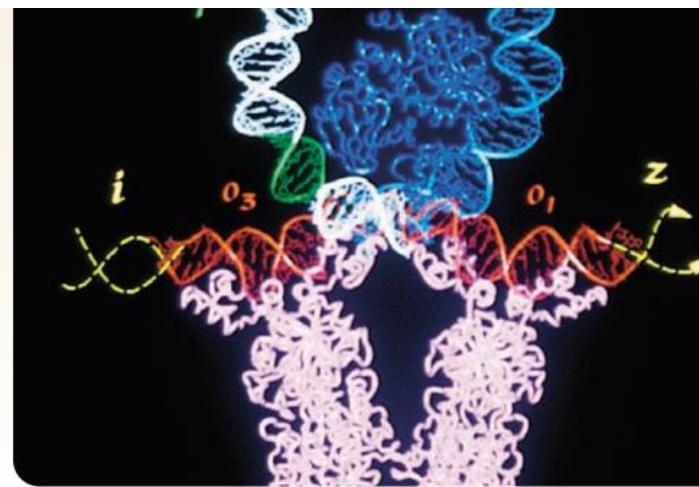


12

Microbial Genetics: Regulation of Gene Expression



Lactose operon activity is under the control of repressor and activator proteins. The *lac* repressor (pink) and catabolite activator protein (blue) are bound to the *lac* operon. The repressor blocks transcription when bound to the operators (red).

PREVIEW

- The long-term regulation of metabolism, behavior, and morphology is brought about by control of gene expression. This can occur at many levels including transcription initiation, transcription elongation, translation, and posttranslation.
- In *Bacteria*, control at the level of transcription initiation is often achieved by regulatory proteins. Some block transcription and others promote transcription. Furthermore, transcription can be terminated prematurely by a process called attenuation, in which ribosome behavior affects RNA polymerase activity. Translation in *Bacteria* can be regulated by small molecules that bind the leader of mRNA. The conformation changes that result block ribosome binding. Translation can also be blocked by antisense RNA molecules.
- Microorganisms must be able to respond rapidly to changing environmental conditions. Their responses often involve many genes or operons. The simultaneous control of many genes or operons is called global control. Important examples of bacterial global control systems are catabolite repression, quorum sensing, and endospore formation.
- Eucaryotic gene expression involves more steps than does bacterial gene expression; thus there are more points in the process where regulation can occur.
- Although archaeal genome organization is similar to that seen in *Bacteria*, the machinery used by the *Archaea* during information flow is more like that of the *Eucarya*. Thus archaeal regulatory mechanisms may be similar to those observed in the other domains of life.

The gram-positive soil bacterium *Bacillus subtilis* senses that the nutrient levels in its environment are decreasing, and it must determine if it should initiate sporulation. An *Escherichia coli* cell is in an environment rich in carbon and energy sources, and it must determine which to use and when to use them. A pathogen is transmitted from a stream to the intestinal tract of its animal host, and it must adjust to the warmer temperature, increased nutrient supply, and defenses of the host. These are just a few examples of situations to which microbes must respond. To make the most efficient use of the resources in the current environment and their own cellular machinery, microbes must respond to changes by altering physiological and behavioral processes. How is this accomplished?

The control of cellular processes by regulation of the activity of enzymes and other proteins is a fine-tuning mechanism: it acts rapidly to adjust metabolic activity from moment to moment. Microorganisms also are able to control the expression of their genome, although over longer intervals. For example, the *E. coli* chromosome can code for about 4500 polypeptides, yet not all proteins are produced at the same time. Regulation of gene expression serves to conserve energy and raw materials, to maintain balance between the amounts of various cell proteins, and to adapt to long-term environmental change. Thus control of gene expression complements the regulation of enzyme activity. [Control of enzyme activity \(section 8.10\)](#)

The particular field which excites my interest is the division between the living and the non-living, as typified by, say, proteins, viruses, bacteria and the structure of chromosomes. The eventual goal, which is somewhat remote, is the description of these activities in terms of their structure, i.e., the spatial distribution of their constituent atoms, in so far as this may prove possible. This might be called the chemical physics of biology.

—Francis Crick

In this chapter we explore the various mechanisms organisms used to regulate gene expression. We begin with a brief discussion of the many levels at which regulation can occur. We then introduce some important examples of the regulation of transcription initiation, transcription elongation, and translation. Finally, we examine how cells use these various regulatory mechanisms to control suites of genes in response to changes in their environments.

12.1 LEVELS OF REGULATION OF GENE EXPRESSION

Figure 12.1 summarizes the expression of bacterial, archaeal, and eucaryotic genes and highlights points in the process where regulation often occurs. Although the overall processes of tran-

scription and translation in the three domains of life are similar, there are differences that affect gene expression. For instance, chromatin structure varies. Bacterial chromosomes lack histones, whereas eucaryotic chromosomes and some archaeal chromosomes are associated with histones. DNA condensed by histones is less accessible to RNA polymerase, and expression of eucaryotic genes involves the additional step of opening up the chromatin to expose promoters. It is also important to remember that in prokaryotes, genes of related function are often transcribed from a single promoter, giving rise to a polycistronic mRNA. In addition, transcription and translation are tightly coupled in prokaryotes. Eucaryotic mRNA molecules, on the other hand, are monocistronic and are the product of RNA processing, which adds the 5' cap and poly-A tail and removes introns. Fur-

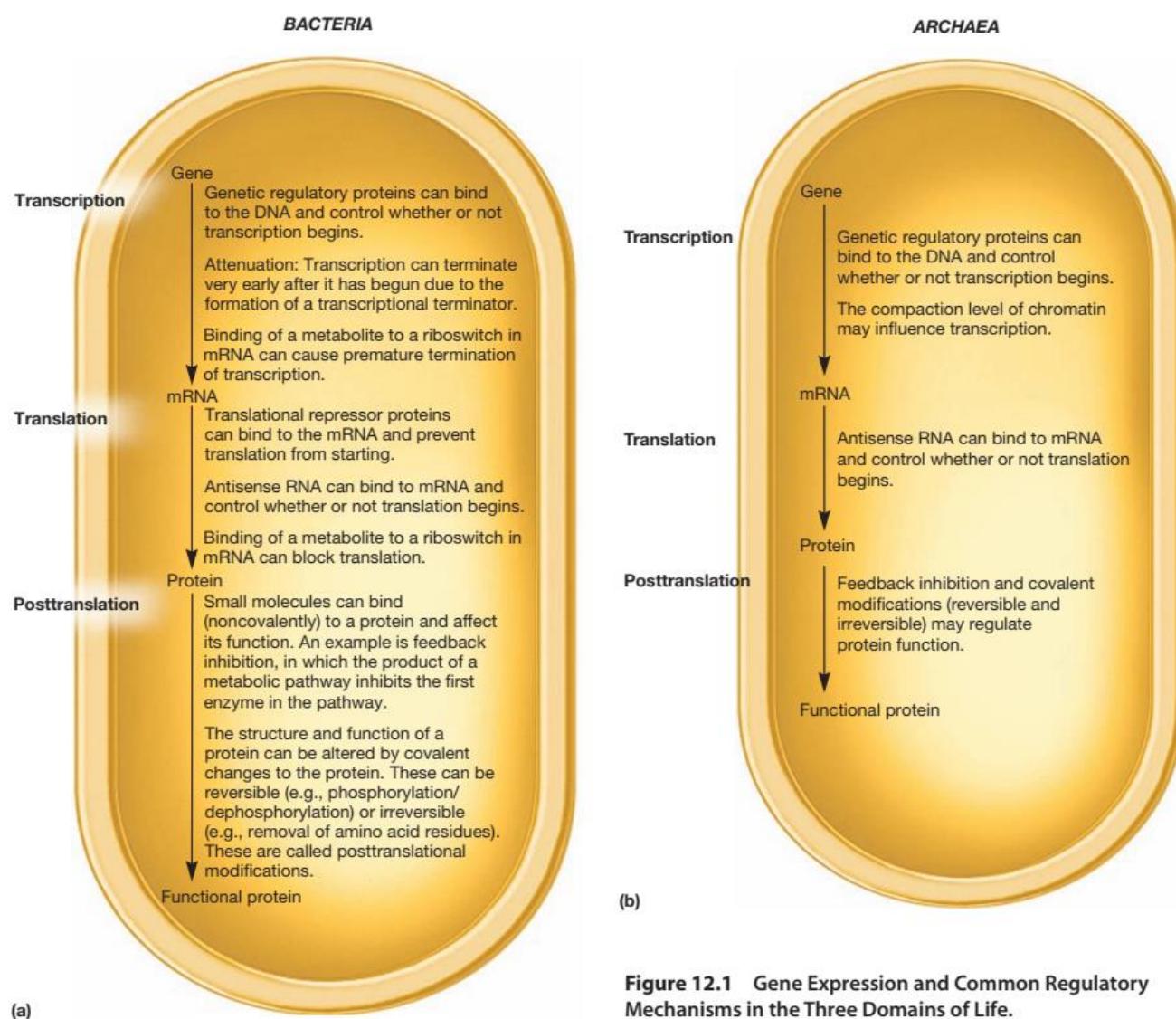
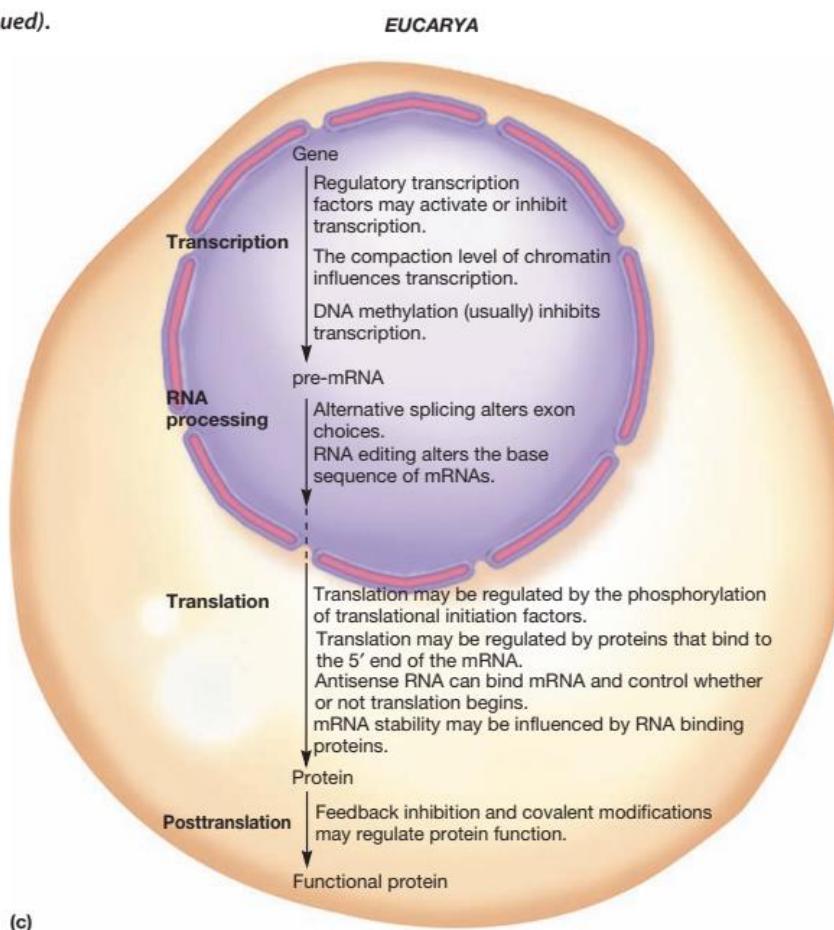


Figure 12.1 Gene Expression and Common Regulatory Mechanisms in the Three Domains of Life.

Figure 12.1 (Continued).

thermore, although genes are organized in a similar fashion in the *Bacteria* and the *Archaea*, the archaeal enzymes, molecules, and signaling sequences that function in transcription and translation are more like those of the *Eucarya*.

Because of these differences, regulation of gene expression is somewhat different in each domain of life. Our focus in this chapter is on well-understood bacterial regulatory processes. We begin our discussion by introducing two phenomena: induction of enzyme synthesis and repression of enzyme synthesis. Induction and repression provided the first models for gene regulation (**Historical Highlights 12.1**). These early models involved the action of regulatory proteins, and the notion that gene expression is regulated solely by proteins persisted for many years. Eventually it was clearly demonstrated that RNA molecules also can have regulatory functions. Induction and repression also demonstrate the regulation of transcription initiation. Although many regulatory processes occur at this level, there are numerous regulatory mechanisms occurring at other levels. We describe some of these mechanisms as well.

12.2 REGULATION OF TRANSCRIPTION INITIATION

Induction and repression are historically important, as they were the first regulatory processes to be understood in any detail. In this section, we first describe these phenomena and then examine the underlying regulatory events.

Induction and Repression of Enzyme Synthesis

Many enzymes are produced almost all of the time because they catalyze reactions in the cell that are needed routinely. These enzymes include those of the central metabolic pathways. Their functions are often referred to as “housekeeping functions” and the genes that encode them are often referred to as **housekeeping genes**. Those housekeeping genes that are expressed continuously are said to be **constitutive genes**. Many genes, however, are expressed only when needed. The β -galactosidase gene is an example of a regulated gene.

β -galactosidase catalyzes the hydrolysis of the disaccharide sugar lactose to glucose and galactose (**figure 12.2**). When *E. coli*



Historical Highlights

12.1 The Discovery of Gene Regulation

The ability of microorganisms to adapt to their environments by adjusting enzyme levels was first discovered by Emil Duclaux, a colleague of Louis Pasteur. He found that the fungus *Aspergillus niger* would produce the enzyme that hydrolyzes sucrose (invertase) only when grown in the presence of sucrose. In 1900 F. Dienert found that yeast contained the enzymes for galactose metabolism only when grown with lactose or galactose and would lose these enzymes upon transfer to a glucose medium. Such a response made sense because the yeast cells would not need enzymes for galactose metabolism when using glucose as its carbon and energy source. Further examples of adaptation were discovered, and by the 1930s H. Karström divided enzymes into two classes: (1) adaptive enzymes that are formed only in the presence of their substrates, and (2) constitutive enzymes that are always present. It was originally thought that enzymes might be formed from inactive precursors and that the presence of the substrate simply shifted the equilibrium between precursor and enzyme toward enzyme formation.

In 1942 Jacques Monod, working at the Pasteur Institute in Paris, began a study of adaptation in the bacterium *E. coli*. It was already known that the enzyme β -galactosidase, which hydrolyzes the sugar lactose to glucose and galactose, was present only when *E. coli* was grown in the presence of lactose. Monod discovered that nonmetabolizable analogues of β -galactosides, such as thiomethyl-galactoside, also could induce enzyme production. This discovery made it possible to study induction in cells growing on carbon and energy sources other than lactose so that the growth rate and inducer concentration would not depend on the lactose supply. He next demonstrated that induction involved the synthesis of new enzyme, not just the conversion of already available precursor. Monod accomplished this by making *E. coli* proteins radioactive with ^{35}S , then transferring the labeled bacteria to nonradioactive medium and adding inducer. The newly formed β -galactosidase was nonradioactive and must have been synthesized after addition of inducer.

