Mechanisms of Cell Communication

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To make a multicellular organism, cells must communicate, just as humans must communicate if they are to organize themselves into a complex society. And just as human communication involves more than the passage of noises from mouth to ear, so cell–cell communication involves more than the transmission of chemical signals across the space between one cell and another. Complex intracellular mechanisms are needed to control which signals are emitted at what time and to enable the signal-receiving cell to interpret those signals and use them to guide its behavior. According to the fossil record, sophisticated multicellular organisms did not appear on Earth until unicellular organisms resembling present-day procaryotes had already been in existence for about 2.5 billion years. The long delay may reflect the difficulty of evolving the language systems of animal, plant, and fungal cells—the machinery that would enable cells sharing the same genome to collaborate and coordinate their behavior, specializing in different ways and subordinating their individual chances of survival to the interests of the multicellular organism as a whole. These highly evolved mechanisms of cell–cell communication are the topic of this chapter.

Communication between cells is mediated mainly by **extracellular signal molecules**. Some of these operate over long distances, signaling to cells far away; others signal only to immediate neighbors. Most cells in multicellular organisms both emit and receive signals. Reception of the signals depends on *receptor proteins*, usually (but not always) at the cell surface, which bind the signal molecule. The binding activates the receptor, which in turn activates one or more *intracellular signaling pathways*. These relay chains of molecules—mainly *intracellular signaling proteins*—process the signal inside the receiving cell and distribute it to the appropriate intracellular targets. These targets are generally *effector proteins*, which are altered when the signaling pathway is activated and implement the appropriate change of cell behavior. Depending on the signal and the nature and state of the receiving cell, these effectors can be gene regulatory proteins, ion channels, components of a metabolic pathway, or parts of the cytoskeleton—among other things (**Figure 15–1**).

We begin this chapter by discussing the general principles of cell communication. We then consider, in turn, the main families of cell-surface receptor proteins and the principal intracellular signaling pathways they activate. The main focus of the chapter is on animal cells, but we end by considering the special features of cell communication in plants.

In This Chapter

GENERAL PRINCIPLES OF CELL COMMUNICATION

Long before multicellular organisms appeared on Earth, unicellular organisms had developed mechanisms for responding to physical and chemical changes in their environment. These almost certainly included mechanisms for response to the presence of other cells. Evidence comes from studies of present-day unicellular organisms such as bacteria and yeasts. Although these cells largely lead independent lives, they can communicate and influence one another's behavior. Many bacteria, for example, respond to chemical signals that are secreted by their neighbors and increase in concentration with increasing population density. This

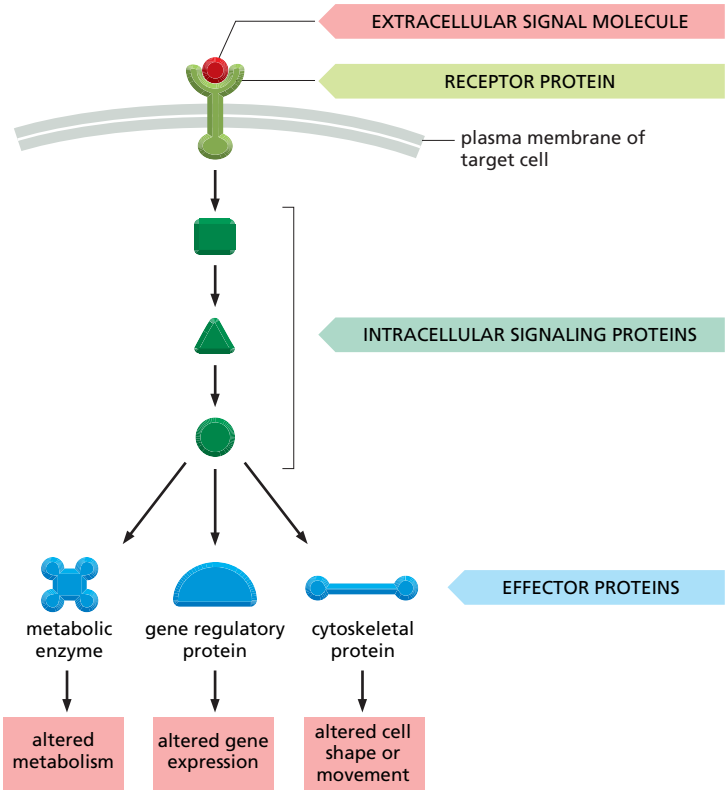

Figure 15–1 A simple intracellular signaling pathway activated by an extracellular signal molecule. The signal molecule usually binds to a receptor protein that is embedded in the plasma membrane of the target cell and activates one or more intracellular signaling pathways mediated by a series of signaling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

process, called *quorum sensing*, allows bacteria to coordinate their behavior, including their motility, antibiotic production, spore formation, and sexual conjugation.

Similarly, yeast cells communicate with one another in preparation for mating. The budding yeast *Saccharomyces cerevisiae* provides a well-studied example: when a haploid individual is ready to mate, it secretes a peptide *mating factor* that signals cells of the opposite mating type to stop proliferating and prepare to mate (**Figure 15–2**). The subsequent fusion of two haploid cells of opposite mating type produces a diploid cell, which can then undergo meiosis and sporulate, generating haploid cells with new assortments of genes (see Figure 21–3B). The reshuffling of genes through sexual reproduction helps a species survive in an unpredictably variable environment (as discussed in Chapter 21).

Studies of yeast mutants that are unable to mate have identified many proteins that are required in the signaling process. These proteins form a signaling network that includes cell-surface receptor proteins, GTP-binding proteins, and protein kinases, and each of these categories has close relatives among the receptors and intracellular signaling proteins in animal cells. Through gene duplication and divergence, however, the signaling systems in animals have become much more elaborate than those in yeasts; the human genome, for example, contains more than 1500 genes that encode receptor proteins, and the number of different receptor proteins is further increased by alternative RNA splicing and post-translational modifications.

The large numbers of signal proteins, receptors, and intracellular signaling proteins used by animals can be grouped into a much smaller number of protein families, most of which have been highly conserved in evolution. Flies, worms, and mammals all use essentially similar machinery for cell communication, and many of the key components and signaling pathways were first discovered through analysis of mutations in *Drosophila* and *C. elegans*.

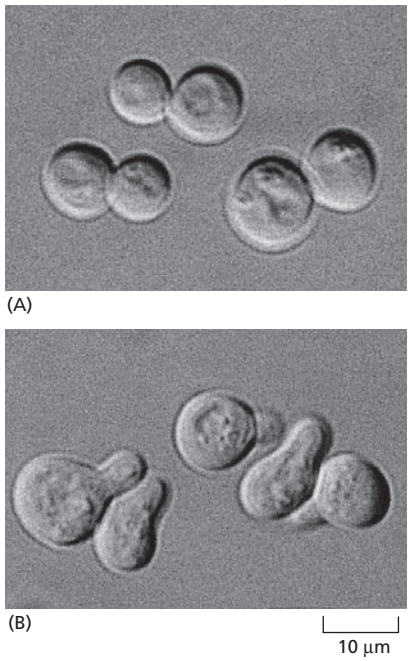

Figure 15–2 Budding yeast cells responding to a mating factor. (A) The cells are normally spherical. (B) In response to the mating factor secreted by neighboring yeast cells, they put out a protrusion toward the source of the factor in preparation for mating. (Courtesy of Michael Snyder.)

Extracellular Signal Molecules Bind to Specific Receptors

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

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

Regardless of the nature of the signal, the *target cell* responds by means of a **receptor**, which specifically binds the signal molecule and then initiates a response in the target cell. The extracellular signal molecules often act at very low concentrations (typically ≤ 10^{-8} M), and the receptors that recognize them usually bind them with high affinity (affinity constant $K_a \geq 10^8$ liters/mole; see Figure 3–43).

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

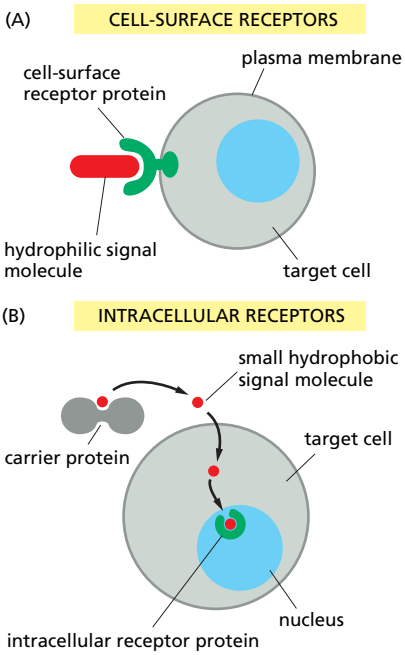

Figure 15–3 The binding of extracellular signal molecules to either cell-surface or intracellular receptors. (A) Most signal molecules are hydrophilic and are therefore unable to cross the target cell's plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate signals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

Extracellular Signal Molecules Can Act Over Either Short or Long Distances

Many signal molecules remain bound to the surface of the signaling cell and influence only cells that contact it (**Figure 15–4**A). Such **contact-dependent signaling** is especially important during development and in immune responses. Contact-dependent signaling during development can sometimes operate over relatively large distances, where the communicating cells extend long processes to make contact with one another.

In most cases, however, signaling cells secrete signal molecules into the extracellular fluid. The secreted molecules may be carried far afield to act on distant target cells, or they may act as **local mediators**, affecting only cells in the local environment of the signaling cell. The latter process is called **paracrine signaling** (Figure 15–4B). Usually, the signaling and target cells in paracrine signaling are of different cell types, but cells may also produce signals that they themselves respond to: this is referred to as *autocrine signaling*. Cancer cells, for example, often use this strategy to stimulate their own survival and proliferation.

For paracrine signals to act only locally, the secreted molecules must not be allowed to diffuse too far; for this reason they are often rapidly taken up by neighboring target cells, destroyed by extracellular enzymes, or immobilized by the extracellular matrix. *Heparan sulfate proteoglycans* (discussed in Chapter 19), either in the extracellular matrix or attached to cell surfaces, often play a part in localizing the action of secreted signal proteins. They contain long polysaccharide side chains that bind the signal proteins and immobilize them. They may also control the stability of these proteins, their transport through the extracellular space, or their interaction with cell-surface receptors. Secreted protein *antagonists* also affect the distance over which a paracrine signal protein acts. These antagonists bind to either the signal molecule itself or its cell-surface receptor and block its activity, and they play an important part in restricting the effective range of secreted signal proteins that influence the developmental decisions that embryonic cells make (discussed in Chapter 22).

Large, complex, multicellular organisms need long-range signaling mechanisms to coordinate the behavior of cells in remote parts of the body. Thus, they have evolved cell types specialized for intercellular communication over large distances. The most sophisticated of these are nerve cells, or neurons, which typically extend long branching processes (axons) that enable them to contact target cells far away, where the processes terminate at the specialized sites of

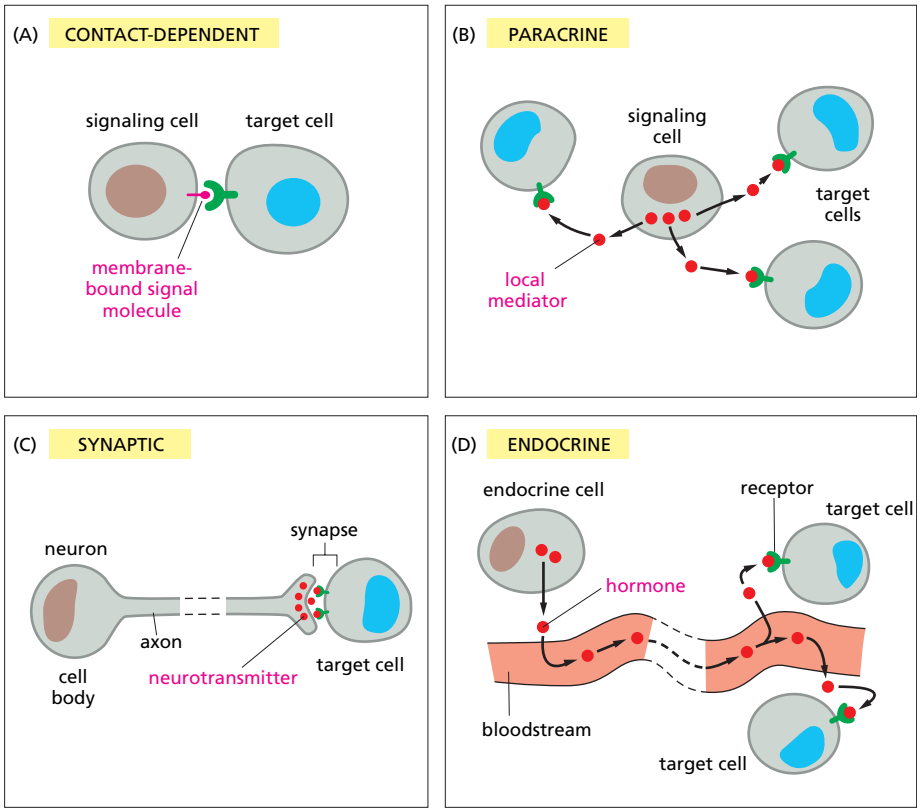

Figure 15–4 Four forms of intercellular signaling. (A) Contact-dependent signaling requires cells to be in direct membrane–membrane contact. (B) Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells. (C) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the neuronal cell body. (D) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream for distribution throughout the body. Many of the same types of signaling molecules are used in paracrine, synaptic, and endocrine signaling; the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

signal transmission known as *chemical synapses*. When activated by stimuli from the environment or from other nerve cells, the neuron sends electrical impulses (action potentials) rapidly along its axon; when such an impulse reaches the synapse at the end of the axon, it triggers secretion of a chemical signal that acts as a **neurotransmitter**. The tightly organized structure of the synapse ensures that the neurotransmitter is delivered specifically to receptors on the postsynaptic target cell (Figure 15–4C). The details of this **synaptic signaling** process are discussed in Chapter 11.

A quite different strategy for signaling over long distances makes use of **endocrine cells**. These secrete their signal molecules, called **hormones**, into the bloodstream, which carries the molecules far and wide, allowing them to act on target cells that may lie anywhere in the body (Figure 15–4D).

