Cell Communication

16

Individual cells, like multicellular organisms, need to sense and respond to their environment. A typical free-living cell—even a primitive 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 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, a large variety of signals coordinate the animal's growth and its day-to-day physiology and behavior. In plants, too, cells are in constant communication with one another. Their interactions allow the plant to respond to the conditions of light, dark, and temperature that guide the cycle of its growth, flowering, and fruiting, and to coordinate what happens in its roots, stems, and leaves.

In this chapter, we examine some of the most important means by which cells communicate, and we discuss how cells send signals and interpret the messages they receive. Although we concentrate on the mechanisms of signal reception and interpretation in animal cells, we also present a brief review of what is known about signaling pathways in plant cells. We begin our discussion with an overview of the general principles of cell signaling and then consider two of the main systems animal cells use to receive and interpret signals.

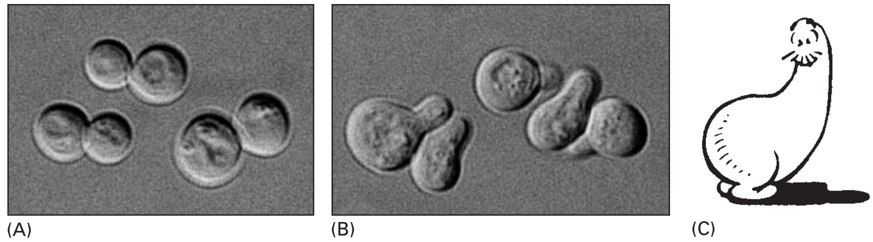


Figure 16–1 Yeast cells respond to mating factor. Budding yeast (*Saccharomyces cerevisiae*) cells are normally spherical (A), but when exposed to mating factor produced by neighboring yeast cells they extend a protrusion toward the source of the factor (B). Cells that adopt this shape in response to the mating signal are called "shmoos" after a classic 1940s cartoon character created by Al Capp (C). (A and B, courtesy of Michael Snyder; C, © Capp Enterprises, Inc., all rights reserved.)

General Principles of Cell Signaling

Information can come in a variety of forms, and communication frequently involves converting information signals from one form to another. When you phone a friend, for instance, the sound waves of your voice are converted to electrical signals that travel over a telephone wire. The critical points in this relay occur where the message is converted from one form to another. This process of conversion is called **signal transduction** (Figure 16–2).

The signals that pass between cells are far simpler than the sorts of messages that humans ordinarily exchange. In a typical communication between cells, the *signaling cell* produces a particular type of *signal molecule* that is detected by the *target cell*. The target cells possess *receptor proteins* that recognize and respond specifically to the signal molecule. Signal transduction begins when the receptor protein on the target cell receives an incoming extracellular signal and converts it to the intracellular signals that direct cell behavior. Most of this chapter will be 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 the different types of signals that cells send to one another.

Signals Can Act over Long or Short Range

Single cells and cells in multicellular organisms use hundreds of kinds of extracellular molecules to send signals to one another—proteins, peptides, amino acids, nucleotides, steroids, fatty acid derivatives, and even dissolved gases—but they rely on only a handful of basic styles of communication for getting the message across (Figure 16–3).

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

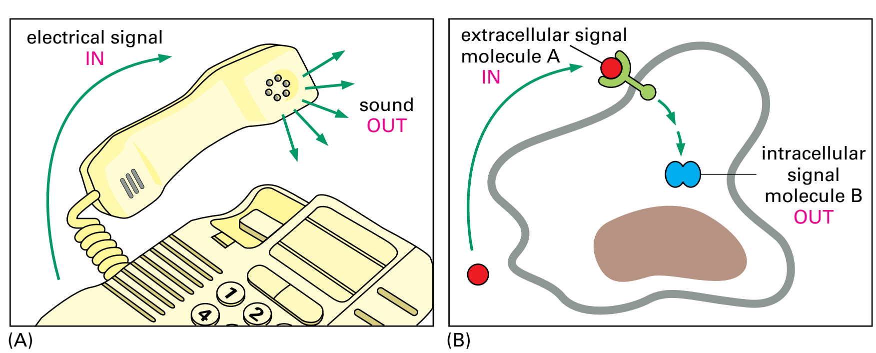


Figure 16–2 Signal transduction is the process whereby one type of signal is converted to another. (A) A telephone receiver converts an electrical signal into a sound signal. (B) A target cell converts an extracellular signal (molecule A) into an intracellular signal (molecule B).

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 medium, remaining in the neighborhood of 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 control cell proliferation in a healing wound function in this way.

Neuronal signaling constitutes a third form of cell communication. Like endocrine cells, neurons can deliver messages across long distances. In the case of neuronal signaling, however, a message is not broadcast widely but is delivered quickly and specifically to individual target cells through private lines (Figure 16–3C). 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. The axons that connect a person's spinal cord and big toe, for example, can be more than 1 m 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 chemical signal called a **neurotransmitter**. These neurotransmitters then diffuse across the narrow (< 100 nm) gap between the axon-terminal membrane and the membrane of the target cell 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 contact through signaling molecules lodged in their plasma membranes. The message is delivered when a signal molecule anchored in the plasma membrane of the signaling cell binds to a receptor molecule embedded in the plasma membrane of the target cell (Figure 16–3D). In embryonic development, for example, such *contact-dependent signaling* plays an important part in tissues in which adjacent cells that are initially similar are destined to become specialized in different ways (Figure 16–4).

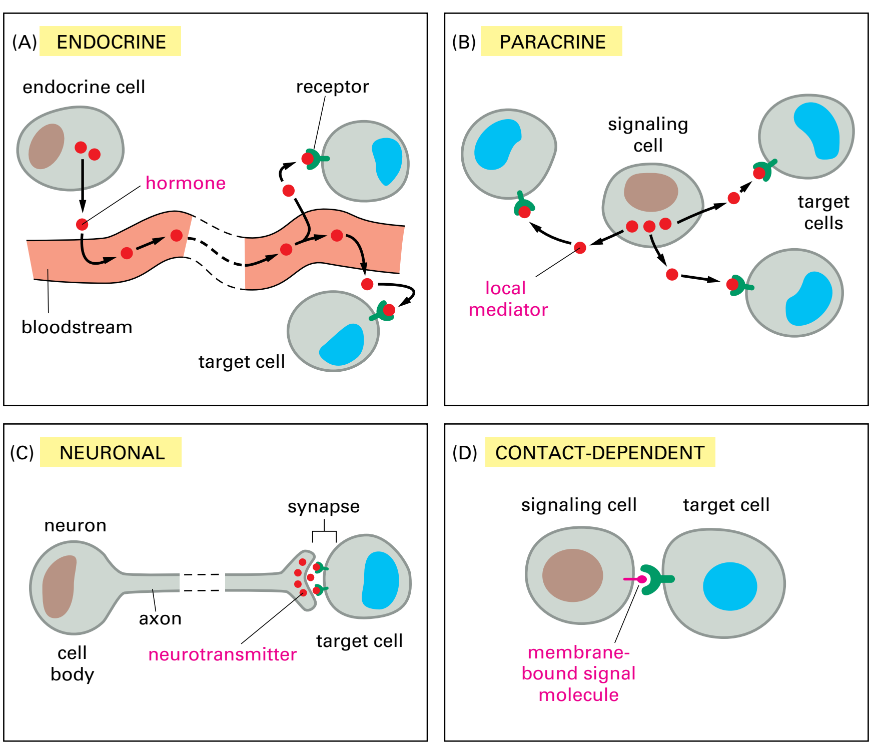


Figure 16–3 Animal cells can signal to one another in various ways.
(A) Hormones produced in endocrine glands are secreted into the bloodstream and are often distributed widely throughout the body. (B) Paracrine signals are released by cells into the extracellular medium in their neighborhood and act locally. (C) Neuronal signals are transmitted along axons to remote target cells. (D) Cells that maintain an intimate membrane-to-membrane interface can engage in contact-dependent signaling. 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.

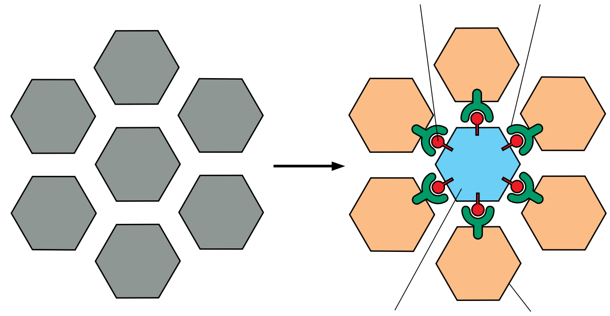


Figure 16–4 Contact-dependent signaling controls nerve-cell production. The nervous system originates in the embryo from a sheet of epithelial cells. Isolated cells in this sheet begin to specialize as neurons, while their neighbors remain nonneuronal and maintain the epithelial structure of the sheet. 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. Both the signal molecule (in this case, Delta) and the receptor molecule (called Notch) are transmembrane proteins. The same mechanism, mediated by essentially the same molecules, controls the detailed pattern of differentiated cell types in various other tissues, in both vertebrates and invertebrates. In mutants where the mechanism fails, some cell types (such as neurons) are produced in great excess at the expense of others.

To relate these different signaling styles, imagine trying to advertise a potentially stimulating lecture—or a concert or football game. An endocrine signal would be akin to broadcasting the information over a radio station. A flyer posted on select notice boards would be the equivalent of a localized paracrine signal. Neuronal signals—long-distance but personal—would be similar to a phone call or an 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 action of several of these is discussed in more detail later in this chapter.

Question 16–1

To remain a local stimulus, 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.

Each Cell Responds to a Limited Set of Signals

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 cells rest, or bound to the surfaces of neighboring cells. Each cell must respond selectively to this mixture of signals, disregarding some and reacting to others, according to the cells' specialized function.

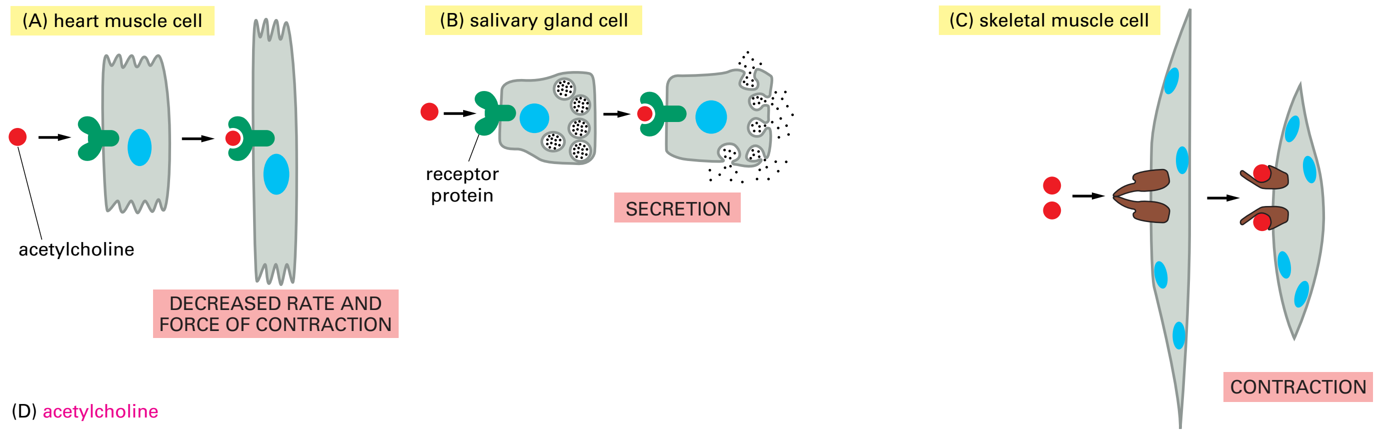
Whether a cell responds to a signal molecule depends first of all on whether it possesses a receptor for that signal. Without the appropriate receptor, a cell will be deaf to the signal and will not react. By producing only a limited set of receptors out of the thousands that are possible, the cell restricts the range of signals that can affect it. But this limited range of signals can still be used to control the behavior of the cell in complex ways. The complexity is of two sorts.

First, one signal, binding to one type of receptor protein, can cause a multitude of effects in the target cell: it can alter the cell's shape, movement, metabolism, and gene expression. As we shall see, the signal from a cell-surface receptor is generally conveyed into the cell interior via a set of interacting molecular mediators that are capable of producing widespread effects in the cell. Furthermore, this intracellular relay system and the intracellular targets 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 muscle cell is exposed to the neurotransmitter *acetylcholine*, the rate and force of its contractions decrease, but when a salivary gland is exposed to the same signal, it secretes components of saliva (Figure 16–5). These responses occur rapidly—within seconds to minutes—because the signal affects the activity of proteins and other molecules that are already present inside the cells.

The second kind of complexity arises because a typical cell possesses a whole collection of different receptors—tens to hundreds of thousands of receptors of a few dozen types. Such variety makes the cell simultaneously sensitive to many extracellular signals. These signals, by acting together, can evoke responses that are more than just the sum of the effects that each signal would evoke on its own. The intracellular relay systems for the different signals interact, so that the presence of one signal modifies the responses to another. Thus one combination of signals may simply enable a cell to survive; another may drive it to differentiate in some specialized way; and another may cause it to divide (Figure 16–6). In the absence of any signals, most animal cells are programmed to kill themselves. Because the execution of such a complex

Table 16–1 Some Examples of Signal Molecules

SIGNAL MOLECULE	SITE OF ORIGIN	CHEMICAL NATURE	SOME ACTIONS
Hormones			
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
Glucagon	α cells of pancreas	peptide	stimulates glucose synthesis, glycogen breakdown, and lipid breakdown, e.g., in liver and fat cells
Insulin	β cells of pancreas	protein	stimulates glucose uptake, protein synthesis, and lipid synthesis, e.g., in liver cells
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 of 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 of certain classes of neurons; promotes growth of their axons
Transforming growth factor-β (TGF-β)	many cell types	protein	inhibits cell proliferation; stimulates extracellular matrix production
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 Signaling Molecules			
Delta	prospective neurons; various other developing cell types	transmembrane protein	inhibits neighboring cells from becoming specialized in same way as the signaling cell



(D) acetylcholine

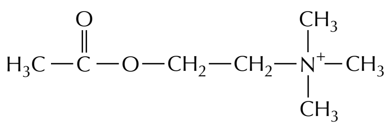


Figure 16–5 The same signal molecule can induce different responses in different target cells. Different cell types are configured to respond to the neurotransmitter acetylcholine in different ways. Acetylcholine binds to similar receptor proteins on heart muscle cells (A) and salivary gland cells (B),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

program often requires the synthesis of new proteins, it may take the cell hours to fully respond to the incoming signals. Overall, the integration of extracellular cues allows a relatively small number of signal molecules, used in different combinations, to exert subtle and complex control over cell behavior.

Receptors Relay Signals via Intracellular Signaling Pathways

Signal reception begins at the point where a signal originating outside the target cell encounters a target molecule belonging to the cell itself. In virtually every case, the target molecule is a **receptor protein** (also called a **receptor**), and each receptor is usually activated by only one type of signal. The receptor protein performs the primary transduction step: it receives an external signal and generates a new intracellular signal in response (see Figure 16–2B). As a rule, this is only the first event in a chain of intracellular signal transduction processes. In this game of molecular tag, the message is passed from one *intracellular signaling molecule* to another, each activating or generating the next signaling molecule in line, until, say, a metabolic enzyme is kicked into action, a

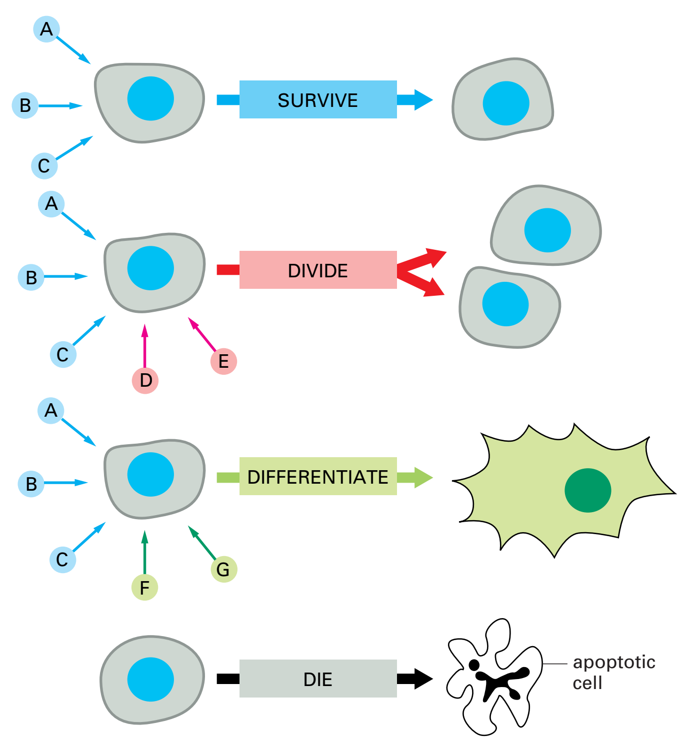


Figure 16–6 An animal cell depends on multiple extracellular signals. Every cell type displays a set of receptor proteins that enables it to respond to a specific set of signal molecules produced by other cells. These signal molecules work in combinations to regulate the behavior of the cell. As shown here, cells may require multiple signals *(blue arrows)* to survive, additional signals *(red arrows)* to divide, and still other signals *(green arrows)* to differentiate. If deprived of survival signals, most cells undergo a form of cell suicide known as programmed cell death, or apoptosis (discussed in Chapter 20).

