CHAPTER **SIXTEEN** 16

Cell Signaling

Individual cells, like multicellular organisms, need to sense and respond to their environment. A free-living cell—even a humble bacterium—must be able to track down nutrients, tell the difference between light and dark, and avoid poisons and predators. And if such a cell is to have any kind of "social life," it must be able to communicate with other cells. When a yeast cell is ready to mate, for example, it secretes a small protein called a mating factor. Yeast cells of the opposite "sex" detect this chemical mating call and respond by halting their progress through the cell-division cycle and reaching out toward the cell that emitted the signal (**Figure 16–1**).

In a multicellular organism, things are much more complicated. Cells must interpret the multitude of signals they receive from other cells to help coordinate their behaviors. During animal development, for example, cells in the embryo exchange signals to determine which specialized role each cell will adopt, what position it will occupy in the animal, and whether it will survive, divide, or die. Later in life, a large variety of signals coordinates the animal's growth and its day-to-day physiology and behavior. In plants as well, cells are in constant communication with one another. These cell–cell interactions allow the plant to coordinate what happens in its roots, stems, and leaves.

In this chapter, we examine some of the most important mechanisms by which cells send signals and interpret the signals they receive. First, we present an overview of the general principles of cell signaling. We then consider two of the main systems animal cells use to receive and interpret signals. Finally, we describe a few signaling mechanisms that work in a different way—including one that operates in plants—before

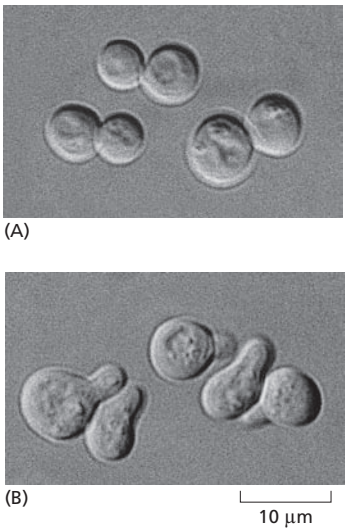


Figure 16–1 Yeast cells respond to mating factor. Budding yeast (*Saccharomyces cerevisiae*) cells are (A) normally spherical, but (B) when they are exposed to an appropriate mating factor produced by neighboring yeast cells, they extend a protrusion toward the source of the factor. (Courtesy of Michael Snyder.)

considering how these intricate signaling networks ultimately interact to control complex behaviors.

GENERAL PRINCIPLES OF CELL SIGNALING

Information can come in a variety of forms, and communication frequently involves converting the signals that carry that information from one form to another. When you receive a call from a friend on your mobile phone, for instance, the phone converts radio signals, which travel through the air, into sound waves, which you hear. This process of conversion is called **signal transduction** (Figure 16–2).

The signals that pass between cells are simpler than the sorts of messages that humans ordinarily exchange. In a typical communication between cells, the *signaling cell* produces a particular type of *extracellular signal molecule* that is detected by the *target cell*. As in human conversation, most animal cells both send and receive signals, and they can therefore act as both signaling cells and target cells.

Target cells possess proteins called *receptors* that recognize and respond specifically to the signal molecule. Signal transduction begins when the receptor on a target cell receives an incoming extracellular signal and then produces *intracellular signaling molecules* that alter cell behavior. Most of this chapter is concerned with signal reception and transduction—the events that cell biologists have in mind when they refer to **cell signaling**. First, however, we look briefly at a few of the different types of extracellular signals that cells send to one another—and what happens when target cells receive those signals.

Signals Can Act over a Long or Short Range

Cells in multicellular organisms use hundreds of kinds of **extracellular signal molecules** to communicate with one another. The signal molecules can be proteins, peptides, amino acids, nucleotides, steroids, fatty acid derivatives, or even dissolved gases—but they all rely on just a handful of basic styles of communication for getting the message across.

In multicellular organisms, the most "public" style of cell–cell communication involves broadcasting the signal throughout the whole body by secreting it into an animal's bloodstream or a plant's sap. Extracellular signal molecules used in this way are called **hormones**, and, in animals, the cells that produce hormones are called *endocrine* cells (Figure 16–3A). Part of the pancreas, for example, is an endocrine gland that produces several hormones—including insulin, which regulates glucose uptake in cells all over the body.

Somewhat less public is the process known as *paracrine signaling*. In this case, rather than entering the bloodstream, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighborhood of

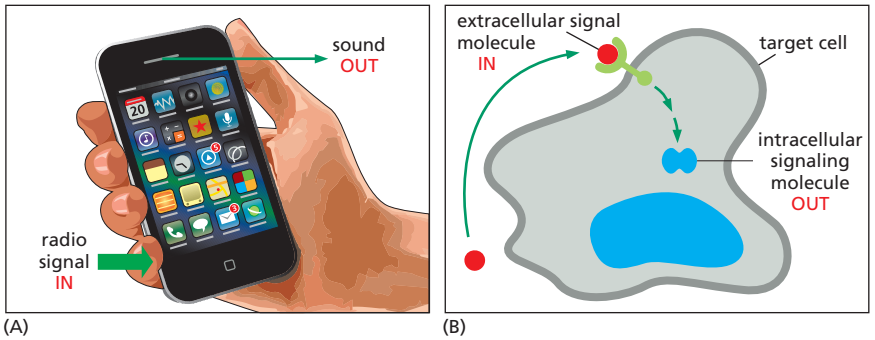


Figure 16–2 Signal transduction is the process whereby one type of signal is converted into another. (A) When a mobile telephone receives a radio signal, it converts it into a sound signal; when transmitting a signal, it does the reverse. (B) A target cell converts an extracellular signal into an intracellular signal.

the cell that secretes them. Thus, they act as **local mediators** on nearby cells (**Figure 16–3B**). Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound function in this way. In some cases, cells can respond to the local mediators that they themselves produce, a form of paracrine communication called *autocrine signaling*; cancer cells sometimes promote their own survival and proliferation in this way.

Neuronal signaling is a third form of cell communication. Like endocrine cells, nerve cells (neurons) can deliver messages over long distances. In the case of neuronal signaling, however, a message is not broadcast widely but is instead delivered quickly and specifically to individual target cells through private lines. As described in Chapter 12, the axon of a neuron terminates at specialized junctions (*synapses*) on target cells that can lie far from the neuronal cell body (**Figure 16–3C**). The axons that extend from the spinal cord to the big toe in an adult human, for example, can be more than a meter in length. When activated by signals from the environment or from other nerve cells, a neuron sends electrical impulses racing along its axon at speeds of up to 100 m/sec. On reaching the axon terminal, these electrical signals are converted into a chemical form: each electrical impulse stimulates the nerve terminal to release a pulse of an extracellular signal molecule called a **neurotransmitter**. The neurotransmitter then diffuses across the narrow (<100 nm) gap that separates the membrane of the axon terminal from that of the target cell, reaching its destination in less than 1 msec.

A fourth style of signal-mediated cell–cell communication—the most intimate and short-range of all—does not require the release of a secreted molecule. Instead, the cells make direct physical contact through signal molecules lodged in the plasma membrane of the signaling cell and receptor proteins embedded in the plasma membrane of the target cell (**Figure 16–3D**). During embryonic development, for example, such

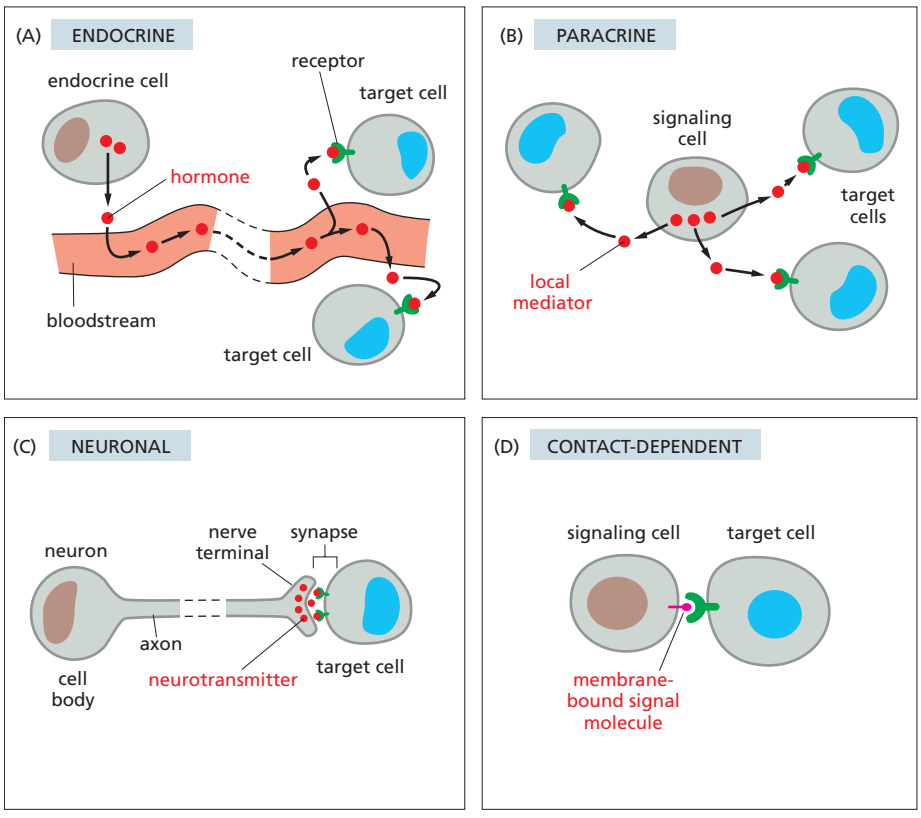


Figure 16–3 Animal cells use extracellular signal molecules to communicate with one another in various ways. (A) Hormones produced in endocrine glands are secreted into the bloodstream and are distributed widely throughout the body. (B) Paracrine signals are released by cells into the extracellular fluid in their neighborhood and act locally. (C) Neuronal signals are transmitted electrically along a nerve cell axon. When this electrical signal reaches the nerve terminal, it causes the release of neurotransmitters onto adjacent target cells. (D) In contact-dependent signaling, a cell-surface-bound signal molecule binds to a receptor protein on an adjacent cell. Many of the same types of signal molecules are used for endocrine, paracrine, and neuronal signaling. The crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

contact-dependent signaling allows adjacent cells that are initially similar to become specialized to form different cell types, as we discuss later in the chapter.

To get a better feel for these different signaling styles, imagine trying to publicize a potentially stimulating lecture—or a concert or sporting event. An endocrine signal would be akin to broadcasting the information over the radio. A more localized paracrine signal would be the equivalent of posting a flyer on selected notice boards near the arena—with an autocrine signal being a reminder you add to your own personal calendar.

Neuronal signals—long-distance but personal—would be similar to a phone call, text message, or e-mail, and contact-dependent signaling would be like a good old-fashioned, face-to-face conversation.

Table 16–1 lists some examples of hormones, local mediators, neurotransmitters, and contact-dependent signal molecules. The actions of several of these are discussed in greater detail throughout the chapter.

TABLE 16–1 SOME EXAMPLES OF SIGNAL MOLECULES

Signal Molecule	Site of Origin	Chemical Nature	Some Actions
Hormones			
Epinephrine (adrenaline)	adrenal gland	derivative of the amino acid tyrosine	increases blood pressure, heart rate, and metabolism
Cortisol	adrenal gland	steroid (derivative of cholesterol)	affects metabolism of proteins, carbohydrates, and lipids in most tissues
Estradiol	ovary	steroid (derivative of cholesterol)	induces and maintains secondary female sexual characteristics
Insulin	β cells of pancreas	protein	stimulates glucose uptake, protein synthesis, and lipid synthesis in various cell types
Testosterone	testis	steroid (derivative of cholesterol)	induces and maintains secondary male sexual characteristics
Thyroid hormone (thyroxine)	thyroid gland	derivative of the amino acid tyrosine	stimulates metabolism in many cell types
Local Mediators			
Epidermal growth factor (EGF)	various cells	protein	stimulates epidermal and many other cell types to proliferate
Platelet-derived growth factor (PDGF)	various cells, including blood platelets	protein	stimulates many cell types to proliferate
Nerve growth factor (NGF)	various innervated tissues	protein	promotes survival and axonal growth of certain classes of neurons
Histamine	mast cells	derivative of the amino acid histidine	causes blood vessels to dilate and become leaky, helping to cause inflammation
Nitric oxide (NO)	nerve cells; endothelial cells lining blood vessels	dissolved gas	causes smooth muscle cells to relax; regulates nerve-cell activity
Neurotransmitters			
Acetylcholine	nerve terminals	derivative of choline	excitatory neurotransmitter at many nerve–muscle synapses and in central nervous system
γ-Aminobutyric acid (GABA)	nerve terminals	derivative of the amino acid glutamic acid	inhibitory neurotransmitter in central nervous system
Contact-dependent Signal Molecules			
Delta	prospective neurons; various other developing cell types	transmembrane protein	inhibits neighboring cells from becoming specialized in same way as the signaling cell

A Limited Set of Extracellular Signals Can Produce a Huge Variety of Cell Behaviors

A typical cell in a multicellular organism is exposed to hundreds of different signal molecules in its environment. These may be free in the extracellular fluid, embedded in the extracellular matrix in which many cells reside, or bound to the surface of neighboring cells. Each cell must respond very selectively to this mixture of signals, disregarding some and reacting to others, according to the cell's specialized function.

Whether a cell responds to a signal molecule depends, first of all, on whether it possesses a **receptor** for that signal. Each receptor is usually activated by only one type of signal. Without the appropriate receptor, a cell will be deaf to the signal and will not respond to it.

Extracellular signal molecules can be divided into two major classes, depending on the type of receptor with which they interact. The first and largest class of signals consists of molecules that are too large or too hydrophilic to cross the plasma membrane of the target cell. These signal molecules rely on receptors on the surface of the target cell to relay their message across the plasma membrane (**Figure 16–4A**). The second class of signals consists of molecules that are small enough or hydrophobic enough to pass through the plasma membrane and into the cytosol of the target cell, where they bind to intracellular receptor proteins (**Figure 16–4B**). Here, we focus primarily on signaling through cell-surface receptors, but we will briefly describe signaling through intracellular receptors later in the chapter.

By producing only a limited set of receptors out of the thousands that are possible, a cell restricts the types of signals that can affect it. Of course, even this restricted set of extracellular signal molecules can change the behavior of a target cell in a large variety of ways, altering its shape, movement, metabolism, gene expression, or some combination of these. How a cell reacts to a signal depends on the set of intracellular signaling molecules each cell-surface receptor produces and how these molecules alter the activity of *effector proteins*, which have a direct effect on the behavior of the target cell. This intracellular relay system and the intracellular effector proteins on which it acts vary from one type of specialized cell to another, so that different types of cells respond to the same signal in different ways. For example, when a heart pacemaker cell is exposed to the neurotransmitter *acetylcholine*, its rate of firing decreases. When a salivary gland cell is exposed to the same signal, it secretes components of saliva, even though the receptors on both cell types are the same. In skeletal muscle, acetylcholine binds to a different receptor protein, causing the muscle cell to contract (**Figure 16–5**). Thus, the extracellular signal molecule alone is not the message: the information conveyed by the signal depends on how the target cell receives and interprets the signal.

A typical cell possesses many sorts of receptors—each present in tens to hundreds of thousands of copies. Such variety makes the cell simultaneously sensitive to many different extracellular signals and allows

QUESTION 16–1

To keep their action local, paracrine signal molecules must be prevented from straying too far from their points of origin. Suggest different ways by which this could be accomplished. Explain your answers.

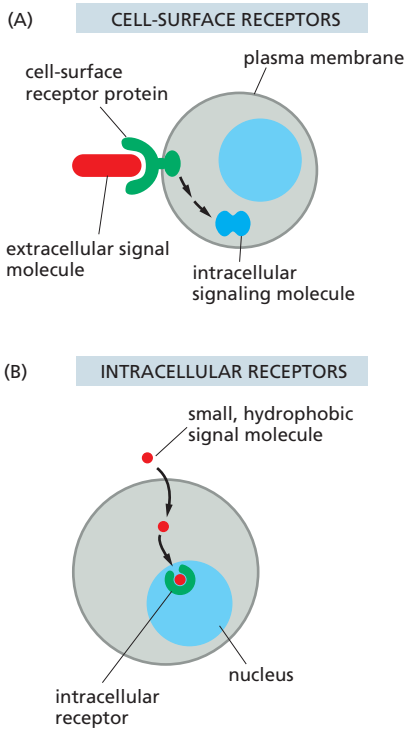


Figure 16–4 Extracellular signal molecules bind either to cell-surface receptors or to intracellular receptors. (A) Most extracellular signal molecules are large and hydrophilic and are therefore unable to cross the plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate one or more intracellular signaling molecules in the target cell. (B) Some small, hydrophobic, extracellular signal molecules pass through the target cell's plasma membrane and bind to intracellular receptors—in the cytosol or in the nucleus (as shown here)—that then regulate gene transcription or other functions.

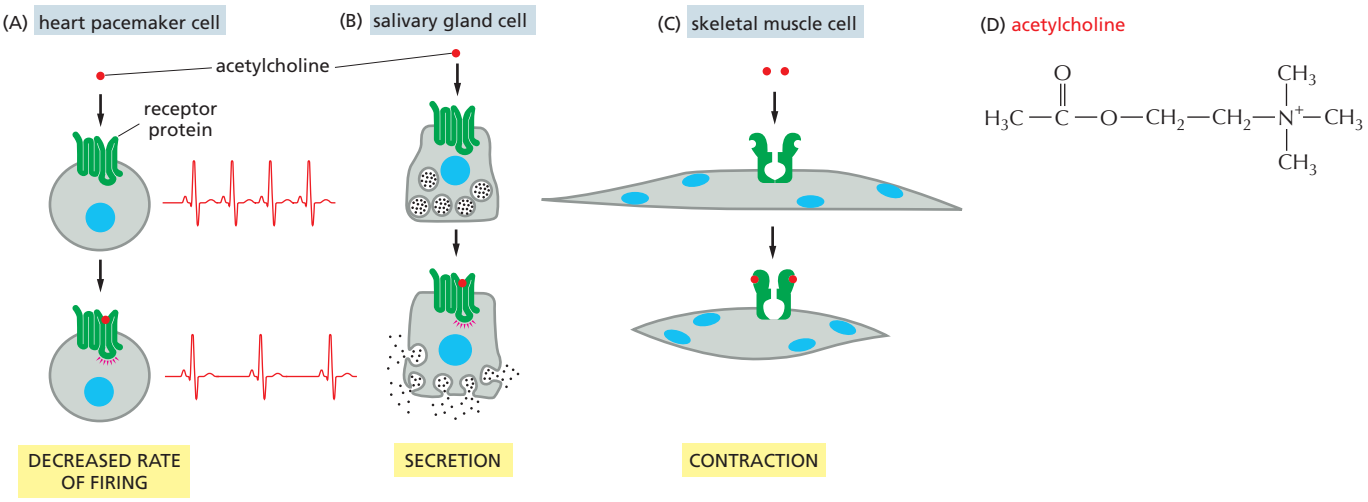


Figure 16–5 The same signal molecule can induce different responses in different target cells. Different cell types are configured to respond to the neurotransmitter acetylcholine in different ways. Acetylcholine binds to similar receptor proteins on (A) heart pacemaker cells and (B) salivary gland cells, but it evokes different responses in each cell type. (C) Skeletal muscle cells produce a different type of receptor protein for the same signal molecule. (D) For such a versatile molecule, acetylcholine has a fairly simple chemical structure.

a relatively small number of signal molecules, used in different combinations, to exert subtle and complex control over cell behavior. A combination of signals can evoke a response that is different from the sum of the effects that each signal would trigger on its own. As we discuss later, this "tailoring" of a cell's response occurs, in part, because the intracellular relay systems activated by the different signals interact. Thus the presence of one signal will often modify the effects of another. One combination of signals might enable a cell to survive; another might drive it to differentiate in some specialized way; and another might cause it to divide. In the absence of the proper signals, most animal cells are programmed to kill themselves (Figure 16–6).

A Cell's Response to a Signal Can Be Fast or Slow

The length of time a cell takes to respond to an extracellular signal can vary greatly, depending on what needs to happen once the message has

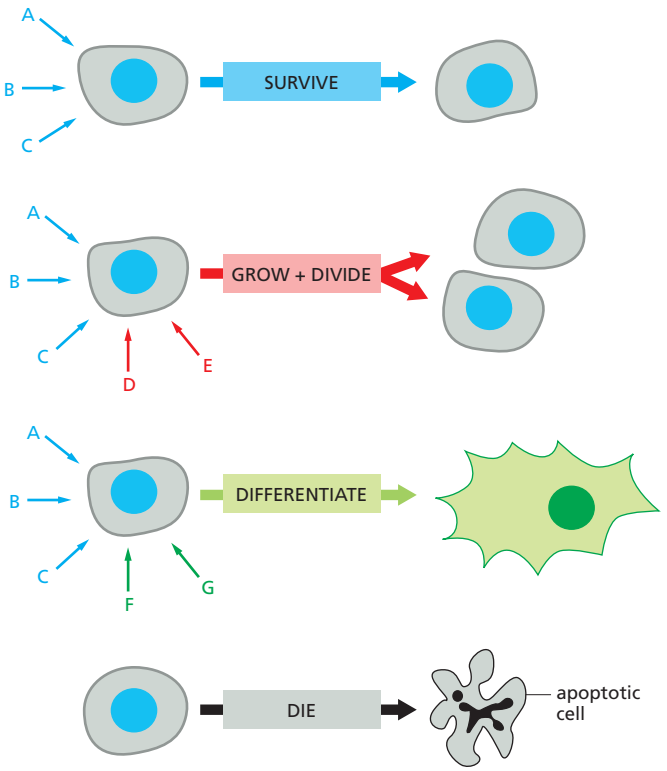


Figure 16–6 An animal cell depends on multiple extracellular signals. Every cell type displays a set of receptor proteins that enables it to respond to a specific set of extracellular signal molecules produced by other cells. These signal molecules work in combinations to regulate the behavior of the cell. As shown here, cells may require multiple signals (*blue* arrows) to survive, additional signals (*red* arrows) to grow and divide, and still other signals (*green* arrows) to differentiate. If deprived of the necessary survival signals, most cells undergo a form of cell suicide known as apoptosis (discussed in Chapter 18).

