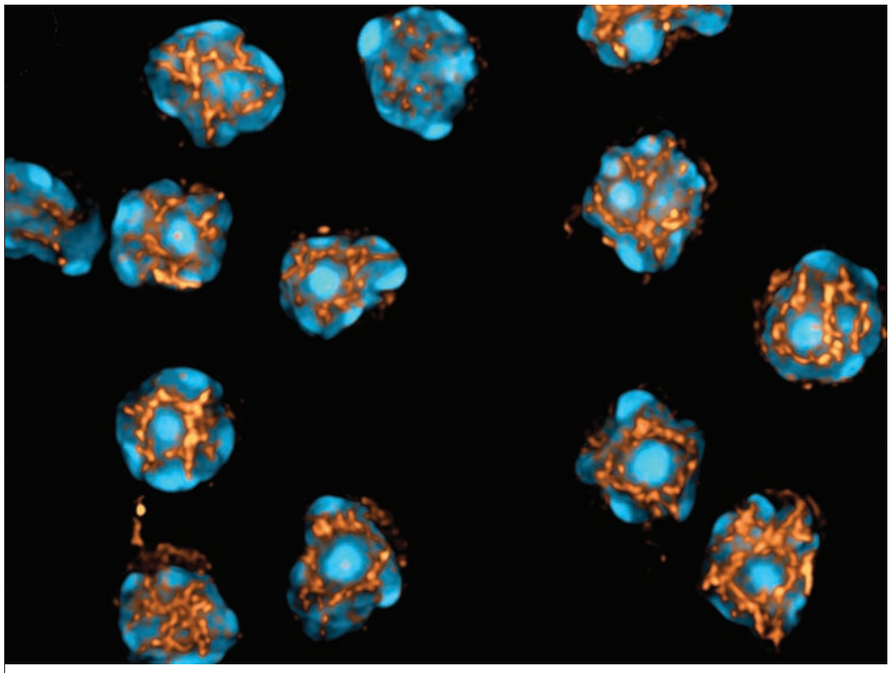


Abby Dernburg and Terumi Kohwi-Shigematsu/Lawrence Berkeley National Laboratory

Chromatin remodeling proteins (gold) binding to chromatin (blue). Chromatin remodeling, a change in chromosome structure in the region of a gene, is a key step in the activation of genes in eukaryotes.

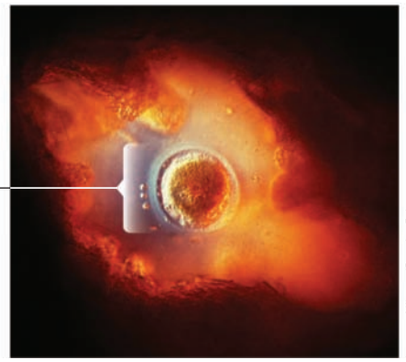
Study Plan

16 Control of Gene Expression

Why It Matters

A human egg cell is almost completely inactive metabolically when it is released from the ovary. It remains quiescent as it begins its travel down a fallopian tube leading from the ovary to the uterus, carried along by movements of cilia lining the walls of the tube **(Figure 16.1).** It is here, in the fallopian tube, that egg and sperm cells meet and embryonic development begins. Within seconds after the cells unite, the fertilized egg breaks its quiescent state and begins a series of divisions that continues as it moves through the fallopian tube and enters the uterus. Subsequent divisions produce specialized cells that *differentiate* into the distinct types tailored for specific functions in the body, such as muscle cells and cells of the nervous system.

All the nucleated cells of the developing embryo retain the same set of genes. The structural and functional differences in the cell types are determined not by the presence or absence of certain genes but rather through differences in *gene activity.* Some genes, known as housekeeping genes, are active in almost all cells; other genes are turned on or off ("expressed" or "not expressed") depending on the cell type. Each differentiated cell is characterized by genes that are active in only that cell type. For example, all mammalian cells carry



Lennart Nilsson/Bonnier Fakta

Figure 16.1
A human egg at the time of its release from the ovary. The outer layer appearing light blue in color is a coat of polysaccharides and glycoproteins that surrounds the egg. Within the egg, genes and regulatory proteins are poised to enter the pathways initiating embryonic development.

the genes for hemoglobin, but these genes are active only in cells that give rise to red blood cells. Specific regulatory events that take place only in red blood cells activate the hemoglobin genes in those cells. Those genes are not activated in other cell types.

The fundamental mechanisms that control gene activity are common to all multicellular eukaryotes. Even single-celled eukaryotes and prokaryotes have systems that turn genes on or off when required. With few exceptions, however, prokaryotic systems are limited almost exclusively to short-term responses to environmental changes; eukaryotic cells exhibit both short-term response and long-term differentiation.

The processes that directly control gene activity are known collectively as **transcriptional regulation.** Transcriptional regulation, the fundamental level of control, determines which genes are transcribed into mRNA. Additional controls fine-tune regulation by affecting the processing of mRNA *(posttranscriptional regulation)*, its translation into proteins *(translational regulation)*, and the life span and activity of the proteins themselves *(posttranslational regulation)*.

These levels of regulation ultimately affect more than proteins, because among the proteins are enzymes that determine the types and kinds of all other molecules made in the developing cell. So, effectively, these regulatory mechanisms tailor the production of all cellular molecules. The entire spectrum of controls constitutes an exquisitely sensitive mechanism regulating when, where, and what kinds and numbers of cellular molecules are produced.

In this chapter we examine the mechanisms of transcriptional regulation and its fine-tuning by additional controls at the posttranscriptional, translational, and posttranslational levels. Our discussion begins with bacterial systems, where researchers first discovered a mechanism for transcriptional regulation, and then moves to eukaryotic systems where the regulation of gene activity is more complicated. How genes regulate development is discussed in Chapter 48.

16.1 Regulation of Gene Expression in Prokaryotes

Transcription and translation are closely regulated in prokaryotes in ways that reflect prokaryotic life histories. Prokaryotes are relatively simple, single-celled organisms with generations that come and go in a matter of minutes. Rather than the complex patterns of long-term cell differentiation and development typical of multicellular eukaryotes, prokaryotic cells typically undergo rapid and reversible alterations in biochemical pathways that allow them to adapt quickly to changes in their environment.

The human intestinal bacterium *Escherichia coli*, for example, can metabolize a wide range of nutrients including lactose (milk sugar). When lactose is present, *E. coli* makes enzymes for metabolizing the sugar, but it does not make those enzymes when lactose is absent. The versatile and responsive control system allows the bacterium to use the nutrients available in the surrounding medium with maximum efficiency.

The Operon Is the Unit of Transcription in Prokaryotes

When the environment in which a bacterium lives changes, some metabolic processes are stopped and others are started. Typically, this involves turning off the genes for the metabolic processes not needed and turning on the genes for the new metabolic processes. For each metabolic process, there are a few to many genes involved, and the regulation of those genes must be coordinated. For example, three genes encode enzymes for the metabolism of lactose by *E. coli*. In the absence of lactose, the three genes are not expressed, while in the presence of lactose, the genes are expressed. That is, the control of these genes is at the transcription level.

In 1961, François Jacob and Jacques Monod of the Pasteur Institute in Paris proposed the *operon model* for the control of the expression of genes for lactose metabolism in *E. coli*. Subsequently, the *operon model* has been shown to be widely applicable to the regulation of gene expression in bacteria and their viruses. Jacob and Monod received the Nobel Prize in 1965 for their discovery and explanation of bacterial operons and their regulation by repressors.

An **operon** is a cluster of prokaryotic genes and the DNA sequences involved in their regulation. One of those DNA sequences is the **promoter,** which is the site to which RNA polymerase binds as it starts transcription of the gene. Each operon, which can contain several to many genes, is transcribed as a unit from the promoter into a single mRNA, and as a result the mRNA contains codes for several proteins. The cluster of genes transcribed into a single mRNA is called a **transcription unit.** A ribosome translates the mRNA from one end to the other, sequentially making each protein encoded in the mRNA. Typically, the proteins encoded by an operon catalyze steps in the same function, such as enzymes acting in sequence in a biochemical pathway.

The other DNA regulatory sequence in the operon is the **operator,** a short segment to which a regulatory protein binds. The regulatory protein is encoded by a gene separate from the operon the protein controls. Some operons are controlled by a regulatory protein termed a **repressor,** which, when active, prevents the operon genes

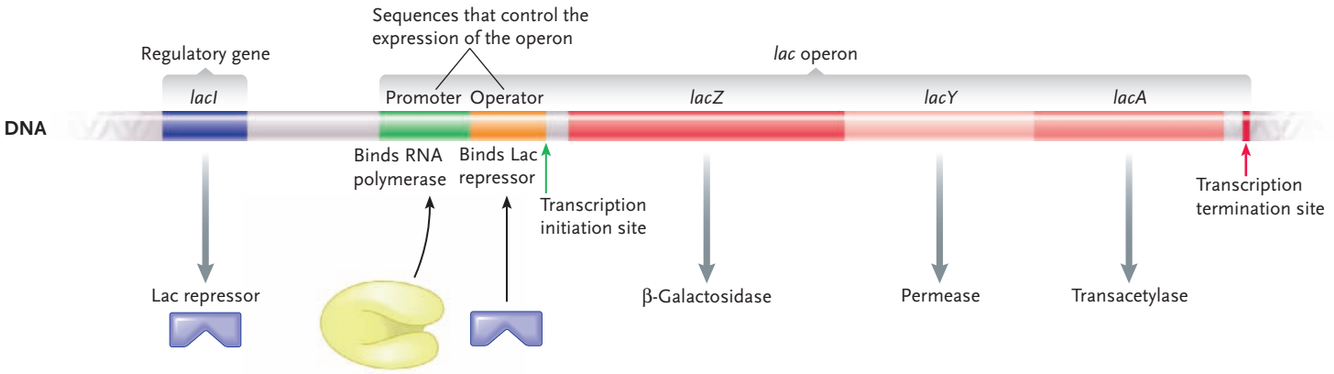


Figure 16.2
The *E. coli lac* operon. The *lacZ*, *lacY*, and *lacA* genes encode the enzymes taking part in lactose metabolism. The separate regulatory gene, *lacI*, encodes the Lac repressor, which plays a pivotal role in the control of the operon. The promoter binds RNA polymerase, and the operator binds activated Lac repressor. The transcription unit, which extends from the transcription initiation site to the transcription termination site, contains the genes.

from being expressed. Other operons are controlled by a regulatory protein termed an *activator,* which, when active, turns on the expression of the genes.

Many operons are controlled by more than one regulatory mechanism, and a number of the repressors or activators control more than one operon. The result is a complex network of superimposed controls that provides total regulation of transcription and allows almost instantaneous responses to changing environmental conditions.

The *lac* Operon for Lactose Metabolism Is Transcribed When an Inducer Inactivates a Repressor

Jacob and Monod researched the genetic control of lactose metabolism in *E. coli*. Lactose is a sugar that, when metabolized, provides energy for the cell. Jacob and Monod used genetic and biochemical approaches to study the genetic control of lactose metabolism in *E. coli*. Their genetic studies showed that three genes are involved: *lacZ, lacY,* and *lacA,* for lactose metabolism **(Figure 16.2).** These three genes are adjacent to one another on the chromosome in the order *Z-Y-A*. The genes are transcribed as a unit into a single mRNA starting with the *lacZ* gene; the promoter for the transcription unit is upstream of *lacZ*. The *lacZ* gene encodes the enzyme β-galactosidase, which catalyzes the conversion of the disaccharide sugar, lactose, into the monosaccharide sugars, glucose and galactose. These sugars are then metabolized by other enzymes, producing energy for the cell. The *lacY* gene encodes a permease enzyme that transports lactose actively into the cell, and the *lacA* gene encodes a transacetylase enzyme, the function of which is unknown.

Jacob and Monod called the cluster of genes and adjacent sequences that control their expression the *lac operon* (see Figure 16.2). They coined the name *operon* from a key DNA sequence they discovered for regulating transcription of the operon—the **operator.** The operator was named because it controls the operation of the genes adjacent to it. For the *lac* operon, the operator is a short DNA sequence between the promoter and the *lacZ* gene.

The two investigators found that the *lac* operon was controlled by a regulatory protein that they termed the *Lac repressor.* The Lac repressor is encoded by the regulatory gene *lacI,* which is nearby but separate from the *lac* operon (see Figure 16.2), and is synthesized in active form. When lactose is absent from the medium, active Lac repressor binds to the operator, thereby blocking the RNA polymerase from binding to the promoter; as a result, transcription cannot occur **(Figure 16.3a).** Actually, the repressor occasionally falls off, allowing transcription to occur—but at a very slow rate, leading to just a few molecules of each encoded enzyme in the cell.

When lactose is added to the medium, the *lac* operon is turned on and all three enzymes are synthesized rapidly **(Figure 16.3b).** How does this occur? Lactose enters the cell and the β-galactosidase molecules already present convert some of it to *allolactose,* an isomer of lactose. Allolactose is an **inducer** for the *lac* operon—the isomer turns on the three genes in the operon. Allolactose does this by binding to the Lac repressor, inactivating it by altering its shape so that it no longer can bind to the operator. With the repressor out of the way, RNA polymerase then is able to bind to the promoter, and it transcribes the three genes. The *lac* operon is called an **inducible operon** because an inducer molecule increases its expression.

