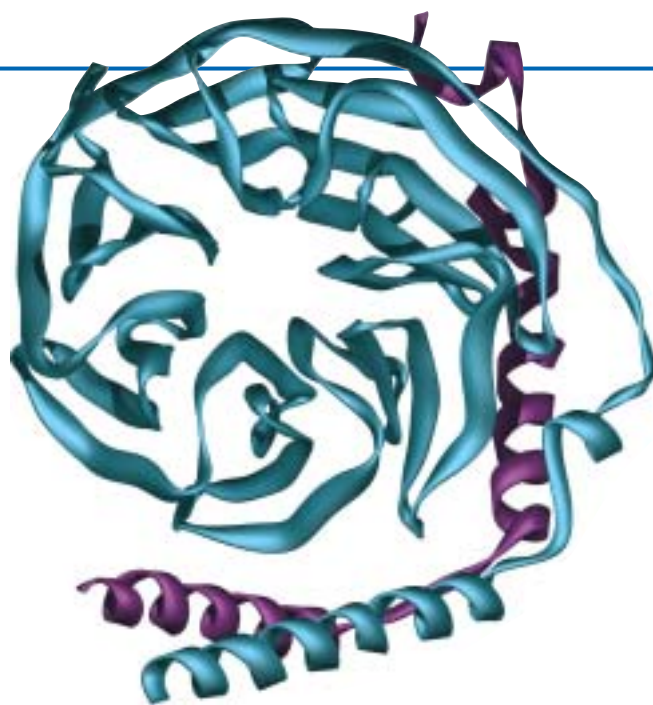


13

SIGNALING AT THE CELL SURFACE



Three-dimensional structure of the G protein β (blue) and γ (purple) complex as obtained by x-ray crystallography.

No cell lives in isolation. In eukaryotic microorganisms such as yeast, slime molds, and protozoans, secreted molecules called **pheromones** coordinate the aggregation of free-living cells for sexual mating or differentiation under certain environmental conditions. Yeast mating-type factors are a well-understood example of pheromone-mediated cell-to-cell signaling (Chapter 22). More important in plants and animals are extracellular **signaling molecules** that function *within* an organism to control metabolic processes within cells, the growth and differentiation of tissues, the synthesis and secretion of proteins, and the composition of intracellular and extracellular fluids. Adjacent cells often communicate by direct cell-cell contact. For example, gap junctions in the plasma membranes of adjacent cells permit them to exchange small molecules and to coordinate metabolic responses. Other junctions between adjacent cells determine the shape and rigidity of many tissues; other interactions adhere cells to the extracellular matrix. Such cell-cell and cell-matrix interactions, which are covered in Chapter 6, may also initiate intracellular signaling via pathways similar to those discussed in this and subsequent chapters.

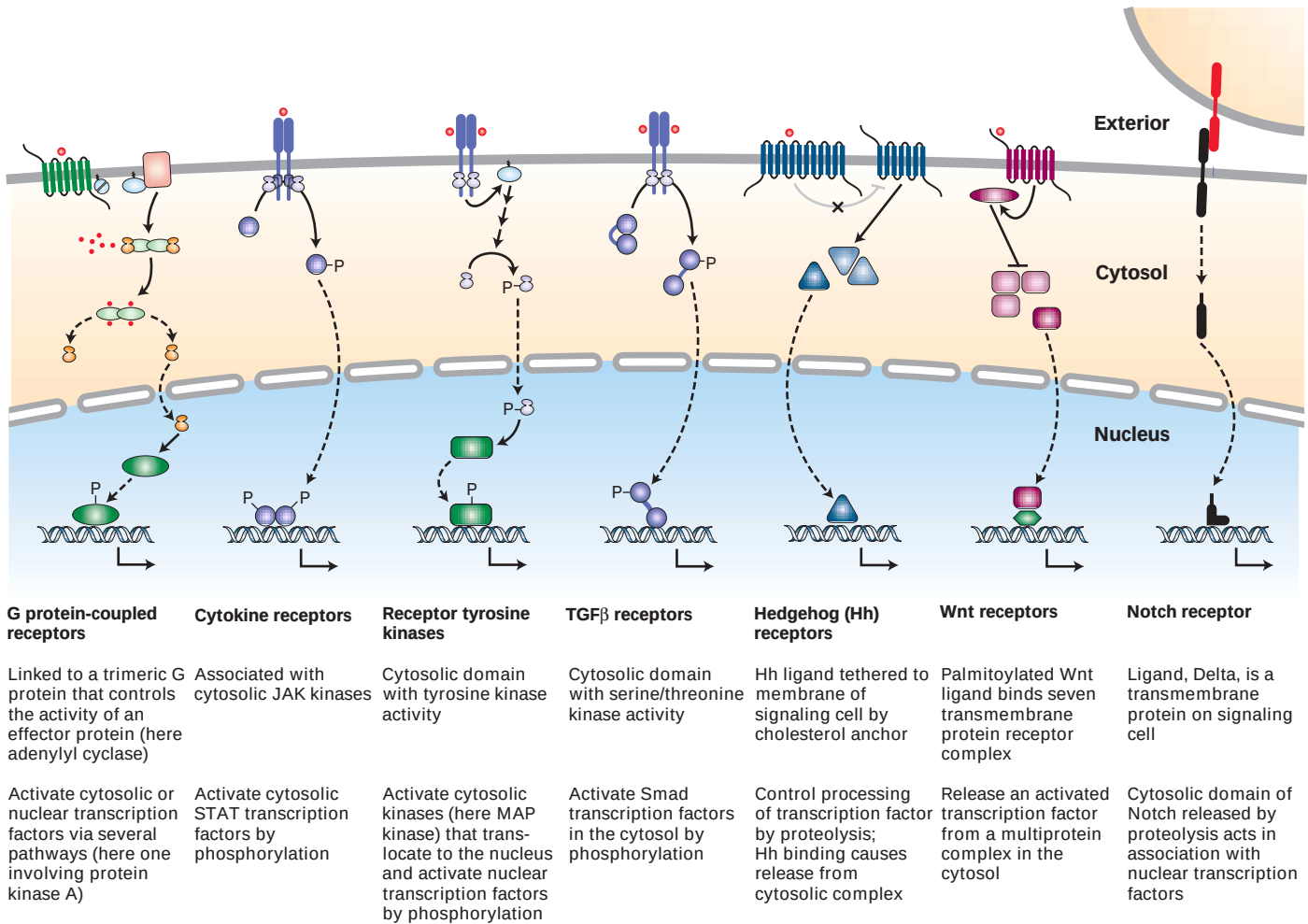
Extracellular signaling molecules are synthesized and released by *signaling cells* and produce a specific response only in *target cells* that have **receptors** for the signaling molecules. In multicellular organisms, an enormous variety of chemicals, including small molecules (e.g., amino acid or lipid derivatives, acetylcholine), peptides, and proteins, are used in this type of cell-to-cell communication. Some signaling molecules, especially hydrophobic molecules such as steroids, retinoids, and thyroxine, spontaneously diffuse through the

plasma membrane and bind to intracellular receptors. Signaling from such intracellular receptors is discussed in Chapter 11.

In this and the next two chapters, we focus on signaling from a diverse group of receptor proteins located in the plasma membrane (Figure 13-1). The signaling molecule acts as a **ligand**, which binds to a structurally complementary site on the extracellular or membrane-spanning domains of the receptor. Binding of a ligand to its receptor causes a conformational change in the cytosolic domain or domains of the receptor that ultimately induces specific cellular responses. The overall process of converting signals into cellular responses, as well as the individual steps in this

OUTLINE

- 13.1 Signaling Molecules and Cell-Surface Receptors
- 13.2 Intracellular Signal Transduction
- 13.3 G Protein–Coupled Receptors That Activate or Inhibit Adenylyl Cyclase
- 13.4 G Protein–Coupled Receptors That Regulate Ion Channels
- 13.5 G Protein–Coupled Receptors That Activate Phospholipase C
- 13.6 Activation of Gene Transcription by G Protein–Coupled Receptors



▲ **FIGURE 13-1 Overview of seven major classes of cell-surface receptors discussed in this book.** In many signaling pathways, ligand binding to a receptor leads to activation of transcription factors in the cytosol, permitting them to translocate into the nucleus and stimulate (or occasionally repress) transcription of their target genes. Alternatively, receptor

stimulation may lead to activation of cytosolic protein kinases that then translocate into the nucleus and regulate the activity of nuclear transcription factors. Some activated receptors, particularly certain G protein-coupled receptors, also can induce changes in the activity of preexisting proteins. [After A. H. Brivanlou and J. Darnell, 2002, *Science* **295**:813.]

process, is termed **signal transduction**. As we will see, signal-transduction pathways may involve relatively few or many components.

We begin this chapter with two sections that describe general principles and techniques that are relevant to most signaling systems. In the remainder of the chapter, we concentrate on the huge class of cell-surface receptors that activate **trimeric G proteins**. Receptors of this type, commonly called **G protein-coupled receptors (GPCRs)**, are found in all eukaryotic cells from yeast to man. The human genome, for instance, encodes several thousand G protein-coupled receptors. These include receptors in the visual, olfactory (smell), and gustatory (taste) systems, many neurotransmitter receptors, and most of the receptors for hormones that control carbohydrate, amino acid, and fat metabolism.

13.1 Signaling Molecules and Cell-Surface Receptors

Communication by extracellular signals usually involves the following steps: (1) synthesis and (2) release of the signaling molecule by the signaling cell; (3) transport of the signal to the target cell; (4) binding of the signal by a specific receptor protein leading to its activation; (5) initiation of one or more intracellular signal-transduction pathways by the activated receptor; (6) specific changes in cellular function, metabolism, or development; and (7) removal of the signal, which often terminates the cellular response (see Figure 13-1). The vast majority of receptors are activated by binding of secreted or membrane-bound molecules (e.g., hormones, growth factors, neurotransmitters, and pheromones).

Some receptors, however, are activated by changes in the concentration of a metabolite (e.g., oxygen or nutrients) or by physical stimuli (e.g., light, touch, heat). In *E. coli*, for instance, receptors in the cell-surface membrane trigger signaling pathways that help the cell respond to changes in the external level of phosphate and other nutrients (see Figure 4-18).

Signaling Molecules in Animals Operate over Various Distances

In animals, signaling by soluble extracellular molecules can be classified into three types—endocrine, paracrine, or autocrine—based on the distance over which the signal acts. In addition, certain membrane-bound proteins act as signals.

In **endocrine** signaling, the signaling molecules, called **hormones**, act on target cells distant from their site of synthesis by cells of the various endocrine organs. In animals, an endocrine hormone usually is carried by the blood or by other extracellular fluids from its site of release to its target.

In **paracrine** signaling, the signaling molecules released by a cell affect target cells only in close proximity. The conduction by a neurotransmitter of a signal from one nerve cell to another or from a nerve cell to a muscle cell (inducing or inhibiting muscle contraction) occurs via paracrine signaling (Chapter 7). Many **growth factors** regulating development in multicellular organisms also act at short range. Some of these molecules bind tightly to the extracellular matrix, unable to signal, but subsequently can be released in an active form. Many developmentally important signals diffuse away from the signaling cell, forming a concentration gradient and inducing various cellular responses depending on their concentration at a particular target cell (Chapter 15).

In **autocrine** signaling, cells respond to substances that they themselves release. Some growth factors act in this fashion, and cultured cells often secrete growth factors that stimulate their own growth and proliferation. This type of signaling is particularly common in tumor cells, many of which overproduce and release growth factors that stimulate inappropriate, unregulated proliferation of themselves as well as adjacent nontumor cells; this process may lead to formation of a tumor mass.

Signaling molecules that are integral membrane proteins located on the cell surface also play an important role in development. In some cases, such membrane-bound signals on one cell bind receptors on the surface of an adjacent target cell to trigger its differentiation. In other cases, proteolytic cleavage of a membrane-bound signaling protein releases the exoplasmic region, which functions as a soluble signaling protein.

Some signaling molecules can act both short range and long range. **Epinephrine**, for example, functions as a neurotransmitter (paracrine signaling) and as a systemic hormone (endocrine signaling). Another example is epidermal growth factor (EGF), which is synthesized as an integral plasma-membrane protein. Membrane-bound EGF can bind to and

signal an adjacent cell by direct contact. Cleavage by an extracellular protease releases a soluble form of EGF, which can signal in either an autocrine or a paracrine manner.

Receptors Activate a Limited Number of Signaling Pathways

The number of receptors and signaling pathways that we discuss throughout this book initially may seem overwhelming. Moreover, the terminology for designating pathways can be confusing. Pathways commonly are named based on the general class of receptor involved (e.g., GPCRs, receptor tyrosine kinases), the type of ligand (e.g., TGF β , Wnt, Hedgehog), or a key intracellular signal transduction component (e.g., NF- κ B). In some cases, the same pathway may be referred to by different names. Fortunately, as researchers have discovered the molecular details of more and more receptors and pathways, some principles and mechanisms are beginning to emerge. These shared features can help us make sense of the wealth of new information concerning cell-to-cell signaling.

First, external signals induce two major types of cellular responses: (1) changes in the activity or function of specific pre-existing proteins and (2) changes in the amounts of specific proteins produced by a cell, most commonly as the result of modification of transcription factors leading to activation or repression of gene transcription. In general, the first type of response occurs more rapidly than the second type. Signaling from G protein-coupled receptors, described in later sections, often results in changes in the activity of preexisting proteins, although activation of these receptors on some cells also can induce changes in gene expression.

The other classes of receptors depicted in Figure 13-1 operate primarily to modulate gene expression. In some cases, the activated receptor directly activates a **transcription factor** in the cytosol (e.g., TGF β and cytokine receptor pathways) or assembles an intracellular signaling complex that activates a cytosolic transcription factor (e.g., Wnt pathways). In yet other pathways, specific proteolytic cleavage of an activated cell-surface receptor or cytosolic protein releases a transcription factor (e.g., Hedgehog, Notch, and NF- κ B pathways). Transcription factors activated in the cytosol by these pathways move into the nucleus, where they stimulate (or occasionally inhibit) transcription of specific target genes. Signaling from receptor tyrosine kinases leads to activation of several cytosolic protein kinases that translocate into the nucleus and regulate the activity of nuclear transcription factors. We consider these signaling pathways, which regulate transcription of many genes essential for cell division and for many cell differentiation processes, in the following two chapters.

Second, some classes of receptors can initiate signaling via more than one intracellular signal-transduction pathway, leading to different cellular responses. This complication is typical of G protein-coupled receptors, receptor tyrosine kinases, and cytokine receptors.

Third, despite the huge number of different kinds of ligands and their specific receptors, a relatively small number of signal-transduction mechanisms and highly conserved intracellular proteins play a major role in intracellular signaling pathways. Our knowledge of these common themes has advanced greatly in recent years. For instance, we can trace the entire signaling pathway from binding of ligand to receptors in several classes to the final cellular response.

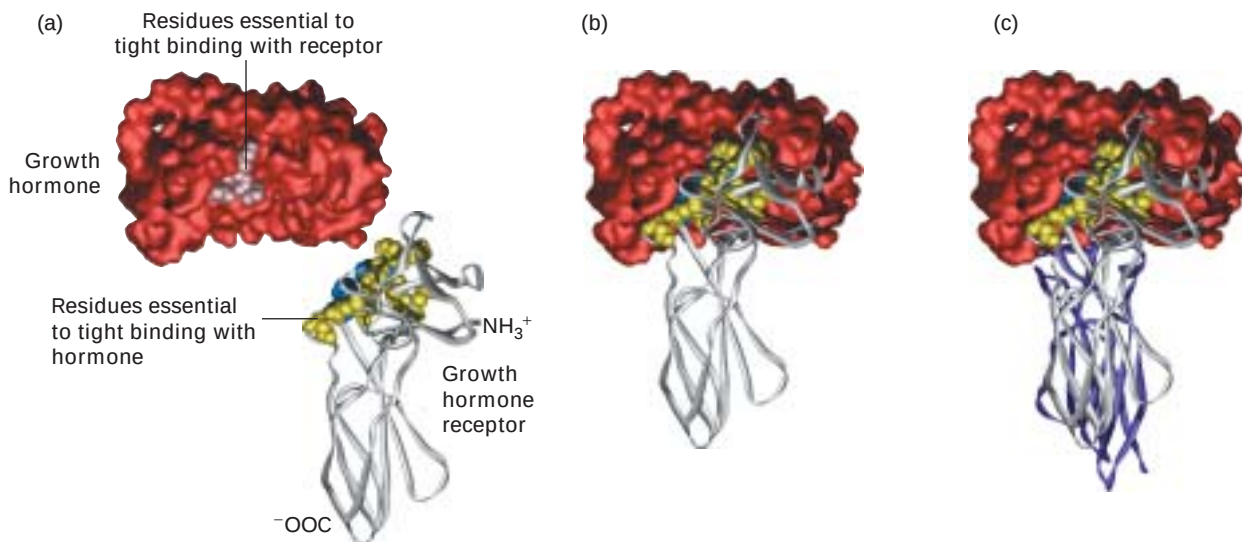
Before delving into the particulars of individual signaling pathways, we discuss the basic properties of cell-surface receptors, as well as methods for identifying and studying them, in the remainder of this section; important general features of intracellular signal transduction are presented in Section 13.2.

Receptor Proteins Exhibit Ligand-Binding and Effector Specificity

The response of a cell or tissue to specific external signals is dictated by the particular receptors it possesses, by the signal-transduction pathways they activate, and by the intracellular processes ultimately affected. Each receptor generally binds only a single signaling molecule or a group of very

closely related molecules (Figure 13-2). In contrast, many signaling molecules bind to multiple types of receptors, each of which can activate different intracellular signaling pathways and thus induce different cellular responses. For instance, different types of acetylcholine receptors are found on the surface of striated muscle cells, heart muscle cells, and pancreatic acinar cells. Release of acetylcholine from a neuron adjacent to a striated muscle cell triggers contraction by activating a ligand-gated ion channel, whereas release adjacent to a heart muscle slows the rate of contraction via activation of a G protein-coupled receptor. Release adjacent to a pancreatic acinar cell triggers exocytosis of secretory granules that contain digestive enzymes. Similarly, epinephrine binds to several different G protein-coupled receptors, each of which induces a distinct cellular response. Thus each receptor protein is characterized by *binding specificity* for a particular ligand, and the resulting receptor-ligand complex exhibits *effector specificity* (i.e., mediates a specific cellular response).

On the other hand, different receptors of the same class that bind different ligands often induce the same cellular responses in a cell. In liver cells, for instance, the hormones epinephrine, glucagon, and ACTH bind to different members of the G protein-coupled receptor family, but all these



▲ **EXPERIMENTAL FIGURE 13-2** **Mutational studies have identified the patches of amino acids in growth hormone and its receptor that determine their highly specific mutual interaction.** The outer surface of the plasma membrane is toward the bottom of the figure, and each receptor is anchored to the membrane by a hydrophobic membrane-spanning alpha helix that is not shown. As determined from the three-dimensional structure of the growth hormone–growth hormone receptor complex, 28 amino acids in the hormone are at the binding interface with one receptor. Each of these amino acids was mutated, one at a time, to alanine, and the effect on receptor binding was determined. (a) From this study it was found that only eight amino acids on growth hormone (pink) contribute 85 percent of the binding energy;

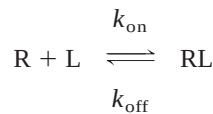
these amino acids are distant in the primary sequence but adjacent in the folded protein. Similar studies showed that two tryptophan residues (blue) in the receptor contribute most of the energy for binding growth hormone, although other amino acids at the interface with the hormone (yellow) are also important. (b) Binding of growth hormone to one receptor molecule is followed by (c) binding of a second receptor to the opposing side of the hormone; this involves the same set of yellow and blue amino acids on the receptor but different residues on the hormone. As we see in the following chapter, such hormone-induced receptor dimerization is a common mechanism for receptor activation. [After B. Cunningham and J. Wells, 1993, *J. Mol. Biol.* **234**:554, and T. Clackson and J. Wells, 1995, *Science* **267**:383.]

receptors activate the same signal-transduction pathway, one that promotes synthesis of **cyclic AMP (cAMP)**. This small signaling molecule in turn regulates various metabolic functions, including glycogen breakdown. As a result, all three hormones have the same effect on liver-cell metabolism.

Maximal Cellular Response to a Signaling Molecule May Not Require Activation of All Receptors

As we've seen, activation of a cell-surface receptor and subsequent signal transduction are triggered by binding of a signaling molecule (ligand) to the receptor. This binding depends on weak, noncovalent forces (i.e., ionic, van der Waals, and hydrophobic interactions) and **molecular complementarity** between the interacting surfaces of a receptor and ligand (Chapter 2). The specificity of a receptor refers to its ability to distinguish closely related substances. The insulin receptor, for example, binds insulin and a related hormone called insulinlike growth factor 1, but no other peptide hormones.

Ligand binding usually can be viewed as a simple reversible reaction,



which can be described by the equation

$$K_d = \frac{[\text{R}][\text{L}]}{[\text{RL}]} \quad (13-1)$$

where $[\text{R}]$ and $[\text{L}]$ are the concentrations of free receptor and ligand, respectively, at equilibrium, and $[\text{RL}]$ is the concentration of the receptor-ligand complex. K_d , the **dissociation constant** of the receptor-ligand complex, measures the *affinity* of the receptor for the ligand. This equilibrium binding equation can be rewritten as

$$\frac{[\text{RL}]}{R_T} = \frac{1}{1 + \frac{K_d}{[\text{L}]}} \quad (13-2)$$

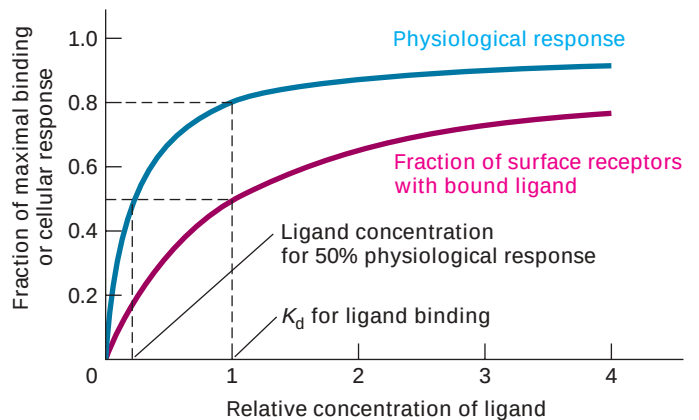
where $R_T = [\text{R}] + [\text{RL}]$, the total concentration of free and bound receptors; therefore, $[\text{RL}]/R_T$ is the fraction of receptors that have a bound ligand. The lower the K_d value, the higher the affinity of a receptor for its ligand. The K_d value is equivalent to the concentration of ligand at which half the receptors contain bound ligand. If $[\text{L}] = K_d$, then from Equation 13-2 we can see that $[\text{RL}] = 0.5 R_T$. Equation 13-2 has the same general form as the Michaelis-Menten equation, which describes simple one-substrate enzymatic reactions (Chapter 3). The K_d for a binding reaction is equivalent to the Michaelis constant K_m , which reflects the affinity of an enzyme for its substrate.

