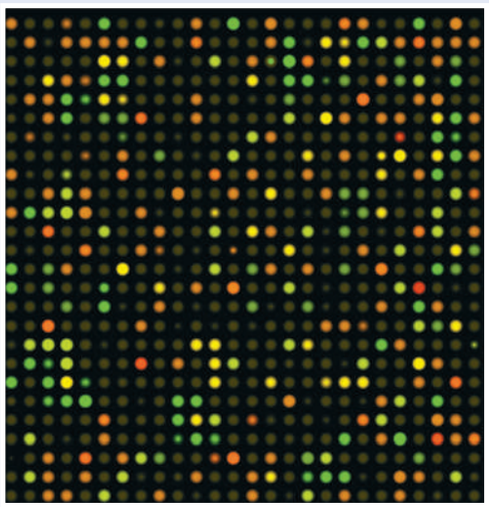
# 11 How Genes Are Controlled

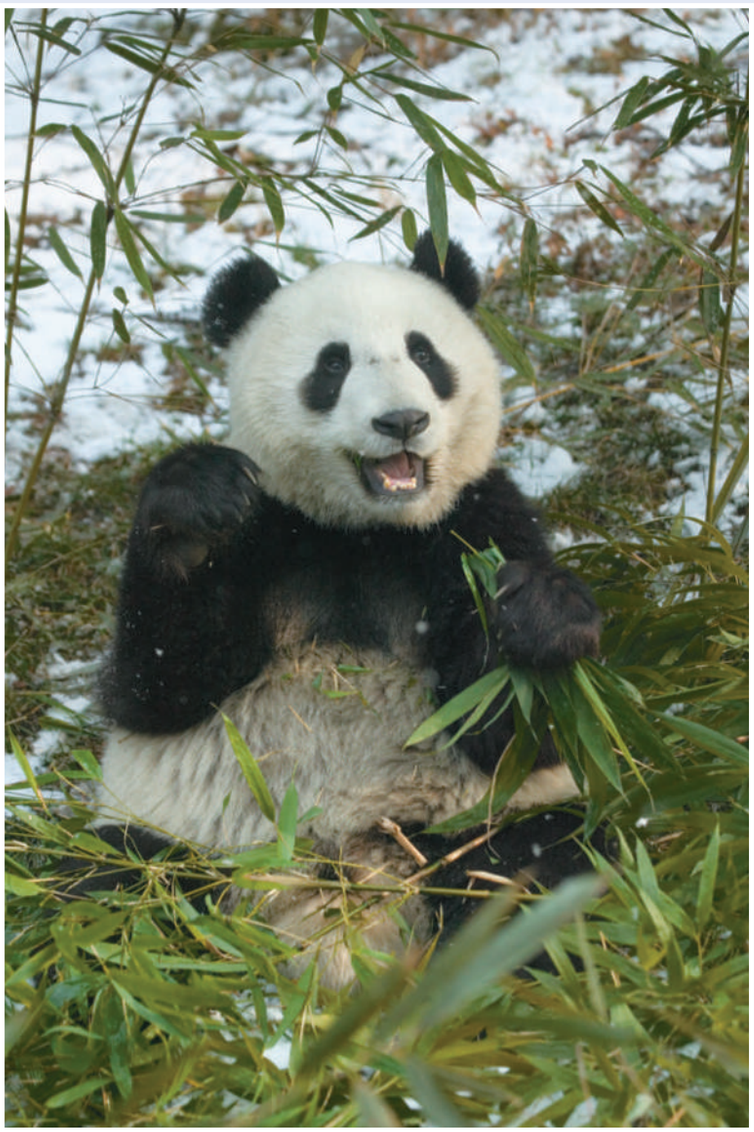
## Why Gene Regulation Matters

Someday a DNA chip might report on the activities of all your genes. ►



▲ Lifestyle choices you make can dramatically affect your risk of cancer.

▼ Cloning may help save the giant panda from extinction.



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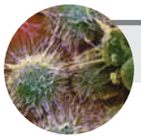
CHAPTER THREAD

Cancer



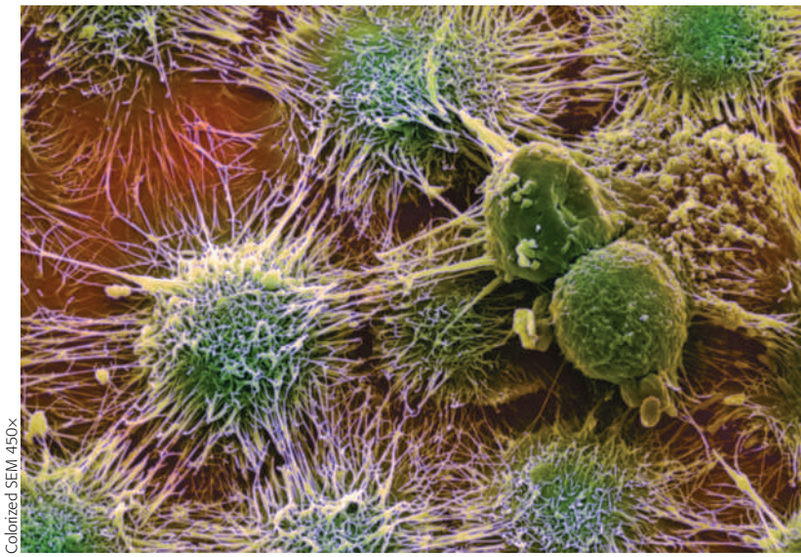


Cancer BIOLOGY AND SOCIETY

# Tobacco's Smoking Gun

When European explorers returned from their first voyages to the Americas, they brought back tobacco, a common trade item among Native Americans. The novelty of smoking tobacco quickly spread through Europe. To keep up with demand, the southern United States soon became a major tobacco producer. Over time, smoking increased in popularity around the world, and by the 1950s, about half of all Americans smoked more than a pack of cigarettes each day. In these early days, little attention was paid to purported health risks; in fact, cigarette advertising often touted the "health benefits" of tobacco, claiming that use of the product had a soothing effect on the throat and helped the smoker remain calm and lose weight.

By the 1960s, however, doctors began to notice a disturbing trend: The rate of lung cancer had increased dramatically. Although the disease was rare in 1930, by 1955 it had become the deadliest form of cancer among American men. In fact, by 1990, lung cancer was killing more than twice as many men each year as any other type of cancer. But a few vocal skeptics, mostly tied to groups with an economic interest in the tobacco industry, doubted the link between smoking and cancer. They pointed out that the evidence was purely statistical or based on animal studies; no direct proof, they said, had been found that tobacco smoke causes cancer in humans.



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**Human cancer cells.** These tumor cells have lost the ability to control their growth.

The "smoking gun" of proof was found in 1996 when researchers added one component of tobacco smoke, called BPDE, to human lung cells growing in the lab. The researchers showed that BPDE binds to a gene within these cells called *p53*. That gene codes for a protein that helps suppress the formation of tumors. The researchers showed that BPDE causes mutations in the *p53* gene that deactivate the protein; with this important tumor-suppressor protein deactivated, tumors grow. This work directly linked a chemical in tobacco smoke to the formation of human lung tumors. Since that time, a mountain of experimental data and statistical studies has removed any scientific doubt of the link between smoking and cancer.

How can a mutation in a gene lead to cancer? It turns out that many cancer-associated genes encode proteins that turn other genes on or off. When these proteins malfunction, the cell may become cancerous. In fact, the ability to properly control which genes are active at any given time is crucial to normal cell function. How genes are controlled and how the regulation of genes affects cells and organisms—including ways this topic affects your own life—are the subjects of this chapter.

# How and Why Genes Are Regulated

Every cell in your body—and, indeed, all the cells in the body of every sexually reproducing organism—was produced through successive rounds of mitosis starting from the zygote, the original cell that formed after fusion of sperm and egg. Mitosis exactly duplicates the chromosomes. Therefore, every cell in your body has the same DNA as the zygote. To put it another way: Every somatic (body) cell contains every gene. However, the cells in your body are specialized in structure and function; a neuron, for example, looks and acts nothing like a red blood cell. But if every cell contains identical genetic instructions, how do cells develop differently from one another? To help you understand this idea, imagine that every restaurant in your hometown uses the same cookbook. If that were the case, how could each restaurant develop a unique menu? The answer is obvious: Even though each restaurant has the same cookbook, different restaurants pick and choose different recipes from this book to prepare. Similarly, cells with the same genetic information can develop into different types of cells through **gene regulation**, mechanisms that turn on certain genes while other genes remain turned off. Regulating gene activity allows for specialization of cells within the body, just as regulating which recipes are used allows for varying menus in multiple restaurants.

As an example of gene regulation, consider the development of a unicellular zygote into a multicellular organism. During embryonic growth, groups of cells follow different paths, and each group becomes a particular kind of tissue. In the mature organism, each cell type—neuron or red blood cell, for instance—has a different pattern of turned-on genes.

What does it mean to say that genes are turned on or off? Genes determine the nucleotide sequence of specific mRNA molecules, and mRNA in turn determines the sequence of amino acids in proteins (in summary: DNA → RNA → protein; see Chapter 10). A gene that is turned on is being transcribed into mRNA, and that message is being translated into specific proteins. The overall process by which genetic information flows from genes to proteins is called **gene expression**.

As an illustration of this principle, **Figure 11.1** shows the patterns of gene expression for four genes in three different specialized cells of an adult human. Note that the genes for "housekeeping" enzymes, such as those that provide energy via glycolysis, are "on" in all the cells. In contrast, the genes for some proteins, such as insulin and hemoglobin, are expressed only by particular kinds of cells. One protein, hemoglobin, is not expressed in any of the cell types shown in the figure. ☑

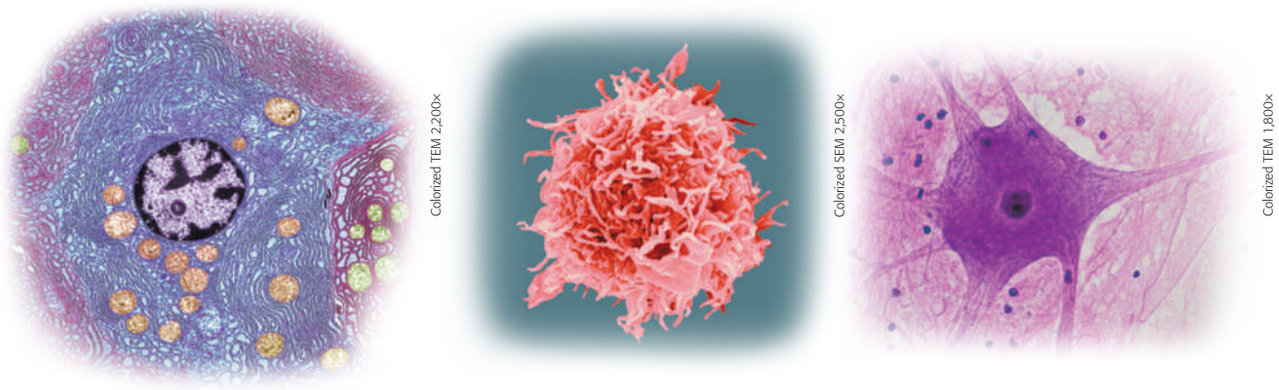
**☑ CHECKPOINT**

If your blood cells and skin cells have the same genes, how can they be so different?

