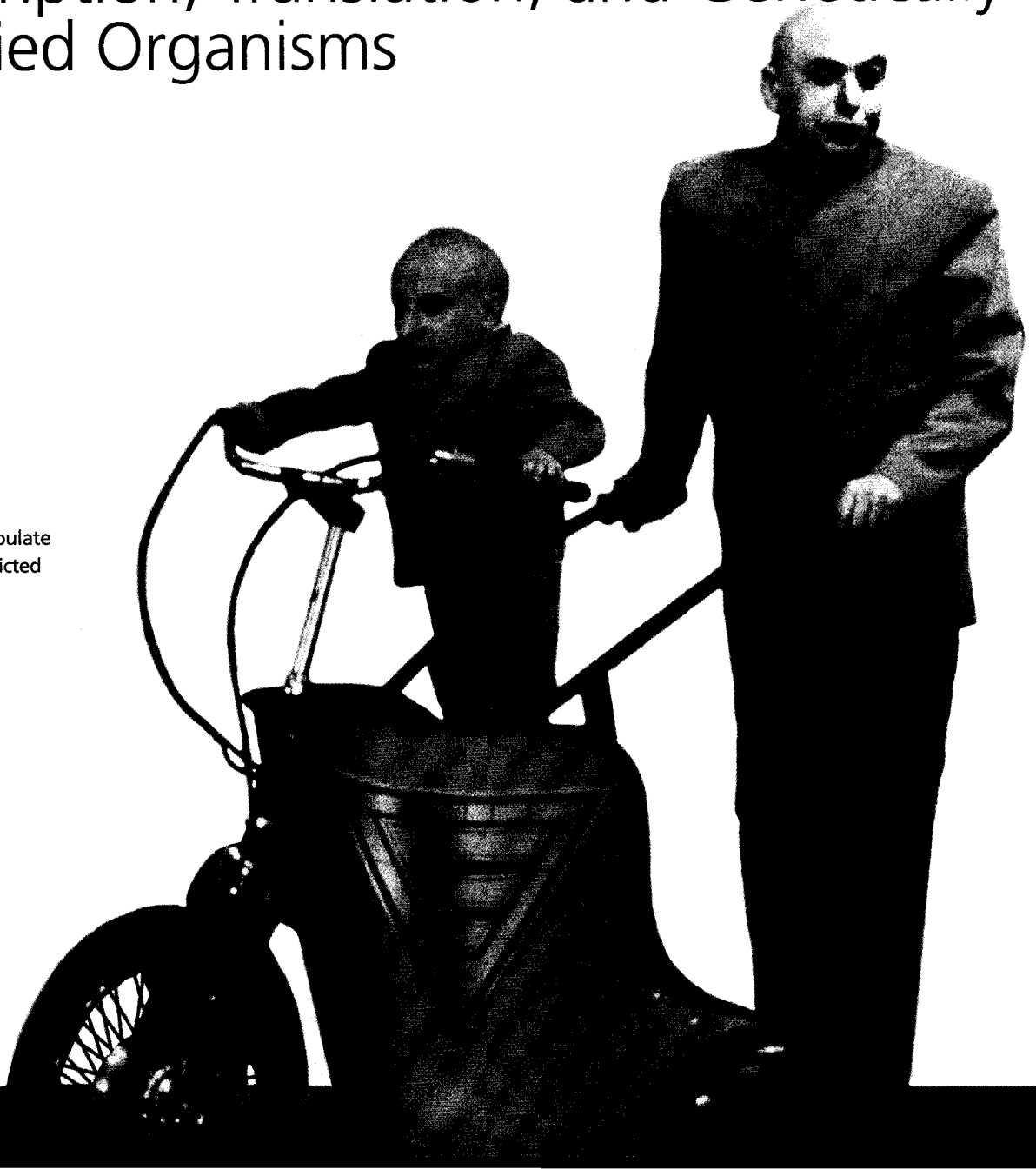
CHAPTER 8

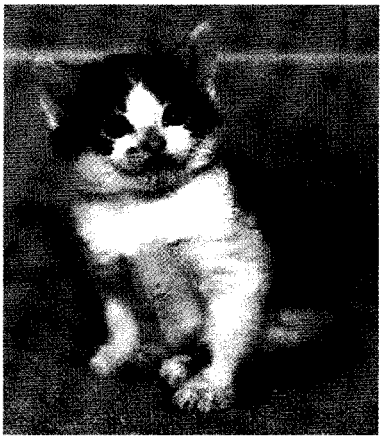
Genetic Engineering

Transcription, Translation, and Genetically Modified Organisms



Scientists who manipulate genes are often depicted as mad scientists.

Can scientists bring extinct species back to life, as seen in the movie *Jurassic Park*?



Scientists can already clone pets such as Cc the cat.

Will they one day be able to clone humans as in the movie Multiplicity?

You hear about them all the time. They are often depicted in cartoons, comic books, movies, and science fiction as mad scientists. These are the scientists who take a gene from one organism and place it into an unrelated organism. These are the scientists who make hormones that farmers inject into the cows that produce the milk we drink. These are the scientists who modify the crops we eat, creating what some people call "Frankenfoods." You may have wondered if it might soon be possible to replace a beloved family member or pet, bring back extinct species through cloning, or even clone yourself. You might worry about a future where parents unwilling to fix their children's "genetic defects" face discrimination.

Who are these scientists? Who pays them? Is anyone regulating their work? Is anyone trying to determine if it is unhealthy to eat these modified foods, whether genetically modified plants will cause environmental problems, or if genetically modified animals are less healthy than their counterparts?

With all kinds of unreliable information coming from so many different sources, it is often hard to separate fact from fiction. To help you sort this out, let us first look at the scientists who are involved in manipulating genes and then learn how they do what they do, as well as about the regulations affecting their work. Finally, we will examine the real prospects and perils of genetic engineering.

8.1 Genetic Engineers

Genetic engineers are scientists who manipulate genes. They make their living working at colleges and universities, for the government, and for private companies. Most of them have had extensive training in genetics. The manipulations that genetic engineers perform include changing a gene, changing how a gene is regulated (turned on or off), or moving a gene from one organism to another.

The training for the typical genetic engineer involves many years of schooling. After completing an undergraduate degree, some will obtain a master's degree, which takes two to three years and requires course work as well as a thesis research project. If the student does not continue past the master's level, he or she will probably work in a laboratory under the supervision of a more senior scientist.

Students who want to continue their education can apply to graduate schools with Ph.D. (doctor of philosophy) programs. Scientists holding a Ph.D. have the title of "Doctor" because they have a doctorate in their chosen field (a medical doctor, or M.D., has a doctorate in medicine). A Ph.D. program involves more course work and an expanded research component; the results of this research must also be published in a peer-reviewed journal. Most Ph.D. scientists have gone to school five or more years after earning their undergraduate degree.

In scientific fields, graduate students generally get paid a small salary and have their tuition waived by the university. In exchange for tuition and salary, students work as teaching assistants overseeing undergraduate laboratory courses. If your biology course has a laboratory component, then you may have had experience with a teaching assistant who is a pursuing an advanced degree in biology.

Most colleges and universities, especially the larger ones, expect faculty members to combine teaching with research. In this way, college professors not only pass information to the next generation; they also add to the knowledge base of their field.

The federal government employs many of these biologists—for example, the National Cancer Institute (NCI) employs genetic engineers. In addition, many genetic engineers work in private industry, which tends to focus on for-profit product and drug development.

Genetic engineers in academia, government, and industry are involved in many different research projects. These projects vary from trying to produce a protein in the laboratory, to changing the genetic characteristics of crop plants, to trying to understand how human genes interact. One of the first genetic engineering projects to seize the attention of the public was the genetic engineering of a protein normally produced by cows.

8.2 Protein Synthesis and Gene Expression

During the early 1980s, genetic engineers at Monsanto® Company began to produce **recombinant bovine growth hormone (*r*BGH)** in their laboratories. Recombinant (*r*) bovine growth hormone is a protein that has been made by genetically engineered bacteria. These bacterial cells have had

their DNA manipulated so that it carries the instructions for, or encodes, a cow growth hormone that can be produced in the laboratory. Hormones are substances that are secreted from specialized glands and travel through the bloodstream to affect their target organs. Growth hormones act on many different organs to increase the overall size of the body and, in cows, to increase milk production.

Before the advent of genetic technologies, growth hormone was extracted from the pituitary glands of slaughtered cows and then injected into live cows (Figure 8.1). It is also possible to obtain human growth hormone from the pituitary glands of human cadavers. When the human growth hormone is injected into humans who have a condition called **pituitary dwarfism**, their size increases. However, harvesting growth hormone from the pituitary glands of cows and humans is laborious, and many cadavers are necessary to obtain small amounts of the protein.

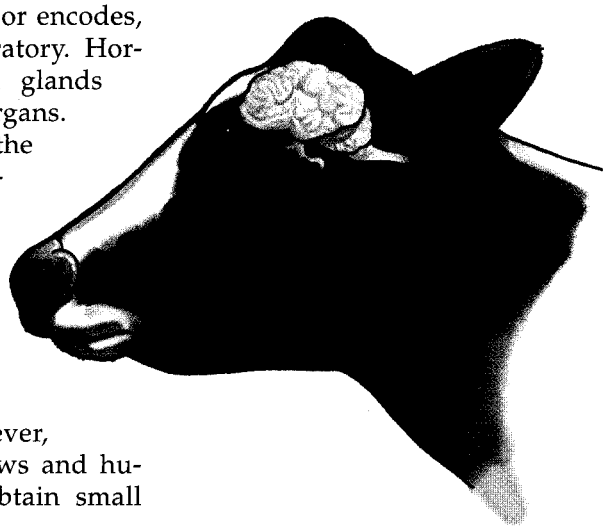


Figure 8.1 Bovine growth hormone (BGH). Bovine growth hormone is a protein produced by the pituitary gland (in red) of the cow brain.

Genetic engineers at Monsanto realized that genetic technology would allow them to produce large quantities of bovine growth hormone in the laboratory, inject it into dairy cows, and increase their milk production, completely bypassing the less-efficient, surgical isolation of BGH. These scientists understood that if they were successful, Monsanto would stand to make a healthy profit from the dairy farmers who would buy the engineered growth hormone to increase the milk yield of their herds. Let us first examine how cells normally use DNA instructions to produce proteins and then how scientists manipulate this process to have bacteria produce proteins normally made by other organisms.

From Gene to Protein

Protein synthesis involves using the instructions carried by a gene to build a particular protein. Genes do not build proteins directly; instead, they carry the instructions that dictate how a protein should be built. Understanding protein synthesis requires that we review a few basics about DNA, genes, and RNA. First, DNA is a polymer of nucleotides that make chemical bonds with each other based on their complementarity (A to T, and C to G). Second, a gene is a sequence of DNA that encodes a protein. Proteins are large molecules composed of amino acids. Each protein has a unique function that is dictated by its particular structure. The structure of a protein is the result of the order of amino acids that comprise it because the chemical properties of amino acids cause a protein to fold in a particular manner.

Before a protein can be built, the instructions carried by a gene are first copied. When the gene is copied, the copy is comprised not of DNA (deoxyribonucleic acid) but of **RNA (ribonucleic acid)**. Therefore, it is important to understand the differences between DNA and RNA.

RNA is also a polymer of nucleotides. A nucleotide is composed of a sugar, a phosphate group, and a nitrogen-containing base. For DNA nucleotides, the sugar is deoxyribose, and the nitrogenous bases are adenine (A), cytosine (C), guanine (G), and thymine (T). The nucleotides that join together to produce RNA are composed of the sugar ribose, a phosphate group, and the nitrogenous bases A, C, G, and U (uracil). There are no thymines (T) in RNA because uracil (U) replaces them. In addition, RNA is usually single stranded,

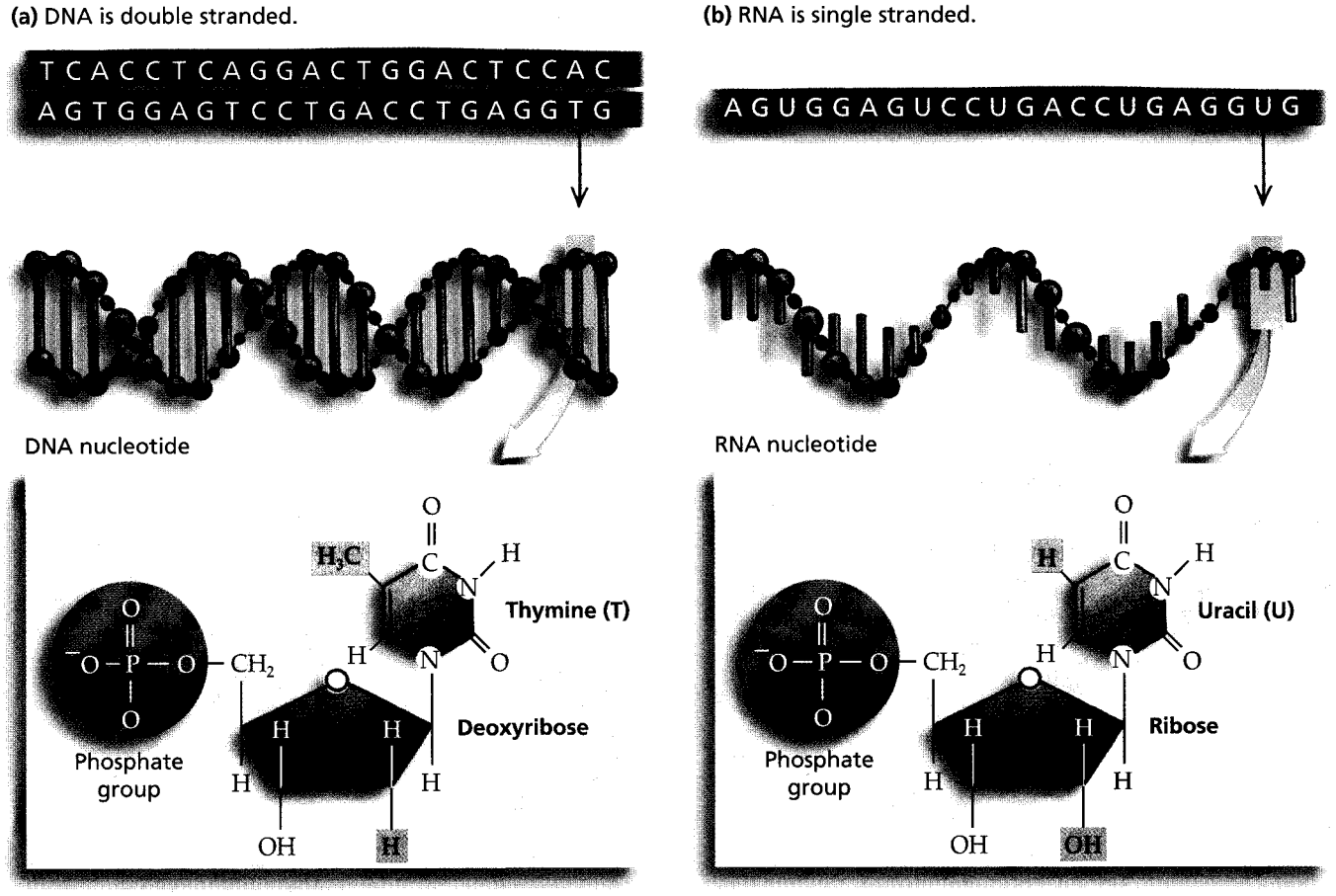


Figure 8.2 DNA and RNA. (a) DNA is double stranded. Each DNA nucleotide is composed of the sugar deoxyribose, a phosphate group and a nitrogen containing base (A, G, C, or T). (b) RNA is single stranded. RNA nucleotides are composed of the sugar ribose, a phosphate group and a nitrogen containing base (A, G, C, or U). Note the difference in the sugars is shown in the pink boxes.

not double stranded like DNA (Figure 8.2). When a cell requires a particular protein, a strand of RNA is produced by using DNA as a template. RNA nucleotides are able to make base pairs with DNA nucleotides. C and G make a base pair, and U pairs with A.