When the lactose is used up from the medium, the regulatory system again switches the *lac* operon off. That is, the absence of lactose means that there are no allolactose inducer molecules to inactivate the repressor; the again-active repressor binds to the operator, blocking transcription of the operon. The controls are aided by

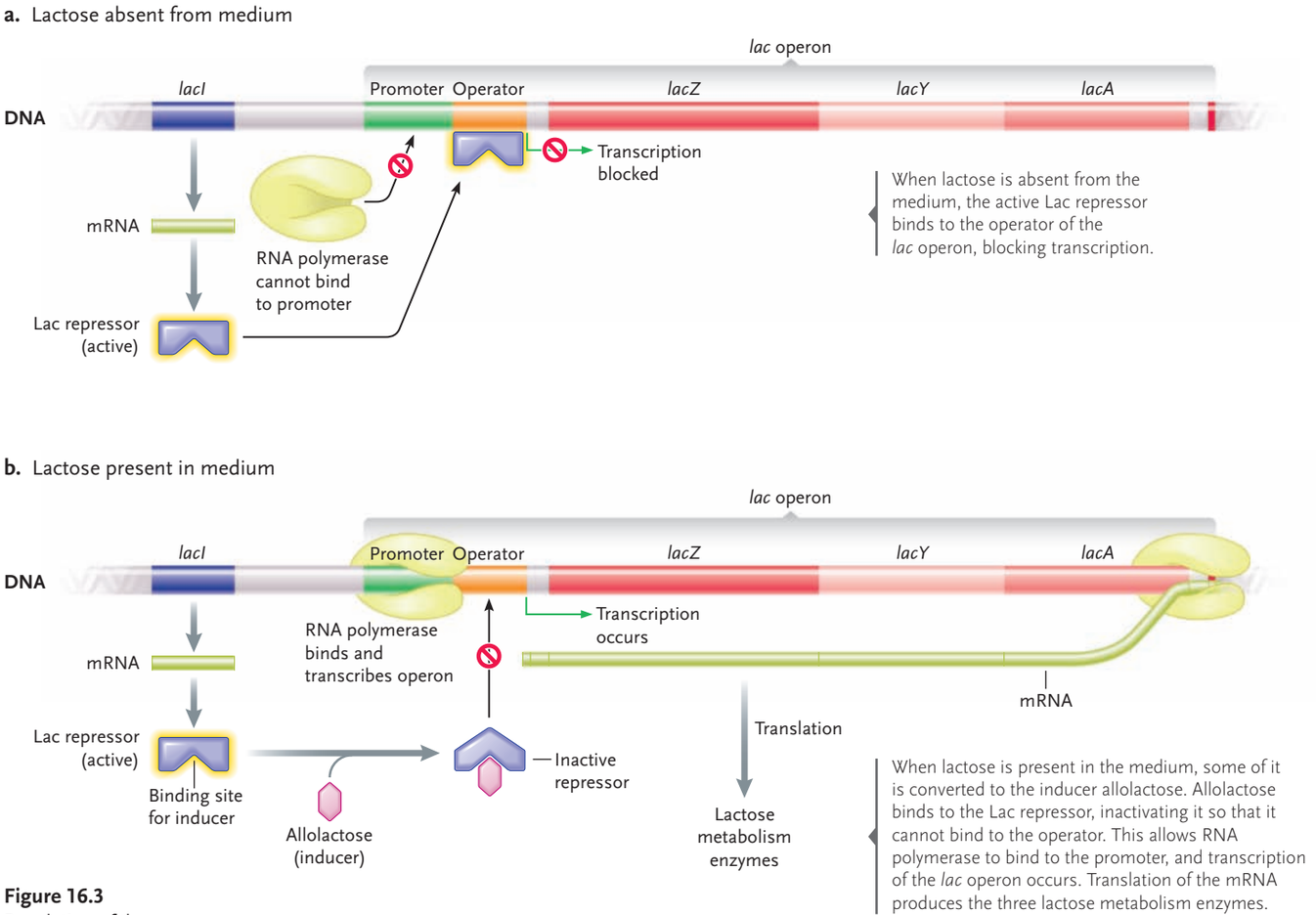


Figure 16.3
Regulation of the inducible *lac* operon by the Lac repressor in the absence **(a)** and presence **(b)** of lactose.

the fact that bacterial mRNAs are very short-lived, about 3 minutes on the average. This quick turnover permits the cytoplasm to be cleared quickly of the mRNAs transcribed from an operon. The enzymes themselves also have short lifetimes, and are quickly degraded.

Transcription of the *trp* Operon Genes for Tryptophan Biosynthesis Is Repressed When Tryptophan Activates a Repressor

Tryptophan is an amino acid that is used in the synthesis of proteins. If tryptophan is absent from the medium, *E. coli* must make tryptophan so that it can synthesize its proteins. If tryptophan is present in the medium, then the cell will use that source of the amino acid rather than making its own.

Tryptophan biosynthesis also involves an operon, the *trp* operon **(Figure 16.4).** The five genes in this operon, *trpA–trpE,* encode the enzymes for the steps in the tryptophan biosynthesis pathway. Upstream of the *trpE* gene are the operon's promoter and operator sequences. Expression of the *trp* operon is controlled by the Trp repressor, a regulatory protein encoded by the *trpR* gene, which is located elsewhere in the genome (not nearby as was the case for the repressor gene for the *lac* operon). In contrast to the Lac repressor, though, the Trp repressor is synthesized in an inactive form in which it cannot bind to the operator.

When tryptophan is absent from the medium and must be made by the cell, the *trp* operon genes are expressed (see Figure 16.4a). This is the default state: since the Trp repressor is inactive and cannot bind to the operator, RNA polymerase can bind to the promoter and transcribe the operon. The resulting mRNA is translated to produce the five tryptophan biosynthetic enzymes that catalyze the reactions for tryptophan synthesis.

If tryptophan is present in the medium, there is no need for the cell to make tryptophan, so the *trp* operon is shut off (see Figure 16.4b). This occurs by a mechanism in which the tryptophan entering the cell binds to the Trp repressor and activates it. The active Trp repressor then binds to the operator of the *trp* operon and blocks RNA polymerase from binding to the promoter—the operon cannot be transcribed.

For the *trp* operon, then, the presence of tryptophan represses the expression of the tryptophan biosynthesis genes; hence, this operon is an example of a **repressible operon.** Here, tryptophan acts as a **corepressor,** a regulatory molecule that combines with a repressor to activate it and shut off the operon.

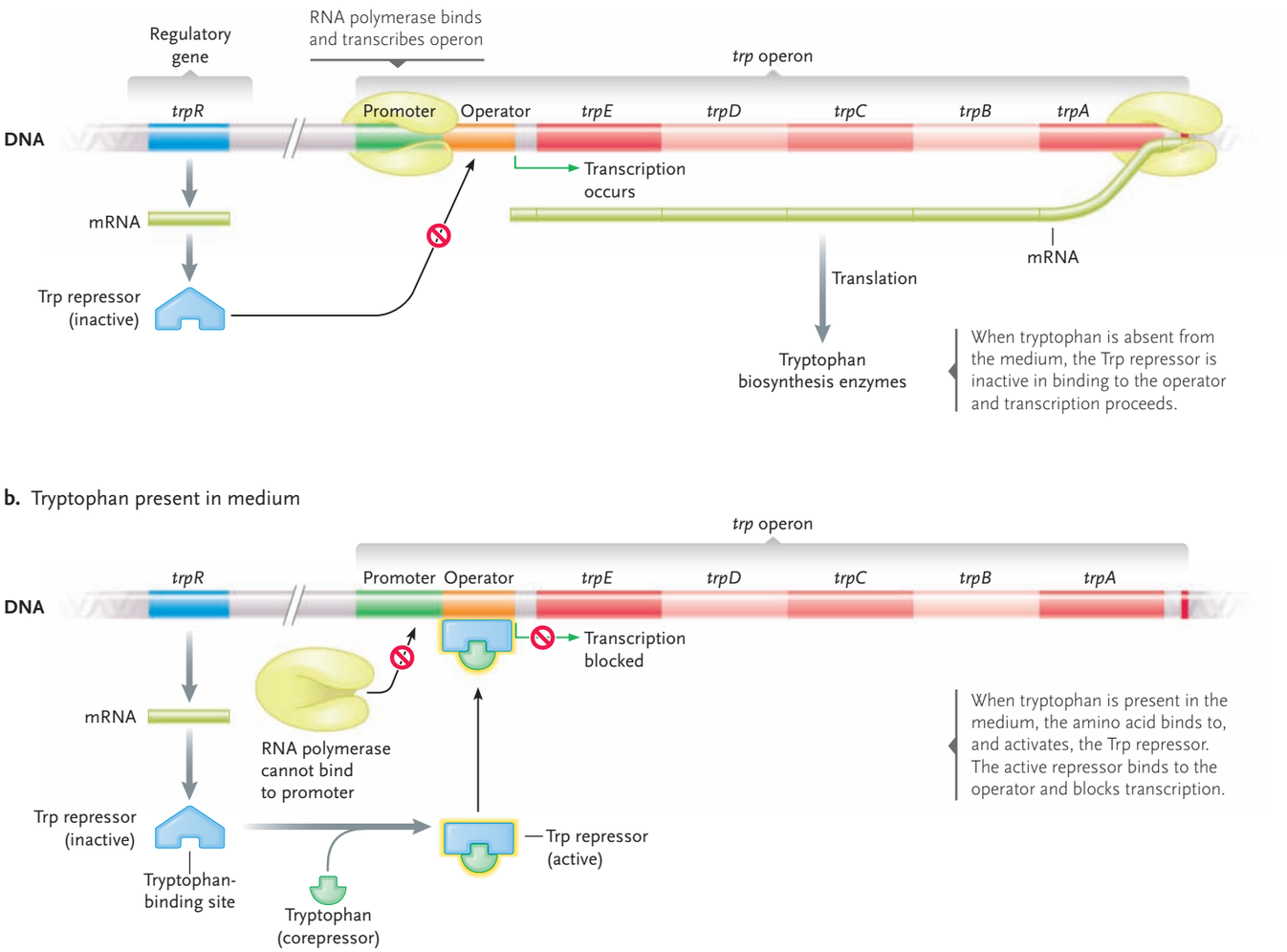


Figure 16.4
Regulation of the repressible *trp* operon by the Trp repressor in the absence **(a)** and presence **(b)** of tryptophan.

To compare and contrast the two operons we have discussed: (1) In the *lac* operon, the repressor is synthesized in an active form. When the inducer (allolactose) is present, it binds to the repressor and inactivates it. The operon is then transcribed. (2) In the *trp* operon, the repressor is synthesized in an inactive form. When the corepressor (tryptophan) is present, it binds to the repressor and activates it. The active repressor blocks transcription of the operon.

Inducible and repressible operons illustrate two types of *negative gene regulation* because both are regulated by a repressor that turns off gene expression when it is in active form. Genes are expressed only when the repressor is in inactive form.

Transcription of the *lac* Operon Is Also Controlled by a Positive Regulatory System

Several years after Jacob and Monod proposed their operon model for the lactose metabolism genes, researchers found a *positive gene regulation* system that also regulates the *lac* operon. This system ensures that the *lac* operon is transcribed if lactose is provided as an energy source, but not if glucose is present in addition to lactose. This is because glucose is a more efficient source of energy than is lactose. Glucose can be used directly in the glycolysis pathway to produce energy for the cell (see Chapter 8). Lactose, on the other hand, must first be converted into glucose and galactose, and the galactose then converted into glucose. These conversions require energy from the cell. Thus the cell gains more net energy by metabolizing glucose than by metabolizing lactose, or for that matter any other sugar.