*Answer: Each cell type expresses different genes than the other cell type.*

## Gene Regulation in Bacteria

To understand how a cell can regulate gene expression, consider the relatively simple case of bacteria. In the course of their lives, bacteria must regulate their genes in response to environmental changes. For example,




► **Figure 11.1 Patterns of gene expression in three types of human cells.** Different types of cells express different combinations of genes. The specialized proteins whose genes are represented here are an enzyme involved in glucose digestion; an antibody, which aids in fighting infection; insulin, a hormone made in the pancreas; and the oxygen transport protein hemoglobin, which is expressed only in red blood cells.

when a nutrient is plentiful, bacteria do not squander valuable resources to make the nutrient from scratch. Bacterial cells that can conserve resources and energy have a survival advantage over cells that are unable to do so. Thus, natural selection has favored bacteria that express only the genes whose products are needed by the cell.

Imagine an *Escherichia coli* bacterium living in your intestines. It will be bathed in various nutrients, depending on what you eat. If you drink a milk shake, for example, there will be a sudden rush of the sugar lactose. In response, *E. coli* will express three genes for enzymes that enable the bacterium to absorb and digest this sugar. After the lactose is gone, these genes are turned off; the bacterium does not waste its energy continuing to produce these enzymes when they are not needed. Thus, a bacterium can adjust its gene expression to changes in the environment.

How does a bacterium "know" if lactose is present or not? In other words, how does the presence or absence of lactose influence the activity of the genes that code for the lactose enzymes? The key is the way the three lactose-digesting genes are organized: They are adjacent in the DNA and turned on and off as a single unit. This regulation is achieved through short stretches of DNA that help turn all three genes on and off at once, coordinating their expression. Such a cluster of related genes and sequences that control them is called an **operon** (Figure 11.2). The operon considered here, the *lac* (short for lactose) operon, illustrates principles of gene regulation that apply to a wide variety of prokaryotic genes.

How do DNA control sequences turn genes on or off? One control sequence, called a **promoter** (green in the figure), is the site where the enzyme RNA polymerase attaches and initiates transcription—in our example, transcription of the genes for lactose-digesting enzymes. Between the promoter and the enzyme genes, a DNA segment called an **operator** (yellow) acts as a switch that is turned on or off, depending on whether a specific protein is bound there. The operator and protein together determine whether RNA polymerase can attach to the promoter and start transcribing the genes (light blue). In the *lac* operon, when the operator switch is turned on, all the enzymes needed to metabolize lactose are made at once.

The top half of Figure 11.2 shows the *lac* operon in "off" mode, its status when there is no lactose available. Transcription is turned off because 1 a protein called a **repressor** ( ) binds to the operator ( ) and 2 physically blocks the attachment of RNA polymerase ( ) to the promoter ( ).

The bottom half of Figure 11.2 shows the operon in "on" mode, when lactose is present. The lactose ( ) interferes with attachment of the *lac* repressor to the operator by 1 binding to the repressor and 2 changing the repressor's shape. In its new shape ( ), the repressor cannot bind to the operator, and the operator switch remains on. 3 RNA polymerase is no longer blocked, so it can now bind to the promoter and from there 4 transcribe the genes for the lactose enzymes into mRNA. 5 Translation produces all three lactose enzymes (purple).

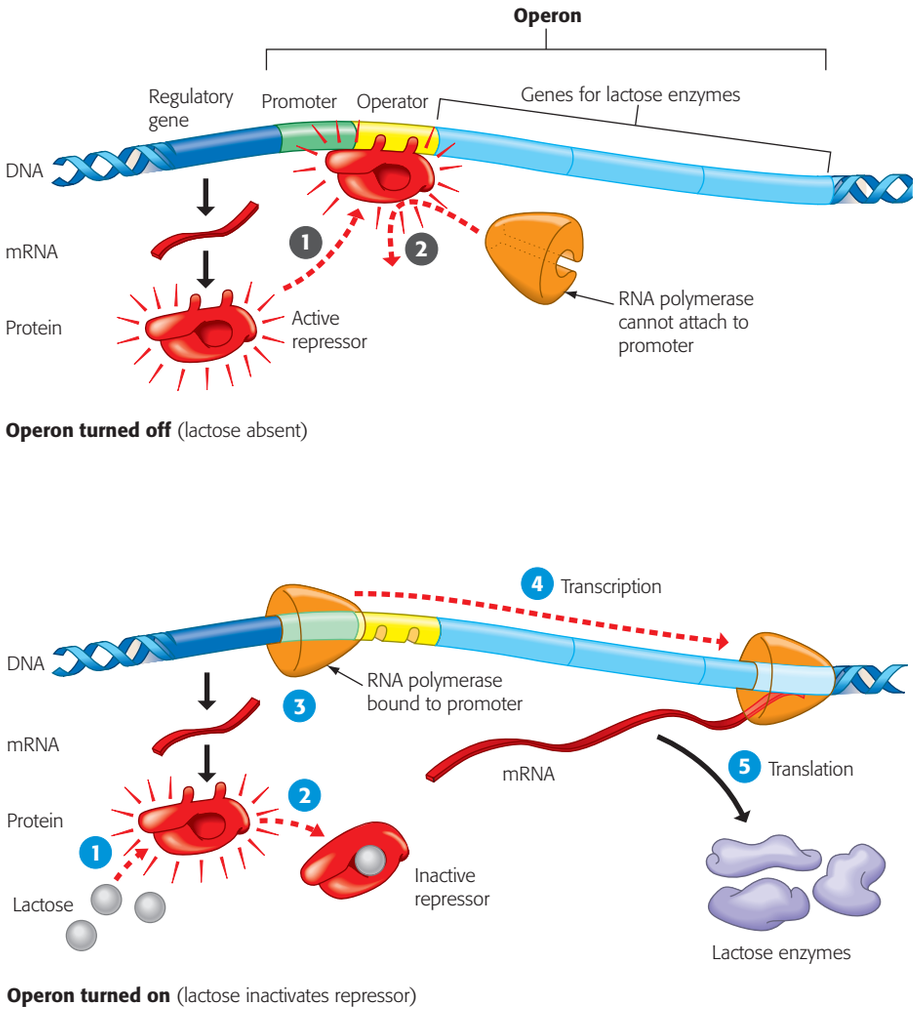
Many operons have been identified in bacteria. Some are quite similar to the *lac* operon, whereas others have somewhat different mechanisms of control. For example, operons that control amino acid synthesis cause bacteria to stop making these molecules when they are already present in the environment, saving materials and energy for the cells. In these cases, the amino acid *activates* the repressor. Armed with a variety of operons, *E. coli* and other prokaryotes can thrive in frequently changing environments. ✓

**✓ CHECKPOINT**

A mutation in *E. coli* makes the *lac* operator unable to bind the active repressor. How would this mutation affect the cell? Why would this effect be a disadvantage?

*Answer: The cell would wastefully produce the enzymes for lactose metabolism continuously, even in the absence of lactose.*

**Figure 11.2 The *lac* operon of *E. coli*.**




**Operon turned off** (lactose absent)

**Operon turned on** (lactose inactivates repressor)

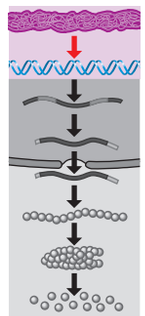
# Gene Regulation in Eukaryotic Cells

Eukaryotes, especially multicellular ones, have more sophisticated mechanisms than bacteria for regulating the expression of their genes. This is not surprising because a prokaryote, being a single cell, does not require the elaborate regulation of gene expression that leads to cell specialization in multicellular eukaryotic organisms. A bacterium does not have neurons that need to be different from blood cells, for example.

The pathway from gene to protein in eukaryotic cells is a long one, providing a number of points where the process can be turned on or off, speeded up or slowed down. Picture the series of pipes that carry water from your local reservoir to a faucet in your home. At various points, valves control the flow of water. We use this analogy in **Figure 11.3** to illustrate the flow of genetic information from a eukaryotic chromosome—a reservoir of genetic information—to an active protein that has been made in the cell's cytoplasm. The multiple mechanisms that control gene expression are analogous to the control valves in your water pipes. In the figure, each control knob indicates a gene expression "valve." All these knobs represent possible control points, although only one or a few control points are likely to be important for a typical protein.

Using a reduced version of Figure 11.3 as a guide, we will explore several ways that eukaryotes can control gene expression, starting within the nucleus.

## The Regulation of DNA Packing



Eukaryotic chromosomes may be in a more or less condensed state, with the DNA and accompanying proteins more or less tightly wrapped together (see Figure 8.4). DNA packing tends to prevent gene expression by preventing RNA polymerase and other transcription proteins from binding to the DNA.

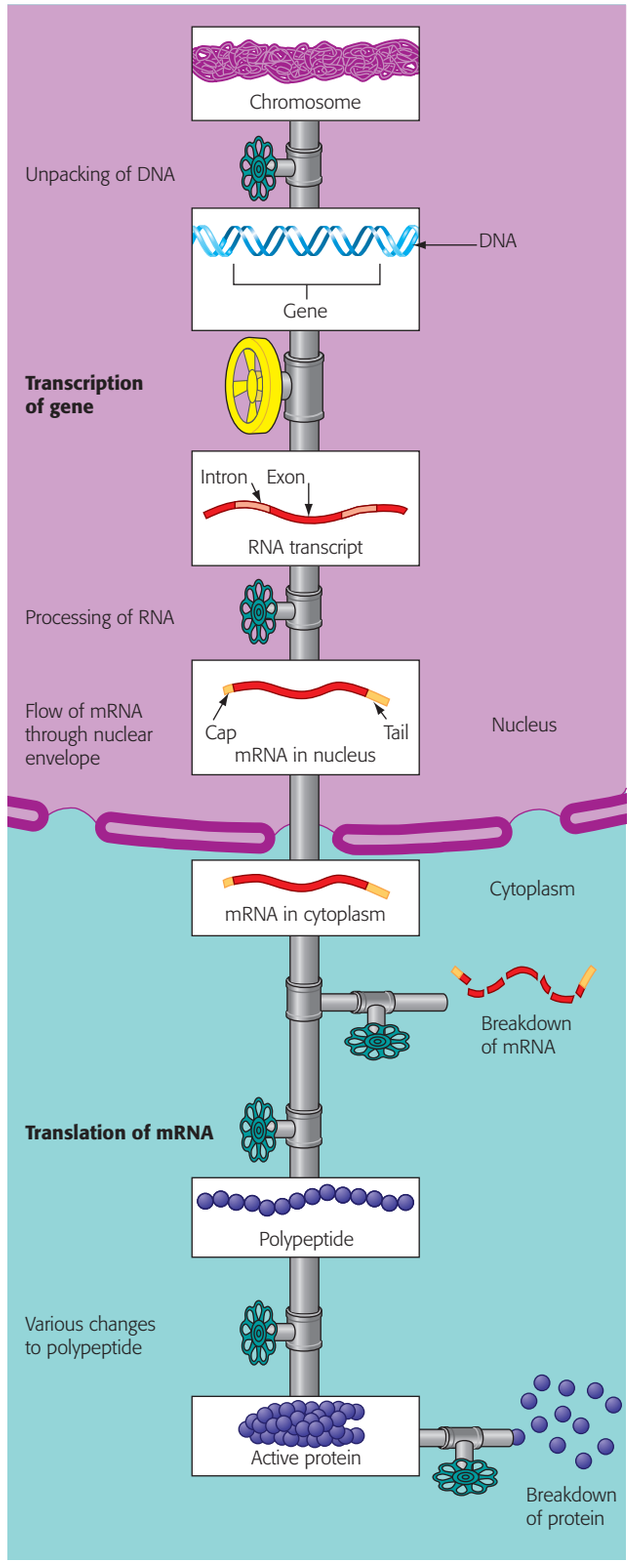
Cells may use DNA packing for the long-term inactivation of genes. One intriguing case is seen in female mammals, where one X chromosome in each somatic cell is highly compacted and almost entirely inactive. This **X chromosome inactivation** first takes place early in embryonic development, when one of the two X chromosomes in each cell is inactivated at random. After one X chromosome is inactivated in each embryonic cell, all of that cell's descendants will have the same X chromosome turned off. Consequently, if a female has different versions of a gene on each of her X chromosomes, about half of her cells will express one version, while the other half will express the alternate version **(Figure 11.4)**. ☑