A study of the genetics of lactose induction in *E. coli* was begun by Joshua Lederberg a few years after Monod had started his work. Lederberg isolated not only mutants lacking β -galactosidase but also a constitutive mutant in which synthesis of the enzyme proceeded in the absence of an inducer (*lacI⁻*). During bacterial conjugation, genes from the donor bacterium enter the recipient to temporarily form an organism with two copies of those genes provided by the donor. When Arthur Pardee, François Jacob, and Monod transferred the gene for inducibility to a constitutive recipient not sensitive to inducers, the newly acquired gene made the recipient bacterium sensitive to inducer again. This functional gene was not a part of the recipient's chromosome. Thus the special gene directed the synthesis of a cytoplasmic product that inhibited the formation of β -galactosidase in the absence of the inducer. In 1961 Jacob and Monod named this special product the repressor and suggested that it was a protein. They further proposed that the repressor protein exerted its effects by binding to the operator, a special site next to the structural genes. They provided genetic evidence for their hypothesis. The name operon was given to the complex of the operator and the genes it controlled. Several years later in 1967, Walter Gilbert and Benno Müller-Hill managed to isolate the lac repressor and show that it was indeed a protein and did bind to a specific site in the *lac* operon. [Bacterial conjugation \(section 13.7\)](#)

The existence of repression was discovered by Monod and G. Cohen-Bazire in 1953 when they found that the presence of the amino acid tryptophan would repress the synthesis of tryptophan synthetase, the final enzyme in the pathway for tryptophan biosynthesis. Subsequent research in many laboratories showed that induction and repression were operating by quite similar mechanisms, each involving repressor proteins that bound to operators on the genome. Jacob, Monod, and Lederberg all became Nobel laureates for their work on gene regulation.

grows with lactose as its only carbon source, each cell contains about 3,000 β -galactosidase molecules, but it has less than three molecules in the absence of lactose. The enzyme β -galactosidase is an **inducible enzyme**—that is, its level rises in the presence of a small **effector molecule** called an **inducer** (in this case the lactose derivative allolactose). Likewise the genes that encode inducible enzymes such as β -galactosidase are referred to as **inducible genes**.

β -galactosidase is an enzyme that functions in a catabolic pathway and many catabolic enzymes are inducible enzymes. The genes for enzymes involved in the biosynthesis of amino acids and other substances, on the other hand, are often called **repressible enzymes**. For instance, an amino acid present in the surroundings may inhibit the formation of enzymes responsible for its biosynthesis. This makes good sense because the microorganism does not need the biosynthetic enzymes for a particular substance if it is already available. Generally, repressible enzymes are necessary for synthesis and always are present unless the end product of their pathway is available. Inducible enzymes,

in contrast, are required only when their substrate is available; they are missing in the absence of the inducer.

Although variations in enzyme levels could be due to changes in the rates of enzyme degradation, most enzymes are relatively stable in growing bacteria. Induction and repression result principally from changes in the rate of transcription. When *E. coli* is growing in the absence of lactose, it lacks mRNA molecules coding for the synthesis of β -galactosidase. In the presence of lactose, however, each cell has 35 to 50 β -galactosidase mRNA molecules. The synthesis of mRNA is dramatically influenced by the presence of lactose.

Control of Transcription Initiation by Regulatory Proteins

The action of regulatory proteins is most often responsible for induction and repression. Regulatory proteins can exert either negative or positive control. **Negative transcriptional control** occurs

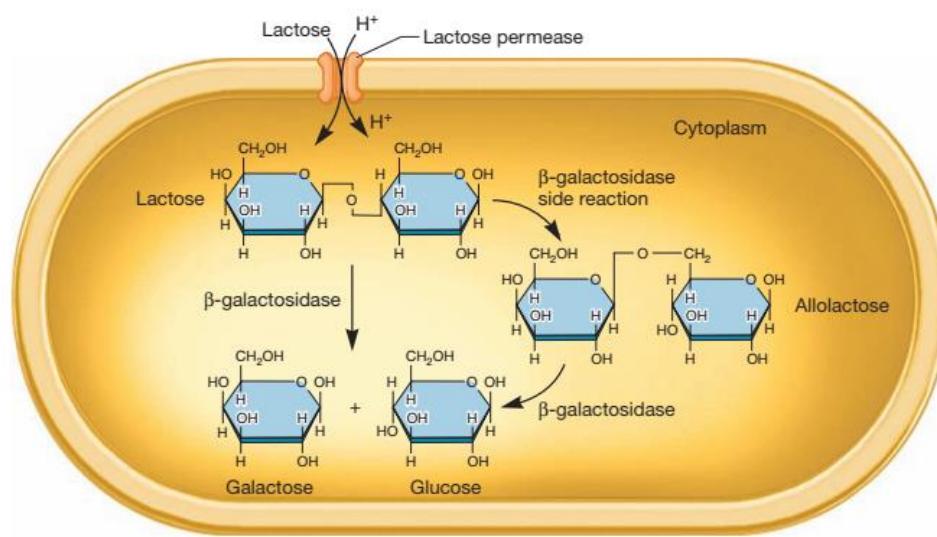


Figure 12.2 The Reactions of β-Galactosidase. The main reaction catalyzed by β-galactosidase is the hydrolysis of lactose, a disaccharide, into the monosaccharides galactose and glucose. The enzyme also catalyzes a minor reaction that converts lactose to allolactose. Allolactose acts as the inducer of β-galactosidase synthesis.

when the protein inhibits initiation of transcription. Regulatory proteins that act in this fashion are called **repressor proteins**. **Positive transcriptional control** occurs when the protein promotes transcription initiation. These proteins are called **activator proteins**.

Repressor and activator proteins usually act by binding DNA at specific sites. Repressor proteins bind a region called the **operator**, which usually overlaps or is downstream of the promoter (i.e., closer to the coding region) (figure 12.3a,b). When bound, the repressor protein either blocks binding of RNA polymerase to the promoter or prevents its movement. Activator proteins bind **activator-binding sites** (figure 12.3c,d). These are often upstream of the promoter (i.e., farther away from the coding region). Binding of an activator to its regulatory site generally promotes RNA polymerase binding.

Repressor and activator proteins must exist in both active and inactive forms if transcription initiation is to be controlled appropriately. The activity of regulatory proteins is modified by small effector molecules, most of which bind the regulatory protein noncovalently. Figure 12.3 shows the four basic ways in which the interactions of an effector and a regulatory protein can affect transcription: (1) For negatively controlled inducible genes (e.g., those encoding enzymes needed for catabolism of a sugar), the repressor protein is active and prevents transcription when the substrate of the pathway is not available (figure 12.3a). It is inactivated by binding of the inducer (e.g., the substrate of the pathway). (2) For negatively controlled repressible genes (e.g., those encoding enzymes needed for the synthesis of an amino acid), the repressor protein is initially synthesized in an inactive form called the **aporepressor**. It is activated by binding of the **corepressor** (figure 12.3b). For repressible enzymes that function in a biosynthetic pathway, the corepressor is often the prod-

uct of the pathway (e.g., an amino acid). (3) The activator of a positively regulated inducible gene is activated by the inducer (figure 12.3c); whereas (4) the activator of a positively regulated repressible gene is inactivated by an inhibitor (figure 12.3d).

Control of enzyme activity: Allosteric regulation (section 8.10)

Recall that functionally related bacterial and archaeal genes are often transcribed from a single promoter. The **structural genes**—the genes coding for polypeptides—are simply lined up together on the DNA, and a single, polycistronic mRNA carries all the messages. The sequence of bases coding for one or more polypeptides, together with the promoter and operator or activator-binding sites, is called an **operon**. Many operons have been discovered and studied. Three well studied operons are discussed next. They demonstrate different ways that regulatory proteins can be used to control gene expression at the level of transcription initiation.

The Lactose Operon: Negative Transcriptional Control of Inducible Genes

The best-studied negative control system is the lactose (*lac*) operon of *E. coli*. The *lac* operon contains three structural genes controlled by the *lac* repressor, which is encoded by *lacI* (figure 12.4). One gene codes for β-galactosidase; a second gene directs the synthesis of β-galactoside permease, the protein responsible for lactose uptake. The third gene codes for the enzyme β-galactoside transacetylase, whose function still is uncertain. The presence of the first two genes in the same operon ensures that the rates of lactose uptake and breakdown will vary together.

Before we describe the regulation of the *lac* operon, we must consider two general aspects of regulation. The first is that gene expression is rarely an all-or-nothing phenomenon; it is a continuum.

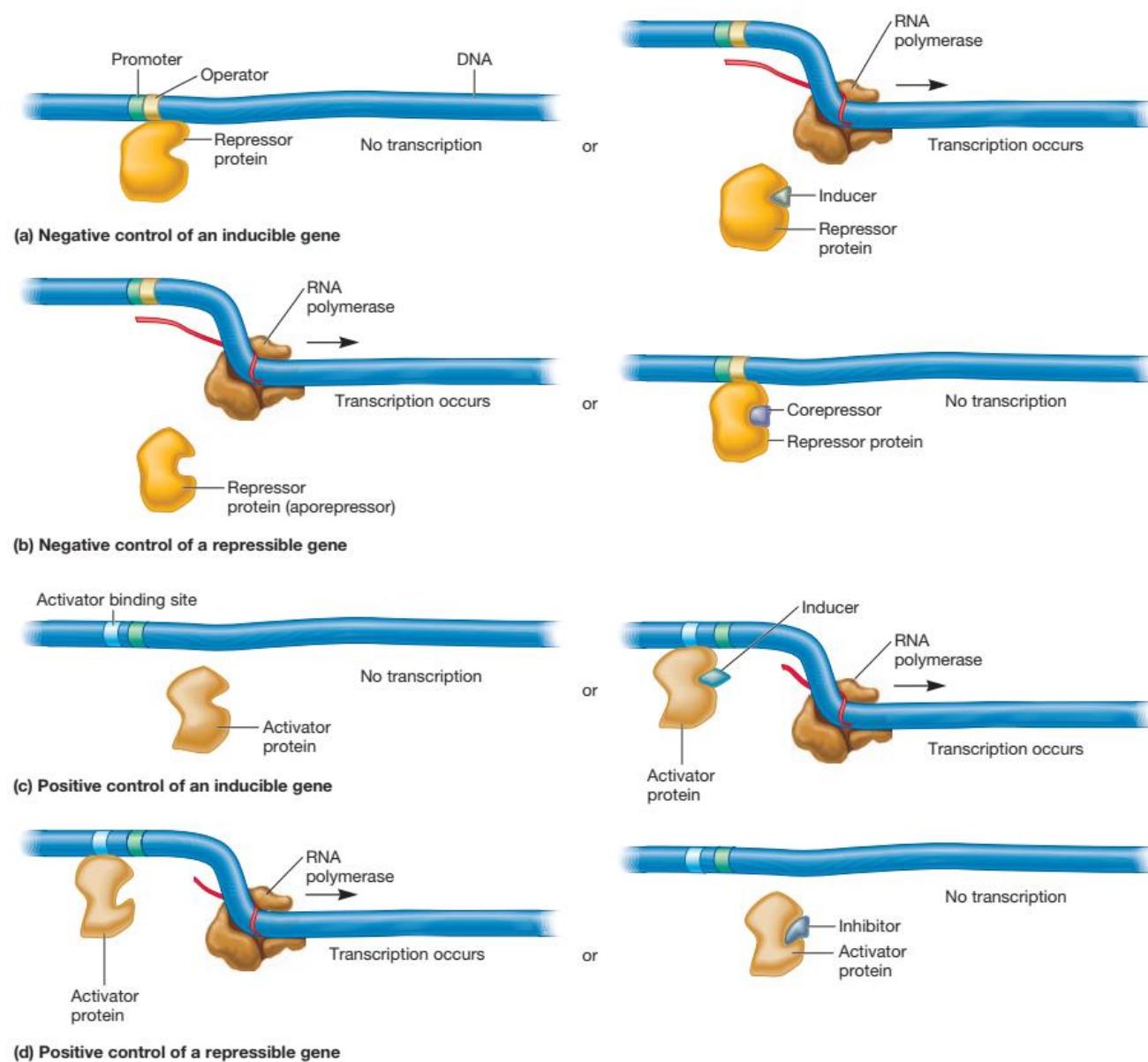


Figure 12.3 Action of Bacterial Regulatory Proteins. Bacterial regulatory proteins have two binding sites—one for a small effector molecule and one for DNA. The binding of the effector molecule changes the regulatory protein's ability to bind DNA. **(a)** In the absence of inducer, the repressor protein blocks transcription. The presence of inducer prevents the repressor from binding DNA and transcription occurs. **(b)** In the absence of a corepressor, the repressor is unable to bind DNA and transcription occurs. When the corepressor is bound to the repressor, the repressor is able to bind DNA and transcription is blocked. **(c)** The activator protein is able to bind DNA and activate transcription only when it is bound to the inducer. **(d)** The activator binds DNA and promotes transcription unless the inhibitor is present. When inhibitor is present, the activator undergoes a conformational change that prevents it from binding DNA; this inhibits transcription.

Inhibition of transcription usually does not mean that genes are “turned off” (though this terminology is frequently used). Rather it means the level of mRNA synthesis is decreased significantly, and in most cases is occurring at very low levels. In other words, many promoters of regulated genes and operons are considered “leaky,”

in that there is always some low, **basal level of transcription**. The second aspect of regulation to be considered is the “decision-making” process used by microbial cells. Consider the regulatory decisions made by an *E. coli* cell. It need only synthesize the enzymes of a specific catabolic pathway if the substrate of the pathway is

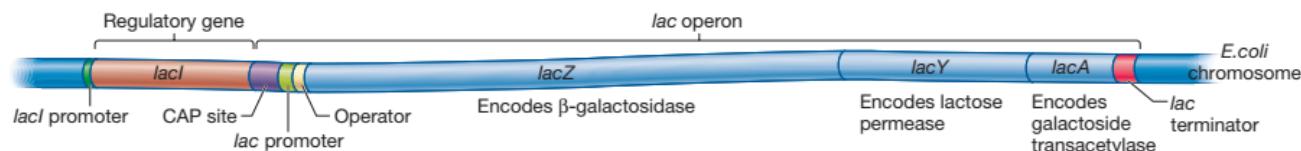


Figure 12.4 The *lac* Operon. The *lac* operon consists of three genes: *lacZ*, *lacY*, and *lacA*, which are transcribed as a single unit from the *lac* promoter. The operon is regulated both negatively and positively. Negative control is brought about by the *lac* repressor, which is the product of the *lacI* gene. The operator is the site of *lac* repressor binding. Positive control results from the action of CAP. CAP binds the CAP site located just upstream from the *lac* promoter. CAP is, in part, responsible for a phenomenon called catabolite repression, an example of a global control network, in which numerous operons are controlled by a single protein.

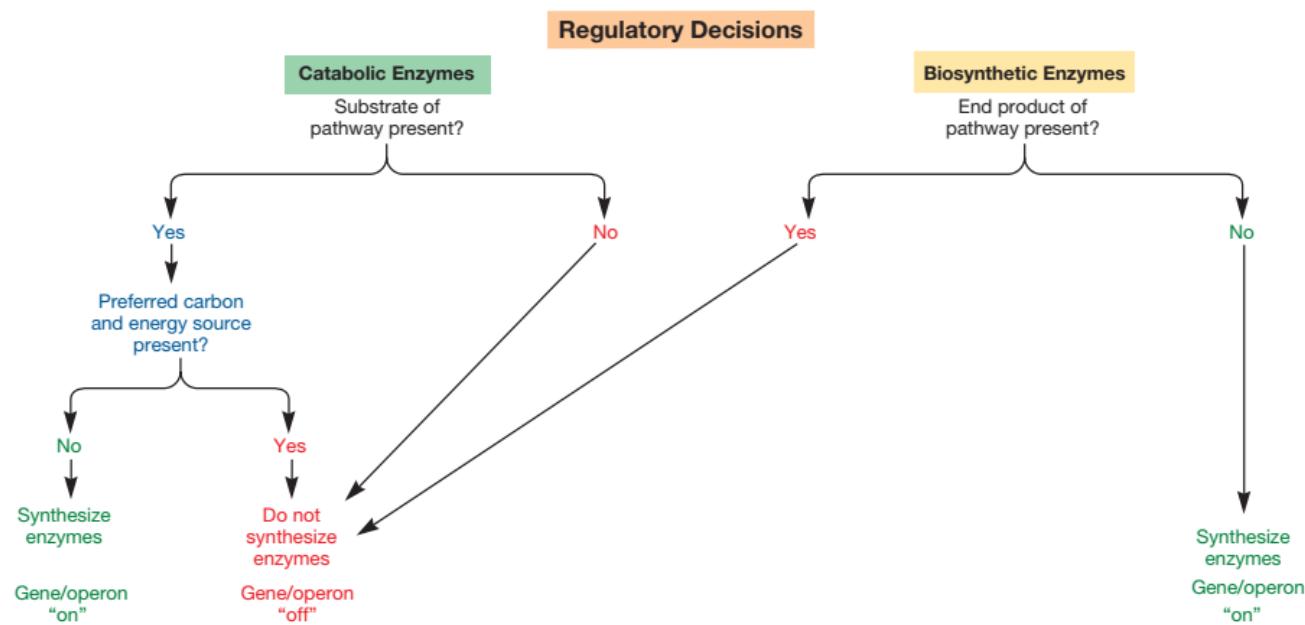


Figure 12.5 Examples of Regulatory Decisions Made by Cells.

present in the environment and a preferred carbon source (e.g., glucose) is not (figure 12.5). Conversely, synthesis of the enzymes involved in biosynthetic pathways is inhibited when the end product of the pathway is present.