The RNA copy then serves as a blueprint that tells the cell which amino acids to join together to produce a protein. Thus, the flow of genetic information in a eukaryotic cell is from DNA to RNA to protein (Figure 8.3).

How does this flow of information actually take place in a cell? Going from gene to protein involves two steps. The first step, called **transcription**, involves producing the copy of the required gene. In the same way that a transcript of a speech is a written version of the words spoken by the speak-

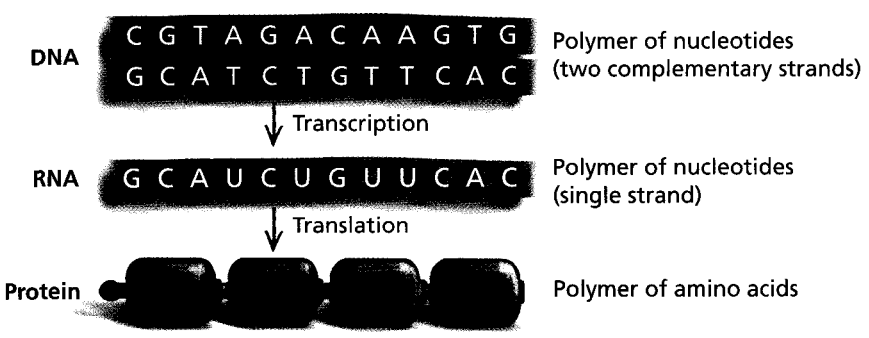


Figure 8.3 The flow of genetic information. Genetic information flows from a DNA to an RNA copy of the DNA gene, to the amino acids that are joined together to produce the protein coded for by the gene.

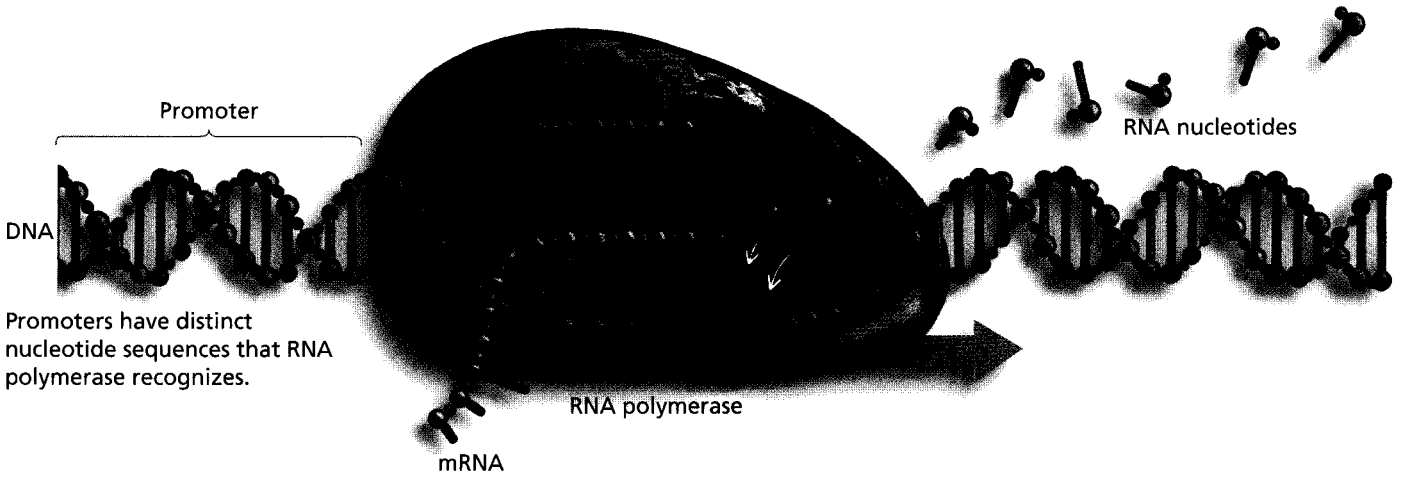


Figure 8.4 Transcription. After locating the start site of the gene, a region called the promoter, the enzyme RNA polymerase ties together nucleotides within the growing RNA strand as they bind to their complementary base on the DNA. Only when a complementary base pair is made between DNA and RNA does the polymerase add an RNA nucleotide to the growing strand. Complementary bases are formed via hydrogen bonding of A with U and G with C. Note that one strand of the DNA is used as a template for the synthesis of the mRNA. When the RNA polymerase reaches the end of the gene, the mRNA transcript is released.

er, transcription inside a cell is a process that produces a copy with the RNA nucleotides substituted for DNA nucleotides. The second step, called **translation**, involves decoding the copied RNA sequence and producing the protein for which it codes. In the same way that a translator helps determine the meaning of words in two different languages, translation in a cell involves moving from the language of nucleotides (DNA and RNA) to the language of amino acids and proteins.

Transcription

Transcription is the copying of a DNA gene into RNA (Figure 8.4). The copy is synthesized by an enzyme called **RNA polymerase**. To begin transcription, the RNA polymerase binds to a nucleotide sequence at the beginning of every gene, called the **promoter**. Once the RNA polymerase has located the beginning of the gene by binding to the promoter, it then rides along the strand of the DNA helix that comprises the gene. As it is traveling along the gene, the RNA polymerase unzips the DNA double helix and ties together RNA nucleotides that are complementary to the DNA strand it is using as a template. This results in the production of a single-stranded RNA molecule that is complementary to the DNA sequence of the gene. This complementary RNA copy of the DNA gene is called **messenger RNA (mRNA)**, since it carries the message of the gene that is to be expressed.

Translation

The second step from gene to protein requires that the mRNA be used to produce the actual protein for which the gene encodes through a process called translation. For translation to occur, a cell needs mRNA, a supply of amino acids to join in the proper order, and some energy in the form of ATP. Translation also requires structures called ribosomes and transfer RNA molecules.

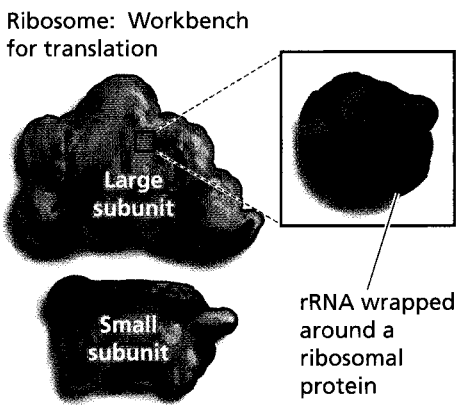


Figure 8.5 Ribosome. Ribosomes are composed of two subunits. Each subunit, in turn, is composed of rRNA and protein. Ribosomes are the site of protein synthesis.

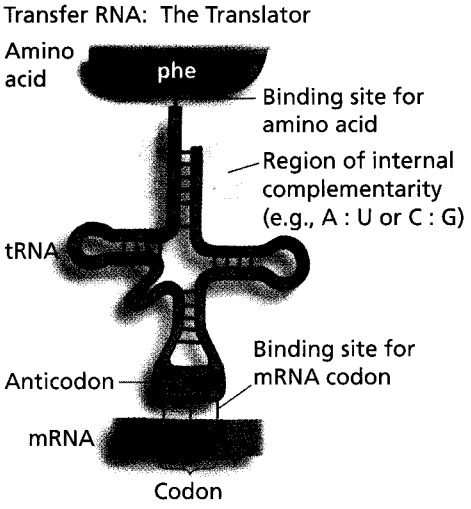


Figure 8.6 Transfer RNA (tRNA). Transfer RNA molecules are composed of an RNA strand that has regions of internal complementarity. Each ribosome has a characteristic 3-nucleotide sequence, called the anticodon, that binds to the mRNA codon. Each tRNA also carries the amino acid corresponding to the mRNA codon to which it binds. Transfer RNAs translate the language of nucleotides into the language of amino acids.

Ribosomes. **Ribosomes** are subcellular, globular structures (Figure 8.5) that are composed of another kind of RNA called **ribosomal RNA (rRNA)**, which is wrapped around many different proteins. Each ribosome is composed of two subunits—one large and one small. When assembled in this fashion, the mRNA can be threaded through the ribosome. In addition, the ribosome can bind to structures called **transfer RNA (tRNA)** that carry amino acids.

Transfer RNA (tRNA). Transfer RNA (Figure 8.6) is yet another type of RNA found in cells (in addition to mRNA and rRNA). Transfer RNA is single stranded but has regions of internal complementarity, where complementary nucleotides (A and U; G and C) bind to each other, resulting in a structure that is single stranded in some regions and double stranded in others. Even though there are some regions of internal complementarity, transfer RNA as a whole is a single strand of nucleotides that folds on itself in some isolated regions. Individual transfer RNAs carry specific amino acids. As mRNA moves through the ribosome, small sequences of mRNA are exposed. These sequences of mRNA are 3 nucleotides long and encode an amino acid; they are called **codons**. Transfer RNAs bind to codons through interactions between the RNA nucleotides at the base of the tRNA, a region called the **anticodon**, and the mRNA codon. The anticodon on a particular tRNA binds to the complementary mRNA codon. Thus, the codon calls for the incorporation of a specific amino acid. When a tRNA anticodon binds to a mRNA codon, the ribosome adds the amino acid that the tRNA is carrying to the growing chain of amino acids that will eventually constitute the finished protein. Therefore, the transfer RNA functions as a sort of cellular translator, fluent in both the language of nucleotides and the language of amino acids.

In this manner, the sequence of bases in the DNA dictates the sequence of bases in the RNA, which in turn dictates the order of amino acids that will be joined together to produce a protein. Protein synthesis ends when a codon that does not code for an amino acid, called a **stop codon**, moves through the ribosome. When a stop codon is present in the ribosome, no new amino acid can be added, and the growing protein is released. Once released, the protein folds up on itself and moves to where it is required in the cell. A summary of the process of translation is shown in Figure 8.7.

The process of translation allows cells to determine which amino acid sequence a particular gene encodes. Scientists can determine the sequence of amino acids that a gene calls for by looking at a chart called the **genetic code**.

Genetic Code. The genetic code shows which mRNA codons code for which amino acids (Table 8.1 on page 200). As Table 8.1 shows, there are 64 codons, 61 of which code for amino acids. Three of the codons are stop codons that occur near the end of a mRNA. Since stop codons do not code for an amino acid, protein synthesis ends when a stop codon enters the ribosome. In the table, you can see that the codon AUG functions both as a start codon (and thus is found near the beginning of each mRNA) and as a codon dictating that the amino acid methionine (met) be incorporated into the protein being synthesized. Notice also that there are many examples of situations when the same amino acid can be coded for by more than one codon. For example, the amino acid threonine (thr) is incorporated into a protein in response to the codons ACU, ACC, ACA, and ACG. The fact that more than one codon can code for the same amino acid is referred to as **redundancy** in the genetic code. There is, however, no situation where a given codon can call for more than one amino acid. For example, AGU codes for serine (ser) and nothing else. Therefore, there is no **ambiguity** in the genetic code as to what amino acid any codon will call for. The genetic code is also **universal** in the sense that different organisms typically decode the same gene to produce the same protein.

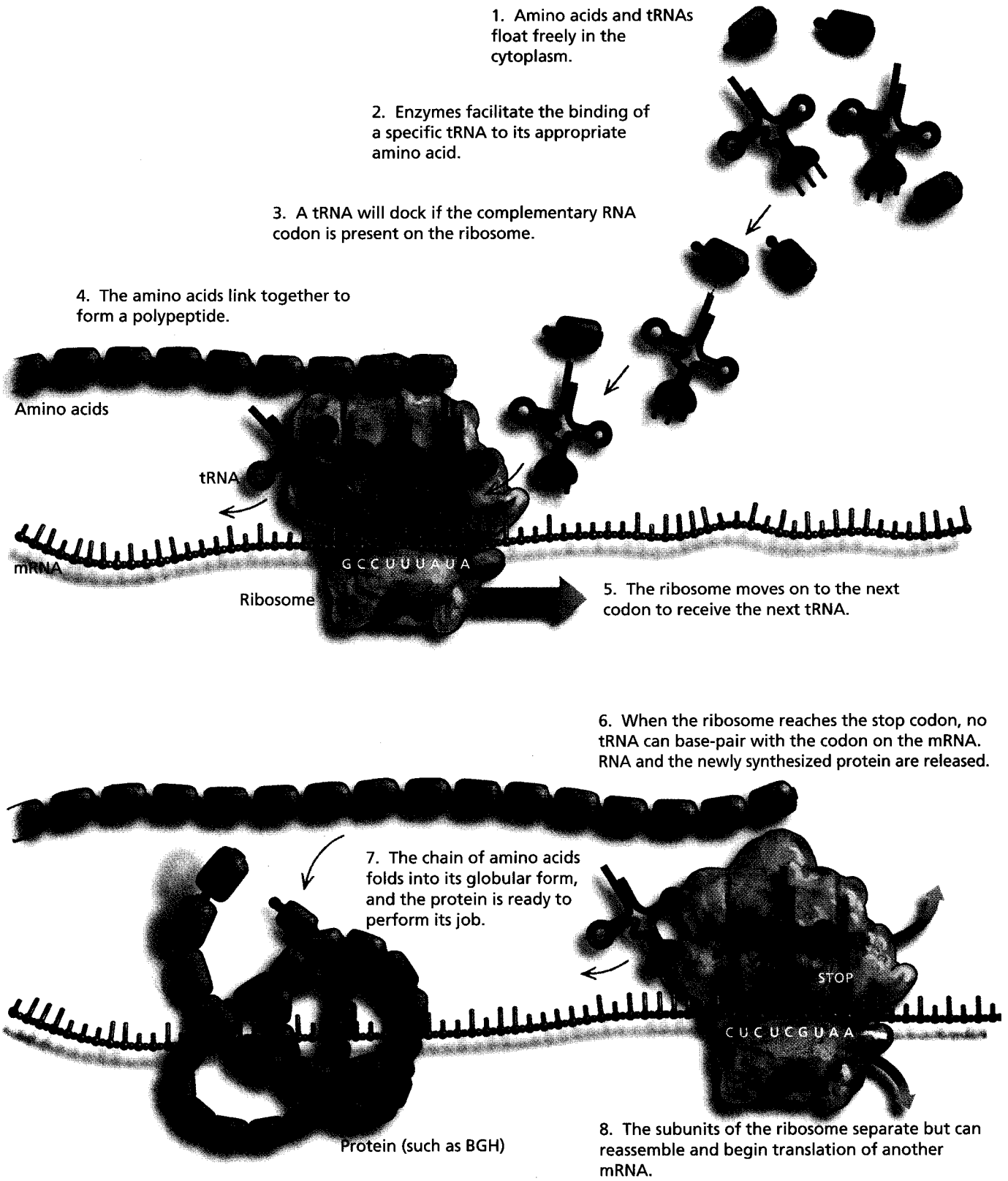


Figure 8.7 Translation. During translation, mRNA directs the synthesis of a protein. The mRNA codon that is exposed in the ribosome binds to its complementary tRNA molecule, which carries the amino acid coded for by the DNA gene. When many amino acids are joined together, the required protein is produced. When the translation machinery reaches a stop codon, the newly synthesized protein is released into the cytoplasm.