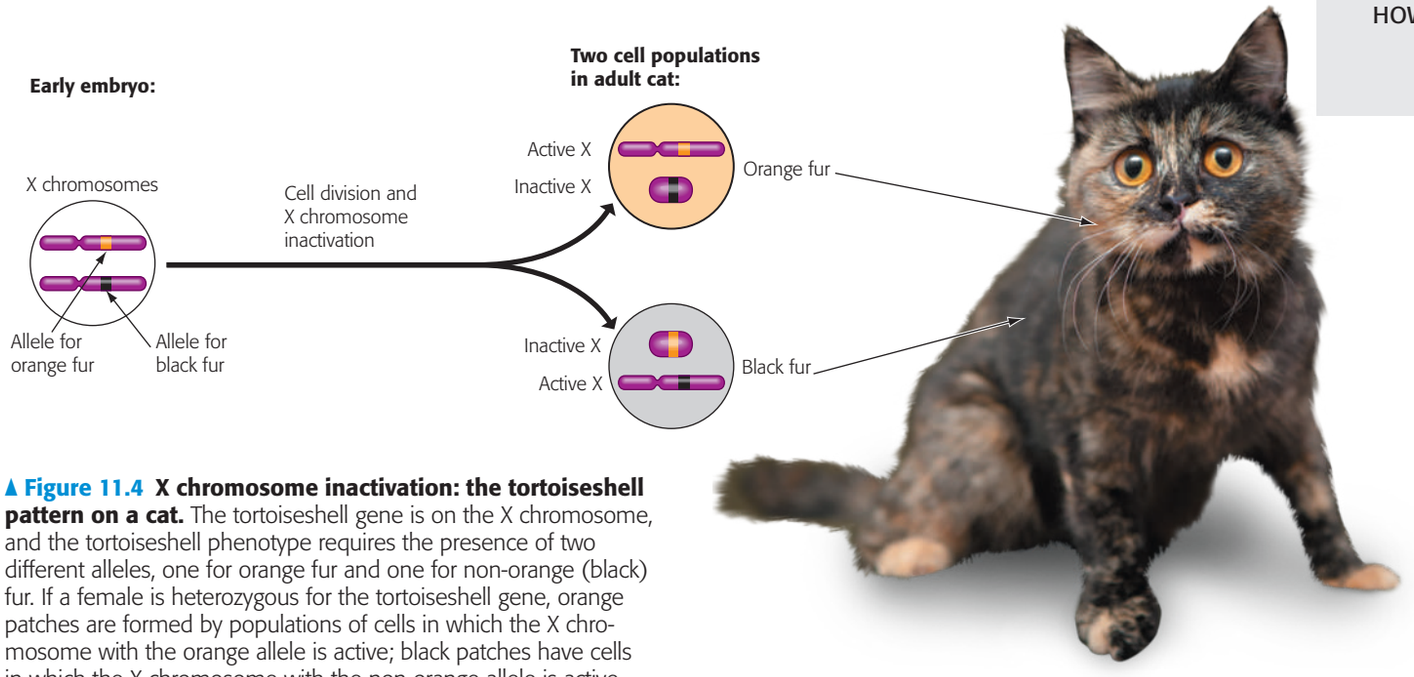
**☑ CHECKPOINT**

Would a gene on the X chromosome be expressed more in human females (who have two copies of the X chromosome) than in human males (who have one copy)?

*Answer: No, because in females one of the X chromosomes in each cell is inactivated.*

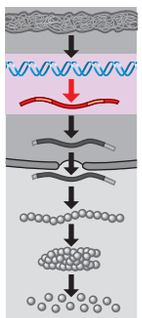
**▼ Figure 11.3 The gene expression "pipeline" in a eukaryotic cell.** Each valve in the pipeline represents a stage at which the pathway from chromosome to functioning protein can be regulated, turned on or off, or speeded up or slowed down. Throughout this discussion we will use a miniature version of this figure to keep track of the stages as they are discussed.






**▲ Figure 11.4 X chromosome inactivation: the tortoiseshell pattern on a cat.** The tortoiseshell gene is on the X chromosome, and the tortoiseshell phenotype requires the presence of two different alleles, one for orange fur and one for non-orange (black) fur. If a female is heterozygous for the tortoiseshell gene, orange patches are formed by populations of cells in which the X chromosome with the orange allele is active; black patches have cells in which the X chromosome with the non-orange allele is active.

## The Initiation of Transcription

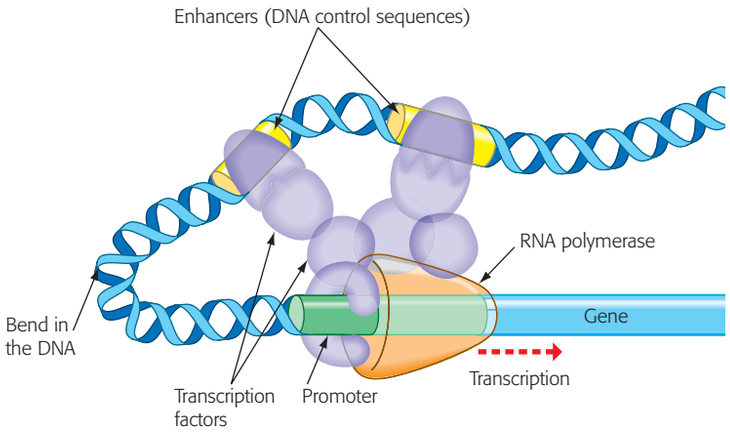


The initiation of transcription (whether transcription starts or not) is the most important stage for regulating gene expression. In both prokaryotes and eukaryotes, regulatory proteins bind to DNA and turn the transcription of genes on and off. Unlike prokaryotic genes, however, most eukaryotic genes are not grouped into operons. Instead, each eukaryotic gene usually has its own promoter and other control sequences.

As illustrated in **Figure 11.5**, transcriptional regulation in eukaryotes is complex, typically involving many proteins (collectively called **transcription factors**; shown in purple in the figure) acting in concert to bind to DNA sequences called **enhancers** (yellow) and to the promoter (green). The DNA-protein assembly promotes the binding of RNA polymerase (orange) to the promoter. Genes coding for related enzymes, such as those in a metabolic pathway, may share a specific kind of enhancer (or collection of enhancers), allowing these genes to be activated at the same time. Not shown in the figure are repressor proteins, which may bind to DNA sequences called **silencers**, inhibiting the start of transcription.

In fact, repressor proteins that turn genes off are less common in eukaryotes than **activators**, proteins that turn genes on by binding to DNA. Activators act by making it easier for RNA polymerase to bind to the promoter. The use of activators is efficient because a typical animal or plant cell needs to turn on (transcribe) only a small percentage of its genes, those required for the cell's specialized structure and function. The "default" state for most genes in multicellular eukaryotes seems to be off, with the exception of "housekeeping" genes for routine activities such as the digestion of glucose. ☑

**▼ Figure 11.5 A model for turning on a eukaryotic gene.** A large assembly of transcription factors (proteins shown in purple) and several control sequences in the DNA are involved in initiating the transcription of a eukaryotic gene.

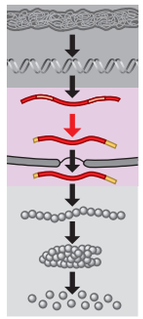



**☑ CHECKPOINT**

Of all the control points of DNA expression shown in Figure 11.3, which is under the tightest regulation?

Answer: the initiation of transcription

## RNA Processing and Breakdown



Within a eukaryotic cell, transcription occurs in the nucleus, where RNA transcripts are processed into mRNA before moving to the cytoplasm for translation by the ribosomes (see Figure 10.19). RNA processing includes the addition of a cap and a tail, as well as the removal of any introns—noncoding DNA segments that interrupt the genetic message—and the splicing together of the remaining exons.

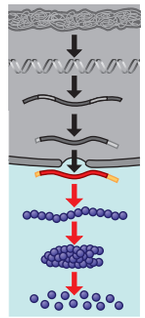
Within a cell, exon splicing can occur in more than one way, generating different mRNA molecules from the same starting RNA molecule. Notice in **Figure 11.6**, for example, that one mRNA ends up with the green exon and the other with the brown exon. With this sort of **alternative RNA splicing**, an organism can produce more than one type of polypeptide from a single gene. A typical human gene contains about ten exons; nearly all genes are spliced in at least two different ways, and some are spliced hundreds of different ways.

After an mRNA is produced in its final form, its "lifetime" can be highly variable, from hours to weeks to months. Controlling the timing of mRNA breakdown provides another opportunity for regulation. But all mRNAs are eventually broken down and their parts recycled.

## microRNAs

Recent research has established an important role for a variety of small single-stranded RNA molecules, called microRNAs (miRNAs), that can bind to complementary sequences on mRNA molecules in the cytoplasm. After binding, some miRNAs trigger breakdown of their target mRNA, whereas others block translation. It has been estimated that miRNAs may regulate the expression of one half of all human genes, a striking figure given that miRNAs were unknown 20 years ago. New techniques attempt to exploit miRNAs via a technique called RNA interference, the injection of small RNA molecules into a cell to turn off specific genes. By understanding the natural process of information flow through a cell, biologists may soon be able to artificially control gene expression in humans.

