Chapter 14

Gene Expression and Signal Transduction

PLANT BIOLOGISTS MAY BE FORGIVEN for taking abiding satisfaction in the fact that Mendel's classic studies on the role of heritable factors in development were carried out on a flowering plant: the garden pea. The heritable factors that Mendel discovered, which control such characteristics as flower color, flower position, pod shape, stem length, seed color, and seed shape, came to be called genes. Genes are the DNA sequences that encode the RNA molecules directly involved in making the enzymes and structural proteins of the cell. Genes are arranged linearly on **chromosomes**, which form linkage groups—that is, genes that are inherited together. The total amount of DNA or genetic information contained in a cell, nucleus, or organelle is termed its **genome**.

Since Mendel's pioneering discoveries in his garden, the principle has become firmly established that the growth, development, and environmental responses of even the simplest microorganism are determined by the programmed expression of its genes. Among multicellular organisms, turning genes on (**gene expression**) or off alters a cell's complement of enzymes and structural proteins, allowing cells to differentiate. In the chapters that follow, we will discuss various aspects of plant development in relation to the regulation of gene expression.

Various internal signals are required for coordinating the expression of genes during development and for enabling the plant to respond to environmental signals. Such internal (as well as external) signaling agents typically bring about their effects by means of sequences of biochemical reactions, called **signal transduction pathways**, that greatly amplify the original signal and ultimately result in the activation or repression of genes.

As will be described in the Growth and Development section of the text, much progress has been made in the study of signal transduction pathways in plants. However, before discussing these pathways in plants, we will review the fundamental features of gene expression and signal transduction in a variety of other organisms, including bacteria, yeasts, and animals, making reference to plant systems wherever appro-

priate. These models will provide a framework for understanding the signaling pathways controlling growth and development in plants.

Genome Size, Organization, and Complexity

As might be expected, the size of the genome bears some relation to the complexity of the organism. For example, the genome size of *E. coli* is 4.7×10^6 base pairs (bp), that of the fruit fly is 2×10^8 bp per haploid cell, and that of a human is 3×10^9 bp per haploid cell. However, genome size in eukaryotes is an unreliable indicator of complexity because not all of the DNA encodes genes.

In prokaryotes, nearly all of the DNA consists of **unique sequences** that encode proteins or functional RNA molecules. In addition to unique sequences, however, eukaryotic chromosomes contain large amounts of noncoding DNA whose main functions appear to be chromosome organization and structure. Much of this noncoding DNA consists of multicopy sequences called **repetitive DNA**. The remainder of the noncoding DNA is made up of single-copy sequences called **spacer DNA**. Together, repetitive and spacer DNA can make up the majority of the total genome in some eukaryotes. For example, in humans only about 5% of the total DNA consists of genes, the unique sequences that code for RNA and protein synthesis.

The genome size in plants is more variable than in any other group of eukaryotes. In angiosperms, the haploid genome ranges from about 1.25×10^8 bp for *Arabidopsis thaliana* (slightly smaller than that of the fruit fly) to 1×10^{11} bp for the monocot *Trillium*, which is considerably larger than the human genome. Even closely related beans of the genus *Vicia* exhibit genomic DNA contents that vary over a 20-fold range. Why are plant genomes so variable in size?

Studies of plant molecular biology have shown that most of the DNA in plants with large genomes is repetitive DNA. Arabidopsis has the smallest genome of any plant because only 10% of its nuclear DNA is repetitive DNA. The genome size of rice is estimated to be about five times that of Arabidopsis, yet the total amount of unique sequence DNA in the rice genome is about the same as in Arabidopsis. Thus the difference in genome size between Arabidopsis and rice is due mainly to repetitive and spacer DNA.

Most plant haploid genomes contain 20,000 to 30,000 genes

Until entire genomes were sequenced, the total number of genes in an organism's genome was difficult to assess. Such numbers are now becoming available for an increasing number of organisms, although precise values are still lacking. The number of genes in bacteria varies from 469 (*Mycoplasma genitalium*) to 7464 (*Nostoc punctiforme*) and overlaps with the number of genes in many simple unicellular eukaryotes. For example, the yeast genome appears to contain about 6000 genes. More complex eukaryotes, such as protozoans, flies, and worms, all seem to have gene numbers in the range of 12,000 to 19,000. The fruit fly (*Drosophila*) genome contains about 14,000 genes, while the nematode worm *Caenorhabditis elegans* has about 19,000 genes.

The current view is that it takes a minimum of ~12,000 genes to form a eukaryotic organism, although values as high as 43,000 genes are common in eukaryotes as a result of multiple copies of certain genes, or **multigene families**. The best-studied plant genome is that of *A. thaliana*. Arabidopsis contains roughly 26,000 genes, approximately the same number as in the human genome. In contrast, the rice (*Oryza sativa*) genome has been estimated to contain between 35,000 and 55,000 genes, far more than are in the human genome.

Some of these genes encode proteins that perform housekeeping functions—basic cellular processes that go on in all the different kinds of cells. Such genes are permanently turned on; that is, they are **constitutively expressed**. Other genes are highly **regulated**, being turned on or off at specific stages of development or in response to specific environmental stimuli.

Prokaryotic Gene Expression

The first step in gene expression is **transcription**, the synthesis of an mRNA copy of the DNA template that encodes a protein (Alberts et al. 2002; Lodish et al. 2004). Transcription is followed by **translation**, the synthesis of the protein on the ribosome. Developmental studies have shown that each plant organ contains large numbers of organ-specific mRNAs. Transcription is controlled by proteins that bind DNA, and these DNA-binding proteins are themselves subject to various types of regulation.

Much of our understanding of the basic elements of transcription is derived from early work on bacterial systems; hence we precede our discussion of eukaryotic gene expression with a brief overview of transcriptional regulation in prokaryotes. However, it is now clear that gene regulation in eukaryotes is far more complex than in prokaryotes. The added complexity of gene expression in eukaryotes is what allows cells and tissues to differentiate and makes possible the diverse life cycles of plants and animals.

DNA-binding proteins regulate transcription in prokaryotes

In prokaryotes, genes are arranged in **operons**, sets of contiguous genes that include **structural genes** and **regulatory sequences**. A famous example is the *E. coli* lactose (*lac*) operon, which was first described in 1961 by François Jacob

and Jacques Monod of the Pasteur Institute in Paris. The *lac* operon is an example of an **inducible** operon—that is, one in which a key metabolic intermediate induces the transcription of the genes.

The *lac* operon is responsible for the production of three proteins involved in utilization of the disaccharide lactose. This operon consists of three structural genes and three regulatory sequences. The structural genes (*z*, *y*, and *a*) code for the sequence of amino acids in three proteins: β-galactosidase, the enzyme that catalyzes the hydrolysis of lactose to glucose and galactose; permease, a carrier protein for the membrane transport of lactose into the cell; and transacetylase, the significance of which is unknown.

The three regulatory sequences (*i*, *p*, and *o*) control the transcription of mRNA for the synthesis of these proteins (Figure 14.1). Gene *i* is responsible for the synthesis of a **repressor protein** that recognizes and binds to a specific nucleotide sequence, the **operator**. The operator, *o*, is located downstream (i.e., on the 3′ side) of the **promoter** sequence, *p*, where RNA polymerase attaches to the operon to initiate transcription, and immediately upstream (i.e., on the 5′ side) of the transcription start site, where transcription begins. (The initiation site is considered to be at the 5′ end of the gene, even though the RNA polymerase transcribes from the 3′ end to the 5′ end along the opposite strand. This convention was adopted so that the sequence of the mRNA would match the DNA sequence of the gene.)

In the absence of lactose, the lactose repressor forms a tight complex with the operator sequence and blocks the interaction of RNA polymerase with the transcription start site, effectively preventing transcription (see Figure 14.1A).

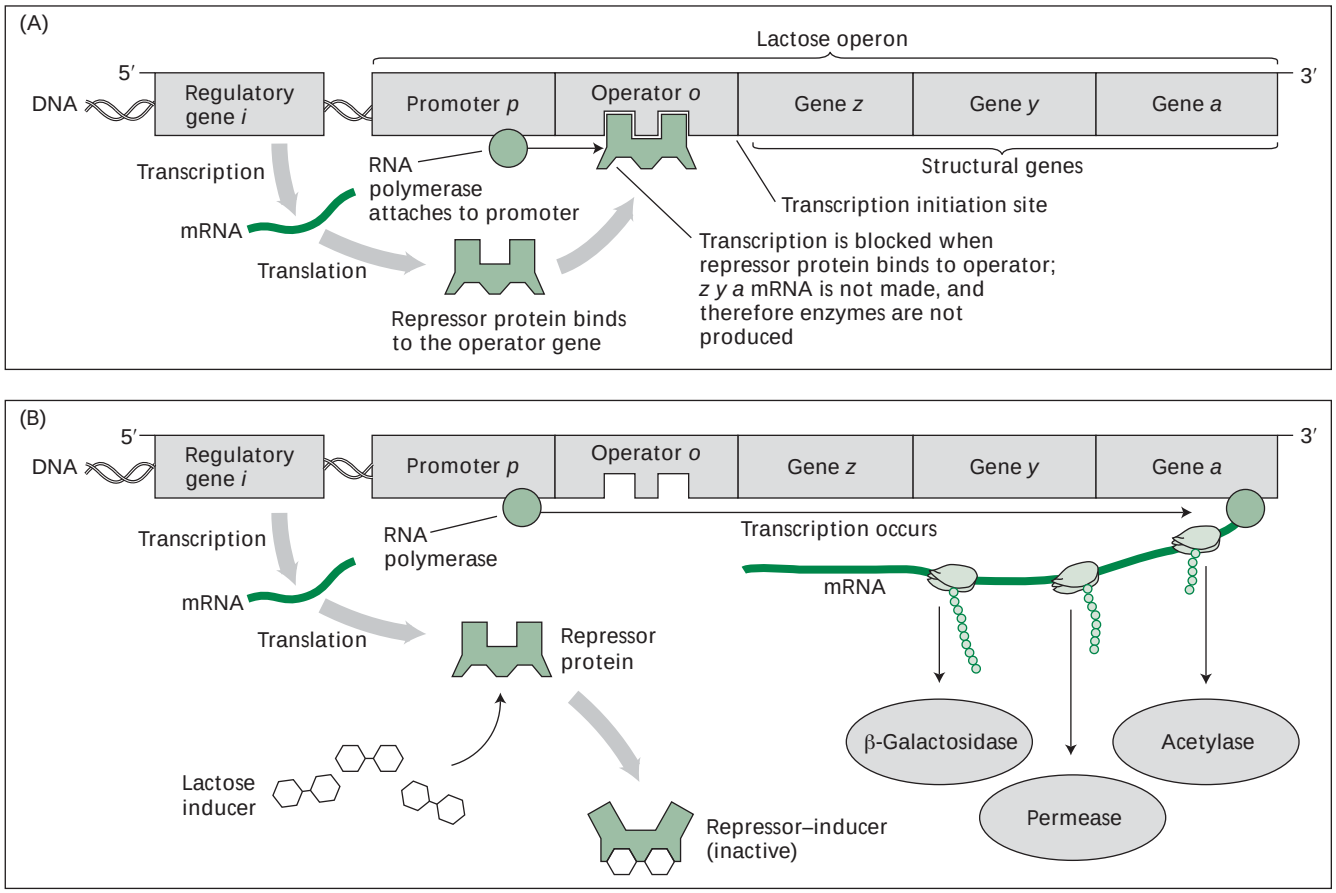


Figure 14.1 The *lac* operon of *E. coli* uses negative control. (A) The regulatory gene *i*, located upstream of the operon, is transcribed to produce an mRNA that encodes a repressor protein. The repressor protein binds to the operator gene *o*. The operator is a short stretch of DNA located between the promoter sequence *p* (the site of RNA polymerase attachment to the DNA) and the three structural genes, *z*, *y*, and *a*. Upon binding to the operator, the repressor prevents RNA polymerase from binding to the transcription initiation site. (B) When lactose (inducer) is added to the medium and is taken up by the cell, it binds to the repressor and inactivates it. The inactivated repressor is unable to bind to *o*, and transcription and translation can proceed. The mRNA produced is termed "polycistronic" because it encodes multiple genes. Note that translation begins while transcription is still in progress.

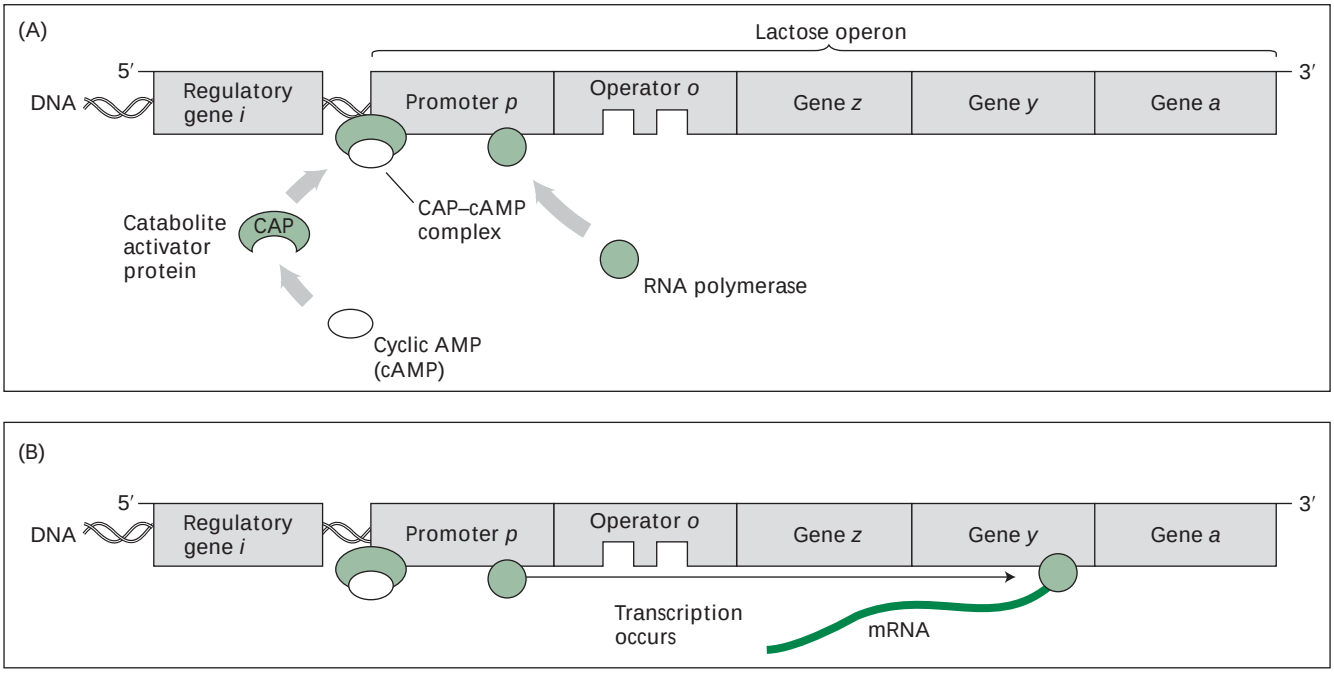


Figure 14.2 Stimulation of transcription by the catabolite activator protein (CAP) and cyclic AMP (cAMP). CAP has no effect on transcription until cAMP binds to it. (A) The CAP–cAMP complex binds to a specific DNA sequence near the promoter region of the *lac* operon. (B) Binding of the CAP–cAMP complex makes the promoter region more accessible to RNA polymerase, and transcription rates are enhanced.

When present, lactose binds to the repressor, causing it to undergo a conformational change (see Figure 14.1B). The *lac* repressor is thus an allosteric protein whose conformation is determined by the presence or absence of an **effector** molecule, in this case lactose. As a result of the conformational change due to binding lactose, the *lac* repressor detaches from the operator. When the operator sequence is unobstructed, the RNA polymerase can move along the DNA, synthesizing a continuous mRNA. The translation of this mRNA yields the three proteins, and lactose is said to induce their synthesis.

The *lac* repressor is an example of **negative control**, since the repressor blocks transcription upon binding to the operator region of the operon. The *lac* operon is also regulated by **positive control**, which was discovered in connection with a phenomenon called the *glucose effect*. If glucose is added to a nutrient medium that includes lactose, the *E. coli* cells metabolize the glucose and ignore the lactose. Glucose suppresses expression of the *lac* operon and prevents synthesis of the enzymes needed to degrade lactose. Glucose exerts this effect by lowering the cellular concentration of cyclic AMP (cAMP). When glucose levels are low, cAMP levels are high. Cyclic AMP binds to an **activator protein**, the *catabolite activator protein* (CAP), which recognizes and binds to a specific nucleotide sequence immediately upstream of the *lac* operator and promoter sites (Figure 14.2).

In contrast to the behavior of the lactose repressor protein, when the CAP is complexed with its effector, cAMP, its affinity for its DNA-binding site is dramatically *increased* (hence the reference to positive control). The ternary complex formed by CAP, cAMP, and the lactose operon DNA sequences induces bending of the DNA, which activates transcription of the lactose operon structural genes by increasing the affinity of RNA polymerase for the neighboring promoter site. Bacteria synthesize cyclic AMP when they exhaust the glucose in their growth medium. The lactose operon genes are thus under opposing regulation by the absence of glucose (high levels of cyclic AMP) and the presence of lactose, since glucose is a catabolite of lactose.

In bacteria, metabolites can also serve as *co-repressors*, activating a repressor protein that blocks transcription. Repression of enzyme synthesis is often involved in the regulation of biosynthetic pathways in which one or more enzymes are synthesized only if the end product of the pathway—an amino acid, for example—is not available. In such a case the amino acid acts as a co-repressor: It com-

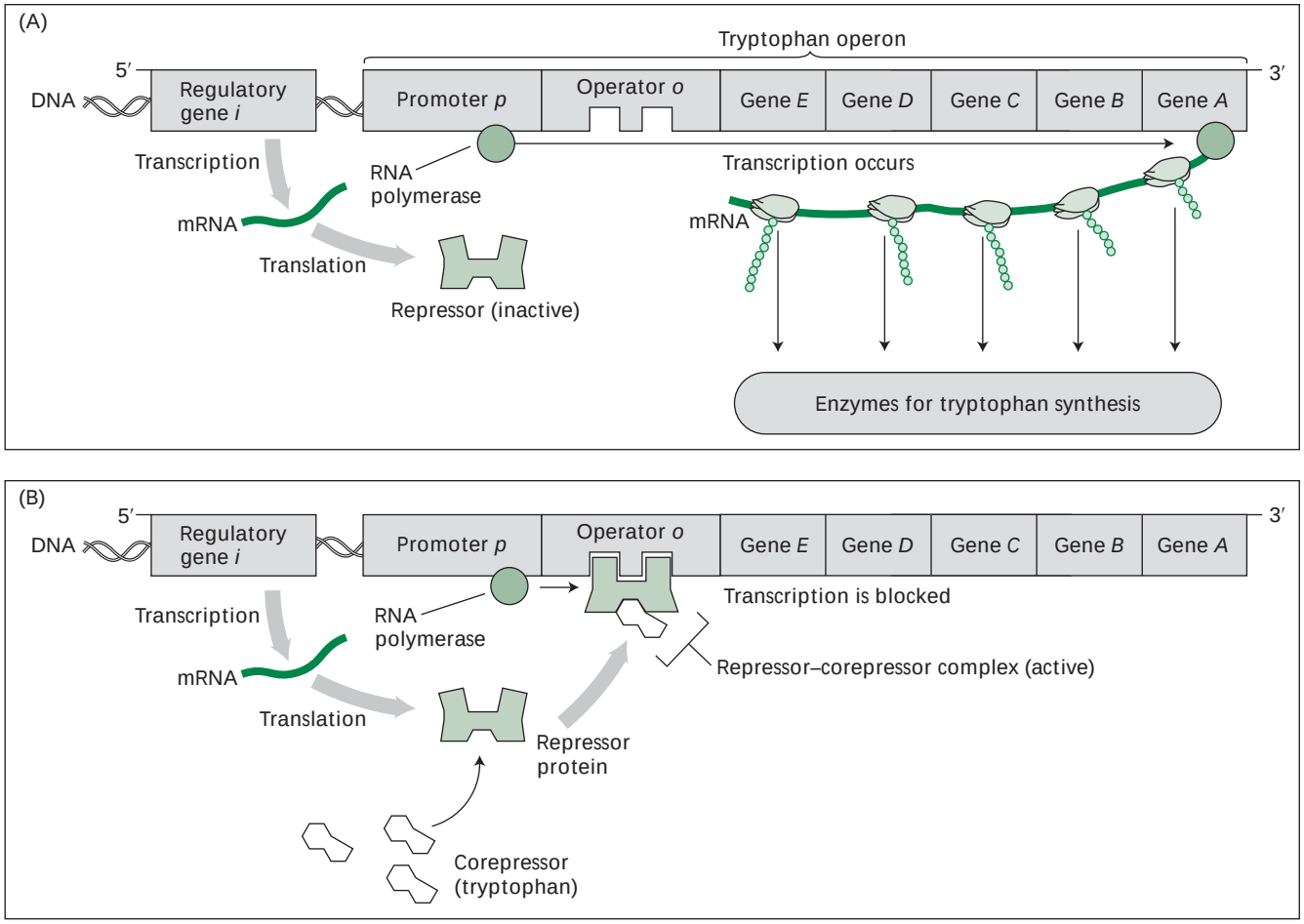


FIGURE 14.3 The tryptophan (*trp*) operon of *E. coli.* Tryptophan (Trp) is the end product of the pathway catalyzed by tryptophan synthetase and other enzymes. Transcription of the repressor genes results in the production of a repressor protein. However, the repressor is inactive until it forms a complex with its co-repressor, Trp. (A) In the absence of Trp, transcription and translation proceed. (B) In the presence of Trp, the activated repressor–co-repressor complex blocks transcription by binding to the operator sequence.

plexes with the repressor protein, and this complex attaches to the operator DNA, preventing transcription. The tryptophan (*trp*) operon in *E. coli* is an example of an operon that works by co-repression (Figure 14.3).