Table 8.1 The genetic code. It is possible to determine which amino acid is coded for by each mRNA codon using a chart called the genetic code. Look at the left-hand side of the chart for the first-base nucleotide in the codon; there are 4 rows, one for each possible RNA nucleotide—A, C, G, or U. By then looking at the intersection of the second-base columns at the top of the chart and the first-base rows, you can narrow your search for the codon to 4 different codons. Finally, the third-base nucleotide in the codon on the right-hand side of the chart determines the amino acid that a given mRNA codon codes for. Note the 3 codons, UAA, UAG, and UGA, that do not code for an amino acid; these are stop codons. The codon AUG is a start codon, found at the beginning of most protein-coding sequences.

Second base

First base	U	C	A	G	Third base
U	UUU, UUC Phenylalanine (phe) UUA, UUG Leucine (leu)	UCU, UCC, UCA, UCG Serine (ser)	UAU, UAC Tyrosine (tyr) UAA Stop codon UAG Stop codon	UGU, UGC Cysteine (cys) UGA Stop codon UGG Tryptophan (trp)	U C A G
C	CUU, CUC, CUA, CUG Leucine (leu)	CCU, CCC, CCA, CCG Proline (pro)	CAU, CAC Histidine (his) CAA, CAG Glutamine (gln)	CGU, CGC, CGA, CGG Arginine (arg)	U C A G
A	AUU, AUC, AUA Isoleucine (ile) AUG Methionine (met) **Start codon**	ACU, ACC, ACA, ACG Threonine (thr)	AAU, AAC Asparagine (asn) AAA, AAG Lysine (lys)	AGU, AGC Serine (ser) AGA, AGG Arginine (arg)	U C A G
G	GUU, GUC, GUA, GUG Valine (val)	GCU, GCC, GCA, GCG Alanine (ala)	GAU, GAC Aspartic acid (asp) GAA, GAG Glutamic acid (glu)	GGU, GGC, GGA, GGG Glycine (gly)	U C A G

Mutations

Sometimes changes to the DNA sequence, called **mutations**, can affect the order of amino acids incorporated into a protein during translation. Mutations to a gene can result in the production of different forms, or alleles, of a gene. Different alleles result from changes in the DNA that alter the amino acid order of the encoded protein. Mutations can result in the production of either no functional protein or a protein different from the one previously called for. If this protein does not have the same amino acid composition, it may not be able to perform the same job (Figure 8.8). Chapter 6 indicates that a substitution of a single nucleotide results in the incorporation of a new amino acid in the hemoglobin protein and compromises the ability of cells to carry oxygen, producing sickle-cell disease.

There are also cases in which a mutation has no effect on a protein. They may occur when changes to the DNA result in the production of a mRNA

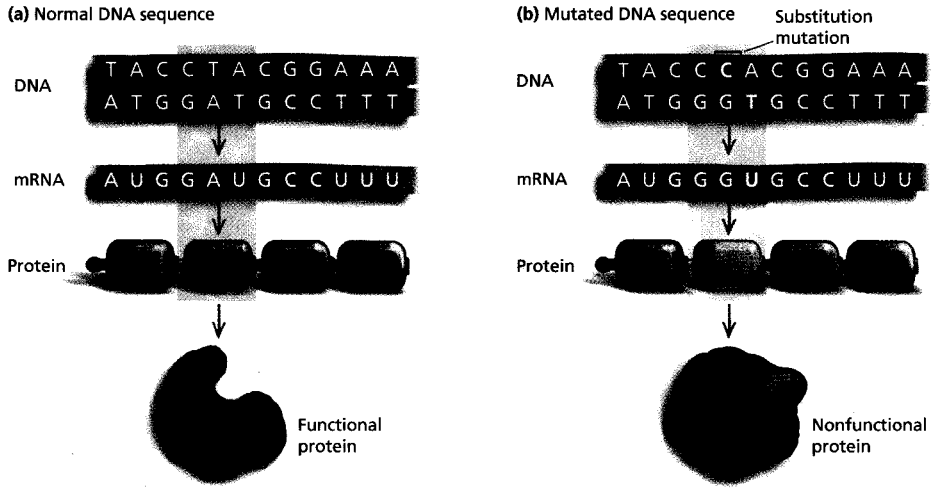


Figure 8.8 Mutation. A single nucleotide change from the normal sequence (a) to the mutated sequence (b) can result in the incorporation of a different amino acid. If the substituted amino acid has chemical properties different from those of the original amino acid, then the protein may assume a different shape and thus lose its ability to perform its job.

codon that codes for the same amino acid as was originally called for. Due to the redundancy of the genetic code, a mutation that changes the mRNA codon from ACU to ACC will have no impact because both of these codons code for the amino acid threonine. This is called a **neutral mutation** (Figure 8.9a). In addition, mutations can result in the substitution of one amino acid for another with similar chemical properties, which may have little or no effect on the protein.

Inserting or deleting a single nucleotide can have a severe impact since the addition (or deletion) of a nucleotide can change the groupings of nucleotides in every codon that follows (Figure 8.9b). Changing the triplet groupings is called altering the **reading frame**. All nucleotides located after an insertion or deletion will be regrouped into different codons, producing a **frameshift mutation**. For example, inserting an extra letter "H" after the fourth letter of the sentence, "The cat ate his dog," could change the reading frame to the nonsensical statement, "The cHa tat ehi sdo g." Inside cells, this often results in the incorporation of a stop codon and the production of a shortened, nonfunctional protein.

To help you understand protein synthesis, let us consider its similarity to an everyday process such as baking a cake. To bake a cake, you would consult a recipe book (genome) for the specific recipe (gene) to make your cake (protein). You may copy the recipe (mRNA) out of the book so that the original recipe (gene) does not become stained or damaged. The original recipe (gene) is left in the book (genome) on a shelf (nucleus), so that you can make another copy when you need it. The original recipe (gene) can be copied again and again. The copy of the recipe (mRNA) is placed on the kitchen counter (ribosome) while you assemble the ingredients (amino acids). The ingredients (amino acids) for your cake (protein) include flour, sugar, butter, milk, and eggs. The ingredients are measured in

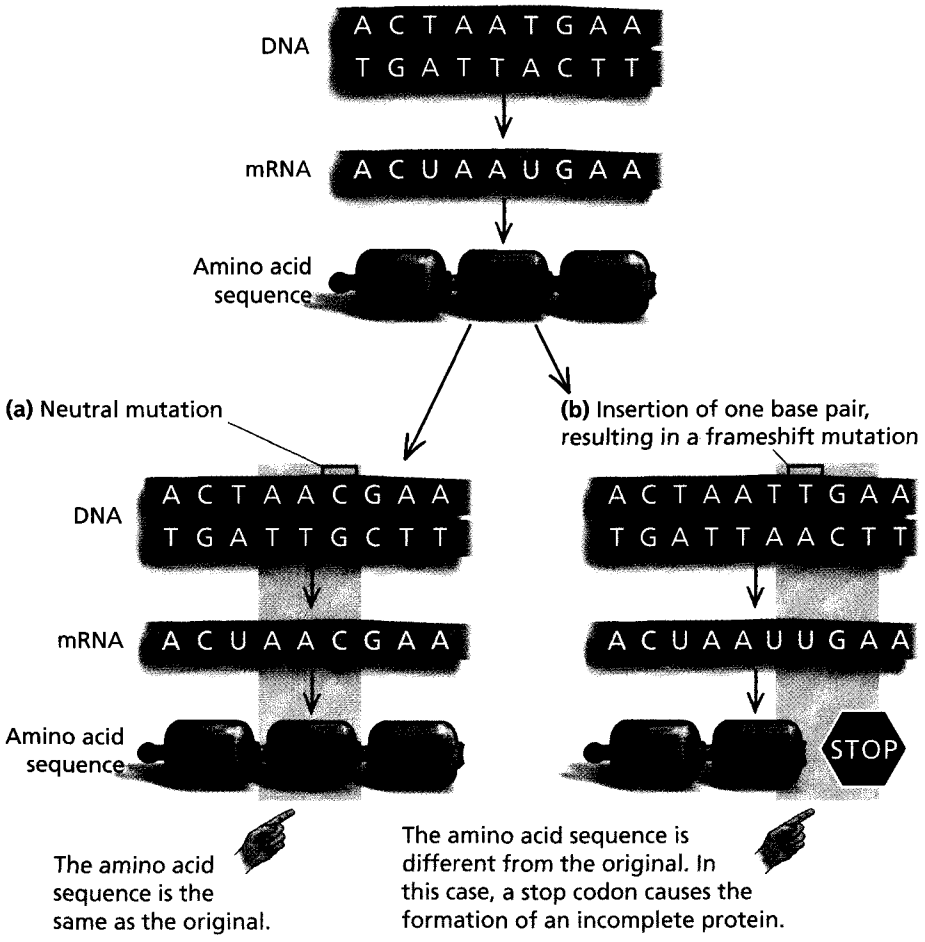


Figure 8.9 Neutral and frameshift mutations. (a) Neutral mutations are changes to the DNA that, due to the redundancy of the genetic code, result in the incorporation of the same amino acid that was originally called for. (b) The insertion (or deletion) of a nucleotide can result in the production of an mRNA that produces the wrong protein or one that terminates translation too soon. This type of mutation is called a frameshift mutation.

measuring spoons and cups (tRNAs) that are dedicated to one specific ingredient. Like the amino acids that are combined in different orders to produce a specific protein, the ingredients in a cake can be used in many ways to produce a variety of foods. The ingredients (amino acids) are always added according to the instructions specified by the original recipe (gene). Changes to the original recipe (mutations) can result in a different or inedible cake being produced.

All cells in all organisms undergo this process of protein synthesis, with different cell types selecting different genes from which to produce proteins. Figure 8.10a shows the coordination of these two processes as they occur in cells with nuclei, that is, eukaryotic cells. In eukaryotic cells, transcription and translation are spatially separate with transcription occurring in the nucleus and translation occurring in the cytoplasm. Cells lacking a membrane-bound nucleus and organelles are called prokaryotic cells. Prokaryotic cells (such as bacterial cells) also undergo protein synthesis, but transcription and translation occur simultaneously in the same location instead of occurring in separate places. As a mRNA is being transcribed, ribosomes attach and begin translating (Figure 8.10b).

Regulating Gene Expression

Different cell types transcribe and translate different genes. Each cell in your body, except sperm or egg cells, has the same complement of genes you inherited from your parents but expresses only a small percentage of those genes. For example, since your liver and pancreas perform a specialized suite of jobs, the cells of your liver turn on or express one suite of genes and the cells of your pancreas, another. Turning a gene on or off, or modulating it more subtly, is called **regulating gene expression**. The expression of a given gene is regulated so that it is turned on and turned off in response to the cell's needs.

Regulation of Transcription. Gene expression is most commonly regulated by controlling the rate of transcription. Regulation of transcription can occur at the promoter, the sequence of nucleotides adjacent to a gene to which the RNA polymerase binds in order to initiate transcription. When a cell requires a particular protein, the RNA polymerase enzyme binds to the promoter for

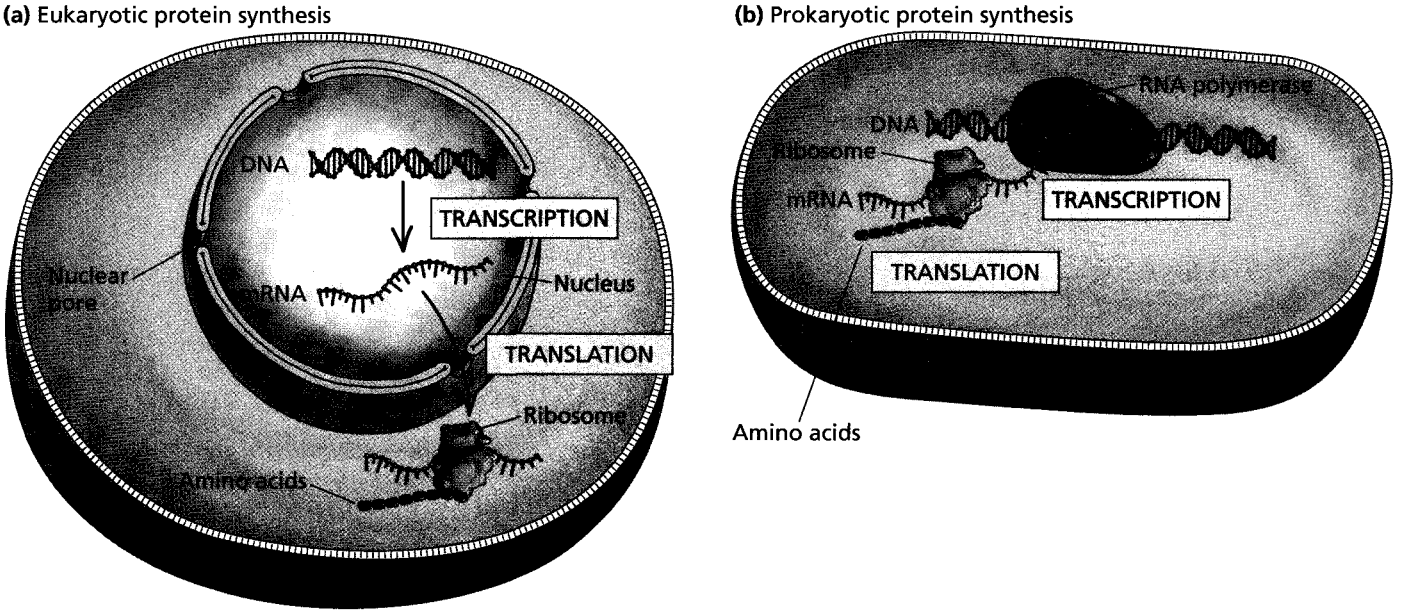


Figure 8.10 Protein synthesis in eukaryotic and prokaryotic cells. (a) In eukaryotes, transcription occurs in the nucleus and translation in the cytoplasm. (b) In prokaryotic cells, which lack nuclei, transcription and translation occur simultaneously in the same location.

that particular gene and transcribes the gene. Prokaryotic and eukaryotic cells both regulate gene expression by regulating transcription but have different strategies for doing so. Prokaryotic cells typically regulate gene expression by blocking transcription via proteins called **repressors** that bind to the promoter and prevent the RNA polymerase from binding. When the gene needs to be expressed, the repressor will be released from the promoter so that the RNA polymerase can bind (Figure 8.11a). This is the main mechanism by which simple single-celled prokaryotes regulate gene expression.

