CHAPTER 15

Cell Signaling

IN THIS CHAPTER

When things change, cells respond. Every cell, from the humble bacterium to the most sophisticated eukaryotic cell, monitors its intracellular and extracellular environment, processes the information it gathers, and responds accordingly. Unicellular organisms, for example, modify their behavior in response to changes in environmental nutrients or toxins. The cells of multicellular organisms detect and respond to countless internal and extracellular signals that control their growth, division, and differentiation during development, as well as their behavior in adult tissues. At the heart of all these communication systems are regulatory proteins that produce chemical signals, which are sent from one place to another in the body or within a cell, usually being processed along the way and integrated with other signals to provide clear and effective communication.

The study of cell signaling has traditionally focused on the mechanisms by which eukaryotic cells communicate with each other using *extracellular signal molecules* such as hormones and growth factors. In this chapter, we describe the features of some of these cell–cell communication systems, and we use them to illustrate the general principles by which any regulatory system, inside or outside the cell, is able to generate, process, and respond to signals. Our main focus is on animal cells, but we end by considering the special features of cell signaling in plants.

PRINCIPLES OF CELL SIGNALING

Long before multicellular creatures roamed the Earth, unicellular organisms had developed mechanisms for responding to physical and chemical changes in their environment. These almost certainly included mechanisms for responding to the presence of other cells. Evidence comes from studies of present-day unicellular organisms such as bacteria and yeasts. Although these cells lead mostly independent lives, they can communicate and influence one another's behavior. Many bacteria, for example, respond to chemical signals that are secreted by their neighbors and accumulate at higher population density. This process, called *quorum sensing*, allows bacteria to coordinate their behavior, including their motility, antibiotic production, spore formation, and sexual conjugation. Similarly, yeast cells communicate with one another in preparation for mating. The budding yeast *Saccharomyces cerevisiae* provides a well-studied example: when a haploid individual is ready to mate, it secretes a peptide *mating factor* that signals cells of the opposite mating type to stop proliferating and prepare to mate. The subsequent fusion of two haploid cells of opposite mating type produces a diploid zygote.

Intercellular communication achieved an astonishing level of complexity during the evolution of multicellular organisms. These organisms are tight-knit societies of cells, in which the well-being of the individual cell is often set aside for the benefit of the organism as a whole. Complex systems of intercellular communication have evolved to allow the collaboration and coordination of different tissues and cell types. Bewildering arrays of signaling systems govern every conceivable feature of cell and tissue function during development and in the adult.

Communication between cells in multicellular organisms is mediated mainly by **extracellular signal molecules**. Some of these operate over long distances,

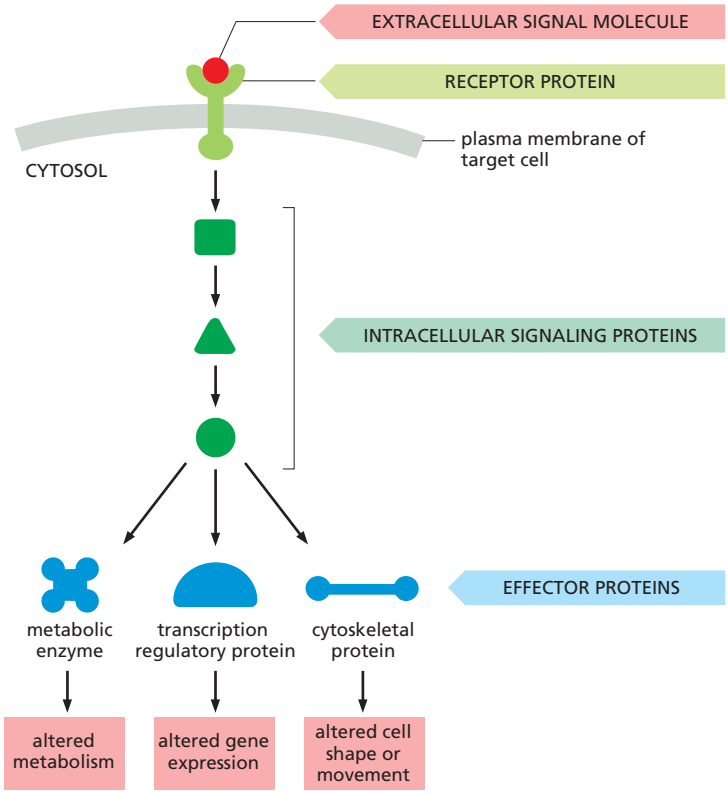


Figure 15–1 A simple intracellular signaling pathway activated by an extracellular signal molecule. The signal molecule usually binds to a receptor protein that is embedded in the plasma membrane of the target cell. The receptor activates one or more intracellular signaling pathways, involving a series of signaling proteins. Finally, one or more of the intracellular signaling proteins alters the activity of effector proteins and thereby the behavior of the cell.

signaling to cells far away; others signal only to immediate neighbors. Most cells in multicellular organisms both emit and receive signals. Reception of the signals depends on *receptor proteins*, usually (but not always) at the cell surface, which bind the signal molecule. The binding activates the receptor, which in turn activates one or more *intracellular signaling pathways* or *systems*. These systems depend on *intracellular signaling proteins*, which process the signal inside the receiving cell and distribute it to the appropriate intracellular targets. The targets that lie at the end of signaling pathways are generally called *effector proteins*, which are altered in some way by the incoming signal and implement the appropriate change in cell behavior. Depending on the signal and the type and state of the receiving cell, these effectors can be transcription regulators, ion channels, components of a metabolic pathway, or parts of the cytoskeleton (**Figure 15–1**).

The fundamental features of cell signaling have been conserved throughout the evolution of the eukaryotes. In budding yeast, for example, the response to mating factor depends on cell-surface receptor proteins, intracellular GTP-binding proteins, and protein kinases that are clearly related to functionally similar proteins in animal cells. Through gene duplication and divergence, however, the signaling systems in animals have become much more elaborate than those in yeasts; the human genome, for example, contains more than 1500 genes that encode receptor proteins, and the number of different receptor proteins is further increased by alternative RNA splicing and post-translational modifications.

Extracellular Signals Can Act Over Short or Long Distances

Many extracellular signal molecules remain bound to the surface of the signaling cell and influence only cells that contact it (**Figure 15–2**A). Such **contact-dependent signaling** is especially important during development and in immune responses. Contact-dependent signaling during development can sometimes operate over relatively large distances if the communicating cells extend long thin processes to make contact with one another.

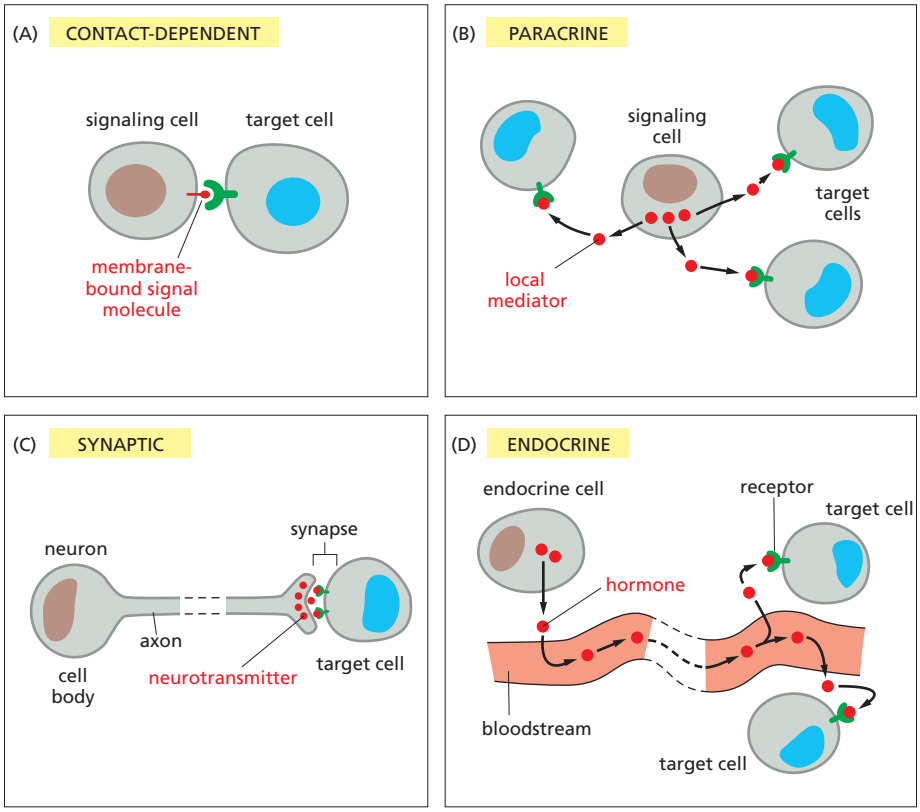


Figure 15–2 Four forms of intercellular signaling. (A) Contact-dependent signaling requires cells to be in direct membrane–membrane contact. (B) Paracrine signaling depends on local mediators that are released into the extracellular space and act on neighboring cells. (C) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the neuronal cell body. (D) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream for distribution throughout the body. Many of the same types of signaling molecules are used in paracrine, synaptic, and endocrine signaling; the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

In most cases, however, signaling cells secrete signal molecules into the extracellular fluid. Often, the secreted molecules are **local mediators**, which act only on cells in the local environment of the signaling cell. This is called **paracrine signaling** (Figure 15–2B). Usually, the signaling and target cells in paracrine signaling are of different cell types, but cells may also produce signals that they themselves respond to: this is referred to as *autocrine signaling*. Cancer cells, for example, often produce extracellular signals that stimulate their own survival and proliferation.

Large multicellular organisms like us need long-range signaling mechanisms to coordinate the behavior of cells in remote parts of the body. Thus, they have evolved cell types specialized for intercellular communication over large distances. The most sophisticated of these are nerve cells, or neurons, which typically extend long, branching processes (axons) that enable them to contact target cells far away, where the processes terminate at the specialized sites of signal transmission known as *chemical synapses*. When a neuron is activated by stimuli from other nerve cells, it sends electrical impulses (action potentials) rapidly along its axon; when the impulse reaches the synapse at the end of the axon, it triggers secretion of a chemical signal that acts as a **neurotransmitter**. The tightly organized structure of the synapse ensures that the neurotransmitter is delivered specifically to receptors on the postsynaptic target cell (Figure 15–2C). The details of this **synaptic signaling** process are discussed in Chapter 11.

A quite different strategy for signaling over long distances makes use of **endocrine cells**, which secrete their signal molecules, called **hormones**, into the bloodstream. The blood carries the molecules far and wide, allowing them to act on target cells that may lie anywhere in the body (Figure 15–2D).

Extracellular Signal Molecules Bind to Specific Receptors

Cells in multicellular animals communicate by means of hundreds of kinds of extracellular signal molecules. These include proteins, small peptides, amino

acids, nucleotides, steroids, retinoids, fatty acid derivatives, and even dissolved gases such as nitric oxide and carbon monoxide. Most of these signal molecules are released into the extracellular space by exocytosis from the signaling cell, as discussed in Chapter 13. Some, however, are emitted by diffusion through the signaling cell's plasma membrane, whereas others are displayed on the external surface of the cell and remain attached to it, providing a signal to other cells only when they make contact. Transmembrane signal proteins may operate in this way, or their extracellular domains may be released from the signaling cell's surface by proteolytic cleavage and then act at a distance.

Regardless of the nature of the signal, the *target cell* responds by means of a **receptor**, which binds the signal molecule and then initiates a response in the target cell. The binding site of the receptor has a complex structure that is shaped to recognize the signal molecule with high specificity, helping to ensure that the receptor responds only to the appropriate signal and not to the many other signaling molecules surrounding the cell. Many signal molecules act at very low concentrations (typically $\leq 10^{-8}$ M), and their receptors usually bind them with high affinity (dissociation constant $K_d \leq 10^{-8}$ M; see Figure 3–44).

In most cases, receptors are transmembrane proteins on the target-cell surface. When these proteins bind an extracellular signal molecule (a *ligand*), they become activated and generate various intracellular signals that alter the behavior of the cell. In other cases, the receptor proteins are inside the target cell, and the signal molecule has to enter the cell to bind to them: this requires that the signal molecule be sufficiently small and hydrophobic to diffuse across the target cell's plasma membrane (**Figure 15–3**). This chapter focuses primarily on signaling through cell-surface receptors, but we will briefly describe signaling through intracellular receptors later in the chapter.

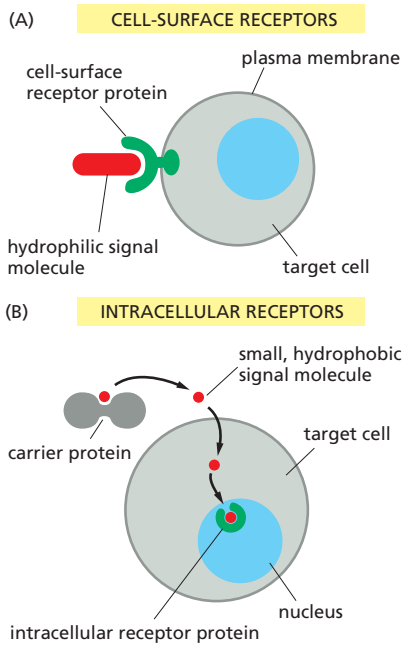


Figure 15–3 The binding of extracellular signal molecules to either cell-surface or intracellular receptors. (A) Most signal molecules are hydrophilic and are therefore unable to cross the target cell's plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate signals inside the target cell (see Figure 15–1). (B) Some small signal molecules, by contrast, diffuse across the plasma membrane and bind to receptor proteins inside the target cell—either in the cytosol or in the nucleus (as shown here). Many of these small signal molecules are hydrophobic and poorly soluble in aqueous solutions; they are therefore transported in the bloodstream and other extracellular fluids bound to carrier proteins, from which they dissociate before entering the target cell.

Each Cell Is Programmed to Respond to Specific Combinations of Extracellular Signals

A typical cell in a multicellular organism is exposed to hundreds of different signal molecules in its environment. The molecules can be soluble, bound to the extracellular matrix, or bound to the surface of a neighboring cell; they can be stimulatory or inhibitory; they can act in innumerable different combinations; and they can influence almost any aspect of cell behavior. The cell responds to this blizzard of signals selectively, in large part by expressing only those receptors and intracellular signaling systems that respond to the signals that are required for the regulation of that cell.

Most cells respond to many different signals in the environment, and some of these signals may influence the response to other signals. One of the key challenges in cell biology is to determine how a cell integrates all of this signaling information in order to make decisions—to divide, to move, to differentiate, and so on. Many cells, for example, require a specific combination of extracellular survival factors to allow the cell to continue living; when deprived of these signals, the cell activates a suicide program and kills itself—usually by *apoptosis*, a form of *programmed cell death*, as discussed in Chapter 18. Cell proliferation often depends on a combination of signals that promote both cell division and survival, as well as signals that stimulate cell growth (**Figure 15–4**). On the other hand, differentiation into a nondividing state (called *terminal differentiation*) frequently requires a different combination of survival and differentiation signals that must override any signal to divide.

In principle, the hundreds of signal molecules that an animal makes can be used in an almost unlimited number of combinations to control the diverse behaviors of its cells in highly specific ways. Relatively small numbers of types of signal molecules and receptors are sufficient. The complexity lies in the ways in which cells respond to the combinations of signals that they receive.

A signal molecule often has different effects on different types of target cells. The neurotransmitter acetylcholine (**Figure 15–5**A), for example, decreases the

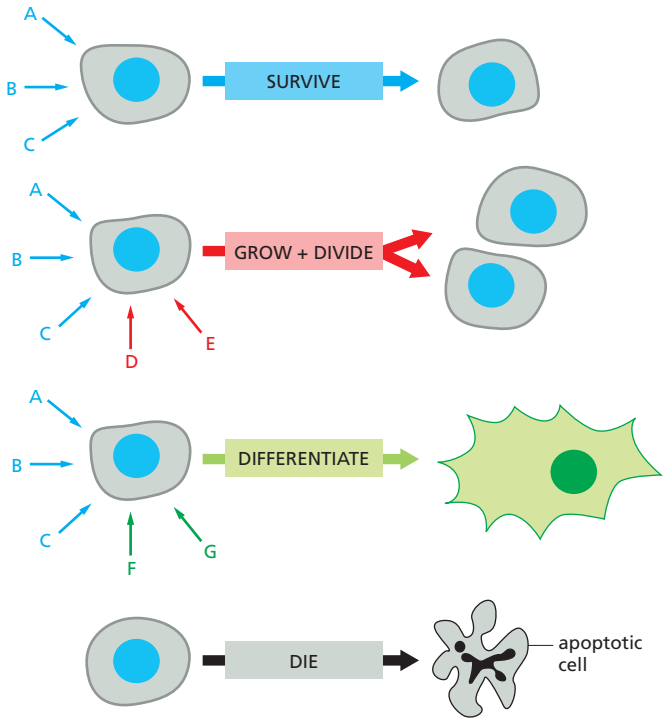


Figure 15–4 An animal cell's dependence on multiple extracellular signal molecules. Each cell type displays a set of receptors that enables it to respond to a corresponding set of signal molecules produced by other cells. These signal molecules work in various combinations to regulate the behavior of the cell. As shown here, an individual cell often requires multiple signals to survive *(blue arrows)* and additional signals to grow and divide *(red arrows)* or differentiate *(green arrows)*. If deprived of appropriate survival signals, a cell will undergo a form of cell suicide known as apoptosis. The actual situation is even more complex. Although not shown, some extracellular signal molecules act to inhibit these and other cell behaviors, or even to induce apoptosis.

rate of action potential firing in heart pacemaker cells (Figure 15–5B) and stimulates the production of saliva by salivary gland cells (Figure 15–5C), even though the receptors are the same on both cell types. In skeletal muscle, acetylcholine causes the cells to contract by binding to a different receptor protein (Figure 15–5D). The different effects of acetylcholine in these cell types result from differences in the intracellular signaling proteins, effector proteins, and genes that are activated. Thus, an extracellular signal itself has little information content; it simply induces the cell to respond according to its predetermined state, which depends on the cell's developmental history and the specific genes it expresses.

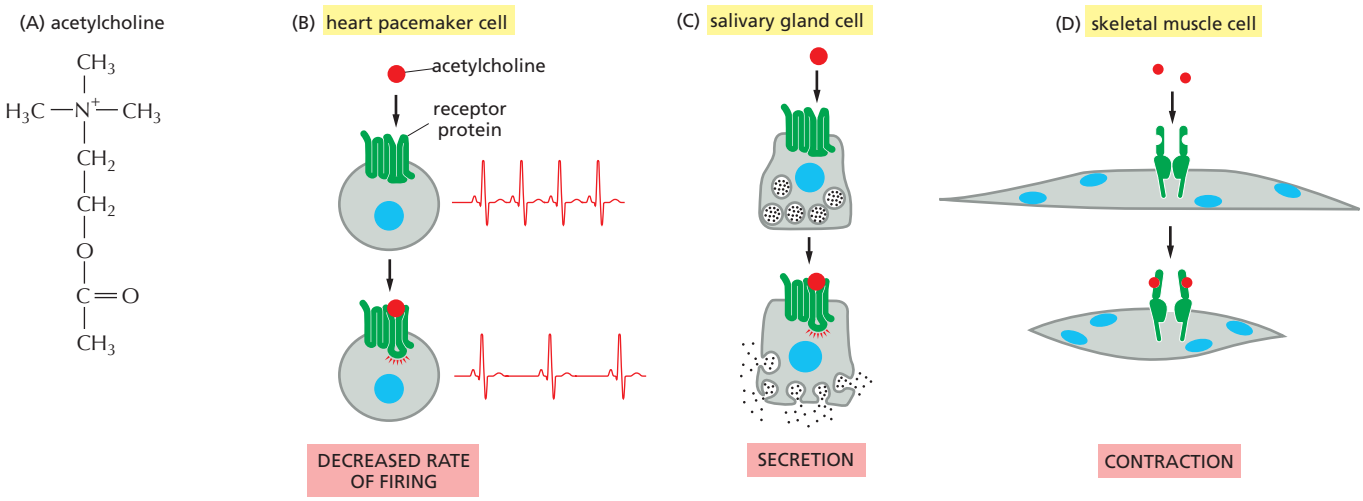


Figure 15–5 Various responses induced by the neurotransmitter acetylcholine. (A) The chemical structure of acetylcholine. (B–D) Different cell types are specialized to respond to acetylcholine in different ways. In some cases (B and C), acetylcholine binds to similar receptor proteins (G-protein-coupled receptors; see Figure 15–6), but the intracellular signals produced are interpreted differently in cells specialized for different functions. In other cases (D), the receptor protein is also different (here, an ion-channel-coupled receptor; see Figure 15–6).

There Are Three Major Classes of Cell-Surface Receptor Proteins

Most extracellular signal molecules bind to specific receptor proteins on the surface of the target cells they influence and do not enter the cytosol or nucleus. These cell-surface receptors act as *signal transducers* by converting an extracellular ligand-binding event into intracellular signals that alter the behavior of the target cell.

Most cell-surface receptor proteins belong to one of three classes, defined by their transduction mechanism. **Ion-channel-coupled receptors**, also known as *transmitter-gated ion channels* or *ionotropic receptors,* are involved in rapid synaptic signaling between nerve cells and other electrically excitable target cells such as nerve and muscle cells (**Figure 15–6**A). This type of signaling is mediated by a small number of neurotransmitters that transiently open or close an ion channel formed by the protein to which they bind, briefly changing the ion permeability of the plasma membrane and thereby changing the excitability of the postsynaptic target cell. Most ion-channel-coupled receptors belong to a large family of homologous, multipass transmembrane proteins. Because they are discussed in detail in Chapter 11, we will not consider them further here.

G-protein-coupled receptors act by indirectly regulating the activity of a separate plasma-membrane-bound target protein, which is generally either an enzyme or an ion channel. A *trimeric GTP-binding protein* (*G protein*) mediates the interaction between the activated receptor and this target protein (Figure 15–6B). The activation of the target protein can change the concentration of one or

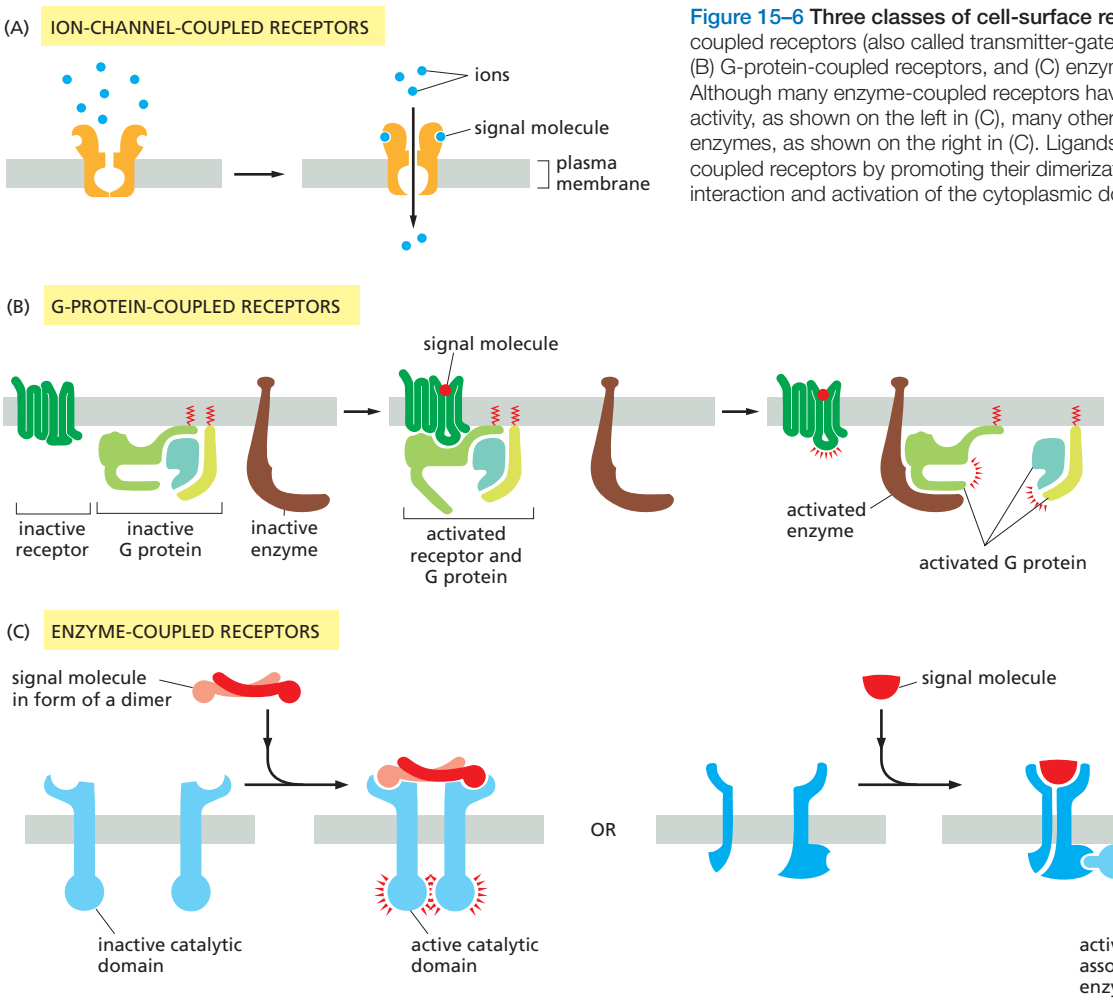


Figure 15–6 Three classes of cell-surface receptors. (A) Ion-channel-coupled receptors (also called transmitter-gated ion channels), (B) G-protein-coupled receptors, and (C) enzyme-coupled receptors. Although many enzyme-coupled receptors have intrinsic enzymatic activity, as shown on the left in (C), many others rely on associated enzymes, as shown on the right in (C). Ligands activate most enzyme-coupled receptors by promoting their dimerization, which results in the interaction and activation of the cytoplasmic domains.

more small intracellular signaling molecules (if the target protein is an enzyme), or it can change the ion permeability of the plasma membrane (if the target protein is an ion channel). The small intracellular signaling molecules act in turn to alter the behavior of yet other signaling proteins in the cell.

Enzyme-coupled receptors either function as enzymes or associate directly with enzymes that they activate (Figure 15–6C). They are usually single-pass transmembrane proteins that have their ligand-binding site outside the cell and their catalytic or enzyme-binding site inside. Enzyme-coupled receptors are heterogeneous in structure compared with the other two classes; the great majority, however, are either protein kinases or associate with protein kinases, which phosphorylate specific sets of proteins in the target cell when activated.

There are also some types of cell-surface receptors that do not fit easily into any of these classes but have important functions in controlling the specialization of different cell types during development and in tissue renewal and repair in adults. We discuss these in a later section, after we explain how G-protein-coupled receptors and enzyme-coupled receptors operate. First, we continue our general discussion of the principles of signaling via cell-surface receptors.

Cell-Surface Receptors Relay Signals Via Intracellular Signaling Molecules

Numerous intracellular signaling molecules relay signals received by cell-surface receptors into the cell interior. The resulting chain of intracellular signaling events ultimately alters effector proteins that are responsible for modifying the behavior of the cell (see Figure 15–1).

Some intracellular signaling molecules are small chemicals, which are often called **second messengers** (the "first messengers" being the extracellular signals). They are generated in large amounts in response to receptor activation and diffuse away from their source, spreading the signal to other parts of the cell. Some, such as *cyclic AMP* and Ca^{2+}, are water-soluble and diffuse in the cytosol, while others, such as *diacylglycerol*, are lipid-soluble and diffuse in the plane of the plasma membrane. In either case, they pass the signal on by binding to and altering the behavior of selected signaling or effector proteins.

Most intracellular signaling molecules are proteins, which help relay the signal into the cell by either generating second messengers or activating the next signaling or effector protein in the pathway. Many of these proteins behave like *molecular switches*. When they receive a signal, they switch from an inactive to an active state, until another process switches them off, returning them to their inactive state. The switching off is just as important as the switching on. If a signaling pathway is to recover after transmitting a signal so that it can be ready to transmit another, every activated molecule in the pathway must return to its original, unactivated state.

The largest class of molecular switches consists of proteins that are activated or inactivated by **phosphorylation** (discussed in Chapter 3). For these proteins, the switch is thrown in one direction by a **protein kinase**, which covalently adds one or more phosphate groups to specific amino acids on the signaling protein, and in the other direction by a **protein phosphatase**, which removes the phosphate groups (**Figure 15–7**A). The activity of any protein regulated by phosphorylation depends on the balance between the activities of the kinases that phosphorylate it and of the phosphatases that dephosphorylate it. About 30–50% of human proteins contain covalently attached phosphate, and the human genome encodes about 520 protein kinases and about 150 protein phosphatases. A typical mammalian cell makes use of hundreds of distinct types of protein kinases at any moment.

Protein kinases attach phosphate to the hydroxyl group of specific amino acids on the target protein. There are two main types of protein kinase. The great majority are **serine/threonine kinases**, which phosphorylate the hydroxyl groups of serines and threonines in their targets. Others are **tyrosine kinases**, which

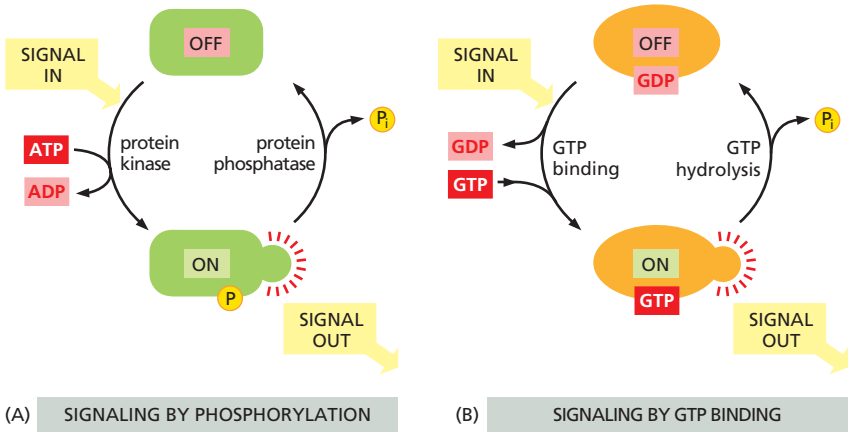


Figure 15–7 Two types of intracellular signaling proteins that act as molecular switches. (A) A protein kinase covalently adds a phosphate from ATP to the signaling protein, and a protein phosphatase removes the phosphate. Although not shown, many signaling proteins are activated by dephosphorylation rather than by phosphorylation. (B) A GTP-binding protein is induced to exchange its bound GDP for GTP, which activates the protein; the protein then inactivates itself by hydrolyzing its bound GTP to GDP.

phosphorylate proteins on tyrosines. The two types of protein kinase are closely related members of a large family, differing primarily in the structure of their protein substrate binding sites.

Many intracellular signaling proteins controlled by phosphorylation are themselves protein kinases, and these are often organized into **kinase cascades**. In such a cascade, one protein kinase, activated by phosphorylation, phosphorylates the next protein kinase in the sequence, and so on, relaying the signal onward and, in some cases, amplifying it or spreading it to other signaling pathways.

The other important class of molecular switches consists of **GTP-binding proteins** (discussed in Chapter 3). These proteins switch between an "on" (actively signaling) state when GTP is bound and an "off" state when GDP is bound. In the "on" state, they usually have intrinsic GTPase activity and shut themselves off by hydrolyzing their bound GTP to GDP (Figure 15–7B). There are two major types of GTP-binding proteins. Large, *trimeric GTP-binding proteins* (also called *G proteins*) help relay signals from G-protein-coupled receptors that activate them (see Figure 15–6B). Small **monomeric GTPases** (also called *monomeric GTP-binding proteins*) help relay signals from many classes of cell-surface receptors.

Specific regulatory proteins control both types of GTP-binding proteins. **GTPase-activating proteins** (**GAPs**) drive the proteins into an "off" state by increasing the rate of hydrolysis of bound GTP. Conversely, **guanine nucleotide exchange factors** (**GEFs**) activate GTP-binding proteins by promoting the release of bound GDP, which allows a new GTP to bind. In the case of trimeric G proteins, the activated receptor serves as the GEF. **Figure 15–8** illustrates the regulation of monomeric GTPases.

Not all molecular switches in signaling systems depend on phosphorylation or GTP binding. We see later that some signaling proteins are switched on or off by the binding of another signaling protein or a second messenger such as cyclic AMP or Ca^{2+}, or by covalent modifications other than phosphorylation or dephosphorylation, such as ubiquitylation (discussed in Chapter 3).

For simplicity, we often portray a signaling pathway as a series of activation steps (see Figure 15–1). It is important to note, however, that most signaling pathways contain inhibitory steps, and a sequence of two inhibitory steps can have the same effect as one activating step (**Figure 15–9**). This *double-negative* activation is very common in signaling systems, as we will see when we describe specific pathways later in this chapter.

Intracellular Signals Must Be Specific and Precise in a Noisy Cytoplasm

Ideally, an activated intracellular signaling molecule should interact only with the appropriate downstream targets, and, likewise, the targets should only be activated by the appropriate upstream signal. In reality, however, intracellular

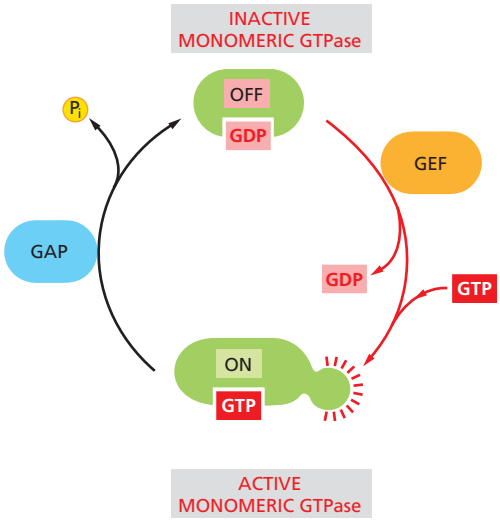


Figure 15–8 The regulation of a monomeric GTPase. GTPase-activating proteins (GAPs) inactivate the protein by stimulating it to hydrolyze its bound GTP to GDP, which remains tightly bound to the inactivated GTPase. Guanine nucleotide exchange factors (GEFs) activate the inactive protein by stimulating it to release its GDP; because the concentration of GTP in the cytosol is 10 times greater than the concentration of GDP, the protein rapidly binds GTP and is thereby activated.

signaling molecules share the cytoplasm with a crowd of closely related signaling molecules that control a diverse array of cellular processes. It is inevitable that an occasional signaling molecule will bind or modify the wrong partner, potentially creating unwanted cross-talk and interference between signaling systems. How does a signal remain strong, precise, and specific under these noisy conditions?

The first line of defense comes from the high affinity and specificity of the interactions between signaling molecules and their correct partners compared to the relatively low affinity of the interactions between inappropriate partners. The binding of a signaling molecule to the correct target is determined by precise and complex interactions between complementary surfaces on the two molecules. Protein kinases, for example, contain active sites that recognize a specific amino acid sequence around the phosphorylation site on the correct target protein, and they often contain additional *docking sites* that promote a specific, high-affinity interaction with the target. These and related mechanisms help provide a strong and persistent interaction between the correct partners, reducing the likelihood of inappropriate interactions with other proteins.

Another important way that cells avoid responses to unwanted background signals depends on the ability of many downstream target proteins to simply ignore such signals. These proteins respond only when the upstream signal reaches a high concentration or activity level. Consider a signaling pathway in which a protein kinase activates some downstream target protein by phosphorylation. If a response is triggered only when more than half of the target proteins are phosphorylated, then there will be little harm done if a small number of them are occasionally phosphorylated by some inappropriate protein kinase. Furthermore, constitutively active protein phosphatases will further reduce the impact of background phosphorylation by rapidly removing much of it. In these and other ways, intracellular signaling systems filter out noise, generating little or no response to low levels of stimuli.

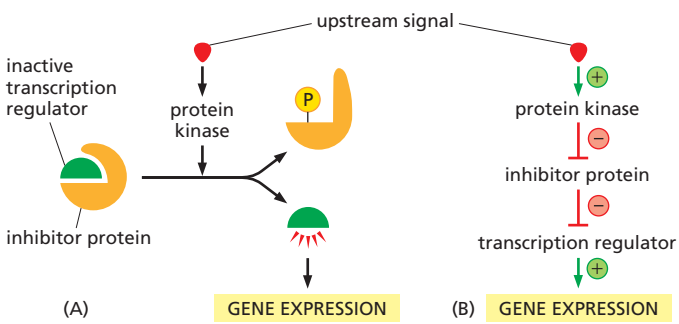


Figure 15–9 A sequence of two inhibitory signals produces a positive signal. (A) In this simple signaling system, a transcription regulator is kept in an inactive state by a bound inhibitor protein. In response to some upstream signal, a protein kinase is activated and phosphorylates the inhibitor, causing its dissociation from the transcription regulator and thereby activating gene expression. (B) This signaling pathway can be diagrammed as a sequence of four steps, including two sequential inhibitory steps that are equivalent to a single activating step.

Cells in a population often exhibit random variation in the concentration or activity of their intracellular signaling molecules. Similarly, individual molecules in a large population of molecules vary in their activity or interactions with other molecules. This *signal variability* introduces another form of noise that can interfere with the precision and efficiency of signaling. Most signaling systems, however, are built to generate remarkably robust and precise responses even when upstream signals are variable or even when some components of the system are disabled. In many cases, this *robustness* depends on the presence of backup mechanisms: for example, a signal might employ two parallel pathways to activate a single common downstream target protein, allowing the response to occur even if one pathway is crippled.

Intracellular Signaling Complexes Form at Activated Receptors

One simple and effective strategy for enhancing the specificity of interactions between signaling molecules is to localize them in the same part of the cell or even within large protein complexes, thereby ensuring that they interact only with each other and not with inappropriate partners. Such mechanisms often involve **scaffold proteins**, which bring together groups of interacting signaling proteins into *signaling complexes*, often before a signal has been received (**Figure 15–10**A). Because the scaffold holds the proteins in close proximity, they can interact at high local concentrations and be sequentially activated rapidly, efficiently, and selectively in response to an appropriate extracellular signal, avoiding unwanted cross-talk with other signaling pathways.

In other cases, such signaling complexes form only transiently in response to an extracellular signal and rapidly disassemble when the signal is gone. They often assemble around a receptor after an extracellular signal molecule has activated it. In many of these cases, the cytoplasmic tail of the activated receptor is phosphorylated during the activation process, and the phosphorylated amino acids then serve as docking sites for the assembly of other signaling proteins (Figure 15–10B). In yet other cases, receptor activation leads to the production of modified phospholipid molecules (called phosphoinositides) in the adjacent plasma membrane, which then recruit specific intracellular signaling proteins to this region of membrane, where they are activated (Figure 15–10C).

Modular Interaction Domains Mediate Interactions Between Intracellular Signaling Proteins

Simply bringing intracellular signaling proteins together into close proximity is sometimes sufficient to activate them. Thus, *induced proximity*, where a signal triggers assembly of a signaling complex, is commonly used to relay signals from protein to protein along a signaling pathway. The assembly of such signaling complexes depends on various highly conserved, small **interaction domains**, which are found in many intracellular signaling proteins. Each of these compact protein modules binds to a particular structural motif in another protein or lipid. The recognized motif in the interacting protein can be a short peptide sequence, a covalent modification (such as a phosphorylated amino acid), or another protein domain. The use of modular interaction domains presumably facilitated the evolution of new signaling pathways; because it can be inserted at many locations in a protein without disturbing the protein's folding or function, a new interaction domain added to an existing signaling protein could connect the protein to additional signaling pathways.

There are many types of interaction domains in signaling proteins. *Src homology 2* (*SH2*) *domains* and *phosphotyrosine-binding* (*PTB*) *domains*, for example, bind to phosphorylated tyrosines in a particular peptide sequence on activated receptors or intracellular signaling proteins. *Src homology 3* (*SH3*) domains bind to short, proline-rich amino acid sequences. Some *pleckstrin homology* (*PH*) domains bind to the charged head groups of specific phosphoinositides that are produced in the plasma membrane in response to an extracellular signal; they

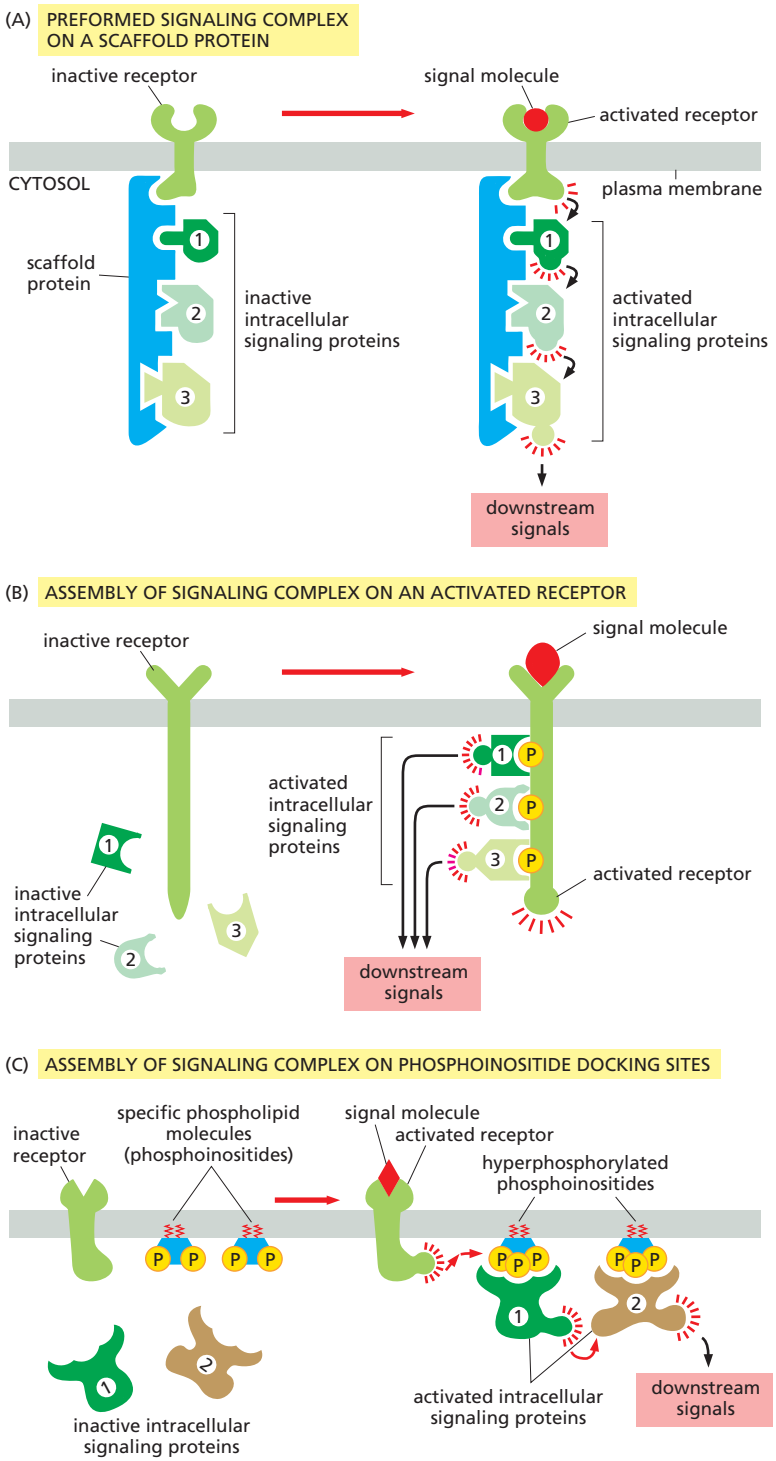


Figure 15–10 Three types of intracellular signaling complexes. (A) A receptor and some of the intracellular signaling proteins it activates in sequence are preassembled into a signaling complex on the inactive receptor by a large scaffold protein. (B) A signaling complex assembles transiently on a receptor only after the binding of an extracellular signal molecule has activated the receptor; here, the activated receptor phosphorylates itself at multiple sites, which then act as docking sites for intracellular signaling proteins. (C) Activation of a receptor leads to the increased phosphorylation of specific phospholipids (phosphoinositides) in the adjacent plasma membrane; these then serve as docking sites for specific intracellular signaling proteins, which can now interact with each other.

enable the protein they are part of to dock on the membrane and interact with other similarly recruited signaling proteins (see Figure 15–10C). Some signaling proteins consist solely of two or more interaction domains and function only as **adaptors** to link two other proteins together in a signaling pathway.