Eukaryotic Gene Expression

The study of bacterial gene expression has provided models that can be tested in eukaryotes. However, the details of the process are quite different and more complex in eukaryotes. In prokaryotes, translation is coupled to transcription: As the mRNA transcripts elongate, they bind to ribosomes and begin synthesizing proteins (translation). In eukaryotes, however, the nuclear envelope separates the genome from the translational machinery. The transcripts must first be transported to the cytoplasm, adding another level of control.

Eukaryotic nuclear transcripts require extensive processing

Eukaryotes differ from prokaryotes also in the organization of their genomes. In most eukaryotic organisms, each gene encodes a single polypeptide. The eukaryotic nuclear genome contains no operons, with one notable exception.* Furthermore, eukaryotic genes are divided into coding regions called **exons** and noncoding regions called **introns** (Figure 14.4). Since the primary transcript, or pre-mRNA, contains both exon and intron sequences, the pre-mRNA must be processed to remove the introns.

RNA processing involves multiple steps. The newly synthesized pre-mRNA is immediately packaged into a string

*About 15% of all *C. elegans* genes are in operons ranging from two to eight genes long.

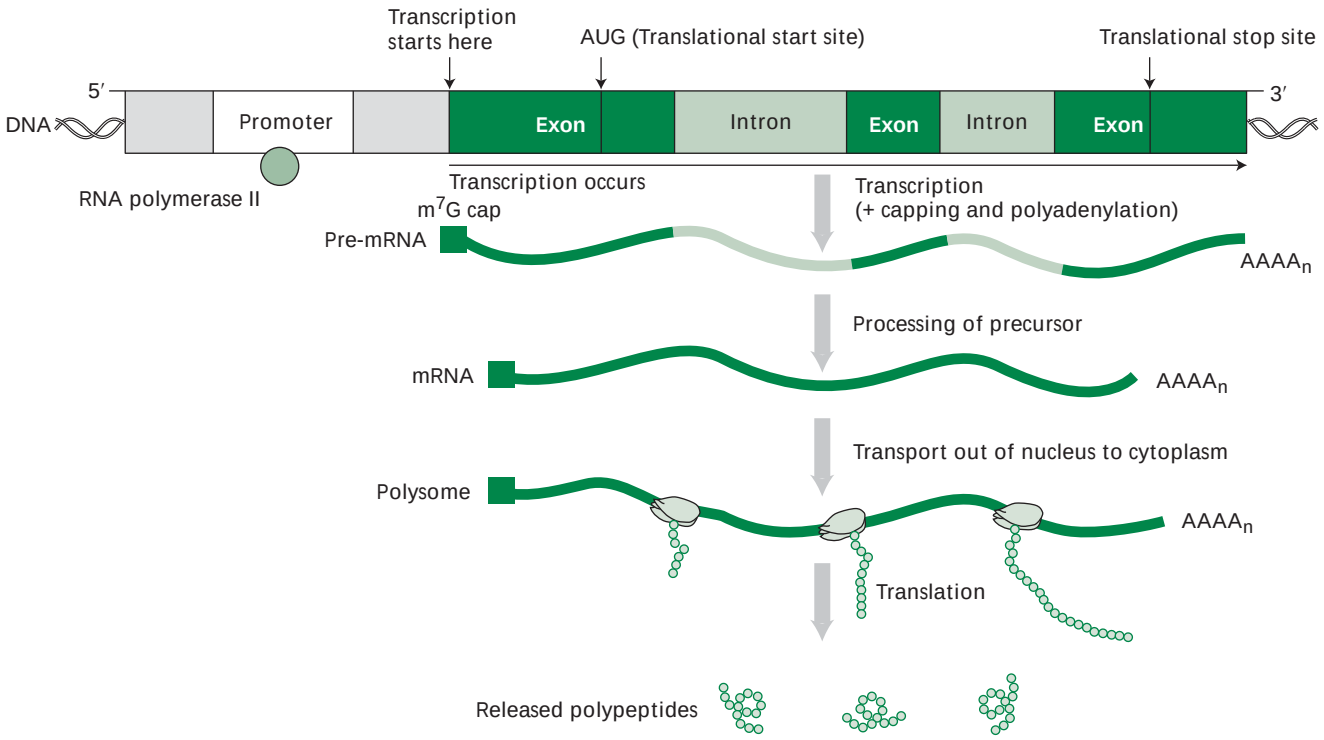


FIGURE 14.4 Gene expression in eukaryotes. RNA polymerase II binds to the promoter of genes that encode proteins. Unlike prokaryotic genes, eukaryotic genes are not clustered in operons, and each is divided into introns and exons. Transcription from the template strand proceeds in the 3′-to-5′ direction at the transcription start site, and the growing RNA chain extends one nucleotide at a time in the 5′-to-3′ direction. Translation begins with the first AUG encoding methionine, as in prokaryotes, and ends with the stop codon. The pre-mRNA transcript is first "capped" by the addition of 7-methylguanylate (m^7G) to the 5′ end. The 3′ end is shortened slightly by cleavage at a specific site, and a poly-A tail is added. The capped and polyadenylated pre-mRNA is then spliced by a spliceosome complex, and the introns are removed. The mature mRNA exits the nucleus through the pores and initiates translation on ribosomes in the cytosol. As each ribosome progresses toward the 3′ end of the mRNA, new ribosomes attach at the 5′ end and begin translating, leading to the formation of polysomes.

of small protein-containing particles, called **heterogeneous ribonucleoprotein particles**, or **hnRNP particles**. Some of these particles are composed of proteins and small nuclear RNAs, and are called **small nuclear ribonucleoproteins**, or **snRNPs** (pronounced "snurps"). Various snRNPs assemble into **spliceosome complexes** at exon–intron boundaries of the pre-mRNA and carry out the splicing reaction.

In some cases, the primary transcript can be spliced in different ways, a process called **alternative RNA splicing**. For example, an exon that is present in one version of a processed transcript may be spliced out of another version. In this way, the same gene can give rise to different polypeptide chains. Approximately 15% of human genes are processed by alternative splicing. Although alternative splicing is rare in plants, it is involved in the synthesis of rubisco activase, RNA polymerase II, and the gene product of a rice homeobox gene (discussed later in the chapter), as well as other proteins (Golovkin and Reddy 1996).

Before splicing, the pre-mRNA is modified in two important ways. First it is **capped** by the addition of 7-methylguanylate to the 57 end of the transcript via a 5′-to-5′ linkage. The pre-mRNA is capped almost immediately after the initiation of mRNA synthesis. One of the functions of the 5′ cap is to protect the growing RNA transcript from degradation by RNases. At a later stage in the synthesis of the primary transcript, the 3′ end is cleaved at a specific site, and a **poly-A tail**, usually consisting of about 100 to 200 adenylic acid residues, is added by the enzyme poly-A polymerase (see Figure 14.4).

The poly-A tail has several functions: (1) It protects against RNases and therefore increases the stability of mRNA molecules in the cytoplasm, (2) both it and the 5′ cap are required for transit through the nuclear pore, and (3) it increases the efficiency of translation on the ribosomes. The requirement of eukaryotic mRNAs to have both a 5′ cap and a poly-A tail ensures that only properly processed transcripts will reach the ribosome and be translated.

Each step in eukaryotic gene expression can potentially regulate the amount of gene product in the cell at any given time (Figure 14.5). Like transcription initiation, splicing may be regulated. Export from the nucleus is also regulated. For example, to exit the nucleus an mRNA must

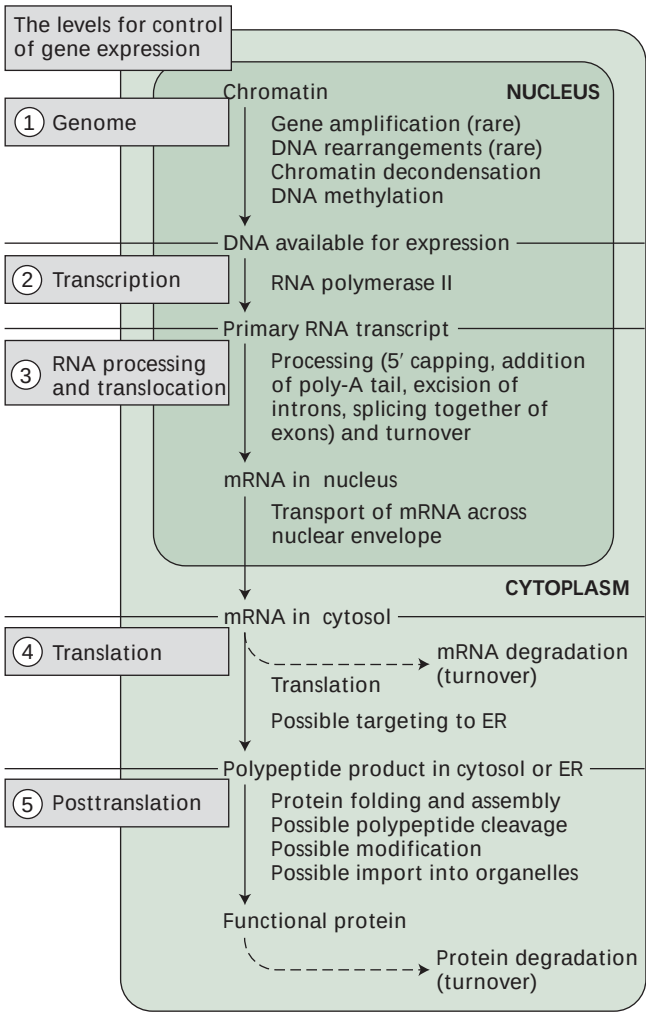


Figure 14.5 Eukaryotic gene expression can be regulated at multiple levels: (1) genomic regulation, by gene amplification, DNA rearrangements, chromatin decondensation or condensation, or DNA methylation; (2) transcriptional regulation; (3) RNA processing, and RNA turnover in the nucleus and translocation out of the nucleus; (4) translational control (including binding to ER in some cases); (5) posttranslational control, including mRNA turnover in the cytosol, and the folding, assembly, modification, and import of proteins into organelles. (After Becker et al. 1996.)

possess a 5′ cap and a poly-A tail, and it must be properly spliced. Incompletely processed transcripts remain in the nucleus and are degraded.

Various posttranscriptional regulatory mechanisms have been identified

The stabilities or **turnover rates** of mRNA molecules differ from one another, and may vary from tissue to tissue, depending on the physiological conditions. For example, in fava bean (*Vicia faba*), fungal infection causes the rapid degradation of the mRNA that encodes the proline-rich protein PvPRP1 of the bean cell wall. Another example of the regulation of gene expression by RNA degradation is the regulation of expression of one of the genes for the small subunit of rubisco in roots of the aquatic duckweed *Lemna gibba*. *Lemna* roots are photosynthetic and therefore express genes for the small subunit of rubisco, but the expression of one of the genes (*SSU5B*) is much lower in roots than in the fronds (leaves). The low level of SSU5B in the roots is due to a high rate of turnover of the *SSU5B* pre-mRNA in the nucleus (Peters and Silverthorne 1995).

In addition to RNA turnover, the **translatability** of mRNA molecules is variable. For example, RNAs fold into molecules with varying secondary and tertiary structures that can influence the accessibility of the translation initiation codon (the first AUG sequence) to the ribosome. Another factor that can influence translatability of an mRNA is codon usage. There is redundancy in the triplet codons that specify a given amino acid during translation, and each cell has a characteristic ratio of the different aminoacylated tRNAs available, known as **codon bias**. If a message contains a large number of triplet codons that are rare for that cell, the small number of charged tRNAs available for those codons will slow translation. Finally, the **cellular location** at which translation occurs seems to affect the rate of gene expression. Free polysomes may translate mRNAs at very different rates from those at which polysomes bound to the endoplasmic reticulum do; even within the endoplasmic reticulum, there may be differential translation rates.

Although examples of posttranscriptional regulation have been demonstrated for each of the steps described above and summarized in Figure 14.5, *the expression of most eukaryotic genes, like their prokaryotic counterparts, appears to be regulated at the level of transcription.*

Transcription in eukaryotes is controlled by *cis*-acting regulatory sequences

The synthesis of most eukaryotic proteins is regulated at the level of transcription. However, transcription in eukaryotes is much more complex than in prokaryotes. First, there are three different RNA polymerases in eukaryotes: I, II, and III. RNA polymerase I is located in the nucleolus and functions in the synthesis of most ribosomal RNAs. RNA polymerase II, located in the nucleoplasm, is responsible for pre-mRNA synthesis. RNA polymerase III, also located in the nucleoplasm, synthesizes small RNAs, such as tRNA and 5S rRNA.

A second important difference between transcription in prokaryotes and in eukaryotes is that the RNA polymerases of eukaryotes require additional proteins called **general transcription factors** to position them at the correct start site. While prokaryotic RNA polymerases also require accessory polypeptides called sigma factors (σ), these polypeptides are considered to be subunits of the RNA polymerase. In contrast, eukaryotic general transcription factors make up a large, multisubunit **transcription initia-**

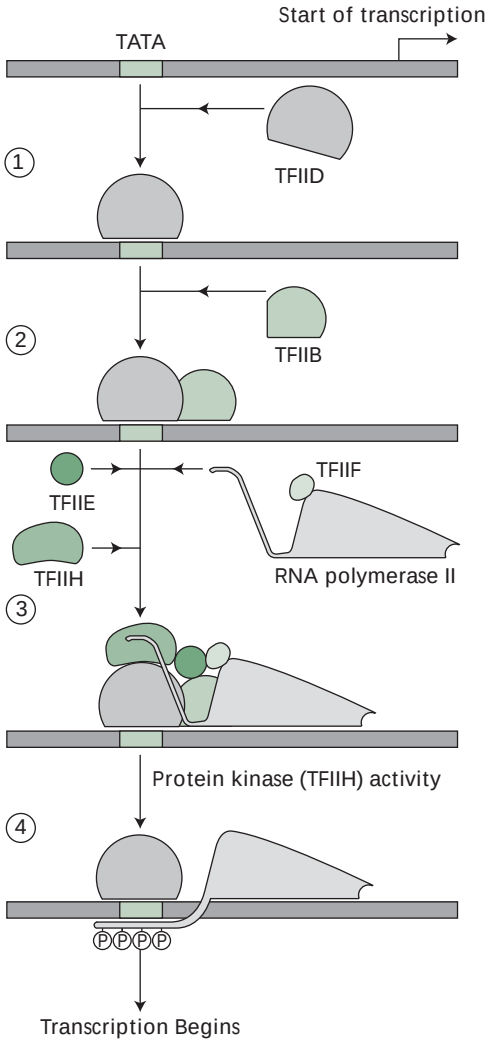


Figure 14.6 Ordered assembly of the general transcription factors required for transcription by RNA polymerase II. (1) TFIID, a multisubunit complex, binds to the TATA box via the TATA-binding protein. (2) TFIIB joins the complex. (3) TFIIF bound to RNA polymerase II associates with the complex, along with TFIIE and TFIIH. The assembly of proteins is referred to as the transcription initiation complex. (4) TFIIH, a protein kinase, phosphorylates the RNA polymerase, some of the general transcription factors are released, and transcription begins.

tion complex. For example, seven general transcription factors constitute the initiation complex of RNA polymerase II, each of which must be added in a specific order during assembly (Figure 14.6).

Transcription is initiated when the final transcription factor, TFIIH (*t*ranscription *f*actor for RNA polymerase *II* protein *H*), joins the complex and causes phosphorylation of the RNA polymerase. RNA polymerase II then separates from the initiation complex and proceeds along the antisense strand in the 3′-to-5′ direction. While some of the general transcription factors dissociate from the complex at this point, others remain to bind another RNA polymerase molecule and initiate another round of transcription.

A third difference between transcription in prokaryotes and in eukaryotes is in the complexity of the promoters, the sequences upstream (5′) of the initiation site that regulate transcription. We can divide the structure of the eukaryotic promoter into two parts, the **core** or **minimum promoter**, consisting of the minimum upstream sequence required for gene expression, and the additional **regulatory sequences**, which control the activity of the core promoter.

Each of the three RNA polymerases has a different type of promoter. An example of a typical RNA polymerase II promoter is shown schematically in Figure 14.7A. The minimum promoter for genes transcribed by RNA polymerase II typically extends about 100 bp upstream of the transcription initiation site and includes several sequence elements referred to as **proximal promoter sequences**. About 25 to 35 bp upstream of the transcriptional start site is a short sequence called the **TATA box**, consisting of the sequence TATAAA(A). The TATA box plays a crucial role in transcription because it serves as the site of assembly of the transcription initiation complex. Approximately 85% of the plant genes sequenced thus far contain TATA boxes.

In addition to the TATA box, the minimum promoters of eukaryotes also contain two additional regulatory sequences: the **CAAT box** and the **GC box** (see Figure 14.7A). These two sequences are the sites of binding of **transcription factors**, proteins that enhance the rate of transcription by facilitating the assembly of the initiation complex. The DNA sequences themselves are termed ***cis*-acting sequences**, since they are adjacent to the transcription units they are regulating. The transcription factors that bind to the *cis*-acting sequences are called ***trans*-acting factors**, since the genes that encode them are located elsewhere in the genome.

Numerous other *cis*-acting sequences located farther upstream of the proximal promoter sequences can exert either positive or negative control over eukaryotic promoters. These sequences are termed the **distal regulatory sequences** and they are usually located within 1000 bp of the transcription initiation site. As with prokaryotes, the positively acting transcription factors that bind to these sites are called **activators**, while those that inhibit transcription are called **repressors**.

As we will see in Chapters 19 and 20, the regulation of gene expression by the plant hormones and by phytochrome is thought to involve the deactivation of repressor proteins. *Cis*-acting sequences involved in gene regulation by hormones and other signaling agents are called **response elements**. As will be discussed in Chapters 17 and 19 through 24 (on phytochrome and the plant hormones), numerous response elements that regulate gene expression have been identified in plants.