The more complex eukaryotic cells have evolved more complex mechanisms to control gene expression. To control transcription, eukaryotic cells more commonly enhance gene expression using proteins called **activators** that help the RNA polymerase bind to the promoter, thus facilitating gene expression (Figure 8.11b). The rate at which the polymerase binds to the promoter is also affected by substances that are present in the cell. For example, the presence of alcohol in a liver cell might result in increased transcription of a gene involved in the breakdown of alcohol.

Regulation by Chromosome Condensation. It is also possible to regulate gene expression by condensing all or part of a chromosome. This prevents RNA polymerase from being able to access genes. Essay 7.3 outlines how the inactivation of an X chromosome turns off the expression of X-linked genes in organisms that have two X chromosomes. Entire chromosomes are also inactivated when they condense during mitosis.

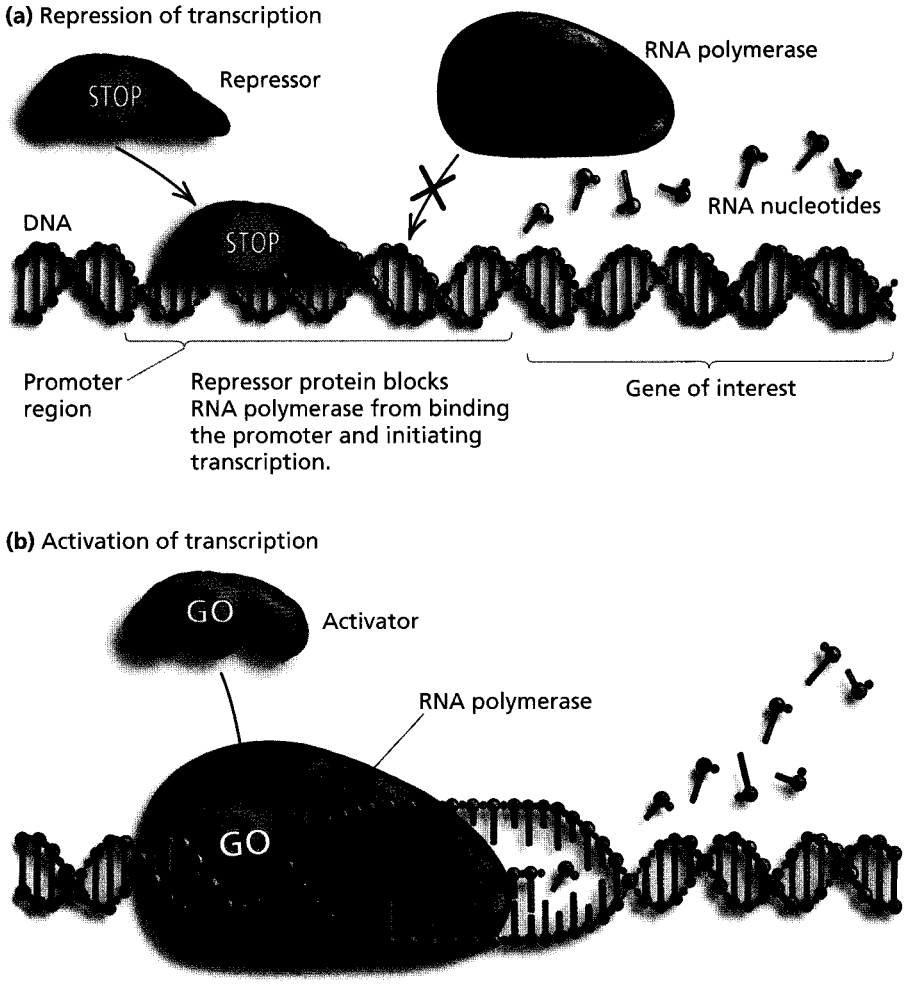


Figure 8.11 Regulation of gene expression. (a) Gene expression can be regulated by (a) repression, during which repressors prevent RNA polymerase from binding the promoter or (b) activation, during which activators help RNA polymerase bind the promoter.

Regulation by mRNA Degradation. Eukaryotic cells can also regulate the expression of a gene by regulating how long a messenger RNA is present in the cytoplasm. Enzymes called **nucleases** roam the cytoplasm, cutting RNA molecules by binding to one end and breaking the bonds between nucleotides. If a particular mRNA has a long "tail," it will survive longer in the cytoplasm and be translated more times. All mRNAs are eventually degraded in this manner; otherwise, once a gene had been transcribed one time, it would be expressed forever.

Regulation of Translation. It is also possible to regulate many of the steps of translation. For example, the binding of the mRNA to the ribosome can be slowed or hastened, as can the movement of the mRNA through the ribosome.

Regulation of Protein Degradation. Once a protein is synthesized, it will persist in the cell for a characteristic amount of time. Like the mRNA that provided the instructions for its synthesis, the life of a protein can be affected by enzymes inside the cell that degrade the protein. Speeding up or slowing down the activities of these enzymes can change the amount of time that a protein is able to be active inside a cell.

The problem of regulating gene expression is easily solved in the case of *r*BGH. Farmers can simply decide how much protein to inject into the bloodstream of a cow.

8.3 Producing Recombinant Proteins

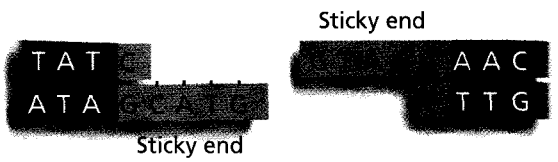
The first step in the production of the *r*BGH protein is to transfer the *BGH* gene from the nucleus of a cow cell into a bacterial cell. Bacteria are single-celled prokaryotes that copy themselves very rapidly. They can thrive in the laboratory if they are allowed to grow in a liquid broth containing the nutrients necessary for survival. Bacteria with the *BGH* gene can serve as factories to produce millions of copies of this gene and its protein product. Making many copies of a gene is called **cloning** the gene.

Cloning a Gene Using Bacteria

The following three steps are involved in moving a *BGH* gene into a bacterial cell (Figure 8.12).

Step 1. Remove the Gene from the Cow Chromosome. The gene is sliced out of the cow chromosome on which it resides by exposing the cow DNA to enzymes that cut DNA. These enzymes, called **restriction enzymes**, act like highly specific molecular scissors. Restriction enzymes cut DNA only at specific sequences, called palindromes, such as:

Note that the bottom middle sequence is the reverse of the top sequence. Many restriction enzymes cut the DNA in a staggered pattern, leaving "sticky ends" such as:



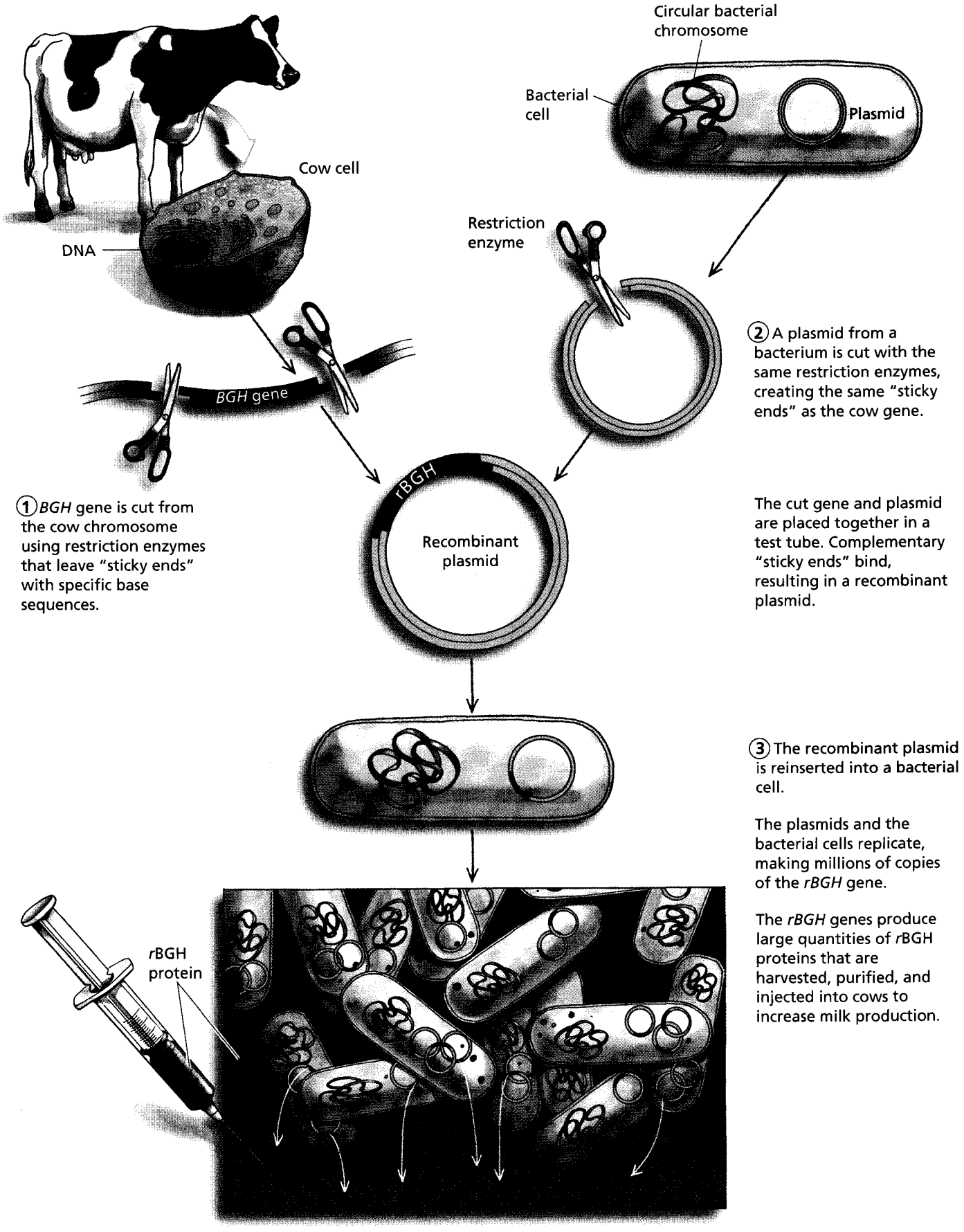


Figure 8.12 Cloning genes using bacteria. Bacteria can be used as factories for the production of human or other animal proteins.

The unpaired bases form bonds with any complementary bases with which they come in contact. The enzyme selected by the scientist cuts on both ends of the *BGH* gene but not inside the gene.

Since different individual restriction enzymes cut DNA only at specific points, scientists need some information about the entire suite of genes present in a particular organism, called the **genome**, to determine which restriction enzyme cutting sites surround the gene of interest. Cutting the DNA generates many different fragments, only one of which will carry the gene of interest.

Step 2. Insert the *BGH* Gene into the Bacterial Plasmid. Once the gene is removed from the cow genome, it is inserted into a bacterial structure called a **plasmid**. A plasmid is a circular piece of DNA that normally exists separate from the bacterial chromosome and can replicate independently of the bacterial chromosome. Think of the plasmid as a ferry that carries the gene into the bacterial cell where it can be replicated. To incorporate the *BGH* gene into the plasmid, the plasmid is also cut with the same restriction enzyme used to cut the gene. Cutting both the plasmid and gene with the same enzyme allows the "sticky ends" that are generated to base-pair with each other (A to T and G to C). When the cut plasmid and the cut gene are placed together in a test tube, they reform into a circular plasmid with the extra gene incorporated.

The bacterial plasmid has now been genetically engineered to carry a cow gene. At this juncture, the *BGH* gene, is referred to as the *rBGH* gene, with the *r* indicating that this product is genetically engineered, or recombinant, because it has been removed from its original location in the cow genome and recombined with the plasmid DNA.

Step 3. Insert the Recombinant Plasmid into a Bacterial Cell. The recombinant plasmid is now inserted into a bacterial cell. Bacteria can be treated so that their cell membranes become porous. When they are placed into a suspension of plasmids, the bacterial cells allow the plasmids back into the cytoplasm of the cell. Once inside the cell, the plasmids replicate themselves, as does the bacterial cell, making thousands of copies of the *rBGH* gene. Using this procedure, scientists can grow large amounts of bacteria capable of producing BGH.

Once scientists successfully clone the *BGH* gene into bacterial cells, the bacteria produce the protein encoded by the gene. Bacteria can be genetically engineered to produce many proteins of importance to humans. For example, bacteria are now used to produce the clotting protein missing from people with hemophilia as well as human insulin for people with diabetes.

Scientists at Monsanto engineered the bacteria so that they could synthesize the *r*BGH protein by placing the growth hormone gene from cows into a bacterial plasmid. The plasmid was placed back into the bacterial cells, which then transcribed the gene and translated the protein. Then the scientists were able to break open the bacterial cells, isolate the BGH protein, and inject it into cows.

Close to one-third of all dairy cows in the United States now undergo daily injections with recombinant bovine growth hormone. These injections increase the volume of milk that each cow produces by around 20%.

Prior to marketing the recombinant protein to dairy farmers, the Monsanto Company had to demonstrate that its product would not be harmful to cows or to humans who consume the cows' milk. This involved obtaining approval from the U.S. Food and Drug Administration (FDA).

FDA Regulations

The FDA is the governmental organization charged with ensuring the safety of all domestic and imported foods and food ingredients (except for meat and poultry, which are regulated by the United States Department of Agriculture). The manufacturer of any new food that is not **generally recognized as safe (GRAS)** must obtain FDA approval before marketing its product. Adding substances to foods also requires FDA approval, unless the additive is GRAS.

According to both the FDA and Monsanto, there is no detectable difference between milk from treated and untreated cows and no way to distinguish between the two. Even if there were increased levels of *r*BGH in the milk of treated cows, there should be no effect on the humans consuming the milk because we drink the milk and do not inject it. Drinking the milk ensures that any protein in it will be digested by the body, just like any other protein that is present in food. Therefore, in 1993, the FDA deemed the milk from *r*BGH-treated cows as safe for human consumption.

In addition, since the milk from treated and untreated cows is indistinguishable, the FDA does not require that milk obtained from *r*BGH-treated cows be labeled in any manner. Vermont is the only state that requires labeling of *r*BGH-treated milk. However, many distributors of milk from untreated cows label their milk as "hormone free," even though there is no evidence of the hormone in milk from treated cows.

It is not unusual that most of this work was performed for a corporation (Monsanto), not a university. There are some fundamental differences between the types of research performed by scientists in industry as compared to the work being done at universities and colleges.

Basic Versus Applied Research

Scientists in academia often seek answers to questions for which there is no profit motive or direct commercial application. This type of research, for which there is not necessarily a commercial application, is called **basic research** and is largely funded by taxpayers through government agencies such as the National Institute of Health (NIH) or the National Science Foundation (NSF). The premise behind basic research is that scientists cannot always predict which kinds of scientific understanding will be valuable to society in the future. For instance, scientists might study transcription or translation simply to better understand these processes. Genetic engineers may spend their entire careers trying to understand the conditions under which a particular protein is synthesized.