Interaction domains enable signaling proteins to bind to one another in multiple specific combinations. Like Lego® bricks, the proteins can form linear or branching chains or three-dimensional networks, which determine the route followed by the signaling pathway. As an example, **Figure 15–11** illustrates how some interaction domains mediate the formation of a large signaling complex around the receptor for the hormone *insulin*.

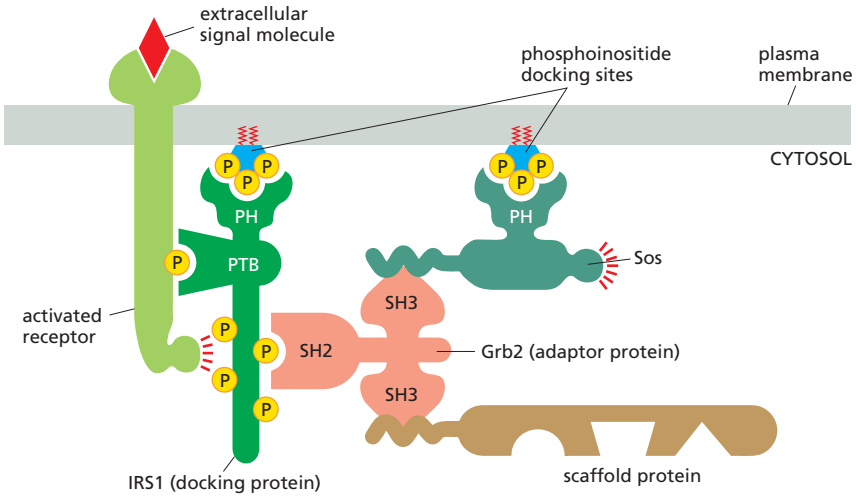


Figure 15–11 A specific signaling complex formed using modular interaction domains. This example is based on the insulin receptor, which is an enzyme-coupled receptor (a receptor tyrosine kinase, discussed later). First, the activated receptor phosphorylates itself on tyrosines, and one of the phosphotyrosines then recruits a docking protein called insulin receptor substrate-1 (IRS1) via a PTB domain of IRS1; the PH domain of IRS1 also binds to specific phosphoinositides on the inner surface of the plasma membrane. Then, the activated receptor phosphorylates IRS1 on tyrosines, and one of these phosphotyrosines binds the SH2 domain of the adaptor protein Grb2. Next, Grb2 uses one of its two SH3 domains to bind to a proline-rich region of a protein called Sos, which relays the signal downstream by acting as a GEF (see Figure 15–8) to activate a monomeric GTPase called Ras (not shown). Sos also binds to phosphoinositides in the plasma membrane via its PH domain. Grb2 uses its other SH3 domain to bind to a proline-rich sequence in a scaffold protein. The scaffold protein binds several other signaling proteins, and the other phosphorylated tyrosines on IRS1 recruit additional signaling proteins that have SH2 domains (not shown).

Another way of bringing receptors and intracellular signaling proteins together is to concentrate them in a specific region of the cell. An important example is the **primary cilium** that projects like an antenna from the surface of most vertebrate cells (discussed in Chapter 16). It is usually short and nonmotile and has microtubules in its core, and a number of surface receptors and signaling proteins are concentrated there. We shall see later that light and smell receptors are also highly concentrated in specialized cilia.

The Relationship Between Signal and Response Varies in Different Signaling Pathways

The function of an intracellular signaling system is to detect and measure a specific stimulus in one location of a cell and then generate an appropriately timed and measured response at another location. The system accomplishes this task by sending information in the form of molecular "signals" from the sensor to the target, often through a series of intermediaries that do not simply pass the signal along but process it in various ways. All signaling systems do not work in precisely the same way: each has evolved specialized behaviors that produce a response that is appropriate for the cell function that system controls. In the following paragraphs, we list some of these behaviors and describe how they vary in different systems, as a foundation for more detailed discussions later.

1. *Response timing* varies dramatically in different signaling systems, according to the speed required for the response. In some cases, such as synaptic signaling (see Figure 15–2C), the response can occur within milliseconds. In other cases, as in the control of cell fate by morphogens during development, a full response can require hours or days.
2. *Sensitivity* to extracellular signals can vary greatly. Hormones tend to act at very low concentrations on their distant target cells, which are therefore highly sensitive to low concentrations of signal. Neurotransmitters, on the other hand, operate at much higher concentrations at a synapse, reducing the need for high sensitivity in postsynaptic receptors. Sensitivity is often controlled by changes in the number or affinity of the receptors on the target cell. A particularly important mechanism for increasing the sensitivity of a signaling system is signal *amplification*, whereby a small number of activated cell-surface receptors evoke a large intracellular response either by producing large amounts of a second messenger or by activating many copies of a downstream signaling protein.
3. *Dynamic range* of a signaling system is related to its sensitivity. Some systems, like those involved in simple developmental decisions, are responsive

over a narrow range of extracellular signal concentrations. Other systems, like those controlling vision or the metabolic response to some hormones, are highly responsive over a much broader range of signal strengths. We will see that broad dynamic range is often achieved by *adaptation* mechanisms that adjust the responsiveness of the system according to the prevailing amount of signal.

4. *Persistence* of a response can vary greatly. A transient response of less than a second is appropriate in some synaptic responses, for example, while a prolonged or even permanent response is required in cell fate decisions during development. Numerous mechanisms, including positive feedback, can be used to alter the duration and reversibility of a response.
5. *Signal processing* can convert a simple signal into a complex response. In many systems, for example, a gradual increase in an extracellular signal is converted into an abrupt, switchlike response. In other cases, a simple input signal is converted into an oscillatory response, produced by a repeating series of transient intracellular signals. Feedback usually lies at the heart of biochemical switches and oscillators, as we describe later.
6. *Integration* allows a response to be governed by multiple inputs. As discussed earlier, for example, specific combinations of extracellular signals are generally required to stimulate complex cell behaviors such as cell survival and proliferation (see Figure 15–4). The cell therefore has to integrate information coming from multiple signals, which often depends on intracellular *coincidence detectors*; these proteins are equivalent to *AND gates* in the microprocessor of a computer, in that they are only activated if they receive multiple converging signals (**Figure 15–12**).
7. *Coordination* of multiple responses in one cell can be achieved by a single extracellular signal. Some extracellular signal molecules, for example, stimulate a cell to both grow and divide. This coordination generally depends on mechanisms for distributing a signal to multiple effectors, by creating branches in the signaling pathway. In some cases, the branching of signaling pathways can allow one signal to *modulate* the strength of a response to other signals.

Given the complexity that arises from behaviors like signal integration, distribution, and feedback, it is clear that signaling systems rarely depend on a simple linear sequence of steps but are often more like a network, in which information flows not just forward but in multiple directions—and sometimes even backward. A major research challenge is to understand the nature of these networks and the response behaviors they can achieve.

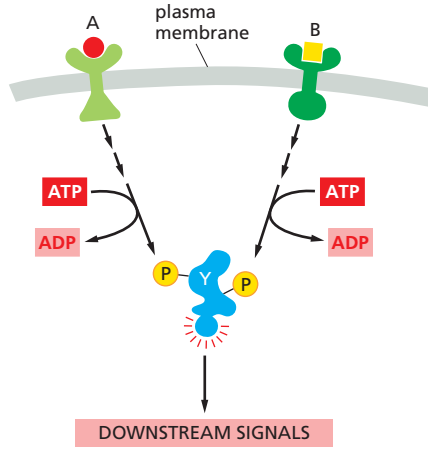


Figure 15–12 Signal integration. Extracellular signals A and B activate different intracellular signaling pathways, each of which leads to the phosphorylation of protein Y but at different sites on the protein. Protein Y is activated only when both of these sites are phosphorylated, and therefore it becomes active only when signals A and B are simultaneously present. Such proteins are often called coincidence detectors.

The Speed of a Response Depends on the Turnover of Signaling Molecules

The speed of any signaling response depends on the nature of the intracellular signaling molecules that carry out the target cell's response. When the response requires only changes in proteins already present in the cell, it can occur very rapidly: an allosteric change in a neurotransmitter-gated ion channel (discussed in Chapter 11), for example, can alter the plasma membrane electrical potential in milliseconds, and responses that depend solely on protein phosphorylation can occur within seconds. When the response involves changes in gene expression and the synthesis of new proteins, however, it usually requires many minutes or hours, regardless of the mode of signal delivery (**Figure 15–13**).

It is natural to think of intracellular signaling systems in terms of the changes produced when an extracellular signal is delivered. But it is just as important to consider what happens when the signal is withdrawn. During development, transient extracellular signals often produce lasting effects: they can trigger a change in the cell's development that persists indefinitely through cell memory mechanisms, as we discuss later (and in Chapters 7 and 22). In most cases in adult tissues, however, the response fades when a signal ceases. Often the effect is transient

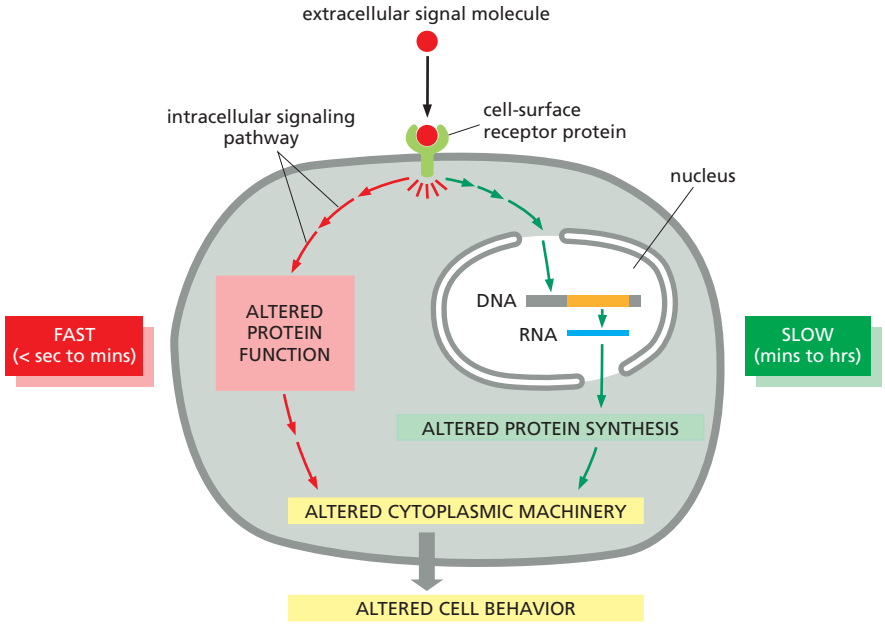


Figure 15–13 Slow and rapid responses to an extracellular signal. Certain types of signal-induced cellular responses, such as increased cell growth and division, involve changes in gene expression and the synthesis of new proteins; they therefore occur slowly, often starting an hour or more after the signal is received. Other responses—such as changes in cell movement, secretion, or metabolism—need not involve changes in gene transcription and therefore occur much more quickly, often starting in seconds or minutes; they may involve the rapid phosphorylation of effector proteins in the cytoplasm, for example. Synaptic responses mediated by changes in membrane potential are even quicker and can occur in milliseconds (not shown). Some signaling systems generate both rapid and slow responses as shown here, allowing the cell to respond quickly to a signal while simultaneously initiating a more long-term, persistent response.

because the signal exerts its effects by altering the concentrations of intracellular molecules that are short-lived (unstable), undergoing continual turnover. Thus, once the extracellular signal is gone, the degradation of the old molecules quickly wipes out all traces of the signal's action. It follows that the speed with which a cell responds to signal removal depends on the rate of destruction, or turnover, of the intracellular molecules that the signal affects.

It is also true, although much less obvious, that this turnover rate can determine the promptness of the response when an extracellular signal arrives. Consider, for example, two intracellular signaling molecules, X and Y, both of which are normally maintained at a steady-state concentration of 1000 molecules per cell. The cell synthesizes and degrades molecule Y at a rate of 100 molecules per second, with each molecule having an average lifetime of 10 seconds. Molecule X has a turnover rate that is 10 times slower than that of Y: it is both synthesized and degraded at a rate of 10 molecules per second, so that each molecule has an average lifetime in the cell of 100 seconds. If a signal acting on the cell causes a tenfold increase in the synthesis rates of both X and Y with no change in the molecular lifetimes, at the end of 1 second the concentration of Y will have increased by nearly 900 molecules per cell ($10 \times 100 - 100$), while the concentration of X will have increased by only 90 molecules per cell. In fact, after a molecule's synthesis rate has been either increased or decreased abruptly, the time required for the molecule to shift halfway from its old to its new equilibrium concentration is equal to its half-life—that is, equal to the time that would be required for its concentration to fall by half if all synthesis were stopped (**Figure 15–14**).

The same principles apply to proteins and small molecules, whether the molecules are in the extracellular space or inside cells. Many intracellular proteins have short half-lives, some surviving for less than 10 minutes. In most cases, these are key regulatory proteins whose concentrations are rapidly controlled in the cell by changes in their rates of synthesis.

As we have seen, many cell responses to extracellular signals depend on the conversion of intracellular signaling proteins from an inactive to an active form, rather than on their synthesis or degradation. Phosphorylation or the binding of GTP, for example, commonly activates signaling proteins. Even in these cases, however, the activation must be rapidly and continuously reversed (by dephosphorylation or GTP hydrolysis to GDP, respectively, in these examples) to make rapid signaling possible. These inactivation processes play a crucial part in determining the magnitude, rapidity, and duration of the response.

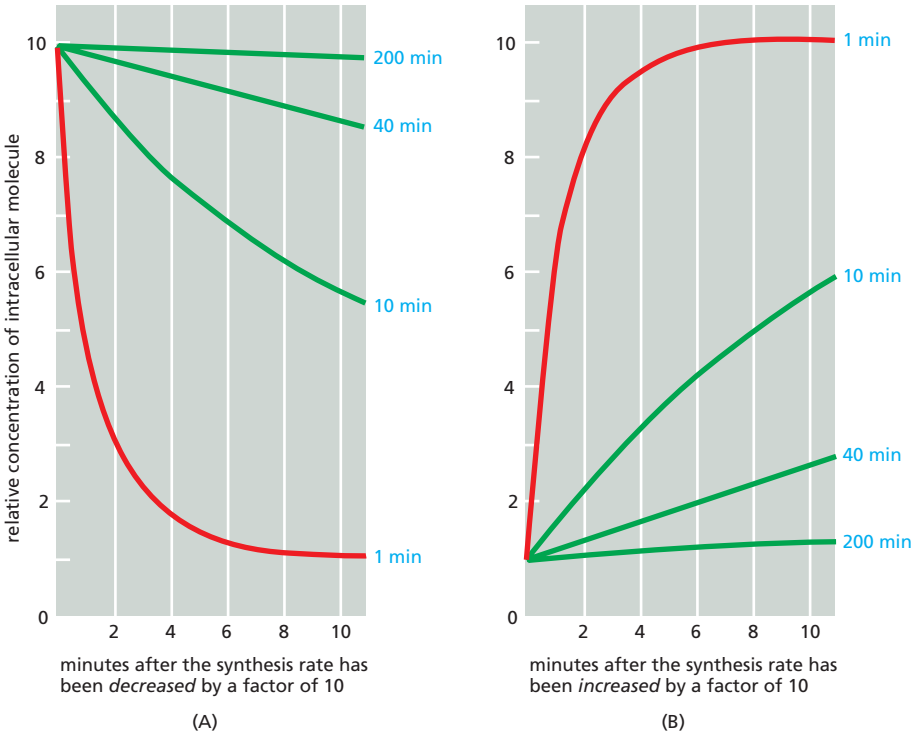


Figure 15–14 The importance of rapid turnover. The graphs show the predicted relative rates of change in the intracellular concentrations of molecules with differing turnover times when their synthesis rates are either (A) decreased or (B) increased suddenly by a factor of 10. In both cases, the concentrations of those molecules that are normally degraded rapidly in the cell *(red lines)* change quickly, whereas the concentrations of those that are normally degraded slowly *(green lines)* change proportionally more slowly. The numbers (in *blue)* on the right are the half-lives assumed for each of the different molecules.

Cells Can Respond Abruptly to a Gradually Increasing Signal

Some signaling systems are capable of generating a smoothly graded response over a wide range of extracellular signal concentrations (**Figure 15–15**, *blue* line); such systems are useful, for example, in the fine tuning of metabolic processes by some hormones. Other signaling systems generate significant responses only when the signal concentration rises beyond some threshold value. These abrupt responses are of two types. One is a *sigmoidal* response, in which low concentrations of stimulus do not have much effect, but then the response rises steeply and continuously at intermediate stimulus levels (Figure 15–15, *red* line). Such systems provide a filter to reduce inappropriate responses to low-level background signals but respond with high sensitivity when the stimulus falls within a small range of physiological signal concentrations. A second type of abrupt response is the *discontinuous* or *all-or-none* response, in which the response switches on completely (and often irreversibly) when the signal reaches some threshold concentration (Figure 15–15, *green* line). Such responses are particularly useful for controlling the choice between two alternative cell states, and they generally involve positive feedback, as we describe in more detail shortly.

Cells use a variety of molecular mechanisms to produce a sigmoidal response to increasing signal concentrations. In one mechanism, more than one intracellular signaling molecule must bind to its downstream target protein to induce a response. As we discuss later, for example, four molecules of the second messenger cyclic AMP must bind simultaneously to each molecule of *cyclic-AMP-dependent protein kinase* (*PKA*) to activate the kinase. A similar sharpening of response is seen when the activation of an intracellular signaling protein requires phosphorylation at more than one site. Such responses become sharper as the number of required molecules or phosphate groups increases, and if the number is large enough, responses become almost all-or-none (**Figure 15–16**).

Responses are also sharpened when an intracellular signaling molecule activates one enzyme and also inhibits another enzyme that catalyzes the opposite reaction. A well-studied example of this common type of regulation is the stimulation of glycogen breakdown in skeletal muscle cells induced by the hormone *adrenaline* (epinephrine). Adrenaline's binding to a G-protein-coupled

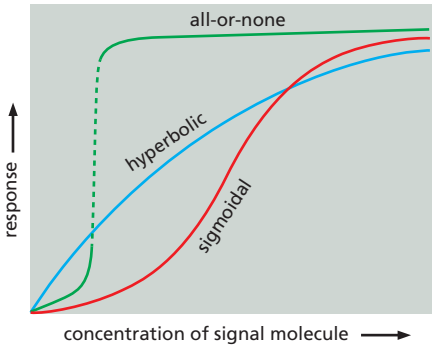


Figure 15–15 Signal processing can produce smoothly graded or switchlike responses. Some cell responses increase gradually as the concentration of extracellular signal molecule increases, eventually reaching a plateau as the signaling pathway is saturated, resulting in a *hyperbolic* response curve *(blue line)*. In other cases, the signaling system reduces the response at low signal concentrations and then produces a steeper response at some intermediate signal concentration—resulting in a *sigmoidal* response curve *(red line)*. In still other cases, the response is more abrupt and switchlike; the cell switches completely between a low and high response, without any stable intermediate response *(green line)*.

cell-surface receptor increases the intracellular concentration of cyclic AMP, which both activates an enzyme that promotes glycogen breakdown and inhibits an enzyme that promotes glycogen synthesis.

Positive Feedback Can Generate an All-or-None Response

Like intracellular metabolic pathways (discussed in Chapter 2) and the systems controlling gene activity (Chapter 7), most intracellular signaling systems incorporate feedback loops, in which the output of a process acts back to regulate that same process. We discussed the mathematical analysis of feedback loops in Chapter 8. In *positive feedback*, the output stimulates its own production; in *negative feedback*, the output inhibits its own production (**Figure 15–17**). Feedback loops are of great general importance in biology, and they regulate many chemical and physical processes in cells. Those that regulate cell signaling can either operate exclusively within the target cell or involve the secretion of extracellular signals. Here, we focus on those feedback loops that operate entirely within the target cell; even the simplest of these loops can produce complex and interesting effects.

Positive feedback in a signaling pathway can transform the behavior of the responding cell. If the positive feedback is of only moderate strength, its effect will be simply to steepen the response to the signal, generating a sigmoidal response like those described earlier; but if the feedback is strong enough, it can produce an all-or-none response (see Figure 15–15). This response goes hand in hand with a further property: once the responding system has switched to the high level of activation, this condition is often self-sustaining and can persist even after the

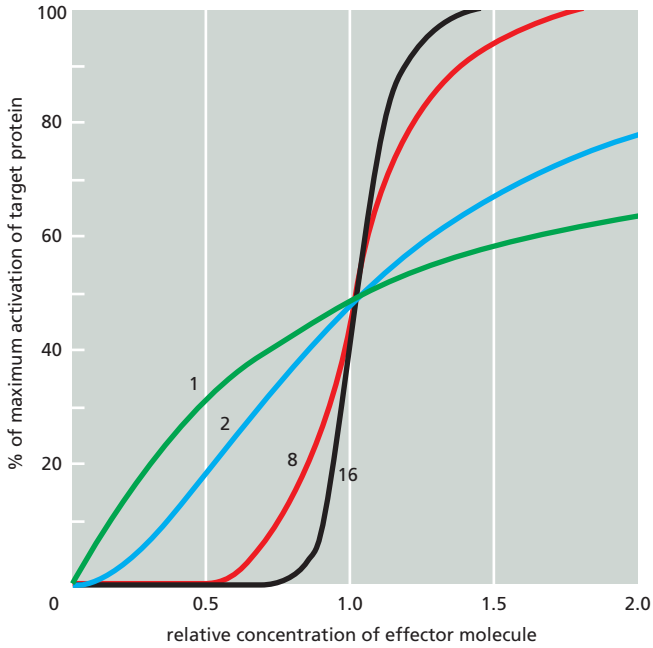


Figure 15–16 Activation curves for an allosteric protein as a function of effector molecule concentration. The curves show how the sharpness of the activation response increases with an increase in the number of allosteric effector molecules that must be bound simultaneously to activate the target protein. The curves shown are those expected, under certain conditions, if the activation requires the simultaneous binding of 1, 2, 8, or 16 effector molecules.

signal strength drops back below its critical value. In such a case, the system is said to be *bistable*: it can exist in either a "switched-off" or a "switched-on" state, and a transient stimulus can flip it from the one state to the other (**Figure 15–18**A and B).

Through positive feedback, a transient extracellular signal can induce long-term changes in cells and their progeny that can persist for the lifetime of the organism. The signals that trigger muscle-cell specification, for example, turn on the transcription of a series of genes that encode muscle-specific transcription regulatory proteins, which stimulate the transcription of their own genes, as well as genes encoding various other muscle-cell proteins; in this way, the decision to become a muscle cell is made permanent. This type of cell memory, which depends on positive feedback, is one of the basic ways in which a cell can undergo a lasting change of character without any alteration in its DNA sequence.

Studies of signaling responses in large populations of cells can give the false impression that a response is smoothly graded, even when strong positive feedback is causing an abrupt, discontinuous switch in the response in individual cells. Only by studying the response in single cells is it possible to see its all-or-none character (**Figure 15–19**). The misleading smooth response in a cell population is due to the random, intrinsic variability in signaling systems that we described earlier: all cells in a population do not respond identically to the same concentration of extracellular signal, especially at intermediate signal concentrations where the receptor is only partially occupied.

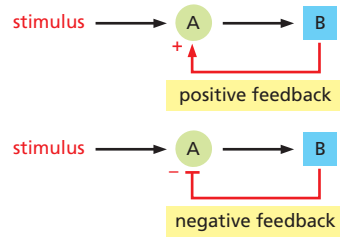


Figure 15–17 Positive and negative feedback. In these simple examples, a stimulus activates protein A, which, in turn, activates protein B. Protein B then acts back to either increase or decrease the activity of A.

Negative Feedback is a Common Motif in Signaling Systems

By contrast with positive feedback, negative feedback counteracts the effect of a stimulus and thereby abbreviates and limits the level of the response, making the system less sensitive to perturbations (see Chapter 8). As with positive feedback, however, qualitatively different responses can be obtained when the feedback

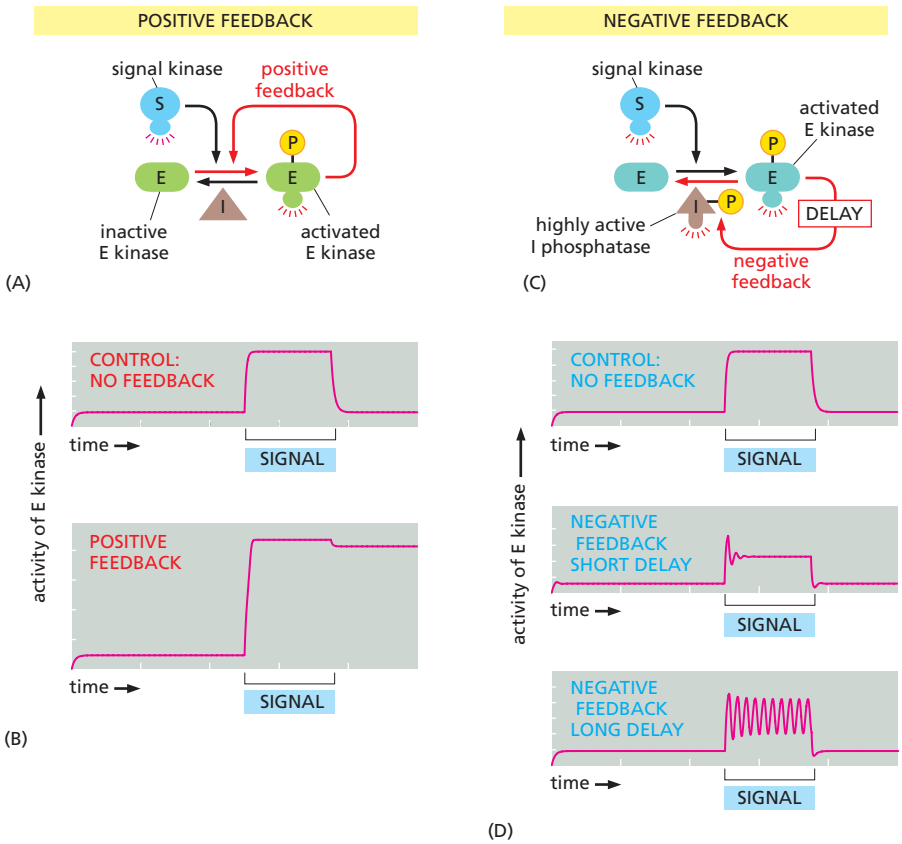


Figure 15–18 Some effects of simple feedback. The graphs show the computed effects of simple positive and negative feedback loops (see Chapter 8). In each case, the input signal is an activated protein kinase (S) that phosphorylates and thereby activates another protein kinase (E); a protein phosphatase (I) dephosphorylates and inactivates the activated E kinase. In the graphs, the *red* line indicates the activity of the E kinase over time; the underlying *blue* bar indicates the time for which the input signal (activated S kinase) is present. (A) Diagram of the positive feedback loop, in which the activated E kinase acts back to promote its own phosphorylation and activation; the basal activity of the I phosphatase dephosphorylates activated E at a steady, low rate. (B) The top graph shows that, without feedback, the activity of the E kinase is simply proportional (with a short lag) to the level of stimulation by the S kinase. The bottom graph shows that, with the positive feedback loop, the transient stimulation by S kinase switches the system from an "off" state to an "on" state, which then persists after the stimulus has been removed. (C) Diagram of the negative feedback loop, in which the activated E kinase phosphorylates and activates the I phosphatase, thereby increasing the rate at which the phosphatase dephosphorylates and inactivates the phosphorylated E kinase. (D) The top graph shows, again, the response in E kinase activity without feedback. The other graphs show the effects on E kinase activity of negative feedback operating after a short or long delay. With a short delay, the system shows a strong, brief response when the signal is abruptly changed, and the feedback then drives the response back down to a lower level. With a long delay, the feedback produces sustained oscillations for as long as the stimulus is present.

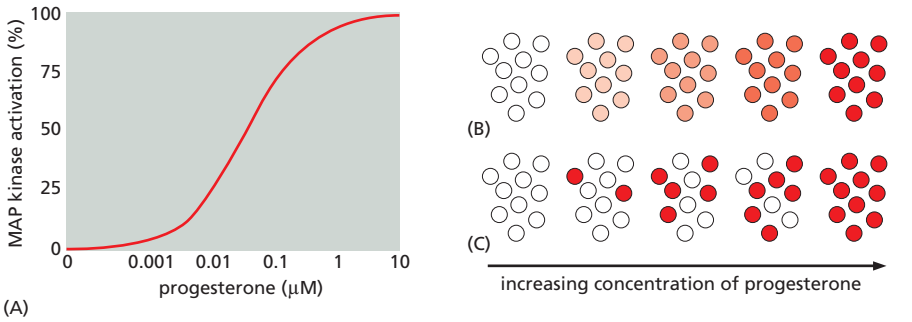


Figure 15–19 The importance of examining individual cells to detect all-or-none responses to increasing concentrations of an extracellular signal. In these experiments, immature frog eggs (oocytes) were stimulated with increasing concentrations of the hormone progesterone. The response was assessed by analyzing the activation of *MAP kinase* (discussed later), which is one of the protein kinases activated by phosphorylation in the response. The amount of phosphorylated (activated) MAP kinase in extracts of the oocytes was assessed biochemically. In (A), extracts of populations of stimulated oocytes were analyzed, and the activation of MAP kinase appeared to increase progressively with increasing progesterone concentration. There are two possible ways of explaining this result: (B) MAP kinase could have increased gradually in each individual cell with increasing progesterone concentration; or (C) individual cells could have responded in an all-or-none way, with the gradual increase in total MAP kinase activation reflecting the increasing number of cells responding with increasing progesterone concentration. When extracts of individual oocytes were analyzed, it was found that cells had either very low amounts or very high amounts, but not intermediate amounts, of the activated kinase, indicating that the response was essentially all-or-none at the level of individual cells, as diagrammed in (C). Subsequent studies revealed that this all-or-none response is due in part to strong positive feedback in the progesterone signaling system. (Adapted from J.E. Ferrell and E.M. Machleder, *Science* 280:895–898, 1998. With permission from AAAS.)

operates more powerfully. A delayed negative feedback with a long enough delay can produce responses that oscillate. The oscillations may persist for as long as the stimulus is present (Figure 15–18C and D) or they may even be generated spontaneously, without need of an external signal to drive them. Many such oscillators also contain positive feedback loops that generate sharper oscillations. Later in this chapter, we will encounter specific examples of oscillatory behavior in the intracellular responses to extracellular signals; all of them depend on negative feedback, generally accompanied by positive feedback.

If negative feedback operates with a short delay, the system behaves like a change detector. It gives a strong response to a stimulus, but the response rapidly decays even while the stimulus persists; if the stimulus is suddenly increased, however, the system responds strongly again, but, again, the response rapidly decays. This is the phenomenon of *adaptation*, which we now discuss.

Cells Can Adjust Their Sensitivity to a Signal

In responding to many types of stimuli, cells and organisms are able to detect the same percentage of change in a signal over a wide range of stimulus strengths. The target cells accomplish this through a reversible process of **adaptation**, or **desensitization**, whereby a prolonged exposure to a stimulus decreases the cells' response to that level of stimulus. In chemical signaling, adaptation enables cells to respond to *changes* in the concentration of an extracellular signal molecule (rather than to the absolute concentration of the signal) over a very wide range of signal concentrations. The underlying mechanism is negative feedback that operates with a short delay: a strong response modifies the signaling machinery involved, such that the machinery resets itself to become less responsive to the same level of signal (see Figure 15–18D, middle graph). Owing to the delay, however, a sudden increase in the signal is able to stimulate the cell again for a short period before the negative feedback has time to kick in.

Adaptation to a signal molecule can occur in various ways. It can result from inactivation of the receptors themselves. The binding of signal molecules to cell-surface receptors, for example, may induce the endocytosis and temporary sequestration of the receptors in endosomes. In some cases, such signal-induced receptor endocytosis leads to the destruction of the receptors in lysosomes, a process referred to as *receptor down-regulation* (in other cases, however, activated receptors continue to signal after they have been endocytosed). Receptors can also become inactivated on the cell surface—for example, by becoming phosphorylated—with a short delay following their activation. Adaptation can also occur at sites downstream of the receptors, either by a change in intracellular signaling proteins involved in transducing the extracellular signal or by the production of an inhibitor protein that blocks the signal transduction process. These various adaptation mechanisms are compared in **Figure 15–20**.

Though bewildering in their complexity, the multiple cross-regulatory signaling pathways and feedback loops that we describe in this chapter are not just a haphazard tangle, but a highly evolved system for processing and interpreting

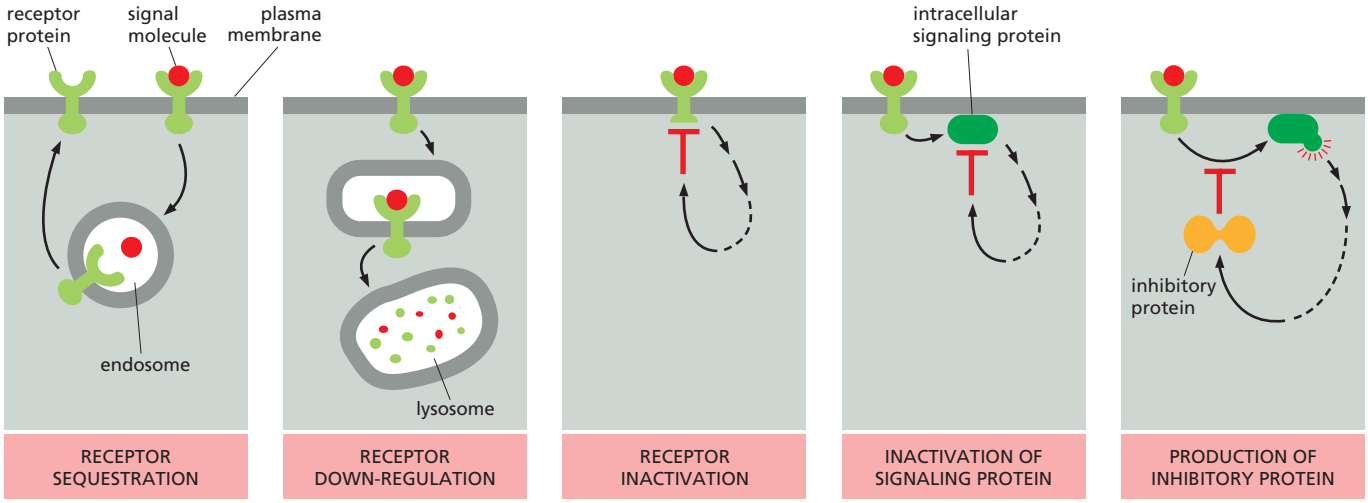


Figure 15–20 Some ways in which target cells can become adapted (desensitized) to an extracellular signal molecule. The mechanisms shown here that operate at the level of the receptor often involve phosphorylation or ubiquitylation of the receptor proteins.

the vast number of signals that impinge upon animal cells. The whole molecular control network, leading from the receptors at the cell surface to the genes in the nucleus, can be viewed as a computing device; and, like that other biological computing device, the brain, it presents one of the hardest problems in biology. We can identify the components and discover how they work individually. We can understand how small subsets of components work together as regulatory modules, noise filters, or adaptation mechanisms, as we have seen. However, it is a much more difficult task to understand how the system works as a whole. This is not only because the system is complex; it is also because the way in which it behaves is strongly dependent on the quantitative details of the molecular interactions, and, for most animal cells, we have only rough qualitative information. A major challenge for the future of signaling research is to develop more sophisticated quantitative and computational methods for the analysis of signaling systems, as described in Chapter 8.

Summary

Each cell in a multicellular animal is programmed to respond to a specific set of extracellular signal molecules produced by other cells. The signal molecules act by binding to a complementary set of receptor proteins expressed by the target cells. Most extracellular signal molecules activate cell-surface receptor proteins, which act as signal transducers, converting the extracellular signal into intracellular ones that alter the behavior of the target cell. Activated receptors relay the signal into the cell interior by activating intracellular signaling proteins. Some of these signaling proteins transduce, amplify, or spread the signal as they relay it, while others integrate signals from different signaling pathways. Some function as switches that are transiently activated by phosphorylation or GTP binding. Large signaling complexes form by means of modular interaction domains in the signaling proteins, which allow the proteins to form functional signaling networks.

Target cells use various mechanisms, including feedback loops, to adjust the ways in which they respond to extracellular signals. Positive feedback loops can help cells to respond in an all-or-none fashion to a gradually increasing concentration of an extracellular signal and to convert a short-lasting signal into a long-lasting, or even irreversible, response. Negative feedback allows cells to adapt to a signal molecule, which enables them to respond to small changes in the concentration of the signal molecule over a large concentration range.

SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

G-protein-coupled receptors (**GPCRs**) form the largest family of cell-surface receptors, and they mediate most responses to signals from the external world, as well as signals from other cells, including hormones, neurotransmitters, and local mediators. Our senses of sight, smell, and taste depend on them. There are more than 800 GPCRs in humans, and in mice there are about 1000 concerned with the sense of smell alone. The signal molecules that act on GPCRs are as varied in structure as they are in function and include proteins and small peptides, as well as derivatives of amino acids and fatty acids, not to mention photons of light and all the molecules that we can smell or taste. The same signal molecule can activate many different GPCR family members; for example, adrenaline activates at least 9 distinct GPCRs, acetylcholine another 5, and the neurotransmitter serotonin at least 14. The different receptors for the same signal are usually expressed in different cell types and elicit different responses.

Despite the chemical and functional diversity of the signal molecules that activate them, all GPCRs have a similar structure. They consist of a single polypeptide chain that threads back and forth across the lipid bilayer seven times, forming a cylindrical structure, often with a deep ligand-binding site at its center (**Figure 15–21**). In addition to their characteristic orientation in the plasma membrane, they all use G proteins to relay the signal into the cell interior.

The GPCR superfamily includes *rhodopsin*, the light-activated protein in the vertebrate eye, as well as the large number of olfactory receptors in the vertebrate nose. Other family members are found in unicellular organisms: the receptors in yeasts that recognize secreted mating factors are an example. It is likely that the GPCRs that mediate cell–cell signaling in multicellular organisms evolved from the sensory receptors in their unicellular eukaryotic ancestors.

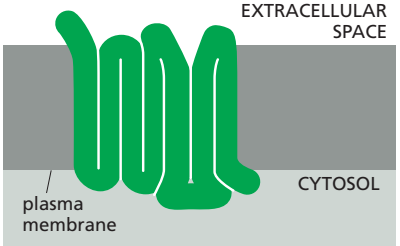
It is remarkable that almost half of all known drugs work through GPCRs or the signaling pathways GPCRs activate. Of the many hundreds of genes in the human genome that encode GPCRs, about 150 encode orphan receptors, for which the ligand is unknown. Many of them are likely targets for new drugs that remain to be discovered.

Trimeric G Proteins Relay Signals From GPCRs

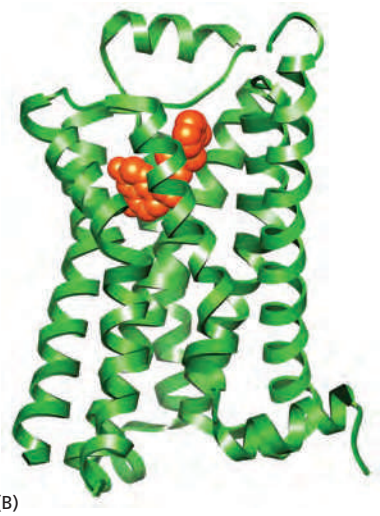
When an extracellular signal molecule binds to a GPCR, the receptor undergoes a conformational change that enables it to activate a **trimeric GTP-binding protein** (**G protein**), which couples the receptor to enzymes or ion channels in the membrane. In some cases, the G protein is physically associated with the receptor before the receptor is activated, whereas in others it binds only after receptor activation. There are various types of G proteins, each specific for a particular set of GPCRs and for a particular set of target proteins in the plasma membrane. They all have a similar structure, however, and operate similarly.

G proteins are composed of three protein subunits—α, β, and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive (**Figure 15–22**). When a GPCR is activated, it acts like a guanine nucleotide exchange factor (GEF) and induces the α subunit to release its bound GDP, allowing GTP to bind in its place. GTP binding then causes an activating conformational change in the Gα subunit, releasing the G protein from the receptor and triggering dissociation of the GTP-bound Gα subunit from the Gβγ pair—both of which then interact with various targets, such as enzymes and ion channels in the plasma membrane, which relay the signal onward (**Figure 15–23**).

The α subunit is a GTPase and becomes inactive when it hydrolyzes its bound GTP to GDP. The time required for GTP hydrolysis is usually short because the GTPase activity is greatly enhanced by the binding of the α subunit to a second protein, which can be either the target protein or a specific **regulator of G protein signaling** (**RGS**). RGS proteins act as α-subunit-specific GTPase-activating proteins (GAPs) (see Figure 15–8), and they help shut off G-protein-mediated responses in all eukaryotes. There are about 25 RGS proteins encoded in the human genome, each of which interacts with a particular set of G proteins.