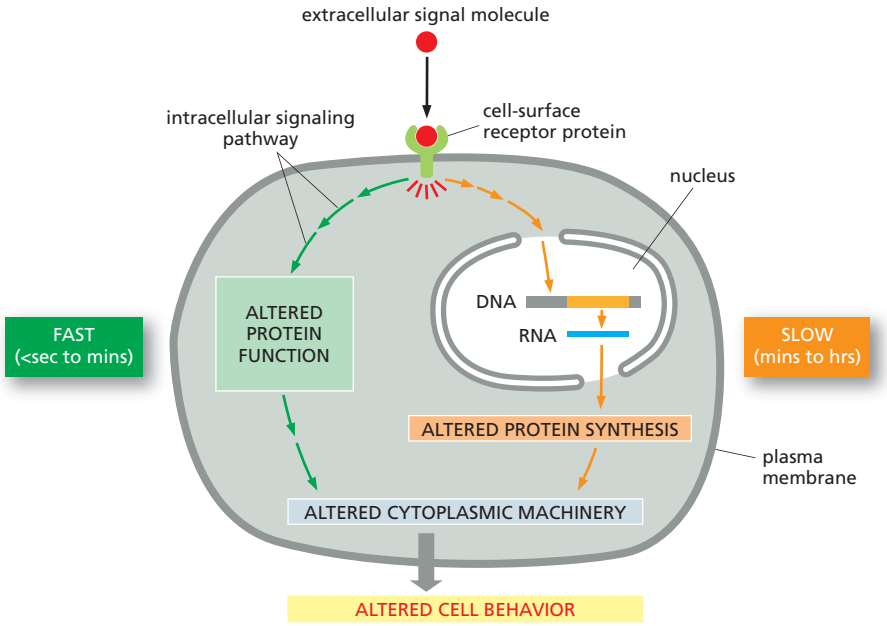


Figure 16–7 Extracellular signals can act slowly or rapidly. Certain types of cell responses—such as cell differentiation or increased cell growth and division (see Figure 16–6)—involve changes in gene expression and the synthesis of new proteins; they therefore occur relatively slowly. Other responses—such as changes in cell movement, secretion, or metabolism—need not involve changes in gene expression and therefore occur more quickly (see Figure 16–5).

been received. Some extracellular signals act swiftly: acetylcholine can stimulate a skeletal muscle cell to contract within milliseconds and a salivary gland cell to secrete within a minute or so. Such rapid responses are possible because, in each case, the signal affects the activity of proteins that are already present inside the target cell, awaiting their marching orders.

Other responses take more time. Cell growth and cell division, when triggered by the appropriate signal molecules, can take many hours to execute. This is because the response to these extracellular signals requires changes in gene expression and the production of new proteins (**Figure 16–7**). We will encounter additional examples of both fast and slow responses—and the signal molecules that stimulate them—later in the chapter.

Cell-Surface Receptors Relay Extracellular Signals via Intracellular Signaling Pathways

The majority of extracellular signal molecules are proteins, peptides, or small, hydrophilic molecules that bind to cell-surface receptors that span the plasma membrane (see Figure 16–4A). Transmembrane receptors detect a signal on the outside and relay the message, in a new form, across the membrane into the interior of the cell.

The receptor protein performs the primary step in signal transduction: it recognizes the extracellular signal and generates new intracellular signals in response (see Figure 16–2B). The resulting intracellular signaling process usually works like a molecular relay race, in which the message is passed "downstream" from one intracellular signaling molecule to another, each activating or generating the next signaling molecule in the pathway, until a metabolic enzyme is kicked into action, the cytoskeleton is tweaked into a new configuration, or a gene is switched on or off. This final outcome is called the response of the cell (**Figure 16–8**).

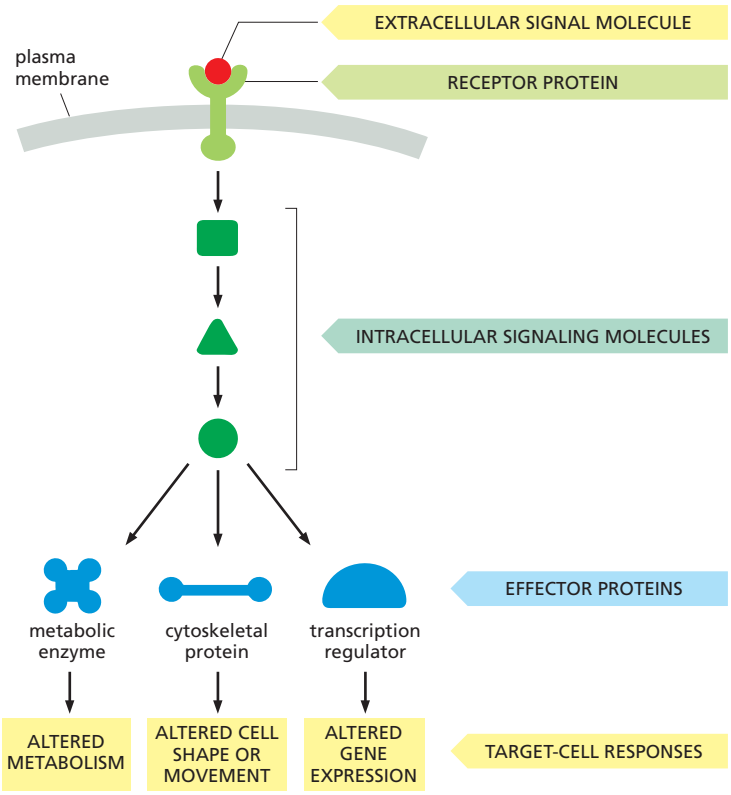


Figure 16–8 Many extracellular signals activate intracellular signaling pathways to change the behavior of the target cell. A cell-surface receptor protein activates one or more intracellular signaling pathways, each mediated by a series of intracellular signaling molecules, which can be proteins or small messenger molecules; only one pathway is shown. Signaling molecules eventually interact with specific *effector* proteins, altering them to change the behavior of the cell in various ways.

QUESTION 16–2

In principle, how might an intracellular signaling protein amplify a signal as it relays it onward?

The components of these **intracellular signaling pathways** perform one or more crucial functions (**Figure 16–9**):

1. They can *relay* the signal onward and thereby help spread it through the cell.
2. They can *amplify* the signal received, making it stronger, so that a few extracellular signal molecules are enough to evoke a large intracellular response.
3. They can detect signals from more than one intracellular signaling pathway and *integrate* them before relaying a signal onward.
4. They can *distribute* the signal to more than one effector protein, creating branches in the information flow diagram and evoking a complex response.
5. They can modulate the response to the signal by regulating the activity of components upstream in the signaling pathway, a process known as *feedback*.

Feedback regulation, although it is last on our list, is actually a very important feature of cell signaling. It can occur anywhere in the signaling pathway and can either boost or weaken the response to the signal. In positive feedback, a component that lies downstream in the pathway acts on an earlier component in the same pathway to enhance the response to the initial signal; in negative feedback, a downstream component acts to inhibit an earlier component in the pathway to diminish the response to the initial signal (**Figure 16–10**). Such feedback regulation is very common in biological systems and can lead to sophisticated responses: positive feedback can generate all-or-none, switchlike responses, for example, whereas negative feedback can generate responses that oscillate on and off as the activities or concentrations of the inhibitory components rise and fall.

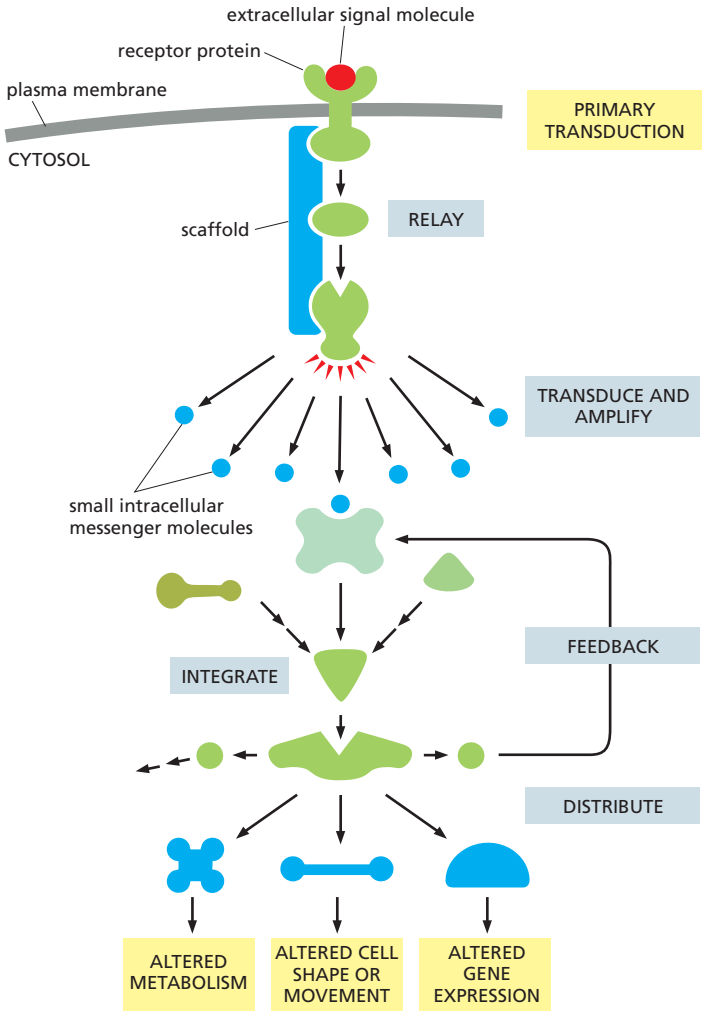


Figure 16–9 Intracellular signaling proteins can relay, amplify, integrate, distribute, and modulate via feedback an incoming signal. In this example, a receptor protein located on the cell surface transduces an extracellular signal into an intracellular signal, which initiates one or more intracellular signaling pathways that relay the signal into the cell interior. Each pathway includes intracellular signaling proteins that can function in one of the various ways shown; some, for example, integrate signals from other intracellular signaling pathways. Many of the steps in the process can be modulated via feedback by other molecules or events in the cell. Note that some proteins in the pathway may be held in close proximity by a scaffold protein, which allows them to be activated at a specific location in the cell and with greater speed, efficiency, and selectivity (discussed in Chapter 4; see Figure 4–52). We review the production and function of small intracellular messenger molecules, more commonly called second messenger molecules, later in the chapter.

Some Intracellular Signaling Proteins Act as Molecular Switches

Many intracellular signaling proteins behave as **molecular switches**: receipt of a signal causes them to toggle from an inactive to an active state. Once activated, these proteins can stimulate—or in some cases suppress—other proteins in the signaling pathway. They then persist in an active state until some other process switches them off again.

The importance of the switching-off process is often underappreciated: imagine the consequences if a signaling pathway that boosts your heart rate were to remain active indefinitely. If a signaling pathway is to recover after transmitting a signal and make itself ready to transmit another, every activated protein in the pathway must be reset to its original,

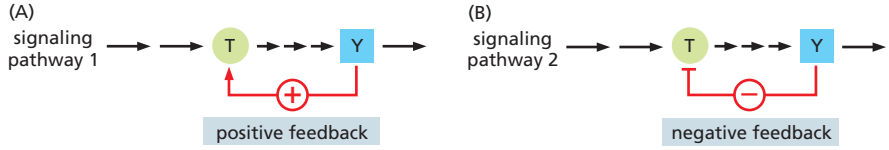


Figure 16–10 Feedback regulation within an intracellular signaling pathway can adjust the response to an extracellular signal. (A) In this simple example, a downstream protein in a signaling pathway, protein Y, acts to increase the activity of the protein that activated it—a form of positive feedback. Positive feedback loops can ignite an explosive response, such as the activation of the proteins that trigger cell division (discussed in Chapter 18). (B) In a simple example of negative feedback, protein Y inhibits the protein that activated it. Negative feedback loops can generate oscillations, similar to the way that populations of predators and prey can seesaw: an increase in prey (here, protein T) would promote the expansion of predators (protein Y); as the number of predators increases, the availability of prey will fall (via negative feedback), which will ultimately cause the predator population to decline. As the predators disappear, the prey populations will recover and multiply, providing food for more predators, and so on.

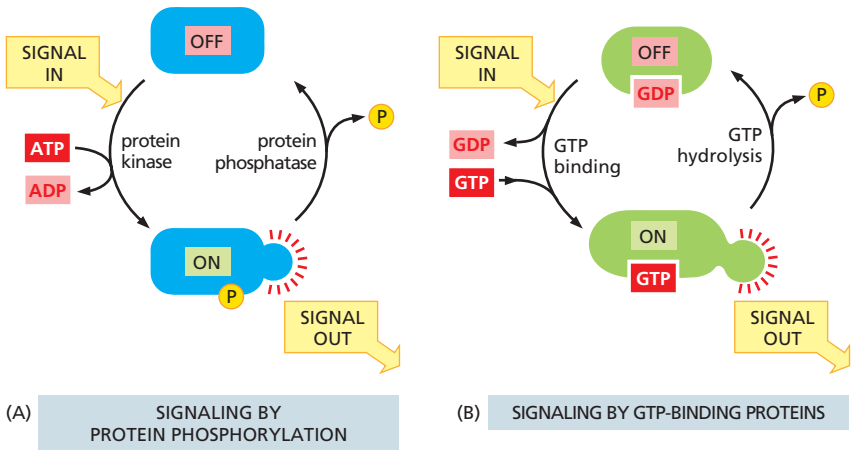


Figure 16–11 Many intracellular signaling proteins act as molecular switches. These proteins can be activated—or in some cases inhibited—by the addition or removal of a phosphate group. (A) In one class of switch protein, the phosphate is added covalently by a protein kinase, which transfers the terminal phosphate group from ATP to the signaling protein; the phosphate is then removed by a protein phosphatase. (B) In the other class of switch protein, a GTP-binding protein is activated when it exchanges its bound GDP for GTP (which, in a sense, adds a phosphate to the protein); the protein then switches itself off by hydrolyzing its bound GTP to GDP.

unstimulated state. Thus, for every activation step along the pathway, there exists an inactivation mechanism. The two are equally important for a signaling pathway to be useful.

Proteins that act as molecular switches fall mostly into one of two classes. The first—and by far the largest—class consists of proteins that are activated or inactivated by phosphorylation, a chemical modification discussed in Chapter 4 (see Figure 4–46). For these molecules, the switch is thrown in one direction by a **protein kinase**, which covalently attaches a phosphate group onto the switch protein, and in the opposite direction by a **protein phosphatase**, which takes the phosphate off again (**Figure 16–11A**). The activity of any protein that is regulated by phosphorylation depends—moment by moment—on the balance between the activities of the protein kinases that phosphorylate it and the protein phosphatases that dephosphorylate it.

Many of the switch proteins controlled by phosphorylation are themselves protein kinases, and these are often organized into *phosphorylation cascades*: one protein kinase, activated by phosphorylation, phosphorylates the next protein kinase in the sequence, and so on, transmitting the signal onward and, in the process, amplifying, distributing, and regulating it. Two main types of protein kinases operate in intracellular signaling pathways: the most common are **serine/threonine kinases**, which—as the name implies—phosphorylate proteins on serines or threonines; others are **tyrosine kinases**, which phosphorylate proteins on tyrosines.

The other class of switch proteins involved in intracellular signaling pathways are **GTP-binding proteins**. These toggle between an active and an inactive state depending on whether they have GTP or GDP bound to them, respectively (**Figure 16–11B**). Once activated by GTP binding, many of these proteins have intrinsic GTP-hydrolyzing (*GTPase*) activity, and they shut themselves off by hydrolyzing their bound GTP to GDP.

Two main types of GTP-binding proteins participate in intracellular signaling. The first type—the large, *trimeric GTP-binding proteins* (also called *G proteins*)—relay messages from *G-protein-coupled receptors*. We discuss this major class of GTP-binding proteins in detail shortly.

Other cell-surface receptors rely on a second type of GTP-binding protein—the small, *monomeric GTPases*—to help relay their signals. These switch proteins are generally aided by two sets of regulatory proteins that help them bind and hydrolyze GTP: *guanine nucleotide exchange factors* (*GEFs*) activate the switches by promoting the exchange of GDP for GTP, and *GTPase-activating proteins* (*GAPs*) turn them off by promoting GTP hydrolysis (**Figure 16–12**).

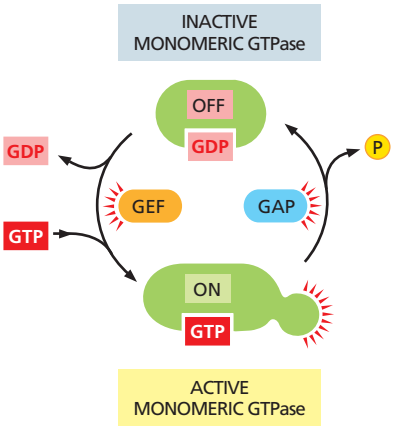


Figure 16–12 The activity of monomeric GTPases is controlled by two types of regulatory proteins. Guanine nucleotide exchange factors (GEFs) promote the exchange of GDP for GTP, thereby switching the protein on. GTPase-activating proteins (GAPs) stimulate the hydrolysis of GTP to GDP, thereby switching the protein off.

Cell-Surface Receptors Fall into Three Main Classes

All cell-surface receptor proteins bind to an extracellular signal molecule and transduce its message into one or more intracellular signaling molecules that alter the cell's behavior. Most of these receptors belong to one of three large classes, which differ in the transduction mechanism they use.

1. *Ion-channel-coupled receptors* change the permeability of the plasma membrane to selected ions, thereby altering the membrane potential and, if the conditions are right, producing an electrical current (**Figure 16–13A**).
2. *G-protein-coupled receptors* activate membrane-bound, trimeric GTP-binding proteins (G proteins), which then activate (or inhibit) an enzyme or an ion channel in the plasma membrane, initiating an intracellular signaling cascade (**Figure 16–13B**).
3. *Enzyme-coupled receptors* either act as enzymes or associate with enzymes inside the cell (**Figure 16–13C**); when stimulated, the enzymes can activate a wide variety of intracellular signaling pathways.