For a simple binding reaction, $K_d = k_{\text{off}}/k_{\text{on}}$, where k_{off} is the rate constant for dissociation of a ligand from its receptor, and k_{on} is the rate constant for formation of a receptor-

ligand complex from free ligand and receptor. The lower k_{off} is relative to k_{on} , the more stable the RL complex, and thus the lower the value of K_d . Like all equilibrium constants, however, the value of K_d does not depend on the *absolute* values of k_{off} and k_{on} , only on their ratio. For this reason, binding of ligand by two different receptors can have the same K_d values but very different rate constants.

In general, the K_d value of a cell-surface receptor for a circulating hormone is greater than the normal (unstimulated) blood level of that hormone. Under this circumstance, changes in hormone concentration are reflected in proportional changes in the fraction of receptors occupied. Suppose, for instance, that the normal concentration of a hormone in the blood is 10^{-9} M and that the K_d for its receptor is 10^{-7} M; by substituting these values into Equation 13-2, we can calculate the fraction of receptors with bound hormone, $[\text{RL}]/R_T$, at equilibrium as 0.0099. Thus about 1 percent of the total receptors will be filled with hormone. If the hormone concentration rises tenfold to 10^{-8} M, the concentration of receptor-hormone complex will rise proportionately, so that about 10 percent of the total receptors would have bound hormone. If the extent of the induced cellular response parallels the amount of RL, as is often the case, then the cellular responses also will increase tenfold.

In many cases, however, the maximal cellular response to a particular ligand is induced when less than 100 percent of its receptors are bound to the ligand. This phenomenon can be revealed by determining the extent of the response and of receptor-ligand binding at different concentrations of ligand (Figure 13-3). For example, a typical erythroid progenitor cell



▲ EXPERIMENTAL FIGURE 13-3 The maximal physiological response to many external signals occurs when only a fraction of the receptor molecules are occupied by ligand. In this situation, plots of the extent of ligand binding and of physiological response at different ligand concentrations differ. In the example shown here, 50 percent of the maximal physiological response is induced at a ligand concentration at which only 18 percent of the receptors are occupied. Likewise, 80 percent of the maximal response is induced when the ligand concentration equals the K_d value, at which 50 percent of the receptors are occupied.

has ≈ 1000 surface receptors for erythropoietin, which induces progenitor cells to proliferate and differentiate into red blood cells. Because only 100 of these receptors need to bind erythropoietin to induce division of a target cell, the ligand concentration needed to induce 50 percent of the maximal cellular response is proportionally lower than the K_d value for binding. In such cases, a plot of the percentage of maximal binding versus ligand concentration differs from a plot of the percentage of maximal cellular response versus ligand concentration.

Sensitivity of a Cell to External Signals Is Determined by the Number of Surface Receptors

Because the cellular response to a particular signaling molecule depends on the number of receptor-ligand complexes, the fewer receptors present on the surface of a cell, the less *sensitive* the cell is to that ligand. As a consequence, a higher ligand concentration is necessary to induce the usual physiological response than would be the case if more receptors were present.

To illustrate this important point, let's extend our example of a typical erythroid progenitor cell. The K_d for binding of erythropoietin (Epo) to its receptor is about 10^{-10} M. As we noted above, only 10 percent of the ≈ 1000 cell-surface erythropoietin receptors on the surface of a cell must be bound to ligand to induce the maximal cellular response. We can determine the ligand concentration, $[L]$, needed to induce the maximal response by rewriting Equation 13-2 as follows:

$$[L] = \frac{K_d}{\frac{R_T}{[RL]} - 1} \quad (13-3)$$

If $R_T = 1000$ (the total number of Epo receptors per cell), $K_d = 10^{-10}$ M, and $[RL] = 100$ (the number of Epo-occupied receptors needed to induce the maximal response), then an Epo concentration of 1.1×10^{-11} M will elicit the maximal response. If R_T is reduced to 200/cell, then a ninefold higher Epo concentration (10^{-10} M) is required to occupy 100 receptors and induce the maximal response. If R_T is further reduced to 120/cell, an Epo concentration of 5×10^{-10} M, a 50-fold increase, is necessary to generate the same cellular response.

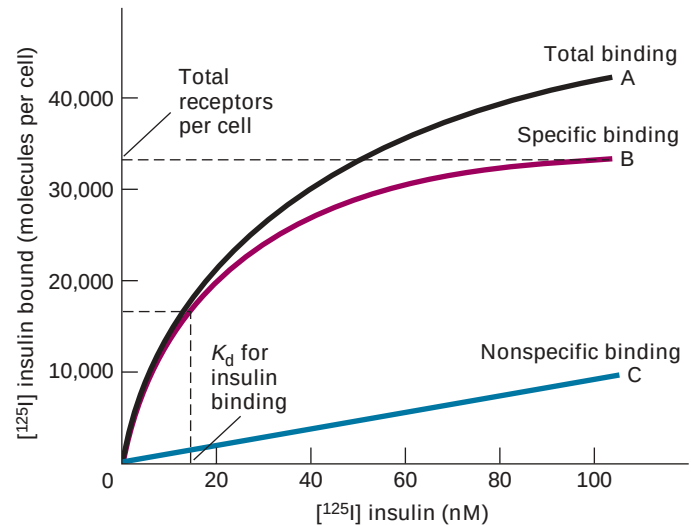
Regulation of the number of receptors for a given signaling molecule expressed by a cell and thus its sensitivity to that signal plays a key role in directing physiological and developmental events. Alternatively, endocytosis of receptors on the cell surface can sufficiently reduce the number present to terminate the usual cellular response at the prevailing signal concentration.

Binding Assays Are Used to Detect Receptors and Determine Their K_d Values

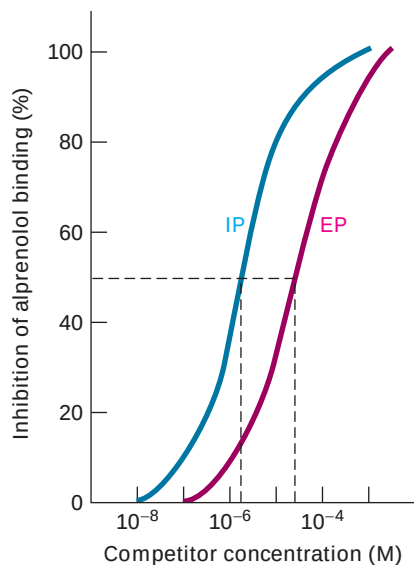
Cell-surface receptors are difficult to identify and purify, mainly because they are present in such minute amounts. The receptor for a particular signaling molecule commonly con-

stitutes only $\approx 10^{-6}$ of the total protein in the cell, or $\approx 10^{-4}$ of the plasma-membrane protein. Purification is also difficult because these integral membrane proteins first must be solubilized with a nonionic detergent so they can be separated from other proteins (see Figure 5-40).

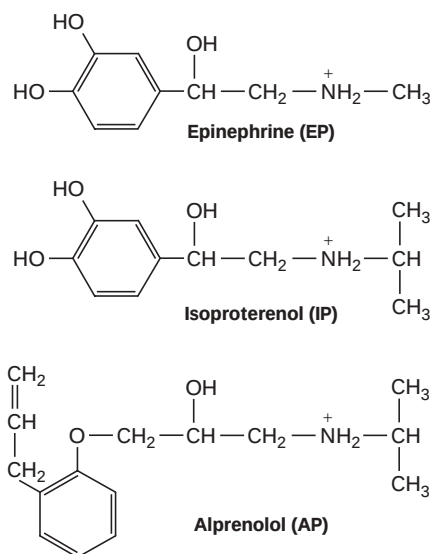
Usually, receptors are detected and measured by their ability to bind radioactive ligands to cells or to cell fragments. The results of such a *binding assay* are illustrated and explained in Figure 13-4. Both the number of ligand-binding sites per cell and the K_d value are easily determined from the specific binding curve (Figure 13-4, curve B), which is described by Equation 13-2. Since each receptor generally binds just one ligand molecule, the number of ligand-binding sites equals the number of active receptors per cell. Straight binding assays like the one in Figure 13-4 are feasible with receptors that have a strong affinity for their ligands, such as the erythropoietin receptor ($K_d = 1 \times 10^{-10}$ M) and the insulin receptor on liver cells ($K_d = 1.4 \times 10^{-8}$ M).



▲ **EXPERIMENTAL FIGURE 13-4 Binding assays for cell-surface receptors can determine the K_d for high-affinity ligands and the number of receptors per cell.** Shown here are data for insulin-specific receptors on the surface of liver cells. A suspension of cells is incubated for 1 hour at 4°C with increasing concentrations of ^{125}I -labeled insulin; the low temperature is used to prevent endocytosis of the cell-surface receptors. The cells are separated from unbound insulin, usually by centrifugation, and the amount of radioactivity bound to them is measured. The total binding curve A represents insulin specifically bound to high-affinity receptors as well as insulin nonspecifically bound with low affinity to other molecules on the cell surface. The contribution of nonspecific binding to total binding is determined by repeating the binding assay in the presence of a 100-fold excess of unlabeled insulin, which saturates all the specific high-affinity sites. In this case, all the labeled insulin binds to nonspecific sites, yielding curve C. The specific binding curve B is calculated as the difference between curves A and C. From curve B, the K_d for insulin binding ($\approx 1.4 \times 10^{-8}$ M, or 14 nM) and the number of receptor molecules per cell ($\approx 33,000$) can be determined. [Adapted from A. Ciechanover et al., 1983, *Cell* **32**:267.]



▲ **EXPERIMENTAL FIGURE 13-5 Binding of low-affinity ligands to cell-surface receptors can be detected in competition assays.** In this example, the synthetic ligand alprenolol, which binds with high affinity to the epinephrine receptor on liver cells ($K_d \approx 3 \times 10^{-9}$ M), is used to detect the binding of two low-affinity ligands, the natural hormone epinephrine (EP) and a synthetic ligand called isoproterenol (IP). Assays are performed as described in Figure 13-4 but with a constant amount of [3 H]alprenolol to which increasing amounts of unlabeled epinephrine or isoproterenol are added. At each competitor concentration, the amount of bound



labeled alprenolol is determined. In a plot of the inhibition of [3 H]alprenolol binding versus epinephrine or isoproterenol concentration, such as shown here, the concentration of the competitor that inhibits alprenolol binding by 50 percent approximates the K_d value for competitor binding. Note that the concentrations of competitors are plotted on a logarithmic scale. The K_d for binding of epinephrine to its receptor on liver cells is only $\approx 5 \times 10^{-5}$ M and would not be measurable by a direct binding assay with [3 H]epinephrine. The K_d for binding of isoproterenol, which induces the normal cellular response, is more than tenfold lower.

Many ligands, however, bind to their receptors with much lower affinity. If the K_d for binding is greater than $\approx 1 \times 10^{-7}$ M, any ligand bound to receptors is likely to dissociate in the few seconds it takes to separate the cells (e.g., by centrifugation) from free (unbound) ligand and measure the amount of bound ligand. One way to detect weak binding of a ligand to its receptor is in a *competition assay* with another ligand that binds to the same receptor with high affinity (low K_d value). In this type of assay, increasing amounts of an unlabeled, low-affinity ligand (the competitor) are added to a cell sample with a constant amount of the radiolabeled, high-affinity ligand (Figure 13-5). Binding of unlabeled competitor blocks binding of the radioactive ligand to the receptor; the concentration of competitor required to inhibit binding of half the radioactive ligand approximates the K_d value for binding of the competitor to the receptor.



Synthetic analogs of natural hormones are widely used in research on cell-surface receptors and as drugs. These analogs fall into two classes: **agonists**, which mimic the function of a natural hormone by binding to its receptor and inducing the normal response, and **antagonists**, which bind to the receptor but induce no response. By occupying ligand-binding sites on a receptor, an antagonist can block binding of the natural hormone (or agonist) and thus reduce the usual physiological activity of the hormone.

For instance, addition of two methyl groups to epinephrine generates isoproterenol, an agonist that binds to epinephrine receptors on bronchial smooth muscle cells about tenfold more strongly than does epinephrine (see Figure 13-5). Because ligand binding to these receptors promotes relaxation of bronchial smooth muscle and thus opening of the air passages in the lungs, isoproterenol is used in treating bronchial asthma, chronic bronchitis, and emphysema. Activation of epinephrine receptors on cardiac muscle cells increases the contraction rate. The antagonist alprenolol and related compounds, referred to as **beta-blockers**, have a very high affinity for these epinephrine receptors. Such antagonists are used to slow heart contractions in the treatment of cardiac arrhythmias and angina. ■

Receptors Can Be Purified by Affinity Techniques or Expressed from Cloned Genes

Cell-surface receptors often can be identified and followed through isolation procedures by *affinity labeling*. In this technique, cells are mixed with an excess of a radiolabeled ligand for the receptor of interest. After unbound ligand is washed away, the cells are treated with a chemical agent that covalently cross-links bound labeled ligand molecules and receptors on the cell surface. Once a radiolabeled ligand is covalently cross-linked to its receptor, it remains bound even in the presence of detergents and other denaturing agents

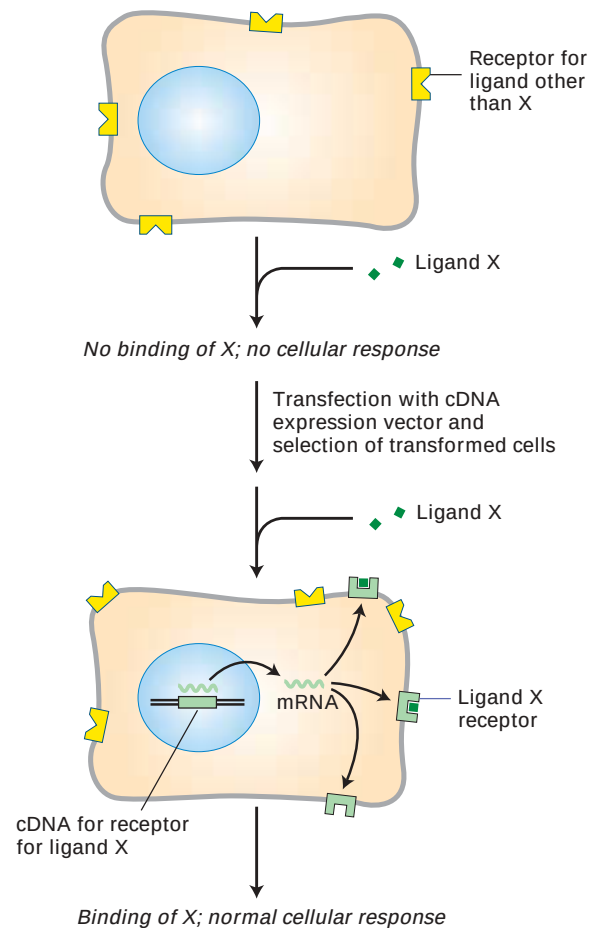
that are used to solubilize receptor proteins from the cell membrane. The labeled ligand provides a means for detecting the receptor during purification procedures.

Another technique often used in purifying cell-surface receptors that retain their ligand-binding ability when solubilized by detergents is similar to *affinity chromatography* using antibodies (see Figure 3-34). To purify a receptor by this technique, a ligand for the receptor of interest, rather than an antibody, is chemically linked to the beads used to form a column. A crude, detergent-solubilized preparation of membrane proteins is passed through the column; only the receptor binds, and other proteins are washed away. Passage of an excess of the soluble ligand through the column causes the bound receptor to be displaced from the beads and eluted from the column. In some cases, a receptor can be purified as much as 100,000-fold in a single affinity chromatographic step.

Once a receptor is purified, its properties can be studied and its gene cloned. A *functional expression assay* of the cloned cDNA in a mammalian cell that normally lacks the encoded receptor can provide definitive proof that the proper protein indeed has been obtained (Figure 13-6). Such expression assays also permit investigators to study the effects of mutating specific amino acids on ligand binding or on “downstream” signal transduction, thereby pinpointing the receptor amino acids responsible for interacting with the ligand and/or with critical signal-transduction proteins.

The cell-surface receptors for many signaling molecules are present in such small amounts that they cannot be purified by affinity chromatography and other conventional biochemical techniques. These low-abundance receptor proteins can now be identified and cloned by various recombinant DNA techniques, eliminating the need to isolate and purify them from cell extracts. In one technique, cloned cDNAs prepared from the entire mRNA extracted from cells that produce the receptor are inserted into expression vectors by techniques described in Chapter 9. The recombinant vectors then are transfected into cells that normally do not synthesize the receptor of interest, as in Figure 13-6. Only the very few transfected cells that contain the cDNA encoding the desired receptor synthesize it; other transfected cells produce irrelevant proteins. The rare cells expressing the desired receptor can be detected and purified by various techniques such as fluorescence-activated cell sorting using a fluorescent-labeled ligand for the receptor of interest (see Figure 5-34). Once a cDNA clone encoding the receptor is identified, the sequence of the cDNA can be determined and that of the receptor protein deduced from the cDNA sequence.

Genomics studies coupled with functional expression assays are now being used to identify genes for previously unknown receptors. In this approach, stored DNA sequences are analyzed for similarities with sequences known to encode receptor proteins (Chapter 9). Any putative receptor genes that are identified in such a search then can be tested for their ability to bind a signaling molecule or induce a response in cultured cells by a functional expression assay.



▲ EXPERIMENTAL FIGURE 13-6 Functional expression assay can identify a cDNA encoding a cell-surface receptor.

Target cells lacking receptors for a particular ligand (X) are stably transfected with a cDNA expression vector encoding the receptor. The design of the expression vector permits selection of transformed cells from those that do not incorporate the vector into their genome (see Figure 9-29b). Providing that these cells already express all the relevant signal-transduction proteins, the transfected cells exhibit the normal cellular response to X if the cDNA in fact encodes the functional receptor.

KEY CONCEPTS OF SECTION 13.1

Signaling Molecules and Cell-Surface Receptors

- Extracellular signaling molecules regulate interactions between unicellular organisms and are critical regulators of physiology and development in multicellular organisms.
- Binding of extracellular signaling molecules to cell-surface receptors triggers intracellular signal-transduction pathways that ultimately modulate cellular metabolism, function, or gene expression (Figure 13-1).
- External signals include membrane-anchored and secreted proteins and peptides, small lipophilic molecules (e.g., steroid hormones, thyroxine), small hydrophilic mol-

ecules derived from amino acids (e.g., epinephrine), gases (e.g., nitric oxide), and physical stimuli (e.g., light).

- Signals from one cell can act on nearby cells (paracrine), on distant cells (endocrine), or on the signaling cell itself (autocrine).
- Receptors bind ligands with considerable specificity, which is determined by noncovalent interactions between a ligand and specific amino acids in the receptor protein (see Figure 13-2).
- The maximal response of a cell to a particular ligand generally occurs at ligand concentrations at which most of its receptors are still not occupied (see Figure 13-3).
- The concentration of ligand at which half its receptors are occupied, the K_d , can be determined experimentally and is a measure of the affinity of the receptor for the ligand (see Figure 13-4).
- Because the amount of a particular receptor expressed is generally quite low (ranging from ≈ 2000 to 20,000 molecules per cell), biochemical purification may not be feasible. Genes encoding low-abundance receptors for specific ligands often can be isolated from cDNA libraries transfected into cultured cells.
- Functional expression assays can determine if a cDNA encodes a particular receptor and are useful in studying the effects on receptor function of specific mutations in its sequence (see Figure 13-6).

13.2 Intracellular Signal Transduction

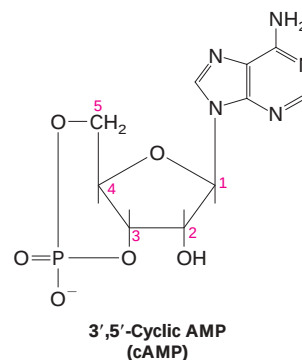
The various intracellular pathways that transduce signals downstream from activated cell-surface receptors differ in their complexity and in the way they transduce signals. We describe the components and operation of many individual pathways later in this chapter and in other chapters. Some general principles of signal transduction, applicable to different pathways, are covered in this section.

Second Messengers Carry Signals from Many Receptors

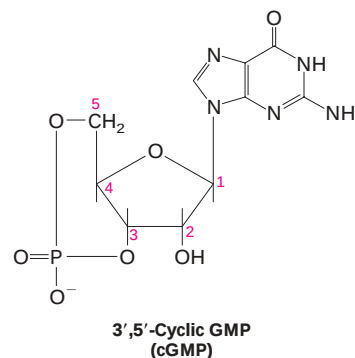
The binding of ligands (“first messengers”) to many cell-surface receptors leads to a short-lived increase (or decrease) in the concentration of certain low-molecular-weight intracellular signaling molecules termed **second messengers**. These molecules include 3',5'-cyclic AMP (cAMP), 3',5'-cyclic GMP (cGMP), 1,2-diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (IP₃), whose structures are shown in Figure 13-7. Other important second messengers are Ca²⁺ and various inositol phospholipids, also called *phosphoinositides*, which are embedded in cellular membranes.

The elevated intracellular concentration of one or more second messengers following binding of an external signaling molecule triggers a rapid alteration in the activity of one or

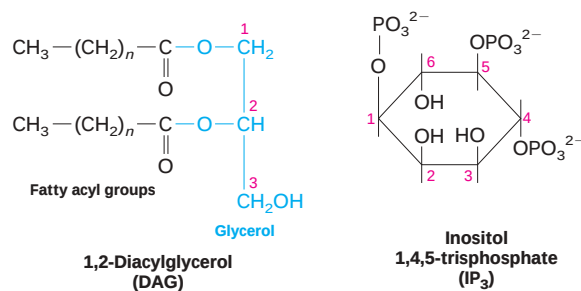
more enzymes or nonenzymatic proteins. In muscle, a signal-induced rise in cytosolic Ca^{2+} triggers contraction (see Figure 19-28); a similar increase in Ca^{2+} induces exocytosis of secretory vesicles in endocrine cells and of neurotransmitter-containing vesicles in nerve cells (see Figure 7-43). Similarly, a rise in cAMP induces various changes in cell metabolism that differ in different types of human cells. The



Activates protein kinase A (PKA)



Activates protein kinase G (PKG)
and opens cation channels in
rod cells



Activates protein kinase C
(PKC)

Opens Ca^{2+} channels in the endoplasmic reticulum

▲ FIGURE 13-7 Four common intracellular second messengers. The major direct effect or effects of each compound are indicated below its structural formula. Calcium ion (Ca^{2+}) and several membrane-bound phosphoinositides also act as second messengers.

mode of action of cAMP and other second messengers is discussed in later sections.