Figure 16.5a shows the positive gene regulation system working when lactose is present and glucose is absent in the growth medium. In essence, we are adding to the model shown earlier in Figure 16.3b. Lactose is metabolized to the inducer, allolactose, which binds to and inactivates the Lac repressor. RNA polymerase is then recruited to the promoter by active *CAP (catabolite activator protein)* at the *CAP site,* a DNA sequence immediately upstream of the pro-

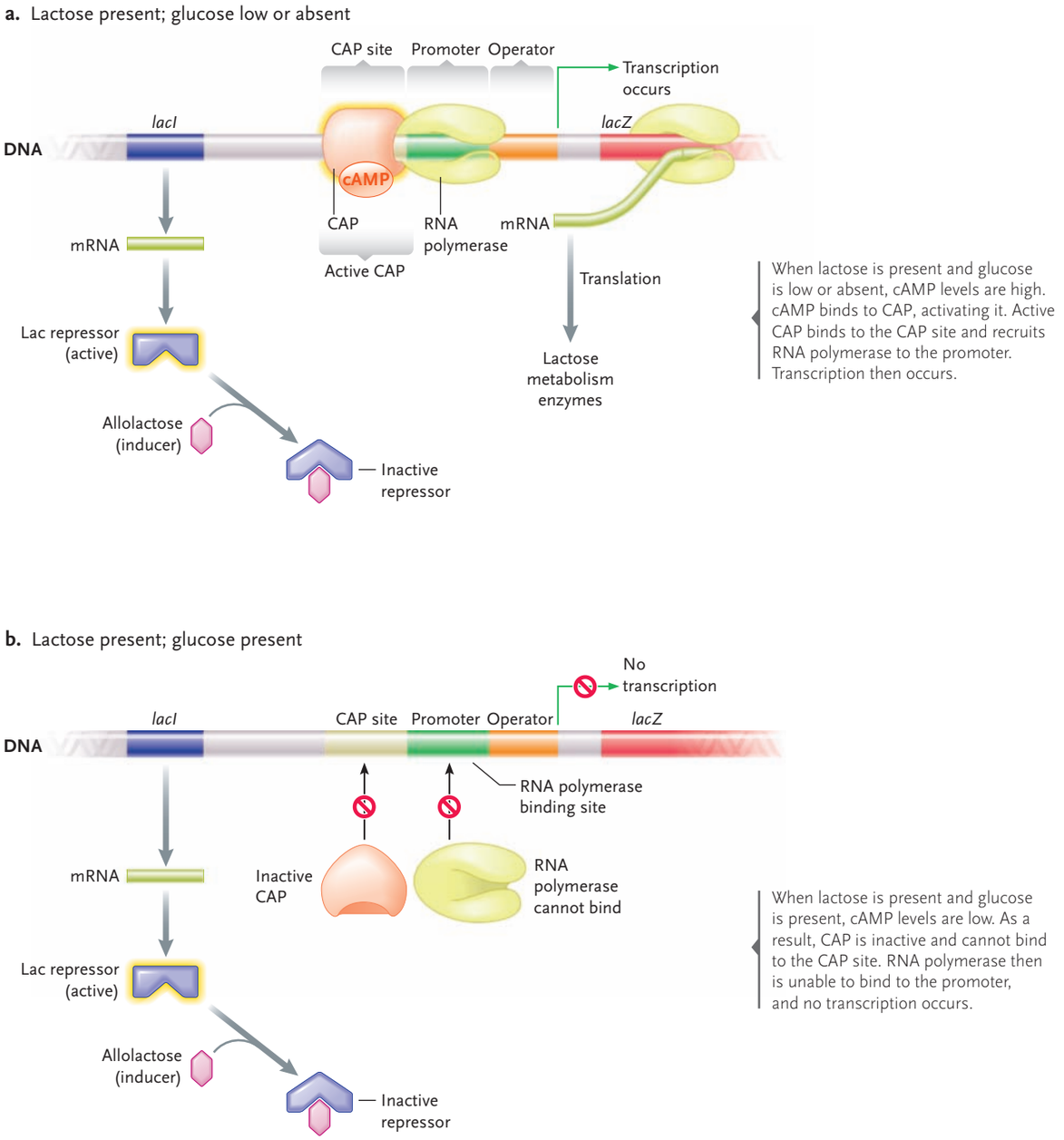


Figure 16.5
Positive regulation of the *lac* operon by the CAP activator. Other operons involved in the metabolism of various sugars are regulated in the same way.

moter. CAP is an **activator,** a regulatory protein that stimulates gene expression. It is synthesized in *inactive* form and is activated when cAMP (cyclic AMP) binds to it (cAMP is a nucleotide that plays a role in regulating cellular processes in both prokaryotes in eukaryotes; see Section 7.4). When glucose is absent from the medium, cAMP is abundant in the cell, so CAP is active under these conditions and can bind to the CAP site.

If both lactose and glucose are present in the medium, the *lac* operon is not transcribed **(Figure 16.5b).** Metabolism of the incoming glucose triggers a series of events leading to inactivation of adenylyl cyclase, the enzyme that catalyzes the synthesis of cAMP from ATP. The level of cAMP drops drastically, reaching a point when it is too low to activate CAP. Without active CAP bound to the CAP site, RNA polymerase is unable to bind to the promoter, and the operon cannot be transcribed. In short, gene expression cannot be activated under these conditions. When the glucose is depleted from the medium, the bacteria then shift to metabolizing the lactose in the medium. Inactivation of adenylyl cyclase is reversed, cAMP levels rise again, and CAP is activated. The events of Figure 16.5a then occur.

The same positive gene regulation system using CAP and cAMP regulates a large number of other operons that control the metabolism of many sugars. In each case, the system functions so that glucose, if

it is present in the growth medium, is metabolized first.

In sum, regulation of gene expression in prokaryotes occurs primarily at the transcription level. There are also some examples of regulation at the translation level. For example, some proteins can bind to the mRNAs that produce them and modulate their translation. This serves as a feedback mechanism to fine-tune the amounts of the proteins in the cell. In the remainder of the chapter we discuss the regulation of gene expression in eukaryotes. You will see that regulation occurs at several points between the gene and the protein, and that regulatory mechanisms are more complex than those in prokaryotes.

Study Break

1. Suppose the *lacI* gene is mutated so that the Lac repressor is not made. How does this mutation affect the regulation of the *lac* operon?
2. Answer the equivalent question for the *trp* operon: How would a mutation that prevents the Trp repressor from being made affect the regulation of the *trp* operon?

16.2 Regulation of Transcription in Eukaryotes

As you just learned, gene expression in prokaryotes is commonly regulated at the transcription level with genes organized in functional units called operons. The molecular mechanisms in operon function are a simple means of coordinating synthesis of proteins with related functions. In eukaryotes, the coordinated synthesis of proteins with related functions also occurs, but the genes involved usually are scattered around the genomes; that is, they are not organized into operons. Nonetheless, like operons, individual eukaryotic genes also consist of protein-coding sequences and adjacent regulatory sequences.

There are two general categories of eukaryotic gene regulation. Short-term regulation involves regulatory events in which gene sets are quickly turned on or off in response to changes in environmental or physiological conditions in the cell's or organism's environment. This type of regulation is most similar to prokaryotic gene regulation. Long-term gene regulation involves regulatory events required for an organism to develop and differentiate. Long-term gene regulation occurs in multicellular eukaryotes and not in simpler, unicellular eukaryotes. The mechanisms we discuss in this and the next section are applicable to both short-term and long-term regulation. The specific molecules and genes involved are different and, of course, so is the outcome to the cell or organism.

In Eukaryotes, Regulation of Gene Expression Occurs at Several Levels

The regulation of gene expression is more complicated in eukaryotes than in prokaryotes because eukaryotic cells are more complex, because the nuclear DNA is organized with histones into chromatin, and because multicellular eukaryotes produce large numbers and types of cells. Further, the eukaryotic nuclear envelope separates the processes of transcription and translation, whereas in prokaryotes translation can start on an mRNA that is still being made. Consequently, gene expression in eukaryotes is regulated at more levels. That is, there is transcriptional regulation, posttranscriptional regulation, translational regulation, and posttranslational regulation **(Figure 16.6).** The most important of these is transcriptional regulation.

Chromatin Structure Plays an Important Role in Whether a Gene Is Active or Inactive

Eukaryotic DNA is organized into chromatin by combination with histone proteins (discussed in Section 14.5). Recall that DNA is wrapped around a core of two molecules each of histones H2A, H2B, H3, and H4, forming the nucleosome (see Figure 14.18). Higher levels of chromatin organization occur when histone H1 links adjacent nucleosomes.

Genes in regions of the DNA that are tightly wound around histones in chromatin are inactive, because their promoters are not accessible to the proteins that initiate transcription. Activating a gene involves changing the state of the chromatin so that the proteins that initiate transcription can bind to their promoters, a process called **chromatin remodeling.** In one type of chromatin remodeling, an activator binds to a regulatory sequence upstream of the gene's promoter and recruits a *remodeling complex,* a protein complex that displaces a nucleosome from the chromatin, exposing the promoter **(Figure 16.7).** In a second type of chromatin remodeling, an activator binds to a regulatory sequence upstream of the gene's promoter, and recruits an enzyme that acetylates (adds acetyl groups: CH_3CO—) to histones in the nucleosome where the promoter is located. Acetylation causes the histones to loosen their association with DNA, and the promoter becomes accessible. This type of remodeling is reversed by deacetylation enzymes that remove the acetyl groups from the histones. Many activators use both of these chromatin remodeling mechanisms to regulate gene activity.

Regulation of Transcription Initiation Involves the Effects of Proteins Binding to a Gene's Promoter and Regulatory Sites

Chromatin remodeling is a crucial initial event in facilitating gene expression. Remodeling opens the way for transcription initiation to occur. Transcription ini-

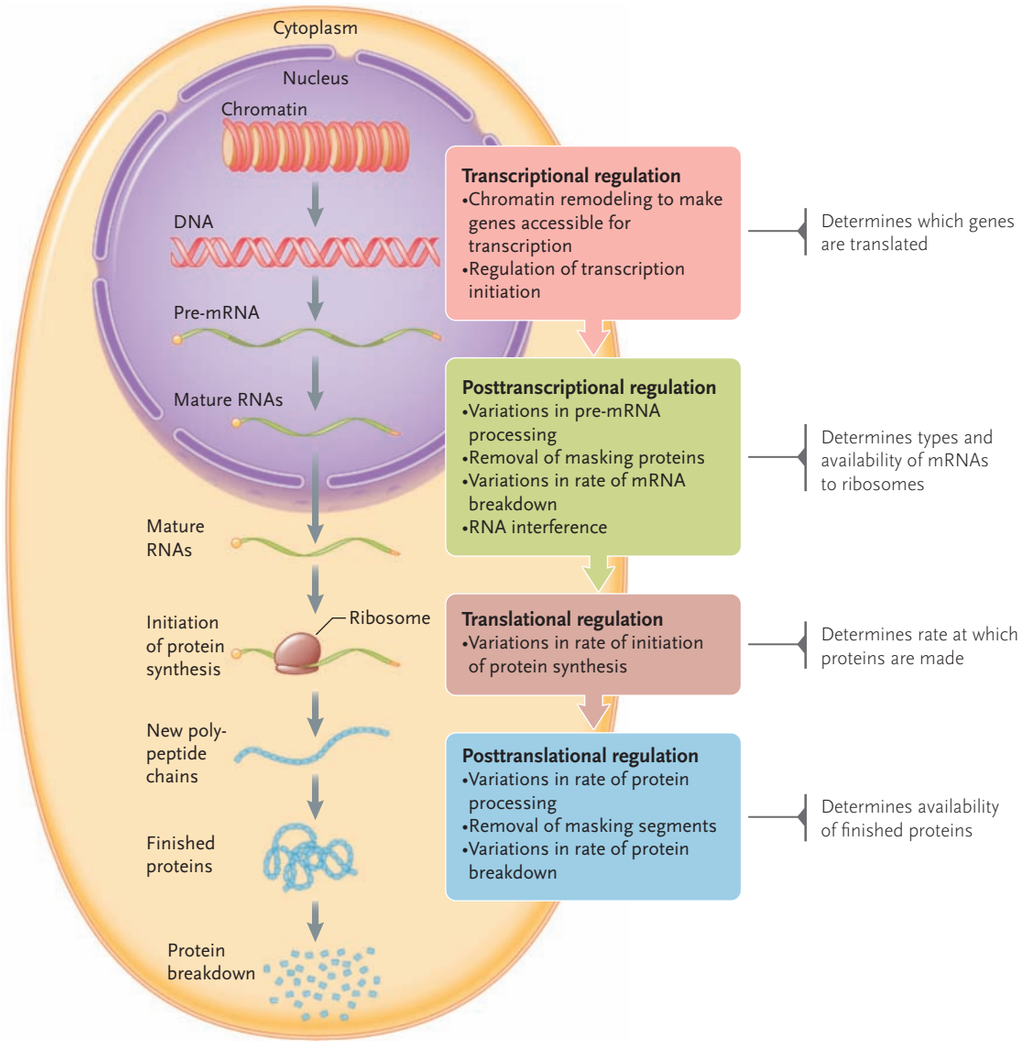


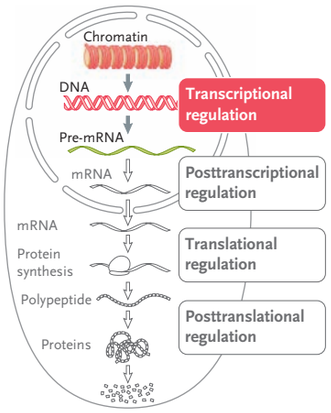
Figure 16.6
Steps in transcriptional, posttranscriptional, translational, and posttranslational regulation of gene expression in eukaryotes.

tiation is the most important level at which the regulation of gene expression takes place.