The number of different types of receptors in each of these three classes is even greater than the number of extracellular signals that act on them. This is because for many extracellular signal molecules there is more than one type of receptor, and these may belong to different receptor classes. The neurotransmitter acetylcholine, for example, acts on skeletal muscle cells via an ion-channel-coupled receptor, whereas in heart cells it acts through a G-protein-coupled receptor. These two types of receptors generate different intracellular signals and thus enable the two types

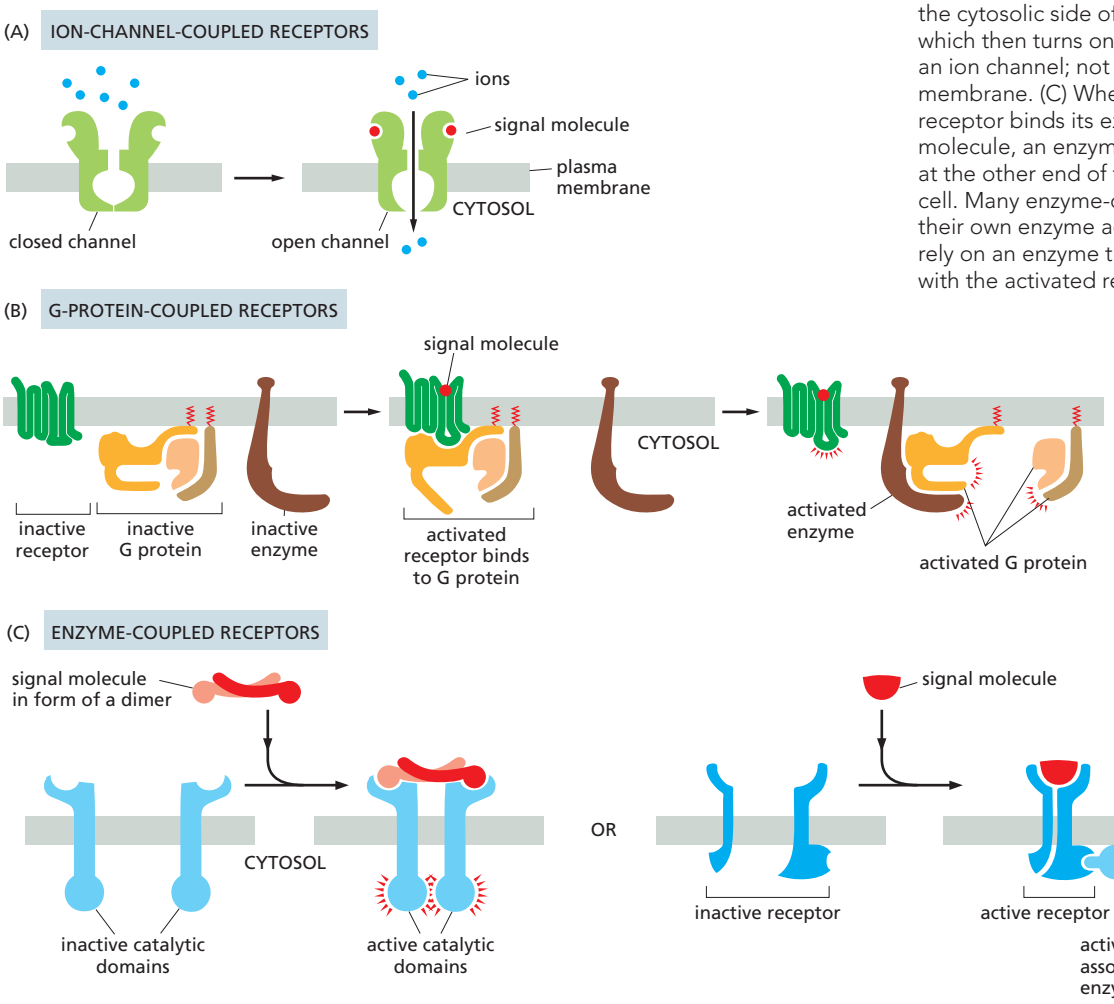


Figure 16–13 Cell-surface receptors fall into one of three main classes. (A) An ion-channel-coupled receptor opens in response to binding an extracellular signal molecule. These channels are also called transmitter-gated ion channels. (B) When a G-protein-coupled receptor binds its extracellular signal molecule, the activated receptor signals to a trimeric G protein on the cytosolic side of the plasma membrane, which then turns on (or off) an enzyme (or an ion channel; not shown) in the same membrane. (C) When an enzyme-coupled receptor binds its extracellular signal molecule, an enzyme activity is switched on at the other end of the receptor, inside the cell. Many enzyme-coupled receptors have their own enzyme activity (*left*), while others rely on an enzyme that becomes associated with the activated receptor (*right*).

TABLE 16–2 SOME FOREIGN SUBSTANCES THAT ACT ON CELL–SURFACE RECEPTORS

Substance	Normal Signal	Receptor Action	Effect
Barbiturates and benzodiazepines (Valium and Ambien)	γ-aminobutyric acid (GABA)	stimulate GABA-activated ion-channel-coupled receptors	relief of anxiety; sedation
Nicotine	acetylcholine	stimulates acetylcholine-activated ion-channel-coupled receptors	constriction of blood vessels; elevation of blood pressure
Morphine and heroin	endorphins and enkephalins	stimulate G-protein-coupled opiate receptors	analgesia (relief of pain); euphoria
Curare	acetylcholine	blocks acetylcholine-activated ion-channel-coupled receptors	blockage of neuromuscular transmission, resulting in paralysis
Strychnine	glycine	blocks glycine-activated ion-channel-coupled receptors	blockage of inhibitory synapses in spinal cord and brain, resulting in seizures and muscle spasm
Capsaicin	heat	stimulates temperature-sensitive ion-channel-coupled receptors	induces painful, burning sensation; prolonged exposure paradoxically leads to pain relief
Menthol	cold	stimulates temperature-sensitive ion-channel-coupled receptors	in moderate amounts, induces a cool sensation; in higher doses, can cause burning pain

of cells to react to acetylcholine in different ways, increasing contraction in skeletal muscle and decreasing the rate of contractions in the heart (see Figure 16–5A and C).

This plethora of cell-surface receptors also provides targets for many foreign substances that interfere with our physiology, from heroin and nicotine to tranquilizers and chili peppers. These substances either block or overstimulate the receptor's natural activity. Many drugs and poisons act in this way (**Table 16–2**), and a large part of the pharmaceutical industry is devoted to producing drugs that will exert a precisely defined effect by binding to a specific type of cell-surface receptor.

Ion-Channel-Coupled Receptors Convert Chemical Signals into Electrical Ones

Of all the types of cell-surface receptors, **ion-channel-coupled receptors** (also known as transmitter-gated ion channels) function in the simplest and most direct way. As we discuss in detail in Chapter 12, these receptors are responsible for the rapid transmission of signals across synapses in the nervous system. They transduce a chemical signal, in the form of a pulse of secreted neurotransmitter molecules delivered to a target cell, directly into an electrical signal, in the form of a change in voltage across the target cell's plasma membrane (see Figure 12–41). When the neurotransmitter binds to ion-channel-coupled receptors on the surface of a target cell, the receptor alters its conformation so as to open a channel in the target cell membrane, rendering it permeable to specific types of ions, such as Na^+, K^+, or Ca^{2+} (see Figure 16–13A and **Movie 16.1**). Driven by their electrochemical gradients, the ions rush into or out of the cell, creating a change in the membrane potential within milliseconds. This change in potential may trigger a nerve impulse or make it easier (or harder) for other neurotransmitters to do so. As we discuss later, the opening of Ca^{2+} channels has additional important effects, as changes in the Ca^{2+} concentration in the target-cell cytosol can profoundly alter the activities of many Ca^{2+}-responsive proteins.

Whereas ion-channel-coupled receptors are especially important in nerve cells and other electrically excitable cells such as muscle cells, G-protein-coupled receptors and enzyme-coupled receptors are important for practically every cell type in the body. Most of the remainder of this chapter deals with these two receptor families and with the signal transduction processes that they use.

G-PROTEIN-COUPLED RECEPTORS

G-protein-coupled receptors (**GPCRs**) form the largest family of cell-surface receptors. There are more than 700 GPCRs in humans, and mice have about 1000 involved in the sense of smell alone. These receptors mediate responses to an enormous diversity of extracellular signal molecules, including hormones, local mediators, and neurotransmitters. The signal molecules that bind GPCRs are as varied in structure as they are in function: they can be proteins, small peptides, or derivatives of amino acids or fatty acids, and for each one of them there is a different receptor or set of receptors. Because GPCRs are involved in such a large variety of cell processes, they are an attractive target for the development of drugs to treat many disorders. About one-third of all drugs used today work through GPCRs.

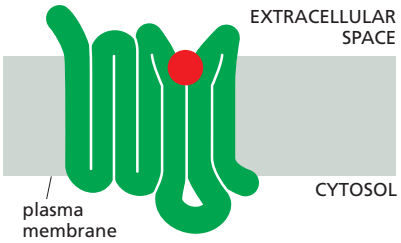
Despite the diversity of the signal molecules that bind to them, all GPCRs have a similar structure: each is made of a single polypeptide chain that threads back and forth across the lipid bilayer seven times (**Figure 16–14**). The GPCR superfamily includes rhodopsin (the light-activated photoreceptor protein in the vertebrate eye), the olfactory (smell) receptors in the vertebrate nose, and the receptors that participate in the mating rituals of single-celled yeasts (see Figure 16–1). Evolutionarily speaking, GPCRs are ancient: even prokaryotes possess structurally similar membrane proteins—such as the bacteriorhodopsin that functions as a light-driven H^+ pump (see Figure 11–28). Although they resemble eukaryotic GPCRs, these prokaryotic proteins do not act through G proteins, but are coupled to other signal transduction systems.

We begin this section with a discussion of how G proteins are activated by GPCRs. We then consider how activated G proteins stimulate ion channels and how they regulate membrane-bound enzymes that control the concentrations of small intracellular messenger molecules, including cyclic AMP and Ca^{2+}, which in turn control the activity of important intracellular signaling proteins. We end with a discussion of how light-activated GPCRs in photoreceptors in our eyes enable us to see.

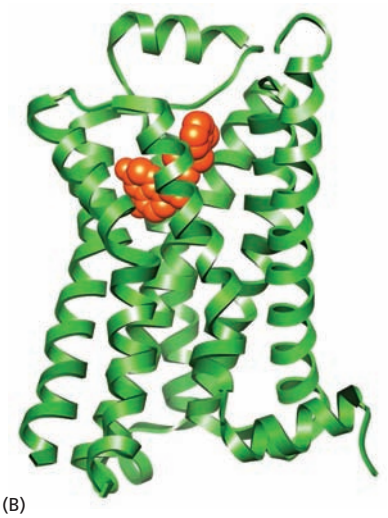
Stimulation of GPCRs Activates G-Protein Subunits

When an extracellular signal molecule binds to a GPCR, the receptor protein undergoes a conformational change that enables it to activate a **G protein** located on the other side of the plasma membrane. To explain how this activation leads to the transmission of a signal, we must first consider how G proteins are constructed and how they operate.

There are several varieties of G proteins. Each is specific for a particular set of receptors and for a particular set of target enzymes or ion channels in the plasma membrane. All of these G proteins, however, have a similar general structure and operate in a similar way. They are composed of three protein subunits—α, β, and γ—two of which are tethered to the plasma membrane by short lipid tails. In the unstimulated state, the α subunit has GDP bound to it, and the G protein is idle (**Figure 16–15A**). When an extracellular signal molecule binds to its receptor, the altered receptor activates a G protein by causing the α subunit to decrease its



(A)



(B)

Figure 16–14 All GPCRs possess a similar structure. The polypeptide chain traverses the membrane as seven α helices. The cytoplasmic portions of the receptor bind to a G protein inside the cell. (A) For receptors that recognize small signal molecules, such as acetylcholine or epinephrine, the ligand (*red*) usually binds deep within the plane of the membrane to a pocket that is formed by amino acids from several transmembrane segments. Receptors that recognize signal molecules that are proteins usually have a large, extracellular domain that, together with some of the transmembrane segments, binds the protein ligand (not shown). (B) Shown here is the structure of a GPCR that binds to epinephrine (*red*). Stimulation of this receptor by epinephrine makes the heart beat faster.

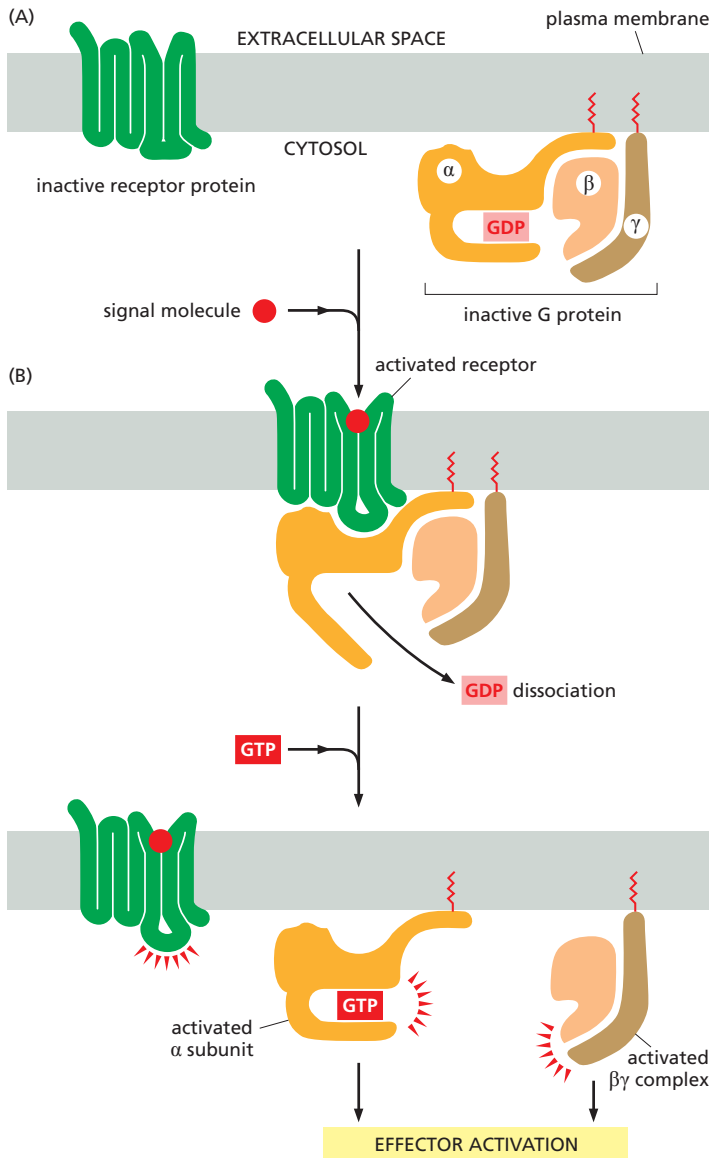


Figure 16–15 An activated GPCR activates G proteins by encouraging the α subunit to expel its GDP and pick up GTP. (A) In the unstimulated state, the receptor and the G protein are both inactive. Although they are shown here as separate entities in the plasma membrane, in some cases they are associated in a preformed complex. (B) Binding of an extracellular signal molecule to the receptor changes the conformation of the receptor, which in turn alters the conformation of the bound G protein. The alteration of the α subunit of the G protein allows it to exchange its GDP for GTP. This exchange triggers an additional conformational change that activates both the α subunit and a βγ complex, which dissociate to interact with their preferred target proteins in the plasma membrane (**Movie 16.2**). The receptor stays active as long as the external signal molecule is bound to it, and it can therefore activate many molecules of G protein. Note that both the α and γ subunits of the G protein have covalently attached lipid molecules (*red*) that help anchor the subunits to the plasma membrane.

affinity for GDP, which is then exchanged for a molecule of GTP. In some cases, this activation breaks up the G-protein subunits, so that the activated α subunit, clutching its GTP, detaches from the βγ complex, which is also activated (**Figure 16–15B**). The two activated parts of the G protein—the α subunit and the βγ complex—can then each interact directly with target proteins in the plasma membrane, which in turn may relay the signal to other destinations in the cell. The longer these target proteins remain bound to an α subunit or a βγ complex, the more prolonged the relayed signal will be.

The amount of time that the α subunit and βγ complex remain "switched on"—and hence available to relay signals—also determines how long a response lasts. This timing is controlled by the behavior of the α subunit. The α subunit has an intrinsic GTPase activity, and it eventually hydrolyzes its bound GTP to GDP, returning the whole G protein to its original, inactive conformation (**Figure 16–16**). GTP hydrolysis and inactivation usually occur within seconds after the G protein has been activated. The inactive G protein is then ready to be reactivated by another activated receptor.

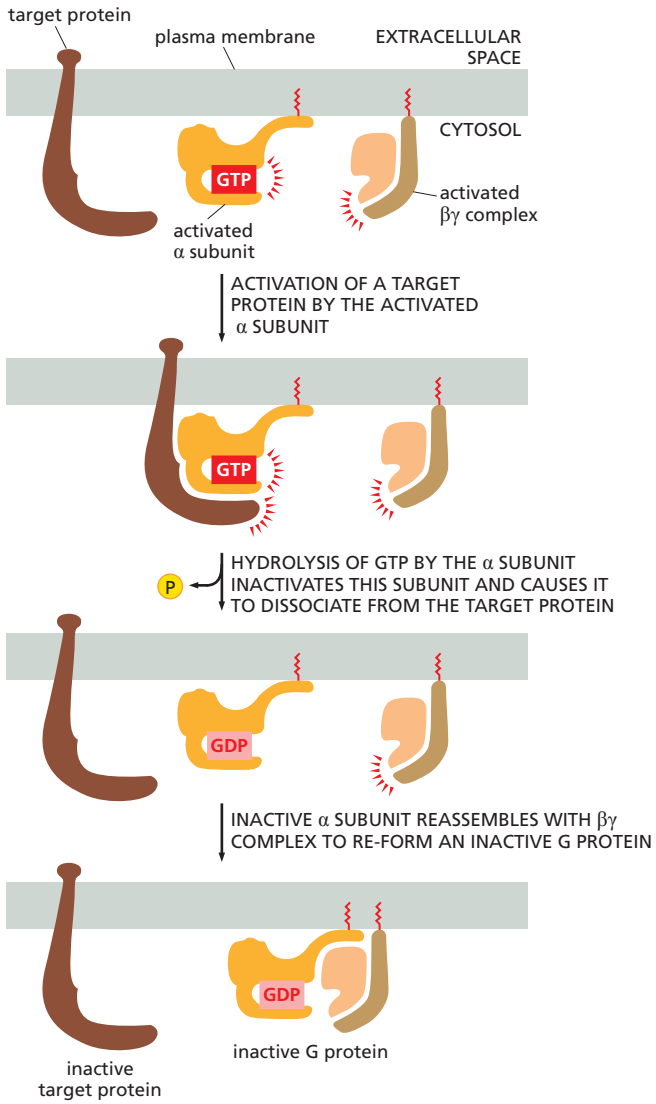


Figure 16–16 The G protein α subunit switches itself off by hydrolyzing its bound GTP to GDP. When an activated α subunit interacts with its target protein, it activates that target protein for as long as the two remain in contact. (In some cases, the α subunit instead inactivates its target; not shown.) The α subunit then hydrolyzes its bound GTP to GDP—an event that takes place usually within seconds of G-protein activation. The hydrolysis of GTP inactivates the α subunit, which dissociates from its target protein and—if the α subunit had separated from the βγ complex (as shown)—reassociates with a βγ complex to re-form an inactive G protein. The G protein is now ready to couple to another activated receptor, as in Figure 16–15B. Both the activated α subunit and the activated βγ complex can interact with target proteins in the plasma membrane. See also **Movie 16.2**.

Some Bacterial Toxins Cause Disease by Altering the Activity of G Proteins

G proteins offer a striking example of the importance of being able to shut down a signal, as well as turn it on. Disrupting the activation—and deactivation—of G proteins can have dire consequences for a cell or organism. Consider cholera, for example. The disease is caused by a bacterium that multiplies in the human intestine, where it produces a protein called *cholera toxin*. This protein enters the cells that line the intestine and modifies the α subunit of a G protein called G_s—so named because it *stimulates* the enzyme adenylyl cyclase, which we discuss shortly. The modification prevents G_s from hydrolyzing its bound GTP, thus locking the G protein in an active state, in which it continuously stimulates adenylyl cyclase. In intestinal cells, this stimulation causes a prolonged and excessive outflow of Cl^- and water into the gut, resulting in catastrophic diarrhea and dehydration. The condition often leads to death unless urgent steps are taken to replace the lost water and ions.

A similar situation occurs in whooping cough (pertussis), a common respiratory infection against which infants are now routinely vaccinated. In this case, the disease-causing bacterium colonizes the lung, where it produces a protein called *pertussis toxin*. This protein alters the α subunit of

QUESTION 16–3

GPCRs activate G proteins by reducing the strength of GDP binding to the G protein. This results in rapid dissociation of bound GDP, which is then replaced by GTP, because GTP is present in the cytosol in much higher concentrations than GDP. What consequences would result from a mutation in the α subunit of a G protein that caused its affinity for GDP to be reduced without significantly changing its affinity for GTP? Compare the effects of this mutation with the effects of cholera toxin.

a different type of G protein, called G_i because it *inhibits* adenylyl cyclase. In this case, however, modification by the toxin disables the G protein by locking it into its inactive GDP-bound state. Inhibiting G_i, like activating G_s, results in the prolonged and inappropriate activation of adenylyl cyclase, which, in this case, stimulates coughing. Both the diarrhea-producing effects of cholera toxin and the cough-provoking effects of pertussis toxin help the disease-causing bacteria move from host to host.

Some G Proteins Directly Regulate Ion Channels

There are about 20 different types of mammalian G proteins, each activated by a particular set of cell-surface receptors and dedicated to activating a particular set of target proteins. These target proteins are either enzymes or ion channels in the plasma membrane. Thus, the binding of an extracellular signal molecule to a GPCR leads to changes in the activities of a specific subset of the possible target proteins in the plasma membrane, producing a response that is appropriate for that signal and that type of cell.

We look first at an example of direct G-protein regulation of ion channels. The heartbeat in animals is controlled by two sets of nerves: one speeds the heart up, the other slows it down. The nerves that signal a slowdown in heartbeat do so by releasing acetylcholine (see Figure 16–5A), which binds to a GPCR on the surface of the heart pacemaker cells. This GPCR activates the G protein, G_i. In this case, the βγ complex binds to the intracellular face of a K^+ channel in the plasma membrane of the pacemaker cell, forcing the ion channel into an open conformation (**Figure 16–17A and B**). This channel opening slows the heart rate by increasing the plasma membrane's permeability to K^+, which makes it more difficult to electrically activate, as explained in Chapter 12. The original signal is terminated—and the K^+ channel recloses—when the α subunit inactivates itself by hydrolyzing its bound GTP, returning the G protein to its inactive state (**Figure 16–17C**).