Funding for basic research is important because no one knows where the next piece of invaluable information will come from. When scientists first began studying the genes in the single-celled eukaryote, *Saccharomyces cerevisiae* (baker's yeast), they probably had no idea that most of the genes present in this yeast were also present in humans. Today, scientists manipulate the environmental conditions of yeast to better understand how genes are regulated in humans. Likewise, scientists interested in studying the diversity of tropical plants (Chapter 12) did not suspect that their work would assist the development of many pharmaceutical agents, including some anticancer agents.

Scientists in industry typically seek to answer questions that will have an immediate and profitable application, like the production of *r*BGH. This **applied research** is important for scientists in industry because new products and improvements to existing ones increase profitability, which in turn determines the success or failure of the business. One example of applied research that has proven to be very lucrative has been the genetic engineering of crop plants.

8.4 Genetic Engineers Can Modify Foods

Whether you realize it or not, you have probably been eating genetically modified foods for your entire life. Some of these modifications have occurred over the last several thousand years due to farmers' use of selective breeding techniques—breeding those cattle that produce the most milk or crossing crop plants that are easiest to harvest. While this artificial selection does not involve moving a gene from one organism to another, it does change the overall frequency of certain alleles for a gene in the population.

The genetic engineering techniques described earlier have allowed scientists to move genes between organisms. Unless you eat only certified organic foods, you have been eating food that has been modified in this way for some time. This may lead you to wonder why and how plants are genetically modified, whether eating them is bad for your health, and whether growing them is bad for the environment.

Why Genetically Modify Crop Plants?

Crop plants are genetically modified to increase their shelf life, yield, and nutritive value. The first genetically engineered fresh produce, tomatoes, became available in American grocery stores in 1994. These tomatoes were engineered to soften and ripen more slowly. The longer ripening time meant that tomatoes would stay on the vine longer, thus making them taste better. The slower ripening also increased the amount of time that tomatoes could be left on grocery store shelves without becoming overripe and mushy. An enzyme called **pectinase** mediates the ripening process in some produce, including tomatoes. This enzyme breaks down pectin, a naturally occurring substance found in plant cells. When the enzyme pectinase is active, it helps break down the pectin, and the produce softens.

To genetically modify tomatoes, genetic engineers inserted a gene that produces a mRNA transcript complementary to the mRNA produced by the transcription of a pectinase gene. In double-stranded DNA, one of the two strands codes for the protein. The mRNA produced by this template strand is called the **sense** RNA. Transcription of the non-template strand produces a version of the mRNA called the **antisense** strand. When the antisense version of the pectinase gene is transcribed, it produces a mRNA that is complementary to the mRNA normally transcribed from the pectinase gene. When the mRNA from the genetically engineered antisense gene is produced, it pairs with its naturally occurring pectinase mRNA complement. Binding the antisense and sense mRNAs leaves less of the sense pectinase mRNA available for translation. Thus, less of the pectinase enzyme is produced, and ripening occurs more slowly (Figure 8.13).

Increasing the economic return on crop plants by improving yield has been the driving force behind the vast majority of genetic engineering projects. Yield can be increased when plants are engineered to be resistant to pesticides, herbicides, drought, and freezing.

Many people believe that improving farmers' yields may help decrease world hunger problems. Others argue that, since there is already enough food being produced to feed the entire population, it might make more sense to use less technological approaches to feeding the hungry. Significant numbers of people around the world are malnourished or starving, not due to a shortage of food but because access to food is tied to money or land. However, as the population increases, it may become imperative to increase the yield of crop plants in order to feed all of the world's people.

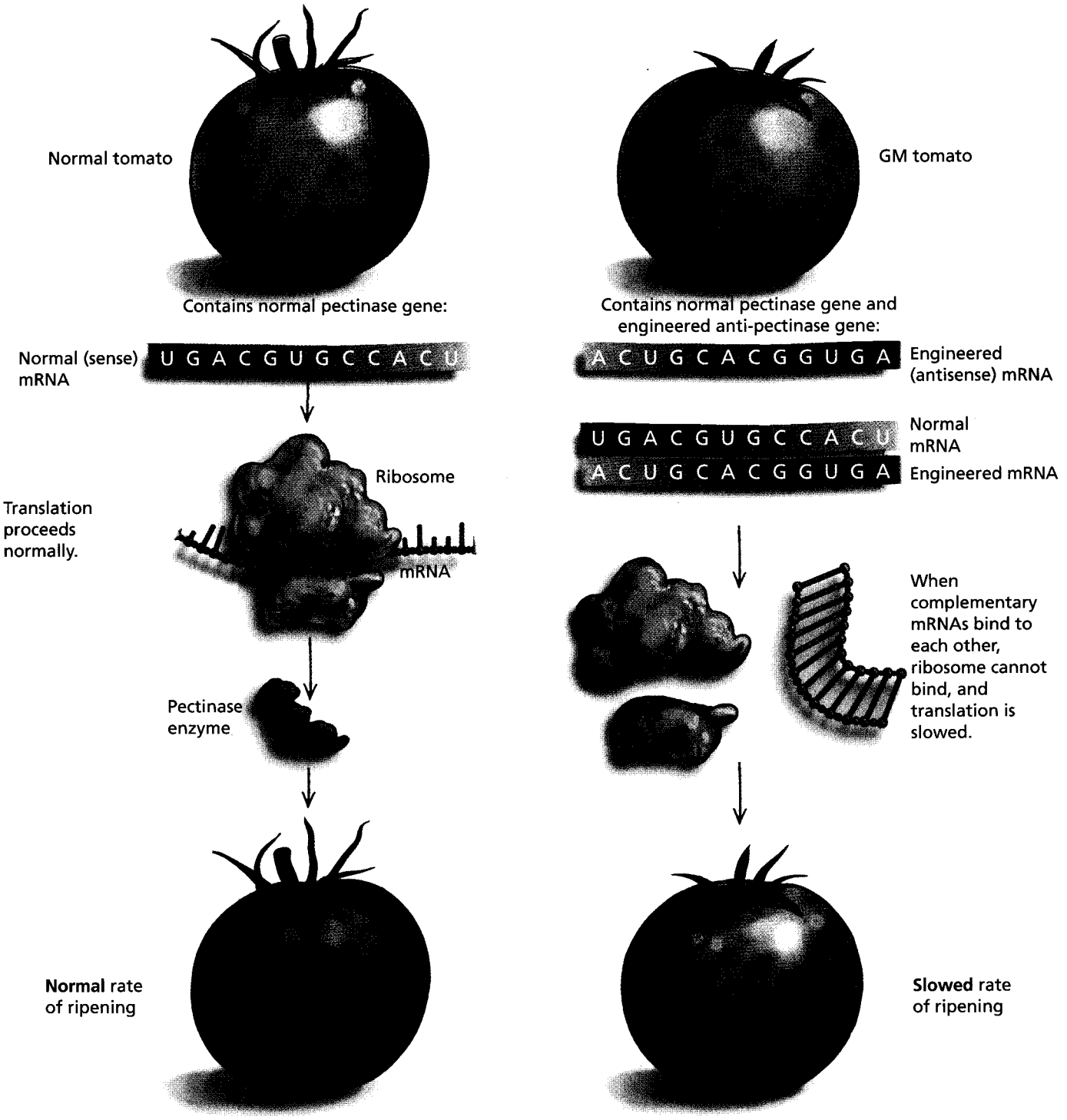


Figure 8.13 Genetically modified tomatoes. Genetically modified tomatoes produce mRNA that decreases the effects of the pectinase enzyme. When the pectinase gene is transcribed and translated, ripening occurs. When the sense pectinase mRNA is bound to the engineered antisense version, translation occurs more slowly, and ripening is slowed.

Genetic engineers may also be able to increase the nutritive value of crops. Some genetic engineers have increased the amount of beta-carotene in rice, a staple food for many of the world's people. Scientists hope that the engineered rice will help decrease the number of people who become blind in underdeveloped nations because cells require beta-carotene in order to synthesize vitamin A, a vitamin required for proper vision. Therefore, eating this genetically modified rice, called golden rice, increases a person's ability to

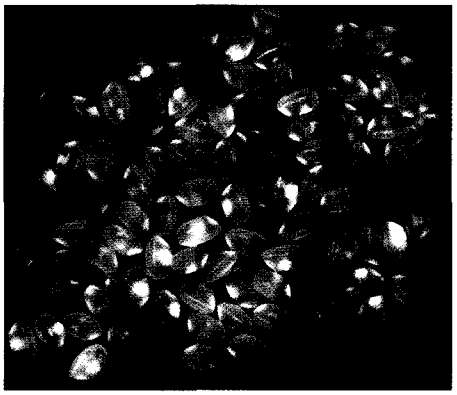


Figure 8.14 Golden rice. Golden rice has been genetically engineered to produce more β-carotene. The increased concentration of β-carotene makes the rice look more gold in color than unmodified rice does.

synthesize vitamin A (Figure 8.14). However, golden rice is not yet approved for human consumption, and there is debate about how effective the rice will actually be in preventing blindness.

Modifying Crop Plants with the Ti Plasmid and Gene Gun

To modify crop plants, the gene must be able to gain access to the plant cell, which means it must be able to move through the plant's rigid, outer cell wall. One "ferry" for moving genes into flowering plants is a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens*. In nature, this bacterium infects plants and causes tumors called **galls** (Figure 8.15a). The tumors are induced by a plasmid, called **Ti plasmid** (for tumor-inducing).

Genes from different organisms can be inserted into the Ti plasmid by (1) using the same restriction enzyme to cut the Ti plasmid and the gene, resulting in identical "sticky ends"; (2) connecting the gene and plasmid together; and (3) reinserting the recombinant plasmid into a bacterium. The bacterium, *A. tumefaciens*, with the recombinant Ti plasmid, is then used to infect plant cells. During infection, the recombinant plasmid is transferred into the host-plant cell (Figure 8.15b). For genetic engineering purposes, scientists use only the portion of a plasmid that does not cause tumor formation.

Moving genes into other agricultural crops such as corn, barley, and rice can also be accomplished by using a device called a **gene gun**. A gene gun shoots metal-coated pellets covered with foreign DNA into plant cells (Figure 8.16). A small percentage of these genes may be incorporated into the plant's genome. The gene gun is often used by companies that do not want to pay licensing fees to Monsanto, holder of the *A. tumefaciens* patent.

When a gene from one organism is incorporated into the genome of another organism, a **transgenic organism** is produced. A transgenic organism is more commonly referred to as a **genetically modified organism (GMO)**.

Many people have raised concerns about genetically modified (GM) crop plants. One concern is that large corporations that own many farms, called **agribusiness** corporations, are profiting so much from GM crop production that they will put owners of family farms out of business. Other concerns focus on the impact of GMOs on human health and the environment (Figure 8.17).

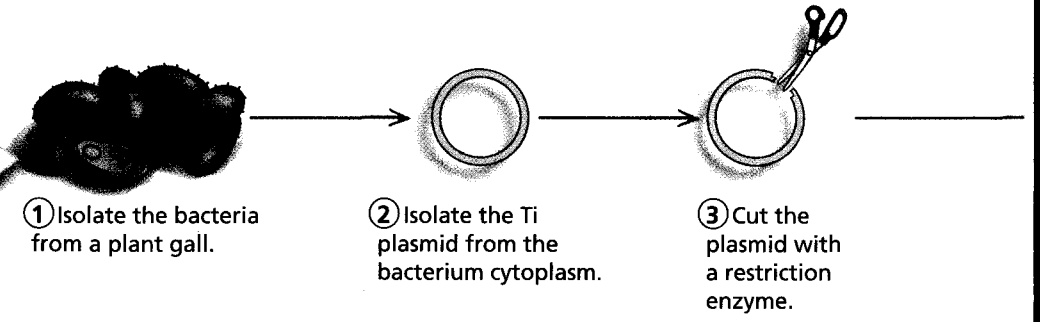
Effect of GMOs on Health

Concerns about the potential negative health effects of consuming GM crops have led some citizens to fight for legislation requiring that genetically modified foods be labeled, enabling consumers to make informed decisions about

Figure 8.15 Genetically modifying plants using the Ti plasmid. (a) Plants infected by *Agrobacterium tumefaciens* in nature show evidence of the infection by producing tumorous galls. (b) The Ti plasmid from *A. tumefaciens* serves as a shuttle for incorporating genes into plant cells. The recombinant plasmid is then used to infect developing plant cells, producing a genetically modified plant. When the plant cell reproduces, it may pass on the engineered gene to its offspring.

(a) Gall caused by *A tumefaciens*

(b) Using the Ti plasmid



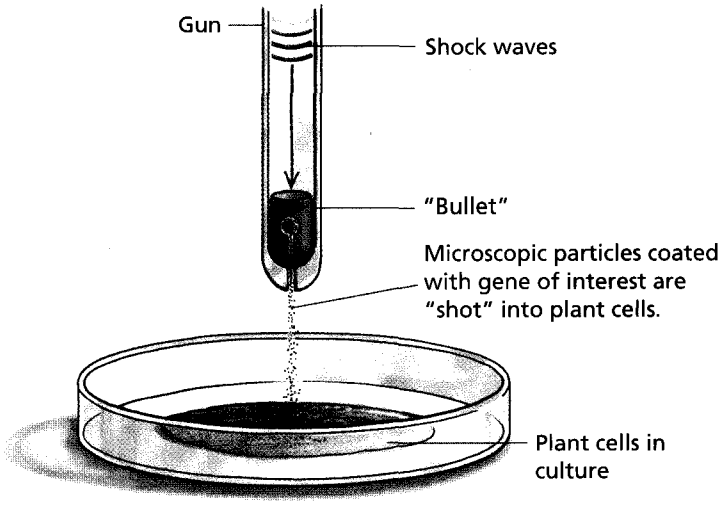


Figure 8.16 Genetically modifying plants using a gene gun. A gene gun shoots a plastic bullet loaded with tiny metal pellets (coated with DNA) into a plant cell. The bullet shells are prevented from leaving the gun, but the DNA-covered pellets penetrate the cell wall, cell membrane, and nuclear membrane of some cells.

the foods they eat. Manufacturers of GM crops argue that labeling foods is expensive and will be viewed by consumers as a warning, even in the absence of any proven risk. Those manufacturers believe that GM food labeling will decrease sales and curtail further innovation.

Genetically Modified Foods in the U.S. Diet. As the labeling controversy rages, most of us are already eating GM foods. Scientists estimate that over half of all foods in U.S. markets contain at least small amounts of GM foods. Twelve different GM plants have been approved for production in the United States. Over 80% of all soybeans grown in the United States are genetically modified for herbicide resistance. Soybean-based ingredients, including oil and flour, are often produced from genetically modified plants and comprise one or more ingredients in many different processed foods.

Close to 40% of the U.S. corn crop is genetically modified to produce its own pesticide against caterpillar pests. Because GM corn is not separated from non-GM corn by farmers or food processors and because many processed food ingredients are corn-based including corn starch and corn syrup, GM corn is thought to be present in most of our processed foods. The percentage of fresh corn that is GM is thought to be closer to 4%.