## The Initiation of Translation



The process of translation—in which an mRNA is used to make a protein—offers additional opportunities for control by regulatory molecules. Red blood cells, for instance, have a protein that prevents the translation of hemoglobin mRNA unless the cell has a supply of heme, an iron-containing chemical group essential for hemoglobin function.

## Protein Activation and Breakdown

The final opportunities for regulating gene expression occur after translation. For example, the hormone insulin is synthesized as one long, inactive polypeptide that must be chopped into pieces before it comes active **(Figure 11.7)**. Other proteins require chemical modification before they become active.

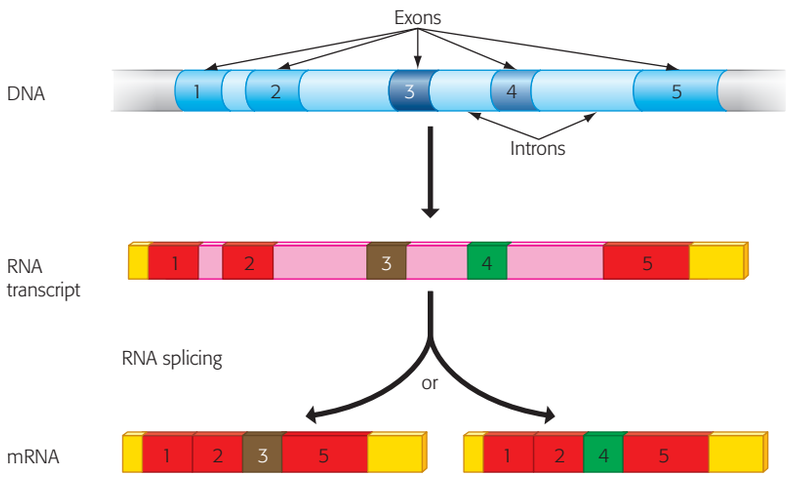
Another control mechanism operating after translation is the selective breakdown of proteins. Some proteins that trigger metabolic changes in cells are broken down within a few minutes or hours. This regulation allows a cell to adjust the kinds and amounts of its proteins in response to changes in its environment. ☑

### ☑ CHECKPOINT

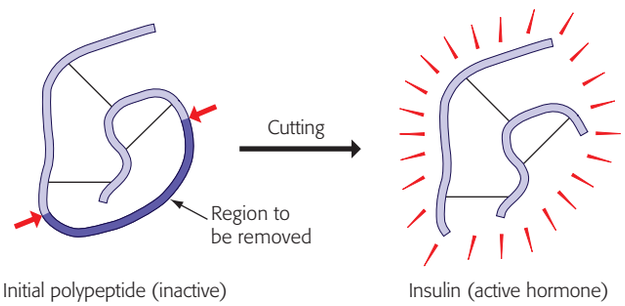
After a gene is transcribed in the nucleus, how is the transcript modified to become mRNA? After the mRNA reaches the cytoplasm, what are four control mechanisms that can regulate the amount of active protein in the cell?

*Answer: by RNA processing, including the addition of cap and tail and RNA splicing; control by microRNAs, initiation of translation, activation of the protein, and breakdown of the protein*

**▼ Figure 11.6 Alternative RNA splicing: producing multiple mRNAs from the same gene.** Two different cells can use the same DNA gene to synthesize different mRNAs and proteins. In this example, one mRNA has ended up with exon 3 (brown) and the other with exon 4 (green). These mRNAs, which are just two of many possible outcomes, can then be translated into different proteins.



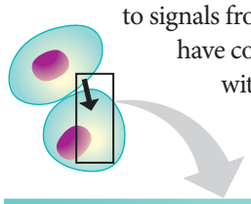

**▼ Figure 11.7 The formation of an active insulin molecule.** Only in its final form, with a central region removed, does insulin act as a hormone.



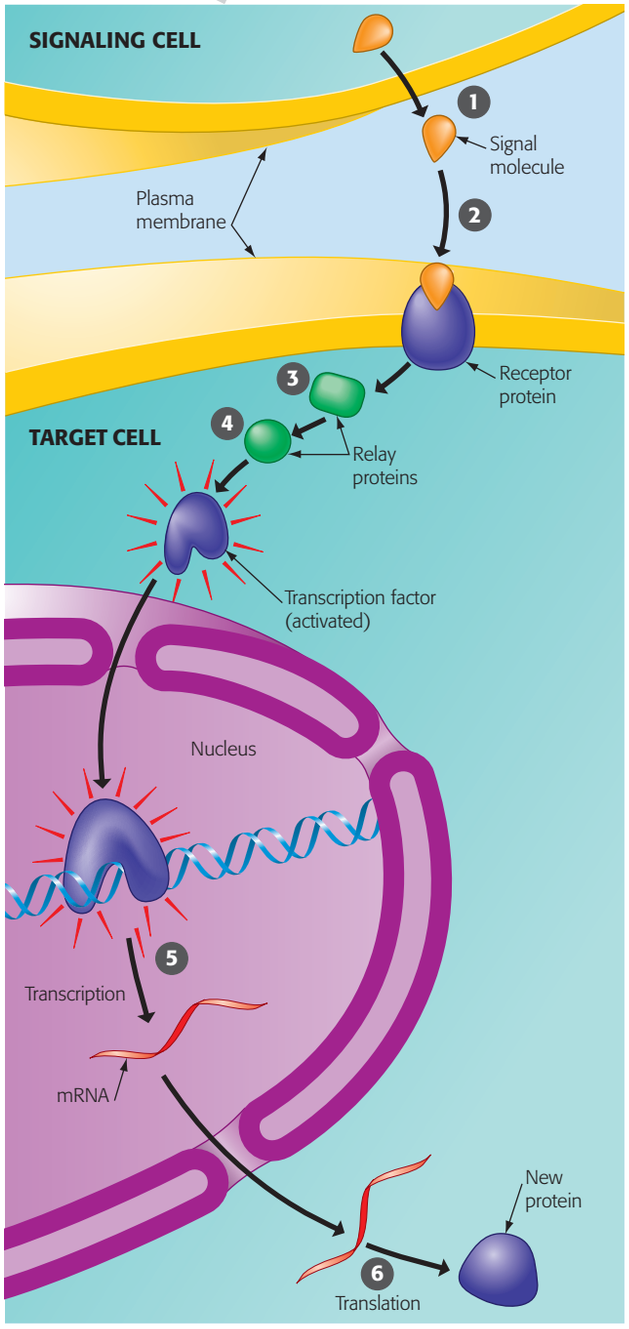
Information Flow

# Cell Signaling

The control of gene expression is a good example of one of biology's important themes: information flow. Through regulation, a cell can alter its activities in response to signals from the environment. So far, we have considered gene regulation only within a single cell. In a multicellular organism, the process can cross cell boundaries, allowing information to be communicated between and among cells. For example, a cell can produce and secrete chemicals, such as hormones, that affect gene regulation in another cell. Consider an analogy from your own experience: In grade school, did you ever station a classmate near the door to signal the teacher's return? Information from outside the room (the teacher's approach) was used to alter behavior within the classroom (stop messing around!). In a similar way, cells use protein "lookouts" to convey information into the cell, resulting in changes to cellular functions.



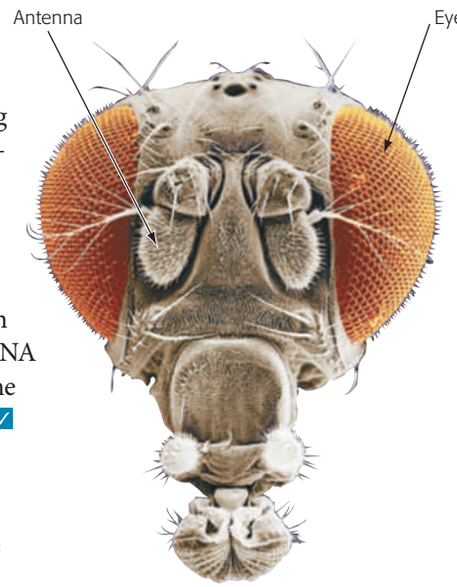
A signal molecule can act by binding to a receptor protein and initiating a **signal transduction pathway**, a series of molecular changes that converts a signal received outside a cell to a specific response inside the target cell. Figure 11.8 shows an example of cell-to-cell signaling in which the target cell's response is the transcription (turning on) of a gene. 1 First, the signaling cell secretes the signal molecule ( ). 2 This molecule binds to a specific receptor protein ( ) embedded in the target cell's plasma membrane. 3 The binding activates a signal transduction pathway consisting of a series of relay proteins (green) within the target cell. Each relay molecule activates the next. 4 The last relay molecule in the series activates a transcription factor ( ) that 5 triggers the transcription of a specific gene. 6 Translation of the mRNA produces a protein that can then perform the function originally called for by the signal. ☑



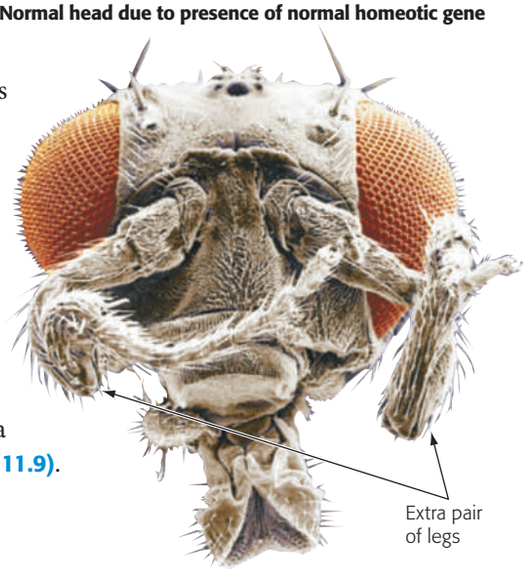

▲ **Figure 11.8 A cell-signaling pathway that turns on a gene.** The coordination of cellular activities in a multicellular organism depends on cell-to-cell signaling that helps regulate genes.

## Homeotic Genes

Cell-to-cell signaling and the control of gene expression are particularly important during early embryonic development, when a single-celled zygote develops into a multicellular organism. Master control genes called **homeotic genes** regulate groups of other genes that determine what body parts will develop in which locations. For example, one set of homeotic genes in fruit flies instructs cells in the midbody to form legs. Elsewhere, these homeotic genes remain turned off, while others are turned on. Mutations in homeotic genes can produce bizarre effects. For example, fruit flies with mutations in homeotic genes may have extra sets of legs growing from their head (Figure 11.9).




**Normal head due to presence of normal homeotic gene**




**Head with extra legs growing due to presence of mutant homeotic gene**

► **Figure 11.9 The effect of homeotic genes.** The strange mutant fruit fly shown at the bottom results from a mutation in a homeotic (master control) gene.

### ☑ CHECKPOINT

How can a signal molecule from one cell alter gene expression in a target cell without entering the target cell?

*Answer: by binding to a receptor protein in the membrane of the target cell and triggering a signal transduction pathway that activates transcription factors*

**CHECKPOINT**

How can a mutation in just one homeotic gene drastically affect an organism's physical appearance?