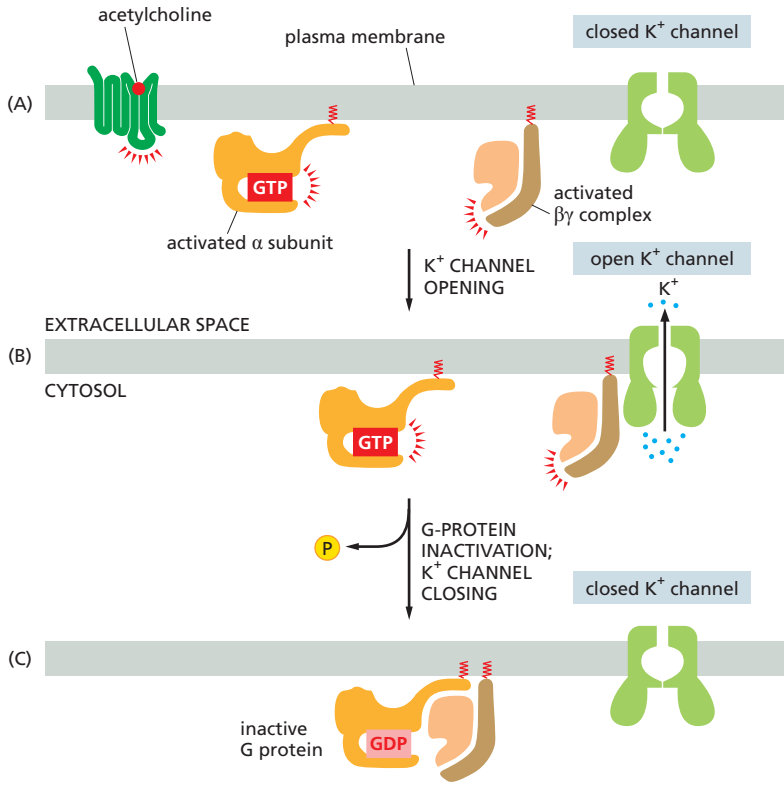


Figure 16–17 A G_i protein directly couples receptor activation to the opening of K^+ channels in the plasma membrane of heart pacemaker cells. (A) Binding of the neurotransmitter acetylcholine to its GPCR on the heart cells results in the activation of the G protein, G_i. (B) The activated βγ complex directly opens a K^+ channel in the plasma membrane, increasing its permeability to K^+ and thereby making the membrane harder to activate and slowing the heart rate. (C) Inactivation of the α subunit by hydrolysis of its bound GTP returns the G protein to its inactive state, allowing the K^+ channel to close.

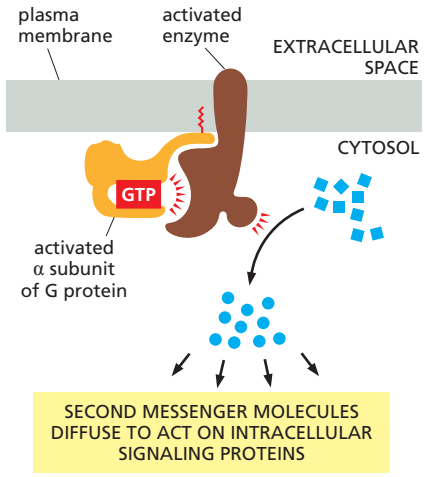


Figure 16–18 Enzymes activated by G proteins increase the concentrations of small intracellular signaling molecules. Because each activated enzyme generates many molecules of these second messengers, the signal is greatly amplified at this step in the pathway (see Figure 16–28). The signal is relayed onward by the second messenger molecules, which bind to specific signaling proteins in the cell and influence their activity.

Many G Proteins Activate Membrane-bound Enzymes That Produce Small Messenger Molecules

When G proteins interact with ion channels they cause an immediate change in the state and behavior of the cell. The interaction of activated G proteins with enzymes, in contrast, has consequences that are less rapid and more complex, as they lead to the production of additional intracellular signaling molecules. The two most frequent target enzymes for G proteins are *adenylyl cyclase*, which produces a small molecule called *cyclic AMP*, and *phospholipase C*, which generates small molecules called *inositol trisphosphate* and *diacylglycerol*. Inositol trisphosphate, in turn, promotes the accumulation of cytosolic Ca^{2+}—yet another intracellular signaling molecule.

Adenylyl cyclase and phospholipase C are activated by different types of G proteins, allowing cells to couple the production of the small molecules to different extracellular signals. Although the coupling may be either stimulatory or inhibitory—as we saw in our discussion of the actions of cholera toxin and pertussis toxin—we concentrate here on G proteins that stimulate enzyme activity.

The small molecules generated by these enzymes are often called *second messengers*—the "first messengers" being the extracellular signals that activated the enzymes in the first place. Once activated, the enzymes generate large quantities of second messengers, which rapidly diffuse away from their source, thereby amplifying and spreading the intracellular signal (**Figure 16–18**).

Different second messenger molecules produce different responses. We first examine the consequences of an increase in the cytosolic concentration of cyclic AMP. This will take us along one of the main types of signaling pathways that lead from the activation of GPCRs. We then discuss the actions of three other second messenger molecules—inositol trisphosphate, diacylglycerol, and Ca^{2+}—which will lead us along a different signaling route.

The Cyclic AMP Signaling Pathway Can Activate Enzymes and Turn On Genes

Many extracellular signals acting via GPCRs affect the activity of the enzyme **adenylyl cyclase** and thus alter the intracellular concentration of the second messenger molecule **cyclic AMP**. Most commonly, the activated G protein α subunit switches on the adenylyl cyclase, causing a dramatic and sudden increase in the synthesis of cyclic AMP from ATP (which is always present in the cell). To help terminate the signal, a second enzyme, called *cyclic AMP phosphodiesterase*, rapidly converts cyclic AMP to ordinary AMP (**Figure 16–19**). One way that caffeine acts as a stimulant is by inhibiting this phosphodiesterase in the nervous system, blocking cyclic AMP degradation and thereby keeping the concentration of this second messenger high.

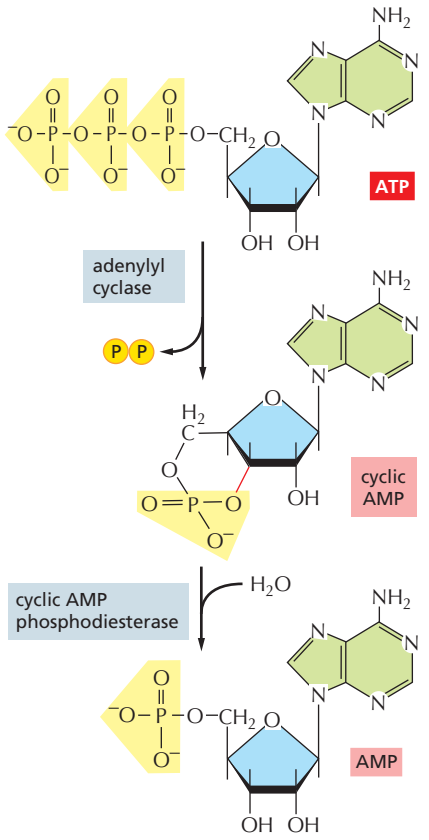


Figure 16–19 Cyclic AMP is synthesized by adenylyl cyclase and degraded by cyclic AMP phosphodiesterase. Cyclic AMP (abbreviated cAMP) is formed from ATP by a cyclization reaction that removes two phosphate groups from ATP and joins the "free" end of the remaining phosphate group to the sugar part of the AMP molecule (*red* bond). The degradation reaction breaks this new bond, forming AMP.

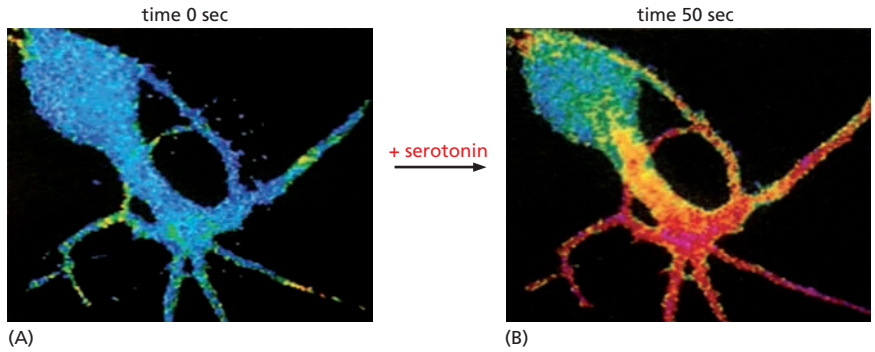


Figure 16–20 The concentration of cyclic AMP rises rapidly in response to an extracellular signal. A nerve cell in culture responds to the binding of the neurotransmitter serotonin to a GPCR by synthesizing cyclic AMP. The concentration of intracellular cyclic AMP was monitored by injecting into the cell a fluorescent protein whose fluorescence changes 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 concentration is about 5×10^{-8} M. (B) Fifty seconds after adding serotonin to the culture medium, the intracellular concentration of cyclic AMP has risen more than twentyfold (to $>10^{-6}$ M) in the parts of the cell where the serotonin receptors are concentrated. (From B.J. Bacskai et al. *Science* 260:222–226, 1993.)

Cyclic AMP phosphodiesterase is continuously active inside the cell. Because it eliminates cyclic AMP so quickly, the cytosolic concentration of this second messenger can change rapidly in response to extracellular signals, rising or falling tenfold in a matter of seconds (**Figure 16–20**). Cyclic AMP is water-soluble, so it can, in some cases, carry the signal throughout the cell, traveling from the site on the membrane where it is synthesized to interact with proteins located in the cytosol, in the nucleus, or on other organelles.

Cyclic AMP exerts most of its effects by activating the enzyme **cyclic-AMP-dependent protein kinase** (**PKA**). This enzyme is normally held inactive in a complex with a regulatory protein. The binding of cyclic AMP to the regulatory protein forces a conformational change that releases the inhibition and unleashes the active kinase. Activated PKA then catalyzes the phosphorylation of particular serines or threonines on specific intracellular proteins, thus altering the activity of these target proteins. In different cell types, different sets of proteins are available to be phosphorylated, which largely explains why the effects of cyclic AMP vary with the type of target cell.

Many kinds of cell responses are mediated by cyclic AMP; a few are listed in **Table 16–3**. As the table shows, different target cells respond very differently to extracellular signals that change intracellular cyclic AMP concentrations. When we are frightened or excited, for example, the adrenal gland releases the hormone *epinephrine* (also called *adrenaline*), which circulates in the bloodstream and binds to a class of GPCRs called adrenergic receptors (see Figure 16–14B), which are present on many types of cells. The consequences of epinephrine binding vary from one cell type to another, but all the cell responses help prepare the body for

TABLE 16–3 SOME CELL RESPONSES MEDIATED BY CYCLIC AMP

Extracellular Signal Molecule*	Target Tissue	Major Response
Epinephrine	heart	increase in heart rate and force of contraction
Epinephrine	skeletal muscle	glycogen breakdown
Epinephrine, glucagon	fat	fat breakdown
Adrenocorticotropic hormone (ACTH)	adrenal gland	cortisol secretion

*Although all of the signal molecules listed here are hormones, some responses to local mediators and to neurotransmitters are also mediated by cyclic AMP.

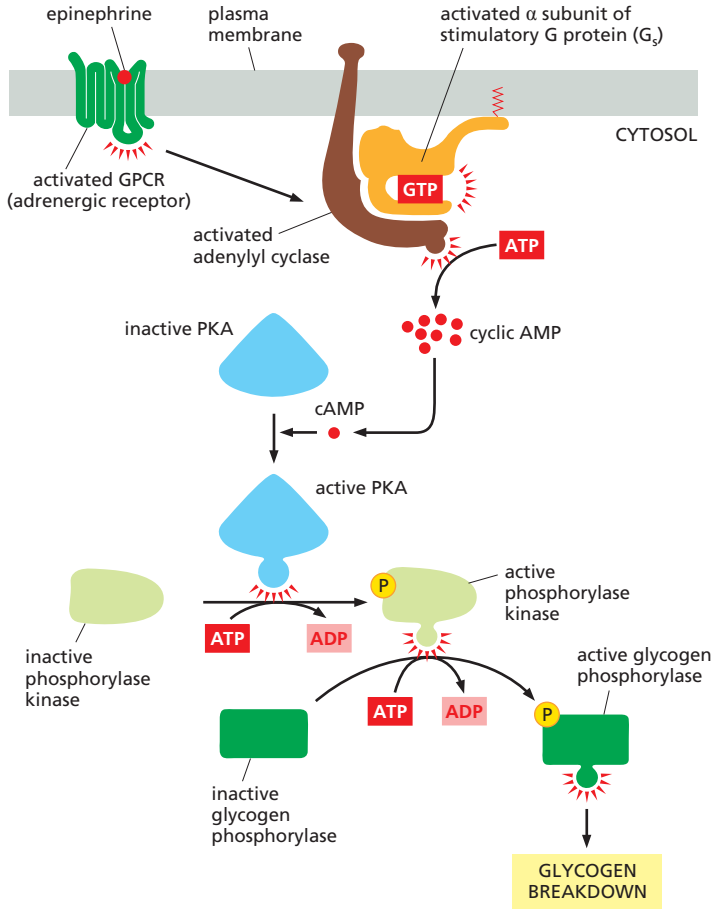


Figure 16–21 Epinephrine stimulates glycogen breakdown in skeletal muscle cells. The hormone activates a GPCR, which turns on a G protein (G_s) that activates adenylyl cyclase to boost the production of cyclic AMP. The increase in cyclic AMP activates PKA, which phosphorylates and activates an enzyme called phosphorylase kinase. This kinase activates glycogen phosphorylase, the enzyme that breaks down glycogen (see Figure 13–22). Because these reactions do not involve changes in gene transcription or new protein synthesis, they occur rapidly.

sudden action. In skeletal muscle, for instance, epinephrine increases intracellular cyclic AMP, causing the breakdown of glycogen—the polymerized storage form of glucose. It does so by activating PKA, which leads to both the activation of an enzyme that promotes glycogen breakdown (**Figure 16–21**) and the inhibition of an enzyme that drives glycogen synthesis. By stimulating glycogen breakdown and inhibiting its synthesis, the increase in cyclic AMP maximizes the amount of glucose available as fuel for anticipated muscular activity. Epinephrine also acts on fat cells, stimulating the breakdown of fat to fatty acids. These fatty acids can then be exported to fuel ATP production in other cells.

In some cases, the effects of increasing cyclic AMP are rapid; in skeletal muscle, for example, glycogen breakdown occurs within seconds of epinephrine binding to its receptor (see Figure 16–21). In other cases, cyclic AMP responses involve changes in gene expression that take minutes or hours to develop. In these slow responses, PKA typically phosphorylates transcription regulators, proteins that activate the transcription of selected genes (as discussed in Chapter 8). For example, an increase in cyclic AMP in certain neurons in the brain controls the production of proteins involved in some forms of learning. **Figure 16–22** illustrates a typical cyclic-AMP-mediated pathway from the plasma membrane to the nucleus.

We now turn to the other enzyme-mediated signaling pathway that leads from GPCRs—the pathway that begins with the activation of the membrane-bound enzyme *phospholipase C* and leads to an increase in the second messengers diacylglycerol, inositol trisphosphate, and Ca^{2+}.

QUESTION 16–4

Explain why cyclic AMP must be broken down rapidly in a cell to allow rapid signaling.

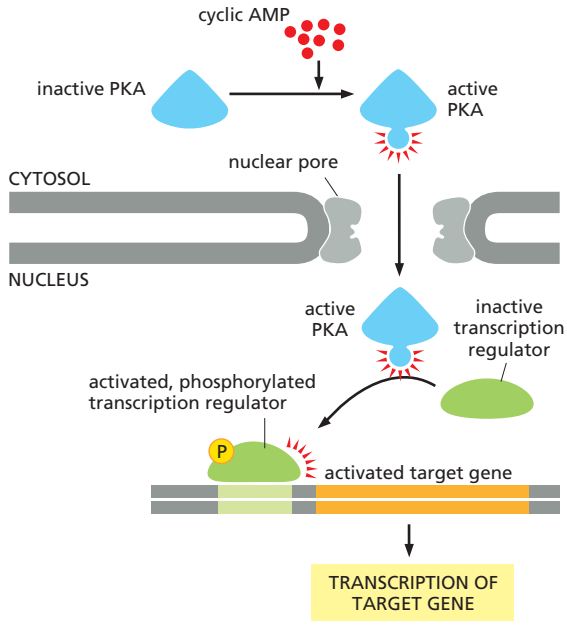


Figure 16–22 A rise in intracellular cyclic AMP can activate gene transcription. PKA, activated by a rise in intracellular cyclic AMP, can enter the nucleus and phosphorylate specific transcription regulators. Once phosphorylated, these proteins stimulate the transcription of a whole set of target genes (Movie 16.3). This type of signaling pathway controls many processes in cells, ranging from hormone synthesis in endocrine cells to the production of proteins involved in long-term memory in the brain. Activated PKA can also phosphorylate and thereby regulate other proteins and enzymes in the cytosol, as shown in Figure 16–21.

The Inositol Phospholipid Pathway Triggers a Rise in Intracellular Ca^{2+}

Some GPCRs exert their effects through a G protein called G_q, which activates the membrane-bound enzyme **phospholipase C** instead of adenylyl cyclase. Examples of signal molecules that act through phospholipase C are given in Table 16–4.

Once activated, phospholipase C propagates the signal by cleaving a lipid molecule that is a component of the plasma membrane. The molecule is an **inositol phospholipid** (a phospholipid with the sugar inositol attached to its head) that is present in small quantities in the cytosolic leaflet of the membrane lipid bilayer (see Figure 11–19). Because of the involvement of this phospholipid, the signaling pathway that begins with the activation of phospholipase C is often referred to as the *inositol phospholipid pathway*. It operates in almost all eukaryotic cells and regulates a large number of different effector proteins.

The cleavage of a membrane inositol phospholipid by phospholipase C generates two second messenger molecules: **inositol 1,4,5-trisphosphate** (**IP_3**) and **diacylglycerol** (**DAG**). Both molecules play a crucial part in relaying the signal (Figure 16–23).

IP_3 is a water-soluble sugar phosphate that is released into the cytosol; there it binds to and opens Ca^{2+} channels that are embedded in the endoplasmic reticulum (ER) membrane. Ca^{2+} stored inside the ER rushes out

TABLE 16–4 SOME CELL RESPONSES MEDIATED BY PHOSPHOLIPASE C ACTIVATION

Signal Molecule	Target Tissue	Major Response
Vasopressin (a peptide hormone)	liver	glycogen breakdown
Acetylcholine	pancreas	secretion of amylase (a digestive enzyme)
Acetylcholine	skeletal muscle	contraction
Thrombin (a proteolytic enzyme)	blood platelets	aggregation

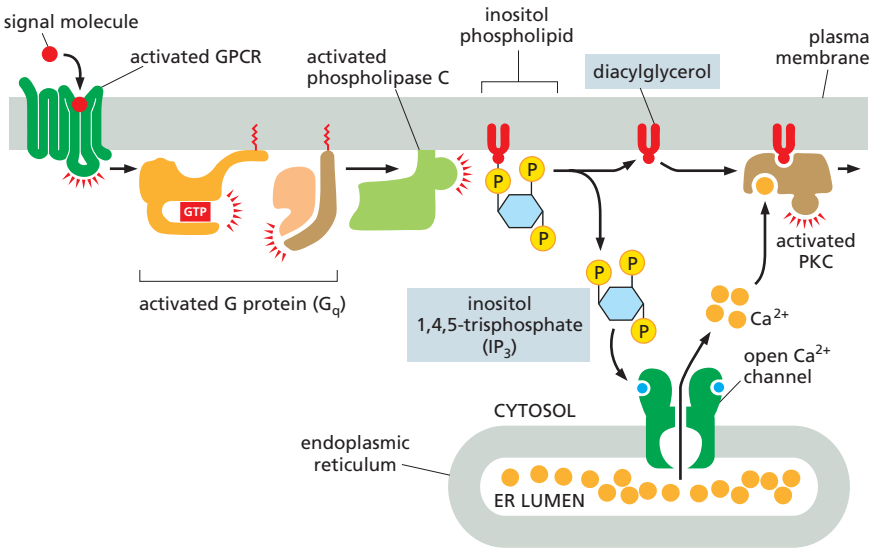


Figure 16–23 Phospholipase C activates two signaling pathways. Two messenger molecules are produced when a membrane inositol phospholipid is hydrolyzed by activated phospholipase C. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and triggers the release of Ca^{2+} from the ER by binding to and opening special Ca^{2+} channels in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to rush out of the ER and into the cytosol. Diacylglycerol remains in the plasma membrane and, together with Ca^{2+}, helps activate the enzyme protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane (Movie 16.4). PKC then phosphorylates its own set of intracellular proteins, further propagating the signal. At the start of the pathway, both the α subunit and the βγ complex of the G protein G_q are involved in activating phospholipase C.

into the cytosol through these open channels, causing a sharp rise in the cytosolic concentration of free Ca^{2+}, which is normally kept very low. This Ca^{2+} in turn signals to other proteins, as we discuss shortly.

Diacylglycerol is a lipid that remains embedded in the plasma membrane after it is produced by phospholipase C; there, it helps recruit and activate a protein kinase, which translocates from the cytosol to the plasma membrane. This enzyme is called **protein kinase C** (**PKC**) because it also needs to bind Ca^{2+} to become active (see Figure 16–23). Once activated, PKC phosphorylates a set of intracellular proteins that varies depending on the cell type.