In addition to having regulatory sequences within the promoter itself, eukaryotic genes can be regulated by control elements located tens of thousands of base pairs away

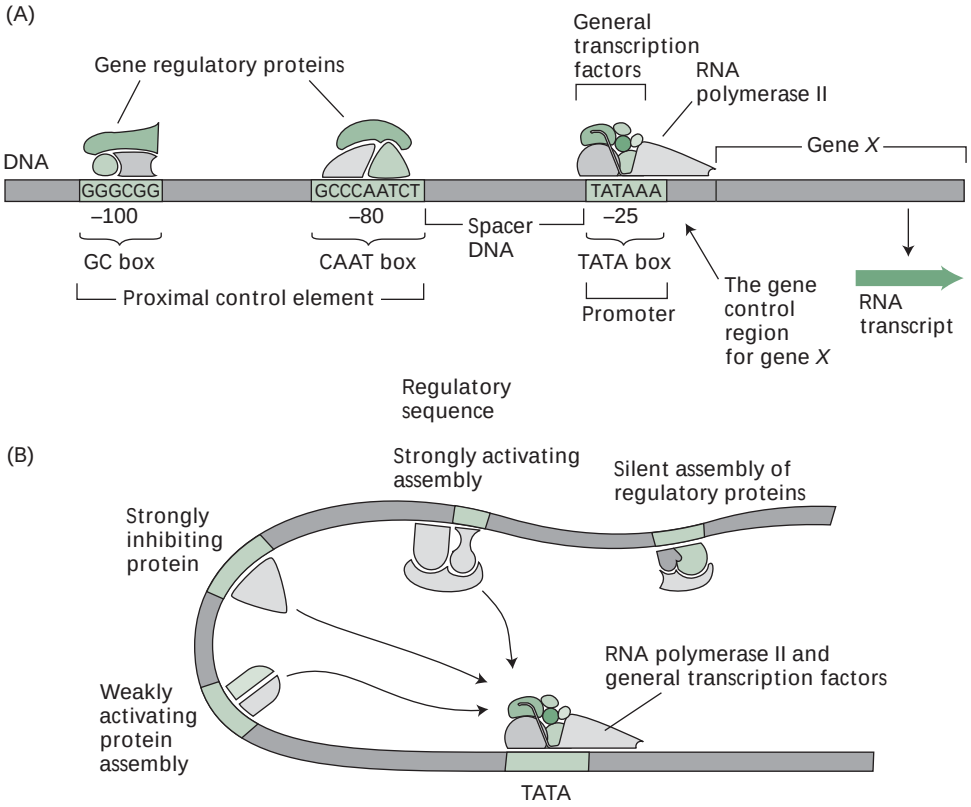


Figure 14.7 Organization and regulation of a typical eukaryotic gene. (A) Features of a typical eukaryotic RNA polymerase II minimum promoter and proteins that regulate gene expression. RNA polymerase II is situated at the TATA box in association with the general transcription factors about 25 bp upstream of the transcription start site. Two *cis*-acting regulatory sequences that enhance the activity of RNA polymerase II are the CAAT box and the GC box, located at about 80 and 100 bp upstream, respectively, of the transcription start site. The DNA proteins that bind to these elements are indicated. (B) Regulation of transcription by distal regulatory sequences and *trans*-acting factors. The *trans*-acting factors bound to distal regulatory sequences can act in concert to activate transcription by making direct physical contact with the transcription initiation complex. The details of this process are not well understood.

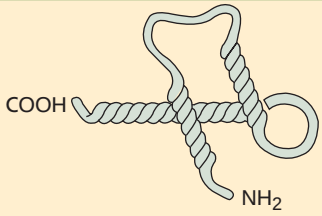
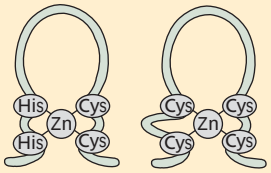
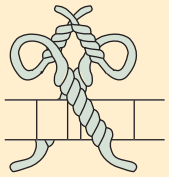
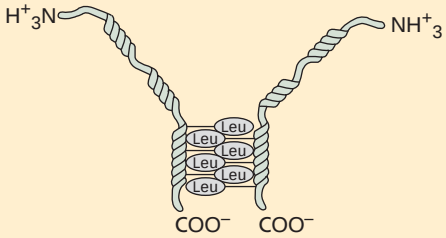
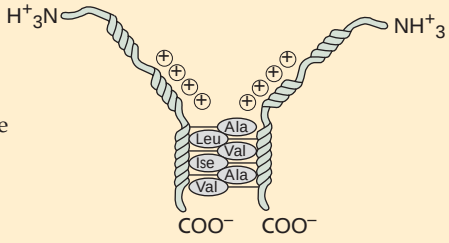
from the start site. Distantly located positive regulatory sequences are called **enhancers**. Enhancers may be located either upstream or downstream from the promoter. In plants, most gene promoters are relatively compact, although enhancer elements are more common in genes of the flavonoid biosynthesis pathway (Zhang and Peterson 2005) (see Chapter 13).

How do all the DNA-binding proteins on the *cis*-acting sequences regulate transcription? During formation of the initiation complex, the DNA between the core promoter and the most distally located control elements loops out in such a way as to allow all of the transcription factors bound to that segment of DNA to make physical contact with the initiation complex (see Figure 14.7B). Through this physical contact the transcription factor exerts its control, either positive or negative, over transcription. Given the large number of control elements that can modify the activity of a single promoter, the possibilities for differential gene regulation in eukaryotes are nearly infinite.

Transcription factors contain specific structural motifs

Transcription factors generally have three structural features: a DNA-binding domain, a transcription-activating domain, and a ligand-binding domain. To bind to a specific sequence of DNA, the DNA-binding domain must have extensive interactions with the double helix through the formation of hydrogen, ionic, and hydrophobic bonds. Although the particular combination and spatial distribution of such interactions are unique for each sequence, analyses of many DNA-binding proteins have led to the identification of a small number of highly conserved DNA-binding structural motifs, which are summarized in Table 14.1.

TABLE 14.1
DNA-Binding Motifs

Name	Examples of proteins	Key structural features	Illustration
Helix-turn-helix	Transcription factors that regulate genes in anthocyanin biosynthesis pathway	Two α helices separated by a turn in the polypeptide chain; function as dimers	
Zinc finger	COP1 in Arabidopsis	Various structures in which zinc plays an important structural role; bind to DNA either as monomers or as dimers	
Helix-loop-helix	GT element–binding protein of phytochrome-regulated genes	A short α helix connected by a loop to a longer a helix; function as dimers	
Leucine zipper	Fos and Jun	An α helix of about 35 amino acids containing leucine at every seventh position; dimerization occurs along the hydrophobic surface	
Basic zipper (bZip)	Opaque 2 protein in maize, G box factors of phytochrome-regulated genes, transcription factors that bind ABA response elements	Variation of the leucine zipper motif in which other hydrophobic amino acids substitute for leucine and the DNA-binding domain contains amino acids	

Most of the transcription factors characterized thus far in plants belong to the basic zipper (bZIP) class of DNA-binding proteins. DNA-binding proteins containing the zinc finger domain are relatively rare in plants.

Homeodomain proteins are a special class of helix-turn-helix proteins

The term "homeodomain protein" is derived from a group of *Drosophila* (fruit fly) genes called **selector genes** or **homeotic genes**. *Drosophila* homeotic genes encode transcription factors that determine which structures develop at specific locations on the fly's body; that is, they act as major developmental switches that activate a large number of genes that constitute the entire genetic program for a particular structure. Mutations in homeotic genes cause **homeosis**, the transformation of one body part into another. For example, a homeotic mutation in the *ANTENNAPEDIA* gene causes a leg to form in place of an antenna. When the sequences of various homeotic genes in *Drosophila* were compared, the proteins were all found to contain a highly conserved stretch of 60 amino acids called the **homeobox**.

Homologous homeobox sequences have now been identified in developmentally important genes of vertebrates and plants. As will be discussed in Chapter 16, the *KNOTTED* (*KN1*) gene of maize encodes a homeodomain protein that can affect cell fate during development. Maize plants with the *kn1* mutation exhibit abnormal cell divisions in the vascular tissues, giving rise to the "knotted" appearance of the leaf surface. However, the *kn1* mutation is not a homeotic mutation, since it does not involve the substitution of one

entire structure for another. Rather, the plant homeodomain protein, KN1, is involved in the regulation of cell division. Thus, not all genes that encode homeodomain proteins are homeotic genes, and vice versa. The Arabidopsis genome contains over 100 homeodomain sequences, and several of them (for example, *KNOX*, *BELL*, *STM*, *KNAT1*, and *WUS*) regulate the development of plant organs, although they are not homeotic genes (see Chapter 16).

As will be discussed in Chapter 25, four of the floral homeotic genes in plants encode proteins with the DNA-binding helix-turn-helix motif called the **MADS domain**.

Eukaryotic genes can be coordinately regulated

Although eukaryotic nuclear genes are not arranged into operons, they are often coordinately regulated in the cell. For example, in yeast, many of the enzymes involved in galactose metabolism and transport are inducible and coregulated, even though the genes are located on different chromosomes. Incubation of wild-type yeast cells in galactose-containing media results in more than a thousandfold increase in the mRNA levels for all of these enzymes.

The six yeast genes that encode the enzymes in the galactose metabolism pathway are under both positive and negative control (Figure 14.8). Most yeast genes are regulated by a single proximal control element called an *upstream activating sequence* (UAS). The *GAL4* gene encodes a transcription factor that binds to UAS elements located about 200 bp upstream of the transcription start sites of all six genes. The UAS of each of the six genes, while not identical, consists of one or more copies of a similar 17 bp repeated sequence. The GAL4 protein can bind to each of them and activate transcription. *In this way a single transcription factor can control the expression of many genes.*

Protein–protein interactions can modify the effects of DNA-binding transcription factors. Another gene on a dif-

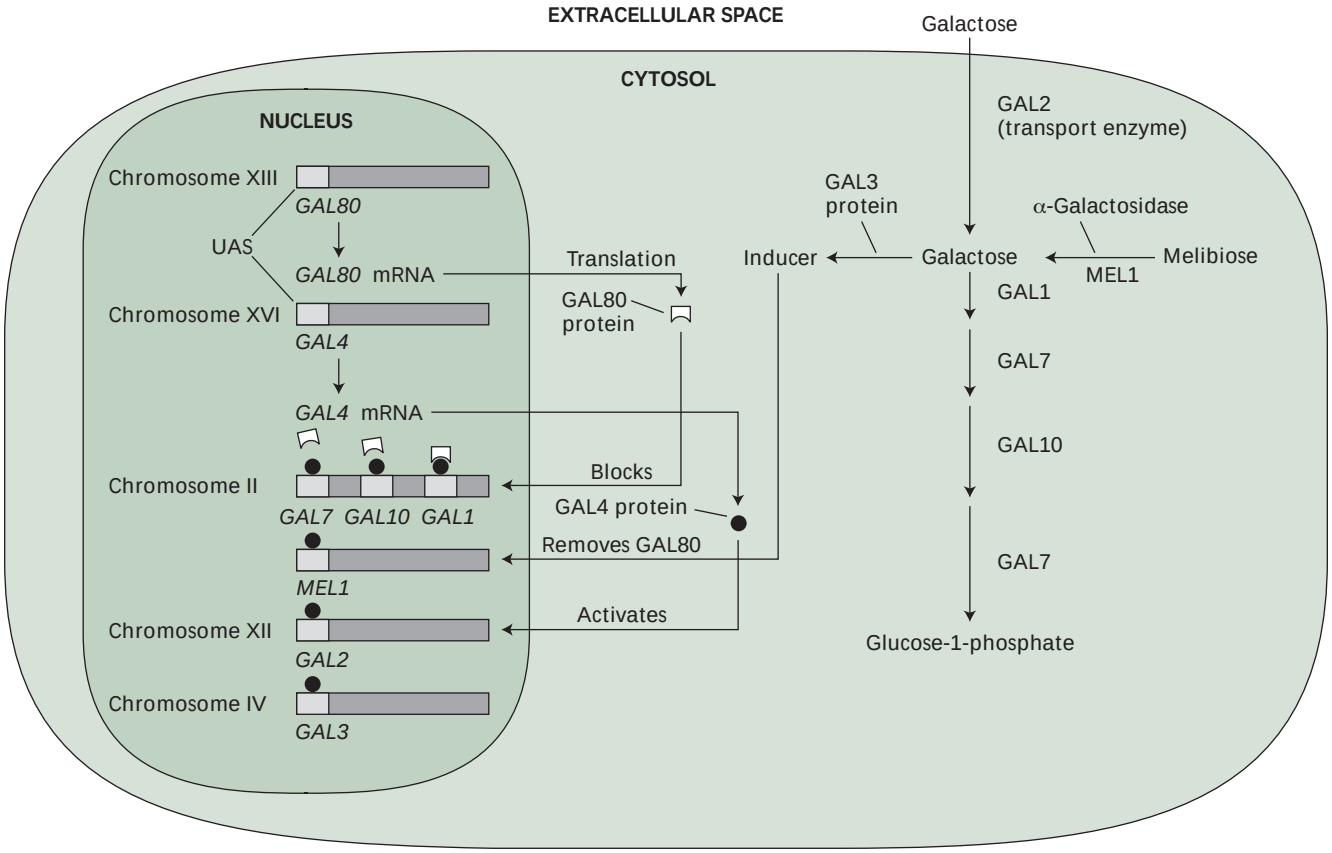


Figure 14.8 Model for eukaryotic gene induction: the galactose metabolism pathway of the yeast *Saccharomyces cerevisiae*. Several enzymes involved in galactose transport and metabolism are induced by a metabolite of galactose. The genes *GAL7*, *GAL10*, *GAL1*, and *MEL1* are located on chromosome II; *GAL2* is on chromosome XII; *GAL3* is on chromosome IV. *GAL4* and *GAL80*, located on two other chromosomes, encode positive and negative *trans*-acting regulatory proteins, respectively. The GAL4 protein binds to an upstream activating sequence located upstream of each of the genes in the pathway, indicated by the hatched lines. The GAL80 protein forms an inhibitory complex with the GAL4 protein. In the presence of galactose, the metabolite formed by the GAL3 gene product diffuses to the nucleus and stimulates transcription by causing dissociation of the GAL80 protein from the complex.

ferent yeast chromosome, *GAL80*, encodes a negative transcription regulator that forms a complex with the GAL4 protein when it is bound to the UAS. When the GAL80 protein is complexed with GAL4, transcription is blocked. In the presence of galactose, however, the metabolite formed by the enzyme that is encoded by the *GAL3* gene acts as an inducer by causing the dissociation of GAL80 from GAL4 (Johnston 1987; Mortimer et al. 1989).

The GAL4 transcription factor consists of an N-terminal DNA-binding domain and a C-terminal activation domain, separated by a flexible protein domain. The DNA-binding domain binds to a specific DNA sequence element, while the activation domain initiates transcription of the gene by interacting with other proteins. Most eukaryotic transcription factors have the same basic domain structure as GAL4. The presence of a flexible protein domain between the DNA-binding and activation domains allows the transcription factor to function even when the distance between the binding sites and activation sites on the DNA varies between different related genes.

There are many other examples of coordinate regulation of genes in eukaryotes. In plants, the developmental effects induced by light and hormones (see Chapters 17 through 24), as well as the adaptive responses caused by various types of stress (see Chapter 26), involve the coordinate regulation of groups of genes that share a common response element upstream of the promoter. In addition, genes that act as major developmental switches, such as the homeotic genes, encode transcription factors that bind to a common regulatory sequence that is present on dozens, or even hundreds, of genes scattered throughout the genome (see Chapters 16 and 25).

Small RNAs are posttranscriptional repressors of gene expression

After exiting the nucleus, newly synthesized mRNA associates with ribosomes and initiates translation. Several factors can affect the rate of gene expression posttranscriptionally. Two of these, mRNA turnover and inhibition of translation, can be regulated by small RNAs called microRNA and short interfering RNA (Baulcombe 2004).

MicroRNAs (**miRNAs**) were first discovered as mutations in genes of *C. elegans* that did not encode proteins. Instead, the products of these genes were short RNAs, about 21 nucleotides long, that could inhibit the translation of specific target mRNAs by hybridizing to their 3′ untranslated regions. MiRNAs have since been found to play an important role in regulating gene expression in both plants and animals. In plants, however, miRNAs function primarily by directing the cleavage of the target mRNA, rather than by inhibiting translation on the ribosome.

All miRNAs arise from a 70 nucleotide primary transcript called the **pri-miRNA**. In animals, pri-miRNA itself is the result of processing of an even longer RNA transcript in the nucleus by the enzyme Drosha. Thus far, no Drosha homolog has been found in plants, suggesting that the early steps of pri-miRNA production may be different in plants. It has been estimated that the Arabidopsis genome contains approximately 100 miRNA loci. As we will see in Chapter 16, miRNAs have been implicated in the regulation of many important developmental events in plants.

The 70-nucleotide pri-miRNA contains palindromic sequences at its two ends (inverted repeat sequences) that are complementary and therefore can base pair with each other. When base-pairing occurs, the pri-miRNA forms **hairpin loops**. In animals there are a few mismatches in the base-pairing of the stem of the hairpin structure (Figure 14.9). These mismatches contribute to the ability of miRNA to inhibit translation. In plants, however, the base-pairing in the stem of the pri-miRNA hairpin loop is more precise. Accordingly, plant miRNAs repress gene expression mainly by promoting RNA degradation rather than by inhibiting translation.

The next step involves the processing of the pri-miRNA stem-loop structure by a ribonuclease that can cleave double-stranded RNA. In animal cells, this enzyme is called Dicer, and it is located in the cytoplasm. Dicer cleaves the pri-miRNA into a double-stranded RNA fragment, 21–23 nucleotides in length, with overhanging two-nucleotide tails at the 3′ ends of each strand (see Figure 14.9). Plant cells contain **Dicer-like proteins** (for example, **DCL1**), but unlike Dicer in animal cells, DCL1 is localized to the nucleus, suggesting that the cleavage of pri-miRNA to form the 21- to 23-nucleotide miniduplexes occurs in the nucleus in plant cells. As described below, the short strands from these miniduplexes serve as guides to target a ribonuclease complex to specific mRNA molecules. This part of the pathway is similar to that of a second type of small RNA called *short interfering RNA*.

Short interfering RNA (**siRNA**) is responsible for the phenomenon of *RNA interference* (*RNAi*). The phenomenon of RNA interference was first observed in Petunia (Napoli et al. 1990), although the mechanism was not understood at the time. In an attempt to produce Petunia flowers with a deep purple color, petunia plants were transformed with extra copies of the gene for chalcone synthase, a key enzyme in the synthesis of anthocyanin pigments (see Chapter 13). But instead of dark purple flowers, the transformants with extra gene copies produced only white flowers. The tendency of extra copies of a gene to induce the suppression of the native gene was termed *cosuppression*.

A related phenomenon was discovered by plant virologists studying viral resistance mechanisms. The genomes of most plant viruses consist of single-stranded RNA (ssRNA). It was known that plants expressing viral proteins exhibited increased resistance to viruses, but it was subsequently found that even plants expressing short, noncoding regions of viral RNA sequences became resistant to the virus. The short viral sequences were somehow able to attack the incoming viruses.

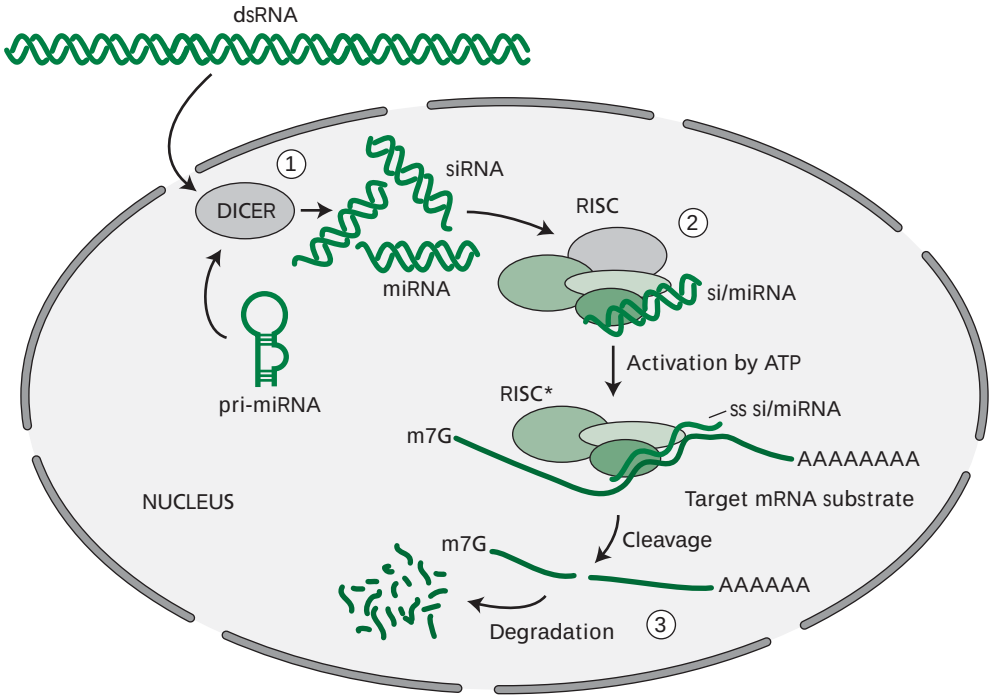


Figure 14.9 Model for the repression of gene expression by siRNA and miRNA. (1) Pri-miRNA stem-loop structures and double-stranded RNA generated by the action of RNA-dependent RNA polymerases are processed by the enzyme DICER (or Dicer-like proteins in plants) to 21- to 23-nucleotide sequences, termed miRNA and siRNA, respectively. (2) The RNA-induced silencing complex (RISC) associates with the miRNA or siRNA and, after activation by ATP, releases one of the RNA strands. The remaining RNA strand serves as a "guide" that directs the complex to its target RNA molecule. (3) The target RNA is cleaved by the RISC ribonuclease, and the cleavage products are further degraded by other cellular ribonucleases.