How do these two aspects of regulation affect expression of the *lac* operon? Lactose is one of many organic molecules *E. coli* can use as a carbon and energy source. It is wasteful to synthesize enzymes of the *lac* operon when lactose is not available. Therefore the cell only expresses this operon at high levels when lactose is the only carbon and energy source present in the environment; the *lac* repressor is responsible for inhibiting transcription when there is no lactose.

The *lac* repressor is a tetramer composed of four identical subunits. The tetramer is formed when two dimers interact. When lactose catabolism is not required, each dimer recognizes and tightly binds one of three different *lac* operator sites: O_1 , O_2 , and O_3 (figure 12.6a). O_1 is the main operator site and must be bound

by the repressor if transcription is to be inhibited. When one dimer is at O_1 and another is at one of the two other operator sites, the dimers bring the two operator sites close together, with a loop of DNA forming between them. The binding of *lac* repressor is a two-step process. First, the repressor binds nonspecifically to DNA. Then it rapidly slides along the DNA until it reaches an operator site. A portion of the repressor fits into the major groove of operator-site DNA (figure 12.6b). Thus the shape of the repressor is ideally suited for specific binding to the DNA double helix.

How does the repressor inhibit transcription? The promoter to which RNA polymerase binds is located near the *lac* operator sites. When there is no lactose, the repressor binds O_1 and one of the other operator sites, bending the DNA in the promoter region. This prevents initiation of transcription either because RNA polymerase cannot access the promoter or because it is blocked from moving into the coding region (figure 12.7a). When lactose is available, it is taken up by the lactose permease. Once inside the

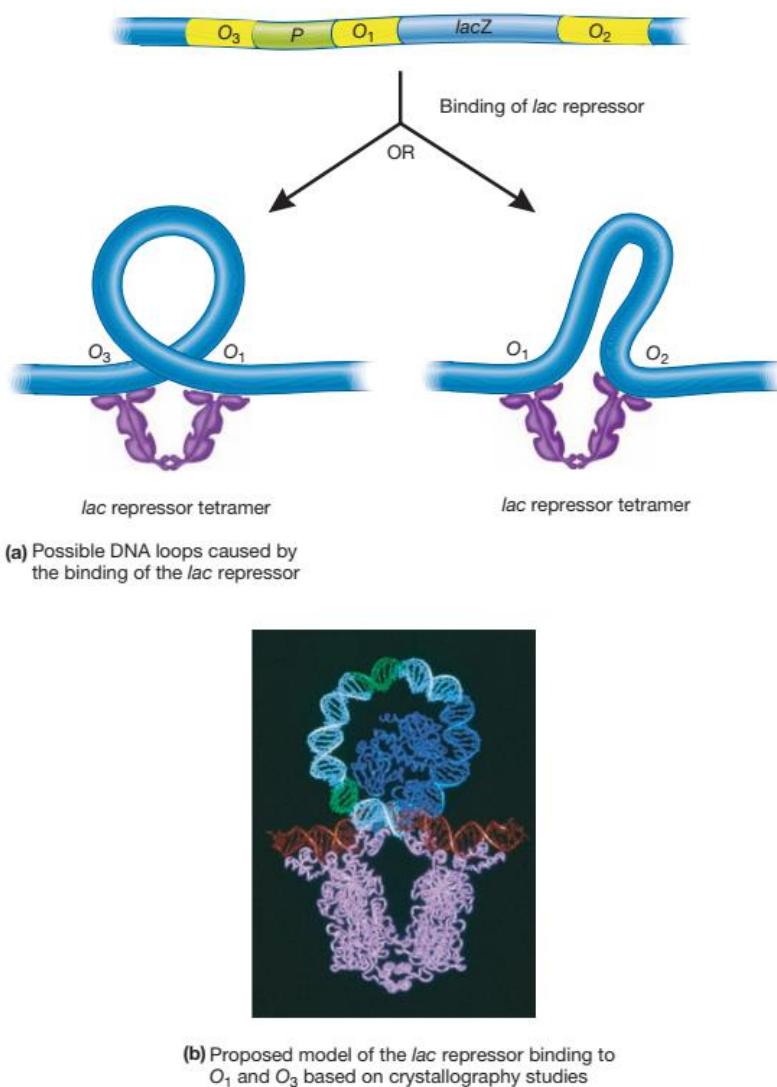


Figure 12.6 The *lac* Operator Sites. The *lac* operon has three operator sites: *O*₁, *O*₂, and *O*₃ (a). *O*₁ is the same operator shown in figure 12.4. As shown in (a) and (b), the *lac* repressor (violet) binds *O*₁ and one of the other operator sites (red) to block transcription, forming a DNA loop. The DNA loop contains the -35 and -10 binding sites (green) recognized by RNA polymerase. Thus these sites are inaccessible and transcription is blocked. The DNA loop also contains the CAP binding site and CAP (blue) is shown bound to the DNA (b). When the *lac* repressor is bound to the operator, CAP is unable to activate transcription.

cell, β -galactosidase converts lactose to allolactose, the inducer of the operon (figure 12.2). This occurs because, as noted previously, there is always a low level of permease and β -galactosidase synthesis. Allolactose binds to the *lac* repressor and causes the repressor to change to an inactive shape that is unable to bind any operator sites. The inactivated repressor leaves the DNA and transcription occurs (figure 12.7b).

Close examination of figures 12.4 and 12.6 clearly shows that the regulation of the *lac* operon is not as simple as has just

been described. That is because the *lac* operon is regulated by a second regulatory protein called CAP. CAP functions in a global regulatory network that allows *E. coli* to use glucose preferentially over all other carbon and energy sources by a mechanism called catabolite repression. The use of two different regulatory proteins to control the synthesis of an operon illustrates a point that is important in the discussion of the regulation of gene expression—there are often layers of regulation of any operon. As described in section 12.5, the use of two

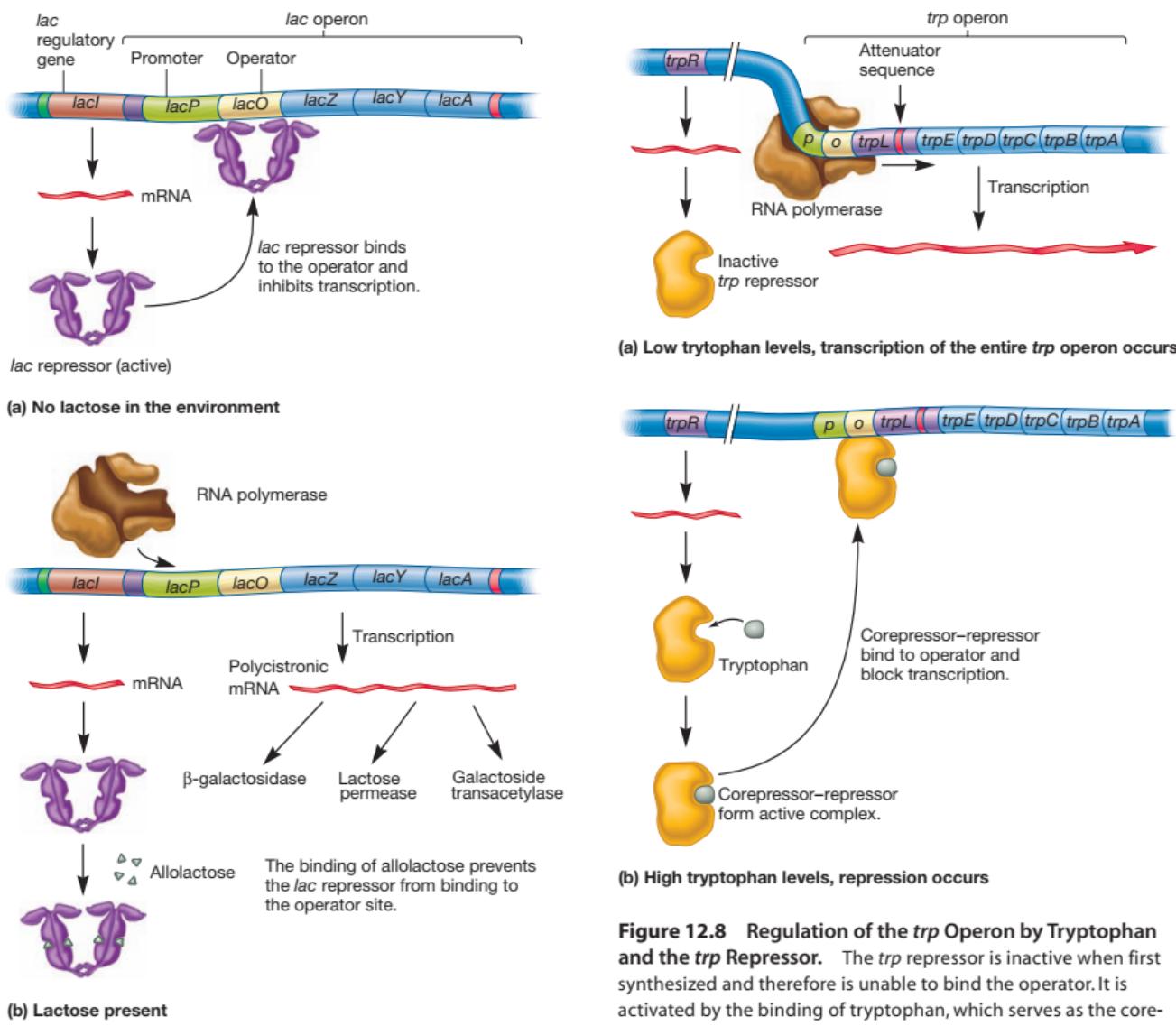


Figure 12.7 Regulation of the lac Operon by the lac Repressor. (a) The lac repressor is active and can bind the operator as long as the inducer of the operon, allolactose, is not present. Binding of the repressor to the operator inhibits transcription of the operon by RNA polymerase. (b) When lactose is available, some of it is converted to allolactose by β -galactosidase. When sufficient amounts of allolactose are present, it binds and inactivates the lac repressor. The repressor leaves the operator and RNA polymerase is free to initiate transcription.

regulatory proteins generates a continuum of expression levels. The highest levels of transcription occur when lactose is available and glucose is not; the lowest levels occur when lactose is not available and glucose is.

Figure 12.8 Regulation of the trp Operon by Tryptophan and the trp Repressor. The trp repressor is inactive when first synthesized and therefore is unable to bind the operator. It is activated by the binding of tryptophan, which serves as the corepressor. (a) When tryptophan levels are low, the repressor is inactive and transcription occurs. The enzymes encoded by the operon catalyze the reactions needed for tryptophan biosynthesis. (b) When tryptophan levels are sufficiently high, it binds the repressor. The repressor-corepressor complex binds the operator and transcription of the operon is inhibited.

The Tryptophan Operon: Negative Transcriptional Control of Repressible Genes

The tryptophan (*trp*) operon of *E. coli* consists of five structural genes that encode enzymes needed for synthesis of the amino acid tryptophan (figure 12.8). It is regulated by the *trp* repressor, which is encoded by the *trpR* gene. Because the enzymes encoded by the *trp* operon function in a biosynthetic pathway, it is wasteful to make the enzymes needed for tryptophan synthesis when

tryptophan is readily available. Therefore, the operon functions only when tryptophan is not present and must be made *de novo* from precursor molecules (figure 12.5). To accomplish this regulatory goal, the *trp* repressor is synthesized in an inactive form that cannot bind the *trp* operator as long as tryptophan levels are low (figure 12.8a). When tryptophan levels increase, tryptophan acts as a corepressor, binding the repressor and activating it. The repressor-corepressor complex then binds the operator, blocking transcription initiation (figure 12.8b).

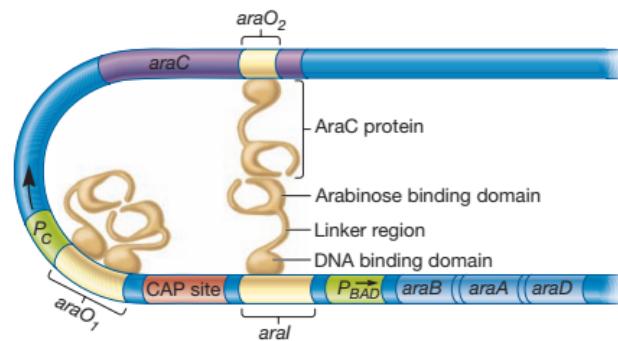
Like the *lac* operon, the *trp* operon is subject to another layer of regulation. In addition to being controlled at the level of transcription initiation by the *trp* repressor, expression of the *trp* operon is also controlled at the level of transcription elongation by a process called attenuation. This mode of regulation is discussed in section 12.3.

The Arabinose Operon: Transcriptional Control by a Protein that Acts Both Positively and Negatively

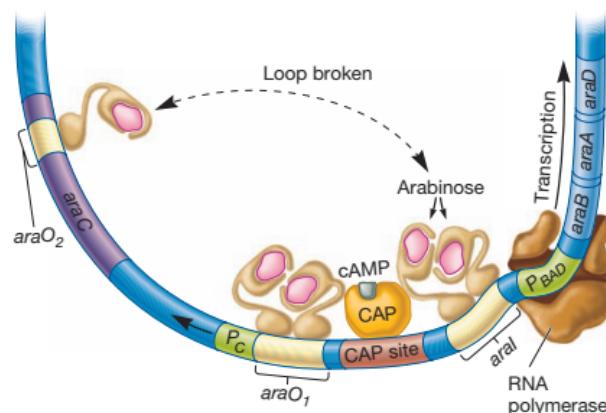
Many regulatory proteins are versatile and can function as repressors for one operon and activators for others. The regulation of the *E. coli* arabinose (*ara*) operon illustrates how the same protein can function either positively or negatively depending on the environmental conditions. The *ara* operon encodes enzymes needed for the catabolism of arabinose to xylulose 5-phosphate, an intermediate of the pentose phosphate pathway. The *ara* operon is regulated by AraC, which can bind three different regulatory sequences: *araO₂*, *araO₁*, and *araI* (figure 12.9). When arabinose is not present, one molecule of AraC binds *araI*, and another binds *araO₂*. The two AraC proteins interact, causing the DNA to bend. This prevents RNA polymerase from binding to the promoter of the *ara* operon, thereby blocking transcription. In these conditions, AraC acts as a repressor (figure 12.9a). However, when arabinose is present, it binds AraC and prevents AraC molecules from interacting. This breaks the DNA loop. Furthermore, binding of two AraC-arabinose complexes to the *araI* site promotes transcription. Thus when arabinose is present, AraC acts as an activator (figure 12.9b). The *ara* operon, like the *lac* operon, is also subject to catabolite repression (see section 12.5). [The breakdown of glucose to pyruvate: The pentose phosphate pathway \(section 9.3\)](#)

Two-Component Regulatory Systems and Phosphorelay Systems

The activity levels of the *lac* repressor, *trp* repressor, and AraC protein are controlled by metabolites of those pathways. However, many environmental conditions do not produce a metabolite that can interact directly with a regulatory protein. These include temperature, osmolarity, and oxygen levels. How do organisms sense and respond to such stimuli? Many genes and operons are turned on or switched off in response to these types of signals by regulatory proteins that are part of a **two-component signal transduction system**. These systems link events occurring outside the cell to the regulation of gene expression. Some of the best-studied signal transduction systems are found in mul-



(a) Operon inhibited in the absence of arabinose



(b) Operon activated in the presence of arabinose

Figure 12.9 Regulation of the *ara* Operon by the AraC Protein. The AraC protein can act both as a repressor and as an activator, depending on the presence or absence of arabinose. **(a)** When arabinose is not available, the protein acts as a repressor. Two AraC proteins are involved. One binds the *araI* site and the other binds the *araO₂* site. The two proteins interact in such a way that the DNA between the two operator sites is bent, making it inaccessible to RNA polymerase. **(b)** When arabinose is present, it binds AraC, disrupting the interaction between the two AraC proteins. Subsequently, two AraC proteins, each bound to arabinose, form a dimer, which binds to the *araI* site. The AraC dimer functions as an activator and transcription occurs.

ticellular eucaryotes. However, important signal transduction systems have been identified in prokaryotes. They serve as models for understanding the more complex systems of eucaryotes as well as the mechanisms by which many pathogens regulate genes encoding virulence factors. Some of these prokaryotic signal transduction systems are the focus of our discussion here.