A Ca^{2+} Signal Triggers Many Biological Processes

Ca^{2+} has such an important and widespread role as an intracellular messenger that we will digress to consider its functions more generally. A surge in the cytosolic concentration of free Ca^{2+} is triggered by many kinds of cell stimuli, not only those that act through GPCRs. When a sperm fertilizes an egg cell, for example, Ca^{2+} channels open, and the resulting rise in cytosolic Ca^{2+} triggers the egg to start development (**Figure 16–24**); for muscle cells, a signal from a nerve triggers a rise in cytosolic Ca^{2+} that initiates muscle contraction (discussed in Chapter 17; see Figure 17–45); and in many secretory cells, including nerve cells, Ca^{2+} triggers secretion (discussed in Chapter 12; see Figure 12–40). Ca^{2+} stimulates all these responses by binding to and influencing the activity of various Ca^{2+}-responsive proteins.

The concentration of free Ca^{2+} in the cytosol of an unstimulated cell is extremely low (10^{-7} M) compared with its concentration in the extracellular fluid (about 10^{-3} M) and in the ER. These differences are maintained

QUESTION 16–5

Why do you suppose cells have evolved intracellular Ca^{2+} stores for signaling even though there is abundant extracellular Ca^{2+}?

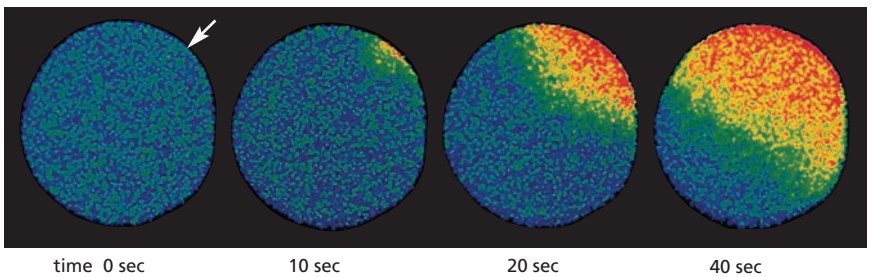


Figure 16–24 Fertilization of an egg by a sperm triggers an increase in cytosolic Ca^{2+} in the egg. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. When a sperm enters the egg, a wave of cytosolic Ca^{2+} (*red*)—released from the ER—sweeps across the egg from the site of sperm entry (*arrow*). This Ca^{2+} wave provokes a change in the egg surface, preventing entry of other sperm, and it also initiates embryonic development. To catch this Ca^{2+} wave, go to Movie 16.5. (Adapted from S. Stricker, *Dev. Bio.* 166:34–58, 1994.)

by membrane-embedded Ca^{2+} pumps that actively remove Ca^{2+} from the cytosol, sending it either into the ER or across the plasma membrane and out of the cell. As a result, a steep electrochemical gradient of Ca^{2+} exists across both the ER membrane and the plasma membrane (discussed in Chapter 12). When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes down its electrochemical gradient into the cytosol, where it triggers changes in Ca^{2+}-responsive proteins. The same Ca^{2+} pumps that normally operate to keep cytosolic Ca^{2+} concentrations low also help to terminate the Ca^{2+} signal.

The effects of Ca^{2+} in the cytosol are largely indirect, in that they are mediated through the interaction of Ca^{2+} with various kinds of Ca^{2+}-responsive proteins. The most widespread and common of these is **calmodulin**, which is present in the cytosol of all eukaryotic cells that have been examined, including those of plants, fungi, and protozoa. When Ca^{2+} binds to calmodulin, the protein undergoes a conformational change that enables it to interact with a wide range of target proteins in the cell, altering their activities (Figure 16–25). One particularly important class of targets for calmodulin is the **Ca^{2+}/calmodulin-dependent protein kinases** (**CaM-kinases**). When these kinases are activated by binding to calmodulin complexed with Ca^{2+}, they influence other processes in the cell by phosphorylating selected proteins. In the mammalian brain, for example, a neuron-specific CaM-kinase is abundant at synapses, where it is thought to play an important part in some forms of learning and memory. This CaM-kinase is activated by the pulses of Ca^{2+} signals that occur during neural activity, and mutant mice that lack the kinase show a marked inability to remember where things are.

A GPCR Signaling Pathway Generates a Dissolved Gas That Carries a Signal to Adjacent Cells

Second messengers like cyclic AMP and calcium are hydrophilic molecules that generally act within the cell where they are produced. But some molecules produced in response to GPCR activation are small enough or hydrophobic enough to pass across the membrane and carry a signal directly to nearby cells. An important example is the gas **nitric oxide** (**NO**), which acts as a signaling molecule in many tissues. NO diffuses readily from its site of synthesis and slips into neighboring cells. The distance the gas diffuses is limited by its reaction with oxygen and water in the extracellular environment, which converts NO into nitrates and nitrites within seconds.

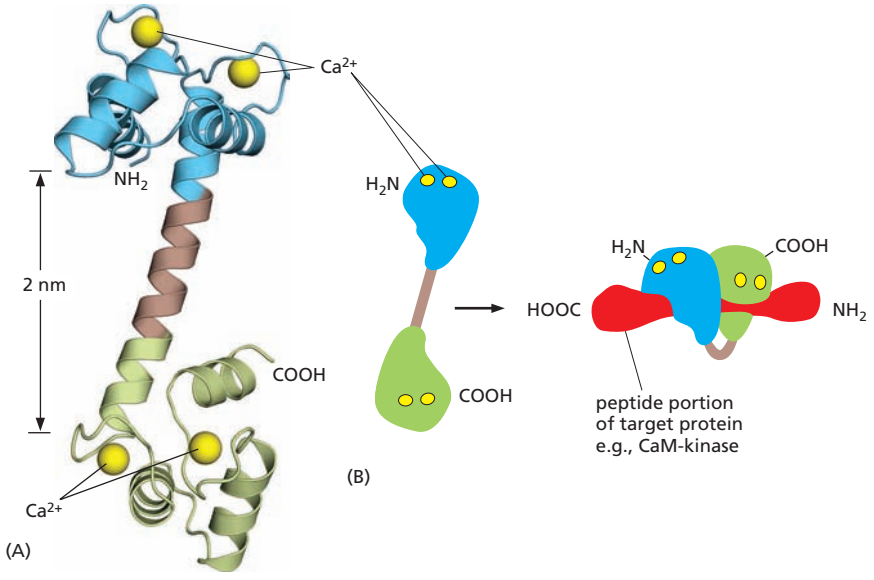


Figure 16–25 Calcium binding changes the shape of the calmodulin protein. (A) Calmodulin has a dumbbell shape, with two globular ends connected by a long α helix. Each of the globular ends has two Ca^{2+}-binding sites. (B) Simplified representation of the structure, showing the conformational changes that occur when Ca^{2+}-bound calmodulin interacts with an isolated segment of a target protein (*red*). In this conformation, the α helix jackknifes to surround the target (Movie 16.6). (B, adapted from W.E. Meador, A.R. Means, and F.A. Quiocho, *Science* 257:1251–1255, 1992, and M. Ikura et al., *Science* 256:632–638, 1992.)

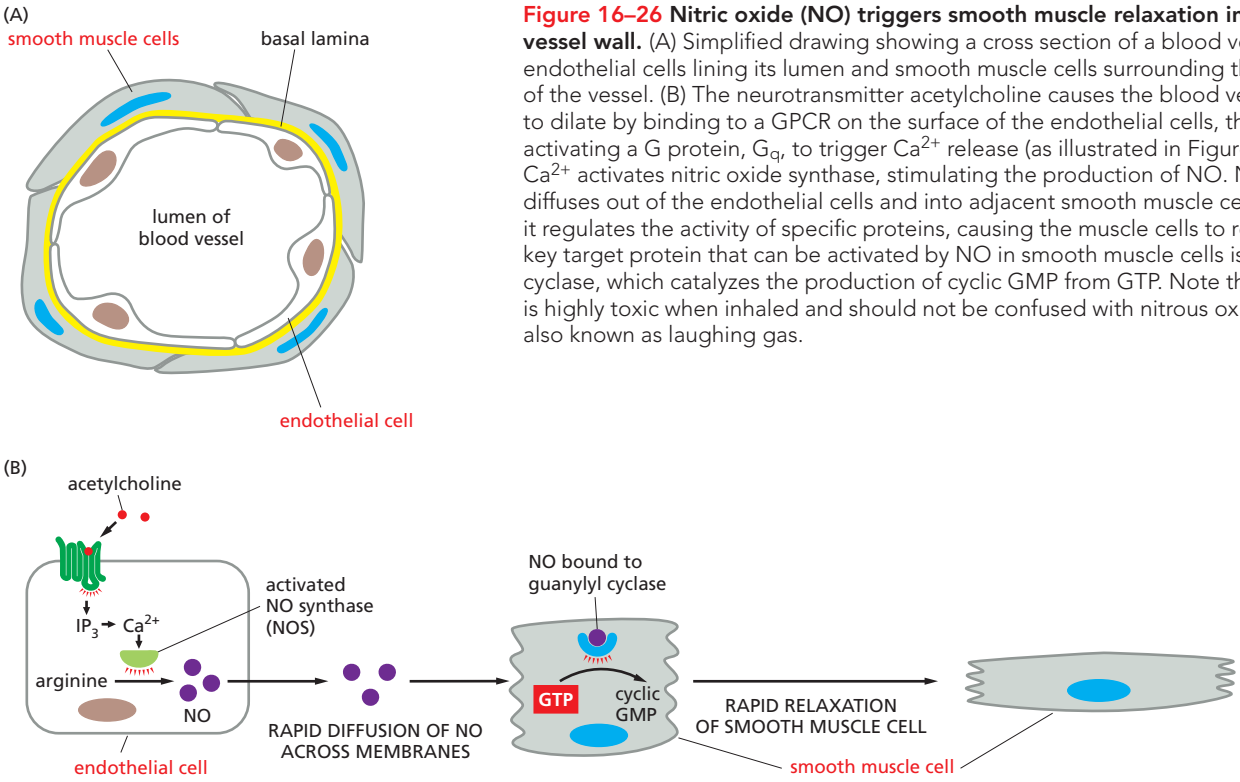


Figure 16–26 Nitric oxide (NO) triggers smooth muscle relaxation in a blood-vessel wall. (A) Simplified drawing showing a cross section of a blood vessel with endothelial cells lining its lumen and smooth muscle cells surrounding the outside of the vessel. (B) The neurotransmitter acetylcholine causes the blood vessel to dilate by binding to a GPCR on the surface of the endothelial cells, thereby activating a G protein, G_q, to trigger Ca^{2+} release (as illustrated in Figure 16–23). Ca^{2+} activates nitric oxide synthase, stimulating the production of NO. NO then diffuses out of the endothelial cells and into adjacent smooth muscle cells, where it regulates the activity of specific proteins, causing the muscle cells to relax. One key target protein that can be activated by NO in smooth muscle cells is guanylyl cyclase, which catalyzes the production of cyclic GMP from GTP. Note that NO gas is highly toxic when inhaled and should not be confused with nitrous oxide (N_2O), also known as laughing gas.

Endothelial cells—the flattened cells that line every blood vessel—release NO in response to acetylcholine secreted by nearby nerve endings. Acetylcholine binds to a GPCR on the endothelial cell surface, resulting in activation of G_q and the release of Ca^{2+} inside the cell (see Figure 16–23). Ca^{2+} then stimulates nitric oxide synthase, which produces NO from the amino acid arginine. This NO diffuses into smooth muscle cells in the adjacent vessel wall, causing the cells to relax; this relaxation allows the vessel to dilate, so that blood flows through it more freely (**Figure 16–26**). The effect of NO on blood vessels accounts for the action of nitroglycerin, which has been used for almost 100 years to treat patients with angina—pain caused by inadequate blood flow to the heart muscle. In the body, nitroglycerin is converted to NO, which rapidly relaxes blood vessels, thereby reducing the workload on the heart and decreasing the muscle's need for oxygen-rich blood. Many nerve cells also use NO to signal neighboring cells: NO released by nerve terminals in the penis, for instance, acts as a local mediator to trigger the blood-vessel dilation responsible for penile erection.

Inside many target cells, NO binds to and activates the enzyme *guanylyl cyclase*, stimulating the formation of *cyclic GMP* from the nucleotide GTP (see Figure 16–26B). Cyclic GMP, a second messenger similar in structure to cyclic AMP, is a key link in the NO signaling chain. The drug Viagra enhances penile erection by blocking the enzyme that degrades cyclic GMP, prolonging the NO signal.

GPCR-Triggered Intracellular Signaling Cascades Can Achieve Astonishing Speed, Sensitivity, and Adaptability

The steps in the *signaling cascades* associated with GPCRs take a long time to describe, but they often take only seconds to execute. Consider how quickly a thrill can make your heart race (when epinephrine stimulates

the GPCRs in your cardiac pacemaker cells), or how fast the smell of food can make your mouth water (through the GPCRs for odors in your nose and the GPCRs for acetylcholine in salivary cells, which stimulate secretion). Among the fastest of all responses mediated by a GPCR, however, is the response of the eye to light: it takes only 20 msec for the most quickly responding photoreceptor cells of the retina (the cone photoreceptors, which are responsible for color vision in bright light) to produce their electrical response to a sudden flash of light.

This exceptional speed is achieved in spite of the necessity to relay the signal over the multiple steps of an intracellular signaling cascade. But photoreceptors also provide a beautiful illustration of the advantages of intracellular signaling cascades: in particular, such cascades allow spectacular amplification of the incoming signal and also allow cells to adapt so as to be able to detect signals of widely varying intensity. The quantitative details have been most thoroughly analyzed for the rod photoreceptor cells in the eye, which are responsible for noncolor vision in dim light (**Figure 16–27**). In this photoreceptor cell, light is sensed by rhodopsin, a G-protein-coupled light receptor. Rhodopsin, when stimulated by light, activates a G protein called transducin. The activated α subunit of transducin then activates an intracellular signaling cascade that causes cation channels to close in the plasma membrane of the photoreceptor cell. This produces a change in the voltage across the cell membrane, which alters neurotransmitter release and ultimately leads to a nerve impulse being sent to the brain.

The signal is repeatedly amplified as it is relayed along this intracellular signaling pathway (**Figure 16–28**). When lighting conditions are dim, as on a moonless night, the amplification is enormous: as few as a dozen photons absorbed across the entire retina will cause a perceptible signal to be delivered to the brain. In bright sunlight, when photons flood through each photoreceptor cell at a rate of billions per second, the signaling cascade undergoes a form of *adaptation*, stepping down the amplification more than 10,000-fold, so that the photoreceptor cells are not overwhelmed and can still register increases and decreases in the strong light. The adaptation depends on negative feedback: an intense response in the photoreceptor cell decreases the cytosolic Ca^{2+} concentration, inhibiting the enzymes responsible for signal amplification.

Adaptation frequently occurs in intracellular signaling pathways that respond to extracellular signal molecules, allowing cells to respond to fluctuations in the concentration of such molecules regardless of whether they are present in small or large amounts. By taking advantage of positive and negative feedback mechanisms (see Figure 16–10), adaptation thus allows a cell to respond equally well to the signaling equivalents of shouts and whispers.

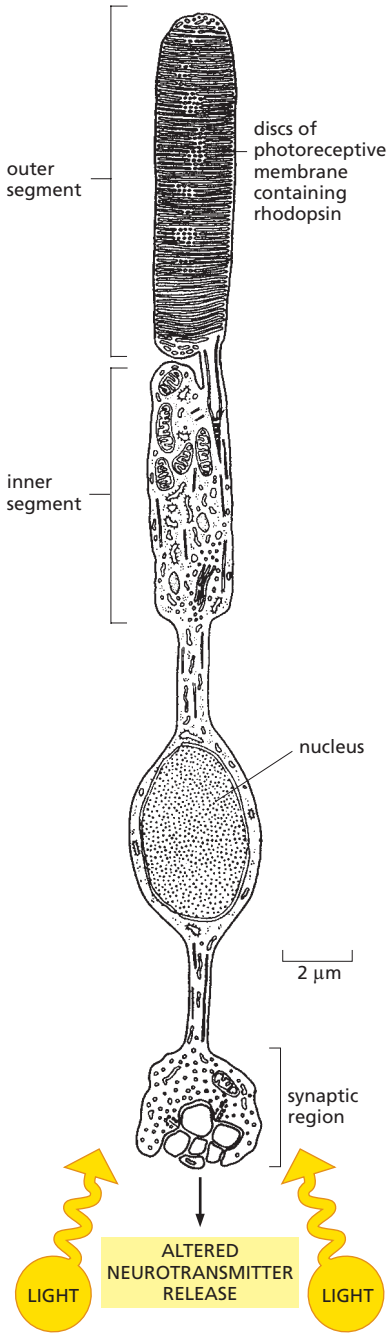


Figure 16–27 A rod photoreceptor cell from the retina is exquisitely sensitive to light. The light-absorbing rhodopsin proteins are embedded in many pancake-shaped vesicles (discs) of membrane inside the outer segment of the photoreceptor cell. When the rod cell is stimulated by light, a signal is relayed from the rhodopsin molecules in the discs, through the cytosol, to ion channels that allow positive ions to flow through the plasma membrane of the outer segment. These cation channels close in response to the cytosolic signal, producing a change in the membrane potential of the rod cell. By mechanisms similar to those that control neurotransmitter release in ordinary nerve cells, the change in membrane potential alters the rate of neurotransmitter release from the synaptic region of the cell. Released neurotransmitters then act on retinal nerve cells that pass the signal on to the brain. (From T.L. Lentz, *Cell Fine Structure*. Philadelphia: Saunders, 1971. With permission from Elsevier.)

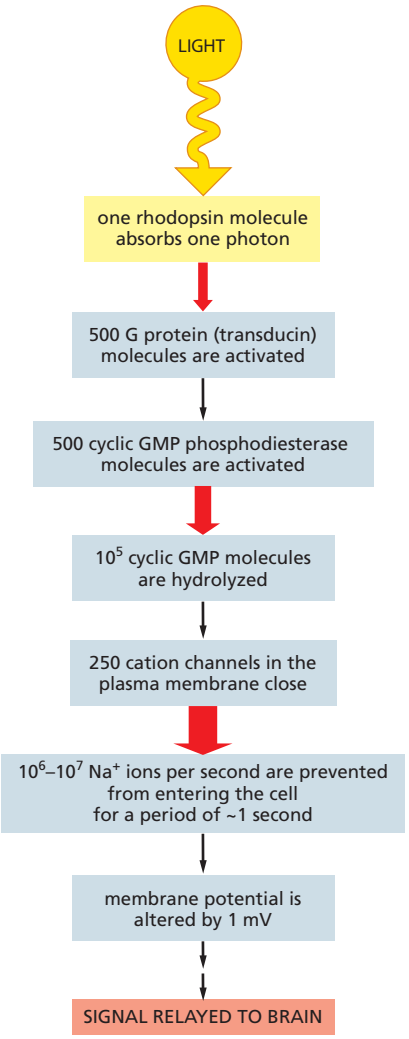


Figure 16–28 The light-induced signaling cascade in rod photoreceptor cells greatly amplifies the light signal. When rod photoreceptors are adapted for dim light, the signal amplification is enormous. The intracellular signaling pathway from the G protein transducin uses components that differ from the ones in previous figures. The cascade functions as follows. In the absence of a light signal, the second messenger molecule cyclic GMP is continuously produced by guanylyl cyclase in the cytosol of the photoreceptor cell. The cyclic GMP then binds to cation channels in the photoreceptor cell plasma membrane, keeping them open. Activation of rhodopsin by light results in the activation of transducin α subunits. These turn on an enzyme called cyclic GMP phosphodiesterase, which breaks down cyclic GMP to GMP (much as cyclic AMP phosphodiesterase breaks down cyclic AMP; see Figure 16–19). The sharp fall in the cytosolic concentration of cyclic GMP reduces the amount of cyclic GMP bound to the cation channels, which therefore close. Closing these channels decreases the influx of Na^+, thereby altering the voltage gradient (membrane potential) across the plasma membrane and, ultimately, the rate of neurotransmitter release, as described in Chapter 12. The *red* arrows indicate the steps at which amplification occurs, with the thickness of the arrow roughly indicating the magnitude of the amplification.

Taste and smell also depend on GPCRs. It seems likely that this mechanism of signal reception, invented early in evolution, has its origins in the basic and universal need of cells to sense and respond to their environment. Of course, GPCRs are not the only receptors that activate intracellular signaling cascades. We now turn to another major class of cell-surface receptors—enzyme-coupled receptors—which play a key part in controlling cell numbers, cell differentiation, and cell movement in multicellular animals, especially during development.

ENZYME-COUPLED RECEPTORS

Like GPCRs, **enzyme-coupled receptors** are transmembrane proteins that display their ligand-binding domains on the outer surface of the plasma membrane (see Figure 16–13C). Instead of associating with a G protein, however, the cytoplasmic domain of the receptor either acts as an enzyme itself or forms a complex with another protein that acts as an enzyme. Enzyme-coupled receptors were discovered through their role in responses to extracellular signal proteins that regulate the growth, proliferation, differentiation, and survival of cells in animal tissues (see Table 16–1, p. 536, for examples). Most of these signal proteins function as local mediators and can act at very low concentrations (about 10^{-9} to 10^{-11} M). Responses to them are typically slow (on the order of hours), and their effects may require many intracellular transduction steps that usually lead to a change in gene expression.