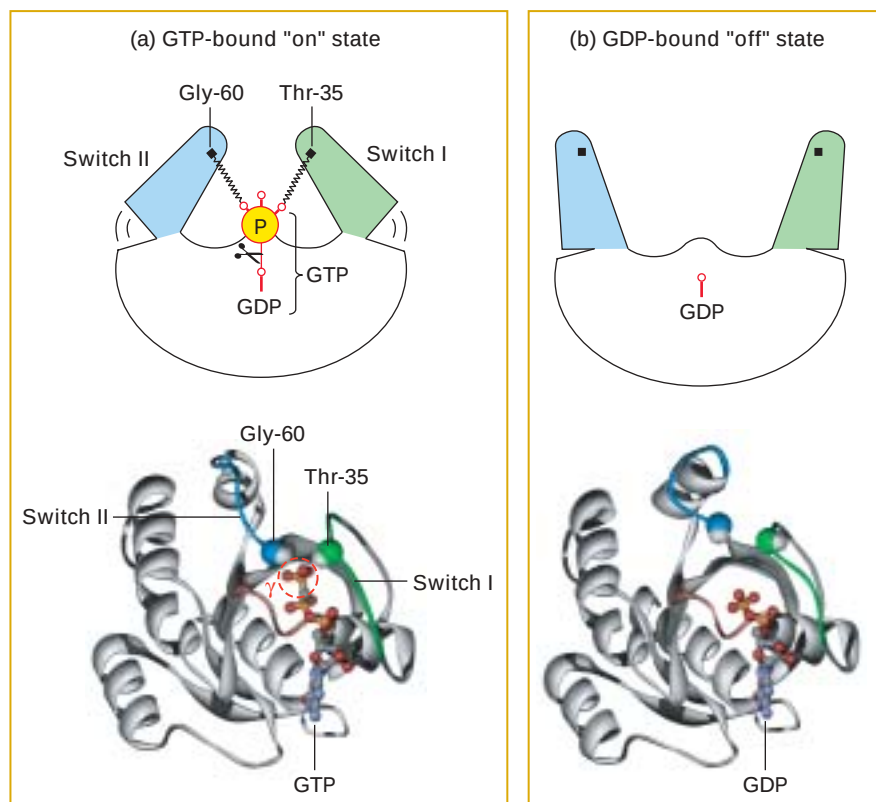
Many Conserved Intracellular Proteins Function in Signal Transduction

In addition to cell-surface receptors and second messengers, two groups of evolutionary conserved proteins function in signal-transduction pathways stimulated by extracellular signals. Here we briefly consider these intracellular signaling proteins; their role in specific pathways is described elsewhere.

GTPase Switch Proteins We introduced the large group of intracellular switch proteins that form the **GTPase superfamily** in Chapter 3. These guanine nucleotide-binding proteins are turned “on” when bound to GTP and turned “off” when bound to GDP (see Figure 3-29). Signal-induced conversion of the inactive to active state is mediated by a *guanine nucleotide-exchange factor (GEF)*, which causes release of GDP from the switch protein. Subsequent binding

of GTP, favored by its high intracellular concentration, induces a conformational change in two segments of the protein, termed switch I and switch II, allowing the protein to bind to and activate other downstream signaling proteins (Figure 13-8). The intrinsic GTPase activity of the switch proteins then hydrolyzes the bound GTP to GDP and P_i , thus changing the conformation of switch I and switch II from the active form back to the inactive form. The rate of GTP hydrolysis frequently is enhanced by a *GTPase-accelerating protein (GAP)*, whose activity also may be controlled by extracellular signals. The rate of GTP hydrolysis regulates the length of time the switch protein remains in the active conformation and able to signal downstream.

There are two classes of GTPase switch proteins: *trimeric* (large) G proteins, which as noted already directly bind to and are activated by certain receptors, and *monomeric* (small) G proteins such as Ras and various Ras-like proteins. Ras is linked indirectly to receptors via adapter proteins and GEF proteins discussed in the next chapter. All G proteins contain regions like switch I and switch II that modulate the



► **FIGURE 13-8 Switching mechanism for monomeric and trimeric G proteins.** The ability of a G protein to interact with other proteins and thus transduce a signal differs in the GTP-bound “on” state and GDP-bound “off” state. (a) In the active “on” state, two domains, termed switch I (green) and switch II (blue), are bound to the terminal γ phosphate of GTP through interactions with the backbone amide groups of a conserved threonine and glycine residue. (b) Release of the

γ phosphate by GTPase-catalyzed hydrolysis causes switch I and switch II to relax into a different conformation, the inactive “off” state. Shown here as ribbon models are both conformations of Ras, a monomeric G protein. A similar spring-loaded mechanism switches the α subunit in trimeric G proteins between the active and inactive conformations. [Adapted from I. Vetter and A. Wittinghofer, 2001, *Science* **294**:1299.]

activity of specific effector proteins by direct protein-protein interactions when the G protein is bound to GTP. Despite these similarities, these two classes of GTP-binding proteins are regulated in very different ways.

Protein Kinases and Phosphatases Activation of all cell-surface receptors leads directly or indirectly to changes in protein phosphorylation through the activation of protein **kinases** or protein **phosphatases**. Animal cells contain two types of protein kinases: those that add phosphate to the hydroxyl group on tyrosine residues and those that add phosphate to the hydroxyl group on serine or threonine (or both) residues. Phosphatases, which remove phosphate groups, can act in concert with kinases to switch the function of various proteins on or off (see Figure 3-30). At last count the human genome encodes 500 protein kinases and 100 different phosphatases. In some signaling pathways, the receptor itself possesses intrinsic kinase or phosphatase activity; in other pathways, the receptor interacts with cytosolic or membrane-associated kinases.

In general, each protein kinase phosphorylates specific residues in a set of target proteins whose patterns of expression generally differ in different cell types. Many proteins are substrates for multiple kinases, and each phosphorylation event, on a different amino acid, modifies the activity of a particular target protein in different ways, some activating its function, others inhibiting it. The catalytic activity of a protein kinase itself commonly is modulated by phosphorylation by other kinases, by direct binding to other proteins, or by changes in the levels of various second messengers. The activity of all protein kinases is opposed by the activity of protein phosphatases, some of which are themselves regulated by extracellular signals. Thus the activity of a protein in a cell can be a complex function of the activities of the usually multiple kinases and phosphatases that act on it. Several examples of this phenomenon that occur in regulation of the cell cycle are described in Chapter 21.

Some Receptors and Signal-Transduction Proteins Are Localized

Although the epinephrine receptors expressed by adipose (fat-storage) cells appear to be uniformly distributed on the surface of these spherical cells, such a uniform distribution probably is rare. More common is the clustering of receptors and other membrane-associated signaling proteins to a particular region of the cell surface. In this section, we show how multiple protein-protein and protein-lipid interactions can cluster signaling proteins in the plasma membrane and discuss some advantages conferred by such clustering. Other instances of localization of signaling proteins are described elsewhere.

Clustering of Membrane Proteins Mediated by Adapter Domains

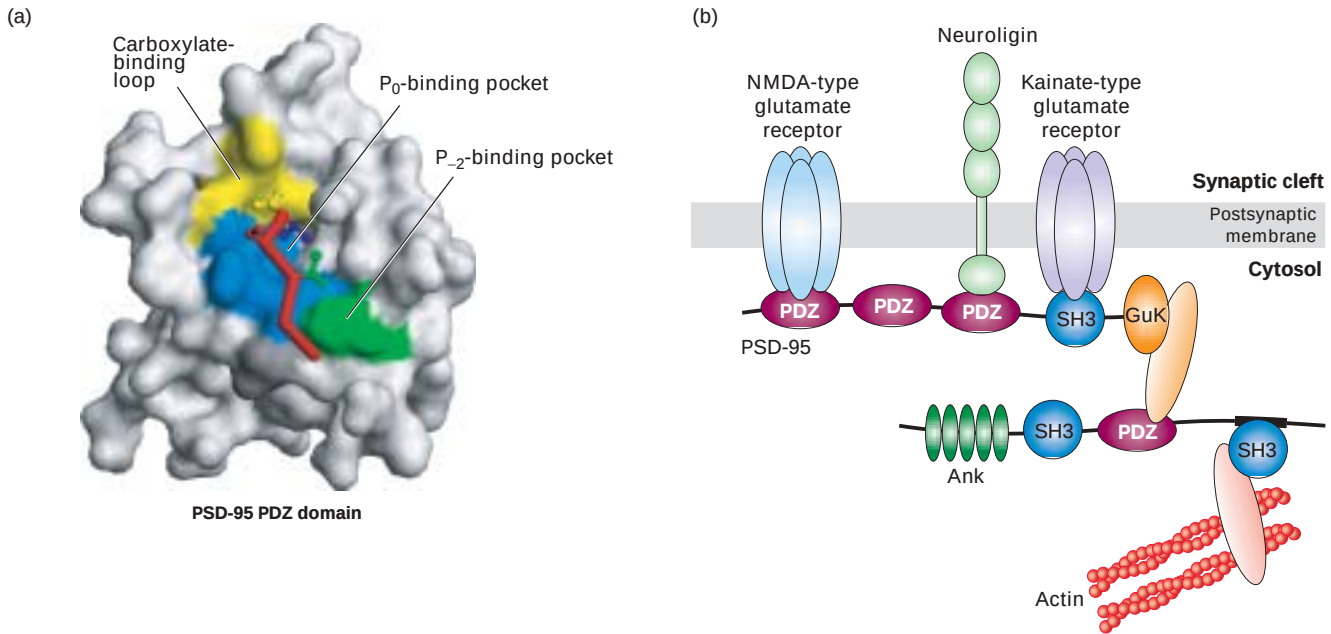
Perhaps the best example of clustering of recep-

tors and other membrane proteins is the chemical synapse. Recall that synaptic junctions are highly specialized structures at which chemical signals (neurotransmitters) are released from a presynaptic cell and bind receptors on an adjacent postsynaptic cell (see Figure 7-31). Clustering of neurotransmitter receptors in the region of the postsynaptic plasma membrane adjacent to the presynaptic cell promotes rapid and efficient signal transmission. Other proteins in the membrane of the postsynaptic cell interact with proteins in the extracellular matrix in order to “lock” the cell into the synapse.

Proteins containing *PDZ domains* play a fundamental role in organizing the plasma membrane of the postsynaptic cell. The PDZ domain was identified as a common element in several cytosolic proteins that bind to integral plasma-membrane proteins. It is a relatively small domain, containing about 90 amino acid residues, that binds to three-residue sequences at the C-terminus of target proteins (Figure 13-9a). Some PDZ domains bind to the sequence Ser/Thr-X-Φ, where X denotes any amino acid and Φ denotes a hydrophobic amino acid; others bind to the sequence Φ-X-Φ.

Most cell-surface receptors and transporters contain multiple subunits, each of which can bind to a PDZ domain. Likewise, many cytosolic proteins contain multiple PDZ domains as well as other types of domains that participate in protein-protein interactions, and thus can bind to multiple membrane proteins at the same time. These interactions permit the clustering of different membrane proteins into large complexes (Figure 13-9b). Other protein-protein interactions enable these complexes to bind to actin filaments that line the underside of the plasma membrane. Since a single actin filament can bind many clusters of the type depicted in Figure 13-9b, even larger numbers of plasma-membrane proteins can be clustered together specifically. This is one of the mechanisms by which many receptors, binding the same or different ligands, are localized to a specific region of the membrane in postsynaptic cells and other cells as well.

Protein Clustering in Lipid Rafts In Chapter 5, we saw that certain lipids in the plasma membrane, particularly cholesterol and sphingolipids, are organized into aggregates, called **lipid rafts**, that also contain specific proteins (see Figure 5-10). In mammalian cells, lipid rafts termed *caveolae* are of particular interest because they have been found to contain several different receptors and other signal-transducing proteins. These rafts are marked by the presence of *caveolin*, a family of ≈25-kDa proteins. Caveolin proteins have a central hydrophobic segment that is thought to span the membrane twice, and both the N- and C-termini face the cytosol. Large oligomers of caveolin form a proteinaceous coat that is visible on the cytosolic surface of caveolae in the electron microscope. Precisely how certain signaling proteins are anchored in caveolae is unclear. Nonetheless, the proximity of signaling proteins to one another within caveolae may facilitate their interaction, thereby promoting certain signaling pathways that otherwise would operate inefficiently.



▲ **FIGURE 13-9 Clustering of membrane proteins mediated by cytosolic adapter proteins containing multiple protein-binding domains.** The PDZ domain, which binds to certain C-terminal sequences, and the SH3 domain, which binds to proline-rich sequences, are two of several conserved domains that participate in protein-protein interactions. (a) Three-dimensional surface structure of a PDZ domain showing the backbone of the bound target peptide in red. Regions in the PDZ domain that bind the COO⁻ group and side chain of the C-terminal residue are colored yellow and blue, respectively. The binding pocket for the residue two distant from the C-terminus (P₋₂) is green. (b) Schematic diagram of protein-protein interactions that cluster several different membrane proteins in a postsynaptic segment of a nerve cell and anchor the resulting

complex to cytoskeletal actin filaments. Within the adapter protein PSD-95, two of the three PDZ domains shown and one SH3 domain bind three different membrane proteins into one complex. The guanylate kinase (GuK) domain of the PSD-95 protein links the complex, via several intervening adapter proteins (including one also containing PDZ and SH3 domains), to fibrous actin underlying the plasma membrane. Neuroligin is an adhesive protein that interacts with components of the extracellular matrix. Ank = ankyrin repeats. Other multibinding adapter proteins localize and cluster different receptors in the synaptic region of the plasma membrane. [Part (a) adapted from B. Harris and W. A. Lim, 2001, *J. Cell Sci.* **114**:3219; part (b) adapted from C. Garner, J. Nash, and R. Huganir, 2000, *Trends Cell Biol.* **10**:274.]

Appropriate Cellular Responses Depend on Interaction and Regulation of Signaling Pathways

In this chapter and the next, we focus primarily on simple signal-transduction pathways triggered by ligand binding to a single type of receptor. Activation of a single type of receptor, however, often leads to production of multiple second messengers, which have different effects. Moreover, the same cellular response (e.g., glycogen breakdown) may be induced by activation of multiple signaling pathways. Such interaction of different signaling pathways permits the fine-tuning of cellular activities required to carry out complex developmental and physiological processes.

The ability of cells to respond appropriately to extracellular signals also depends on regulation of signaling pathways themselves. For example, once the concentration of an external signal decreases, signaling via some intracellular pathways is terminated by degradation of a second messenger; in other pathways, signaling is terminated by deactivation of a signal-transduction protein. Another important mechanism for as-

suming appropriate cellular responses is *desensitization* of receptors at high signal concentrations or after prolonged exposure to a signal. The sensitivity of a cell to a particular signaling molecule can be down-regulated by **endocytosis** of its receptors, thus decreasing the number on the cell surface, or by modifying their activity so that the receptors either cannot bind ligand or form a receptor-ligand complex that does not induce the normal cellular response. Such modulation of receptor activity often results from phosphorylation of the receptor, binding of other proteins to it, or both. We examine the details of various mechanisms for regulating signaling pathways in our discussion of individual pathways.

KEY CONCEPTS OF SECTION 13.2 Intracellular Signal Transduction

- The level of second messengers, such as Ca²⁺, cAMP, and IP₃, increases or occasionally decreases in response to binding of ligand to cell-surface receptors (see Figure 13-7). These nonprotein intracellular signaling molecules,

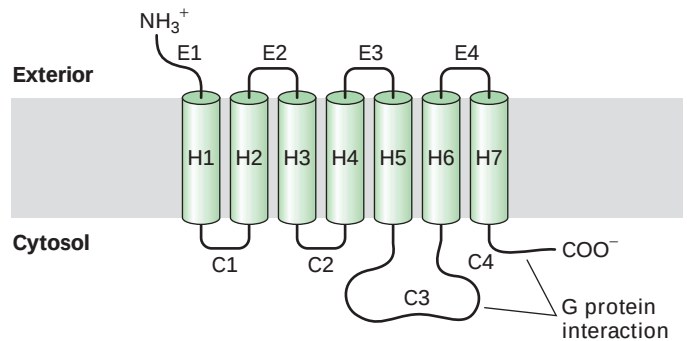
in turn, regulate the activities of enzymes and nonenzymatic proteins.

- Conserved proteins that act in many signal-transduction pathways include monomeric and trimeric G proteins (see Figure 13-8) and protein kinases and phosphatases.
- Cytosolic proteins that contain multiple PDZ or other protein-binding domains cluster receptors and other proteins within the plasma membrane, as occurs in post-synaptic cells (see Figure 13-9).
- Many receptors and signal-transduction proteins cluster in caveolin-containing lipid rafts. Such clustering may facilitate interaction between signaling proteins, thus enhancing signal transduction.
- Rapid termination of signaling once a particular ligand is withdrawn and receptor desensitization at high ligand concentrations or after prolonged exposure help cells respond appropriately under different circumstances.

13.3 G Protein–Coupled Receptors That Activate or Inhibit Adenylyl Cyclase

We now turn our attention to the very large group of cell-surface receptors that are coupled to signal-transducing trimeric G proteins. All G protein–coupled receptors

(GPCRs) contain seven membrane-spanning regions with their N-terminal segment on the exoplasmic face and their C-terminal segment on the cytosolic face of the plasma membrane (Figure 13-10). The GPCR family includes receptors for numerous hormones and neurotransmitters, light-activated receptors (rhodopsins) in the eye, and literally thousands of odorant receptors in the mammalian nose.



▲ **FIGURE 13-10 Schematic diagram of the general structure of G protein–coupled receptors.** All receptors of this type have the same orientation in the membrane and contain seven transmembrane α -helical regions (H1–H7), four extracellular segments (E1–E4), and four cytosolic segments (C1–C4). The carboxyl-terminal segment (C4), the C3 loop, and, in some receptors, also the C2 loop are involved in interactions with a coupled trimeric G protein.

TABLE 13-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

G $_{\alpha}$ Class	Associated Effector	2nd Messenger	Receptor Examples
G $_{s\alpha}$	Adenylyl cyclase	cAMP (increased)	β -Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
G $_{i\alpha}$	Adenylyl cyclase K $^{+}$ channel (G $_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_1 -Adrenergic receptor Muscarinic acetylcholine receptor
G $_{olf\alpha}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
G $_{q\alpha}$	Phospholipase C	IP $_3$, DAG (increased)	α_2 -Adrenergic receptor
G $_{o\alpha}$	Phospholipase C	IP $_3$, DAG (increased)	Acetylcholine receptor in endothelial cells
G $_{t\alpha}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G $_{\alpha}$ subclass may be associated with more than one effector protein. To date, only one major G $_{s\alpha}$ has been identified, but multiple G $_{q\alpha}$ and G $_{i\alpha}$ proteins have been described. Effector proteins commonly are regulated by G $_{\alpha}$ but in some cases by G $_{\beta\gamma}$ or the combined action of G $_{\alpha}$ and G $_{\beta\gamma}$. IP $_3$ = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.

SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

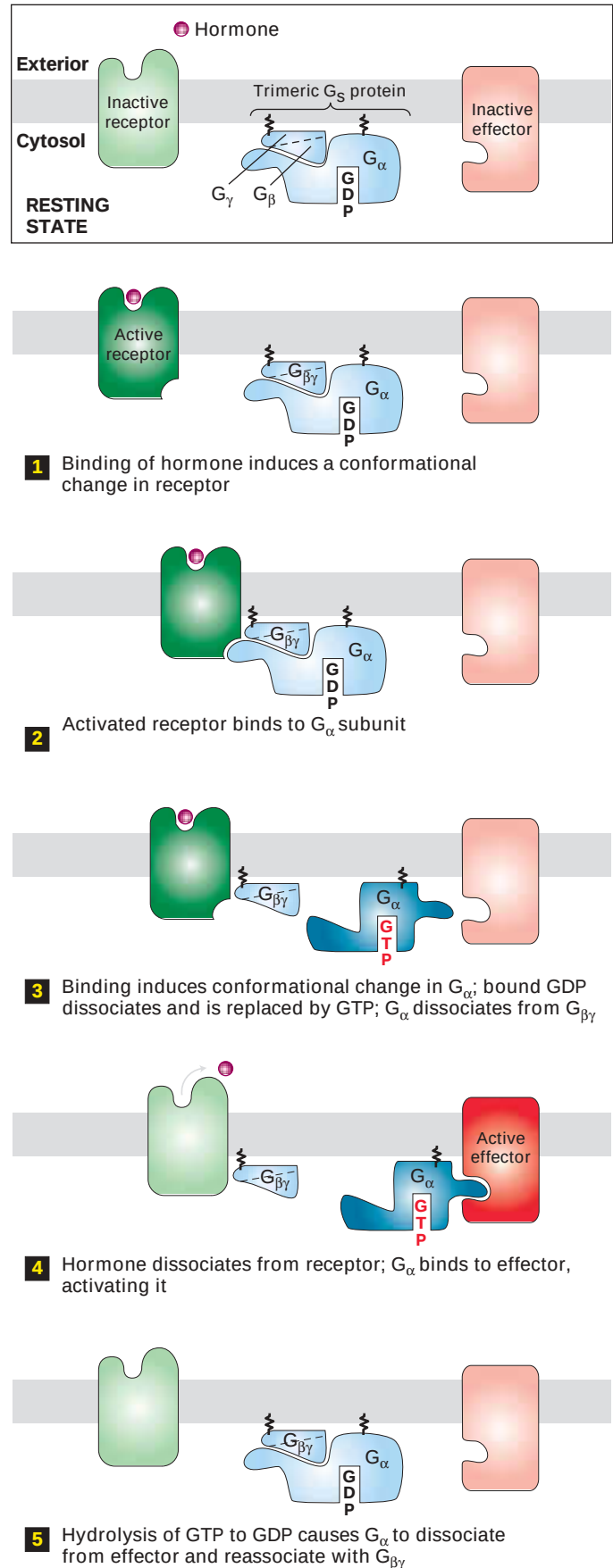
The signal-transducing G proteins contain three subunits designated α , β , and γ . During intracellular signaling the β and γ subunits remain bound together and are usually referred to as the $G_{\beta\gamma}$ subunit. The G_{α} subunit is a GTPase switch protein that alternates between an active (on) state with bound GTP and an inactive (off) state with bound GDP (see Figure 13-8). Stimulation of a coupled receptor causes activation of the G protein, which in turn modulates the activity of an associated *effector protein*. Although the effector protein most commonly is activated by $G_{\alpha}\cdot\text{GTP}$, in some cases it is inhibited. Moreover, depending on the cell and ligand, the $G_{\beta\gamma}$ subunit, rather than $G_{\alpha}\cdot\text{GTP}$, may transduce the signal to the effector protein. In addition, the activity of several different effector proteins is controlled by different GPCR-ligand complexes. All effector proteins, however, are either membrane-bound ion channels or enzymes that catalyze formation of second messengers (e.g., cAMP, DAG, and IP_3). These variations on the theme of GPCR signaling arise because multiple G proteins are encoded in eukaryotic genomes. The human genome, for example, encodes 27 different G_{α} , 5 G_{β} , and 13 G_{γ} subunits. So far as is known, the different $G_{\beta\gamma}$ subunits function similarly. Table 13-1 summarizes the functions of the major classes of G proteins with different G_{α} subunits.

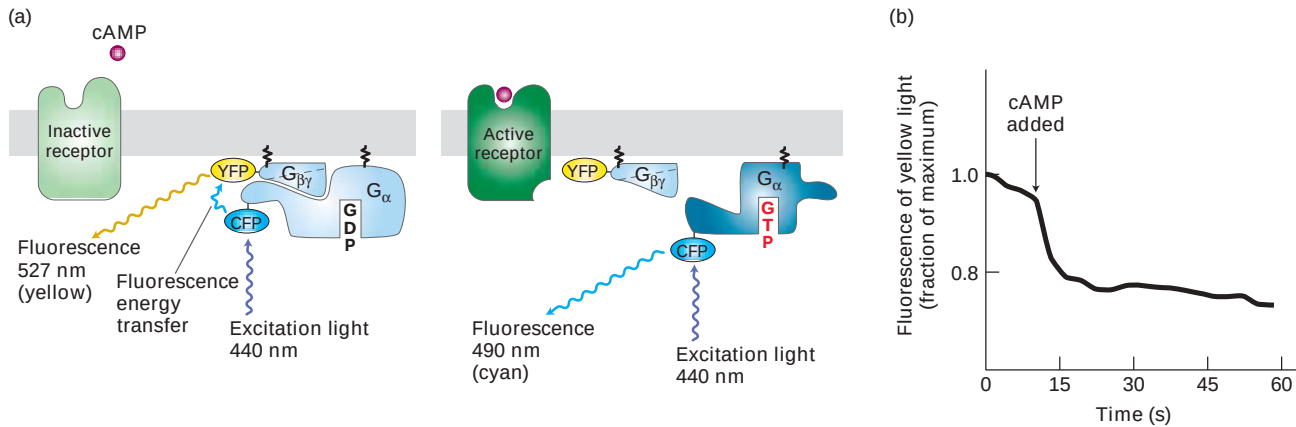
In this section, we first discuss how GPCR signals are transduced to an effector protein, a process that is similar for all receptors of this type. Then we focus on pathways in which cAMP is the second messenger, using the epinephrine-stimulated degradation of glycogen as an example.