Figure 15–5 compares the mechanisms that allow endocrine cells and nerve cells to coordinate cell behavior over long distances in animals. Because endocrine signaling relies on diffusion and blood flow, it is relatively slow. Synaptic signaling, by contrast, is much faster, as well as more precise. Nerve cells can transmit information over long distances by electrical impulses that travel at speeds of up to 100 meters per second; once released from a nerve terminal, a neurotransmitter has to diffuse less than 100 nm to the target cell, a process that takes less than a millisecond. Another difference between endocrine and synaptic signaling is that, whereas hormones are greatly diluted in the bloodstream and interstitial fluid and therefore must be able to act at very low concentrations (typically $< 10^{-8}$ M), neurotransmitters are diluted much less and can achieve high local concentrations. The concentration of *acetylcholine* in the synaptic cleft of an active neuromuscular junction, for example, is about 5×10^{-4} M. Correspondingly, neurotransmitter receptors have a relatively low affinity for their ligand, which means that the neurotransmitter can dissociate rapidly from the receptor to help terminate a response. Moreover, after its release from a nerve terminal, a neurotransmitter is quickly removed from the synaptic cleft, either by specific hydrolytic enzymes that destroy it or by specific membrane transport proteins

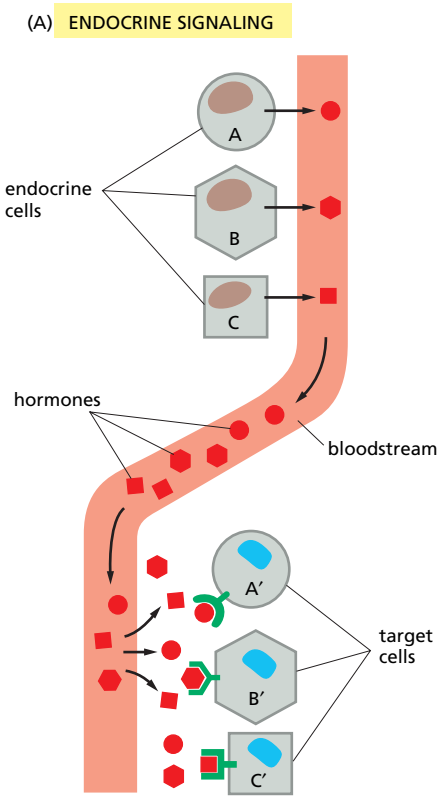

Figure 15–5 The contrast between endocrine and neuronal strategies for long-range signaling. In complex animals, endocrine cells and nerve cells work together to coordinate the activities of cells in widely separated parts of the body. Whereas different endocrine cells must use different hormones to communicate specifically with their target cells, different nerve cells can use the same neurotransmitter and still communicate in a highly specific manner. (A) Endocrine cells secrete hormones into the blood, and these act only on those target cells that carry the appropriate receptors: the receptors bind the specific hormone, which the target cells thereby "pull" from the extracellular fluid. (B) In synaptic signaling, by contrast, specificity arises from the synaptic contacts between a nerve cell and the specific target cells it signals. <CTGA> Usually, only a target cell that is in synaptic communication with a nerve cell is exposed to the neurotransmitter released from the nerve terminal (although some neurotransmitters act in a paracrine mode, serving as local mediators that influence multiple target cells in the area).

that pump it back into either the nerve terminal or neighboring glial cells. Thus, synaptic signaling is much more precise than endocrine signaling, both in time and in space.

The speed of a response to an extracellular signal depends not only on the mechanism of signal delivery but also on the nature of the target cell's response. When the response requires only changes in proteins already present in the cell, it can occur very rapidly: an allosteric change in a neurotransmitter-gated ion channel (discussed in Chapter 11), for example, can alter the plasma membrane electrical potential in milliseconds, and responses that depend solely on protein phosphorylation can occur within seconds. When the response involves changes in gene expression and the synthesis of new proteins, however, it usually requires many minutes or hours, regardless of the mode of signal delivery (**Figure 15–6**).

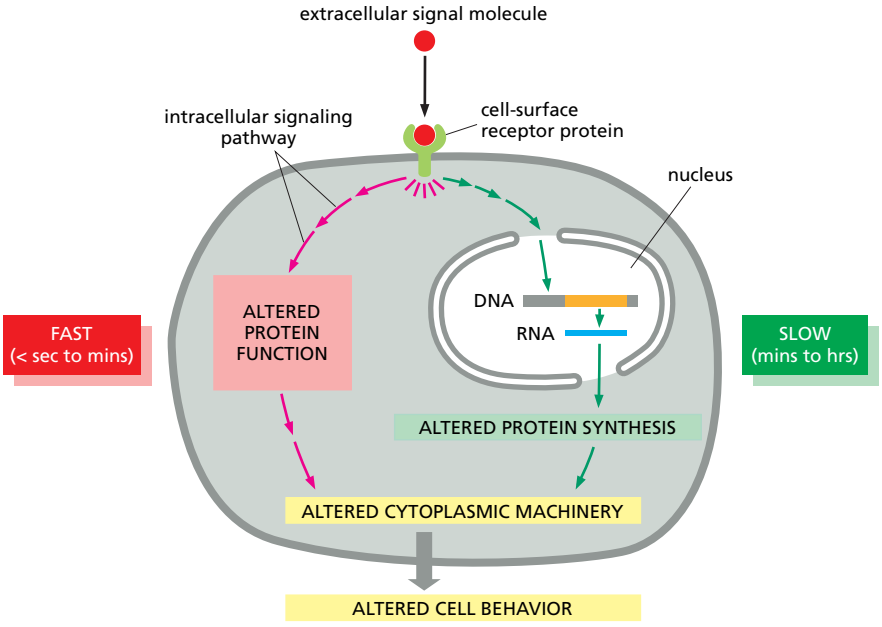

Figure 15–6 Extracellular signals can act slowly or rapidly to change the behavior of a target cell. Certain types of signaled responses, such as increased cell growth and division, involve changes in gene expression and the synthesis of new proteins; they therefore occur slowly, often starting after an hour or more. Other responses—such as changes in cell movement, secretion, or metabolism—need not involve changes in gene transcription and therefore occur much more quickly, often starting in seconds or minutes; they may involve the rapid phosphorylation of effector proteins in the cytoplasm, for example. Synaptic responses mediated by changes in membrane potential can occur in milliseconds (not shown).

Gap Junctions Allow Neighboring Cells to Share Signaling Information

Gap junctions are narrow water-filled channels that directly connect the cytoplasms of adjacent epithelial cells, as well as of some other cell types (see Figure 19–34). The channels allow the exchange of inorganic ions and other small water-soluble molecules, but not of macromolecules such as proteins or nucleic acids. Thus, cells connected by gap junctions can communicate with each other directly, without having to surmount the barrier presented by the intervening plasma membranes (**Figure 15–7**). In this way, gap junctions provide for the most intimate of all forms of cell communication, short of cytoplasmic bridges (see Figure 21–31) or cell fusion.

In contrast with other modes of cell signaling, gap junctions generally allow communications to pass in both directions symmetrically, and their typical effect is to homogenize conditions in the communicating cells. They can also be important in spreading the effect of extracellular signals that act through small intracellular mediators such as Ca^{2+} and cyclic AMP (discussed later), which pass readily through the gap-junctional channels. In the liver, for example, a fall in blood glucose levels releases *noradrenaline* (norepinephrine) from sympathetic nerve endings. The noradrenaline stimulates hepatocytes in the liver to increase glycogen breakdown and to release glucose into the blood, a response that depends on an increase in intracellular cyclic AMP. Not all of the hepatocytes are innervated by sympathetic nerves, however. By means of the gap junctions that connect hepatocytes, the innervated hepatocytes transmit the signal to the noninnervated ones, at least in part by the movement of cyclic AMP through gap junctions. As expected, mice with a mutation in the major gap junction gene expressed in the liver fail to mobilize glucose normally when blood glucose levels fall. Gap junctions are discussed in detail in Chapter 19.

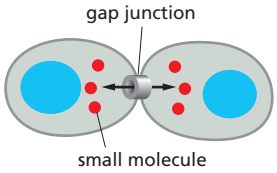

Figure 15–7 Signaling via gap junctions. Cells connected by gap junctions share small molecules, including small intracellular signaling molecules such as cyclic AMP and Ca^{2+}, and can therefore respond to extracellular signals in a coordinated way.

Each Cell Is Programmed to Respond to Specific Combinations of Extracellular Signal Molecules

A typical cell in a multicellular organism may be exposed to hundreds of different signal molecules in its environment. The molecules can be soluble, bound to the extracellular matrix, or bound to the surface of a neighboring cell; they can be stimulatory or inhibitory; they can act in innumerable different combinations; and they can influence almost any aspect of cell behavior. The cell must respond to this babel of signals selectively, according to its own specific character. This character is acquired through progressive cell specialization in the course of development. A cell may respond to one combination of signals by differentiating, to another combination by growing and dividing, and to yet another by performing some specialized function such as contraction or secretion. One of the great challenges in cell biology is to understand how a cell integrates all of this signaling information in order to make its crucial decisions—to divide, to move, to differentiate, and so on. For most of the cells in animal tissues, even the decision to continue living depends on correct interpretation of a specific combination of signals required for survival. When deprived of these signals (in a culture dish, for example), the cell activates a suicide program and kills itself—usually by *apoptosis,* a form of *programmed cell death* (**Figure 15–8**), as discussed in Chapter 18. Because different types of cells require different combinations of survival signals, each cell type is restricted to a specific set of environments in the body. Many epithelial cells, for example, require survival signals from the basal lamina on which they sit (discussed in Chapter 19); they die by apoptosis if they lose contact with this sheet of matrix.

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

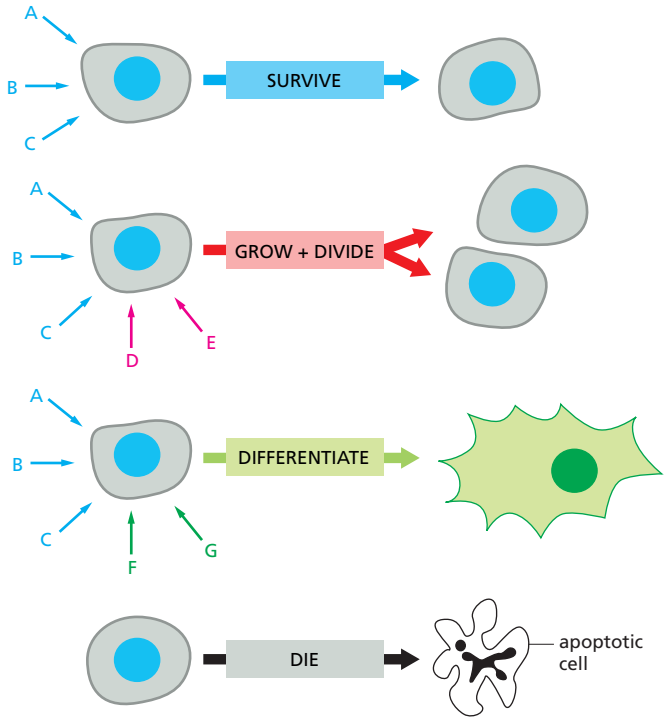

Figure 15–8 An animal cell's dependence on multiple extracellular signal molecules. Each cell type displays a set of receptors that enables it to respond to a corresponding set of signal molecules produced by other cells. These signal molecules work in combinations to regulate the behavior of the cell. As shown here, an individual cell often requires multiple signals to survive *(blue arrows)* and additional signals to grow and divide *(red arrow)* or differentiate *(green arrows)*. If deprived of appropriate survival signals, a cell will undergo a form of cell suicide known as apoptosis. The actual situation is even more complex. Although not shown, some extracellular signal molecules act to inhibit these and other cell behaviors, or even to induce apoptosis.

Different Types of Cells Usually Respond Differently to the Same Extracellular Signal Molecule

A cell's response to extracellular signals depends not only on the receptor proteins it possesses but also on the intracellular machinery by which it integrates and interprets the signals it receives. Thus, a single signal molecule usually has different effects on different types of target cells. The neurotransmitter acetylcholine (**Figure 15–9**A), for example, decreases the rate and force of contraction in heart muscle cells (Figure 15–9B), but it stimulates skeletal muscle cells to contract (see Figure 15–9C). In this case, the acetylcholine receptor proteins on skeletal muscle cells differ from those on heart muscle cells. But receptor

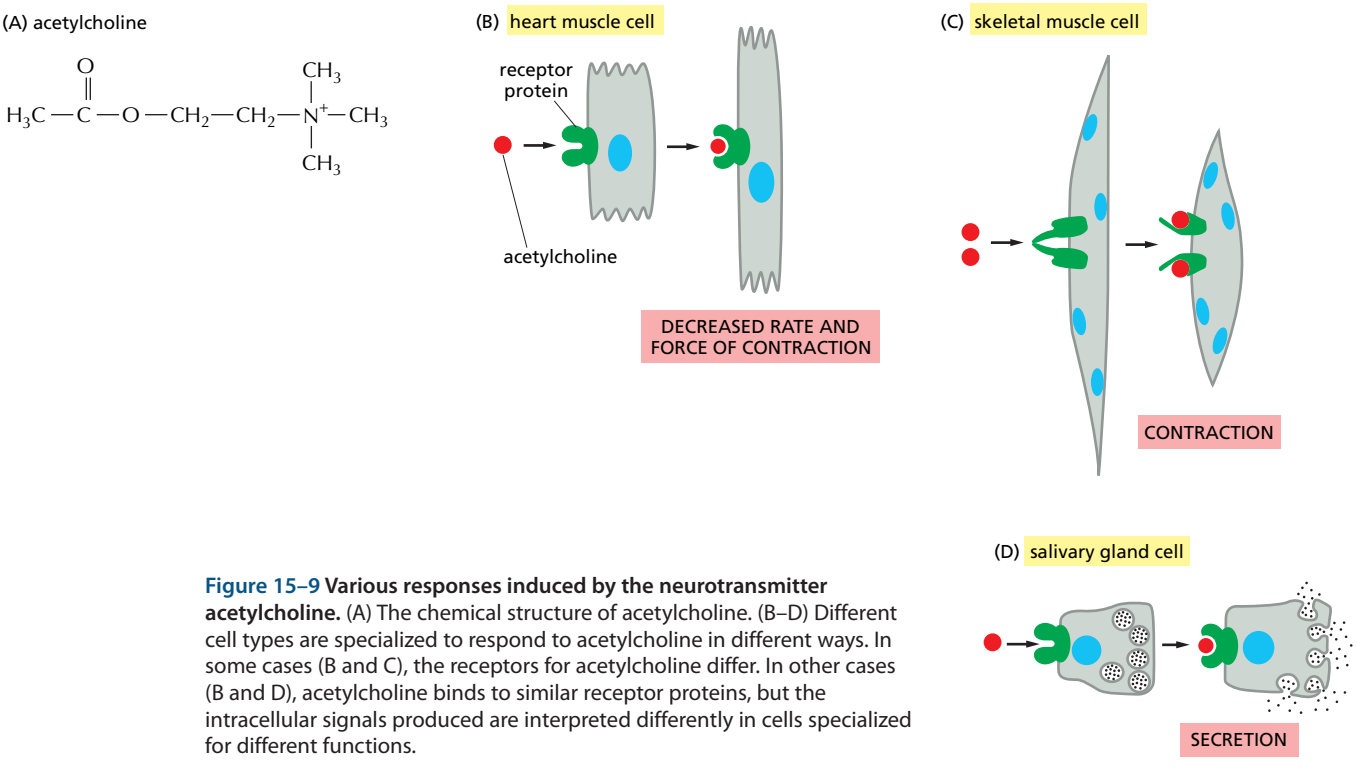

Figure 15–9 Various responses induced by the neurotransmitter acetylcholine. (A) The chemical structure of acetylcholine. (B–D) Different cell types are specialized to respond to acetylcholine in different ways. In some cases (B and C), the receptors for acetylcholine differ. In other cases (B and D), acetylcholine binds to similar receptor proteins, but the intracellular signals produced are interpreted differently in cells specialized for different functions.

differences are usually not the explanation for the different effects. The same signal molecule binding to identical receptor proteins usually produces very different responses in different types of target cells, as in the case of acetylcholine binding to heart muscle and to salivary gland cells (compare Figures 15–9B and D). In some cases, this reflects differences in the intracellular signaling proteins activated, whereas in others it reflects differences in the effector proteins or genes activated. Thus, an extracellular signal itself has little information content; it simply induces the cell to respond according to its predetermined state, which depends on the cell's developmental history and the specific genes it expresses.

