granted for the field testing of the recombinant *P. syringae* (**Figure 19.2**).

During the first test on several thousand strawberry plants, the plants were ripped out by vandals. In a second field test, the ability of *Ice*$^-$ bacteria to protect potato plants was tested. Although some of the plants were destroyed by vandals, the results of this field experiment showed that the *Ice*$^-$ bacteria did protect potato plants from frost damage. In addition, soil sampling showed that the recombinant bacteria were contained at the field site and did not proliferate into surrounding areas. Even so, the release of recombinant microorganisms into the environment remains controversial. Since this first test, relatively few recombinant strains have been released. In the case of *Ice*$^-$ bacteria, further research was discouraged by a variety of factors, including governmental regulation and the expense of doing additional experiments. The recombinant *Ice*$^-$ bacteria were never commercialized.

Microorganisms Can Reduce Environmental Pollutants

The term **bioremediation** refers to the use of living organisms or their products to decrease pollutants in the environment. As its name suggests, this is a biological remedy for pollution. During bioremediation via microorganisms, enzymes produced by a microorganism modify a toxic pollutant by altering or transforming its structure. This event is called **biotransformation.** In many cases, biotransformation results in **biodegradation,** in which the toxic pollutant is degraded, yielding less complex, nontoxic metabolites. Alternatively, biotransformation without biodegradation can also occur. For example, toxic heavy metals can often be rendered less toxic by oxidation or reduction reactions carried out by microorganisms. Another way to alter the toxicity of organic pollutants is by promoting polymerization. In many cases, polymerized toxic compounds are less likely to leach from the soil and, therefore, are less environmentally toxic than their parent compounds.

Since the early 1900s, microorganisms have been used in the treatment and degradation of sewage. More recently, the field of bioremediation has expanded into the treatment of hazardous and refractory chemical wastes—chemicals that are difficult to degrade and usually associated with industrial activity. These pollutants include petroleum hydrocarbons, halogenated organic compounds, pesticides, herbicides, and organic solvents. Many new applications that use microorganisms to degrade these pollutants are being tested. The field of bioremediation has been fostered, to a large extent, by better knowledge of how pollutants are degraded by microorganisms, the identification of new and useful strains of microbes, and the ability to enhance bioremediation through genetic engineering.

Molecular genetic technology is key in identifying genes that encode enzymes involved in bioremediation. The characterization of the relevant genes greatly enhances our understanding of how microbes can modify toxic pollutants. In addition, recombinant strains created in the laboratory can be more efficient at degrading certain types of pollutants.

In 1980, in a landmark case (*Diamond v. Chakrabarty*), the U.S. Supreme Court ruled that a live, recombinant microorganism is patentable as a "manufacture or composition of matter." The first recombinant microorganism to be patented was an "oil-eating" bacterium that contained a laboratory-constructed plasmid. This strain can oxidize the hydrocarbons commonly found in petroleum. It grew faster on crude oil than did any of the natural isolates tested. However, it has not been a commercial success because this recombinant strain metabolizes only a limited number of toxic compounds, a fraction of the more than 3000 actually present in crude oil. Unfortunately, the recombinant strain

FIGURE 19.2   **The release of recombinant *P. syringae* in a field test.**

did not degrade many higher-molecular-weight compounds, which tend to persist in the environment.

Thus far, most bioremediation has involved the use of natural microorganisms rather than recombinant ones. Currently, bioremediation should be considered a developing industry. Many studies are currently underway aimed at elucidating the mechanisms whereby microorganisms degrade toxic pollutants. In the future, recombinant microorganisms may provide an effective way to decrease the levels of toxic chemicals within our environment. However, this approach will require careful studies to demonstrate that recombinant organisms are effective at reducing pollutants, and are safe and able to survive when released into the environment.

19.2 GENETICALLY MODIFIED ANIMALS

As mentioned at the beginning of this chapter, transgenic organisms contain recombinant DNA from another species that has been integrated into their genome. A dramatic example of this is shown in **Figure 19.3**. In this case, the gene that encodes human growth hormone was introduced into the genome of a mouse. The larger mouse on the right is a transgenic mouse that expresses the human growth hormone gene to a very high level.

The production of transgenic animals is a relatively new area of biotechnology. In recent years, a few transgenic species have
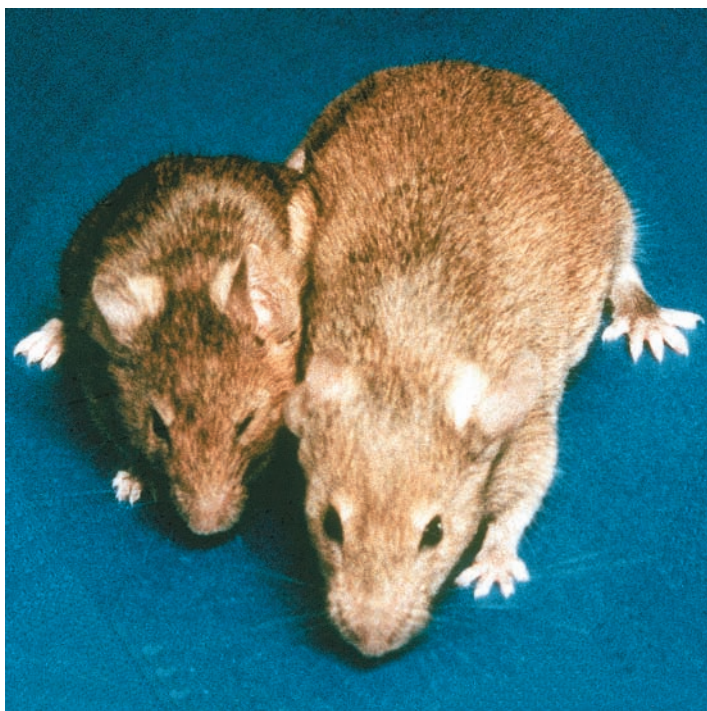
FIGURE 19.3   A comparison between a normal mouse and a transgenic mouse that carries a growth hormone gene.

Genes → Traits   The transgenic mouse (on the right) carries a gene encoding human growth hormone. The introduction of the human gene into the mouse's genome has caused it to grow larger.

reached the stage of commercialization. Many researchers believe that this technology holds great promise for innovations in biotechnology. However, the degree to which this potential may be realized depends, in part, on the public's concern about the production and consumption of transgenic species.

In this section, we begin by examining the mechanisms through which cloned DNA becomes integrated into the chromosomal DNA of animal cells. We will then explore the current techniques used to produce transgenic animals and their potential uses.
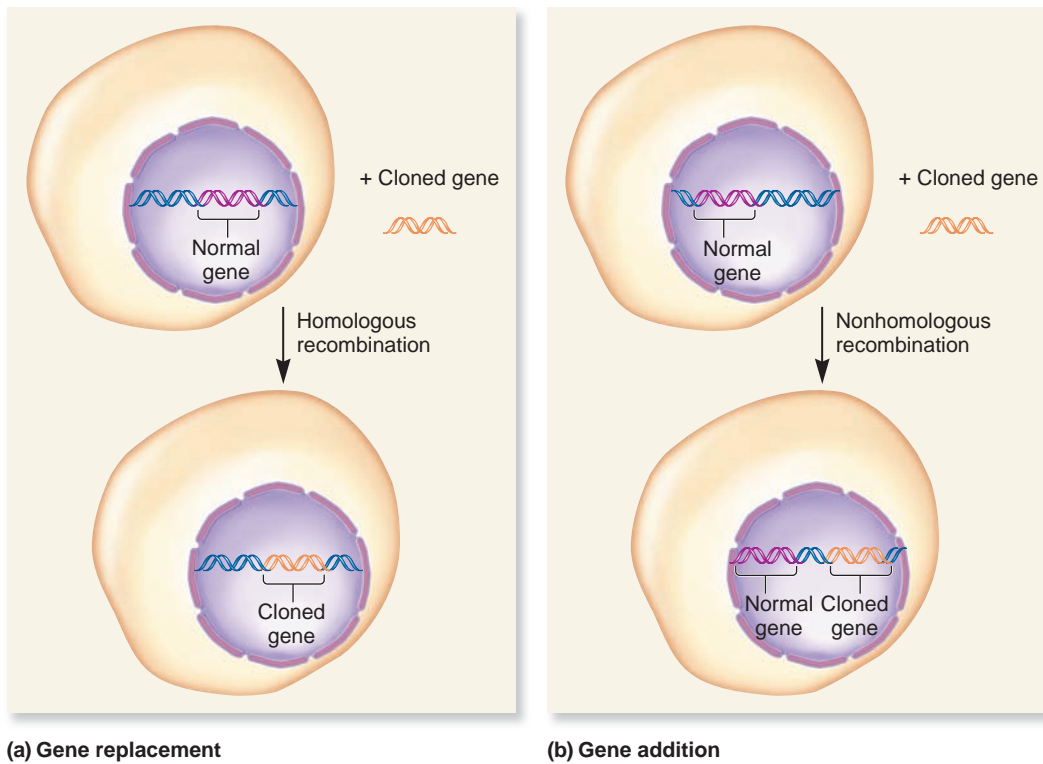
The Integration of a Cloned Gene into a Chromosome Can Result in Gene Replacement or Gene Addition

In Chapter 18, we considered methods to clone genes. A common approach is to insert a chromosomal gene into a vector and then propagate the vector in living microorganisms such as bacteria or yeast cells. Cloned genes can also be introduced into animal and plant cells. However, to be inherited stably from generation to generation, the cloned gene must become integrated into one (or more) of the chromosomes that reside in the cell nucleus. The integration of cloned DNA into a chromosome occurs by recombination, which is described in Chapter 17.

Figure 19.4 illustrates how a cloned gene can integrate into a chromosome by recombination. If the genome of the host cell carries the same type of gene and if the cloned gene is swapped with the normal chromosomal gene by homologous recombination, then the cloned gene will replace the normal gene within the chromosome (Figure 19.4a). This process is termed **gene replacement.** If the cloned gene has been rendered inactive by mutation and replaces the normal gene, researchers can study how the loss of normal gene function affects the organism. This is called a **gene knockout.** More commonly, a cloned gene may be introduced into a cell and become integrated into the genome by nonhomologous recombination in a process known as **gene addition** (Figure 19.4b). As shown here, both the cloned and normal genes are present following gene addition.

Researchers may also introduce a cloned gene that is not already present in a particular species' genome. An interesting example of this type of gene addition involves the production of aquarium fish that "glow," the aptly named **GloFish.** The GloFish is a brand of transgenic zebrafish (*Danio rerio*) that glows with bright green, red, or yellow fluorescent color (**Figure 19.5**). How were these fish produced? In 1999, Zhiyuan Gong and his colleagues started with a gene from jellyfish that encodes a green fluorescent protein and inserted it into the zebrafish genome by gene addition, causing the zebrafish to glow green. By placing the gene next to a gene promoter that would turn the gene on in the presence of certain environmental toxins, their goal was to eventually develop a fish that could be used to detect water pollution. The researchers subsequently collaborated with a company to market the fish for aquarium use. They developed a red fluorescent zebrafish by adding a gene from a sea coral, and a yellow fluorescent zebrafish by adding a variant of the jellyfish gene. In 2003, the GloFish became the first genetically modified organism to be sold as a pet. GloFish have been successfully marketed in

FIGURE 19.4 **The introduction of a cloned gene into a cell can lead to gene replacement or gene addition.** In these two examples, the cloned gene is similar to a normal gene already present in the genome of a cell. **(a)** When the cloned gene undergoes homologous recombination and replaces the normal gene, this is called gene replacement. **(b)** Alternatively, the cloned gene may recombine nonhomologously at some other chromosomal location, leading to gene addition.

several countries, including the United States, although the sale of GloFish is banned in California.

Molecular Biologists Can Produce Mice That Contain Gene Replacements

In bacteria and yeast, which have relatively small genomes, homologous recombination between cloned genes and the host cell chromosome occurs at a relatively high rate, so gene replacement is commonly achieved. Gene replacement is useful when a researcher or biotechnologist wants to make a gene mutation in the laboratory and then introduce the mutant gene into a living organism to compare the effects of the normal and mutant genes on the phenotype. However, in more complex eukaryotes with very large genomes, the introduction of cloned genes into cells is much more likely to result in gene addition rather than gene replacement. For example, when a cloned gene is introduced into a mouse cell, it undergoes homologous recombination less than 0.1% of the time. Most of the time (over 99.9%), gene addition occurs.