(A)



(B)

Figure 15–21 A G-protein-coupled receptor (GPCR). (A) GPCRs that bind small ligands such as adrenaline have small extracellular domains, and the ligand usually binds deep within the plane of the membrane to a site that is formed by amino acids from several transmembrane segments. GPCRs that bind protein ligands have a large extracellular domain (not shown here) that contributes to ligand binding. (B) The structure of the β_2-adrenergic receptor, a receptor for the neurotransmitter adrenaline, illustrates the typical cylindrical arrangement of the seven transmembrane helices in a GPCR. The ligand *(orange)* binds in a pocket between the helices, resulting in conformational changes on the cytoplasmic surface of the receptor that promote G-protein activation (not shown). (PDB code: 3P0G.)

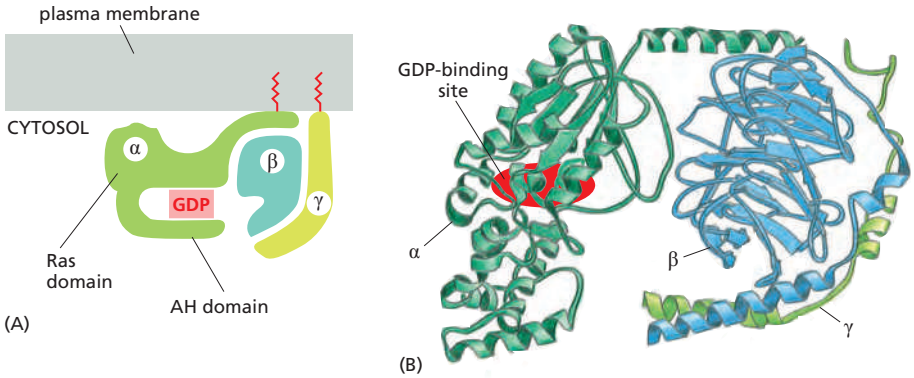


Figure 15–22 The structure of an inactive G protein. (A) Note that both the α and the γ subunits have covalently attached lipid molecules *(red tails)* that help bind them to the plasma membrane, and the α subunit has GDP bound. (B) The three-dimensional structure of the inactive, GDP-bound form of a G protein called G_s, which interacts with numerous GPCRs, including the β_2-adrenergic receptor shown in Figures 15–21 and 15–23. The α subunit contains the GTPase domain and binds to one side of the β subunit. The γ subunit binds to the opposite side of the β subunit, and the β and γ subunits together form a single functional unit. The GTPase domain of the α subunit contains two major subdomains: the "Ras" domain, which is related to other GTPases and provides one face of the nucleotide-binding pocket; and the alpha-helical or "AH" domain, which clamps the nucleotide in place. (B, based on D.G. Lombright et al., *Nature* 379:311–319, 1996. With permission from Macmillan Publishers Ltd.)

Some G Proteins Regulate the Production of Cyclic AMP

Cyclic AMP (cAMP) acts as a second messenger in some signaling pathways. An extracellular signal can increase cAMP concentration more than twentyfold in seconds (**Figure 15–24**). As explained earlier (see Figure 15–14), such a rapid response requires balancing a rapid synthesis of the molecule with its rapid breakdown or removal. Cyclic AMP is synthesized from ATP by an enzyme called

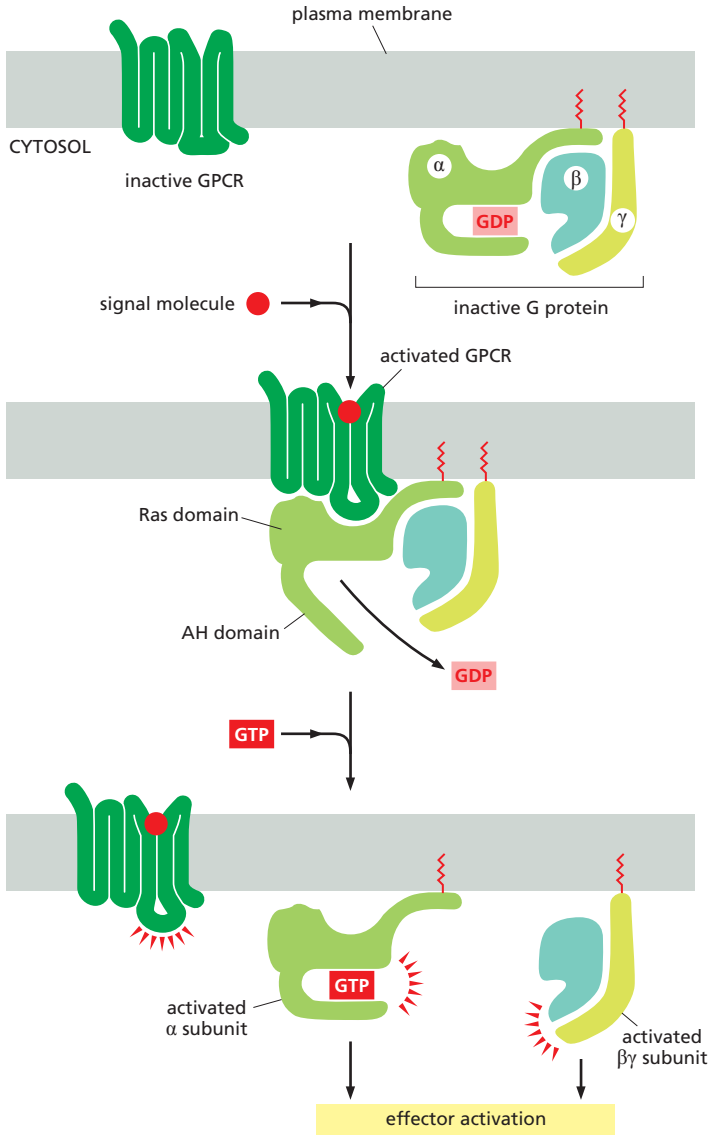


Figure 15–23 Activation of a G protein by an activated GPCR. Binding of an extracellular signal molecule to a GPCR changes the conformation of the receptor, which allows the receptor to bind and alter the conformation of a trimeric G protein. The AH domain of the G protein α subunit moves outward to open the nucleotide-binding site, thereby promoting dissociation of GDP. GTP binding then promotes closure of the nucleotide-binding site, triggering conformational changes that cause dissociation of the α subunit from the receptor and from the βγ complex. The GTP-bound α subunit and the βγ complex each regulate the activities of downstream signaling molecules (not shown). The receptor stays active while the extracellular signal molecule is bound to it, and it can therefore catalyze the activation of many G-protein molecules (**Movie 15.1**).

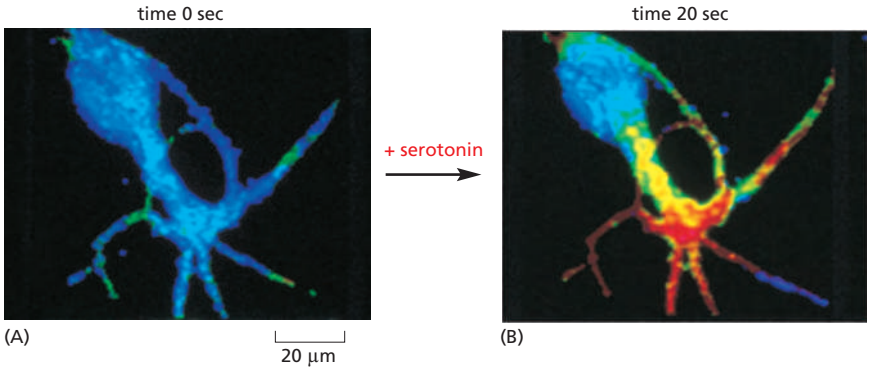


Figure 15–24 An increase in cyclic AMP in response to an extracellular signal. This nerve cell in culture is responding to the neurotransmitter serotonin, which acts through a GPCR to cause a rapid rise in the intracellular concentration of cyclic AMP. To monitor the cyclic AMP level, the cell has been loaded with a fluorescent protein that changes its fluorescence when it binds cyclic AMP. *Blue* indicates a low level of cyclic AMP, *yellow* an intermediate level, and *red* a high level. (A) In the resting cell, the cyclic AMP level is about 5×10^{-8} M. (B) Twenty seconds after the addition of serotonin to the culture medium, the intracellular level of cyclic AMP has increased to more than 10^{-6} M in the relevant parts of the cell, an increase of more than twentyfold. (From B.J. Bacskai et al., *Science* 260:222–226, 1993. With permission from AAAS.)

adenylyl cyclase, and it is rapidly and continuously destroyed by **cyclic AMP phosphodiesterases** (Figure 15–25). Adenylyl cyclase is a large, multipass transmembrane protein with its catalytic domain on the cytosolic side of the plasma membrane. There are at least eight isoforms in mammals, most of which are regulated by both G proteins and Ca^{2+}.

Many extracellular signals work by increasing cAMP concentrations inside the cell. These signals activate GPCRs that are coupled to a **stimulatory G protein** ($\mathbf{G_s}$). The activated α subunit of G_s binds and thereby activates adenylyl cyclase. Other extracellular signals, acting through different GPCRs, reduce cAMP levels by activating an **inhibitory G protein** ($\mathbf{G_i}$), which then inhibits adenylyl cyclase.

Both G_s and G_i are targets for medically important bacterial toxins. *Cholera toxin*, which is produced by the bacterium that causes cholera, is an enzyme that catalyzes the transfer of ADP ribose from intracellular NAD^+ to the α subunit of G_s. This ADP ribosylation alters the α subunit so that it can no longer hydrolyze its bound GTP, causing it to remain in an active state that stimulates adenylyl cyclase indefinitely. The resulting prolonged elevation in cAMP concentration within intestinal epithelial cells causes a large efflux of Cl^- and water into the gut, thereby causing the severe diarrhea that characterizes cholera. *Pertussis toxin*, which is made by the bacterium that causes pertussis (whooping cough), catalyzes the ADP ribosylation of the α subunit of G_i, preventing the protein from interacting with receptors; as a result, the G protein remains in the inactive GDP-bound state and is unable to regulate its target proteins. These two toxins are widely used in experiments to determine whether a cell's GPCR-dependent response to a signal is mediated by G_s or by G_i.

Some of the responses mediated by a G_s-stimulated increase in cAMP concentration are listed in Table 15–1. As the table shows, different cell types respond differently to an increase in cAMP concentration. Some cell types, such as fat cells, activate adenylyl cyclase in response to multiple hormones, all of which thereby stimulate the breakdown of triglyceride (the storage form of fat) to fatty acids. Individuals with genetic defects in the G_s α subunit show decreased responses to certain hormones, resulting in metabolic abnormalities, abnormal bone development, and mental retardation.

Cyclic-AMP-Dependent Protein Kinase (PKA) Mediates Most of the Effects of Cyclic AMP

In most animal cells, cAMP exerts its effects mainly by activating **cyclic-AMP-dependent protein kinase** (**PKA**). This kinase phosphorylates specific serines or

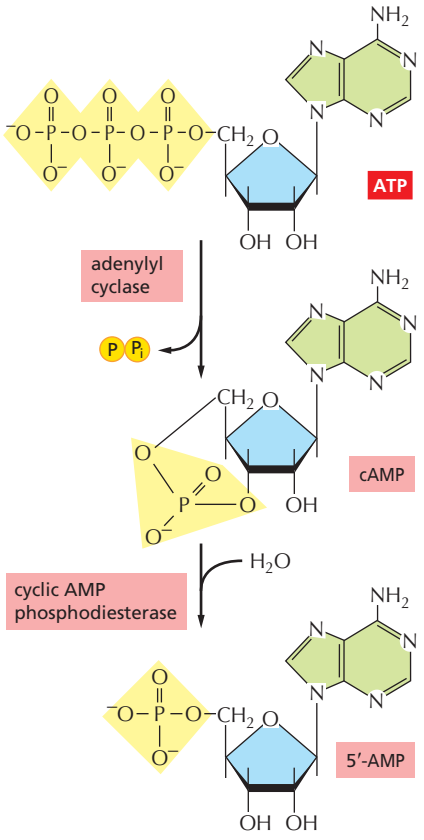


Figure 15–25 The synthesis and degradation of cyclic AMP. In a reaction catalyzed by the enzyme adenylyl cyclase, cyclic AMP (cAMP) is synthesized from ATP through a cyclization reaction that removes two phosphate groups as pyrophosphate (PP_i); a pyrophosphatase drives this synthesis by hydrolyzing the released pyrophosphate to phosphate (not shown). Cyclic AMP is short-lived (unstable) in the cell because it is hydrolyzed by specific phosphodiesterases to form 5′-AMP, as indicated.

TABLE 15–1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP

Target tissue	Hormone	Major response
Thyroid gland	Thyroid-stimulating hormone (TSH)	Thyroid hormone synthesis and secretion
Adrenal cortex	Adrenocorticotrophic hormone (ACTH)	Cortisol secretion
Ovary	Luteinizing hormone (LH)	Progesterone secretion
Muscle	Adrenaline	Glycogen breakdown
Bone	Parathormone	Bone resorption
Heart	Adrenaline	Increase in heart rate and force of contraction
Liver	Glucagon	Glycogen breakdown
Kidney	Vasopressin	Water resorption
Fat	Adrenaline, ACTH, glucagon, TSH	Triglyceride breakdown

threonines on selected target proteins, including intracellular signaling proteins and effector proteins, thereby regulating their activity. The target proteins differ from one cell type to another, which explains why the effects of cAMP vary so markedly depending on the cell type (see Table 15–1).

In the inactive state, PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cAMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex. The released catalytic subunits are thereby activated to phosphorylate specific target proteins (**Figure 15–26**). The regulatory subunits of PKA (also called A-kinase) are important for localizing the kinase inside the cell: special *A-kinase anchoring proteins* (*AKAPs*) bind both to the regulatory subunits and to a component of the cytoskeleton or a membrane of an organelle, thereby tethering the enzyme complex to a particular subcellular compartment. Some AKAPs also bind other signaling proteins, forming a signaling complex. An AKAP located around the nucleus of heart muscle cells, for example, binds both PKA and a phosphodiesterase that hydrolyzes cAMP. In unstimulated cells, the phosphodiesterase keeps the local cAMP concentration low, so that the bound PKA is inactive; in stimulated cells, cAMP concentration rapidly rises, overwhelming the phosphodiesterase and activating the PKA. Among the target proteins that PKA phosphorylates and activates in these cells is the adjacent phosphodiesterase, which rapidly lowers the cAMP concentration again. This negative feedback arrangement converts what might otherwise be a prolonged PKA response into a brief, local pulse of PKA activity.

Whereas some responses mediated by cAMP occur within seconds (see Figure 15–24), others depend on changes in the transcription of specific genes and take hours to develop fully. In cells that secrete the peptide hormone *somatostatin*,

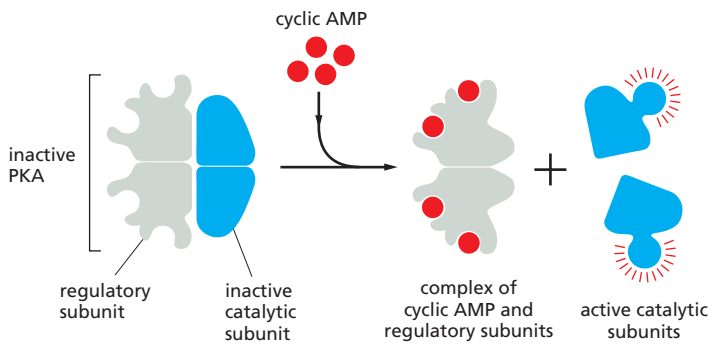


Figure 15–26 The activation of cyclic-AMP-dependent protein kinase (PKA). The binding of cAMP to the regulatory subunits of the PKA tetramer induces a conformational change, causing these subunits to dissociate from the catalytic subunits, thereby activating the kinase activity of the catalytic subunits. The release of the catalytic subunits requires the binding of more than two cAMP molecules to the regulatory subunits in the tetramer. This requirement greatly sharpens the response of the kinase to changes in cAMP concentration, as discussed earlier (see Figure 15–16). Mammalian cells have at least two types of PKAs: type I is mainly in the cytosol, whereas type II is bound via its regulatory subunits and special anchoring proteins to the plasma membrane, nuclear membrane, mitochondrial outer membrane, and microtubules. In both types, once the catalytic subunits are freed and active, they can migrate into the nucleus (where they can phosphorylate transcription regulatory proteins), while the regulatory subunits remain in the cytoplasm.

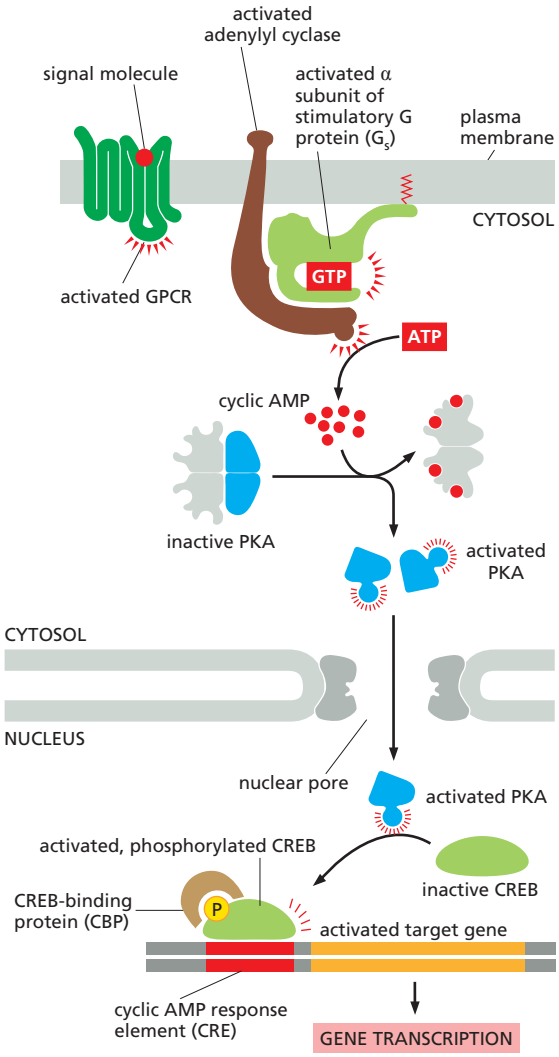


Figure 15–27 How a rise in intracellular cyclic AMP concentration can alter gene transcription. The binding of an extracellular signal molecule to its GPCR activates adenylyl cyclase via G_s and thereby increases cAMP concentration in the cytosol. This rise activates PKA, and the released catalytic subunits of PKA can then enter the nucleus, where they phosphorylate the transcription regulatory protein CREB. Once phosphorylated, CREB recruits the coactivator CBP, which stimulates gene transcription. In some cases, at least, the inactive CREB protein is bound to the cyclic AMP response element (CRE) in DNA before it is phosphorylated (not shown). See Movie 15.2.

for example, cAMP activates the gene that encodes this hormone. The regulatory region of the somatostatin gene contains a short *cis*-regulatory sequence, called the *cyclic AMP response element* (*CRE*), which is also found in the regulatory region of many other genes activated by cAMP. A specific transcription regulator called **CRE-binding (CREB) protein** recognizes this sequence. When PKA is activated by cAMP, it phosphorylates CREB on a single serine; phosphorylated CREB then recruits a transcriptional coactivator called *CREB-binding protein* (*CBP*), which stimulates the transcription of the target genes (Figure 15–27). Thus, CREB can transform a short cAMP signal into a long-term change in a cell, a process that, in the brain, is thought to play an important part in some forms of learning and memory.

Some G Proteins Signal Via Phospholipids

Many GPCRs exert their effects through G proteins that activate the plasma-membrane-bound enzyme **phospholipase C-β (PLCβ)**. Table 15–2 lists some examples of responses activated in this way. The phospholipase acts on a phosphorylated inositol phospholipid (a *phosphoinositide*) called **phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2]**, which is present in small amounts in the inner half of the plasma membrane lipid bilayer (Figure 15–28). Receptors that activate this **inositol phospholipid signaling pathway** mainly do so via a G protein called $\mathbf{G_q}$, which activates phospholipase C-β in much the same way that G_s activates adenylyl cyclase. The activated phospholipase then cleaves the PI(4,5)P_2 to generate two

TABLE 15–2 Some Cell Responses in Which GPCRs Activate PLCβ

Target tissue	Signal molecule	Major response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Muscle contraction
Blood platelets	Thrombin	Platelet aggregation

products: **inositol 1,4,5-trisphosphate** (IP_3) and **diacylglycerol**. At this step, the signaling pathway splits into two branches.

IP_3 is a water-soluble molecule that leaves the plasma membrane and diffuses rapidly through the cytosol. When it reaches the endoplasmic reticulum (ER), it binds to and opens **IP_3-gated Ca^{2+}-release channels** (also called **IP_3 receptors**) in the ER membrane. Ca^{2+} stored in the ER is released through the open channels, quickly raising the concentration of Ca^{2+} in the cytosol (**Figure 15–29**). The increase in cytosolic Ca^{2+} propagates the signal by influencing the activity of Ca^{2+}-sensitive intracellular proteins, as we describe shortly.

At the same time that the IP_3 produced by the hydrolysis of $PI(4,5)P_2$ is increasing the concentration of Ca^{2+} in the cytosol, the other cleavage product of the $PI(4,5)P_2$, diacylglycerol, is exerting different effects. It also acts as a second messenger, but it remains embedded in the plasma membrane, where it has several potential signaling roles. One of its major functions is to activate a protein kinase called **protein kinase C** (**PKC**), so named because it is Ca^{2+}-dependent. The initial rise in cytosolic Ca^{2+} induced by IP_3 alters the PKC so that it translocates from the cytosol to the cytoplasmic face of the plasma membrane. There it is activated by the combination of Ca^{2+}, diacylglycerol, and the negatively charged membrane phospholipid phosphatidylserine (see Figure 15–29). Once activated, PKC phosphorylates target proteins that vary depending on the cell type. The principles are the same as discussed earlier for PKA, although most of the target proteins are different.

Diacylglycerol can be further cleaved to release arachidonic acid, which can either act as a signal in its own right or be used in the synthesis of other small lipid signal molecules called *eicosanoids*. Most vertebrate cell types make eicosanoids, including *prostaglandins*, which have many biological activities. They participate

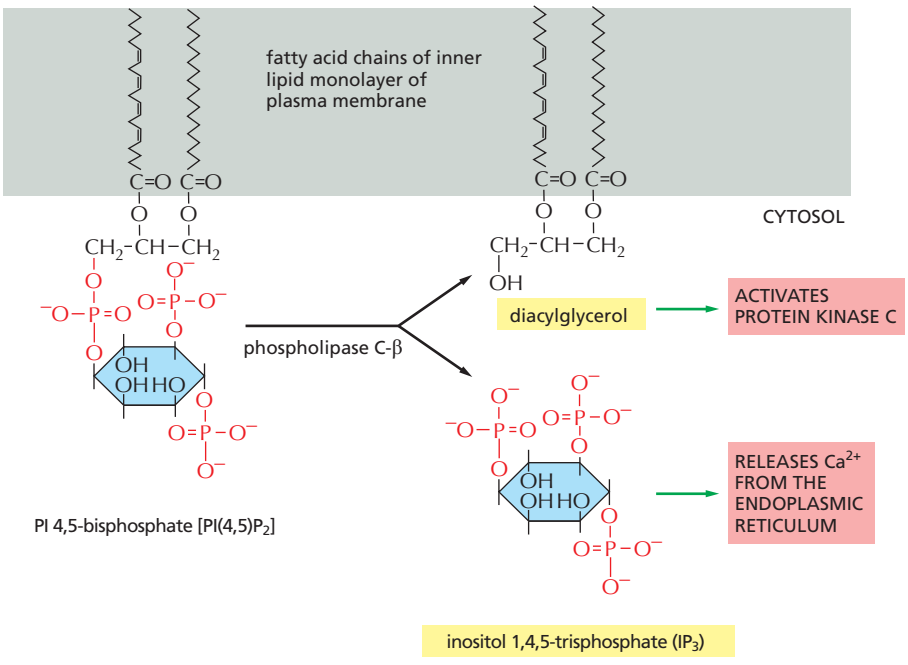


Figure 15–28 The hydrolysis of PI(4,5)P_2 by phospholipase C-β. Two second messengers are produced directly from the hydrolysis of $PI(4,5)P_2$: inositol 1,4,5-trisphosphate (IP_3), which diffuses through the cytosol and releases Ca^{2+} from the endoplasmic reticulum, and diacylglycerol, which remains in the membrane and helps to activate protein kinase C (PKC; see Figure 15–29). There are several classes of phospholipase C: these include the β class, which is activated by GPCRs; as we see later, the γ class is activated by a class of enzyme-coupled receptors called receptor tyrosine kinases (RTKs).

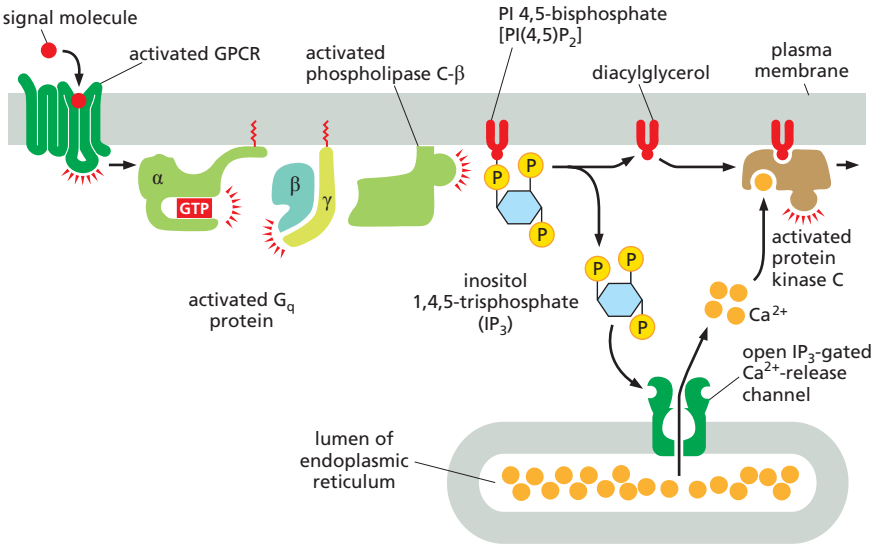


Figure 15–29 How GPCRs increase cytosolic Ca^{2+} and activate protein kinase C. The activated GPCR stimulates the plasma-membrane-bound phospholipase C-β (PLCβ) via a G protein called G_q. The α subunit and βγ complex of G_q are both involved in this activation. Two second messengers are produced when $PI(4,5)P_2$ is hydrolyzed by activated PLCβ. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and releases Ca^{2+} from the ER by binding to and opening IP_3-gated Ca^{2+}-release channels (IP_3 receptors) in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to escape into the cytosol when the release channels are opened. Diacylglycerol remains in the plasma membrane and, together with phosphatidylserine (not shown) and Ca^{2+}, helps to activate protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane. Of the 10 or more distinct isoforms of PKC in humans, at least 4 are activated by diacylglycerol (Movie 15.3).

in pain and inflammatory responses, for example, and many anti-inflammatory drugs (such as aspirin, ibuprofen, and cortisone) act in part by inhibiting their synthesis.

Ca^{2+} Functions as a Ubiquitous Intracellular Mediator

Many extracellular signals, and not just those that work via G proteins, trigger an increase in cytosolic Ca^{2+} concentration. In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including nerve cells, it triggers secretion. Ca^{2+} has numerous other functions in a variety of cell types. Ca^{2+} is such an effective signaling mediator because its concentration in the cytosol is normally very low (~10^{-7} M), whereas its concentration in the extracellular fluid (~10^{-3} M) and in the lumen of the ER [and sarcoplasmic reticulum (SR) in muscle] is high. Thus, there is a large gradient tending to drive Ca^{2+} into the cytosol across both the plasma membrane and the ER or SR membrane. When a signal transiently opens Ca^{2+} channels in these membranes, Ca^{2+} rushes into the cytosol, and the resulting 10–20-fold increase in the local Ca^{2+} concentration activates Ca^{2+}-responsive proteins in the cell.

Some stimuli, including membrane depolarization, membrane stretch, and certain extracellular signals, activate Ca^{2+} channels in the plasma membrane, resulting in Ca^{2+} influx from outside the cell. Other signals, including the GPCR-mediated signals described earlier, act primarily through IP_3 receptors to stimulate Ca^{2+} release from intracellular stores in the ER (see Figure 15–29). The ER membrane also contains a second type of regulated Ca^{2+} channel called the **ryanodine receptor** (so called because it is sensitive to the plant alkaloid ryanodine), which opens in response to rising Ca^{2+} levels and thereby amplifies the Ca^{2+} signal, as we describe shortly.

Several mechanisms rapidly terminate the Ca^{2+} signal and are also responsible for keeping the concentration of Ca^{2+} in the cytosol low in resting cells. Most importantly, there are Ca^{2+}-pumps in the plasma membrane and the ER membrane that use the energy of ATP hydrolysis to pump Ca^{2+} out of the cytosol. Cells such as muscle and nerve cells, which make extensive use of Ca^{2+} signaling, have an additional Ca^{2+} transporter (a Na^+-driven Ca^{2+} exchanger) in their plasma membrane that couples the efflux of Ca^{2+} to the influx of Na^+.

Feedback Generates Ca^{2+} Waves and Oscillations

The IP_3 receptors and ryanodine receptors of the ER membrane have an important feature: they are both stimulated by low to moderate cytoplasmic Ca^{2+} concentrations. This *Ca^{2+}-induced calcium release* (*CICR*) results in positive feedback,

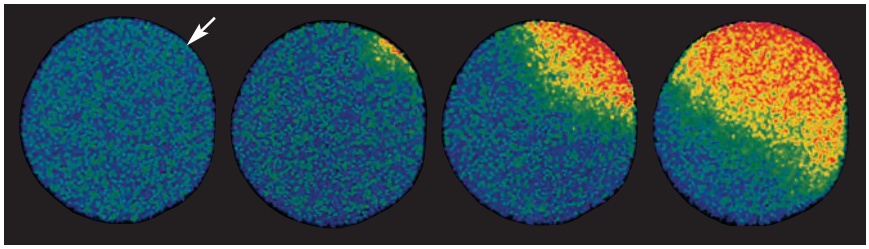


Figure 15–30 The fertilization of an egg by a sperm triggers a wave of cytosolic Ca^{2+}. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. A wave of cytosolic Ca^{2+} *(red)*, released from the ER, sweeps across the egg from the site of sperm entry *(arrow)*. This Ca^{2+} wave changes the egg cell surface, preventing the entry of other sperm, and it also initiates embryonic development (**Movie 15.5**). The initial increase in Ca^{2+} is thought to be caused by a sperm-specific form of PLC (PLCζ) that the sperm brings into the egg cytoplasm when it fuses with the egg; the PLCζ cleaves $PI(4,5)P_2$ to produce IP_3, which releases Ca^{2+} from the egg ER. The released Ca^{2+} stimulates further Ca^{2+} release from the ER, producing the spreading wave, as we explain in Figure 15–31. (Courtesy of Stephen A. Stricker.)

which has a major impact on the properties of the Ca^{2+} signal. The importance of this feedback is seen clearly in studies with Ca^{2+}-sensitive fluorescent indicators, such as *aequorin* or *fura-2* (discussed in Chapter 9), which allow researchers to monitor cytosolic Ca^{2+} in individual cells under a microscope (**Figure 15–30** and **Movie 15.4**).

When cells carrying a Ca^{2+} indicator are treated with a small amount of an extracellular signal molecule that stimulates IP_3 production, tiny bursts of Ca^{2+} are seen in one or more discrete regions of the cell. These Ca^{2+} puffs or sparks reflect the local opening of small groups of IP_3-gated Ca^{2+}-release channels in the ER. Because various Ca^{2+}-binding proteins act as Ca^{2+} buffers and restrict the diffusion of Ca^{2+}, the signal often remains localized to the site where the Ca^{2+} enters the cytosol. If the extracellular signal is sufficiently strong and persistent, however, the local Ca^{2+} concentration can reach a sufficient level to activate nearby IP_3 receptors and ryanodine receptors, resulting in a regenerative wave of Ca^{2+} release that moves through the cytosol (**Figure 15–31**), much like an action potential in an axon.

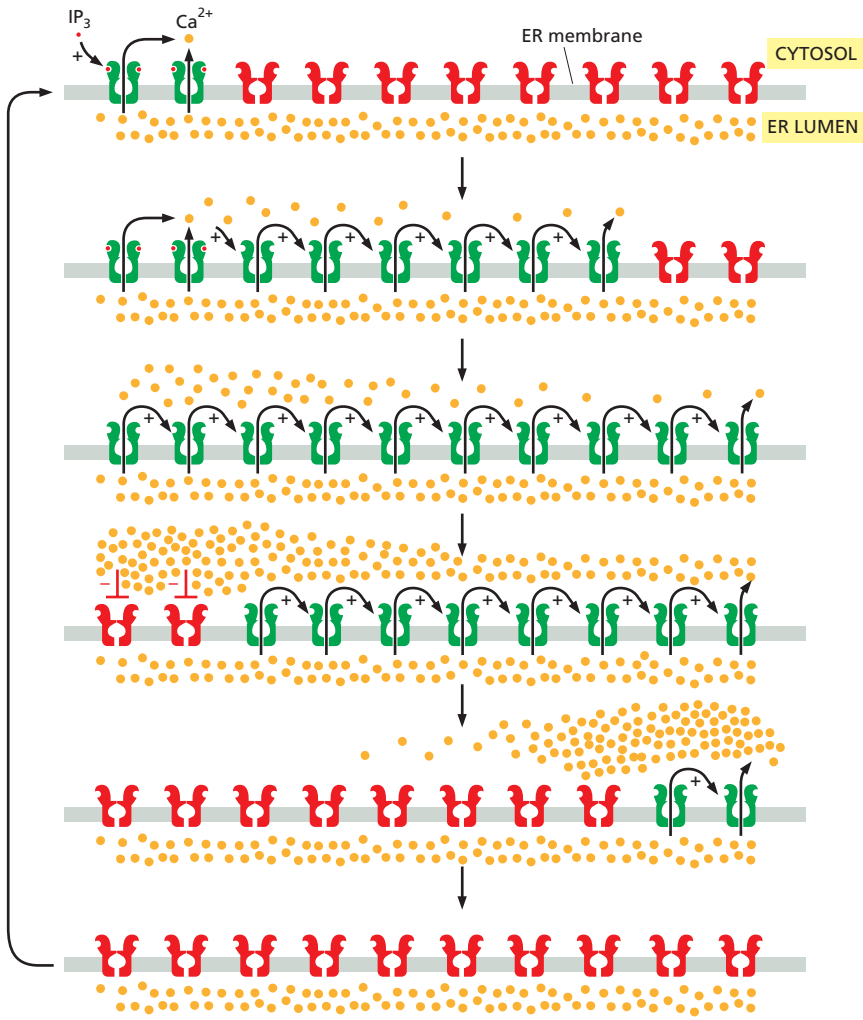


Figure 15–31 Positive and negative feedback produce Ca^{2+} waves and oscillations. This diagram shows IP_3 receptors and ryanodine receptors on a portion of the ER membrane: active receptors are in *green*; inactive receptors are in *red*. When a small amount of cytosolic IP_3 activates a cluster of IP_3 receptors at one site on the ER membrane (*top*), the local release of Ca^{2+} promotes the opening of nearby IP_3 and ryanodine receptors, resulting in more Ca^{2+} release. This positive feedback (indicated by *positive* signs) produces a regenerative wave of Ca^{2+} release that spreads across the cell (see Figure 15–30). These waves of Ca^{2+} release move more quickly across the cell than would be possible by simple diffusion. Also, unlike a diffusing burst of Ca^{2+} ions, which will become more dilute as it spreads, the regenerative wave produces a high Ca^{2+} concentration across the entire cell. Eventually, the local Ca^{2+} concentration inactivates IP_3 receptors and ryanodine receptors (*middle*; indicated by *red* negative signs), shutting down the Ca^{2+} release. Ca^{2+}-pumps reduce the local cytosolic Ca^{2+} concentration to its normal low levels. The result is a Ca^{2+} spike: positive feedback drives a rapid rise in cytosolic Ca^{2+}, and negative feedback sends it back down again. The Ca^{2+} channels remain refractory to further stimulation for some period of time, delaying the generation of another Ca^{2+} spike *(bottom)*. Eventually, however, the negative feedback wears off, allowing IP_3 to trigger another Ca^{2+} wave. The end result is repeated Ca^{2+} oscillations (see Figure 15–32). Under some conditions, these oscillations can be seen as repeating narrow waves of Ca^{2+} moving across the cell.

Another important property of IP_3 receptors and ryanodine receptors is that they are inhibited, after some delay, by high Ca^{2+} concentrations (a form of negative feedback). Thus, the rise in Ca^{2+} in a stimulated cell leads to inhibition of Ca^{2+} release; because Ca^{2+} pumps remove the cytosolic Ca^{2+}, the Ca^{2+} concentration falls (see Figure 15–31). The decline in Ca^{2+} eventually relieves the negative feedback, allowing cytosolic Ca^{2+} to rise again. As in other cases of delayed negative feedback (see Figure 15–18), the result is an oscillation in the Ca^{2+} concentration. These oscillations persist for as long as receptors are activated at the cell surface, and their frequency reflects the strength of the extracellular stimulus (**Figure 15–32**). The frequency, amplitude, and breadth of oscillations can also be modulated by other signaling mechanisms, such as phosphorylation, which influence the Ca^{2+} sensitivity of Ca^{2+} channels or affect other components in the signaling system.

The frequency of Ca^{2+} oscillations can be translated into a frequency-dependent cell response. In some cases, the frequency-dependent response itself is also oscillatory: in hormone-secreting pituitary cells, for example, stimulation by an extracellular signal induces repeated Ca^{2+} spikes, each of which is associated with a burst of hormone secretion. In other cases, the frequency-dependent response is non-oscillatory: in some types of cells, for instance, one frequency of Ca^{2+} spikes activates the transcription of one set of genes, while a higher frequency activates the transcription of a different set. How do cells sense the frequency of Ca^{2+} spikes and change their response accordingly? The mechanism presumably depends on Ca^{2+}-sensitive proteins that change their activity as a function of Ca^{2+}-spike frequency. A protein kinase that acts as a molecular memory device seems to have this remarkable property, as we discuss next.

Ca^{2+}/Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

Various Ca^{2+}-binding proteins help to relay the cytosolic Ca^{2+} signal. The most important is **calmodulin**, which is found in all eukaryotic cells and can constitute as much as 1% of a cell's total protein mass. Calmodulin functions as a multipurpose intracellular Ca^{2+} receptor, governing many Ca^{2+}-regulated processes. It consists of a highly conserved, single polypeptide chain with four high-affinity Ca^{2+}-binding sites (**Figure 15–33**A). When activated by Ca^{2+} binding, it undergoes a conformational change. Because two or more Ca^{2+} ions must bind before

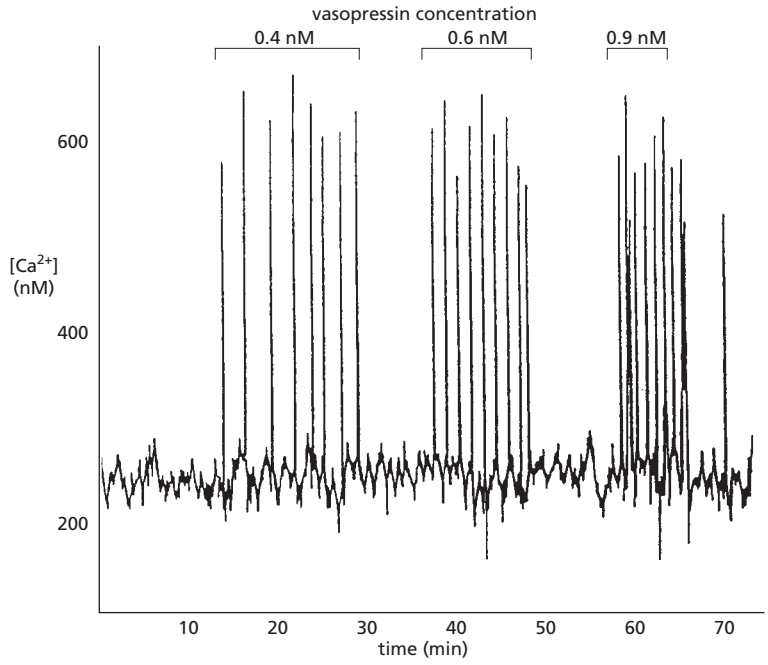


Figure 15–32 Vasopressin-induced Ca^{2+} oscillations in a liver cell. The cell was loaded with the Ca^{2+}-sensitive protein aequorin and then exposed to increasing concentrations of the peptide signal molecule *vasopressin*, which activates a GPCR and thereby PLCβ (see Table 15–2). Note that the frequency of the Ca^{2+} spikes increases with an increasing concentration of vasopressin but that the amplitude of the spikes is not affected. Each spike lasts about 7 seconds. (Adapted from N.M. Woods, K.S.R. Cuthbertson and P.H. Cobbold, *Nature* 319:600–602, 1986. With permission from Macmillan Publishers Ltd.)

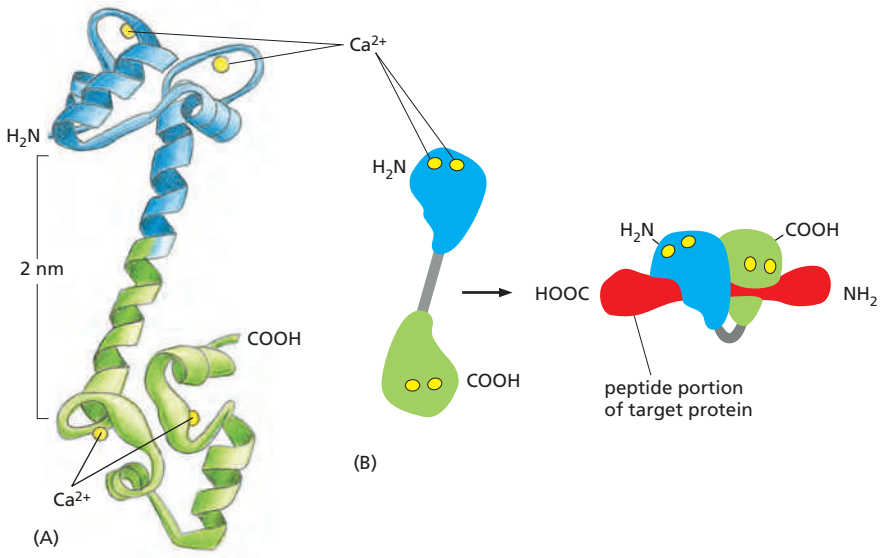


Figure 15–33 The structure of Ca^{2+}/calmodulin. (A) The molecule has a dumbbell shape, with two globular ends, which can bind to many target proteins. The globular ends are connected by a long, exposed α helix, which allows the protein to adopt a number of different conformations, depending on the target protein it interacts with. Each globular head has two Ca^{2+}-binding sites (**Movie 15.6**). (B) Shown is the major structural change that occurs in Ca^{2+}/calmodulin when it binds to a target protein (in this example, a peptide that consists of the Ca^{2+}/calmodulin-binding domain of a Ca^{2+}/calmodulin-dependent protein kinase). Note that the Ca^{2+}/calmodulin has "jack-knifed" to surround the peptide. When it binds to other targets, it can adopt different conformations. (A, based on x-ray crystallographic data from Y.S. Babu et al., *Nature* 315:37–40, 1985. With permission from Macmillan Publishers Ltd; B, based on x-ray crystallographic data from W.E. Meador, A.R. Means, and F.A. Quiocho, *Science* 257:1251–1255, 1992, and on nuclear magnetic resonance (NMR) spectroscopy data from M. Ikura et al., *Science* 256:632–638, 1992.)

calmodulin adopts its active conformation, the protein displays a sigmoidal response to increasing concentrations of Ca^{2+} (see Figure 15–16).