Most of the canola oil in the United States is extracted from GM rapeseed plants, which are engineered for herbicide resistance. Canola oil is used in many different products, including vegetable oil, salad dressing, margarine, fried foods, chips, cookies, and pastries.

Figure 8.17 Protesters at a World Trade Organization meeting in Seattle. These people are concerned about how GMOs may affect humans and the environment.

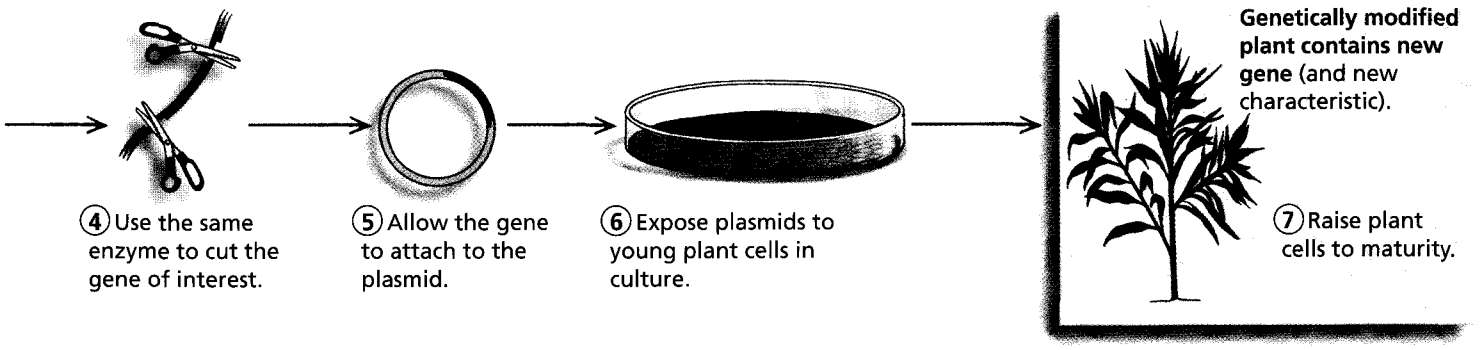


Figure 8.18 "No GMO" labelling. Many food manufacturers and consumers consider the use of unmodified foods to be a selling point for their products.

Genetically modified cotton varieties resistant to caterpillars now account for over 70% of the cotton crop. While cotton is more often used for clothing than for foods, cottonseed oil is used in cooking oils, salad dressing, peanut butter, chips, crackers, and cookies.

Of the 12 different GM plants approved for production, 8 are not commonly grown. Very few farmers are growing GM potatoes, squash, papaya, tomato, sugar beets, rice, flax, and radicchio, likely in response to consumer fears about the health consequences of eating these foods. Products that do not contain GMOs are often labeled to promote that fact (Figure 8.18).

How Are GM Foods Evaluated for Safety? Genetically modified crop plants must be approved by the Environmental Protection Agency (EPA) prior to their release into the environment. The FDA becomes involved in testing the GM crop only when the food from which the gene comes has never been tested, or when there is reason to be concerned that the newly inserted gene may encode a protein that will prove to be a toxin or an allergen.

Allergy is a serious problem for the close to 8% of Americans who experience allergic reactions to foods. Symptoms of food allergy range from a mild upset stomach to sudden death. Genetic engineers must be vigilant about testing foods with known allergens; a person who knows he must avoid eating peanuts may not know to avoid a food that has been genetically modified to contain a peanut gene that may cause a reaction—although no such food currently exists.

If the gene being shuffled from one organism to another is not known to be toxic or cause an allergic reaction, the FDA considers the GM food to be substantially equivalent to the foods from which it was derived; that is, the GM food is GRAS. If a modified crop contains a gene derived from a food that has been shown to cause a toxic or allergic reaction in humans, then it must undergo testing prior to being marketed.

This method of determining potential hazards worked well in the case of a modified soybean that carried a gene from the Brazil nut. This engineering was done in an effort to increase the protein content of soybeans. Since Brazil nuts were known to cause allergic reactions in some people, the modified beans were tested and did indeed cause an allergic reaction in susceptible people. The product was withdrawn, and no one was harmed.

Proponents of genetic engineering cite this as an example of the efficacy of the FDA rules. Opponents of genetically modifying foods wonder whether it will always be possible to predict which foods to test. They point out that it is possible for a protein encoded by a gene with one apparent function to interact with substances in its new environment in unpredictable ways and cause unpredictable effects. For example, those proteins that do not normally cause allergic reactions may be modified in a manner that transforms them into allergens. If a protein originally produced by bacteria (which do not modify proteins in the same manner as plants or other eukaryotic cells do) were inserted into plant cells, the plant cell could modify the protein in such a way that the protein becomes an allergen.

In evaluating toxicity, scientists focus on the protein produced by the modified plant and not the actual gene that is inserted. This is because the gene itself is digested and broken down into its component nucleotides when it is eaten and therefore will not be transcribed and translated inside human cells.

Many plants contain low levels of natural plant toxins. Because plants cannot defend themselves against predation by moving away or physically resisting, they have evolved to rely on these chemical defenses. In fact, the leaves and roots of many plant species are not edible due to the presence of these toxins. When early farmers domesticated plants, selective breeding led to the production of crop plants with reduced levels of toxins. This means that the plants we eat today have a much lower concentration of toxins. This is also part of the reason that modern plants are so susceptible to disease. If an inserted gene were to disrupt the regulation of a toxin gene whose activities had been diminished by selective breeding, it might increase the production of the toxin.

Concern about GM foods is not limited to their consumption. Many people are also concerned about the effects of GM crop plants on the environment.

GM Crops and the Environment

Many genetically modified crops have been engineered in order to increase their yield. For centuries, farmers have tried to increase yields by killing the pests that damage crops and by controlling the growth of weeds that compete for nutrients, rain, and sunlight. In the United States, farmers typically spray chemical pesticides and herbicides directly onto their fields. This practice concerns people worried about the health effects of eating foods that have been treated by these often toxic or cancer-causing chemicals. In addition, both pesticides and herbicides may leach through the soil and contaminate drinking water.

To help decrease farmers' reliance on pesticides, agribusiness companies have engineered plants that are genetically resistant to pests. For example, corn plants have been engineered to kill the European corn borer (Figure 8.19a). To do this, scientists transferred a gene that produces a toxin from the soil bacterium *Bacillus thuringiensis* (Bt) into corn. The *Bt* toxin gene encodes a protein that is lethal to corn borers but not to humans (Figure 8.19b). The idea of using this bacterium for pest control actually came from organic farmers, who have sprayed unengineered *B. thuringiensis* on crop plants for many years. Genetically modified Bt corn has proven to be so successful at resisting the corn borer that close to one-half of all corn currently grown in the United States is engineered with this gene.

Effects on Nontarget Organisms. Shortly after the arrival of Bt corn, concern arose about its impact on organisms in the surrounding areas that are not pests—that is, nontarget organisms. One laboratory study showed that milkweed, a plant commonly found on the edges of cornfields that had been dusted

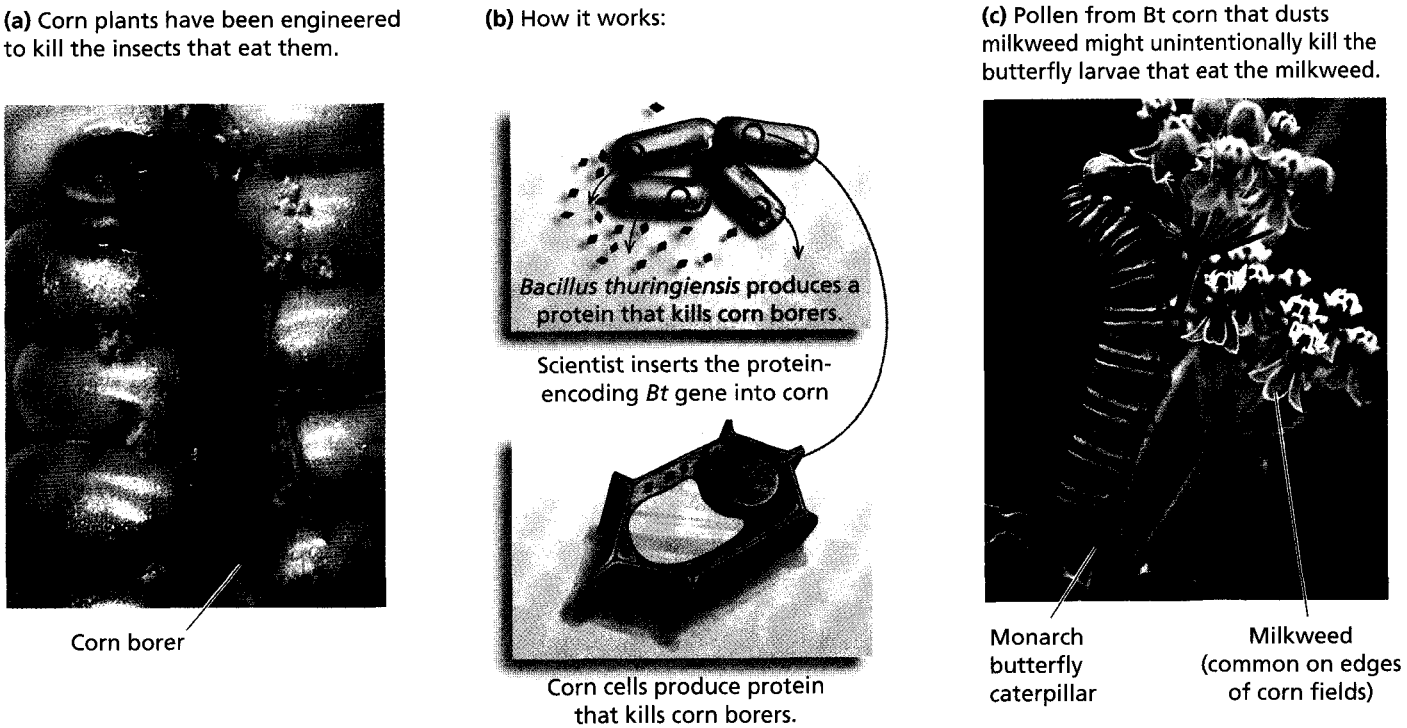


Figure 8.19 The European corn borer. (a) The European corn borer damages corn and decreases yields. (b) Genetic modification of corn plants provides resistance to the pest. (c) Some researchers have shown that Bt corn may be harmful to other organisms.

with pollen from Bt corn, was lethal to monarch butterfly caterpillars, for which milkweed is the only source of food (Figure 8.19c). This research was performed in a laboratory and has not been shown to occur on farmers' fields, but results of this study indicate there may be cause for concern about how GM crops will affect other organisms.

Modified corn also caused controversy in 2000 when a variety of corn called StarLink™ was found in Taco Bell® taco shells. StarLink, containing a modified gene that was resistant to heat and did not break down during digestion, had not been approved by the EPA for human consumption. As a result, there was a massive recall of the taco shells as well as numerous other cornmeal-based products. This incident raised serious concerns about the ability of regulators, farmers, and food processors to keep unapproved GM products out of the nation's food supply.

Evolution of Resistant Pests. Critics of Bt corn point out that it is only a matter of time before corn borers evolve resistance to Bt corn. Corn borers with genetic variations that give them a preexisting resistance to the toxin will be more likely to survive and pass on their resistance genes, creating a population of resistant insects. This in turn will require the development of new varieties of genetically engineered corn. The same is true for pesticides applied to crops; pests evolve resistance because application of a pesticide does not always kill all of the targeted organisms. The few pests that have preexisting resistance genes and are not susceptible survive and produce resistant offspring. Eventually, widespread resistance develops, and a new pesticide must be developed and applied.

The problem of accelerated evolution of *Bt* resistance is particularly vexing for the organic farmers, who were the first to use *B. thuringiensis* for controlling the corn borer but who did so in a targeted way. If corn borers develop resistance to *Bt* toxin due to widespread use of Bt corn, organic farmers will have lost a powerful tool for controlling this pest.

The continued need for the development of new pesticides in farming is paralleled by farmers' reliance on herbicides. Herbicide-resistant crop plants, such as Roundup Ready® soybeans, have been engineered to be resistant to Roundup® herbicide, used to control weeds in soybean fields. Farmers can now spray their fields of genetically engineered soybeans with herbicides that will kill everything but the crop plant. Some people worry that this resistance gene will allow farmers to spray more herbicide on their crops since there is no chance of killing the GM plant, thereby exposing consumers and the environment to even more herbicide.

Transfer of Genetic Material. There is also concern that GM crop plants may transfer engineered genes from modified crop plants to their wild or weedy relatives. Wind, rain, birds, and bees carry genetically modified pollen to related plants near fields containing GM crops (or even to farms where no GM crops are being grown). Many cultivated crops have retained the ability to interbreed with their wild relatives; in these cases, genes from farm crops can mix with genes from the wild crops. This is unlikely to happen with corn or soybeans, which do not have weedy relatives in North America. However, it has already been demonstrated that GM canola has transferred genes to its weedy relatives, and the same is likely to happen with squash and rice. Thus the herbicide is rendered ineffective since both the crop plant and its weedy relative share the same resistance gene. It may become impossible to determine whether weed plants surrounding fields of engineered crops have been pollinated with pollen containing the modified gene, and there could be unintended consequences for the ecology of the surrounding environment. Also, if pollen from GM crop plants drifts to farms that are not growing modified crops, it becomes impossible to determine whether a crop plant has engineered genes. This would be disastrous in the event of a recall of the genetically modified seed.

Additional Problems. Genetic manipulation could lead to decreasing variation within a species, and this too can have evolutionary consequences. Most GM corn, in addition to carrying the *Bt* resistance gene, has also been selectively bred to mature all at once, produce uniform ears, and have a particular nutrient profile. Because GM varieties do increase production or reduce the cost of inputs, they have often become extremely popular, meaning that most of the nation's corn and soybean crops are nearly genetically identical. If an unforeseen disease or pest were to sweep through an area containing this corn or soybean variety, the disease would probably devastate a large portion of the crop.

Most, but not all, of the genetic engineering that occurs to produce crop plants resistant to pesticides and herbicides is performed by private companies and is designed to maximize profits. For example, Roundup Ready soybeans are purchased by farmers who then apply Roundup herbicide; both the GM soybean and the herbicide are sold by Monsanto. Crops engineered for a more altruistic purpose, such as the golden rice described earlier, were developed in academic research centers and thus far have not proved financially viable for profit-making companies. Someday the techniques pioneered by agribusiness firms may be used to help solve the problem of world hunger and disease, but this has not been the case to date.

While there is hope that genetic engineers will be able to help solve hunger problems by making farming more productive, there are also concerns about any negative health and environmental effects of GM foods. It remains to be seen whether genetic engineering will constitute a lasting improvement to agriculture.