The challenge of understanding how a cell integrates, processes, and reacts to the various inputs it receives is analogous in many ways to the challenge of understanding how the brain integrates and processes information to control behavior. In both cases, we need more than just a list of the components and connections in the system to understand how the process works. As a first step, we need to consider some basic principles concerning the way a cell responds to a simple signal of a given type.

The Fate of Some Developing Cells Depends on Their Position in Morphogen Gradients

The same signal acting on the same cell type can have qualitatively different effects depending on the signal's concentration. As we discuss in Chapter 22, such different responses of a cell to different concentrations of the same signal molecule are crucial in animal development, when cells are becoming different from one another.

The extracellular signal molecule in these cases during development is called a **morphogen**, and, in the simplest cases, it diffuses out from a localized cellular source (a *signaling center*), generating a signal concentration gradient. The responding cells adopt different cell fates in accordance with their position in the gradient: those cells closest to the signaling center that encounter the highest concentration of the morphogen have the highest number of receptors activated and follow one pathway of development, whereas those slightly further away follow another, and so on (**Figure 15–10**). As we discuss later (and in Chapter 22), the different levels of receptor activation lead to differences in the concentration or activity of one or more gene regulatory proteins in the nucleus of each cell, which in turn results in different patterns of gene expression. Further, more local signaling interactions between the cells in the gradient often help determine and stabilize the different fate choices.

A Cell Can Alter the Concentration of an Intracellular Molecule Quickly Only If the Lifetime of the Molecule Is Short

It is natural to think of signaling systems in terms of the changes produced when an extracellular signal is delivered. But it is just as important to consider what happens when the signal is withdrawn. During development, transient extracellular signals often produce lasting effects: they can trigger a change in the cell's development that persists indefinitely through cell memory mechanisms, as we discuss later (and in Chapters 7 and 22). In most cases in adult tissues, however, the response fades when a signal ceases. Often the effect is transient because the signal exerts its effects by altering the concentrations of intracellular molecules that are short-lived (unstable), undergoing continual turnover. Thus, once the extracellular signal is gone, the replacement of the old molecules by new ones wipes out all traces of the signal's action. It follows that the speed with which a cell responds to signal removal depends on the rate of destruction, or turnover, of the intracellular molecules that the signal affects.

It is also true, although much less obvious, that this turnover rate can determine the promptness of the response when an extracellular signal arrives. Consider, for example, two intracellular signaling molecules X and Y, both of which are normally maintained at a concentration of 1000 molecules per cell. The cell

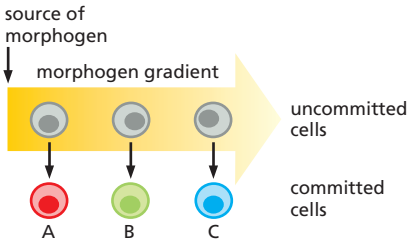

Figure 15–10 Cells adopt different fates depending on their position in a morphogen gradient. The different concentrations of morphogen induce the expression of different sets of genes, resulting in different cell fates (indicated by different letters and colors).

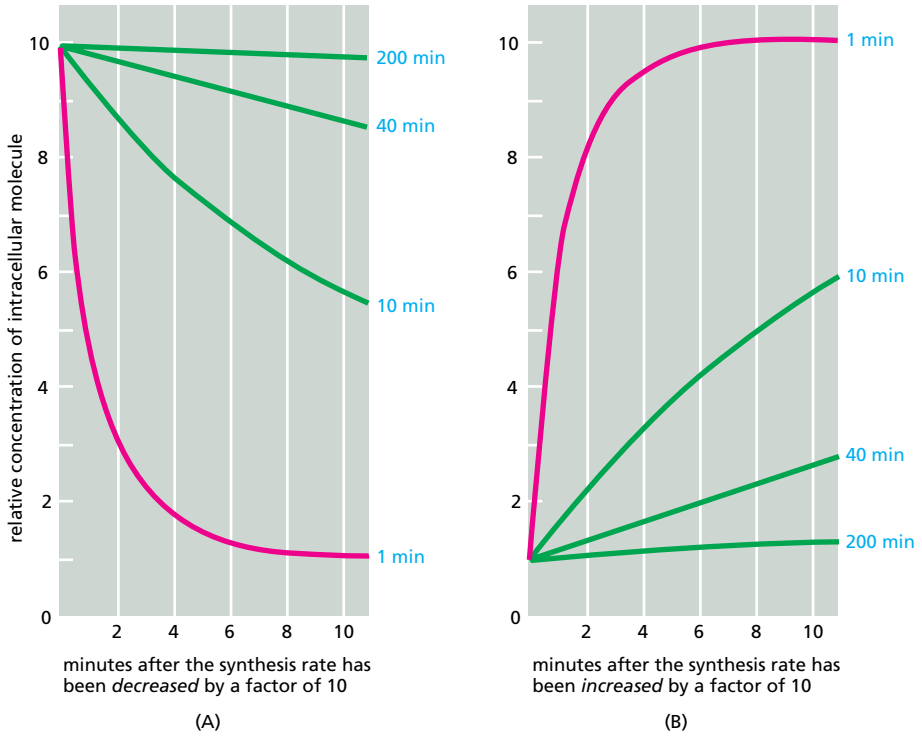

Figure 15–11 The importance of rapid turnover. The graphs show the predicted relative rates of change in the intracellular concentrations of molecules with differing turnover times when their synthesis rates are either (A) decreased or (B) increased suddenly by a factor of 10. In both cases, the concentrations of those molecules that are normally degraded rapidly in the cell *(red lines)* change quickly, whereas the concentrations of those that are normally degraded slowly *(green lines)* change proportionally more slowly. The numbers (in *blue)* on the right are the half-lives assumed for each of the different molecules.

synthesizes and degrades molecule Y at a rate of 100 molecules per second, with each molecule having an average lifetime of 10 seconds. Molecule X has a turnover rate that is 10 times slower than that of Y: it is both synthesized and degraded at a rate of 10 molecules per second, so that each molecule has an average lifetime in the cell of 100 seconds. If a signal acting on the cell causes a tenfold increase in the synthesis rates of both X and Y with no change in the molecular lifetimes, at the end of 1 second the concentration of Y will have increased by nearly 900 molecules per cell ($10 \times 100 - 100$), while the concentration of X will have increased by only 90 molecules per cell. In fact, after a molecule's synthesis rate has been either increased or decreased abruptly, the time required for the molecule to shift halfway from its old to its new equilibrium concentration is equal to its half-life—that is, equal to the time that would be required for its concentration to fall by half if all synthesis were stopped (**Figure 15–11**).

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

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

Nitric Oxide Gas Signals by Directly Regulating the Activity of Specific Proteins Inside the Target Cell

Most of this chapter is concerned with signaling pathways activated by cell-surface receptors. Before discussing these receptors and pathways, however, we briefly consider some important signal molecules that activate *intracellular receptors*. These molecules include nitric oxide and steroid hormones, which we discuss in turn. Although most extracellular signal molecules are hydrophilic

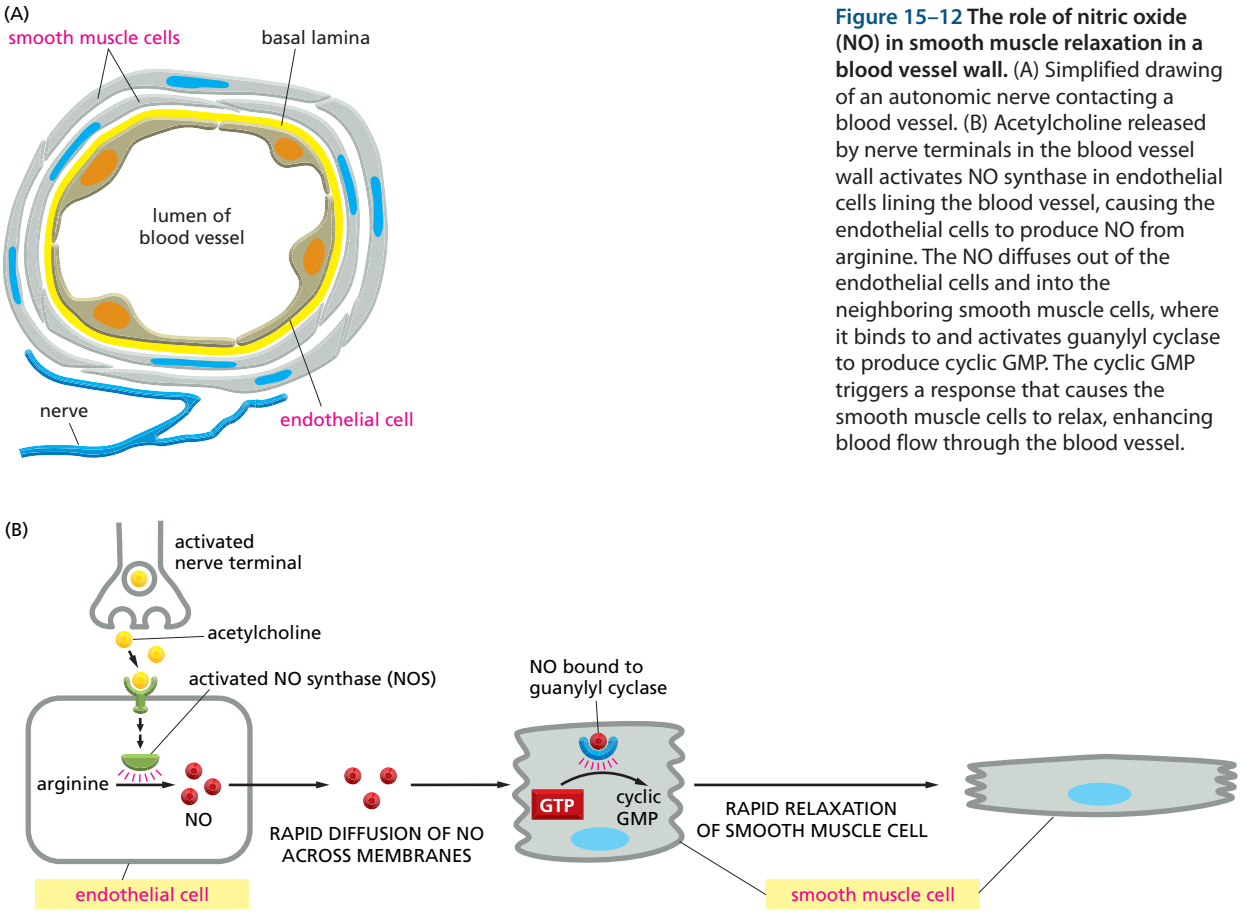

Figure 15–12 The role of nitric oxide (NO) in smooth muscle relaxation in a blood vessel wall. (A) Simplified drawing of an autonomic nerve contacting a blood vessel. (B) Acetylcholine released by nerve terminals in the blood vessel wall activates NO synthase in endothelial cells lining the blood vessel, causing the endothelial cells to produce NO from arginine. The NO diffuses out of the endothelial cells and into the neighboring smooth muscle cells, where it binds to and activates guanylyl cyclase to produce cyclic GMP. The cyclic GMP triggers a response that causes the smooth muscle cells to relax, enhancing blood flow through the blood vessel.

and bind to receptors on the surface of the target cell, some are hydrophobic enough, small enough, or both, to pass readily across the target cell's plasma membrane. Once inside, they directly regulate the activity of specific intracellular proteins. An important and remarkable example is the gas **nitric oxide (NO)**, which acts as a signal molecule in both animals and plants. Even some bacteria can detect a very low concentration of NO and move away from it.

In mammals, one of NO's many functions is to relax smooth muscle. It has this role in the walls of blood vessels, for example (**Figure 15–12**A). Autonomic nerves in the vessel wall release acetylcholine; the acetylcholine acts on the nearby endothelial cells that line the interior of the vessel; and the endothelial cells respond by releasing NO, which relaxes the smooth muscle cells in the wall, allowing the vessel to dilate. This effect of NO on blood vessels provides an explanation for the mechanism of action of nitroglycerine, which has been used for about 100 years to treat patients with angina (pain resulting from inadequate blood flow to the heart muscle). The nitroglycerine is converted to NO, which relaxes blood vessels. This reduces the workload on the heart and, as a consequence, reduces the oxygen requirement of the heart muscle.

Many types of nerve cells use NO more directly to signal to their neighbors. NO released by autonomic nerves in the penis, for example, causes the local blood vessel dilation that is responsible for penile erection. NO is also produced by activated macrophages and neutrophils to help them to kill invading microorganisms. In plants, NO is involved in the defensive responses to injury or infection.

NO is made by the deamination of the amino acid arginine, catalyzed by enzymes called **NO synthases** (**NOS**) (Figure 15–12B). The NOS in endothelial cells is called *eNOS,* while that in nerve and muscle cells is called *nNOS.* Nerve and muscle cells constitutively make nNOS, which is activated to produce NO by an influx of Ca^{2+} when the cells are stimulated. Macrophages, by contrast, make yet another NOS, called inducible NOS (*iNOS*) because they make it only when they are activated, usually in response to an infection.

Because dissolved NO passes readily across membranes, it rapidly diffuses out of the cell where it is produced and into neighboring cells (see Figure 15–12B). It acts only locally because it has a short half-life—about 5–10 seconds—in the extracellular space before oxygen and water convert it to nitrates and nitrites.