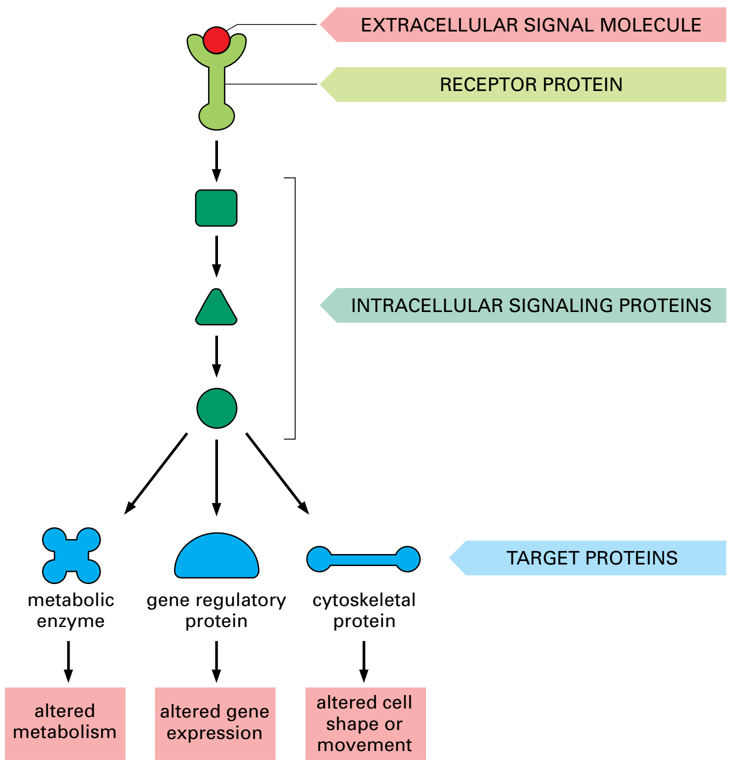


Figure 16–7 Extracellular signals alter the activity of a variety of cell proteins to change the behavior of the cell. In this case, the signal molecule binds to a cell-surface receptor protein. The receptor protein activates an intracellular signaling pathway that is mediated by a series of intracellular signaling proteins. Some of these signaling proteins interact with target proteins, altering them to change the behavior of the cell.

gene is switched on, or the cytoskeleton is tweaked into a new configuration. This final outcome is called the *response* of the cell (Figure 16–7).

These relay chains, or **signaling cascades**, of intracellular signaling molecules have several crucial functions (Figure 16–8):

1. They *transform,* or *transduce,* the signal into a molecular form suitable for passing the signal along or stimulating a response.
2. They *relay* the signal from the point in the cell at which it is received to the point at which the response is produced.
3. In many cases, signaling cascades also *amplify* the signal received, making it stronger, so that a few extracellular signal molecules are enough to evoke a large intracellular response.

Question 16–2

When a single photon of light is absorbed by a rhodopsin photo-receptor, it activates about 500 individual molecules of an intracellular signaling protein called transducin. Each molecule, in turn, binds to and activates an enzyme, phosphodiesterase, that hydrolyzes about 4000 molecules of cyclic GMP per second. Cyclic GMP is a small molecule, similar to cyclic AMP, that in the cytosol of rod photoreceptor cells binds to the Na^+ channels in the plasma membrane and keeps them in their open conformation, as we discuss later (see Figure 16–28). If you only consider the decrease in cyclic GMP, what is the degree of signal amplification if each transducin molecule remains active for 100 milliseconds?

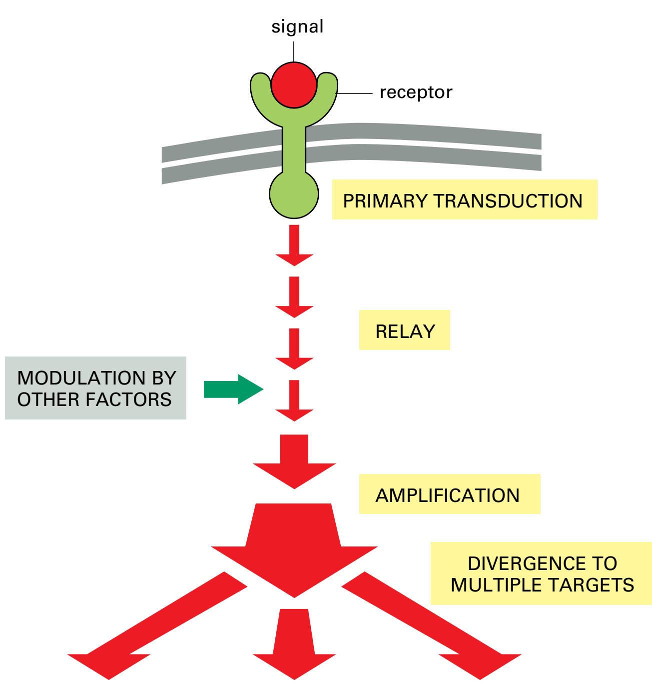


Figure 16–8 Cellular signaling cascades can follow a complex path. A receptor protein located on the cell surface transduces an extracellular signal into an intracellular signal, initiating a signaling cascade that transfers the signal into the cell interior, amplifying and distributing it en route. Many of the steps in the cascade can be modulated by other molecules or events in the cell.

4. The signaling cascades can also *distribute* the signal so as to influence several processes in parallel: at any step in the pathway, the signal can *diverge* and be relayed to a number of different intracellular targets, creating branches in the information flow diagram and evoking a complex response.
5. Each step in this signaling cascade is open to *modulation* by other factors, including other external signals, so that the effects of the signal can be tailored to the conditions prevailing inside or outside the cell.

Most signaling pathways trace a long and branching route, and enlist many molecular players, as they relay information from receptors at the cell surface to appropriate machinery in the cell's interior. But some signaling pathways are simpler and more direct, as we discuss in the next two sections.

Nitric Oxide Crosses the Plasma Membrane and Activates Intracellular Enzymes Directly

Extracellular signal molecules in general fall into two classes. 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. They rely on receptors on the surface of the target cell to relay their message across the membrane (Figure 16–9A). The second, and smaller, class of signals consists of molecules that are small enough or hydrophobic enough to slip easily through the plasma membrane (Figure 16–9B). Once inside, these signal molecules either activate intracellular enzymes or bind to intracellular receptor proteins that regulate gene expression.

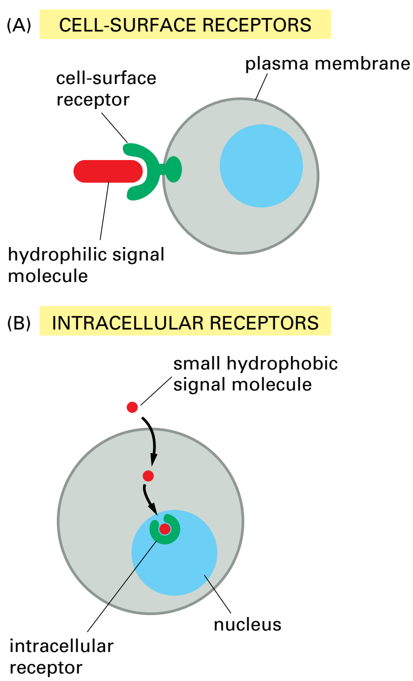
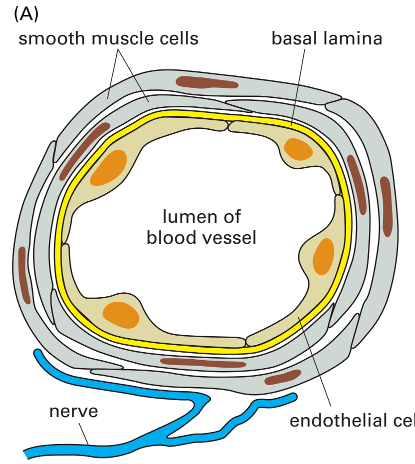


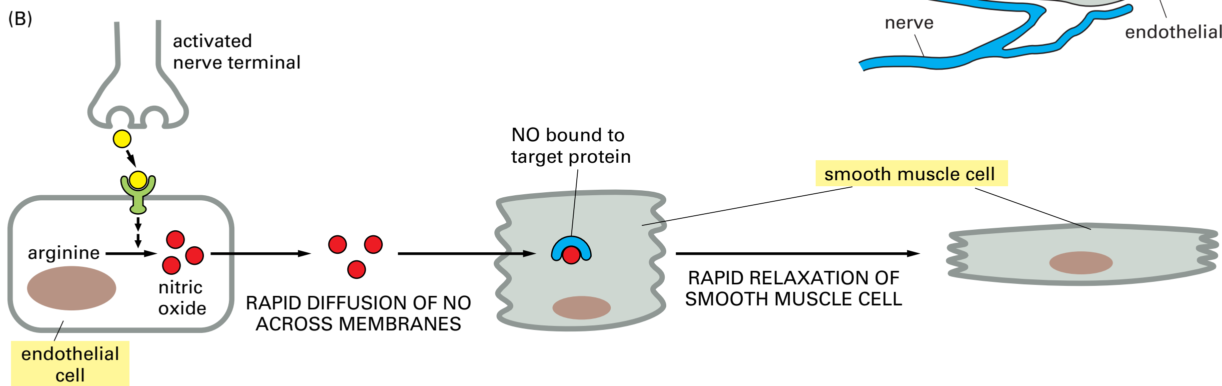
Figure 16–9 Extracellular signal molecules bind either to cell-surface receptors or to intracellular enzymes or receptors. (A) Most signal molecules are large and hydrophilic and are therefore unable to cross the plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate one or more signals inside the target cell (as shown in Figure 16–7). (B) Some small hydrophobic signal molecules, by contrast, diffuse across the target cell's plasma membrane and activate enzymes or bind to intracellular receptors—either in the cytosol (as shown) or in the nucleus.

For an extracellular signal to alter a cell within a few seconds or minutes, direct activation of an enzyme is an effective strategy. **Nitric oxide (NO)** is just such a signal. This dissolved gas diffuses readily out of the cell that generates it and enters neighboring cells. NO is made from the amino acid arginine and operates as a local mediator in many tissues. The gas acts only locally because it is quickly converted to nitrates and nitrites (with a half-life of about 5–10 seconds) by reaction with oxygen and water outside cells. Endothelial cells—the flattened cells that line every blood vessel—release NO in response to stimulation by nerve endings. This NO signal causes smooth muscle cells in the vessel wall to relax, allowing the vessel to dilate, so that blood flows through it more freely (Figure 16–10). The effect of NO on blood vessels accounts for the action of nitroglycerine, 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, nitroglycerine is converted to NO, which relaxes coronary blood vessels and increases blood flow to the heart. Many nerve cells also use NO to signal neighboring cells: NO released by nerve terminals in the penis, for instance, triggers the local blood-vessel dilation that is responsible for penile erection.

Inside many target cells, NO binds to the enzyme *guanylyl cyclase,* stimulating the formation of *cyclic GMP* from the nucleotide GTP. Cyclic GMP itself is a small intracellular signaling molecule that forms the next link in the signaling chain that leads to the cell's ultimate response. The impotence drug Viagra enhances penile erection by blocking the degradation of cyclic GMP, prolonging the NO signal. Cyclic GMP is very similar in its structure and mechanism of action to *cyclic AMP,* a much more commonly used intracellular messenger molecule whose actions we discuss later.

Figure 16–10 Nitric oxide (NO) triggers smooth muscle relaxation in a blood-vessel wall. (A) Drawing of a blood vessel. (B) Sequence of events leading to dilation of the blood vessel. Acetylcholine released by nerve terminals in the blood-vessel wall stimulates endothelial cells lining the blood vessel to make and release NO. The NO diffuses out of the endothelial cells and into adjacent smooth muscle cells, causing the muscle cells to relax. Note that NO gas is highly toxic when inhaled and should not be confused with nitrous oxide (N_2O), also known as laughing gas.





Some Hormones Cross the Plasma Membrane and Bind to Intracellular Receptors

Gases such as NO are not the only signal molecules that can cross the plasma membrane. Hydrophobic signal molecules such as the **steroid hormones**—including *cortisol, estradiol,* and *testosterone*—and the *thyroid hormones* such as *thyroxine* (Figure 16–11) all pass through the plasma membrane of the target cell. Instead of activating intracellular enzymes, however, they bind to receptor proteins located either in the cytosol or in the nucleus. These hormone receptors are proteins capable of regulating gene transcription, but they are typically present in an inactive form in unstimulated cells. When a hormone binds, the receptor protein undergoes a large conformational change that activates the protein, allowing it to promote or inhibit the transcription of a selected set of genes (Figure 16–12). Each hormone binds a different receptor

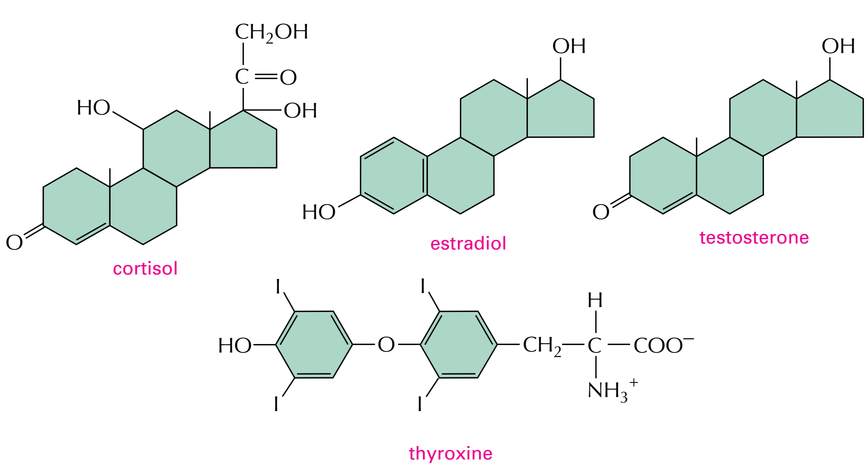
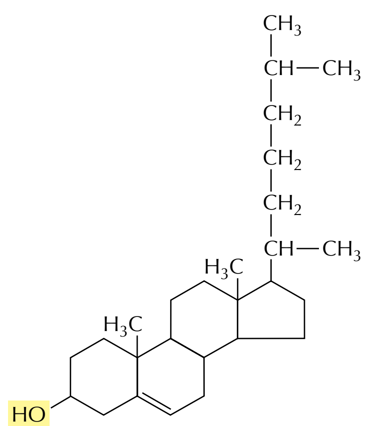


Figure 16–11 Some small hydrophobic hormones bind to intracellular receptors that act as gene regulatory proteins. Although these signaling molecules differ in their chemical structure and function, they all act by binding to intracellular receptor proteins. Their receptors are not identical, but they are evolutionarily related, belonging to the *nuclear receptor superfamily* of gene regulatory proteins. The sites of origin and functions of these hormones are given in Table 16–1 (p.).

Question 16–3

Consider the structure of cholesterol (Figure Q16–3), a small hydrophobic molecule with a sterol backbone similar to that of three of the hormones shown in Figure 16–11, 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?



cholesterol

Figure Q16–3

protein, and each receptor acts at a different set of regulatory sites in DNA (discussed in Chapter 8). Because the hormones regulate different sets of genes, they evoke a variety of physiological responses (see also Table 16–1, p.).

Steroid hormone receptors play an essential role in human physiology, as illustrated by the dramatic consequences of a lack of the receptor for testosterone in humans. The male sex hormone testosterone shapes the formation of the external genitalia and influences brain development in the fetus; at puberty, it 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 the hormone, but their cells cannot respond to it. As a result, these individuals develop the appearance and behavior of females, which is the pathway of sexual and brain development that would occur if no male or female hormones were produced. This demonstrates the key role of the testosterone receptor in sexual development, and 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.

Cell-Surface Receptors Fall into Three Main Classes

In contrast to NO and the steroid and thyroid hormones, the vast majority of signal molecules are too large or hydrophilic to cross the plasma membrane of the target cell. These proteins, peptides, and other bulky, water-soluble molecules bind to receptor proteins that span the plasma membrane (Figure 16–13). The 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.

Most cell-surface receptor proteins belong to one of three large families: *ion-channel–linked receptors, G-protein–linked receptors,* or *enzyme-linked receptors.* These families differ in the nature of the intra-

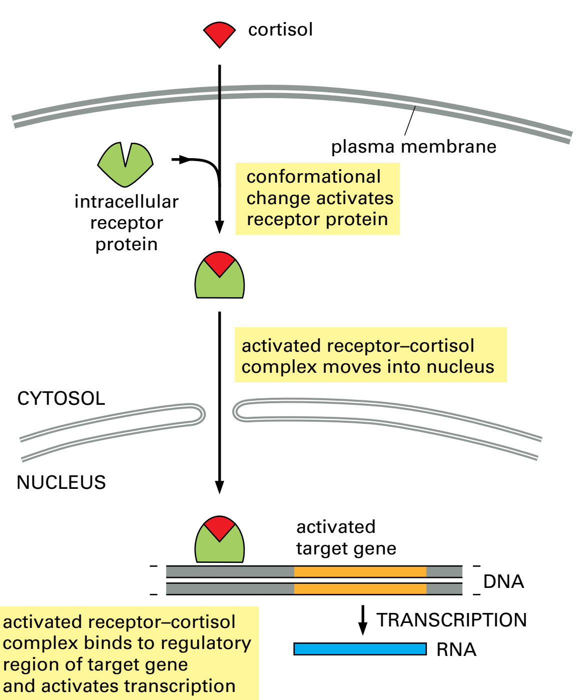


Figure 16–12 The steroid hormone cortisol acts by activating a gene regulatory protein. Cortisol diffuses directly across the plasma membrane and binds to its receptor protein, which is located in the cytosol. The hormone–receptor complex is then transported into the nucleus via the nuclear pores. Cortisol binding activates the receptor protein, which is then able to bind to specific regulatory sequences in the DNA and activate gene transcription. The receptors for cortisol and some other steroid hormones are located in the cytosol; those for the other signal molecules of this family are already bound to DNA in the nucleus.

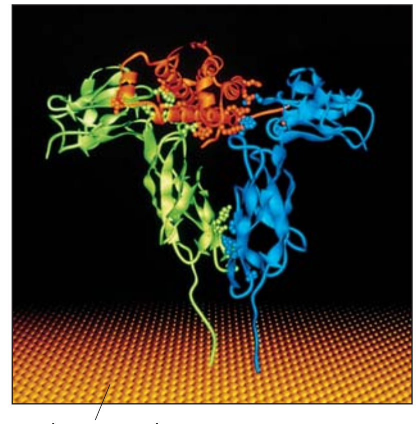


Figure 16–13 Most signal molecules bind to receptor proteins on the target cell surface. Shown here, the three-dimensional structure of human growth hormone *(red)* bound to its receptor. Binding of the hormone brings together two identical receptor proteins (one shown in *green*, the other in *blue).* The structures shown were determined by X-ray crystallographic studies of complexes formed between the hormone and extracellular receptor domains produced by recombinant DNA technology. Hormone binding activates cytoplasmic tyrosine kinases that are tightly bound to the cytosolic tails of the transmembrane receptors (not shown). (From A.M. deVos, M. Ultsch, and A.A. Kossiakoff, *Science* 255:306–312, 1992. © AAAS.)

cellular signal that they generate when the extracellular signal molecule binds to them. For ion-channel–linked receptors, the resulting signal is a flow of ions across the membrane, which produces an electrical current (Figure 16–14A). G-protein–linked receptors activate a class of membrane-bound protein (a *trimeric GTP-binding protein* or *G protein*), which is then released to migrate in the plane of the plasma membrane, initiating a cascade of other effects (Figure 16–14B). And enzyme-linked receptors, when activated, act as enzymes or are associated with enzymes inside the cell (Figure 16–14C). Switching on this enzymatic activity then generates a host of additional signals, including small molecules that are released into the cytosol.