Two-component signal transduction systems are found in both the *Archaea* and the *Bacteria* and are named after the two proteins that govern the regulatory pathway. The first is a **sensor**

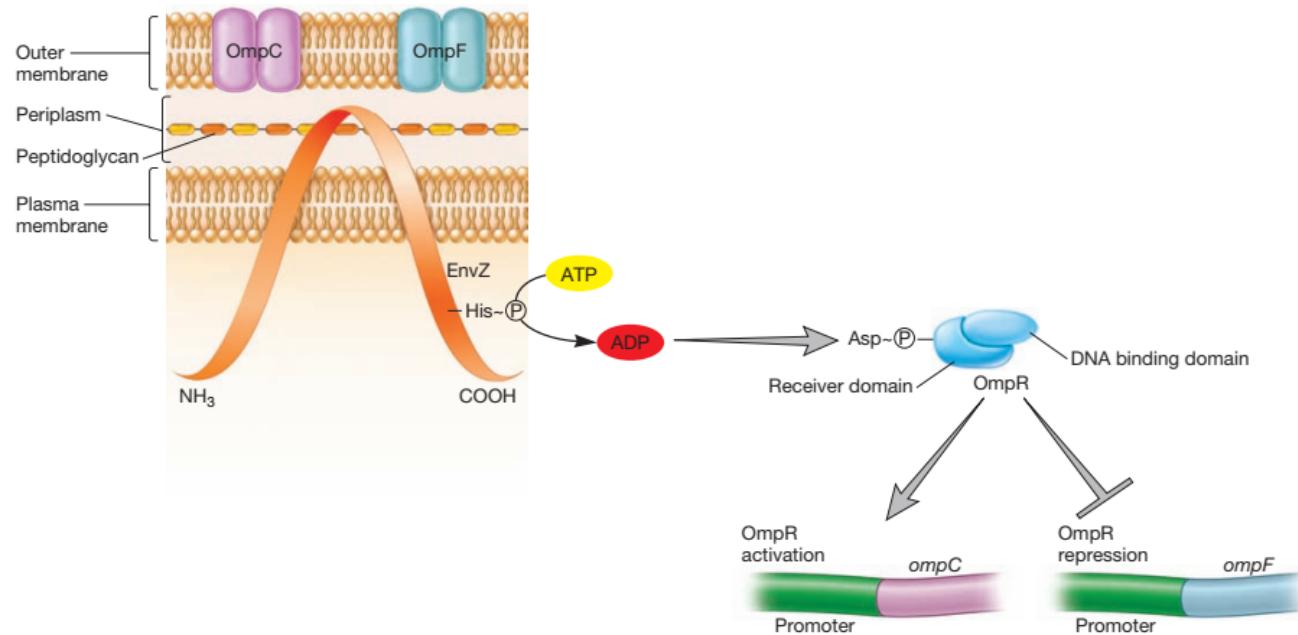


Figure 12.10 Two Component Signal Transduction System and the Regulation of Porin Proteins. In this system, the sensor kinase protein EnvZ loops through the cytoplasmic membrane so that both its C- and N-termini are in the cytoplasm. When EnvZ senses an increase in osmolarity, it autophosphorylates a histidine residue at its C-terminus. EnvZ then passes the phosphoryl group to the response regulator OmpR, which accepts it on an aspartic acid residue located in its N-terminus. This activates OmpR so that it is able to bind DNA and repress *ompF* expression and enhance that of *ompC*.

kinase protein that spans the cytoplasmic membrane so that part of it is exposed to the extracellular environment (periplasm, in gram-negative bacteria) while another part is exposed to the cytoplasm (**figure 12.10**). In this way, it can sense specific changes in the environment and communicate information to the cell's interior. The second component is the **response-regulator protein**, a DNA-binding protein that, when activated by the sensor kinase, promotes transcription of genes or operons whose expression is needed for adaptation to the detected environmental stimulus. The response-regulator protein may also inhibit transcription of genes or operons that are not needed under the current environmental conditions.

The regulation of the ratio of **OmpF:OmpC porin proteins** in *E. coli* is one of the best-understood two-component signal transduction systems (figure 12.10). Recall that the outer membrane of gram-negative bacteria contains channels made of porin proteins. The two most important porins in *E. coli* are OmpF and OmpC (Omp for *outer membrane protein*). OmpC pores are slightly smaller and are made when the bacterium grows at high osmotic pressures. It is the dominant porin when *E. coli* is in the intestinal tract. The larger OmpF pores are favored when *E. coli* grows in a dilute environment; OmpF allows solutes to diffuse into the cell more readily. The cell must maintain a constant level of porin protein in the membrane, but the relative levels of the two porins change to correspond with the osmolarity of the medium. Clearly,

the cell must have a way of sensing increases in osmolarity so that *ompF* expression is repressed and *ompC* transcription is enhanced.

The sensor kinase in the OmpF:OmpC two-component regulatory system is the EnvZ protein (*env* for *cell envelope*). It is an integral membrane protein anchored to the membrane by two membrane-spanning domains. EnvZ is looped through the membrane such that a central domain protrudes into the periplasm, while the amino and carboxyl termini are exposed to the cytoplasm. The second component, OmpR, is a soluble, cytoplasmic protein that regulates the transcription of the *ompF* and *ompC* structural genes. The N-terminal end of OmpR is called the receiver domain because it possesses a specific aspartic acid residue that accepts the signal (a phosphoryl group) from the sensor kinase. Upon receipt of the signal, the C-terminal end of OmpR is able to regulate transcription by binding DNA. At low osmolarity, EnvZ is inactive, but when EnvZ senses that osmolarity has increased, EnvZ phosphorylates itself (**autophosphorylation**) on a specific histidine residue. This phosphoryl group is quickly transferred to the N-terminus of OmpR. Once OmpR is phosphorylated, it is able to regulate transcription of the porin genes so that *ompF* transcription is repressed and *ompC* transcription is activated.

Two-component signal transduction systems are simple in design: the signal recognized by the sensor kinase is directly transduced (sent) to the response regulator that mediates the required

changes in gene expression; in many cases numerous genes and operons may be regulated by the same response regulator. Thus two-component systems often function in global regulatory networks. The effectiveness of two-component systems is illustrated by their abundance: most prokaryotic cells use a variety of two-component signal transduction systems to respond to an array of environmental stresses. For example, the morphologically complex genus *Streptomyces* has over 50 such systems!

Two-component signal transduction systems involve a simple phosphorelay where the sensor kinase transfers its phosphoryl group directly to the response-regulator protein. However, there are instances when more proteins participate in the transfer of phosphoryl groups. These longer pathways are called **phosphorelay systems**. An important and well-studied phosphorelay system functions during sporulation in *Bacillus subtilis* and is described in section 12.5. It should be noted that some phosphorelay systems control protein activity rather than gene transcription. An example of this type of system is chemotaxis in *E. coli*, which is described in chapter 8.

1. Many genes and operons are regulated at the level of transcription initiation. Why do you think this is the case?
2. What are induction and repression? How do bacteria use them to respond to changing nutrient supplies?
3. Define negative control and positive control of transcription initiation. Describe how regulatory proteins function in these regulatory mechanisms. Define repressor protein, activator protein, operator, activator-binding site, inducer, corepressor, structural gene, and operon.
4. Using figure 12.5 as a guide, trace the “decision-making” pathway of an *E. coli* cell that is growing in a medium containing arabinose but lacking tryptophan.
5. Describe a two-component signal transduction system. How does it differ from a phosphorelay system? How are the *lac* repressor, the *trp* repressor, and the AraC protein similar to the response regulators of two-component and phosphorelay systems? How are they different?

12.3 REGULATION OF TRANSCRIPTION ELONGATION

Organisms can also regulate transcription by controlling the termination of transcription. In this type of regulation, transcription is initiated but prematurely stopped depending on the environmental conditions and the needs of the organism. The first demonstration of this level of regulation, called attenuation, occurred in the 1970s in studies of the *trp* operon. More recently, riboswitches have been discovered. These regulatory sequences in the leader of an mRNA both sense and respond to environmental conditions by either prematurely terminating transcription or blocking translation. Both attenuation and riboswitches are described in this section.

Attenuation

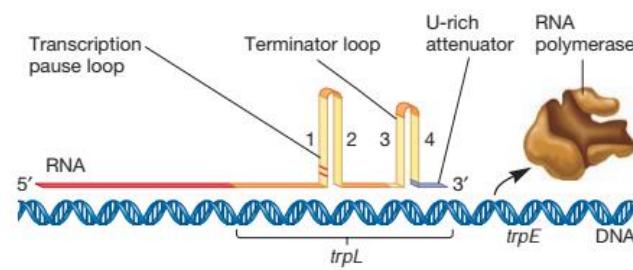
As noted earlier, the tryptophan (*trp*) operon of *E. coli* is under the control of a repressor protein, and excess tryptophan inhibits transcription of operon genes by acting as a corepressor and acti-

vating the repressor protein. Although the operon is regulated mainly by repression, the continuation of transcription also is controlled. That is, there are two decision points involved in transcriptional control, the initiation of transcription and the continuation of transcription past the leader region.

This additional level of control serves to adjust levels of transcription in a more subtle fashion. When the repressor is not active, RNA polymerase begins transcription of the leader region but it often does not progress to the first structural gene in the operon. Instead, transcription is terminated within the leader region; this is called **attenuation**. The ability to attenuate transcription is based on the nucleotide sequences in the leader region and on the fact that in prokaryotes, transcription is coupled with translation (see figure 11.39). The leader of the *trp* operon mRNA is unusual in that it is translated. The product, which has never been isolated, is called the leader peptide. In addition to encoding the leader peptide, the leader contains **attenuator** sequences (figure 12.11). When transcribed, these sequences form stem-loop secondary structures in the newly formed mRNA. We define these sequences numerically (regions 1, 2, 3, and 4). When regions 1 and 2 pair with one another (1:2; figure 12.11a), they form a secondary structure called the **pause loop**, which causes RNA polymerase to slow down. The pause loop forms just prior to the formation of a second structure called the **terminator loop**, which is made when regions 3 and 4 base pair (3:4; figure 12.11a). A poly(U) sequence follows the 3:4 terminator loop, just as it does in other rho-independent transcriptional terminators (see figure 11.31). However, in this case, the terminator is in the leader rather than at the end of the gene. Another stem-loop structure can be formed in the leader region by the pairing of regions 2 and 3 (2:3; figure 12.11b). The formation of this **antiterminator loop** prevents the generation of both the 1:2 pause and 3:4 terminator loops.

How do these various loops control transcription termination? Three scenarios describe the process. In the first, translation is not coupled to transcription because protein synthesis is not occurring. In other words, no ribosome is associated with the mRNA. In this scenario, the pause and terminator loops form, stopping transcription before RNA polymerase reaches the *trpE* gene (figure 12.11a).

In the next two scenarios, translation and transcription are coupled; that is, a ribosome associates with the leader mRNA as the rest of the mRNA is being synthesized. The interaction between RNA polymerase and the nearest ribosome determines which stem-loop structures are formed. As a ribosome translates the mRNA, it will follow the RNA polymerase. Among the first several nucleotides of region 1 are two tryptophan (trp) codons; this is unusual because normally there is only one trp per 100 amino acids in *E. coli* proteins. If tryptophan levels are low, the ribosome will stall when it encounters the two trp codons. It stalls because the paucity of charged tRNA^{trp} molecules delays the filling of the A site of the ribosome (figure 12.11b). Meanwhile the RNA polymerase continues to transcribe mRNA, moving away from the stalled ribosome. The presence of the ribosome on region 1 will prevent it from base pairing with region 2. As RNA polymerase continues, region 3 is transcribed, enabling the formation of the 2:3 antiterminator loop. This prevents the formation of the



(a) No translation occurring

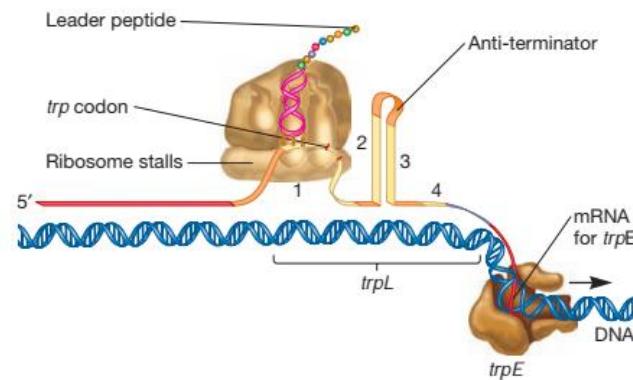
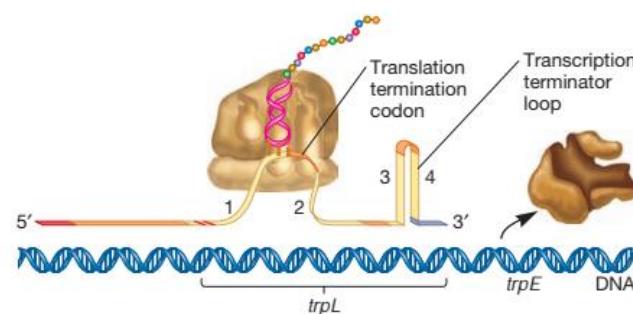
(b) Translation occurring, low tryptophan levels,
2:3 forms → transcription continues(c) Translation occurring, high tryptophan levels,
3:4 forms → transcription is terminated

Figure 12.11 Attenuation of the *trp* Operon. (a) When protein synthesis has slowed, transcription and translation are not tightly coupled. Under these conditions, the most stable form of the mRNA occurs when region 1 hydrogen bonds to region 2 (RNA polymerase pause loop) and region 3 hydrogen bonds to region 4 (transcription terminator or attenuator loop). The formation of the transcription terminator causes transcription to stop just beyond *trpL* (*trp* leader). (b) When protein synthesis is occurring, transcription and translation are coupled, and the behavior of the ribosome on *trpL* influences transcription. If tryptophan levels are low, the ribosome pauses at the *trp* codons in *trpL* because of insufficient amounts of charged tRNA^{Trp}. This blocks region 1 of the mRNA, so that region 2 can hydrogen bond only with region 3. Because region 3 is already hydrogen bonded to region 2, the 3:4 terminator loop cannot form. Transcription proceeds and the *trp* biosynthetic enzymes are made. (c) If tryptophan levels are high, translation of *trpL* progresses to the stop codon, blocking region 2. Regions 3 and 4 can hydrogen bond and transcription terminates.