*Answer: Because homeotic genes control many other genes, a single change can affect the expression of many of the proteins that control appearance.*

One of the most significant biological discoveries in recent years uncovered the fact that similar homeotic genes help direct embryonic development in nearly every eukaryotic organism examined so far, including yeasts, plants, earthworms, frogs, chickens, mice, and humans. These similarities suggest that these homeotic genes arose very early in the history of life and that the genes have remained remarkably unchanged over eons of animal evolution. ✓

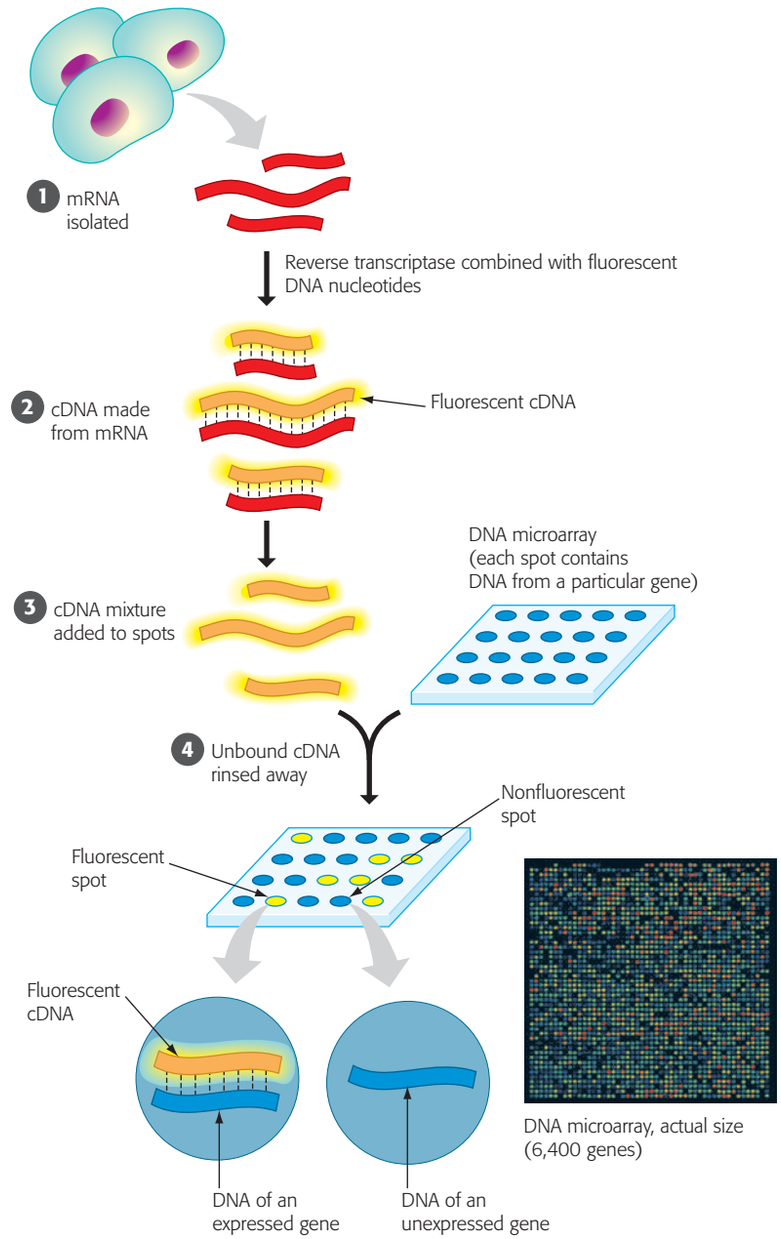
Someday a DNA chip might report on the activities of all your genes.

which genes were being transcribed in the starting cells. Researchers can thus learn which genes are active in different tissues, at different times, or in tissues from individuals in different states of health. Such information may contribute to a better understanding of diseases and suggest new therapies. For example, comparing patterns of gene expression in breast cancer tumors with noncancerous breast tissue has resulted in more effective treatment protocols.

## DNA Microarrays: Visualizing Gene Expression

Scientists who study gene regulation often want to determine which genes are switched on or off in a particular cell. A **DNA microarray** is a slide with thousands of different kinds of single-stranded DNA fragments attached in a tightly spaced array (grid). Each DNA fragment is obtained from a particular gene; a single microarray thus carries DNA from thousands of genes, perhaps even all the genes of an organism.

**Figure 11.10** outlines how microarrays are used. 1 A researcher collects all of the mRNA transcribed in a particular type of cell at a given moment. This collection of mRNA is mixed with reverse transcriptase, a viral enzyme that 2 produces DNA that is complementary to each mRNA sequence. This **complementary DNA (cDNA)** is synthesized using nucleotides that have been modified to fluoresce (glow). The fluorescent cDNA collection thus represents all of the genes being actively transcribed in the cell. 3 A small amount of the fluorescently labeled cDNA mixture is added to the DNA fragments of the microarray. If a molecule in the cDNA mixture is complementary to a DNA fragment at a particular location on the grid, the cDNA molecule binds to it, becoming fixed there. 4 After unbound cDNA is rinsed away, the remaining cDNA glows in the microarray. The pattern of glowing spots enables the researcher to determine




▲ **Figure 11.10 Visualizing gene expression using a DNA microarray.**

# Cloning Plants and Animals

Now that we have examined how gene expression is regulated, we will devote the rest of this chapter to discussing how gene regulation affects two important processes: cloning and cancer.

## The Genetic Potential of Cells

One of the most important take-home lessons from this chapter is that all body cells contain a complete complement of genes, even if they are not expressing all of them. If you've ever grown a plant from a small cutting, you've seen evidence of this yourself: A single differentiated plant cell can undergo cell division and give rise to a complete adult plant. On a larger scale, the technique described in **Figure 11.11** can be used to produce hundreds or thousands of genetically identical organisms—clones—from the cells of a single plant.

Plant cloning is now used extensively in agriculture. For some plants, such as orchids, cloning is the only commercially practical means of reproducing plants. In other cases, cloning has been used to reproduce a plant with specific desirable traits, such as high fruit yield or resistance to disease. Seedless plants (such as seedless grapes, watermelons, and oranges) cannot reproduce sexually, leaving cloning as the sole means of mass-producing these common foods.

Is this sort of cloning possible in animals? A good indication that some animal cells can also tap into their full genetic potential is **regeneration**, the regrowth of lost body parts. When a salamander loses a tail, for example, certain cells in the tail stump reverse their differentiated state, divide, and then differentiate again to give rise to a new tail. Many other animals, especially among the invertebrates (sea stars and sponges, for example), can regenerate lost parts, and isolated pieces of a few relatively simple animals can dedifferentiate and then develop into an entirely new organism (see Figure 8.1).


► **Figure 11.11 Test-tube cloning of an orchid.** Tissue removed from the stem of an orchid plant and placed in growth medium may begin dividing and eventually grow into an adult plant. The new plant is a genetic duplicate of the parent plant. This process proves that mature plant cells can reverse their differentiation and develop into all the specialized cells of an adult plant.

# Reproductive Cloning of Animals

Animal cloning is achieved through a procedure called **nuclear transplantation** **(Figure 11.12)**. First performed in the 1950s on frog embryos and in the 1990s on adult mammals, nuclear transplantation involves replacing the nucleus of an egg cell or a zygote with a nucleus removed from an adult body cell. If properly stimulated, the recipient cell may then begin to divide. Repeated cell divisions form a hollow ball of about 100 cells. At this point, the cells may be used for different purposes, as indicated by the two branches in Figure 11.12.

If the animal to be cloned is a mammal, further development requires implanting the early embryo into the uterus of a surrogate mother (Figure 11.12, upper branch). The resulting animal will be a "clone" (genetic copy) of the donor. This type of cloning is called **reproductive cloning** because it results in the birth of a new animal.

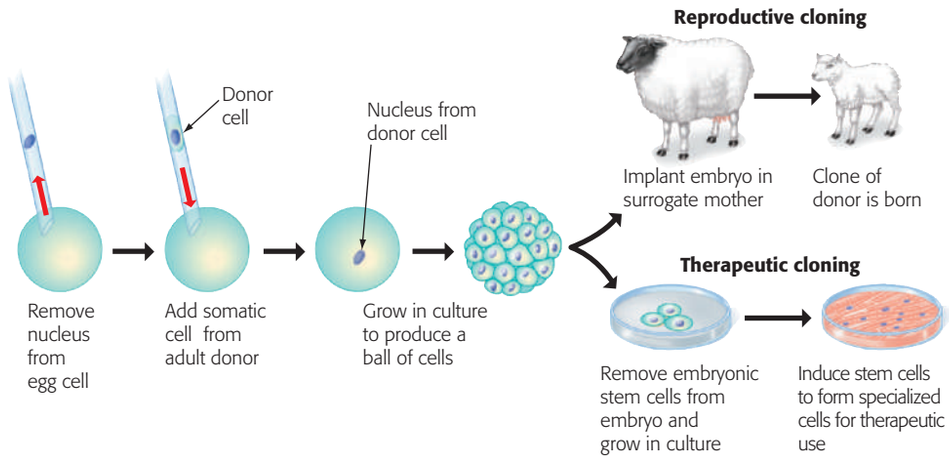
In 1996, researchers used reproductive cloning to produce the first mammal cloned from an adult cell, a sheep named Dolly. The researchers fused specially treated sheep cells with eggs from which they had removed the nuclei. After several days of growth, the resulting embryos were implanted in the uteruses of surrogate mothers. One of the embryos developed into Dolly—and, as expected, Dolly resembled the nucleus donor, not the egg donor or the surrogate mother.

Cloning may help save the giant panda from extinction.

## Practical Applications of Reproductive Cloning

Since the first success in 1996, researchers have cloned many species of mammals, including mice, horses, dogs, mules, cows, pigs, rabbits, ferrets, camels, goats, and cats **(Figure 11.13a)**. Why would anyone want to do this? In agriculture, farm animals with specific sets of desirable traits might be cloned to produce identical herds. In research, genetically identical animals can provide perfect "control animals" for experiments. The pharmaceutical industry is experimenting with cloning animals for potential medical use **(Figure 11.13b)**. For example, researchers have produced pig clones that lack a gene for a protein that can cause immune system rejection in humans. Organs from such pigs may one day be used in human patients who require life-saving transplants.

Perhaps the most intriguing use of reproductive cloning is to restock populations of endangered animals. Among the rare animals that have been cloned are a wild mouflon (a small European sheep), a gaur (an Asian ox), and gray wolves **(Figure 11.13c)**, and many others are being attempted. In 2003, a banteng (a Javanese cow whose numbers have dwindled to just a few in the wild) was cloned using frozen cells from a zoo-raised banteng that had died 23 years prior. Scientists obtained banteng skin tissue from "The Frozen Zoo," a facility in San Diego, California, where samples from rare or endangered animals are stored for conservation. The scientists transplanted nuclei from the frozen cells




▲ **Figure 11.12 Cloning by nuclear transplantation.** In nuclear transplantation, a nucleus from an adult body cell is injected into a nucleus-free egg cell. The resulting embryo may then be used to produce a new organism (reproductive cloning, shown in the upper branch) or to provide stem cells (therapeutic cloning, lower branch).

into nucleus-free eggs from dairy cows. The resulting embryos were implanted into surrogate cows, leading to the birth of a healthy baby banteng. This success shows that it is possible to produce a baby even when a female of the donor species is unavailable. Scientists may someday be able to use similar cross-species methods to clone an animal from a recently extinct species.