The relationship between cosuppression and viral resistance in plants became apparent with the discovery of **RNA-dependent RNA polymerases** (**RDRs** or **RdRPs**) (Baulcombe 2004). RDRs, which form RNA duplexes by synthesizing antisense RNA using a sense RNA strand as a template, are required for both cosuppresion and viral resistance. During cosuppression in Petunia, the presence of extra chalcone synthase mRNAs triggers the production of RDRs, which quickly generate RNA duplexes. These duplexes are then cleaved throughout their length into 21- to 23-nucleotide double-stranded fragments by Dicer-like proteins in the nucleus.

Viral resistance in plants also depends on RDRs. Upon entering the cytoplasm, the viral ssRNA is converted to double-stranded RNA by plant RDRs. The resulting double stranded intermediate then serves as a substrate for Dicer-like proteins, which generate the canonical 21- to 23-nucleotide double-stranded fragments involved in the degradation of the virus (see below). Similar conclusions about the role of double-stranded RNA in gene suppression have been reached in studies with other organisms, including fungi and animals.

After formation of the 21- to 23-nucleotide miRNAs or siRNAs by the Dicer-like proteins, the duplex associates with a large ribonuclease complex called the **RNA-induced silencing complex** (**RISC**) (see Figure 14.9). In a reaction requiring ATP, the RISC then unwinds the duplex and releases the sense RNA stand. The RISC then uses the remaining antisense strand as a guide to bind to a target mRNA or viral sequence, much as the anticodon of tRNA recognizes the codon of mRNA on the ribosome. In plants, the miRNA is either perfectly complementary, or nearly complementary, to the target mRNA sequence, and the RNA-induced silencing complex typically cleaves the target RNA in the middle of the binding site. Cleavage renders the two halves of the mRNA (or virus) susceptible to further degradation by other cellular ribonucleases.

The ubiquitin pathway regulates protein turnover

A protein, once synthesized, has a finite lifetime in the cell, ranging from a few minutes to several hours. Hence, steady-state levels of cellular enzymes are attained as the result of an equilibrium between protein synthesis and protein degradation, or turnover. Protein turnover plays an important role in development. In etiolated seedlings, for example, the red-light photoreceptor phytochrome is regulated by proteolysis. The phytochrome synthesized in the dark is highly stable and accumulates in the cells to high concentrations. Upon exposure to red light, however, the phytochrome is converted to its active form and simulta-

neously becomes highly susceptible to degradation by proteases (see Chapter 17). The auxin receptor appears to be an enzyme that participates in the turnover of proteins in the nucleus. By inducing the turnover of repressor proteins, auxin stimulates the expression of genes important in cell growth and differentiation (see Chapter 19).

In both plant and animal cells there are two distinct pathways of protein turnover, one in specialized lytic vacuoles (called lysosomes in animal cells) and the other in the soluble matrix of the cytoplasm and nucleus. Protein turnover by lytic vacuoles was discussed in Chapter 1. Autophagosomes containing engulfed cytoplasm can fuse directly with a lytic vacuole, initiating degradation of the protein within. Alternatively, the prevacuolar compartment containing membranes from endocytotic vesicles may also fuse with a lytic vacuole (see Chapter 1).

The nonvacuolar pathway of protein turnover involves the ATP-dependent formation of a covalent bond to a small, 76-amino-acid polypeptide called **ubiquitin**. Ubiquitination of an enzyme molecule apparently marks it for destruction by a large ATP-dependent proteolytic complex (**proteasome**) that specifically recognizes the "tagged" molecule (Figure 14.10) (Coux et al. 1996). More than 90% of the short-lived proteins in eukaryotic cells are degraded via the ubiquitin pathway (Lam 1997). The ubiquitin pathway regulates cytosolic and nuclear protein turnover in plant cells as well (Shanklin et al. 1987):

- Before it can take part in protein tagging, free ubiquitin must be activated.
- The **ubiquitin-activating enzyme** (**E1**) catalyzes the ATP-dependent adenylylation of the C terminus of ubiquitin.
- The adenylylated ubiquitin is then transferred to a cysteine residue on a second enzyme, the **ubiquitin-conjugating enzyme** (**E2**).
- Proteins destined for turnover form complexes with a third protein, **ubiquitin ligase** (**E3**).
- Finally, the E2–ubiquitin conjugate is used to transfer ubiquitin to the lysine residues of proteins bound to E3. This process can occur multiple times to form a polymer of ubiquitin.
- The ubiquitinated protein is then targeted to the proteasome for degradation.

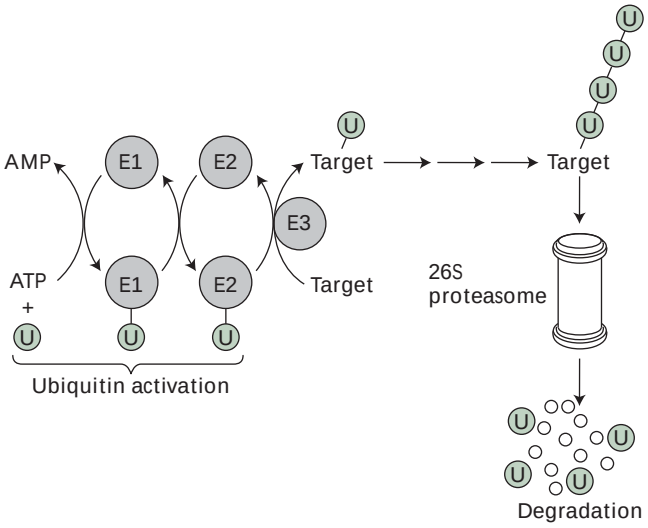


Figure 14.10 Diagram of the ubiquitin (U) pathway of protein degradation in the cytosol. ATP is required for the initial activation of E1. E1 transfers ubiquitin to E2. E3 mediates the final transfer of ubiquitin to a target protein, which may be ubiquitinated multiple times. The ubiquitinated target protein is then degraded by the 26S proteasome.

As we shall see later in the book, a variety of developmental phenomena in plants are regulated by protein turnover. In fact, the auxin receptor involved in gene regulation has now been shown to be a ubiquitin protein ligase (see Chapter 19).

Signal Transduction in Prokaryotes

Prokaryotic cells could not have survived billions of years of evolution without an exquisitely developed ability to sense their environment. As we have seen, bacteria respond to the presence of a nutrient by synthesizing the proteins involved in the uptake and metabolism of that nutrient. Bacteria can also respond to nonnutrient signals, both physical and chemical. Motile bacteria can adjust their movements according to the prevailing gradients of light, oxygen, osmolarity, temperature, and toxic chemicals in the medium.

The basic mechanisms that enable bacteria to sense and to respond to their environment are common to all cell sensory systems and include stimulus *detection*, signal *amplification*, and the appropriate *output responses.* Many bacterial signaling pathways have been shown to consist of modular units called *transmitters* and *receivers*. These modules form the basis of the so-called two-component regulatory systems.

Bacteria employ two-component regulatory systems to sense extracellular signals

Bacteria sense chemicals in the environment by means of a small family of cell surface receptors, each involved in the response to a defined group of chemicals (hereafter referred to as ligands). A protein in the plasma membrane of bacteria binds directly to a ligand, or binds to a soluble protein that has already attached to the ligand, in the **periplasmic space** between the plasma membrane and the cell wall. Upon binding, the membrane protein undergoes a conformational change that is propagated across the membrane to the cytosolic domain of the receptor protein. This conformational change initiates the signaling pathway that leads to the response.

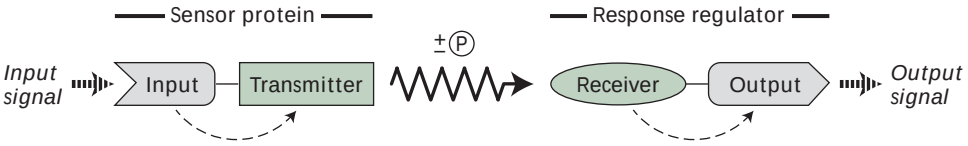


FIGURE 14.11 Signaling via bacterial two-component systems. The sensor protein detects the stimulus via the input domain and transfers the signal to the transmitter domain by means of a conformational change (indicated by the first dashed arrow). The transmitter domain of the sensor then communicates with the response regulator by protein phosphorylation of the receiver domain. Phosphorylation of the receiver domain induces a conformational change (second dashed arrow) that activates the output domain and brings about the cellular response.

A broad spectrum of responses in bacteria, including osmoregulation, chemotaxis, and sporulation, are regulated by two-component systems. **Two-component regulatory systems** are composed of a **sensor protein** and a **response regulator protein** (Figure 14.11) (Parkinson 1993). The function of the sensor is to receive the signal and to pass the signal on to the response regulator, which brings about the cellular response, typically gene expression. Sensor proteins have two domains, an **input domain**, which receives the environmental signal, and a **transmitter domain**, which transmits the signal to the response regulator. The response regulator also has two domains, a **receiver domain**, which receives the signal from the transmitter domain of the sensor protein, and an **output domain**, such as a DNA-binding domain, which brings about the response.

The signal is passed from transmitter domain to receiver domain via protein phosphorylation. Transmitter domains have the ability to phosphorylate themselves, using ATP, on a specific histidine residue near the amino terminus (Figure 14.12A). For this reason, sensor proteins containing transmitter domains are called **autophosphorylating histidine kinases**. These proteins normally function as dimers in which the catalytic site of one subunit phosphorylates the acceptor site on the other.

Immediately after the transmitter domain becomes autophosphorylated on a histidine residue, the phosphate is transferred to a specific aspartate residue near the middle of the receiver domain of the response regulator protein (see Figure 14.12A). As a result, a specific aspartate residue of the response regulator becomes phosphorylated (see Figure 14.12B). Phosphorylation of the aspartate residue causes the response regulator to undergo a conformational change that results in its activation.

Osmolarity is detected by a two-component system

An example of a relatively simple bacterial two-component system is the signaling system involved in sensing osmolarity in *E. coli*. *E. coli* is a gram-negative bacterium and thus has two cell membranes, an inner membrane and an outer membrane, separated by a cell wall. The inner membrane is the primary permeability barrier of the cell. The outer membrane contains large pores composed of two types of porin proteins, **OmpF** and **OmpC**. Pores made with OmpF are larger than those made with OmpC.

When *E. coli* is subjected to high osmolarity in the medium, it synthesizes more OmpC than OmpF, resulting in smaller pores on the outer membrane. These smaller pores filter out the solutes from the periplasmic space, shielding the inner membrane from the effects of the high solute concentration in the external medium. When the bacterium is placed in a medium with low osmolarity, more OmpF is synthesized, and the average pore size increases.

As Figure 14.13 shows, expression of the genes that encode the two porin proteins is regulated by a two-component system. The sensor protein, **EnvZ**, is located on the inner membrane. It consists of an N-terminal periplasmic input domain that detects the osmolarity changes in the medium, flanked by two membrane-spanning segments, and a C-terminal cytoplasmic transmitter domain.

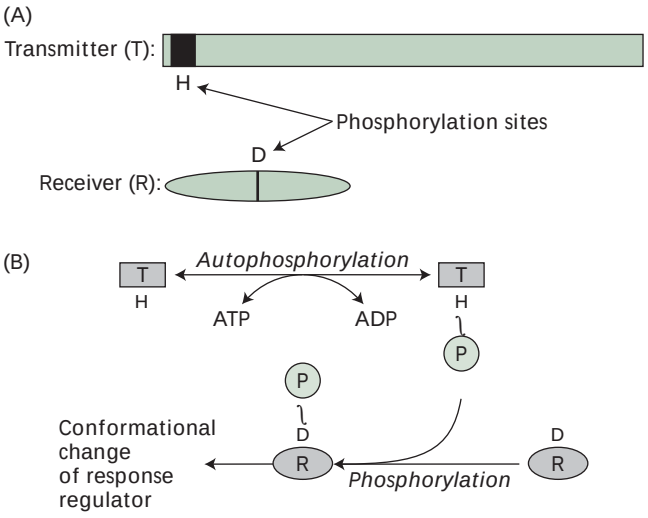


FIGURE 14.12 Phosphorylation signaling mechanism of bacterial two-component systems. (A) The transmitter domain of the sensor protein contains a conserved histidine (H) at its N-terminal end, while the receiver domain of the response regulator contains a conserved aspartate (D). (B) The transmitter phosphorylates itself at its conserved histidine and transfers the phosphate to the aspartate of the response regulator. The response regulator then undergoes a conformational change leading to the response. (After Parkinson 1993.)

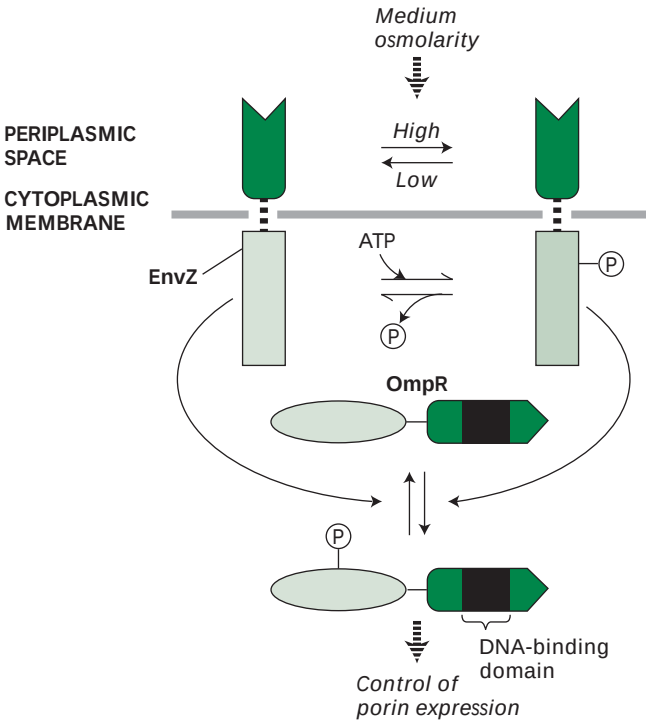


Figure 14.13 *E. coli* two-component system for osmoregulation. When the osmolarity of the medium is high, the membrane sensor protein, EnvZ (in the form of a dimer), acts as an autophosphorylating histidine kinase. The phosphorylated EnvZ then phosphorylates the response regulator, OmpR, which has a DNA-binding domain. Phosphorylated OmpR binds to the promoters of the two porin genes, *ompC* and *ompF*, enhancing expression of the former and repressing expression of the latter. When the osmolarity of the medium is low, EnvZ acts as a protein phosphatase instead of a kinase and dephosphorylates OmpR. When the nonphosphorylated form of OmpR binds to the promoters of the two porin genes, *ompC* expression is repressed and *ompF* expression is stimulated. (From Parkinson 1993.)

When the osmolarity of the medium increases, the input domain undergoes a conformational change that is transduced across the membrane to the transmitter domain. The transmitter then autophosphorylates its histidine residue. The phosphate is rapidly transferred to an aspartate residue of the receiver domain of the response regulator, **OmpR**. The N terminus of OmpR consists of a DNA-binding domain. When activated by phosphorylation, this domain interacts with RNA polymerase at the promoters of the porin genes, enhancing the expression of *ompC* and repressing the expression of *ompF*. Under conditions of low osmolarity in the medium, the nonphosphorylated form of OmpR stimulates *ompF* expression and represses *ompC* expression. In this way the osmolarity stimulus is communicated to the genes.

Related two-component systems have been identified in eukaryotes

Recently, combination sensor–response regulator proteins related to the bacterial two-component systems have been discovered in yeast and in plants. For example, The *SLN1* gene of the yeast *Saccharomyces cerevisiae* encodes a 134-kilodalton protein that has sequence similarities to both the transmitter and the receiver domains of bacteria and appears to function in osmoregulation (Ota and Varshavsky 1993).

There is increasing evidence that several plant signaling systems evolved from bacterial two-component systems. For example, the red/far-red–absorbing pigment, phytochrome, is present in cyanobacteria, and it appears to be related to bacterial sensor proteins (see Chapter 17). In addition, the genes that encode putative receptors for two plant hormones, cytokinin and ethylene, both contain autophosphorylating histidine kinase domains, as well as contiguous response regulator motifs. These proteins will be discussed in more detail in Chapters 21 and 22.

Signal Transduction in Eukaryotes

Many eukaryotic microorganisms use chemical signals in cell–cell communication. For example, in the slime mold *Dictyostelium*, starvation induces certain cells to secrete cyclic AMP (cAMP). The secreted cAMP diffuses across the substrate and induces nearby cells to aggregate into a slug-like colony. Yeast mating-type factors are another example of chemical communication between the cells of simple microorganisms. Around a billion years ago, however, cell signaling took a great leap in complexity when eukaryotic cells began to associate together as multicellular organisms. After the evolution of multicellularity came a trend toward ever-increasing cell specialization, as well as the development of tissues and organs to perform specific functions.

Coordination of the development and environmental responses of complex multicellular organisms required an array of signaling mechanisms. Two main systems evolved in animals: the nervous system and the endocrine system. Plants, lacking motility, never developed a nervous system, but they did evolve hormones as chemical messengers. As photosynthesizing organisms, plants also evolved mechanisms for adapting their growth and development to the amount and quality of light.

In the sections that follow we will explore some of the basic mechanisms of signal transduction in animals, emphasizing pathways that may have some parallel in plants. However, keep in mind that plant signal transduction pathways may differ in significant ways from those of animals. To illustrate this point, we end the chapter with an overview of some of the known plant-specific transmembrane receptors.

Two classes of hormonal signals define two classes of receptors

Hormones fall into two classes based on their ability to move across the plasma membrane: *lipophilic hormones*, which diffuse readily across the hydrophobic bilayer of the plasma membrane; and *water-soluble hormones*, which are unable to enter the cell. Lipophilic hormones bind mainly to receptors in the cytoplasm or nucleus; water-soluble hormones bind to receptors located on the cell surface. In either case, ligand binding alters the receptor, typically by causing a conformational change.

Some receptors, such as the steroid hormone receptors (see the next section), can regulate gene expression directly. In the vast majority of cases, however, the receptor initiates one or more sequences of biochemical reactions that connect the stimulus to a cellular response. Such a sequence of reactions is called a **signal transduction pathway**. Typically, the end result of signal transduction pathways is to regulate transcription factors, which in turn regulate gene expression.

Signal transduction pathways often involve the generation of **second messengers**, transient secondary signals inside the cell that greatly amplify the original signal. For example, a single hormone molecule might lead to the activation of an enzyme that produces hundreds of molecules of a second messenger. Among the most common second messengers are 3′,5′-cyclic AMP (cAMP); 3′,5′-cyclic GMP (cGMP); nitric oxide (NO); cyclic ADP-ribose (cADPR); 1,2-diacylglycerol (DAG); inositol 1,4,5-trisphosphate (IP_3); and Ca^{2+} (Figure 14.14). Hormone binding normally causes elevated levels of one or more of these second messengers, resulting in the activation or inactivation of enzymes or regulatory proteins. Protein kinases and phosphatases are nearly always involved.

The proteins that bind to second messengers function as **switch proteins**, because of their ability to undergo a marked conformational change when they bind to their ligand. Once activated, switch proteins go on to regulate the activity of numerous other proteins in the cell.

Most steroid receptors act as transcription factors

The steroid hormones, thyroid hormones, retinoids, and vitamin D all pass freely across the plasma membrane because of their hydrophobic nature, and they bind to *intracellular* receptor proteins. When activated by binding to their ligand, these proteins function as transcription factors. All such intracellular receptor proteins have DNA-binding domains. The ligands fall into two classes based on whether the receptor is localized to the cytoplasm or the nucleus.