3:4 terminator loop. Because the terminator loop is not formed, RNA polymerase is not ejected from the DNA and transcription continues into the *trp* biosynthetic genes. If, on the other hand, there is plenty of tryptophan in the cell, there will be an abundance of charged tRNA^{Trp} and the ribosome will translate these two *trp* codons in the leader peptide sequence without hesitation. Thus the ribosome remains close to the RNA polymerase. As RNA polymerase and the ribosome continue through the leader region, regions 1 and 2 are transcribed and readily form a pause loop. Then regions 3 and 4 are transcribed, the terminator loop forms, and RNA polymerase is ejected from the DNA template. Finally, the presence of a UGA stop codon between regions 1 and 2 will cause early termination of translation (figure 12.11c). Although the leader peptide will be synthesized, it appears to be rapidly degraded. [The genetic code \(section 11.7\)](#)

Attenuation's usefulness is apparent. If the bacterium is deficient in an amino acid other than tryptophan, protein synthesis will slow and tryptophanyl-tRNA will accumulate. Transcription of the tryptophan operon will be inhibited by attenuation. When the bacterium begins to synthesize protein rapidly, tryptophan may be scarce and the concentration of tryptophanyl-tRNA may be low. This would reduce attenuation activity and stimulate operon transcription, resulting in larger quantities of the tryptophan biosynthetic enzymes. Acting together, repression and attenuation can coordinate the rate of synthesis of amino acid biosynthetic enzymes with the availability of amino acid end products and with the overall rate of protein synthesis. When tryptophan is present at high concentrations, any RNA polymerases not blocked by the activated repressor protein probably will not get past the attenuator sequence. Repression decreases transcription about seventyfold and attenuation slows it another eight- to tenfold; when both mechanisms operate together, transcription can be slowed about 600-fold.

Attenuation is important in regulating at least five other operons that include amino acid biosynthetic enzymes. In all cases, the leader peptide sequences resemble the tryptophan system in

organization. For example, the leader peptide sequence of the histidine operon codes for seven histidines in a row and is followed by an attenuator that is a terminator sequence.

Riboswitches

Regulation by riboswitches, or sensory RNAs, is a specialized form of transcription attenuation that does not involve ribosome behavior. In this case, the leader region of the mRNA can fold in different ways. If folded one way, transcription continues; if folded another, transcription is terminated. This leader region is called a **riboswitch** because it turns transcription on or off. What makes riboswitches unique and exciting is that they alter their folding pattern in direct response to the binding of an effector molecule—a capability previously thought to be associated only with proteins.

One of the first discoveries of this type of regulation was the riboflavin (*rib*) biosynthetic operon of *B. subtilis*. The synthesis of riboflavin biosynthetic enzymes is repressed by flavin mononucleotide (FMN), which is derived from riboflavin. When transcription of the *rib* operon begins, sequences in the leader region of the mRNA fold into a structure called the RFN-element. This element binds FMN, and in doing so alters the folding of the leader region, creating a terminator that stops transcription (figure 12.12).

It now appears that controlling transcription attenuation with sensory RNAs is an important method used by gram-positive bacteria to regulate amino acid-related genes. As in the case of the *rib* operon, the leader regions of these mRNAs contain a regulatory element. In this case, the region is called the T box. T box sequences give rise to competing terminator and antiterminator loops. The development of either a terminator or an antiterminator is determined by the binding of uncharged tRNA corresponding to the relevant amino acid (i.e., tRNA that is not carrying its cognate amino acid). For instance, expression of a tyrosyl-tRNA synthetase gene (i.e., a gene that encodes the enzyme that links tyrosine to a tRNA molecule) is governed by the presence of tRNA^{Tyr}. When the level

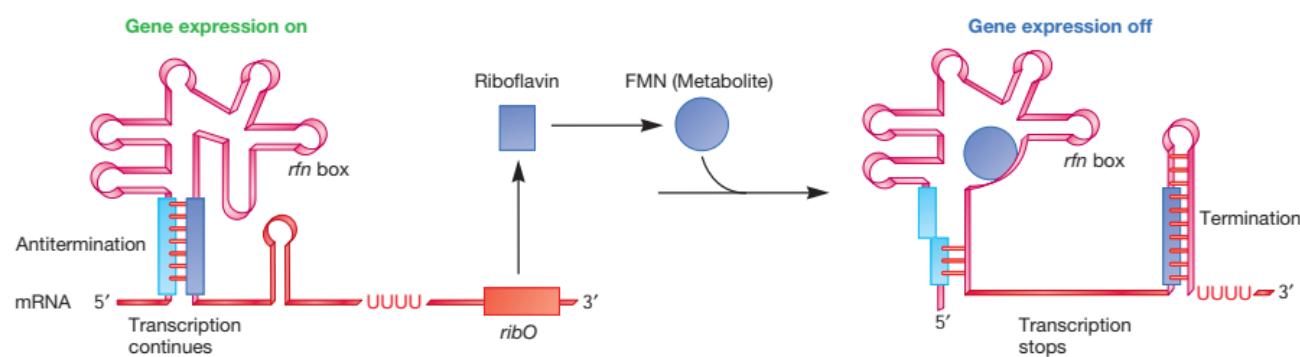


Figure 12.12 Riboswitch Control of the Riboflavin (*rib*) Operon of *Bacillus subtilis*. The *rib* operon produces enzymes needed for the synthesis of riboflavin, a component of flavin mononucleotide (FMN). Binding of FMN to the *rfn* (rifampin) box in the leader of *rib* mRNA causes a change in mRNA folding, which results in the formation of a transcription terminator and cessation of transcription.

Table 12.1 Regulation of Gene Expression by Riboswitches

System	Microbe (s)	Target genes encode:	Effector & Regulatory Response
T box	Many gram-positive bacteria	Amino acid biosynthetic enzymes	Uncharged tRNA; anticodon base pairs to 5' end of mRNA, preventing formation of transcriptional terminator
Vitamin B ₁₂ element	<i>E. coli</i>	Cobalamine biosynthetic enzymes	Adenosylcobalamine (AdoCbl) binds to <i>btuB</i> mRNA and blocks translation
THI box	<i>Rhizobium etli</i> <i>E. coli</i> <i>B. subtilis</i>	Thiamin (Vitamin B ₁) biosynthetic and transport proteins	Thiamin pyrophosphate (TPP) causes either premature transcriptional termination (<i>R. etli</i> , <i>B. subtilis</i>) or blocks ribosome binding (<i>E. coli</i>)
RFN-element	<i>B. subtilis</i>	Riboflavin biosynthetic enzymes	Flavin mononucleotide (FMN) causes premature transcriptional termination
S box	Low G + C gram-positive bacteria	Methionine biosynthetic enzymes	S-adenosylmethionine (SAM) causes premature transcriptional termination

of charged tRNA^{Tyr} falls, the anticodon of an uncharged tRNA binds directly to the “specifier sequence” codon in the leader of the mRNA. At the same time, the antiterminator loop is stabilized by base pairing between sequences in the loop and the acceptor end of the tRNA, which normally binds the amino acid. This prevents formation of the terminator structure, and transcription of the tyrosyl-tRNA synthetase gene continues. Genomic analysis now suggests that the T box mechanism may be involved in regulating over 300 genes and/or operons; some other genes that bear sensory RNA in their leader regions are listed in **table 12.1**. Other riboswitches have been shown to function at the level of translation. They are described in the next section.

12.4 REGULATION AT THE LEVEL OF TRANSLATION

It appears that in general, the riboswitches found in gram-positive bacteria function by transcriptional termination, while the riboswitches discovered in gram-negative bacteria regulate the translation of mRNA. Translation is usually regulated by blocking its initiation. As noted previously, some riboswitches work at this level. In addition, some small RNA molecules can control translation initiation. Both are described in this section.

Regulation of Translation by Riboswitches

Similar to the riboswitches described earlier, riboswitches that function at the translational level contain effector-binding elements at the 5' end of the mRNA. Binding of the effector molecule alters the folding pattern of the leader region of the mRNA, which often results in occlusion of the Shine-Dalgarno sequence and other elements of the ribosome-binding site. This inhibits ribosome binding and initiation of translation (**figure 12.13**). An example of this type of regulation is observed for the thiamine biosynthetic operons of numerous bacteria and some archaea. The leader regions of thiamine operons contain a structure called the THI-element, which can bind thiamin pyrophosphate. Bind-

ing of thiamin pyrophosphate to the THI-element causes a conformational change in the leader region that sequesters the Shine-Dalgarno sequence and blocks translation initiation.

Regulation of Translation by Small RNA Molecules

A large number of RNA molecules have been discovered that do not function as mRNAs, tRNAs, or rRNAs. Microbiologists often refer to them as **small RNAs (sRNAs)** or as noncoding RNAs (ncRNAs). In *E. coli*, there are more than 40 sRNAs, ranging in size from around 40 to 400 nucleotides. It is thought that eukaryotes may have hundreds to thousands of sRNAs with lengths from 21 to over 10,000 nucleotides. Although some sRNAs have been implicated in the regulation of DNA replication and transcription, many function at the level of translation.

In *E. coli*, most sRNAs regulate translation by base pairing to the leader region of a target mRNA. Thus they are complementary to the mRNA and are called **antisense RNAs**. It seems intuitive that by binding to the leader, antisense RNAs would block ribosome binding and inhibit translation. Indeed, many antisense RNAs work in this manner. However, some antisense RNAs actually promote translation upon binding to the mRNA. Whether inhibitory or activating, most *E. coli* antisense RNAs work with a protein called Hfq to regulate their target RNAs. The Hfq protein is an RNA **chaperone**—that is, it interacts with RNA to promote changes in its structure. In addition, the Hfq protein may promote RNA-RNA interactions.

The regulation of synthesis of OmpF and OmpC porin proteins provides an example of translation control by an antisense RNA. In addition to regulation by the OmpR protein described previously (figure 12.10), expression of the *ompF* gene is regulated by an antisense RNA called MicF RNA, which is the product of the *micF* gene (mic for mRNA-interfering complementary RNA). The MicF RNA is complementary to *ompF* at the translation initiation site (**figure 12.14**). It base pairs with *ompF* mRNA and represses translation. MicF RNA is produced under conditions such as high

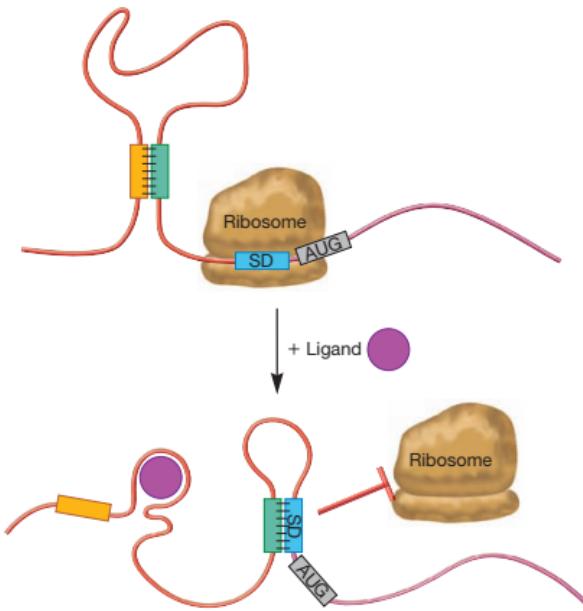


Figure 12.13 Regulation of Translation by a Riboswitch. In the absence of a relevant metabolite, an effector binding site is formed in the leader of the mRNA (red) when complementary sequences (orange box and green box) hydrogen bond. This folding pattern exposes important sequences in the ribosome-binding site (e.g., the Shine-Dalgarno sequence; blue box) and translation occurs. When the appropriate effector molecule is present, it binds the leader, disrupting the existing structure and creating a new structure with the ribosome-binding-site sequences. Thus the ribosome-binding site becomes inaccessible and translation is blocked.

osmotic pressure or the presence of some toxic material, both of which favor *ompC* expression. Production of MicF RNA helps ensure that OmpF protein is not produced at high levels at the same time as OmpC protein. Some other antisense RNAs are listed in **table 12.2**.

1. Define attenuation. What are the functions of the leader region and ribosome in attenuation?
2. Of what practical importance is attenuation in coordinating the synthesis of amino acids and proteins? Describe how attenuation activity would vary when protein synthesis is suddenly accelerated, then later rapidly decelerated.
3. What are riboswitches? How are they similar to attenuation as described for the *trp* operon? How do they differ?

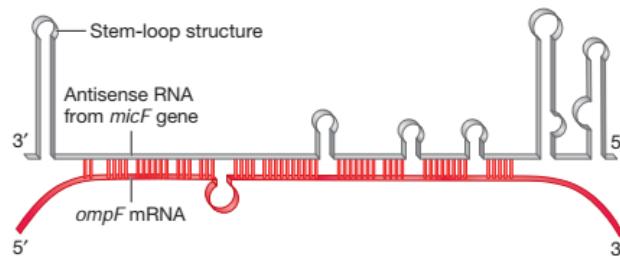


Figure 12.14 Regulation of Translation by Antisense RNA. The *ompF* mRNA encodes the porin OmpF. Translation of this message is regulated by the antisense RNA MicF, the product of the *micF* gene. MicF is complementary to the *ompF* mRNA and, when bound to it, prevents translation from occurring.

Table 12.2 Regulation of Gene Expression by Small Regulatory RNAs			
Small RNA	Size	Bacterium	Function
RhyB	90 nt ¹	<i>E. coli</i>	Represses translation of iron-containing proteins (e.g., <i>sodB</i>) when iron availability is low
Spot 42	109 nt	<i>E. coli</i>	Inhibits translation of <i>galK</i> (encodes galactokinase)
RprA	105 nt	<i>E. coli</i>	Promotes translation of <i>rpoS</i> mRNA; antisense repressor of global negative regulator H-NS (involved in stress responses)
MicF	109 nt	<i>E. coli</i>	Inhibits <i>ompF</i> mRNA translation
OxyS	109 nt	<i>E. coli</i>	Inhibits translation of transcriptional regulator <i>fhlA</i> mRNA and <i>rpoS</i> mRNA (encodes σ^s , a stationary phase sigma factor)
DsrA	85 nt	<i>E. coli</i>	Increases translation of <i>rpoS</i> mRNA
CsrB	366 nt	<i>E. coli</i>	Inhibits CsrA, a translational regulatory protein that positively regulates flagella synthesis, acetate metabolism, and glycolysis
RNAIII	512 nt	<i>Staphylococcus aureus</i>	Activates genes encoding secreted proteins (e.g., α hemolysin)
			Represses genes encoding surface proteins
RNA α	650 nt	<i>Vibrio anguillarum</i>	Decreased expression of Fat, an iron uptake protein
RsmB'	259 nt	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Stabilizes mRNA of virulence proteins (e.g., cellulases, proteases, pectinolytic enzymes)

¹ nt: nucleotides

12.5 GLOBAL REGULATORY SYSTEMS

Thus far, we have been considering the function of isolated operons. However, organisms must respond rapidly to a wide variety of changing environmental conditions and be able to cope with such stressors as nutrient deprivation, dessication, and major temperature fluctuations. They also have to compete successfully with other organisms for scarce nutrients and use these nutrients efficiently. These challenges require a regulatory system that can rapidly control many operons at the same time. Regulatory systems that affect many genes and pathways simultaneously are called **global regulatory systems**.