The allosteric activation of calmodulin by Ca^{2+} is analogous to the activation of PKA by cyclic AMP, except that Ca^{2+}/calmodulin has no enzymatic activity itself but instead acts by binding to and activating other proteins. In some cases, calmodulin serves as a permanent regulatory subunit of an enzyme complex, but usually the binding of Ca^{2+} instead enables calmodulin to bind to various target proteins in the cell to alter their activity.

When an activated molecule of Ca^{2+}/calmodulin binds to its target protein, the calmodulin further changes its conformation, the nature of which depends on the specific target protein (Figure 15–33B). Among the many targets calmodulin regulates are enzymes and membrane transport proteins. As one example, Ca^{2+}/calmodulin binds to and activates the plasma membrane Ca^{2+}-pump that uses ATP hydrolysis to pump Ca^{2+} out of cells. Thus, whenever the concentration of Ca^{2+} in the cytosol rises, the pump is activated, which helps to return the cytosolic Ca^{2+} level to resting levels.

Many effects of Ca^{2+}, however, are more indirect and are mediated by protein phosphorylations catalyzed by a family of protein kinases called **Ca^{2+}/calmodulin-dependent kinases** (**CaM-kinases**). Some CaM-kinases phosphorylate transcription regulators, such as the CREB protein (see Figure 15–27), and in this way activate or inhibit the transcription of specific genes.

One of the best-studied CaM-kinases is **CaM-kinase II**, which is found in most animal cells but is especially enriched in the nervous system. It constitutes up to 2% of the total protein mass in some regions of the brain, and it is highly concentrated in synapses. CaM-kinase II has several remarkable properties. To begin with, it has a spectacular quaternary structure: twelve copies of the enzyme are assembled into a stacked pair of rings, with kinase domains on the outside linked to a central hub (**Figure 15–34**). This structure helps the enzyme function as a molecular memory device, switching to an active state when exposed to Ca^{2+}/calmodulin and then remaining active even after the Ca^{2+} signal has decayed. This is because adjacent kinase subunits can phosphorylate each other (a process called *autophosphorylation*) when Ca^{2+}/calmodulin activates them (Figure 15–34). Once a kinase subunit is autophosphorylated, it remains active even in the absence of Ca^{2+}, thereby prolonging the duration of the kinase activity beyond that of the initial activating Ca^{2+} signal. The enzyme maintains this activity until a protein phosphatase removes the autophosphorylation and shuts the kinase off. CaM-kinase II activation can thereby serve as a memory trace of a prior Ca^{2+} pulse, and it seems to have a role in some types of memory and learning in the vertebrate nervous system. Mutant mice that lack a brain-specific form of the enzyme have specific defects in their ability to remember where things are.

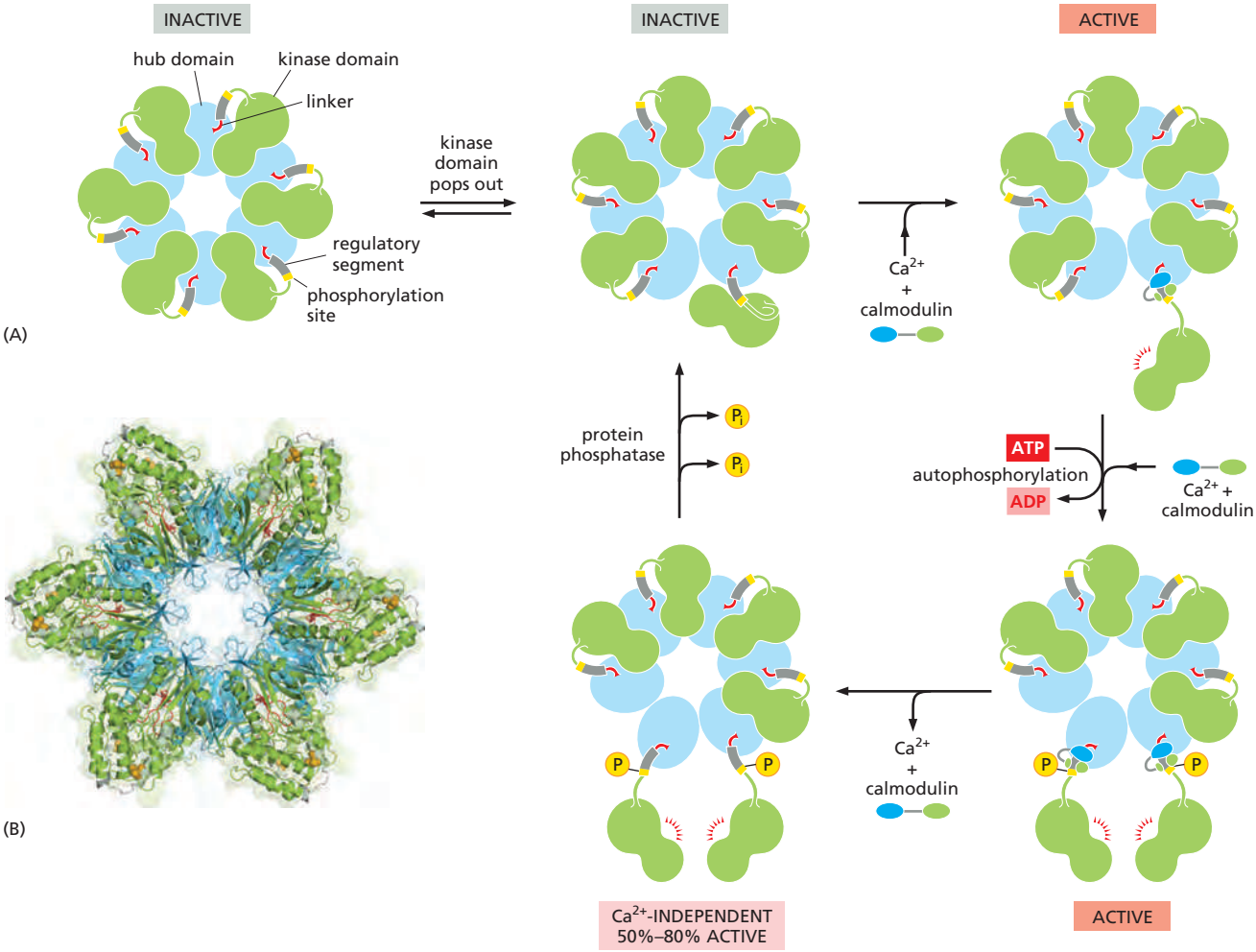


Figure 15–34 The stepwise activation of CaM-kinase II. (A) Each CaM-kinase II protein has two major domains: an amino-terminal kinase domain *(green)* and a carboxyl-terminal hub domain *(blue)*, linked by a regulatory segment. Six CaM-kinase II proteins are assembled into a giant ring in which the hub domains interact tightly to produce a central structure that is surrounded by kinase domains. The complete enzyme contains two stacked rings, for a total of 12 kinase proteins, but only one ring is shown here for clarity. When the enzyme is inactive, the ring exists in a dynamic equilibrium between two states. The first (*upper left*) is a compact state, in which the kinase domains interact with the hub, so that the regulatory segment is buried in the kinase active site and thereby blocks catalytic activity. In the second inactive state (*upper middle*), a kinase domain has popped out and is linked to the central hub by its regulatory segment, which continues to inhibit the kinase but is now accessible to Ca^{2+}/calmodulin. If present, Ca^{2+}/calmodulin will bind the regulatory segment and prevent it from inhibiting the kinase, thereby locking the kinase in an active state (*upper right*). If the adjacent kinase subunit also pops out from the hub, it will also be activated by Ca^{2+}/calmodulin, and the two kinases will then phosphorylate each other on their regulatory segments (*lower right*). This autophosphorylation further activates the enzyme. It also prolongs the activity of the enzyme in two ways. First, it traps the bound Ca^{2+}/calmodulin so that it does not dissociate from the enzyme until cytosolic Ca^{2+} levels return to basal values for at least 10 seconds (not shown). Second, it converts the enzyme to a Ca^{2+}-independent form, so that the kinase remains active even after the Ca^{2+}/calmodulin dissociates from it (*lower left*). This activity continues until the action of a protein phosphatase overrides the autophosphorylation activity of CaM-kinase II. (B) This structural model of the enzyme is based on x-ray crystallographic analysis.

The remarkable dodecameric structure of the enzyme allows it to achieve a broad range of intermediate activity states in response to different Ca^{2+} oscillation frequencies: higher frequencies tend to cause more subunits in the enzyme to reach the phosphorylated active state (see Figure 15–35). The behavior of CaM-kinase II is also controlled by the length of the linker segment between the kinase and hub domains. The linker is longer in some isoforms of the enzyme; in these isoforms, the kinase domains tend to pop out of the ring more frequently, making it more sensitive to Ca^{2+}. These and other mechanisms allow the cell to tailor the responsiveness of the enzyme to the needs of different types of neurons. (Adapted from L.H. Chao et al., *Cell* 146:732–745, 2011. PDB code: 3SOA.)

Another remarkable property of CaM-kinase II is that the enzyme can use its intrinsic memory mechanism to decode the frequency of Ca^{2+} oscillations. This property is thought to be especially important at a nerve cell synapse, where changes in intracellular Ca^{2+} levels in a postsynaptic cell as a result of neural activity can lead to long-term changes in the subsequent effectiveness of that synapse

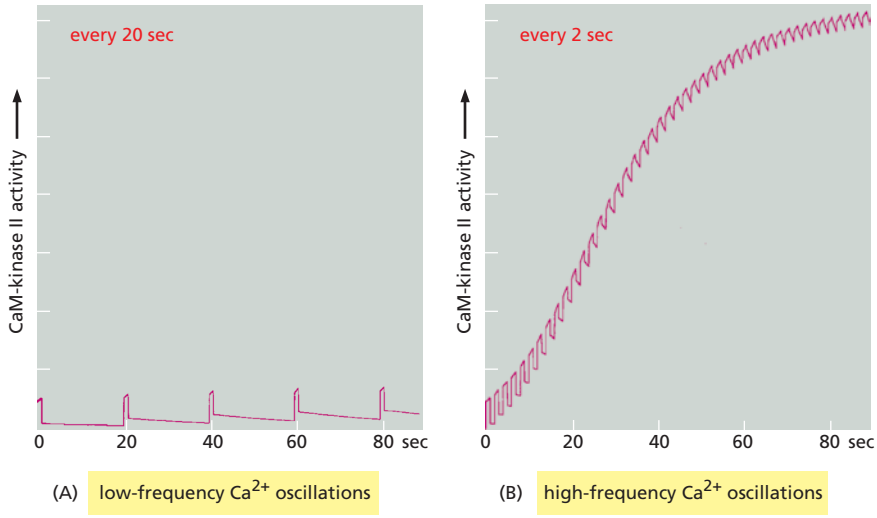


Figure 15–35 CaM-kinase II as a frequency decoder of Ca^{2+} oscillations. (A) At low frequencies of Ca^{2+} spikes, the enzyme becomes inactive after each spike, as the autophosphorylation induced by Ca^{2+}/calmodulin binding does not maintain the enzyme's activity long enough for the enzyme to remain active until the next Ca^{2+} spike arrives. (B) At higher spike frequencies, however, the enzyme fails to inactivate completely between Ca^{2+} spikes, so its activity ratchets up with each spike. If the spike frequency is high enough, this progressive increase in enzyme activity will continue until the enzyme is autophosphorylated on all subunits and is therefore maximally activated. Although not shown, once enough of its subunits are autophosphorylated, the enzyme can be maintained in a highly active state even with a relatively low frequency of Ca^{2+} spikes (a form of cell memory). The binding of Ca^{2+}/calmodulin to the enzyme is enhanced by the CaM-kinase II autophosphorylation (an additional form of positive feedback), helping to generate a more switchlike response to repeated Ca^{2+} spikes. (From P.I. Hanson, T. Meyer, L. Stryer, and H. Schulman, *Neuron* 12:943–956, 1994. With permission from Elsevier.)

(discussed in Chapter 11). When CaM-kinase II is exposed to both a protein phosphatase and repetitive pulses of Ca^{2+}/calmodulin at different frequencies that mimic those observed in stimulated cells, the enzyme's activity increases steeply as a function of pulse frequency (**Figure 15–35**).

Some G Proteins Directly Regulate Ion Channels

G proteins do not act exclusively by regulating the activity of membrane-bound enzymes that alter the concentration of cyclic AMP or Ca^{2+} in the cytosol. The α subunit of one type of G protein (called G_{12}), for example, activates a guanine nucleotide exchange factor (GEF) that activates a monomeric GTPase of the *Rho family* (discussed later and in Chapter 16), which regulates the actin cytoskeleton.

In some other cases, G proteins directly activate or inactivate ion channels in the plasma membrane of the target cell, thereby altering the ion permeability—and hence the electrical excitability—of the membrane. As an example, acetylcholine released by the vagus nerve reduces the heart rate (see Figure 15–5B). This effect is mediated by a special class of acetylcholine receptors that activate the G_i protein discussed earlier. Once activated, the α subunit of G_i inhibits adenylyl cyclase (as described previously), while the βγ subunits bind to K^+ channels in the heart muscle cell plasma membrane and open them. The opening of these K^+ channels makes it harder to depolarize the cell and thereby contributes to the inhibitory effect of acetylcholine on the heart. (These acetylcholine receptors, which can be activated by the fungal alkaloid muscarine, are called *muscarinic acetylcholine receptors* to distinguish them from the very different *nicotinic acetylcholine receptors*, which are ion-channel-coupled receptors on skeletal muscle and nerve cells that can be activated by the binding of nicotine, as well as by acetylcholine.)

Other G proteins regulate the activity of ion channels less directly, either by stimulating channel phosphorylation (by PKA, PKC, or CaM-kinase, for example) or by causing the production or destruction of cyclic nucleotides that directly activate or inactivate ion channels. These *cyclic-nucleotide-gated ion channels* have a crucial role in both smell (olfaction) and vision, as we now discuss.

Smell and Vision Depend on GPCRs That Regulate Ion Channels

Humans can distinguish more than 10,000 distinct smells, which they detect using specialized olfactory receptor neurons in the lining of the nose. These cells use specific GPCRs called **olfactory receptors** to recognize odors; the receptors are displayed on the surface of the modified cilia that extend from each cell (**Figure 15–36**). The receptors act through cAMP. When stimulated by odorant binding,

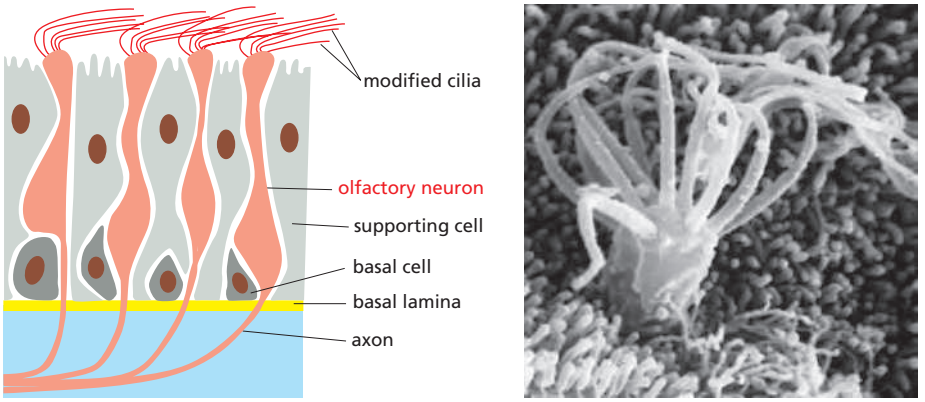


Figure 15–36 Olfactory receptor neurons. (A) A section of olfactory epithelium in the nose. Olfactory receptor neurons possess modified cilia, which project from the surface of the epithelium and contain the olfactory receptors, as well as the signal transduction machinery. The axon, which extends from the opposite end of the receptor neuron, conveys electrical signals to the brain when an odorant activates the cell to produce an action potential. In rodents, at least, the basal cells act as stem cells, producing new receptor neurons throughout life, to replace the neurons that die. (B) A scanning electron micrograph of the cilia on the surface of an olfactory neuron. (B, from E.E. Morrison and R.M. Costanzo, *J. Comp. Neurol.* 297:1–13, 1990. With permission from Wiley-Liss.)

they activate an olfactory-specific G protein (known as G_{olf}), which in turn activates adenylyl cyclase. The resulting increase in cAMP opens *cyclic-AMP-gated cation channels*, thereby allowing an influx of Na^+, which depolarizes the olfactory receptor neuron and initiates a nerve impulse that travels along its axon to the brain.

There are about 1000 different olfactory receptors in a mouse and about 350 in a human, each encoded by a different gene and each recognizing a different set of odorants. Each olfactory receptor neuron produces only one of these receptors; the neuron responds to a specific set of odorants by means of the specific receptor it displays, and each odorant activates its own characteristic set of olfactory receptor neurons. The same receptor also helps direct the elongating axon of each developing olfactory neuron to the specific target neurons that it will connect to in the brain. A different set of GPCRs acts in a similar way in some vertebrates to mediate responses to *pheromones*, chemical signals detected in a different part of the nose that are used in communication between members of the same species. Humans, however, are thought to lack functional pheromone receptors.

Vertebrate vision employs a similarly elaborate, highly sensitive, signal-detection process. Cyclic-nucleotide-gated ion channels are also involved, but the crucial cyclic nucleotide is **cyclic GMP** (Figure 15–37) rather than cAMP. As with cAMP, a continuous rapid synthesis (by *guanylyl cyclase*) and rapid degradation (by *cyclic GMP phosphodiesterase*) controls the concentration of cyclic GMP in the cytosol.

In visual transduction responses, which are the fastest G-protein-mediated responses known in vertebrates, the receptor activation stimulated by light causes a fall rather than a rise in the level of the cyclic nucleotide. The pathway has been especially well studied in **rod photoreceptors** (**rods**) in the vertebrate retina. Rods are responsible for noncolor vision in dim light, whereas **cone photoreceptors** (**cones**) are responsible for color vision in bright light. A rod photoreceptor is a highly specialized cell with outer and inner segments, a cell body, and a synaptic region where the rod passes a chemical signal to a retinal nerve cell (Figure 15–38). This nerve cell relays the signal to another nerve cell in the retina, which in turn relays it to the brain.

The phototransduction apparatus is in the outer segment of the rod, which contains a stack of *discs*, each formed by a closed sac of membrane that is densely packed with photosensitive **rhodopsin** molecules. The plasma membrane surrounding the outer segment contains *cyclic-GMP-gated cation channels.* Cyclic GMP bound to these channels keeps them open in the dark. Paradoxically, light causes a hyperpolarization (which inhibits synaptic signaling) rather than a depolarization of the plasma membrane (which would stimulate synaptic signaling). Hyperpolarization (that is, the membrane potential moves to a more negative value—discussed in Chapter 11) results because the light-induced activation of rhodopsin molecules in the disc membrane decreases the cyclic GMP concentration and closes the cation channels in the surrounding plasma membrane (Figure 15–39).

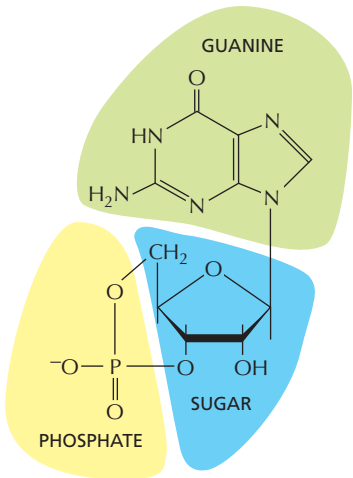
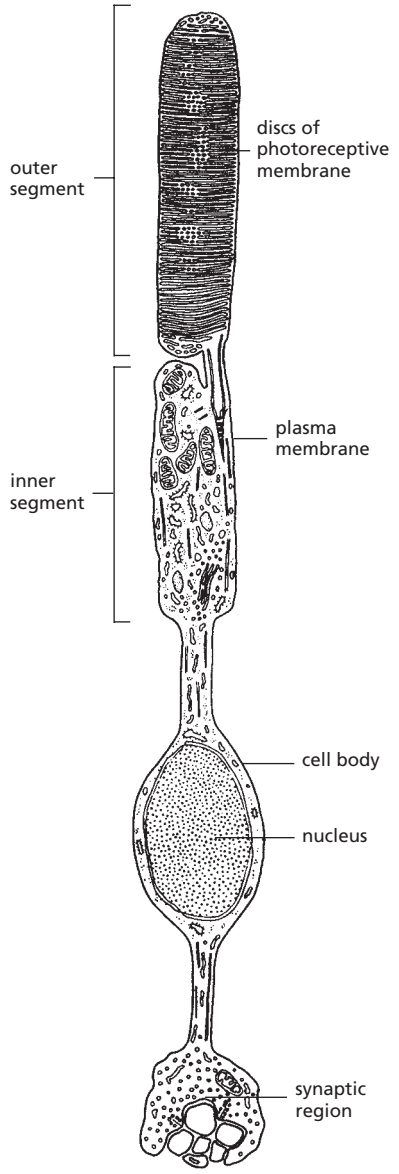


Figure 15–37 Cyclic GMP.

Figure 15–38 A rod photoreceptor cell. There are about 1000 discs in the outer segment. The disc membranes are not connected to the plasma membrane. The inner and outer segments are specialized parts of a *primary cilium* (discussed in Chapter 16). A primary cilium extends from the surface of most vertebrate cells, where it serves as a signaling organelle.



Rhodopsin is a member of the GPCR family, but the activating extracellular signal is not a molecule but a photon of light. Each rhodopsin molecule contains a covalently attached chromophore, 11-*cis* retinal, which isomerizes almost instantaneously to all-*trans* retinal when it absorbs a single photon. The isomerization alters the shape of the retinal, forcing a conformational change in the protein (opsin). The activated rhodopsin molecule then alters the conformation of the G protein *transducin* (G_t), causing the transducin α subunit to activate **cyclic GMP phosphodiesterase**. The phosphodiesterase then hydrolyzes cyclic GMP, so that cyclic GMP levels in the cytosol fall. This drop in cyclic GMP concentration decreases the amount of cyclic GMP bound to the plasma membrane cation channels, allowing more of these cyclic-GMP-sensitive channels to close. In this way, the signal quickly passes from the disc membrane to the plasma membrane, and a light signal is converted into an electrical one, through a hyperpolarization of the rod cell plasma membrane.

Rods use several negative feedback loops to allow the cells to revert quickly to a resting, dark state in the aftermath of a flash of light—a requirement for perceiving the shortness of the flash. A rhodopsin-specific protein kinase called *rhodopsin kinase* (*RK*) phosphorylates the cytosolic tail of activated rhodopsin on multiple serines, partially inhibiting the ability of the rhodopsin to activate transducin. An inhibitory protein called *arrestin* (discussed later) then binds to the phosphorylated rhodopsin, further inhibiting rhodopsin's activity. Mice or humans with a mutation that inactivates the gene encoding RK have a prolonged light response.

At the same time as arrestin shuts off rhodopsin, an RGS protein (discussed earlier) binds to activated transducin, stimulating the transducin to hydrolyze its bound GTP to GDP, which returns transducin to its inactive state. In addition, the cation channels that close in response to light are permeable to Ca^{2+}, as well as

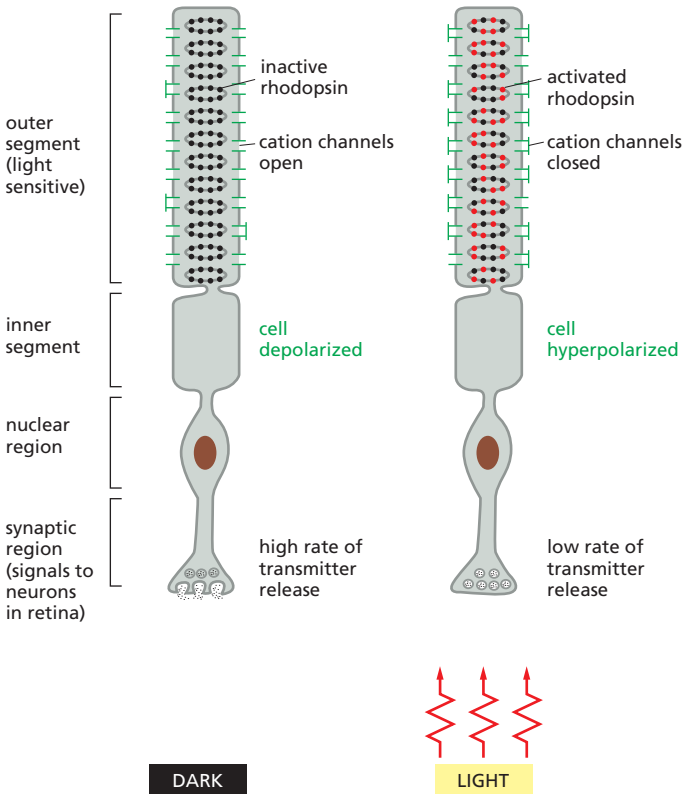


Figure 15–39 The response of a rod photoreceptor cell to light. Rhodopsin molecules in the outer-segment discs absorb photons. Photon absorption closes cation channels in the plasma membrane, which hyperpolarizes the membrane and reduces the rate of neurotransmitter release from the synaptic region. Because the neurotransmitter inhibits many of the postsynaptic retinal neurons, illumination serves to free the neurons from inhibition and thus, in effect, excites them. The neural connections of the retina lie between the light source and the outer segment, and so the light must pass through the synapses and rod cell nucleus to reach the light sensors.

to Na^+, so that when they close, the normal influx of Ca^{2+} is inhibited, causing the Ca^{2+} concentration in the cytosol to fall. The decrease in Ca^{2+} concentration stimulates guanylyl cyclase to replenish the cyclic GMP, rapidly returning its level to where it was before the light was switched on. A specific Ca^{2+}-sensitive protein mediates the activation of guanylyl cyclase in response to the fall in Ca^{2+} levels. In contrast to calmodulin, this protein is inactive when Ca^{2+} is bound to it and active when it is Ca^{2+}-free. It therefore stimulates the cyclase when Ca^{2+} levels fall following a light response.

Negative feedback mechanisms do more than just return the rod to its resting state after a transient light flash; they also help the rod to *adapt*, stepping down the response when the rod is exposed to light continuously. Adaptation, as we discussed earlier, allows the receptor cell to function as a sensitive detector of *changes* in stimulus intensity over an enormously wide range of baseline levels of stimulation. It is why we can see faint stars in a dark sky, or a camera flash in bright sunlight.

The various trimeric G proteins we have discussed in this chapter fall into four major families, as summarized in **Table 15–3**.

Nitric Oxide Is a Gaseous Signaling Mediator That Passes Between Cells

Signaling molecules like cyclic nucleotides and calcium are hydrophilic small molecules that generally act within the cell where they are produced. Some signaling molecules, however, are hydrophobic enough, small enough, or both, to pass readily across the plasma membrane and carry signals to nearby cells. An important and remarkable example is the gas **nitric oxide** (**NO**), which acts as a signal molecule in many tissues of both animals and plants.

In mammals, one of NO's many functions is to relax smooth muscle in the walls of blood vessels. The neurotransmitter acetylcholine stimulates NO synthesis by

TABLE 15–3 Four Major Families of Trimeric G Proteins*

Family	Some family members	Subunits that mediate action	Some functions
I	G_s	α	Activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	Activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	Inhibits adenylyl cyclase
		βγ	Activates K^+ channels
	G_o	βγ	Activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	Activates phospholipase C-β
	G_t (transducin)	α	Activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	Activates phospholipase C-β
IV	$G_{12/13}$	α	Activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

activating a GPCR on the membranes of the endothelial cells that line the interior of the vessel. The activated receptor triggers IP_3 synthesis and Ca^{2+} release (see Figure 15–29), leading to stimulation of an enzyme that synthesizes NO. Because dissolved NO passes readily across membranes, it diffuses out of the cell where it is produced and into neighboring smooth muscle cells, where it causes muscle relaxation and thereby vessel dilation (**Figure 15–40**). It acts only locally because it has a short half-life—about 5–10 seconds—in the extracellular space before oxygen and water convert it to nitrates and nitrites.

The effect of NO on blood vessels provides an explanation for the mechanism of action of nitroglycerine, which has been used for about 100 years to treat patients with angina (pain resulting from inadequate blood flow to the heart muscle). The nitroglycerine is converted to NO, which relaxes blood vessels. This reduces the workload on the heart and, as a consequence, reduces the oxygen requirement of the heart muscle.

NO is made by the deamination of the amino acid arginine, catalyzed by enzymes called **NO synthases** (**NOS**) (see Figure 15–40). The NOS in endothelial cells is called *eNOS*, while that in nerve and muscle cells is called *nNOS*. Both eNOS and nNOS are stimulated by Ca^{2+}. Macrophages, by contrast, make yet another NOS, called inducible NOS (*iNOS*), that is constitutively active but synthesized only when the cells are activated, usually in response to an infection.

In some target cells, including smooth muscle cells, NO binds reversibly to iron in the active site of guanylyl cyclase, stimulating synthesis of cyclic GMP. NO can increase cyclic GMP in the cytosol within seconds, because the normal rate of turnover of cyclic GMP is high: rapid degradation to GMP by a phosphodiesterase constantly balances the production of cyclic GMP by guanylyl cyclase. The drug Viagra® and its relatives inhibit the cyclic GMP phosphodiesterase in the penis, thereby increasing the amount of time that cyclic GMP levels remain elevated in the smooth muscle cells of penile blood vessels after NO production is induced by local nerve terminals. The cyclic GMP, in turn, keeps the blood vessels relaxed and thereby the penis erect. NO can also signal cells independently of cyclic GMP. It can, for example, alter the activity of an intracellular protein by covalently nitrosylating thiol (–SH) groups on specific cysteines in the protein.

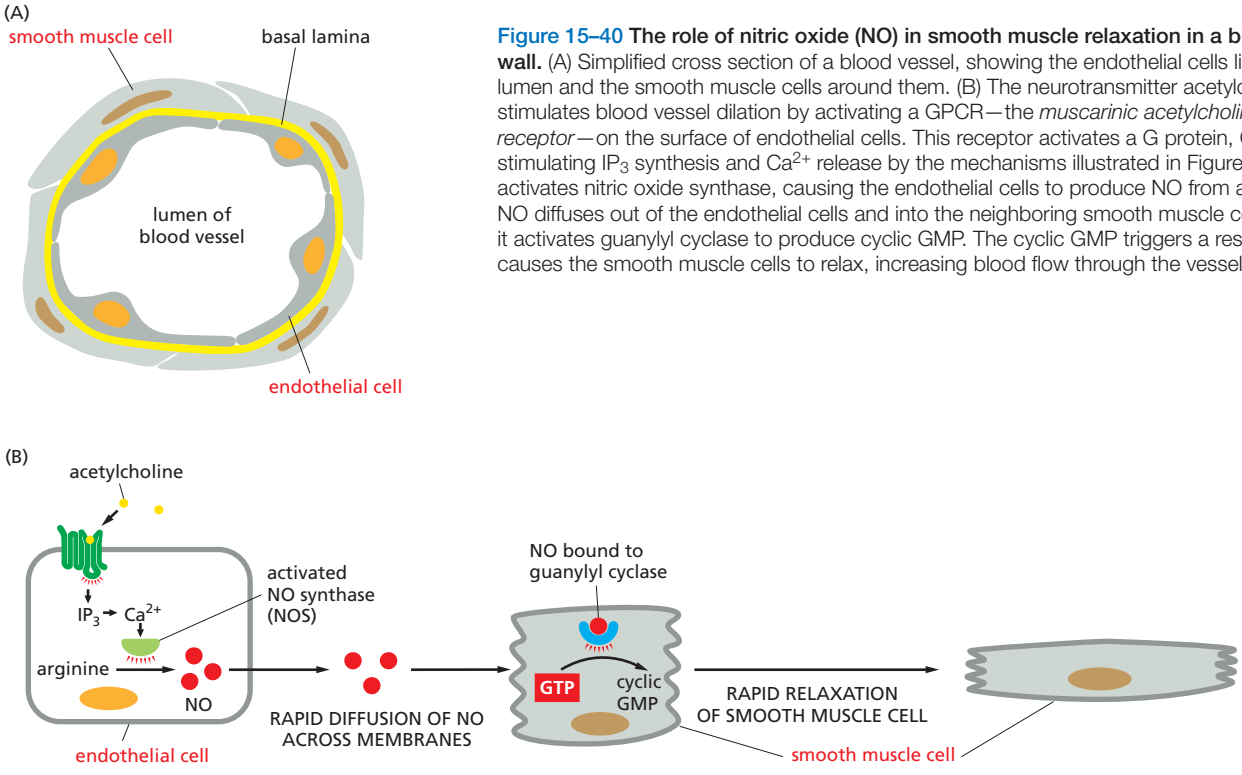


Figure 15–40 The role of nitric oxide (NO) in smooth muscle relaxation in a blood vessel wall. (A) Simplified cross section of a blood vessel, showing the endothelial cells lining the lumen and the smooth muscle cells around them. (B) The neurotransmitter acetylcholine stimulates blood vessel dilation by activating a GPCR—the *muscarinic acetylcholine receptor*—on the surface of endothelial cells. This receptor activates a G protein, G_q, thereby stimulating IP_3 synthesis and Ca^{2+} release by the mechanisms illustrated in Figure 15–29. Ca^{2+} activates nitric oxide synthase, causing the endothelial cells to produce NO from arginine. The NO diffuses out of the endothelial cells and into the neighboring smooth muscle cells, where it activates guanylyl cyclase to produce cyclic GMP. The cyclic GMP triggers a response that causes the smooth muscle cells to relax, increasing blood flow through the vessel.

Second Messengers and Enzymatic Cascades Amplify Signals

Despite the differences in molecular details, the different intracellular signaling pathways that GPCRs trigger share certain features and obey similar general principles. They depend on relay chains of intracellular signaling proteins and second messengers. These relay chains provide numerous opportunities for amplifying the responses to extracellular signals. In the visual transduction cascade, for example, a single activated rhodopsin molecule catalyzes the activation of hundreds of molecules of transducin at a rate of about 1000 transducin molecules per second. Each activated transducin molecule activates a molecule of cyclic GMP phosphodiesterase, each of which hydrolyzes about 4000 molecules of cyclic GMP per second. This catalytic cascade lasts for about 1 second and results in the hydrolysis of more than 10^5 cyclic GMP molecules for a single quantum of light absorbed, and the resulting drop in the concentration of cyclic GMP in turn transiently closes hundreds of cation channels in the plasma membrane (**Figure 15–41**). As a result, a rod cell can respond to even a single photon of light in a way that is highly reproducible in its timing and magnitude.

Likewise, when an extracellular signal molecule binds to a receptor that indirectly activates adenylyl cyclase via G_s, each receptor protein may activate many molecules of G_s protein, each of which can activate a cyclase molecule. Each cyclase molecule, in turn, can catalyze the conversion of a large number of ATP molecules to cAMP molecules. A similar amplification operates in the IP_3 signaling pathway. In these ways, a nanomolar (10^{-9} M) change in the concentration of an extracellular signal can induce micromolar (10^{-6} M) changes in the concentration of a second messenger such as cAMP or Ca^{2+}. Because these messengers function as allosteric effectors to activate specific enzymes or ion channels, a single extracellular signal molecule can alter many thousands of protein molecules within the target cell.

Any such amplifying cascade of stimulatory signals requires counterbalancing mechanisms at every step of the cascade to restore the system to its resting state when stimulation ceases. As emphasized earlier, the response to stimulation can be rapid only if the inactivating mechanisms are also rapid. Cells therefore have efficient mechanisms for rapidly degrading (and resynthesizing) cyclic nucleotides and for buffering and removing cytosolic Ca^{2+}, as well as for inactivating the responding enzymes and ion channels once they have been activated. This is not only essential for turning a response off, but is also important for defining the resting state from which a response begins.

Each protein in the signaling relay chain can be a separate target for regulation, including the receptor itself, as we discuss next.

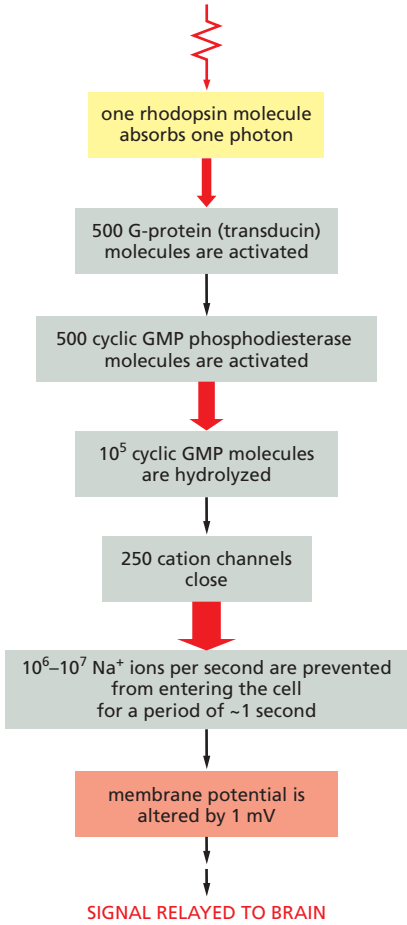


Figure 15–41 Amplification in the light-induced catalytic cascade in vertebrate rods. The *red arrows* indicate the steps where amplification occurs, with the thickness of the arrow roughly indicating the magnitude of the amplification.

GPCR Desensitization Depends on Receptor Phosphorylation

As discussed earlier, when target cells are exposed to a high concentration of a stimulating ligand for a prolonged period, they can become *desensitized*, or *adapted*, in several different ways. An important class of adaptation mechanisms depends on alteration of the quantity or condition of the receptor molecules themselves.

For GPCRs, there are three general modes of adaptation (see Figure 15–20): (1) In *receptor sequestration*, they are temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand. (2) In *receptor down-regulation*, they are destroyed in lysosomes after internalization. (3) In *receptor inactivation*, they become altered so that they can no longer interact with G proteins.

In each case, the desensitization of the GPCRs depends on their phosphorylation by PKA, PKC, or a member of the family of **GPCR kinases** (**GRKs**), which includes the rhodopsin-specific kinase RK involved in rod photoreceptor desensitization discussed earlier. The GRKs phosphorylate multiple serines and threonines on a GPCR, but they do so only after ligand binding has activated the receptor, because it is the activated receptor that allosterically activates the GRK.

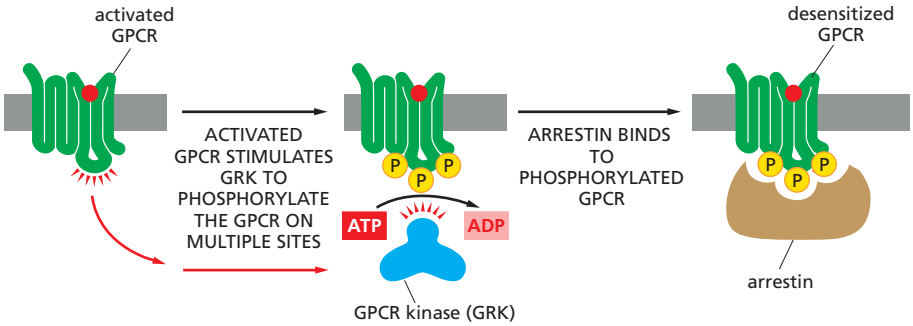


Figure 15–42 The roles of GPCR kinases (GRKs) and arrestins in GPCR desensitization. A GRK phosphorylates only activated receptors because it is the activated GPCR that activates the GRK. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and also directs its endocytosis (not shown). Mice that are deficient in one form of arrestin fail to desensitize in response to morphine, for example, attesting to the importance of arrestins for desensitization.

As with rhodopsin, once a receptor has been phosphorylated by a GRK, it binds with high affinity to a member of the **arrestin** family of proteins (**Figure 15–42**).

The bound arrestin can contribute to the desensitization process in at least two ways. First, it prevents the activated receptor from interacting with G proteins. Second, it serves as an adaptor protein to help couple the receptor to the clathrin-dependent endocytosis machinery (discussed in Chapter 13), inducing receptor-mediated endocytosis. The fate of the internalized GPCR–arrestin complex depends on other proteins in the complex. In some cases, the receptor is dephosphorylated and recycled back to the plasma membrane for reuse. In others, it is ubiquitylated, endocytosed, and degraded in lysosomes (discussed later).

Receptor endocytosis does not necessarily stop the receptor from signaling. In some cases, the bound arrestin recruits other signaling proteins to relay the signal onward from the internalized GPCRs along new pathways.

Summary

GPCRs can indirectly activate or inactivate either plasma-membrane-bound enzymes or ion channels via G proteins. When an activated receptor stimulates a G protein, the G protein undergoes a conformational change that activates its α subunit, thereby triggering release of a βγ complex. Either component can then directly regulate the activity of target proteins in the plasma membrane. Some GPCRs either activate or inactivate adenylyl cyclase, thereby altering the intracellular concentration of the second messenger cyclic AMP. Others activate a phosphoinositide-specific phospholipase C (PLCβ), which generates two second messengers. One is inositol 1,4,5-trisphosphate (IP_3), which releases Ca^{2+} from the ER and thereby increases the concentration of Ca^{2+} in the cytosol. The other is diacylglycerol, which remains in the plasma membrane and helps activate protein kinase C (PKC). An increase in cytosolic cyclic AMP or Ca^{2+} levels affects cells mainly by stimulating cAMP-dependent protein kinase (PKA) and Ca^{2+}/calmodulin-dependent protein kinases (CaM-kinases), respectively.