Organization of a Eukaryotic Protein-Coding Gene. **Figure 16.8** shows a eukaryotic gene, emphasizing the regulatory sites involved in its expression. Immediately upstream of the transcription unit is the promoter, a short region often containing the TATA box. The TATA box plays an important role in transcription initiation. RNA polymerase II itself cannot recognize the promoter sequence. Instead, proteins called transcription factors recognize and bind to the TATA box and then recruit the polymerase. Once the RNA polymerase II–transcription factor complex forms, the polymerase unwinds the DNA and transcription begins. Adjacent to the promoter, further upstream, is the **promoter proximal region**, which contains regulatory sequences called *promoter proximal elements*. Promoter proximal elements are part of a regulatory system for increasing the rate of transcription. More distant from the beginning of the gene is the **enhancer**, which contains regulatory sequences that determine whether the gene is transcribed at its maximum possible rate.

Activation of Transcription. To initiate transcription, proteins called **general transcription factors** (also called *basal transcription factors*) bind to the promoter in the area of the TATA box **(Figure 16.9).** These factors recruit the enzyme RNA polymerase II, which alone cannot bind to the promoter, and orient the enzyme to start transcription at the correct place. The combination of general transcription factors with RNA polymerase II is the **transcription initiation complex.** On its own, this complex brings about only a low rate of transcription initiation, which leads to just a few mRNA transcripts.

Activators—regulatory proteins that control the expression of one or more genes—bind to the promoter proximal elements to increase the rate of transcription. When bound, activators interact directly with



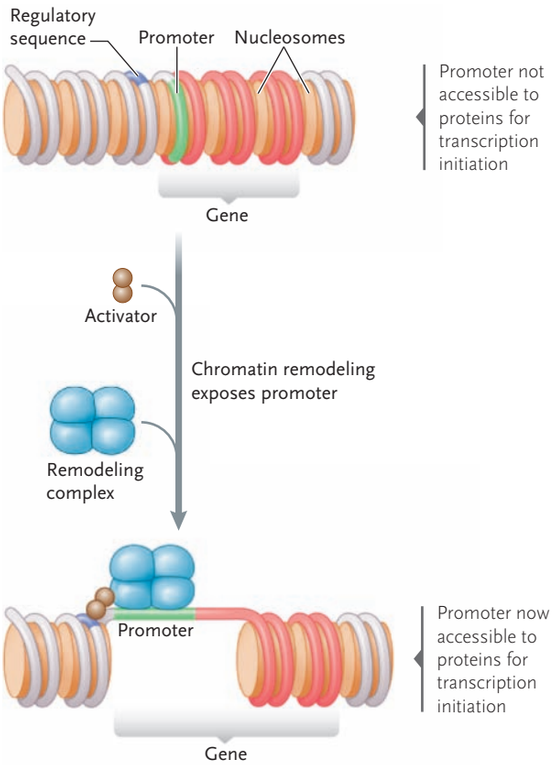
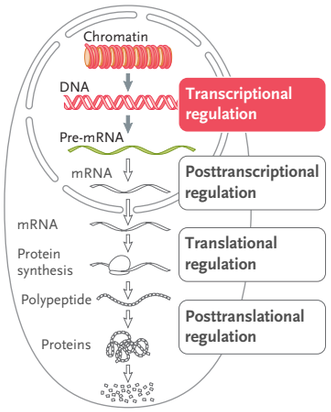


Figure 16.7
Exposing a gene's promoter by chromatin remodeling.



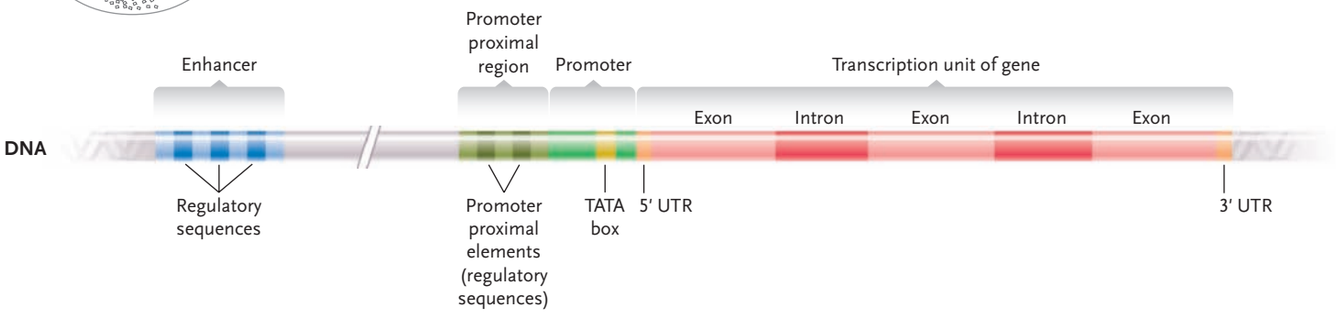


Figure 16.8
Organization of a eukaryotic gene. The transcription unit is the segment that is transcribed into the pre-mRNA; it contains the 5′ UTR (untranslated region), exons, introns, and 3′ UTR. Immediately upstream of the transcription unit is the promoter, which often contains the TATA box. Adjacent to the promoter and further upstream of the transcription unit is the promoter proximal region, which contains regulatory sequences called promoter proximal elements. More distant from the gene is the enhancer, which contains regulatory sequences that control the rate of transcription of the gene.

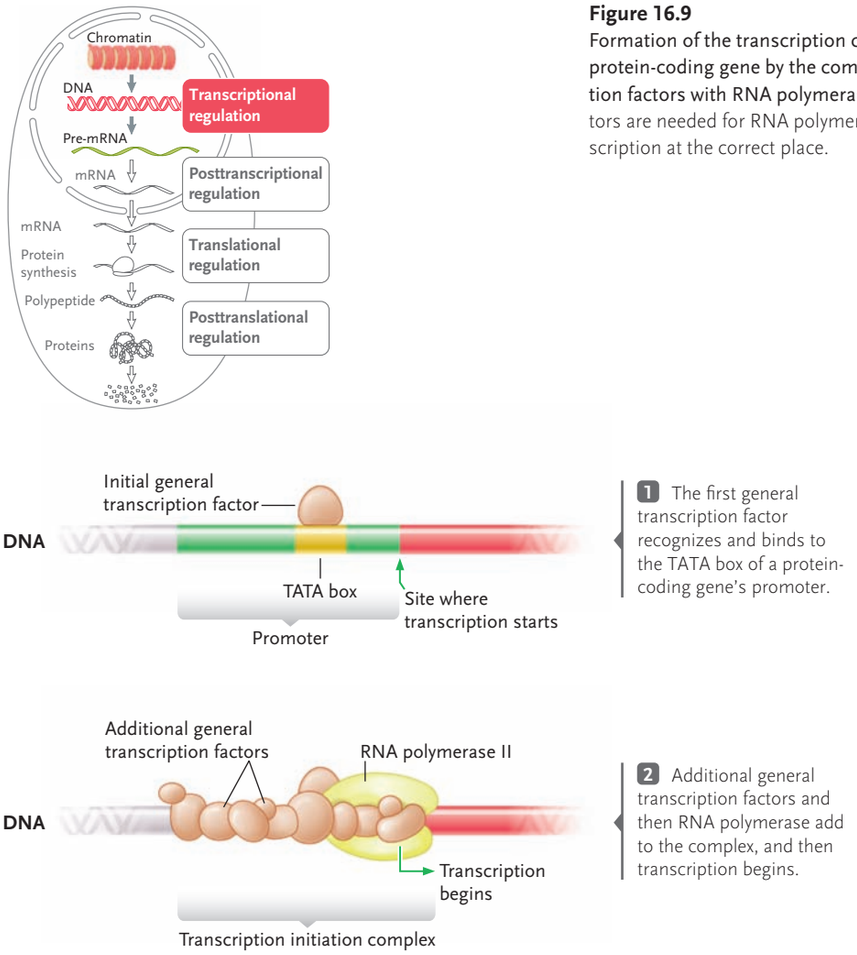


Figure 16.9
Formation of the transcription complex on the promoter of a protein-coding gene by the combination of general transcription factors with RNA polymerase. The general transcription factors are needed for RNA polymerase to bind and initiate transcription at the correct place.

the general transcription factors to stimulate transcription initiation, so many more transcripts are synthesized in a given time. Housekeeping genes—genes that are expressed in all cell types for basic cellular functions such as glucose metabolism—have promoter proximal elements that are recognized by activators present in all cell types. By contrast, genes expressed only in particular cell types or at particular times have promoter proximal elements that are recognized by activators found only in those cell types or at those times. To turn this around, the particular set of activators present within a cell at a given time is responsible for determining which genes in that cell are expressed to a significant level.

Events at the enhancer determine whether a gene is transcribed at its maximal rate **(Figure 16.10).** Particular activators bind to the regulatory sequences within the enhancer. A **coactivator** (also called a *mediator*), a large multiprotein complex, forms a bridge between the activators at the enhancer and the proteins at the promoter and promoter proximal region, and causes the DNA to loop around on itself. The interactions between the coactivator, the proteins at the promoter, and the RNA polymerase stimulate transcription to its maximal rate.

Repression of Transcription. In some genes, repressors oppose the effect of activators, thereby blocking or reducing the rate of transcription. The final rate of transcription then depends upon the "battle" between the activation signal and the repression signal.

Repressors in eukaryotes work in various ways. Some repressors bind to the same regulatory sequence to which activators bind (often in the enhancer), thereby preventing activators from binding to that site. Other repressors bind to their own specific site in the DNA near where the activator binds and interact with the activator so that it cannot interact with the coactivator. Yet other repressors recruit histone deacetylation enzymes that modify histones, leading to chromatin compaction and making a gene's promoter inaccessible to the transcription machinery.

Combinatorial Gene Regulation. Let us review the key elements of regulation of transcription of a protein-coding gene. General transcription factors bind to cer-

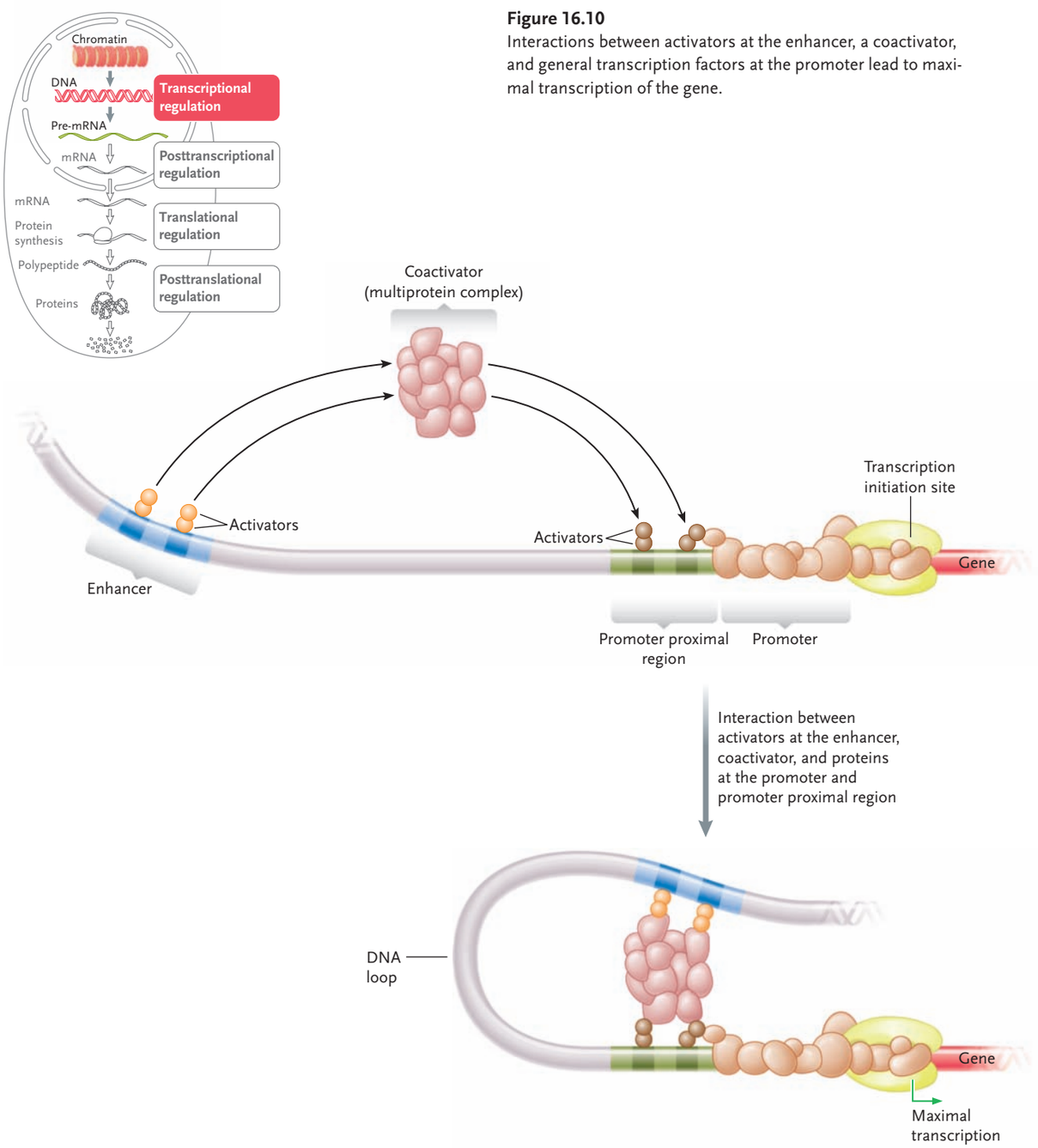


Figure 16.10
Interactions between activators at the enhancer, a coactivator, and general transcription factors at the promoter lead to maximal transcription of the gene.

tain promoter sequences such as the TATA box and recruit RNA polymerase II; this results in a basal level of transcription. Specific activators bind to promoter proximal elements and stimulate the rate of transcription initiation. Activators also bind to the enhancer to give maximal transcription of the gene.