Steroids (testosterone, estrogen, cortisol, etc.) make up the type I ligands. Type I ligands bind to their receptors in the cytosol, where they are associated with heat shock proteins. Upon binding to its ligand, the receptor dissociates from its heat shock proteins and becomes activated. The activated receptor-hormone complex then moves into the

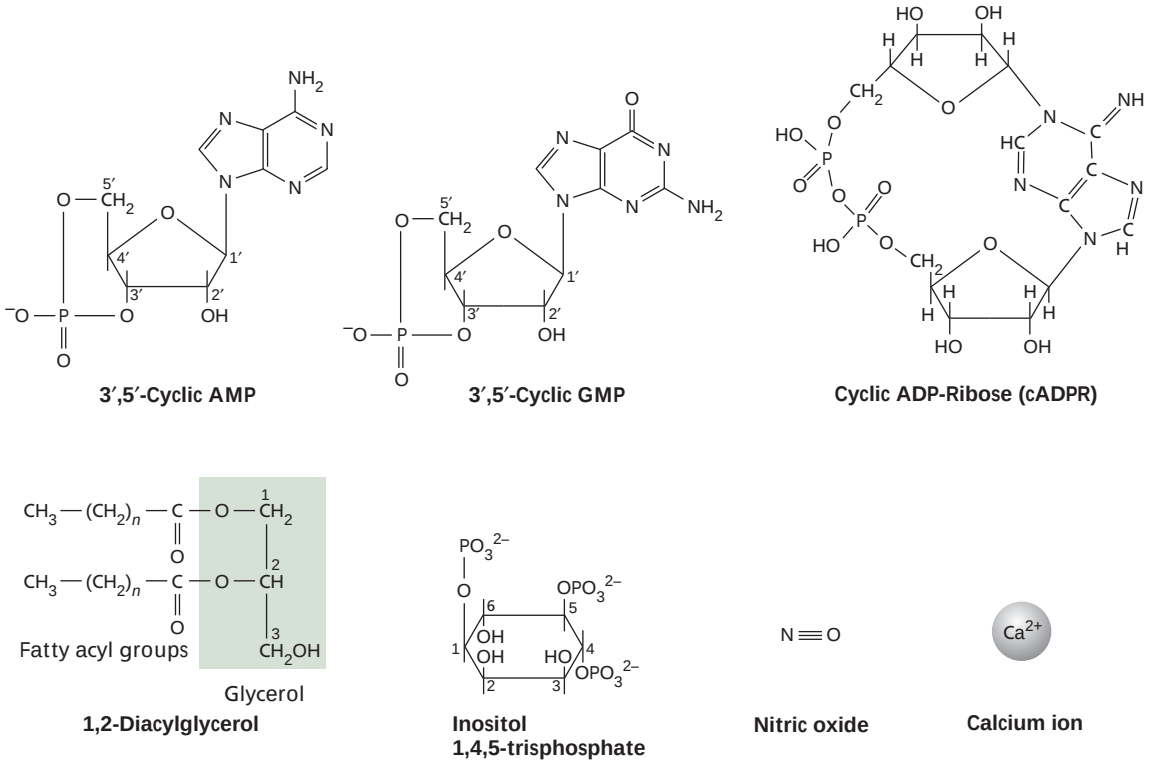


Figure 14.14 Structure of seven eukaryotic second messengers.

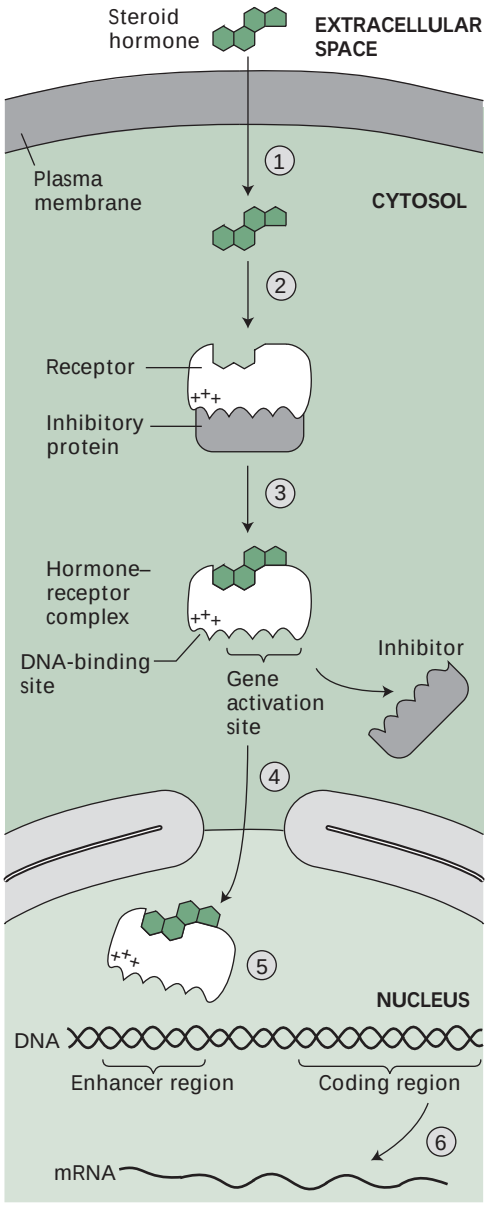


Figure 14.15 Glucocorticoid steroid receptors are transcription factors. (1) Glucocorticoid hormone is lipophilic and diffuses readily through the membrane to the cytosol. (2) Once in the cytosol, the hormone binds to its cytosolic receptor, (3) causing the release of an inhibitory protein from the receptor. (4) The activated receptor then diffuses into the nucleus. (5) In the nucleus, the receptor–hormone complex binds to the enhancer regions of steroid-regulated genes. (6) Transcription of the genes is stimulated.

nucleus where it dimerizes and binds to a specific **hormone response element** (**HRE**). Steroid DNA response elements are typically located in enhancer regions of steroid-stimulated genes. Binding of the hormone–receptor complex to the HRE either represses or activates gene expression (Figure 14.15).

Type II ligands include vitamin D, thyroid hormones, and retinoids. These ligands bind to their receptors in the nucleus. The activated hormone–receptor complex may dimerize before binding to its DNA response element, stimulating gene transcription.

Although it was long thought that plants lacked steroid hormones, **brassinosteroids** are now known to function as hormones in plant growth and development. In contrast to animals, however, the plant steroid receptor is a cell surface receptor. As will be discussed in Chapter 24, the **brassinosteroid receptor** is a type of transmembrane protein called a *leucine-rich repeat receptor*, which will be described at the end of this chapter.

Cell surface receptors can interact with G proteins

All water-soluble mammalian hormones bind to cell surface receptors. Members of the largest class of mammalian cell surface receptors interact with signal-transducing, GTP-binding regulatory proteins called **heterotrimeric G proteins**. These proteins act as *GTPase switch proteins*, because the binding of GTP causes a conformational change resulting in activation. The activated G proteins, in turn, activate an **effector enzyme**. The activated effector enzyme then generates an intracellular second messenger, which stimulates a variety of cellular processes.

Receptors using heterotrimeric G proteins are structurally similar and functionally diverse. Their overall structure is similar to that of bacteriorhodopsin, the purple pigment involved in photosynthesis in bacteria of the genus *Halobacterium*, and to that of rhodopsin, the visual pigment of the vertebrate eye. The olfactory receptors of the vertebrate nose also belong to this group. The receptor proteins consist of **seven-transmembrane α helices** (Figure 14.16). These receptors are sometimes referred to as *seven-spanning*, *seven-pass*, or *serpentine* receptors.

Heterotrimeric G proteins cycle between active and inactive forms

The G proteins that transduce the signals from the seven-spanning receptors are called *heterotrimeric* G proteins because they are composed of three different subunits: α, β, and γ (gamma). They are distinct from the monomeric G proteins, which will be discussed later. Heterotrimeric G proteins cycle between active and inactive forms, thus acting as molecular switches. The β and γ subunits form a tight complex that anchors the trimeric G protein to the membrane on the cytoplasmic side (see Figure 14.16). The G protein becomes activated upon binding to the ligand-activated seven-spanning receptor. In its inactive form, G exists as a trimer with GDP bound to the α subunit. Binding to the receptor–ligand complex induces the α subunit to exchange GDP for GTP. This exchange causes the α subunit to dissociate from β and γ, allowing α to associate instead with an effector enzyme.

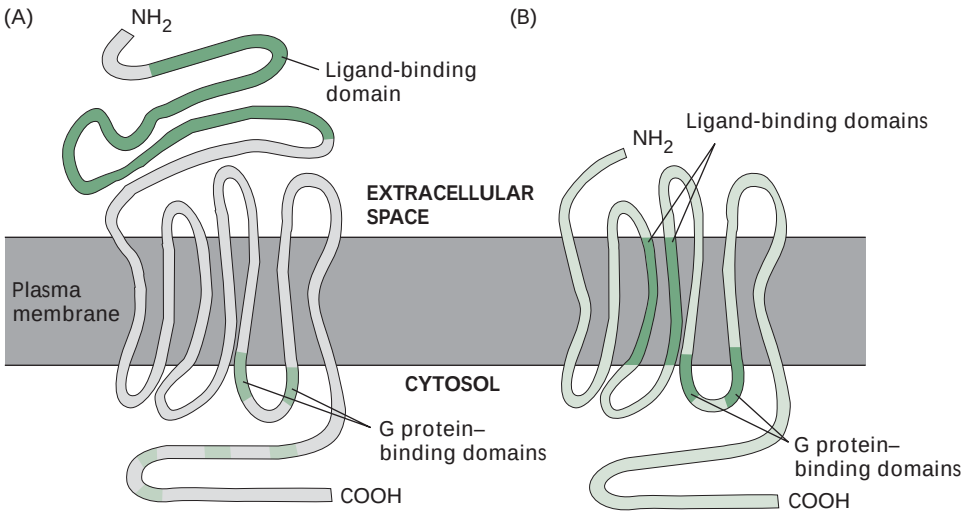


Figure 14.16 Schematic drawing of two types of seven-spanning receptors. (A) Large extracellular ligand-binding domains are characteristic of seven-spanning receptors that bind proteins. The region of the intracellular domain that interacts with the heterotrimeric G protein is indicated. (B) Small extracellular domains are characteristic of seven-spanning receptors that bind to small ligands such as epinephrine. The ligand-binding site is usually formed by several of the transmembrane helices within the bilayer.

The α subunit has a GTPase activity that is activated when it binds to the effector enzyme, in this case **adenylyl cyclase** (also called *adenylate cyclase*) (Figure 14.17). GTP is hydrolyzed to GDP, thereby inactivating the α subunit, which in turn inactivates adenylyl cyclase. The α subunit bound to GDP reassociates with the β and γ subunits and can then be reactivated by associating with the hormone–receptor complex.

Activation of adenylyl cyclase increases the level of cyclic AMP

Cyclic AMP is an important signaling molecule in both prokaryotes and animal cells, and increasing evidence suggests that cAMP plays a similar role in plant cells. In vertebrates, adenylyl cyclase is an integral membrane protein that contains two clusters of six membrane-spanning domains separating two catalytic domains that extend into the cytoplasm. Activation of adenylyl cyclase by heterotrimeric G proteins raises the concentration of cAMP in the cell, which is normally maintained at a low level by the action of **cyclic AMP phosphodiesterase**, which hydrolyzes cAMP to 5′-AMP.

Nearly all the effects of cAMP in animal cells are mediated by the enzyme **protein kinase A** (**PKA**). In unstimulated cells, PKA is in the inactive state because of the presence of a pair of inhibitory subunits. Cyclic AMP binds to these inhibitory subunits, causing them to dissociate from the two catalytic subunits, thereby activating the catalytic subunits. The activated catalytic subunits then are able to phosphorylate specific serine or threonine residues of selected proteins, which may also be protein kinases. An example of an enzyme that is phosphorylated by PKA is *glycogen phosphorylase kinase*. When phosphorylated by PKA, glycogen phosphorylase kinase phosphorylates (activates) *glycogen phosphorylase*, the enzyme that breaks down glycogen in muscle cells to glucose-1-phosphate.

In cells in which cAMP regulates gene expression, PKA phosphorylates a transcription factor called **CREB** (*c*yclic AMP *r*esponse *e*lement–*b*inding protein). Upon activation by PKA, CREB binds to the cAMP response element (CRE), which is located in the promoter regions of genes that are regulated by cAMP.

In addition to activating PKA, cAMP can interact with specific cAMP-gated cation channels. For example, in olfactory receptor neurons, cAMP binds to and opens Na^+ channels on the plasma membrane, resulting in Na^+ influx and membrane depolarization.

Because of the extremely low levels of cyclic AMP that have been detected in plant tissue extracts, the role of cAMP in plant signal transduction has been highly controversial (Assmann 1995). Nevertheless, various lines of evidence supporting a role of cAMP in plant cells have accumulated. For example, genes that encode homologs of CREB have been identified in plants (Kategiri et al. 1989). The Arabidopsis genome encodes 20 putative members of a cyclic nucleotide-gated channel family.

Pollen tube growth in lily has been shown to be stimulated by concentrations of cAMP as low as 10 n*M* (Tezuka et al. 1993). Li and colleagues (1994) showed that cAMP activates K^+ channels in the plasma membrane of fava bean

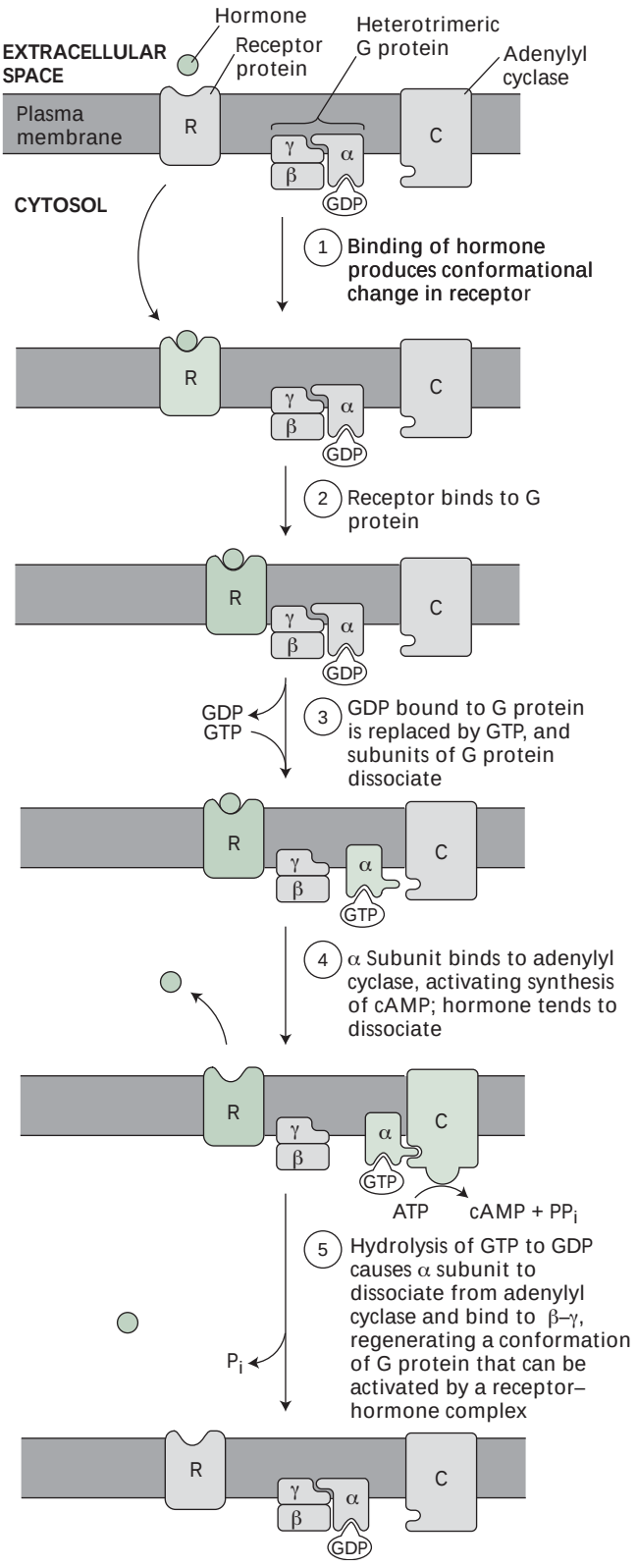


Figure 14.17 Hormone-induced activation of an effector enzyme is mediated by the α subunit of a heterotrimeric G protein. (1) Upon binding to its hormonal ligand, the seven-spanning receptor undergoes a conformational change. (2) The receptor binds to the heterotrimeric G protein. (3) Contact with the receptor induces the α subunit of the heterotrimeric G protein to exchange GDP for GTP, and the α subunit then dissociates from the complex. (4) The G protein α subunit associates with the effector protein (adenylyl cyclase) in the membrane, causing its activation. At the same time the hormone is released from its receptor. (5) The effector enzyme becomes inactivated when GTP is hydrolyzed to GDP. The α subunit then reassociates with the heterotrimeric G protein and is ready to be reactivated by a second hormonal stimulus.

(*V. faba*) mesophyll cells. Ichikawa and coworkers (1997) identified possible genes for adenylyl cyclase in tobacco (*Nicotiana tabacum*) and Arabidopsis. Studies using a cyclic nucleotide fluorescent indicator injected into pollen tubes have reported cAMP levels as high as ~150 nM. Moreover, the presence of cAMP (as well as cGMP) in plant cells has now been confirmed by tandem mass spectrometry (Richards et al. 2002). Thus, despite years of doubt, the universality of both cAMP and cGMP as second messengers in living organisms, including plants, seems likely.

Activation of phospholipase C initiates the IP_3 pathway

Calcium serves as a second messenger for a wide variety of cell signaling events. This role of calcium is well established in animal cells, and as we will see in later chapters, circumstantial evidence suggests a role for calcium in signal transduction in plants as well. The concentration of free Ca^{2+} in the cytosol normally is maintained at extremely low levels (1×10^{-7} *M*). Ca^{2+}-ATPases on the plasma membrane and on the endoplasmic reticulum pump calcium ions out of the cell and into the lumen of the ER, respectively. In plant cells, most of the calcium of the cell accumulates in the vacuole. The proton electrochemical gradient across the vacuolar membrane that is generated by tonoplast proton pumps drives calcium uptake via Ca^{2+}–H^+ antiporters (see Chapter 6).

In animal cells, certain hormones can induce a transient rise in the cytosolic Ca^{2+} concentration to about 5×10^{-6} *M*. This increase may occur even in the absence of extracellular calcium, indicating that the Ca^{2+} is being released from intracellular compartments by the opening of intracellular calcium channels. However, the coupling of hormone binding to the opening of intracellular calcium channels is mediated by yet another second messenger, inositol trisphosphate (IP_3).

Phosphatidylinositol (PI) is a minor phospholipid component of cell membranes (see Chapter 11). PI can be converted to the polyphosphoinositides PI phosphate (PIP) and **PI bisphosphate** (**PIP_2**) by kinases (Figure 14.18). Although PIP_2 is even less abundant in the membrane than PI is, it plays a central role in signal transduction. In animal cells, binding of a hormone, such as vasopressin, to its receptor leads to the activation of heterotrimeric G proteins. The α subunit then dissociates from G and activates a

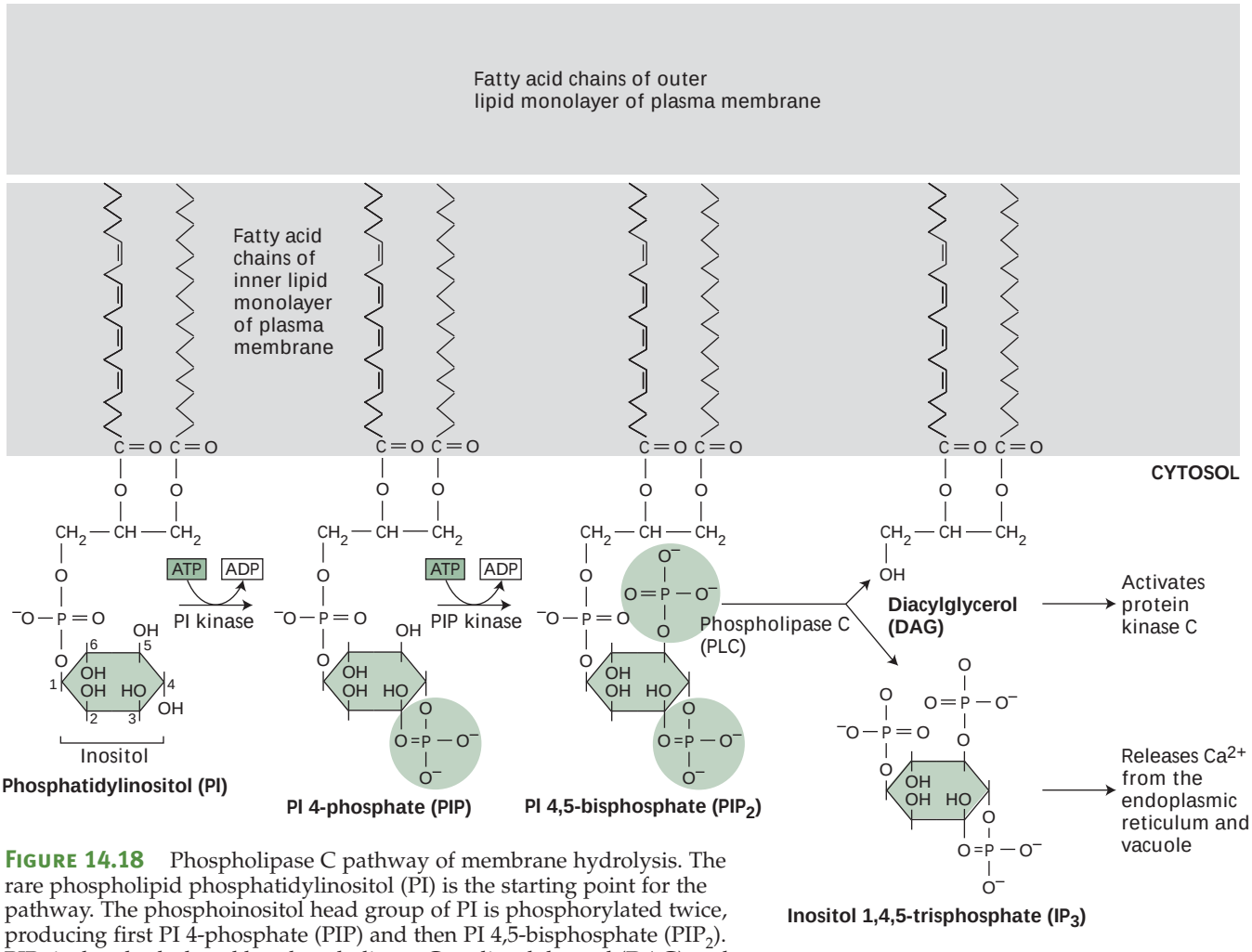


FIGURE 14.18 Phospholipase C pathway of membrane hydrolysis. The rare phospholipid phosphatidylinositol (PI) is the starting point for the pathway. The phosphoinositol head group of PI is phosphorylated twice, producing first PI 4-phosphate (PIP) and then PI 4,5-bisphosphate (PIP_2). PIP_2 is then hydrolyzed by phospholipase C to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3).

phosphoinositide-specific phospholipase, **phospholipase C** (**PLC**). The activated PLC rapidly hydrolyzes PIP_2, generating **inositol trisphosphate** (**IP_3**) and **diacylglycerol** (**DAG**) as products. Each of these two molecules plays an important role in cell signaling.