To produce mice with gene replacements, molecular biologists have devised laboratory procedures to preferentially select cells in which homologous recombination has occurred. One approach is shown in **Figure 19.6**. The gene of interest is found
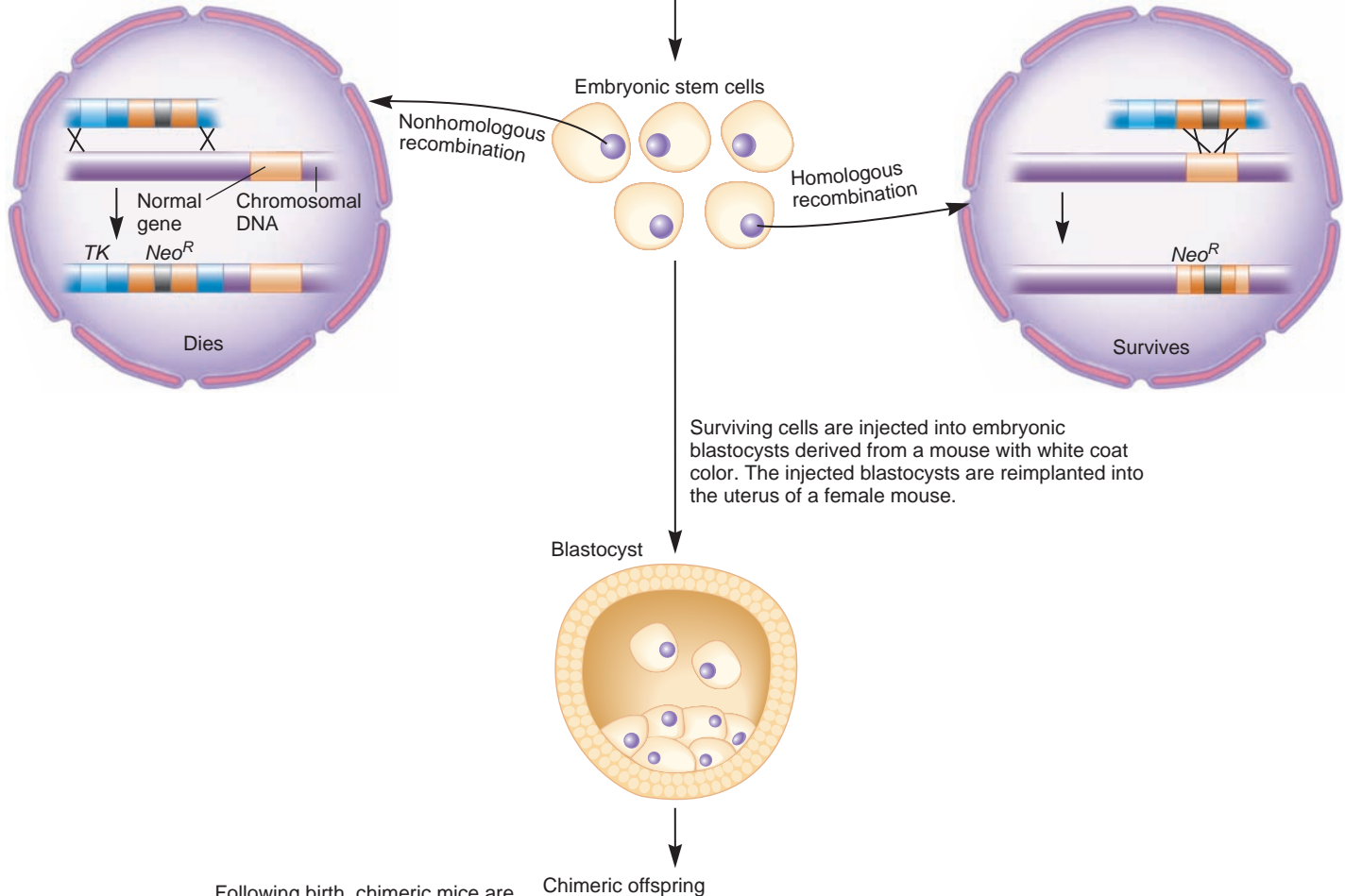

FIGURE 19.5 **The use of gene addition to produce fish that glow.** The aquarium fish shown here, which are named GloFish, are transgenic organisms that have received a gene from jellyfish or sea corals that encodes a fluorescent protein, causing them to glow green, red, or yellow.

The gene of interest has been cloned. A neomycin resistance gene is inserted into the center of this gene, and a thymidine kinase gene is inserted next to the gene.

TK Neo^R
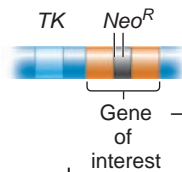
Gene – Same gene as normal,
of   chromosomal gene except
interest   it has Neo^R inserted into it.

This cloned DNA is then introduced into embryonic stem cells. In this case, the cells were derived from a mouse with dark fur color. The cells are grown in the presence of neomycin and gancyclovir. Only those cells that contain the Neo^R gene but are lacking the TK gene will survive.

Embryonic stem cells

Nonhomologous recombination

Homologous recombination

Normal gene
Chromosomal DNA

TK Neo^R

Dies

Neo^R

Survives

Surviving cells are injected into embryonic blastocysts derived from a mouse with white coat color. The injected blastocysts are reimplanted into the uterus of a female mouse.

Blastocyst

Following birth, chimeric mice are identified as those that contain a coat with both dark and white fur. The appropriate crosses are made in order to produce mice that have two copies of the target gene.

Chimeric offspring
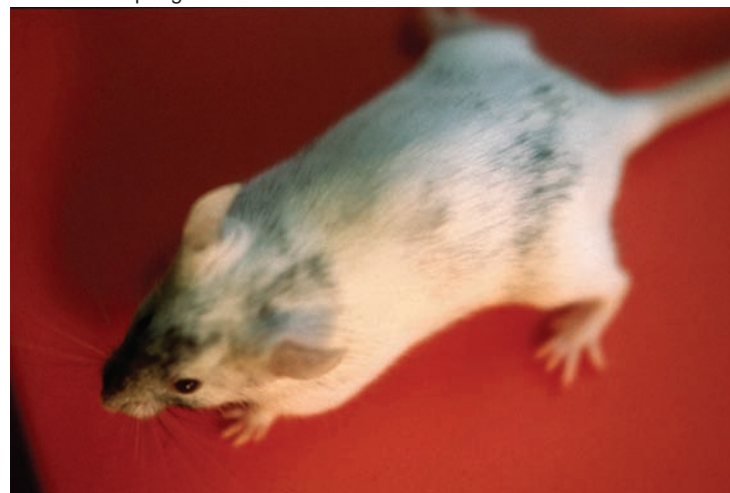
FIGURE 19.6 **Producing a gene replacement in mice.** The bottom of this figure shows a photograph of a chimeric mouse. Note the patches of black and white fur.

in a mouse chromosome and is cloned so that it can be manipulated in vitro. The cloned gene is shown at the top of Figure 19.6. The cloning procedure involves two selectable marker genes that influence whether or not mouse embryonic cells can grow in the presence of certain drugs. First, the gene of interest is inactivated by inserting a neomycin-resistance gene (called Neo^R) into the center of its coding sequence. Neo^R provides cells with resistance to neomycin. Next, a thymidine kinase gene, designated *TK*, is inserted adjacent to the gene of interest but not within the gene itself. The *TK* gene renders cells sensitive to killing by a drug called gancyclovir.

After the cloned gene has been modified in vitro, it is introduced into mouse embryonic stem cells. How do researchers identify the cells in which homologous recombination has occurred? When the cells are grown in the presence of neomycin and gancyclovir, most nonhomologous recombinants are killed because they also carry the *TK* gene. In contrast, homologous recombinants in which the normal gene has been partially replaced with the cloned gene contain only the Neo^R gene, so they are resistant to both drugs. The surviving embryonic cells can then be injected into blastocysts, early embryos that are obtained from a pregnant mouse. In the example shown in Figure 19.6, the embryonic cells are from a mouse with dark fur, and the blastocysts are from a mouse with white fur. The embryonic cells can mix with the blastocyst cells to create a **chimera,** an organism that contains cells from two different individuals. To identify chimeras, the injected blastocysts are reimplanted into the uterus of a female mouse and allowed to develop. When this mouse gives birth, chimeras are easily identified because they contain patches of white and dark fur (see Figure 19.6).

Chimeric animals that contain a single-gene replacement can then be mated to other mice to produce offspring that carry the mutant gene. Because mice are diploid, researchers must make two or more subsequent crosses to create a strain of mice that contains both copies of the mutant target gene. However, homozygous strains for a gene knockout cannot be produced if the mutant gene is lethal in the homozygous state.

An alternative method for producing genetically modified mice is to inject the desired gene into a fertilized egg. To conduct this type of experiment, researchers obtain mouse eggs and fertilize them in vitro. Immediately following fertilization, the cloned gene is injected into the sperm pronucleus—the haploid nucleus that has not yet fused with the egg nucleus. The cloned DNA then integrates into the genome, and the two pronuclei fuse to form the diploid nucleus of a zygote. The zygote begins to divide and is introduced into the uterus of a female mouse, where it becomes implanted and grows. As discussed next, genetically modified mice are used in basic research and to study human diseases.

Gene Knockouts and Knockins Are Produced in Mice to Understand Gene Function and Human Disease

As we have seen, researchers can replace a normal mouse gene with one that has been inactivated by the insertion of an antibiotic-resistance gene. As mentioned, when a mouse is homozygous for an inactivated gene, this condition is called a gene

knockout. The inactive mutant gene has replaced both copies of the normal gene. In other words, the function of the normal gene has been "knocked out." By creating gene knockouts, researchers can study how the loss of normal gene function affects the organism. Gene knockouts frequently have specific effects on the phenotype of a mouse, which helps researchers to determine that the function of a gene is critical within a particular tissue or during a specific stage of development. In many cases, however, a gene knockout produces no obvious phenotypic effect. One explanation is that a single gene may make such a small contribution to an organism's phenotype that its loss may be difficult to detect. Alternatively, another possible explanation for a lack of observable phenotypic change in a knockout mouse may involve **gene redundancy.** This means that when one type of gene is inactivated, another gene with a similar function may be able to compensate for the inactive gene.

A particularly exciting avenue of gene knockout research is its application in the study and treatment of human disease. How is this useful? Knocking out the function of a gene may provide clues about what that gene normally does. Because humans share many genes with mice, observing the characteristics of knockout mice gives researchers information that can be used to better understand how a similar gene may cause or contribute to a disease in humans. Examples of research areas in which knockout mice have been useful include cancer, obesity, heart disease, diabetes, and many inherited disorders. The use of knockouts in the area of functional genomics is also discussed in Chapter 21.

In contrast to knockouts, researchers may introduce genes into the mouse genome to study the effects of gene overexpression or to examine the effects of particular alleles, such as those that may cause disease in humans. To accomplish this, researchers can produce a **gene knockin.** A gene knockin is a gene addition in which a gene of interest has been added to a particular site in the mouse genome (**Figure 19.7**). In this example, the cloned gene is inserted into the middle of a segment of DNA from a noncritical site in the mouse genome. The noncritical segment is very long, which allows the transgene to be targeted to that specific, noncritical integration site by homologous recombination after it is introduced into mouse cells. Such gene knockins tend to result in a more consistent level of expression of the transgene compared to gene additions that may occur randomly in another place in the genome. Also, because a targeted transgene is not interfering with a critical locus, the researcher can be more certain that any resulting phenotypic effect is due to the expression of the transgene.

To study human diseases, researchers have produced strains of transgenic mice that harbor both gene knockouts and gene knockins. A strain of mice engineered to carry a mutation that is analogous to a disease-causing mutation in a human gene is termed a mouse model. As an example, let's consider sickle cell disease, which is due to a mutation in the human β-globin gene (refer back to Figure 4.7). This gene encodes a polypeptide called β globin; adult hemoglobin is composed of both α-globin and β-globin polypeptides. When researchers produced a gene knockin by introducing the mutant human β-globin gene into mice, the resulting mice showed only mild symptoms of the disease. However, Chris Pászty and Edward Rubin produced a mouse model with multiple gene knockins
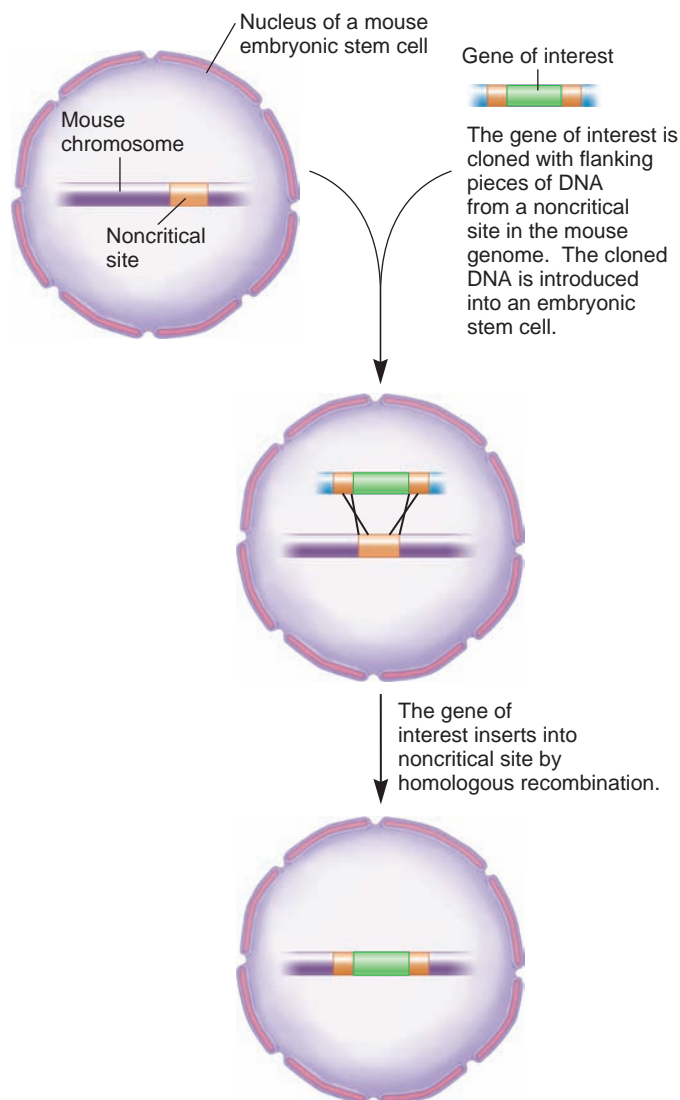
FIGURE 19.7 **Producing a gene knockin in mice.** This simplified diagram illustrates the strategy of producing a gene knockin. The gene of interest is cloned, and the cloned gene is flanked by DNA sequences that are homologous to a noncritical site in the mouse genome. When introduced into a mouse embryonic stem cell, the gene inserts into the genome by homologous recombination, which is an example of a targeted gene addition.

and gene knockouts. In particular, the mice had gene knockins for the normal human α-globin gene and the mutant β-globin gene from patients with sickle cell anemia. The strain also had gene knockouts of the mouse α-globin gene and β-globin gene:

> normal human α-globin gene knockin;
> mutant human β-globin gene knockin;
> mouse α-globin gene knockout;
> mouse β-globin gene knockout.

Therefore, these mice made adult hemoglobin just like people with sickle cell disease, but they did not produce any normal mouse hemoglobin. These transgenic mice exhibit the major features of sickle cell disease—sickled red blood cells, anemia, and multiorgan pathology. They have been useful as a model for studying the disease and testing potential therapies.

Biotechnology Holds Promise in Producing Transgenic Livestock

The technology for creating transgenic mice has been extended to other animals, and much research is under way to develop transgenic species of livestock, including fish, sheep, pigs, goats, and cattle. For some farmers, the ability to modify the characteristics of livestock via the introduction of cloned genes is an exciting prospect. In addition, work is currently under way to produce genetically modified pigs that are expected to be resistant to rejection mechanisms that occur following organ transplantation to humans. These strains may in time become a source of organs or cells for patients.