How are these events coordinated in regulating gene expression? Characteristic of any given gene is the number and types of promoter proximal elements. In some genes there may be only one regulatory element, but genes under complex regulatory control have many regulatory elements. Similarly, the number and types of regulatory sequences in the enhancer is specific for each gene.

Both promoter proximal regions and enhancers are important in regulating the transcription of a gene. Each different regulatory sequence in those two regions binds a specific regulatory protein. Since some regulatory proteins are activators and others are repressors, the overall effect of regulatory sequences on transcription depends on the particular proteins that bind to them. If activators bind to both the regulatory sequences in the promoter proximal region and to the enhancer, transcription is activated maximally, mean-

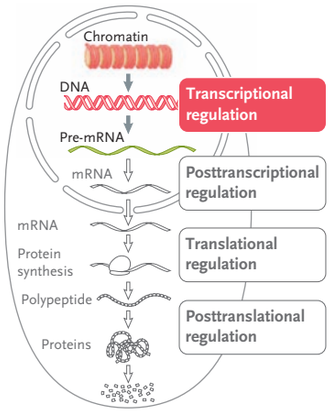
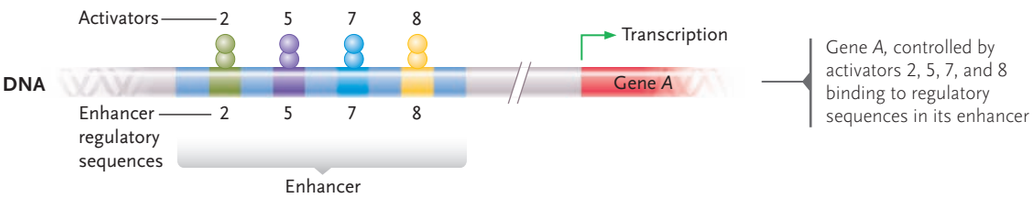
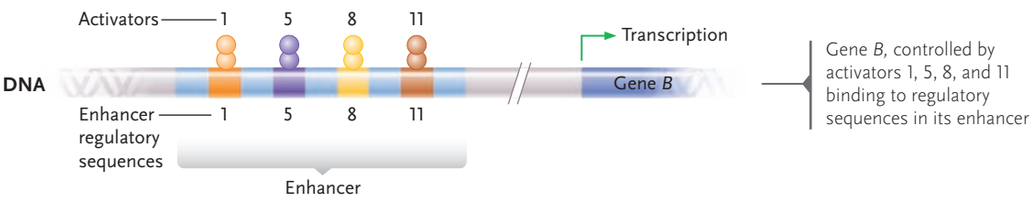


Figure 16.11
Combinatorial gene regulation. A relatively small number of regulatory proteins control transcription of all protein-coding genes. Different combinations of activators bind to enhancer regulatory sequences to control the rate of transcription of each gene.

a. A unique combination of activators controls gene *A*.



b. A different combination of activators controls gene *B*.



ing a high rate of transcription and therefore the production of a high level of the mRNA encoded by the gene. But, if a repressor binds to the enhancer and an activator binds to the promoter proximal element, the amount of gene expression depends upon the relative strengths of those two regulatory proteins. For example, if the repressor is strong, gene expression, in terms of the rate of transcription and the consequent level of the mRNA encoded by the gene, will be reduced.

A relatively small number of regulatory proteins (activators and repressors) control transcription of all protein-coding genes. By combining a few regulatory proteins in particular ways, the transcription of a wide array of genes can be controlled, and a large number of cell types can be specified. The process is called **combinatorial gene regulation.** Let us consider a theoretical example of two genes, each with activators already bound to the respective promoter proximal elements **(Figure 16.11).** Maximal transcription of gene *A* requires activators 2, 5, 7, and 8 binding to their regulatory sequences in the enhancer, whereas maximal transcription of gene *B* requires activators 1, 5, 8, and 11 binding to its enhancer. That is, both genes require activators 5 and 8 for full activation in combination with different other activators.

This operating principle solves a basic dilemma in gene regulation—if each gene were regulated by a single, distinct protein, the number of genes encoding regulatory proteins would have to equal the number of genes to be regulated. Regulating the regulators would require another set of genes of equal number, and so on until the coding capacity of any chromosome set, no matter how large, would be exhausted. But because different genes require different combinations of regulatory proteins, the number of genes encoding regulatory proteins can be much lower than the number of genes they control.

Coordinated Regulation of Transcription of Genes with Related Functions. In the discussion of prokaryotic operons, you learned that genes with related function are often clustered *and* they are transcribed from one promoter onto a single mRNA. That mRNA is translated from one end to the other to produce the several proteins

encoded by the genes. There are no operons in eukaryotes, yet the transcription of genes with related function is coordinately controlled. How is this accomplished?

The answer is that all genes that are coordinately regulated have the same regulatory sequences associated with them. Therefore, with one signal, the transcription of all of the genes can be controlled simultaneously. Let us consider an example of this: the control of gene expression by steroid hormones in mammals. A **hormone** is a molecule produced by one tissue and transported via the bloodstream to another specific tissue to alter its physiological activity. A **steroid** is a type of lipid derived from cholesterol (see Section 3.4). Examples of steroid hormones are testosterone and glucocorticoid. Testosterone regulates the expression of a large number of genes associated with the maintenance of primary and secondary male characteristics. Glucocorticoid, among other actions, regulates the expression of genes involved in the maintenance of the concentration of glucose and other fuel molecules in the blood.

A steroid hormone acts on specific target tissues in the body because only cells in those tissues have *steroid hormone receptors* in their cytoplasm that recognize and bind the hormone (see Section 7.5). The steroid hormone moves through the plasma membrane into the cytoplasm and the receptor binds to it **(Figure 16.12).** The hormone-receptor complex then enters the nucleus and binds to specific regulatory sequences adjacent to the genes whose expression is controlled by the hormone. This binding activates transcription of those genes, and proteins encoded by the genes are made rapidly.

All genes regulated by a specific steroid hormone have the same DNA sequence to which the hormone-receptor complex binds. This sequence is called a **steroid hormone response element.** For example, all genes controlled by glucocorticoid have a glucocorticoid response element associated with them. Therefore, the release of glucocorticoid into the bloodstream coordinately activates the transcription of genes with that response element.

Methylation of DNA Can Control Gene Transcription

DNA methylation, in which a methyl group (—CH_3) is added enzymatically to cytosine bases in the DNA, can regulate transcription of a gene. Specifically, methylation of cytosines in promoters inhibits transcription and turns the genes off, a phenomenon called **silencing.**

For example, genes encoding the blood protein hemoglobin are highly methylated and inactive in most vertebrate body cells. In the cell lines giving rise to red blood cells, however, enzymes remove the methyl groups from the hemoglobin genes, which are then transcribed.

DNA methylation in some cases silences large blocks of genes, or even chromosomes. For example, in body cells of female placental mammals, including humans, one of the two X chromosomes packs tightly into a mass known as a Barr body, in which essentially all the genes of the X chromosome are turned off. As part of this general inactivation, which also includes chromatin modifications, cytosines in the DNA become methylated.

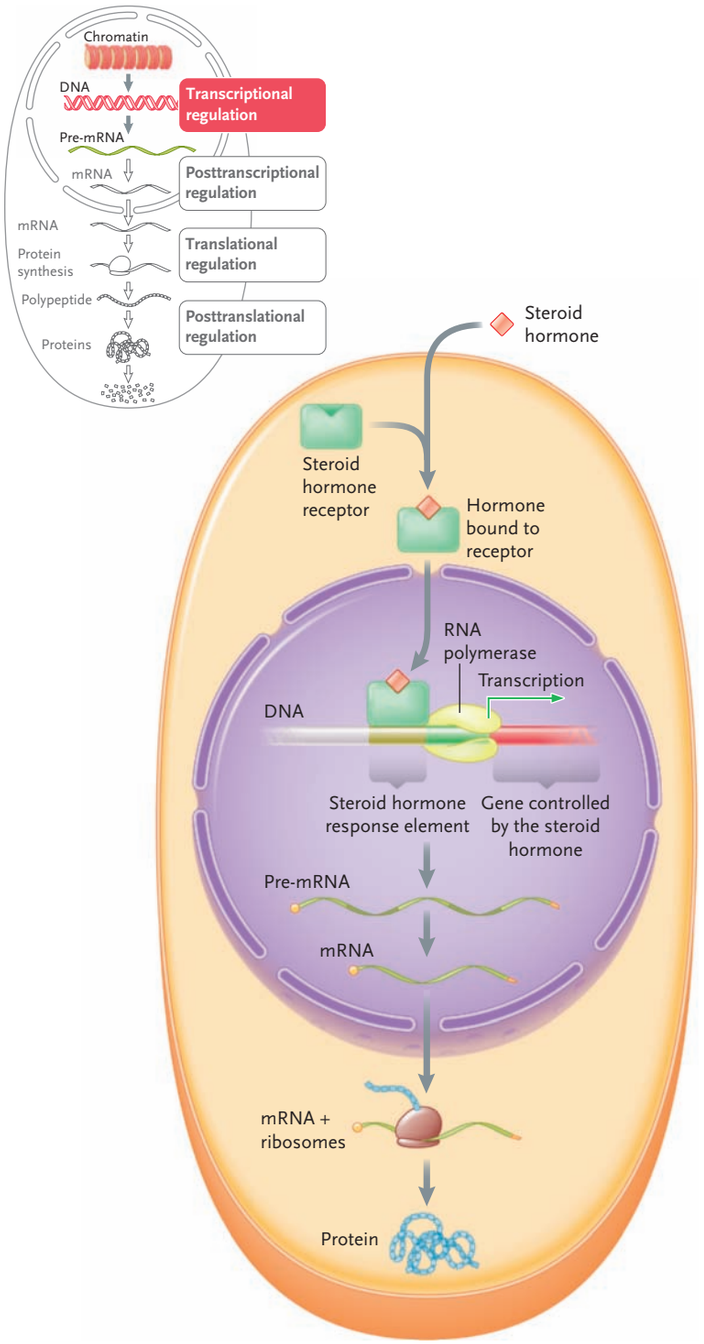


Figure 16.12
Steroid hormone regulation of gene expression. A steroid hormone enters the cell and forms a complex in the cytoplasm with a steroid hormone receptor that is specific to the hormone. Steroid hormone-receptor complexes migrate to the nucleus, bind to the steroid hormone response element next to each gene they control (one such gene is shown in the figure), and affect transcription of those genes.

DNA methylation underlies **genomic imprinting**, in which methylation permanently silences transcription of either the inherited maternal or paternal allele of a particular gene (see Section 13.5). The methylation occurs during gametogenesis in a parent. An inherited methylated allele is not expressed—it is silenced. That allele is known as the *imprinted allele*. The expression of the gene involved therefore depends upon expression of the nonimprinted allele inherited from the other parent. The methylation of the parental allele is maintained as the DNA is replicated, so that the silenced allele remains inactive in progeny cells. Some examples of genomic imprinting were presented in Section 13.5. In one of those examples, the mammalian *Igf2* (insulin growth factor 2) gene is inherited with the paternally derived allele nonmethylated and, therefore, active, and with the maternally derived allele methylated and, therefore, silenced.

Once mRNAs are transcribed from active genes, further regulation occurs at each of the major steps in the pathway from genes to proteins: during pre-mRNA processing and the movement of finished mRNAs to the cytoplasm (posttranslational regulation), during protein synthesis (translational regulation), and after translation is complete (posttranslational regulation). The next section takes up the regulatory mechanisms operating at each of these steps.

Study Break

1. What is the role of histones in gene expression? How does acetylation of the histones affect gene expression?
2. What are the roles of general transcription factors, activators, and coactivators in transcription of a protein-coding gene?

16.3 Posttranscriptional, Translational, and Posttranslational Regulation

Transcriptional regulation determines which genes are copied into mRNAs. This basic level of regulation is fine-tuned by posttranscriptional, translational, and posttranslational controls, the subjects of this section (refer again to Figure 16.6).

Posttranscriptional Regulation Controls mRNA Availability

Posttranscriptional regulation regulates translation by controlling the availability of mRNAs to ribosomes. The controls work by several mechanisms, including changes in pre-mRNA processing and the rate at which mRNAs are degraded.