The number of different types of receptors in these three classes is even greater than the number of extracellular signals that act on them, because for many extracellular signal molecules there is more than one type of receptor. The neurotransmitter acetylcholine, for example, acts

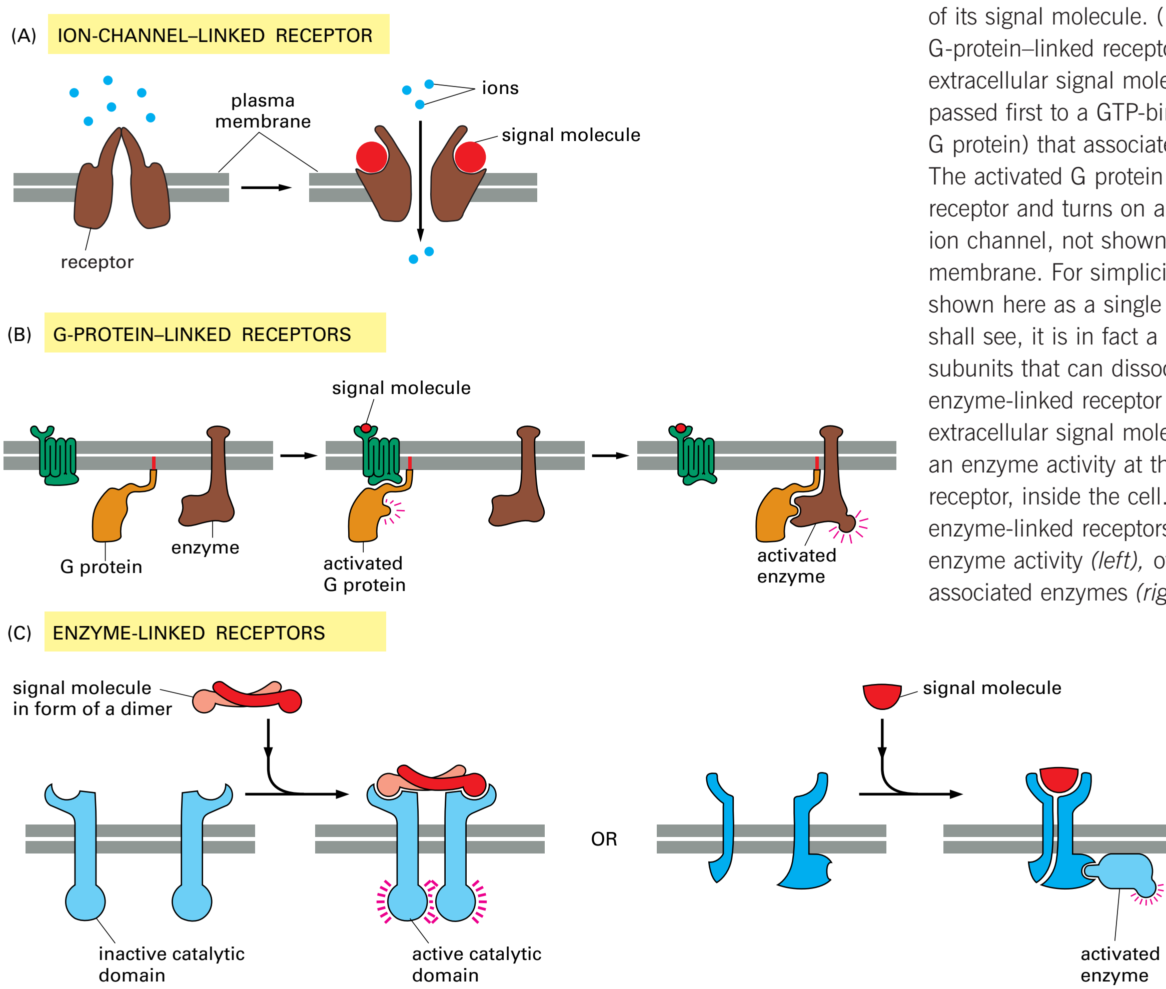


Figure 16–14 Cell-surface receptors fall into three basic classes. (A) An ion-channel–linked receptor opens (or closes, not shown) in response to binding of its signal molecule. (B) When a G-protein–linked receptor binds its extracellular signal molecule, the signal is passed first to a GTP-binding protein (a G protein) that associates with the receptor. The activated G protein then leaves the receptor and turns on a target enzyme (or ion channel, not shown) in the plasma membrane. For simplicity, the G protein is shown here as a single molecule; as we shall see, it is in fact a complex of three subunits that can dissociate. (C) An enzyme-linked receptor binds its extracellular signal molecule, switching on an enzyme activity at the other end of the receptor, inside the cell. Although many enzyme-linked receptors have their own enzyme activity *(left),* others rely on associated enzymes *(right).*

Table 16–2 Some Substances That Mimic Natural Signal Molecules

MIMIC	SIGNAL MOLECULE	RECEPTOR ACTION	EFFECT
Valium and barbiturates	γ-aminobutyric acid (GABA)	stimulate GABA-activated ion-channel–linked receptors	relief of anxiety; sedation
Nicotine	acetylcholine	stimulates acetylcholine-activated ion-channel–linked receptors	constriction of blood vessels; elevation of blood pressure
Morphine and heroin	endorphins and enkephalins	stimulate G-protein–linked opiate receptors	analgesia (relief of pain); euphoria
Curare	acetylcholine	inhibits acetylcholine-activated ion-channel–linked receptors	blockage of neuromuscular transmission, resulting in paralysis
Strychnine	glycine	blocks glycine-activated ion-channel–linked receptors	seizures and muscle spasm

on skeletal muscle cells via an ion-channel–linked receptor, whereas in heart muscle cells it acts through a G-protein–linked receptor (see Figure 16–5A and C). These two types of receptors generate different intracellular signals, and thus enable the two types of muscle cells to react to acetylcholine in different ways, increasing contraction in skeletal muscle and decreasing the frequency of contractions in heart.

Question 16–4

The signaling mechanisms used by a steroid hormone receptor and by an ion-channel–linked receptor are both very simple and have very few components. Can they lead to an amplification of the initial signal, and, if so, how?

The multitude of different cell-surface receptors that the body requires for its own signaling purposes are also targets for many foreign substances that interfere with our physiology and sensations, from heroin and nicotine to tranquilizers and chili peppers. These substances either mimic the natural ligand for a receptor, occupying the normal ligand-binding site, or bind to the receptor at some other site, blocking or overstimulating 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 the search for substances that will exert a precisely defined effect by binding to a specific type of cell-surface receptor.

Ion-channel–linked Receptors Convert Chemical Signals into Electrical Ones

Of all the cell-surface receptor types, *ion-channel–linked receptors* (also known as *transmitter-gated ion channels*) function in the simplest and most direct way. 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 neurotransmitter delivered to the outside of the target cell, directly into an electrical signal, in the form of a change in voltage across the target cell's plasma membrane. When the neurotransmitter binds, this type of receptor alters its conformation so as to open or close a channel for the flow of specific types of ions—such as Na^+, K^+, Ca^{2+}, or Cl^-—across the membrane (see Figure 16–14A). Driven by an electrochemical gradient, the ions rush into or out of the cell, creating a change in the membrane potential within a millisecond or so. This change in potential may trigger a nerve impulse, or alter the ability of other signals to do so. As we discuss later in this chapter, the opening of Ca^{2+} channels has special effects, as changes in the intracellular Ca^{2+} concentration can profoundly alter the activities of many enzymes. The function of ion-channel–linked receptors is discussed in greater detail in Chapter 12.

Whereas ion-channel–linked receptors are a specialty of the nervous system and of other electrically excitable cells such as muscle, G-protein–linked receptors and enzyme-linked receptors are used by practically every cell type of the body. Most of the remainder of this chapter will deal with these receptor families and with the signal transduction processes that they initiate.

Many Intracellular Signaling Proteins Act as Molecular Switches

Signals received via G-protein–linked or enzyme-linked receptors are transmitted to elaborate relay systems formed from cascades of intracellular signaling molecules. Apart from a few small molecules (such as cyclic GMP, cyclic AMP, and Ca^{2+}), these intracellular signaling molecules are proteins. Some serve as chemical transducers: in response to one type of chemical signal they generate another. Others serve as messengers, receiving a signal in one part of the cell and moving to another to exert an effect; and so on (see Figure 16–8).

Most of the key intracellular signaling proteins behave as **molecular switches**: receipt of a signal switches them from an inactive to an active state. Once activated, these proteins can turn on other proteins in the 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. If a signaling pathway is to recover after transmitting a signal and make itself ready to transmit another, every molecular switch must be reset to its original, unstimulated state. Thus, at every step, for every activation mechanism there has to be an inactivation mechanism. The two are equally important for the function of the system.

Proteins that act as molecular switches mostly fall into one of two main classes. The first and by far the largest class consists of proteins whose activity is turned on or off by phosphorylation, as discussed in Chapter 4 (see Figure 4–41). For these, the switch is thrown in one direction by a protein kinase, which tacks a phosphate onto the switch protein, and in the other direction by a protein phosphatase, which plucks the phosphate off the switch protein (Figure 16–15A). 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 it, distributing it, and modulating it.

The other main class of switch proteins involved in signaling consists of GTP-binding proteins. These switch between an active and an

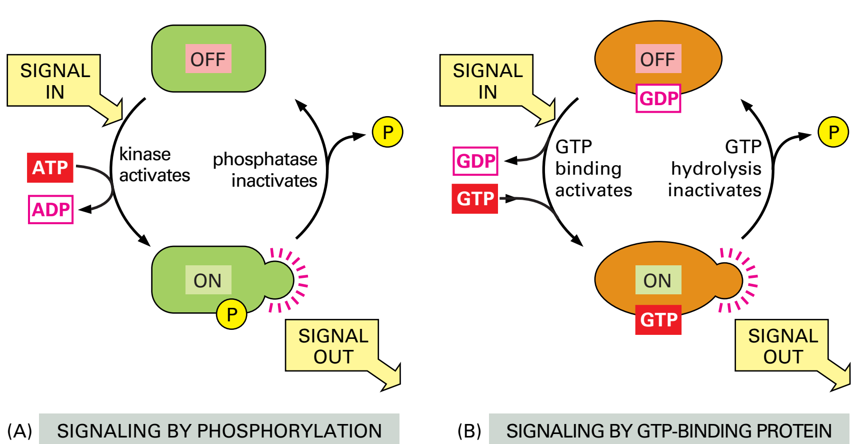


Figure 16–15 Intracellular signaling proteins act as molecular switches. Intracellular signaling proteins can be activated by the addition of a phosphate group and inactivated by the removal of the phosphate. In some cases, the phosphate is added covalently to the protein by a protein kinase that transfers the terminal phosphate group from ATP to the signaling protein; the phosphate is then removed by a protein phosphatase (A). In other cases, a GTP-binding signaling protein is induced to exchange its bound GDP for GTP; hydrolysis of the bound GTP to GDP then switches the protein off (B).

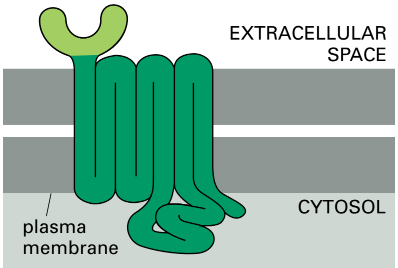


Figure 16–16 All G-protein–linked receptors possess a similar structure. The cytoplasmic portions of the receptor are responsible for binding to the G protein inside the cell. Receptors that bind to signal molecules that are proteins usually have a large extracellular ligand-binding domain *(light green).* This domain, together with some of the transmembrane segments, binds the protein ligand. Receptors that recognize small signal molecules such as adrenaline, however, have small extracellular domains, and the ligand usually binds deep within the plane of the membrane to a site that is formed by amino acids from several transmembrane segments (not shown).

inactive state according to whether they have GTP or GDP bound to them (Figure 16–15B). The mechanisms that control the switch on and the switch off will be described in the next section. GTP-binding proteins are important in several signaling pathways. One type, the G proteins, have a central role in signaling via G-protein–linked receptors, to which we now turn.

G-protein–linked Receptors

G-protein–linked receptors form the largest family of cell-surface receptors, with hundreds of members already identified in mammalian cells. They mediate responses to an enormous diversity of extracellular signal molecules, including hormones, local mediators, and neurotransmitters. These signal molecules 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.

Despite the diversity of the signal molecules that bind to them, all G-protein–linked receptors that have been analyzed possess a similar structure: each is made of a single polypeptide chain that threads back and forth across the lipid bilayer seven times (Figure 16–16). This superfamily of *seven-pass transmembrane receptor proteins* 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. Evolutionarily speaking, G-protein–linked receptors are ancient: even bacteria possess structurally similar membrane proteins—such as the bacteriorhodopsin that functions as a light-driven H^+ pump (discussed in Chapter 11). Although they resemble eucaryotic G-protein–linked receptors, these bacterial receptors do not act through G proteins; instead they are coupled to different signal transduction systems.

Stimulation of G-protein–linked Receptors Activates G-Protein Subunits

When an extracellular signaling molecule binds to a seven-pass transmembrane receptor, the receptor protein undergoes a conformational change that enables the receptor to activate a G protein located on the underside 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 function.

There are several varieties of G proteins. Each is specific for a particular set of receptors and a particular set of downstream target proteins, as we discuss shortly. 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 (Figure 16–17A), and the G protein is idle. When an extracellular ligand binds to its receptor, the altered receptor activates a G protein by causing the α subunit to lose some of its affinity for GDP, which it exchanges for a molecule of GTP. This activation breaks up the G protein subunits: the “switched-on” α subunit, clutching its GTP, detaches from the βγ complex, giving rise to two separate molecules that now roam independently along the plasma membrane (Figure 16–17B and C). The two activated parts of a G protein—the α subunit and the βγ complex—can both interact directly with target proteins located in the plasma membrane, which in turn may relay the signal to yet other destinations. The longer these target proteins have an α or a βγ subunit bound to them, the stronger and more prolonged the relayed signal will be.

The amount of time that the α and βγ subunits remain dissociated—and hence available to relay signals—is limited by the behavior of the α subunit. The α subunit has an intrinsic GTP-hydrolyzing (*GTPase*) activity, and it eventually hydrolyzes its bound GTP back to GDP; the α subunit then reassociates with a βγ complex and the signal is shut off (Figure 16–18). This reunion generally occurs within seconds after the G protein has been activated. The reconstituted G protein is now ready to be reactivated by another activated receptor.

Again, this system demonstrates a general principle of cell signaling: the mechanisms that shut a signal off are as important as the mechanisms that turn it on (see Figure 16–15B). They offer as many opportunities for control, and as many dangers of mishap. Take cholera, for example. The disease is caused by a bacterium that multiplies in the 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, because it *stimulates* the enzyme adenylyl cyclase, discussed later) in such a way that it can no longer hydrolyze its bound GTP. The altered α subunit thus remains in the active state indefinitely, continuously transmitting a signal to its target proteins. In intestinal cells, this 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 a different type of G protein (called G_i, because it *inhibits*

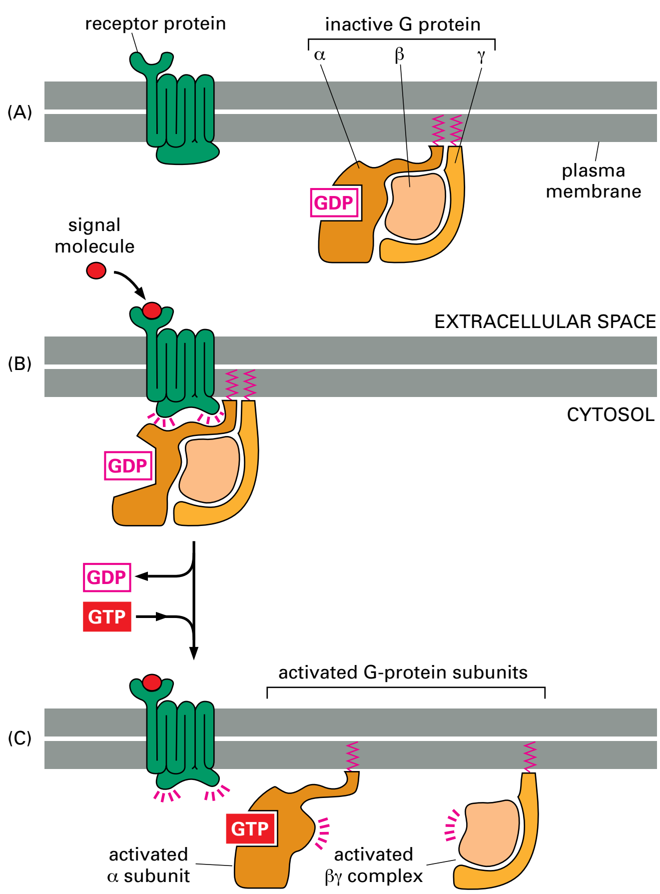
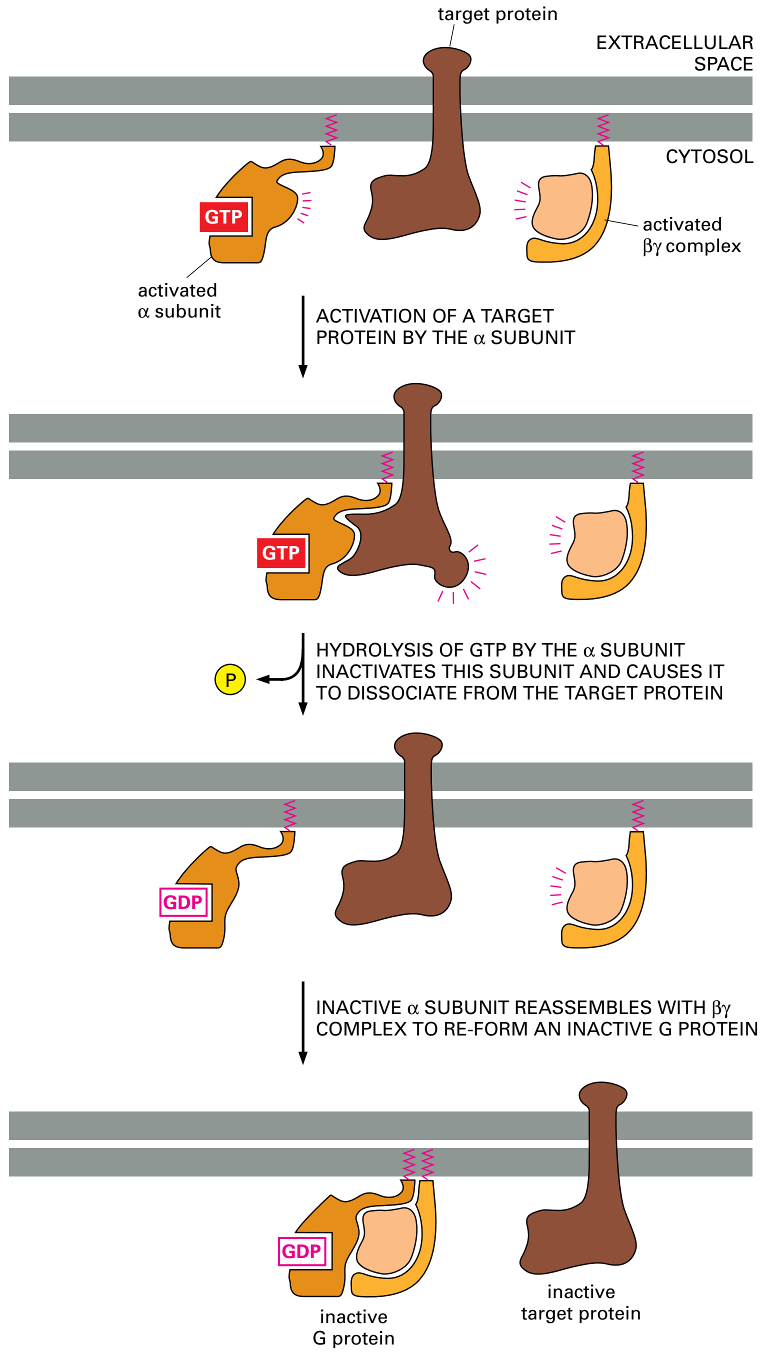