The use of cloning to repopulate endangered species holds tremendous promise. However, cloning may also create new problems. Conservationists argue that cloning may detract from efforts to preserve natural habitats. They correctly point out that cloning does not increase genetic diversity and is therefore not as beneficial to endangered species as natural reproduction. In addition, an increasing body of evidence suggests that cloned animals are less healthy than animals produced via fertilization: Many cloned animals exhibit defects such as susceptibility to obesity, pneumonia, liver failure, and premature death. Dolly the cloned sheep, for example, was euthanized in 2003 after suffering complications from a lung disease usually seen only in much older sheep. She was 6 years old, while her breed has a life expectancy of 12 years. Some evidence suggests that chromosomal changes in the cloned animals are the cause, but the effects of cloning on animal health are still being investigated.

## Human Cloning

The cloning of various mammals has heightened speculation that humans could be cloned. Critics point out the many practical and ethical objections to human cloning. Practically, cloning of mammals is extremely difficult and inefficient. Only a small percentage of cloned embryos (usually less than 10%) develop normally, and they appear less healthy than naturally born kin. Ethically, the discussion about whether or not people should be cloned—and if so, under what circumstances—is far from settled. Meanwhile, the research and the debate continue. ☑

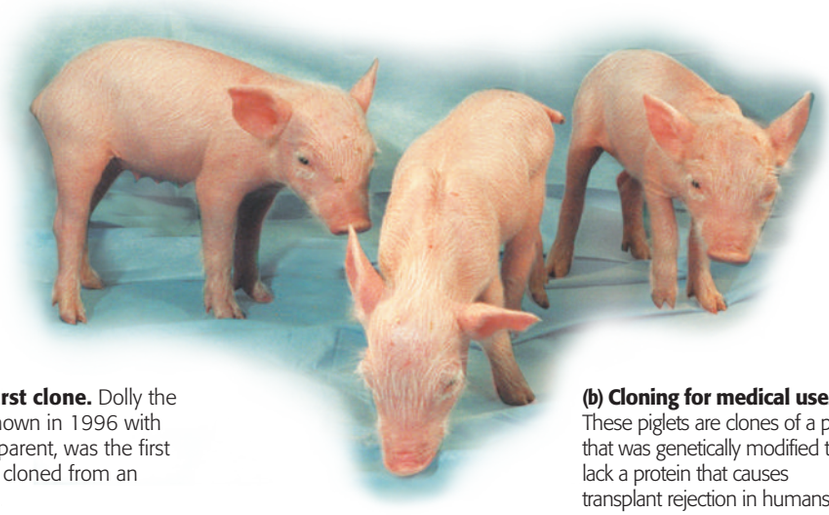
### ☑ CHECKPOINT

Imagine that mouse coat color is always passed down from parent to offspring. Suppose a nucleus from an adult body cell of a black mouse is injected into an egg removed from a white mouse, and then the embryo is implanted into a brown mouse. What would be the color of the resulting cloned mice?

*Answer: black, the color of the nucleus donor*

**▼ Figure 11.13 Reproductive cloning of mammals.**

**(a) The first clone.** Dolly the sheep, shown in 1996 with her lone parent, was the first mammal cloned from an adult cell.



**(b) Cloning for medical use.** These piglets are clones of a pig that was genetically modified to lack a protein that causes transplant rejection in humans.

**(c) Clones of endangered animals**

Mouflon lamb with mother

Banteng

Gaur

# Therapeutic Cloning and Stem Cells

The lower branch of Figure 11.12 shows the process of **therapeutic cloning**. The purpose of this procedure is not to produce a living organism but rather to produce embryonic stem cells.

## Embryonic Stem Cells

In mammals, **embryonic stem cells (ES cells)** are obtained by removing cells from an early embryo and growing them in laboratory culture. Embryonic stem cells can divide indefinitely, and under the right conditions—such as the presence of certain growth-stimulating proteins—can (hypothetically) develop into a wide variety of different specialized cells **(Figure 11.14)**. If scientists can discover the right conditions, they may be able to grow cells for the repair of injured or diseased organs. Some people speculate, for example, that ES cells may one day be used to replace cells damaged by spinal cord injuries or heart attacks. The use of embryonic stem cells in therapeutic cloning is controversial, however, because the removal of ES cells destroys the embryo.

## Umbilical Cord Blood Banking

Another source of stem cells is blood collected from the umbilical cord and placenta at birth **(Figure 11.15)**. Such stem cells appear to be partially differentiated. In 2005, doctors reported that an infusion of umbilical cord blood stem cells appeared to cure some babies of Krabbe disease, a fatal inherited disorder of the nervous system. Other people have received cord blood as a treatment for leukemia. To date, however, most attempts at umbilical cord blood therapy have not been successful. At present, the American Academy of Pediatrics recommends cord blood banking only for babies born into families with a known genetic risk.

## Adult Stem Cells

Embryonic stem cells are not the only stem cells available to researchers. **Adult stem cells** can also generate replacements for some of the body's cells. Adult stem cells are further along the road to differentiation than ES cells and can therefore give rise to only a few related types of specialized cells. For example, stem cells in bone marrow generate different kinds of blood cells. Adult stem cells from donor bone marrow have long been used as a source of immune system cells in patients whose own immune systems have been destroyed by disease or cancer treatments.

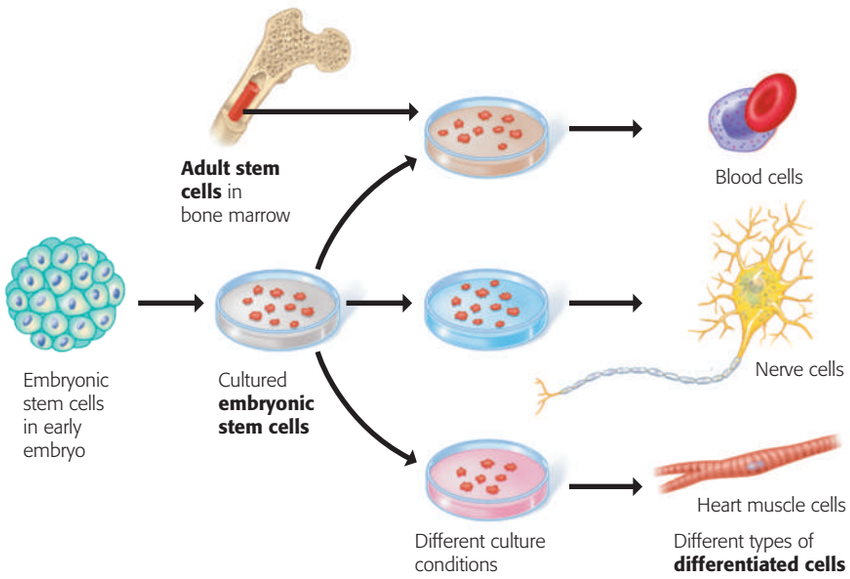
Because no embryonic tissue is involved in their harvest, adult stem cells are less ethically problematic than ES cells. However, many researchers hypothesize that only the more versatile ES cells are likely to lead to groundbreaking advances in human health. Recent research has shown that some adult cells, such as human skin cells, may be reprogrammed to act like ES cells. In the near future, such cells may prove to be both therapeutically useful and ethically clear. ☑

**☑ CHECKPOINT**

How do the results of reproductive cloning and therapeutic cloning differ?

*Answer: Reproductive cloning results in the production of a live individual; therapeutic cloning produces stem cells.*

**▼ Figure 11.14 Differentiation of embryonic stem cells in culture.** Scientists hope to someday discover growth conditions that will stimulate cultured stem cells to differentiate into specialized cells.




**▼ Figure 11.15 Umbilical cord blood banking.** Just after birth, a doctor inserts a needle into the umbilical cord and extracts ¼ to ½ cup of blood. The umbilical cord blood (inset), rich in stem cells, is frozen and kept in a blood bank, where it is available if needed for medical treatment.

# The Genetic Basis of Cancer

Cancer includes a variety of diseases in which cells escape from the control mechanisms that normally limit their growth and division (as introduced in Chapter 8). This escape involves changes in gene expression.

## Genes That Cause Cancer

One of the earliest clues to the role of genes in cancer was the discovery in 1911 of a virus that causes cancer in chickens. Viruses that cause cancer can become permanent residents in host cells by inserting their nucleic acid into the DNA of host chromosomes. Over the last century, researchers have identified a number of viruses that harbor cancer-causing genes. One example is the human papillomavirus (HPV), which can be transmitted through sexual contact and is associated with several types of cancer, including cervical cancer.

### Oncogenes and Tumor-Suppressor Genes

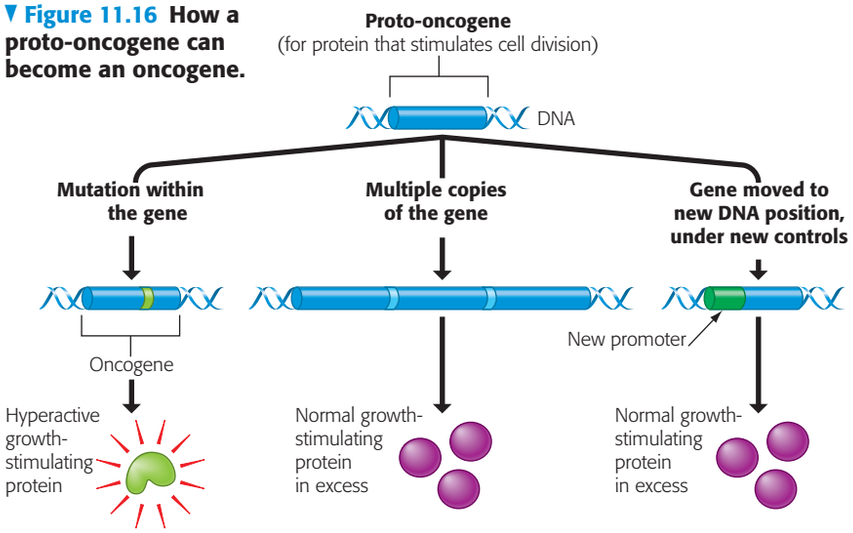
In 1976, American molecular biologists J. Michael Bishop, Harold Varmus, and their colleagues made a startling discovery. They found that a cancer-causing chicken virus contains a cancer-causing gene that is an altered version of a normal chicken gene. A gene that causes cancer is called an **oncogene** ("tumor gene"). Subsequent research has shown that the chromosomes of many animals, including humans, contain genes that can be converted to oncogenes. A normal gene with the potential to become an oncogene is called a **proto-oncogene**. (These terms can be confusing, so let's repeat them: A *proto-oncogene* is a normal, healthy gene that, if changed, can become a cancer-causing *oncogene*.) A cell can acquire an oncogene from a virus or from the mutation of one of its own proto-oncogenes.