IP_3 opens calcium channels on the ER and on the tonoplast

The IP_3 generated by the activated phospholipase C is water soluble and diffuses through the cytosol until it encounters IP_3-binding sites on the ER and (in plants) on the tonoplast. These binding sites are IP_3-gated Ca^{2+} channels that open when they bind IP_3 (Figure 14.19). Since these organelles maintain internal Ca^{2+} concentrations in the millimolar range, calcium diffuses rapidly into the cytosol down a steep concentration gradient. The response is terminated when IP_3 is broken down by specific phosphatases or when the released calcium is pumped out of the cytoplasm by Ca^{2+}-ATPases.

Studies with Ca^{2+}-sensitive fluorescent indicators, such as fura-2 and aequorin, have shown that the calcium signal often originates in a localized region of the cell and propagates as a wave throughout the cytosol. Repeated waves called *calcium oscillations* can follow the original signal, each lasting from a few seconds to several minutes. The biological significance of calcium oscillation is still unclear, although it has been suggested that it is a mechanism for avoiding the toxicity that might result from a sustained elevation in cytosolic levels of free calcium. Such wavelike oscillations have recently been detected in plant stomatal guard cells (McAinsh et al. 1995).

Cyclic ADP-ribose mediates intracellular Ca^{2+} release independently of IP_3 signaling

Cyclic ADP-Ribose (cADPR) acts as a second messenger that can release calcium from intracellular stores, independent of the IP_3 signaling pathway. Like cAMP, cADPR is a cyclic nucleotide, but whereas cAMP brings about its

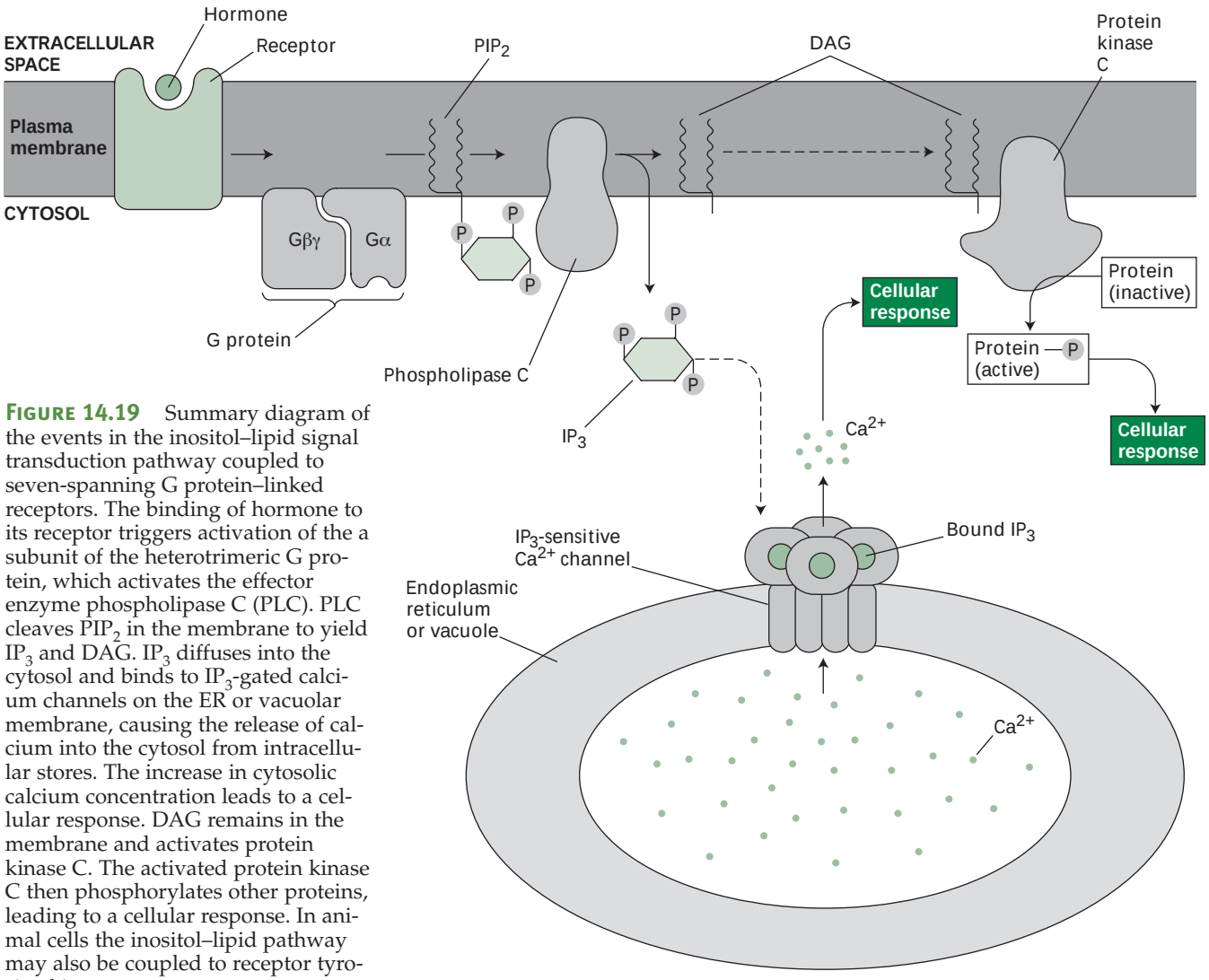


FIGURE 14.19 Summary diagram of the events in the inositol–lipid signal transduction pathway coupled to seven-spanning G protein–linked receptors. The binding of hormone to its receptor triggers activation of the a subunit of the heterotrimeric G protein, which activates the effector enzyme phospholipase C (PLC). PLC cleaves PIP_2 in the membrane to yield IP_3 and DAG. IP_3 diffuses into the cytosol and binds to IP_3-gated calcium channels on the ER or vacuolar membrane, causing the release of calcium into the cytosol from intracellular stores. The increase in cytosolic calcium concentration leads to a cellular response. DAG remains in the membrane and activates protein kinase C. The activated protein kinase C then phosphorylates other proteins, leading to a cellular response. In animal cells the inositol–lipid pathway may also be coupled to receptor tyrosine kinases.

effects by activating protein kinase A, cADPR binds to and activates specific calcium channels, called type-3 ryanodine receptors (ryanodine is a calcium channel blocker). These ryanodine receptor/calcium channels are located on the membranes of calcium-storing organelles, such as sarcoplasmic reticulum of animal cells or the vacuoles of plant cells. By stimulating the release of calcium into the cytosol, cADPR helps to regulate calcium oscillations that bring about physiological effects. Abscisic acid-induced stomatal closure is an example of the roles of cADPR and calcium oscillations in plants (see Chapter 23).

Some protein kinases are activated by calcium–calmodulin complexes

As we have seen with IP_3-gated channels, calcium can activate some proteins, such as channels, by binding directly to them. However, most of the effects of calcium result from the binding of calcium to the regulatory protein **calmodulin** (Figure 14.20). Calmodulin is a highly conserved protein that is abundant in all eukaryotic cells, but it appears to be absent from prokaryotic cells. The same calcium-binding site is found in a wide variety of calcium-binding proteins and is called an EF hand. The name is derived from the two α helices, E and F, that are part of the calcium-binding domain of the protein parvalbumin (Kretsinger 1980).

Each calmodulin molecule binds four Ca^{2+} ions and changes conformation, enabling it to bind to and activate other proteins. Calmodulin thus acts as a *calcium switch protein*. The Ca^{2+}–calmodulin complex can stimulate some enzymes directly, such as the plasma membrane Ca^{2+}-ATPase, which pumps calcium out of the cell. Most of the effects of calcium, however, are brought about by activation of **Ca^{2+}–calmodulin-dependent protein kinases** (**CaM**

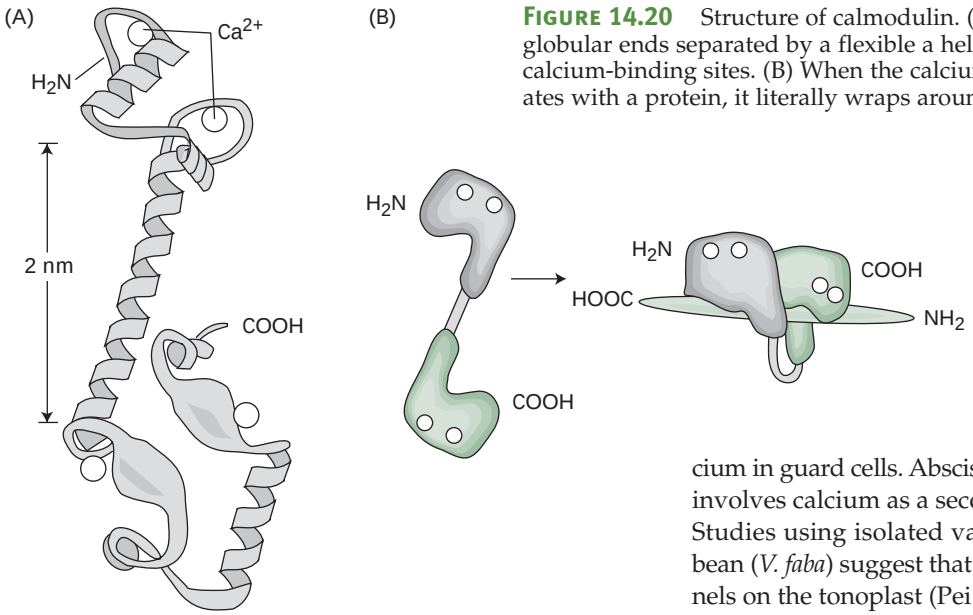


Figure 14.20 Structure of calmodulin. (A) Calmodulin consists of two globular ends separated by a flexible a helix. Each globular end has two calcium-binding sites. (B) When the calcium–calmodulin complex associates with a protein, it literally wraps around it.

kinases). CaM kinases phosphorylate serine or threonine residues of their target enzymes, causing enzyme activation. Thus, the effect that calcium has on a particular cell depends to a large extent on which CaM kinases are expressed in that cell.

Calcium signaling has been strongly implicated in many developmental processes in plants, ranging from the regulation of development by phytochrome (see Chapter 17) to the regulation of stomatal guard cells by abscisic acid (see Chapter 23). Thus far, however, there have been few reports of CaM kinase activity in plants. Recently, however, a gene that codes for a CaM kinase has been cloned from lily and shown to be specifically expressed in anthers. The lily CaM kinase is a serine/threonine kinase that phosphorylates various protein substrates in vitro in a Ca^{2+}–calmodulin-dependent manner (Takezawa et al. 1996). A calmodulin-binding receptor-like cytoplasmic kinase (CRCK1) from Arabidopsis has also been identified (Yang et al 2004).

Plants contain calcium-dependent protein kinases

The most abundant calcium-regulated protein kinases in plants appear to be the **calcium-dependent protein kinases** (**CDPKs**) (Harper et al. 1991; Roberts and Harmon 1992). CDPKs are strongly activated by calcium, but are insensitive to calmodulin. The proteins are characterized by two domains: a catalytic domain that is similar to those of the animal CaM kinases, and a calmodulin-like domain. The presence of a calmodulin-like domain may explain why the enzyme does not require calmodulin for activity.

CDPKs are widespread in plants and are encoded by multigene families. About 34 CDPK genes have been identified in the Arabidopsis genome. In plants, CDPKs have been implicated in environmental stress responses and hormone signaling. CDPKs may also mediate the effects of calcium in guard cells. Abscisic acid–induced stomatal closure involves calcium as a second messenger (see Chapter 23). Studies using isolated vacuoles from guard cells of fava bean (*V. faba*) suggest that CDPKs can regulate anion channels on the tonoplast (Pei et al. 1996).

In addition, eight putative **CDPK-related protein kinases** (**CRKs**) have also been identified in the Arabidopsis genome based on the sequence homology to CDPKs.

Diacylglycerol activates protein kinase C

Cleavage of PIP_2 by phospholipase C produces diacylglycerol (DAG) in addition to IP_3 (see Figure 14.18). Whereas IP_3 is hydrophilic and diffuses rapidly into the cytoplasm, DAG is a lipid and remains in the membrane. In animal cells, DAG can associate with and activate the serine/threonine kinase **protein kinase C** (**PKC**). The inactive form of PKC is a soluble enzyme that is located in the cytosol. Upon binding to calcium, the soluble, inactive PKC undergoes a conformational change and associates with a PKC receptor protein that transports it to the inner surface of the plasma membrane, where it encounters DAG.

PKCs have been shown to phosphorylate ion channels, transcription factors, and enzymes in animal cells. One of the enzymes phosphorylated by PKC is another protein kinase that regulates cell proliferation and differentiation, *MAP kinase kinase kinase* (discussed later in the chapter). G proteins, phospholipase C, and various protein kinases have been identified in plant membranes (Millner and Causier 1996). PKC activity has also been detected in plants (Elliott and Kokke 1987; Chen et al. 1996), and a plant gene encoding the PKC receptor protein that transports the soluble enzyme to the membrane has recently been cloned (Kwak et al. 1997). However, there is as yet no evidence that activation of PKC by DAG plays a role in plant signal transduction.

Phospholipase A_2 and phospholipase D generate other membrane-derived signaling agents

In animals, the **endocrine system** is involved in signaling between hormone-producing cells at one location of the body and hormone-responding cells at another location; in

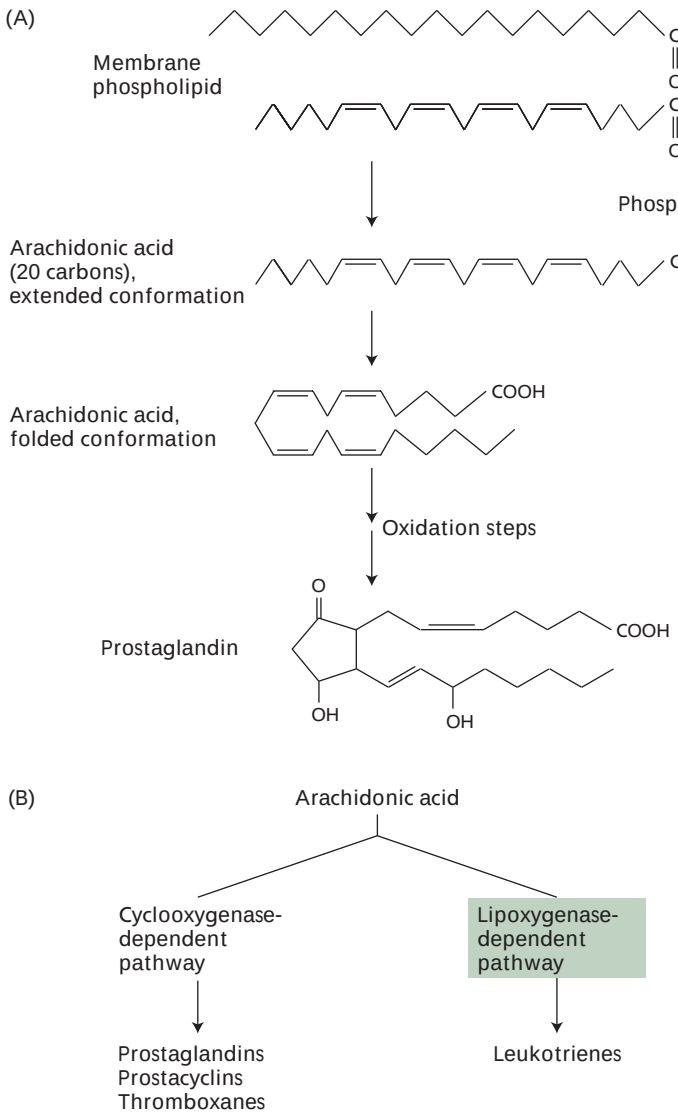


Figure 14.21 Eicosanoid biosynthetic pathway. (A) The first step is the hydrolysis of 20-carbon fatty acid chains containing at least three double bonds from a membrane phospholipid by the enzyme phospholipase A_2, producing arachidonic acid, which can be oxidized by prostaglandin. (B) Arachidonic acid is further metabolized by two pathways: one cyclooxygenase dependent, the other lipoxygenase dependent.

contrast, the **autocrine system** involves cells sending signals to themselves and their immediate neighbors. One type of autocrine signaling system that plays important roles in pain and inflammatory responses, as well as platelet aggregation and smooth-muscle contraction, is called the **eicosanoid pathway**.

There are four major classes of eicosanoids: prostaglandins, prostacyclins, thromboxanes, and leukotrienes. All are derived from the breakdown of membrane phospholipids, and in this respect the eicosanoid pathway resembles the IP_3 pathway. There the resemblance ends, however. For whereas the IP_3 pathway begins with the cleavage of IP_3 from PIP_2 by phospholipase C, the eicosanoid pathway is initiated by the cleavage of the 20-carbon fatty acid **arachidonic acid** from phosphatidylcholine by the enzyme **phospholipase A_2** (**PLA_2**) (see Figure 14.21A). Two oxidative pathways—one cyclooxygenase dependent, the other lipoxygenase dependent—then convert arachidonic acid to the four eicosanoids (see Figure 14.21B). Higher plants generally have negligible amounts of arachidonic acid in their membranes, although the level is higher in certain mosses.

In addition to generating arachidonic acid, PLA_2 produces **lysophosphatidylcholine** (**LPC**) as a breakdown product of phosphatidylcholine. LPC has detergent properties, and it has been shown to regulate ion channels through its effects on protein kinases. For example, LPC has been shown to modulate the sodium currents in cardiac-muscle cells by signal transduction pathways that involve the activation of both protein kinase C and a tyrosine kinase (Watson and Gold 1997). Protein kinase C is activated by LPC independently of the phospholipase C pathway.

In recent years plant biologists have become increasingly interested in the eicosanoid pathway because it now appears that an important signaling agent in plant defense responses, **jasmonic acid**, is produced by a similar pathway, which was described in Chapter 13.

A third phospholipase, **phospholipase D** (**PLD**), reacts with phosphatidylcholine to produce two products: IP_3 and phosphatidic acid (PA). Evidence is accumulating that PA may act as a second messenger in plants. The level of PA increases in the cell within minutes of a variety of stress treatments, and PA has been implicated in the action of the plant hormone abscisic acid (see Chapter 23).