Although it is usually possible to regulate all the genes of a metabolic pathway in a single operon, there are good reasons for more complex global systems. Some processes involve too many genes to be accommodated in a single operon. For example, the machinery required for protein synthesis is composed of 150 or more gene products, and coordination requires a regulatory network that controls many separate operons. Sometimes two levels of regulation are required because individual operons must be controlled independently and also cooperate with other operons. Regulation of sugar catabolism in *E. coli* is a good example. *E. coli* uses glucose when it is available; in such a case, operons for other catabolic pathways are repressed. If glucose is unavailable and another nutrient is present, the appropriate operon is activated.

Global regulatory systems are so complex that a specialized nomenclature is used to describe the various kinds. Perhaps the most basic type is the **regulon**. A regulon is a collection of genes or operons that is controlled by a common regulatory protein. Usually the operons are associated with a single pathway or function (e.g., the production of heat-shock proteins or the catabolism of glycerol). A somewhat more complex situation is seen with a **modulon**. This is an operon network under the control of a common global regulatory protein, but whose constituent operons also are controlled separately by their own regulators. A good example of a modulon is catabolite repression, which is discussed on page 308. The most complex global systems are referred to as **stimulons**. A stimulon is a regulatory system in which all operons respond together in a coordinated way to an environmental stimulus. It may contain several regulons and modulons, and some of these may not share regulatory proteins. For instance, the genes involved in a response to phosphate limitation are scattered among several regulons and are part of one stimulon.

Mechanisms Used for Global Regulation

Global regulation is complex and often involves more than one regulatory mechanism. Most global regulatory networks are controlled by one or more regulatory proteins. Two-component regulatory systems and phosphorelay systems also play important roles in global control. In *Bacteria*, many global regulatory networks make use of **alternate sigma factors**, which can immediately change expression of many genes as they direct RNA polymerase to specific subsets of a bacterium's genome. This is possible because RNA polymerase core enzyme needs the assistance of a sigma factor to bind a promoter and initiate transcription. Each sigma factor recognizes promoters that differ in sequence, especially at the -10 and -35 positions. The specific sequences recognized by a given sigma factor are called its **consensus sequences**. When a complex process requires a radical change in transcription or a precisely timed sequence of transcription, it may be regulated by a series of sigma factors. [Transcription \(section 11.6\)](#)

E. coli synthesizes several sigma factors (**table 12.3**). Under normal conditions, a sigma factor called σ^{70} directs RNA polymerase activity. (The superscript number or letter indicates the size or function of the sigma factor; 70 stands for 70,000 Da.) When flagella and chemotactic proteins are needed, *E. coli* produces σ^F (σ^{28}). σ^F then binds its consensus sequences in promoters of genes whose products are needed for flagella biosynthesis and chemotaxis. If the temperature rises too high, σ^H (σ^{32}) is produced and stimulates the formation of around 17 heat-shock proteins that protect the cell from thermal destruction. Importantly, each sigma factor has its own set of promoters to which it binds.

In the discussions that follow, we describe three global regulatory networks. The first relatively simple example is the catabolite repression modulon, which involves regulation of transcription by both repressors and activators. The second is quorum sensing, which was introduced in chapter 6. Although the example we discuss regulates a single operon, many quorum-sensing systems regulate the transcription of suites of genes and operons. Finally, we examine a more complex process, that of sporulation in the gram-positive bacterium *B. subtilis*. Regulation of endospore formation involves numerous control mechanisms, including phosphorelay and sequential use of sigma factors.

Table 12.3 *E. coli* Sigma Factors

Sigma Factor	Genes Transcribed
σ^{70}	Genes needed during exponential growth
σ^S	Genes needed during the general stress response and during stationary phase
σ^E	Genes needed to restore membrane integrity and the proper folding of membrane proteins
σ^H (σ^{32})	Genes needed to protect against heat shock and other stresses, including chaperones that help maintain or restore proper folding of cytoplasmic proteins and proteases that degrade damaged proteins
FecI sigma factor	Genes that encode the iron citrate transport machinery in response to iron starvation and the availability of iron citrate
σ^F (σ^{28})	Genes involved in flagellum assembly
σ^{60}	Genes involved in nitrogen metabolism

Catabolite Repression

If *E. coli* grows in a medium containing both glucose and lactose, it uses glucose preferentially until the sugar is exhausted. Then after a short lag, growth resumes with lactose as the carbon source (figure 12.15). This biphasic growth pattern or response is called **diauxic growth**. The cause of diauxic growth or diauxie is complex and not completely understood, but **catabolite repression** plays a part. The enzymes for glucose catabolism are constitutive. However, operons that encode enzymes required for the catabolism of carbon sources that must first be modified before entering glycolysis (e.g., the *lac* operon) are regulated by catabolite repression. These include the *ara*, *mal* (maltose), and *gal* (galactose) operons, as well as the *lac* operon. Collectively, these can be called catabolite operons, and their expression is coordinately (or globally) repressed when glucose is plentiful and activated when it is not.

The coordinated regulation of catabolite operons is brought about by **catabolite activator protein (CAP)**, which is also called **cyclic AMP receptor protein (CRP)**. CAP exists in two states: it is active when the small cyclic nucleotide **3', 5'-cyclic adenosine monophosphate (cAMP)** (figure 12.16) is bound, and it is inactive when it is free of cAMP. The levels of cAMP are controlled by the enzyme adenyl cyclase, which converts ATP to cAMP and PP_i. Adenyl cyclase is active only when little or no glucose is available. Thus the level of cAMP varies inversely with that of glucose: when glucose is unavailable and the catabolism of another sugar might be needed, the amount of cAMP in the cell increases allowing cAMP to bind to and activate CAP.

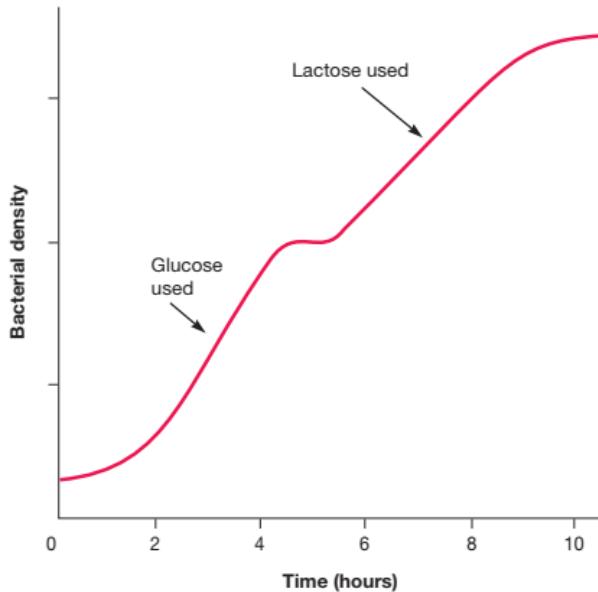


Figure 12.15 Diauxic Growth. The diauxic growth curve of *E. coli* grown with a mixture of glucose and lactose. Glucose is first used, then lactose. A short lag in growth is present while the bacteria synthesize the enzymes needed for lactose use.

All catabolite operons contain a CAP binding site, and CAP must be bound to this site before RNA polymerase can bind the promoter and begin transcription. Upon binding, CAP bends the DNA within two helical turns (figure 12.6b and figure 12.17). Interaction of CAP with RNA polymerase then stimulates transcription. Thus all catabolite operons are controlled by two regulatory proteins: the regulatory protein specific to each operon (e.g., *lac* repressor and AraC protein) and CAP. In the case of the *lac* operon, if glucose is absent and lactose is present, the inducer allolactose will bind to and inactivate the *lac* repressor protein, CAP will be in the active form (with cAMP bound), and transcription will proceed (figure 12.18a). However, if glucose and lactose are both in short supply, even though CAP binds to the *lac* promoter, transcription will be inhibited by the presence of the repressor protein, which remains bound to the operator in the absence of inducer (figure 12.18c). Dual control ensures that the *lac* operon is expressed only when lactose catabolic genes are needed.

We have seen how CAP controls catabolite operons; now let us turn our attention to the regulation of the levels of cAMP. The decrease in cAMP levels that occurs when glucose is present is due to the effect of the phosphoenolpyruvate: phosphotransferase system (PTS) on the activity of adenyl cyclase. Recall from chap-

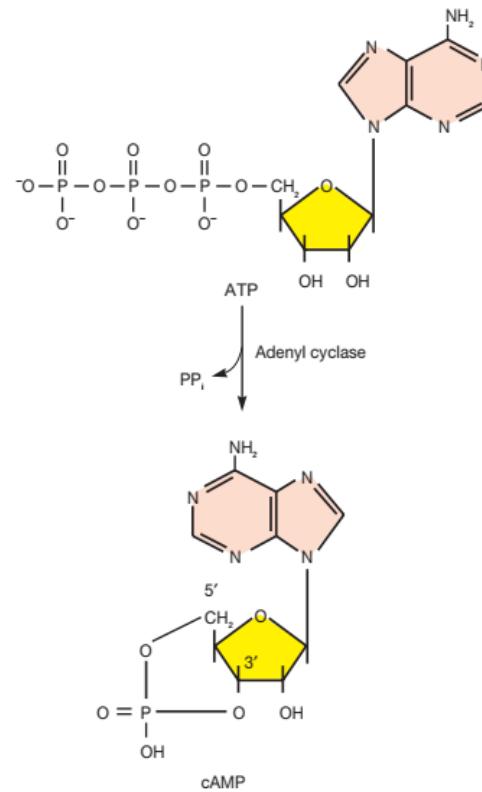


Figure 12.16 Cyclic Adenosine Monophosphate (cAMP). The phosphate group extends between the 3' and 5' hydroxyls of the ribose sugar. The enzyme adenyl cyclase forms cAMP from ATP.

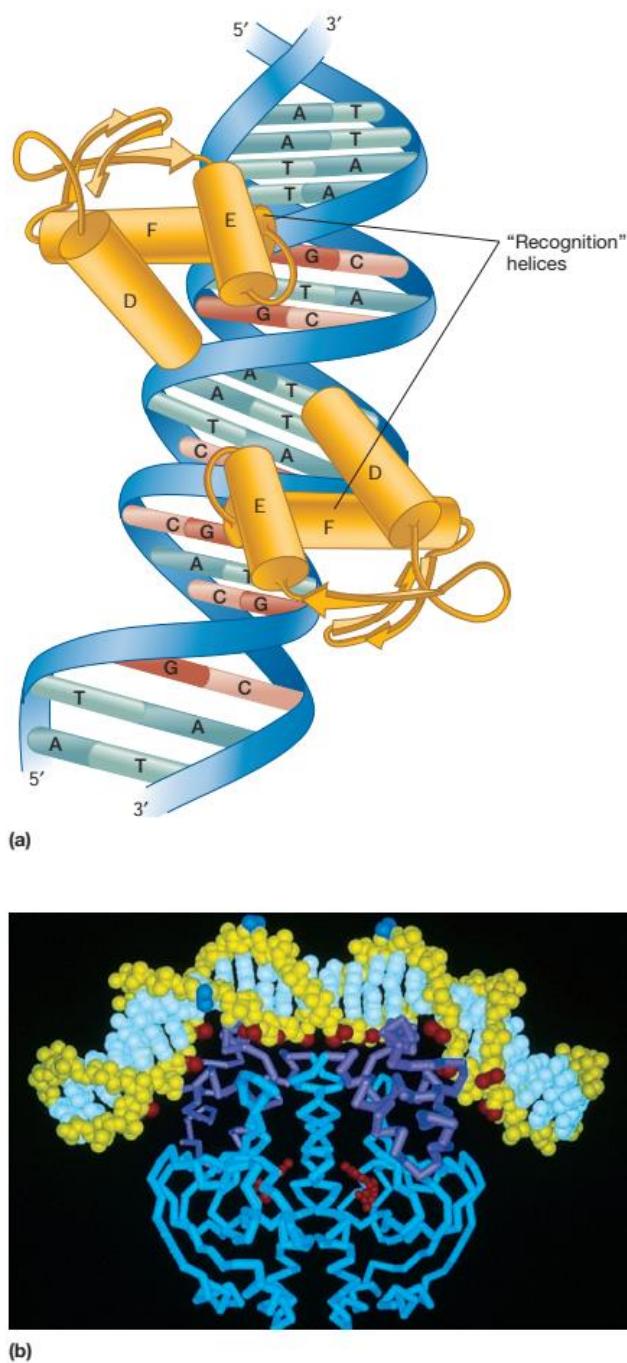


Figure 12.17 CAP Structure and DNA Binding. (a) The CAP dimer binding to DNA at the *lac* operon promoter. The recognition helices fit into two adjacent major grooves on the double helix. (b) A model of the *E. coli* CAP-DNA complex derived from crystal structure studies. The cAMP-binding domain is in blue and the DNA-binding domain, in purple. The cAMP molecules bound to CAP are in red. Note that the DNA is bent when complexed with CAP.

ter 5 that in the PTS, a phosphoryl group is transferred by a series of proteins from phosphoenolpyruvate (PEP) to glucose, which then enters the cell as glucose 6-phosphate. When glucose is present, enzyme IIA transfers the phosphoryl group to enzyme IIB, which phosphorylates glucose. Because enzyme IIA rapidly transfers phosphoryl groups, it exists largely in an unphosphorylated state. Unphosphorylated enzyme IIA inhibits the permeases for many sugars, and in doing so inhibits sugar uptake. However, when glucose is absent, the phosphoryl groups from PEP are transferred to enzyme IIA, but are not transferred to enzyme IIB. The phosphorylated form of enzyme IIA accumulates. This form of the enzyme activates adenyl cyclase, stimulating cAMP production. [Uptake of nutrients by the cell: Group translocation \(section 5.6\)](#)

Catabolite repression is of considerable advantage to the bacterium. It will use the most easily catabolized sugar (glucose) first rather than synthesize the enzymes necessary for catabolism of another carbon and energy source. These control mechanisms are present in a variety of bacteria and metabolic pathways.

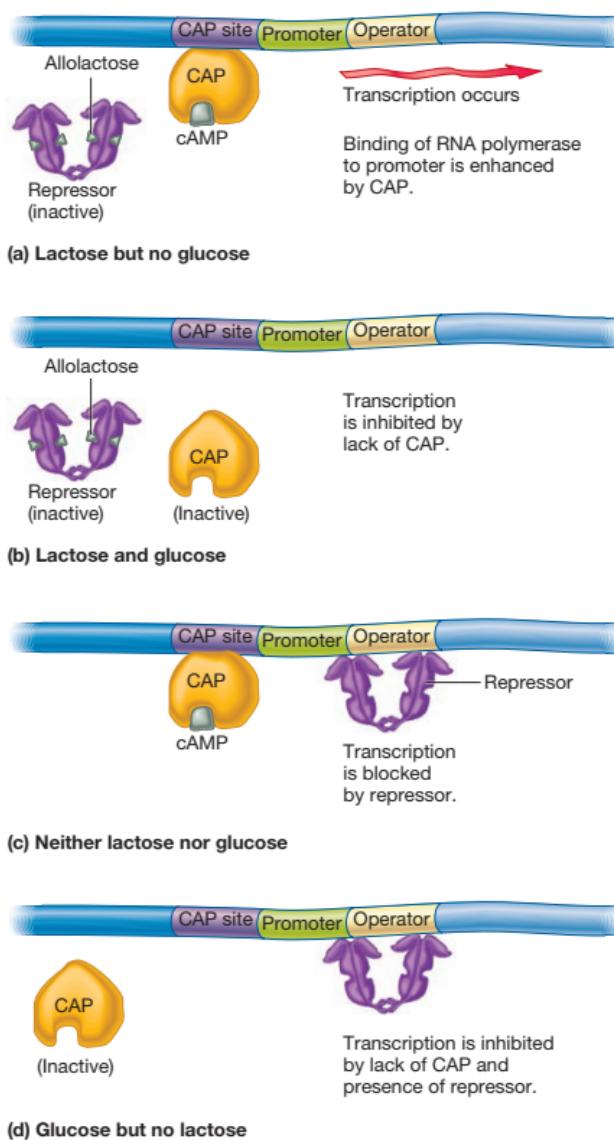
Quorum Sensing

Cell to cell communication among prokaryotes occurs by the exchange of small molecules often termed signals or signaling molecules. The exchange of signaling molecules is essential in the coordination of gene expression in microbial populations. This was first recognized in the marine bioluminescent bacterium *Vibrio fischeri*, which produces light only if cells are at high density. It has since been discovered that intercellular communication plays an essential role in the regulation of genes whose products are needed for the establishment of virulence, symbiosis, biofilm production, plasmid transfer, and morphological differentiation in a wide range of microorganisms. Here we describe how signals that are secreted by one group of cells can regulate the genetic expression of another. Our focus is on the regulation of a single operon. However, it should be kept in mind that **quorum sensing** can regulate multiple genes and operons. [Microbial growth in natural environments: Cell-cell communication within microbial populations \(section 6.6\)](#)

Quorum sensing in *V. fischeri* and many other gram-negative bacteria uses an **N-acyl homoserine lactone (AHL)** signal ([figure 12.19](#)). Synthesis of this small molecule is catalyzed by an enzyme called AHL synthase, the product of the *luxI* gene. The *luxI* gene is subject to positive autoregulation. That is to say, transcription of *luxI* increases as AHL accumulates in the cell. This is accomplished through a transcriptional activator, LuxR, which is active only when it binds AHL ([figure 12.19](#)). Thus a simple feedback loop is created. Without AHL-activated LuxR, the *luxI* gene will be transcribed only at basal levels. AHL freely diffuses out of the cell and accumulates in the environment. As cell density increases, the concentration of AHL outside the cell eventually exceeds that inside the cell, and the concentration gradient is reversed. As AHL flows back into the cell, it binds and activates LuxR. LuxR can now activate high-level transcription of *luxI* and the genes whose products are needed for bioluminescence (*luxCDABEG*). Quorum sensing is often called **autoinduction** and the AHL signal is termed **autoinducer** to reflect the autoregulatory nature of this system.