The G_{α} Subunit of G Proteins Cycles Between Active and Inactive Forms

Figure 13-11 illustrates how G protein–coupled receptors transduce signals from extracellular hormones to associated effector proteins. Both the G_{α} and G_{γ} subunits are linked to the membrane by covalently attached lipids. In the resting state, when no ligand is bound to the receptor, the G_{α} subunit is bound to GDP and complexed with $G_{\beta\gamma}$. Binding of the normal hormonal ligand (e.g., epinephrine) or an ago-

► **FIGURE 13-11 Operational model for ligand-induced activation of effector proteins associated with G protein–coupled receptors.** The G_{α} and $G_{\beta\gamma}$ subunits of trimeric G proteins are tethered to the membrane by covalently attached lipid molecules (wiggly black lines). Following ligand binding, dissociation of the G protein, and exchange of GDP with GTP (steps 1–3), the free $G_{\alpha}\cdot\text{GTP}$ binds to and activates an effector protein (step 4). Hydrolysis of GTP terminates signaling and leads to reassembly of the trimeric form, returning the system to the resting state (step 5). Binding of another ligand molecule causes repetition of the cycle. In some pathways, the effector protein is activated by the free $G_{\beta\gamma}$ subunit.





▲ EXPERIMENTAL FIGURE 13-12 Receptor-mediated activation of coupled G proteins occurs within a few seconds of ligand binding in living cells.

The amoeba *Dictyostelium discoideum* was transfected with genes encoding two fusion proteins: a G α fused to cyan fluorescent protein (CFP), a mutant form of green fluorescent protein (GFP), and a G β fused to another GFP variant, yellow fluorescent protein (YFP). CFP normally fluoresces 490-nm light; YFP, 527-nm light. (a) When CFP and YFP are nearby, as in the resting G α -G $\beta\gamma$ complex, fluorescence energy transfer can occur between CFP and YFP (left). As a result, irradiation of resting cells with 440-nm light (which directly excites CFP but not YFP) causes emission of 527-nm (yellow) light, char-

acteristic of YFP. However, if ligand binding leads to dissociation of the G α and G $\beta\gamma$ subunits, then fluorescence energy transfer cannot occur. In this case, irradiation of cells at 440 nm causes emission of 490-nm light (cyan) characteristic of CFP (right). (b) Plot of the emission of yellow light (527 nm) from a single transfected amoeba cell before and after addition of cyclic AMP (arrows), the extracellular ligand for the GPCR in these cells. The drop in fluorescence, which results from the dissociation of the G α -CFP fusion protein from the G $\beta\gamma$ -YFP fusion protein, occurs within seconds of cAMP addition. [Adapted from C. Janetopoulos et al., 2001, *Science* **291**:2408.]

nist (e.g., isoproterenol) to the receptor changes its conformation, causing it to bind to the G α subunit in such a way that GDP is displaced from G α and GTP becomes bound. Thus the activated ligand-bound receptor functions as a GEF for the G α subunit (see Figure 3-29).

Once the exchange of nucleotides has occurred, the G α -GTP complex dissociates from the G $\beta\gamma$ subunit, but both remain anchored in the membrane. In most cases, G α -GTP then interacts with and activates an associated effector protein, as depicted in Figure 13-11. This activation is short-lived, however, because GTP bound to G α is hydrolyzed to GDP in seconds, catalyzed by a GTPase enzyme that is an intrinsic part of the G α subunit. The resulting G α -GDP quickly reassociates with G $\beta\gamma$, thus terminating effector activation. In many cases, a protein termed *RGS* (regulator of G protein signaling) accelerates GTP hydrolysis by the G α subunit, reducing the time during which the effector remains activated.

Early evidence supporting the model shown in Figure 13-11 came from studies with compounds that can bind to G α subunits as well as GTP does, but cannot be hydrolyzed by the intrinsic GTPase. In these compounds the P–O–P phosphodiester linkage connecting the β and γ phosphates of GTP is replaced by a nonhydrolyzable P–CH₂–P or P–NH–P linkage. Addition of such a GTP analog to a plasma-membrane preparation in the presence of the natural ligand or an agonist for a particular receptor results in a much longer-lived activation of the associated effector protein than

occurs with GTP. That is because once the GDP bound to G α is displaced by the nonhydrolyzable GTP analog, it remains permanently bound to G α . Because this complex is as functional as the normal G α -GTP complex in activating the effector protein, the effector remains permanently active.

The GPCR-mediated dissociation of trimeric G proteins recently has been detected in living cells. These studies have exploited the phenomenon of *fluorescence energy transfer*, which can change the wavelength of emitted fluorescence when two fluorescent proteins interact. Figure 13-12 shows how this experimental approach has demonstrated the dissociation of the G α -G $\beta\gamma$ complex within a few seconds of ligand addition, providing further evidence for the model of G protein cycling. This general experimental protocol can be used to follow the formation and dissociation of other protein-protein complexes in living cells.

Epinephrine Binds to Several Different G Protein–Coupled Receptors

Epinephrine is particularly important in mediating the body's response to stress, such as fright or heavy exercise, when all tissues have an increased need to catabolize glucose and fatty acids to produce ATP. These principal metabolic fuels can be supplied to the blood in seconds by the rapid breakdown of glycogen to glucose in the liver (*glycogenolysis*) and of triacylglycerols to fatty acids in adipose cells (*lipolysis*).

In mammals, the liberation of glucose and fatty acids can be triggered by binding of epinephrine (or norepinephrine) to β -adrenergic receptors on the surface of hepatic (liver) and adipose cells. Epinephrine bound to β -adrenergic receptors on heart muscle cells increases the contraction rate, which increases the blood supply to the tissues. In contrast, epinephrine stimulation of β -adrenergic receptors on smooth muscle cells of the intestine causes them to relax. Another type of epinephrine receptor, the α_2 -adrenergic receptor, is found on smooth muscle cells lining the blood vessels in the intestinal tract, skin, and kidneys. Binding of epinephrine to these receptors causes the arteries to constrict, cutting off circulation to these peripheral organs. These diverse effects of epinephrine are directed to a common end: supplying energy for the rapid movement of major locomotor muscles in response to bodily stress.

Although all epinephrine receptors are G protein-coupled receptors, the different types are coupled to different G proteins. Thus in addition to their physiological importance, these receptors are of interest because they trigger different intracellular signal-transduction pathways. Both subtypes of β -adrenergic receptors, termed β_1 and β_2 , are coupled to a *stimulatory G protein* (G_s) that activates the membrane-bound enzyme **adenylyl cyclase** (see Table 13-1). Once activated, adenylyl cyclase catalyzes synthesis of the second messenger cAMP. That binding of epinephrine to β -adrenergic receptors induces a rise in cAMP has been demonstrated in functional expression assays like that depicted in Figure 13-6. When cloned cDNA encoding the β -adrenergic receptor is transfected into receptor-negative cells, the transfected cells accumulate cAMP in response to epinephrine stimulation. Similar experiments in which mutant receptors are expressed have helped to define the functions of specific amino acids in binding hormones and activating different G proteins.

The two subtypes of α -adrenergic receptors, α_1 and α_2 , are coupled to different G proteins. The α_1 -adrenergic receptor is coupled to a G_i protein that inhibits adenylyl cyclase, the same effector enzyme associated with β -adrenergic receptors. In contrast, the G_q protein coupled to the α_2 -adrenergic receptor activates a different effector enzyme that generates different second messengers (see Section 13.5).



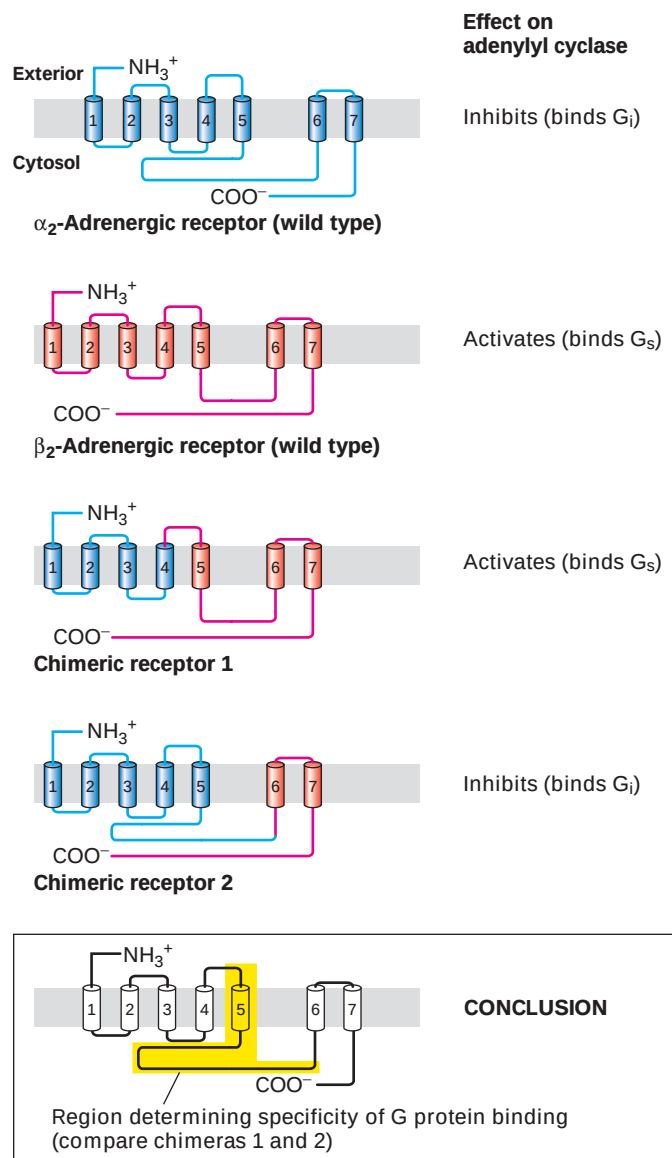
Some bacterial toxins contain a subunit that penetrates the plasma membrane of cells and catalyzes a chemical modification of $G_{s\alpha}$ ·GTP that prevents hydrolysis of bound GTP to GDP. As a result, $G_{s\alpha}$ remains in the active state, continuously activating adenylyl cyclase in the absence of hormonal stimulation. Cholera toxin produced by the bacterium *Vibrio cholera* and enterotoxins produced by certain strains of *E. coli* act in this way on intestinal epithelial cells. The resulting excessive rise in intracellular cAMP leads to the loss of electrolytes and water into the intestinal lumen, producing the watery diarrhea characteristic of infection by these bacteria.

Bordetella pertussis, a bacterium that commonly infects the respiratory tract, is the cause of whooping cough. Pertussis toxin catalyzes a modification of $G_{i\alpha}$ that prevents re-

lease of bound GDP, thus locking $G_{i\alpha}$ in the inactive state. This inactivation of G_i leads to an increase in cAMP in epithelial cells of the airways, promoting loss of fluids and electrolytes and mucus secretion. ■

Critical Functional Domains in Receptors and Coupled G Proteins Have Been Identified

As noted already, all G protein-coupled receptors contain seven transmembrane α helices and presumably have a similar three-dimensional structure. Studies with chimeric adrenergic receptors, like those outlined in Figure 13-13, suggest that the long C3 loop between α helices 5 and 6 is important for interactions between a receptor and its coupled G protein. Presumably, ligand binding causes these helices to move relative to each other. As a result, the conformation of the C3 loop connecting these two helices changes in a way that allows the loop to bind and

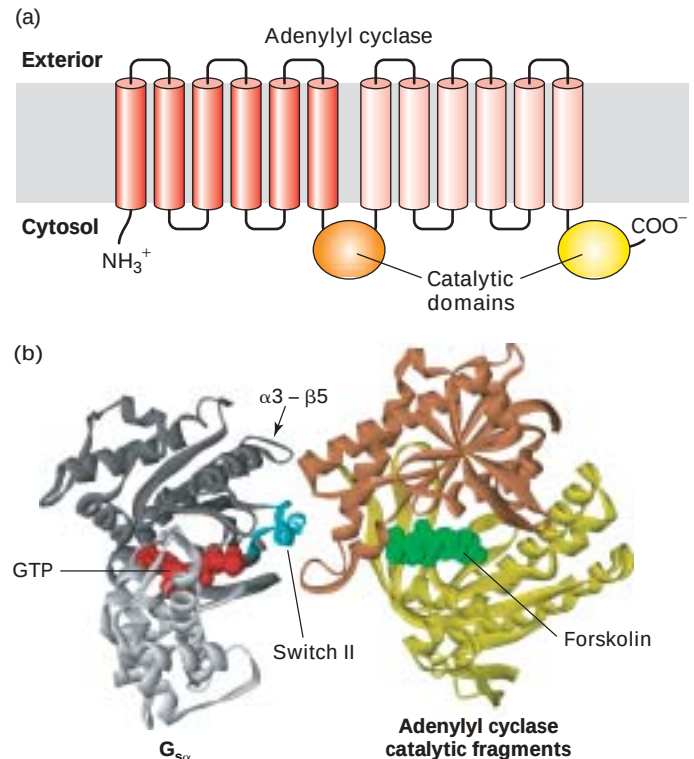


activate the transducing G_{α} subunit. Specific regions within the C3 loop are thought to assume a unique three-dimensional structure in all receptors that bind the same G protein (e.g., G_s or G_i). Other evidence indicates that the C2 loop, joining helices 3 and 4, also contributes to the interaction of some receptors with a G protein and that residues in at least four transmembrane helices participate in ligand binding.

X-ray crystallographic analysis has pinpointed the regions in $G_{s\alpha}$ ·GTP that interact with adenylyl cyclase. This enzyme is a multipass transmembrane protein with two large cytosolic segments containing the catalytic domains (Figure 13-14a). Because such transmembrane proteins are notoriously difficult to crystallize, scientists prepared two protein fragments encompassing the catalytic domains of adenylyl cyclase and allowed them to associate in the presence of $G_{s\alpha}$ ·GTP and forskolin, which stabilizes the catalytic adenylyl cyclase fragments in their active conformations. The complex that formed was catalytically active and showed pharmacological and biochemical properties similar to those of intact full-length adenylyl cyclase. In this complex, two regions of $G_{s\alpha}$ ·GTP, the switch II helix and the $\alpha 3$ - $\beta 5$ loop, contact the adenylyl cyclase fragments (Figure 13-14b). Recall that switch II is one of the segments of a G protein whose conformation is different in the GTP-bound and GDP-bound states (see Figure 13-8). The GTP-induced conformation of $G_{s\alpha}$ that favors its dissociation from $G_{\beta\gamma}$ is precisely the conformation essential for binding of $G_{s\alpha}$ to adenylyl cyclase. Other studies indicate that $G_{i\alpha}$ binds to a different region of adenylyl cyclase, accounting for its different effect on the effector.

To understand how binding of $G_{s\alpha}$ ·GTP promotes adenylyl cyclase activity, scientists will first have to solve the structure of the adenylyl cyclase catalytic domains in their unactivated conformations (i.e., in the absence of bound $G_{s\alpha}$ ·GTP). One hypothesis is that binding of the switch II helix to a cleft in one catalytic domain of adenylyl cyclase leads to rotation of the other catalytic domain. This rotation is proposed to lead to a stabilization of the transition state, thereby stimulating catalytic activity.

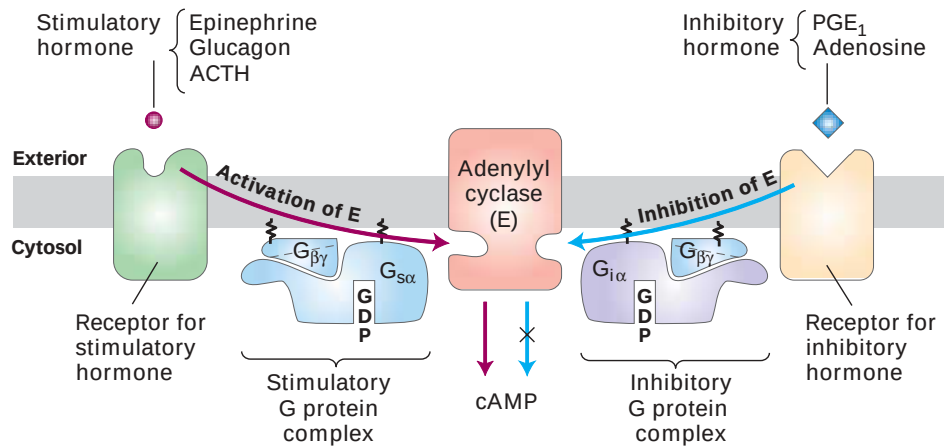
◀ **EXPERIMENTAL FIGURE 13-13 Studies with chimeric adrenergic receptors identify the long C3 loop as critical to interaction with G proteins.** *Xenopus* oocytes were microinjected with mRNA encoding a wild-type α_2 -adrenergic, β_2 -adrenergic, or chimeric α - β receptor. Although *Xenopus* oocytes do not normally express adrenergic receptors, they do express G proteins that can couple to the foreign receptors expressed on the surface of microinjected oocytes. The adenylyl cyclase activity of the injected cells in the presence of epinephrine agonists was determined and indicated whether the adrenergic receptor bound to the stimulatory (G_s) or inhibitory (G_i) type of oocyte G protein. Comparison of chimeric receptor 1, which interacts with G_s , and chimeric receptor 2, which interacts with G_i , shows that the G protein specificity is determined primarily by the source of the cytosol-facing C3 loop (yellow) between α helices 5 and 6. [See B. Kobilka et al., 1988, *Science* **240**:1310.]



▲ **FIGURE 13-14 Structure of mammalian adenylyl cyclases and their interaction with $G_{s\alpha}$ ·GTP.** (a) Schematic diagram of mammalian adenylyl cyclases. The membrane-bound enzyme contains two similar catalytic domains on the cytosolic face of the membrane and two integral membrane domains, each of which is thought to contain six transmembrane α helices. (b) Three-dimensional structure of $G_{s\alpha}$ ·GTP complexed with two fragments encompassing the catalytic domain of adenylyl cyclase determined by x-ray crystallography. The $\alpha 3$ - $\beta 5$ loop and the helix in the switch II region (blue) of $G_{s\alpha}$ ·GTP interact simultaneously with a specific region of adenylyl cyclase. The darker-colored portion of $G_{s\alpha}$ is the GTPase domain, which is similar in structure to Ras (see Figure 13-8); the lighter portion is a helical domain. The two adenylyl cyclase fragments are shown in orange and yellow. Forskolin (green) locks the cyclase fragments in their active conformations. [Part (a) see W.-J. Tang and A. G. Gilman, 1992, *Cell* **70**:869; part (b) adapted from J. J. G. Tesmer et al., 1997, *Science* **278**:1907]

Adenylyl Cyclase Is Stimulated and Inhibited by Different Receptor-Ligand Complexes

The versatile trimeric G proteins enable different receptor-hormone complexes to modulate the activity of the same effector protein. In the liver, for instance, glucagon and epinephrine bind to different receptors, but both receptors interact with and activate the same G_s , which activates adenylyl cyclase, thereby triggering the same metabolic responses. Activation of adenylyl cyclase, and thus the cAMP level, is proportional to the total concentration of $G_{s\alpha}$ ·GTP resulting from binding of both hormones to their respective receptors.



▲ **FIGURE 13-15 Hormone-induced activation and inhibition of adenylyl cyclase in adipose cells.** Ligand binding to G_s -coupled receptors causes activation of adenylyl cyclase, whereas ligand binding to G_i -coupled receptors causes inhibition of the enzyme. The $G_{\beta\gamma}$ subunit in both stimulatory and inhibitory G proteins is identical; the G_α subunits and their corresponding

receptors differ. Ligand-stimulated formation of active $G_\alpha \cdot \text{GTP}$ complexes occurs by the same mechanism in both G_s and G_i proteins (see Figure 13-11). However, $G_{s\alpha} \cdot \text{GTP}$ and $G_{i\alpha} \cdot \text{GTP}$ interact differently with adenylyl cyclase, so that one stimulates and the other inhibits its catalytic activity. [See A. G. Gilman, 1984, *Cell* **36**:577.]

Positive and negative regulation of adenylyl cyclase activity occurs in some cell types, providing fine-tuned control of the cAMP level. For example, stimulation of adipose cells by epinephrine, glucagon, or ACTH activates adenylyl cyclase, whereas prostaglandin PGE1 or adenosine inhibits the enzyme (Figure 13-15). The receptors for PGE1 and adenosine interact with inhibitory G_i , which contains the same β and γ subunits as stimulatory G_s but a different α subunit ($G_{i\alpha}$). In response to binding of an inhibitory ligand to its receptor, the associated G_i protein releases its bound GDP and binds GTP; the active $G_{i\alpha} \cdot \text{GTP}$ complex then dissociates from $G_{\beta\gamma}$ and inhibits (rather than stimulates) adenylyl cyclase.

cAMP-Activated Protein Kinase A Mediates Various Responses in Different Cells

In multicellular animals virtually all the diverse effects of cAMP are mediated through **protein kinase A (PKA)**, also called *cAMP-dependent protein kinase*. As discussed in Chapter 3, inactive PKA is a tetramer consisting of two regulatory (R) subunits and two catalytic (C) subunits. Each R subunit has two distinct cAMP-binding sites; binding of cAMP to both sites in an R subunit leads to release of the associated C subunit, unmasking its catalytic site and activating its kinase activity (see Figure 3-27a). Binding of cAMP by an R subunit occurs in a cooperative fashion; that is, binding of the first cAMP molecule lowers the K_d for binding of the second. Thus small changes in the level of cytosolic cAMP can cause proportionately large changes in the amount of dissociated C subunits and, hence, in kinase activity. Rapid activation of an enzyme by hormone-triggered dissociation of an inhibitor is a common feature of various signaling pathways.

Most mammalian cells express receptors coupled to G_s protein. Stimulation of these receptors by various hormones leads to activation of PKA, but the resulting cellular response depends on the particular PKA isoform and on the PKA substrates expressed by the cell. For instance, the effects of epinephrine on glycogen metabolism, which are mediated via cAMP and PKA, are confined mainly to liver and muscle cells, which express enzymes for making and degrading glycogen. In adipose cells, epinephrine-induced activation of PKA promotes phosphorylation and activation of the phospholipase that catalyzes hydrolysis of stored triglycerides to yield free fatty acids and glycerol. These fatty acids are released into the blood and taken up as an energy source by cells in other tissues such as the kidney, heart, and muscles. Likewise, stimulation of G protein-coupled receptors on ovarian cells by certain pituitary hormones leads to activation of PKA, which in turn promotes synthesis of two steroid hormones, estrogen and progesterone, crucial to the development of female sex characteristics.