A novel avenue of research involves the production of medically important proteins in the mammary glands of livestock. This approach is sometimes called **molecular pharming.** (The term is also used to describe the manufacture of medical products by agricultural plants.) As shown in **Table 19.3**, several human proteins have been successfully produced in the milk of domestic livestock. Compared with the production of proteins in bacteria, one advantage is that certain proteins are more likely to function properly when expressed in mammals. This may be due to covalent modifications, such as the attachment of carbohydrate groups, which occur in eukaryotes but not in bacteria. In addition, certain proteins may be degraded rapidly or folded improperly when expressed in bacteria. Furthermore, the yield of recombinant proteins in milk can be quite large. Dairy cows, for example, produce about 10,000 liters of milk per year per cow. In some cases, a transgenic cow can produce approximately 1 g of the transgenic protein per liter of milk.

To introduce a human gene into an animal so the encoded protein will be secreted into its milk, the strategy is to insert the gene next to a milk-specific promoter. Eukaryotic genes often are expressed in a tissue-specific fashion. In mammals, certain

TABLE **19.3**		
Proteins That Can Be Produced in the Milk of Domestic Animals		
Protein	**Host**	**Use**
Lactoferrin	Cattle	Used as an iron supplement in infant formula
Tissue plasminogen activator (TPA)	Goat	Dissolves blood clots
Antibodies	Cattle	Used to combat specific infectious diseases
α_1-Antitrypsin	Sheep	Treatment of emphysema
Factor IX	Sheep	Treatment of certain inherited forms of hemophilia
Insulin-like growth factor	Cattle	Treatment of diabetes

genes are expressed specifically within the mammary gland so their protein product is secreted into the milk. Examples of milk-specific genes include genes that encode milk proteins such as β-lactoglobulin, casein, and whey acidic protein. To express a human gene that encodes a protein hormone into a domestic animal's milk, the promoter for a milk-specific gene is linked to the coding sequence for the human gene (**Figure 19.8**). The DNA is then injected into an oocyte, where it is integrated into the genome. The fertilized oocyte is then implanted into the uterus of a female animal, which later gives birth to a transgenic offspring. If the offspring is a female, the protein hormone encoded by the human gene is expressed within the mammary gland and secreted into the milk. The milk can then be obtained from the animal, and the human hormone isolated.

19.3 REPRODUCTIVE CLONING AND STEM CELLS

The previous section focused on the area of biotechnology in which cloned genes are introduced into animals. Another aspect of biotechnology involves the cloning of whole organisms or the manipulation of stem cells. In this section, we will consider mammalian cloning and stem cell research. These topics have received enormous public attention due to the complex ethical issues they raise.

Researchers Have Succeeded in Cloning Mammals from Somatic Cells

The term "cloning" has several different meanings. In Chapter 18, we discussed gene cloning, which involves methods that produce many copies of a gene. The cloning of an entire organism is a different matter. **Reproductive cloning** refers to methods that produce two or more genetically identical individuals. This happens occasionally in nature; identical twins are genetic clones that began from the same fertilized egg. Similarly, researchers can take mammalian embryos at an early stage of development (e.g., the two-cell to eight-cell stage), separate the cells, implant them into the uterus, and obtain multiple births of genetically identical individuals.
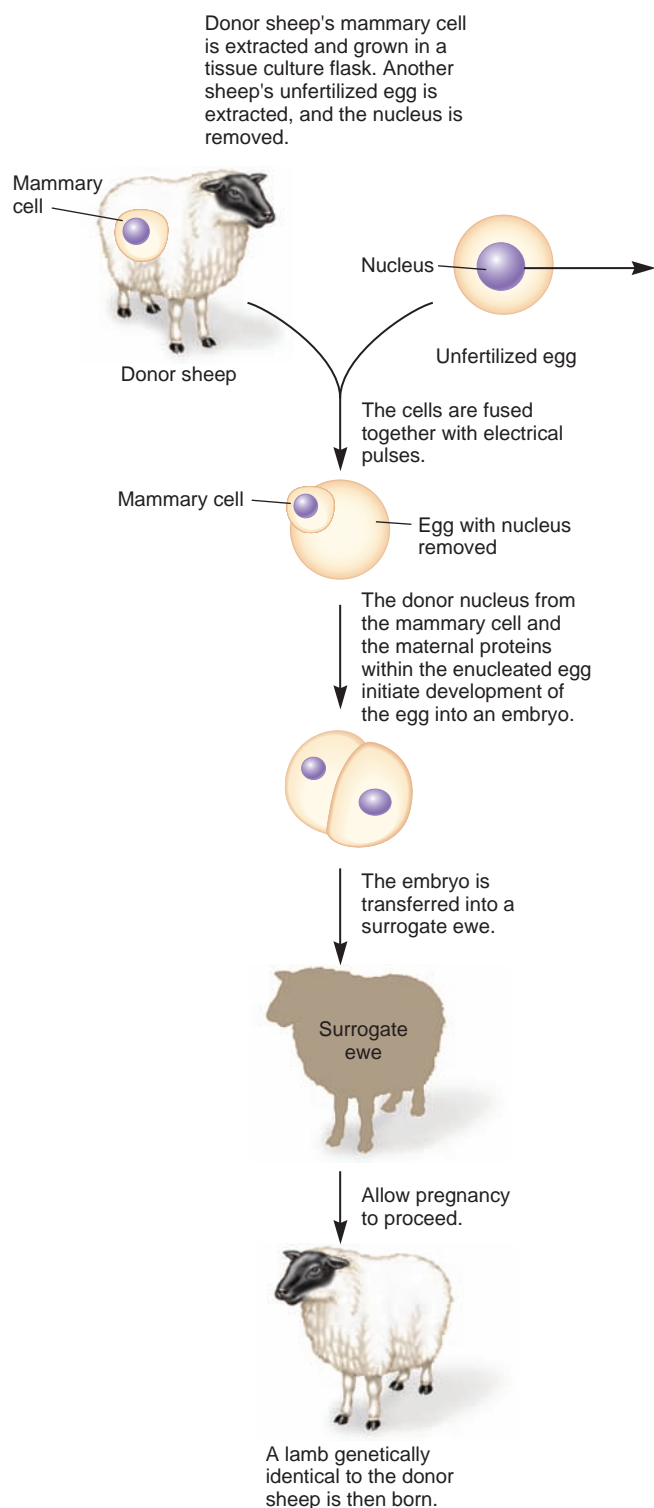
In the case of plants, cloning is an easier undertaking, as we will explore later in the chapter. Plants can be cloned from somatic cells. In most cases, it is relatively easy to take a cutting from a plant, expose it to growth hormones, and obtain a separate plant that is genetically identical to the original. However, this approach has not been possible with mammals. For several decades, scientists believed that chromosomes within the somatic cells of mammals had incurred irreversible genetic changes that render them unsuitable for cloning. However, this hypothesis has proven to be incorrect. In 1997, Ian Wilmut and his colleagues at the Roslin Institute in Scotland announced that a sheep, named Dolly, had been cloned using the genetic material from somatic cells.

How was Dolly produced? As shown in **Figure 19.9**, the researchers removed mammary cells from an adult female sheep
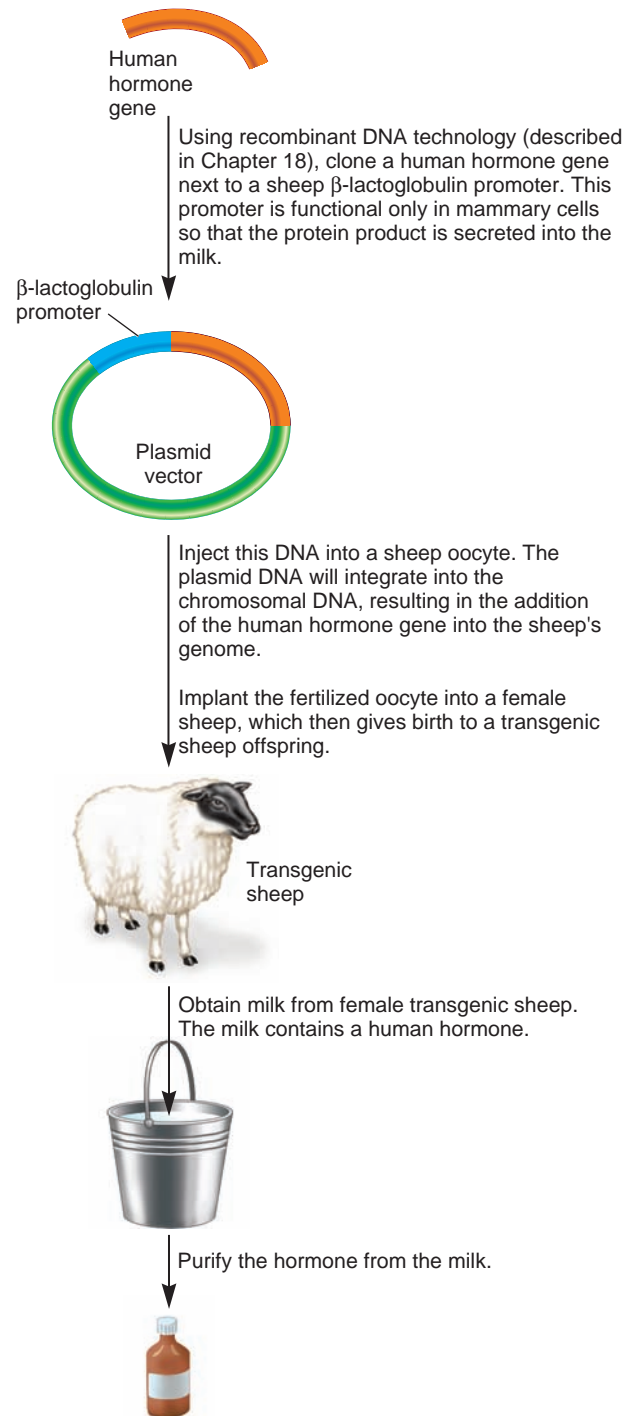
FIGURE 19.8 **Strategy for expressing human genes in a domestic animal's milk.** The β-lactoglobulin gene is normally expressed in mammary cells, whereas the human hormone gene is not. To express the human hormone gene in milk, the promoter from the milk-specific gene in sheep is linked to the coding sequence of the human hormone gene. In addition to the promoter, a short signal sequence may also be necessary so the protein is secreted from the mammary cells and into the milk.

Genes → Traits By using genetic engineering, researchers can give sheep the trait of producing a human hormone in their milk. This hormone can be purified from the milk and used to treat humans.

Donor sheep's mammary cell is extracted and grown in a tissue culture flask. Another sheep's unfertilized egg is extracted, and the nucleus is removed.

Mammary cell

Nucleus

Donor sheep

Unfertilized egg

The cells are fused together with electrical pulses.

Mammary cell

Egg with nucleus removed

The donor nucleus from the mammary cell and the maternal proteins within the enucleated egg initiate development of the egg into an embryo.

The embryo is transferred into a surrogate ewe.

Surrogate ewe

Allow pregnancy to proceed.

A lamb genetically identical to the donor sheep is then born.

FIGURE 19.9   **Protocol for the successful cloning of sheep.**
Genes → Traits Dolly was (almost) genetically identical to the sheep that donated a mammary cell to create her. Dolly and the donor sheep were (almost) genetically identical in the same way that identical twins are; they carried the same set of genes and looked remarkably similar. However, they may have had minor genetic differences due to possible variation in their mitochondrial DNA and may have exhibited some phenotypic differences due to maternal effect or imprinted genes.

and grew them in the laboratory. The researchers then extracted the nucleus from an egg cell of a different sheep and used electrical pulses to fuse the diploid mammary cell with the enucleated egg cell. After fusion, the zygote began embryonic development, and the resulting embryo was implanted into the uterus of a surrogate mother sheep. One hundred and forty-eight days later, Dolly was born.

Although Dolly was clearly a clone of the initial adult female sheep, tests conducted when she was 3 years old suggested that she was "genetically older" than her actual age indicated. As mammals age, chromosomes in somatic cells tend to shorten from the telomeres—the ends of eukaryotic chromosomes. Therefore, older individuals have shorter chromosomes in their somatic cells than younger ones do. This shortening does not seem to occur in the cells of the germ line, however. When researchers analyzed the chromosomes in Dolly's somatic cells when she was about 3 years old, the lengths of her chromosomes were consistent with a sheep that was significantly older, say, 9 or 10 years old. The sheep that donated the somatic cell that produced Dolly was 6 years old, and her mammary cells had been grown in culture for several cell doublings before a mammary cell was fused with an oocyte. This led researchers to postulate that Dolly's shorter telomeres were a result of chromosome shortening in the somatic cells of the sheep that donated the nucleus. In 2003, the Roslin Institute announced the decision to euthanize 6-year-old Dolly after an examination showed progressive lung disease. Her death has raised concerns among experts that the techniques used to produce Dolly could have caused premature aging.

With regard to telomere length, research in mice and cattle has shown different results; the telomeres of these cloned animals appear to be the correct length. For example, cloning was conducted on mice via the method described in Figure 19.9 for six consecutive generations. The cloned mice of the sixth generation had normal telomeres. Further research is necessary to determine if cloning via somatic cells has an effect on the length of telomeres in subsequent generations. However, other studies in mice point to various types of genetic flaws in cloned animals. For example, Rudolf Jaenisch and his colleagues used DNA microarray technology (described in Chapter 21) to analyze the transcription patterns of over 10,000 genes in cloned mice. As much as 4% of those genes were not expressed normally. Furthermore, research has shown that cloned mice die at a younger age than their naturally bred counterparts.