In some target cells, including smooth muscle cells, NO reversibly binds to iron in the active site of the enzyme *guanylyl cyclase,* stimulating this enzyme to produce the small intracellular signaling molecule *cyclic GMP,* which we discuss later. Thus, guanylyl cyclase acts both as an intracellular receptor for NO and as an intracellular signaling protein (see Figure 15–12B). NO can increase cyclic GMP in the cytosol within seconds, because the normal rate of turnover of cyclic GMP is high: a rapid degradation to GMP by a *phosphodiesterase* constantly balances the production of cyclic GMP from GTP by the guanylyl cyclase. The drug Viagra and its newer relatives inhibit the cyclic GMP phosphodiesterase in the penis, thereby increasing the amount of time that cyclic GMP levels remain elevated in the smooth muscle cells of penile blood vessels after NO production is induced by local nerve terminals. The cyclic GMP, in turn, keeps the blood vessels relaxed and thereby the penis erect.

NO can also signal cells independently of cyclic GMP. It can, for example, alter the activity of an intracellular protein by covalently nitrosylating thiol (–SH) groups on specific cysteines in the protein.

Carbon monoxide (CO) is another gas that is used as an extracellular signal molecule and like NO can act by stimulating guanylyl cyclase. But, these gases are not the only signal molecules that can pass directly across the target-cell plasma membrane, as we discuss next.

Nuclear Receptors Are Ligand-Modulated Gene Regulatory Proteins

Various small hydrophobic signal molecules diffuse directly across the plasma membrane of target cells and bind to intracellular receptors that are gene regulatory proteins. These signal molecules include steroid hormones, thyroid hormones, retinoids, and vitamin D. Although they differ greatly from one another in both chemical structure (**Figure 15–13**) and function, they all act by a similar mechanism. They bind to their respective intracellular receptor proteins and alter the ability of these proteins to control the transcription of specific genes. Thus, these proteins serve both as intracellular receptors and as intracellular effectors for the signal.

The receptors are all structurally related, being part of the very large **nuclear receptor superfamily**. Many family members have been identified by DNA sequencing only, and their ligand is not yet known; these proteins are therefore referred to as *orphan nuclear receptors.* Currently, more than half of the 48 nuclear receptors encoded in the human genome are orphans, as are 17 out of

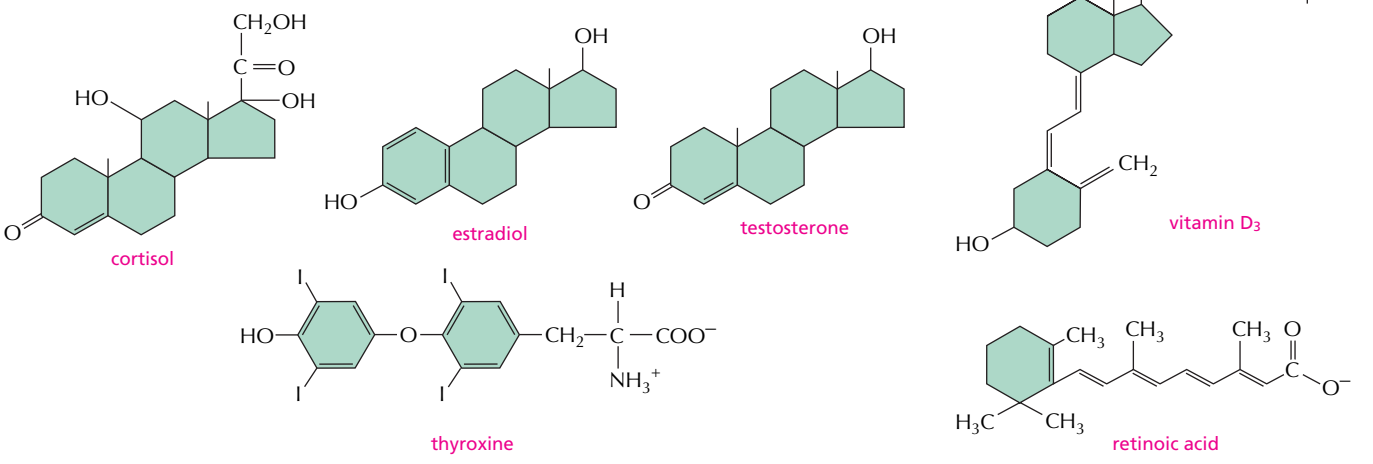

Figure 15–13 Some nongaseous signal molecules that bind to intracellular receptors. Note that all of them are small and hydrophobic. The active, hydroxylated form of vitamin D_3 is shown. Estradiol and testosterone are steroid sex hormones.

18 in *Drosophila* and all 278 in the nematode *C. elegans* (see Figure 4–85). Some mammalian nuclear receptors are regulated by intracellular metabolites rather than by secreted signal molecules; the *peroxisome proliferation-activated receptors (PPARs),* for example, bind intracellular lipid metabolites and regulate the transcription of genes involved in lipid metabolism and fat cell differentiation (discussed in Chapter 23). It seems likely that the nuclear receptors for hormones evolved from such receptors for intracellular metabolites, which would help explain their intracellular location.

Steroid hormones—which include cortisol, the steroid sex hormones, vitamin D (in vertebrates), and the molting hormone *ecdysone* (in insects)—are all made from cholesterol. *Cortisol* is produced in the cortex of the adrenal glands and influences the metabolism of many types of cells. The *steroid sex hormones* are made in the testes and ovaries, and they are responsible for the secondary sex characteristics that distinguish males from females. *Vitamin D* is synthesized in the skin in response to sunlight; after it has been converted to its active form in the liver or kidneys, it regulates Ca^{2+} metabolism, promoting Ca^{2+} uptake in the gut and reducing its excretion in the kidneys. The *thyroid hormones,* which are made from the amino acid tyrosine, act to increase the metabolic rate of many cell types, while the *retinoids,* such as retinoic acid, are made from vitamin A and have important roles as local mediators in vertebrate development. Although all of these signal molecules are relatively insoluble in water, they are made soluble for transport in the bloodstream and other extracellular fluids by binding to specific carrier proteins, from which they dissociate before entering a target cell (see Figure 15–3B).

The nuclear receptors bind to specific DNA sequences adjacent to the genes that the ligand regulates. Some of the receptors, such as those for cortisol, are located primarily in the cytosol and enter the nucleus only after ligand binding; others, such as the thyroid and retinoid receptors, are bound to DNA in the nucleus even in the absence of ligand. In either case, the inactive receptors are usually bound to inhibitory protein complexes. Ligand binding alters the conformation of the receptor protein, causing the inhibitory complex to dissociate, while also causing the receptor to bind coactivator proteins that stimulate gene transcription (**Figure 15–14**). In other cases, however, ligand binding to a nuclear receptor inhibits transcription: some thyroid hormone receptors, for example, act as transcriptional activators in the absence of their hormone and become transcriptional repressors when hormone binds.

The transcriptional response usually takes place in multiple steps. In the cases in which ligand binding activates transcription, for example, the direct stimulation of a small number of specific genes occurs within about 30 minutes and constitutes the *primary response;* the protein products of these genes in turn activate other genes to produce a delayed, *secondary response;* and so on. In addition, some of the proteins produced in the primary response may act back to inhibit the transcription of primary response genes, thereby limiting the response—an example of negative feedback, which we discuss later. In this way, a simple hormonal trigger can cause a very complex change in the pattern of gene expression (**Figure 15–15**).

The responses to steroid and thyroid hormones, vitamin D, and retinoids are determined as much by the nature of the target cell as by the nature of the signal molecule. Many types of cells have the identical intracellular receptor, but the set of genes that the receptor regulates differs in each cell type. This is because more than one type of gene regulatory protein generally must bind to a eucaryotic gene to regulate its transcription. An intracellular receptor can therefore regulate a gene only if there is the right combination of other gene regulatory proteins, and many of these are cell-type specific.

In summary, each of these hydrophobic signal molecules induces a characteristic set of responses in an animal for two reasons. First, only certain types of cells have receptors for it. Second, each of these cell types contains a different combination of other cell-type-specific gene regulatory proteins that collaborate with the activated receptor to influence the transcription of specific sets of genes. This principle applies to all signaled responses that depend on gene regulatory proteins, including the many other examples we discuss in this chapter.

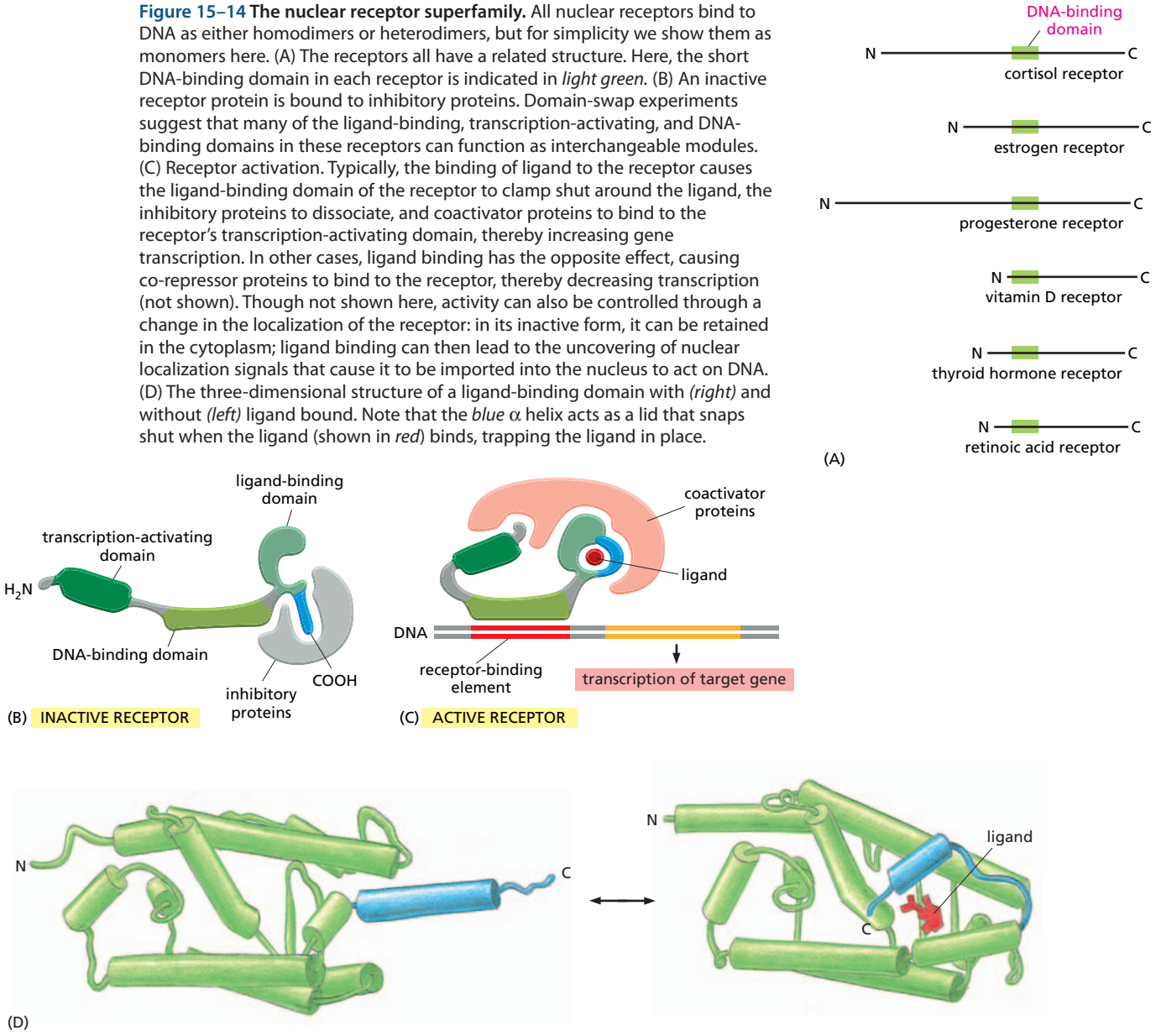

Figure 15–14 The nuclear receptor superfamily. All nuclear receptors bind to DNA as either homodimers or heterodimers, but for simplicity we show them as monomers here. (A) The receptors all have a related structure. Here, the short DNA-binding domain in each receptor is indicated in *light green.* (B) An inactive receptor protein is bound to inhibitory proteins. Domain-swap experiments suggest that many of the ligand-binding, transcription-activating, and DNA-binding domains in these receptors can function as interchangeable modules. (C) Receptor activation. Typically, the binding of ligand to the receptor causes the ligand-binding domain of the receptor to clamp shut around the ligand, the inhibitory proteins to dissociate, and coactivator proteins to bind to the receptor's transcription-activating domain, thereby increasing gene transcription. In other cases, ligand binding has the opposite effect, causing co-repressor proteins to bind to the receptor, thereby decreasing transcription (not shown). Though not shown here, activity can also be controlled through a change in the localization of the receptor: in its inactive form, it can be retained in the cytoplasm; ligand binding can then lead to the uncovering of nuclear localization signals that cause it to be imported into the nucleus to act on DNA. (D) The three-dimensional structure of a ligand-binding domain with *(right)* and without *(left)* ligand bound. Note that the *blue* α helix acts as a lid that snaps shut when the ligand (shown in *red*) binds, trapping the ligand in place.

The molecular details of how nuclear receptors and other gene regulatory proteins control specific gene transcription are discussed in Chapter 7.