Variations in Pre-mRNA Processing. In Chapter 15 we noted that mRNAs are transcribed initially as pre-mRNA molecules. These pre-mRNAs are processed to produce the finished mRNAs, which then enter protein synthesis. Variations in pre-mRNA processing can regulate *which* proteins are made in cells. As described in Section 15.3, pre-mRNAs can processed by *alternative splicing*. Alternative splicing produces different mRNAs from the same pre-mRNA by removing different combinations of exons (the amino acid–coding segments) along with the introns (the noncoding spacers). The resulting mRNAs are translated to produce a family of related proteins with various combinations of amino acid sequences derived from the exons. Alternative splicing itself is under regulatory control. Regulatory proteins specific to the type of cell control which exons are removed from pre-mRNA molecules by binding to regulatory sequences within those molecules. The outcome of alternative splicing is that appropriate proteins within a family are synthesized in cell types or tissues in which they are optimally functional. Perhaps three-quarters of human genes are alternatively spliced at the pre-mRNA level.

Posttranscriptional Control by Masking Proteins. Some posttranscriptional controls operate by means of "masking" proteins that bind to mRNAs and make them unavailable for protein synthesis. These controls are important in many animal eggs, in which they keep mRNAs in an inactive form until the egg has been fertilized and embryonic development is under way. When an mRNA is to become active, other factors—other proteins, made as part of the developmental pathway—remove the masking proteins and allow the mRNA to enter protein synthesis.

Variations in the Rate of mRNA Breakdown. The rate at which eukaryotic mRNAs break down can also be controlled posttranscriptionally. The mechanism involves a regulatory molecule, such as a steroid hormone, directly or indirectly affecting the mRNA breakdown steps, either slowing or increasing the rate of those steps. For example, in the mammary gland of the rat, the mRNA for casein (a milk protein) has a half-life of about 5 hours (meaning that it takes 5 hours for half of the mRNA present at a given time to break down). The half-life of casein mRNA changes to about 92 hours if the peptide hormone prolactin is present. Prolactin is synthesized in the brain and in other tissues, including the breast. The most important effect of prolactin is to stimulate the mammary glands to produce milk (that is, it stimulates lactation). During milk production, a large amount of casein must be synthesized, and this is accomplished in part by radically decreasing the rate of breakdown of the casein mRNA.

Nucleotide sequences in the 5′ UTR (untranslated region; see Section 15.3) appear also to be important in determining mRNA half life. If the 5′ UTR is transferred experimentally from one mRNA to another, the

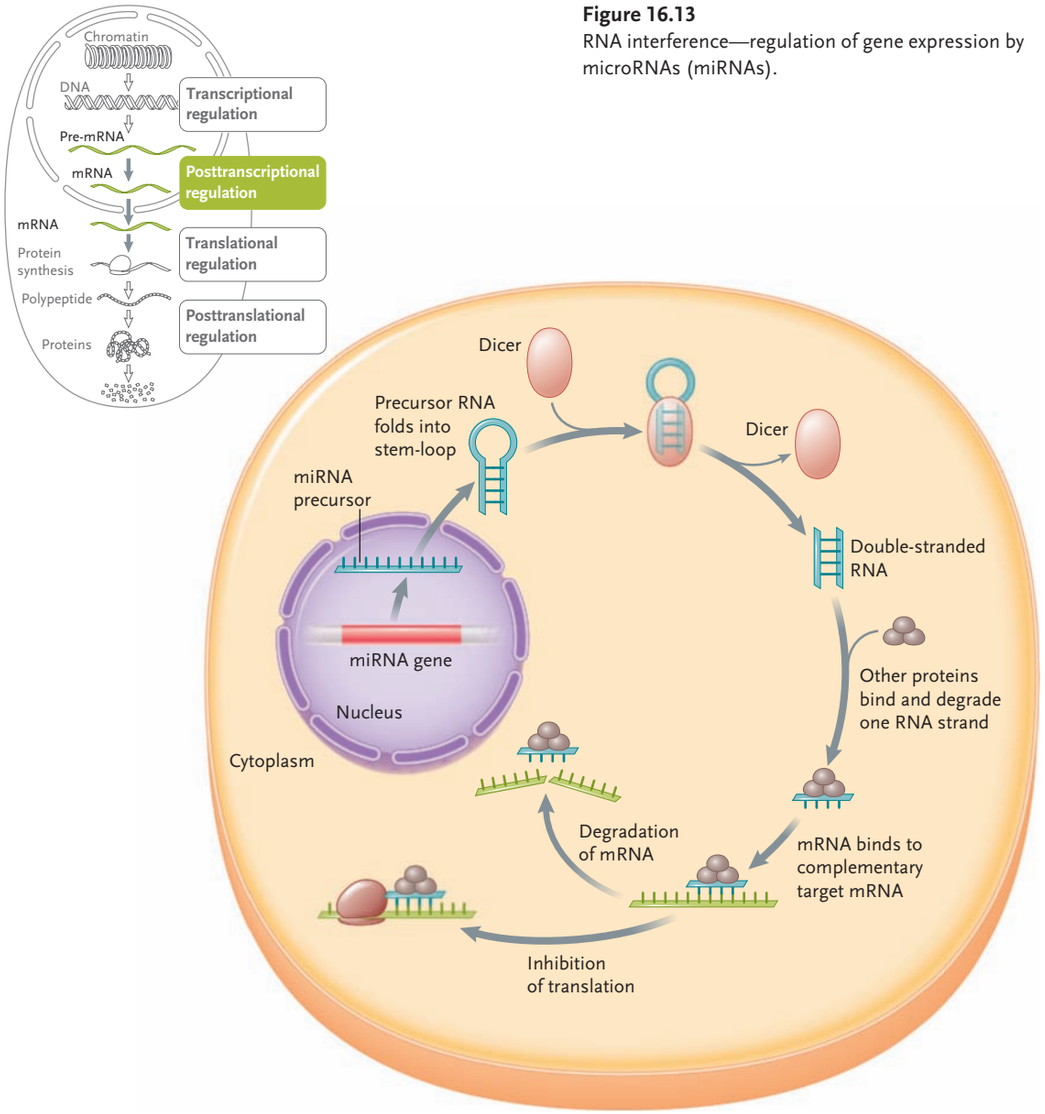


Figure 16.13
RNA interference—regulation of gene expression by microRNAs (miRNAs).

half-life of the receiving mRNA becomes the same as that of the donor mRNA. The controlling sequences in the 5′ UTR of an mRNA might be recognized by proteins that regulate its stability.

Regulation of Gene Expression by Small RNAs. The relatively recent discovery of *micro-RNAs* (miRNAs) has revolutionized our understanding of gene control. miRNAs are small, single-stranded RNAs found in organisms as diverse as worms, flies, plants, and mammals, where they regulate important processes such as development, growth, and behavior. What are miRNAs and how do they work?

Each miRNAs is encoded by a non-protein-coding gene. Transcription of the gene produces an RNA that is the precursor to the miRNA **(Figure 16.13).** The precursor RNA folds and base-pairs with itself, forming a stem-loop structure. An enzyme named Dicer cuts the stem-loop to produce a double-stranded RNA, about 21–22 base pairs long. A protein complex then binds to the double-stranded RNA and degrades one of the two RNA strands, leaving a small single-stranded RNA—the miRNA. Still bound to the protein complex, the miRNA binds to any mRNA that has a complementary sequence. Gene expression is then silenced in one of two ways: either the proteins in the complex cleave the mRNA where the miRNA is bound to it, or the double-stranded segment formed between the miRNA and the mRNA blocks ribosomes from translating the mRNA.

Researchers think that there are 120 genes for miRNAs in worms and 250 genes in humans. Many of these miRNAs are expressed in developmentally regulated patterns. The targets of the miRNA's action are often mRNAs for regulatory proteins that control the development of the organism.

The phenomenon of silencing a gene posttranscriptionally by a small, single-stranded RNA that is

complementary to part of an mRNA is termed **RNA interference (RNAi).** miRNAs are one class of single-stranded RNAs that cause RNAi; another class is known as **small interfering RNA (siRNA).** Whereas miRNA is produced from RNA that is encoded in the cell's genome, siRNA is produced from double-stranded RNA that is *not* encoded by nuclear genes. For example, the life cycle and replication of many viruses involves a double-stranded RNA stage. Viral double-stranded RNA enters the RNAi process as described for miRNAs: double-stranded RNA is cut by Dicer into short double-stranded RNA molecules, and then a protein complex binds to the molecules and degrades one of the RNA strands to produce siRNA. The protein complex is the same one that acts on the double-stranded RNA precursors of miRNAs. In the RNAi process, siRNA acts exactly like microRNA—mRNAs complementary to the siRNA are targeted and either they are degraded or their translation is blocked. In our viral example, the targeted mRNAs would be mRNAs for proteins needed for viral genome replication and the production of new virus particles.

Any gene can be silenced experimentally by RNAi. To silence a gene, researchers introduce into the cell a double-stranded RNA that can be processed by Dicer and the protein complex into an siRNA complementary to the mRNA transcribed from that gene. Indeed, RNAi has become a powerful new technique for silencing specific genes experimentally in a variety of organisms. Andrew Fire of the Massachusetts Institute of Technology and Craig Mello of Harvard University received a Nobel Prize in 2006 for their discovery of RNA interference.

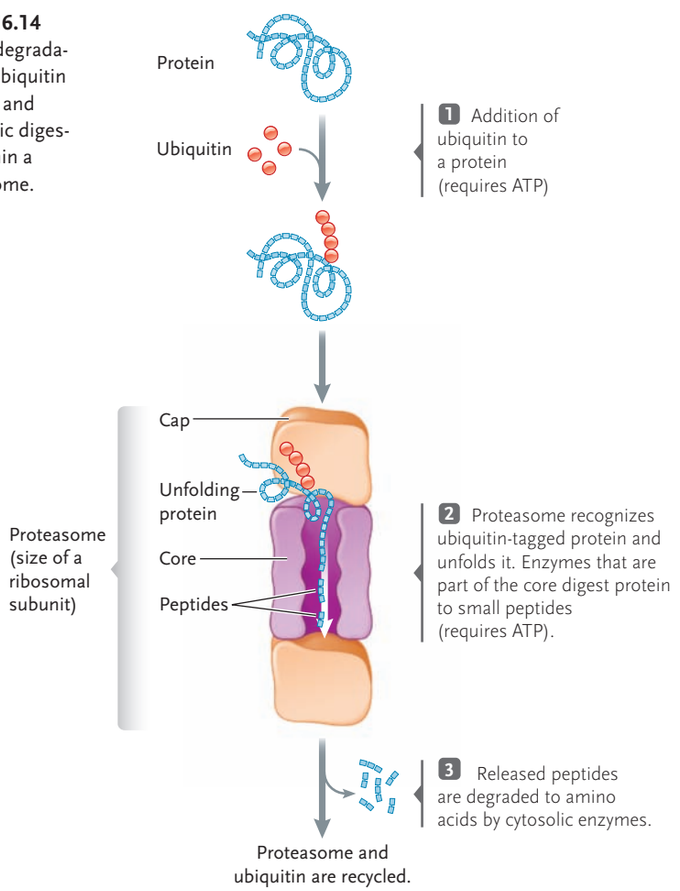


Figure 16.14
Protein degradation by ubiquitin addition and enzymatic digestion within a proteasome.

Translational Regulation Controls the Rate of Protein Synthesis

At the next regulatory level, translational regulation controls the rate at which mRNAs are used in protein synthesis. Translational regulation occurs in essentially all cell types and species. For example, translational regulation is involved in cell cycle control in all eukaryotes and in many processes during development in multicellular eukaryotes, such as red blood cell differentiation in animals. Significantly, many viruses exploit translational regulation to control their infection of cells and to shut off the host cell's own genes.

Let us consider the general role of translational regulation in animal development. During early development of most animals, little transcription occurs. The changes in protein synthesis patterns seen in developing cell types and tissues instead derive from the activation, repression, or degradation of maternal mRNAs, the mRNAs that were in the mother's egg before fertilization. One important mechanism for translational regulation involves adjusting the length of the poly(A) tail of the mRNA. (Recall from Section 15.3 that the poly(A) tail—a string of adenine-containing nucleotides—is added to the 3′ end of pre-mRNA and is retained on the mRNA produced from the pre-mRNA after introns are removed.) That is, enzymes can change the length of the poly(A) tail on an mRNA in the cytoplasm in either direction: by shortening it or lengthening it. Increases in poly(A) tail length result in increased translation; decreases in length result in decreased translation. For example, during embryogenesis (the formation of the embryo) of the fruit fly, *Drosophila,* key proteins are synthesized when the poly(A) tails on the mRNAs for those proteins are lengthened in a regulated way. Evidence for this came from experiments in which poly(A) tail lengthening was blocked; the result was that embryogenesis was inhibited. But, while researchers know that the length of poly(A) tails is regulated in the cytoplasm, how this process occurs is not completely understood.

Posttranslational Regulation Controls the Availability of Functional Proteins

Posttranslational regulation controls the availability of functional proteins mainly in three ways: chemical modification, processing, and degradation. Chemical modification involves the addition or removal of chemical groups, which reversibly alters the activity of the protein. For example, you saw in Section 7.2 how the addition of phosphate groups to proteins involved in signal transduction pathways either stimulates or in-

hibits the activity of those proteins. Further, in Section 10.4 you learned how the addition of phosphate groups to target proteins plays a crucial role in regulating how a cell progresses through the cell division cycle. And, in Section 16.2 you saw how acetylation of histones altered the properties of the nucleosome, loosening its association with DNA in chromatin.