PKC, PKA, and CaM-kinases phosphorylate specific target proteins and thereby alter the activity of the proteins. Each type of cell has its own characteristic set of target proteins that is regulated in these ways, enabling the cell to make its own distinctive response to the second messengers. The intracellular signaling cascades activated by GPCRs greatly amplify the responses, so that many thousands of target protein molecules are changed for each molecule of extracellular signaling ligand bound to its receptor. The responses mediated by GPCRs are rapidly turned off when the extracellular signal is removed, and activated GPCRs are inactivated by phosphorylation and association with arrestins.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

Like GPCRs, **enzyme-coupled receptors** are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane. Instead of having a cytosolic domain that associates with a trimeric G protein, however, their cytosolic domain either has intrinsic enzyme activity or associates directly with an enzyme. Whereas a GPCR has seven transmembrane segments, each subunit of an enzyme-coupled receptor typically has only one. GPCRs and enzyme-coupled receptors often activate some of the same signaling pathways. In this section, we describe some of the important features of signaling by enzyme-coupled receptors, with an emphasis on the most common class of these proteins, the *receptor tyrosine kinases*.

Activated Receptor Tyrosine Kinases (RTKs) Phosphorylate Themselves

Many extracellular signal proteins act through **receptor tyrosine kinases** (**RTKs**). These include many secreted and cell-surface-bound proteins that control cell behavior in developing and adult animals. Some of these signal proteins and their RTKs are listed in **Table 15–4**.

There are about 60 human RTKs, which can be classified into about 20 structural subfamilies, each dedicated to its complementary family of protein ligands. **Figure 15–43** shows the basic structural features of a number of the families that operate in mammals. In all cases, the binding of the signal protein to the ligand-binding domain on the extracellular side of the receptor activates the tyrosine kinase domain on the cytosolic side. This leads to phosphorylation of tyrosine side chains on the cytosolic part of the receptor, creating phosphotyrosine docking sites for various intracellular signaling proteins that relay the signal.

How does the binding of an extracellular ligand activate the kinase domain on the other side of the plasma membrane? For a GPCR, ligand binding is thought to change the relative orientation of several of the transmembrane α helices, thereby shifting the position of the cytoplasmic loops relative to one another. It is unlikely, however, that a conformational change could propagate across the lipid bilayer

TABLE 15–4 Some Signal Proteins That Act Via RTKs

Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

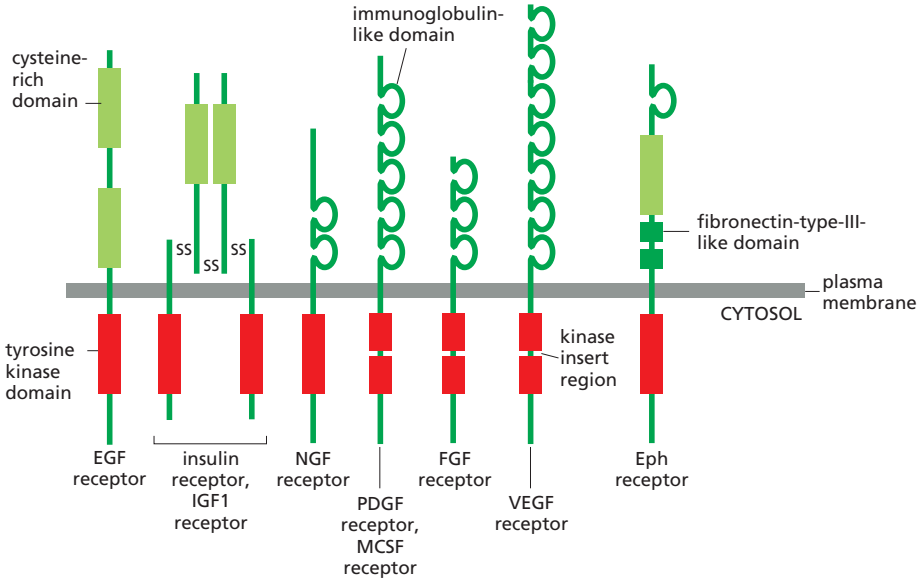


Figure 15–43 Some subfamilies of RTKs. Only one or two members of each subfamily are indicated. Note that in some cases, the tyrosine kinase domain is interrupted by a "kinase insert region" that is an extra segment emerging from the folded kinase domain. The functions of most of the cysteine-rich, immunoglobulin-like, and fibronectin-type-III-like domains are not known. Some of the ligands for the receptors shown are listed in Table 15–4, along with some representative responses that they mediate.

through a single transmembrane α helix. Instead, for most RTKs, ligand binding causes the receptors to dimerize, bringing the two cytoplasmic kinase domains together and thereby promoting their activation (Figure 15–44).

Dimerization stimulates kinase activity by a variety of mechanisms. In many cases, such as the insulin receptor, dimerization simply brings the kinase domains close to each other in an orientation that allows them to phosphorylate each other on specific tyrosines in the kinase active sites, thereby promoting conformational changes that fully activate both kinase domains. In other cases, such as the receptor for *epidermal growth factor* (*EGF*), the kinase is not activated by phosphorylation but by conformational changes brought about by interactions between the two kinase domains outside their active sites (Figure 15–45).

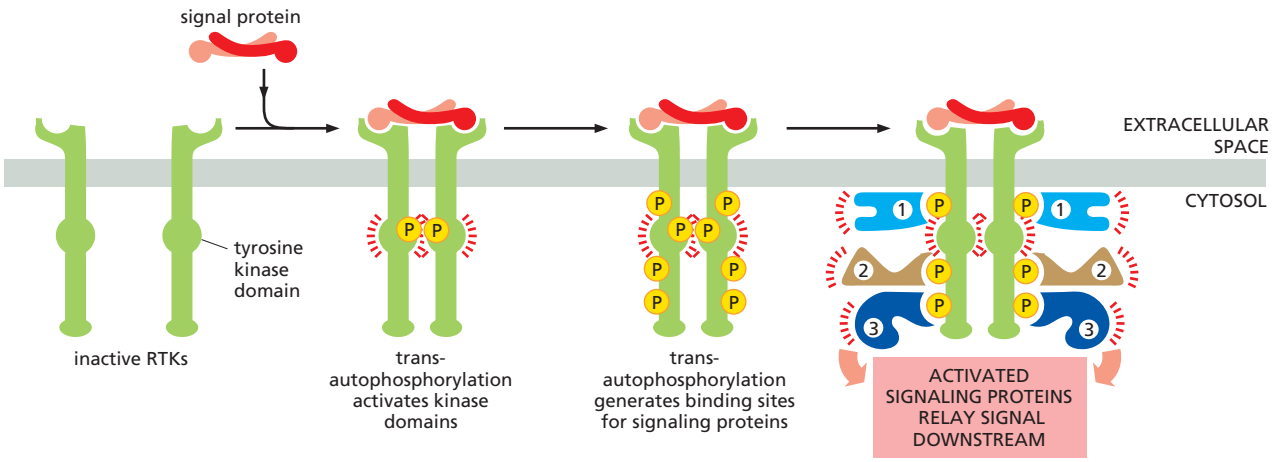


Figure 15–44 Activation of RTKs by dimerization. In the absence of extracellular signals, most RTKs exist as monomers in which the internal kinase domain is inactive. Binding of ligand brings two monomers together to form a dimer. In most cases, the close proximity in the dimer leads the two kinase domains to phosphorylate each other, which has two effects. First, phosphorylation at some tyrosines in the kinase domains promotes the complete activation of the domains. Second, phosphorylation at tyrosines in other parts of the receptors generates docking sites for intracellular signaling proteins, resulting in the formation of large signaling complexes that can then broadcast signals along multiple signaling pathways.

Mechanisms of dimerization vary widely among different RTK family members. In some cases, as shown here, the ligand itself is a dimer and brings two receptors together by binding them simultaneously. In other cases, a monomeric ligand can interact with two receptors simultaneously to bring them together, or two ligands can bind independently on two receptors to promote dimerization. In some RTKs—notably those in the insulin receptor family—the receptor is always a dimer (see Figure 15–43), and ligand binding causes a conformational change that brings the two internal kinase domains closer together. Although many RTKs are activated by transautophosphorylation as shown here, there are some important exceptions, including the EGF receptor illustrated in Figure 15–45.

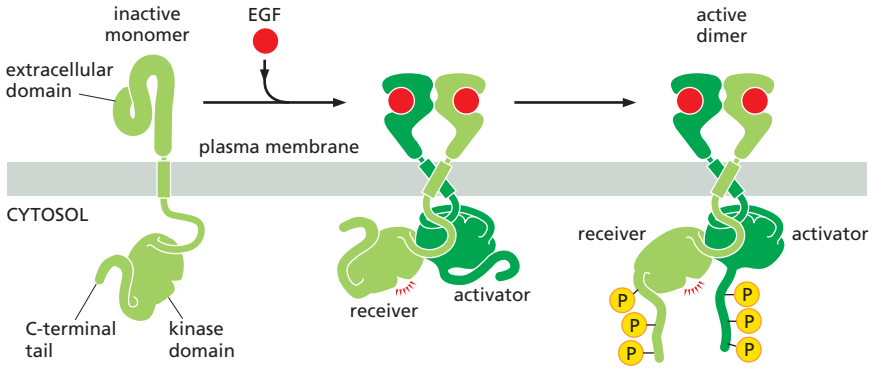


Figure 15–45 Activation of the EGF receptor kinase. In the absence of ligand, the EGF receptor exists primarily as an inactive monomer. EGF binding results in a conformational change that promotes dimerization of the external domains. The receptor kinase domain, unlike that of many RTKs, is not activated by transautophosphorylation. Instead, dimerization orients the internal kinase domains into an asymmetric dimer, in which one kinase domain (the "activator") pushes against the other kinase domain (the "receiver"), thereby causing an activating conformational change in the receiver. The active receiver domain then phosphorylates multiple tyrosines in the C-terminal tails of both receptors, generating docking sites for intracellular signaling proteins (see Figure 15–44).

Phosphorylated Tyrosines on RTKs Serve as Docking Sites for Intracellular Signaling Proteins

Once the kinase domains of an RTK dimer are activated, they phosphorylate multiple additional sites in the cytosolic parts of the receptors, typically in disordered regions outside the kinase domain (see Figure 15–44). This phosphorylation creates high-affinity docking sites for intracellular signaling proteins. Each signaling protein binds to a particular phosphorylated site on the activated receptors because it contains a specific phosphotyrosine-binding domain that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine.

Once bound to the activated RTK, a signaling protein may become phosphorylated on tyrosines and thereby activated. In many cases, however, the binding alone may be sufficient to activate the docked signaling protein, by either inducing a conformational change in the protein or simply bringing it near the protein that is next in the signaling pathway. Thus, receptor phosphorylation serves as a switch to trigger the assembly of an intracellular signaling complex, which can then relay the signal onward, often along multiple routes, to various destinations in the cell. Because different RTKs bind different combinations of these signaling proteins, they activate different responses.

Some RTKs use additional docking proteins to enlarge the signaling complex at activated receptors. Insulin and IGF1 receptor signaling, for example, depend on a specialized docking protein called *insulin receptor substrate 1* (*IRS1*). IRS1 associates with phosphorylated tyrosines on the activated receptor and is then phosphorylated at multiple sites, thereby creating many more docking sites than could be accommodated on the receptor alone (see Figure 15–11).

Proteins with SH2 Domains Bind to Phosphorylated Tyrosines

A whole menagerie of intracellular signaling proteins can bind to the phosphotyrosines on activated RTKs (or on docking proteins such as IRS1). They help to relay the signal onward, mainly through chains of protein–protein interactions mediated by modular *interaction domains*, as discussed earlier. Some of the docked proteins are enzymes, such as **phospholipase C-γ (PLCγ)**, which functions in the same way as phospholipase C-β—activating the inositol phospholipid signaling pathway discussed earlier in connection with GPCRs (see Figures 15–28 and 15–29). Through this pathway, RTKs can increase cytosolic Ca^{2+} levels and activate PKC. Another enzyme that docks on these receptors is the cytoplasmic tyrosine kinase *Src*, which phosphorylates other signaling proteins on tyrosines. Yet another is *phosphoinositide 3-kinase* (*PI 3-kinase*), which phosphorylates lipids rather than proteins; as we discuss later, the phosphorylated lipids then serve as docking sites to attract various signaling proteins to the plasma membrane.

The intracellular signaling proteins that bind to phosphotyrosines have varied structures and functions. However, they usually share highly conserved phosphotyrosine-binding domains. These can be either **SH2 domains** (for *Src homology*

region) or, less commonly, *PTB domains* (for *phosphotyrosine-binding*). By recognizing specific phosphorylated tyrosines, these small interaction domains enable the proteins that contain them to bind to activated RTKs, as well as to many other intracellular signaling proteins that have been transiently phosphorylated on tyrosines (**Figure 15–46**). As discussed previously, many signaling proteins also contain other interaction domains that allow them to interact specifically with other proteins as part of the signaling process. These domains include the *SH3 domain*, which binds to proline-rich motifs in intracellular proteins (see Figure 15–11).

Not all proteins that bind to activated RTKs via SH2 domains help to relay the signal onward. Some act to decrease the signaling process, providing negative feedback. One example is the *c-Cbl protein*, which can dock on some activated receptors and catalyze their ubiquitylation, covalently adding one or more ubiquitin molecules to specific sites on the receptor. This promotes the endocytosis and degradation of the receptors in lysosomes—an example of receptor down-regulation (see Figure 15–20). Endocytic proteins that contain *ubiquitin-interaction motifs (UIMs)* recognize the ubiquitylated RTKs and direct them into clathrin-coated vesicles and, ultimately, into lysosomes (discussed in Chapter 13). Mutations that inactivate c-Cbl-dependent RTK down-regulation cause prolonged RTK signaling and thereby promote the development of cancer.

As is the case for GPCRs, ligand-induced endocytosis of RTKs does not always decrease signaling. In some cases, RTKs are endocytosed with their bound signaling proteins and continue to signal from endosomes or other intracellular compartments. This mechanism, for example, allows *nerve growth factor* (*NGF*) to bind to its specific RTK (called TrkA) at the end of a long nerve cell axon and signal to the cell body of the same cell a long distance away. Here, signaling endocytic

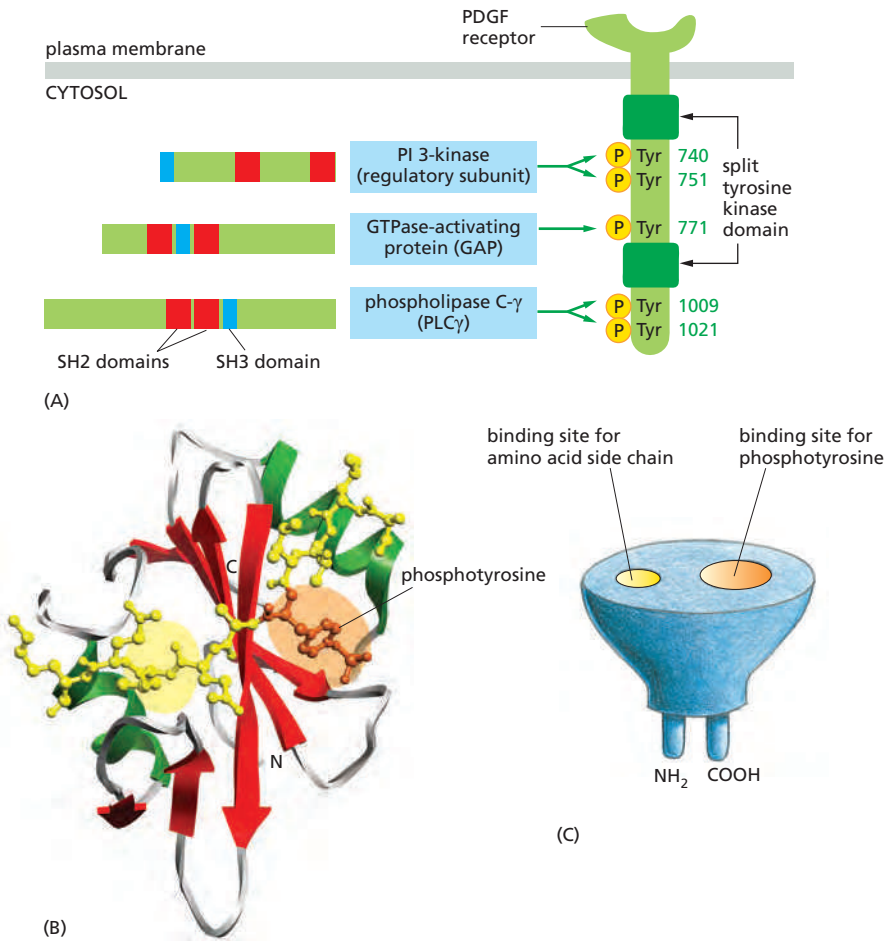


Figure 15–46 The binding of SH2-containing intracellular signaling proteins to an activated RTK. (A) This drawing of a receptor for *platelet-derived growth factor* (*PDGF*) shows five phosphotyrosine docking sites, three in the kinase insert region and two on the C-terminal tail, to which the three signaling proteins shown bind as indicated. The numbers on the right indicate the positions of the tyrosines in the polypeptide chain. These binding sites have been identified by using recombinant DNA technology to mutate specific tyrosines in the receptor. Mutation of tyrosines 1009 and 1021, for example, prevents the binding and activation of PLCγ, so that receptor activation no longer stimulates the inositol phospholipid signaling pathway. The locations of the SH2 *(red)* and SH3 *(blue)* domains in the three signaling proteins are indicated. (Additional phosphotyrosine docking sites on this receptor are not shown, including those that bind the cytoplasmic tyrosine kinase Src and two adaptor proteins.) It is unclear how many signaling proteins can bind simultaneously to a single RTK. (B) The three-dimensional structure of an SH2 domain, as determined by x-ray crystallography. The binding pocket for phosphotyrosine is shaded in *orange* on the right, and a pocket for binding a specific amino acid side chain (isoleucine, in this case) is shaded in *yellow* on the left. The RTK polypeptide segment that binds the SH2 domain is shown in *yellow* (see also Figure 3–40). (C) The SH2 domain is a compact, "plug-in" module, which can be inserted almost anywhere in a protein without disturbing the protein's folding or function (discussed in Chapter 3). Because each domain has distinct sites for recognizing phosphotyrosine and for recognizing a particular amino acid side chain, different SH2 domains recognize phosphotyrosine in the context of different flanking amino acid sequences. (B, based on data from G. Waksman et al., *Cell* 72:779–790, 1993. With permission from Elsevier. PDB code: 2SRC.)

vesicles containing TrkA, with NGF bound on the lumenal side and signaling proteins docked on the cytosolic side, are transported along the axon to the cell body, where they signal the cell to survive.

Some signaling proteins are composed almost entirely of SH2 and SH3 domains and function as *adaptors* to couple tyrosine-phosphorylated proteins to other proteins that do not have their own SH2 domains (see Figure 15–11). Adaptor proteins of this type help to couple activated RTKs to the important signaling protein *Ras*, a monomeric GTPase that, in turn, can activate various downstream signaling pathways, as we now discuss.

The GTPase Ras Mediates Signaling by Most RTKs

The **Ras superfamily** consists of various families of monomeric GTPases, but only the Ras and Rho families relay signals from cell-surface receptors (**Table 15–5**). By interacting with different intracellular signaling proteins, a single Ras or Rho family member can coordinately spread the signal along several distinct downstream signaling pathways, thereby acting as a *signaling hub*.

There are three major, closely related Ras proteins in humans: H-, K-, and N-Ras (see Table 15–5). Although they have subtly different functions, they are thought to work in the same way, and we will refer to them simply as **Ras**. Like many monomeric GTPases, Ras contains one or more covalently attached lipid groups that help anchor the protein to the cytoplasmic face of the membrane, from where it relays signals to other parts of the cell. Ras is often required, for example, when RTKs signal to the nucleus to stimulate cell proliferation or differentiation, both of which require changes in gene expression. If Ras function is inhibited by various experimental approaches, the cell proliferation or differentiation responses normally induced by the activated RTKs do not occur. Conversely, 30% of human tumors express hyperactive mutant forms of Ras, which contribute to the uncontrolled proliferation of the cancer cells.

Like other GTP-binding proteins, Ras functions as a molecular switch, cycling between two distinct conformational states—active when GTP is bound and inactive when GDP is bound (**Movie 15.7**). As discussed earlier for monomeric GTPases in general, two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states (see Figure 15–8).

TABLE 15–5 The Ras Superfamily of Monomeric GTPases

Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3–67.

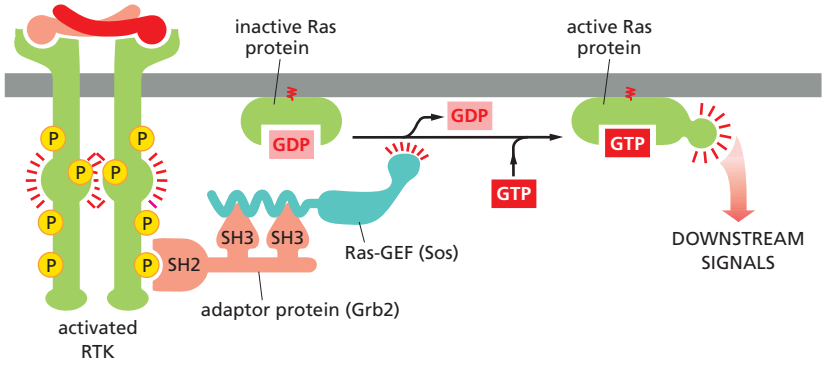


Figure 15–47 How an RTK activates Ras. Grb2 recognizes a specific phosphorylated tyrosine on the activated receptor by means of an SH2 domain and recruits Sos by means of two SH3 domains. Sos stimulates the inactive Ras protein to replace its bound GDP by GTP, which activates Ras to relay the signal downstream.

Ras guanine nucleotide exchange factors (**Ras-GEFs**) stimulate the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras. *Ras GTPase-activating proteins* (**Ras-GAPs**) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras. Hyperactive mutant forms of Ras are resistant to GAP-mediated GTPase stimulation and are locked permanently in the GTP-bound active state, which is why they promote the development of cancer.

But how do RTKs normally activate Ras? In principle, they could either activate a Ras-GEF or inhibit a Ras-GAP. Even though some GAPs bind directly (via their SH2 domains) to activated RTKs (see Figure 15–46A), it is the indirect coupling of the receptor to a Ras-GEF that drives Ras into its active state. The loss of function of a Ras-GEF has a similar effect to the loss of function of Ras itself. Activation of the other Ras superfamily proteins, including those of the Rho family, also occurs through the activation of GEFs. The particular GEF determines in which membrane the GTPase is activated and, by acting as a scaffold, it can also determine which downstream proteins the GTPase activates.

The GEF that mediates Ras activation by RTKs was discovered by genetic studies of eye development in *Drosophila*, where an RTK called *Sevenless* (*Sev*) is required for the formation of a photoreceptor cell called R7. Genetic screens for components of this signaling pathway led to the discovery of a Ras-GEF called *Son-of-sevenless* (*Sos*). Further genetic screens uncovered another protein, now called *Grb2*, which is an adaptor protein that links the Sev receptor to the Sos protein; the SH2 domain of the Grb2 adaptor binds to the activated receptor, while its two SH3 domains bind to Sos. Sos then promotes Ras activation. Biochemical and cell biological studies have shown that Grb2 and Sos also link activated RTKs to Ras in mammalian cells, revealing that this is a highly conserved mechanism in RTK signaling (**Figure 15–47**). Once activated, Ras activates various other signaling proteins to relay the signal downstream, as we discuss next.

Ras Activates a MAP Kinase Signaling Module

Both the tyrosine phosphorylations and the activation of Ras triggered by activated RTKs are usually short-lived (**Figure 15–48**). *Tyrosine-specific protein phosphatases* quickly reverse the phosphorylations, and Ras-GAPs induce activated

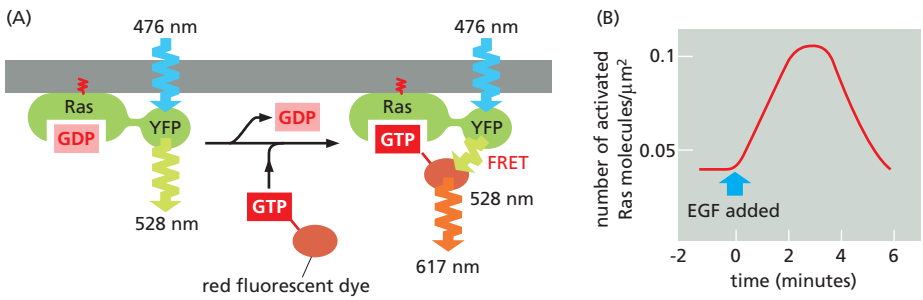


Figure 15–48 Transient activation of Ras revealed by single-molecule fluorescence resonance energy transfer (FRET). (A) Schematic drawing of the experimental strategy. Cells of a human cancer cell line are genetically engineered to express a Ras protein that is covalently linked to yellow fluorescent protein (YFP). GTP that is labeled with a red fluorescent dye is microinjected into some of the cells. The cells are then stimulated with the extracellular signal protein EGF, and single fluorescent molecules of Ras-YFP at the inner surface of the plasma membrane are followed by video fluorescence microscopy in individual cells. When a fluorescent Ras-YFP molecule becomes activated, it exchanges unlabeled GDP for fluorescently labeled GTP; the energy emitted by the YFP now activates the fluorescent GTP to emit red light (called fluorescence resonance energy transfer, or FRET; see Figure 9–26). Thus, the activation of single Ras molecules can be followed by the emission of red fluorescence from a previously yellow-green fluorescent spot at the plasma membrane. As shown in (B), activated Ras molecules can be detected after about 30 seconds of EGF stimulation. The red signal peaks at about 3 minutes and then decreases to baseline by 6 minutes. As Ras-GAP is found to be recruited to the same spots at the plasma membrane as Ras, it presumably plays a major part in rapidly shutting off the Ras signal. (Modified from H. Murakoshi et al., *Proc. Natl Acad. Sci. USA* 101:7317–7322, 2004. With permission from National Academy of Sciences.)

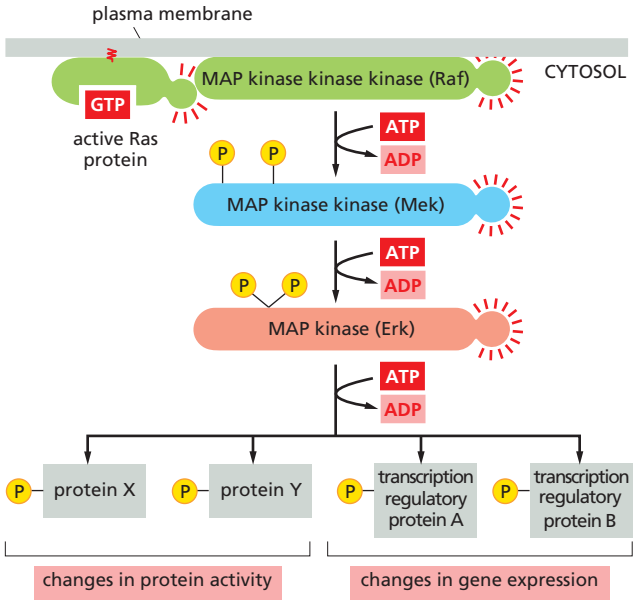


Figure 15–49 The MAP kinase module activated by Ras. The three-component module begins with a MAP kinase kinase kinase called *Raf*. Ras recruits Raf to the plasma membrane and helps activate it. Raf then activates the MAP kinase kinase *Mek*, which then activates the MAP kinase *Erk*. Erk in turn phosphorylates a variety of downstream proteins, including other protein kinases, as well as transcription regulators in the nucleus. The resulting changes in protein activities and gene expression cause complex changes in cell behavior.

Ras to inactivate itself by hydrolyzing its bound GTP to GDP. To stimulate cells to proliferate or differentiate, these short-lived signaling events must be converted into longer-lasting ones that can sustain the signal and relay it downstream to the nucleus to alter the pattern of gene expression. One of the key mechanisms used for this purpose is a system of proteins called the *mitogen-activated protein kinase module* (**MAP kinase module**) (Figure 15–49). The three components of this system form a functional signaling module that has been remarkably well conserved during evolution and is used, with variations, in many different signaling contexts.

The three components are all protein kinases. The final kinase in the series is called simply MAP kinase (MAPK). The next one upstream from this is MAP kinase kinase (MAPKK): it phosphorylates and thereby activates MAP kinase. Next above that, receiving an activating signal directly from Ras, is MAP kinase kinase kinase (MAPKKK): it phosphorylates and thereby activates MAPKK. In the mammalian **Ras–MAP-kinase signaling pathway**, these three kinases are known by shorter names: Raf (= MAPKKK), Mek (= MAPKK), and Erk (=MAPK).

Once activated, the MAP kinase relays the signal downstream by phosphorylating various proteins in the cell, including transcription regulators and other protein kinases (see Figure 15–49). Erk, for example, enters the nucleus and phosphorylates one or more components of a transcription regulatory complex. This activates the transcription of a set of *immediate early genes*, so named because they turn on within minutes after an RTK receives an extracellular signal, even if protein synthesis is experimentally blocked with drugs. Some of these genes encode other transcription regulators that turn on other genes, a process that requires both protein synthesis and more time. In this way, the Ras–MAP-kinase signaling pathway conveys signals from the cell surface to the nucleus and alters the pattern of gene expression. Among the genes activated by this pathway are some that stimulate cell proliferation, such as the genes encoding G_1 *cyclins* (discussed in Chapter 17).

Extracellular signals usually activate MAP kinases only transiently, and the period during which the kinase remains active influences the response. When EGF activates its receptors in a neural precursor cell line, for example, Erk MAP kinase activity peaks at 5 minutes and rapidly declines, and the cells later go on to divide. By contrast, when NGF activates its receptors on the same cells, Erk activity remains high for many hours, and the cells stop proliferating and differentiate into neurons.

Many factors influence the duration and other features of the signaling response, including positive and negative feedback loops, which can combine to

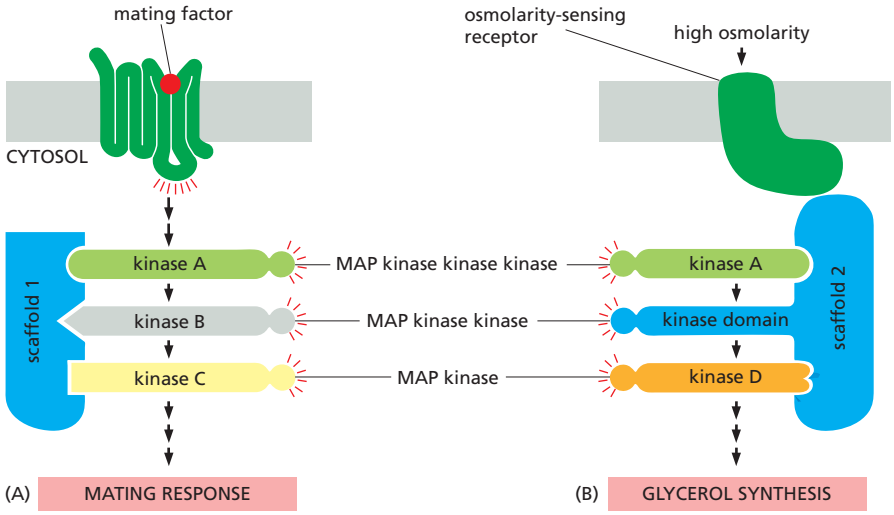


Figure 15–50 The organization of two MAP kinase modules by scaffold proteins in budding yeast. Budding yeast have at least six three-component MAP kinase modules involved in a variety of biological processes, including the two responses illustrated here—a mating response and the response to high osmolarity. (A) The mating response is triggered when a mating factor secreted by a yeast of opposite mating type binds to a GPCR. This activates a G protein, the βγ complex of which indirectly activates the MAPKKK (kinase A), which then relays the response onward. Once activated, the MAP kinase (kinase C) phosphorylates and thereby activates several proteins that mediate the mating response, in which the yeast cell stops dividing and prepares for fusion. The three kinases in this module are bound to scaffold protein 1. (B) In a second response, a yeast cell exposed to a high-osmolarity environment is induced to synthesize glycerol to increase its internal osmolarity. This response is mediated by an osmolarity-sensing receptor protein and a different MAP kinase module bound to a second scaffold protein. (Note that the kinase domain of scaffold 2 provides the MAPKK activity of this module.) Although both pathways use the same MAPKKK (kinase A, *green*), there is no cross-talk between them because the kinases in each module are bound to different scaffold proteins, and the osmosensor is bound to the same scaffold protein as the particular kinase it activates.

give responses that are either graded or switchlike and either brief or long lasting. In an example illustrated earlier, in Figure 15–19, MAP kinase activates a complex positive feedback loop to produce an all-or-none, irreversible response when frog oocytes are stimulated to mature by a brief exposure to the extracellular signal molecule progesterone. In many cells, MAP kinases activate a negative feedback loop by increasing the concentration of a protein phosphatase that removes the phosphate from MAP kinase. The increase in the phosphatase results from both an increase in the transcription of the phosphatase gene and the stabilization of the enzyme against degradation. In the Ras–MAP-kinase pathway shown in Figure 15–49, Erk also phosphorylates and inactivates Raf, providing another negative feedback loop that helps shut off the MAP kinase module.

Scaffold Proteins Help Prevent Cross-talk Between Parallel MAP Kinase Modules

Three-component MAP kinase signaling modules operate in all eukaryotic cells, with different modules mediating different responses in the same cell. In budding yeast, for example, one such module mediates the response to mating pheromone, another the response to starvation, and yet another the response to osmotic shock. Some of these MAP kinase modules use one or more of the same kinases and yet manage to activate different effector proteins and hence different responses. As discussed earlier, one way in which cells avoid cross-talk between the different parallel signaling pathways and ensure that each response is specific is to use scaffold proteins (see Figure 15–10A). In budding yeast cells, such scaffolds bind all or some of the kinases in each MAP kinase module to form a complex and thereby help to ensure response specificity (**Figure 15–50**).

Mammalian cells also use this scaffold strategy to prevent cross-talk between different MAP kinase modules. At least five parallel MAP kinase modules can operate in a mammalian cell. These modules make use of at least 12 MAP kinases, 7 MAPKKs, and 7 MAPKKKs. Two of these modules (terminating in MAP kinases called JNK and p38) are activated by different kinds of cell stresses, such as ultraviolet (UV) irradiation, heat shock, and osmotic stress, as well as by inflammatory cytokines; others mainly mediate responses to signals from other cells.

Although the scaffold strategy provides precision and avoids cross-talk, it reduces the opportunities for amplification and spreading of the signal to different parts of the cell, which require at least some of the components to be diffusible. It is unclear to what extent the individual components of MAP kinase modules can dissociate from the scaffold during the activation process to permit amplification.

Rho Family GTPases Functionally Couple Cell-Surface Receptors to the Cytoskeleton

Besides the Ras proteins, the other class of Ras superfamily GTPases that relays signals from cell-surface receptors is the large **Rho family** (see Table 15–5). Rho family monomeric GTPases regulate both the actin and microtubule cytoskeletons, controlling cell shape, polarity, motility, and adhesion (discussed in Chapter 16); they also regulate cell-cycle progression, gene transcription, and membrane transport. They play a key part in the guidance of cell migration and nerve axon outgrowth, mediating cytoskeletal responses to the activation of a special class of guidance receptors. We focus on this aspect of Rho family function here.

The three best-characterized family members are **Rho** itself, **Rac**, and **Cdc42**, each of which affects multiple downstream target proteins. In the same way as for Ras, GEFs activate and GAPs inactivate the Rho family GTPases; there are more than 80 Rho-GEFs and more than 70 Rho-GAPs in humans. Some of the GEFs and GAPs are specific for one particular family member, whereas others are less specific. Unlike Ras, which is membrane-associated even when inactive (with GDP bound), inactive Rho family GTPases are often bound to *guanine nucleotide dissociation inhibitors* (*GDIs*) in the cytosol, which prevent the GTPases from interacting with their Rho-GEFs at the plasma membrane.

Signaling by extracellular signaling proteins of the **ephrin** family provides an example of how RTKs can activate a Rho GTPase. Ephrins bind and thereby activate members of the *Eph* family of RTKs (see Figure 15–43). One member of the Eph family is found on the surface of motor neurons and helps guide the migrating tip of the axon (called a *growth cone*) to its muscle target. The binding of a cell-surface *ephrin* protein activates the Eph receptor, causing the growth cones to collapse, thereby repelling them from inappropriate regions and keeping them on track. The response depends on a Rho-GEF called *ephexin*, which is stably associated with the cytosolic tail of the Eph receptor. When ephrin binding activates the Eph receptor, the receptor activates a cytoplasmic tyrosine kinase that phosphorylates ephexin on a tyrosine, enhancing the ability of ephexin to activate the Rho protein RhoA. The activated RhoA (RhoA-GTP) then regulates various downstream target proteins, including some effector proteins that control the actin cytoskeleton, causing the growth cone to collapse (**Figure 15–51**).

Having considered how RTKs use GEFs and monomeric GTPases to relay signals into the cell, we now consider a second major strategy that RTKs use that depends on a quite different intracellular relay mechanism.

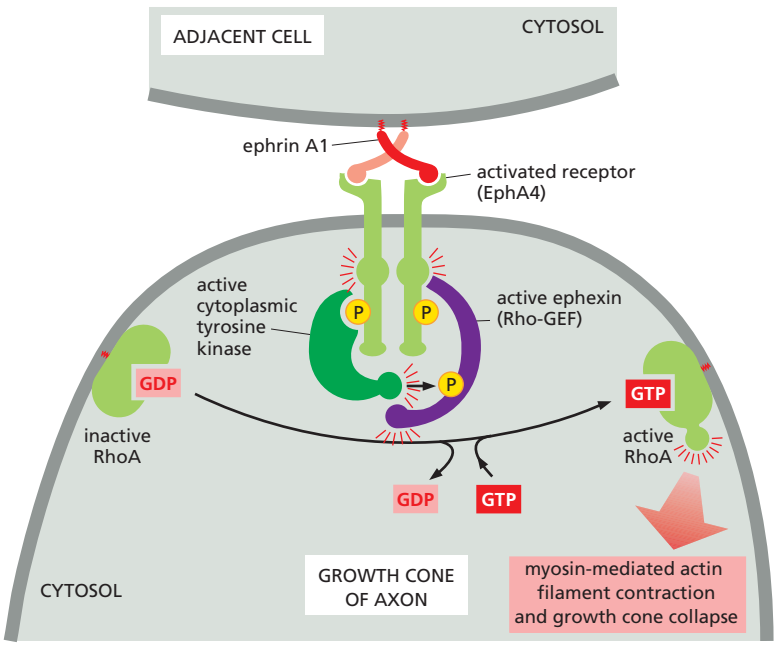


Figure 15–51 Growth cone collapse mediated by Rho family GTPases. The binding of ephrin A1 proteins on an adjacent cell activates EphA4 RTKs on the growth cone. Phosphotyrosines on the activated Eph receptors recruit and activate a cytoplasmic tyrosine kinase to phosphorylate the receptor-associated Rho-GEF ephexin on a tyrosine. This enhances the ability of the ephexin to activate RhoA. RhoA then induces the growth cone to collapse by stimulating the myosin-dependent contraction of the actin cytoskeleton.

PI 3-Kinase Produces Lipid Docking Sites in the Plasma Membrane

As mentioned earlier, one of the proteins that binds to the intracellular tail of RTK molecules is the plasma-membrane-bound enzyme **phosphoinositide 3-kinase** (**PI 3-kinase**). This kinase principally phosphorylates inositol phospholipids rather than proteins, and both RTKs and GPCRs can activate it. It plays a central part in promoting cell survival and growth.

Phosphatidylinositol (*PI*) is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites on its inositol head group to generate a variety of phosphorylated PI lipids called **phosphoinositides**. When activated, PI 3-kinase catalyzes phosphorylation at the 3 position of the inositol ring to generate several phosphoinositides (**Figure 15–52**). The production of PI(3,4,5)P_3 matters most because it can serve as a docking site for various intracellular signaling proteins, which assemble into signaling complexes that relay the signal into the cell from the cytosolic face of the plasma membrane (see Figure 15–10C).

Notice the difference between this use of phosphoinositides and their use described earlier, in which PI(4,5)P_2 is cleaved by PLCβ (in the case of GPCRs) or PLCγ (in the case of RTKs) to generate soluble IP_3 and membrane-bound diacylglycerol (see Figures 15–28 and 15–29). By contrast, PI(3,4,5)P_3 is not cleaved by either PLC. It is made from PI(4,5)P_2 and then remains in the plasma membrane until specific *phosphoinositide phosphatases* dephosphorylate it. Prominent among these is the *PTEN* phosphatase, which dephosphorylates the 3 position of the inositol ring. Mutations in PTEN are found in many cancers: by prolonging signaling by PI 3-kinase, they promote uncontrolled cell growth.

There are various types of PI 3-kinases. Those activated by RTKs and GPCRs belong to class I. These are heterodimers composed of a common catalytic subunit and different regulatory subunits. RTKs activate *class Ia PI 3-kinases*, in which the regulatory subunit is an adaptor protein that binds to two phosphotyrosines on activated RTKs through its two SH2 domains (see Figure 15–46A). GPCRs activate *class Ib PI 3-kinases*, which have a regulatory subunit that binds to the βγ complex of an activated trimeric G protein (G_q) when GPCRs are activated by their extracellular ligand. The direct binding of activated Ras can also activate the common class I catalytic subunit.

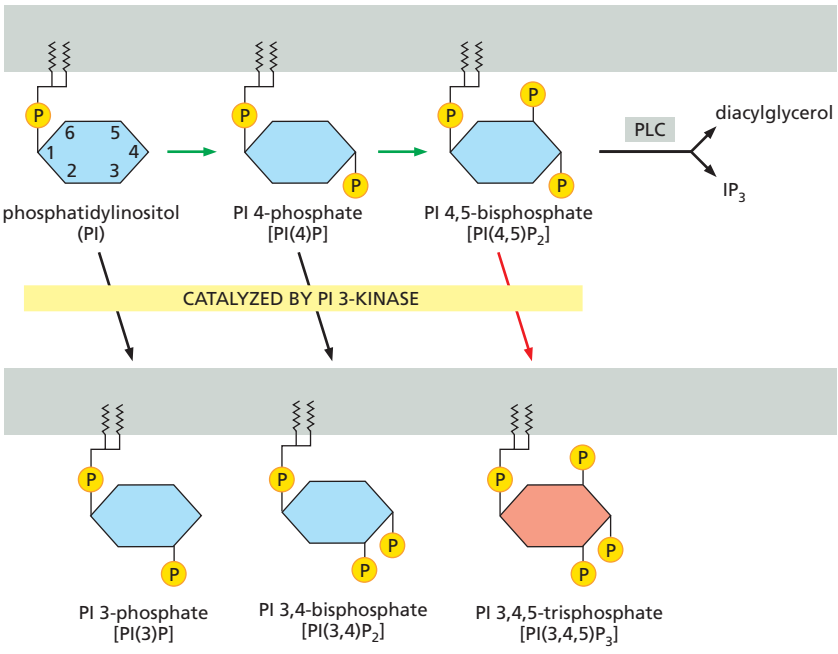


Figure 15–52 The generation of phosphoinositide docking sites by PI 3-kinase. PI 3-kinase phosphorylates the inositol ring on carbon atom 3 to generate the phosphoinositides shown at the bottom of the figure (diverting them away from the pathway leading to IP_3 and diacylglycerol; see Figure 15–28). The most important phosphorylation (indicated in *red*) is of PI(4,5)P_2 to PI(3,4,5)P_3, which can serve as a docking site for signaling proteins with PI(3,4,5)P_3-binding PH domains. Other inositol phospholipid kinases (not shown) catalyze the phosphorylations indicated by the *green arrows*.