Nuclear receptor proteins are sometimes also present on the cell surface, where they function by mechanisms entirely different from that just described. In the remainder of the chapter, we consider various ways in which cell-surface receptors convert extracellular signals into intracellular ones, a process called *signal transduction.*

The Three Largest Classes of Cell-Surface Receptor Proteins Are Ion-Channel-Coupled, G-Protein-Coupled, and Enzyme-Coupled Receptors

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

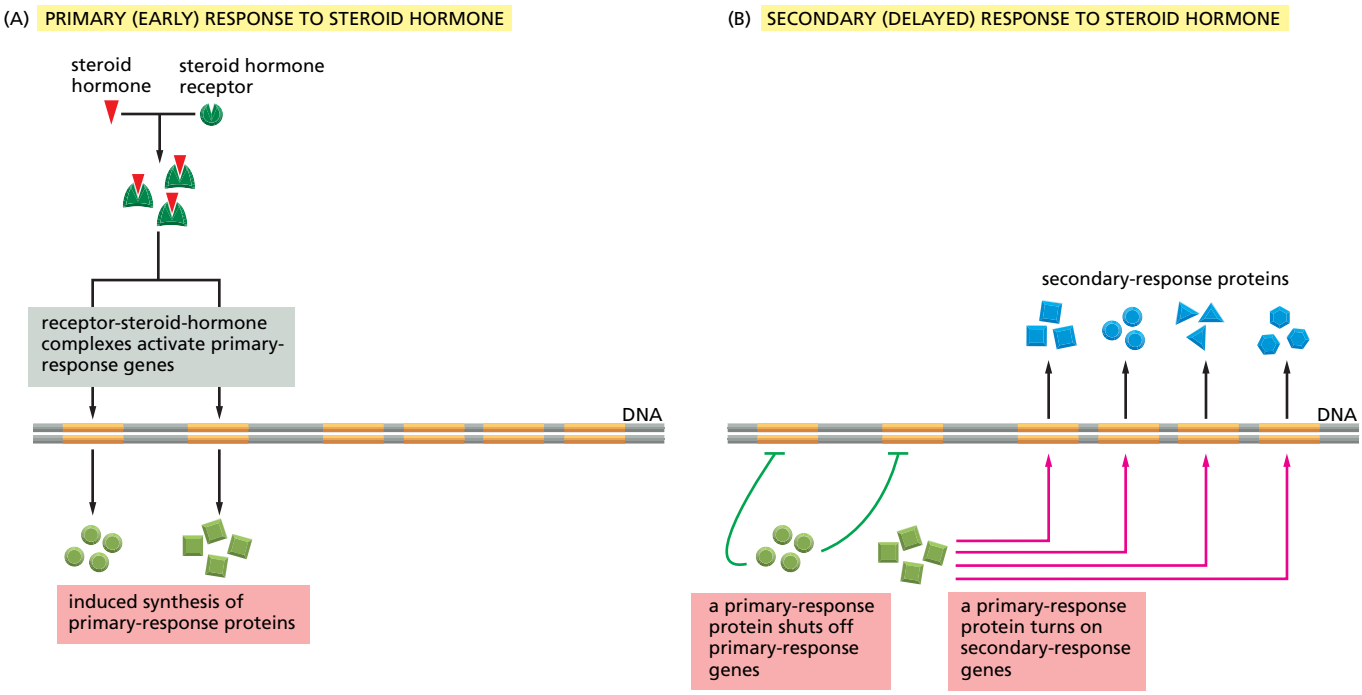

Figure 15–15 An example of primary and secondary responses induced by the activation of a nuclear hormone receptor. (A) Early primary response and (B) delayed secondary response. Some of the primary-response proteins turn on secondary-response genes, while others turn off the primary-response genes. The actual number of primary-response and secondary-response genes is greater than shown. Drugs that inhibit protein synthesis suppress the transcription of secondary-response genes but not primary-response genes, allowing these two classes of gene transcription responses to be readily distinguished. The figure shows the responses to a steroid hormone, but the same principles apply for many other ligands that activate nuclear receptor proteins.

Most cell-surface receptor proteins belong to one of three classes, defined by their transduction mechanism. **Ion-channel-coupled receptors**, also known as *transmitter-gated ion channels* or *ionotropic receptors,* are involved in rapid synaptic signaling between nerve cells and other electrically excitable target cells such as nerve and muscle cells (**Figure 15–16**A). This type of signaling is mediated by a small number of neurotransmitters that transiently open or close an ion channel formed by the protein to which they bind, briefly changing the ion permeability of the plasma membrane and thereby the excitability of the postsynaptic target cell. Most ion-channel-coupled receptors belong to a large family of homologous, multipass transmembrane proteins. Because they are discussed in detail in Chapter 11, we shall not consider them further here.

G-protein-coupled receptors act by indirectly regulating the activity of a separate plasma-membrane-bound target protein, which is generally either an enzyme or an ion channel. A *trimeric GTP-binding protein (G protein)* mediates the interaction between the activated receptor and this target protein (Figure 15–16B). The activation of the target protein can change the concentration of one or more small intracellular mediators (if the target protein is an enzyme), or it can change the ion permeability of the plasma membrane (if the target protein is an ion channel). The small intracellular mediators act in turn to alter the behavior of yet other signaling proteins in the cell. All of the G-protein-coupled receptors belong to a large family of homologous, multipass transmembrane proteins.

Enzyme-coupled receptors either function directly as enzymes or associate directly with enzymes that they activate (Figure 15–16C). They are usually single-pass transmembrane proteins that have their ligand-binding site outside the cell and their catalytic or enzyme-binding site inside. Enzyme-coupled receptors are heterogeneous in structure compared with the other two classes. The great majority, however, are either protein kinases or associate with protein kinases, which phosphorylate specific sets of proteins in the target cell when activated.

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

Most Activated Cell-Surface Receptors Relay Signals Via Small Molecules and a Network of Intracellular Signaling Proteins

A combination of small and large *intracellular signaling molecules* relays signals received at the cell surface by either G-protein-coupled or enzyme-coupled receptors into the cell interior. The resulting chain of intracellular signaling events ultimately alters effector proteins that are responsible for modifying the behavior of the cell (see Figure 15–1).

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

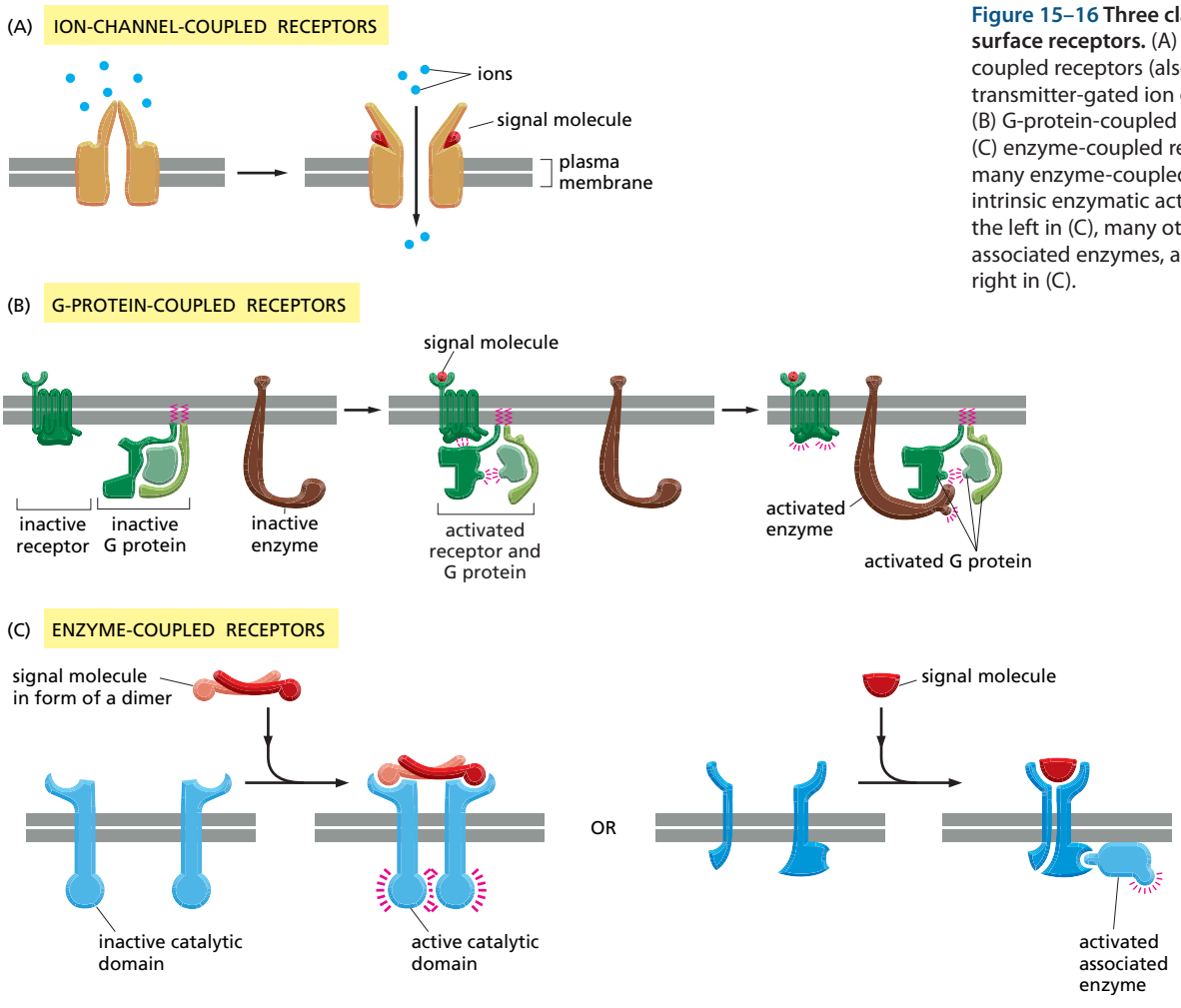

Figure 15–16 Three classes of cell-surface receptors. (A) Ion-channel-coupled receptors (also called transmitter-gated ion channels), (B) G-protein-coupled receptors, and (C) enzyme-coupled receptors. Although many enzyme-coupled receptors have intrinsic enzymatic activity, as shown on the left in (C), many others rely on associated enzymes, as shown on the right in (C).

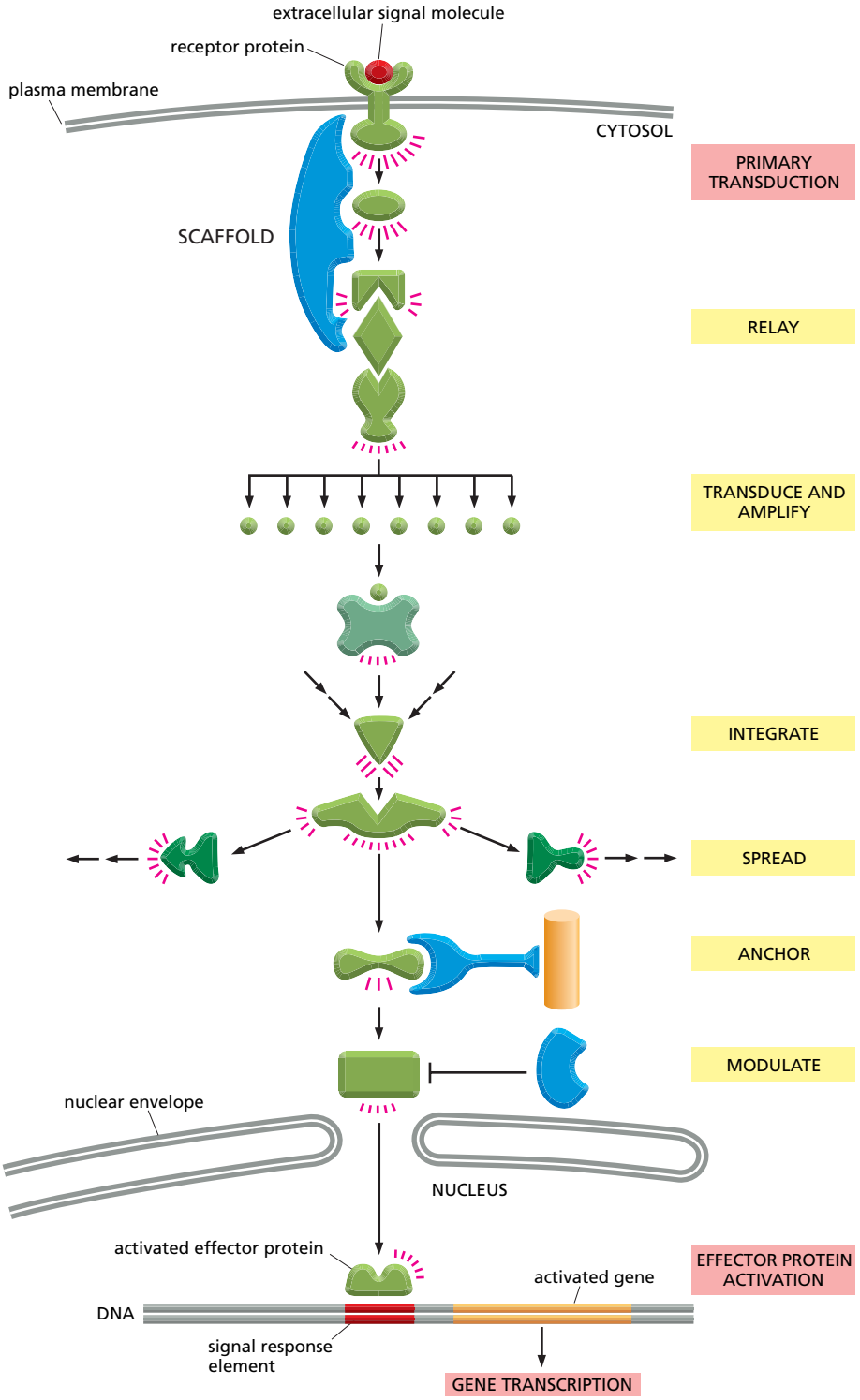

Figure 15–17 A hypothetical intracellular signaling pathway from a cell-surface receptor to the nucleus. In this example, a series of signaling proteins and small intracellular mediators relay the extracellular signal into the nucleus, causing a change in gene expression. The signal is altered (transduced), amplified, distributed, and modulated *en route*. Because many of the steps can be affected by other extracellular and intracellular signals, the final effect of one extracellular signal depends on multiple factors affecting the cell (see Figure 15–8). Ultimately, the signaling pathway activates (or inactivates) effector proteins that alter the cell's behavior. In this example, the effector is a gene regulatory protein that activates gene transcription. Although the figure shows individual signaling proteins performing a single function, in actuality they often have more than one function; scaffold proteins, for example, often also serve to anchor several signaling proteins to a particular intracellular structure.

Most signaling pathways to the nucleus are more direct than this one, which is not based on a known pathway.

The large intracellular signaling molecules are **intracellular signaling proteins**, which help relay the signal into the cell by either generating small intracellular mediators or activating the next signaling or effector protein in the pathway. These proteins form a functional network, in which each protein helps to process the signal in one or more of the following ways as it spreads the signal's influence through the cell (**Figure 15–17**):

1. The protein may simply *relay* the signal to the next signaling component in the chain.
2. It may act as a *scaffold* to bring two or more signaling proteins together so that they can interact more quickly and efficiently.

3. It may transform, or *transduce,* the signal into a different form, which is suitable for either passing the signal along or stimulating a cell response.
4. It may *amplify* the signal it receives, either by producing large amounts of a small intracellular mediator or by activating many copies of a downstream signaling protein. In this way, a small number of extracellular signal molecules can evoke a large intracellular response. When there are multiple amplification steps in a relay chain, the chain is often referred to as a **signaling cascade**.
5. It may receive signals from two or more signaling pathways and *integrate* them before relaying a signal onward. A protein that requires input from two or more signaling pathways to become activated, is often referred to as a coincidence detector.
6. It may *spread* the signal from one signaling pathway to another, creating branches in the signaling stream, thereby increasing the complexity of the response.
7. It may *anchor* one or more signaling proteins in a pathway to a particular structure in the cell where the signaling proteins are needed.
8. It may *modulate* the activity of other signaling proteins and thereby regulate the strength of signaling along a pathway.