In processing, proteins are synthesized as inactive precursors, which are converted to an active form under regulatory control. For example, you saw in Section 15.4 that the digestive enzyme pepsin is synthesized as pepsinogen, an inactive precursor that activates by removal of a segment of amino acids. Similarly, the glucose-regulating hormone insulin is synthesized as a precursor called proinsulin; processing of the precursor removes a central segment but leaves the insulin molecule, which consists of two polypeptide chains linked by disulfide bridges.

The rate of degradation of proteins is also under regulatory control. Some proteins in eukaryotic cells last for the lifetime of the individual, while others persist only for minutes. Proteins with relatively short cellular lives include many of the proteins regulating transcription. Typically, these short-lived proteins are marked for breakdown by enzymes that attach a "doom tag" consisting of a small protein called *ubiquitin* (**Figure 16.14,** step 1). The protein is given this name because it is indeed ubiquitous—present in almost the same form in essentially all eukaryotes. The ubiquitin tag labels the doomed proteins so that they are recognized and attacked by a *proteasome,* a large cytoplasmic complex of a number of different proteins (step 2). The proteasome unfolds the protein, and protein-digesting enzymes within the core digest the protein into small peptides. The peptides are released from the proteasome and cytosolic enzymes further digest the peptides into individual amino acids, which are recycled for use in protein synthesis or oxidized as an energy source (step 3). The ubiquitin protein and proteasome are also recycled. Aaron Ciechanover and Avram Herhsko, both of the Israel Institute of Technology, Haifa, Israel, and Irwin Rose of the University of California, Irvine, received a Nobel Prize in 2004 for the discovery of ubiquitin-mediated protein degradation.

Control of protein breakdown is the last of the opportunities for control of gene expression. We will now look at cancer, a disease in which control of gene expression goes awry.

Study Break

1. How does a microRNA silence gene expression?
2. If the poly(A) tail on a mRNA was removed, what would likely be the effect on the translation of that mRNA?

16.4 The Loss of Regulatory Controls in Cancer

The cell division cycle of all eukaryotic cells from single-celled microorganisms to cells that are components of multicellular organisms is controlled by genes. The types of genes exerting this control are basically the same in terms of functions in all eukaryotes. Mutations in these genes can disrupt normal cell growth and division. The effects of such mutations are more significant and profound in complex multicellular organisms, particularly mammals. For example, occasionally, dividing and differentiating cells deviate from their normal genetic program and give rise to tissue masses called *tumors*. In other words, the cells lose their normal regulatory controls and revert partially or completely to an embryonic developmental state, in a process called *dedifferentiation*. If the deviant cells stay together in a single mass, the tumor is said to be *benign*. Benign tumors usually are not life threatening, and their surgical removal generally results in a complete cure.

If the cells of a tumor invade and disrupt surrounding tissues, the tumor is said to be *malignant* and is called a cancer (**Figure 16.15** shows a cancer cell). Sometimes, cells from malignant tumors break off and move through the blood system or lymphatic system, forming new tumors at other locations in the body. The spreading of a malignant tumor is called *metastasis* (meaning "change of state"). Malignant tumors can result in debilitation and death in various ways, including damage to critical organs, metabolic problems, hemorrhage, and secondary malignancies. In some cases, malignant tumors can be eliminated from the body by surgery or destroyed by chemicals *(chemotherapy)* or radiation.

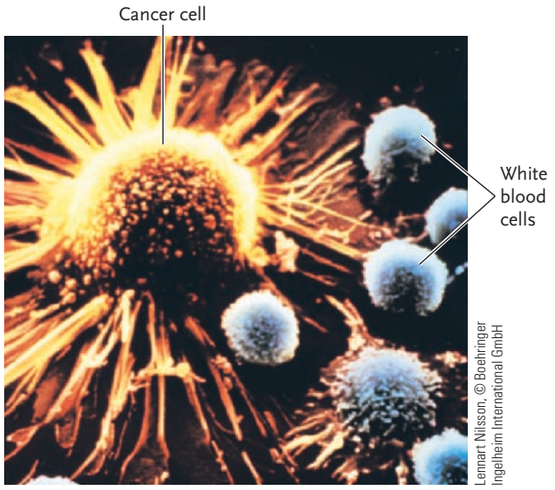


Figure 16.15
A scanning electron micrograph of a cancer cell surrounded by several white blood cells.

Insights from the Molecular Revolution

A Viral Tax on Transcriptional Regulation

The human *T-cell leukemia virus (HTLV)* causes a virulent form of cancer by triggering rapid, uncontrolled division of white blood cells. It does so by speeding up a pathway that triggers division at normal rates in uninfected cells. The pathway is a G-protein coupled receptor-response pathway involving cyclic AMP (cAMP) as a second messenger (see Section 7.4).

Normally, the pathway is triggered by cAMP released when an infection takes place. In the pathway, a specific activator called CREB (CRE-binding protein) is activated by phosphorylation and binds to CRE (cAMP-response element), in the enhancers for a group of genes that controls cell division. The binding turns on the genes and leads to the rapid division of white blood cells characteristic of the immune response.

HTLV takes advantage of the pathway by means of a short sequence CGTCA, in its DNA that mimics part of the human CRE enhancer sequence TGACGTCA. When the host cell's CREB is activated, it binds avidly to the enhancer sequence in the virus, turns on the viral genes, and leads to reproduction of the virus. Unfortunately for someone infected with HTLV, CREB in an infected cell also binds more avidly to the enhancers of cell division genes, leading to the uncontrolled division of white cells and, hence, to leukemia.

How HTLV produced these effects on cell division was puzzling because, in a test tube, CREB binds only weakly to the viral mimic of the CRE enhancer. An explanation was provided by Susanne Wagner and Michael R. Green at the University of Massachusetts Medical Center in Worcester, who found that HTLV uses one of its own proteins, called *Tax,* to get around the problem of weak binding. When Tax is present, CREB binds strongly to the viral CRE imitation. Tax also greatly increases the ability of CREB to bind to the normal CRE enhancer, leading to rapid and uncontrolled growth of infected white cells and to leukemia.

How does the Tax protein accomplish this feat? The CREB activator interacts with DNA as a dimer (a pair of molecules that together form the functional form). Wagner and Green found that the Tax protein greatly increases the ability of CREB to form dimers and thus to bind to the viral CRE enhancer. Evidently, Tax acts as a sort of molecular "safety pin" that holds the dimer together with either the viral imitation or the cell's CRE enhancer.

The Tax protein thus compensates for the imperfection of the viral sequence that imitates CRE by greatly increasing the ability of CREB to form dimers and bind to it. Although Tax provides a major advantage to HTLV, it may also open a chink in its armor. If a means can be found to interfere with Tax or its synthesis, it may be possible to stop the uncontrolled growth of white cells, and thus the leukemia caused by the virus.

Most Cancers Are Caused by Genes That Have Lost Their Normal Controls

All the characteristics of cancer cells—dedifferentiation, uncontrolled division, and metastasis—reflect changes in gene activity. Many of the genes that become altered encode proteins that control the cell division cycle of normal cells. That is, healthy cells grow and divide only when the balance of stimulatory and inhibitory signals received from outside the cell favors cell division. A cancer cell, by contrast, does not respond properly to the usual signals and divides without the usual constraints.

Two main types of genes commonly show altered activities as cells become cancer cells. One class is the **proto-oncogenes** (*oncos* = bulk or mass), genes in normal cells that encode various kinds of proteins that stimulate cell division. In cancer cells, the proto-oncogenes are altered to become **oncogenes**, genes that stimulate the cell to progress to the cancerous state. Among the mechanisms that can convert proto-oncogenes to oncogenes:

- Mutations in a gene's promoter or other control sequences may disrupt normal regulatory controls, making the gene abnormally active. The mutations can occur spontaneously or be induced by radiation or by particular chemicals.
- Mutations in the coding segment of the gene may produce an altered form of the encoded protein that is abnormally active.
- Translocation, in which a segment of a chromosome breaks off and attaches to a different chromosome (discussed in Section 13.3), may move a gene that controls cell division to a new location near the promoter or enhancer sequence of a highly active gene, making the cell division gene overactive.
- Infecting viruses may introduce genes to regions in the chromosomes where the expression of the genes disrupts cell cycle control or alters regulatory proteins to turn genes on. (*Insights from the Molecular Revolution* describes a virus that causes a blood cancer by altering a transcription factor.)

For example, translocation may affect *MYC,* a proto-oncogene controlling cell division. The activity of *MYC* is normally tightly regulated. However, *MYC* lies in a chromosome region that often breaks, causing a translocation that places *MYC* near the enhancer and promoter of a highly active antibody gene. The placement

makes *MYC* continuously active, converting it into an oncogene that triggers rapid and uncontrolled cell division.

Several proto-oncogenes encode cell surface receptors that bind extracellular signal molecules such as peptide hormones or growth factors. In general, the oncogene forms of these receptors are continually activated, whether they are bound to the external signal molecule or not. As a result, the internal pathways they trigger, including those that cause cells to divide, are also continually active.

Another key group of proto-oncogenes encodes enzymes forming parts of the internal reaction pathways triggered by surface receptors (see Chapter 7). Most important are genes encoding the protein kinases, which regulate the activity of other proteins by adding phosphate groups to them. Some of the proteins phosphorylated by the protein kinases directly take part in gene regulation or initiation of cell division; others form parts of the cellular response pathways linked to surface receptors. The protein kinases encoded by the oncogene forms of the genes are continually active, constantly phosphorylating the control protein so that cell division continues at high and uncontrolled rates.

The other main class of genes that shows altered activity in cancer cells is the **tumor-suppressor genes**, which, in normal cells, encode proteins that inhibit cell division. Both alleles of a tumor suppressor gene must be inactivated for inhibitory activity to be lost in cancer cells. The best known of these genes is *TP53*, so called because its encoded protein, p53, has a molecular weight of 53,000 daltons. Among other activities, normal p53 stops cell division by combining with and inhibiting cyclin-dependent protein kinases that trigger entry into critical stages of DNA replication and mitosis (discussed in Section 10.4). Without the normal form of the p53 protein, the cyclin-dependent protein kinases are continually active in triggering cell division. Inactive *TP53* genes are found in many types of cancers.

Cancer Develops Gradually by Multiple Steps

Cancer rarely develops by alteration of a single proto-oncogene to an oncogene, or inactivation of a single tumor-suppressor gene. Instead, in almost all cancers, successive alterations in several to many genes gradually accumulate to tilt normal cells to cancer cells. This gradual mechanism is called the *multistep progression of cancer* **(Figure 16.16).** The gradual nature of the process explains why smokers, for example, may not develop cancer until years after the first mutations caused by chemicals in tobacco smoke may occur, soon after smoking begins. It also offers some hope to those who quit smoking, for stopping the exposure to the carcinogenic smoke may halt multistep progression before it reaches its deadly conclusion in cancer.

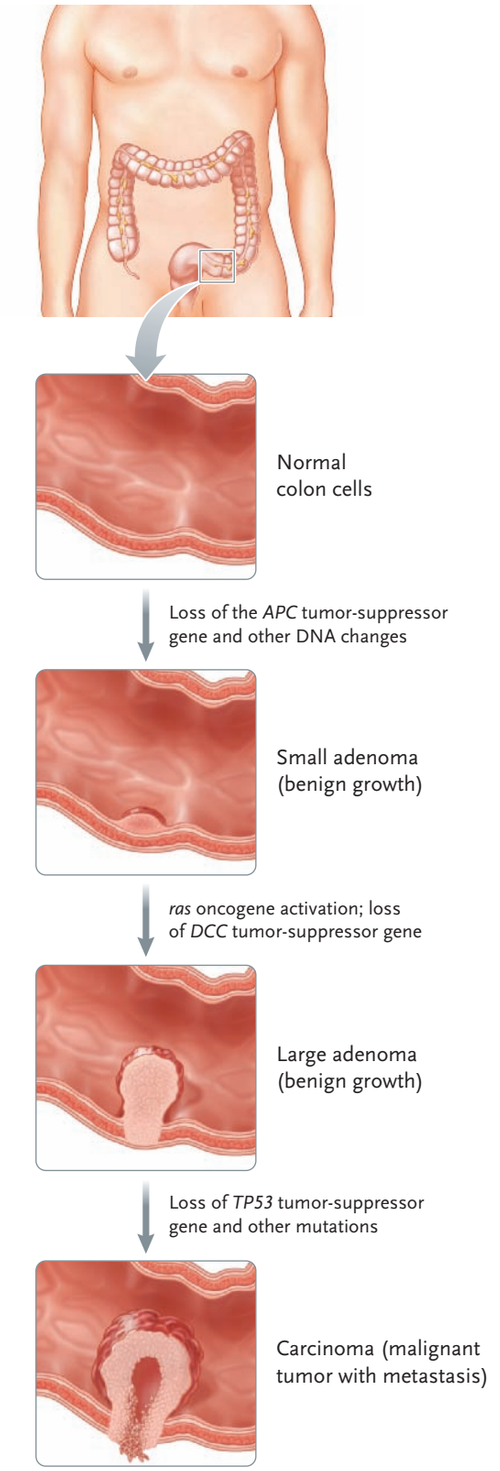


Figure 16.16
A multistep model for the development of a type of colorectal cancer.