How can a change in a gene cause cancer? Searching for the normal roles of proto-oncogenes in the cell, researchers found that many of these genes code for **growth factors**—proteins that stimulate cell division—or for other proteins that affect the cell cycle. When all these proteins are functioning normally, in the right amounts at the right times, they help keep the rate of cell division at an appropriate level. When they malfunction—if a growth factor becomes hyperactive, for example—cancer (uncontrolled cell growth) may result.

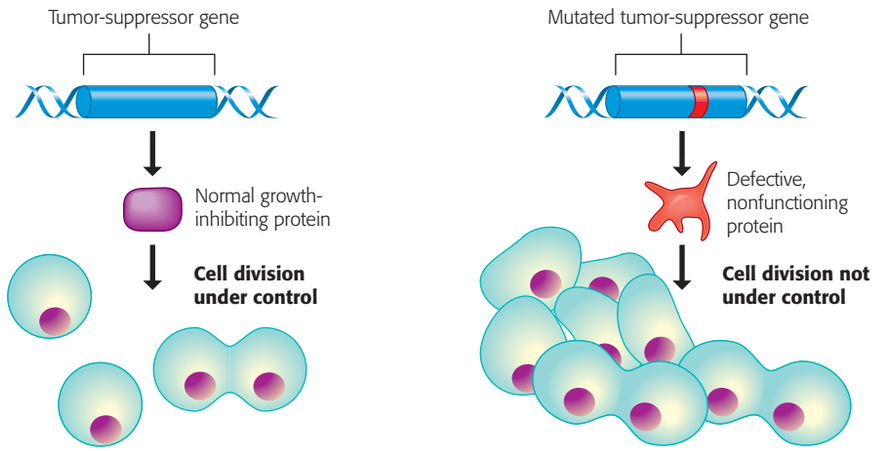
For a proto-oncogene to become an oncogene, a mutation must occur in the cell's DNA. **Figure 11.16** illustrates three kinds of changes in DNA that can produce active oncogenes. In all three cases, abnormal gene expression stimulates the cell to divide excessively.

Changes in genes whose products inhibit cell division are also involved in cancer. These genes are called **tumor-suppressor genes** because the proteins they encode normally help prevent uncontrolled cell growth **(Figure 11.17)**. Any mutation that keeps a growth-inhibiting protein from being made or from functioning may contribute to the development of cancer. Researchers have identified many mutations in both tumor-suppressor and growth factor genes that are associated with cancer, as we'll discuss next.

**▼ Figure 11.16 How a proto-oncogene can become an oncogene.**

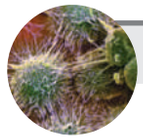



**▼ Figure 11.17 Tumor-suppressor genes.**




**(a) Normal cell growth.** A tumor-suppressor gene normally codes for a protein that inhibits cell growth and division. Such genes help prevent cancerous tumors from arising or spreading.

**(b) Uncontrolled cell growth (cancer).** When a mutation in a tumor-suppressor gene makes its protein defective, cells that are usually under the control of the normal protein may divide excessively, forming a tumor.



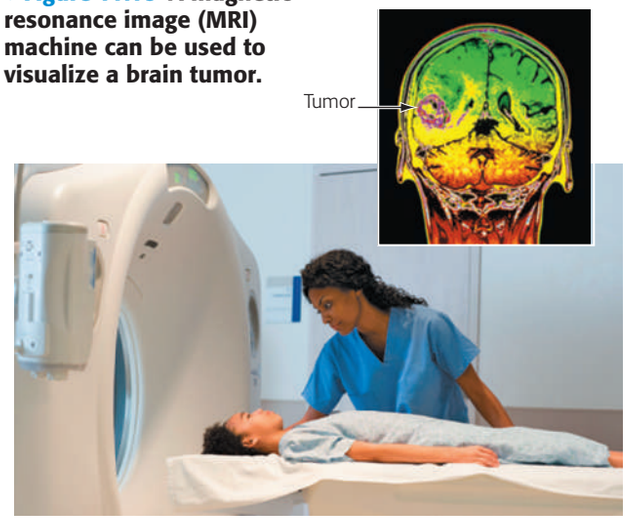
## Cancer THE PROCESS OF SCIENCE

# Are Childhood Tumors Different?

Medical researchers have made many **observations** of specific mutations that can lead to cancer. They therefore **question** whether different kinds of cancers are associated with specific mutations. A large research team led by the Johns Hopkins Kimmel Cancer Center in Baltimore formed the **hypothesis** that young patients with medulloblastoma (MB)—the most common pediatric brain cancer and the deadliest form of childhood cancer **(Figure 11.18)**—harbor unique mutations. They made the **prediction** that a genetic map of MB cells from childhood tumors would have cancer-associated mutations not found in adult brain cancer tissue.

The **experiment** involved sequencing all the genes in tumors removed from 22 pediatric MB patients and comparing them with normal tissue from these same patients. Their **results** showed that each tumor had an average of 11 mutations. Although this may seem like a lot, it is actually five to ten times fewer than the number of mutations associated with MB in adult patients. Young MB patients therefore seem to have fewer, but deadlier, mutations. When they investigated the role that the mutated genes play, the research team found that some help control DNA packing, whereas others play a role in the development of organs. The researchers hope that this new knowledge about the genetic basis of MB may be used to develop new therapies for this often fatal disease.

**▼ Figure 11.18 A magnetic resonance image (MRI) machine can be used to visualize a brain tumor.**

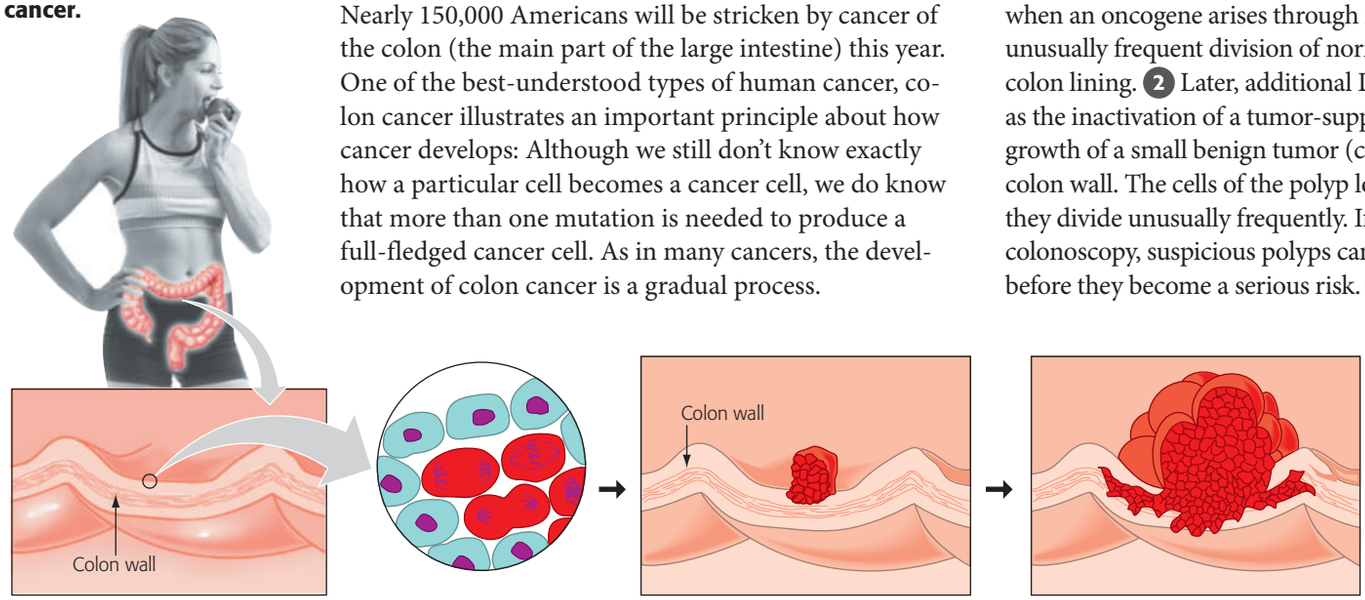



## The Progression of a Cancer

Nearly 150,000 Americans will be stricken by cancer of the colon (the main part of the large intestine) this year. One of the best-understood types of human cancer, colon cancer illustrates an important principle about how cancer develops: Although we still don't know exactly how a particular cell becomes a cancer cell, we do know that more than one mutation is needed to produce a full-fledged cancer cell. As in many cancers, the development of colon cancer is a gradual process.

As shown in **Figure 11.19**, ❶ colon cancer begins when an oncogene arises through mutation, causing unusually frequent division of normal-looking cells in the colon lining. ❷ Later, additional DNA mutations (such as the inactivation of a tumor-suppressor gene) cause the growth of a small benign tumor (called a polyp) in the colon wall. The cells of the polyp look normal, although they divide unusually frequently. If detected during a colonoscopy, suspicious polyps can usually be removed before they become a serious risk. ❸ Further mutations

**▼ Figure 11.19 Stepwise development of colon cancer.**



eventually lead to formation of a malignant tumor—a tumor that has the potential to metastasize (spread). It typically takes at least six DNA mutations (usually creating at least one active oncogene and disabling at least one tumor-suppressor gene) before a cell becomes fully cancerous.

The development of a malignant tumor is accompanied by a gradual accumulation of mutations that convert proto-oncogenes to oncogenes and knock out tumor-suppressor genes **(Figure 11.20)**. The requirement for several DNA mutations—usually four or more—explains why cancers can take a long time to develop. This requirement may also help explain why the incidence of cancer increases with age; the longer we live, the more likely we are to accumulate mutations that cause cancer.

## Inherited Cancer

The fact that multiple genetic changes are required to produce a cancer cell helps explain the observation that cancers can run in families. An individual inheriting an oncogene or a mutant version of a tumor-suppressor gene is one step closer to accumulating the necessary mutations for cancer to develop than is an individual without any such mutations. Geneticists are therefore devoting much effort to identifying inherited cancer mutations so that predisposition to certain cancers can be detected early in life.