We now consider in more detail some of the strategies that intracellular signaling proteins use in processing the signal as it is relayed along signaling pathways. We encounter these general strategies again in later sections of the chapter, when we discuss specific receptor classes and the signaling pathways they activate.

Many Intracellular Signaling Proteins Function as Molecular Switches That Are Activated by Phosphorylation or GTP Binding

Many intracellular signaling proteins behave like *molecular switches.* When they receive a signal, they switch from an inactive to an active conformation, until another process switches them off, returning them to their inactive conformation. As we mentioned earlier, the switching off is just as important as the switching on. If a signaling pathway is to recover after transmitting a signal so that it can be ready to transmit another, every activated molecule in the pathway must return to its original, unactivated state.

Two important classes of molecular switches that operate in intracellular signaling pathways depend on the gain or loss of phosphate groups for their activation or inactivation, although the way in which the phosphate is gained and lost is very different in the two classes. The largest class consists of proteins that are activated or inactivated by **phosphorylation** (discussed in Chapter 3). For these proteins, the switch is thrown in one direction by a **protein kinase**, which covalently adds one or more phosphate groups to the signaling protein, and in the other direction by a **protein phosphatase**, which removes the phosphate groups (**Figure 15–18**A). The activity of any protein regulated by

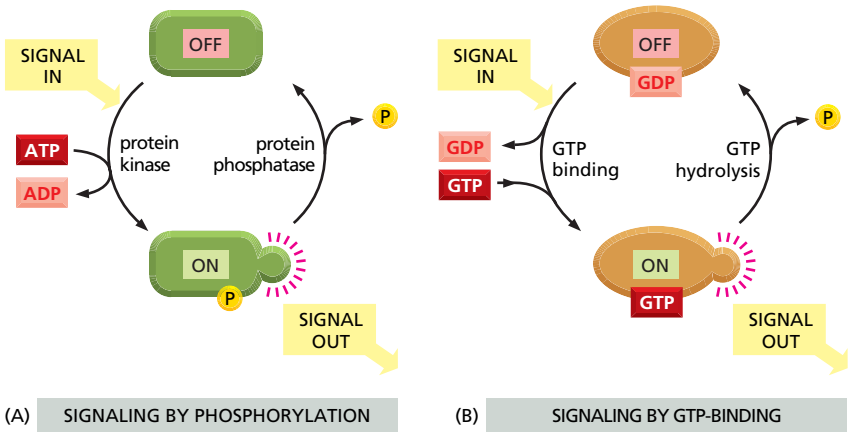

Figure 15–18 Two types of intracellular signaling proteins that act as molecular switches. Although one type is activated by phosphorylation and the other by GTP binding, in both cases the addition of a phosphate group switches the activation state of the protein and the removal of the phosphate switches it back again. (A) A protein kinase covalently adds a phosphate from ATP to the signaling protein, and a protein phosphatase removes the phosphate. Although not shown, some signaling proteins are activated by dephosphorylation rather than by phosphorylation. (B) A GTP-binding protein is induced to exchange its bound GDP for GTP, which activates the protein; the protein then inactivates itself by hydrolyzing its bound GTP to GDP.

phosphorylation depends on the balance at any instant between the activities of the kinases that phosphorylate it and of the phosphatases that dephosphorylate it. About 30% of human proteins contain covalently attached phosphate, and the human genome encodes about 520 protein kinases and about 150 protein phosphatases. It is thought that a typical mammalian cell makes use of hundreds of distinct types of protein kinases at any one time.

Many signaling proteins controlled by phosphorylation are themselves protein kinases, and these are often organized into **phosphorylation cascades**. In such a cascade, one protein kinase, activated by phosphorylation, phosphorylates the next protein kinase in the sequence, and so on, relaying the signal onward and, in the process, amplifying it and sometimes spreading it to other signaling pathways. Two main types of protein kinases operate as intracellular signaling proteins. The great majority are **serine/threonine kinases**, which phosphorylate proteins on serines and (less often) threonines. Others are **tyrosine kinases**, which phosphorylate proteins on tyrosines. An occasional kinase can do both.

The other important class of molecular switches that function by gaining and losing phosphate groups consists of **GTP-binding proteins** (discussed in Chapter 3). These proteins switch between an "on" (actively signaling) state when GTP is bound and an "off" state when GDP is bound. In the "on" state, they have intrinsic GTPase activity and shut themselves off by hydrolyzing their bound GTP to GDP (Figure 15–18B). There are two major types of GTP-binding proteins. Large *trimeric GTP-binding proteins* (also called *G proteins*) help relay signals from G-protein-coupled receptors that activate them (see Figure 15–16B). Small **monomeric GTPases** (also called *monomeric GTP-binding proteins*) help relay signals from many classes of cell-surface receptors.

Specific regulatory proteins control both types of GTP-binding proteins. **GTPase-activating proteins** (**GAPs**) drive the proteins into an "off" state by increasing the rate of hydrolysis of bound GTP; the GAPs that function in this way are also called *regulators of G-protein signaling (RGS)* proteins. Conversely, G-protein-coupled receptors activate trimeric G proteins, and **guanine nucleotide exchange factors** (**GEFs**) activate monomeric GTPases, by promoting the release of bound GDP in exchange for binding of GTP. **Figure 15–19** illustrates the regulation of monomeric GTPases.

Both trimeric G proteins and monomeric GTPases also participate in many other processes in eucaryotic cells, including the regulation of vesicular traffic and aspects of cell division.

As discussed earlier, specific combinations of extracellular signals, rather than one signal molecule acting alone, are generally required to stimulate complex cell behaviors, such as cell survival and cell growth and proliferation (see Figure 15–8). The cell therefore has to integrate information coming from multiple signals if it is to make an appropriate response; many mammalian cells, for example, require both soluble signals and signals from the extracellular matrix

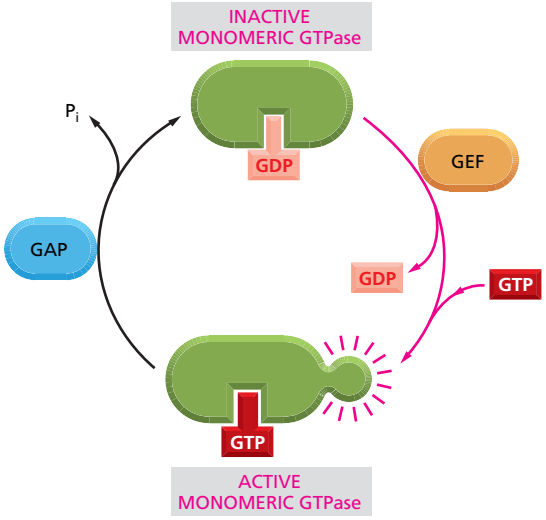

Figure 15–19 The regulation of a monomeric GTPase. GTPase-activating proteins (GAPs) inactivate the protein by stimulating it to hydrolyze its bound GTP to GDP, which remains tightly bound to the inactivated GTPase. Guanine nucleotide exchange factors (GEFs) activate the inactive protein by stimulating it to release its GDP; because the concentration of GTP in the cytosol is 10 times greater than the concentration of GDP, the protein rapidly binds GTP once it ejects GDP and is thereby activated.

(discussed in Chapter 19) to grow and proliferate. The integration depends in part on intracellular *coincidence detectors*, which are equivalent to *AND gates* in the microprocessor of a computer, in that they are only activated if they receive multiple converging signals (see Figure 15–17). **Figure 15–20** illustrates a simple hypothetical example of such a protein.

Not all molecular switches in signaling pathways depend on phosphorylation or GTP binding, however. We see later that some signaling proteins are switched on or off by the binding of another signaling protein or a small intracellular mediator such as cyclic AMP or Ca^{2+}, or by covalent modifications other than phosphorylation or dephosphorylation, such as ubiquitylation. Moreover, not all intracellular signaling proteins act as switches when they are phosphorylated or otherwise reversibly modified. As we discuss later, in many cases the covalently added group simply marks the protein so that it can interact with other signaling proteins that recognize the modification.

Intracellular Signaling Complexes Enhance the Speed, Efficiency, and Specificity of the Response

Even a single type of extracellular signal acting through a single type of cell-surface receptor often activates multiple parallel signaling pathways and can thereby influence multiple aspects of cell behavior—such as shape, movement, metabolism, and gene expression. Given the complexity of signal-response systems, which often involve multiple interacting relay chains of signaling proteins, how does an individual cell manage to make specific responses to so many different combinations of extracellular signals? The question is especially puzzling because many of the signals are closely related to one another and bind to closely related types of receptors. The same type of intracellular relay protein may couple one receptor subtype to one set of effectors and another receptor subtype to another set of effectors. In such cases, how is it possible to achieve specificity and avoid cross-talk? One strategy makes use of **scaffold proteins** (see Figure 15–17), which bind together groups of interacting signaling proteins into *signaling complexes*, often before a signal has been received (**Figure 15–21**A). Because the scaffold holds the signaling proteins in close proximity, the components can interact at high local concentrations and be sequentially activated speedily, efficiently, and selectively in response to an appropriate extracellular signal, avoiding unwanted cross-talk with other signaling pathways.

In other cases, signaling complexes form only transiently in response to an extracellular signal and rapidly disassemble when the signal is gone. Such transient complexes often assemble around a receptor after an extracellular signal molecule has activated it. In many of these cases, the cytoplasmic tail of the activated receptor is phosphorylated during the activation process, and the phosphorylated amino acids then serve as docking sites for the assembly of other signaling proteins (Figure 15–21B). In yet other cases, receptor activation leads to the production of modified phospholipid molecules (called phosphoinositides) in the adjacent plasma membrane, which then recruit specific intracellular signaling proteins to this region of membrane, where they are activated (Figure 15–21C). We discuss the roles of phosphoinositides in membrane trafficking events in Chapter 13.

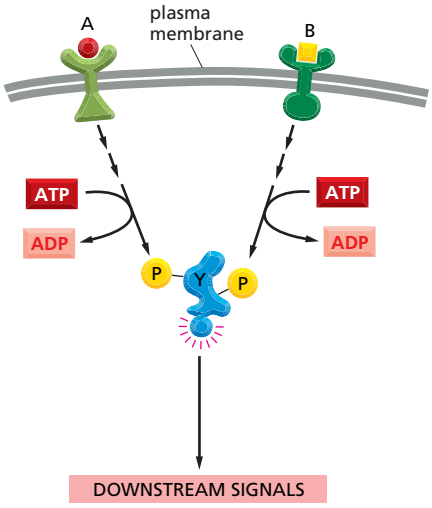

Figure 15–20 Signal integration. Extracellular signals A and B activate different intracellular signaling pathways, each of which leads to the phosphorylation of protein Y but at different sites on the protein. Protein Y is activated only when both of these sites are phosphorylated, and therefore it becomes active only when signals A and B are simultaneously present. Such proteins are often called coincidence detectors.

Modular Interaction Domains Mediate Interactions Between Intracellular Signaling Proteins

Simply bringing intracellular signaling proteins together into close proximity is sometimes sufficient to activate them. Thus, *induced proximity*, where a signal triggers assembly of a signaling complex, is commonly used to relay signals from protein to protein along a signaling pathway. The assembly of such signaling complexes depends on various highly conserved, small **interaction domains,** which are found in many intracellular signaling proteins. Each of these compact

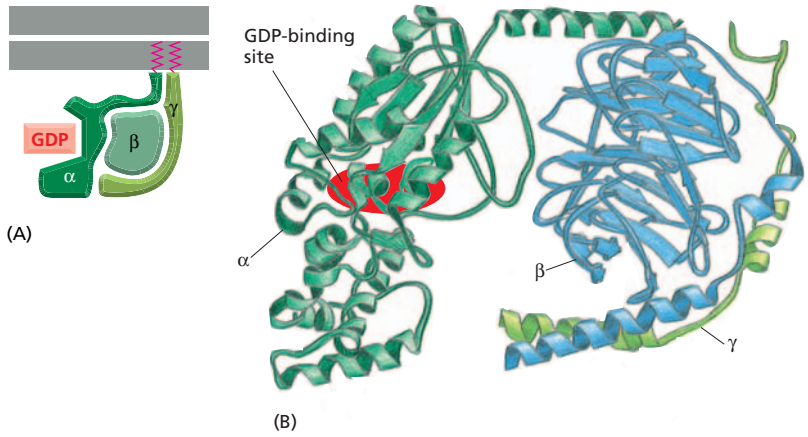

Figure 15–31 The structure of an inactive G protein. (A) Note that both the α and the γ subunits have covalently attached lipid molecules *(red)* that help bind them to the plasma membrane, and the α subunit has GDP bound. (B) The three-dimensional structure of an inactive G protein, based on transducin, the G protein that operates in visual transduction (discussed later). The α subunit contains the GTPase domain and binds to one side of the β subunit, which locks the GTPase domain in an inactive conformation that binds GDP. The γ subunit binds to the opposite side of the β subunit, and the β and γ subunits together form a single functional unit. (B, based on D.G. Lombright et al., *Nature* 379:311–319, 1996. With permission from Macmillan Publishers Ltd.)

Trimeric G Proteins Relay Signals from GPCRs

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

G proteins are composed of three protein subunits—α, β, and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive (**Figure 15–31**). When a GPCR is activated, it acts like a guanine nucleotide exchange factor (GEF) and induces the α subunit to release its bound GDP, allowing GTP to bind in its place. This exchange causes a large conformational change in the G protein, which activates it. It was originally thought that the activation always causes the trimer to dissociate into two activated components—an *α subunit* and a *βγ complex*. However, there is now evidence that, in some cases at least, the conformational change exposes previously buried surfaces between the α subunit and the βγ complex, so that the α subunit and βγ complex can each now interact with their targets without requiring the subunits to dissociate (**Figure 15–32**). These targets are either enzymes or ion channels in the plasma membrane that relay the signal onward.

The α subunit is a GTPase, and once it hydrolyzes its bound GTP to GDP it becomes inactive. The time for which the G protein remains active depends on how quickly the α subunit hydrolyzes its bound GTP. This time is usually short because the GTPase activity is greatly enhanced by the binding of the α subunit to a second protein, which can be either the target protein or a specific **regulator of G protein signaling** (**RGS**). RGS proteins act as α-subunit-specific GTPase-activating proteins (GAPs) (see Figure 15–19), and they help shut off G-protein-mediated responses in all eucaryotes. There are about 25 RGS proteins encoded in the human genome, each of which interacts with a particular set of G proteins.