Enzyme-coupled receptors, however, can also mediate direct, rapid reconfigurations of the cytoskeleton, changing the cell's shape and the way that it moves. The extracellular signals that induce such changes are often not diffusible signal proteins, but proteins attached to the surfaces over which a cell is crawling.

The largest class of enzyme-coupled receptors consists of receptors with a cytoplasmic domain that functions as a tyrosine kinase, which phosphorylates particular tyrosines on specific intracellular signaling proteins. These receptors, called **receptor tyrosine kinases** (**RTKs**), will be the main focus of this section.

We begin with a discussion of how RTKs are activated in response to extracellular signals. We then consider how activated RTKs transmit the signal along two major intracellular signaling pathways that terminate at various effector proteins in the target cell. Finally, we describe how some

QUESTION 16–6

One important feature of any intracellular signaling pathway is its ability to be turned off. Consider the pathway shown in Figure 16–28. Where would off switches be required? Which ones do you suppose would be the most important?

enzyme-coupled receptors bypass such intracellular signaling cascades and use a more direct mechanism to regulate gene transcription.

Abnormal cell growth, proliferation, differentiation, survival, and migration are fundamental features of a cancer cell, and abnormalities in signaling via RTKs and other enzyme-coupled receptors have a major role in the development of most cancers.

Activated RTKs Recruit a Complex of Intracellular Signaling Proteins

To do its job as a signal transducer, an enzyme-coupled receptor has to switch on the enzyme activity of its intracellular domain (or of an associated enzyme) when an external signal molecule binds to its extracellular domain. Unlike GPCRs, enzyme-coupled receptor proteins usually have only one transmembrane segment, which spans the lipid bilayer as a single α helix. Because a single α helix is poorly suited to transmit a conformational change across the bilayer, enzyme-coupled receptors have a different strategy for transducing the extracellular signal. In many cases, the binding of an extracellular signal molecule causes two receptor molecules to come together in the plasma membrane, forming a dimer. This pairing brings the two intracellular tails of the receptors together and activates their kinase domains, such that each receptor tail phosphorylates the other. In the case of RTKs, the phosphorylations occur on specific tyrosines.

This tyrosine phosphorylation then triggers the assembly of a transient but elaborate intracellular signaling complex on the cytosolic tails of the receptors. The newly phosphorylated tyrosines serve as docking sites for a whole zoo of intracellular signaling proteins—perhaps as many as 10 or 20 different molecules (**Figure 16–29**). Some of these proteins become phosphorylated and activated on binding to the receptors, and they then propagate the signal; others function solely as scaffolds, which couple the receptors to other signaling proteins, thereby helping to build the active signaling complex (see Figure 16–9). All of these docked intracellular signaling proteins possess a specialized *interaction domain*, which recognizes specific phosphorylated tyrosines on the receptor tails. Other

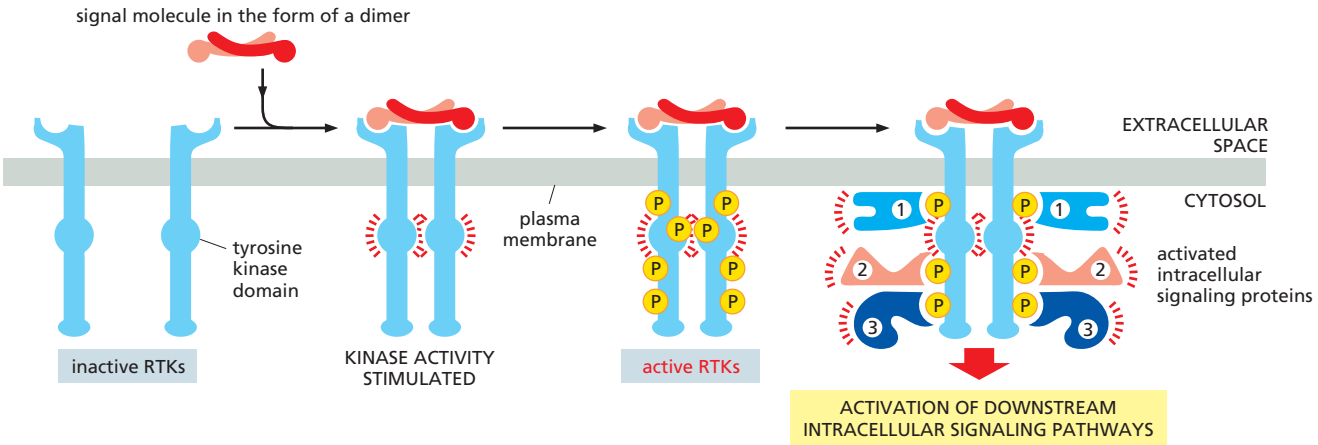


Figure 16–29 Activation of an RTK stimulates the assembly of an intracellular signaling complex. Typically, the binding of a signal molecule to the extracellular domain of an RTK causes two receptor molecules to associate into a dimer. The signal molecule shown here is itself a dimer and thus can physically cross-link two receptor molecules; other signal molecules induce a conformational change in the RTKs, causing the receptors to dimerize (not shown). In either case, dimer formation brings the kinase domain of each cytosolic receptor tail into contact with the other; this activates the kinases to phosphorylate the adjacent tail on several tyrosines. Each phosphorylated tyrosine serves as a specific docking site for a different intracellular signaling protein, which then helps relay the signal to the cell's interior; these proteins contain a specialized interaction domain—in this case, a module called an SH2 domain—that recognizes and binds to specific phosphorylated tyrosines on the cytosolic tail of an activated RTK or on another intracellular signaling protein.

interaction domains allow intracellular signaling proteins to recognize phosphorylated lipids that are produced on the cytosolic side of the plasma membrane in response to certain signals, as we discuss later.

As long as they remain together, the signaling protein complexes assembled on the cytosolic tails of the RTKs can transmit a signal along several routes simultaneously to many destinations in the cell, thus activating and coordinating the numerous biochemical changes that are required to trigger a complex response such as cell proliferation or differentiation. To help terminate the response, the tyrosine phosphorylations are reversed by *tyrosine phosphatases*, which remove the phosphates that were added to the tyrosines of both the RTKs and other intracellular signaling proteins in response to the extracellular signal. In some cases, activated RTKs (as well as some GPCRs) are inactivated in a more brutal way: they are dragged into the interior of the cell by endocytosis and then destroyed by digestion in lysosomes (as discussed in Chapter 15).

Different RTKs recruit different collections of intracellular signaling proteins, producing different effects; however, certain components are used by most RTKs. These include, for example, a phospholipase C that functions in the same way as the phospholipase C activated by GPCRs to trigger the inositol phospholipid signaling pathway discussed earlier (see Figure 16–23). Another intracellular signaling protein that is activated by almost all RTKs is a small GTP-binding protein called Ras, as we discuss next.

Most RTKs Activate the Monomeric GTPase Ras

As we have seen, activated RTKs recruit and activate many kinds of intracellular signaling proteins, leading to the formation of large signaling complexes on the cytosolic tail of the RTK. One of the key members of these signaling complexes is **Ras**—a small GTP-binding protein that is bound by a lipid tail to the cytosolic face of the plasma membrane. Virtually all RTKs activate Ras, including platelet-derived growth factor (PDGF) receptors, which mediate cell proliferation in wound healing, and nerve growth factor (NGF) receptors, which play an important part in the development of certain vertebrate neurons.

The Ras protein is a member of a large family of small GTP-binding proteins, often called **monomeric GTPases** to distinguish them from the trimeric G proteins that we encountered earlier. Ras resembles the α subunit of a G protein and functions as a molecular switch in much the same way. It cycles between two distinct conformational states—active when GTP is bound and inactive when GDP is bound. Interaction with an activating protein called Ras-GEF encourages Ras to exchange its GDP for GTP, thus switching Ras to its activated state (**Figure 16–30**); after a delay, Ras is switched off by a GAP called Ras-GAP (see Figure 16–12), which promotes the hydrolysis of its bound GTP to GDP (**Movie 16.7**).

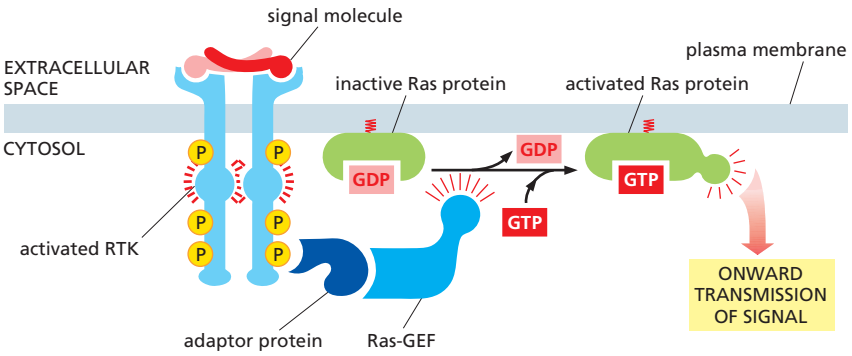


Figure 16–30 RTKs activate Ras. An adaptor protein docks on a particular phosphotyrosine on the activated receptor (the other signaling proteins that would be bound to the receptor, as shown in Figure 16–29, have been omitted for simplicity). The adaptor recruits a Ras guanine nucleotide exchange factor (Ras-GEF) that stimulates Ras to exchange its bound GDP for GTP. The activated Ras protein can now stimulate several downstream signaling pathways, one of which is shown in Figure 16–31. Note that the Ras protein contains a covalently attached lipid group (*red*) that helps anchor the protein to the inside of the plasma membrane.

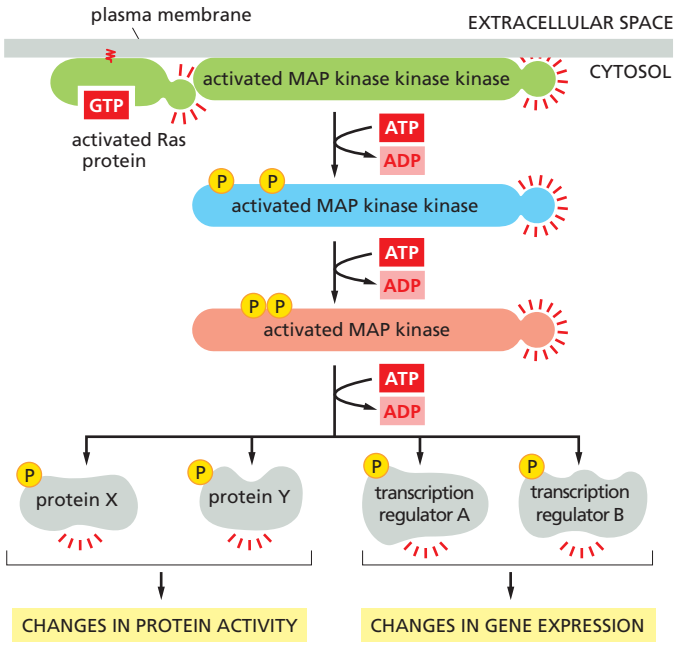


Figure 16–31 Ras activates a MAP-kinase signaling module. The Ras protein, activated by the process shown in Figure 16–30, activates a three-kinase signaling module, which relays the signal onward. The final kinase in the module, MAP kinase, phosphorylates various downstream signaling or effector proteins.

In its active state, Ras initiates a phosphorylation cascade in which a series of serine/threonine kinases phosphorylate and activate one another in sequence, like an intracellular game of dominoes. This relay system, which carries the signal from the plasma membrane to the nucleus, includes a three-kinase module called the **MAP-kinase signaling module**, in honor of the final enzyme in the chain, the mitogen-activated protein kinase, or **MAP kinase**. (As we discuss in Chapter 18, *mitogens* are extracellular signal molecules that stimulate cell proliferation.) In this pathway, outlined in **Figure 16–31**, MAP kinase is phosphorylated and activated by an enzyme called, logically enough, MAP kinase kinase. This protein is itself switched on by a MAP kinase kinase kinase (which is activated by Ras). At the end of the MAP-kinase cascade, MAP kinase phosphorylates various effector proteins, including certain transcription regulators, altering their ability to control gene transcription. The resulting change in the pattern of gene expression may stimulate cell proliferation, promote cell survival, or induce cell differentiation: the precise outcome will depend on which other genes are active in the cell and what other signals the cell receives. How biologists unravel such complex signaling pathways is discussed in **How We Know**, pp. 563–564.

Before Ras was discovered in normal cells, a mutant form of the protein was found in human cancer cells. The mutation inactivates the GTPase activity of Ras, so that the protein cannot shut itself off, promoting uncontrolled cell proliferation and the development of cancer. About 30% of human cancers contain such activating mutations in a *Ras* gene; of the cancers that do not, many have mutations in genes that encode proteins that function in the same signaling pathway as Ras. Many of the genes that encode normal intracellular signaling proteins were initially identified in the hunt for cancer-promoting *oncogenes* (discussed in Chapter 20).

RTKs Activate PI 3-Kinase to Produce Lipid Docking Sites in the Plasma Membrane

Many of the extracellular signal proteins that stimulate animal cells to survive and grow, including signal proteins belonging to the insulin-like

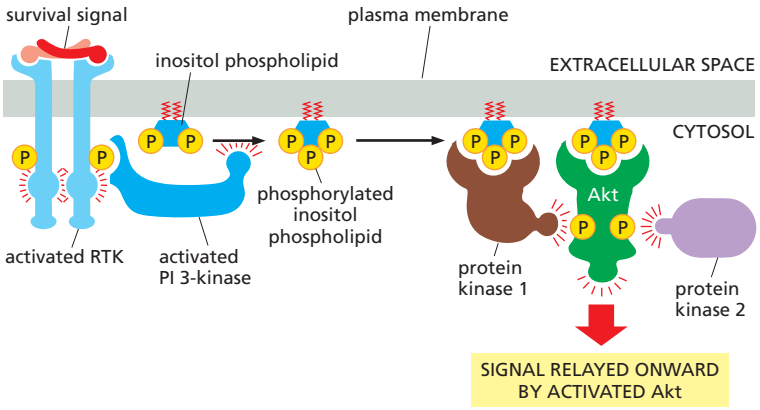


Figure 16–32 Some RTKs activate the PI-3-kinase–Akt signaling pathway. An extracellular survival signal, such as IGF, activates an RTK, which recruits and activates PI 3-kinase. PI 3-kinase then phosphorylates an inositol phospholipid that is embedded in the cytosolic side of the plasma membrane. The resulting phosphorylated inositol phospholipid attracts intracellular signaling proteins that have a special domain that recognizes it. One of these signaling proteins, Akt, is a protein kinase that is activated at the membrane by phosphorylation mediated by two other protein kinases (here called protein kinases 1 and 2); protein kinase 1 is also recruited by the phosphorylated lipid docking sites. Once activated, Akt is released from the plasma membrane and phosphorylates various downstream proteins on specific serines and threonines (not shown).

growth factor (IGF) family, act through RTKs. One crucially important signaling pathway that these RTKs activate to promote cell growth and survival involves the enzyme **phosphoinositide 3-kinase** (**PI 3-kinase**), which phosphorylates inositol phospholipids in the plasma membrane. These phosphorylated lipids serve as docking sites for specific intracellular signaling proteins, which relocate from the cytosol to the plasma membrane, where they can activate one another. One of the most important of these relocated signaling proteins is the serine/threonine kinase *Akt* (**Figure 16–32**).

Akt, also called protein kinase B (PKB), promotes the growth and survival of many cell types, often by inactivating the signaling proteins it phosphorylates. For example, Akt phosphorylates and inactivates a cytosolic protein called Bad. In its active state, Bad encourages the cell to kill itself by indirectly activating a cell-suicide program called apoptosis (discussed in Chapter 18). Phosphorylation by Akt thus promotes cell survival by inactivating a protein that otherwise promotes cell death (**Figure 16–33**).

In addition to promoting cell survival, the *PI-3-kinase–Akt signaling pathway* stimulates cells to grow in size. It does so by indirectly activating

QUESTION 16–7

Would you expect to activate RTKs by exposing the exterior of cells to antibodies that bind to the respective proteins? Would your answer be different for GPCRs? (Hint: review Panel 4–2, on pp. 140–141, regarding the properties of antibody molecules.)

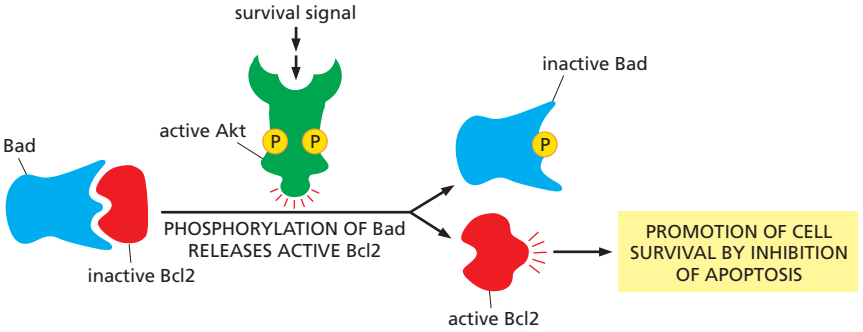


Figure 16–33 Activated Akt promotes cell survival. One way it does so is by phosphorylating and inactivating a protein called Bad. In its unphosphorylated state, Bad promotes apoptosis (a form of cell death) by binding to and inhibiting a protein, called Bcl2, which otherwise suppresses apoptosis. When Bad is phosphorylated by Akt, Bad releases Bcl2, which now blocks apoptosis, thereby promoting cell survival.

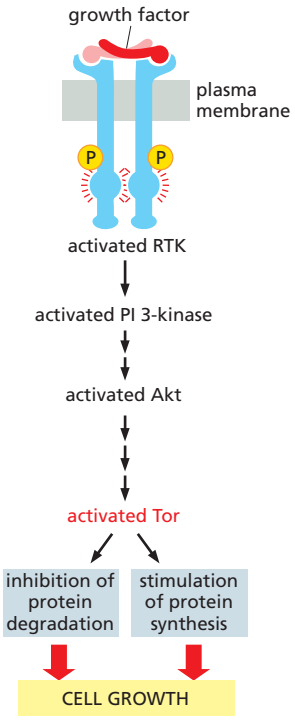


Figure 16–34 Akt stimulates cells to grow in size by activating the serine/threonine kinase Tor. The binding of a growth factor to an RTK activates the PI-3-kinase–Akt signaling pathway (as shown in Figure 16–32). Akt then indirectly activates Tor by phosphorylating and inhibiting a protein that helps to keep Tor shut down (not shown). Tor stimulates protein synthesis and inhibits protein degradation by phosphorylating key proteins in these processes (not shown). The anticancer drug rapamycin slows cell growth by inhibiting Tor. In fact, the Tor protein derives its name from the fact that it is a target of rapamycin.

a large serine/threonine kinase called *Tor*. Tor stimulates cells to grow both by enhancing protein synthesis and by inhibiting protein degradation (**Figure 16–34**). The anticancer drug rapamycin works by inactivating Tor, indicating the importance of this signaling pathway in regulating cell growth and survival—and the consequences of its disregulation in cancer.

The main intracellular signaling cascades activated by GPCRs and RTKs are summarized in **Figure 16–35**. As dauntingly complex as such pathways may seem, the complexity of cell signaling is actually much greater still. First, we have not discussed all of the intracellular signaling pathways that operate in cells. Second, although we depict these signaling pathways as being relatively linear and self-contained, they do not operate entirely independently. We will return to this concept of signal integration at the chapter's conclusion. But first, we take a brief detour to introduce a few important types of signaling systems that we have thus far overlooked.

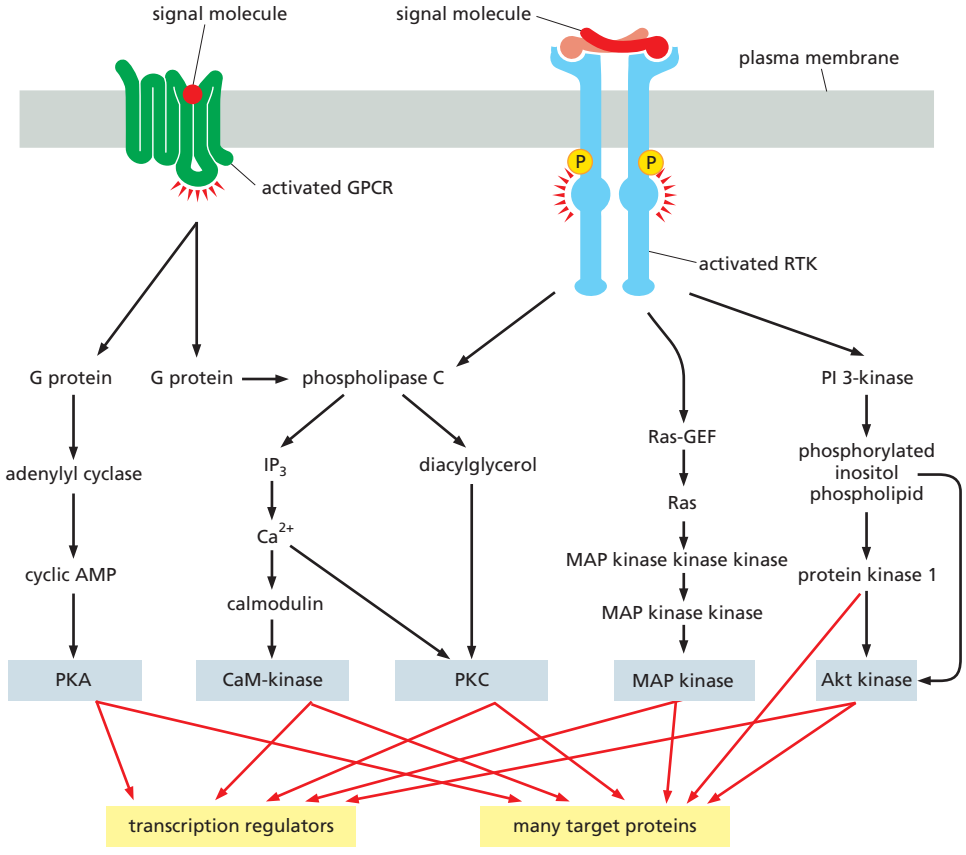


Figure 16–35 Both GPCRs and RTKs activate multiple intracellular signaling pathways. The figure reviews five of these pathways: two leading from GPCRs—through adenylyl cyclase and through phospholipase C—and three leading from RTKs—through phospholipase C, Ras, and PI 3-kinase. Each pathway differs from the others, yet they use some common components to transmit their signals. Because all five eventually activate protein kinases (*gray* boxes), it seems that each is capable in principle of regulating practically any process in the cell.