Although PKA acts on different substrates in different types of cells, it always phosphorylates a serine or threonine residue that occurs within the same sequence motif: X-Arg-(Arg/Lys)-X-(Ser/Thr)- Φ , where X denotes any amino acid and Φ denotes a hydrophobic amino acid. Other serine/threonine kinases phosphorylate target residues within different sequence motifs.

Glycogen Metabolism Is Regulated by Hormone-Induced Activation of Protein Kinase A

The first cAMP-mediated cellular response to be discovered—the release of glucose from **glycogen**—occurs in muscle and liver cells stimulated by epinephrine or other

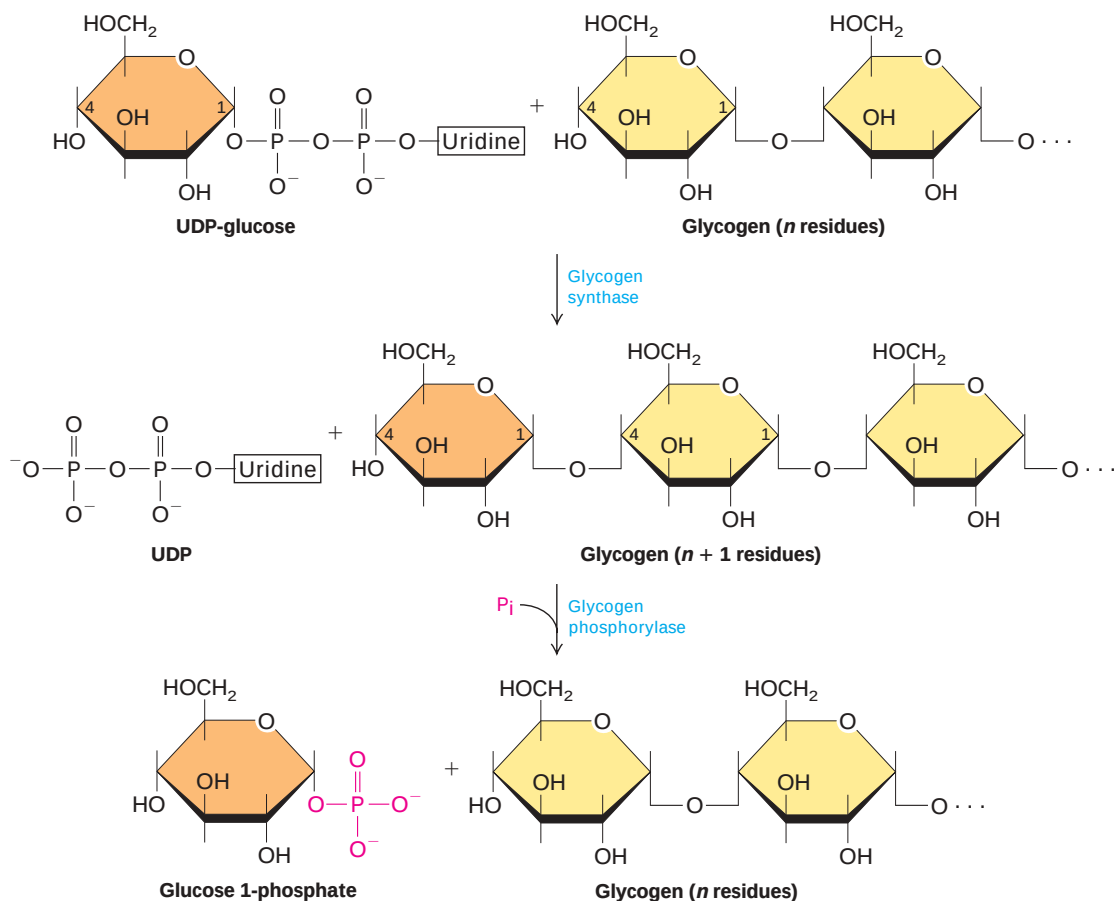
hormones whose receptors are coupled to G_s protein. This response exemplifies how activation of PKA can coordinate the activity of a group of intracellular enzymes toward a common purpose.

Glycogen, a large glucose polymer, is the major storage form of glucose in animals. Like all biopolymers, glycogen is synthesized by one set of enzymes and degraded by another (Figure 13-16). Three enzymes convert glucose into uridine diphosphoglucose (UDP-glucose), the primary intermediate in glycogen synthesis. The glucose residue of UDP-glucose is transferred by *glycogen synthase* to the free hydroxyl group on carbon 4 of a glucose residue at the end of a growing glycogen chain. Degradation of glycogen involves the stepwise removal of glucose residues from the same end by a phosphorolysis reaction, catalyzed by *glycogen phosphorylase*, yielding glucose 1-phosphate.

In both muscle and liver cells, glucose 1-phosphate produced from glycogen is converted to glucose 6-phosphate. In muscle cells, this metabolite enters the glycolytic pathway and is metabolized to generate ATP for use in powering muscle contraction (Chapter 8). Unlike muscle cells, liver cells

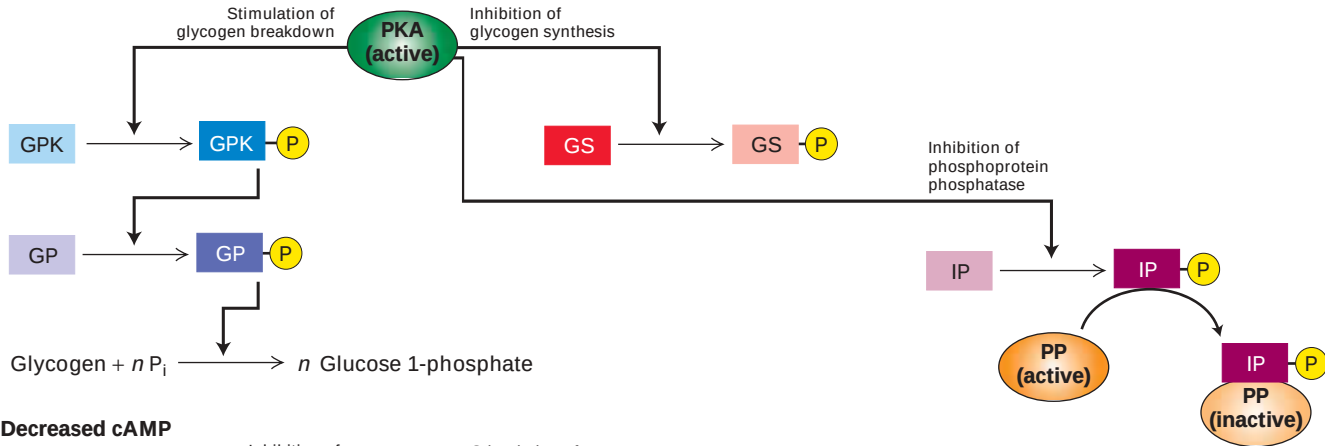
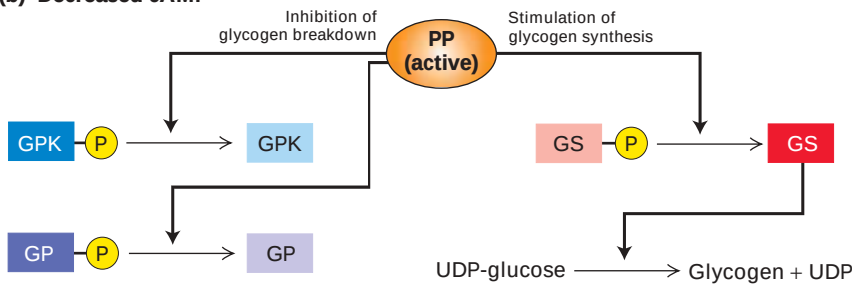
contain a phosphatase that hydrolyzes glucose 6-phosphate to glucose, which is exported from these cells in part by a glucose transporter (GLUT2) in the plasma membrane (Chapter 7). Thus glycogen stores in the liver are primarily broken down to glucose, which is immediately released into the blood and transported to other tissues, particularly the muscles and brain.

The epinephrine-stimulated increase in cAMP and subsequent activation of PKA enhance the conversion of glycogen to glucose 1-phosphate in two ways: by *inhibiting* glycogen synthesis and by *stimulating* glycogen degradation (Figure 13-17a). PKA phosphorylates and thus inactivates glycogen synthase, the enzyme that synthesizes glycogen. PKA promotes glycogen degradation indirectly by phosphorylating and thus activating an intermediate kinase, glycogen phosphorylase kinase (GPK), that in turn phosphorylates and activates glycogen phosphorylase, the enzyme that degrades glycogen. The entire process is reversed when epinephrine is removed and the level of cAMP drops, inactivating PKA. This reversal is mediated by *phosphoprotein phosphatase*, which removes the phosphate residues



▲ **FIGURE 13-16 Synthesis and degradation of glycogen.** Incorporation of glucose from UDP-glucose into glycogen is catalyzed by glycogen synthase. Removal of glucose units from

glycogen is catalyzed by glycogen phosphorylase. Because two different enzymes catalyze the formation and degradation of glycogen, the two reactions can be independently regulated.

(a) Increased cAMP**(b) Decreased cAMP****Abbreviations:**

PKA	Protein kinase A
PP	Phosphoprotein phosphatase
GPK	Glycogen phosphorylase kinase
GP	Glycogen phosphorylase
GS	Glycogen synthase
IP	Inhibitor of phosphoprotein phosphatase

▲ FIGURE 13-17 Regulation of glycogen metabolism by cAMP in liver and muscle cells. Active enzymes are highlighted in darker shades; inactive forms, in lighter shades. (a) An increase in cytosolic cAMP activates PKA, which inhibits glycogen synthesis directly and promotes glycogen degradation via a protein kinase cascade. At high cAMP, PKA also phosphorylates an inhibitor of phosphoprotein phosphatase

(PP). Binding of the phosphorylated inhibitor to PP prevents this phosphatase from dephosphorylating the activated enzymes in the kinase cascade or the inactive glycogen synthase. (b) A decrease in cAMP inactivates PKA, leading to release of the active form of phosphoprotein phosphatase. The action of this enzyme promotes glycogen synthesis and inhibits glycogen degradation.

from the inactive form of glycogen synthase, thereby activating it, and from the active forms of glycogen phosphorylase kinase and glycogen phosphorylase, thereby inactivating them (Figure 13-17b).

Phosphoprotein phosphatase itself is regulated by PKA. Activated PKA phosphorylates an inhibitor of phosphoprotein phosphatase; the phosphorylated inhibitor then binds to phosphoprotein phosphatase, inhibiting its activity (see Figure 13-17a). At low cAMP levels, when PKA is inactive, the inhibitor is not phosphorylated and phosphoprotein phosphatase is active. As a result, the synthesis of glycogen by glycogen synthase is enhanced and the degradation of glycogen by glycogen phosphorylase is inhibited.

Epinephrine-induced glycogenolysis thus exhibits dual regulation: activation of the enzymes catalyzing glycogen degradation and inhibition of enzymes promoting glycogen synthesis. Such coordinate regulation of stimulatory and inhibitory pathways provides an efficient mechanism for achieving a particular cellular response and is a common phenomenon in regulatory biology.

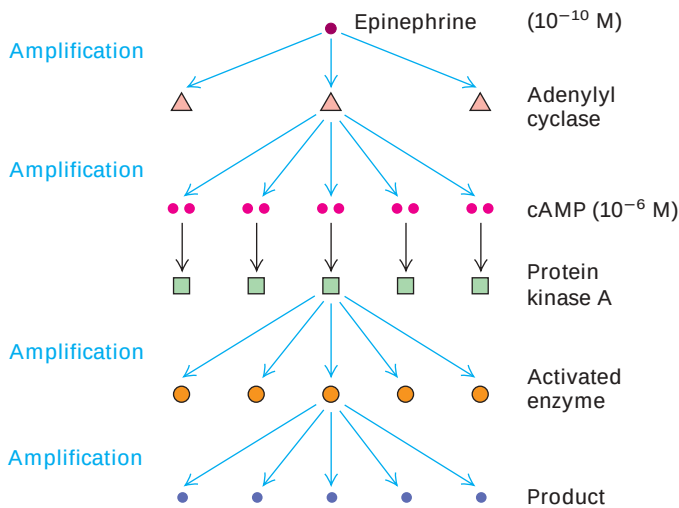
Signal Amplification Commonly Occurs Downstream from Cell-Surface Receptors

The cellular responses induced by G protein-coupled receptors that activate adenylyl cyclase may require tens of thousands or even millions of cAMP molecules per cell. Thus the hormone signal must be amplified in order to generate sufficient second messenger from the few thousand receptors for a particular hormone present on a cell. *Signal amplification* is possible because both receptors and G proteins can diffuse rapidly in the plasma membrane. A single receptor-hormone complex causes conversion of up to 100 inactive $G_{s\alpha}$ molecules to the active form. Each active $G_{s\alpha}\cdot GTP$, in turn, probably activates a single adenylyl cyclase molecule, which then catalyzes synthesis of many cAMP molecules during the time $G_{s\alpha}\cdot GTP$ is bound to it. Although the exact extent of this amplification is difficult to measure, binding of a single hormone molecule to one receptor molecule can result in the synthesis of at least several hundred cAMP molecules before the receptor-hormone complex dissociates and

activation of adenylyl cyclase ceases. Similar amplification probably occurs in signaling from receptors coupled to other G proteins and some other types of receptors whose activation induces synthesis of second messengers.

A second level of amplification is illustrated by the cAMP-mediated stimulation of glycogenolysis. As we just discussed, cAMP promotes glycogen degradation via a three-stage *cascade*, that is, a series of reactions in which the enzyme catalyzing one step is activated (or inhibited) by the product of a previous step (see Figure 13-17a). The amplification that occurs in such a cascade depends on the number of steps in it.

Both levels of amplification are depicted in Figure 13-18. For example, blood levels of epinephrine as low as 10^{-10} M can stimulate liver glycogenolysis and release of glucose. An epinephrine stimulus of this magnitude generates an intracellular cAMP concentration of 10^{-6} M, an amplification of 10^4 . Because three more catalytic steps precede the release of glucose, another 10^4 amplification can occur. In striated muscle, the concentrations of the three successive enzymes in the glycogenolytic cascade—protein kinase A, glycogen phosphorylase kinase, and glycogen phosphorylase—are in a 1:10:240 ratio, which dramatically illustrates the amplification of the effects of epinephrine and cAMP.



▲ **FIGURE 13-18 Amplification of an external signal downstream from a cell-surface receptor.** In this example, binding of a single epinephrine molecule to one G_s protein–coupled receptor molecule induces synthesis of a large number of cAMP molecules, the first level of amplification. Four molecules of cAMP activate two molecules of protein kinase A (PKA), but each activated PKA phosphorylates and activates multiple product molecules. This second level of amplification may involve several sequential reactions in which the product of one reaction activates the enzyme catalyzing the next reaction. The more steps in such a cascade, the greater the signal amplification possible.

Although such a cascade may seem overcomplicated, it not only greatly amplifies an external signal but also allows an entire group of enzyme-catalyzed reactions to be coordinately regulated by a single type of signaling molecule. In addition, the multiple steps between stimulus and final response offer possibilities for regulation by other signaling pathways, thereby fine-tuning the cellular response. We will encounter other examples of cascades in signaling pathways discussed in the next chapter.

Several Mechanisms Regulate Signaling from G Protein–Coupled Receptors

Several factors contribute to termination of the response to hormones recognized by β -adrenergic receptors and other receptors coupled to G_s . First, the affinity of the receptor for hormone decreases when the GDP bound to G_{sc} is replaced with a GTP following hormone binding. This increase in the K_d of the receptor-hormone complex enhances dissociation of the hormone from the receptor. Second, the GTP bound to G_{sc} is quickly hydrolyzed, reversing the activation of adenylyl cyclase and production of cAMP (see Figure 13-11). Third, *cAMP phosphodiesterase* acts to hydrolyze cAMP to 5'-AMP, terminating the cellular response. Thus the continuous presence of hormone at a high enough concentration is required for continuous activation of adenylyl cyclase and maintenance of an elevated cAMP level. Once the hormone concentration falls sufficiently, the cellular response quickly terminates.

When a G_s protein–coupled receptor is exposed to hormonal stimulation for several hours, several serine and threonine residues in the cytosolic domain of the receptor become phosphorylated by protein kinase A (PKA). The phosphorylated receptor can bind its ligand, but ligand binding leads to reduced activation of adenylyl cyclase; thus the receptor is desensitized. This is an example of *feedback suppression*, in which the end product of a pathway (here activated PKA) blocks an early step in the pathway (here, receptor activation). Because the activity of PKA is enhanced by the high cAMP level induced by any hormone that activates G_s , prolonged exposure to one such hormone, say, epinephrine, causes desensitization not only of β -adrenergic receptors but also of G_s protein–coupled receptors that bind different ligands (e.g., glucagon). This cross-regulation is called *heterologous desensitization*.

Additional residues in the cytosolic domain of the β -adrenergic receptor are phosphorylated by a receptor-specific enzyme called *β -adrenergic receptor kinase (BARK)*, but only when epinephrine or an agonist is bound to the receptor. Because BARK phosphorylates only activated β -adrenergic receptors, this process is called *homologous desensitization*. Prolonged treatment of cells with epinephrine results in extensive phosphorylation and hence desensitization of the β -adrenergic receptor by both PKA and BARK.

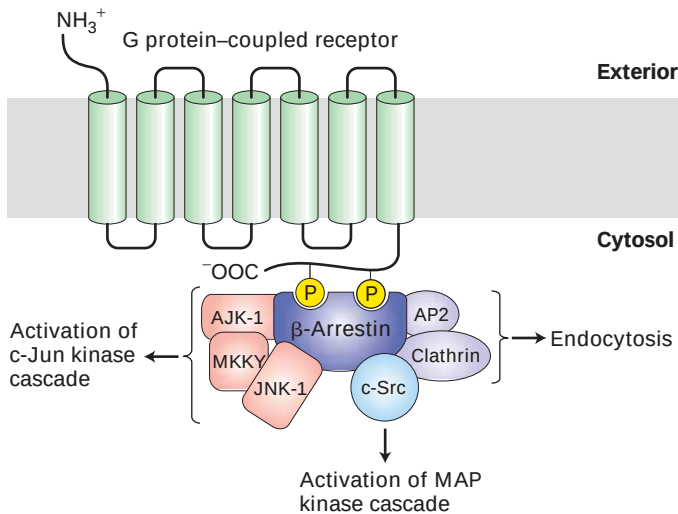
Phosphorylated (desensitized) receptors are constantly being resensitized owing to dephosphorylation by constitutive

phosphatases. Thus the number of phosphates per receptor molecule reflects how much ligand has been bound in the recent past (e.g., 1–10 minutes). This means that if a cell is constantly being exposed to a certain concentration of a hormone, that hormone concentration will eventually cease to stimulate the receptor. If the hormone concentration is now increased to a new value, the receptor will activate downstream signaling pathways but to a lesser extent than would occur if the cell were switched from a medium without hormone to one with this hormone level. If the hormone is then completely removed, the receptor becomes completely dephosphorylated and “reset” to its maximum sensitivity, in which case it can respond to very low levels of hormone. Thus a feedback loop involving receptor phosphorylation and dephosphorylation modulates the activity of β -adrenergic and related G_s protein-coupled receptors, permitting a cell to adjust receptor sensitivity to the hormone level at which it is being stimulated.

Another key participant in regulation of β -adrenergic receptors is β -arrestin. This cytosolic protein binds to receptors extensively phosphorylated by BARK and completely inhibits their interaction with and ability to activate G_s . An

additional function of β -arrestin in regulating cell-surface receptors initially was suggested by the observation that loss of cell surface β -adrenergic receptors in response to ligand binding is stimulated by overexpression of BARK and β -arrestin. Subsequent studies revealed that β -arrestin binds not only to phosphorylated receptors but also to clathrin and an associated protein termed AP2, two essential components of the coated vesicles that are involved in one type of endocytosis. These interactions promote the formation of coated pits and endocytosis of the associated receptors, thereby decreasing the number of receptors exposed on the cell surface (Figure 13-19). Eventually the internalized receptors become dephosphorylated in endosomes, β -arrestin dissociates, and the resensitized receptors recycle to the cell surface, similar to recycling of the LDL receptor (Chapter 17). Regulation of other G protein-coupled receptors also is thought to involve endocytosis of ligand-occupied receptors and their sequestration inside the cell.

As we discuss later, β -arrestin also functions as an adapter protein in transducing signals from G_s protein-coupled receptors to the nucleus. The multiple functions of β -arrestin illustrate the importance of adapter proteins in both regulating signaling and transducing signals from cell-surface receptors.



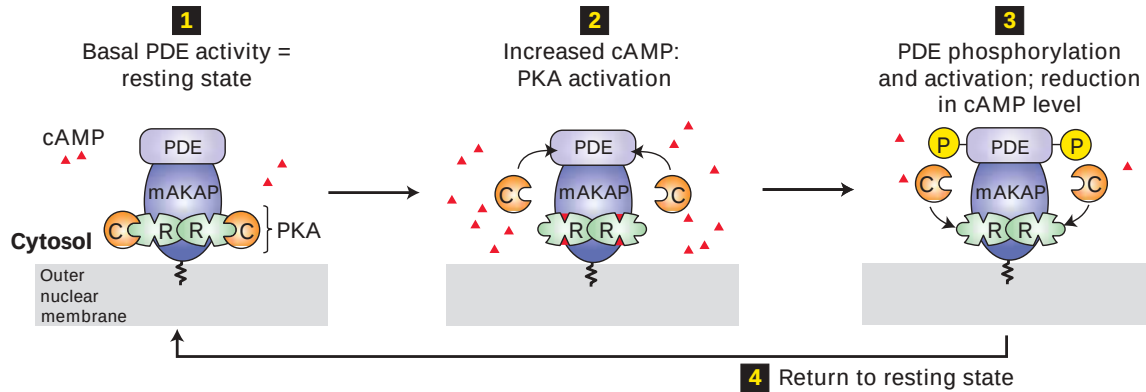
▲ **FIGURE 13-19 Role of β -arrestin in GPCR desensitization and signal transduction.** β -Arrestin binds to phosphorylated serine and tyrosine residues in the C-terminal segment of G protein-coupled receptors (GPCRs). Clathrin and AP2, two other proteins bound by β -arrestin, promote endocytosis of the receptor. β -Arrestin also functions in transducing signals from activated receptors by binding to and activating several cytosolic protein kinases. c-Src activates the MAP kinase pathway, leading to phosphorylation of key transcription factors (Chapter 14). Interaction of β -arrestin with three other proteins, including JNK-1 (a Jun N-terminal kinase), results in phosphorylation and activation of the c-Jun transcription factor. [Adapted from W. Miller and R. J. Lefkowitz, 2001, *Curr. Opin. Cell Biol.* **13**:139, and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.]

Anchoring Proteins Localize Effects of cAMP to Specific Subcellular Regions

In many cell types, a rise in the cAMP level may produce a response that is required in one part of the cell but is unwanted, perhaps deleterious, in another part. A family of anchoring proteins localizes PKA isoforms to specific subcellular locations, thereby restricting cAMP-dependent responses to these locations. These proteins, referred to as *A kinase-associated proteins* (AKAPs), have a two-domain structure with one domain conferring a specific subcellular location and another that binds to the regulatory subunit of protein kinase A.