Mammalian cloning is still at an early stage of development. Nevertheless, the breakthrough of creating Dolly has shown that it is technically possible. In recent years, cloning from somatic cells has been achieved in several mammalian species, including sheep, cattle, mice, goats, and pigs. In 2002, the first pet was cloned. She was named Carbon Copy, also called Copy Cat (**Figure 19.10**). Mammalian cloning may potentially have many practical applications. Cloning livestock would enable farmers to use the somatic cells from their best individuals to create genetically homogeneous herds, which could increase agricultural yield. However, such a genetically homogeneous herd may be more susceptible to rare diseases.
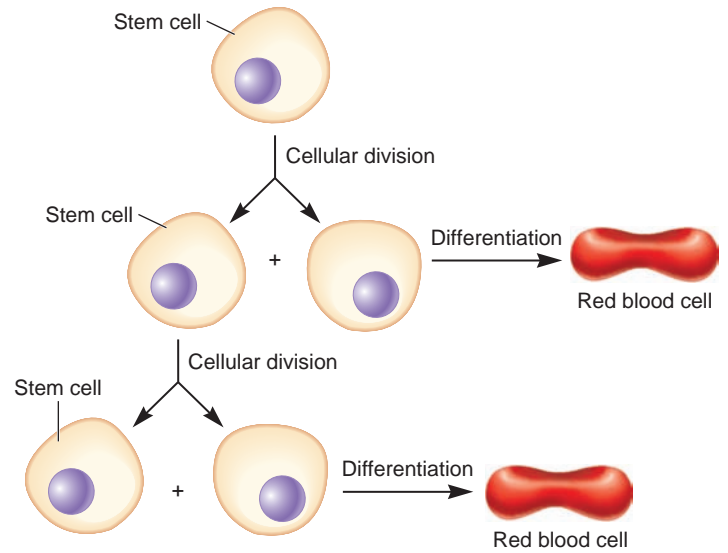
FIGURE 19.10   Carbon Copy, the first cloned pet. The animal shown here was produced using a procedure similar to the one shown in Figure 19.9.

FIGURE 19.11   Growth pattern of stem cells. The two main traits that stem cells exhibit are an ability to divide and an ability to differentiate. When a stem cell divides, one of the two cells remains a stem cell, and the other daughter cell differentiates into a specialized cell type.

Though some people are concerned about the practical uses of cloning agricultural species, a majority have become very concerned with the possibility of human cloning. This prospect has raised a host of serious ethical questions. For example, some people feel that it is morally wrong and threatens the basic fabric of parenthood and family. Others feel that it is a technology that could offer a new avenue for reproduction, one that could be offered to infertile couples, for example. In the public sector, the sentiment toward human cloning has been generally negative. Many countries have issued an all-out ban on human cloning, but others permit limited research in this area. Because the technology for cloning exists, our society will continue to wrestle with the legal and ethical aspects of cloning as it applies not only to animals but also to people.

Stem Cells Have the Ability to Divide and Differentiate into Different Cell Types

Stem cells supply the cells that construct our bodies from a fertilized egg. In adults, stem cells also replenish worn-out or damaged cells. To accomplish this task, stem cells have two common characteristics. First, they have the capacity to divide, and second, they can differentiate into one or more specialized cell types. As shown in **Figure 19.11**, the two daughter cells produced from the division of a stem cell can have different fates. One of the cells may remain an undifferentiated stem cell, while the other daughter cell can differentiate into a specialized cell type. With this
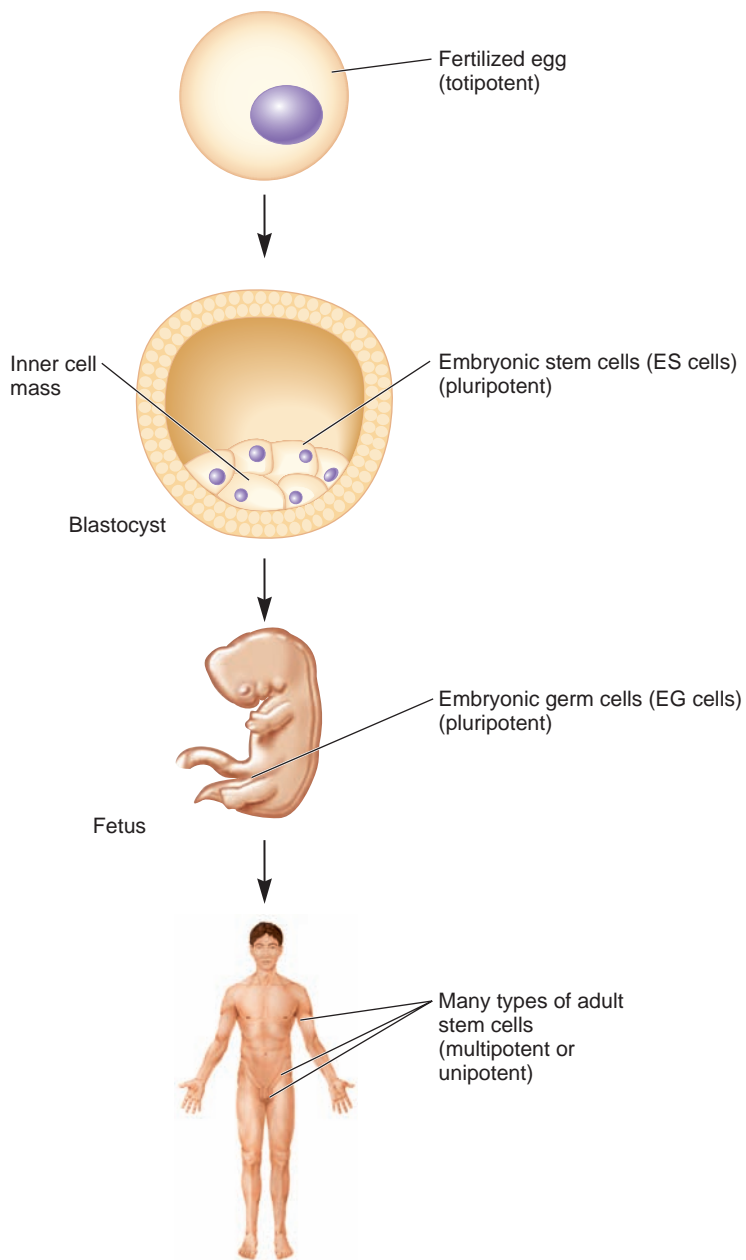
type of asymmetrical division/differentiation pattern, the population of stem cells remains relatively constant, yet the stem cells provide a population of specialized cells. In the adult, this type of mechanism is needed to replenish cells that have a finite life span, such as skin epithelial cells and red blood cells.

In mammals, stem cells are commonly categorized according to their developmental stage and their ability to differentiate (**Figure 19.12**). The ultimate stem cell is the fertilized egg, which, via multiple cellular divisions, can give rise to an entire organism. A fertilized egg is considered **totipotent,** because it can give rise to all the cell types in the adult organism. The early mammalian embryo contains **embryonic stem cells (ES cells),** which are found in the inner cell mass of the blastocyst. The blastocyst is the stage of embryonic development prior to uterine implantation—the preimplantation embryo. ES cells are **pluripotent,** which means they can differentiate into almost every cell type of the body. However, a single ES cell has lost the ability to produce an entire, intact individual.

During the early fetal stage of development, the germ-line cells found in the gonads also are pluripotent. These cells are called **embryonic germ cells (EG cells).** Interestingly, certain types of human cancers called teratocarcinomas arise from cells that are pluripotent. These bizarre tumors contain a variety of tissues including cartilage, neuroectoderm, muscle, bone, skin, ganglionic structures, and primitive glands. Due to the seemingly embryonic origin of teratocarcinoma cells, these cells are termed **embryonic carcinoma cells (EC cells).**

As mentioned, adults also contain stem cells, but these are thought to be multipotent or unipotent. A **multipotent** stem cell can differentiate into several cell types but far fewer than an ES cell. For example, hematopoietic stem cells (HSCs) found in the

Fertilized egg
(totipotent)

Inner cell
mass

Embryonic stem cells (ES cells)
(pluripotent)

Blastocyst

Embryonic germ cells (EG cells)
(pluripotent)

Fetus

Many types of adult
stem cells
(multipotent or
unipotent)

FIGURE 19.12   **Occurrence of stem cells at different stages of development.**

bone marrow can supply cells that populate two different tissues, namely, the blood and lymphoid tissues (**Figure 19.13**). Furthermore, each of these tissues contains several cell types. Multipotent HSCs can follow a pathway in which cell division produces a myeloid progenitor cell, which can then differentiate into a red blood cell, megakaryocyte, basophil, monocyte, eosinophil, neutrophil, or dendritic cell. Alternatively, an HSC can follow a path in which it becomes a lymphoid progenitor cell, which then differentiates into a T cell, B cell, natural killer cell, or dendritic cell. Other stem cells found in the adult seem to be **unipotent.** For example, primordial germ cells in the testis differentiate only into a single cell type, the sperm.

Stem Cells Have the Potential to Treat a Variety of Diseases

What are the potential uses of stem cells? Interest in stem cells centers on two main areas. Because stem cells have the capacity to differentiate into multiple cell types, the study of stem cells may help us to understand basic genetic mechanisms that underlie the process of development, the details of which are described in Chapter 23. A second compelling reason why people have become interested in stem cells is their potential to treat human diseases or injuries that cause cell and tissue damage. This application has already become a reality in certain cases. For example, bone marrow transplantation is used to treat patients with certain forms of cancers. Such patients may be given radiation treatments that destroy their immune systems. When these patients are injected with bone marrow from a healthy person, the stem cells within the transplanted marrow have the ability to proliferate and differentiate within their bodies and provide them with a functioning immune system.

Renewed interest in the use of stem cells in the potential treatment of many other diseases was fostered in 1998 by studies of two separate teams, headed by James Thomson and John Gearhart, showing that embryonic cells, either ES or EG cells, can be successfully propagated in the laboratory. As mentioned, ES and EG cells are pluripotent and therefore have the capacity to produce many different kinds of tissue. As shown in **Table 19.4**, embryonic cells could potentially be used to treat a wide variety of diseases associated with cell and tissue damage. By comparison, it would be difficult, based on our modern knowledge, to treat these diseases with adult stem cells because of the inability to locate most types of adult stem cells within the body and successfully grow them in the laboratory. Even HSCs are elusive. In the bone marrow, about 1 cell in 10,000 is a stem cell, yet that is enough to populate all of the blood and lymphoid cells of the body. The stem cells of most other adult tissues are equally difficult to locate, if not more so. In addition, with the exception of stem cells in the blood, other types of stem cells in the adult body are difficult to remove in sufficient numbers for transplantation. By comparison, ES and EG cells are easy to identify and have the great advantage of rapid growth in the laboratory. For these reasons, ES and EG cells offer a greater potential for transplantation, based on our current knowledge of stem cell biology.

For ES or EG cells to be used in transplantation, researchers need to derive methods that cause them to differentiate into the appropriate type of tissue. For example, if the goal was to repair a spinal cord injury, ES or EG cells would need the appropriate cues that cause them to differentiate into neural tissue. At present, much research is needed to understand and potentially control the fate of ES or EG cells. Currently, researchers speculate that a complex variety of factors determines the developmental fates of stem cells. These include internal factors within the stem cells themselves, as well as external factors such as the properties of neighboring cells and the presence of hormones and growth factors in the environment.
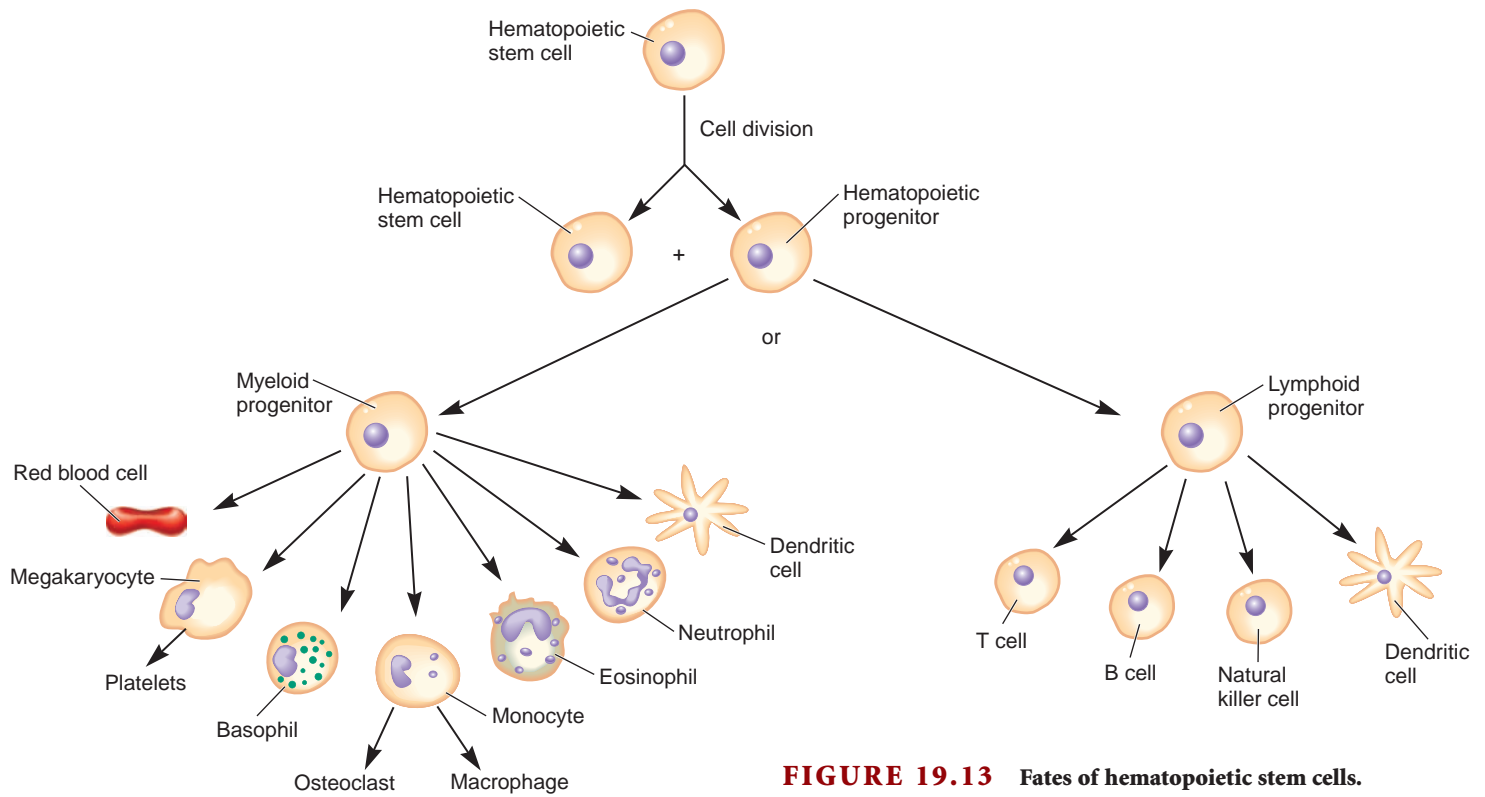
FIGURE 19.13   Fates of hematopoietic stem cells.