GPCRs activate various intracellular signaling pathways, including some that are also activated by enzyme-coupled receptors. In this section, however, we focus on those GPCR-activated pathways that use small intracellular mediators.

Some G Proteins Regulate the Production of Cyclic AMP

Cyclic AMP (cAMP) acts as a small intracellular mediator in all procaryotic and animal cells that have been studied. Its normal concentration in the cytosol is about 10^{-7} M, but an extracellular signal can increase this concentration more than twentyfold in seconds (**Figure 15–33**). As explained earlier (see Figure

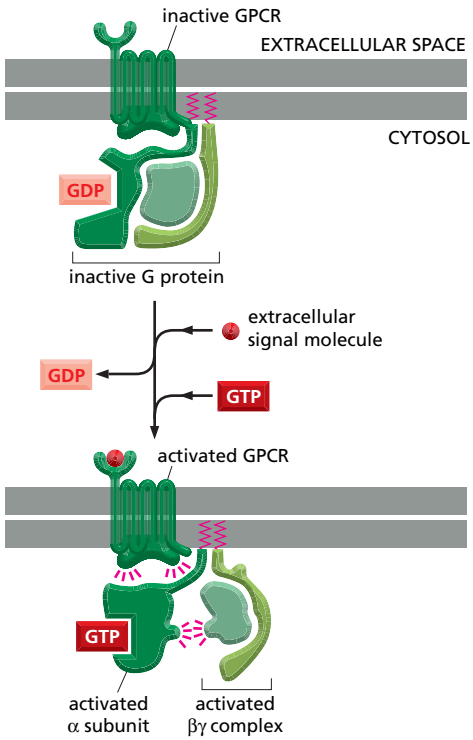

Figure 15–32 Activation of a G protein by an activated GPCR. Binding of an extracellular signal to a GPCR changes the conformation of the receptor, which in turn alters the conformation of the G protein. The alteration of the α subunit of the G protein allows it to exchange its GDP for GTP, activating both the α subunit and the 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, which dissociate from the receptor once activated (not shown). In some cases, the α subunit and the βγ complex dissociate from each other when the G protein is activated.

15–11), such a rapid response requires balancing a rapid synthesis of the molecule with its rapid breakdown or removal. Cyclic AMP is synthesized from ATP by a plasma-membrane-bound enzyme **adenylyl cyclase**, and it is rapidly and continuously destroyed by **cyclic AMP phosphodiesterases** that hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP) (**Figure 15–34**).

Many extracellular signal molecules work by increasing cyclic AMP concentration, and they do so by increasing the activity of adenylyl cyclase against a steady background of phosphodiesterase activity. Adenylyl cyclase is a large multipass transmembrane protein with its catalytic domain on the cytosolic side of the plasma membrane. There are at least eight isoforms in mammals, most of which are regulated by both G proteins and Ca^{2+}. GPCRs that act by increasing cyclic AMP are coupled to a **stimulatory G protein (G_s)**, which activates adenylyl cyclase and thereby increases cyclic AMP concentration. Another G protein, called **inhibitory G protein (G_i)**, inhibits adenylyl cyclase, but it acts mainly by directly regulating ion channels (as we discuss later).

Both G_s and G_i are targets for some medically important bacterial toxins. *Cholera toxin*, which is produced by the bacterium that causes cholera, is an enzyme that catalyzes the transfer of ADP ribose from intracellular NAD^+ to the α subunit of G_s. This ADP ribosylation alters the α subunit so that it can no longer hydrolyze its bound GTP, causing it to remain in an active state that stimulates adenylyl cyclase indefinitely. The resulting prolonged elevation in cyclic AMP concentration within intestinal epithelial cells causes a large efflux of Cl^- and water into the gut, thereby causing the severe diarrhea that characterizes cholera. *Pertussis toxin*, which is made by the bacterium that causes pertussis

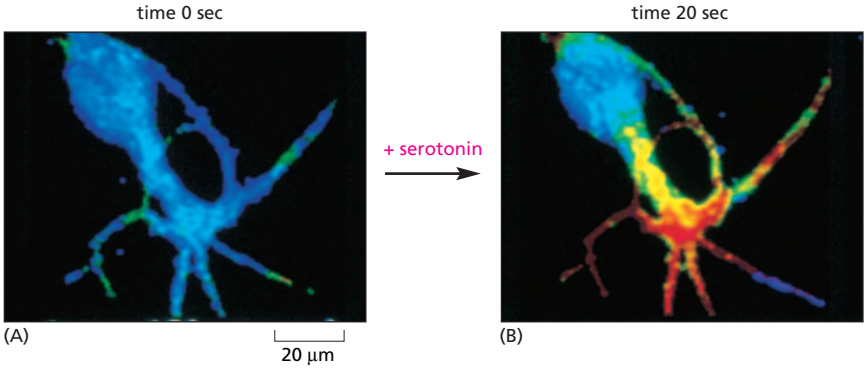

Figure 15–33 An increase in cyclic AMP in response to an extracellular signal. This nerve cell in culture is responding to the neurotransmitter serotonin, which acts through a GPCR to cause a rapid rise in the intracellular concentration of cyclic AMP. To monitor the cyclic AMP level, the cell has been loaded with a fluorescent protein that changes its fluorescence when it binds cyclic AMP. *Blue* indicates a low level of cyclic AMP, *yellow* an intermediate level, and *red* a high level. (A) In the resting cell, the cyclic AMP level is about 5×10^{-8} M. (B) Twenty seconds after the addition of serotonin to the culture medium, the intracellular level of cyclic AMP has increased to more than 10^{-6} M in the relevant parts of the cell, an increase of more than twentyfold. (From Brian J. Bacskai et al., *Science* 260:222–226, 1993. With permission from AAAS.)

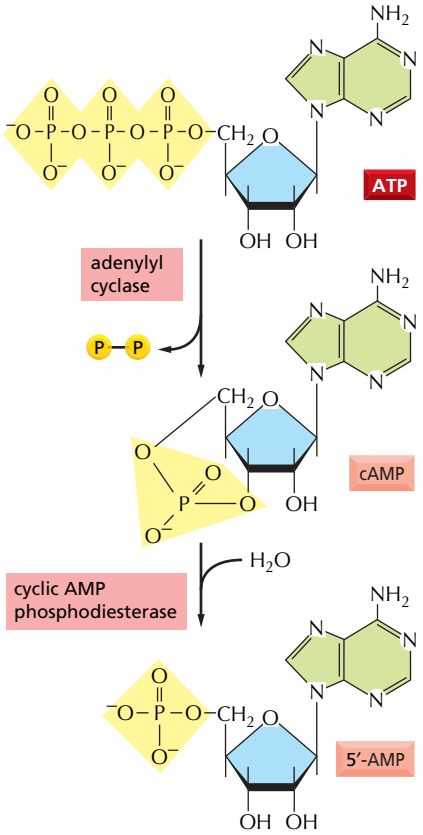

Figure 15–34 The synthesis and degradation of cyclic AMP. In a reaction catalyzed by the enzyme adenylyl cyclase, cyclic AMP (cAMP) is synthesized from ATP through a cyclization reaction that removes two phosphate groups as pyrophosphate (Ⓟ – Ⓟ); a pyrophosphatase drives this synthesis by hydrolyzing the released pyrophosphate to phosphate (not shown). Cyclic AMP is short-lived (unstable) in the cell because it is hydrolyzed by specific phosphodiesterases to form 5′-AMP, as indicated.

(whooping cough), catalyzes the ADP ribosylation of the α subunit of G_i, preventing the protein from interacting with receptors; as a result, the G protein retains its bound GDP and is unable to regulate its target proteins. These two toxins are widely used in experiments to determine whether a cell's GPCR-dependent response to a signal is mediated by G_s or by G_i.

Some of the responses mediated by a G_s-stimulated increase in cyclic AMP concentration are listed in **Table 15–1**. As the table shows, different cell types respond differently to an increase in cyclic AMP concentration, and one cell type often responds in the same way to such an increase, regardless of the extracellular signal that causes it. At least four hormones activate adenylyl cyclase in fat cells, for example, and all of them stimulate the breakdown of triglyceride (the storage form of fat) to fatty acids (see Table 15–1).

Individuals who are genetically deficient in a particular G_s α subunit show decreased responses to certain hormones. As a consequence, these people display metabolic abnormalities, have abnormal bone development, and are mentally retarded.

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

TARGET TISSUE	HORMONE	MAJOR RESPONSE
Thyroid gland	thyroid-stimulating hormone (TSH)	thyroid hormone synthesis and secretion
Adrenal cortex	adrenocorticotrophic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone (LH)	progesterone secretion
Muscle	adrenaline	glycogen breakdown
Bone	parathormone	bone resorption
Heart	adrenaline	increase in heart rate and force of contraction
Liver	glucagon	glycogen breakdown
Kidney	vasopressin	water resorption
Fat	adrenaline, ACTH, glucagon, TSH	triglyceride breakdown

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

In most animal cells, cyclic AMP exerts its effects mainly by activating **cyclic-AMP-dependent protein kinase (PKA)**. This kinase phosphorylates specific serines or threonines on selected target proteins, including intracellular signaling proteins and effector proteins, thereby regulating their activity. The target proteins differ from one cell type to another, which explains why the effects of cyclic AMP vary so markedly depending on the cell type (see Table 15–1).

In the inactive state, PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cyclic AMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex. The released catalytic subunits are thereby activated to phosphorylate specific target proteins (**Figure 15–35**). The regulatory subunits of PKA (also called A-kinase) are important for localizing the kinase inside the cell: special *A-kinase anchoring proteins (AKAPs)* bind both to the regulatory subunits and to a component of the cytoskeleton or a membrane of an organelle, thereby tethering the enzyme complex to a particular subcellular compartment. Some AKAPs also bind other signaling proteins, forming a complex that functions as a signaling module. An AKAP located around the nucleus of heart muscle cells, for example, binds both PKA and a phosphodiesterase that hydrolyzes cyclic AMP. In unstimulated cells, the phosphodiesterase keeps the local cyclic AMP concentration low, so that the bound PKA is inactive; in stimulated cells, cyclic AMP concentration rapidly rises, overwhelming the phosphodiesterase and activating the PKA. Among the target proteins that PKA phosphorylates and activates in these cells is the adjacent phosphodiesterase, which rapidly lowers the cyclic AMP concentration again. This arrangement converts what might otherwise be a weak and prolonged PKA response into a strong, brief, local pulse of PKA activity.

Whereas some responses mediated by cyclic AMP occur within seconds and do not depend on changes in gene transcription (see Figure 15–33), others do depend on changes in the transcription of specific genes and take hours to develop fully. In cells that secrete the peptide hormone *somatostatin,* for example, cyclic AMP activates the gene that encodes this hormone. The regulatory region of the somatostatin gene contains a short DNA sequence, called the *cyclic AMP response element (CRE),* which is also found in the regulatory region of many other genes activated by cyclic AMP. A specific gene regulatory protein called **CRE-binding (CREB) protein** recognizes this sequence. When PKA is activated by cAMP, it phosphorylates CREB on a single serine; phosphorylated CREB then recruits a transcriptional coactivator called *CREB-binding protein (CBP),* which stimulates the transcription of the target genes (**Figure 15–36**). Thus, CREB can transform a short cyclic AMP signal into a long-term change in a cell, a process that, in the brain, is thought to play an important part in some forms of learning and memory.

PKA does not mediate all the effects of cyclic AMP in cells. As we discuss later, in olfactory neurons (responsible for the sense of smell), cyclic AMP also directly activates special ion channels in the plasma membrane. Moreover, in some other

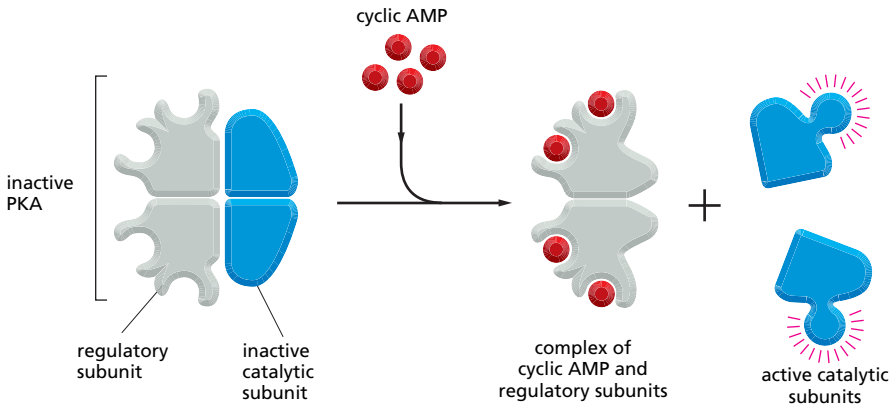

Figure 15–35 The activation of cyclic-AMP-dependent protein kinase (PKA). The binding of cyclic AMP to the regulatory subunits of the PKA tetramer induces a conformational change, causing these subunits to dissociate from the catalytic subunits, thereby activating the kinase activity of the catalytic subunits. The release of the catalytic subunits requires the binding of more than two cyclic AMP molecules to the regulatory subunits in the tetramer. This requirement greatly sharpens the response of the kinase to changes in cyclic AMP concentration, as discussed earlier (see Figure 15–25). Mammalian cells have at least two types of PKAs: type I is mainly in the cytosol, whereas type II is bound via its regulatory subunits and special anchoring proteins to the plasma membrane, nuclear membrane, mitochondrial outer membrane, and microtubules. In both types, once the catalytic subunits are freed and active, they can migrate into the nucleus (where they can phosphorylate gene regulatory proteins), while the regulatory subunits remain in the cytoplasm. The three-dimensional structure of the protein kinase domain of the PKA catalytic subunit is shown in Figure 3–65.