8.5 Genetic Engineers Can Modify Humans

Some genetic engineers are attempting to modify humans. These modifications may one day include replacing defective or nonfunctional alleles of a gene with a functional copy of the gene. If this happens, it might be possible for physicians to diagnose genetic defects in early embryos and fix them, allowing the embryo to develop into an adult without any genetic diseases. Recent developments that have helped scientists to better understand the human genome may make this scenario more likely.

The Human Genome Project

The **Human Genome Project** involves sequencing or determining the nucleotide-base sequence (A, C, G, or T) of the entire human genome and the location of each of the 20,000 to 25,000 human genes. In 1990, the Office of Health and Environmental Research of the U.S. Department of Energy (DOE), along with the National Institute of Health (NIH) and scientists from around the world, undertook this project. At the time, scientists involved in the project proposed to have a complete accounting of all the genes present in humans by the year 2005. However, the race to complete the sequencing of the human genome was drastically accelerated by technological advances and the involvement of a private company named Celera Genomics™. At stake were the rights to patent the gene sequences. Initially, Celera wanted to retain the rights to the DNA sequences, but government scientists were making sequences available to the public. Eventually, the two groups worked together to publish the entire sequence of billions of nucleotide pairs that comprise the human genome in 2003.

The scientists involved in this multinational effort also sequenced the genomes of the mouse, the fruit fly, a roundworm, bakers' yeast, and a common intestinal bacterium named *E. coli*. Scientists thought it was important to sequence the genomes of organisms other than humans because these **model organisms** are easy to manipulate in genetic studies and because important

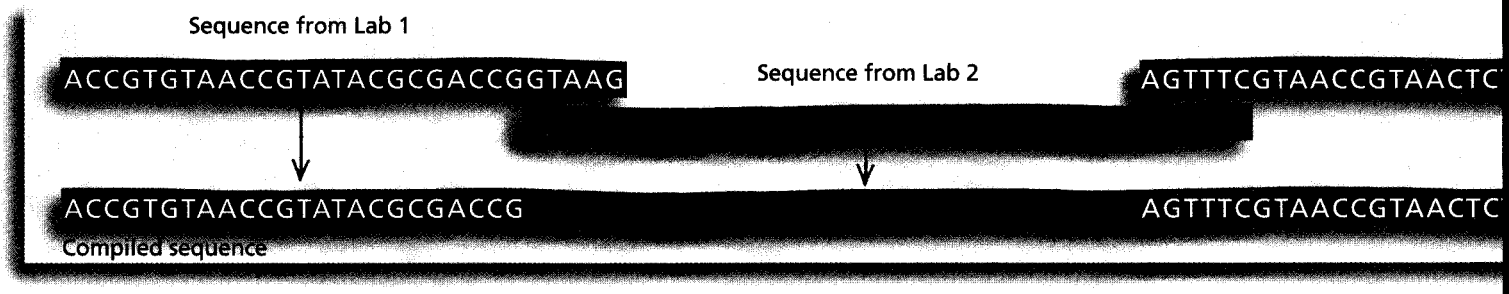


Figure 8.20 Chromosome walking. Scientists can determine the location of genes on chromosomes using DNA sequence information uploaded to the Internet from laboratories around the world. Workers search for areas that overlap and fill in the gaps, much like assembling a jigsaw puzzle.

genes are often found in many different organisms. In fact, 90% of human genes are also present in mice; 50% are in fruit flies, and 31% are in bakers' yeast. Therefore, understanding how a certain gene functions in a model organism helps us understand how the same gene functions in humans.

To sequence the human genome, scientists first isolated DNA from white blood cells. They then cleaved the chromosomes into more manageable sizes using restriction enzymes, cloned them into plasmids, and determined the base sequence using automated DNA sequencers. These sequencing machines distinguish between nucleotides based on structural differences in the nitrogenous bases. Sequence information was then uploaded to the Internet. Scientists working on this, or any other project, could search for regions of sequence information that overlapped with known sequences. Using overlapping regions, scientists in laboratories all over the world worked together to patch together DNA sequence information. In this manner, scientists sequenced entire chromosomes by "walking" from one end of a chromosome to the other (Figure 8.20). DNA sequence information obtained by means of the Human Genome Project may someday enable medical doctors to take blood samples from patients and determine which genetic diseases are likely to affect them.

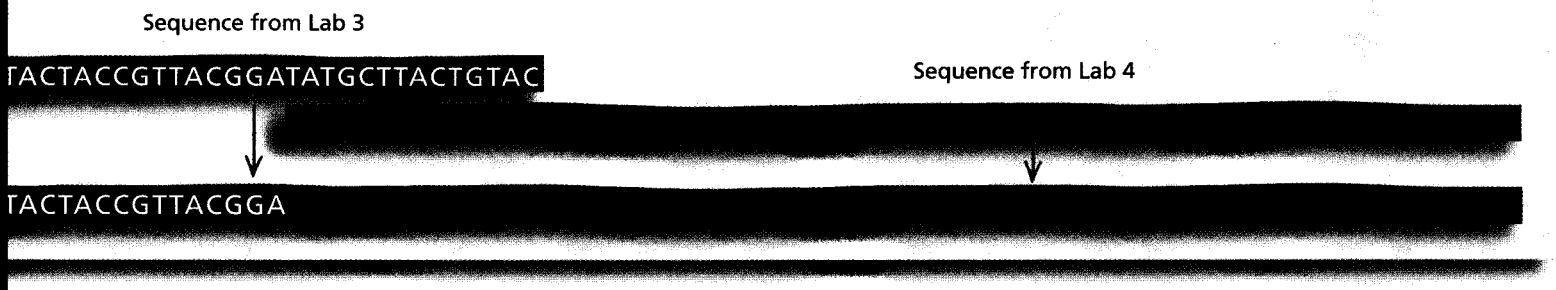
Many people worry about having these types of tests performed because this personal information may get back to their insurance companies or employers, but there is a positive side to having all of this information available. Once the genetic basis of a disease has been worked out—that is, how the gene of a healthy person differs from the gene of a person with a genetic disease—the information can be used to develop treatments or cures.

Gene Therapy

Scientists who try to replace defective human genes (or their protein products) with functional genes are performing **gene therapy**. Gene therapy may someday enable scientists to fix genetic diseases in an embryo. To do so, the scientists would supply the embryo with a normal version of a defective gene; this so-called **germ-line gene therapy** would ensure that the embryo and any cells produced by cell division would replicate the new, functional version of the gene. Thus, most of the cells would have the corrected version of the gene, and when these genetically modified individuals have children, they will pass on the corrected version of the gene. If scientists can fix genetic defects in early embryos, some genetic diseases can be prevented.

Another type of gene therapy, called **somatic cell gene therapy**, can be performed on body cells to fix or replace the defective protein in only the affected cells. Using this method, scientists introduce a functional version of a defective gene into an affected individual cell in the laboratory, allow the cell to reproduce, and then place the copies of the cell bearing the corrected gene into the diseased person.

This treatment may seem like science fiction, but it is likely that this method of treating genetic diseases will be considered a normal procedure in the not-too-distant future. In fact, genetic engineers already have successfully treated a genetic disorder called **severe combined immunodeficiency (SCID)**, a disease caused



by a genetic mutation that results in the absence of an important enzyme and severely weakens the individual's immune system. Because their immune systems are compromised, people with SCID are incapable of fighting off any infection, and they often suffer severe brain damage from the high temperatures associated with unabated infection. Any exposure to infection can kill or disable someone with SCID, so most patients must stay inside their homes and often live inside protective bubbles that separate them from everyone, even family members.

To devise a successful treatment for SCID, or any disease treated with gene therapy, scientists had to overcome a major obstacle—getting the therapeutic gene to the right place.

Proteins break down easily and are difficult to deliver to the proper cells, so it is more effective to replace a defective gene than to continually replace a defective protein. Delivering a normal copy of a defective gene only to the cell type that requires it is a difficult task. SCID, a disorder that has been treated successfully, was chosen by early gene therapists in part because defective immune system cells could be removed from the body, treated, and returned to the body.

Immune system cells that require the enzyme missing in SCID patients circulate in the bloodstream. Blood removed from a child with SCID is infected with nonpathogenic (non-disease-causing) versions of a virus. This virus is first engineered to carry a normal copy of the defective gene in SCID patients. After the immune system cells are infected with the virus, these recombinant cells, which now bear copies of the functional gene, are returned to the SCID patient (Figure 8.21a).

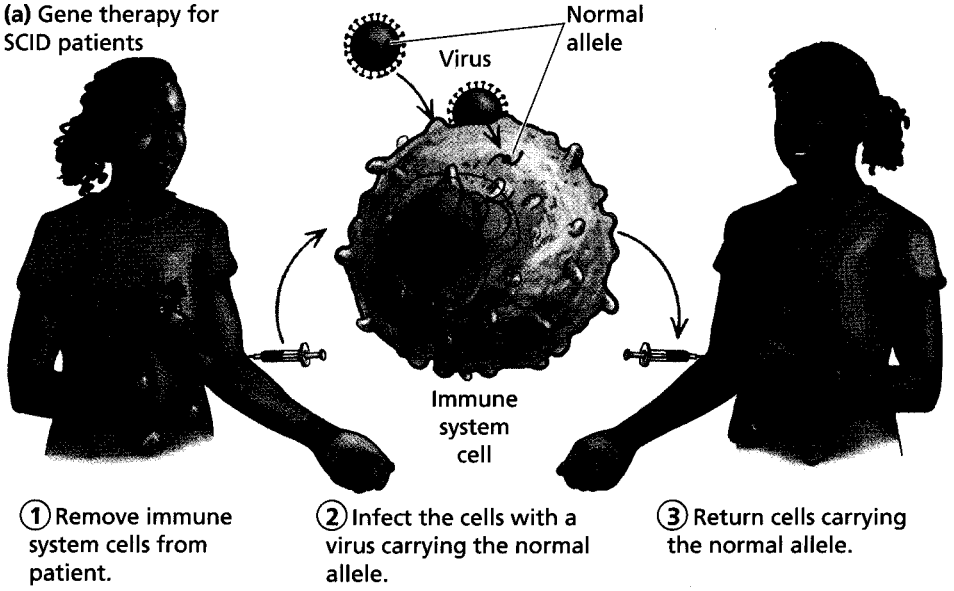


Figure 8.21 Gene therapy in a SCID patient. (a) A virus carrying the normal gene is allowed to infect immune system cells that have been removed from a person with SCID. The virus inserts the normal copy of the gene into some of the cells, and these cells are then injected into the SCID patient. (b) Ashi DiSilva, the first gene therapy patient.

In 1990, a 4-year-old girl named Ashi DiSilva (Figure 8.21b) was the first patient to receive gene therapy for SCID. Ashi's parents were willing to face the unknown risks to their daughter because they were already far too familiar with the risks of SCID—the couple's two other children also had SCID and were severely disabled. Ashi is now a healthy adult with an immune system that is able to fight off most infections.

However, Ashi must continue to receive treatments because blood cells, whether genetically engineered or not, have limited life spans. When most of Ashi's engineered blood cells have broken down, she must be treated again; thus, she undergoes this gene therapy a few times each year. Since Ashi's gene therapy turned out well, many other SCID patients have been successfully treated and can live normal lives. Unfortunately, Ashi's gene therapy does not prevent her from passing on the defective allele to her biological children because this therapy is not "fixing" the allele in her ovaries.

Although things worked out well for Ashi, successful gene therapy is far from routine. Two of 11 French boys treated with gene therapy for SCID developed leukemia that is thought to be related to their treatment, and an American teenager died from complications of experimental gene therapy meant to cure his relatively mild genetic disorder.

In addition to the risks involved in conducting any experimental therapy, not many genetic diseases can be treated with gene therapy. Gene therapy to date has focused on diseases caused by single genes for which defective cells can be removed from the body, treated, and reintroduced to the body. Most genetic diseases are caused by many genes, affect cells that cannot be removed and replaced, and are influenced by the environment.

Most people support the research of genetic engineers in their attempts to find better methods for delivering gene sequences to the required locations and for regulating the genes once they are in place. A far more controversial type of genetic engineering involves making an exact copy of an entire organism by a process called **cloning**.

Cloning Humans

Human cloning occurs commonly in nature via the spontaneous production of identical twins. These clones arise when an embryo subdivides itself into two separate embryos early in development. This is not the type of cloning that many people find objectionable; people are more likely to be upset by cloning that involves selecting which traits an individual will possess. Natural cloning of an early embryo to make identical twins does not allow any more selection for specific traits than does fertilization. However, in the future it may be possible to select adult humans who possess desired traits and clone them. Since cloning does not actually alter an individual's genes, it is more of a reproductive technology than a genetic engineering technology. However, it may someday be possible to alter the genes of a cloned embryo.

Cloning offspring from adults with desirable traits has been successfully performed on cattle, goats, mice, cats, pigs, rabbits, and sheep. In fact, the animal that brought cloning to the attention of the public was a ewe named Dolly.

Dolly was cloned when Scottish scientists took cells from the mammary gland of an adult female sheep and fused it with an egg cell that had previously had its nucleus removed. Treated egg cells were then placed in the uterus of an adult ewe that had been hormonally treated to support a pregnancy. Scientists had to try many times before this **nuclear transfer** technique worked. In all, 277 embryos were constructed before one was able to develop into a live lamb (Figure 8.22). Dolly was born in 1997.