From an ethical perspective, the primary issue that raises debate is the source of the stem cells for research and potential treatments. Most ES cells have been derived from human embryos that were produced from in vitro fertilization and were subsequently not used. Most EG cells are obtained from aborted fetuses. Some feel that it is morally wrong to use such tissue in research and/or the treatment of disease or they fear that this technology could lead to intentional abortions for the sole purpose of obtaining fetal tissues for transplantation. Alternatively, others feel that the embryos and fetuses that provide the ES and EG cells are not going to become living individuals, and therefore, it is beneficial to study these cells and use them in a positive way to treat human diseases and injury. It is not clear whether these two opposing viewpoints can reach a common ground.

As a compromise, some governments have enacted laws that limit or prohibit the use of embryos or fetuses to obtain stem cells, yet permit the use of stem cell lines that are already available in research laboratories.

If stem cells could be obtained from adult cells and propagated in the laboratory, an ethical dilemma may be avoided because most people do not have serious moral objections to current procedures such as bone marrow transplantation. In 2006, work by Shinya Yamanaka and colleagues showed that adult mouse fibroblasts (a type of connective tissue cell) could become pluripotent via the injection of four different genes that encode transcription factors. In 2007, Yamanaka's laboratory and two other research groups showed that such induced pluripotent stem cells (iPS) can differentiate into all cell types when injected into mouse blastocysts and grown into baby mice. Though further research is still needed, these recent results indicate that adult cells can be reprogrammed to become embryonic stem cells.

TABLE **19.4**	
Potential Uses of Stem Cells to Treat Diseases	
Cell/Tissue Type	**Disease Treatment**
Neural	Implantation of cells into the brain to treat Parkinson disease
	Treatment of injuries such as spinal cord injuries
Skin	Treatment of burn victims and other types of skin disorders
Cardiac	Repair of heart damage associated with heart attacks
Cartilage	Repair of joints damaged by injury or arthritis
Bone	Repair of damaged bone or replacement with new bone
Liver	Repair or replacement of liver tissue that has been damaged by injury or disease
Skeletal muscle	Repair or replacement of damaged muscle

19.4 GENETICALLY MODIFIED PLANTS

As we have seen, researchers have succeeded in making genetically modified animals for a variety of reasons. In this section, we will examine the methods that scientists follow to make transgenic plants.

A large amount of research has been aimed at the use of transgenic species in agriculture. For centuries, agriculture has relied on selective breeding programs to produce plants and animals with desirable characteristics. For agriculturally important species, this often means the production of strains that are larger, are disease-resistant, and yield high-quality food. Agricultural scientists can now complement traditional breeding strategies with modern molecular genetic approaches. In the mid-1990s, genetically modified crops first became commercialized. Since that time, their use has progressively increased. In 2009, roughly 25% of all agricultural crops were transgenic. Worldwide, more than 100 million hectares (247 million acres) of transgenic crops were planted. In this section, we will discuss some current and potential uses of transgenic plants in agriculture.

Agrobacterium tumefaciens and Other Methods Can Be Used to Make Transgenic Plants

As we have seen, the introduction of cloned genes into embryonic cells can produce transgenic animals. The production of transgenic plants is somewhat easier, because some somatic cells are totipotent, which means they are capable of developing into an entire organism. Therefore, a transgenic plant can be made by the introduction of cloned genes into somatic tissue, such as the tissue of a leaf. After the cells of a leaf have become transgenic, an entire plant can be regenerated by the treatment of the leaf with plant growth hormones, which cause it to form roots and shoots.

Molecular biologists can use the bacterium *Agrobacterium tumefaciens*, which naturally infects plant cells, to produce transgenic plants. A plasmid from the bacterium, known as the Ti plasmid (Tumor-inducing plasmid), naturally induces tumor formation after a plant has been infected (**Figure 19.14a**). A segment of the plasmid DNA, known as **T DNA** (for transferred DNA), is transferred from the bacterium to the infected plant cells. The T DNA from the Ti plasmid is integrated into the chromosomal DNA of the plant cell by recombination. After this occurs, genes within the T DNA that encode plant growth hormones cause uncontrolled plant cell growth. This produces a cancerous plant growth known as a crown gall tumor (**Figure 19.14b**).

Because *A. tumefaciens* inserts its T DNA into the chromosomal DNA of plant cells, it can be used as a vector to introduce cloned genes into plants. Molecular geneticists have been able to modify the Ti plasmid to make this an efficient process. Such vectors are called **T-DNA vectors**. The T DNA genes that cause the development of a gall have been identified. Fortunately for genetic engineers, when these genes are deleted, the T DNA is still taken up into plant cells and integrated within the plant chromosomal DNA. However, a gall does not form. In

addition, geneticists have inserted selectable marker genes into the T DNA to allow selection of plant cells that have taken up the T DNA. A gene that provides resistance to the antibiotic kanamycin (kan^R) is a commonly used selectable marker. The T-DNA vectors used in cloning experiments are also modified
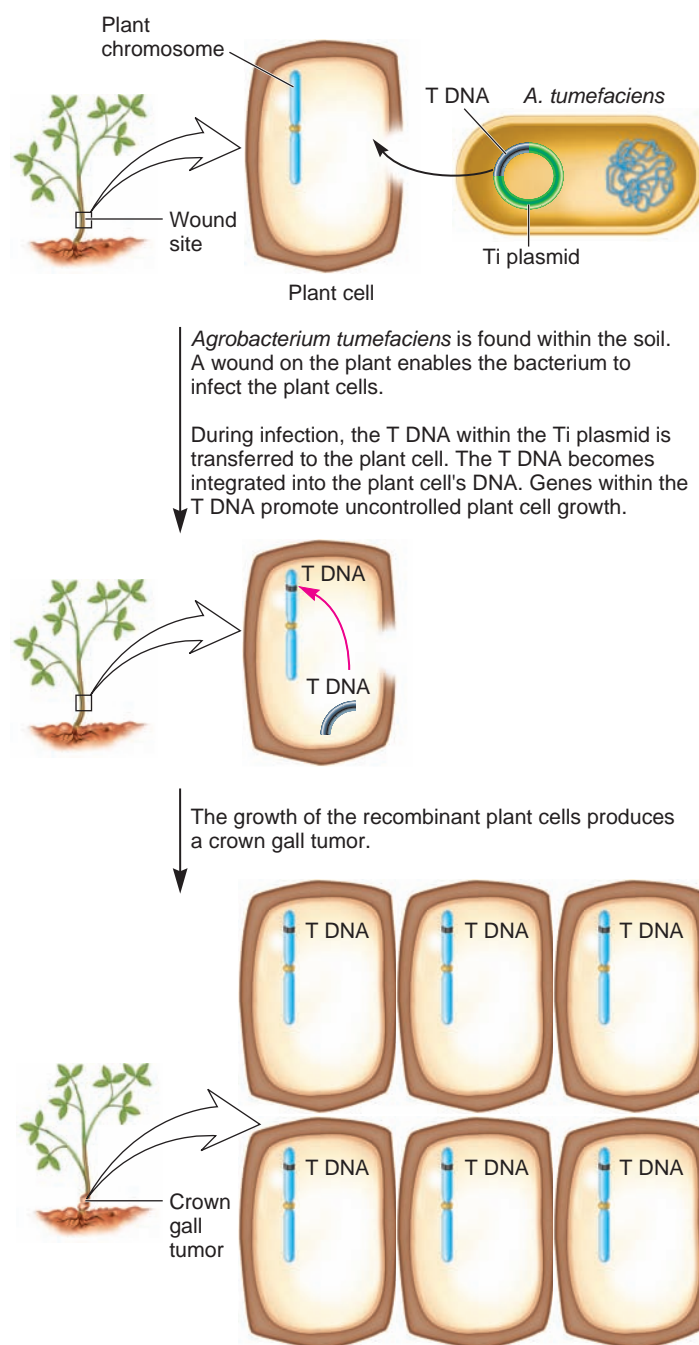
(a) The production of a crown gall tumor by *A. tumefaciens* infection

FIGURE 19.14   *Agrobacterium tumefaciens* **infecting a plant and causing a crown gall tumor.**
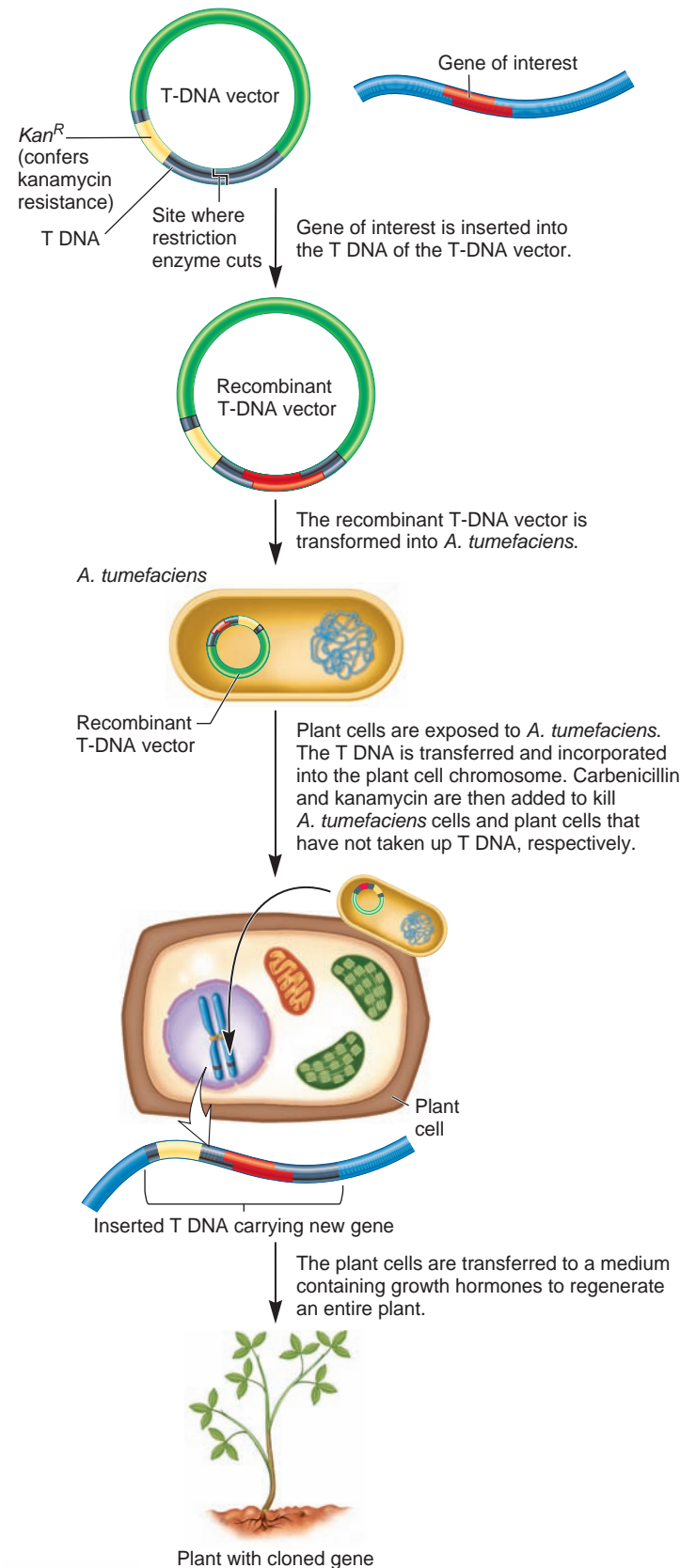
(b) A crown gall tumor on a pecan tree

FIGURE 19.14 *cont.*

to contain unique restriction sites for the convenient insertion of any gene.

Figure 19.15 shows the general strategy for producing transgenic plants via T DNA-mediated gene transfer. A gene of interest is inserted into a genetically engineered T-DNA vector and then transformed into *A. tumefaciens*. Plant cells are exposed to the transformed *A. tumefaciens*. After allowing time for infection, the plant cells are exposed to the antibiotics kanamycin and carbenicillin. Carbenicillin kills *A. tumefaciens*, and kanamycin kills any plant cells that have not taken up the T DNA with the antibiotic-resistance gene. Therefore, the only surviving cells are those plant cells that have integrated the T DNA into their genome. Because the T DNA also contains the cloned gene of interest, the selected plant cells are expected to have received this cloned gene as well. The cells are then transferred to a medium that contains the plant growth hormones necessary for the regeneration of entire plants. These plants can then be analyzed to verify that they are transgenic plants containing the cloned gene.

A. tumefaciens infects a wide range of plant species, including most dicotyledonous plants, most gymnosperms, and some monocotyledonous plants. However, not all plant species are infected by this bacterium. Fortunately, other methods are available for introducing genes into plant cells. Another common way to produce transgenic plants is an approach known as **biolistic gene transfer.** In this method, plant cells are bombarded with high-velocity microprojectiles coated with DNA. When fired upon by this "gene gun," the microprojectiles penetrate the cell wall and membrane, thereby entering the plant cell. The cells that take up the DNA are identified with a selectable marker and regenerated into new plants.

Other methods are also available for introducing DNA into plant cells (and also animal cells). For example, DNA can

Gene of interest is inserted into the T DNA of the T-DNA vector.

The recombinant T-DNA vector is transformed into *A. tumefaciens*.