One anchoring protein (AKAP15) is tethered to the cytosolic face of the plasma membrane near a particular type of gated Ca^{2+} channel in certain heart muscle cells. In the heart, activation of β -adrenergic receptors by epinephrine (as part of the fight-or-flight response) leads to PKA-catalyzed phosphorylation of these Ca^{2+} channels, causing them to open; the resulting influx of Ca^{2+} increases the rate of heart muscle contraction. The interaction of AKAP15 with PKA localizes PKA next to these channels, thereby reducing the time that otherwise would be required for diffusion of PKA catalytic subunits from their sites of generation to their Ca^{2+} -channel substrate.

Another A kinase-associated protein (mAAP) in heart muscle anchors both PKA and cAMP phosphodiesterase (PDE) to the outer nuclear membrane. Because of the close proximity of PDE to PKA, a negative feedback loop provides



▲ **FIGURE 13-20 Localization of protein kinase A (PKA) to the nuclear membrane in heart muscle.** This A kinase–associated protein mAKAP anchors both PKA and cAMP phosphodiesterase (PDE) to the nuclear membrane, maintaining them in a negative feedback loop that provides close local control of the cAMP level. Step **1**: The basal level of PDE activity in the absence of hormone (resting state) keeps cAMP levels below those necessary for PKA activation. Step **2**: Activation of β -adrenergic receptors causes an increase in cAMP level in

excess of that which can be degraded by PDE. The resulting binding of cAMP to the regulatory (R) subunits of PKA releases the active catalytic (C) subunits. Step **3**: Subsequent phosphorylation of PDE by PKA stimulates its catalytic activity, thereby driving cAMP levels back to basal and causing reformation of the inactive PKA. Subsequent dephosphorylation of PDE (step **4**) returns the complex to the resting state.

[Adapted from K. L. Dodge et al., 2001, *EMBO J.* **20**:1921.]

close local control of the cAMP level and hence PKA activity (Figure 13-20). The localization of PKA near the nuclear membrane also facilitates entry of the catalytic subunits into the nucleus, where they phosphorylate and activate certain transcription factors (Section 13.6).

KEY CONCEPTS OF SECTION 13.3

G Protein–Coupled Receptors That Activate or Inhibit Adenylyl Cyclase

- Trimeric G proteins transduce signals from coupled cell-surface receptors to associated effector proteins, which are either enzymes that form second messengers or cation channel proteins (see Table 13-1).
- Signals most commonly are transduced by G_{α} , a GTPase switch protein that alternates between an active (“on”) state with bound GTP and inactive (“off”) state with GDP. The β and γ subunits, which remain bound together, occasionally transduce signals.
- Hormone-occupied receptors act as GEFs for G_{α} proteins, catalyzing dissociation of GDP and enabling GTP to bind. The resulting change in conformation of switch regions in G_{α} causes it to dissociate from the $G_{\beta\gamma}$ subunit and interact with an effector protein (see Figure 13-11).
- $G_{s\alpha}$, which is activated by multiple types of GPCRs, binds to and activates adenylyl cyclase, enhancing the synthesis of 3',5'-cyclic AMP (cAMP).

- cAMP-dependent activation of protein kinase A (PKA) mediates the diverse effects of cAMP in different cells. The substrates for PKA and thus the cellular response to hormone-induced activation of PKA vary among cell types.
- In liver and muscle cells, activation of PKA induced by epinephrine and other hormones exerts a dual effect, inhibiting glycogen synthesis and stimulating glycogen breakdown via a kinase cascade (see Figure 13-17).
- Signaling pathways involving second messengers and kinase cascades amplify an external signal tremendously (see Figure 13-18).
- BARK phosphorylates ligand-bound β -adrenergic receptors, leading to the binding of β -arrestin and endocytosis of the receptors. The consequent reduction in cell-surface-receptor numbers renders the cell less sensitive to additional hormone.
- Localization of PKA to specific regions of the cell by anchoring proteins restricts the effects of cAMP to particular subcellular locations.

13.4 G Protein–Coupled Receptors That Regulate Ion Channels

As we learned in Chapter 7, many neurotransmitter receptors are ligand-gated ion channels. These include some types of glutamate and serotonin receptors, as well as the nicotinic acetylcholine receptor found at nerve-muscle synapses. Many

neurotransmitter receptors, however, are G protein–coupled receptors. The effector protein for some of these is a Na^+ or K^+ channel; neurotransmitter binding to these receptors causes the associated ion channel to open or close, leading to changes in the membrane potential. Other neurotransmitter receptors, as well as odorant receptors in the nose and photoreceptors in the eye, are G protein–coupled receptors that indirectly modulate the activity of ion channels via the action of second messengers. In this section, we consider two G protein–coupled receptors that illustrate the direct and indirect mechanisms for regulating ion channels: the muscarinic acetylcholine and G_i -coupled receptors.

Cardiac Muscarinic Acetylcholine Receptors Activate a G Protein That Opens K^+ Channels

Binding of acetylcholine to nicotinic acetylcholine receptors in striated muscle cells generates an action potential that triggers muscle contraction (see Figure 7-45). In contrast, the *muscarinic acetylcholine receptors* in cardiac muscle are inhibitory. Binding of acetylcholine to these receptors slows the

rate of heart muscle contraction by causing a long-lived (several seconds) hyperpolarization of the muscle cell membrane. This can be studied experimentally by direct addition of acetylcholine to heart muscle in culture.

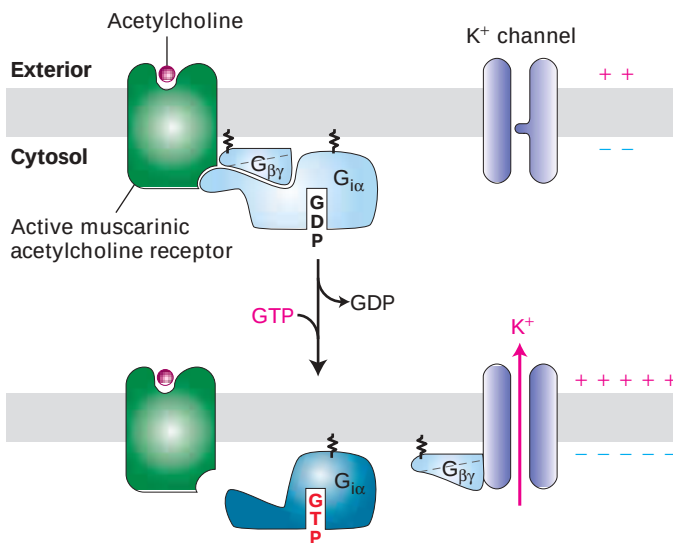
Activation of the muscarinic acetylcholine receptor, which is coupled to a G_i protein, leads to opening of associated K^+ channels; the subsequent efflux of K^+ ions causes hyperpolarization of the plasma membrane. As depicted in Figure 13-21, the signal from activated receptors is transduced to the effector protein by the released $\text{G}_{\beta\gamma}$ subunit rather than by $\text{G}_{\alpha}\cdot\text{GTP}$. That $\text{G}_{\beta\gamma}$ directly activates the K^+ channel was demonstrated by patch-clamping experiments, which can measure ion flow through a single ion channel in a small patch of membrane (see Figure 7-17). When purified $\text{G}_{\beta\gamma}$ protein was added to the cytosolic face of a patch of heart muscle plasma membrane, K^+ channels opened immediately, even in the absence of acetylcholine or other neurotransmitters.

G_i -Coupled Receptors Are Activated by Light

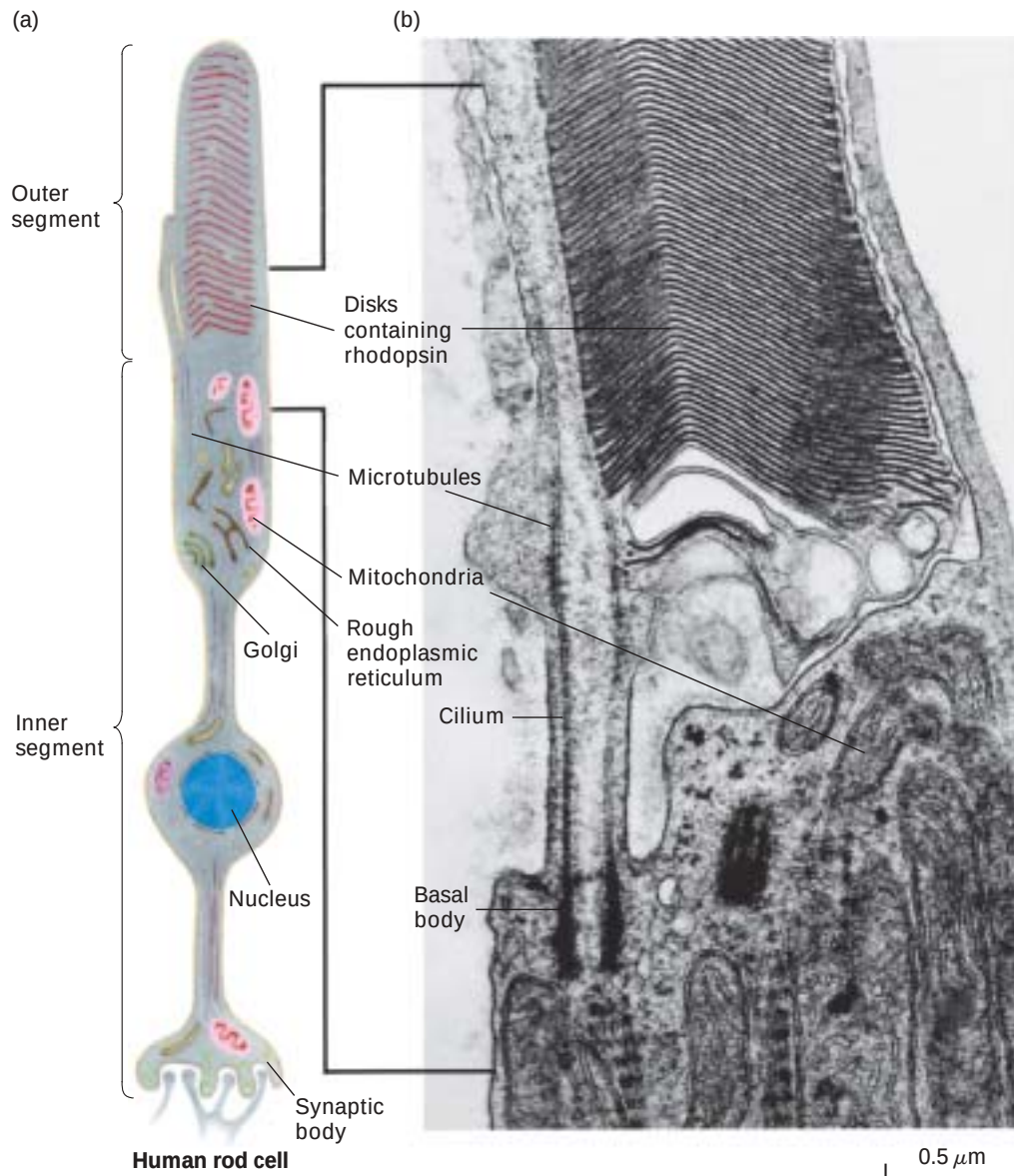
The human retina contains two types of photoreceptors, *rods* and *cones*, that are the primary recipients of visual stimulation. Cones are involved in color vision, while rods are stimulated by weak light like moonlight over a range of wavelengths. The photoreceptors synapse on layer upon layer of interneurons that are innervated by different combinations of photoreceptor cells. All these signals are processed and interpreted by the part of the brain called the *visual cortex*.

Rhodopsin, a G protein–coupled receptor that is activated by light, is localized to the thousand or so flattened membrane disks that make up the outer segment of rod cells (Figure 13-22). The trimeric G protein coupled to rhodopsin, often called *transducin* (G_t), is found only in rod cells. A human rod cell contains about 4×10^7 molecules of rhodopsin, which consists of the seven-spanning protein *opsin* to which is covalently bound the light-absorbing pigment 11-*cis*-retinal. Upon absorption of a photon, the retinal moiety of rhodopsin is very rapidly converted to the all-*trans* isomer, causing a conformational change in the opsin portion that activates it (Figure 13-23). This is equivalent to the conformational change that occurs upon ligand binding by other G protein–coupled receptors. The resulting form in which opsin is covalently bound to all-*trans*-retinal is called *meta-rhodopsin II*, or *activated opsin*. Analogous to other G protein–coupled receptors, this light-activated form of rhodopsin interacts with and activates an associated G protein (i.e., G_t). Activated opsin is unstable and spontaneously dissociates into its component parts, releasing opsin and all-*trans*-retinal, thereby terminating visual signaling. In the dark, free all-*trans*-retinal is converted back to 11-*cis*-retinal, which can then rebind to opsin, re-forming rhodopsin.

In the dark, the membrane potential of a rod cell is about -30 mV, considerably less than the resting potential (-60 to



▲ **FIGURE 13-21 Operational model of muscarinic acetylcholine receptor in the heart muscle plasma membrane.** These receptors are linked via a trimeric G protein to K^+ channels. Binding of acetylcholine triggers activation of the G_{α} subunit and its dissociation from the $\text{G}_{\beta\gamma}$ subunit in the usual way (see Figure 13-11). In this case, the released $\text{G}_{\beta\gamma}$ subunit (rather than $\text{G}_{\alpha}\cdot\text{GTP}$) binds to and opens the associated effector, a K^+ channel. The increase in K^+ permeability hyperpolarizes the membrane, which reduces the frequency of heart muscle contraction. Though not shown here, activation is terminated when the GTP bound to G_{α} is hydrolyzed to GDP and $\text{G}_{\alpha}\cdot\text{GDP}$ recombines with $\text{G}_{\beta\gamma}$. [See K. Ho et al., 1993, *Nature* **362**:31, and Y. Kubo et al., 1993, *Nature* **362**:127.]

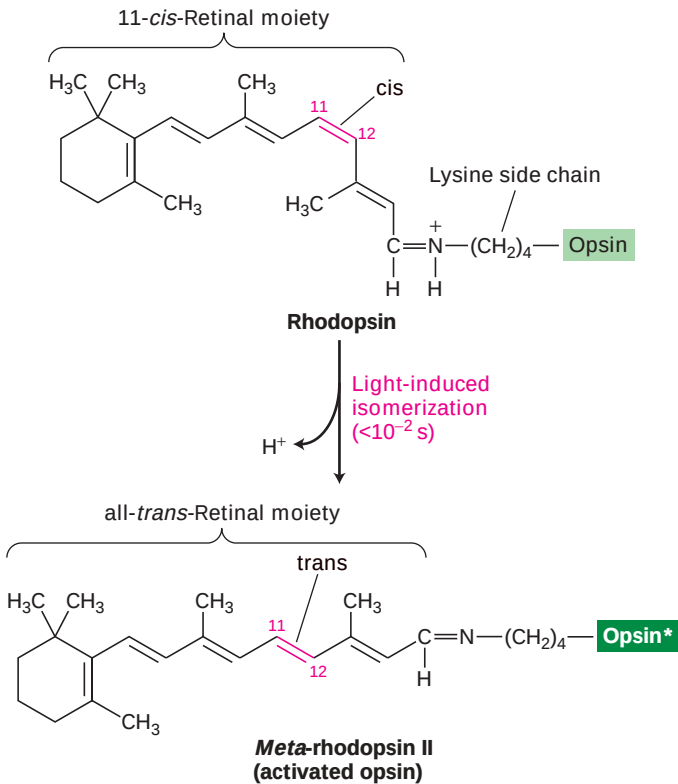


▲ **FIGURE 13-22 Human rod cell.** (a) Schematic diagram of an entire rod cell. At the synaptic body, the rod cell forms a synapse with one or more bipolar interneurons. Rhodopsin, a light-sensitive G protein–coupled receptor, is located in the flattened membrane disks of the outer segment. (b) Electron micrograph of the

region of the rod cell indicated by the bracket in (a). This region includes the junction of the inner and outer segments. [Part (b) from R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company, p. 91.]

–90 mV) typical of neurons and other electrically active cells. As a consequence of this depolarization, rod cells in the dark are constantly secreting neurotransmitters, and the bipolar interneurons with which they synapse are continually being stimulated. The depolarized state of the plasma membrane of resting rod cells is due to the presence of a large number of open *nonselective* ion channels that admit Na^+ and Ca^{2+} , as well as K^+ . Absorption of light by rhodopsin leads to closing of these channels, causing the membrane potential to become *more* negative.

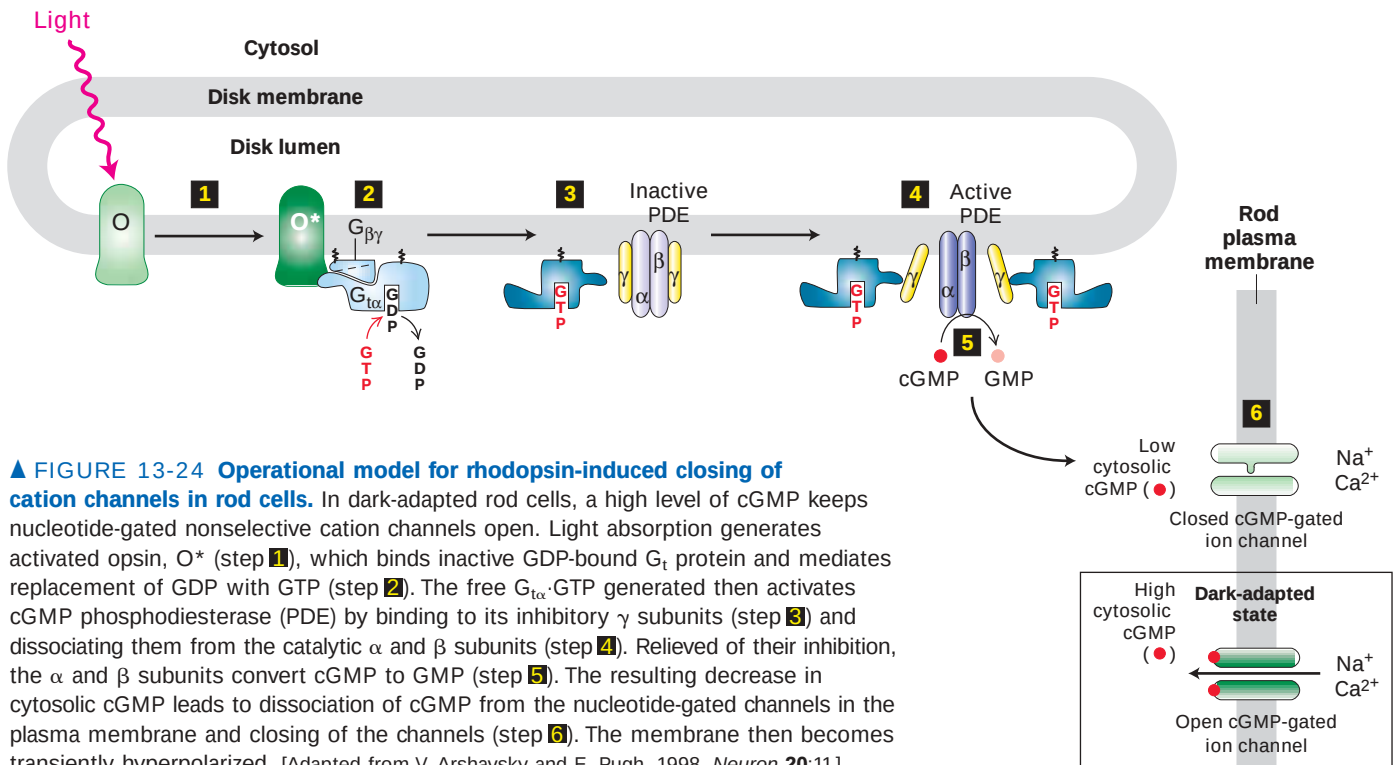
The more photons absorbed by rhodopsin, the more channels are closed, the fewer Na^+ ions cross the membrane from the outside, the more negative the membrane potential becomes, and the less neurotransmitter is released. This change is transmitted to the brain where it is perceived as light. Remarkably, a single photon absorbed by a resting rod cell produces a measurable response, a decrease in the membrane potential of about 1 mV, which in amphibians lasts a second or two. Humans are able to detect a flash of as few as five photons.



Activation of Rhodopsin Induces Closing of cGMP-Gated Cation Channels

The key transducing molecule linking activated opsin to the closing of cation channels in the rod-cell plasma membrane is the second messenger **cyclic GMP (cGMP)**. Rod outer segments contain an unusually high concentration (≈ 0.07 mM) of cGMP, which is continuously formed from GTP in a reaction catalyzed by guanylyl cyclase that appears to be unaffected by light. However, light absorption by rhodopsin induces activation of a *cGMP phosphodiesterase*, which hydrolyzes cGMP to 5'-GMP. As a result, the cGMP concentration decreases upon illumination. The high level of cGMP

◀ **FIGURE 13-23 The light-triggered step in vision.** The light-absorbing pigment 11-*cis*-retinal is covalently bound to the amino group of a lysine residue in opsin, the protein portion of rhodopsin. Absorption of light causes rapid photoisomerization of the *cis*-retinal to the all-*trans* isomer, forming the unstable intermediate *meta*-rhodopsin II, or activated opsin, which activates G_t proteins. Within seconds all-*trans*-retinal dissociates from opsin and is converted by an enzyme back to the *cis* isomer, which then rebinds to another opsin molecule. [See J. Nathans, 1992, *Biochemistry* 31:4923.]



▲ **FIGURE 13-24 Operational model for rhodopsin-induced closing of cation channels in rod cells.** In dark-adapted rod cells, a high level of cGMP keeps nucleotide-gated nonselective cation channels open. Light absorption generates activated opsin, O^* (step 1), which binds inactive GDP-bound G_t protein and mediates replacement of GDP with GTP (step 2). The free G_{α} -GTP generated then activates cGMP phosphodiesterase (PDE) by binding to its inhibitory γ subunits (step 3) and dissociating them from the catalytic α and β subunits (step 4). Relieved of their inhibition, the α and β subunits convert cGMP to GMP (step 5). The resulting decrease in cytosolic cGMP leads to dissociation of cGMP from the nucleotide-gated channels in the plasma membrane and closing of the channels (step 6). The membrane then becomes transiently hyperpolarized. [Adapted from V. Arshavsky and E. Pugh, 1998, *Neuron* 20:11]

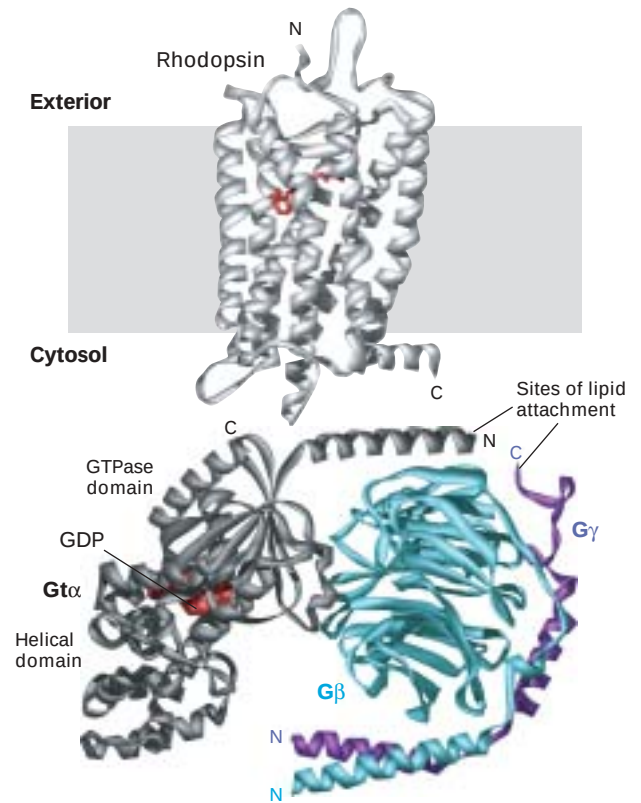
present in the dark acts to keep *cGMP-gated cation channels* open; the light-induced decrease in cGMP leads to channel closing, membrane hyperpolarization, and reduced neurotransmitter release.