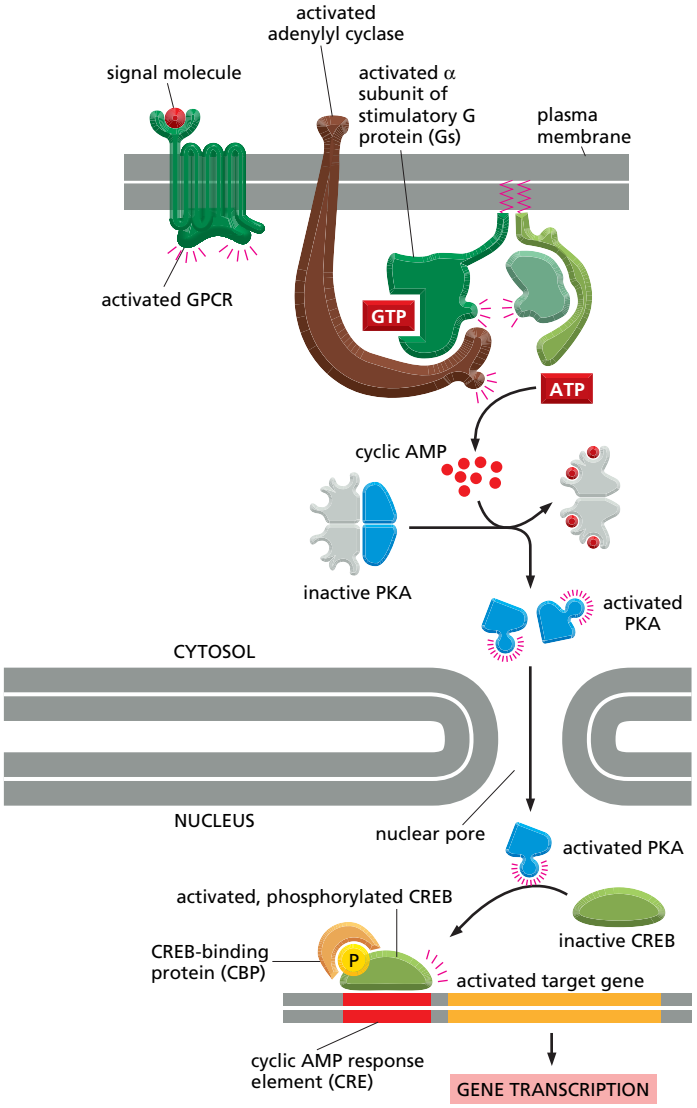

Figure 15–36 How a rise in intracellular cyclic AMP concentration can alter gene transcription. <AGAT> The binding of an extracellular signal molecule to its GPCR activates adenylyl cyclase via G_s and thereby increases cyclic AMP concentration in the cytosol. The rise in cyclic AMP concentration activates PKA, and the released catalytic subunits of PKA can then enter the nucleus, where they phosphorylate the gene regulatory protein CREB. Once phosphorylated, CREB recruits the coactivator CBP, which stimulates gene transcription. In some cases, at least, the inactive CREB protein is bound to the cyclic AMP response element (CRE) in DNA before it is phosphorylated (not shown).

This signaling pathway controls many processes in cells, ranging from hormone synthesis in endocrine cells to the production of proteins required for the induction of long-term memory in the brain. We will see later that CREB can also be activated by some other signaling pathways, independent of cAMP.

cells, it directly activates a guanine nucleotide exchange factor (GEF) that, in turn, activates a monomeric GTPase called *Rap1*, which often leads to increased cell adhesion through the activation of cell-surface *integrins* (discussed in Chapter 19).

Having discussed how trimeric G proteins link activated GPCRs to adenylyl cyclase, we now consider how they couple activated GPCRs to another crucial enzyme, *phospholipase C*. The activation of this enzyme increases the concentration of several small intracellular mediators, including Ca^{2+}, which help relay the signal onward. Ca^{2+} is even more widely used than cyclic AMP as a small intracellular mediator.

Some G Proteins Activate An Inositol Phospholipid Signaling Pathway by Activating Phospholipase C-β

Many GPCRs exert their effects mainly via G proteins that activate the plasma-membrane-bound enzyme **phospholipase C-β (PLCβ)**. **Table 15–2** lists several examples of responses activated in this way. The phospholipase acts on a phosphorylated inositol phospholipid (a *phosphoinositide*) called **phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2, or PIP_2]**, which is present in small amounts in the inner half of the plasma membrane lipid bilayer (**Figure 15–37**). Receptors that activate this **inositol phospholipid signaling pathway** mainly do so via a G protein called $\mathbf{G_q}$, which activates phospholipase C-β in much the same way that

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

TARGET TISSUE	SIGNAL MOLECULE	MAJOR RESPONSE
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	muscle contraction
Blood platelets	thrombin	platelet aggregation

G_s activates adenylyl cyclase. The activated phospholipase then cleaves the PIP_2 to generate two products: *inositol 1,4,5-trisphosphate (IP_3)* and *diacylglycerol* (**Figure 15–38**). At this step, the signaling pathway splits into two branches.

Inositol 1,4,5-trisphosphate (IP_3) is a water-soluble molecule that acts as a small intracellular mediator. It leaves the plasma membrane and diffuses rapidly through the cytosol. When it reaches the endoplasmic reticulum (ER), it binds to and opens **IP_3-gated Ca^{2+}-release channels** (also called **IP_3 receptors**) in the ER membrane. Ca^{2+} stored in the ER is released through the open channels, quickly raising the concentration of Ca^{2+} in the cytosol (**Figure 15–39**). Once the ER Ca^{2+} stores have been depleted, they are refilled by the activation of *store-operated Ca^{2+} channels* in the plasma membrane and a Ca^{2+} sensor protein in the ER membrane, in regions where the two membranes are closely apposed.

We discuss later how the increase in cytosolic Ca^{2+} propagates the signal by influencing the activity of Ca^{2+}-sensitive intracellular proteins. Several mechanisms operate to terminate the initial Ca^{2+} response: (1) IP_3 is rapidly dephosphorylated by specific lipid phosphatases to form IP_2; (2) IP_3 is phosphorylated by specific lipid kinases to form IP_4 (which may function as another small intracellular mediator); and (3) Ca^{2+} that enters the cytosol is rapidly pumped out, mainly to the exterior of the cell (see Figure 15–41).

At the same time that the IP_3 produced by the hydrolysis of PIP_2 is increasing the concentration of Ca^{2+} in the cytosol, the other cleavage product of the PIP_2, **diacylglycerol**, is exerting different effects. It also acts as a small intracellular mediator, but it remains embedded in the plasma membrane, where it has several potential signaling roles. It can be further cleaved to release arachidonic acid, which can either act as a signal in its own right or be used in the synthesis of other small lipid signal molecules called *eicosanoids*. Most vertebrate cell

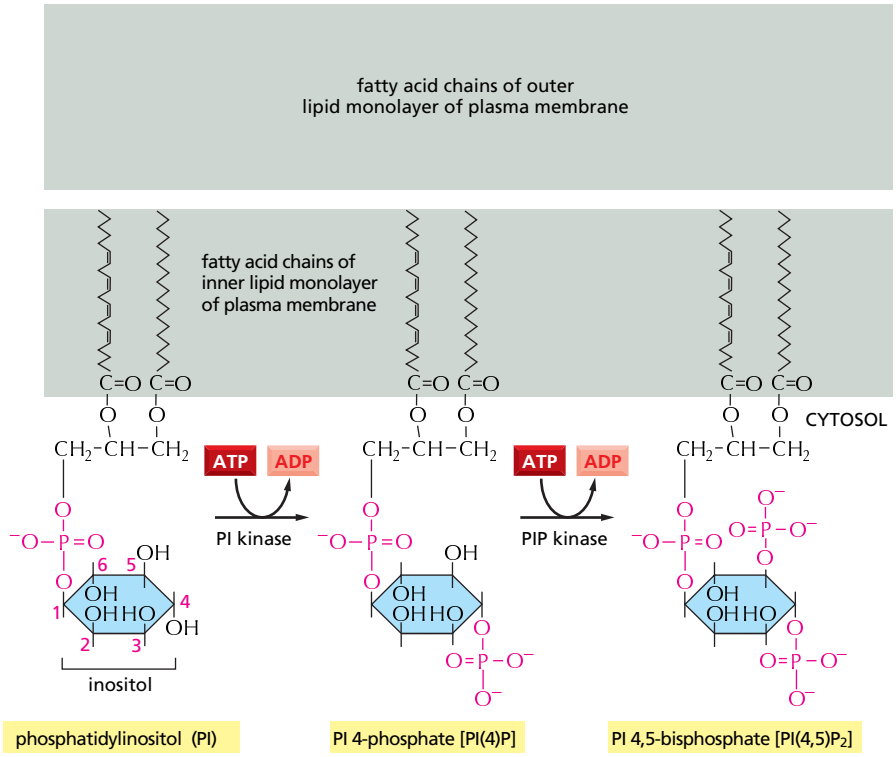

Figure 15–37 The synthesis of PI(4,5)P_2. The phosphoinositides PI(4)P and PI(4,5)P_2 are produced by the phosphorylation of phosphatidylinositol (PI) and PI(4)P, respectively. Although all three inositol phospholipids may be broken down in a signaling response, it is the breakdown of PI(4,5)P_2 that predominates and is most critical because it generates two intracellular mediators, as shown in Figures 15–38 and 15–39. Nevertheless, PI(4,5)P_2 is the least abundant, constituting less than 10% of the total inositol phospholipids and about 1% of the lipids in the plasma membrane. The conventional numbering of the carbon atoms in the inositol ring is shown in *red numbers* on the PI molecule.

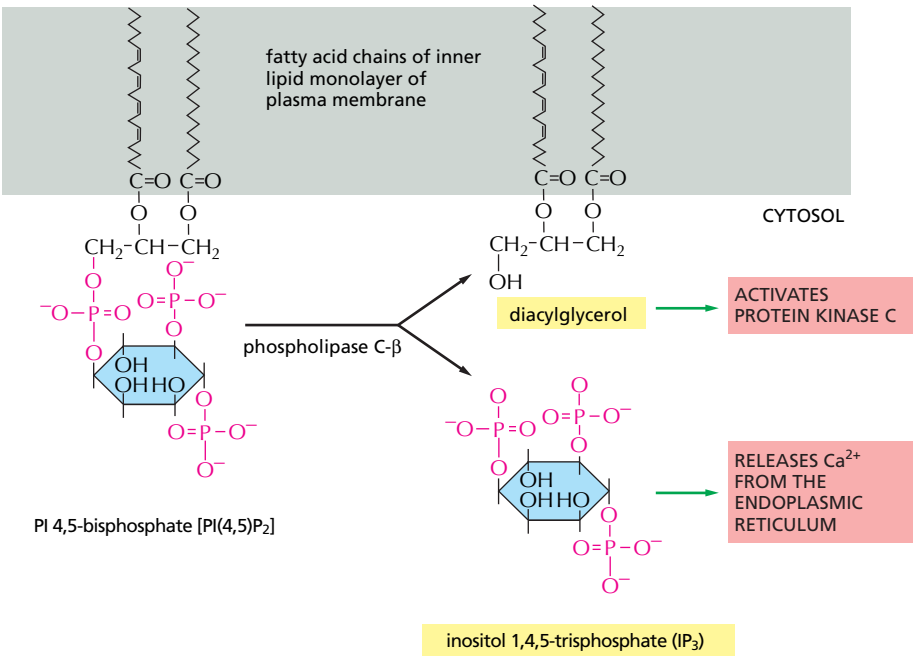

Figure 15–38 The hydrolysis of PI(4,5)P_2 by phospholipase C-β. Two small intracellular mediators are produced directly from the hydrolysis of the PIP_2: inositol 1,4,5-trisphosphate (IP_3), which diffuses through the cytosol and releases Ca^{2+} from the endoplasmic reticulum, and diacylglycerol, which remains in the membrane and helps to activate the enzyme protein kinase C (PKC; see Figure 15–39). There are several classes of phospholipase C; these include the β class, which is activated by GPCRs; as we see later, the γ class is activated by a class of enzyme-coupled receptors called receptor tyrosine kinases (RTKs).

types make eicosanoids, including *prostaglandins,* which have many biological activities. They participate in pain and inflammatory responses, for example, and most anti-inflammatory drugs (such as aspirin, ibuprofen, and cortisone) act—in part, at least—by inhibiting their synthesis.

A second function of diacylglycerol is to activate a crucial serine/threonine protein kinase called **protein kinase C (PKC)**, so named because it is Ca^{2+}-dependent. The initial rise in cytosolic Ca^{2+} induced by IP_3 alters the PKC so that it translocates from the cytosol to the cytoplasmic face of the plasma membrane. There it is activated by the combination of Ca^{2+}, diacylglycerol, and the negatively charged membrane phospholipid phosphatidylserine (see Figure 15–39). Once activated, PKC phosphorylates target proteins that vary depending on the cell type. The principles are the same as discussed earlier for PKA, although most of the target proteins are different.

There are various classes of PKCs, only some of which (called *conventional PKCs*) are activated by Ca^{2+} and diacylglycerol; the others are called *atypical PKCs.* Different PKCs phosphorylate different substrates mainly because different anchoring or scaffold proteins tether them to different compartments in the cell.

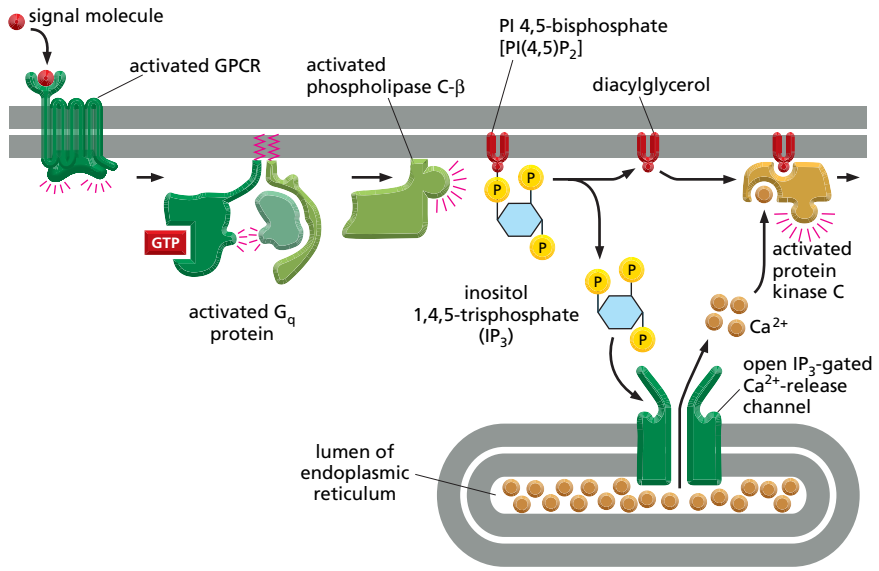

Figure 15–39 How GPCRs increase cytosolic Ca^{2+} and activate PKC. The activated GPCR stimulates the plasma-membrane-bound phospholipase PLCβ via a G protein. Depending on the isoform of the PLCβ, it may be activated by the α subunit of G_q as shown, by the βγ subunits of another G protein, or by both. Two small intracellular messenger molecules are produced when PI(4,5)P_2 is hydrolyzed by activated PLCβ. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and releases Ca^{2+} from the ER by binding to and opening IP_3-gated Ca^{2+}-release channels (IP_3 receptors) in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to escape into the cytosol when the release channels are open. Diacylglycerol remains in the plasma membrane and, together with phosphatidylserine (not shown) and Ca^{2+}, helps to activate protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane. Of the 10 or more distinct isoforms of PKC in humans, at least 4 are activated by diacylglycerol.