Unanswered Questions

How are specific patterns of gene expression generated and maintained in a developing eukaryotic organism?

You learned in this chapter that chromatin remodeling is necessary to "open the door" for the transcription machinery to assemble at a promoter. However, researchers do not understand completely how the chromatin remodeling complexes are targeted to particular genes and regulated to give specific patterns of gene expression in a eukaryotic cell, or throughout the development of multicellular eukaryotic organism to maturity, and then through subsequent life. Since mutations in genes encoding chromatin remodeling components are directly linked to human cancers, understanding such basic functions of the complexes is a highly important goal.

Can RNA interference silence disease?

RNA interference (RNAi) is the process in which a small RNA in a complex with several proteins interferes with the expression of an mRNA, either by cleaving it or by blocking its translation. Potentially, RNAi therapy will have many clinical applications, including the treatment of cancers by targeting out-of-control oncogenes. Let us consider two RNAi therapies that are in the works.

Macular degeneration treatment. Some human genetic diseases such as macular degeneration, the leading cause of blindness among those age 55 and older in the United States, are characterized by an overabundance of a protein called VEGF (vascular endothelial growth factor), which promotes blood vessel growth. In macular degeneration patients, the overabundance of VEGF leads to excess blood vessels behind the retina. These blood vessels leak, leading to clouded and often complete loss of vision. Researchers are investigating whether RNAi could be an effective therapy for such diseases. In fact, two biotechnology companies have recently starting testing an RNAi therapy for macular degeneration, targeting the expression of the gene for VEGF. Practically speaking, such research involves first understanding the expression of the disease gene and then working out the way to deliver the small interfering RNA to the diseased cells to eliminate the gene product or decrease its level.

Anti-viral treatment. Similarly, important research is being done to see if RNAi can be an effective therapy for viral infections. Viral targets being investigated by research groups include HIV (the virus that causes AIDS), and hepatitis B and hepatitis C (viruses that cause liver disease). This research involves developing an effective small interfering RNA that can block expression of a vital viral gene, and then perfecting a system to deliver it to patients. In fact, geneticists Anton McCaffrey and Mark Kay of Stanford University have had success in using RNAi to control hepatitis C in laboratory mice. However, the method used to introduce the RNAi is not feasible with humans and, hence, other delivery approaches are being explored.

Peter J. Russell

The ravages of cancer, probably more than any other example, bring home the critical extent to which humans and all other multicellular organisms depend on the mechanisms controlling gene expression to develop and live normally. In a sense, the most amazing thing about these control mechanisms is that, in spite of their complexity, they operate without failures throughout most of the lives of all eukaryotes.

In the next chapter, you will learn about the molecular genetics of bacteria and their phages and about DNA sequences in prokaryotic and eukaryotic genomes that have the ability to move to different chromosomal locations.

Study Break

1. What is the normal function of a tumor-suppressor gene? How do mutations in tumor-suppressor genes contribute to the onset of cancer?
2. What is the normal function of a proto-oncogene? How can mutations in proto-oncogenes contribute to the onset of cancer?

Review

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16.1 Regulation of Gene Expression in Prokaryotes

- Transcriptional control in prokaryotes involves short-term changes that turn specific genes on or off in response to changes in environmental conditions. The changes in gene activity are controlled by regulatory proteins that recognize operators of operons (Figure 16.2).
- Regulatory proteins may be repressors, which slow the rate of transcription of operons, or activators, which increase the rate of transcription.
- Some repressors are made in an active form, in which they bind to the operator of an operon and inhibit its transcription. Combination with an inducer blocks the activity of the repressor and allows the operon to be transcribed (Figure 16.3).
- Other repressors are made in an inactive form, in which they are unable to inhibit transcription of an operon unless they combine with a corepressor (Figure 16.4).

- Activators typically are made in inactive form, in which they cannot bind to their binding site next to an operon. Combining with another molecule, often a nucleotide, converts the activator into the form in which it binds with its binding site and recruits RNA polymerase, thereby stimulating transcription of the operon (Figure 16.5).

Animation: The lactose operon

Animation: Negative control of the lactose operon

16.2 Regulation of Transcription in Eukaryotes

- Operons are not found in eukaryotes. Instead, genes that encode proteins with related functions typically are scattered through the genome, while being regulated in a coordinated manner.
- Two general types of gene regulation occur in eukaryotes. Short-term regulation involves relatively rapid changes in gene expression in response to changes in environmental or physiological conditions. Long-term regulation involves changes in gene expression associated with the development and differentiation of an organism.
- Gene expression in eukaryotes is regulated at the transcriptional level (where most regulation occurs) and at posttranscriptional, translational, and posttranslational levels (Figure 16.6).
- Transcriptionally active genes have a looser chromatin structure than transcriptionally inactive genes. The change in chromatin structure that accompanies the activation of transcription of a gene involves chromatin remodeling—specific histone modifications—particularly in the region of a gene's promoter (Figure 16.7).
- Regulation of transcription initiation involves proteins binding to a gene's promoter and regulatory sites. At the promoter, general transcription factors bind and recruit RNA polymerase II, giving a very low level of transcription. Activator proteins bind to promoter proximal elements and increase the rate of transcription. Other activators bind to the enhancer and, through interaction with a coactivator, which binds also to the proteins at the promoter, greatly stimulate the rate of transcription (Figures 16.8–16.10).
- The overall control of transcription of a gene depends on the particular regulatory proteins that bind to promoter proximal elements and enhancers. The regulatory proteins are cell-type specific and may be activators or repressors. This gene regulation is achieved by a relatively low number of regulatory proteins, acting in various combinations (Figure 16.11).
- The coordinate expression of genes with related functions is achieved by each of the related genes having the same regulatory sequences associated with them.
- Sections of chromosomes or whole chromosomes can be inactivated by DNA methylation, a phenomenon called silencing. DNA methylation is also involved in genomic imprinting, in which transcription of either the inherited maternal or paternal allele of a gene is inhibited permanently.

Animation: Controls of eukaryotic gene expression

Animation: X-chromosome inactivation

16.3 Posttranscriptional, Translational, and Posttranslational Regulation

- Posttranscriptional, translational, and posttranslational controls operate primarily to regulate the quantities of proteins synthesized in cells (Figure 16.6).
- Posttranscriptional controls regulate pre-mRNA processing, mRNA availability for translation, and the rate at which mRNAs are degraded. In alternative splicing, different mRNAs are derived from the same pre-mRNA. In another process, small single-stranded RNAs complexed with proteins bind to mRNAs that have complementary sequences, and either the mRNA is cleaved or translation is blocked (Figure 16.13).
- Translational regulation controls the rate at which mRNAs are used by ribosomes in protein synthesis.
- Posttranslational controls regulate the availability of functional proteins. Mechanisms of regulation include the alteration of protein activity by chemical modification, protein activation by processing of inactive precursors, and affecting the rate of degradation of a protein.

16.4 The Loss of Regulatory Controls in Cancer

- In cancer, cells partially or completely dedifferentiate, divide rapidly and uncontrollably, and break loose to form additional tumors in other parts of the body.
- Proto-oncogenes and tumor-suppressor genes typically are altered in cancer cells. Proto-oncogenes encode proteins that stimulate cell division. Their altered forms, oncogenes, are abnormally active. Tumor-suppressor genes in their normal form encode proteins that inhibit cell division. Mutated forms of these genes lose this inhibitory activity.
- Most cancers develop by multistep progression involving the successive alteration of several to many genes (Figure 16.16).

Questions

Self-Test Questions

1. The control of the delivery of mRNA to the cytoplasm is an example of:
   a. translational regulation.
   b. posttranslational regulation.
   c. transcriptional regulation.
   d. posttranscriptional regulation.
   e. deoxyribonucleic regulation.
2. For the *E. coli lac* operon, when glucose is absent and lactose is added:
   a. allolactose binds to the operator.
   b. the *lac* gene cannot make Lac repressor protein.
   c. allolactose binds the Lac repressor protein to remove it from the operator.
   d. the genes *lacZ, lacY,* and *lacA* are turned off.
   e. β-galactosidase decreases in the cell.
3. For the *E. coli lac* operon, when lactose is present:
   a. and glucose is absent, cAMP binds and activates catabolic activator protein (CAP).
   b. and glucose is absent, the level of cAMP decreases.
   c. activated CAP binds the repressor protein to remove it from the operator gene.
   d. the cell prefers lactose over glucose.
   e. RNA polymerase cannot bind to the promoter.
4. For the *trp* operon:
   a. tryptophan is an inducer.
   b. when end-product tryptophan binds to the Trp repressor, it stops transcription of the tryptophan biosynthesis genes.
   c. Trp repressor is synthesized in active form.

d. low levels of tryptophan bind to the *trp* operator and block transcription of the tryptophan biosynthesis genes.
e. high levels of tryptophan activate RNA polymerase and induce transcription.

5. Chromatin remodeling activates gene expression when it:
   a. allows proteins initiating transcription to disengage from the promoter.
   b. winds genes tightly around histones.
   c. deacetylates histones.
   d. inserts nucleosomes into chromatin.
   e. recruits a protein complex that displaces nucleosome from the promoter.
6. Which statement about activation of transcription is *not* correct?
   a. A transcription factor binds the TATA box.
   b. A coactivator called a mediator forms a bridge between the promoter and the gene to be transcribed.
   c. Transcription factors bind the promoter and RNA polymerase.
   d. Activators bind to the enhancer region on DNA.
   e. RNA is transcribed downstream from the promoter region.
7. Which of the following statements does not support the idea of combinatorial gene regulation?
   a. Promoter proximal regions and enhancers regulate transcription of genes.
   b. A few regulatory genes can control a large number of transcribable genes.
   c. If repressor binding to enhancer is strong, gene expression is reduced.
   d. Genes requiring complex regulation have a single regulatory element.
   e. The number and types of regulatory sequences in the enhancer vary with each gene.
8. Normal ears in a certain mammal are perky; mutants have droopy ears. In males of these mammals, the gene encoding perky ears is transcribed only from the female parent. This is because the gene from the male parent is silenced by methylation. If the maternal gene is mutated:
   a. male offspring have droopy ears.
   b. male offspring have perky ears.
   c. male offspring have one droopy ear and one perky ear.
   d. the genetic mechanism is called alternative splicing.
   e. this is an example of posttranscriptional regulation.
9. Which of the following statements does not describe microRNA?
   a. MicroRNA is encoded by non-protein-coding genes.
   b. MicroRNA has a precursor that is folded and then cut by a Dicer enzyme.
   c. MicroRNA is an example of a molecule that induces RNA interference or gene silencing.
   d. MicroRNA is synthesized in vitro but not in vivo.
   e. MicroRNA has a similar function to that of small interfering RNAs.
10. Which of the following is not a characteristic of cancer cells?
    a. proto-oncogenes converting to active oncogenes
    b. the position of the *MYC* gene near a repressor gene
    c. the mutation of the *TP53* gene
    d. their stepwise developmental stages
    e. amplification of growth factors and growth factor receptors

Questions for Discussion

1. In a mutant strain of *E. coli*, the CAP protein is unable to combine with its target region of the *lac* operon. How would you expect the mutation to affect transcription when cells of this strain are subjected to the following conditions?
   a. lactose and glucose are both available
   b. lactose is available but glucose is not
   c. both lactose and glucose are unavailable
2. Duchenne muscular dystrophy, an inherited genetic disorder, affects boys almost exclusively. Early in childhood, muscle tissue begins to break down in affected individuals, who typically die in their teens or early twenties as a result of respiratory failure. Muscle samples from women who carry the mutation reveal some regions of degenerating muscle tissue adjacent to other regions that are normal. Develop a hypothesis explaining these observations.
3. Eukaryotic transcription is generally controlled by binding of regulatory proteins to DNA sequences rather than by modification of RNA polymerases. Develop a hypothesis explaining why this is so.

Evolution Link

Fruit flies homozygous for a mutation in the tumor-suppressor gene *HIPPO* develop tumors in every organ. Expression of the human gene *MST2* in flies homozygous for *HIPPO* show greatly reduced or no tumors. What does this result suggest about the evolution of tumor suppressor genes in animals?

Experimental Analysis

Design an experiment using rats as the model organism to test the hypothesis that human chorionic gonadotrophin (hCG), a hormone produced during pregnancy, leads to a significant protection against breast cancer.

How Would You Vote?

Some females at high risk of developing breast cancer opt for prophylactic mastectomy, the surgical removal of one or both breasts even before cancer develops. Many of them would never have developed cancer. Should the surgery be restricted to cancer treatment? Go to www.thomsonedu.com/login to investigate both sides of the issue and then vote.