As depicted in Figure 13-24, cGMP phosphodiesterase is the effector protein for G_t . The free $G_{\alpha}\cdot\text{GTP}$ complex that is generated after light absorption by rhodopsin binds to the two inhibitory γ subunits of cGMP phosphodiesterase, releasing the active catalytic α and β subunits, which then convert cGMP to GMP. This is another example of how signal-induced removal of an inhibitor can quickly activate an enzyme, a common mechanism in signaling pathways. A single molecule of activated opsin in the disk membrane can activate 500 G_{α} molecules, each of which in turn activates cGMP phosphodiesterase; this is the primary stage of signal amplification in the visual system. Even though activation of cGMP phosphodiesterase leads to a decrease in a second messenger, cGMP, this activation occurs by the same general mechanism described earlier except that absorption of light by rhodopsin rather than ligand binding is the activating signal (see Figure 13-11).

Conversion of active $G_{\alpha}\cdot\text{GTP}$ back to inactive $G_{\alpha}\cdot\text{GDP}$ is accelerated by a GTPase-activating protein (GAP) specific for $G_{\alpha}\cdot\text{GTP}$. In mammals G_{α} normally remains in the active GTP-bound state for only a fraction of a second. Thus cGMP phosphodiesterase rapidly becomes inactivated, and the cGMP level gradually rises to its original level when the light stimulus is removed. This allows rapid responses of the eye toward moving or changing objects.

Recent x-ray crystallographic studies reveal how the subunits of G_t protein interact with each other and with light-activated rhodopsin and provide clues about how binding of GTP leads to dissociation of G_{α} from $G_{\beta\gamma}$ (Figure 13-25). Two surfaces of G_{α} interact with G_{β} : an N-terminal region near the membrane surface and the two adjacent switch I and switch II regions, which are found in all G_{α} proteins. Although G_{β} and G_{γ} also contact each other, G_{γ} does not contact G_{α} .

Studies with adrenergic receptors discussed earlier indicate that ligand binding to a G protein–coupled receptor causes the transmembrane helices in the receptor to slide relative to one another, resulting in conformational changes in the cytosolic loops that create a binding site for the coupled trimeric G protein. The crystallographic structures in Figure 13-25 suggest that the nucleotide-binding domain of G_{α} , together with the lipid anchors at the C-terminus of G_{γ} and the N-terminus of G_{α} , form a surface that binds to light-activated rhodopsin (O^* in Figure 13-24), promoting the release of GDP from G_{α} and the subsequent binding of GTP. The subsequent conformational changes in G_{α} , particularly those within switches I and II, disrupt the molecular interactions between G_{α} and $G_{\beta\gamma}$, leading to their dissociation. The structural studies with rhodopsin and G_t are consistent with data concerning other G protein–coupled receptors and are thought to be generally applicable to all receptors of this type.



▲ FIGURE 13-25 Structural models of rhodopsin and its associated G_t protein. The structures of rhodopsin and the G_{α} and $G_{\beta\gamma}$ subunits were obtained by x-ray crystallography. The C-terminal segment of rhodopsin is not shown in this model. The orientation of G_{α} with respect to rhodopsin and the membrane is hypothetical; it is based on the charge and hydrophobicity of the protein surfaces and the known rhodopsin-binding sites on G_{α} . As in other trimeric G proteins, the G_{α} and G_{γ} subunits contain covalently attached lipids that are thought to be inserted into the membrane. In the GDP-bound form shown here (GDP, red), the α subunit (gray) and the β subunit (light blue) interact with each other, as do the β and γ (purple) subunits, but the small γ subunit, which contains just two α helices, does not contact the α subunit. Several segments of the α subunit are thought to interact with an activated receptor, causing a conformational change that promotes release of GDP and binding of GTP. Binding of GTP, in turn, induces large conformational changes in the switch regions of G_{α} , leading to dissociation of G_{α} from $G_{\beta\gamma}$. The structure of a $G_{s\alpha}$ subunit in the GTP-bound form, which interacts with an effector protein, is shown in Figure 13-14b. [Adapted from H. Hamm, 2001, *Proc. Nat'l. Acad. Sci. USA* **98**:4819, and D. G. Lambright et al., 1996, *Nature* **379**:311.]

Direct support for the role of cGMP in rod-cell activity has been obtained in patch-clamping studies using isolated patches of rod outer-segment plasma membrane, which contains abundant cGMP-gated cation channels. When cGMP is added to the cytosolic surface of these patches, there is a rapid increase in the number of open ion channels. The effect occurs in the absence of protein kinases or phosphatases, and

cGMP acts directly on the channels to keep them open, indicating that these are nucleotide-gated channels. Like the voltage-gated K^+ channels discussed in Chapter 7, the cGMP-gated channel protein contains four subunits, each of which is able to bind a cGMP molecule (see Figure 7-36a). Three or four cGMP molecules must bind per channel in order to open it; this allosteric interaction makes channel opening very sensitive to small changes in cGMP levels.

Rod Cells Adapt to Varying Levels of Ambient Light

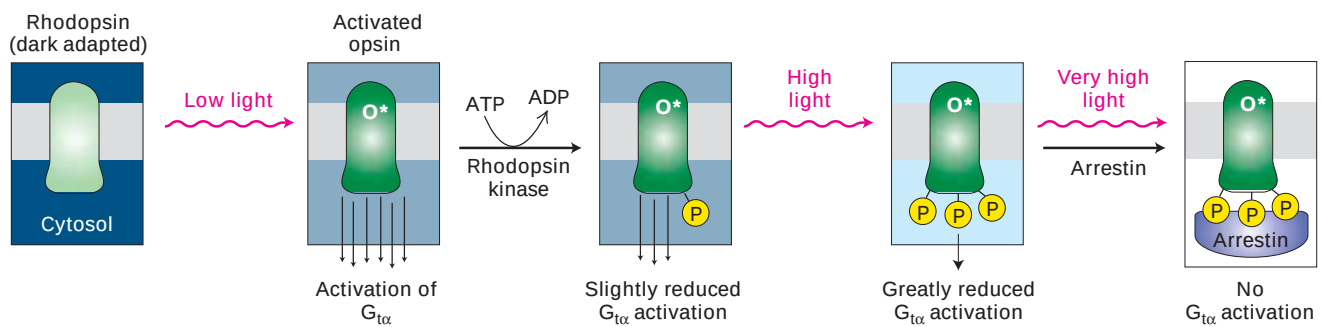
Cone cells are insensitive to low levels of illumination, and the activity of rod cells is inhibited at high light levels. Thus when we move from daylight into a dimly lighted room, we are initially blinded. As the rod cells slowly become sensitive to the dim light, we gradually are able to see and distinguish objects. This process of *visual adaptation* permits a rod cell to perceive contrast over a 100,000-fold range of ambient light levels; as a result, differences in light levels, rather than the absolute amount of absorbed light, are used to form visual images.

One process contributing to visual adaptation involves phosphorylation of activated opsin (O^*) by *rhodopsin kinase* (Figure 13-26). This rod-cell enzyme is analogous to β -adrenergic receptor kinase (BARK) discussed previously. Each opsin molecule has three principal serine phosphorylation sites; the more sites that are phosphorylated, the less able O^* is to activate G_t and thus induce closing of cGMP-gated cation channels. Indeed, rod cells from mice with mutant rhodopsins bearing zero or only one of these serine residues show a much slower than normal rate of deacti-

vation in bright light. Because the extent of opsin phosphorylation is proportional to the amount of time each opsin molecule spends in the light-activated form, it is a measure of the background (ambient) level of light. Under high-light conditions, phosphorylated opsin is abundant and activation of G_t is reduced; thus, a greater increase in light level will be necessary to generate a visual signal. When the level of ambient light is reduced, the opsins become dephosphorylated and the ability to activate G_t increases; in this case, fewer additional photons will be necessary to generate a visual signal.

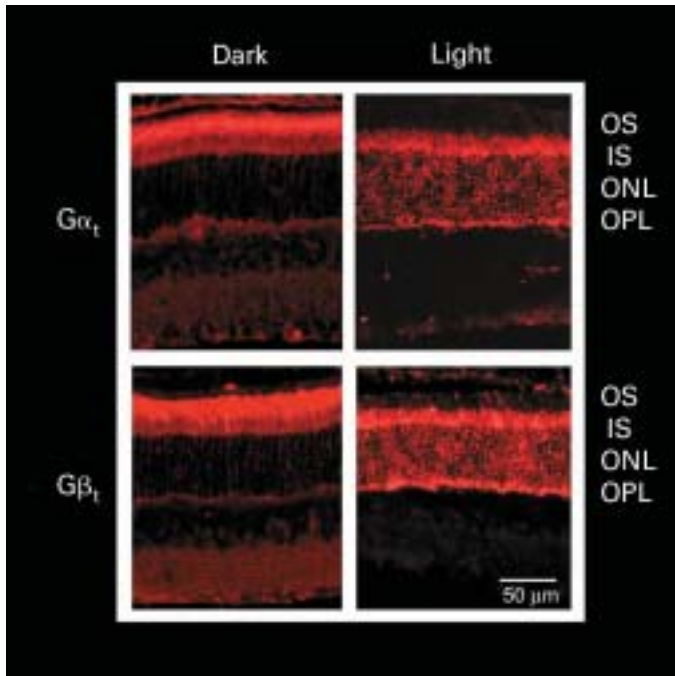
At high ambient light (such as noontime outdoors), the level of opsin phosphorylation is such that the protein β -arrestin binds to the C-terminal segment of opsin. The bound β -arrestin prevents interaction of G_t with O^* , totally blocking formation of the active $G_{t\alpha}$ -GTP complex and causing a shutdown of all rod-cell activity. The mechanism by which rod-cell activity is controlled by rhodopsin kinase and arrestin is similar to adaptation (or desensitization) of other G protein-coupled receptors to high ligand levels.

A second mechanism of visual adaptation appears unique to rod cells. In dark-adapted cells virtually all the $G_{t\alpha}$ and $G_{\beta\gamma}$ subunits are in the outer segments. But exposure for 10 minutes to moderate daytime intensities of light causes over 80 percent of the $G_{t\alpha}$ and $G_{\beta\gamma}$ subunits to move out of the outer segments into other cellular compartments (Figure 13-27). The mechanism by which these proteins move is not yet known, but as a result of this adaptation G_t proteins are physically unable to bind activated opsin. As occurs in other signaling pathways, multiple mechanisms are thus used to inactivate signaling during visual adaptation, presumably to allow strict control of activation of the signaling pathway over broad ranges of illumination.



▲ **FIGURE 13-26 Role of opsin phosphorylation in adaptation of rod cells to changes in ambient light levels.** Light-activated opsin (O^*), but not dark-adapted rhodopsin, is a substrate for rhodopsin kinase. The extent of opsin phosphorylation is directly proportional to the amount of time each opsin molecule spends in the light-activated form and thus to the average ambient light level over the previous few minutes. The ability of O^* to activate

$G_{t\alpha}$ is inversely proportional to the number of phosphorylated residues. Thus the higher the ambient light level, the greater the extent of opsin phosphorylation and the larger the increase in light level needed to activate the same number of $G_{t\alpha}$ (transducin) molecules. At very high light levels, arrestin binds to the completely phosphorylated opsin, forming a complex that cannot activate transducin at all. [See L. Lagnado and D. Baylor, 1992, *Neuron* 8:995, and A. Mendez et al., 2000, *Neuron* 28:153.]



▲ **EXPERIMENTAL FIGURE 13-27 Movement of G_t from outer segments of rod cells contributes to visual adaptation.**

As shown by immunofluorescence staining of retinas of dark-adapted rats, both the α and β subunits of transducin ($G_{\alpha t}$ and $G_{\beta t}$) are localized to the outer segments (OS) of rod cells, where they can be activated by rhodopsin photoreceptors in the membrane disks (see Figure 13-22). After several minutes of bright light most of the transducin α and β subunits have moved to the inner segment (IS) of the rod cells, where they cannot interact with active opsin; this contributes to desensitization of rod cells at high light intensities. [From M. Sokolov et al., 2002, *Neuron* 33:95. Courtesy of Vadim Arshavsky, Harvard Medical School.]

KEY CONCEPTS OF SECTION 13.4

G Protein–Coupled Receptors That Regulate Ion Channels

- The cardiac muscarinic acetylcholine receptor is a GPCR whose effector protein is a K^+ channel. Receptor activation causes release of the $G_{\beta\gamma}$ subunit, which opens K^+ channels (see Figure 13-21). The resulting hyperpolarization of the cell membrane slows the rate of heart muscle contraction.
- Rhodopsin, the photosensitive GPCR in rod cells, comprises the opsin protein linked to 11-*cis*-retinal. The light-induced isomerization of the 11-*cis*-retinal moiety produces activated opsin, which then activates the coupled trimeric G protein transducin (G_t) by catalyzing exchange of free GTP for bound GDP on the $G_{\alpha t}$ subunit.

- The effector protein activated by $G_{\alpha t}$ -GTP is cGMP phosphodiesterase. Reduction in the cGMP level by this enzyme leads to closing of cGMP-gated Na^+/Ca^{2+} channels, hyperpolarization of the membrane, and decreased release of neurotransmitter (see Figure 13-24).

- As with other G_{α} proteins, binding of GTP to $G_{\alpha t}$ causes conformational changes in the protein that disrupt its molecular interactions with $G_{\beta\gamma}$ and enable $G_{\alpha t}$ -GTP to bind to its downstream effector (see Figure 13-25).

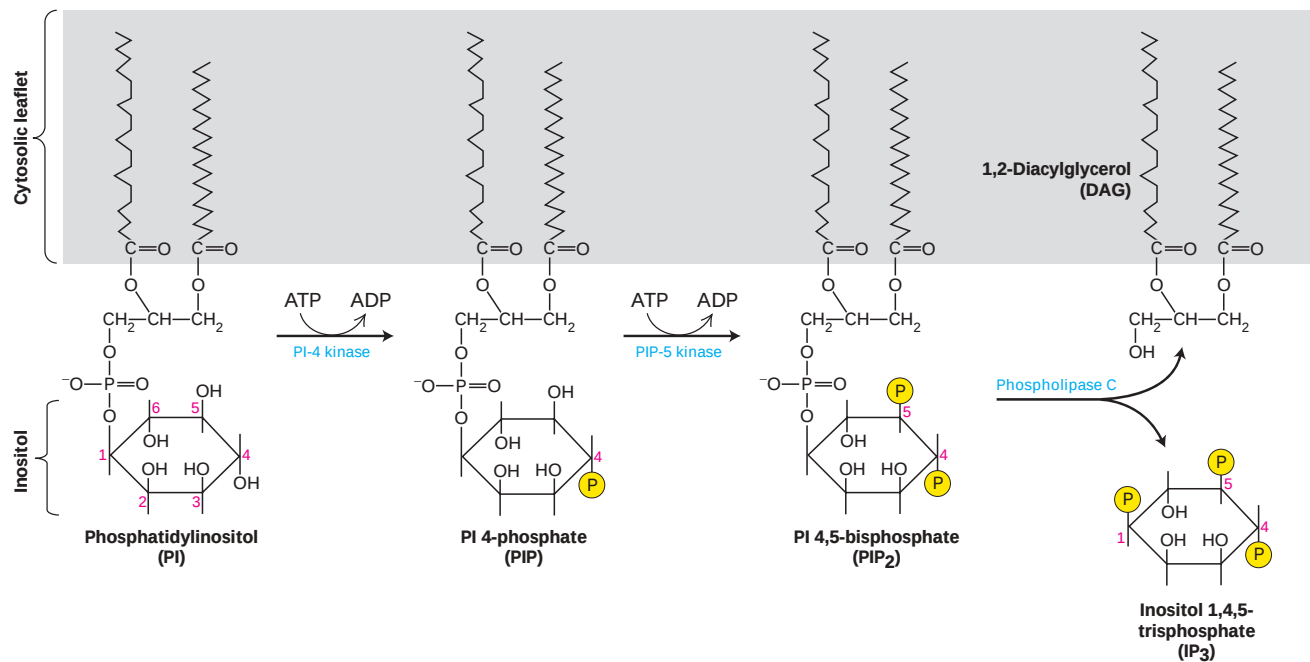
- Phosphorylation of light-activated opsin by rhodopsin kinase and subsequent binding of arrestin to phosphorylated opsin inhibit its ability to activate transducin (see Figure 13-26). This general mechanism of adaptation, or desensitization, is utilized by other GPCRs at high ligand levels.

13.5 G Protein–Coupled Receptors That Activate Phospholipase C

In this section, we discuss GPCR-triggered signal-transduction pathways involving several other second messengers and the mechanisms by which they regulate various cellular activities. A number of these second messengers are derived from *phosphatidylinositol (PI)*. The inositol group in this phospholipid, which extends into the cytosol adjacent to the membrane, can be reversibly phosphorylated at several positions by the combined actions of various kinases and phosphatases. These reactions yield several different membrane-bound **phosphoinositides**, two of which are depicted in Figure 13-28.

The levels of many phosphoinositides in cells are dynamically regulated by extracellular signals, especially those that bind to receptor tyrosine kinases or cytokine receptors, which we cover in the next chapter. The phosphoinositide PIP_2 (PI 4,5-bisphosphate) binds many cytosolic proteins to the plasma membrane. Some of these proteins are required for forming and remodeling the actin cytoskeleton (Chapter 19); others are required for binding of proteins important for endocytosis and vesicle fusions (Chapter 17).

PIP_2 is also cleaved by the plasma-membrane–associated enzyme **phospholipase C (PLC)** to generate two important second messengers: **1,2-diacylglycerol (DAG)**, a lipophilic molecule that remains associated with the membrane, and **inositol 1,4,5-trisphosphate (IP_3)**, which diffuses in the cytosol (see Figure 13-28). We refer to downstream events involving these two second messengers collectively as the *IP_3 /DAG pathway*. Hormone binding to receptors coupled to either a G_o or a G_q protein (see Table 13-1) induces activation of the β isoform of phospholipase C (PLC β) by the general mechanism outlined in Figure 13-11.



▲ **FIGURE 13-28 Synthesis of DAG and IP₃ from membrane-bound phosphatidylinositol (PI).** Each membrane-bound PI kinase places a phosphate (yellow circles) on a specific hydroxyl group on the inositol ring, producing the phosphoinositides

PIP and PIP₂. Cleavage of PIP₂ by phospholipase C (PLC) yields the two important second messengers DAG and IP₃. [See A. Toker and L. C. Cantley, 1997, *Nature* **387**:673, and C. L. Carpenter and L. C. Cantley, 1996, *Curr. Opin. Cell Biol.* **8**:153.]

Inositol 1,4,5-Trisphosphate (IP₃) Triggers Release of Ca²⁺ from the Endoplasmic Reticulum

Most intracellular Ca²⁺ ions are sequestered in the mitochondria and in the lumen of the endoplasmic reticulum (ER) and other vesicles. Cells employ various mechanisms for regulating the concentration of Ca²⁺ ions in the cytosol, which usually is kept below 0.2 μM. For instance, Ca²⁺ ATPases pump cytosolic Ca²⁺ ions across the plasma membrane to the cell exterior or into the lumens of intracellular Ca²⁺-storing compartments (see Figure 7-7). As we discuss below, a small rise in cytosolic Ca²⁺ induces a variety of cellular responses, and thus the cytosolic concentration of Ca²⁺ is carefully controlled.

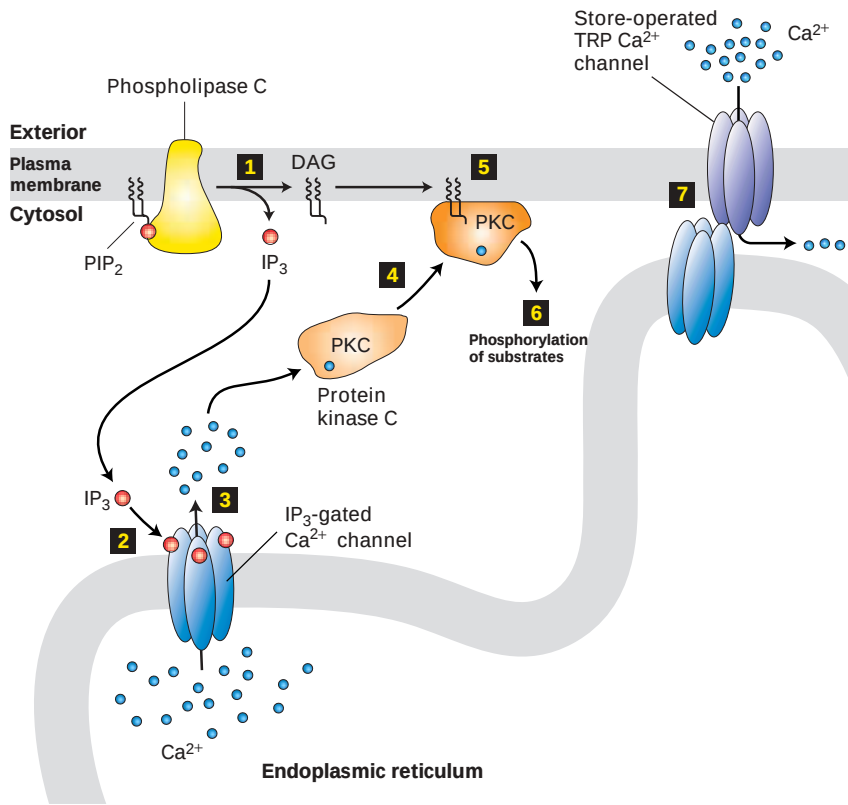
Binding of many hormones to their cell-surface receptors on liver, fat, and other cells induces an elevation in cytosolic Ca²⁺ even when Ca²⁺ ions are absent from the surrounding extracellular fluid. In this situation, Ca²⁺ is released into the cytosol from the ER lumen through operation of the IP₃-gated Ca²⁺ channel in the ER membrane. This large protein is composed of four identical subunits, each containing an IP₃-binding site in the N-terminal cytosolic domain. IP₃ binding induces opening of the channel, allowing Ca²⁺ ions to exit from the ER into the cytosol (Figure 13-29). When various phosphorylated inositols found in cells are added to preparations of ER vesicles, only IP₃ causes release of Ca²⁺ ions from the vesicles. This simple experiment demonstrates the specificity of the IP₃ effect.

The IP₃-mediated rise in the cytosolic Ca²⁺ level is only transient because Ca²⁺ ATPases located in the plasma mem-

brane and ER membrane actively pump Ca²⁺ from the cytosol to the cell exterior and ER lumen, respectively. Furthermore, within a second of its generation, one specific phosphate on IP₃ is hydrolyzed, yielding inositol 1,4-bisphosphate, which does not stimulate Ca²⁺ release from the ER.