UNTANGLING CELL SIGNALING PATHWAYS

Intracellular signaling pathways are never mapped out in a single experiment. Although insulin was first isolated from dog pancreas in the early 1920s, the molecular chain of events that links the binding of insulin to its receptor with the activation of the transporter proteins that take up glucose has taken decades to untangle—and is still not completely understood.

Instead, investigators figure out, piece by piece, how all the links in the chain fit together—and how each contributes to the cell's response to an extracellular signal molecule such as the hormone insulin. Here, we discuss the kinds of experiments that allow scientists to identify individual links and, ultimately, to piece together complex signaling pathways.

Close encounters

Most signaling pathways depend on proteins that physically interact with one another. There are several ways to detect such direct contact. One involves using a protein as "bait." For example, to isolate the receptor that binds to insulin, one could attach insulin to a chromatography column. Cells that respond to the hormone are broken open with detergents that disrupt their membranes, releasing the transmembrane receptor proteins (see Figure 11–27). When this slurry is poured over the chromatography column, the proteins that bind to insulin will stick and can later be eluted and identified (see Figure 4–55).

Protein–protein interactions in a signaling pathway can also be identified by *co-immunoprecipitation*. For example, cells exposed to an extracellular signal molecule can be broken open, and antibodies can be used to grab the receptor protein known to recognize the signal molecule (see Panel 4–2, pp. 140–141, and Panel 4–3, pp. 164–165). If the receptor is strongly associated with other proteins, as shown in Figure 16–29, these will be captured as well. In this way, researchers can identify which proteins interact when an extracellular signal molecule stimulates cells.

Once two proteins are known to bind to each other, an investigator can pinpoint which parts of the proteins are required for the interaction using the DNA technology discussed in Chapter 10. For example, to determine which phosphorylated tyrosine on a receptor tyrosine kinase (RTK) is recognized by a certain intracellular signaling protein, a series of mutant receptors can be constructed, each missing a different tyrosine from its cytoplasmic domain (**Figure 16–36**). In this way, the specific tyrosines required for binding can be determined. Similarly, one can determine whether this phosphotyrosine docking site is required for the receptor to transmit a signal to the cell.

Jamming the pathway

Ultimately, one wants to assess what role a particular protein plays in a signaling pathway. A first test may

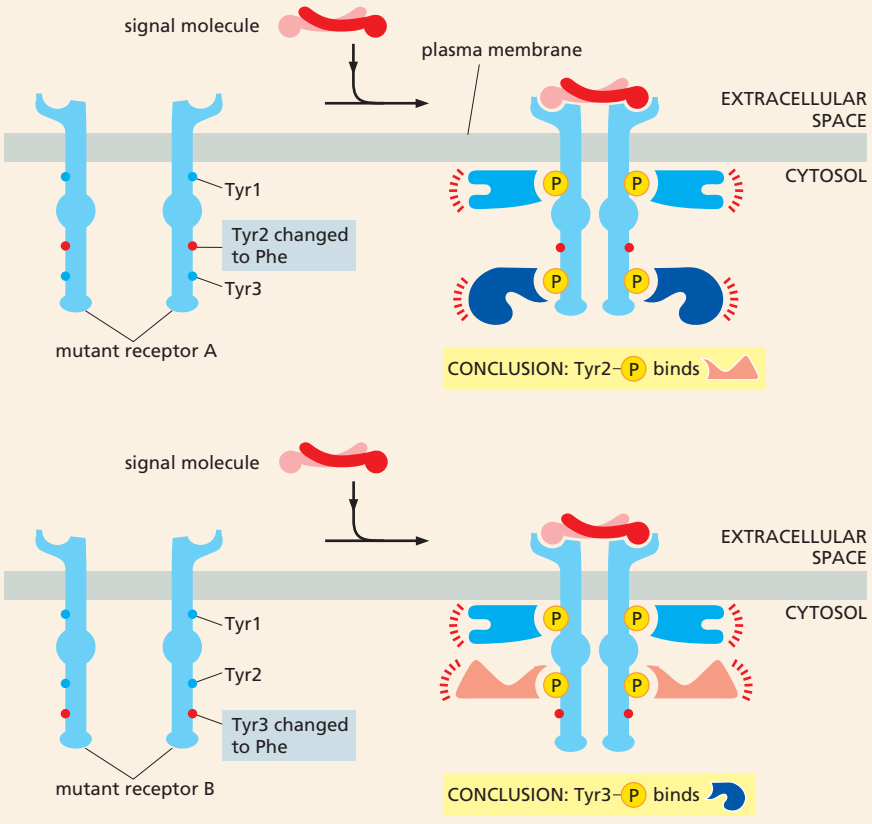


Figure 16–36 Mutant proteins can help to determine exactly where an intracellular signaling molecule binds. As shown in Figure 16–29, on binding their extracellular signal molecule, a pair of RTKs come together and phosphorylate specific tyrosines on each other's cytoplasmic tails. These phosphorylated tyrosines bind different intracellular signaling proteins, which then become activated and pass on the signal. To determine which tyrosine binds to a specific intracellular signaling protein, a series of mutant receptors is constructed. In the mutants shown, tyrosines Tyr2 or Tyr3 have been replaced, one at a time, by phenylalanine (*red*), thereby preventing phosphorylation at that site. As a result, the mutant receptors no longer bind to one of the intracellular signaling proteins shown in Figure 16–29. The effect on the cell's response to the signal can then be determined. It is important that the mutant receptor is tested in a cell that does not have its own normal receptors for the signal molecule.

involve using DNA technology to introduce into cells a gene encoding a constantly active form of the protein, to see if this mimics the effect of the extracellular signal molecule. Consider Ras, for example. The mutant form of Ras involved in human cancers is constantly active because it has lost its ability to hydrolyze the bound GTP that keeps the Ras protein switched on. This continuously active form of Ras can stimulate some cells to proliferate, even in the absence of a proliferation signal.

Conversely, the activity of a specific signaling protein can be inhibited or eliminated. In the case of Ras, for example, one could shut down the expression of the *Ras* gene in cells by RNA interference or CRISPR (see Figure 10–31). Such cells do not proliferate in response to extracellular mitogens, indicating the importance of normal Ras signaling in the proliferative response.

Making mutants

Another powerful strategy that scientists use to determine which proteins participate in cell signaling involves screening tens of thousands of animals—fruit flies or nematode worms, for example (discussed in Chapter 19)—to search for mutants in which a signaling pathway is not functioning properly. By examining enough mutant animals, many of the genes that encode the proteins involved in a signaling pathway can be identified.

Such classical genetic screens can also help sort out the order in which intracellular signaling proteins act in a pathway. Suppose that a genetic screen uncovers a pair of new proteins, X and Y, involved in the Ras signaling pathway. To determine whether these proteins lie upstream or downstream of Ras, one could create cells that express an inactive, mutant form of each protein, and then ask whether these mutant cells can be "rescued" by the addition of a continuously active form of Ras. If the constantly active Ras overcomes the blockage created by the mutant protein, the protein must operate upstream of Ras in the pathway (**Figure 16–37A**). However, if Ras operates upstream of the protein, a constantly active Ras would be unable to transmit a signal past the obstruction caused by the disabled protein (**Figure 16–37B**). Through such experiments, even the most complex intracellular signaling pathways can be mapped out, one step at a time (**Figure 16–37C**).

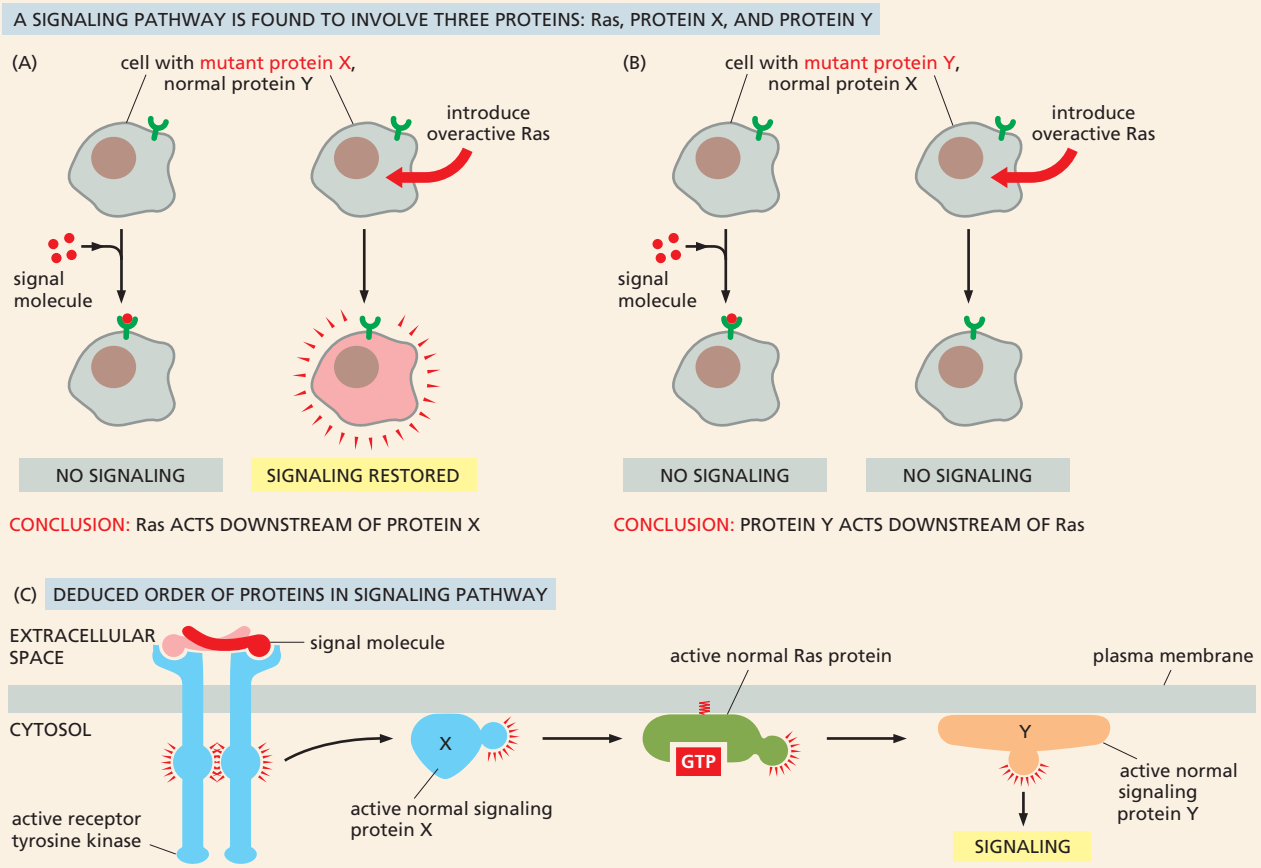


Figure 16–37 The use of mutant cell lines and an overactive form of Ras can help dissect an intracellular signaling pathway. In this hypothetical pathway, Ras, protein X, and protein Y are required for proper signaling. (A) In cells in which protein X has been inactivated, signaling does not occur. However, this signaling blockage can be overcome by the addition of an overactive form of Ras, such that the pathway is active even in the absence of the extracellular signal molecule. This result indicates that Ras acts downstream of protein X in the pathway. (B) Signaling is also disrupted in cells in which protein Y has been inactivated. In this case, introduction of an overactive Ras does not restore normal signaling, indicating that protein Y operates downstream of Ras. (C) Based on these results, the deduced order of the signaling pathway is shown.

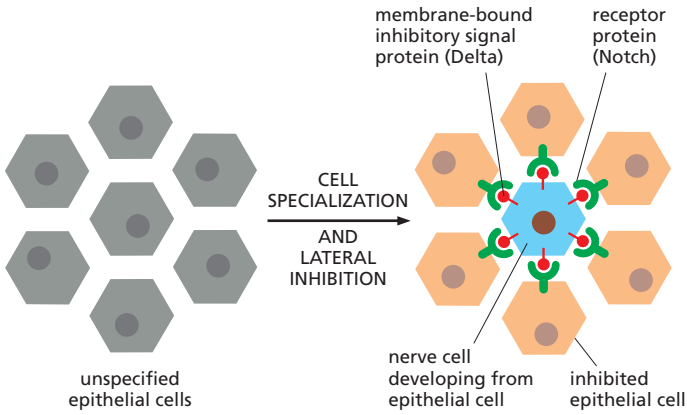


Figure 16–38 Notch signaling controls nerve-cell production in the fruit fly *Drosophila*. The fly nervous system originates in the embryo from a sheet of epithelial cells. Isolated cells in this sheet begin to specialize as neurons (*blue*), while their neighbors remain non-neuronal and maintain the structure of the epithelial sheet. The signals that control this process are transmitted via direct cell–cell contacts: each future neuron delivers an inhibitory signal to the cells next to it, deterring them from specializing as neurons too—a process called lateral inhibition. Both the signal molecule (Delta) and the receptor molecule (Notch) are transmembrane proteins, and the pathway represents a form of contact-dependent signaling (see Figure 16–3D).

Some Receptors Activate a Fast Track to the Nucleus

Not all receptors trigger complex signaling cascades that use multiple components to carry a message to the nucleus. Some take a more direct route to control gene expression. One such receptor is the protein Notch. Notch is a crucially important receptor in all animals, both during development and in adults. Among other things, it controls the development of neural cells in *Drosophila* (**Figure 16–38**).

In this simple signaling pathway, the receptor itself acts as a transcription regulator. When activated by the binding of Delta, a transmembrane signal protein on the surface of a neighboring cell, the Notch receptor is cleaved. This cleavage releases the cytosolic tail of the receptor, which is then free to move to the nucleus, where it helps to activate the appropriate set of Notch-responsive genes (**Figure 16–39**).

Some Extracellular Signal Molecules Cross the Plasma Membrane and Bind to Intracellular Receptors

Another direct route to the nucleus is taken by extracellular signal molecules that rely on intracellular receptor proteins (see Figure 16–4B). These molecules include the **steroid hormones**—*cortisol*, *estradiol*, and *testosterone*—and the thyroid hormones such as *thyroxine* (**Figure 16–40**). All of these hydrophobic molecules pass through the plasma membrane

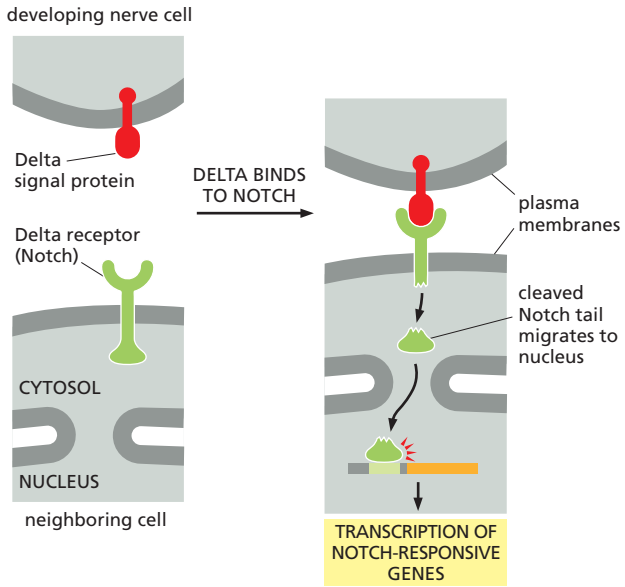


Figure 16–39 The Notch receptor itself is a transcription regulator. When the membrane-bound signal protein Delta binds to its receptor, Notch, on a neighboring cell, the receptor is cleaved by a protease. The released part of the cytosolic tail of Notch migrates to the nucleus, where it activates Notch-responsive genes. One consequence of this signaling process is shown in Figure 16–38.

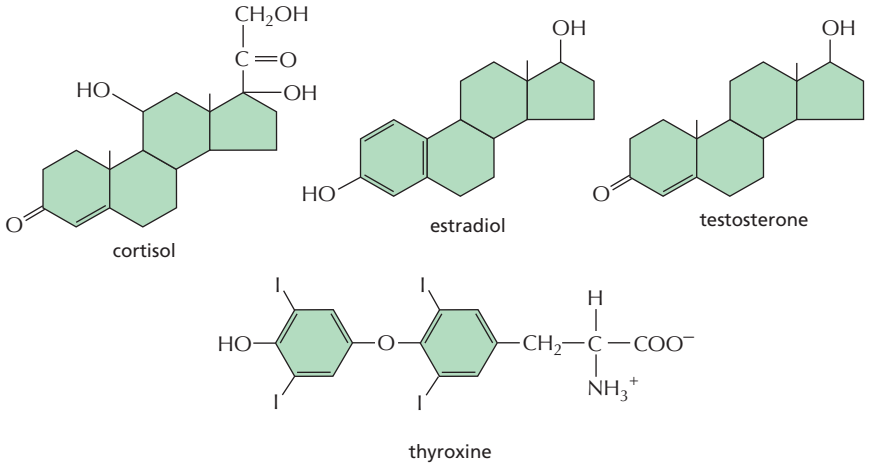


Figure 16–40 Some small, hydrophobic hormones bind to intracellular receptors that act as transcription regulators. Although these signal molecules differ in their chemical structures and functions, they all act by binding to intracellular receptor proteins that act as transcription regulators. Their receptors are not identical, but they are evolutionarily related, belonging to the *nuclear receptor superfamily*. The sites of origin and functions of these hormones are given in Table 16–1 (p. 536).

of the target cell and bind to receptor proteins located in either the cytosol or the nucleus. Regardless of their initial location, these intracellular receptor proteins are referred to as **nuclear receptors** because, when activated by hormone binding, they enter the nucleus, where they regulate the transcription of genes. In unstimulated cells, nuclear receptors are typically present in an inactive form. When a hormone binds, the receptor undergoes a large conformational change that activates the protein, allowing it to promote or inhibit the transcription of specific target genes (Figure 16–41). Each hormone binds to a different nuclear receptor, and each receptor acts at a different set of regulatory sites in DNA (discussed in Chapter 8). Moreover, a given hormone usually regulates different sets of genes in different cell types, thereby evoking different physiological responses in different target cells.

Nuclear receptors and the hormones that activate them have essential roles in human physiology (see Table 16–1, p. 536). Loss of these signaling systems can have dramatic consequences, as illustrated by the effects of mutations that eliminate the receptor for the male sex hormone testosterone. Testosterone in humans shapes the formation of the external genitalia and influences brain development in the fetus; at puberty, the

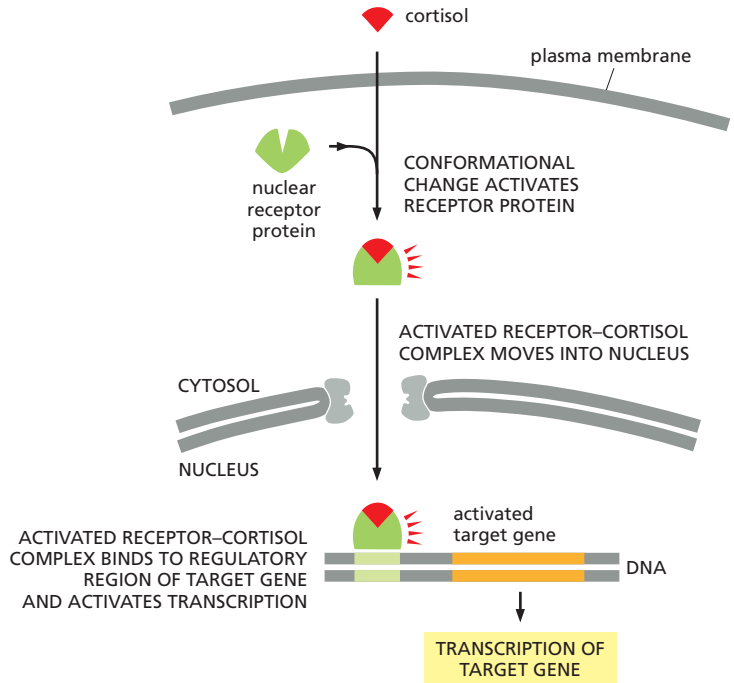


Figure 16–41 The steroid hormone cortisol acts by activating a transcription regulator. Cortisol is one of the hormones produced by the adrenal glands in response to stress. It crosses the plasma membrane of a target cell and binds to its receptor protein, which is located in the cytosol. The receptor–hormone complex is then transported into the nucleus via the nuclear pores. Cortisol binding activates the receptor protein, which is then able to bind to specific regulatory sequences in DNA and activate (or repress, not shown) the transcription of specific target genes. Whereas the receptors for cortisol and some other steroid hormones are located in the cytosol, those for other steroid hormones and for thyroid hormones are already bound to DNA in the nucleus even in the absence of hormone.

hormone triggers the development of male secondary sexual characteristics. Some very rare individuals are genetically male—that is, they have both an X and a Y chromosome—but lack the testosterone receptor as a result of a mutation in the corresponding gene; thus, they make testosterone, but their cells cannot respond to it. As a result, these individuals develop as females, which is the path that sexual and brain development would take if no male or female hormones were produced. Such a sex reversal demonstrates the crucial role of the testosterone receptor in sexual development, and it also shows that the receptor is required not just in one cell type to mediate one effect of testosterone, but in many cell types to help produce the whole range of features that distinguish men from women.