Plant cells are exposed to *A. tumefaciens*. The T DNA is transferred and incorporated into the plant cell chromosome. Carbenicillin and kanamycin are then added to kill *A. tumefaciens* cells and plant cells that have not taken up T DNA, respectively.

The plant cells are transferred to a medium containing growth hormones to regenerate an entire plant.

FIGURE 19.15   The transfer of genes into plants using a T-DNA vector from *A. tumefaciens*.

enter plant cells by **microinjection**—the use of microscopic-sized needles—or by **electroporation**—the use of electrical current to create temporary pores in the plasma membrane. Because the rigid plant cell wall is a difficult barrier for DNA entry, other approaches involve the use of protoplasts, which are plant cells that have had their cell walls removed. DNA can be introduced into protoplasts using a variety of methods, including treatment with polyethylene glycol and calcium phosphate.

The production of transgenic plants has been achieved for many agriculturally important plant species, including alfalfa, corn, cotton, soybean, tobacco, and tomato. Some of the applications of transgenic plants are described next.

TABLE **19.5**

Traits That Have Been Modified in Transgenic Plants

Trait	Examples
Plant Protection	
Resistance to viral, bacterial, and fungal pathogens	Transgenic plants that express the pokeweed antiviral protein are resistant to a variety of viral pathogens.
Resistance to insects	Transgenic plants that express the CryIA protein from *Bacillus thuringiensis* are resistant to a variety of insects (see Figure 19.18).
Resistance to herbicides	Transgenic plants can express proteins that render them resistant to particular herbicides (see Figure 19.16).
Plant Quality	
Improvement in storage	Transgenic plants can express antisense RNA that silences a gene involved in fruit softening (see Figure 19.17).
Change in plant composition	Transgenic strains of canola have been altered with regard to oil composition; the seeds of the Brazil nut have been rendered methionine-rich via transgenic technology.
New Products	
Biodegradable plastics	Transgenic plants have been made that can synthesize polyhydroxyalkanoates, which are used as biodegradable plastics.
Vaccines	Transgenic plants have been modified to produce vaccines in their leaves against many human and animal diseases, including hepatitis B, cholera, and malaria.
Pharmaceuticals	Transgenic plants have been made that produce a variety of medicines, including human interferon-α (to fight viral diseases and cancer), human epidermal growth factor (for wound repair), and human aprotinin (for reducing blood loss during transplantation surgery).
Antibodies	Human antibodies have been made in transgenic plants to battle various diseases such as non-Hodgkin lymphoma.

Transgenic Plants Can Be Given Characteristics That Are Agriculturally Useful

Various traits can be modified in transgenic plants (**Table 19.5**). Frequently, transgenic research has sought to produce plant strains resistant to insects, disease, and herbicides. For example, transgenic plants highly tolerant of particular herbicides have been made. The Monsanto Company has produced transgenic plant strains tolerant of glyphosate, the active agent in the herbicide Roundup. The herbicide remains effective against weeds, but the herbicide-resistant crop is spared (**Figure 19.16**).

Another important approach is to make plant strains that are disease-resistant. In many cases, virus-resistant plants have been developed by introducing a gene that encodes a viral coat protein. When the plant cells express the viral coat protein, they become resistant to infection by that pathogenic virus.

Many transgenic plants have been approved for human consumption. The first example was the Flavr Savr tomato (**Figure 19.17**), which was developed by Calgene Inc., which is now part of Monsanto. In this technique, a tomato plant was given a gene that encodes an antisense RNA complementary to the mRNA that encodes the enzyme polygalacturonase. This enzyme, which is expressed during ripening, digests sugar linkages within the pectin found in plant cell walls and thus softens the tomato. The antisense RNA binds to the polygalacturonase mRNA, preventing it from being translated. In addition, the double-stranded RNA is targeted for degradation (RNA interference), as discussed in Chapter 15 (Figure 15.24). Silencing the expression of polygalacturonase has the practical advantage of preventing the tomatoes from softening as quickly as unmodified tomatoes. Therefore, these tomatoes can be allowed to ripen on the vine

FIGURE 19.16   **Transgenic plants that are resistant to glyphosate.**

Genes → Traits This field of soybean plants has been treated with glyphosate. The plants on the left have been genetically engineered to contain a herbicide-resistance gene. They are resistant to killing by glyphosate. By comparison, the dead plants in the row with the orange stick do not contain this gene.

FIGURE 19.17 **The genetically engineered Flavr Savr tomato.**

Genes → Traits This transgenic tomato plant has been genetically engineered to contain an artificial gene that encodes an antisense RNA that binds to the mRNA that encodes polygalacturonase. This prevents the mRNA from being translated, which inhibits the synthesis of polygalacturonase. Without this enzyme, the Flavr Savr tomato can be left on the vine to ripen longer than traditional tomatoes, thereby improving its flavor.

for a longer period of time, enhancing their flavor and extending their shelf life. Improved taste is an important consideration in the $5-billion annual U.S. tomato market. By comparison, other commercial tomatoes commonly are picked when green and allowed to ripen later in order to maintain their firmness longer.

The Flavr Savr tomato was not a commercial success, however, and its sales were eventually discontinued. The failure of the Flavr Savr has been attributed to a variety of issues. In particular, the variety of tomato that was genetically engineered may have not been the best choice, and the antirotting trait was not as helpful for the tomato business as Calgene had anticipated. Another factor was that the ripe Flavr Savr tomatoes were more delicate and required the use of expensive handling equipment that wasn't needed for unmodified, green tomatoes.

A much more successful example of the use of transgenic plants has involved the introduction of genes from *Bacillus thuringiensis* (Bt). As discussed earlier, this bacterium produces toxins that kill certain types of caterpillars and beetles and has been widely used as a biological control agent for several decades. These toxins are proteins encoded in the genome of *B. thuringiensis*. Researchers have succeeded in cloning toxin genes from *B. thuringiensis* and transferring those genes into plants. Such Bt varieties of plants produce the toxins themselves and therefore are resistant to many types of caterpillars and beetles. Examples of commercialized crops include Bt corn (**Figure 19.18a**) and Bt cotton. Since their introduction in 1996, the commercial use of these two Bt crops has steadily increased (**Figure 19.18b**).

The introduction of transgenic plants into agriculture has been strongly opposed by some people. What are the perceived risks? One potential risk is that transgenes in commercial crops could endanger native species. For example, Bt crops may kill pollinators of native species. Another worry is that the planting of transgenic crops could potentially lead to the proliferation of resistant insects. To prevent this from happening, researchers are producing transgenic strains that carry more than one toxin gene, which makes it more difficult for insect resistance to arise. Despite these and other concerns, many farmers are embracing transgenic crops, and their use continues to rise.

(a) A field of Bt corn
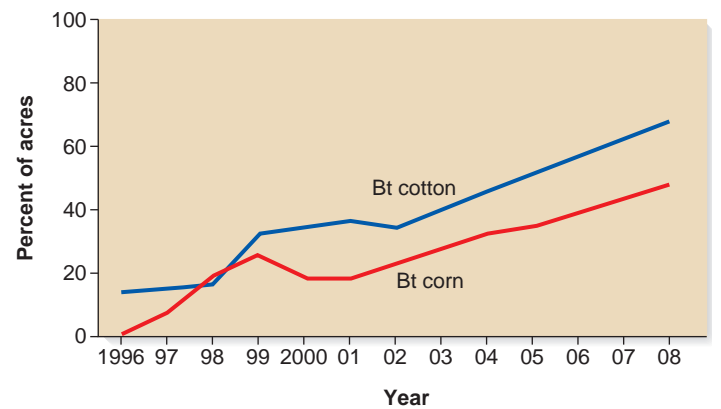
(b) Bt corn and Bt cotton usage since 1996

FIGURE 19.18 **The production of Bt crops. (a)** A field of Bt corn. These corn plants carry an endotoxin gene from *Bacillus thuringiensis* that provides them with resistance to insects such as corn borers, which are a major pest of corn plants. **(b)** A graph showing the increase in usage of Bt corn and Bt cotton in the United States since their commercial introduction in 1996.

19.5 HUMAN GENE THERAPY

Throughout this textbook, we have considered examples in which mutant genes cause human diseases. In Chapter 22, we discuss some examples that include rare inherited disorders and more common diseases such as cancer. Because mutant genes cause disease, geneticists are actively pursuing the goal of using normal, cloned genes to compensate for defects in mutant genes. **Gene therapy** is the introduction of cloned genes into living cells to treat disease. It is a potential method for treating a wide variety of illnesses.

Many current research efforts in gene therapy are aimed at alleviating inherited human diseases. Over 7000 human genetic diseases are known to involve a single gene abnormality. Familiar examples include cystic fibrosis, sickle cell disease, and hemophilia. In addition, gene therapies have also been aimed at treating diseases such as cancer and cardiovascular disease, which may occur later in life. Some scientists are even pursuing research that will use gene therapy to combat infectious diseases such as AIDS. Even though gene therapy is still at an early stage of development, a large amount of research has already been conducted. Unfortunately, success has been limited, and relatively few patients have been treated with gene therapy. Nevertheless, a few results have been somewhat promising. **Table 19.6** describes several types of diseases that are being investigated as potential targets for gene therapy. In this section, we will examine the approaches to gene therapy and how it may be used to treat human disease.

TABLE **19.6**	
Future Prospects in Gene Therapy	
Type of Disease	**Treatment of**
Blood	Sickle cell disease, hemophilia, severe combined immunodeficiency disease (SCID)
Metabolic	Glycogen storage diseases, lysosomal storage diseases, and phenylketonuria
Muscular	Duchenne muscular dystrophy, myotonic muscular dystrophy
Lung	Cystic fibrosis
Cancer	Brain tumors, breast cancer, colorectal cancer, malignant melanoma, ovarian cancer, and several other types of malignancies
Cardiovascular	Atherosclerosis, essential hypertension
Infectious	AIDS, possibly other viral diseases that involve latent infections

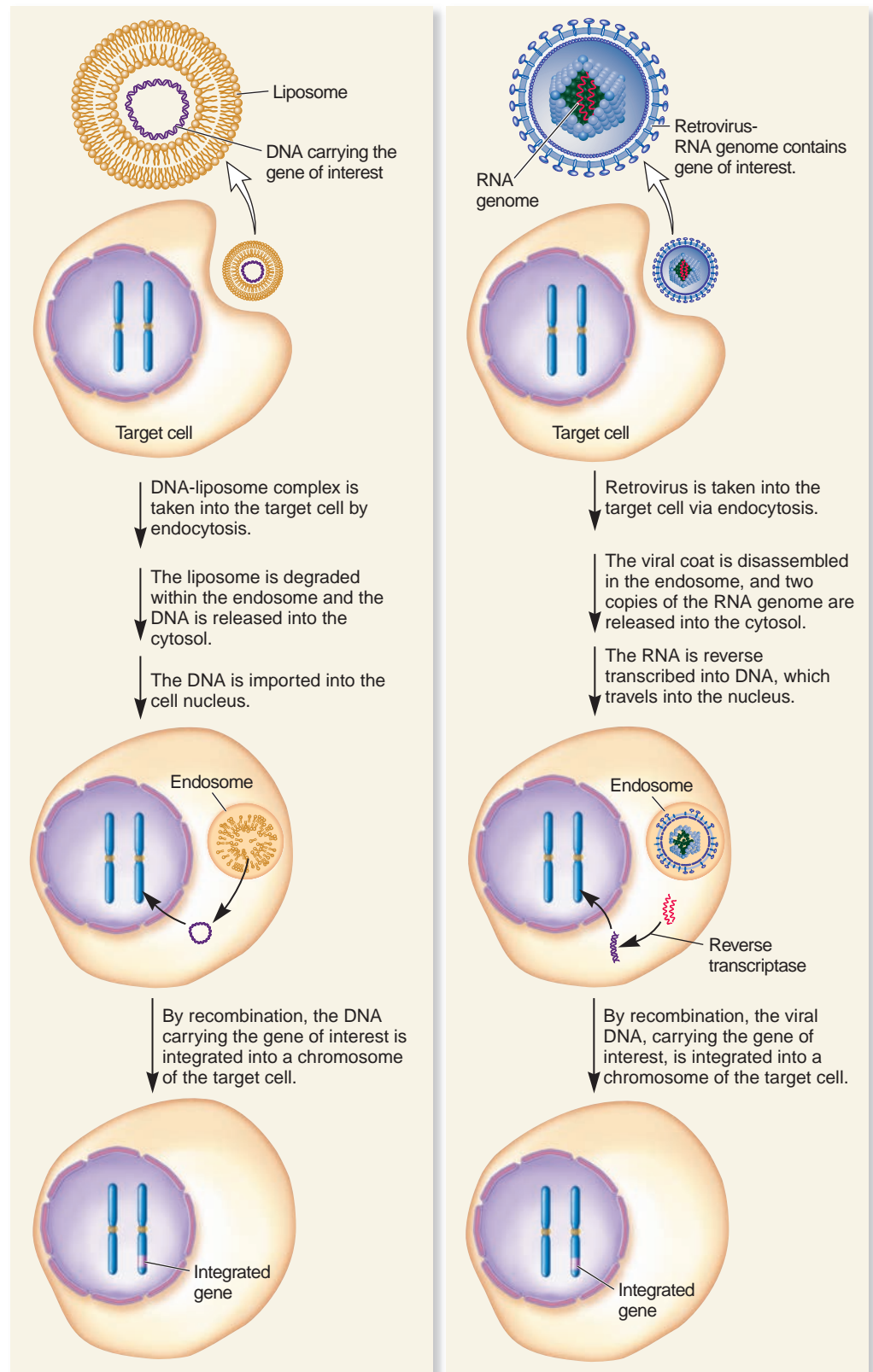## Gene Therapy Involves the Introduction of Cloned Genes into Human Cells

A key step in gene therapy is the introduction of a cloned gene into the cells of people. This is a challenging aspect of gene therapy, because it requires that many cells of a person's body take up the cloned gene and express it. Otherwise, the disease symptoms will not be corrected. The techniques to transfer a cloned gene into human cells can be categorized as nonviral and viral gene transfer methods. The most common nonviral technique involves the use of **liposomes,** which are lipid vesicles (**Figure 19.19a**). The DNA containing the gene of interest is complexed with liposomes that carry a positive charge (i.e., cationic liposomes). The DNA–liposome complexes are taken into cells via endocytosis, in which a portion of the plasma membrane invaginates and creates an intracellular vesicle known as an endosome; the liposome is degraded within the endosome. The DNA is then released into the cytosol, imported into the nucleus, and then integrated into a chromosome of the target cell. An advantage of gene transfer via liposomes is that the liposomes do not elicit an immune response. A disadvantage is that the efficiency of gene transfer may be very low.