In vertebrate vision, a heterotrimeric G protein activates cyclic GMP phosphodiesterase

The human eye contains two types of photoreceptor cells: rods and cones. Rods are responsible for monochromatic vision in dim light; cones are involved in color vision in bright light. Signal transduction in response to light has been studied more intensively in rods. The rod is a highly specialized tubular cell that contains an elongated stack of densely packed membrane sacs called **discs** at the tip, or

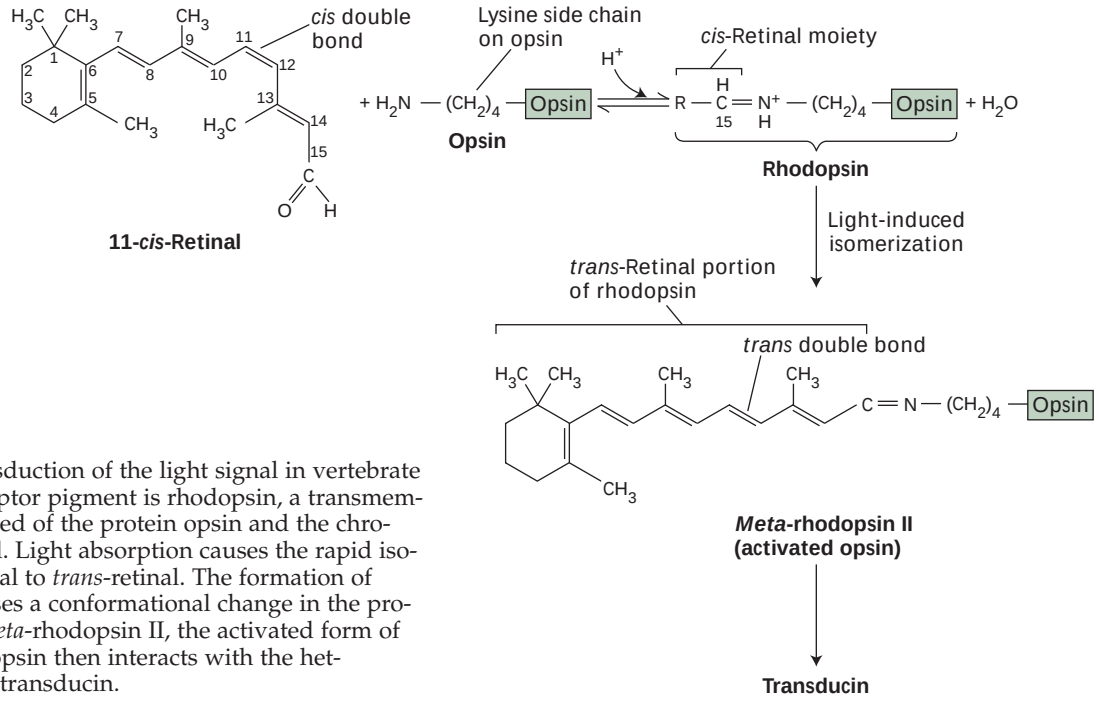


Figure 14.22 Transduction of the light signal in vertebrate vision. The photoreceptor pigment is rhodopsin, a transmembrane protein composed of the protein opsin and the chromophore 11-*cis*-retinal. Light absorption causes the rapid isomerization of *cis*-retinal to *trans*-retinal. The formation of *trans*-retinal then causes a conformational change in the protein opsin, forming *meta*-rhodopsin II, the activated form of opsin. The activated opsin then interacts with the heterotrimeric G protein transducin.

outer segment, reminiscent of the grana stacks of chloroplasts. The disc membranes of rod cells contain the photosensitive protein pigment rhodopsin, a member of the seven-spanning transmembrane family of receptors. **Rhodopsin** consists of the protein opsin covalently bound to the light-absorbing molecule **11-*cis*-retinal**. When 11-*cis*-retinal absorbs a single photon of light (400 to 600 nm) it immediately isomerizes to **all-*trans*-retinal** (Figure 14.22). This change causes a slower conformational change in the protein, converting it to *meta*-rhodopsin II, or **activated opsin**.

Activated opsin, in turn, lowers the concentration of the cyclic nucleotide **3′,5′-cGMP**. Cyclic GMP is synthesized from GTP by the enzyme guanylate cyclase. In the dark, guanylate cyclase activity results in the buildup of a high concentration of cGMP in the rod cells. Because the plasma membrane contains cGMP-gated Na^+ channels, the high cGMP concentration in the cytosol maintains the Na^+ channels in the open position in the absence of light. When the Na^+ channels are open, Na^+ can enter the cell freely, and this passage of Na^+ tends to depolarize the membrane potential.

When opsin becomes activated by light, however, it binds to the heterotrimeric G protein **transducin**. This binding causes the α subunit of transducin to exchange GDP for GTP and dissociate from the complex. The α subunit of transducin then activates the enzyme cyclic GMP phosphodiesterase, which breaks down 3′,5′-cGMP to 5′-GMP (Figure 14.23). Light therefore has the effect of decreasing the concentration of cGMP in the rod cell. A lower concentration of cGMP has the effect of closing the cGMP-gated Na^+ channels on the plasma membrane, which are kept open in the dark by a high cGMP concentration. To give some idea of the signal amplification provided, a single photon may cause the closure of hundreds of Na^+ channels, blocking the uptake of about 10 million Na^+ ions.

By preventing the influx of Na^+, which tends to depolarize the membrane, the membrane polarity increases—that is, becomes hyperpolarized. In this way a light signal is converted into an electric signal. Membrane hyperpolarization, in turn, inhibits neurotransmitter release from the synaptic body of the rod cell. Paradoxically, the nervous system detects light as an *inhibition* rather than a stimulation of neurotransmitter release.

Cyclic GMP, which regulates ion channels and protein kinases in animal cells, appears to be an important regulatory molecule in plant cells as well. Cyclic GMP has been definitively identified in plant extracts by gas chromatography combined with mass spectrometry (Janistyn 1983; Newton and Brown 1992). Moreover, cGMP has been implicated as a second messenger in the responses of phytochrome (see Chapter 17) and gibberellin (see Chapter 20).

Nitric oxide gas stimulates the synthesis of cGMP

The level of 3′,5′-cGMP in cells is controlled by the balance between the rate of cGMP synthesis by the enzyme, **guanylyl** (or **guanylate**) cyclase, and the rate of cGMP degradation by the enzyme cGMP phosphodiesterase. We have seen how light activation of rhodopsin leads to the activation of cGMP phosphodiesterase in vertebrate rod cells, resulting in a reduction in cGMP. In smooth muscle tissue

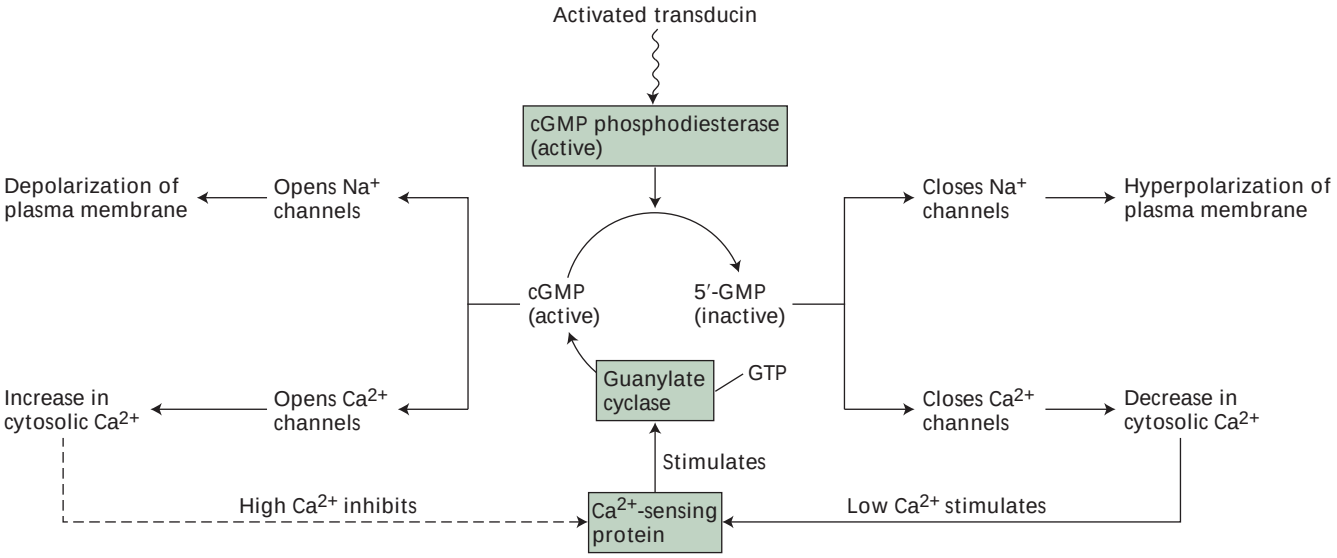


Figure 14.23 The role of cyclic GMP (cGMP) and calcium as second messengers in vertebrate vision. Activation of the heterotrimeric G protein transducin by activated opsin causes the activation of cGMP phosphodiesterase, which lowers the concentration of cGMP in the cell. The reduction in cGMP closes cGMP-activated Na^+ channels. Closure of the Na^+ channels blocks the influx of Na^+, causing membrane hyperpolarization. Cyclic GMP also regulates calcium channels. When the cGMP concentration in the cell is high, the calcium channels open, raising the cytosolic calcium concentration. Guanylate cyclase, the enzyme that synthesizes cGMP from GTP, is inhibited by high levels of calcium. Conversely, when cGMP levels are low, closure of calcium channels lowers the cytosolic calcium concentration. This lowering of the calcium concentration stimulates guanylate cyclase. Calcium thus provides a feedback system for regulating cGMP levels in the cell.

of animal cells, cGMP levels can be increased via the direct activation of guanylyl cyclase by the signaling intermediate, nitric oxide (NO). NO is synthesized from arginine by the enzyme, NO synthase, in a reaction involving oxygen:

$$\text{Arginine} + O_2 \xrightarrow{\text{NO synthase}} \text{Citrulline} + \text{NO}$$

Once produced in animal endothelial cells, dissolved NO passes rapidly across membranes and acts locally on neighboring smooth muscle cells, with a half-life of 5–10 seconds. Guanylyl cyclase contains a heme group that binds NO tightly, and binding of NO causes a conformational change which activates the enzyme. The NO-induced increase in cGMP causes smooth muscle cells to relax. Nitroglycerine, which can be metabolized to yield NO, has long been administered to heart patients to prevent the coronary artery spasms responsible for variant angina. In plants, NO has been implicated as an intermediate in ABA-induced stomatal closure (see Chapter 23).

Cell surface receptors may have catalytic activity

Some cell surface receptors are enzymes themselves or are directly associated with enzymes. Unlike the seven-spanning receptors, the **catalytic receptors**, as these enzyme or enzyme-associated receptors are called, are typically attached to the membrane via a single transmembrane helix and do not interact with heterotrimeric G proteins. The six main categories of catalytic receptors in animals include: (1) receptor tyrosine kinases, (2) receptor tyrosine phosphatases, (3) receptor serine/threonine kinases, (4) tyrosine kinase–linked receptors, (5) receptor guanylate cyclases, and (6) cell surface proteases. Of these, the receptor tyrosine kinases (RTKs) are probably the most abundant in animal cells. Another abundant family of receptors in animals are the transforming growth factor β (TGFβ)-type receptor serine/threonine kinases (RSKs).

Thus far, neither RTKs nor RSKs have been identified in plants. However, plant cells do contain a class of receptors, called **plant receptor kinases** (**PRKs**), that are structurally similar to both the animal RTKs and RSKs, although plant and animal receptor kinases appear to have evolved independently (Cock et al. 2002). Although animal and plant receptor kinases evolved separately, some of the components of the RTK and RSK signaling pathway of animals have been identified in plants.

After first reviewing the animal RTK and RSK pathways, we will examine the PRK receptors of plants.

Ligand binding to receptor tyrosine kinases induces autophosphorylation

The **receptor tyrosine kinases** (**RTKs**) make up the most important class of enzyme-linked cell surface receptors in animal cells, although so far they have not been found in either plants or fungi. Their ligands are soluble or mem-

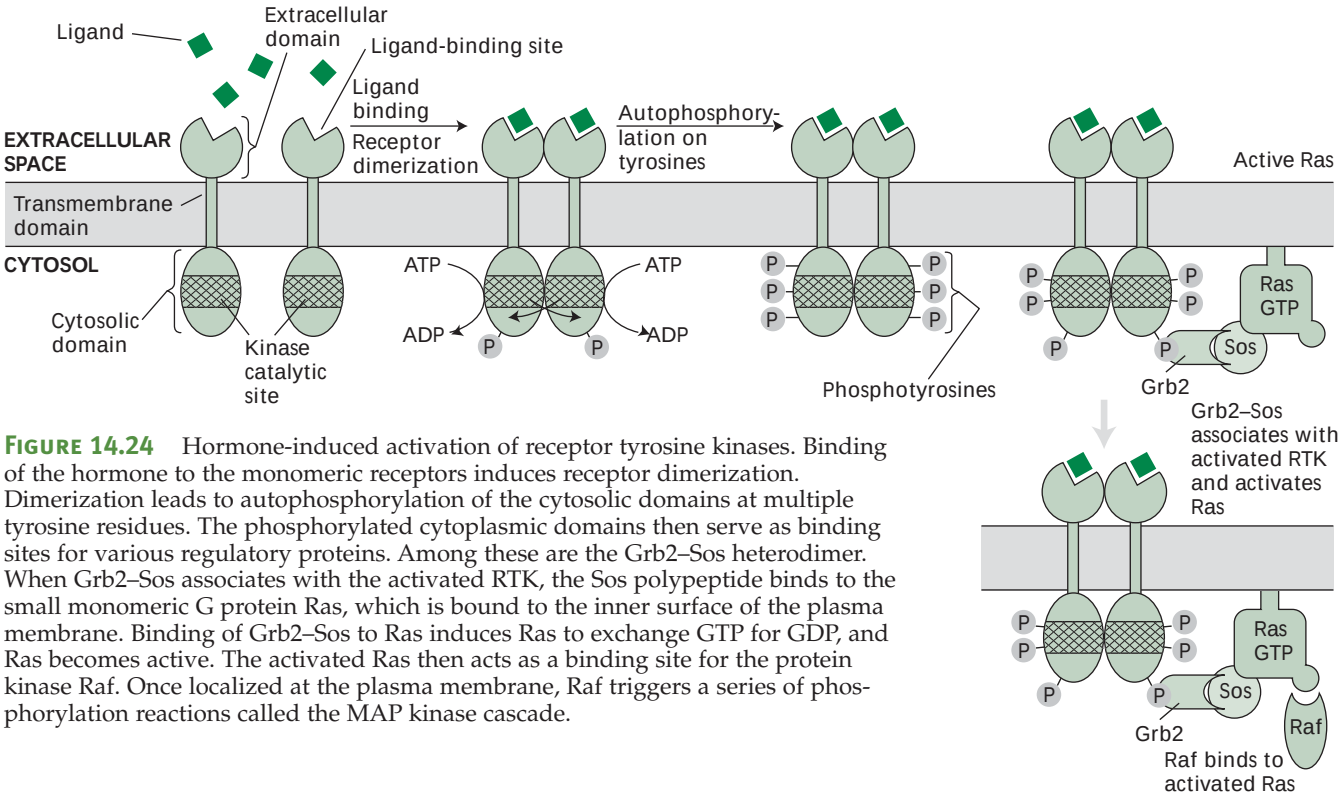


FIGURE 14.24 Hormone-induced activation of receptor tyrosine kinases. Binding of the hormone to the monomeric receptors induces receptor dimerization. Dimerization leads to autophosphorylation of the cytosolic domains at multiple tyrosine residues. The phosphorylated cytoplasmic domains then serve as binding sites for various regulatory proteins. Among these are the Grb2–Sos heterodimer. When Grb2–Sos associates with the activated RTK, the Sos polypeptide binds to the small monomeric G protein Ras, which is bound to the inner surface of the plasma membrane. Binding of Grb2–Sos to Ras induces Ras to exchange GTP for GDP, and Ras becomes active. The activated Ras then acts as a binding site for the protein kinase Raf. Once localized at the plasma membrane, Raf triggers a series of phosphorylation reactions called the MAP kinase cascade.

brane-bound peptide or protein hormones, including insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and several other protein growth factors.

Since the transmembrane domain that separates the hormone-binding site on the outer surface of the membrane from the catalytic site on the cytoplasmic surface consists of only a single α helix, the hormone cannot transmit a signal directly to the cytosolic side of the membrane via a conformational change. Rather, binding of the ligand to its receptor induces dimerization of adjacent receptors, which allows the two catalytic domains to come into contact and phosphorylate each other on multiple tyrosine residues (autophosphorylation) (Figure 14.24). Dimerization may be a general mechanism for activating cell surface receptors that contain single transmembrane domains.

Intracellular signaling proteins that bind to RTKs are activated by phosphorylation

Once autophosphorylated, the catalytic site of the RTKs binds to a variety of cytosolic signaling proteins. After binding to the RTK, the inactive signaling protein is itself phosphorylated on specific tyrosine residues. Some transcription factors are activated in this way, after which they migrate to the nucleus and stimulate gene expression directly. Other signaling molecules take part in a signaling cascade that ultimately results in the activation of transcription factors. The signaling cascade initiated by RTKs begins with the small, monomeric G protein Ras.

THE RAS SUPERFAMILY In addition to possessing heterotrimeric G proteins, eukaryotic cells contain small **monomeric G proteins** that are related to the α subunits of the heterotrimeric G proteins. The three families Ras, Rab, and Rho/Rac all belong to the **RAS superfamily of monomeric GTPases**. Rho and Rac relay signals from surface receptors to the actin cytoskeleton; members of the Rab family of GTPases are involved in regulating intracellular membrane vesicle traffic; the Ras proteins, which are located on the inner surface of the membrane, play a crucial role in initiating the kinase cascade that relays signals from RTKs to the nucleus.

The *RAS* gene was originally discovered as a viral oncogene (cancer-causing gene) and was later shown to be present as a normal gene in animal cells. Ras is a G protein that cycles between an inactive GDP-binding form and an active GTP-binding form. Ras also possesses GTPase activity that hydrolyzes bound GTP to GDP, thus terminating the response. The *RAS* oncogene is a mutant form of the protein that is unable to hydrolyze GTP. As a result, the molecular switch remains in the on position, triggering uncontrolled cell division.

The Ras superfamily of monomeric G proteins comprises over 60 distinct mammalian members that can be divided into several subfamilies (Ras, Rho, Rab, Arf, Ran, and

Gem). The Arabidopsis genome contains 93 genes encoding small GTP-binding proteins. Phylogenetic analysis of these genes shows that plants contain Rab, Rop, Rho, Arf, and Ran GTPases, but no Ras GTPases. The lack of Ras GTPases in plants probably reflects the lack of RTKs in plants, since Ras proteins act downstream of RTKs (Vernoud et al. 2003).

Ras recruits Raf to the plasma membrane

The initial steps in the Ras signaling pathway are illustrated in Figure 14.24. First, binding of the hormone to the RTK induces dimerization followed by autophosphorylation of the catalytic domain. Autophosphorylation of the receptor causes binding to the **Grb2** protein, which is tightly associated with another protein, called **Sos**. As a result, the Grb2–Sos complex attaches to the RTK at the phosphorylation site. The Sos protein then binds to the inactive form of Ras, which is associated with the inner surface of the plasma membrane. Upon binding to Sos, Ras releases GDP and binds GTP instead, which converts Ras to the active form. The activated Ras, in turn, provides a binding site for the soluble serine/threonine kinase **Raf**. The primary function of the activated Ras is thus to recruit Raf to the plasma membrane. Binding to Ras activates Raf and initiates a chain of phosphorylation reactions called the MAPK cascade (see the next section).

As we will see in later chapters, increasing evidence suggests that plant signaling pathways also employ the MAPK cascade. For example, the ethylene receptor, ETR1, probably passes its signal to CTR1, a protein kinase of the Raf family (see Chapter 22).

The activated MAP kinase enters the nucleus

The **MAPK** (*m*itogen-*a*ctivated *p*rotein *k*inase) **cascade** owes its name to a series of protein kinases that phosphorylate each other in a specific sequence, much like runners in a relay race passing a baton (Figure 14.25). The first kinase in the sequence is Raf, referred to in this context as **MAP kinase kinase kinase** (**MAPKKK**). MAPKKK passes the phosphate baton to **MAP kinase kinase** (**MAPKK**), which hands it off to **MAP kinase** (**MAPK**). MAPK, the "anchor" of the relay team, enters the nucleus, where it activates still other protein kinases, specific transcription factors, and regulatory proteins.

The transcription factors that are activated by MAPK are called serum response factor (SRF), because all of the growth factors that bind to RTKs are transported in the serum, and ternary complex factor (TCF). Serum response factors bind to specific nucleotide sequences on the genes they regulate called serum response elements (SREs). The entire process from binding of the growth factor to the receptor to transcriptional activation of gene expression can be very rapid, taking place in a few minutes.