Figure 16–17 G proteins dissociate into two signaling proteins when activated. (A) In the unstimulated state, the receptor and the G protein are both inactive. Although they are shown here as separate entities in the plasma membrane, in some cases, at least, they are associated in a preformed complex. (B) Binding of an extracellular signal to the receptor changes the conformation of the receptor, which in turn alters the conformation of the G protein that is bound to the receptor. (C) The alteration of the α subunit of the G protein allows it to exchange its GDP for GTP. This causes the G protein to break up into two active components—an α subunit and a βγ complex, both of which can regulate the activity of target proteins in the plasma membrane. The receptor stays active while the external signal molecule is bound to it, and it can therefore catalyze the activation of 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 them to the plasma membrane.

Figure 16–18 The G-protein α subunit switches itself off by hydrolyzing its bound GTP. When an activated α subunit encounters and binds its target, it turns on its protein partner (or in some cases inactivates it, not shown) for as long as the two remain in touch. Within seconds, the GTP on the α subunit is hydrolyzed to GDP by the α subunit's intrinsic GTPase activity. This loss of GTP inactivates the α subunit, which dissociates from its target protein and reassociates with a βγ complex to re-form an inactive G protein. The G protein is now ready to couple to another receptor, as in Figure 16–17B. Both the activated α subunit (as shown) and the free βγ complex (not shown) can regulate target proteins.



Question 16–5

G-protein–linked receptors activate G proteins by reducing the strength of GDP binding. This results in rapid dissociation of bound GDP, which is then replaced by GTP, which 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.

adenylyl cyclase). In this case, however, modification by the toxin disables the G protein by locking it into its inactive GDP-bound state. Knocking out G_i, like activating G_s, results in the generation of a prolonged, inappropriate signal. Oddly, although the biochemical effects of cholera and pertussis toxins are known in detail, it is not clear how the bacteria benefit from their actions. In any case, what cholera and pertussis toxins do show us is that, like a car accelerating out of control, intracellular signaling pathways can be rendered dangerously overactive either by gluing down the molecular gas pedal or by cutting the molecular brakes.

Some G Proteins Regulate Ion Channels

The target proteins for G-protein subunits are either ion channels or membrane-bound enzymes. Different targets are affected by different

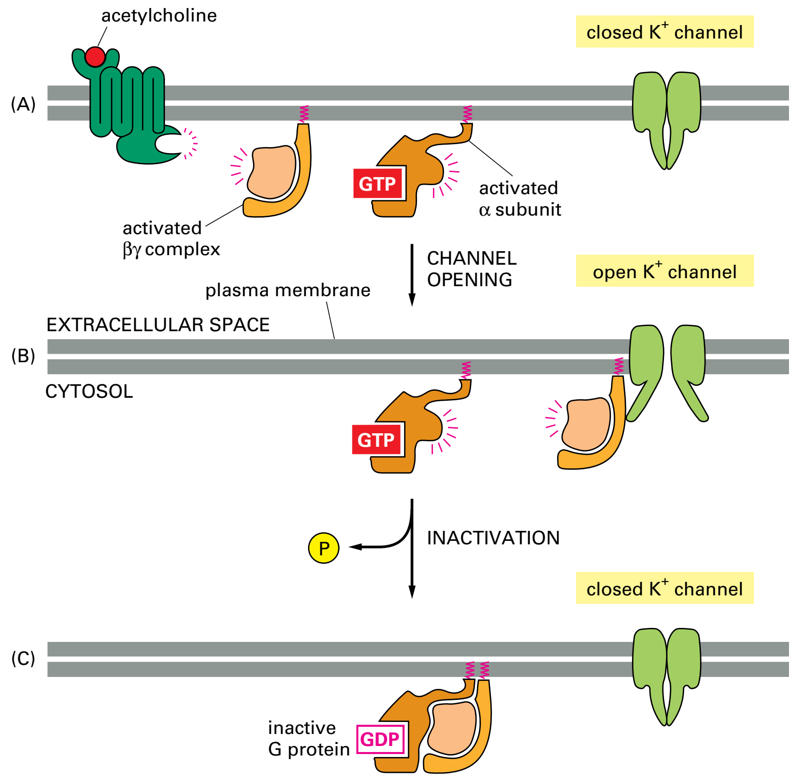


Figure 16–19 G proteins couple receptor activation to the opening of K^+ channels in the plasma membrane of heart muscle cells. (A) Binding of the neurotransmitter acetylcholine to its G-protein–linked receptor on heart muscle cells results in the dissociation of the G protein into an activated βγ complex and an activated α subunit. (B) The activated βγ complex binds to and opens a K^+ channel in the heart cell plasma membrane.
(C) Inactivation of the α subunit by hydrolysis of bound GTP causes it to reassociate with the βγ complex to form an inactive G protein, allowing the K^+ channel to close.

types of G proteins (of which about 20 have so far been discovered in mammalian cells), and these various G proteins are themselves activated by different classes of cell-surface receptors. In this way, binding of an extracellular signal molecule to a G-protein–linked receptor leads to effects on a particular subset of the possible target proteins, eliciting a response that is appropriate for that signal and that type of cell.

We look first at an example of G-protein regulation of ion channels. The heartbeat in animals is controlled by two sets of nerve fibers: one set speeds the heart up, the other slows it down. The nerves that signal a slowdown in heartbeat do so by releasing acetylcholine, which binds to a G-protein–linked receptor on the surface of the heart muscle cells. When acetylcholine binds to this receptor, a G protein (G_i) is activated—dissociating into an α subunit and a βγ complex (Figure 16–19A). In this particular example, the βγ complex is the active signaling component: it binds to the intracellular face of a K^+ channel in the heart muscle cell plasma membrane, forcing the ion channel into an open conformation and allowing K^+ to flow out of the cell (Figure 16–19B). This alters the electrical properties of the heart muscle cell, inhibiting its activity. The signal is shut down—and the K^+ channel recloses—when the α subunit inactivates itself by hydrolyzing its bound GTP and reassociates with the βγ complex to form an inactive G protein (Figure 16–19C).

Some G Proteins Activate Membrane-bound Enzymes

The interactions of G proteins with ion channels cause an immediate change in the state and behavior of the cell. Their interactions with enzyme targets have more complex consequences, leading to the production of additional intracellular signaling molecules. The most frequent target enzymes for G proteins are *adenylyl cyclase,* the enzyme responsible for production of the small intracellular signaling molecule *cyclic AMP*, and *phospholipase C,* the enzyme responsible for production of the small intracellular signaling molecules *inositol trisphosphate* and *diacylglycerol.* These two enzymes are activated by different types

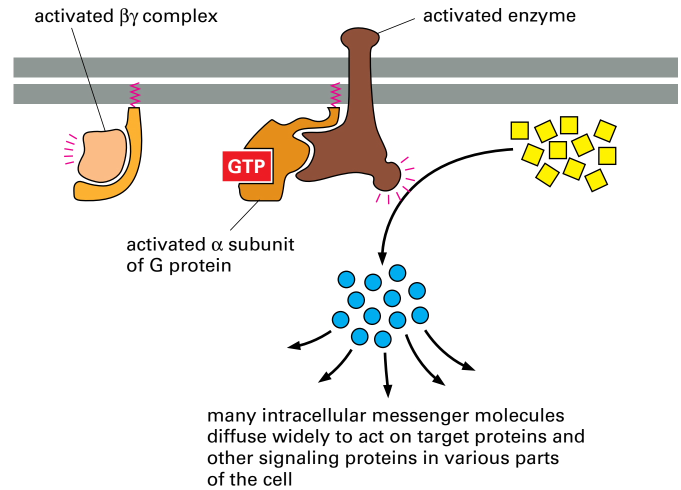


Figure 16–20 Enzymes activated by G proteins catalyze the synthesis of intracellular second-messenger molecules. Because each activated enzyme generates many second-messenger molecules, the signal is greatly amplified at this step in the pathway. The signal is passed on by the messenger molecules, which bind to target proteins and other signaling proteins in the cell and influence their activity.

of G proteins, so that cells are able to couple the production of the small intracellular signaling molecules to different extracellular signals. As we saw earlier, the coupling may be either stimulatory or inhibitory. We concentrate here on G proteins that stimulate enzyme activity. The small intracellular signaling molecules generated in these cascades are often called **second messengers** (the "first messengers" being the extracellular signals); they are produced in large numbers when a membrane-bound enzyme—such as adenylyl cyclase or phospholipase C—is activated, and they rapidly diffuse away from their source, spreading the signal throughout the cell (Figure 16–20).

Different second-messenger molecules, of course, produce different cellular responses. We will first examine the consequences of an increase in the intracellular concentration of cyclic AMP. This will take us along one of the main types of signaling pathways that lead from the activation of G-protein–linked receptors. We then discuss the actions of inositol trisphosphate and diacylglycerol, second-messenger molecules that will lead us along a different molecular route.

The Cyclic AMP Pathway Can Activate Enzymes and Turn On Genes

Many extracellular signals acting via G-protein–linked receptors affect the activity of **adenylyl cyclase** and thus alter the concentration of the messenger molecule **cyclic AMP** inside the cell. 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). Because it stimulates the cyclase, this G protein is called G_S. To eliminate the signal, a second enzyme, called *cyclic AMP phosphodiesterase,* rapidly converts cyclic AMP to ordinary AMP (Figure 16–21). One way that caffeine acts as a stimulant is by inhibiting this enzyme in the nervous system, blocking cyclic AMP degradation and keeping the concentration of this second-messenger molecule high.

Cyclic AMP phosphodiesterase is continuously active inside the cell. Because it breaks cyclic AMP down so quickly, the concentrations

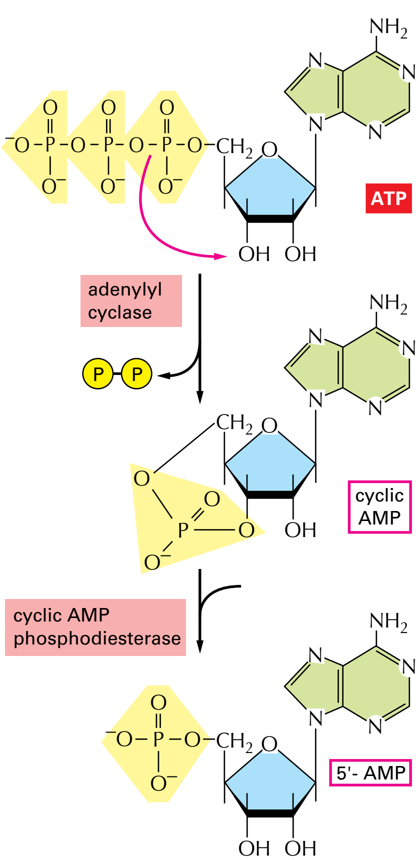


Figure 16–21 Cyclic AMP is synthesized by adenylyl cyclase and degraded by cyclic AMP phosphodiesterase. Cyclic AMP is formed from ATP by a cyclization reaction that removes two phosphate groups from ATP and joins the "free" end of the remaining phosphate group to the sugar part of the ATP molecule. The degradation reaction breaks this second bond, forming AMP.

Figure 16–22 Cyclic AMP concentration rises in response to an extracellular signal. A nerve cell in culture responds to the binding of the neurotransmitter serotonin to a G-protein–linked receptor 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 level is about 5×10^{-8} M. (B) Twenty seconds after adding serotonin to the culture medium, the intracellular concentration of cyclic AMP has risen to more than 10^{-6} M, an increase of more than twentyfold. (Courtesy of Roger Tsien.)

of this second messenger can change rapidly in response to extracellular signals, rising or falling tenfold in a matter of seconds (Figure 16–22). Cyclic AMP is a water-soluble molecule, so it can carry its signal throughout the cell, traveling from the site on the membrane where it is synthesized to interact with proteins located in the cytosol, the nucleus, or other organelles.

Many 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 levels. In many types of animal cells, stimulating cyclic AMP production boosts the rate of consumption of metabolic fuel. When we are frightened or excited, for example, the adrenal gland releases the hormone *adrenaline,* which circulates in the bloodstream and binds to a class of G-protein–linked receptors (adrenergic receptors) that are present on many types of cells. The consequences vary from one cell type to another, but all the cell responses help prepare the body for sudden action. In skeletal muscle, for example, adrenaline triggers a rise in the intracellular level of cyclic AMP, which causes the breakdown of glycogen (the polymerized storage form of glucose). This glycogen breakdown makes more glucose available as fuel for anticipated muscular activity. Adrenaline also acts on fat cells, stimulating the breakdown of triacylglyceride (the storage form of fat) to fatty acids—an immediately usable form of cell fuel (discussed in Chapter 13), which can also be exported to other cells.

Cyclic AMP exerts these various effects mainly by activating the enzyme **cyclic-AMP–dependent protein kinase** (**PKA**). This enzyme is normally held inactive in a complex with another protein. The binding of cyclic AMP forces a conformational change that unleashes the active kinase. Activated PKA then catalyzes the phosphorylation of particular serines or threonines on certain intracellular proteins, thus altering

Table 16–3 Some Hormone-induced Cell Responses Mediated by Cyclic AMP

EXTRACELLULAR SIGNAL MOLECULE*	TARGET TISSUE	MAJOR RESPONSE
Adrenaline	heart	increase in heart rate and force of contraction
Adrenaline	muscle	glycogen breakdown
Adrenaline, ACTH, glucagon	fat	fat breakdown
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.

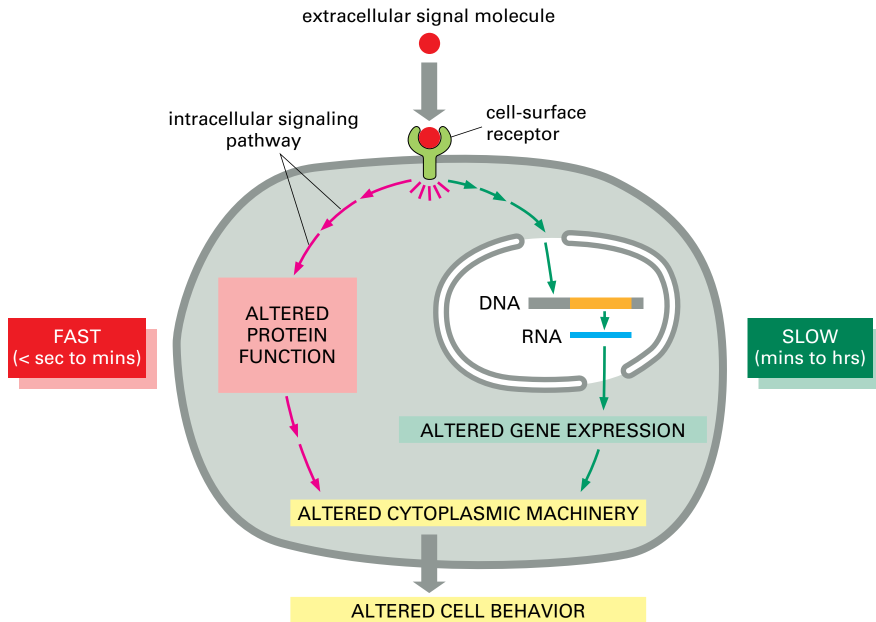


Figure 16–23 Extracellular signals can act slowly or rapidly. Certain types of altered cell behavior, such as increased cell growth and division, involve changes in gene expression and the synthesis of new proteins; they therefore occur relatively slowly. Other responses—such as changes in cell movement, secretion, or metabolism—need not involve the nuclear machinery and therefore occur more quickly; they may involve the rapid phosphorylation of target proteins in the cytoplasm, for example.

Question 16–6

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

their activity. In different cell types, different sets of target proteins are available to be phosphorylated, which explains why the effects of cyclic AMP vary with the target cell.