Intracellular signaling proteins bind to $PI(3,4,5)P_3$ produced by activated PI 3-kinase via a specific interaction domain, such as a **pleckstrin homology (PH) domain**, first identified in the platelet protein pleckstrin. PH domains function mainly as protein–protein interaction domains, and it is only a small subset of them that bind to $PI(3,4,5)P_3$; at least some of these also recognize a specific membrane-bound protein as well as the $PI(3,4,5)P_3$, which greatly increases the specificity of the binding and helps to explain why the signaling proteins with $PI(3,4,5)P_3$-binding PH domains do not all dock at all $PI(3,4,5)P_3$ sites. PH domains occur in about 200 human proteins, including the Ras-GEF Sos discussed earlier (see Figure 15–11).

One especially important PH-domain-containing protein is the serine/threonine protein kinase *Akt*. The *PI-3-kinase–Akt signaling pathway* is the major pathway activated by the hormone *insulin*. It also plays a key part in promoting the survival and growth of many cell types in both invertebrates and vertebrates, as we now discuss.

The PI-3-Kinase–Akt Signaling Pathway Stimulates Animal Cells to Survive and Grow

As discussed earlier, extracellular signals are usually required for animal cells to grow and divide, as well as to survive (see Figure 15–4). Members of the *insulin-like growth factor* (*IGF*) family of signal proteins, for example, stimulate many types of animal cells to survive and grow. They bind to specific RTKs (see Figure 15–43), which activate PI 3-kinase to produce $PI(3,4,5)P_3$. The $PI(3,4,5)P_3$ recruits two protein kinases to the plasma membrane via their PH domains—**Akt** (also called *protein kinase B*, or *PKB*) and *phosphoinositide-dependent protein kinase 1* (*PDK1*), and this leads to the activation of Akt (**Figure 15–53**). Once activated,

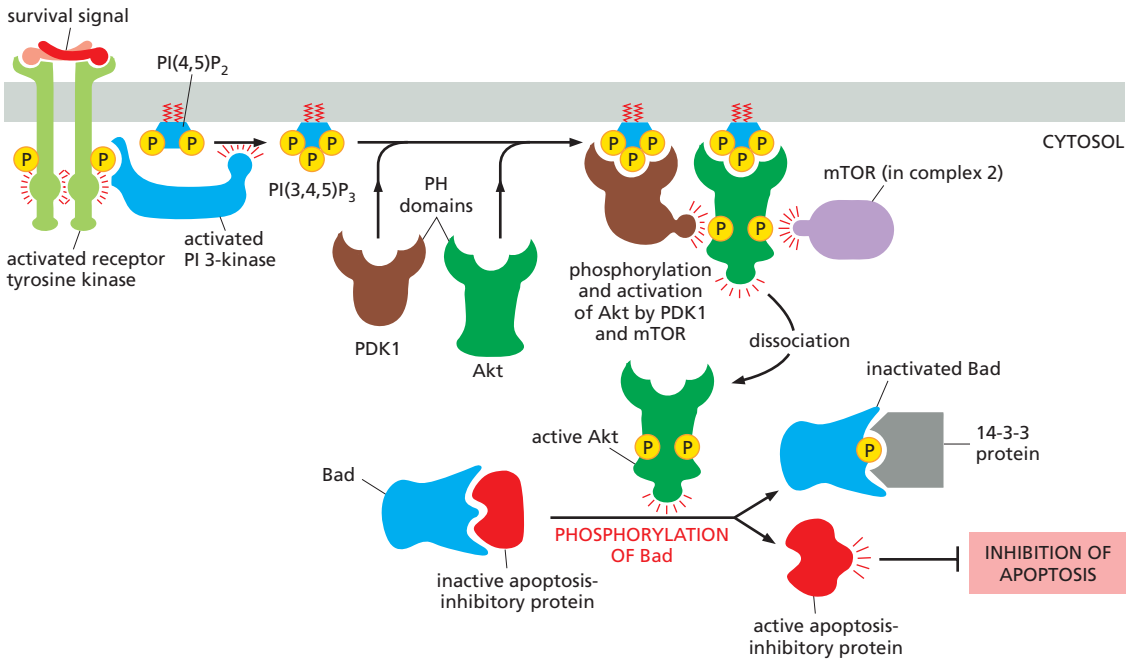


Figure 15–53 One way in which signaling through PI 3-kinase promotes cell survival. An extracellular survival signal activates an RTK, which recruits and activates PI 3-kinase. The PI 3-kinase produces $PI(3,4,5)P_3$, which serves as a docking site for two serine/threonine kinases with PH domains—Akt and the phosphoinositide-dependent kinase PDK1—and brings them into proximity at the plasma membrane. The Akt is phosphorylated on a serine by a third kinase (usually mTOR in complex 2), which alters the conformation of the Akt so that it can be phosphorylated on a threonine by PDK1, which activates the Akt. The activated Akt now dissociates from the plasma membrane and phosphorylates various target proteins, including the Bad protein. When unphosphorylated, Bad holds one or more apoptosis-inhibitory proteins (of the Bcl2 family—discussed in Chapter 18) in an inactive state. Once phosphorylated, Bad releases the inhibitory proteins, which now can block apoptosis and thereby promote cell survival. As shown, the phosphorylated Bad binds to a ubiquitous cytosolic protein called *14-3-3*, which keeps Bad out of action.

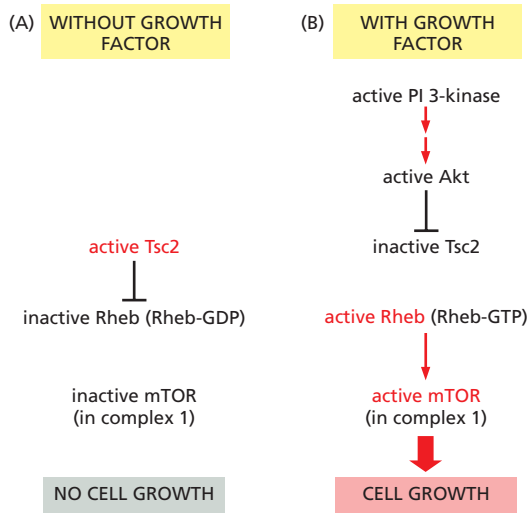


Figure 15–54 Activation of mTOR by the PI-3-kinase–Akt signaling pathway. (A) In the absence of extracellular growth factors, Tsc2 (a Rheb-GAP) keeps Rheb inactive; mTOR in complex 1 is inactive, and there is no cell growth. (B) In the presence of growth factors, activated Akt phosphorylates and inhibits Tsc2, thereby promoting the activation of Rheb. Activated Rheb (Rheb-GTP) helps activate mTOR in complex 1, which in turn stimulates cell growth. Figure 15–53 shows how growth factors (or survival signals) activate Akt. The Erk MAP kinase (see Figure 15–49) can also phosphorylate and inhibit Tsc2 and thereby activate mTOR. Thus, both the PI-3-kinase–Akt and Ras–MAP-kinase signaling pathways converge on mTOR in complex 1 to stimulate cell growth.

Tsc2 is short for *tuberous sclerosis protein 2,* and it is one component of a heterodimer composed of Tsc1 and Tsc2 (not shown); these proteins are so called because mutations in either gene encoding them cause the genetic disease *tuberous sclerosis,* which is associated with benign tumors that contain abnormally large cells.

Akt phosphorylates various target proteins at the plasma membrane, as well as in the cytosol and nucleus. The effect on most of the known targets is to inactivate them; but the targets are such that these actions of Akt all conspire to enhance cell survival and growth, as illustrated for one cell survival pathway in Figure 15–53.

The control of cell growth by the **PI-3-kinase–Akt pathway** depends in part on a large protein kinase called **TOR** (named as the target of *rapamycin*, a bacterial toxin that inactivates the kinase and is used clinically as both an immunosuppressant and anticancer drug). TOR was originally identified in yeasts in genetic screens for rapamycin resistance; in mammalian cells, it is called **mTOR**, which exists in cells in two functionally distinct multiprotein complexes. *mTOR complex 1* contains the protein *raptor*; this complex is sensitive to rapamycin, and it stimulates cell growth—both by promoting ribosome production and protein synthesis and by inhibiting protein degradation. Complex 1 also promotes both cell growth and cell survival by stimulating nutrient uptake and metabolism. *mTOR complex 2* contains the protein *rictor* and is insensitive to rapamycin; it helps to activate Akt (see Figure 15–53), and it regulates the actin cytoskeleton via Rho family GTPases.

The mTOR in complex 1 integrates inputs from various sources, including extracellular signal proteins referred to as *growth factors* and nutrients such as amino acids, both of which help activate mTOR and promote cell growth. The growth factors activate mTOR mainly via the PI-3-kinase–Akt pathway. Akt activates mTOR in complex 1 indirectly by phosphorylating, and thereby inhibiting, a GAP called Tsc2. Tsc2 acts on a monomeric Ras-related GTPase called **Rheb** (see Table 15–5). Rheb in its active form (Rheb-GTP) activates mTOR in complex 1. The net result is that Akt activates mTOR and thereby promotes cell growth (**Figure 15–54**). We discuss how mTOR stimulates ribosome production and protein synthesis in Chapter 17 (see Figure 17–64).

RTKs and GPCRs Activate Overlapping Signaling Pathways

As mentioned earlier, RTKs and GPCRs activate some of the same intracellular signaling pathways. Both, for example, can activate the inositol phospholipid pathway triggered by phospholipase C. Moreover, even when they activate different pathways, the different pathways can converge on the same target proteins. **Figure 15–55** illustrates both of these types of signaling overlaps: it summarizes five parallel intracellular signaling pathways that we have discussed so far—one triggered by GPCRs, two triggered by RTKs, and two triggered by both kinds of receptors. Interactions among these pathways allow different extracellular signal molecules to modulate and coordinate each other's effects.

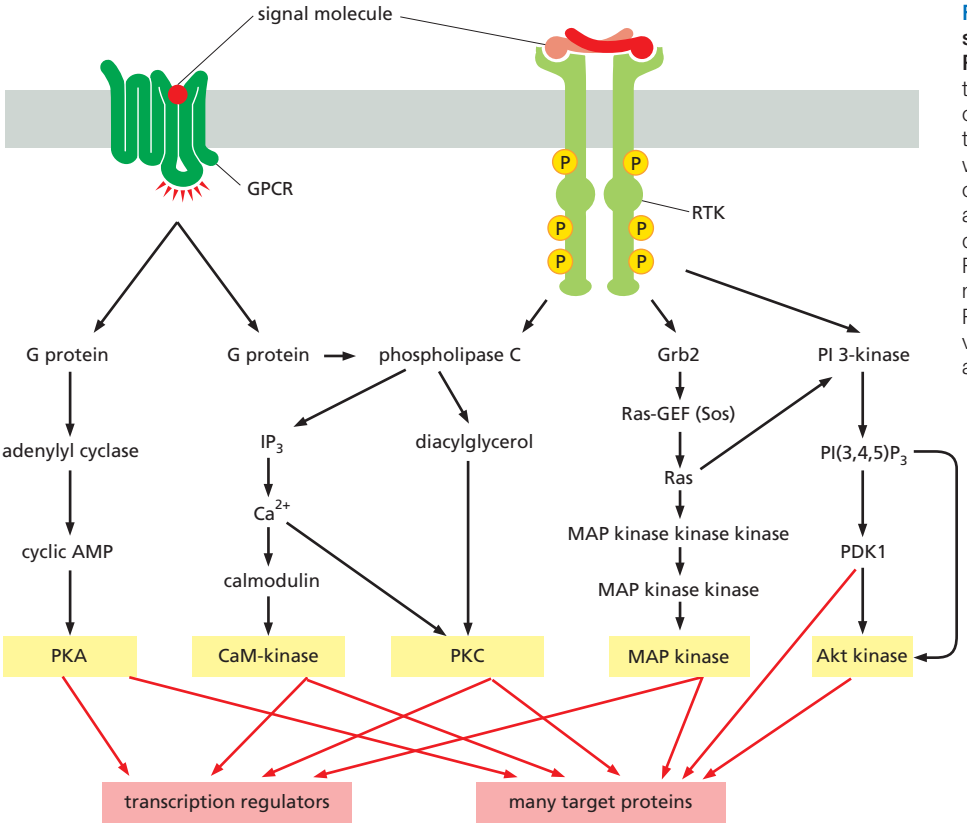


Figure 15–55 Five parallel intracellular signaling pathways activated by GPCRs, RTKs, or both. In this simplified example, the five kinases (shaded *yellow*) at the end of each signaling pathway phosphorylate target proteins (shaded *red*), many of which are phosphorylated by more than one of the kinases. The phospholipase C activated by the two types of receptors is different: GPCRs activate PLCβ, whereas RTKs activate PLCγ (not shown). Although not shown, some GPCRs can also activate Ras, but they do so independently of Grb2, via a Ras-GEF that is activated by Ca^{2+} and diacylglycerol.

Some Enzyme-Coupled Receptors Associate with Cytoplasmic Tyrosine Kinases

Many cell-surface receptors depend on tyrosine phosphorylation for their activity and yet lack a tyrosine kinase domain. These receptors act through **cytoplasmic tyrosine kinases**, which are associated with the receptors and phosphorylate various target proteins, often including the receptors themselves, when the receptors bind their ligand. These **tyrosine-kinase-associated receptors** thus function in much the same way as RTKs, except that their kinase domain is encoded by a separate gene and is noncovalently associated with the receptor polypeptide chain. A variety of receptor classes belong in this category, including the receptors for antigen and interleukins on lymphocytes (discussed in Chapter 24), integrins (discussed in Chapter 19), and receptors for various cytokines and some hormones. As with RTKs, many of these receptors are either preformed dimers or are cross-linked into dimers by ligand binding.

Some of these receptors depend on members of the largest family of mammalian cytoplasmic tyrosine kinases, the **Src family** (see Figures 3–10 and 3–64), which includes *Src*, *Yes*, *Fgr*, *Fyn*, *Lck*, *Lyn*, *Hck*, and *Blk*. These protein kinases all contain SH2 and SH3 domains and are located on the cytoplasmic side of the plasma membrane, held there partly by their interaction with transmembrane receptor proteins and partly by covalently attached lipid chains. Different family members are associated with different receptors and phosphorylate overlapping but distinct sets of target proteins. Lyn, Fyn, and Lck, for example, are each associated with different sets of receptors on lymphocytes. In each case, the kinase is activated when an extracellular ligand binds to the appropriate receptor protein. Src itself, as well as several other family members, can also bind to activated RTKs; in these cases, the receptor and cytoplasmic kinases mutually stimulate each other's catalytic activity, thereby strengthening and prolonging the signal (see Figure 15–51). There are even some G proteins (G_s and G_i) that can activate Src, which is one way that the activation of GPCRs can lead to tyrosine phosphorylation of intracellular signaling proteins and effector proteins.

Another type of cytoplasmic tyrosine kinase associates with *integrins*, the main receptors that cells use to bind to the extracellular matrix (discussed in Chapter 19). The binding of matrix components to integrins activates intracellular signaling pathways that influence the behavior of the cell. When integrins cluster at sites of matrix contact, they help trigger the assembly of cell–matrix junctions called *focal adhesions*. Among the many proteins recruited into these junctions is the cytoplasmic tyrosine kinase called **focal adhesion kinase** (**FAK**), which binds to the cytosolic tail of one of the integrin subunits with the assistance of other proteins. The clustered FAK molecules phosphorylate each other, creating phosphotyrosine docking sites where the Src kinase can bind. Src and FAK then phosphorylate each other and other proteins that assemble in the junction, including many of the signaling proteins used by RTKs. In this way, the two tyrosine kinases signal to the cell that it has adhered to a suitable substratum, where the cell can now survive, grow, divide, migrate, and so on.

The largest and most diverse class of receptors that rely on cytoplasmic tyrosine kinases to relay signals into the cell is the class of *cytokine receptors*, which we consider next.

Cytokine Receptors Activate the JAK–STAT Signaling Pathway

The large family of **cytokine receptors** includes receptors for many kinds of local mediators (collectively called *cytokines*), as well as receptors for some hormones, such as *growth hormone* and *prolactin* (**Movie 15.8**). These receptors are stably associated with cytoplasmic tyrosine kinases called **Janus kinases** (**JAKs**) (after the two-faced Roman god), which phosphorylate and activate transcription regulators called **STATs** (**s**ignal **t**ransducers and **a**ctivators of **t**ranscription). STAT proteins are located in the cytosol and are referred to as *latent transcription regulators* because they migrate into the nucleus and regulate gene transcription only after they are activated.

Although many intracellular signaling pathways lead from cell-surface receptors to the nucleus, where they alter gene transcription (see Figure 15–55), the **JAK–STAT signaling pathway** provides one of the more direct routes. Cytokine receptors are dimers or trimers and are stably associated with one or two of the four known JAKs (JAK1, JAK2, JAK3, and Tyk2). Cytokine binding alters the arrangement so as to bring two JAKs into close proximity so that they phosphorylate each other, thereby increasing the activity of their tyrosine kinase domains. The JAKs then phosphorylate tyrosines on the cytoplasmic tails of cytokine receptors, creating phosphotyrosine docking sites for STATs (**Figure 15–56**). Some adaptor proteins can also bind to some of these sites and couple cytokine receptors to the Ras–MAP-kinase signaling pathway discussed earlier, but these will not be discussed here.

There are at least six STATs in mammals. Each has an SH2 domain that performs two functions. First, it mediates the binding of the STAT protein to a phosphotyrosine docking site on an activated cytokine receptor. Once bound, the JAKs phosphorylate the STAT on tyrosines, causing the STAT to dissociate from the receptor. Second, the SH2 domain on the released STAT now mediates its binding to a phosphotyrosine on another STAT molecule, forming either a STAT homodimer or a heterodimer. The STAT dimer then translocates to the nucleus, where, in combination with other transcription regulatory proteins, it binds to a specific *cis*-regulatory sequence in various genes and stimulates their transcription (see Figure 15–56). In response to the hormone prolactin, for example, which stimulates breast cells to produce milk, activated STAT5 stimulates the transcription of genes that encode milk proteins. **Table 15–6** lists some of the more than 30 cytokines and hormones that activate the JAK–STAT pathway by binding to cytokine receptors.

Negative feedback regulates the responses mediated by the JAK–STAT pathway. In addition to activating genes that encode proteins mediating the cytokine-induced response, the STAT dimers can also activate genes that encode inhibitory proteins that help shut off the response. Some of these proteins bind to

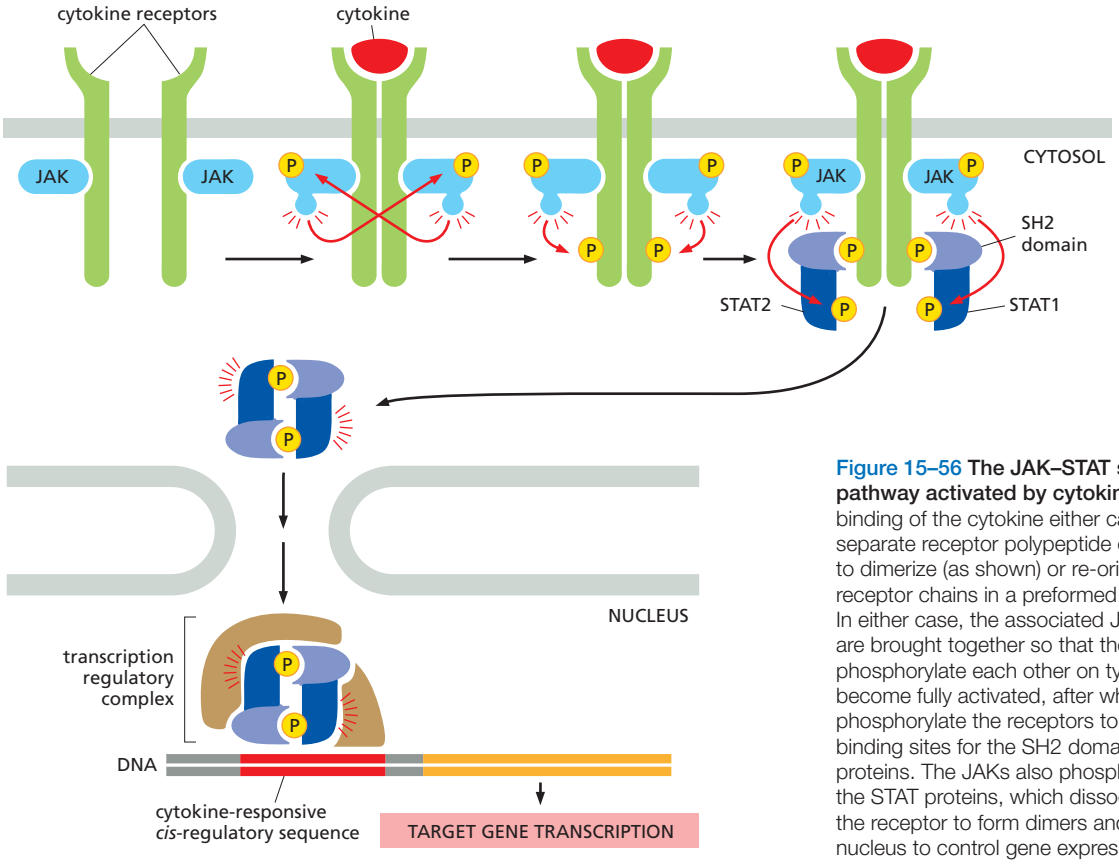


Figure 15–56 The JAK–STAT signaling pathway activated by cytokines. The binding of the cytokine either causes two separate receptor polypeptide chains to dimerize (as shown) or re-orients the receptor chains in a preformed dimer. In either case, the associated JAKs are brought together so that they can phosphorylate each other on tyrosines to become fully activated, after which they phosphorylate the receptors to generate binding sites for the SH2 domains of STAT proteins. The JAKs also phosphorylate the STAT proteins, which dissociate from the receptor to form dimers and enter the nucleus to control gene expression.

and inactivate phosphorylated JAKs and their associated phosphorylated receptors; others bind to phosphorylated STAT dimers and prevent them from binding to their DNA targets. Such negative feedback mechanisms, however, are not enough on their own to turn off the response. Inactivation of the activated JAKs and STATs requires dephosphorylation of their phosphotyrosines.

Protein Tyrosine Phosphatases Reverse Tyrosine Phosphorylations

In all signaling pathways that use tyrosine phosphorylation, the tyrosine phosphorylations are reversed by **protein tyrosine phosphatases**. These phosphatases are as important in the signaling process as the protein tyrosine kinases that add the phosphates. Whereas only a few types of *serine/threonine protein phosphatase*

TABLE 15–6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

Signal protein	Receptor-associated JAKs	STATs activated	Some responses
Interferon-γ (IFNγ)	JAK1 and JAK2	STAT1	Activates macrophages
Interferon-α (IFNα)	Tyk2 and JAK2	STAT1 and STAT2	Increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	Stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	Stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	Stimulates growth by inducing IGF1 production
Granulocyte–Macrophage-Colony-Stimulating Factor (GMCSF)	JAK2	STAT5	Stimulates production of granulocytes and macrophages

catalytic subunits are responsible for removing phosphate groups from phosphorylated serines and threonines on proteins, there are about 100 protein tyrosine phosphatases encoded in the human genome, including some *dual-specificity phosphatases* that also dephosphorylate serines and threonines.

Like tyrosine kinases, the tyrosine phosphatases occur in both cytoplasmic and transmembrane forms. Unlike serine/threonine protein phosphatases, which generally have broad specificity, most tyrosine phosphatases display exquisite specificity for their substrates, removing phosphate groups from only selected phosphotyrosines on a subset of proteins. Together, these phosphatases ensure that tyrosine phosphorylations are short-lived and that the level of tyrosine phosphorylation in resting cells is very low. They do not, however, simply continuously reverse the effects of protein tyrosine kinases; they are often regulated to act only at the appropriate time and place.

Having discussed the crucial role of tyrosine phosphorylation and dephosphorylation in the intracellular signaling pathways activated by many enzyme-coupled receptors, we now turn to a class of enzyme-coupled receptors that rely on serine and threonine phosphorylation. These *receptor serine/threonine kinases* activate an even more direct signaling pathway to the nucleus than does the JAK–STAT pathway. They directly phosphorylate latent transcription regulators called *Smads*, which then translocate into the nucleus to control gene transcription.

Signal Proteins of the TGFβ Superfamily Act Through Receptor Serine/Threonine Kinases and Smads

The **transforming growth factor-β (TGFβ) superfamily** consists of a large number (33 in humans) of structurally related, secreted, dimeric proteins. They act either as hormones or, more commonly, as local mediators to regulate a wide range of biological functions in all animals. During development, they regulate pattern formation and influence various cell behaviors, including proliferation, specification and differentiation, extracellular matrix production, and cell death. In adults, they are involved in tissue repair and in immune regulation, as well as in many other processes. The superfamily consists of the TGFβ/*activin* family and the larger *bone morphogenetic protein* (*BMP*) family.

All of these proteins act through enzyme-coupled receptors that are single-pass transmembrane proteins with a serine/threonine kinase domain on the cytosolic side of the plasma membrane. There are two classes of these **receptor serine/threonine kinases**—*type I* and *type II*—which are structurally similar homodimers. Each member of the TGFβ superfamily binds to a characteristic combination of type-I and type-II receptor dimers, bringing the kinase domains together so that the type-II receptor can phosphorylate and activate the type-I receptor, forming an active tetrameric receptor complex.

Once activated, the receptor complex uses a strategy for rapidly relaying the signal to the nucleus that is very similar to the JAK–STAT strategy used by cytokine receptors. The activated type-I receptor directly binds and phosphorylates a latent transcription regulator of the **Smad family** (named after the first two proteins identified, Sma in *C. elegans* and Mad in *Drosophila*). Activated TGFβ/activin receptors phosphorylate Smad2 or Smad3, while activated BMP receptors phosphorylate Smad1, Smad5, or Smad8. Once one of these *receptor-activated Smads* (*R-Smads*) has been phosphorylated, it dissociates from the receptor and binds to Smad4 (called a *co-Smad*), which can form a complex with any of the five R-Smads. The Smad complex then translocates into the nucleus, where it associates with other transcription regulators and controls the transcription of specific target genes (**Figure 15–57**). Because the partner proteins in the nucleus vary depending on the cell type and state of the cell, the genes affected vary.

Activated TGFβ receptors and their bound ligand are endocytosed by two distinct routes, one leading to further activation and the other leading to inactivation. The activation route depends on clathrin-coated vesicles and leads to early endosomes (discussed in Chapter 13), where most of the Smad activation occurs. An anchoring protein called *SARA* (for *Smad anchor for receptor activation*) has

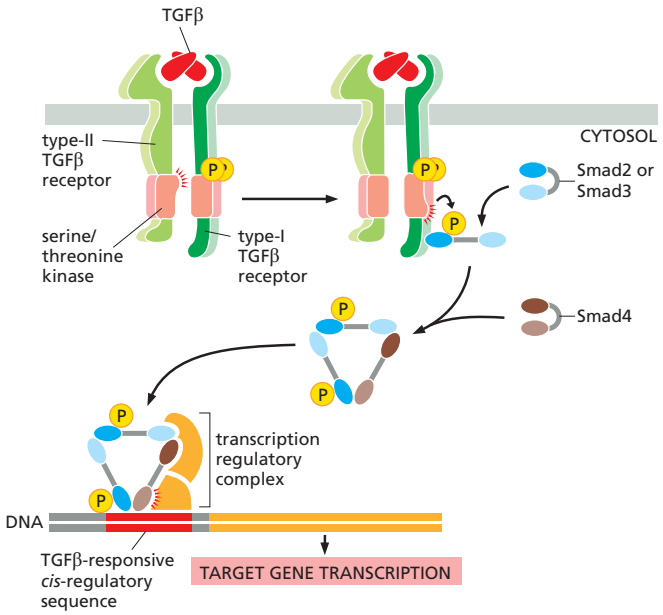


Figure 15–57 The Smad-dependent signaling pathway activated by TGFβ. The TGFβ dimer promotes the assembly of a tetrameric receptor complex containing two copies each of the type-I and type-II receptors. The type-II receptors phosphorylate specific sites on the type-I receptors, thereby activating their kinase domains and leading to phosphorylation of R-Smads such as Smad2 and Smad3. Smads open up to expose a dimerization surface when they are phosphorylated, leading to the formation of a trimeric Smad complex containing two R-Smads and the co-Smad, Smad4. The phosphorylated Smad complex enters the nucleus and collaborates with other transcription regulators to control the transcription of specific target genes.

an important role in this pathway; it is concentrated in early endosomes and binds to both activated TGFβ receptors and Smads, increasing the efficiency of receptor-mediated Smad phosphorylation. The inactivation route depends on *caveolae* (discussed in Chapter 13) and leads to receptor ubiquitylation and degradation in proteasomes.

During the signaling response, the Smads shuttle continuously between the cytoplasm and the nucleus: they are dephosphorylated in the nucleus and exported to the cytoplasm, where they can be rephosphorylated by activated receptors. In this way, the effect exerted on the target genes reflects both the concentration of the extracellular signal and the time the signal continues to act on the cell-surface receptors (often several hours). Cells exposed to a morphogen at high concentration, or for a long time, or both, will switch on one set of genes, whereas cells receiving a lower or more transient exposure will switch on another set.

As in other signaling systems, negative feedback regulates the Smad pathway. Among the target genes activated by Smad complexes are those that encode *inhibitory Smads*, either Smad6 or Smad7. Smad7 (and possibly Smad6) binds to the cytosolic tail of the activated receptor and inhibits its signaling ability in at least three ways: (1) it competes with R-Smads for binding sites on the receptor, decreasing R-Smad phosphorylation; (2) it recruits a ubiquitin ligase called *Smurf*, which ubiquitylates the receptor, leading to receptor internalization and degradation (it is because Smurfs also ubiquitylate and promote the degradation of Smads that they are called *Smad ubiquitylation regulatory factors,* or Smurfs); and (3) it recruits a protein phosphatase that dephosphorylates and inactivates the receptor. In addition, the inhibitory Smads bind to the co-Smad, Smad4, and inhibit it, either by preventing its binding to R-Smads or by promoting its ubiquitylation and degradation.

Although receptor serine/threonine kinases operate mainly through the Smad pathway just described, they can also stimulate other intracellular signaling proteins such as MAP kinases and PI 3-kinase. Conversely, signaling proteins in other pathways can phosphorylate Smads and thereby influence signaling along the Smad pathway.

Summary

There are various classes of enzyme-coupled receptors, the most common of which are receptor tyrosine kinases (RTKs), tyrosine-kinase-associated receptors, and receptor serine/threonine kinases.

Ligand binding to RTKs causes their dimerization, which leads to activation of their kinase domains. These activated kinase domains phosphorylate multiple tyrosines on the receptors, producing a set of phosphotyrosines that serve as docking sites for a set of intracellular signaling proteins, which bind via their SH2 (or PTB) domains. One such signaling protein serves as an adaptor to couple some activated receptors to a Ras-GEF (Sos), which activates the monomeric GTPase Ras; Ras, in turn, activates a three-component MAP kinase signaling module, which relays the signal to the nucleus by phosphorylating transcription regulatory proteins. Another important signaling protein that can dock on activated RTKs is PI 3-kinase, which phosphorylates specific phosphoinositides to produce lipid docking sites in the plasma membrane for signaling proteins with phosphoinositide-binding PH domains, including the serine/threonine protein kinase Akt (PKB), which plays a key part in the control of cell survival and cell growth. Many receptor classes, including some RTKs, activate Rho family monomeric GTPases, which functionally couple the receptors to the cytoskeleton.

Tyrosine-kinase-associated receptors depend on various cytoplasmic tyrosine kinases for their action. These kinases include members of the Src family, which associate with many kinds of receptors, and the focal adhesion kinase (FAK), which associates with integrins at focal adhesions. The cytoplasmic tyrosine kinases then phosphorylate a variety of signaling proteins to relay the signal onward. The largest family of receptors in this class is the cytokine receptor family. When stimulated by ligand binding, these receptors activate JAK cytoplasmic tyrosine kinases, which phosphorylate STATs. The STATs then dimerize, translocate to the nucleus, and activate the transcription of specific genes. Receptor serine/threonine kinases, which are activated by signal proteins of the TGFβ superfamily, act similarly: they directly phosphorylate and activate Smads, which then oligomerize with another Smad, translocate to the nucleus, and regulate gene transcription.

ALTERNATIVE SIGNALING ROUTES IN GENE REGULATION

Major changes in the behavior of a cell tend to depend on changes in the expression of numerous genes. Thus, many extracellular signaling molecules carry out their effects, in whole or in part, by initiating signaling pathways that change the activities of transcription regulators. There are numerous examples of gene regulation in both GPCR and enzyme-coupled receptor pathways (see Figures 15–27 and 15–49). In this section, we describe some of the less common signaling mechanisms by which gene expression can be controlled. We begin with several pathways that depend on *regulated proteolysis* to control the activity and location of latent transcription regulators. We then turn to a class of extracellular signal molecules that do not employ cell-surface receptors but enter the cell and interact directly with transcription regulators to perform their functions. Finally, we briefly discuss some of the mechanisms by which gene expression is controlled by the *circadian rhythm*: the daily cycle of light and dark.

The Receptor Notch Is a Latent Transcription Regulatory Protein

Signaling through the **Notch** receptor protein is used widely in animal development. As discussed in Chapter 22, it has a general role in controlling cell fate choices and regulating pattern formation during the development of most tissues, as well as in the continual renewal of tissues such as the lining of the gut. It is best known, however, for its role in the production of *Drosophila* neural cells, which usually arise as isolated single cells within an epithelial sheet of precursor cells. During this process, when a precursor cell commits to becoming a neural cell, it signals to its immediate neighbors not to do the same; the inhibited cells develop into epidermal cells instead. This process, called *lateral inhibition*, depends on a contact-dependent signaling mechanism that is activated by a single-pass transmembrane signal protein called **Delta**, displayed on the surface of the future neural cell. By binding to the Notch receptor protein on a neighboring cell, Delta

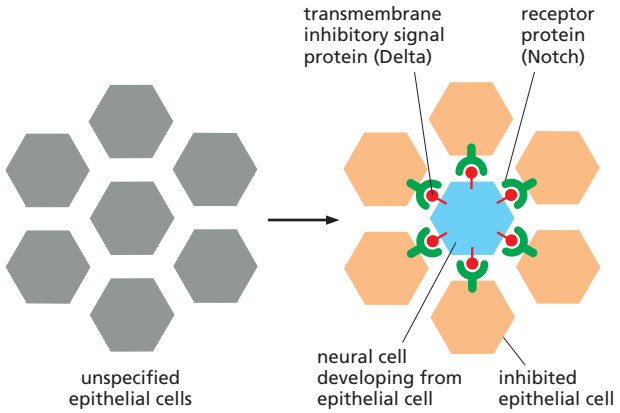


Figure 15–58 Lateral inhibition mediated by Notch and Delta during neural cell development in *Drosophila*. When individual cells in the epithelium begin to develop as neural cells, they signal to their neighbors not to do the same. This inhibitory, contact-dependent signaling is mediated by the ligand Delta, which appears on the surface of the future neural cell and binds to Notch receptor proteins on the neighboring cells. In many tissues, all the cells in a cluster initially express both Delta and Notch, and a competition occurs, with one cell emerging as winner, expressing Delta strongly and inhibiting its neighbors from doing likewise. In other cases, additional factors interact with Delta or Notch to make some cells susceptible to the lateral inhibition signal and others unresponsive to it.

signals to the neighbor not to become neural (**Figure 15–58**). When this signaling process is defective, a huge excess of neural cells is produced at the expense of epidermal cells, which is lethal.

Notch is a single-pass transmembrane protein that requires proteolytic processing to function. It acts as a latent transcription regulator and provides the simplest and most direct signaling pathway known from a cell-surface receptor to the nucleus. When activated by the binding of Delta on another cell, a plasma-membrane-bound protease cleaves off the cytoplasmic tail of Notch, and the released tail translocates into the nucleus to activate the transcription of a set of Notch-response genes. The Notch tail fragment acts by binding to a DNA-binding protein, converting it from a transcriptional repressor into a transcriptional activator.

The Notch receptor undergoes three successive proteolytic cleavage steps, but only the last two depend on Delta binding. As part of its normal biosynthesis, it is cleaved in the Golgi apparatus to form a heterodimer, which is then transported to the cell surface as the mature receptor. The binding of Delta to Notch induces a second cleavage in the extracellular domain, mediated by an extracellular protease. A final cleavage quickly follows, cutting free the cytoplasmic tail of the activated receptor (**Figure 15–59**). Note that, unlike most receptors, the activation of Notch is irreversible; once activated by ligand binding, the protein cannot be used again.

This final cleavage of the Notch tail occurs just within the transmembrane segment, and it is mediated by a protease complex called *γ-secretase*, which is also responsible for the intramembrane cleavage of various other proteins. One of its essential subunits is *Presenilin*, so called because mutations in the gene encoding it are a frequent cause of early-onset, familial Alzheimer's disease, a form of presenile dementia. The protease complex is thought to contribute to this and other forms of Alzheimer's disease by generating extracellular peptide fragments from a transmembrane neuronal protein; the fragments accumulate in excessive amounts and form aggregates of misfolded protein called amyloid plaques, which may injure nerve cells and contribute to their degeneration and loss.

Both Notch and Delta are glycoproteins, and their interaction is regulated by the glycosylation of Notch. The *Fringe family* of glycosyl transferases, in particular, adds extra sugars to the *O*-linked oligosaccharide (discussed in Chapter 13) on Notch, which alters the specificity of Notch for its ligands. This has provided the first example of the modulation of ligand–receptor signaling by differential receptor glycosylation.

Wnt Proteins Bind to Frizzled Receptors and Inhibit the Degradation of β-Catenin

Wnt proteins are secreted signal molecules that act as local mediators and morphogens to control many aspects of development in all animals that have been studied. They were discovered independently in flies and in mice: in *Drosophila*, the *Wingless* (*Wg*) gene originally came to light because of its role as a morphogen

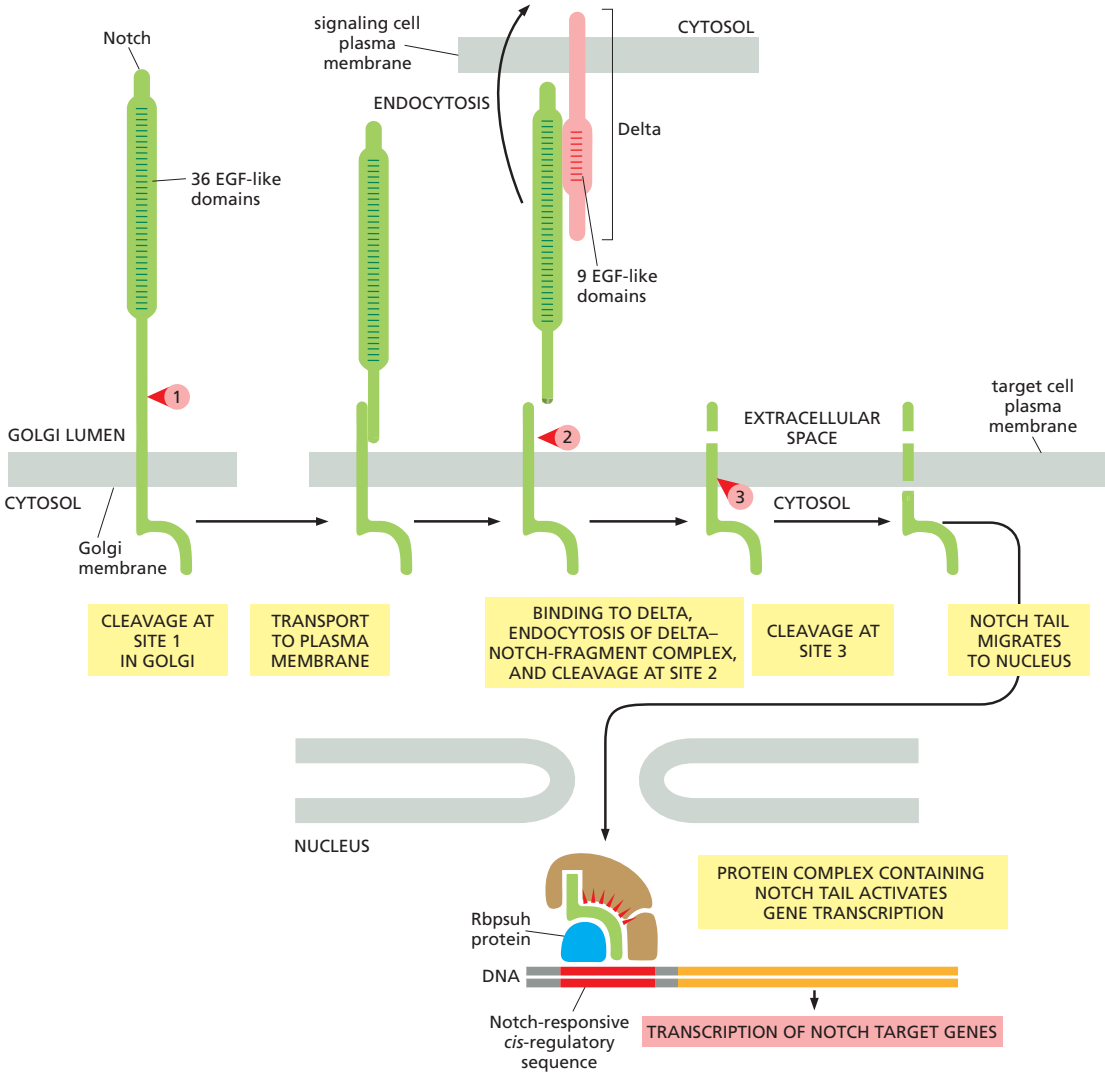


Figure 15–59 The processing and activation of Notch by proteolytic cleavage. The *numbered red arrowheads* indicate the sites of proteolytic cleavage. The first proteolytic processing step occurs within the *trans* Golgi network to generate the mature heterodimeric Notch receptor that is then displayed on the cell surface. The binding to Delta on a neighboring cell triggers the next two proteolytic steps: the complex of Delta and the Notch fragment to which it is bound is endocytosed by the Delta-expressing cell, exposing the extracellular cleavage site in the transmembrane Notch subunit. Note that Notch and Delta interact through their repeated EGF-like domains. The released Notch tail migrates into the nucleus, where it binds to the Rbpsuh protein, which it converts from a transcriptional repressor to a transcriptional activator.

in wing development, while in mice, the *Int1* gene was found because it promoted the development of breast tumors when activated by the integration of a virus next to it. Both of these genes encode Wnt proteins. Wnts are unusual as secreted proteins in that they have a fatty acid chain covalently attached to their N-terminus, which increases their binding to cell surfaces. There are 19 Wnts in humans, each having distinct, but often overlapping, functions.