About 15% of colorectal cancers, for example, involve inherited mutations. There is also evidence that inheritance plays a role in 5–10% of patients with breast cancer, a disease that strikes one out of every ten American women **(Figure 11.21)**. Mutations in either or both of two genes—called *BRCA1* (pronounced "braca-1") and *BRCA2*—are found in at least half of inherited breast cancers. Both *BRCA1* and *BRCA2* are considered tumor-suppressor genes because the normal versions protect against breast cancer. A woman who inherits one mutant *BRCA1* allele has a 60% probability of developing breast cancer before the age of 50, compared with only a 2% probability for an individual lacking the mutations. Tests using DNA sequencing can now detect these mutations. Unfortunately, these tests are of limited use because surgical removal of the breasts and/or ovaries is the only preventive option currently available to women who carry the mutant genes. ☑

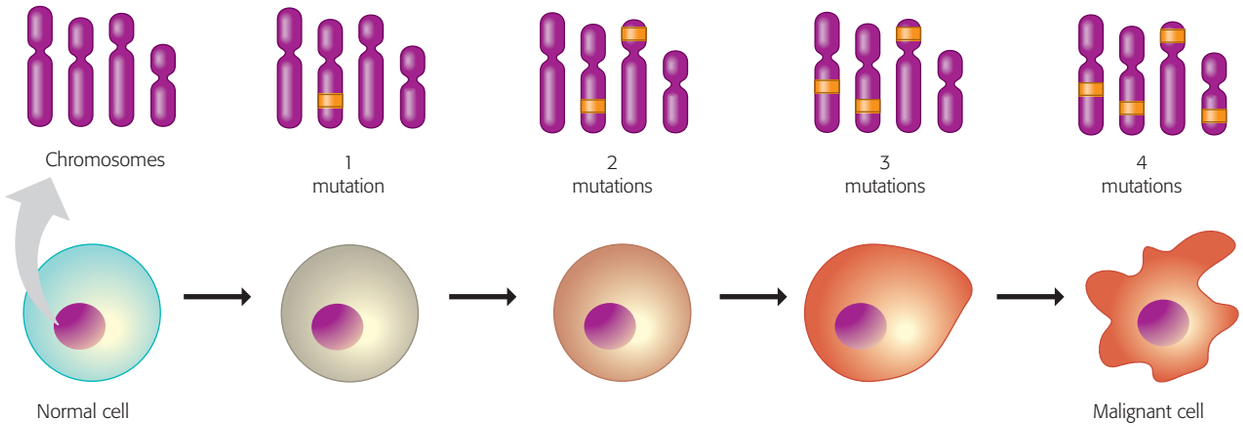
▲ **Figure 11.21 Breast cancer.** In 2013, at age 37, the actress Angelina Jolie underwent a preventive double mastectomy after learning she had a mutant *BRCA1* gene. Jolie's mother, grandmother, and aunt all died young from breast or ovarian cancer.

**☑ CHECKPOINT**

How can a mutation in a tumor-suppressor gene contribute to the development of cancer?

*Answer: A mutated tumor-suppressor gene may produce a defective protein unable to function in a pathway that normally inhibits cell division and therefore normally suppresses tumors.*

▼ **Figure 11.20 Accumulation of mutations in the development of a cancer cell.** Mutations leading to cancer accumulate in a lineage of cells. In this figure, colors distinguish the normal cells from cells with one or more mutations, leading to increased cell division and cancer. Once a cancer-promoting mutation occurs (orange band on chromosome), it is passed to all the descendants of the cell carrying it.



# Cancer Risk and Prevention

Cancer is the second-leading cause of death (after heart disease) in most industrialized countries. Death rates due to certain forms of cancer have decreased in recent years, but the overall cancer death rate is still on the rise, currently increasing at about 1% per decade.

Although some cancers occur spontaneously, most cancers arise from mutations that are caused by **carcinogens**, cancer-causing agents found in the environment. Mutations often result from decades of exposure to carcinogens. One of the most potent carcinogens is ultraviolet (UV) radiation. Excessive exposure to UV radiation from the sun can cause skin cancer, including a deadly type called melanoma. You can decrease your risk by using sun protection (clothing, lotion, hats, etc.).

The one substance known to cause more cases and types of cancer than any other is tobacco. By a wide margin, more people die from lung cancer (nearly 160,000 Americans in 2014) than from any other form of cancer. Most tobacco-related cancers are due to smoking cigarettes, but smoking cigars, inhaling secondhand smoke, and smokeless tobacco also pose risks. As **Table 11.1** indicates, tobacco use, sometimes in combination with alcohol consumption, causes several types of cancer. Exposure to some of the most lethal carcinogens is often a matter of individual choice: Tobacco use, the consumption of alcohol, and excessive time spent in the sun are all avoidable behaviors that affect cancer risk.

Some food choices significantly reduce a person's odds of developing cancer. For instance, eating 20–30 g of plant fiber daily (about the amount found in seven apples), while eating less animal fat may help prevent colon cancer. There is also evidence that certain substances in fruits and vegetables, including vitamins C and E and certain compounds related to vitamin A, may help protect against a variety of cancers. Cabbage and its relatives, such as broccoli and cauliflower, are thought to be especially rich in substances that help prevent cancer, although some of the specific substances have not yet been identified. Determining how diet influences cancer has become an important focus of nutrition research.

**Lifestyle choices you make can dramatically affect your risk of cancer.**

The battle against cancer is being waged on many fronts, and there is reason for optimism in the progress being made. It is especially encouraging that we can help reduce our risk of acquiring some of the most common forms of cancer by the choices we make in our daily lives. ✓

**✓ CHECKPOINT**

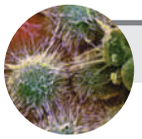
Of all known behavioral factors, which one causes the most cancer cases and deaths?

Answer: tobacco use

**Table 11.1 Cancer in the United States (Ranked by Number of Cases)**

| Cancer | Known or Likely Carcinogens or Factors | Estimated Cases (2014) | Estimated Deaths (2014) |
|---|---|---|---|
| Breast | Estrogen; possibly dietary fat | 235,000 | 40,400 |
| Prostate | Testosterone; possibly dietary fat | 233,000 | 29,500 |
| Lung | Cigarette smoke | 224,000 | 159,000 |
| Colon and rectum | High dietary fat; low dietary fiber | 136,800 | 50,310 |
| Skin | Ultraviolet light | 81,200 | 13,000 |
| Lymphomas | Viruses (for some types) | 80,000 | 20,100 |
| Bladder | Cigarette smoke | 74,700 | 15,600 |
| Uterus | Estrogen | 65,000 | 12,600 |
| Kidney | Cigarette smoke | 63,900 | 13,900 |
| Leukemias | X-rays; benzene; viruses (for some types) | 52,400 | 24,100 |
| Pancreas | Cigarette smoke | 46,400 | 39,600 |
| Liver | Alcohol; hepatitis viruses | 33,200 | 23,000 |
| Brain and nerve | Trauma; X-rays | 23,400 | 14,300 |
| Stomach | Table salt; cigarette smoke | 22,200 | 11,000 |
| Ovary | Large number of ovulation cycles | 22,000 | 14,300 |
| Cervix | Viruses; cigarette smoke | 12,400 | 4,000 |
| All other types | | 259,940 | 101,010 |
| Total | | 1,665,540 | 585,720 |

*Data from: Cancer Facts and Figures 2014 (American Cancer Society Inc.).*



## Cancer EVOLUTION CONNECTION

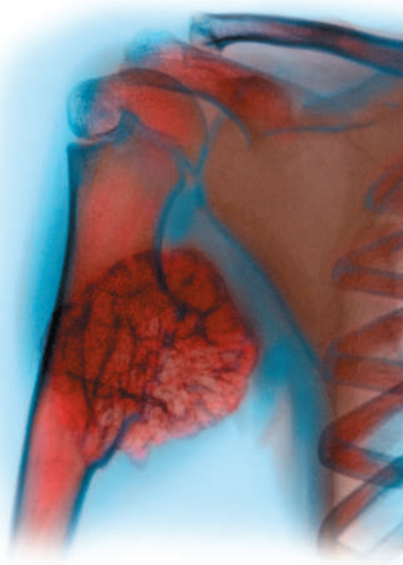
# The Evolution of Cancer in the Body

The theory of evolution describes natural selection acting on populations. Recently, medical researchers have been using an evolutionary perspective to gain insight into the development of tumors, such as the bone tumor shown in **Figure 11.22**. Evolution drives the growth of a tumor—which can be thought of as a population of cancer cells—and also affects how those cells respond to cancer treatments.

Recall that there are several assumptions behind Darwin's theory of natural selection (see Chapter 1). Let's consider how each one can be applied to cancer. First, all evolving populations have the potential to produce more offspring than can be supported by the environment. Cancer cells, with their uncontrolled growth, clearly demonstrate overproduction. Second, there must be variation among individuals of the population. Studies of tumor cell DNA, like the one described in the Process of Science section, show genetic variability within tumors. Third, variations in the population must affect survival and reproductive success. Indeed, the accumulation of mutations in cancer cells renders them less susceptible to normal mechanisms of reproductive control. Mutations that enhance survival of malignant cancer cells are passed on to that cell's descendants. In short, a tumor evolves.

Viewing the progression of cancer through the lens of evolution helps explain why there is no easy "cure" for cancer but may also pave the way for novel therapies. For example, some researchers are attempting to "prime" tumors for treatment by increasing the reproductive success of only those cells that will be susceptible to a chemotherapy drug. Our understanding of cancer, like all other aspects of biology, benefits from an evolutionary perspective.

**▼ Figure 11.22 X-ray of shoulder and upper arm, revealing a large bone tumor.**



# Chapter Review

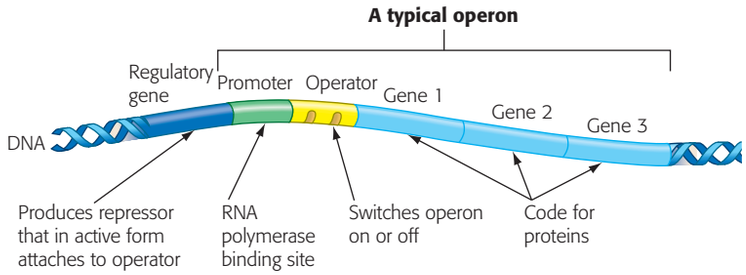
## SUMMARY OF KEY CONCEPTS

### How and Why Genes Are Regulated

The various types of cells in a multicellular organism owe their distinctiveness to different combinations of genes being turned on and off via gene regulation in each cell type.

### Gene Regulation in Bacteria

An operon is a cluster of genes with related functions together with their promoter and other DNA sequences that control their transcription. For example, the *lac* operon allows *E. coli* to produce enzymes for lactose use only when the sugar is present.




### Gene Regulation in Eukaryotic Cells

In the nucleus of eukaryotic cells, there are several possible control points in the pathway of gene expression.

- DNA packing tends to block gene expression by preventing access of transcription proteins to the DNA. An extreme example is X chromosome inactivation in the cells of female mammals.
- The most important control point in both eukaryotes and prokaryotes is at gene transcription. Various regulatory proteins interact with DNA and with each other to turn the transcription of eukaryotic genes on or off.
- There are also opportunities for the control of eukaryotic gene expression after transcription, when introns are cut out of the RNA and a cap and tail are added to process RNA transcripts into mRNA.
- In the cytoplasm, presence of microRNAs may block the translation of an mRNA, and various proteins may regulate the start of translation.
- Finally, the cell may activate the finished protein in various ways (for instance, by cutting out portions or chemical modification). Eventually, the protein may be selectively broken down.