In some cases the effects of activating a cyclic AMP cascade are rapid; in others the effects are slow (Figure 16–23). In skeletal muscle cells, for example, activated PKA phosphorylates enzymes involved in glycogen metabolism, triggering the mechanism that breaks down glycogen to glucose. This response occurs within seconds. At the other extreme, some cyclic AMP responses take minutes or hours to develop. Included in this slow class are responses that involve changes in gene expression, an important means of regulating cell behavior. Thus in some cells, the PKA phosphorylates gene regulatory proteins that then activate the transcription of selected genes, a process that requires minutes or hours. In endocrine cells in the hypothalamus, for example, a rise in the amount of intracellular cyclic AMP stimulates the production and secretion of a peptide hormone called somatostatin. Increases in cyclic AMP concentrations in neurons, by contrast, control the production of proteins involved in long-term memory. Figure 16–24 shows the extensive relay chain for such a pathway from the plasma membrane to the nucleus.

We now turn to the other enzyme-mediated signaling cascade that leads from G-protein–linked receptors—the pathway that begins with the activation of the membrane-bound enzyme *phospholipase C* and leads to the generation of the second messengers inositol trisphosphate and diacylglycerol.

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

Some G-protein–linked receptors exert their effects via a type of G protein that activates the membrane-bound enzyme **phospholipase C** instead of adenylyl cyclase. A few examples are given in Table 16–4.

Once activated, phospholipase C propagates its signal by cleaving a lipid molecule that is a component of the cell membrane. The molecule is an **inositol phospholipid** (a phospholipid that has the sugar inositol attached to its head) that is present in small quantities in the inner half of the plasma membrane lipid bilayer. Because of the involvement of

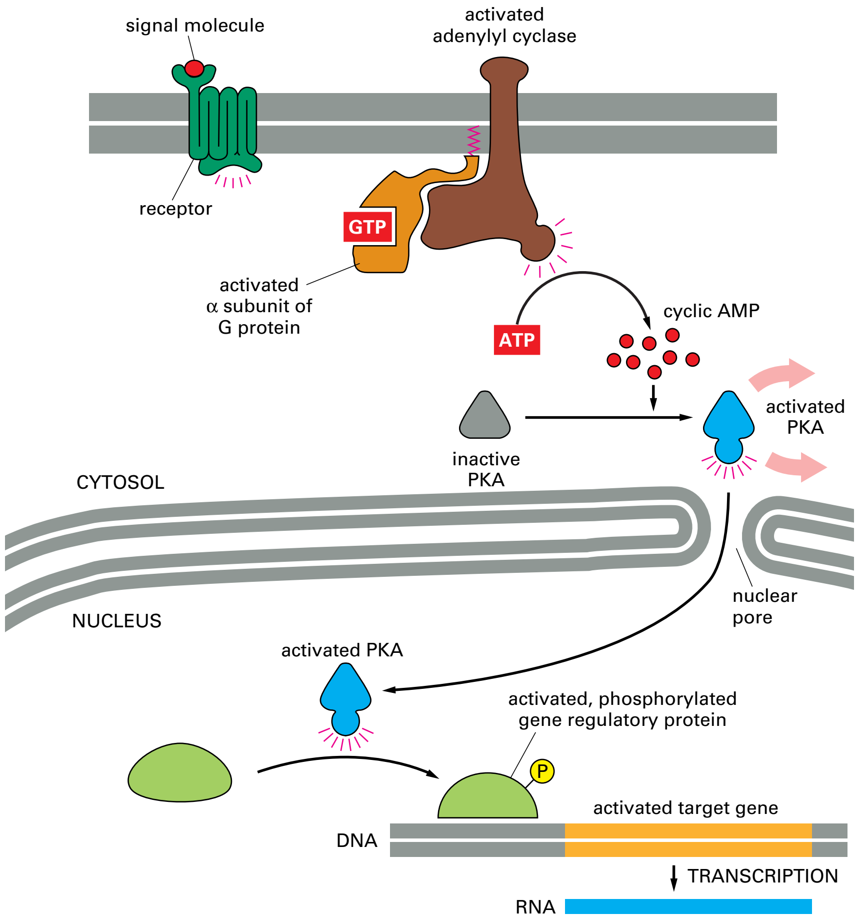


Figure 16–24 A rise in intracellular cyclic AMP can activate gene transcription. Binding of a hormone or neurotransmitter to its G-protein–linked receptor can lead to the activation of adenylyl cyclase and a rise in intracellular cyclic AMP. In the cytosol, cyclic AMP activates PKA, which then moves into the nucleus and phosphorylates specific gene regulatory proteins. Once phosphorylated, these proteins stimulate the transcription of a whole set of target genes. 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 indicated by the *light red arrows).*

this phospholipid, the signaling pathway that begins with the activation of phospholipase C is often known as the *inositol phospholipid pathway*. This signaling cascade occurs in almost all eucaryotic cells and affects a host of different target proteins.

The cascade works in the following way. When phospholipase C chops the sugar–phosphate head off the inositol phospholipid, it generates two small messenger molecules—**inositol 1,4,5-trisphosphate** (**IP_3**) and **diacylglycerol** (**DAG**). IP_3, a hydrophilic sugar phosphate, diffuses into the cytosol, while the lipid DAG remains embedded in the plasma membrane. Both molecules play a crucial part in signaling inside the cell, and we will consider them in turn.

The IP_3 released into the cytosol will eventually encounter the endoplasmic reticulum; there it binds to and opens Ca^{2+} channels that are embedded in the endoplasmic reticulum membrane. Ca^{2+} stored

Table 16–4 Some Responses Mediated by Phospholipase C Activation

SIGNAL MOLECULE	TARGET TISSUE	MAJOR RESPONSE
Vasopressin (a protein hormone)	liver	glycogen breakdown
Acetylcholine	pancreas	secretes amylase (a digestive enzyme)
Acetylcholine	smooth muscle	contraction
Thrombin (a proteolytic enzyme)	blood platelets	aggregation

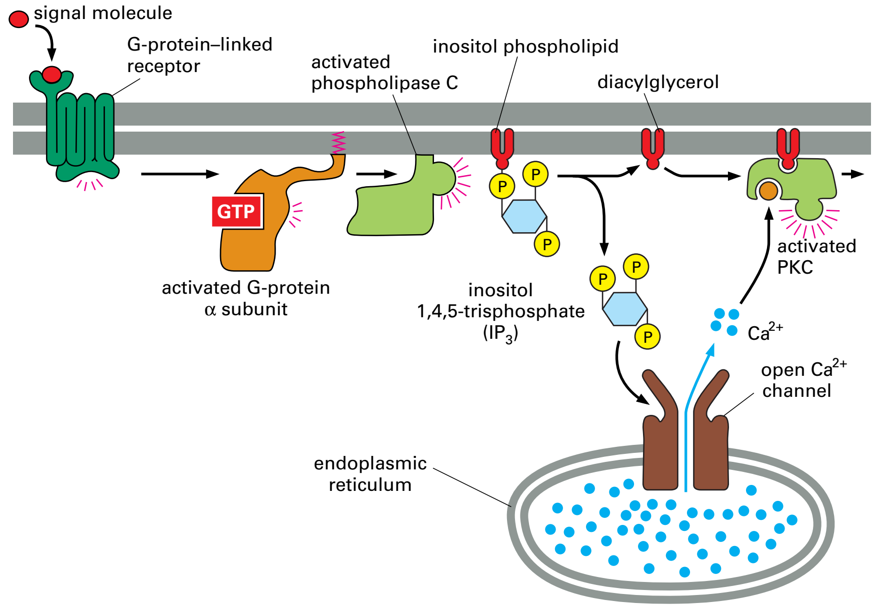


Figure 16–25 Phospholipase C activates two signaling pathways. Two intracellular messenger molecules are produced when a membrane inositol phospholipid is hydrolyzed by activated phospholipase C. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and triggers the release of Ca^{2+} from the endoplasmic reticulum by binding to and opening special Ca^{2+} channels in the endoplasmic reticulum membrane. The large electrochemical gradient for Ca^{2+} causes Ca^{2+} to rush out into the cytosol. Diacylglycerol remains in the plasma membrane and, together with Ca^{2+}, helps to activate the enzyme protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane.

inside the endoplasmic reticulum rushes out into the cytosol through these open channels (Figure 16–25), causing a sharp rise in the cytosolic concentration of free Ca^{2+}, which is normally kept very low.

Together with Ca^{2+}, diacylglycerol 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 16–25). Once activated, PKC phosphorylates a set of intracellular proteins that varies depending on the cell type. PKC operates on the same principle as PKA, although most of its target proteins are different.

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

Ca^{2+} has such an important and widespread role as an intracellular messenger that we must digress to consider its functions more generally. A surge in the cytosolic concentration of free Ca^{2+} is triggered by many different signals, not only those that act through G-protein–linked receptors. When a sperm fertilizes an egg cell, for example, Ca^{2+} channels open, and the resulting rise in cytosolic Ca^{2+} triggers the start of embryonic development (Figure 16–26); for skeletal muscle cells, a signal from a neighboring nerve triggers a rise in cytosolic Ca^{2+} that initiates contraction; and in many secretory cells, including nerve cells, Ca^{2+} triggers secretion. Ca^{2+} stimulates all these responses by binding to and influencing the activity of Ca^{2+}-sensitive 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 and in the endoplasmic reticulum. These differences are maintained by membrane pumps that actively pump Ca^{2+} out of the

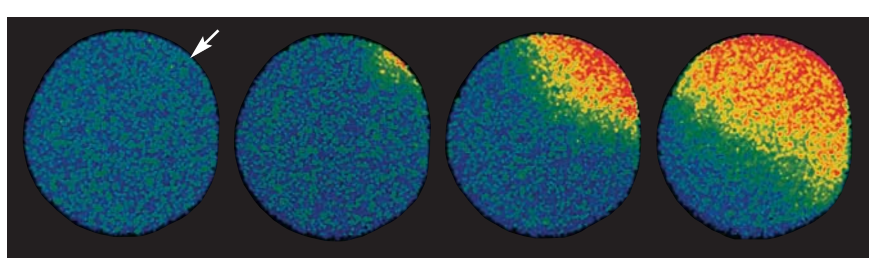


Figure 16–26 Fertilization of an egg by a sperm triggers an increase in cytosolic Ca^{2+} in the egg. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. When the sperm penetrates the egg, a wave of cytosolic Ca^{2+} *(red)*—released from the endoplasmic reticulum—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 initiates embryonic development. (Courtesy of Stephen A. Stricker.)

cytosol—either into the endoplasmic reticulum or across the plasma membrane and out of the cell. As a result, a steep electrochemical gradient of Ca^{2+} exists across the endoplasmic reticulum membrane and across the plasma membrane (discussed in Chapter 12). When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol down its electrochemical gradient, triggering changes in Ca^{2+}-responsive proteins in the cytosol.

The effects of Ca^{2+} in the cytosol are largely indirect: they are mediated through the interaction of Ca^{2+} with various transducer proteins, known collectively as *Ca^{2+}-binding proteins*. The most widespread and common of these is the Ca^{2+}-responsive protein **calmodulin**. Calmodulin is present in the cytosol of all eucaryotic cells that have been examined, including those of plants, fungi, and protozoa. When calmodulin binds to Ca^{2+}, the protein undergoes a conformational change that enables it to wrap around a wide range of target proteins in the cell, altering their activity (Figure 16–27). One particularly important class of targets for calmodulin includes 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 a part in learning and memory. Some memories, it seems, depend on this CaM-kinase and the pulses of Ca^{2+} signals that occur during neural activity: mutant mice that lack the kinase show a marked inability to remember where things are.

Question 16–7

Why do you suppose cells have evolved intracellular Ca^{2+} stores even though extracellular Ca^{2+} is usually not limiting?

Intracellular Signaling Cascades Can Achieve Astonishing Speed, Sensitivity, and Adaptability: A Look at Photoreceptors in the Eye

The steps in the signaling cascades associated with G-protein–linked receptors take a long time to describe, but they often take only seconds to execute. Consider how quickly a thrill can make your heart beat faster (when adrenaline stimulates the G-protein–linked receptors in your heart muscle cells, accelerating your heartbeat), or how fast the smell of food can make you salivate (through the G-protein–linked receptors for odors in your nose and the G-protein–linked receptors for acetylcholine in salivary cells that stimulate secretion). Among the fastest of all

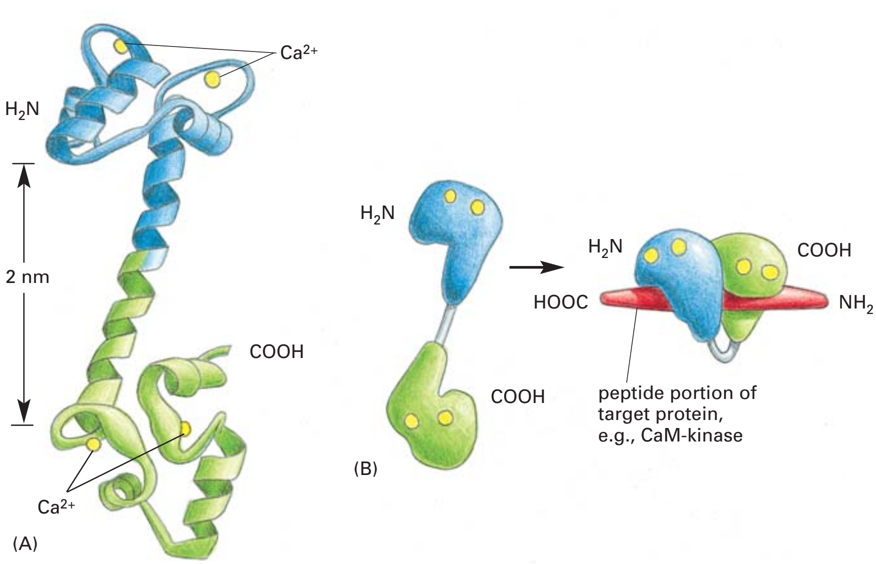


Figure 16–27 X-ray diffraction and NMR studies reveal the structure of Ca^{2+}/calmodulin. (A) The calmodulin molecule has a dumbbell shape, with two globular ends connected by a long, flexible α helix. Each end has two Ca^{2+}-binding domains. (B) Simplified representation of the structure, showing the conformational changes in Ca^{2+}/calmodulin that occur when it binds to a target protein. Note that the α helix has jackknifed to surround the target protein. (A, based on X-ray crystallographic data from Y.S. Babu et al., *Nature* 315:37–40, 1985. © 1985 Macmillan Magazines Ltd.; B, based on X-ray crystallographic data from W.E. Meador, A.R. Means, and F.A. Quiocho, *Science* 257:1251–1255, 1992, and on NMR data from M. Ikura et al., *Science* 256:632–638, 1992. © AAAS.)

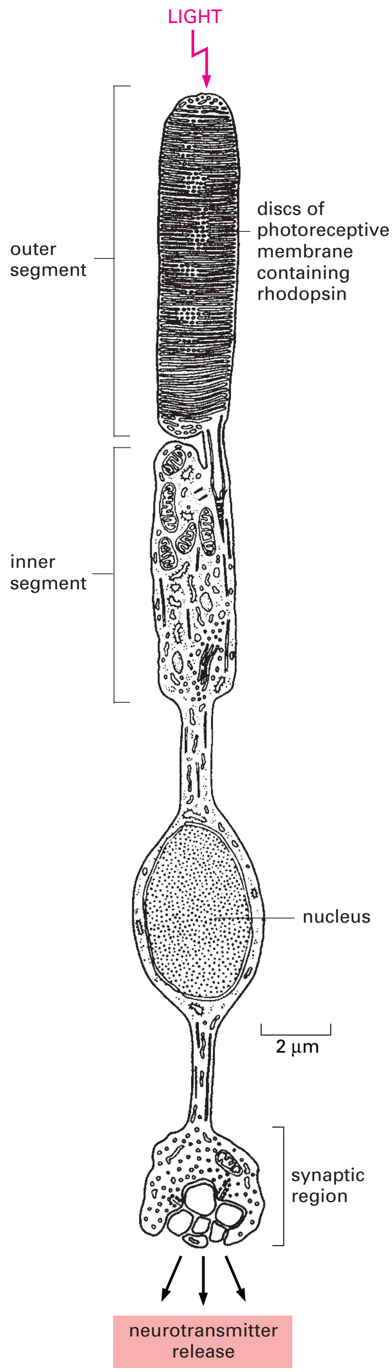


Figure 16–28 A rod photoreceptor cell from the retina is exquisitely sensitive to light. (A) Drawing of a rod photoreceptor. The light-absorbing molecules of rhodopsin are embedded in many pancake-shaped vesicles *(discs)* of membrane inside the outer segment of the cell. Neurotransmitter is released from the opposite end of the cell to control firing of the retinal nerve cells that pass on the signal to the brain. When the rod cell is stimulated by light, a signal is relayed from the rhodopsin molecules in the discs, through the cytosol of the outer segment, to Na^+ channels in the plasma membrane of the outer segment. The Na^+ channels close in response to the 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. (A, Adapted from T.L. Leutz, Cell Fine Structure. Philadelphia: Saunders, 1971.)

responses mediated by a G-protein–linked receptor, however, is the response of the eye to bright light: it takes only 20 msec for the most quickly responding photoreceptor cells of the retina (the cone photoreceptors) to produce their electrical response to a sudden flash of light.

This speed is achieved in spite of the necessity to relay the signal over several steps of an intracellular signaling cascade. But photoreceptors also provide a beautiful illustration of the positive advantages of signaling cascades: in particular, such cascades allow spectacular amplification of the incoming signal and 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 (Figure 16–28). Here, light is harvested by *rhodopsin,* a G-protein–linked light receptor. Light-activated rhodopsin activates a G protein called *transducin.* The activated α subunit of transducin then activates an intracellular signaling cascade that causes Na^+ channels to close in the plasma membrane of the photoreceptor cell. This produces a change in the voltage across the cell membrane, with the ultimate consequence that a nerve impulse is sent to the brain.

The signal is repeatedly amplified as it is relayed along this pathway (Figure 16–29). When lighting conditions are dim (as on a moonless night) the amplification is enormous, and as few as a dozen photons absorbed in 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 *adapts,* 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 generates an intracellular signal (a change in Ca^{2+} concentration) that inhibits the enzymes responsible for signal amplification.

Adaptation also occurs in signaling pathways that respond to chemical signals; again, it allows cells to remain sensitive to changes of signal intensity over a wide range of background levels of stimulation. Adaptation, in other words, allows a cell to respond to both messages that are whispered and those that are shouted.