Some of the genes that are activated encode other transcription factors that regulate the expression of other genes.

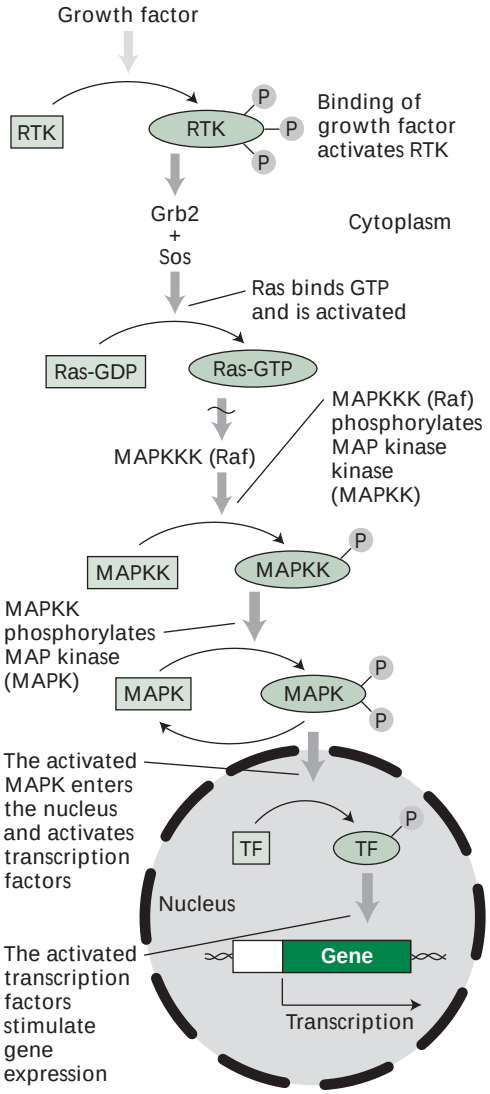


Figure 14.25 The MAPK cascade. Hormonal stimulation of the receptor tyrosine kinase leads to the activation of Raf (see Figure 14.23), also known as MAP kinase kinase kinase (MAPKKK). (1) MAPKKK phosphorylates MAP kinase kinase (MAPKK). (2) MAPKK phosphorylates MAP kinase (MAPK). (3) The activated MAPK enters the nucleus and activates transcription factors (TF). (4) The activated transcription factors stimulate gene expression. (After Karp 1996.)

Because these genes are important for cell proliferation and growth, many of them are proto-oncogenes. For example, one of the genes whose expression is stimulated by MAPK is the proto-oncogene *FOS*. A **proto-oncogene** is a normal gene that potentially can cause malignant tumors when mutated. When the **Fos** protein combines with the phosphorylated **Jun** protein (one of the nuclear proteins that is phosphorylated by MAPK), it forms a heterodimeric transcription factor called **AP-1**, which turns on other genes. Other important proto-oncogenes that encode nuclear tran-

scription factors include ***MYC*** and ***MYB***. Both phytochrome (see Chapter 17) and gibberellin (see Chapter 20) are believed to regulate gene expression via the up-regulation of MYB-like transcription factors.

Plant receptor kinases are structurally similar to animal receptor tyrosine kinases

Plant receptor kinases (PRKs) are structurally similar to the animal RTKs and RSKs. They have a large extracellular domain, span the membrane only once, and contain a catalytic domain on the cytoplasmic side. Like the animal RTKs and RSKs, PRKs typically are active in the dimeric state. Like the RSKs of animal cells, PRKs are autophosphorylating serine/threonine kinases (Cock et al. 2002).

Over 20 distinct types of extracllular domains have been identified in receptors of the PRK superfamily. These include S domains, leucine-rich repeat (LRR) domains, domains with epidermal growth factor (EGF) repeat sequences, and lectin domains. S-locus and LRR receptors are perhaps the best-studied examples.

S-domain receptors are characterized by an extracellular S domain homologous to the self-incompatibility locus (S-locus) glycoprotein, and are called **S receptor kinases** or **SRKs**. The S domain was first identified in a group of secreted glycoproteins, called S locus glycoproteins (SLGs), which regulate self-incompatibility in *Brassica* species. Self-incompatibility is characterized by the failure of pollen tubes to grow when placed on pistils from the same plant, and self-incompatibility loci are genes that regulate this phenotype.

The S domain consists of ten cysteines in a particular arrangement with other amino acids. The high degree of homology between the S domains of SRKs and those of SLGs suggests that they are functionally related and are involved in the recognition pathways involved in pollen tube growth. Consistent with this idea, SRK genes are expressed predominantly in pistils. Several other S domain PRKs with highly divergent sequences have been identified in other species, and each of these may play unique roles in plant cell signaling.

The **leucine-rich repeat** (**LRR**) family of receptors constitute the second group of RLKs. They were first identified as disease resistance genes that may play key roles in the cell surface recognition of ligands produced by pathogens and the subsequent activation of the intracellular defense response (Song et al. 1995; Bent 1996). However, plant LRR receptors have been implicated in normal developmental functions as well. For example, a pollen-specific LRR receptor has been identified in sunflower that may be involved in cell–cell recognition during pollination (Reddy et al. 1995), and the Arabidopsis *ERECTA* gene, which regulates the shape and size of organs originating from the shoot apical meristem, encodes an LRR receptor (Torii et al. 1996). More recently, the receptor for the plant steroid hormone brassinosteroid has been identified as an LRR receptor (see Chapter 24).

The LRR receptors are members of a larger family of LRR proteins that includes soluble forms with lower molecular mass that are widespread in plants and animals. The most conserved element of the LRR domain forms a β sheet with an exposed face that participates in protein–protein interactions (Buchanan and Gay 1996). The small soluble LRR proteins may participate in cell signaling by hydrophobic binding to LRR receptors. For example, in tomato a protein that contains four tandem repeats of a canonical 24-amino-acid leucine-rich repeat motif is up-regulated during virus infection. This protein is apparently secreted into the apoplast along with a protease that digests it to lower molecular weight peptides (Tornero et al. 1996). These peptides could form part of a signaling pathway by interacting with cell surface LRR receptors.

Other developmentally important PRKs include CLAVATA1 (CLV1), which is involved in the maintenance of the apical meristem in Arabidopsis (see Chapter 16), and wall-associated kinases (WAKs), which are covalently bound to the cell wall (primarily to pectin molecules) and help to regulate cell expansion. (For a recent summary of plant hormone receptors, see **Web Essay 14.1**.)

Summary

The size of the genome (the total amount of DNA in a cell, a nucleus, or an organelle) is related to the complexity of the organism. However, not all of the DNA in a genome codes for genes. Prokaryotic genomes consist mainly of unique sequences (genes). Much of the genome in eukaryotes, however, consists of repetitive DNA and spacer DNA. The genome size in plants is highly variable, ranging from 1.5×10^8 bp in Arabidopsis to 1×10^{11} bp in *Trillium*. Plant genomes contain about 25,000 genes; by comparison, the *Drosophila* genome contains about 12,000 genes.

In prokaryotes, structural genes involved in related functions are organized into operons, such as the *lac* operon. Regulatory genes encode DNA-binding proteins that may repress or activate transcription. In inducible systems, the regulatory proteins are themselves activated or inactivated by binding to small effector molecules.

Similar control systems are present in eukaryotic genomes. However, related genes are not clustered in operons, and genes are subdivided into exons and introns. Pre-mRNA transcripts must be processed by splicing, capping, and addition of poly-A tails to produce the mature mRNA, and the mature mRNA must then exit the nucleus to initiate translation in the cytosol. Despite these differences, most eukaryotic genes are regulated at the level of transcription, as in prokaryotes.

Transcription in eukaryotes is characterized by three different RNA polymerases whose activities are modulated by a diverse group of *cis*-acting regulatory sequences. RNA polymerase II is responsible for the synthesis of pre-mRNA. General transcription factors assemble into a tran-

scription initiation complex at the TATA box of the minimum promoter, which lies within 100 bp of the transcription start site of the gene. Additional *cis*-acting regulatory sequences, such as the CAAT box and GC box, bind transcription factors that enhance expression of the gene. Distal regulatory sequences located farther upstream bind to other transcription factors called activators or repressors. Many plant genes are also regulated by enhancers, distantly located positive regulatory sequences.

Despite being scattered throughout the genome, many eukaryotic genes are both inducible and coregulated. Genes that are coordinately regulated have common *cis*-acting regulatory sequences in their promoters. Most transcription factors in plants contain the basic zipper (bZIP) motif. An important group of transcription factors in plants, the floral homeotic genes, contain the MADS domain.

Enzyme concentration is also regulated by protein degradation, or turnover. As yet there is no evidence that plant vacuoles function like animal lysosomes in protein turnover, except during senescence, when the contents of the vacuole are released. However, protein turnover via the covalent attachment of the short polypeptide ubiquitin and subsequent proteolysis is an important mechanism for regulating the cytosolic protein concentration in plants.

Signal transduction pathways coordinate gene expression with environmental conditions and with development. Prokaryotes employ two-component regulatory systems that include a sensor protein and a response regulator protein that facilitates the response, typically gene expression. The sensor and the response regulator communicate via protein phosphorylation. Receptor proteins related to the bacterial two-component systems have recently been identified in yeast and plants.

In multicellular eukaryotes, lipophilic hormones usually bind to intracellular receptors, while water-soluble hormones bind to cell surface receptors. Binding to a receptor initiates a signal transduction pathway, often involving the generation of second messengers, such as cyclic nucleotides, inositol trisphosphate, and calcium, which greatly amplify the original signal and bring about the cellular response. Such pathways normally lead to changes in gene expression. In plants, the receptor for the phytohormone brassinosteroid is a cell surface receptor.

The seven-spanning receptors of animal cells interact with heterotrimeric G proteins, which act as molecular switches by cycling between active (GTP-binding) forms and inactive (GDP-binding) forms. Dissociation of the α subunit from the complex allows it to activate the effector enzyme. Activation of adenylyl cyclase increases cAMP levels, resulting in the activation of protein kinase A. Cyclic AMP can also regulate cation channels directly.

When heterotrimeric G proteins activate phospholipase C, it initiates the IP_3 pathway. IP_3 released from the membrane opens intracellular calcium channels, releasing calcium from the ER and vacuole into the cytosol. The increase in calcium concentration, in turn, activates protein kinases and other enzymes. In plants, calcium dependent protein kinases, which have a calmodulin domain, are activated by calcium directly. The other by-product of phospholipase C, diacylglycerol, can also act as a second messenger by activating protein kinase C.

There is increasing evidence that cyclic GMP operates as a second messenger in plant cells as it does in animal cells. In animal cells, cyclic GMP has been shown to regulate ion channels and protein kinases.

The most common family of cell surface catalytic receptors in animals consists of the receptor tyrosine kinases. RTKs dimerize upon binding to the hormone; then their multiple tyrosine residues are autophosphorylated. The phosphorylated receptor then acts as an assembly site for various protein complexes, including the Ras superfamily of monomeric GTPases. Binding of Ras leads to recruitment of the protein kinase Raf to the membrane. Raf initiates the MAPK cascade. The last kinase to be phosphorylated (activated) is MAP kinase, which enters the nucleus and activates various transcription factors (serum response factors), which bind to *cis*-acting regulatory sequences called serum response elements.

Plants appear to lack RTKs, but they have structurally similar receptors called plant receptor kinases (PRKs), which are serine/threonine kinases. Over 20 different extracellular domains have been identified in the PRK superfamily. The signal transduction pathways of these receptors are currently being elucidated.

Web Material

Web Essay

14.1 Plant Hormone Receptors: Perception Is Everything

A summary of the known plant hormone receptors, emphasizing their common features and interrelationships.

General Reading

*Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. (1994) Molecular Biology of the Cell, 3rd ed. Garland, New York.

*Becker, W. M., Reece, J. B., and Poenie, M. F. (1996) The World of the Cell, 3rd ed. Benjamin/Cummings, Menlo Park, Calif.

Dey, P. M., and Harborne, J. B., eds. (1997) Plant Biochemistry. Academic Press, San Diego.

Fosket, D. E. (1994) Plant Growth and Development: A Molecular Approach. Academic Press, San Diego.

*Karp, G. (1996) Cell and Molecular Biology: Concepts and Experiments. Wiley, New York.

*Lodish, H., Baltimore, D., Berk, A., Zipursky, S., Matsidaira, P., and Darnell, J. (1995) Molecular Cell Biology. Scientific American Books, New York.
Tobin, A. J., and Morel, R. E. (1997) Asking about Cells. Harcourt Brace, Fort Worth, Tex.
*Indicates a reference that is general reading in the field and is also cited in this chapter.

Chapter References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002) *Molecular Biology of the Cell*, 4th ed. Garland Science, New York.
Assmann, A. M. (1995) Cyclic AMP as a second messenger in higher plants: Status and future prospects. *Plant Physiol.* 91: 624–628.
Baulcombe, D. (2004) RNA silencing in plants. *Nature* 431: 356–363.
Bent, A. F. (1996) Plant disease resistance genes: Function meets structure. *Plant Cell* 8: 1757–1771.
Buchanan, S. G., and Gay, N. J. (1996) Structural and functional diversity in the leucine-rich repeat family of proteins. *Prog. Biophys. Mol. Biol.* 65: 1–44.
Chen, X., Xiao Z.-A., and Zhang, C.-H. (1996) A preliminary study on plant protein kinase C. *Acta Phytophysiol. Sinica* 22: 437–440.
Cock, J. M., Vanoosthuyse, V., and Gaude, T. (2002) Receptor kinase signaling in plants and animals: Distinct molecular systems with mechanistic similarities. *Curr. Opin. Cell Biol.* 14: 230–236.
Coux, O., Tanaka, K., and Goldberg, A. L. (1996) Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem.* 65: 2069–2076.
Elliott, D. C., and Kokke, Y. S. (1987) Partial purification and properties of a protein kinase C type enzyme from plants. *Phytochemistry* 26: 2929–2936.
Golovkin, M., and Reddy, A. S. N. (1996) Structure and expression of a plant U1 snRNP 70K gene—Alternative splicing of U1 snRNP pre-mRNA produces two different transcripts. *Plant Cell* 8: 1421–1435.
Harper, J. J., Sussman, M. R., Schaller, G. E., Putnam-Evans, C., Charbonneau, H., and Harmon, A. C. (1991) A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. *Science* 252: 951–954.
Ichikawa, T., Suzuki, Y., Czaja, I., Schommer, C., Lesnick, A., Schell, J., and Walden, R. (1997) Identification and role of adenylyl cyclase in auxin signalling in higher plants. *Nature* 390: 698–701.
Janistyn, B. (1983) Gas chromatographic-mass spectroscopic identification and quantification of cyclic guanosine-3′:5′-monophosphate in maize seedlings (*Zea mays*). *Planta* 159: 382–385.
Johnston, M. (1987) A model fungal gene regulatory mechanism: The GAL genes of *Saccharomyces cerevisiae*. *Microbiol. Rev.* 51: 458–476.
Kategiri, F., Lam, E., and Chua, N.-H. (1989) Two tobacco DNA binding proteins with homology to the nuclear factor CREB. *Nature* 340: 727–730.
Kretsinger, R. (1980) Structure and evolution of calcium-modulated proteins. *CRC Crit. Rev. Biochem.* 8: 119–174.
Kwak, J. M., Kim, S. A., Lee, S. K., Oh, S.-A., Byoun, C.-H., Han, J.-K., and Nam, H. G. (1997) Insulin-induced maturation of *Xenopus* oocytes is inhibited by microinjection of a *Brassica napus* cDNA clone with high similarity to a mammalian receptor for activated protein kinase C. *Planta* 201: 245–251.
Lam, E. (1997) Nucleic acids and proteins. In *Plant Biochemistry*, P. M. Dey and J. B. Harborne, eds., Academic Press, San Diego, pp. 316–352.
Li, W., Luan, S., Schreiber, S. L., and Assmann, S. M. (1994) Cyclic AMP stimulates K^+ channel activity in mesophyll cells of *Vicia faba* L. *Plant Physiol.* 106: 957–961.
Lodish, H., Berk, A., Matsidaira, P., Kaiser, C. A., Krieger, M., Scott, M. P., Zipursky, S., and Darnell, J. (2004) *Molecular Cell Biology*. W. H. Freeman, New York.
McAinsh, M. R., Webb, A. A. R., Taylor, J. E., and Hetherington, A. M. (1995) Stimulus-induced oscillations in guard cell cytosolic free calcium. *Plant Cell* 7: 1207–1219.
Millner, P. A., and Causier, B. E. (1996) G-protein coupled receptors in plant cells. *J. Exp. Bot.* 47: 983–992.
Mortimer, R. K., Schild, D., Contopoulou, C. R., and Kans, J. A. (1989) Genetic map of *Saccharomyces cerevisiae*, Edition l0. *Yeast* 5: 321–403.
Napoli C., Lemieux C., and Jorgensen R. (1990) Introduction of a chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans. *Plant Cell* 2: 279–289.
Newton, R. P., and Brown, E. G. (1992) Analytical procedures for cyclic nucleotides and their associated enzymes in plant tissues. *Phytochem. Anal.* 3: 1–13.
Ota, I. M., and Varshavsky, A. (1993) A yeast protein similar to bacterial two-component regulators. *Science* 262: 566–569.
Parkinson, J. S. (1993) Signal transduction schemes of bacteria. *Cell* 73: 857–871.
Pei, Z.-M., Ward, J. M., Harper, J. F., and Schroeder, J. I. (1996) A novel chloride channel in *Vicia faba* guard cell vacuoles activated by the serine-threonine kinase, CDPK. *EMBO J.* 15: 6564–6574.
Peters, J. L., and Silverthorne, J. (1995) Organ-specific stability of two *Lemna* rbcS mRNAs is determined primarily in the nuclear compartment. *Plant Cell* 7: 131–140.
Reddy, J. T., Dudareva, N., Evrard, J.-L., Krauter, R., Steinmetz, A., and Pillay, D. T. N. (1995) A pollen-specific gene from sunflower encodes a member of the leucine-rich-repeat protein superfamily. *Plant Sci.* 111: 81–93.
Richards, H., Das, S., Smith, C. J., Pereira, L., Geisbrecht, A., Devitt, N. J., Games, D. E., van Geyschem, J., Brenton, A. G., and Newton, R. P. (2002) Cyclic nucleotide content of tobacco BY-2 cells. *Phytochemistry* 61: 531–537.
Roberts, D. M., and Harmon, A. C. (1992) Calcium-modulated protein targets of intracellular calcium signals in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43: 375–414.
Shanklin, J., Jabben, M., and Vierstra, R. D. (1987) Red light induced formation of ubiquitin-phytochrome conjugates: Identification of possible intermediates of phytochrome degradation. *Proc. Natl. Acad. Sci. USA* 84: 359–363.
Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., Zhu, L.-H., et al. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science* 270: 1804–1806.
Takezawa, D., Ramachandiran, S., Paranjape, V., and Poovaiah, B. W. (1996) Dual regulation of a chimeric plant serine-threonine kinase by calcium and calcium-calmodulin. *J. Biol. Chem.* 271: 8126–8132.
Tezuka, T., Hiratsuka, S., and Takahasi, S. Y. (1993) Promotion of the growth of self-incompatible pollen tubes in lily by cAMP. *Plant Cell Physiol.* 27: 193–197.
Torii, K. U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokayama, R., Whittier, R. F., and Komeda, Y. (1996) The *Arabidopsis* ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell* 8: 735–746.

Tornero, P., Mayda, E., Gomez, M. D., Canas, L., Conejero, V., and Vera, P. (1996) Characterization of LRP, a leucine-rich repeat (LRR) protein from tomato plants that is processed during pathogenesis. *Plant J.* 10: 315–330.

Vernoud, V., Horton, A. C., Yang, Z., and Nielsen, E. (2003) Analysis of the small GTPase gene superfamily of Arabidopsis. *Plant Physiol.* 131: 1191–1208.

Watson, C. L., and Gold, M. R. (1997) Lysophosphatidylcholine modulates cardiac I-Na via multiple protein kinase pathways. *Circ. Res.* 81: 387–395.

Yang, T., Chaudhuri, S., Yang, L., Chen, Y., and Poovaiah, B. W. (2004) Calcium/calmodulin up-regulates a cytoplasmic receptor-like kinase in plants. *J. Biol. Chem.* 279: 42552–42559.

Zhang, F. and Peterson, T. (2005) Comparisons of maize pericarp color1 alleles reveal paralogous gene recombination and an organ-specific enhancer region. *Plant Cell* 17: 903–914.