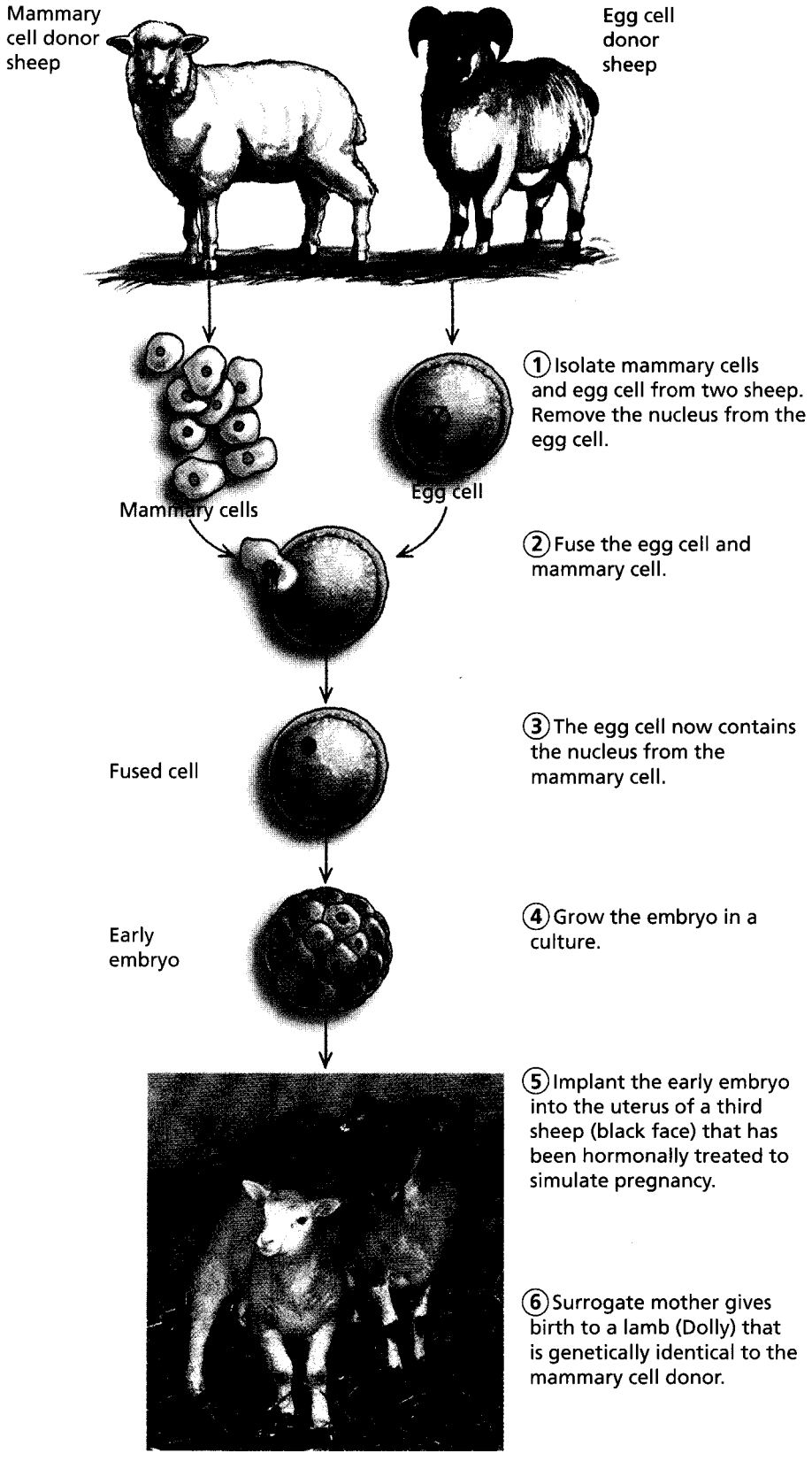


Figure 8.22 Dolly. This sheep is the result of a successful attempt to clone a mammal. She was genetically identical to her cell-donor mother.

The research that led to Dolly's birth was designed to provide a method of ensuring that cloned livestock would have the genetic traits that made them most beneficial to farmers. Sheep that produced the most high-quality wool and cattle that produced the best beef would be cloned.

Essay 8.1 Stem Cells

Genetic engineers in some laboratories are trying to harness the healing powers of human stem cells. These cells, unlike most of the cells in your body, do not perform a specific function; instead, they are able to produce many different kinds of cells and tissues. Because stem cells do not have a particular function, they are said to be **undifferentiated**. Although they are undifferentiated, they can be pressed into service as many different cell types. Imagine that you are remodeling an old home, and you have a type of material that you can mold into anything you might need for the remodeling job—brick, tile, pipe, plaster, and so forth. Having a supply of this material would help you fix many different kinds of damage. Scientists believe that stem cells may serve as this type of all-purpose repair material in the body. If cells are nudged in a particular developmental direction in the laboratory, they can be directed to become a particular tissue or organ. Using stem cells from early embryos to produce healthy tissues as replacements for damaged tissues is called therapeutic cloning. Tissues and organs grown from stem cells in the laboratory may someday be used to replace organs damaged in accidents or organs that are gradually failing due to **degenerative diseases**. Degenerative diseases start with the slow breakdown of an organ and progress to organ failure. Additionally, when one organ is not working properly, other organs are affected. Degenerative diseases include diabetes, liver and lung diseases, heart disease, and Alzheimer's disease.

Stem cells could provide healthy tissue to replace those tissues damaged by spinal cord injury or burns. New heart muscle could be produced to replace muscle damaged during a heart attack. A diabetic could have a new pancreas, and people suffering from some types of arthritis could have replacement cartilage to cushion their joints. Thousands of people waiting for organ transplants might be saved if new organs were grown in the lab.

Stem cells are usually isolated from early embryos that are left over after fertility treatments. **In vitro** (Latin, meaning "in glass") fertilization procedures often result in the production of excess embryos because many egg cells are harvested from a woman who wishes to become pregnant. These egg cells are then mixed with her partner's sperm in a petri dish, resulting in the production of many fertilized eggs that grow into embryos. A few of the embryos are then implanted into the woman's uterus. The remaining embryos are stored so that more attempts can be made if pregnancy does not result or if the couple desires more children. When the couple achieves the desired number of pregnancies, the remaining embryos are discarded or, with the couple's consent, used for stem-cell research.

Early embryonic cells are harvested because stem cells are **totipotent** directly after fertilization; in other words, these stem cells can become any other cell type (Figure E8.1). As the embryo develops, its cells become less and less able to produce other cell types. As a human embryo grows, the early cells start dividing and forming different, specialized cells such as heart cells, bone cells, and muscle cells. Once formed, specialized nonstem cells can divide only to produce replicas of themselves. They cannot backtrack and become a different type of cell.

There is, however, a small supply of stem cells present in adult tissues, probably so that these tissues can repair themselves. Though stem cells exist in adult tissue, they are not present in great numbers, so they can be hard to find and extract for growth. They do not have the limitless replicative potential of embryonic stem cells, making it hard to grow them in large batches. Also, their ability to be transformed into different cell types is limited, making them less useful than embryonic stem cells.

Although embryonic stem cells are easier to work with and have more powerful healing potential than adult stem cells, laws in the United States restrict government funding for embryonic stem cell research. Embryos are destroyed when stem cells are harvested from them—a result that many find objectionable. In 2001, President Bush signed an executive order to ban scientists from using government money for studies involving human embryonic stem cells, unless those cells were created before the 2001 ban.

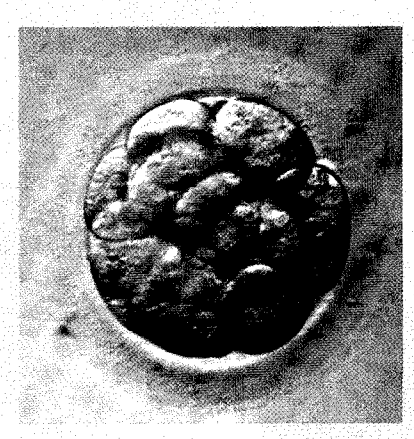


Figure E8.1 Early human embryo in a petri dish.

This technique is more efficient than allowing two prize animals to breed because each animal gives only half of its genes to the offspring. There is no guarantee that the offspring of two prize animals will have the desired traits. Even when a genetic clone is produced, there is no guarantee that the clone produced will be identical in the appearance and behavior as the original.

No one knows if nuclear transfer will work in humans—or if cloning is safe. If Dolly is a representative example, cloning animals may not be safe. In 2003, at age 6 years, Dolly was put to sleep to relieve her from the discomfort of arthritis and a progressive lung disease, conditions usually found only in older sheep. The fact that Dolly developed these conditions has led scientists to question whether she had aged prematurely. Scientists are watching other cloned animals for similar signs of premature aging.

Another type of cloning technology involves the use of early embryos that can be induced to develop into particular tissues or organs to be used for transplants. Called **therapeutic cloning**, this technique involves **stem cells**, a special type of cells, and has proven to be controversial (Essay 8.1).

The debate about human cloning mimics the larger debate about genetic engineering. As a society, we need to determine whether the potential for good outweighs the potential harm for each application of these technologies (Table 8.2). When it comes to human cloning, the potential for abuse could be substantial. Important questions regarding human cloning will not be resolved by developing human cloning techniques. Ideally, these issues will be discussed and legislation enacted *before* it becomes possible to clone humans.

Table 8.2 Pros and cons of genetic engineering.

Why the work of genetic engineers is important
• GM animals and crops may make farms more productive.
• GM crops may be made to taste better, last longer, or contain more nutrients.
• Genetic engineers hope to cure diseases and save lives.
Why the work of genetic engineers is controversial
• GM crops encourage agribusiness, which may close down some small farms.
• GM animals and crops may cause health problems in consumers.
• GM crops might have unexpected adverse effects on the environment.
• Present research might lead to the unethical genetic modification of humans.
• Lack of genetic diversity of GM crops could lead to destruction of food supply worldwide by pest or environmental change.

CHAPTER REVIEW

Summary

8.1 Genetic Engineers

- Genetic engineers manipulate genes for both nonprofit and for-profit reasons (p. 194).

8.2 Protein Synthesis and Gene Expression

- Genetic engineering techniques allow scientists to use bacterial cells to produce proteins for human use (pp. 194–195).
- Genes carry instructions for synthesizing proteins (p. 195).
- Protein synthesis involves the processes of transcription and translation (pp. 196–197).
- Transcription occurs in the nucleus of eukaryotic cells when an RNA polymerase enzyme binds to the promoter, located at the start site of a gene, and makes a mRNA that is complementary to the DNA gene. RNA differs from DNA in that the sugar in RNA is ribose (not deoxyribose) and the nitrogenous bases are adenine, guanine, cytosine, and uracil (no thymines in RNA) (p. 197).
- Translation occurs in the cytoplasm of eukaryotic cells and involves mRNA, ribosomes, and tRNA. Messenger RNA carries the code from the DNA, and ribosomes are the site where amino acids are assembled to synthesize proteins. Transfer RNA (tRNA) carries amino acids, which bind to triplet nucleotide sequences on the mRNA called codons (pp. 197–198).
- A particular tRNA carries a specific amino acid. Each tRNA has its unique anticodon that binds to the codon and carries instructions for its particular amino acid (p. 198).
- The amino acid coded for by a particular codon can be determined using the genetic code (p. 198).
- The flow of genetic information is from the DNA sequence to the mRNA transcript to the encoded protein (pp. 198–199).
- Mutations are changes to DNA sequences that can affect protein structure and function. Neutral mutations are changes to the DNA that do not result in a different amino acid being incorporated. Insertions or deletions of nucleotides can result in frameshift mutations that change the protein drastically (pp. 200–201).
- A given cell type expresses only a small percentage of the genes that an organism possesses (p. 202).
- Turning the expression of a gene up or down is accomplished in different ways in prokaryotes and eukaryotes. Prokaryotes typically block the promoter with a repressor protein to keep

gene expression turned off. Eukaryotes regulate gene expression in any of 5 ways: (1) increasing transcription through the use of proteins that stimulate RNA polymerase binding; (2) varying the time that DNA spends in the uncondensed, active form; (3) altering the mRNA life span; (4) slowing down or speeding up translation; and (5) affecting the protein life span (pp. 202–204).

Web Tutorial 8.1 Transcription
Web Tutorial 8.2 Translation

8.3 Producing Recombinant Proteins

- Bovine growth hormone is a protein produced by the pituitary glands of cows. To increase the quantity of milk that a cow produces, additional growth hormone is injected into the cow (p. 204).
- Modern genetic engineering techniques enable scientists to produce recombinant BGH in the lab by placing the gene for growth hormone into plasmids, which then clone the gene by making millions of copies of it as they replicate themselves inside their bacterial hosts. Bacteria can then express the gene by transcribing a mRNA copy and translating the mRNA into a protein. The recombinant bovine growth hormone is then isolated and injected into cows (pp. 204–206).
- FDA approval is required for any new food or additive that is not generally recognized as safe (p. 207).
- Basic research is research for which there is not a known commercial application. Applied research typically has a more immediate application from which there can be a profit (p. 207).

Web Tutorial 8.3 Producing Bovine Growth Hormone

8.4 Genetic Engineers Can Modify Foods

- Genetic engineering techniques allow for the modification of foods much more quickly than do selective breeding techniques (p. 208).
- Crop plants are genetically modified to increase their shelf life, yield, and nutritive value. Some tomatoes have been modified to slow ripening by the insertion of RNA that is antisense to the ripening-enhancing pectinase gene (pp. 208–209).
- A Ti plasmid or gene gun can be used to insert a particular gene into plant cells (p. 210).
- Although there have been no documented incidents of negative health effects from GM food consumption, there is concern that some GM foods may cause allergic reactions or serve as toxins (p. 212).
- Concerns about GM crops include their impacts on surrounding organisms, the evolution of resistances, transfer of modified genes to wild and weedy relatives, and decreased genetic variation (pp. 213–215).

8.5 Genetic Engineers Can Modify Humans

- Information about genes obtained from the Human Genome Project can be used to help scientists replace genes that are defective or missing in people with genetic diseases (p. 215).
- Gene therapy involves replacing defective genes or their products in an embryo or in the affected adult tissue (p. 216).
- Gene therapy is considered experimental but may hold tremendous promise once scientists determine how to target genes to the right locations and express them in the proper amounts (pp. 216–217).
- Cloning animals with desirable agricultural traits has occurred. It may someday be possible to clone humans, but it is unclear if these humans would be healthy (p. 221).

Learning the Basics

1. List the order of nucleotides on the mRNA that would be transcribed from the following DNA sequence: CGATTACTTA

2. Using the genetic code (Table 8.1 on page 200), list the order of amino acids encoded by the following mRNA nucleotides: CAACGCAUUUUG

3. List the subcellular structures that participate in translation.

4. Transcription _____.

   A. synthesizes new daughter DNA molecules from an existing DNA molecule; **B.** makes a RNA copy of a gene that is to be translated; **C.** pairs thymines (T) with adenines (A); **D.** occurs on ribosomes

5. Transfer RNA (tRNA) _____.

   A. carries monosaccharides to the ribosome for synthesis; **B.** is made of messenger RNA; **C.** has an anticodon region, which is complementary to the mRNA codon; **D.** is the site of protein synthesis

6. During the process of transcription, _____.

   A. DNA serves as a template for the synthesis of more DNA; **B.** DNA serves as a template for the synthesis of RNA; **C.** DNA serves as a template for the synthesis of

proteins; **D.** RNA serves as a template for the synthesis of proteins

7. Translation results in the production of _____.

   A. RNA; **B.** DNA; **C.** protein; **D.** individual amino acids; **E.** transfer RNA molecules

8. The RNA polymerase enzyme binds to _____, initiating transcription.

   A. amino acids; **B.** tRNA; **C.** the promoter sequence; **D.** the ribosome

9. A particular triplet of bases in the coding sequence of DNA is TGA. The anticodon on the tRNA that binds to the mRNA codon is _____.

   A. TGA; **B.** UGA; **C.** UCU; **D.** ACU

10. RNA and DNA are similar because _____.

    A. they are both double-stranded helices; **B.** uracil is found in both of them; **C.** both contain the sugar deoxyribose; **D.** both are made up of nucleotides consisting of a sugar, a phosphate, and a base

Analyzing and Applying the Basics

1. Why are the cells comprising various tissues of your body different, even though they all contain the same genes?

2. Why are Ti plasmids and gene guns used to insert genes into plant cells?

3. Why are some genetic defects more likely to be cured by gene therapy than others are?

Connecting the Science

1. The first "test-tube baby," Louise Brown, was born over 30 years ago. Sperm from her father was combined with an egg cell from her mother. The fertilized egg cell was then placed into the mother's uterus for the period of gestation. At the time of Louise's conception, many people were very concerned about the ethics of scientists performing these in vitro fertilizations. Do you think human cloning will eventually be as commonplace as in vitro fertilizations are now? Why or why not?

2. Do you think it is acceptable to grow genetically modified foods if health risks turn out to be low but environmental effects are high?