In addition to vision, taste and smell also depend on G-protein–linked receptors. It seems likely that this mechanism of signal reception, invented early in the evolution of the eucaryotes, has its origins in the basic and universal need of cells to sense and respond to their environment. Of course, G-protein–linked receptors are not the

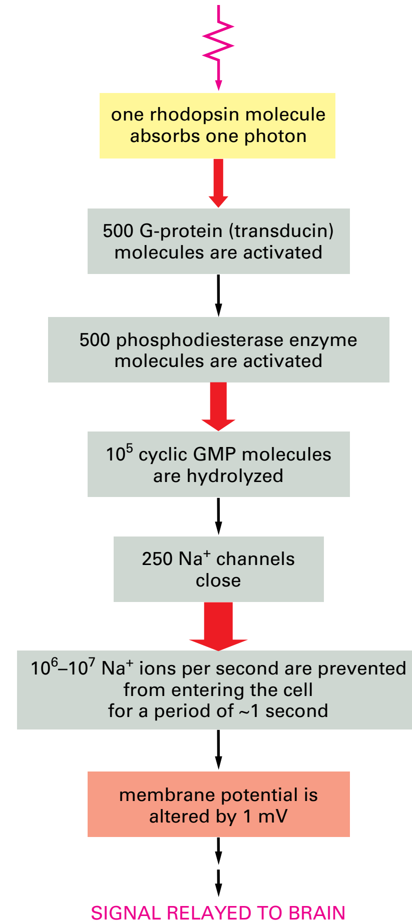


Figure 16–29 The light-induced signaling cascade in rod photoreceptor cells greatly amplifies the light signal. When rod photoreceptors are adapted for dim light, signal amplification is enormous. The intracellular signaling pathway from the G protein transducin uses components that differ from the ones previously described. The cascade functions as follows. In the absence of a light signal, the messenger molecule cyclic GMP (similar to cyclic AMP, with a guanine in place of adenine) is continuously produced in the photoreceptor cell and binds to Na^+ channels in the photoreceptor cell plasma membrane, keeping them open. Activation of rhodopsin by light results in formation of activated transducin α subunits. These activate an enzyme called cyclic nucleotide phosphodiesterase, which breaks down cyclic GMP to GMP. The sharp fall in the intracellular level of cyclic GMP causes the bound cyclic GMP to dissociate from the Na^+ channels, which therefore close. The *red arrows* indicate the steps at which amplification occurs.

only receptors that activate intracellular signaling cascades. We now turn to another class of cell-surface receptors that play a key part in controlling cell numbers, cell differentiation, and cell movement in multicellular animals.

Enzyme-linked Receptors

Like G-protein–linked receptors, enzyme-linked receptors are transmembrane proteins that display their ligand-binding domains on the outer surface of the plasma membrane. Instead of associating with a G protein, however, the cytoplasmic domain of the receptor acts as an enzyme—or forms a complex with another protein that acts as an enzyme. Enzyme-linked receptors (see Figure 16–14C) came to light through their role in responses to *growth factors*—the extracellular signal proteins that regulate the growth, proliferation, differentiation, and survival of cells in animal tissues (see Table 16–1, p. , for examples). Most growth factors act as local mediators and can act at very low concentrations (about 10^{-9} to 10^{-11} M). Responses to growth factors are typically slow (on the order of hours) and require many intracellular transduction steps that eventually lead to changes in gene expression.

Enzyme-linked receptors also mediate direct, rapid reconfigurations of the cytoskeleton, controlling the way a cell moves and changes its shape. The extracellular signals for these architectural alterations are often not diffusible growth factors, but proteins attached to the surfaces over which a cell is crawling. Disorders of cell growth, proliferation, differentiation, survival, and migration are fundamental to cancer, and abnormalities in signaling via enzyme-linked receptors play a major role in the initiation of this class of diseases.

The largest class of enzyme-linked receptors is made up of those with a cytoplasmic domain that functions as a tyrosine protein kinase, phosphorylating tyrosine side chains on selected intracellular proteins. Such receptors are called **receptor tyrosine kinases**. This category includes the great majority of growth factor receptors, on which we concentrate here.

Activated Receptor Tyrosine Kinases Assemble a Complex of Intracellular Signaling Proteins

To do its job as a signal transducer, an enzyme-linked receptor has to switch on the enzyme activity of its intracellular domain (or an associ-

Question 16–8

One important feature of any signaling cascade is its ability to turn off. Consider the cascade shown in Figure 16–29. Where would off switches be required? Which ones do you suppose are the most important?

ated protein) when an external signal molecule binds to its extracellular domain. Unlike the seven-pass G-protein–linked receptors, enzyme-linked receptor proteins usually have only one transmembrane segment, which is thought to span the lipid bilayer as a single α helix. There is, it seems, no way to transmit a conformational change through a single α helix, and so enzyme-linked receptors have a different strategy for transducing the extracellular signal. In many cases, the binding of a signal molecule causes two receptor molecules to come together in the membrane, forming a dimer. Contact between the two adjacent intracellular receptor tails activates their kinase function, with the result that each receptor phosphorylates the other. In the case of receptor tyrosine kinases, the phosphorylations occur on specific tyrosines located on the cytosolic tail of the receptors.

This phosphorylation then triggers the assembly of an elaborate intracellular signaling complex on the receptor tails. The newly phosphorylated tyrosines serve as binding sites for a whole zoo of intracellular signaling proteins—perhaps as many as 10 or 20 different molecules—which themselves can become activated upon binding. While it lasts, this protein complex transmits its signal along several routes simultaneously to many destinations inside the cell, thus activating and coordinating the numerous biochemical changes that are required to trigger a complex response, such as cell proliferation (Figure 16–30). To terminate the activation of the receptor, the cell contains *protein tyrosine phosphatases,* which remove the phosphates that were added in response to the extracellular signal. In other cases, activated receptors are disposed of in a more brutal way: they are dragged into the interior of the cell by endocytosis and then destroyed by digestion in lysosomes.

Different receptor tyrosine kinases recruit different collections of intracellular signaling proteins, producing different effects; but certain components seem to be used quite widely. These include, for example, a phospholipase that functions in the same way as phospholipase C to activate the inositol phospholipid signaling pathway (see Figure 16–25). They also include an important signaling enzyme called *phosphatidylinositol 3-kinase (PI 3-kinase),* which phosphorylates inositol phospholipids in the plasma membrane that then become docking sites for other intracellular signaling proteins. One of these signaling proteins is *protein kinase B (PKB),* which phosphorylates target proteins on serines and threonines and is especially important in signaling cells to survive and grow.

The main signaling pathway from receptor tyrosine kinases to the nucleus, however, takes another route. This pathway has become well known for a sinister reason: mutations that cause a runaway activation

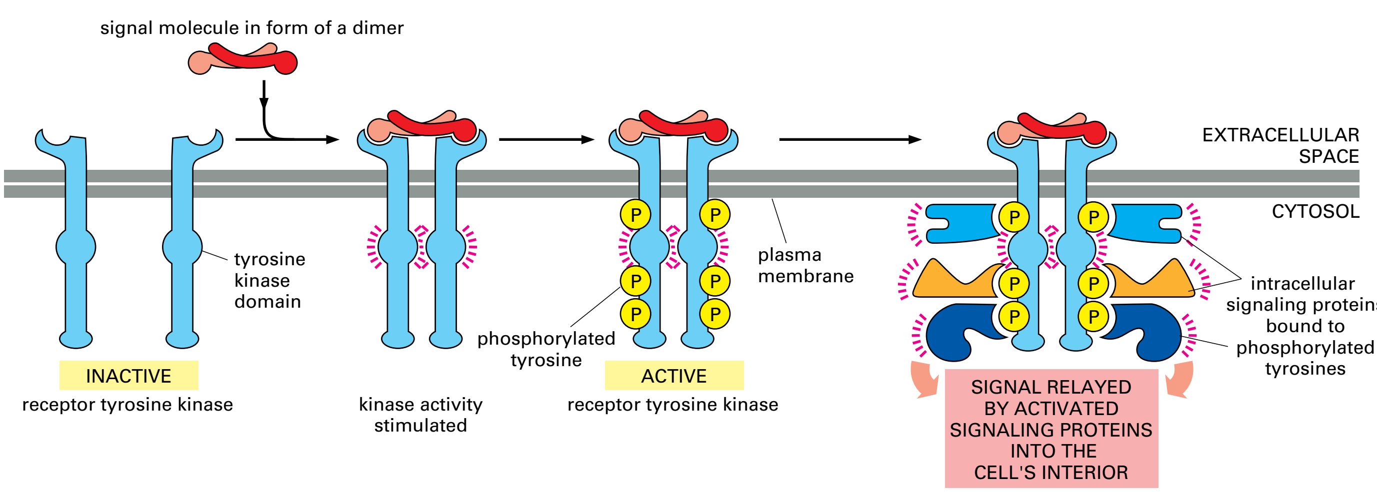


Figure 16–30 Activation of a receptor tyrosine kinase stimulates the assembly of an intracellular signaling complex. Typically, the binding of a signal molecule to the extracellular domain of a receptor tyrosine kinase causes two receptor molecules to associate into a dimer. The signal molecule shown here is itself a dimer and thus can physically cross-link two receptor molecules. In other cases, binding of the signal molecule changes the conformation of the receptor molecules in such a way that they dimerize. Dimer formation brings the kinase domains of each intracellular receptor tail into contact with the other; this activates the kinases and enables them to phosphorylate each other on several tyrosine side chains. Each phosphorylated tyrosine serves as a specific binding site for a different intracellular signaling protein, which then helps relay the signal to the cell's interior.

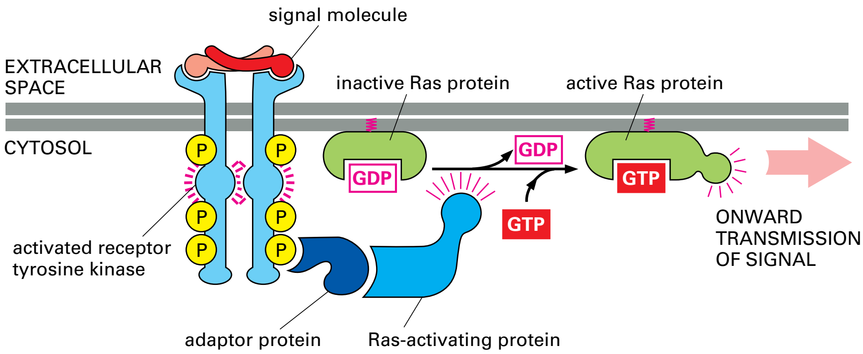


Figure 16–31 Receptor tyrosine kinases activate Ras. An adaptor protein docks on a particular phosphotyrosine on the activated receptor (the other signaling proteins that are shown bound to the receptor in Figure 16–30 are omitted for simplicity). The adaptor recruits and stimulates a protein accomplice that functions as a Ras-activating protein. This protein in turn stimulates Ras to exchange its bound GDP for GTP. The activated Ras protein then stimulates the next steps in the signaling pathway, one of which is shown in Figure 16–32. Note that the Ras protein contains a covalently attached lipid group *(red)* that helps anchor the protein to the plasma membrane.

of this signaling cascade— thereby stimulating cell division inappropriately—help trigger many types of cancers. We shall conclude our discussion of receptor tyrosine kinases by tracing this pathway from the receptor to the nucleus.

Receptor Tyrosine Kinases Activate the GTP-binding Protein Ras

As mentioned earlier, activated receptor tyrosine kinases recruit many kinds of intracellular signaling proteins. Some of these proteins function solely as physical *adaptors;* they help build a large signaling aggregate by coupling the receptor to other proteins, which in turn may bind to and activate yet other proteins that pass the message along. One of the key players in these adaptor-assembled signaling complexes is **Ras**—a small protein that is bound by a lipid tail to the cytoplasmic face of the plasma membrane (Figure 16–31). Virtually all receptor tyrosine kinases activate Ras, from the platelet-derived growth factor (PDGF) receptors that mediate cell proliferation in wound healing to the nerve growth factor (NGF) receptors that prevent certain neurons from dying in the developing nervous system.

The Ras protein is a member of a large family of small, single-subunit GTP-binding proteins, often called the *monomeric GTP-binding proteins* to distinguish them from the *trimeric G proteins* that we encountered earlier in this chapter. 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 (see Figure 16–15B). Interaction with an activating protein encourages Ras to exchange its GDP for GTP, thus switching Ras to its activated state. After a delay, Ras switches itself off again by hydrolyzing its GTP to GDP.

In its active state, Ras promotes the activation of a phosphorylation cascade in which a series of protein kinases phosphorylate and activate one another in sequence, like an intracellular game of dominoes (Figure 16–32). This relay system, which carries the signal from the plasma membrane to the nucleus, is called a **MAP-kinase cascade**, in honor of the final kinase in the chain **MAP-kinase** (*m*itogen-*a*ctivated *p*rotein kinase). In this cascade, MAP-kinase is phosphorylated and activated by an enzyme called, logically enough, *MAP-kinase-kinase*. And this protein is itself switched on by a *MAP-kinase-kinase-kinase* (which is activated by Ras). At the end of the signal cascade, MAP-kinase phosphorylates certain gene regulatory proteins on serines and threonines, altering their ability to control gene transcription and thereby causing a change in the pattern of gene expression. This shift 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

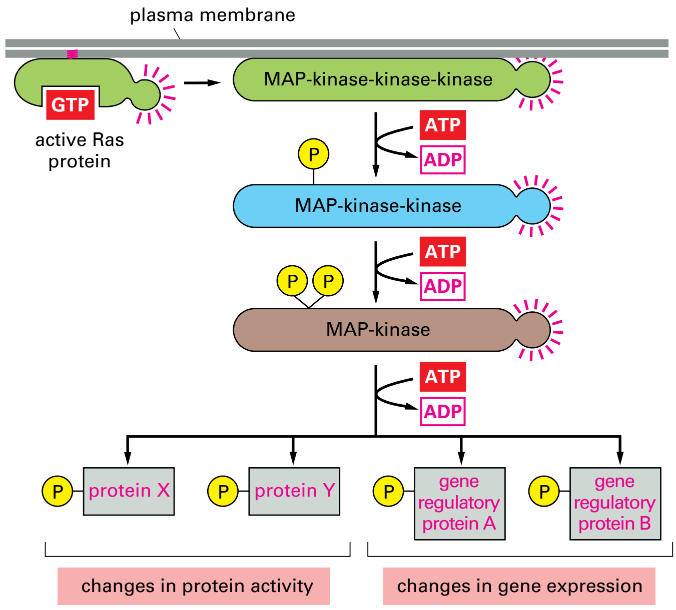


Figure 16–32 Ras activates a MAP-kinase phosphorylation cascade. A Ras protein activated by the process shown in Figure 16–31 triggers a phosphorylation cascade of three protein kinases, which relay and distribute the signal. The final kinase in the cascade, MAP-kinase, phosphorylates various downstream target proteins. These targets can include other protein kinases and, most important, gene regulatory proteins that control gene expression. Changes in gene expression and protein activity result in complex changes in cell behaviors such as proliferation and differentiation—typical outcomes of the Ras/MAP-kinase signaling pathway.

and what other signals the cell receives. How researchers unravel such complex signaling cascades is discussed in How We Know, pp. ●–●.

The importance of Ras has been demonstrated in various ways. If Ras is inhibited by an intracellular injection of Ras-inactivating antibodies, for example, a cell may no longer respond to the growth factors it would normally recognize. Conversely, if Ras activity is permanently switched on, the cell may act as if it is being bombarded continuously by growth factor. Before it was discovered in normal cells, the Ras protein was found in human cancer cells, in which a mutation in the gene for Ras caused the production of such a hyperactive form of Ras. This mutant Ras protein helps stimulate the cells to divide even in the absence of growth factors. The resulting uncontrolled cell proliferation contributes to the formation of cancer.

About 30% of human cancers contain such activating mutations in *ras* genes, and many other cancers have mutations in genes whose products lie in the same signaling pathway as Ras. Many of the genes that encode these intracellular signaling proteins were identified in the quest for cancer-promoting *oncogenes,* which are discussed in Chapter 21. The normal versions of the genes—which encode the signaling proteins essential for proper cell function—are often known as *proto-oncogenes,* because they are capable of being converted into oncogenes by mutation.

Cancer is a disease in which cells in the body behave in a selfish and antisocial way—destroying the harmony of the multicellular organism by proliferating when they should not and invading tissues that they should not enter. The molecular derangements responsible for such unruly behavior are discussed more fully in Chapter 21. But it seems appropriate to note here that the common occurrence in cancer of mutations in genes for cell-signaling components reflects a familiar truth: maintaining order in a complex, integrated community depends above all on good communication.

Question 16–9

Would you expect to activate G-protein–linked receptors and receptor tyrosine kinases by exposing cells to antibodies raised to the respective proteins? (Hint: review Panel 4–6, on pp. ●–●, regarding the properties of antibody molecules.)

Some Enzyme-linked Receptors Activate a Fast Track to the Nucleus

Not all enzyme-linked receptors trigger complex signaling cascades that require the cooperation of a sequence of protein kinases to carry a mes-

How We Know: Untangling Cell Signaling Pathways

Intracellular signaling pathways are never mapped out in a single experiment. 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 such as insulin. The process involves breaking down the broad questions about how a cell responds to the signal into smaller, more manageable questions: Which protein is the insulin receptor? Which intracellular proteins become activated when insulin is present? With which proteins do these activated proteins interact? How does one protein activate another? Here we discuss the kinds of experiments that provide answers to such riddles.

Stimulation

When cells are exposed to an extracellular signal molecule, one result is that a number of proteins become phosphorylated. Some of these will be the intracellular signaling proteins responsible for propagating the message throughout the cell; others will be target proteins responsible for the cell's response. To determine which molecules have been activated by phosphorylation, researchers break open the cells, separate the proteins by size on a gel (see Chapter 4, Panels 4–3 to 4–5), and then use antibodies to detect phosphorylated proteins.

Another common way to visualize newly phosphorylated proteins involves supplying cells with a radioactive version of ATP while they are being exposed to an extracellular signal molecule. Protein kinases activated by the signal will transfer radioactive phosphate from the labeled ATP to their protein substrates. Again, the cell proteins are separated on a gel, but then the radiolabeled proteins are detected by exposing the gel to an X-ray film.