Wnts can activate at least two types of intracellular signaling pathways. Our primary focus here is the *Wnt/β-catenin pathway* (also known as the *canonical Wnt pathway*), which is centered on the latent transcription regulator *β-catenin*. A second pathway, called the *planar polarity pathway*, coordinates the polarization of cells in the plane of a developing epithelium and depends on Rho family GTPases. Both of these pathways begin with the binding of Wnts to **Frizzled**

family cell-surface receptors, which are seven-pass transmembrane proteins that resemble GPCRs in structure but do not generally work through the activation of G proteins. Instead, when activated by Wnt binding, Frizzled proteins recruit the scaffold protein **Dishevelled**, which helps relay the signal to other signaling molecules.

The **Wnt/β-catenin pathway** acts by regulating the proteolysis of the multifunctional protein **β-catenin** (or *Armadillo* in flies). A portion of the cell's β-catenin is located at cell–cell junctions and thereby contributes to the control of cell–cell adhesion (discussed in Chapter 19), while the remaining β-catenin is rapidly degraded in the cytoplasm. Degradation depends on a large protein *degradation complex*, which binds β-catenin and keeps it out of the nucleus while promoting its degradation. The complex contains at least four other proteins: a protein kinase called *casein kinase 1* (*CK1*) phosphorylates the β-catenin on a serine, priming it for further phosphorylation by another protein kinase called *glycogen synthase kinase 3* (*GSK3*); this final phosphorylation marks the protein for ubiquitylation and rapid degradation in proteasomes. Two scaffold proteins called *axin* and *Adenomatous polyposis coli* (*APC*) hold the protein complex together (**Figure 15–60**A). APC gets its name from the finding that the gene encoding it is often mutated in a type of benign tumor (adenoma) of the colon; the tumor projects into the lumen as a polyp and can eventually become malignant. (This APC should not be confused with the anaphase-promoting complex, or APC/C, that plays a central part in selective protein degradation during the cell cycle—see Figure 17–15A.)

Wnt proteins regulate β-catenin proteolysis by binding to both a Frizzled protein and a co-receptor that is related to the low-density lipoprotein (LDL) receptor (discussed in Chapter 13) and is therefore called an **LDL-receptor-related protein** (**LRP**). In a poorly understood process, the activated receptor complex recruits the Dishevelled scaffold and promotes the phosphorylation of the LRP receptor by the two protein kinases, GSK3 and CK1. Axin is brought to the receptor complex and inactivated, thereby disrupting the β-catenin degradation complex in the cytoplasm. In this way, the phosphorylation and degradation of β-catenin are prevented, enabling unphosphorylated β-catenin to accumulate and translocate to the nucleus, where it alters the pattern of gene transcription (Figure 15–60B).

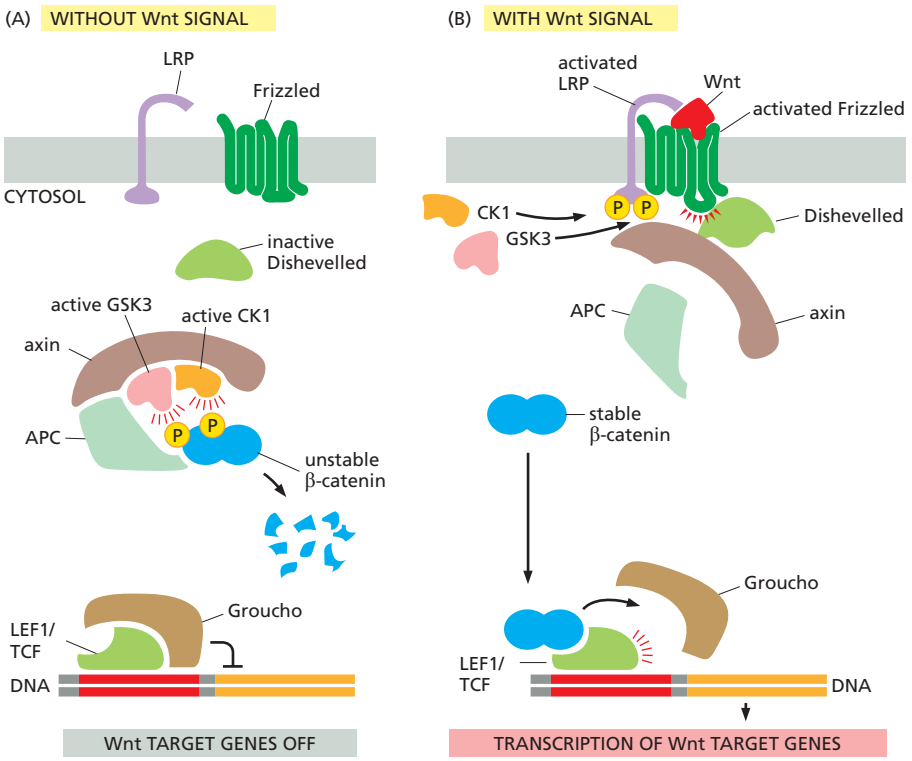


Figure 15–60 The Wnt/β-catenin signaling pathway. (A) In the absence of a Wnt signal, β-catenin that is not bound to cell–cell adherens junctions (not shown) interacts with a degradation complex containing APC, axin, GSK3, and CK1. In this complex, β-catenin is phosphorylated by CK1 and then by GSK3, triggering its ubiquitylation and degradation in proteasomes. Wnt-responsive genes are kept inactive by the Groucho co-repressor protein bound to the transcription regulator LEF1/TCF. (B) Wnt binding to Frizzled and LRP clusters the two co-receptors together, and the cytosolic tail of LRP is phosphorylated by GSK3 and then by CK1. Axin binds to the phosphorylated LRP and is inactivated and/or degraded, resulting in disassembly of the degradation complex. The phosphorylation of β-catenin is thereby prevented, and unphosphorylated β-catenin accumulates and translocates to the nucleus, where it binds to LEF1/TCF, displaces the co-repressor Groucho, and acts as a coactivator to stimulate the transcription of Wnt target genes. The scaffold protein Dishevelled is required for the signaling pathway to operate; it binds to Frizzled and becomes phosphorylated (not shown), but its precise role is unknown.

In the absence of Wnt signaling, Wnt-responsive genes are kept silent by an inhibitory complex of transcription regulatory proteins. The complex includes proteins of the *LEF1/TCF* family bound to a co-repressor protein of the *Groucho* family (see Figure 15–60A). In response to a Wnt signal, β-catenin enters the nucleus and binds to the LEF1/TCF proteins, displacing Groucho. The β-catenin now functions as a coactivator, inducing the transcription of the Wnt target genes (see Figure 15–60B). Thus, as in the case of Notch signaling, Wnt/β-catenin signaling triggers a switch from transcriptional repression to transcriptional activation.

Among the genes activated by β-catenin is *Myc*, which encodes a protein (Myc) that is an important regulator of cell growth and proliferation (discussed in Chapter 17). Mutations of the *Apc* gene occur in 80% of human colon cancers (discussed in Chapter 20). These mutations inhibit the protein's ability to bind β-catenin, so that β-catenin accumulates in the nucleus and stimulates the transcription of *c-Myc* and other Wnt target genes, even in the absence of Wnt signaling. The resulting uncontrolled cell growth and proliferation promote the development of cancer.

Various secreted inhibitory proteins regulate Wnt signaling in development. Some bind to the LRP receptors and promote their down-regulation, whereas others compete with Frizzled receptors for secreted Wnts. In *Drosophila* at least, Wnts activate negative feedback loops, in which Wnt target genes encode proteins that help shut the response off; some of these proteins inhibit Dishevelled, and others are secreted inhibitors.

Hedgehog Proteins Bind to Patched, Relieving Its Inhibition of Smoothened

Hedgehog proteins and Wnt proteins act in similar ways. Both are secreted signal molecules, which act as local mediators and morphogens in many developing invertebrate and vertebrate tissues. Both proteins are modified by covalently attached lipids, depend on secreted or cell-surface-bound heparan sulfate proteoglycans (discussed in Chapter 19) for their action, and activate latent transcription regulators by inhibiting their degradation. They both trigger a switch from transcriptional repression to transcriptional activation, and excessive signaling along either pathway in adult cells can lead to cancer. They even use some of the same intracellular signaling proteins and sometimes collaborate to mediate a response.

The **Hedgehog proteins** were discovered in *Drosophila*, where this protein family has only one member. Mutation of the *Hedgehog* gene produces a larva covered with spiky processes (denticles), like a hedgehog. At least three genes encode Hedgehog proteins in vertebrates—*Sonic*, *Desert*, and *Indian hedgehog*. The active forms of all Hedgehog proteins are covalently coupled to cholesterol, as well as to a fatty acid chain. The cholesterol is added during an unusual processing step, in which a precursor protein cleaves itself to produce a smaller, cholesterol-containing signal protein. Most of what we know about the Hedgehog signaling pathway came initially from genetic studies in flies, and it is the fly pathway that we summarize here.

The effects of Hedgehog are mediated by a latent transcription regulator called **Cubitus interruptus** (**Ci**), the regulation of which is reminiscent of the regulation of β-catenin by Wnts. In the absence of a Hedgehog signal, Ci is ubiquitylated and proteolytically cleaved in proteasomes. Instead of being completely degraded, however, Ci is processed to form a smaller fragment, which accumulates in the nucleus, where it acts as a transcriptional repressor, helping to keep Hedgehog-responsive genes silent. The proteolytic processing of the Ci protein depends on its phosphorylation by three protein kinases—PKA and two kinases also used in the Wnt pathway, namely GSK3 and CK1. As in the Wnt pathway, the proteolytic processing occurs in a multiprotein complex. The complex includes the protein kinase *Fused* and a scaffold protein *Costal2*, which stably associates with Ci, recruits the three other kinases, and binds the complex to microtubules, thereby keeping unprocessed Ci out of the nucleus (**Figure 15–61**A).

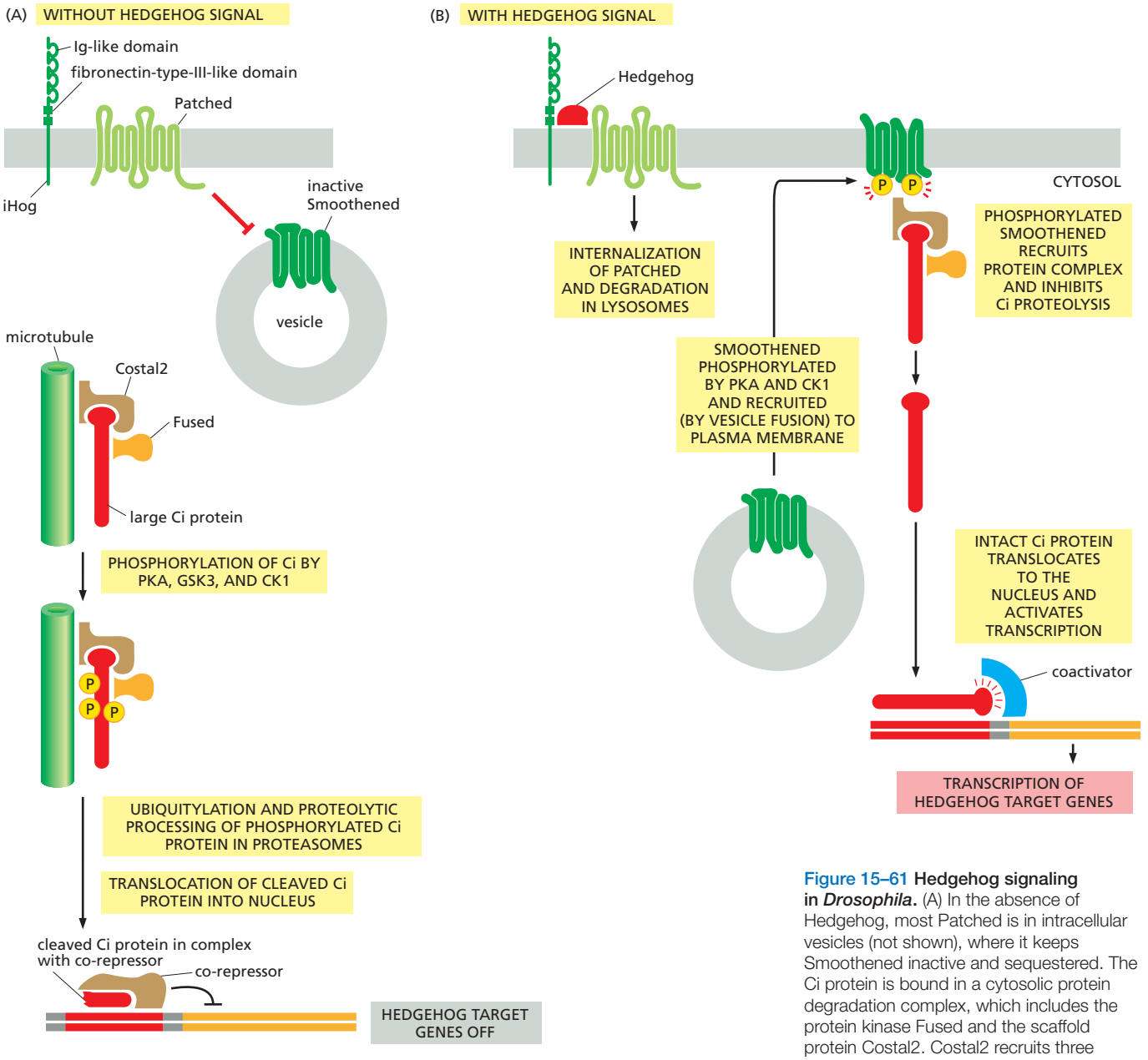


Figure 15–61 Hedgehog signaling in *Drosophila*. (A) In the absence of Hedgehog, most Patched is in intracellular vesicles (not shown), where it keeps Smoothened inactive and sequestered. The Ci protein is bound in a cytosolic protein degradation complex, which includes the protein kinase Fused and the scaffold protein Costal2. Costal2 recruits three other protein kinases (PKA, GSK3, and CK1; not shown), which phosphorylate Ci. Phosphorylated Ci is ubiquitylated and then cleaved in proteasomes (not shown) to form a transcriptional repressor, which accumulates in the nucleus to help keep Hedgehog target genes inactive. (B) Hedgehog binding to iHog and Patched removes the inhibition of Smoothened by Patched. Smoothened is phosphorylated by PKA and CK1 and translocates to the plasma membrane, where it recruits the complex containing Fused, Costal2, and Ci. Costal2 releases unprocessed Ci, which accumulates in the nucleus and activates the transcription of Hedgehog target genes. Many details in the pathway are poorly understood, including the role of Fused.

Hedgehog functions by blocking the proteolytic processing of Ci, thereby changing it into a transcriptional activator. It does this by a convoluted signaling process that depends on three transmembrane proteins: Patched, iHog, and Smoothened. **Patched** is predicted to cross the plasma membrane 12 times, and, although much of it is in intracellular vesicles, some is on the cell surface where it can bind the Hedgehog protein. **iHog** is also on the cell surface and is thought to serve as a co-receptor for Hedgehog. **Smoothened** is a seven-pass transmembrane protein with a structure very similar to a GPCR, but it does not seem to act as a Hedgehog receptor or even as an activator of G proteins; it is controlled by Patched and iHog.

In the absence of a Hedgehog signal, Patched employs an unknown mechanism to keep Smoothened sequestered and inactive in intracellular vesicles (see Figure 15–61A). The binding of Hedgehog to iHog and Patched inhibits the activity of Patched and induces its endocytosis and degradation. The result is that Smoothened is liberated from inhibition and translocates to the plasma membrane, where it recruits the protein complex containing Ci, Fused, and Costal2.

Costal2 is no longer able to bind the other three kinases, and so Ci is no longer cleaved and can now enter the nucleus and activate the transcription of Hedgehog target genes (Figure 15–61B). Among the genes activated by Ci is *Patched* itself; the resulting increase in Patched protein on the cell surface inhibits further Hedgehog signaling—providing another example of negative feedback.

Many gaps remain in our understanding of the Hedgehog signaling pathway. It is not known, for example, how Patched keeps Smoothened inactive and intracellular. As the structure of Patched resembles a transmembrane transporter protein, it has been proposed that it may transport a small molecule into the cell that keeps Smoothened sequestered in vesicles.

Even less is known about the more complex Hedgehog pathway in vertebrate cells. In addition to there being at least three types of vertebrate Hedgehog proteins, there are three Ci-like transcription regulator proteins (*Gli1*, *Gli2*, and *Gli3*) downstream of Smoothened. Gli2 and Gli3 are most similar to Ci in structure and function, and Gli3 has been shown to undergo proteolytic processing like Ci and to act as either a transcriptional repressor or a transcriptional activator. Moreover, in vertebrates, Smoothened, upon activation, becomes localized to the surface of the primary cilium (discussed in Chapter 16), where the Gli proteins are also concentrated, thereby increasing the speed and efficiency of signaling.

Hedgehog signaling can promote cell proliferation, and excessive Hedgehog signaling can lead to cancer. Inactivating mutations in one of the two human *Patched* genes, for example, which lead to excessive Hedgehog signaling, occur frequently in *basal cell carcinoma* of the skin, the most common form of cancer in Caucasians. A small molecule called *cyclopamine*, made by a meadow lily, is being used to treat cancers associated with excessive Hedgehog signaling. It blocks Hedgehog signaling by binding tightly to Smoothened and inhibiting its activity. It was originally identified because it causes severe developmental defects in the progeny of sheep grazing on such lilies; these include the presence of a single central eye (a condition called *cyclopia*), which is also seen in mice that are deficient in Hedgehog signaling.

Many Stressful and Inflammatory Stimuli Act Through an NFκB-Dependent Signaling Pathway

The **NFκB proteins** are latent transcription regulators that are present in most animal cells and are central to many stressful, inflammatory, and innate immune responses. These responses occur as a reaction to infection or injury and help protect stressed multicellular organisms and their cells (discussed in Chapter 24). An excessive or inappropriate inflammatory response in animals can also damage tissue and cause severe pain, and chronic inflammation can lead to cancer; as in the case of Wnt and Hedgehog signaling, excessive NFκB signaling is found in a number of human cancers. NFκB proteins also have important roles during normal animal development: the *Drosophila* NFκB family member *Dorsal*, for example, has a crucial role in specifying the dorsal–ventral axis of the developing fly embryo (discussed in Chapter 22).

Various cell-surface receptors activate the NFκB signaling pathway in animal cells. *Toll receptors* in *Drosophila* and *Toll-like receptors* in vertebrates, for example, recognize pathogens and activate this pathway in triggering innate immune responses (discussed in Chapter 24). The receptors for *tumor necrosis factor α* (*TNFα*) and *interleukin-1* (*IL1*), which are vertebrate cytokines especially important in inducing inflammatory responses, also activate this signaling pathway. The Toll, Toll-like, and IL1 receptors belong to the same family of proteins, whereas TNF receptors belong to a different family; all of them, however, act in similar ways to activate NFκB. When activated, they trigger a multiprotein ubiquitylation and phosphorylation cascade that releases NFκB from an inhibitory protein complex, so that it can translocate to the nucleus and turn on the transcription of hundreds of genes that participate in inflammatory and innate immune responses.

There are five NFκB proteins in mammals (*RelA*, *RelB*, *c-Rel*, *NFκB1*, and *NFκB2*), and they form a variety of homodimers and heterodimers, each of which

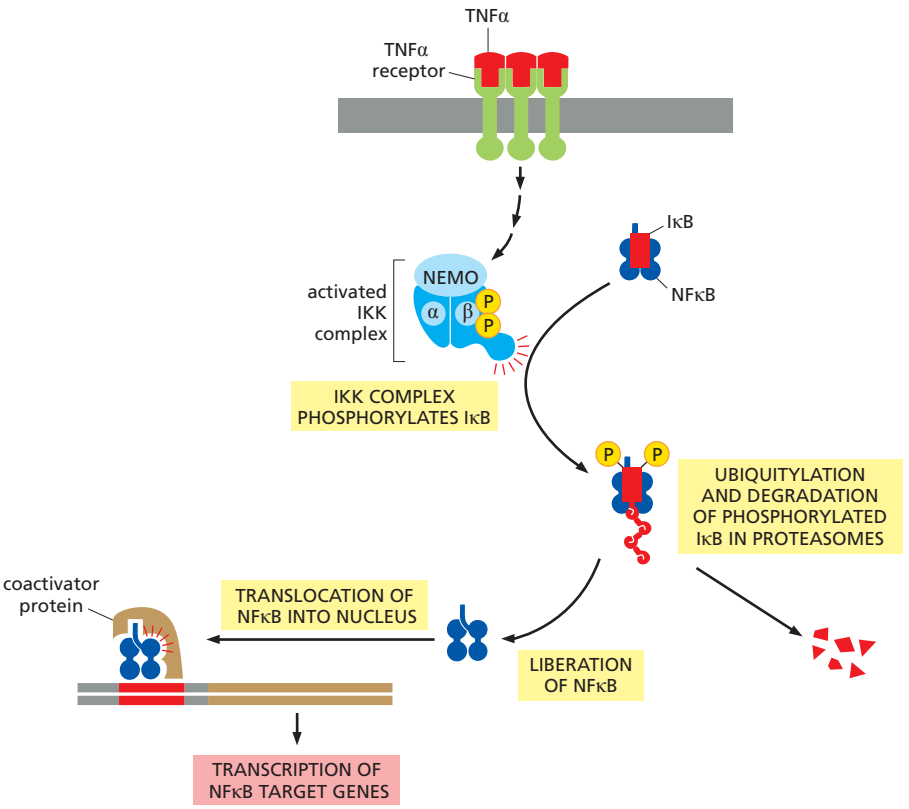


Figure 15–62 The activation of the NFκB pathway by TNFα. Both TNFα and its receptors are trimers. The binding of TNFα causes a rearrangement of the clustered cytosolic tails of the receptors, which now recruit various signaling proteins, resulting in the activation of a protein kinase that phosphorylates and activates IκB kinase kinase (IKK). IKK is a heterotrimer composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit called NEMO. IKKβ then phosphorylates IκB on two serines, which marks the protein for ubiquitylation and degradation in proteasomes. The released NFκB translocates into the nucleus, where, in collaboration with coactivator proteins, it stimulates the transcription of its target genes.

activates its own characteristic set of genes. Inhibitory proteins called **IκB** bind tightly to the dimers and hold them in an inactive state within the cytoplasm of unstimulated cells. There are three major IκB proteins in mammals (IκB α, β, and ε), and the signals that release NFκB dimers do so by triggering a signaling pathway that leads to the phosphorylation, ubiquitylation, and consequent degradation of the IκB proteins (**Figure 15–62**).

Among the genes activated by the released NFκB is the gene that encodes IκBα. This activation leads to increased synthesis of IκBα protein, which binds to NFκB and inactivates it, creating a negative feedback loop (**Figure 15–63**A). Experiments on TNFα-induced responses, as well as computer modeling studies of the responses, indicate that the negative feedback produces two types of NFκB responses, depending on the duration of the TNFα stimulus; importantly, the two types of responses induce different patterns of gene expression (Figure 15–63B, C, and D). The negative feedback through IκBα is required for both types of responses: in cells deficient in IκBα, even a short exposure to TNFα induces a sustained activation of NFκB, without oscillations, and all of the NFκB-responsive genes are activated.

Thus far, we have focused on the mechanisms by which extracellular signal molecules use cell-surface receptors to initiate changes in gene expression. We now turn to a class of extracellular signals that bypasses the plasma membrane entirely and controls, in the most direct way possible, transcription regulatory proteins inside the cell.

Nuclear Receptors Are Ligand-Modulated Transcription Regulators

Various small, hydrophobic signal molecules diffuse directly across the plasma membrane of target cells and bind to intracellular receptors that are transcription regulators. These signal molecules include steroid hormones, thyroid hormones, retinoids, and vitamin D. Although they differ greatly from one another in both

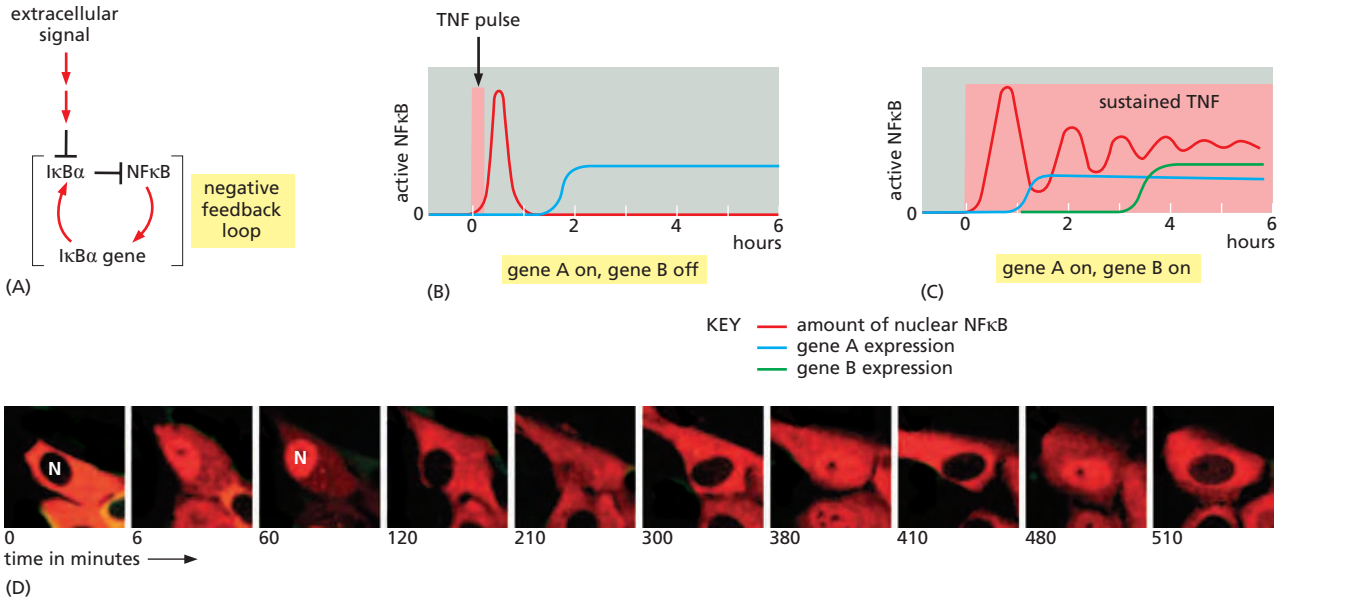


Figure 15–63 Negative feedback in the NFκB signaling pathway induces oscillations in NFκB activation. (A) Drawing showing how activated NFκB stimulates the transcription of the IκBα gene, the protein product of which acts back in the cytoplasm to sequester and inhibit NFκB there; if the stimulus is persistent, the newly made IκBα protein will then be ubiquitylated and degraded, liberating active NFκB again so that it can return to the nucleus and activate transcription (see Figure 15–62). (B) A short exposure to TNFα produces a single, short pulse of NFκB activation, beginning within minutes and ending by 1 hour. This response turns on the transcription of gene A but not gene B. (C) A sustained exposure to TNFα for the entire 6 hours of the experiment produces oscillations in NFκB activation that damp down over time. This response turns on the transcription of both genes; gene B turns on only after several hours, indicating that gene B transcription requires prolonged activation of NFκB, for reasons that are not understood. (D) These time-lapse confocal fluorescence micrographs from a different study of TNFα stimulation show the oscillations of NFκB in a cultured cell, as indicated by its periodic movement into the nucleus (N) of a fusion protein composed of NFκB fused to a red fluorescent protein. In the cell at the center of the micrographs, NFκB is active and in the nucleus at 6, 60, 210, 380, and 480 minutes, but it is exclusively in the cytoplasm at 0, 120, 300, 410, and 510 minutes. (A–C, based on data from A. Hoffmann et al., *Science* 298:1241–1245, 2002, and adapted from A.Y. Ting and D. Endy, *Science* 298:1189–1190, 2002; D, from D.E. Nelson et al., *Science* 306:704–708, 2004. All with permission from AAAS.)

chemical structure (**Figure 15–64**) and function, they all act by a similar mechanism. They bind to their respective intracellular receptor proteins and alter the ability of these proteins to control the transcription of specific genes. Thus, these proteins serve both as intracellular receptors and as intracellular effectors for the signal.

The receptors are all structurally related, being part of the very large **nuclear receptor superfamily**. Many family members have been identified by DNA sequencing only, and their ligand is not yet known; they are therefore referred to as *orphan nuclear receptors*, and they make up large fractions of the nuclear receptors encoded in the genomes of humans, *Drosophila*, and the nematode *C. elegans*. Some mammalian nuclear receptors are regulated by intracellular metabolites rather than by secreted signal molecules; the *peroxisome proliferation-activated receptors* (*PPARs*), for example, bind intracellular lipid metabolites and regulate the transcription of genes involved in lipid metabolism and fat-cell differentiation. It seems likely that the nuclear receptors for hormones evolved from such receptors for intracellular metabolites, which would help explain their intracellular location.

Steroid hormones—which include cortisol, the steroid sex hormones, vitamin D (in vertebrates), and the molting hormone *ecdysone* (in insects)—are all made from cholesterol. *Cortisol* is produced in the cortex of the adrenal glands and influences the metabolism of many types of cells. The *steroid sex hormones* are made in the testes and ovaries and are responsible for the secondary sex characteristics that distinguish males from females. *Vitamin D* is synthesized in the skin in response to sunlight; after it has been converted to its active form in

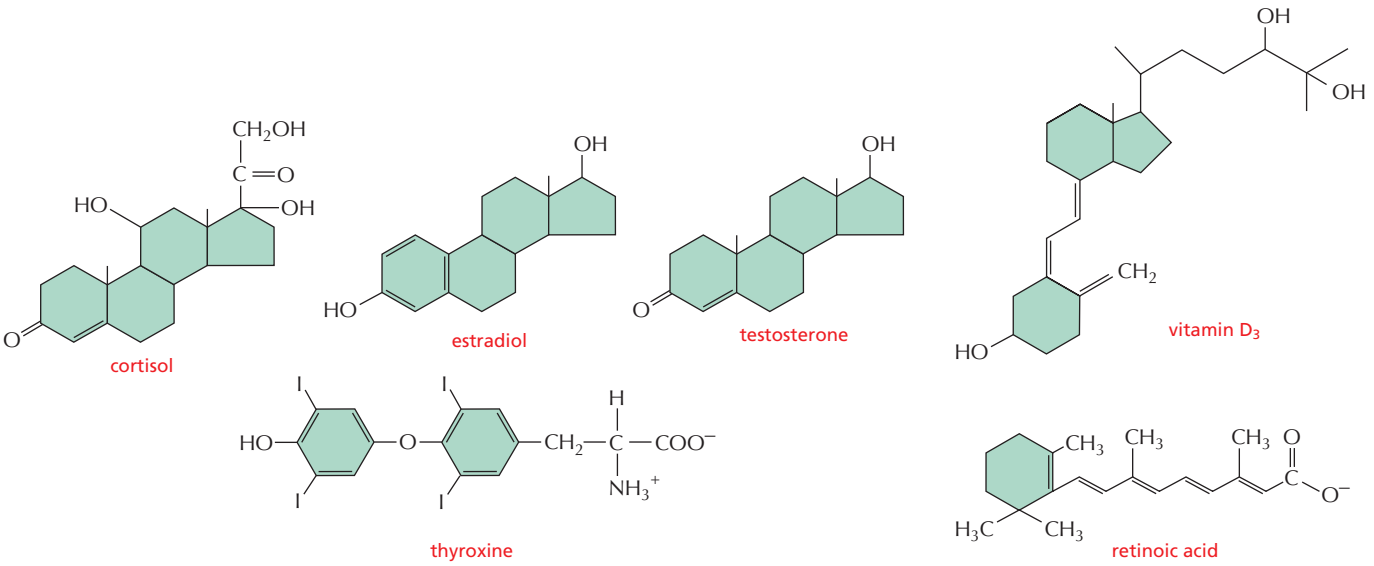


Figure 15–64 Some signal molecules that bind to intracellular receptors. Note that all of them are small and hydrophobic. The active, hydroxylated form of vitamin D_3 is shown. Estradiol and testosterone are steroid sex hormones.

the liver or kidneys, it regulates Ca^{2+} metabolism, promoting Ca^{2+} uptake in the gut and reducing its excretion in the kidneys. The *thyroid hormones*, which are made from the amino acid tyrosine, act to increase the metabolic rate of many cell types, while the *retinoids*, such as retinoic acid, are made from vitamin A and have important roles as local mediators in vertebrate development. Although all of these signal molecules are relatively insoluble in water, they are made soluble for transport in the bloodstream and other extracellular fluids by binding to specific carrier proteins, from which they dissociate before entering a target cell (see Figure 15–3B).

The nuclear receptors bind to specific DNA sequences adjacent to the genes that the ligand regulates. Some of the receptors, such as those for cortisol, are located primarily in the cytosol and enter the nucleus only after ligand binding; others, such as the thyroid and retinoid receptors, are bound to DNA in the nucleus even in the absence of ligand. In either case, the inactive receptors are usually bound to inhibitory protein complexes. Ligand binding alters the conformation of the receptor protein, causing the inhibitory complex to dissociate, while also causing the receptor to bind coactivator proteins that stimulate gene transcription (**Figure 15–65**). In other cases, however, ligand binding to a nuclear receptor inhibits transcription: some thyroid hormone receptors, for example, act as transcriptional activators in the absence of their hormone and become transcriptional repressors when hormone binds.

Thus far, we have focused on the control of gene expression by extracellular signal molecules produced by other cells. We now turn to gene regulation by a more global environmental signal: the cycle of light and darkness that results from the Earth's rotation.

Circadian Clocks Contain Negative Feedback Loops That Control Gene Expression

Life on Earth evolved in the presence of a daily cycle of day and night, and many present-day organisms (ranging from archaea to plants and humans) possess an internal rhythm that dictates different behaviors at different times of day. These behaviors range from the cyclical change in metabolic enzyme activities of a bacterium to the elaborate sleep–wake cycles of humans. The internal oscillators that control such diurnal rhythms are called **circadian clocks**.

Having a circadian clock enables an organism to anticipate the regular daily changes in its environment and take appropriate action in advance. Of course, the internal clock cannot be perfectly accurate, and so it must be capable of being reset by external cues such as the light of day. Thus, circadian clocks keep running

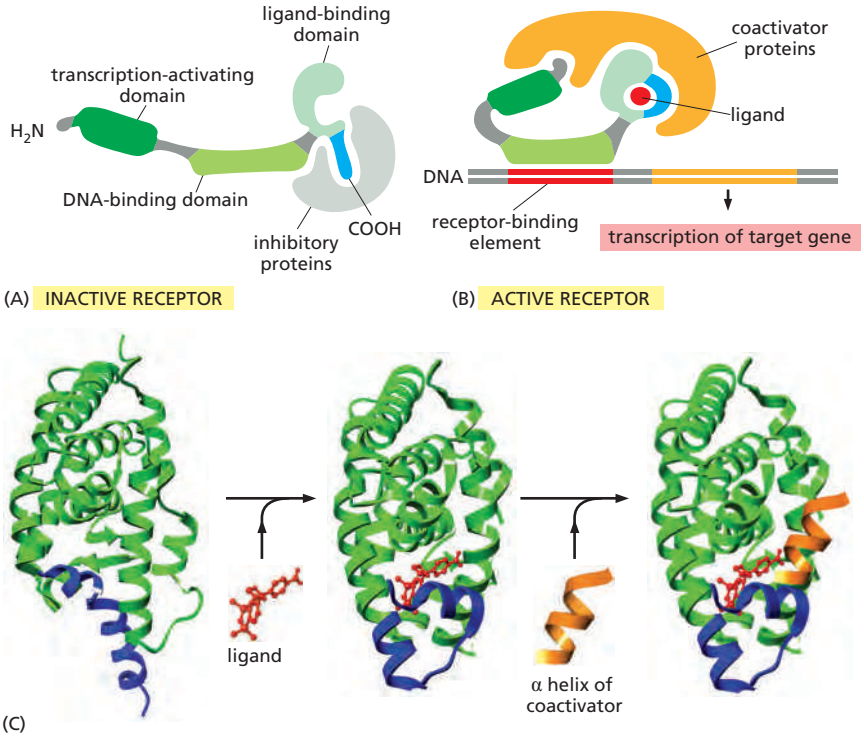


Figure 15–65 The activation of nuclear receptors. All nuclear receptors bind to DNA as either homodimers or heterodimers, but for simplicity we show them as monomers. (A) The receptors all have a related structure, which includes three major domains, as shown. An inactive receptor is bound to inhibitory proteins. (B) Typically, the binding of ligand to the receptor causes the ligand-binding domain of the receptor to clamp shut around the ligand, the inhibitory proteins to dissociate, and coactivator proteins to bind to the receptor's transcription-activating domain, thereby increasing gene transcription. In other cases, ligand binding has the opposite effect, causing co-repressor proteins to bind to the receptor, thereby decreasing transcription (not shown). (C) The structure of the ligand-binding domain of the retinoic acid receptor is shown in the absence *(left)* and presence *(middle)* of ligand (shown in *red*). When ligand binds, the *blue* α helix acts as a lid that snaps shut, trapping the ligand in place. The shift in the conformation of the receptor upon ligand binding also creates a binding site for a small α helix *(orange)* on the surface of coactivator proteins. (PDB codes: 1LBD, 2ZYO, and 2ZXZ.)

even when the environmental cues (changes in light and dark) are removed, but the period of this free-running rhythm is generally a little less or more than 24 hours. External signals indicating the time of day cause small adjustments in the running of the clock, so as to keep the organism in synchrony with its environment. Following more drastic shifts, circadian cycles become gradually reset (entrained) by the new cycle of light and dark, as anyone who has experienced jet lag can attest.

We might expect that the circadian clock would be a complex multicellular device, with different groups of cells responsible for different parts of the oscillation mechanism. Remarkably, however, in almost all multicellular organisms, including humans, the timekeepers are individual cells. Thus, a clock that operates in each member of a specialized group of brain cells (the SCN cells in the suprachiasmatic nucleus of the hypothalamus) controls our diurnal cycles of sleeping and waking, body temperature, and hormone release. Even if these cells are removed from the brain and dispersed in a culture dish, they will continue to oscillate individually, showing a cyclic pattern of gene expression with a period of approximately 24 hours. In the intact body, the SCN cells receive neural cues from the retina, entraining the SCN cells to the daily cycle of light and dark; they also send information about the time of day to another brain area, the pineal gland, which relays the time signal to the rest of the body by releasing the hormone melatonin in time with the clock.

Although the SCN cells have a central role as timekeepers in mammals, almost all the other cells in the mammalian body have an internal circadian rhythm, which has the ability to reset in response to light. Similarly, in *Drosophila*, many different types of cells have a similar circadian clock, which continues to cycle when they have been dissected away from the rest of the fly and can be reset by externally imposed light and dark cycles.

The working of circadian clocks, therefore, is a fundamental problem in cell biology. Although we do not yet understand all the details, studies in a wide variety of organisms have revealed the basic principles and molecular components. The key principle is that circadian clocks generally depend on *negative feedback loops*. As discussed earlier, oscillations in the activity of an intracellular signaling protein can occur if that protein inhibits its own activity with a long delay (see

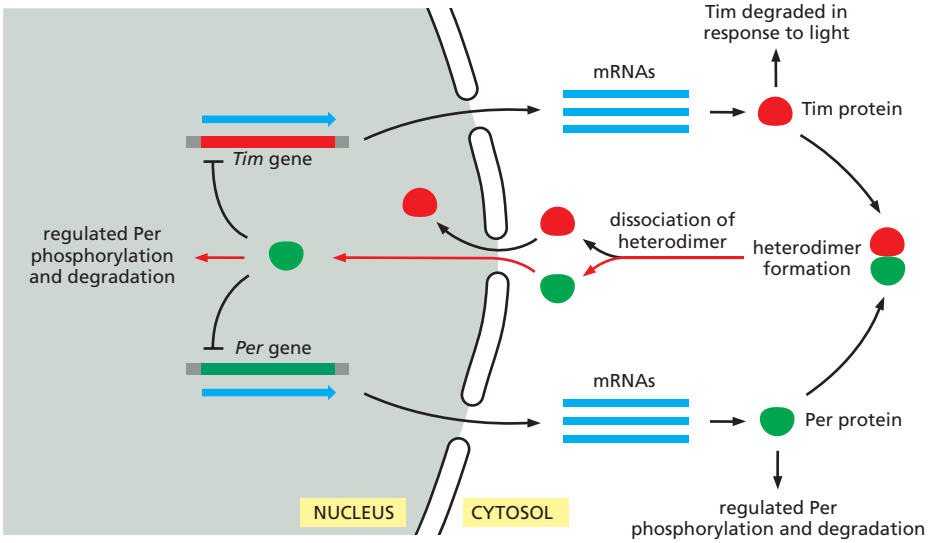


Figure 15–66 Simplified outline of the mechanism of the circadian clock in *Drosophila* cells. A central feature of the clock is the periodic accumulation and decay of two transcription regulatory proteins, Tim (short for timeless, based on the phenotype of a gene mutation) and Per (short for period). The mRNAs encoding these proteins rise gradually during the day and are translated in the cytosol, where the two proteins associate to form a heterodimer. After a time delay, the heterodimer dissociates and Tim and Per are transported into the nucleus, where Per represses the *Tim* and *Per* genes, resulting in negative feedback that causes the levels of Tim and Per to fall. In addition to this transcriptional feedback, the clock depends on numerous other proteins. For example, the controlled degradation of Per indicated in the diagram imposes delays in the accumulation of Tim and Per, which are crucial to the functioning of the clock. Steps at which specific delays are imposed are shown in *red*.

Entrainment (or resetting) of the clock occurs in response to new light–dark cycles. Although most *Drosophila* cells do not have true photoreceptors, light is sensed by intracellular flavoproteins, also called cryptochromes. In the presence of light, these proteins associate with the Tim protein and cause its degradation, thereby resetting the clock. (Adapted from J.C. Dunlap, *Science* 311:184–186, 2006.)

Figure 15–18C and D). In *Drosophila* and many other animals, including humans, the heart of the circadian clock is a delayed negative feedback loop based on transcription regulators: accumulation of certain gene products switches off the transcription of their own genes, but with a delay, so that the cell oscillates between a state in which the products are present and transcription is switched off, and one in which the products are absent and transcription is switched on (**Figure 15–66**). The negative feedback underlying circadian rhythms does not have to be based on transcription regulators. In some cell types, the circadian clock is constructed of proteins that govern their own activities through post-translational mechanisms, as we discuss next.