A second way to transfer genes into human cells is via viruses. Commonly used viruses for gene therapy include retroviruses, adenoviruses, and parvoviruses. The genetic modification of these viral genomes has led to the development of gene therapy vectors with a capacity to infect cells or tissues, much like the ability of wild-type viruses to infect cells. However, in contrast to wild-type viruses, gene therapy viral vectors have been genetically engineered so they can no longer replicate within target cells. Nevertheless, the genetically engineered viruses are naturally taken up by cells via endocytosis (**Figure 19.19b**). The viral coat disassembles, and the viral genome is released into the cytosol. In the case of retroviruses, the genome is RNA, which is reverse-transcribed into DNA. The viral DNA, which carries a gene of interest, travels into the nucleus and is then integrated into a chromosome of the target cell.

A key advantage of viral vectors is their ability to efficiently transfer cloned genes to a variety of human cell types. However, a major disadvantage of viral-mediated gene therapy is the potential to evoke an undesirable immune response when injected into a patient. The inflammatory responses induced by adenovirus particles, for example, can be very strong and even fatal. Therefore, much effort has been aimed at preventing the inflammatory responses mediated by virus particles. These include the application of immunosuppressive drugs and the generation of less immunogenic viral vectors by further genetic modification within the viral genome.

FIGURE 19.19 **Methods of gene transfer used in gene therapy.** (a) In this example, the DNA containing the gene of interest is complexed with cationic liposomes. These complexes are taken into cells by endocytosis, in which a portion of the plasma membrane invaginates and creates an intracellular vesicle known as an endosome. After it is released from the endosome, the DNA may then integrate into the chromosomal DNA via recombination. (b) In this example, the gene of interest is cloned into a retrovirus. When the retrovirus infects a cell, the RNA genome is reverse-transcribed into double-stranded DNA, which then integrates into the chromosome. Viruses used in gene therapy have been genetically altered so they cannot proliferate after entry into the target cell.

Liposome

DNA carrying the gene of interest

Target cell

DNA-liposome complex is taken into the target cell by endocytosis.

The liposome is degraded within the endosome and the DNA is released into the cytosol.

The DNA is imported into the cell nucleus.

Endosome

By recombination, the DNA carrying the gene of interest is integrated into a chromosome of the target cell.

Integrated gene

(a) Nonviral approach

RNA genome

Retrovirus-RNA genome contains gene of interest.

Target cell

Retrovirus is taken into the target cell via endocytosis.

The viral coat is disassembled in the endosome, and two copies of the RNA genome are released into the cytosol.

The RNA is reverse transcribed into DNA, which travels into the nucleus.

Endosome

Reverse transcriptase

By recombination, the viral DNA, carrying the gene of interest, is integrated into a chromosome of the target cell.

Integrated gene

(b) Viral approach

Adenosine Deaminase Deficiency Was the First Inherited Disease Treated with Gene Therapy

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism. If both copies of the *ADA* gene are defective, deoxyadenosine accumulates within the cells of the individual. At high concentrations, deoxyadenosine is particularly toxic to lymphocytes in the immune system, namely, T cells and B cells. In affected individuals, the destruction of T and B cells leads to a form of severe combined immunodeficiency (SCID). If left untreated, SCID is typically fatal at an early age (generally, 1–2 years old), because the immune system of these individuals is severely compromised and cannot fight infections.

Three approaches can be used to treat ADA deficiency. In some cases, a patient may receive a bone marrow transplant from a compatible donor. A second method is to treat SCID patients with purified ADA that is coupled to polyethylene glycol (PEG). This PEG-ADA is taken up by lymphocytes and can correct the ADA deficiency. Unfortunately, these two approaches are not always available and/or successful. A third, more recent approach is to treat ADA patients with gene therapy.

On September 14, 1990, the first human gene therapy was approved for a young girl suffering from ADA deficiency. This work was carried out by a large team of researchers composed of R. Michael Blaese, Kenneth Culver, W. French Anderson, and colleagues. Prior to this clinical trial, the normal gene for ADA had been cloned into a retroviral vector that can infect lymphocytes. The general aim of this therapy was to remove lymphocytes from the blood of the young girl with SCID, introduce the normal *ADA* gene into her cells, and then return them to her bloodstream.

Figure 19.20 outlines the protocol for the experimental treatment. Lymphocytes (i.e., T cells) were removed and cultured in a laboratory. The lymphocytes were then transfected with a nonpathogenic retrovirus that had been genetically engineered to contain the normal *ADA* gene. During the life cycle of a retrovirus, the retroviral genetic material is inserted into the host cell's DNA. Therefore, because this retrovirus contained the normal *ADA* gene, this gene also was inserted into the chromosomal DNA of the girl's lymphocytes. After this had occurred in the laboratory, the cells were reintroduced back into the patient. This approach is called an **ex vivo approach** because the genetic manipulations occur outside the body, and the products are reintroduced into the body.

■ THE HYPOTHESIS

Infecting lymphocytes with a retrovirus containing the normal *ADA* gene will correct the inherited deficiency of the mutant *ADA* gene in patients with ADA deficiency.

■ TESTING THE HYPOTHESIS — FIGURE 19.20   The first human gene therapy for adenosine deaminase deficiency by Blaese and colleagues.

Starting material: A retrovirus carrying the normal *ADA* gene.

Experimental level   **Conceptual level**

1. Remove *ADA*-deficient lymphocytes from the patient with severe combined immunodeficiency disease (SCID).

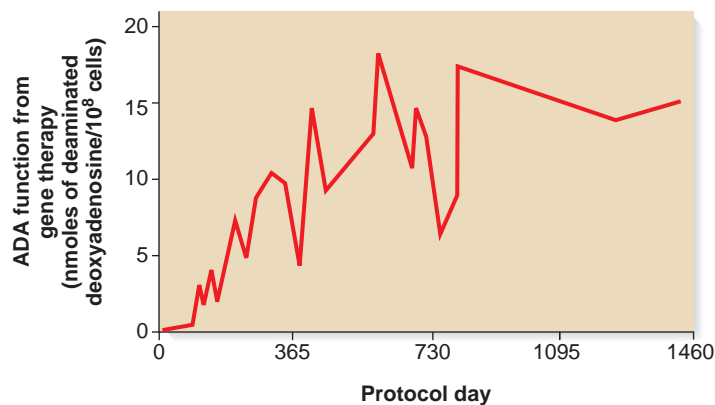Lymphocytes

Lymphocyte

Mutant *ADA* gene

2. Culture the cells in a laboratory.
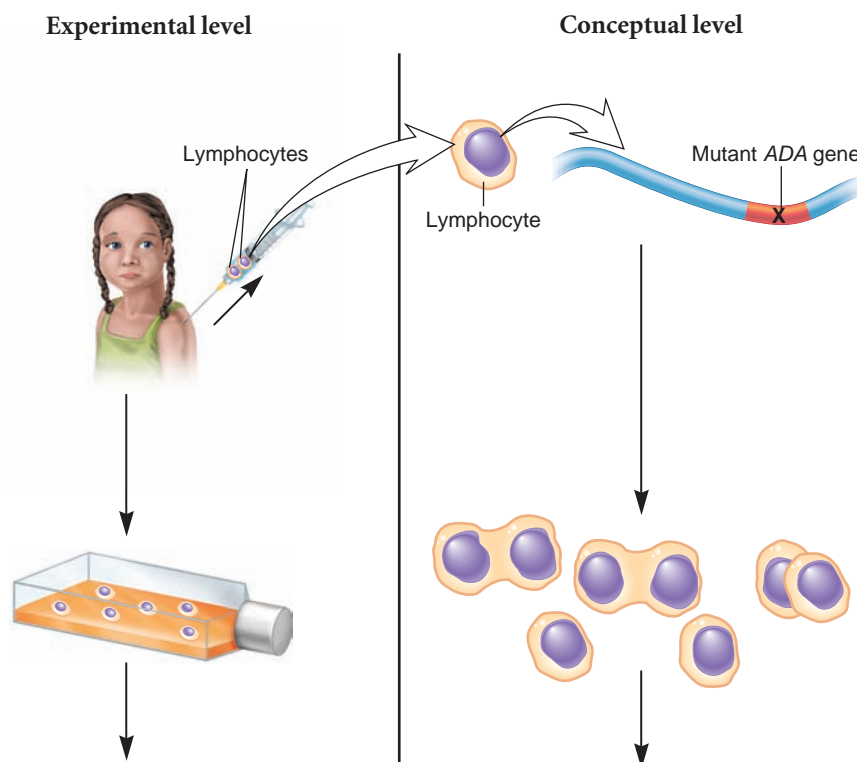
3. Infect the cells with a retrovirus that contains the normal *ADA* gene. Retroviruses insert their DNA into the host cell chromosome as part of their reproductive cycle.

Retrovirus with *ADA* gene

Retrovirus   Lymphocyte

4. Infuse the *ADA*-gene-corrected lymphocytes back into the SCID patient.

Normal *ADA* gene

Retroviral inserted DNA
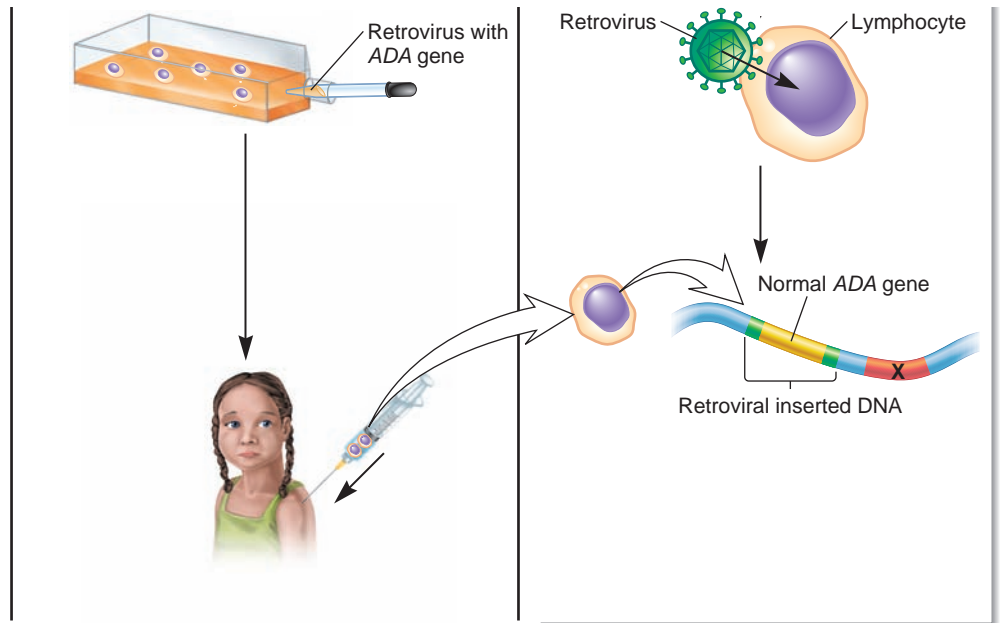
THE DATA

Data from: R.M. Blaese, K.W. Culver, A.D. Miller, et al. (1995) T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. *Science 270*, 475–480.

INTERPRETING THE DATA

In this clinical trial, two patients were enrolled, and a third patient was later treated in Japan. Was the treatment a success? The results of this trial showed that the transfer of DNA into a large number of human cells is feasible. As shown in the data, in at least one patient, T cells carrying the cloned gene were still detectable 8 to 10 years after they had been transferred. However, most of the circulating T cells were not found to contain the cloned gene. Because the individuals also received a low dose of PEG-ADA treatment, researchers could not determine whether or not gene transfer into T cells by itself was of significant clinical benefit.

Another form of SCID, termed SCID-X1, is inherited as an X-linked trait. SCID-X1 is characterized by a block in T-cell growth and differentiation. This block is caused by mutations in the gene encoding the γ_c cytokine receptor, which plays a key role in the recognition of signals that are needed to promote the growth, survival, and differentiation of T cells. A gene therapy trial for SCID-X1 similar to the trial shown in Figure 19.20 was initiated in 2000 in which a normal γ_c cytokine receptor gene was cloned into a retroviral vector and then introduced into SCID-X1 patients' lymphocytes. The lymphocytes were then reintroduced back into their bodies. At a 10-month follow-up, T cells expressing the normal γ_c cytokine receptor were detected in two patients. Most importantly, the T-cell counts in these two patients had risen to levels that were comparable to those in normal individuals. This clinical trial was the first clear demonstration that gene therapy can offer clinical benefit, providing in these cases what seemed to be a complete correction of the disease phenotype. However, in a French study involving 10 SCID-X1 patients, an unexpected and serious side effect occurred. Within 3 years of gene therapy treatment, 3 out of the 10 treated children developed leukemia—a form of cancer involving the proliferation of white blood cells. In these cases, the disease was caused by the integration of the retroviral vector next to a particular gene in the patients' genomes. The development of leukemia in these patients has halted many clinical trials involving gene therapy.

A self-help quiz involving this experiment can be found at www.mhhe.com/brookergenetics4e.

Aerosol Sprays May Be Used to Treat Cystic Fibrosis

Cystic fibrosis (CF) is a rare recessive disorder with debilitating consequences. About 1 in 3000 babies whose parents are of northern European descent are affected with this disorder. CF is caused by a defect in a gene termed the cystic fibrosis transmembrane regulator (*CFTR*), which encodes a protein that functions in the transport of chloride ions across the plasma membrane of epithelial cells, such as cells lining the respiratory and intestinal tracts. A defect in membrane transport leads to an abnormality in salt and water balance, which causes a variety of symptoms, particularly an overaccumulation of mucus in the lungs. Even though great strides have been made in the treatment of the symptoms of CF, this disease remains associated with repeated lung infections and a shortened life span. In most cases, mortality results from chronic lung infections.