Contact

Once the activated proteins have been identified, one can determine which proteins interact with them. To identify interacting proteins, scientists often make use of *immunoprecipitation.* In this technique, antibodies are used to latch onto a specific protein, dragging it out of solution and down to the bottom of a test tube (see Chapter 4, panel 4–6). If the captured protein happens to be bound to other proteins, these will be dragged down as well. In this way, researchers can identify which proteins interact when cells are stimulated by a signal molecule.

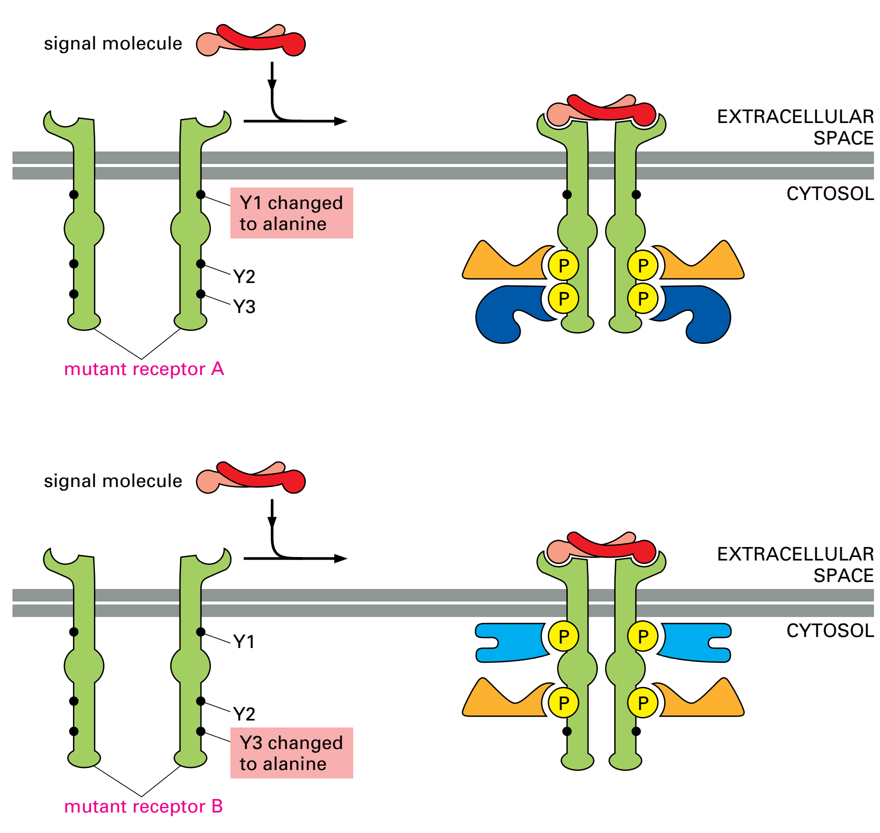


Figure 16–33 Mutant proteins can help to determine exactly where an intracellular signaling molecule binds. As shown in Figure 16–30, on binding their signal molecule, a pair of receptor tyrosine kinases come together and phosphorylate specific tyrosines on each other's cytoplasmic tails. These phosphorylated tyrosines attract different intracellular signaling molecules, which then become activated and pass on the signal. To determine which tyrosine binds to a specific intracellular signaling molecule, a series of mutant receptors are constructed. In the mutants shown single tyrosines (Y1 or Y3) have been substituted by an alanine. As a result, the mutant receptors no longer bind to one of the intracellular signaling proteins. The effect on the cell's response to the signal can then be determined. It is important that the mutant receptor be tested in a cell that does not have its own normal receptors for the signal molecule.

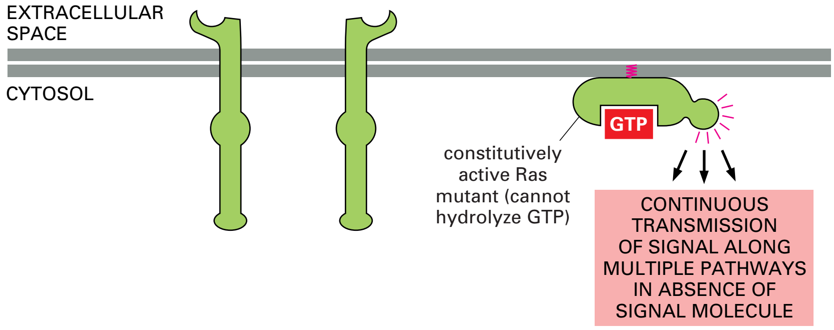


Figure 16–34 A constitutively active form of Ras transmits a signal even in the absence of a signal molecule. As shown in Figure 16–31, the normal Ras protein is activated in response to certain extracellular signals. The overactive form of Ras shown here has lost the ability to hydrolyze GTP. Thus it cannot shut down its activity and, as a result, is constantly active.

Once two proteins are known to bind to each other, the experimenter can proceed to pinpoint which parts of the proteins are required for the interaction. This often involves constructing a set of mutant proteins, each of which differs slightly from the normal one. To determine which phosphorylated tyrosine on a receptor tyrosine kinase a certain intracellular signaling protein binds to, for example, a series of mutant receptors is used, each missing a different tyrosine from its cytoplasmic domain (Figure 16–33). In this way, the specific tyrosines required for binding can be determined. Similarly, one can determine whether this tyrosine docking site is required for the receptor to transmit a signal to the cell.

Response

Ultimately, one wants to assess how important a particular protein is for a signaling process. A first test involves using recombinant 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. Take Ras, for example. The oncogenic form of the protein is constantly active because it has lost its ability to hydrolyze the GTP that keeps it switched on. This continuously active form of Ras can stimulate some cells to proliferate even in the absence of growth factors, which is one way it contributes to cancer (Figure 16–34).

The ultimate test of the importance of an intracellular protein in a signaling pathway is to inactivate the protein or its gene and see if the signaling pathway is affected. In the case of Ras, for example, one can introduce into cells a "dominant negative" mutant form of Ras. This disabled form of Ras clings too tightly to GDP, and therefore cannot be activated. Because it can still bind to other signaling partners in the pathway, it jams the pathway, preventing normal copies of Ras from doing their job. Such stalled cells do not proliferate in response to extracellular growth factors, indicating the importance of normal Ras signaling in the proliferative response.

Assembly

Most signaling pathways take decades to untangle. 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 is still incompletely understood.

One powerful strategy that scientists use to identify proteins that participate in cell signaling involves screening a massive number of animals—usually tens of thousands of fruit flies or nematode worms that have been treated with a mutagen. They are looking for mutants in which a signaling pathway is not functioning properly. Flies and worms are useful because they reproduce rapidly and can be maintained in vast numbers in the laboratory. By examining enough mutant animals, many of the genes that encode the proteins involved in a signaling cascade can be identified—including receptors, protein kinases, gene regulatory proteins, and so on.

Such genetic scans can also reveal the order in which signaling proteins act in a pathway. Imagine that the genetic screen reveals two new proteins X and Y in the Ras signaling pathway (Figure 16–35A). If insertion of a gene that encodes a continuously active version of Ras "rescues" the signaling pathway in cells in which a defective protein X was blocking the pathway, then Ras must operate downstream of X in the signaling cascade (Figure 16–35B). If Ras operates upstream of protein Y in the pathway, a constantly active Ras would be unable to transmit a signal past the obstruction caused by the disabled protein Y (Figure 16–35C).

Used together, these biochemical and genetic techniques allow even the most complex intracellular signaling pathways to be dissected.

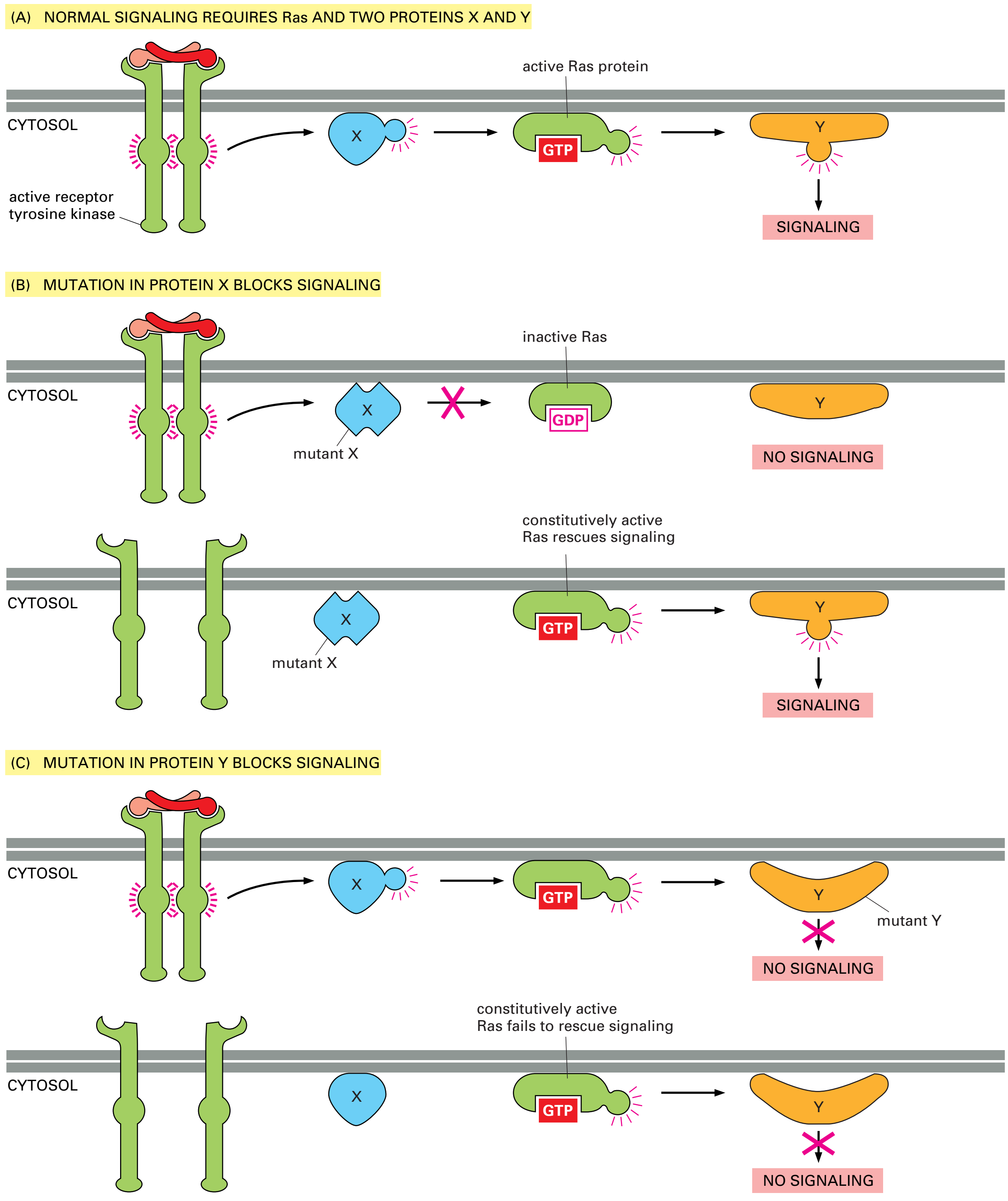


Figure 16–35 Genetic analysis reveals the order in which signaling proteins act in a pathway. A signaling pathway can be inactivated by mutations in any one of its components. Here we show how a Ras signaling pathway (A) can be shut down by a mutation in either protein X (B) or protein Y (C). Addition of a constitutively active form of Ras to these cells can help to unravel where in the pathway the mutant proteins lie. Adding a continuously active Ras to cells with a mutation in X restores activity to the pathway, allowing the signal to be transmitted even in the absence of the signaling molecule (B). An overactive Ras can rescue these cells because Ras lies downstream of the mutant protein X that is jamming the pathway. Adding a continuously active Ras to cells with a mutation in protein Y has no effect, as Ras lies upstream of the blockage (C).

sage to the nucleus. Some receptors use a more direct route to control gene expression.

A few hormones and many local mediators—also called *cytokines*—bind to receptors that can activate gene regulatory proteins that are held in a latent state at the plasma membrane. Once activated, these regulatory proteins head straight for the nucleus, where they stimulate the transcription of specific genes. This direct signaling pathway is used, for example, by *interferons*, which are cytokines that instruct cells to produce proteins that will make them more resistant to viral infection. Unlike the receptor tyrosine kinases that stimulate elaborate signaling cascades, **cytokine receptors** have no intrinsic enzyme activity. Instead, they are associated with cytoplasmic tyrosine kinases called *JAKs* that are activated when a cytokine binds to its receptor. Once activated, the kinases phosphorylate and activate cytoplasmic gene regulatory proteins called STATs, which then migrate to the nucleus, where they stimulate transcription of specific target genes (Figure 16–36).

Different cytokine receptors evoke different cellular responses by activating different STATs. Like any pathway that is turned on by phosphorylation, the cytokine signal is shut off by protein phosphatases that remove the phosphate groups from the activated signaling proteins.

An even more direct signaling pathway is used by another class of enzyme-linked receptors that resemble receptor tyrosine kinases. These are receptor **serine/threonine kinases** that directly phosphorylate and activate cytoplasmic gene regulatory proteins (called SMADs) when stimulated by an extracellular signal molecule (Figure 16–37). The hormones and local mediators that activate these receptors belong to the *TGF-β superfamily* of extracellular proteins, which play an especially important role in animal development.

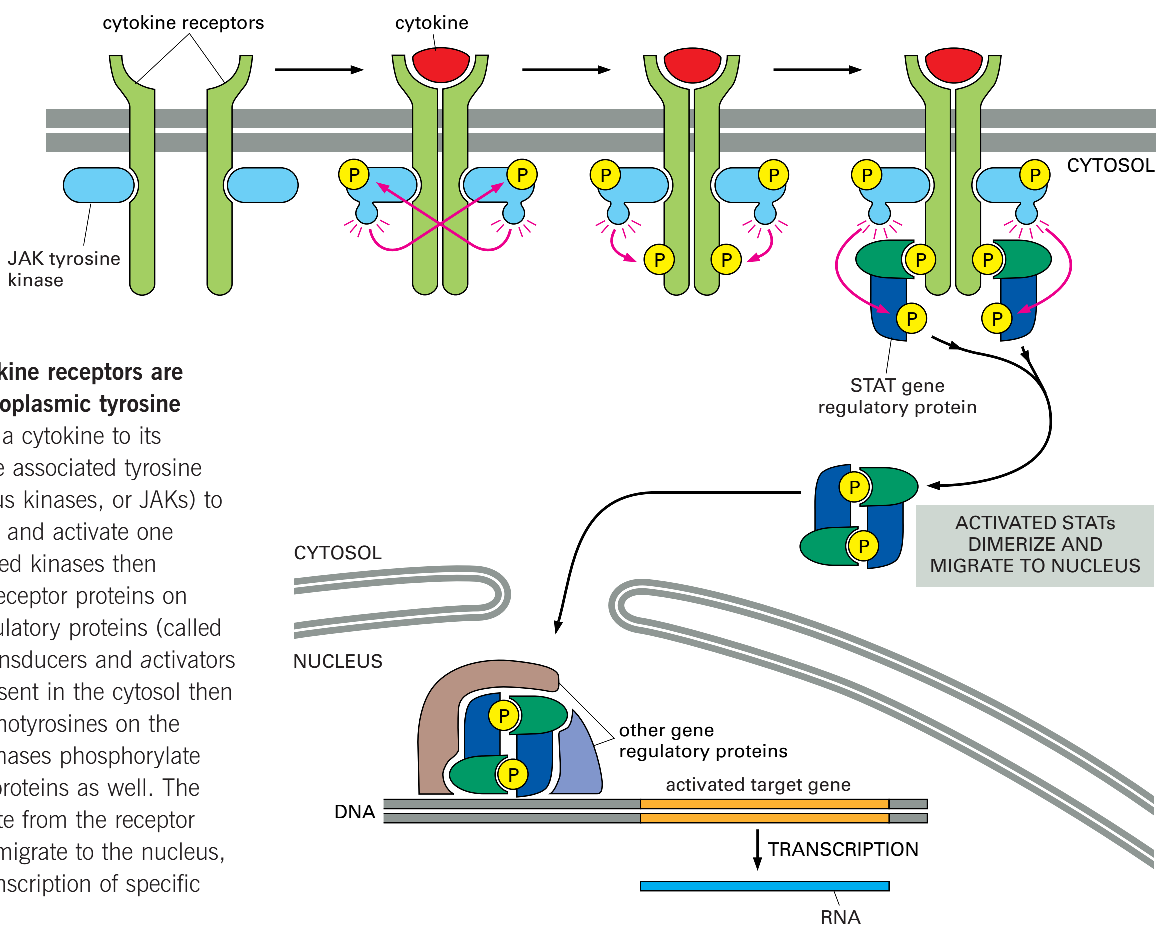


Figure 16–36 Cytokine receptors are associated with cytoplasmic tyrosine kinases. Binding of a cytokine to its receptors causes the associated tyrosine kinases (called Janus kinases, or JAKs) to cross-phosphorylate and activate one another. The activated kinases then phosphorylate the receptor proteins on tyrosines. Gene regulatory proteins (called STATs, for *s*ignal *t*ransducers and *a*ctivators of *t*ranscription) present in the cytosol then attach to the phosphotyrosines on the receptor, and the kinases phosphorylate and activate these proteins as well. The STATs then dissociate from the receptor proteins, dimerize, migrate to the nucleus, and activate the transcription of specific target genes.