Three Proteins in a Test Tube Can Reconstitute a Cyanobacterial Circadian Clock

The best understood circadian clock is found in the photosynthetic cyanobacterium, *Synechococcus elongatus*. The core oscillator in this organism is remarkably simple, being composed of just three proteins—*KaiA, KaiB,* and *KaiC*. The central player is KaiC, a multifunctional enzyme that catalyzes its own phosphorylation and dephosphorylation in a 24-hour cycle: it gradually phosphorylates itself sequentially at two sites during the day and dephosphorylates itself during the night. This timing depends on interactions with the two other Kai proteins: KaiA binds to unphosphorylated KaiC and stimulates KaiC autophosphosphorylation, first at one site and then, with a delay, at the other. The second phosphorylation promotes the binding of the third protein, KaiB, which blocks the stimulatory effect of KaiA and thereby allows KaiC to dephosphorylate itself, bringing KaiC back to its dephosphorylated state. This clock depends on a negative feedback loop: KaiC drives its own phosphorylation until, after a delay, it recruits an inhibitor, KaiB, that stimulates KaiC to dephosphorylate itself. Amazingly, when the three Kai proteins are purified and incubated in a test tube with ATP, KaiC phosphorylation and dephosphorylation occur with roughly 24-hour timing over a period of several days (**Figure 15–67**).

Circadian oscillations in KaiC phosphorylation lead to parallel rhythms in the expression of large numbers of genes involved in controlling metabolic activities and cell division (see Figure 15–67). As a result, many aspects of cell behavior are synchronized with the circadian cycle.

Even in continuous darkness, cyanobacterial cells generate free-running oscillations of KaiC phosphorylation with roughly 24-hour periods. As in other circadian clocks, the cyanobacterial clock is entrained by the environmental light/dark

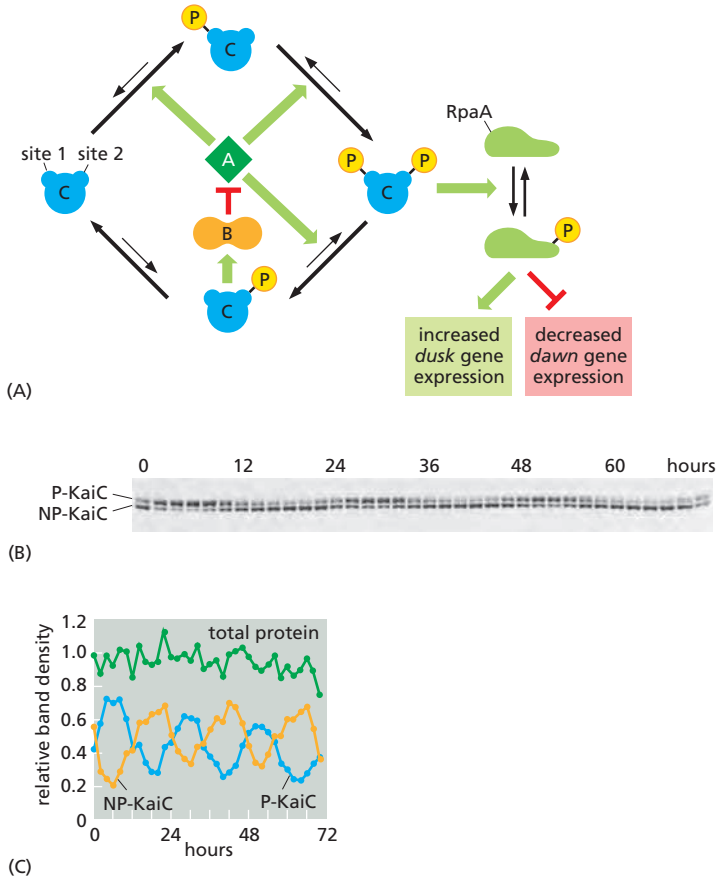


Figure 15–67 The core circadian oscillator of cyanobacteria. (A) KaiC is a combined kinase and phosphatase that phosphorylates and dephosphorylates itself on two adjacent sites. In the absence of other proteins, the phosphatase activity is dominant, and the protein is mostly unphosphorylated. The binding of KaiA to KaiC suppresses the phosphatase activity and promotes the kinase activity, leading to KaiC phosphorylation, first at site 1 and then at site 2, resulting in diphosphorylated KaiC. KaiC then dephosphorylates itself slowly at site 1, even in the presence of KaiA, so that KaiC is phosphorylated only at site 2. This form of KaiC interacts with KaiB, which blocks the stimulatory effects of KaiA, thereby reducing the rate of KaiC phosphorylation and allowing dephosphorylation to occur. Diphosphorylated KaiC increases in abundance during the day and peaks around dusk. It activates other proteins that phosphorylate a transcription regulator (RpaA), which then stimulates expression of some genes (the *dusk* genes that peak in early evening) and inhibits expression of other genes (the *dawn* genes that peak in the morning). When KaiC dephosphorylation gradually occurs during the night, these effects are reversed: *dusk* genes are turned off and dawn genes are turned on.

(B) In this experiment, the three Kai proteins were purified and mixed in a test tube with ATP (which is required for KaiC kinase activity). Every two hours over the next 3 days, the KaiC protein was analyzed by polyacrylamide gel electrophoresis, in which the phosphorylated form of KaiC migrates more slowly *(upper band*, P-KaiC) than the nonphosphorylated form *(lower band*, NP-KaiC). The three different phosphorylated forms of KaiC are not distinguished by this method. The phosphorylation of KaiC oscillates with a roughly 24-hour period. (C) The amount of phosphorylated and unphosphorylated KaiC in the experiment in B is plotted on this graph, along with the amount of total protein. (B and C, from M. Nakajima et al., *Science* 308:414–415, 2005. With permission from AAAS.)

cycle. Light is thought to affect the circadian clock indirectly: the activities of Kai proteins are influenced by changes in intracellular redox potential, which occur as a result of increased photosynthetic activity during the day.

Summary

Some signaling pathways that are especially important in animal development depend on proteolysis to control the activity and location of latent transcription regulatory proteins. Notch receptors are themselves such proteins, which are activated by cleavage when Delta on another cell binds to them; the cleaved cytosolic tail of Notch migrates into the nucleus, where it stimulates the transcription of Notch-responsive genes. In the Wnt/β-catenin signaling pathway, by contrast, the proteolysis of the latent transcription regulatory protein β-catenin is inhibited when a secreted Wnt protein binds to both a Frizzled and LRP receptor protein; as a result, β-catenin accumulates in the nucleus and activates the transcription of Wnt target genes.

Hedgehog signaling in flies works much like Wnt signaling. In the absence of a signal, a bifunctional, cytoplasmic transcription regulator, Ci, is proteolytically cleaved to form a transcriptional repressor that keeps Hedgehog target genes silenced. The binding of Hedgehog to its receptors (Patched and iHog) inhibits the proteolytic processing of Ci; as a result, the intact Ci protein accumulates in the nucleus and activates the transcription of Hedgehog-responsive genes. In Notch, Wnt, and Hedgehog signaling, the extracellular signal triggers a switch from transcriptional repression to transcriptional activation.

Signaling through the latent transcription regulator NFκB also depends on proteolysis. NFκB proteins are normally held in an inactive state by inhibitory IκB proteins in the cytoplasm. A variety of extracellular stimuli, including proinflammatory cytokines, trigger the phosphorylation and ubiquitylation of IκB, marking it for degradation; this enables the NFκB to translocate to the nucleus and activate

the transcription of its target genes. NFκB also activates the transcription of the gene that encodes IκBα, creating a negative feedback loop, which can produce prolonged oscillations in NFκB activity with sustained extracellular signaling.

Some small, hydrophobic signal molecules, including steroid and thyroid hormones, diffuse across the plasma membrane of the target cell and activate intracellular receptor proteins that directly regulate the transcription of specific genes.

In many cell types, gene expression is governed by circadian clocks, in which delayed negative feedback produces 24-hour oscillations in the activities of transcription regulators, anticipating the cell's changing needs during the day and night.

SIGNALING IN PLANTS

In plants, as in animals, cells are in constant communication with one another. Plant cells communicate to coordinate their activities in response to the changing conditions of light, dark, and temperature, which guide the plant's cycle of growth, flowering, and fruiting. Plant cells also communicate to coordinate activities in their roots, stems, and leaves. In this final section, we consider how plant cells signal to one another and how they respond to light. Less is known about the receptors and intracellular signaling mechanisms involved in cell communication in plants than is known in animals, and we will concentrate mainly on how the receptors and intracellular signaling mechanisms differ from those used by animals.

Multicellularity and Cell Communication Evolved Independently in Plants and Animals

Although plants and animals are both eukaryotes, they have evolved separately for more than a billion years. Their last common ancestor is thought to have been a unicellular eukaryote that had mitochondria but no chloroplasts; the plant lineage acquired chloroplasts after plants and animals diverged. The earliest fossils of multicellular animals and plants date from almost 600 million years ago. Thus, it seems that plants and animals evolved multicellularity independently, each starting from a different unicellular eukaryote, some time between 1.6 and 0.6 billion years ago (**Figure 15–68**).

If multicellularity evolved independently in plants and animals, the molecules and mechanisms used for cell communication will have evolved separately and would be expected to be different. There should be some degree of resemblance, however, because the genes in both plants and animals diverged from those contained by their last common unicellular ancestor. Thus, whereas both plants and animals use nitric oxide, cyclic GMP, Ca^{2+}, and Rho family GTPases for signaling, there are no homologs of the nuclear receptor family, Ras, JAK, STAT, TGFβ,

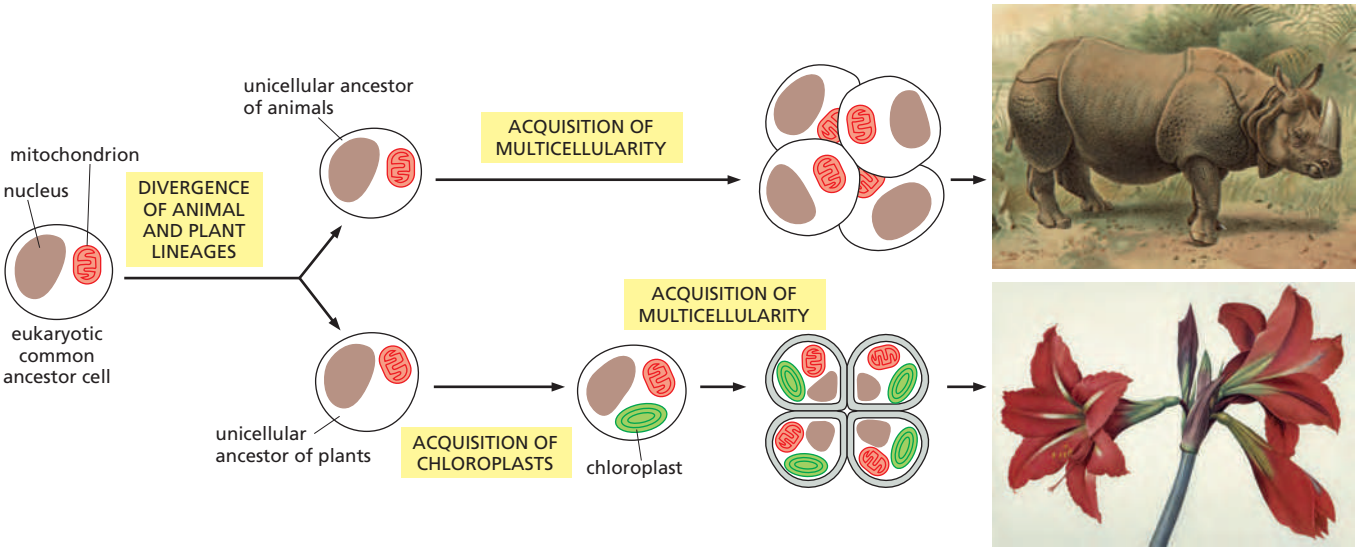


Figure 15–68 The proposed divergence of plant and animal lineages from a common unicellular eukaryotic ancestor. The plant lineage acquired chloroplasts after the two lineages diverged. Both lineages independently gave rise to multicellular organisms—plants and animals. (Paintings courtesy of John Innes Foundation.)

Notch, Wnt, or Hedgehog encoded by the completely sequenced genome of *Arabidopsis thaliana*, the small flowering plant. Similarly, plants do not seem to use cyclic AMP for intracellular signaling. Nevertheless, the general strategies underlying signaling are frequently very similar in plants and animals. Both, for example, use enzyme-coupled cell-surface receptors, as we now discuss.

Receptor Serine/Threonine Kinases Are the Largest Class of Cell-Surface Receptors in Plants

Most cell-surface receptors in plants are enzyme-coupled. However, whereas the largest class of enzyme-coupled receptors in animals is the receptor tyrosine kinase (RTK) class, this type of receptor is extremely rare in plants. Instead, plants rely largely on a great diversity of transmembrane *receptor serine/threonine kinases*, which have a typical serine/threonine kinase cytoplasmic domain and an extracellular ligand-binding domain. The most abundant types of these receptors have a tandem array of extracellular leucine-rich repeat structures and are therefore called **leucine-rich repeat (LRR) receptor kinases**.

There are about 175 LRR receptor kinases encoded by the *Arabidopsis* genome. These include a protein called *Bri1*, which forms part of a cell-surface steroid hormone receptor. Plants synthesize a class of steroids that are called **brassinosteroids** because they were originally identified in the mustard family *Brassicaceae*, which includes *Arabidopsis*. These signal molecules regulate the growth and differentiation of plants throughout their life cycle. Binding of a brassinosteroid to a Bri1 cell-surface receptor kinase initiates an intracellular signaling cascade that uses a GSK3 protein kinase and a protein phosphatase to regulate the phosphorylation and degradation of specific transcription regulatory proteins in the nucleus, and thereby specific gene transcription. Mutant plants that are deficient in the Bri1 receptor kinase are insensitive to brassinosteroids and are therefore dwarfs.

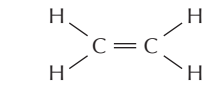
The LRR receptor kinases are only one of many classes of transmembrane receptor serine/threonine kinases in plants. There are at least six additional families, each with its own characteristic set of extracellular domains. The *lectin receptor kinases*, for example, have extracellular domains that bind carbohydrate signal molecules. The *Arabidopsis* genome encodes over 300 receptor serine/threonine kinases, which makes them the largest family of receptors known in plants. Many are involved in defense responses against pathogens.

Ethylene Blocks the Degradation of Specific Transcription Regulatory Proteins in the Nucleus

Various **plant growth regulators** (also called **plant hormones**) help to coordinate plant development. They include *ethylene*, *auxin*, *cytokinins*, *gibberellins*, and *abscisic acid*, as well as brassinosteroids. Growth regulators are all small molecules made by most plant cells. They diffuse readily through cell walls and can either act locally or be transported to influence cells further away. Each growth regulator can have multiple effects. The specific effect depends on environmental conditions, the nutritional state of the plant, the responsiveness of the target cells, and which other growth regulators are acting.

Ethylene is an important example. This small gas molecule (**Figure 15–69**A) can influence plant development in various ways; it can, for example, promote fruit ripening, leaf abscission, and plant senescence. It also functions as a stress signal in response to wounding, infection, flooding, and so on. When the shoot of a germinating seedling, for instance, encounters an obstacle, ethylene promotes a complex response that allows the seedling to safely bypass the obstacle (Figure 15–69B and C).

Plants have various ethylene receptors, which are located in the endoplasmic reticulum and are all structurally related. They are dimeric, multipass transmembrane proteins, with a copper-containing ethylene-binding domain and a domain that interacts with a cytoplasmic protein called *CTR1*, which is closely related



(A)

Figure 15–69 The ethylene-mediated triple response that occurs when the growing shoot of a germinating seedling encounters an obstacle underground. (A) The structure of ethylene. (B) In the absence of obstacles, the shoot grows upward and is long and thin. (C) If the shoot encounters an obstacle, such as a piece of gravel in the soil, the seedling responds to the encounter in three ways. First, it thickens its stem, which can then exert more force on the obstacle. Second, it shields the tip of the shoot (at *top*) by increasing the curvature of a specialized hook structure. Third, it reduces the shoot's tendency to grow away from the direction of gravity, so as to avoid the obstacle. (Courtesy of Melanie Webb.)

in sequence to the Raf MAP kinase kinase kinase discussed earlier (see Figure 15–49). Surprisingly, it is the empty receptors that are active and keep CTR1 active. By an unknown signaling mechanism, active CTR1 stimulates the ubiquitylation and degradation in proteasomes of a nuclear transcription regulator called *EIN3*, which is required for the transcription of ethylene-responsive genes. In this way, the empty but active receptors keep ethylene-response genes off. Ethylene binding inactivates the receptors, altering their conformation so that they no longer activate CTR1. The EIN3 protein is no longer ubiquitylated and degraded and can now activate the transcription of the large number of ethylene-responsive genes (**Figure 15–70**).

Regulated Positioning of Auxin Transporters Patterns Plant Growth

The plant hormone **auxin**, which is generally indole-3-acetic acid (**Figure 15–71**A), binds to receptor proteins in the nucleus. It helps plants grow toward light, grow upward rather than branch out, and grow their roots downward. It also regulates organ initiation and positioning and helps plants flower and bear fruit. Like ethylene (and like some of the animal signal molecules we have described in this chapter), auxin influences gene expression by controlling the degradation of transcription regulators. It works by stimulating the ubiquitylation and degradation of repressor proteins that block the transcription of auxin target genes in unstimulated cells (Figure 15–71B and C).

Auxin is unique in the way that it is transported. Unlike animal hormones, which are usually secreted by a specific endocrine organ and transported to target

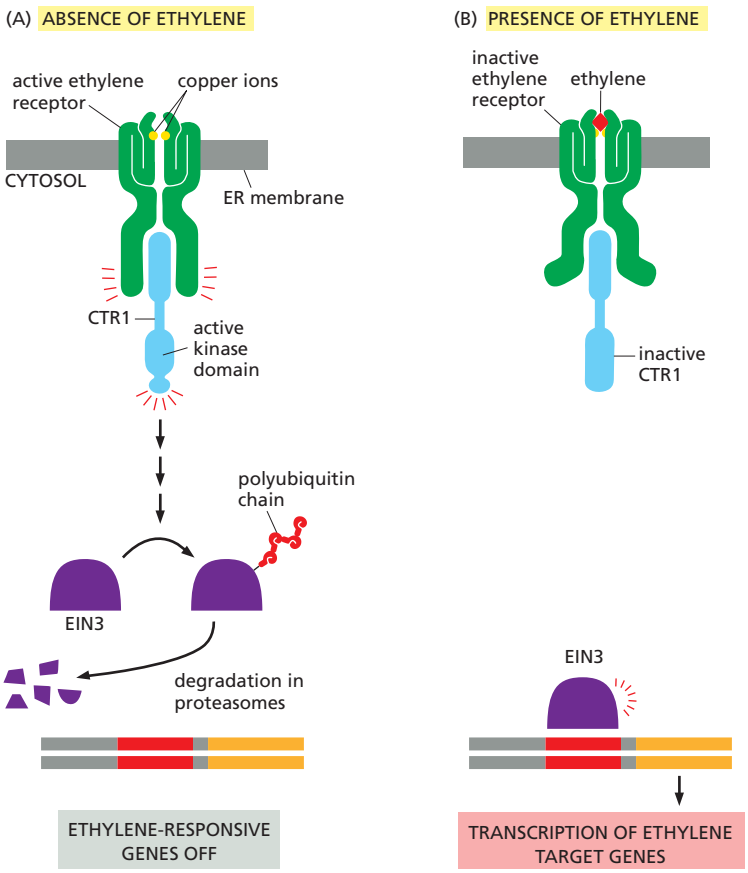


Figure 15–70 The ethylene signaling pathway. (A) In the absence of ethylene, the receptors and CTR1 are active, causing the ubiquitylation and destruction of EIN3, the transcription regulatory protein in the nucleus that is responsible for the transcription of ethylene-responsive genes. (B) The binding of ethylene inactivates the receptors and disrupts the activation of CTR1. The EIN3 protein is not degraded and can therefore activate the transcription of ethylene-responsive genes.

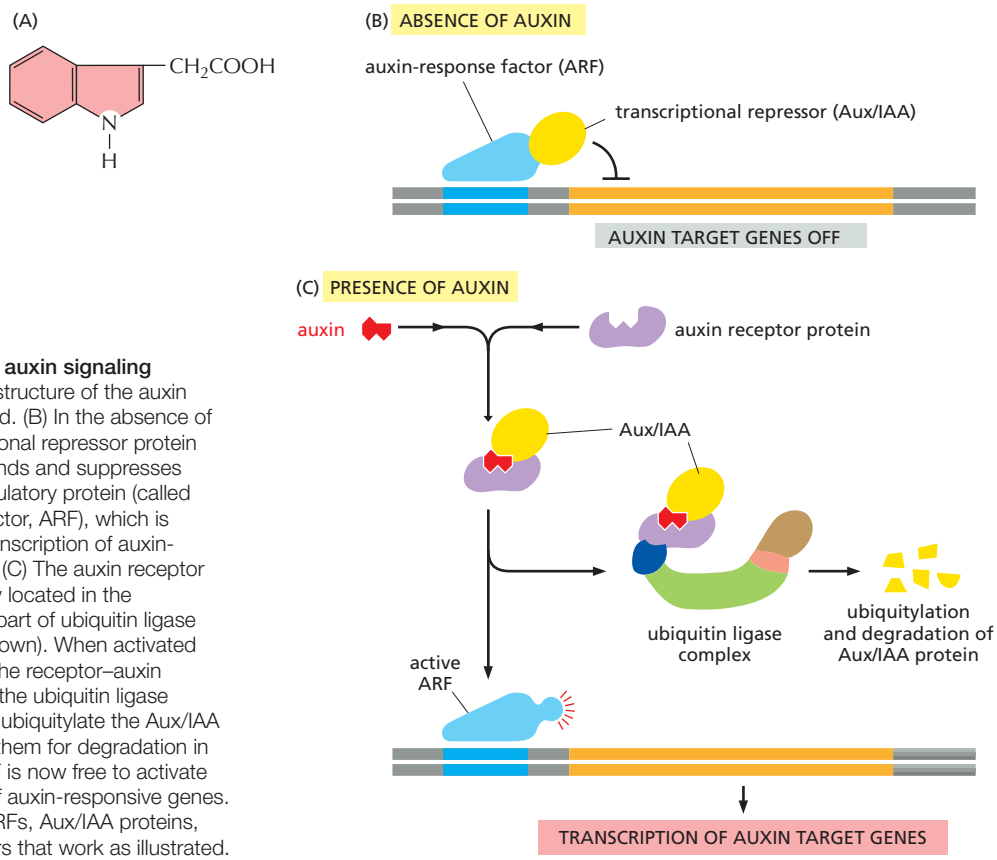


Figure 15–71 The auxin signaling pathway. (A) The structure of the auxin indole-3-acetic acid. (B) In the absence of auxin, a transcriptional repressor protein (called Aux/IAA) binds and suppresses a transcription regulatory protein (called auxin-response factor, ARF), which is required for the transcription of auxin-responsive genes. (C) The auxin receptor proteins are mainly located in the nucleus and form part of ubiquitin ligase complexes (not shown). When activated by auxin binding, the receptor–auxin complexes recruit the ubiquitin ligase complexes, which ubiquitylate the Aux/IAA proteins, marking them for degradation in proteasomes. ARF is now free to activate the transcription of auxin-responsive genes. There are many ARFs, Aux/IAA proteins, and auxin receptors that work as illustrated.

cells via the circulatory system, auxin has its own transport system. Specific plasma-membrane-bound *influx transporter proteins* and *efflux transporter proteins* move auxin into and out of plant cells, respectively. The efflux transporters can be distributed asymmetrically in the plasma membrane to make the efflux of auxin directional. A row of cells with their auxin efflux transporters confined to the basal plasma membrane, for example, will transport auxin from the top of the plant to the bottom.

In some regions of the plant, the localization of the auxin transporters, and therefore the direction of auxin flow, is highly dynamic and regulated. A cell can rapidly redistribute transporters by controlling the traffic of vesicles containing them. The auxin efflux transporters, for example, normally recycle between intracellular vesicles and the plasma membrane. A cell can redistribute these transporters on its surface by inhibiting their endocytosis in one domain of the plasma membrane, causing the transporters to accumulate there. One example occurs in the root, where gravity influences the direction of growth. The auxin efflux transporters are normally distributed symmetrically in the cap cells of the root. Within minutes of a change in the direction of the gravity vector, however, the efflux transporters redistribute to one side of the cells, so that auxin is pumped out toward the side of the root pointing downward. Because auxin inhibits root-cell elongation, this redirection of auxin transport causes the root tip to reorient, so that it grows downward again (**Figure 15–72**).

Phytochromes Detect Red Light, and Cryptochromes Detect Blue Light

Plant development is greatly influenced by environmental conditions. Unlike animals, plants cannot move when conditions become unfavorable; they have to adapt or they die. The most important environmental influence on plants is

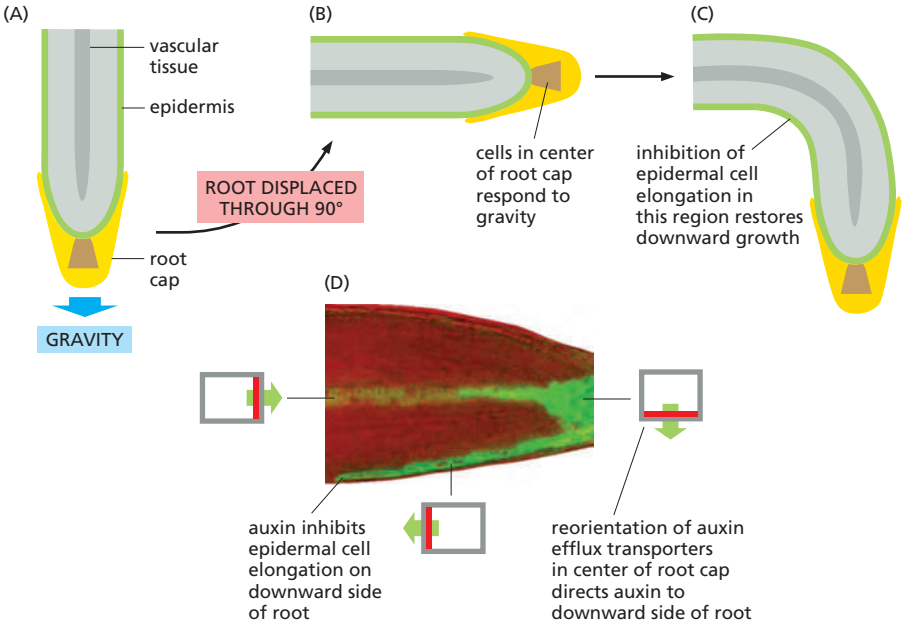


Figure 15–72 Auxin transport and root gravitropism. (A–C) Roots respond to a 90° change in the gravity vector and adjust their direction of growth so that they grow downward again. The cells that respond to gravity are in the center of the root cap, while it is the epidermal cells further back (on the lower side) that decrease their rate of elongation to restore downward growth. (D) The gravity-responsive cells in the root cap redistribute their auxin efflux transporters in response to the displacement of the root. This redirects the auxin flux mainly to the lower part of the displaced root, where it inhibits the elongation of the epidermal cells. The resulting asymmetrical distribution of auxin in the *Arabidopsis* root tip shown here is assessed indirectly, using an auxin-responsive reporter gene that encodes a protein fused to green fluorescent protein (GFP); the epidermal cells on the downward side of the root are green, whereas those on the upper side are not, reflecting the asymmetrical distribution of auxin. The distribution of auxin efflux transporters in the plasma membrane of cells in different regions of the root (shown as *gray rectangles*) is indicated in *red*, and the direction of auxin efflux is indicated by a *green arrow*. (The fluorescence photograph in D is from T. Paciorek et al., *Nature* 435:1251–1256, 2005. With permission from Macmillan Publishers Ltd.)

light, which is their energy source and has a major role throughout their entire life cycle—from germination, through seedling development, to flowering and senescence. Plants have thus evolved a large set of light-sensitive proteins to monitor the quantity, quality, direction, and duration of light. These are usually referred to as *photoreceptors*. However, because the term photoreceptor is also used for light-sensitive cells in the animal retina (see Figure 15–38), we shall use the term *photoprotein* instead.

All photoproteins sense light by means of a covalently attached light-absorbing chromophore, which changes its shape in response to light and then induces a change in the protein's conformation. The best-known plant photoproteins are the **phytochromes**, which are present in all plants and in some algae but are absent in animals. These are dimeric, cytoplasmic serine/threonine kinases, which respond differentially and reversibly to red and far-red light: whereas red light usually activates the kinase activity of the phytochrome, far-red light inactivates it. When activated by red light, the phytochrome is thought to phosphorylate itself and then to phosphorylate one or more other proteins in the cell. In some light responses, the activated phytochrome translocates into the nucleus, where it activates transcription regulators to alter gene transcription (**Figure 15–73**). In other cases, the activated phytochrome activates a latent transcription regulator in the cytoplasm, which then translocates into the nucleus to regulate gene transcription. In still other cases, the photoprotein triggers signaling pathways in the cytosol that alter the cell's behavior without involving the nucleus.

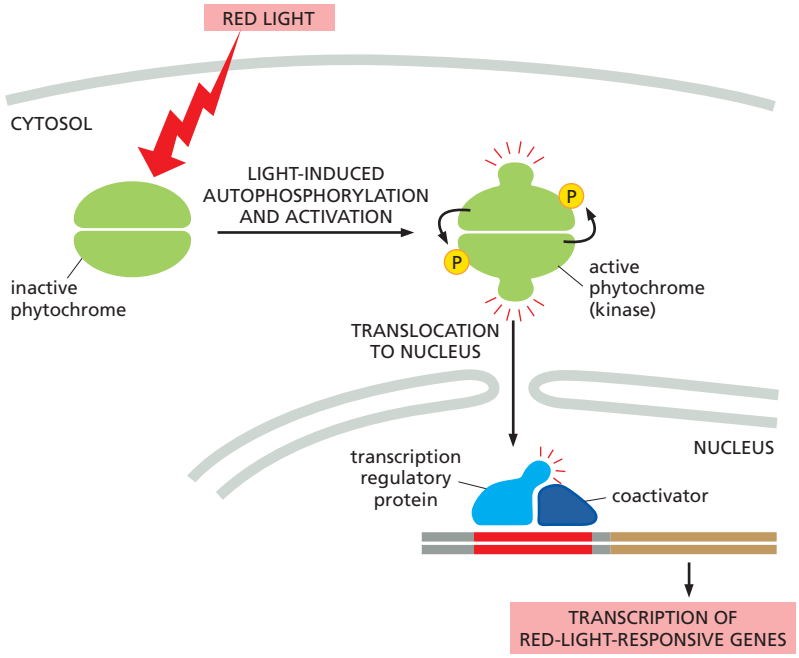


Figure 15–73 One way in which phytochromes mediate a light response in plant cells. When activated by red light, the phytochrome, which is a dimeric protein kinase, phosphorylates itself and then moves into the nucleus, where it activates transcription regulatory proteins to stimulate the transcription of red-light-responsive genes.

Plants sense blue light using photoproteins of two other sorts, phototropin and cryptochromes. **Phototropin** is associated with the plasma membrane and is partly responsible for *phototropism*, the tendency of plants to grow toward light. Phototropism occurs by directional cell elongation, which is stimulated by auxin, but the links between phototropin and auxin are unknown.

Cryptochromes are flavoproteins that are sensitive to blue light. They are structurally related to blue-light-sensitive enzymes called *photolyases*, which are involved in the repair of ultraviolet-induced DNA damage in all organisms, except most mammals. Unlike phytochromes, cryptochromes are also found in animals, where they have an important role in circadian clocks (see Figure 15–66). Although cryptochromes are thought to have evolved from the photolyases, they do not have a role in DNA repair.

Summary

Plants and animals are thought to have evolved multicellularity and cell communication mechanisms independently, each starting from a different unicellular eukaryote, which in turn evolved from a common unicellular eukaryotic ancestor. Not surprisingly, therefore, the mechanisms used to signal between cells in animals and in plants have both similarities and differences. Whereas animals rely heavily on GPCRs and RTKs, plants rely mainly on enzyme-coupled receptors of the receptor serine/threonine kinase type, especially ones with extracellular leucine-rich repeats. Various plant hormones, or growth regulators, including ethylene and auxin, help coordinate plant development. Ethylene acts through intracellular receptors to stop the degradation of specific nuclear transcription regulators, which can then activate the transcription of ethylene-responsive genes. The receptors for some other plant hormones, including auxin, also regulate the degradation of specific transcription regulators, although the details vary. Auxin signaling is unusual in that it has its own highly regulated transport system, in which the dynamic positioning of plasma-membrane-bound auxin transporters controls the direction of auxin flow and thereby the direction of plant growth. Light has an important role in regulating plant development. These light responses are mediated by a variety of light-sensitive photoproteins, including phytochromes, which are responsive to red light, and cryptochromes and phototropin, which are sensitive to blue light.

WHAT WE DON'T KNOW

- How does a cell integrate the information received from its many different cell-surface receptors to make all-or-none decisions?
- Much of what we know about cell signaling comes from biochemical studies of isolated proteins in test tubes. What is the precise quantitative behavior of intracellular signaling networks in an intact cell, or in an intact animal, where countless other signals and cell components might influence signaling specificity and strength?
- How do intracellular signaling circuits generate specific and dynamic signaling patterns such as oscillations and waves, and how are these patterns sensed and interpreted by the cell?
- Scaffold proteins and activated receptor tyrosine kinases nucleate the assembly of large intracellular signaling complexes. What is the dynamic behavior of these complexes, and how does this behavior influence downstream signaling?

PROBLEMS

Which statements are true? Explain why or why not.

15–1 All second messengers are water-soluble and diffuse freely through the cytosol.

15–2 In the regulation of molecular switches, protein kinases and guanine nucleotide exchange factors (GEFs) always turn proteins on, whereas protein phosphatases and GTPase-activating proteins (GAPs) always turn proteins off.

15–3 Most intracellular signaling pathways provide numerous opportunities for amplifying the responses to extracellular signals.

15–4 Binding of extracellular ligands to receptor tyrosine kinases (RTKs) activates the intracellular catalytic domain by propagating a conformational change across the lipid bilayer through a single transmembrane α helix.

15–5 Protein tyrosine phosphatases display exquisite specificity for their substrates, unlike most serine/threonine protein phosphatases, which have rather broad specificity.

15–6 Even though plants and animals independently evolved multicellularity, they use virtually all the same signaling proteins and second messengers for cell–cell communication.

Discuss the following problems.

15–7 Suppose that the circulating concentration of hormone is 10^{-10} M and the K_d for binding to its receptor is 10^{-8} M. What fraction of the receptors will have hormone bound? If a meaningful physiological response occurs when 50% of the receptors have bound a hormone molecule, how much will the concentration of hormone have to rise to elicit a response? The fraction of receptors (R) bound to hormone (H) to form a receptor–hormone complex (R–H) is $[R\text{–}H]/([R] + [R\text{–}H]) = [R\text{–}H]/[R]_{TOT} = [H]/([H] + K_d)$.

15–8 Cells communicate in ways that resemble human communication. Decide which of the following forms of human communication are analogous to autocrine, paracrine, endocrine, and synaptic signaling by cells.

A. A telephone conversation
B. Talking to people at a cocktail party
C. A radio announcement
D. Talking to yourself

15–9 Why do signaling responses that involve changes in proteins already present in the cell occur in milliseconds to seconds, whereas responses that require changes in gene expression require minutes to hours?

15–10 How is it that different cells can respond in different ways to exactly the same signaling molecule even when they have identical receptors?

15–11 Why do you suppose that phosphorylation/dephosphorylation, as opposed to allosteric binding of small molecules, for example, has evolved to play such a prominent role in switching proteins on and off in signaling pathways?

15–12 Consider a signaling pathway that proceeds through three protein kinases that are sequentially activated by phosphorylation. In one case, the kinases are held in a signaling complex by a scaffolding protein; in the other, the kinases are freely diffusible (**Figure Q15–1**). Discuss the properties of these two types of organization in terms of signal amplification, speed, and potential for cross-talk between signaling pathways.

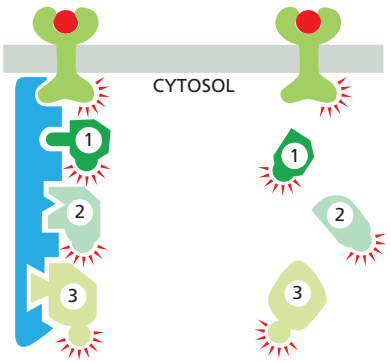


Figure Q15–1 A kinase cascade organized by a scaffolding protein or composed of freely diffusing components (Problem 15–12).

15–13 Describe three ways in which a gradual increase in an extracellular signal can be sharpened by the target cell to produce an abrupt or nearly all-or-none response.

15–14 Activation ("maturation") of frog oocytes is signaled through a MAP kinase signaling module. An increase in the hormone progesterone triggers the module by stimulating the translation of Mos mRNA, which is the frog's MAP kinase kinase kinase (**Figure Q15–2**). Maturation is easy to score visually by the presence of a white spot in the middle of the brown surface of the oocyte (see Figure Q15–2). To determine the dose–response curve for progesterone-induced activation of MAP kinase, you place 16 oocytes in each of six plastic dishes and add various concentrations of progesterone. After an overnight incubation, you crush the oocytes, prepare an extract, and determine the state of MAP kinase phosphorylation (hence, activation) by SDS polyacrylamide-gel electrophoresis (**Figure Q15–3**A). This analysis shows a graded response of MAP kinase to increasing concentrations of progesterone.

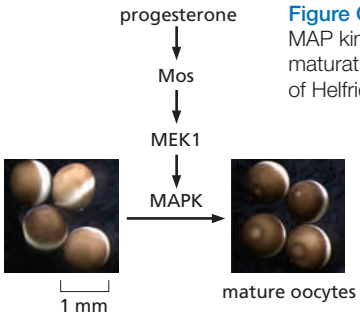


Figure Q15–2 Progesterone-induced MAP kinase activation, leading to oocyte maturation (Problem 15–14). (Courtesy of Helfrid Hochegger.)

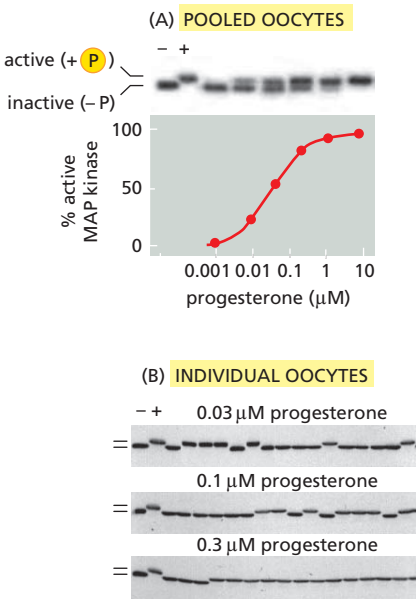


Figure Q15–3 Activation of frog oocytes (Problem 15–14). (A) Phosphorylation of MAP kinase in pooled oocytes. (B) Phosphorylation of MAP kinase in individual oocytes. MAP kinase was detected by immunoblotting using a MAP-kinase-specific antibody. The first two lanes in each gel contain nonphosphorylated, inactive MAP kinase (–) and phosphorylated, active MAP kinase (+). (From J.E. Ferrell, Jr., and E.M. Machleder, *Science* 280:895–898, 1998. With permission from AAAS.)

Before you crushed the oocytes, you noticed that not all oocytes in individual dishes had white spots. Had some oocytes undergone partial activation and not yet reached the white-spot stage? To answer this question, you repeat the experiment, but this time you analyze MAP kinase activation in individual oocytes. You are surprised to find that each oocyte has either a fully activated or a completely inactive MAP kinase (Figure Q15–3B). How can an all-or-none response in individual oocytes give rise to a graded response in the population?

15–15 Propose specific types of mutations in the gene for the regulatory subunit of cyclic-AMP-dependent protein kinase (PKA) that could lead to either a permanently active PKA or a permanently inactive PKA.

15–16 Phosphorylase kinase integrates signals from the cyclic-AMP-dependent and Ca^{2+}-dependent signaling pathways that control glycogen breakdown in liver and muscle cells (**Figure Q15–4**). Phosphorylase kinase is composed of four subunits. One is the protein kinase that catalyzes the addition of phosphate to glycogen phosphorylase to activate it for glycogen breakdown. The other three subunits are regulatory proteins that control the activity of the

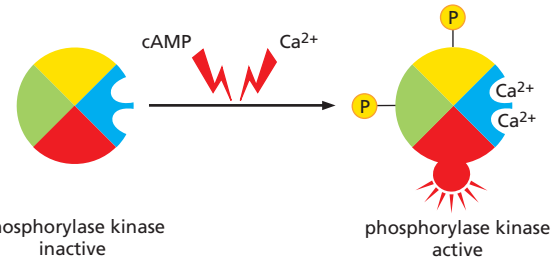


Figure Q15–4 Integration of cyclic-AMP-dependent and Ca^{2+}-dependent signaling pathways by phosphorylase kinase in liver and muscle cells (Problem 15–16).

catalytic subunit. Two contain sites for phosphorylation by PKA, which is activated by cyclic AMP. The remaining subunit is calmodulin, which binds Ca^{2+} when the cytosolic Ca^{2+} concentration rises. The regulatory subunits control the equilibrium between the active and inactive conformations of the catalytic subunit, with each phosphate and Ca^{2+} nudging the equilibrium toward the active conformation. How does this arrangement allow phosphorylase kinase to serve its role as an integrator protein for the multiple pathways that stimulate glycogen breakdown?

15–17 The Wnt planar polarity signaling pathway normally ensures that each wing cell in *Drosophila* has a single hair. Overexpression of the *Frizzled* gene from a heat-shock promoter (hs-*Fz*) causes multiple hairs to grow from many cells (**Figure Q15–5**A). This phenotype is suppressed if hs-*Fz* is combined with a heterozygous deletion (Dsh^{Δ}) of the *Dishevelled* gene (Figure Q15–5B). Do these results allow you to order the action of Frizzled and Dishevelled in the signaling pathway? If so, what is the order? Explain your reasoning.

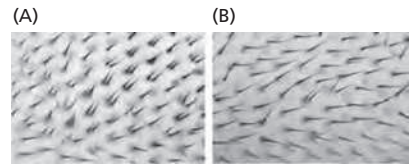


Figure Q15–5 Pattern of hair growth on wing cells in genetically different *Drosophila* (Problem 15–17). (From C.G. Winter et al., *Cell* 105:81–91, 2001. With permission from Elsevier.)

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