Figure 12.18 Regulation of the lac Operon by the lac Repressor and CAP.

A continuum of lac mRNA synthesis is brought about by the action of CAP, an activator protein, and the lac repressor. (a) When lactose is available and glucose is not, the repressor is inactivated and cAMP levels increase. Cyclic AMP binds CAP, activating it. CAP binds the CAP binding site near the lac promoter and facilitates binding of RNA polymerase. Under these conditions, transcription occurs at maximal levels. (b) When both lactose and glucose are available, both CAP and the lac repressor are inactive. Because RNA polymerase cannot bind the promoter efficiently without the aid of CAP, transcription levels are low. (c) When neither glucose nor lactose is available, both CAP and the lac repressor are active. In this situation, both proteins are bound to their regulatory sites. CAP binding enhances the binding of RNA polymerase to the promoter. However, the repressor blocks transcription. Transcription levels are low. (d) When glucose is available and lactose is not, CAP is inactive and the lac repressor is active. Thus RNA polymerase binds inefficiently, and those polymerase molecules that do bind are blocked by the repressor. This condition results in the lowest levels of transcription observed for the lac operon.



Another kind of quorum sensing depends on an elaborate, two-component signal transduction system. It is found in both gram-negative and gram-positive bacteria including (but not limited to) *Staphylococcus aureus*, *Ralstonia solanacearum*, *Salmonella enterica*, *Vibrio cholerae*, and *E. coli*. It has been best studied in the bioluminescent bacterium *Vibrio harveyi*. Unlike *V. fischeri*, *V. harveyi* responds to two autoinducer molecules: AI-1 and AI-2. AI-1 is a homoserine lactone and its synthesis depends on the *luxM* gene. AI-2 is furanosylborate, a small molecule that contains a boron atom—quite an unusual component in an organic molecule. Its synthesis relies on the product of the *luxS* gene. As shown in figure 12.20, AI-1 and AI-2 are secreted by the cell, which then uses separate proteins called LuxN and LuxPQ to detect their presence. At low cell density in the absence of either AI-1 or AI-2, LuxN and

LuxPQ autophosphorylate and converge on a single phosphotransferase protein called LuxU. LuxU accepts phosphates from each sensor kinase and then phosphorylates the response regulator LuxO. Phosphorylated LuxO in turn activates the transcription of genes encoding several small RNAs that destabilize *luxR* mRNA. Because LuxR is a transcriptional activator of the operon *luxCDABE*, which encodes proteins needed for bioluminescence, cells do not make light at low cell density. An interesting thing happens as cell and autoinducer densities increase: Lux N binds AI-1 and LuxPQ binds AI-2, and the proteins switch from functioning as kinases to phosphatases, proteins that dephosphorylate rather than phosphorylate their substrates. The flow of phosphates is now reversed; LuxO is inactivated by dephosphorylation and *luxR* mRNA is translated. LuxR now activates

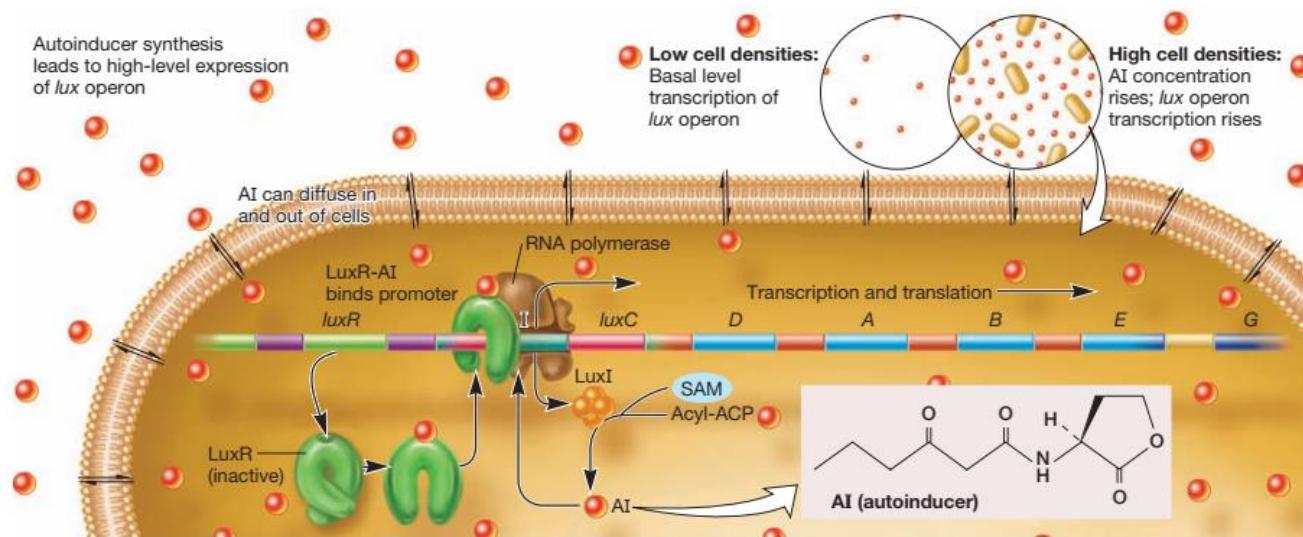


Figure 12.19 Quorum Sensing in *V. fischeri*. The AHL signaling molecule diffuses out of the cell; when cell density is high, the concentration of AHL diffuses back into the cell where it binds to and activates the transcriptional regulator LuxR. Active LuxR then stimulates transcription of the gene coding for AHL synthase (*luxI*) as well as the genes encoding proteins needed for light production.

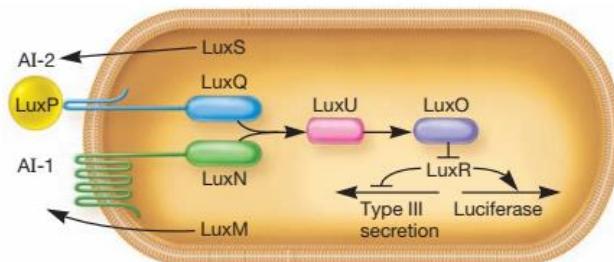


Figure 12.20 Quorum Sensing in *V. harveyi*. Two autoinducing signals, AI-1 and AI-2, are produced. At low cell density, the two component signal transduction system consisting of the signal kinases LuxPQ and LuxN initiate a phosphorelay that results in inhibition of the transcriptional regulator LuxR. Without LuxR, bioluminescence genes are repressed but genes for a type III secretion system (TTSS) are transcribed. At high cell density, LuxPQ and LuxN function as phosphatases, reversing the flow of phosphates. This results in activation of LuxR, transcriptional activation of the bioluminescence operon and repression of the TTSS genes.

transcription of *luxCDABE* and light is produced. Careful inspection of figure 12.20 reveals that another set of genes is controlled by the AI-1, AI-2 system of *V. harveyi*. In this microbe, genes for a type III protein secretion system (TTSS) are controlled in the opposite manner as those for bioluminescence.

LuxS-type autoinducers are found in a number of bacteria, but the precise AI-2 structure is specific for each species. Nonetheless, bacteria of different species can “talk” to each other because the products of the LuxS enzymes spontaneously rearrange. Thus in a

bacterial community consisting of several AI-2-producing bacterial species, AI-2 molecules in the environment interconvert. This means that individual cells may be responding to their own signal and that produced by other species. LuxS-producing bacteria can also interfere with each other’s communication. This has been shown experimentally. When *V. harveyi* and *E. coli* are grown together, *E. coli* consumes AI-2 produced by *V. harveyi*, thereby inhibiting light production and promoting TTSS production at high cell density. *E. coli* can also limit AI-2 regulated gene expression in *V. cholerae* by consuming the vibroid AI-2. Thus although the regulation of gene expression is generally considered in the context of a single cell, or at least a single species, it appears that in nature, microbes are most likely responding not only directly to the environment, but to each other as well.

Sporulation in *Bacillus subtilis*

As discussed in chapter 3, endospore formation is a complex process that involves asymmetric division of the cytoplasm to yield a large mother cell and a smaller forespore, engulfment of the forespore by the mother cell, and construction of additional layers of spore coverings (figure 12.21a and figure 3.49). Sporulation takes approximately 8 hours. It is controlled by phosphorelay, posttranslational modification of proteins, numerous transcription initiation regulatory proteins, and alternate sigma factors. The latter are particularly important. When growing vegetatively, *B. subtilis* RNA polymerase uses sigma factors σ^A and σ^H to recognize genes for normal survival. However, when cells sense a starvation signal, a cascade of events is initiated that results in the production of other sigma factors that are differentially expressed in the developing endospore and mother cell.

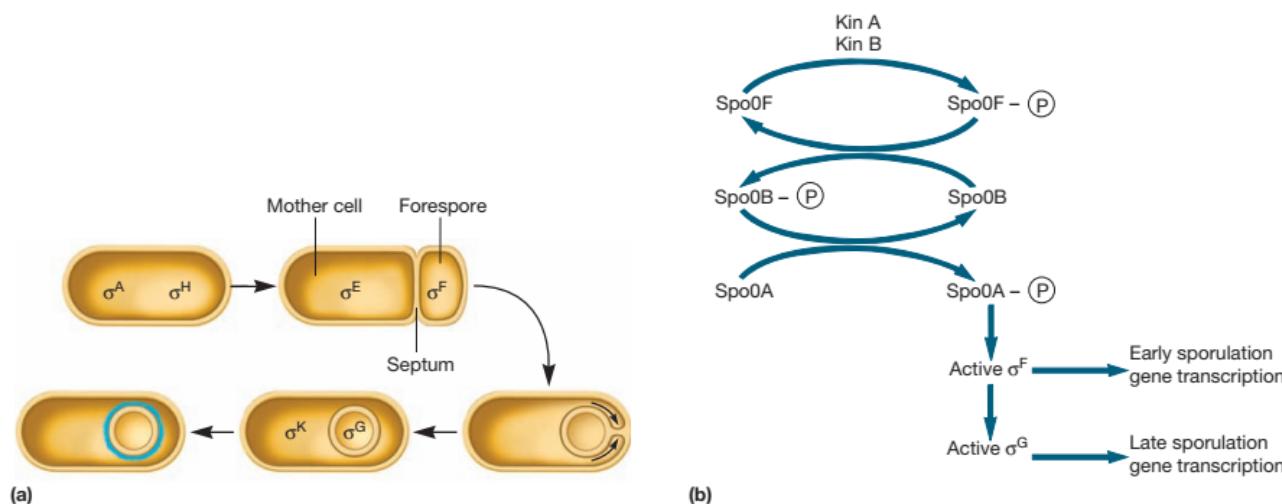


Figure 12.21 Genetic Regulation of Sporulation in *Bacillus subtilis*. (a) The initiation of sporulation is governed in part by the activities of two spatially separated sigma factors. σ^F is located in the forespore, while σ^E is confined to the mother cell. These sigma factors direct the initiation of transcription of genes whose products are needed for early events in sporulation. Later, σ^G and σ^K are localized to the developing endospore and mother cell, respectively. They control the expression of genes whose products are involved in the later steps of sporulation. (b) The activation of σ^F is accomplished through a phosphorelay system that is triggered by the activation of the sensor kinase protein KinA. When KinA senses starvation, it autophosphorylates a specific histidine residue. The phosphoryl group is then passed in relay fashion from Spo0F to Spo0B and finally to Spo0A. See text for details.

Initiation of sporulation is controlled by the protein Spo0A, a response-regulator protein that is part of a phosphorelay system. Sensor kinases associated with this system detect environmental stimuli that trigger sporulation. One of the most important sensor kinases is KinA, which senses nutrient starvation. When *B. subtilis* finds its nutrients are depleted, KinA autophosphorylates a specific histidine residue. The phosphoryl group is then transferred to an aspartic acid residue on Spo0F. However, Spo0F cannot directly regulate gene expression; instead, Spo0F donates the phosphoryl group to a histidine on Spo0B. Spo0B in turn relays the phosphoryl group to Spo0A. Phosphorylated Spo0A positively controls genes needed for sporulation and negatively controls genes that are not needed. In response to Spo0A, the expression of over 500 genes is altered. Among the genes whose expression is stimulated by Spo0A is *sigF*, the gene encoding sigma factor σ^F (figure 12.21b), and *spoIIGB*, the gene encoding an inactive form of σ^E (pro- σ^E).

When sporulation starts, the chromosome has replicated, with one copy remaining in the mother cell and another to be partitioned in the forespore. Shortly after the formation of the spore septum, σ^F is found in the forespore, and pro- σ^E is localized in the mother cell. Pro- σ^E is cleaved by a protease to form active σ^E .

The two sigma factors, σ^F and σ^E , bind to the promoters of genes needed in the forespore and mother cell, respectively. There they direct the expression of genes whose products are needed for the early steps of endospore formation. One of the many genes σ^F regulates is a gene that encodes another sigma factor, σ^G , which will replace σ^F in the developing endospore. Likewise, σ^E directs the transcription of a mother-cell-specific sigma factor, σ^K . Like σ^E , σ^K is first produced in an inactive form, pro- σ^K . Upon activation of pro- σ^K by proteolysis, σ^K ensures that genes encoding late-stage sporulation products are transcribed. Overall, temporal regulation is achieved because σ^F and σ^E direct transcription of genes needed early in the sporulation process, while σ^G and σ^K are needed for the transcription of genes whose products are needed later. In addition, spatial control of gene expression is accomplished because σ^F and σ^G are located in the forespore and σ^E and σ^K are found only in the mother cell.

1. What are global regulatory systems and why are they necessary? Briefly describe regulons, modulons, and stimulons.
2. What is diauxic growth? Explain how catabolite repression causes diauxic growth.