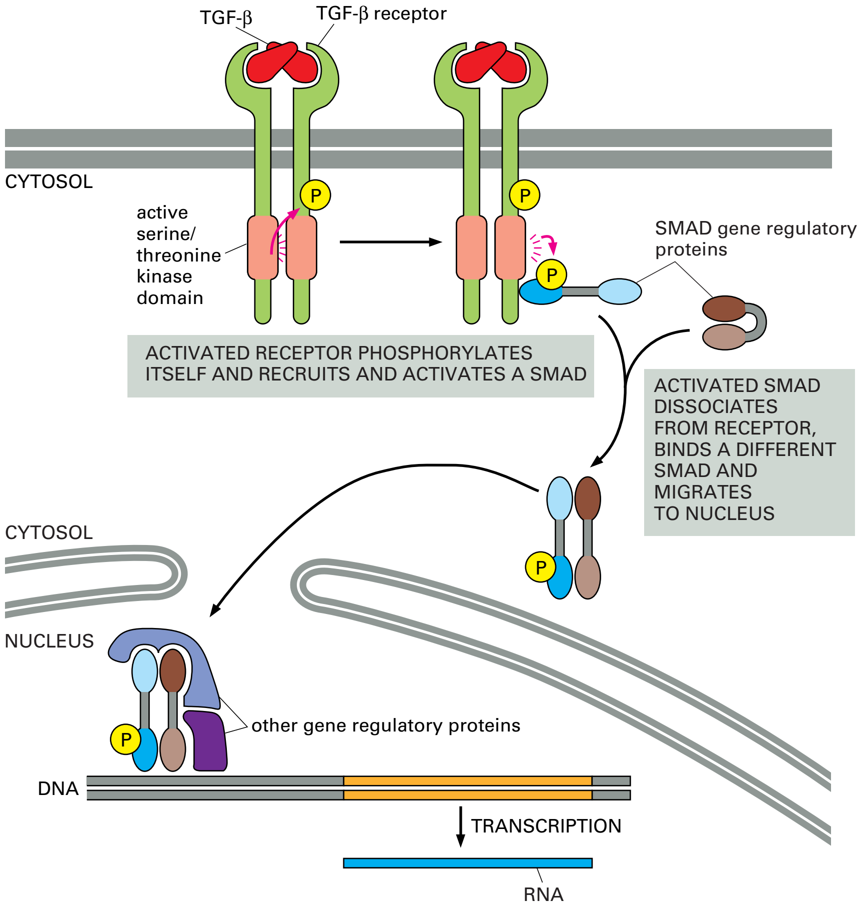


Figure 16–37 TGF-β receptors activate gene regulatory proteins directly at the plasma membrane. These receptor serine/threonine kinases phosphorylate themselves and then recruit and activate cytoplasmic gene regulatory proteins (called SMADs, after the related proteins *Sma* in nematodes and *Mad* in flies). The SMADs then dissociate from the receptors and bind to other SMADs, and the complexes then migrate to the nucleus, where they stimulate transcription of specific target genes. TGF-β stands for transforming growth factor-β.

Protein Kinase Networks Integrate Information to Control Complex Cell Behaviors

In this chapter, we have outlined several major pathways for conveying a signal from the cell surface to the cell interior. Figure 16–38 compares four of these pathways: the routes from G-protein–linked receptors via adenylyl cyclase and via phospholipase C, and the routes from enzyme-linked receptors via phospholipase C and via Ras. Each pathway differs from the others, yet they use common components to transmit their signals. Because all these pathways eventually activate protein kinases, it seems that each is capable in principle of regulating practically any process in the cell.

In fact, the complexity of cell signaling is much greater than we have described. First, we have not presented every intracellular signaling pathway available to cells; second, the major signaling cascades we have introduced interact in ways that we have not described. They are connected by interactions of many sorts, but the most extensive links are those mediated by the protein kinases present in each of the pathways. These kinases often phosphorylate, and hence regulate, components in other signaling pathways in addition to components in the pathway to which they themselves belong. Thus, a certain amount of *cross talk* occurs among the different pathways (see Figure 16–38), and indeed between virtually all the control systems of the cell. To give an idea of the scale of these regulatory systems, genome sequencing studies suggest that about 2% of our genes code for protein kinases; moreover, hundreds of distinct types of protein kinases are thought to be present in a single mammalian cell. How can we make sense of this tangled web of interacting signaling pathways, and what is the function of such complexity?

Question 16–10

If cell-surface receptors can rapidly signal to the nucleus by activating latent transcription factors such as STATs and SMADs at the plasma membrane, why do most cell-surface receptors use long, indirect signaling cascades to influence gene transcription in the nucleus?

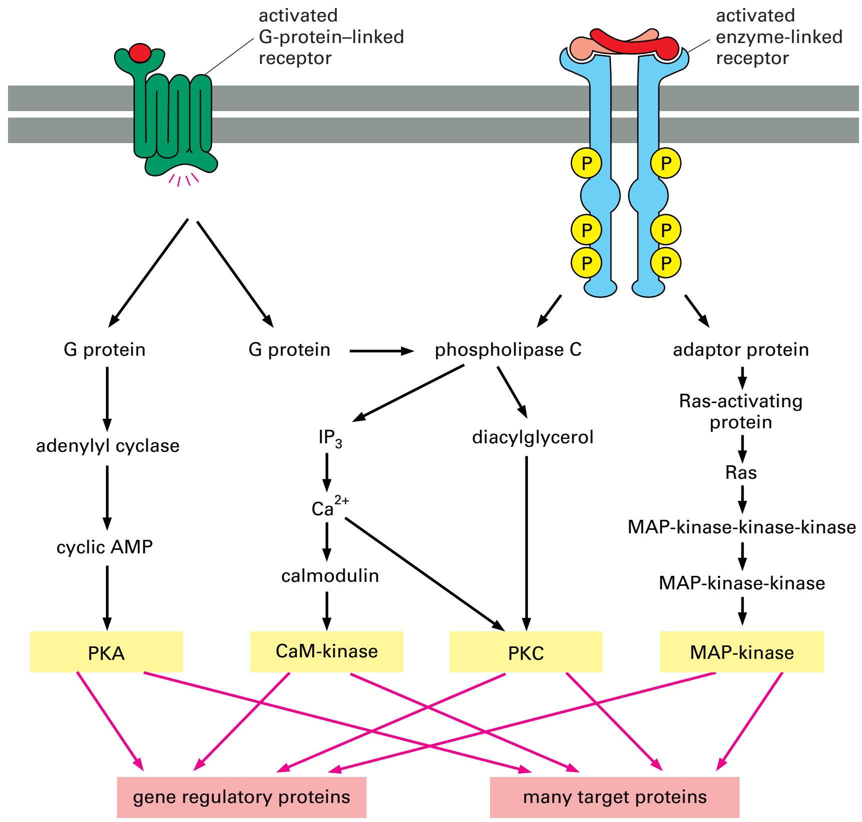


Figure 16–38 Signaling pathways can be highly interconnected. The diagram sketches the pathways from G-protein–linked receptors via adenylyl cyclase and via phospholipase C, and from enzyme-linked receptors via phospholipase C and via Ras. The protein kinases in these pathways phosphorylate many proteins, including proteins belonging to the other pathways. The resulting dense network of regulatory interconnections is symbolized by the *red arrows* radiating from each kinase shaded in *yellow;* some kinases phosphorylate some of the same target proteins.

A cell receives messages from many sources, and it must integrate this information to generate an appropriate response—to live or die, to divide or differentiate, to change shape, relocate, or send out a chemical message of its own. Through the cross talk between signaling pathways, the cell is able to put two or more bits of information together and react to the combination. Thus, some intracellular signaling proteins act as integrating devices, usually by having several potential phosphorylation sites, each of which can be phosphorylated by a different protein kinase. Information received from different sources can converge on such proteins, which then convert the input to a single outgoing signal (Figure 16–39). The 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 exploration of the pathways that cells use to process signals from their environment has led us from receptors on the cell surface to the proteins that form the elaborate control systems that operate deep within the cell's interior. We have examined a large array of signaling networks, which allow cells to combine and process inputs from different sources, store information, and respond in an appropriate manner that benefits the organism. But our understanding of these intricate networks is still evolving: we are still discovering new links in the chain, new signaling partners, new connections, and even new pathways. And while we still have much to learn about signaling pathways in animal cells, we know even less about such pathways in plants.

Multicellularity and Cell Communication Evolved Independently in Plants and Animals

Plants and animals have been evolving independently for more than a billion years, the last common ancestor being a single-celled eucaryote 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 multicellular functioning. Thus, the mechanisms for cell–cell communication in plants and animals evolved separately and would be expected to be quite different. At the same time, however, plants and animals started with a common set of eucaryotic genes—including some used by single-cell organisms to communicate among themselves—and so their signaling systems should show some similarities.

A striking resemblance occurs at the cell surface. Like animals, plants make extensive use of membrane-embedded cell-surface receptors—especially enzyme-linked receptors. The spindly weed *Arabidopsis*—a plant studied by many present-day biologists—has hundreds of genes encoding receptor serine/threonine kinases, which are structurally distinct from those found in animal cells (see Figure 16–37). Such receptors are thought to play an important part in a large variety of plant cell signaling processes, including those governing growth, development, and disease resistance. In contrast to animal cells, plant cells seem not to use receptor tyrosine kinases, steroid-hormone–type nuclear receptors, or cyclic AMP, and they seem to use few G-protein–linked receptors.

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. Ethylene receptors are related to the proteins that bacteria use to locate nutrients or flee from poisons. Like the bacterial proteins, they function as histidine kinases and are unlike any receptor proteins yet found in animal cells. Activated ethylene receptors activate a MAP-kinase cascade that is similar to MAP-kinase cascades found in animal cells—presumably reflecting the common ancestry of plants and animals. For most of the receptor kinases in plants, however, the signal transduction pathways that link receptor activation to a cell response are not yet known.

Unraveling cell signaling pathways is an active area of research, and new discoveries are being made in both plant and animal systems every day. Genome sequencing projects are providing long lists of components involved in signal transduction in a large variety of organisms.

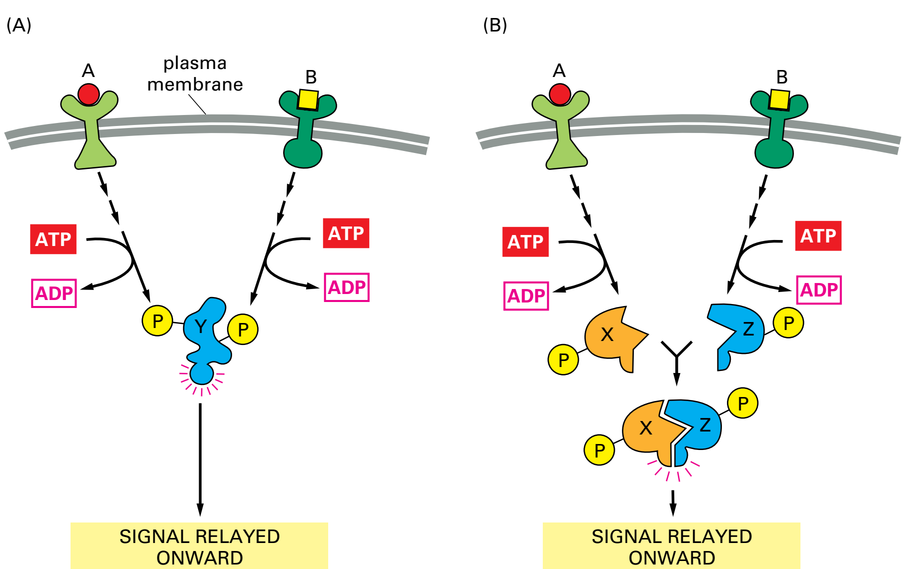


Figure 16–39 Some intracellular signaling proteins serve to integrate incoming signals. Signals A and B may activate different cascades of protein phosphorylations, each of which leads to the phosphorylation of protein Y but at different sites on the protein (A). Protein Y is activated only when both of these sites are phosphorylated, and therefore it is active only when signals A and B are simultaneously present. Alternatively, signals A and B could lead to the phosphorylation of two proteins, X and Z, which then bind to each other to create the active protein XZ (B).

Even when we have identified all the pieces, it will remain a major challenge to figure out exactly how they fit together to allow cells to integrate the diverse array of signals in their environment and respond in appropriate ways.

Essential Concepts

- Cells in multicellular organisms communicate through a large variety of extracellular chemical signals.
- Hormones are carried in the blood to distant target cells, but most other extracellular signal molecules act over only a short range. Neighboring cells often communicate through direct cell-surface contacts.
- Cells are stimulated by an extracellular signal molecule when it binds to and activates a receptor protein. Each receptor protein recognizes a particular signal molecule.
- Receptor proteins act as transducers, converting the signal from one physical form to another.
- Most extracellular signal molecules cannot pass through the plasma membrane; they bind to cell-surface receptor proteins that transduce the extracellular signal into different intracellular signals.
- Small hydrophobic extracellular signal molecules such as steroid hormones and nitric oxide can diffuse directly across the plasma membrane; they activate intracellular receptor proteins, which are either gene regulatory proteins or enzymes.
- There are three main classes of cell-surface receptors: (1) ion-channel–linked receptors, (2) G-protein–linked receptors, and (3) enzyme-linked receptors.
- G-protein–linked receptors and enzyme-linked receptors respond to extracellular signals by initiating cascades of intracellular signaling reactions that alter the behavior of the cell.
- G-protein–linked receptors activate a class of trimeric GTP-binding proteins called G proteins, which act as molecular switches, transmitting the signal onward for a short period and then switching themselves off by hydrolyzing their bound GTP to GDP.
- Some G proteins directly regulate ion channels in the plasma membrane. Others activate the enzyme adenylyl cyclase, increasing the intracellular concentration of cyclic AMP. Still other G proteins activate the enzyme phospholipase C, which generates the messenger molecules inositol trisphosphate (IP_3) and diacylglycerol.
- IP_3 opens ion channels in the membrane of the endoplasmic reticulum, releasing a flood of free Ca^{2+} ions into the cytosol. Ca^{2+} itself acts as an intracellular messenger, altering the activity of a wide range of proteins.
- A rise in cyclic AMP activates protein kinase A (PKA), while Ca^{2+} and diacylglycerol in combination activate protein kinase C (PKC).
- PKA and PKC phosphorylate selected target proteins on serines and threonines, thereby altering protein activity. Different cell types contain different sets of target proteins and are affected in different ways.
- In general, stimulation of G-protein–linked receptors produces rapid and reversible cell responses.
- Many enzyme-linked receptors have intracellular protein domains that function as enzymes; most are receptor tyrosine kinases, which are activated by growth factors and phosphorylate tyrosines on selected intracellular proteins.
- Activated receptor tyrosine kinases cause the assembly of an intracellular signaling complex on the intracellular tail of the receptor; a

part of this complex serves to activate Ras, a small GTP-binding protein, which activates a cascade of protein kinases that relay the signal from the plasma membrane to the nucleus.

- Mutations that stimulate cell proliferation by making Ras constantly active are a common feature of many cancers.
- Some enzyme-linked receptors activate a direct pathway to the nucleus. Instead of activating signaling cascades, they turn on gene regulatory proteins right at the plasma membrane.
- The different intracellular signaling pathways interact, enabling cells to produce an appropriate response to a complex combination of signals. Some combinations of signals enable a cell to survive; other combinations of signals will cause it to proliferate; and in the absence of any signals, most cells will kill themselves by undergoing apoptosis.
- Plants, like animals, use enzyme-linked cell-surface receptors to control their growth and development.

Key Terms

adaptation
adenylyl cyclase
Ca^{2+}/calmodulin-dependent protein kinases (CaM-kinases)
calmodulin
cell signaling
cyclic AMP
cyclic-AMP–dependent protein kinase (PKA)
cytokine receptor
diacylglycerol (DAG)
G-protein–linked receptor
hormone
inositol phospholipid
inositol 1,4,5-trisphosphate (IP_3)
local mediator
MAP-kinase
MAP-kinase cascade
molecular switch
neurotransmitter
nitric oxide (NO)
nuclear receptor
phospholipase C
protein kinase C (PKC)
Ras
receptor
receptor protein
receptor serine/threonine kinase
receptor tyrosine kinase
second messenger
serine/threonine kinase
signal transduction
signaling cascade
steroid hormone
tyrosine kinase

Questions

Question 16–11

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

A. The signal molecule acetylcholine has different effects on different cell types in an animal and binds to different 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 βγ complexes—but not GDP-bound, fully assembled G proteins—activate other molecules downstream of G-protein–linked receptors.

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. *ras* is an oncogene.

H. Tyrosine phosphorylation serves to build binding sites for other proteins to bind to receptor tyrosine kinases.

Question 16–12

The Ras protein functions as a molecular switch that is set to its on state by other proteins that cause it to bind GTP. A GTPase-activating protein resets 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 given a mutant cell that lacks the GTPase-activating protein. What abnormalities would you expect to find in the way that Ras activity responds to extracellular signals?

Question 16–13

A. Compare and contrast signaling by neurons to that carried out by endocrine cells, which secrete hormones.

B. Discuss the relative advantages of the two mechanisms.

Question 16–14

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

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 16–15

"One of the great kings of the past ruled an enormous kingdom that was more beautiful than anywhere else in the world. Every plant glistened as brilliantly as polished jade, and the softly rolling hills were as sleek as the waves of the summer sea. The wisdom of all of his decisions relied on a constant flow of information brought to him daily by messengers who told him about every detail of his kingdom so that he could take quick, appropriate actions whenever in need. Despite all the abundance of beauty and efficiency, his people felt doomed to live under his rule, for he had an adviser who had studied cellular signal transduction and accordingly administered the king's Department of Information. The adviser had implemented the policy that all messengers shall be immediately beheaded whenever spotted by the Royal Guard, because for rapid signaling the lifetime of messengers ought to be short. Their plea 'Don't hurt me, I'm only the messenger!' was to no avail, and the people of the kingdom suffered terribly because of the rapid loss of their sons and daughters." Why is the analogy on which the king's adviser bases his policies inappropriate? Briefly discuss the features that set cell signaling pathways apart from the human communication pathway described in the story.

Question 16–16

In a series of experiments, genes that code for mutant forms of a receptor tyrosine kinase 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 they are expressed at considerably higher levels than the normal gene. What would be the consequences of introducing a mutant gene that codes for a receptor tyrosine kinase (A) lacking its extracellular domain, or (B) lacking its intracellular domain?

Question 16–17

Discuss the following statement: "Membrane proteins that span the membrane many times can undergo a conformation 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 16–18

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 16–19

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

Question 16–20

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 purpose of such regulation?

Question 16–21

The contraction of the myosin–actin system in muscle cells is triggered by a rise in intracellular Ca^{2+}. Muscle cells have specialized Ca^{2+}-release channels—called ryanodine receptors because of their sensitivity to the drug ryanodine—that lie 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 16–25, the ligand that opens ryanodine receptors is Ca^{2+} itself. Discuss the consequences of ryanodine channels for muscle cell contraction.

Question 16–22

Two protein kinases, K1 and K2, function sequentially in an intracellular signaling cascade. 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 when no signal is received by these cells. In the normal signaling pathway, does K1 activate K2 or does K2 activate K1? Explain your answer.

Question 16–23

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 the shortest and most direct pathway from the cell surface to the nucleus.

Question 16–24

Why do you think animal cells and plant cells have such different intracellular signaling mechanisms and yet share some common mechanisms?