Plants Make Use of Receptors and Signaling Strategies That Differ from Those Used by Animals

Plants and animals have been evolving independently for more than a billion years, the last common ancestor being a single-celled eukaryote that most likely lived on its own. Because these kingdoms diverged so long ago—when it was still "every cell for itself"—each has evolved its own molecular solutions to the complex problem of becoming multicellular. Thus the mechanisms for cell–cell communication in plants and animals are in some ways quite different. At the same time, however, plants and animals started with a common set of eukaryotic genes—including some used by single-celled organisms to communicate among themselves—so their signaling systems also show some similarities.

Like animals, plants make extensive use of transmembrane cell-surface receptors—especially enzyme-coupled receptors. The spindly weed *Arabidopsis thaliana* (see Figure 1–33) has hundreds of genes encoding **receptor serine/threonine kinases**. These are, however, structurally distinct from the receptor serine/threonine kinases found in animal cells (which we do not discuss in this chapter). The plant receptors are thought to play an important part in a large variety of cell signaling processes, including those governing plant growth, development, and disease resistance. In contrast to animal cells, plant cells seem not to use RTKs, steroid-hormone-type nuclear receptors, or cyclic AMP, and they seem to use few GPCRs.

One of the best-studied signaling systems in plants mediates the response of cells to ethylene—a gaseous hormone that regulates a diverse array of developmental processes, including seed germination and fruit ripening. Tomato growers use ethylene to ripen their fruit, even after it has been picked. Although ethylene receptors are not evolutionarily related to any of the classes of receptor proteins that we have discussed so far, they function as enzyme-coupled receptors. Surprisingly, it is the empty receptor that is active: in the absence of ethylene, the empty receptor activates an associated protein kinase that ultimately shuts off the ethylene-responsive genes in the nucleus; when ethylene is present, the receptor and kinase are inactive, and the ethylene-responsive genes are transcribed (**Figure 16–42**). This strategy, whereby signals act to relieve transcriptional inhibition, is commonly used in plants.

Protein Kinase Networks Integrate Information to Control Complex Cell Behaviors

Whether part of a plant or an animal, a cell receives messages from many sources, and it must integrate this information to generate an appropriate response: to live or die, to divide, to differentiate, to change shape, to move, to send out a chemical message of its own, and so on (see Figure

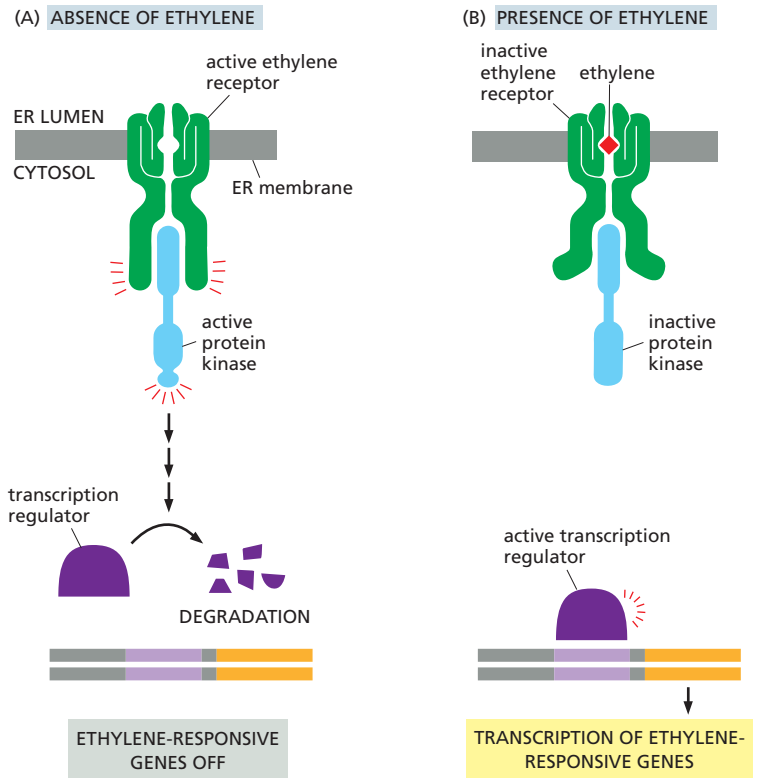


Figure 16–42 The ethylene signaling pathway turns on genes by relieving inhibition. (A) In the absence of ethylene, the receptor directly activates an associated protein kinase, which then indirectly promotes the destruction of the transcription regulator that switches on ethylene-responsive genes. As a result, the genes remain turned off. (B) In the presence of ethylene, the receptor and kinase are both inactive, and the transcription regulator remains intact and stimulates the transcription of the ethylene-responsive genes. The kinase that ethylene receptors interact with is a serine/threonine kinase that is closely related to the MAP kinase kinase kinase found in animal cells (see Figure 16–31). Note that the ethylene receptor is located in the endoplasmic reticulum; because ethylene is hydrophobic, it passes easily into the cell interior to reach its receptor.

16–6, **Movie 16.8**, and **Movie 16.9**). This integration is made possible by connections and interactions that occur between different signaling pathways. Such cross-talk allows the cell to bring together multiple streams of information and react to a rich combination of signals.

The most extensive links among the pathways are mediated by the protein kinases present in each. These kinases often phosphorylate, and hence regulate, components in other signaling pathways, in addition to components in their own pathway (see Figure 16–35). To give an idea of the scale of the complexity, genome sequencing studies suggest that about 2% of our ~19,000 protein-coding genes code for protein kinases; moreover, hundreds of distinct types of protein kinases are thought to be present in a single mammalian cell.

Many intracellular signaling proteins have several potential phosphorylation sites, each of which can be phosphorylated by a different protein kinase. These proteins can thus act as integrating devices. Information received from different intracellular signaling pathways can converge on such proteins, which then convert a multicomponent input to a single outgoing signal (**Figure 16–43**, and see Figure 16–9). These integrating proteins, in turn, can deliver a signal to many downstream targets. In this way, the intracellular signaling system may act like a network of nerve cells in the brain—or like a collection of microprocessors in a computer—interpreting complex information and generating complex responses.

Our understanding of these intricate networks is still evolving: we are still discovering new links in the chains, new signaling partners, new connections, and even new pathways. Unraveling the intracellular signaling pathways—in both animals and plants—is one of the most active areas of research in cell biology, and new discoveries are being made every day. Genome sequencing projects continue to provide long lists of components involved in signal transduction in a large variety of organisms. Yet even if we could identify every single component in this elaborate

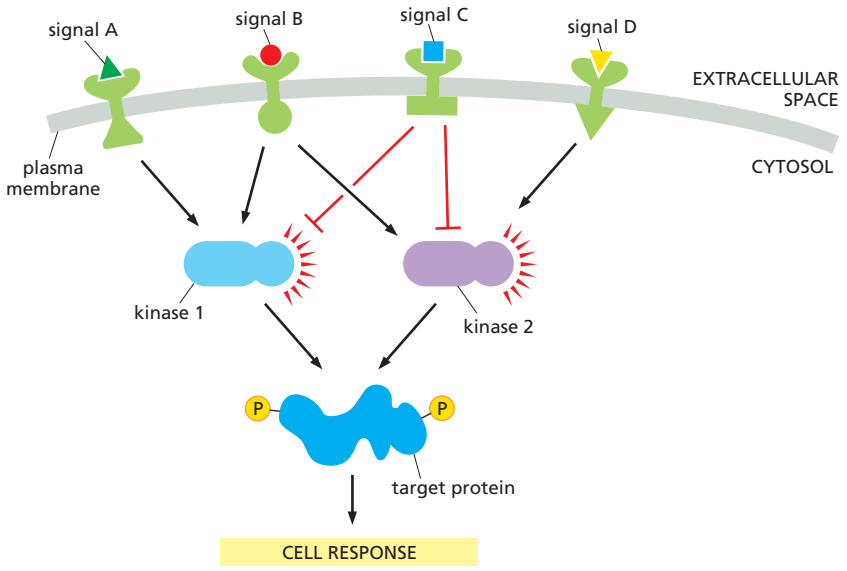


Figure 16–43 Intracellular signaling proteins serve to integrate incoming signals. Extracellular signals A, B, C, and D activate different receptors in the plasma membrane. The receptors act upon two protein kinases, which they either activate (*black* arrow) or inhibit (*red* crossbar). The kinases phosphorylate the same target protein and, when it is fully phosphorylated, this target protein triggers a cell response.

It can be seen that signal molecule B activates both protein kinases and therefore produces a strong output response. Signals A and D each activate a different kinase and therefore produce a response only if they are simultaneously present. Signal molecule C inhibits the cell response and will compete with the other signal molecules. The net outcome will depend both on the numbers of signaling molecules and the strengths of their connections. In a real cell, these parameters would be determined by evolution.

network of signaling pathways, it will remain a major challenge to figure out exactly how they all work together to allow cells—and organisms—to integrate the diverse array of information that inundates them constantly and to respond in a way that enhances their ability to adapt and survive.

ESSENTIAL CONCEPTS

- Cells in multicellular organisms communicate through a huge variety of extracellular chemical signals.
- In animals, hormones are carried in the blood to distant target cells, but most other extracellular signal molecules act over only a short distance. Neighboring cells often communicate through direct cell–cell contact.
- For an extracellular signal molecule to influence a target cell it must interact with a receptor protein on or in the target cell. Each receptor protein recognizes a particular signal molecule.
- Most extracellular signal molecules bind to cell-surface receptor proteins that convert (transduce) the extracellular signal into different intracellular signals, which are usually organized into signaling pathways.
- There are three main classes of cell-surface receptors: (1) ion-channel-coupled receptors, (2) G-protein-coupled receptors (GPCRs), and (3) enzyme-coupled receptors.
- GPCRs and enzyme-coupled receptors respond to extracellular signals by activating one or more intracellular signaling pathways, which, in turn, activate effector proteins that alter the behavior of the cell.
- Turning off signaling pathways is as important as turning them on. Each activated component in a signaling pathway must be subsequently inactivated or removed for the pathway to function again.
- GPCRs activate trimeric GTP-binding proteins called G proteins; these act as molecular switches, transmitting the signal onward for a short period before switching themselves off by hydrolyzing their bound GTP to GDP.
- G proteins directly regulate ion channels or enzymes in the plasma membrane. Some directly activate (or inactivate) the enzyme adenylyl

cyclase, which increases (or decreases) the intracellular concentration of the second messenger molecule cyclic AMP; others directly activate the enzyme phospholipase C, which generates the second messenger molecules inositol trisphosphate (IP_3) and diacylglycerol.

- IP_3 opens Ca^{2+} channels in the membrane of the endoplasmic reticulum, releasing a flood of free Ca^{2+} ions into the cytosol. The Ca^{2+} itself acts as a second messenger, altering the activity of a wide range of Ca^{2+}-responsive proteins. These include calmodulin, which activates various target proteins such as Ca^{2+}/calmodulin-dependent protein kinases (CaM-kinases).
- A rise in cyclic AMP activates protein kinase A (PKA), while Ca^{2+} and diacylglycerol in combination activate protein kinase C (PKC).
- PKA, PKC, and CaM-kinases phosphorylate selected signaling and effector proteins on serines and threonines, thereby altering their activity. Different cell types contain different sets of signaling and effector proteins and are therefore affected in different ways.
- Enzyme-coupled receptors have intracellular protein domains that function as enzymes or are associated with intracellular enzymes. Many enzyme-coupled receptors are receptor tyrosine kinases (RTKs), which phosphorylate themselves and selected intracellular signaling proteins on tyrosines. The phosphotyrosines on RTKs then serve as docking sites for various intracellular signaling proteins.
- Most RTKs activate the monomeric GTPase Ras, which, in turn, activates a three-protein MAP-kinase signaling module that helps relay the signal from the plasma membrane to the nucleus.
- Ras mutations stimulate cell proliferation by keeping Ras (and, consequently, the Ras–MAP kinase signaling pathway) constantly active and are a common feature of many human cancers.
- Some RTKs stimulate cell growth and cell survival by activating PI 3-kinase, which phosphorylates specific inositol phospholipids in the cytosolic leaflet of the plasma membrane lipid bilayer. This inositol phosphorylation creates lipid docking sites that attract specific signaling proteins from the cytosol, including the protein kinase Akt, which becomes active and relays the signal onward.
- Other receptors, such as Notch, have a direct pathway to the nucleus. When activated, part of the receptor migrates from the plasma membrane to the nucleus, where it regulates the transcription of specific genes.
- Some extracellular signal molecules, such as steroid hormones and nitric oxide, are small or hydrophobic enough to cross the plasma membrane and activate intracellular proteins, which are usually either transcription regulators or enzymes.
- Plants, like animals, use enzyme-coupled cell-surface receptors to recognize the extracellular signal molecules that control their growth and development; these receptors often act by relieving the transcriptional repression of specific genes.
- Different intracellular signaling pathways interact, enabling each cell type to produce the appropriate response to a combination of extracellular signals. In the absence of such signals, most animal cells have been programmed to kill themselves by undergoing apoptosis.
- We are far from understanding how a cell integrates all of the many extracellular signals that bombard it to generate an appropriate response.

KEY TERMS

adaptation
adenylyl cyclase
Ca^{2+}/calmodulin-dependent protein kinase (CaM-kinase)
calmodulin
cell signaling
cyclic AMP
cyclic-AMP-dependent protein kinase (PKA)
diacylglycerol (DAG)
enzyme-coupled receptor
extracellular signal molecule
G protein
G-protein-coupled receptor (GPCR)
GTP-binding protein
hormone
inositol 1,4,5-trisphosphate (IP_3)
inositol phospholipid
intracellular signaling pathway
ion-channel-coupled receptor
local mediator
MAP kinase
MAP-kinase signaling module
molecular switch
monomeric GTPase
neurotransmitter
nitric oxide (NO)
nuclear receptor
phosphoinositide 3-kinase (PI 3-kinase)
phospholipase C
protein kinase
protein kinase C (PKC)
protein phosphatase
Ras
receptor
receptor serine/threonine kinase
receptor tyrosine kinase (RTK)
serine/threonine kinase
signal transduction
steroid hormone
tyrosine kinase

QUESTIONS

QUESTION 16–8

Which of the following statements are correct? Explain your answers.

A. The extracellular signal molecule acetylcholine has different effects on different cell types in an animal and often binds to different cell-surface receptor molecules on different cell types.

B. After acetylcholine is secreted from cells, it is long-lived, because it has to reach target cells all over the body.

C. Both the GTP-bound α subunits and nucleotide-free $\beta\gamma$ complexes—but not GDP-bound, fully assembled G proteins—can activate other molecules downstream of GPCRs.

D. IP_3 is produced directly by cleavage of an inositol phospholipid without incorporation of an additional phosphate group.

E. Calmodulin regulates the intracellular Ca^{2+} concentration.

F. Different signals originating from the plasma membrane can be integrated by cross-talk between different signaling pathways inside the cell.

G. Tyrosine phosphorylation serves to build binding sites for other proteins to bind to RTKs.

QUESTION 16–9

The Ras protein functions as a molecular switch that is set to its "on" state by other proteins that cause it to release its bound GDP and bind GTP. A GTPase-activating protein helps reset the switch to the "off" state by inducing Ras to hydrolyze its bound GTP to GDP much more rapidly than it would without this encouragement. Thus, Ras works like a light switch that one person turns on and another turns off. You are studying a mutant cell that lacks the GTPase-activating protein. What abnormalities would you expect to find in the way in which Ras activity responds to extracellular signals?

QUESTION 16–10

A. Compare and contrast signaling by neurons, which secrete neurotransmitters at synapses, with signaling carried out by endocrine cells, which secrete hormones into the blood.

B. Discuss the relative advantages of the two mechanisms.

QUESTION 16–11

Two intracellular molecules, X and Y, are both normally synthesized at a constant rate of 1000 molecules per second per cell. Molecule X is broken down slowly: each molecule of X survives on average for 100 seconds. Molecule Y is broken down 10 times faster: each molecule of Y survives on average for 10 seconds.

A. Calculate how many molecules of X and Y the cell contains at any time.

B. If the rates of synthesis of both X and Y are suddenly increased tenfold to 10,000 molecules per second per cell—without any change in their degradation rates—how many molecules of X and Y will there be after one second?

C. Which molecule would be preferred for rapid signaling?

QUESTION 16–12

In a series of experiments, genes that code for mutant forms of an RTK are introduced into cells. The cells also express their own normal form of the receptor from their normal gene, although the mutant genes are constructed so that the mutant RTK is expressed at considerably higher concentration than the normal RTK. What would be the consequences of introducing a mutant gene that codes for an RTK (A) lacking its extracellular domain, or (B) lacking its intracellular domain?

QUESTION 16–13

Discuss the following statement: "Membrane proteins that span the membrane many times can undergo a conformational change upon ligand binding that can be sensed on the other side of the membrane. Thus, individual protein molecules can transmit a signal across a membrane. In contrast, individual single-span membrane proteins cannot transmit a conformational change across the membrane but require oligomerization."

QUESTION 16–14

What are the similarities and differences between the reactions that lead to the activation of G proteins and the reactions that lead to the activation of Ras?

QUESTION 16–15

Why do you suppose cells use Ca^{2+} (which is kept by Ca^{2+} pumps at a cytosolic concentration of 10^{-7} M) for intracellular signaling and not another ion such as Na^{+} (which is kept by the Na^{+} pump at a cytosolic concentration of 10^{-3} M)?

QUESTION 16–16

It seems counterintuitive that a cell, having a perfectly abundant supply of nutrients available, would commit suicide if not constantly stimulated by signals from other cells (see Figure 16–6). What do you suppose might be the advantages of such regulation?

QUESTION 16–17

The contraction of the myosin–actin system in cardiac muscle cells is triggered by a rise in intracellular Ca^{2+}. Cardiac muscle cells have specialized Ca^{2+} channels—called ryanodine receptors because of their sensitivity to the drug ryanodine—that are embedded in the membrane of the sarcoplasmic reticulum, a specialized form of the endoplasmic reticulum. In contrast to the IP_3-gated Ca^{2+} channels in the endoplasmic reticulum shown in Figure 16–23, the signaling molecule that opens ryanodine receptors is Ca^{2+} itself. Discuss the consequences of this feature of ryanodine channels for cardiac muscle cell contraction.

QUESTION 16–18

Two protein kinases, K1 and K2, function in an intracellular signaling pathway. If either kinase contains a mutation that permanently inactivates its function, no response is seen in cells when an extracellular signal is received. A different mutation in K1 makes it permanently active, so that in cells containing that mutation, a response is observed even in the absence of an extracellular signal. You characterize a double-mutant cell that contains K2 with the inactivating mutation and K1 with the activating mutation. You observe that the response is seen even in the absence of an extracellular signal. In the normal signaling pathway, does K1 activate K2 or does K2 activate K1? Explain your answer.

QUESTION 16–19

A. Trace the steps of a long and indirect signaling pathway from a cell-surface receptor to a change in gene expression in the nucleus.

B. Compare this pathway with an example of a short and direct pathway from the cell surface to the nucleus.

QUESTION 16–20

How does PI 3-kinase activate the Akt kinase after activation of an RTK?

QUESTION 16–21

Consider the structure of cholesterol, a small, hydrophobic molecule with a sterol backbone similar to that of three of the hormones shown in Figure 16–40, but possessing fewer polar groups such as –OH, =O, and $–COO^{-}$. If cholesterol were not normally found in cell membranes, could it be used effectively as a hormone if an appropriate intracellular receptor evolved?

QUESTION 16–22

The signaling mechanisms used by a steroid-hormone-type nuclear receptor and by an ion-channel-coupled receptor are relatively simple as they have few components. Can they lead to an amplification of the initial signal, and, if so, how?

QUESTION 16–23

If some cell-surface receptors, including Notch, can rapidly signal to the nucleus by activating latent transcription regulators at the plasma membrane, why do most cell-surface receptors use long, indirect signaling cascades to influence gene transcription in the nucleus?

QUESTION 16–24

Animal cells and plant cells have some very different intracellular signaling mechanisms but also share some common mechanisms. Why do you think this is so?