3. Describe the events that occur in each of the following growth conditions: *E. coli* in a medium containing glucose, but not lactose; in a medium containing both sugars; in a medium containing lactose but no glucose; and in a medium containing neither sugar.
4. What would be the phenotype of a *V. fischeri* mutant strain that could not regulate *luxL*, so that it was constantly producing autoinducer?
5. Why do you think bacteria use quorum sensing to regulate genes needed for virulence? How might this reason be related to the rationale behind using quorum sensing to establish a symbiotic relationship?
6. Briefly describe how a phosphorelay system and sigma factors are used to control sporulation in *B. subtilis*. Give one example of posttranslational modification as a means to regulate this process.

12.6 REGULATION OF GENE EXPRESSION IN EUCKARYA AND ARCHAEEA

As is the case in *Bacteria*, the regulation of gene expression in *Eucarya* and *Archaea* can occur at transcriptional, translational, and posttranslational levels. However, because of chromatin structure and the additional steps needed to produce a functional protein, regulation of eukaryotic gene expression is even more complex than what has already been discussed. Much of the work on gene regulation in *Eucarya* has focused on transcription initiation. More recently, regulation by small RNA molecules has attracted considerable attention. Our understanding of the regulation of archaeal gene expression unfortunately lags considerably behind what we know for *Eucarya* and *Bacteria*. However, some intriguing discoveries are briefly introduced here.

As noted in chapter 11, transcription initiation in *Eucarya* involves numerous transcription factors (see figure 11.33). Many transcription factors, such as TFIID, are general transcription factors that are part of the machinery common to transcription initiation of all eukaryotic genes. On the other hand, **regulatory transcription factors** are specific to one or more genes and alter the rate of transcription. Regulatory transcription factors are in some ways analogous to bacterial regulatory proteins. As just mentioned, they too alter the rate of transcription of their target genes by binding specific DNA sequences that are usually located near the promoter. In eukaryotes, transcription factors that function as activators bind regulatory sites called **enhancers**, whereas those that function as repressors bind sites called **silencers** (figure 12.22). However, the manner by which regulatory transcription factors control the rate of transcription is not the same as the mechanism used by bacterial regulatory proteins. After binding an enhancer or silencer, regulatory tran-

scription factors act indirectly to increase or decrease the rate of transcription. Many regulatory transcription factors control transcription initiation by interacting with general transcription factors, in particular TFIID (figure 12.22a) and a multisubunit protein complex called mediator (figure 12.22b). Others recruit chromatin-remodeling enzymes to a promoter. These enzymes change the degree of compaction of the DNA, making the promoter more or less accessible to RNA polymerase.

Another regulatory mechanism common to both *Bacteria* and eukaryotes is the use of sRNA molecules to control gene expression. This approach appears to be widely prevalent in eukaryotes. Many sRNAs act as antisense RNAs and function at the level of translation, as previously described. Some eukaryotic antisense RNAs are much smaller than typical bacterial antisense RNAs and are called **microRNAs (miRNAs)**. Some sRNA molecules are important components of the spliceosome. These regulatory RNAs may contribute to the selection of splice sites used during mRNA processing to remove introns. In doing so, different proteins can be made at certain times in the life cycle of the organism by combining different protein-coding sequences.

The regulation of gene expression in the *Archaea* has garnered a great deal of interest. This is because the archaeal transcription and translation machinery is most similar to that of the *Eucarya*, yet functions in cells with typical, *bacteria*-like genome organization. The question being asked is whether archaeal regulation of gene expression is more like bacterial regulation or more like eukaryotic regulation. Thus far, the answer is mixed. Most of the archaeal regulatory proteins function much like bacterial activators and repressors—that is, they bind DNA sites near the promoter and enhance or block binding of RNA polymerase, respectively. However, a few seem to function more like eukaryotic regulatory transcription factors in that they bring about their effects by interacting with a general transcription factor, such as the TATA binding protein. Small RNA molecules also have been identified in some archaea; their role in regulation is still being elucidated. *Introduction to the Archaea: Genetics and molecular biology* (section 20.1)

1. List the differences among the *Bacteria*, *Archaea*, and *Eucarya* that affect the way each regulates gene expression. Which domain has the most levels at which gene expression can be regulated? Why?
2. How are bacterial regulatory proteins and eukaryotic regulatory transcription factors similar? How do they differ?
3. What regulatory sequences in bacterial genomes are analogous to the enhancers and silencers observed in eukaryotic genomes?

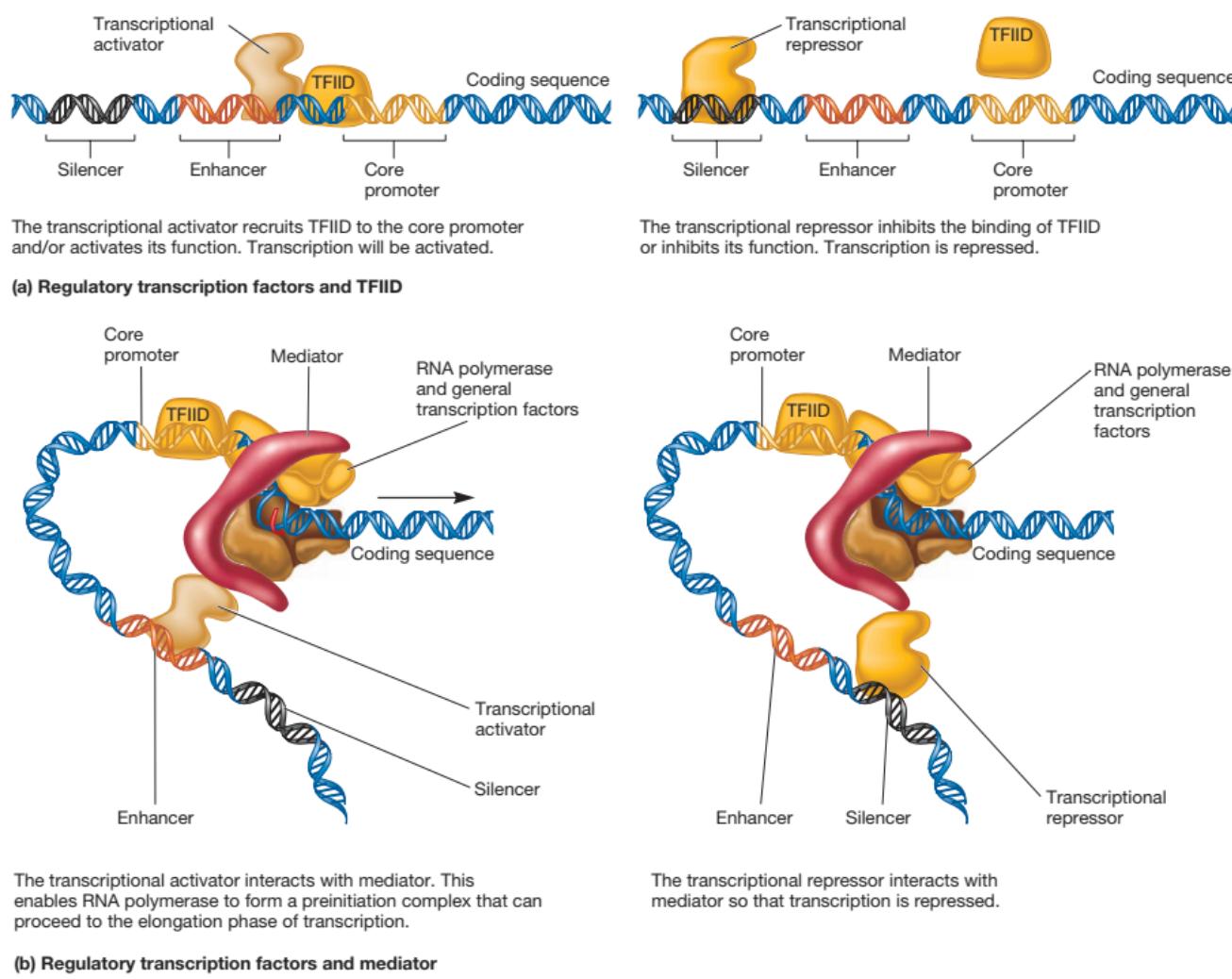


Figure 12.22 The Activity of Eucaryotic Regulatory Transcription Factors. Regulatory transcription factors do not exert their effects directly on RNA polymerase. Instead they act via other proteins, most commonly the general transcription factor TFIID and a protein called mediator. Mediator's role in transcription is to aid RNA polymerase in switching from the initiation stage of transcription to the elongation stage. **(a)** A regulatory protein acting through TFIID. Activators could influence transcription-enhancing TFIID recruitment of RNA polymerase to the promoter. Repressors would inhibit this ability. **(b)** A regulatory protein acting through the mediator protein. An activator would stimulate mediator activity; a repressor would decrease mediator activity.

Summary

12.1 Levels of Regulation of Gene Expression

- Regulation of gene expression can be controlled at many levels, including transcription initiation, transcription elongation, translation, and posttranslation (figure 12.1).
- The three domains of life differ in terms of their genome structure and the steps required to complete gene expression. These differences affect the regulatory mechanisms they use.

12.2 Regulation of Transcription Initiation

- Induction and repression of enzyme activity are two important regulatory phenomena. They usually occur because of the activity of regulatory proteins.
- Regulatory proteins can either inhibit transcription (negative control) or promote transcription (positive control). Their activity is modulated by small effector molecules called inducers, corepressors, and inhibitors (figure 12.3).

- c. Repressors are responsible for negative control. They block transcription by binding an operator and interfering with the binding of RNA polymerase to its promoter. They can also block transcription by blocking the movement of RNA polymerase after it binds DNA.
- d. Activator proteins are responsible for positive control. They bind DNA sequences called activator-binding sites, and in doing so, promote binding of RNA polymerase to its promoter.
- e. The *lac* operon of *E. coli* is an example of a negatively controlled inducible operon. When there is no lactose in the surroundings, the *lac* repressor is active and transcription is blocked. When lactose is available, it is converted to allolactose by the enzyme β -galactosidase. Allolactose acts as the inducer of the *lac* operon by binding the repressor and inactivating it. The inactive repressor cannot bind the operator and transcription occurs (figure 12.7).
- f. The *trp* operon of *E. coli* is an example of a negatively controlled repressible operon. When there is no tryptophan available, the *trp* repressor is inactive and transcription occurs. When tryptophan levels are high, tryptophan acts as a corepressor and binds the *trp* repressor, activating it. The *trp* repressor binds the operator and blocks transcription (figure 12.8).
- g. The *ara* operon of *E. coli* is an example of an inducible operon that is regulated by a dual-function regulatory protein, the AraC protein. AraC functions as a repressor when arabinose is not available. It functions as an activator when arabinose, the inducer, is available (figure 12.9).
- h. Some regulatory proteins are members of two-component signal transduction systems and phosphorelay systems. These systems have a sensor kinase that detects an environmental change. The sensor kinase transduces the environmental signal to the response-regulator protein either directly (two-component system) or indirectly (phosphorelay) by transferring a phosphoryl group to it. The response regulator then activates genes needed to adapt to the new environmental conditions and inhibits expression of those genes that are not needed (figure 12.10).

12.3 Regulation of Transcription Elongation

- a. In the tryptophan operon, a leader region lies between the operator and the first structural gene (figure 12.11). It codes for the synthesis of a leader peptide and contains an attenuator, a rho-independent termination site.
- b. The synthesis of the leader peptide by a ribosome while RNA polymerase is transcribing the leader region regulates transcription; therefore the tryptophan operon is expressed only when there is insufficient tryptophan available. This mechanism of transcription control is called attenuation.
- c. The leader regions of some mRNA molecules can bind metabolites that act as effector molecules. Binding of the metabolite to the mRNA causes a change in the leader structure, which can terminate transcription. This regulatory mechanism is called a riboswitch (figure 12.12).

12.4 Regulation at the Level of Translation

- a. Some riboswitches regulate gene expression at the level of translation. For these riboswitches, the binding of a small molecule to specific sequences in the leader region of the mRNA alters leader structure and prevents ribosome binding (figure 12.13).
- b. Translation can also be controlled by antisense RNAs. These small RNA molecules are noncoding. They base pair to the mRNA and usually inhibit translation (figure 12.14).

12.5 Global Regulatory Systems

- a. Global regulatory systems can control many operons simultaneously and help microbes respond rapidly to a wide variety of environmental challenges.
- b. Global regulatory systems often involve many layers of regulation. Regulatory mechanisms such as regulatory proteins, alternate sigma factors, two-component signal transduction systems, and phosphorelay systems are often used.
- c. Diauxic growth is observed when *E. coli* is cultured in the presence of glucose and another sugar such as lactose (figure 12.15). This growth pattern is the result of catabolite repression, where glucose is used preferentially over other sugars. Operons that are part of the catabolite repression system are regulated by the activator protein CAP. CAP activity is modulated by cAMP, which is produced only when glucose is not available. Thus when there is no glucose, CAP is active and promotes transcription of operons needed for the catabolism of other sugars (figure 12.18).
- d. Quorum sensing is a type of cell-cell communication mediated by small signaling molecules such as N-acyl-homoserine lactone (AHL). Quorum sensing couples cell density to regulation of transcription. Well-studied quorum-sensing systems include the regulation of bioluminescence in *Vibrio* spp. (figures 12.19 and 12.20). Other systems regulate virulence genes and biofilm formation.
- e. Endospore formation in *B. subtilis* is another example of a global regulatory system. Two important regulatory mechanisms used during sporulation are a phosphorelay system that is important in initiation of sporulation and the use of alternate sigma factors (figure 12.21).

12.6 Regulation of Gene Expression in Eucarya and Archaea

- a. Regulatory transcription factors are used by *Eucarya* to control transcription initiation. They can exert either positive or negative control (figure 12.22).
- b. Antisense RNAs are used by *Eucarya* to regulate translation.
- c. Microbiologists know relatively little about archaeal regulation of gene expression. Some archaea use regulatory proteins that are similar to bacterial regulatory systems to control initiation of transcription.

Key Terms

activator protein 295	constitutive gene 293	inducible gene 294	repressor protein 295
activator-binding site 295	corepressor 295	N-acyl homoserine lactone (AHL) 309	response-regulator protein 301
alternate sigma factors 307	3', 5'-cyclic adenosine monophosphate (cAMP) 308	negative transcriptional control 294	riboswitch 304
antisense RNA 305	cyclic AMP receptor protein (CRP) 308	operator 295	sensor kinase protein 300
aporepressor 295	diauxic growth 308	operon 295	silencer 313
attenuation 302	enhancer 313	phosphorelay system 302	small RNAs (sRNAs) 305
attenuator 302	global regulatory systems 307	positive transcriptional control 295	structural gene 295
autoinducer 309	housekeeping gene 293	quorum sensing 309	two-component signal transduction
autoinduction 309	inducer 294	regulatory transcription factor 313	system 300
catabolite activator protein (CAP) 308	inducible enzyme 294	regulon 307	repressible enzyme 294
catabolite repression 308			

Critical Thinking Questions

1. Attenuation affects anabolic pathways, whereas repression can affect either anabolic or catabolic pathways. Provide an explanation for this.
2. Describe the phenotype of the following strains of *E. coli* mutants when grown in two different media: glucose only and lactose only. Explain the reasoning behind your answer.
 - a. A strain with a mutation in the gene encoding the *lac* repressor such that it cannot bind allolactose.
 - b. A strain with a mutation in the gene encoding CAP such that it does not release cAMP.
 - c. A strain in which the Shine-Dalgarno sequence has been deleted from the gene encoding adenyl cyclase.
3. What would be the phenotype of an *E. coli* strain in which the tandem trp codons in the leader region were mutated so that they coded for serine instead?
4. What would be the phenotype of a *B. subtilis* strain whose gene for σ^G has been deleted? Consider the ability of the mutant to survive in nutrient-rich versus nutrient-depleted conditions.
5. Propose a mechanism by which a cell might sense and respond to levels of Na^+ in its environment.

Learn More

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