CF has been the subject of much gene therapy research. Clinical trials have tested the ability of gene therapy to improve the condition of patients suffering from CF. To implement CF gene therapy, it is necessary to deliver the normal *CFTR* gene to the lung cells. Unlike *ADA* gene therapy, in which the lymphocytes can be treated ex vivo, lung epithelial cells cannot be removed and then put back into the individual. Instead, researchers must design innovative approaches that can target the *CFTR* gene directly to the lung cells.

To achieve this goal, CF gene therapy methods have involved the use of an inhaled aerosol spray. In one protocol, the normal *CFTR* gene is cloned into an adenovirus, a virus that normally infects lung epithelial cells and causes a lung infection. This adenovirus, however, has been engineered so it can gain entry into the epithelial cells but not cause a lung infection. In addition, the adenovirus has been engineered to contain the normal *CFTR* gene. In a second approach, the normal *CFTR* gene is complexed with liposomes. When inhaled by the patient via an aerosol spray, the lung epithelial cells take up this liposome complex.

Like *ADA* gene therapy, CF gene therapy is at an early stage of development. Researchers hope that gene therapy eventually will become an effective method of alleviating the symptoms associated with this disease.

CHAPTER SUMMARY

- Biotechnology is the application of technologies that involve the use of living organisms, or products from living organisms, for the development of products that benefit humans. Genetically modified organisms have received genetic material via recombinant DNA technology.

19.1 Uses of Microorganisms in Biotechnology

- Microorganisms have several uses in biotechnology (see Table 19.1).
- Recombinant microorganisms are used to make human hormones, such as insulin (see Table 19.2, Figure 19.1).
- Microorganisms may be used as biological control agents, though their release is sometimes controversial (see Figure 19.2).
- Microorganisms may be used in bioremediation, which is the method of using living organisms to decrease pollutants in the environment.

19.2 Genetically Modified Animals

- A transgenic organism carries a gene from another species (see Figure 19.3).
- The integration of a cloned gene into a chromosome may result in gene replacement or gene addition (see Figures 19.4, 19.5).
- Gene knockouts and knockins in mice are made to study gene function and to produce mouse models for human diseases (see Figures 19.6, 19.7).
- Transgenic livestock may produce human proteins, such as hormones, in their milk (see Table 19.3, Figure 19.8).

19.3 Reproductive Cloning and Stem Cells

- Mammalian reproductive cloning can be achieved using somatic cells and oocytes with their nuclei removed (see Figures 19.9, 19.10).

- Stem cells have the ability to divide and differentiate. Stem cells may be totipotent, pluripotent, multipotent, or unipotent (see Figures 19.11–19.13).
- Stem cells have the potential to treat a variety of human diseases (see Table 19.4).

19.4 Genetically Modified Plants

- *Agrobacterium tumefaciens* transfers T DNA to plants. Researchers have used T-DNA vectors to make genetically modified plants (see Figures 19.14, 19.15).

- Researchers have made many transgenic plants that have traits that are useful to humans, including herbicide and pesticide resistance (see Table 19.5, Figures 19.16–19.18).

19.5 Human Gene Therapy

- Human gene therapy is the introduction of cloned genes into cells in an attempt to treat a disease. Genes may be introduced via liposomes or viruses (see Figure 19.19).
- The first human gene therapy trial was aimed at treating adenine deaminase deficiency (see Figure 19.20).

PROBLEM SETS & INSIGHTS

Solved Problems

S1. Which of the following would appropriately be described as a transgenic organism?

  A. The sheep "Dolly," which was produced by cloning

  B. A sheep that produces human α_1-antitrypsin in its milk

  C. The Flavr Savr strain of tomato

  D. A hybrid strain of corn produced from crossing two inbred strains of corn (The inbred strains were not transgenic.)

Answer:

  A. No, Dolly was not produced using recombinant techniques. Pieces of DNA were not cut and combined in a new way.

  B. Yes

  C. Yes

  D. No, the hybrids simply contain chromosomal genes from two different parental strains.

S2. Describe the strategy for producing human proteins in the milk of livestock.

Answer: Milk proteins are encoded by genes with promoters and regulatory sequences that direct the expression of these genes within the cells of the mammary gland. To get other proteins expressed in the mammary gland, the strategy is to link the promoter and regulatory sequences from a milk-specific gene to the coding sequence of the gene that encodes the human protein of interest. In some cases, it is also necessary to add a signal sequence to the amino-terminal end of the target protein. A signal sequence is a short polypeptide that directs the secretion of a protein from a cell. If the target protein does not already have a signal sequence, it is possible to use a signal sequence from a milk-specific gene to promote the secretion of the target protein from the mammary cells and into the milk. During this process, the signal sequence is cleaved from the secreted protein.

S3. With regard to genetically modified organisms, describe two that have not been successful and two that have.

Answer: Two unsuccessful examples are oil-eating bacteria and the Flavr Savr tomato. Two very successful examples include bacteria that make human insulin and Bt varieties of agricultural crops.

Conceptual Questions

C1. What is a recombinant microorganism? Discuss examples of recombinant microbes.

C2. A conjugation-deficient strain of *A. radiobacter* is used to combat crown gall disease. Explain how this bacterium prevents the disease and the advantage of a conjugation-deficient strain.

C3. What is bioremediation? What is the difference between biotransformation and biodegradation?

C4. What is a biological control agent? Briefly describe three examples.

C5. As described in Table 19.2, several medical agents are now commercially produced by genetically engineered microorganisms. Discuss the advantages and disadvantages of making these agents this way.

C6. What is a mouse model for human disease?

C7. What is a transgenic organism? Describe three examples.

C8. What part of the *A. tumefaciens* DNA gets transferred to the genome of a plant cell during infection?

C9. Explain the difference between gene addition and gene replacement. Are the following descriptions examples of gene addition or gene replacement?

  A. A mouse model to study cystic fibrosis

  B. Introduction of a pesticide-resistance gene into corn using the T-DNA vector of *A. tumefaciens*

C10. As described in Chapter 5, not all inherited traits are determined by nuclear genes (i.e., genes located in the cell nucleus) that are expressed during the life of an individual. In particular, maternal effect genes and mitochondrial genes are notable exceptions. With these ideas in mind, let's consider the cloning of sheep (e.g., Dolly).

  A. With regard to maternal effect genes, is the phenotype of such a cloned animal determined by the animal that donated the enucleated egg or by the animal that donated the somatic cell nucleus? Explain.

B. Does the cloned animal inherit extranuclear traits from the animal that donated the egg or from the animal that donated the somatic cell? Explain.

C. In what ways would you expect this cloned animal to be similar to or different from the animal that donated the somatic cell? Is it accurate to call such an animal a "clone" of the animal that donated the nucleus?

C11. Discuss some of the worthwhile traits that can be modified in transgenic plants.

C12. Discuss the concerns that some people have with regard to the uses of genetically engineered organisms.

Experimental Questions

E1. Recombinant bacteria can produce hormones that are normally produced in humans. Briefly describe how this is accomplished.

E2. *Bacillus thuringiensis* can make toxins that kill insects. This toxin must be applied several times during the growth season to prevent insect damage. As an alternative to repeated applications, one strategy is to apply bacteria directly to leaves. However, *B. thuringiensis* does not survive very long in the field. Other bacteria, such as *Pseudomonas syringae*, do. Propose a way to alter *P. syringae* so that it could be used as an insecticide. Discuss advantages and disadvantages of this approach compared with the repeated applications of the insecticide from *B. thuringiensis*.

E3. In the experiment of Figure 19.1, why was it necessary to link the coding sequence for the A or B chains to the sequence for β-galactosidase? How were the A or B chains separated from β-galactosidase after the fusion protein was synthesized in *E. coli*?

E4. Explain how it is possible to select for homologous recombination in mice. What phenotypic marker is used to readily identify chimeric mice?

E5. To produce transgenic plants, plant tissue is exposed to *Agrobacterium tumefaciens* and then grown in media containing kanamycin, carbenicillin, and plant growth hormones. Explain the purpose behind each of these three agents. What would happen if you left out the kanamycin?

E6. List and briefly describe five methods for the introduction of cloned genes into plants.

E7. What is a gene knockout? Is an animal or plant with a gene knockout a heterozygote or homozygote? What might you conclude if a gene knockout does not have a phenotypic effect?

E8. Nowadays, it is common for researchers to identify genes using cloning methods described in Chapters 18 and 19. A gene can be identified according to its molecular features. For example, a segment of DNA can be identified as a gene because it contains the right combination of sequences: a promoter, exons, introns, and a terminator. Or a gene can be identified because it is transcribed into mRNA. In the study of plants and animals, it is relatively common for researchers to identify genes using molecular techniques without knowing the function of the gene. In the case of mice, the function of the gene can be investigated by making a gene knockout. If the knockout causes a phenotypic change in the mouse, this may provide an important clue regarding the function of a gene. For example, a gene knockout that produced an albino mouse would indicate the gene knocked out probably plays a role in pigment formation. The experimental strategy of first identifying a gene based on its molecular properties and then investigating its function by making a knockout is called reverse genetics. Explain how this approach is opposite (or "in reverse") to the conventional way that geneticists study the function of genes.

E9. According to the methods described in Figure 19.6, can homologous recombination that results in gene replacement cause the integration of both the *TK* and *Neo*^{R} genes? Explain why or why not. Describe how the *TK* gene and *Neo*^{R} gene are used in a selection scheme that favors gene replacement.

E10. What is a chimera? How are chimeras made?

E11. Evidence (see P.G. Shiels, A.J. Kind, K.H. Campbell, et al. (1999) Analysis of telomere lengths in cloned sheep. *Nature 399,* 316–17) suggested that Dolly may have been "genetically older" than her actual age would have suggested. As mammals age, the chromosomes in somatic cells tend to shorten from the telomeres. Therefore, older individuals have shorter chromosomes in their somatic cells than do younger ones. When researchers analyzed the chromosomes in the somatic cells of Dolly when she was about 3 years old, the lengths of her chromosomes were consistent with a sheep that was significantly older, say, 9 or 10 years old. (Note: As described in the chapter, the sheep that donated the somatic cell that produced Dolly was 6 years old, and her mammary cells had been grown in culture for several cell doublings before a mammary cell was fused with an oocyte.)

A. Suggest an explanation why Dolly's chromosomes seemed so old.

B. Let's suppose that Dolly at age 11 gave birth to a lamb named Molly; Molly was produced naturally (by mating Dolly with a normal male). When Molly was 8 years old, a sample of somatic cells was analyzed. How old would you expect Molly's chromosomes to appear, based on the phenomenon of telomere shortening? Explain your answer.

C. Discuss how the observation of chromosome shortening, which was observed in Dolly, might affect the popularity of reproductive cloning.

E12. When transgenic organisms are made, the transgene may integrate into multiple sites within the genome. Furthermore, the integration site may influence the expression of the gene. For example, if a transgene integrates into a heterochromatic (i.e., highly condensed) region of a chromosome, the transgene may not be expressed. For these reasons, it is important for geneticists to analyze transgenic organisms with regard to the number of transgene insertions and the expression levels of the transgenes. Chapter 18 describes three methods (Southern blotting, Northern blotting, and Western blotting) that can be used to detect genes and gene products. Which of these techniques would you use to determine the number of transgenes in a transgenic plant or animal? Why is it important to know the number of transgenes? (Hint: You may want to use a transgenic animal or plant as breeding stock to produce many more transgenic animals or plants.) Which technique would you use to determine the expression levels of transgenes?

E13. What is molecular pharming? Compared with the production of proteins by bacteria, why might it be advantageous?

E14. What is reproductive cloning? Are identical twins in humans considered to be clones? With regard to agricultural species, what are some potential advantages to reproductive cloning?

E15. Researchers have identified a gene in humans that (when mutant) causes severe dwarfism and mental retardation. This disorder is inherited in an autosomal recessive manner, and the mutant allele is known to be a loss-of-function mutation. The same gene has been found in mice, although a mutant version of the gene has not been discovered in mice. To develop drugs and an effective therapy to treat this disorder in humans, it would be experimentally useful to have a mouse model. In other words, it would be desirable to develop a strain of mice that carry the mutant allele in the homozygous condition. Experimentally, how would you develop such a strain?

E16. Treatment of adenosine deaminase (ADA) deficiency is an example of ex vivo gene therapy. Why is this therapy called ex vivo? Can ex vivo gene therapy be used to treat all inherited diseases? Explain.

E17. Describe the targeting methods used in cystic fibrosis gene therapy. Provided the *CFTR* gene gets to the patient's lung cells, would you expect this to be a permanent cure for the patient, or would it be necessary to perform this gene therapy on a regular basis (say, monthly)?

E18. Several research studies are under way that involve the use of gene therapies to inhibit the growth of cancer cells. As discussed in Chapter 22, oncogenes are mutant genes that are overexpressed and cause cancer. New gene therapies are aimed at silencing an oncogene by producing antisense RNA that recognizes the mRNA transcribed from an oncogene. Based on your understanding of antisense RNA (described in Chapters 14 and 15), explain how this strategy would prevent the growth of cancer cells.

Questions for Student Discussion/Collaboration

1. Discuss the advantages and disadvantages of gene therapy. Because a limited amount of funding is available for gene therapy research, make a priority list of the three top diseases for which you would fund research. Discuss your choices.

2. A commercially available strain of *P. syringae* marketed as Frostban B is used to combat frost damage. This is a naturally occurring *Ice⁻* strain. Discuss the advantages and disadvantages of using this strain compared with a recombinant version.

3. Make a list of the types of traits you would like to see altered in transgenic plants and animals. Suggest ways (i.e., what genes would you use?) to accomplish these alterations.

Note: All answers appear at the website for this textbook; the answers to even-numbered questions are in the back of the textbook.

www.mhhe.com/brookergenetics4e

Visit the website for practice tests, answer keys, and other learning aids for this chapter. Enhance your understanding of genetics with our interactive exercises, quizzes, animations, and much more.