Without some means for replenishing depleted stores of intracellular Ca²⁺, a cell would soon be unable to increase the cytosolic Ca²⁺ level in response to hormone-induced IP₃. Patch-clamping studies have revealed that a plasma-membrane Ca²⁺ channel, called the TRP channel or the store-operated channel, opens in response to depletion of ER Ca²⁺ stores (see Figure 13-29). In a way that is not understood, depletion of Ca²⁺ in the ER lumen leads to a conformational change in the IP₃-gated Ca²⁺ channel that allows it to bind to the TRP Ca²⁺ channel in the plasma membrane, causing the latter to open. Indeed, expression in cells of a specific fragment of the ER membrane IP₃-gated Ca²⁺ channel prevents opening of the TRP channel upon depletion of ER Ca²⁺ stores, implicating an interaction between the two Ca²⁺ channels in opening the TRP channel.

Opening of IP₃-gated Ca²⁺ channels is potentiated by cytosolic Ca²⁺ ions, which increase the affinity of these channel receptors for IP₃, resulting in greater release of stored Ca²⁺. Higher concentrations of cytosolic Ca²⁺, however, inhibit IP₃-induced release of Ca²⁺ from intracellular stores by decreasing the affinity of the receptor for IP₃. This complex regulation of IP₃-gated Ca²⁺ channels in ER membranes by cytosolic Ca²⁺ can lead to rapid oscillations in the cytosolic Ca²⁺ level when the IP₃ pathway in cells is stimu-



◀ **FIGURE 13-29 IP₃/DAG pathway and the elevation of cytosolic Ca²⁺.**

This pathway can be triggered by ligand binding to certain G protein–coupled receptors and several other receptor types, leading to activation of phospholipase C. Cleavage of PIP₂ by phospholipase C yields IP₃ and DAG (step 1). After diffusing through the cytosol, IP₃ interacts with and opens Ca²⁺ channels in the membrane of the endoplasmic reticulum (step 2), causing release of stored Ca²⁺ ions into the cytosol (step 3). One of various cellular responses induced by a rise in cytosolic Ca²⁺ is recruitment of protein kinase C (PKC) to the plasma membrane (step 4), where it is activated by DAG (step 5). The activated kinase can phosphorylate various cellular enzymes and receptors, thereby altering their activity (step 6). As endoplasmic reticulum Ca²⁺ stores are depleted, the IP₃-gated Ca²⁺ channels bind to and open store-operated TRP Ca²⁺ channels in the plasma membrane, allowing influx of extracellular Ca²⁺ (step 7). [Adapted from J. W. Putney, 1999, *Proc. Nat'l. Acad. Sci. USA* 96:14669.]

lated. For example, stimulation of hormone-secreting cells in the pituitary by luteinizing hormone–releasing hormone (LHRH) causes rapid, repeated spikes in the cytosolic Ca²⁺ level; each spike is associated with a burst in secretion of luteinizing hormone (LH). The purpose of the fluctuations of Ca²⁺, rather than a sustained rise in cytosolic Ca²⁺, is not understood. One possibility is that a sustained rise in Ca²⁺ may be toxic to cells.

Diacylglycerol (DAG) Activates Protein Kinase C, Which Regulates Many Other Proteins

After its formation by hydrolysis of PIP₂ or other phosphoinositides, DAG remains associated with the plasma membrane. The principal function of DAG is to activate a family of protein kinases collectively termed **protein kinase C (PKC)**. In the absence of hormone stimulation, protein kinase C is present as a soluble cytosolic protein that is catalytically inactive. A rise in the cytosolic Ca²⁺ level causes protein kinase C to bind to the cytosolic leaflet of the plasma membrane, where the membrane-associated DAG can activate it. Thus activation of protein kinase C depends on an increase of both Ca²⁺ ions and DAG, suggesting an interaction between the two branches of the IP₃/DAG pathway (see Figure 13-29).

The activation of protein kinase C in different cells results in a varied array of cellular responses, indicating that it plays a key role in many aspects of cellular growth and metabolism. In liver cells, for instance, protein kinase C helps

regulate glycogen metabolism by phosphorylating and thus inhibiting glycogen synthase. Protein kinase C also phosphorylates various transcription factors; depending on the cell type; these induce synthesis of mRNAs that trigger cell proliferation.

Ca²⁺/Calmodulin Complex Mediates Many Cellular Responses to External Signals

Ligand binding to several types of receptors, in addition to G protein–coupled receptors, can activate a phospholipase C isoform, leading to an IP₃-mediated increase in the cytosolic level of free Ca²⁺. Such localized increases in cytosolic Ca²⁺ in specific cell types are critical to its function as a second messenger. For example, acetylcholine stimulation of G protein–coupled receptors in secretory cells of the pancreas and parotid gland induces an IP₃-mediated rise in Ca²⁺ that triggers the fusion of secretory vesicles with the plasma membrane and release of their contents into the extracellular space. In blood platelets, the rise in Ca²⁺ induced by thrombin stimulation triggers a conformational change in these cell fragments leading to their aggregation, an important step in plugging holes in blood vessels. Secretion of insulin from pancreatic β cells also is triggered by Ca²⁺, although the increase in Ca²⁺ occurs by a different mechanism (see Figure 15-7).

A small cytosolic protein called **calmodulin**, which is ubiquitous in eukaryotic cells, functions as a multipurpose switch protein that mediates many cellular effects of Ca²⁺

ions. Binding of Ca^{2+} to four sites on calmodulin yields a complex that interacts with and modulates the activity of many enzymes and other proteins (see Figure 3-28). Because Ca^{2+} binds to calmodulin in a cooperative fashion, a small change in the level of cytosolic Ca^{2+} leads to a large change in the level of active calmodulin. One well-studied enzyme activated by the Ca^{2+} /calmodulin complex is myosin light-chain kinase, which regulates the activity of myosin in muscle cells (Chapter 19). Another is cAMP phosphodiesterase, the enzyme that degrades cAMP to 5'-AMP and terminates its effects. This reaction thus links Ca^{2+} and cAMP, one of many examples in which two second messengers interact to fine-tune certain aspects of cell regulation.

In certain cells, the rise in cytosolic Ca^{2+} following receptor signaling via PLC-generated IP_3 leads to activation of specific transcription factors. In some cases, Ca^{2+} /calmodulin activates protein kinases that, in turn, phosphorylate transcription factors, thereby modifying their activity and regulating gene expression. In other cases, Ca^{2+} /calmodulin activates a phosphatase that removes phosphate groups from a transcription factor. An important example of this mechanism involves T cells of the immune system in which Ca^{2+} ions enhance the activity of an essential transcription factor, NFAT (nuclear factor of activated T cells). In unstimulated cells, phosphorylated NFAT is located in the cytosol. Following receptor stimulation and elevation of cytosolic Ca^{2+} , the Ca^{2+} /calmodulin complex binds to and activates calcineurin, a protein-serine phosphatase. Activated calcineurin then dephosphorylates key phosphate residues on cytosolic NFAT, exposing a nuclear localization sequence that allows NFAT to move into the nucleus and stimulate expression of genes essential for activation of T cells.

The Ca^{2+} /calmodulin complex also plays a key role in controlling the diameter of blood vessels and thus their ability to deliver oxygen to tissues. This pathway involves a

novel signaling molecule and provides another example of cGMP functioning as a second messenger.

Signal-Induced Relaxation of Vascular Smooth Muscle Is Mediated by cGMP-Activated Protein Kinase G



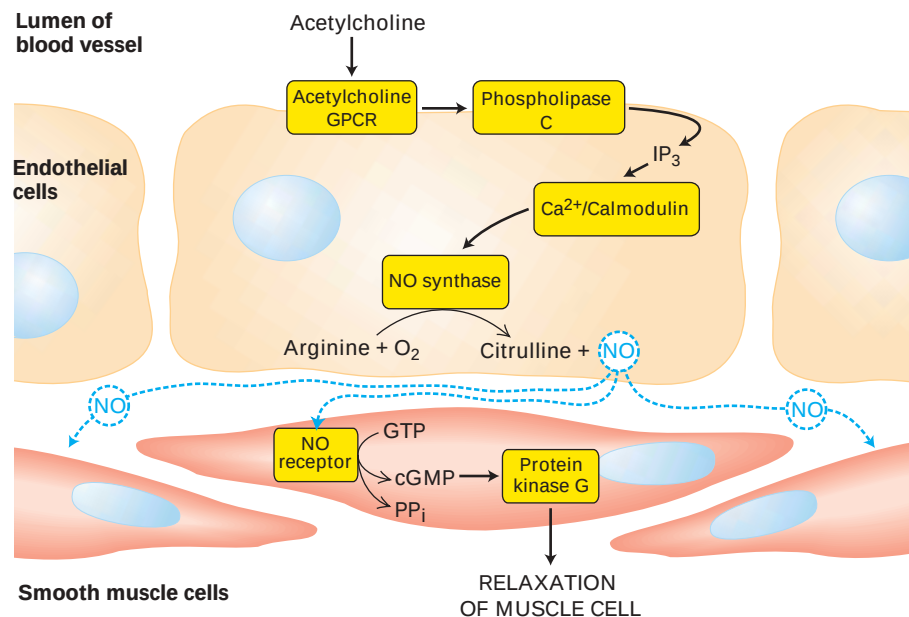
Nitroglycerin has been used for over a century as a treatment for the intense chest pain of angina. It was known to slowly decompose in the body to *nitric oxide* (NO), which causes relaxation of the smooth muscle cells surrounding the blood vessels that “feed” the heart muscle itself, thereby increasing the diameter of the blood vessels and increasing the flow of oxygen-bearing blood to the heart muscle. One of the most intriguing discoveries in modern medicine is that NO, a toxic gas found in car exhaust, is in fact a natural signaling molecule. ■

Definitive evidence for the role of NO in inducing relaxation of smooth muscle came from a set of experiments in which acetylcholine was added to experimental preparations of the smooth muscle cells that surround blood vessels. Direct application of acetylcholine to these cells caused them to contract, the expected effect of acetylcholine on these muscle cells. But addition of acetylcholine to the lumen of small isolated blood vessels caused the underlying smooth muscles to relax, not contract. Subsequent studies showed that in response to acetylcholine the endothelial cells that line the lumen of blood vessels were releasing some substance that in turn triggered muscle cell relaxation. That substance turned out to be NO.

We now know that endothelial cells contain a G_o protein-coupled receptor that binds acetylcholine and activates phospholipase C, leading to an increase in the level of

► FIGURE 13-30 Regulation of contractility of arterial smooth muscle by nitric oxide (NO) and cGMP.

Nitric oxide is synthesized in endothelial cells in response to acetylcholine and the subsequent elevation in cytosolic Ca^{2+} . NO diffuses locally through tissues and activates an intracellular NO receptor with guanylyl cyclase activity in nearby smooth muscle cells. The resulting rise in cGMP leads to activation of protein kinase G (PKG), relaxation of the muscle, and thus vasodilation. The cell-surface receptor for atrial natriuretic factor (ANF) also has intrinsic guanylyl cyclase activity (not shown); stimulation of this receptor on smooth muscle cells also leads to increased cGMP and subsequent muscle relaxation. PP_i = pyrophosphate. [See C. S. Lowenstein et al., 1994, *Ann. Intern. Med.* 120:227]



cytosolic Ca^{2+} . After Ca^{2+} binds to calmodulin, the resulting complex stimulates the activity of NO synthase, an enzyme that catalyzes formation of NO from O_2 and the amino acid arginine. Because NO has a short half-life (2–30 seconds), it can diffuse only locally in tissues from its site of synthesis. In particular NO diffuses from the endothelial cell into neighboring smooth muscle cells, where it triggers muscle relaxation (Figure 13-30).

The effect of NO on smooth muscle is mediated by the second messenger cGMP, which can be formed by an intracellular NO receptor expressed by smooth muscle cells. Binding of NO to the heme group in this receptor leads to a conformational change that increases its intrinsic guanylyl cyclase activity, leading to a rise in the cGMP level. Most of the effects of cGMP are mediated by a cGMP-dependent protein kinase, also known as *protein kinase G* (PKG). In vascular smooth muscle, protein kinase G activates a signaling pathway that results in inhibition of the actin-myosin complex, relaxation of the cell, and dilation of the blood vessel. In this case, cGMP acts indirectly via protein kinase G, whereas in rod cells cGMP acts directly by binding to and thus opening cation channels in the plasma membrane.

Relaxation of vascular smooth muscle also is triggered by binding of atrial natriuretic factor (ANF) and some other peptide hormones to their receptors on smooth muscle cells. The cytosolic domain of these cell-surface receptors, like the intracellular NO receptor, possesses intrinsic guanylyl cyclase activity. When an increased blood volume stretches cardiac muscle cells in the heart atrium, they release ANF. Circulating ANF binds to ANF receptors in smooth muscle cells surrounding blood vessels, inducing activation of guanylyl cyclase activity and formation of cGMP. Subsequent activation of protein kinase G causes dilation of the vessel by the mechanism described above. This vasodilation reduces blood pressure and counters the stimulus that provoked the initial release of ANF.

KEY CONCEPTS OF SECTION 13.5

G Protein–Coupled Receptors That Activate Phospholipase C

- Stimulation of some GPCRs and other cell-surface receptors leads to activation of phospholipase C, which generates two second messengers: diffusible IP_3 and membrane-bound DAG (see Figure 13-28).
- IP_3 triggers opening of IP_3 -gated Ca^{2+} channels in the endoplasmic reticulum and elevation of cytosolic free Ca^{2+} . In response to elevated cytosolic Ca^{2+} , protein kinase C is recruited to the plasma membrane, where it is activated by DAG (see Figure 13-29).
- The Ca^{2+} /calmodulin complex regulates the activity of many different proteins, including cAMP phosphodiesterase, nitric oxide synthase, and protein kinases or phosphatases that control the activity of various transcription factors.

- Stimulation of the acetylcholine GPCR on endothelial cells induces an increase in cytosolic Ca^{2+} and subsequent synthesis of NO. After diffusing into surrounding smooth muscle cells, NO activates intracellular guanylate cyclase to synthesize cGMP (see Figure 13-30).
- Synthesis of cGMP in vascular smooth muscle cells leads to activation of protein kinase G, which triggers a pathway leading to muscle relaxation and vasodilation.
- cGMP is also produced in vascular smooth muscle cells by stimulation of cell-surface receptors that have intrinsic guanylate cyclase activity. These include receptors for atrial natriuretic factor (ANF).

13.6 Activation of Gene Transcription by G Protein–Coupled Receptors

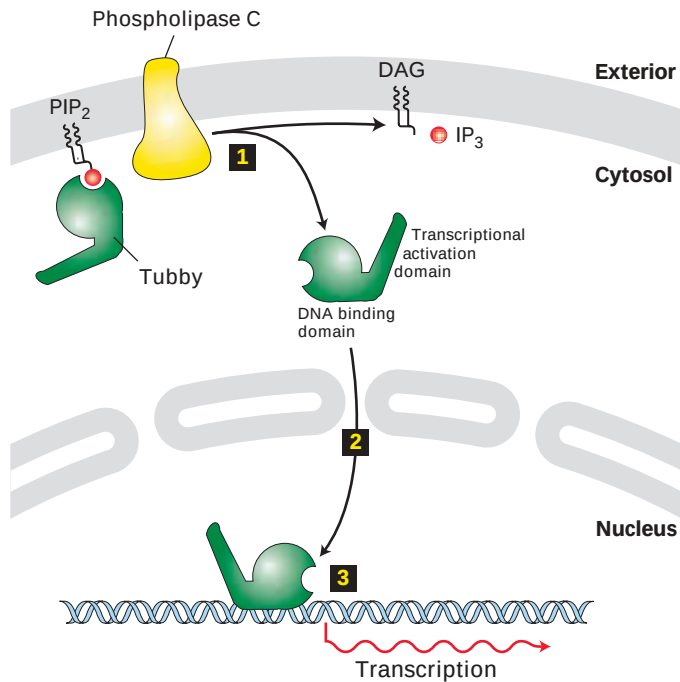
As mentioned early in this chapter, intracellular signal-transduction pathways can have short-term and long-term effects on the cell. Short-term effects (seconds to minutes) result from modulation of the activity of preexisting enzymes or other proteins, leading to changes in cell metabolism or function. Most of the pathways activated by G protein–coupled receptors fall into this category. However, GPCR signaling pathways also can have long-term effects (hours to days) owing to activation or repression of gene transcription, leading in some cases to cell proliferation or to differentiation into a different type of cell. Earlier we discussed how a signal-induced rise in cytosolic Ca^{2+} can lead to activation of transcription factors. Here we consider other mechanisms by which some G protein–coupled receptors regulate gene expression.

Membrane-Localized Tubby Transcription Factor Is Released by Activation of Phospholipase C



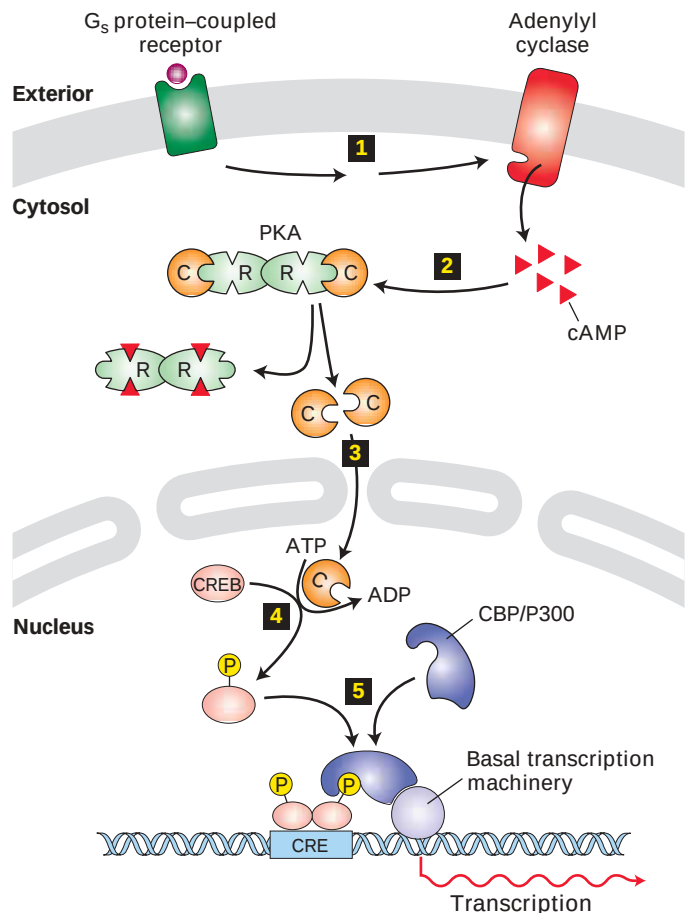
The *tubby* gene, which is expressed primarily in certain areas of the brain involved in control of eating behavior, first attracted attention because of its involvement in obesity. Mice bearing mutations in the *tubby* gene develop adult-onset obesity, and certain aspects of their metabolism resemble that of obese humans.

Sequencing of the cloned *tubby* gene suggested that its encoded protein contains both a DNA-binding domain and a transcription-activation domain (Chapter 11). However, the Tubby protein was found to be localized near the plasma membrane, making it an unlikely candidate as a transcription factor. Subsequent studies revealed that Tubby binds tightly to PIP_2 , anchoring the protein to the plasma membrane (Figure 13-31). Hormone binding to G_α - or G_q -coupled receptors, which activate phospholipase C, leads to hydrolysis of PIP_2 and release of Tubby into the cytosol. Tubby then enters the nucleus and activates transcription of a still unknown gene or genes. Identification of these genes should provide clues about how their encoded proteins relate to obesity. ■



◀ **FIGURE 13-31 Activation of the Tubby transcription factor following ligand binding to receptors coupled to G_o or G_q.** In resting cells, Tubby is bound tightly to PIP₂ in the plasma membrane. Receptor stimulation (not shown) leads to activation of phospholipase C, hydrolysis of PIP₂, and release of Tubby into the cytosol (1). Directed by two functional nuclear localization sequences (NLS) in its N-terminal domain, Tubby translocates into the nucleus (2) and activates transcription of target genes (3). It is not known whether IP₃ remains bound to Tubby. [Adapted from S. Santagata et al., 2001, *Science* **292**:2041.]

▶ **FIGURE 13-32 Activation of gene expression following ligand binding to G_s protein-coupled receptors.** Receptor stimulation (1) leads to activation of PKA (2). Catalytic subunits of PKA translocate to the nucleus (3) and there phosphorylate and activate the transcription factor CREB (4). Phosphorylated CREB associates with the co-activator CBP/P300 (5) to stimulate various target genes controlled by the CRE regulatory element. See the text for details. [See K. A. Lee and N. Masson, 1993, *Biochim. Biophys. Acta* **1174**:221, and D. Parker et al., 1996, *Mol. Cell Biol.* **16**(2):694.]



CREB Links cAMP Signals to Transcription

In mammalian cells, an elevation in the cytosolic cAMP level stimulates the expression of many genes. For instance, increased cAMP in certain endocrine cells induces production of somatostatin, a peptide that inhibits release of various hormones; in liver cells, cAMP induces synthesis of several enzymes involved in converting three-carbon compounds to glucose.

All genes regulated by cAMP contain a cis-acting DNA sequence, the *cAMP-response element (CRE)*, that binds the phosphorylated form of a transcription factor called *CRE-binding (CREB) protein*, which is found only in the nucleus. As discussed previously, binding of neurotransmitters and hormones to G_s protein-coupled receptors activates adenylyl cyclase, leading to an increase in cAMP and subsequent release of the active catalytic subunit of PKA. Some of the catalytic subunits then translocate to the nucleus and phosphorylate serine-133 on CREB protein.

Phosphorylated CREB protein binds to CRE-containing target genes and also interacts with a *co-activator* termed *CBP/300*, which links CREB to the basal transcriptional machinery, thereby permitting CREB to stimulate transcription (Figure 13-32). Earlier studies suggested that phosphorylation induced a conformational change in CREB protein, but more recent work indicates that CBP/P300 binds specifically to phosphoserine-133 in activated CREB. As discussed in Chapter 11, other signal-regulated transcription factors rely on CBP/P300 to exert their activating effect. Thus this co-activator plays an important role in integrating signals from multiple signaling pathways that regulate gene transcription.

GPCR-Bound Arrestin Activates Several Kinase Cascades That Control Gene Expression

We saw earlier that binding of β -arrestin to phosphorylated serines in the cytosolic domain of G protein-coupled receptors both blocks activation of G_{α} and mediates endocytosis of the GPCR-arrestin complex. Perhaps surprisingly, the GPCR-arrestin complex also acts as a scaffold for binding and activating several cytosolic kinases (see Figure 13-19). These include c-Src, which activates the MAP kinase pathway and other pathways leading to transcription of genes needed for cell division. A complex of three arrestin-bound proteins, including a Jun N-terminal kinase (JNK-1), initiates a kinase cascade that ultimately activates the c-Jun transcription factor. Activated c-Jun promotes expression of certain growth-promoting enzymes and other proteins that help cells respond to some stresses.



Binding of epinephrine to the β -adrenergic receptors in heart muscle stimulates glycogenolysis and enhances the rate of muscle contraction. Prolonged treatment with epinephrine, however, induces proliferation of these cardiac muscle cells. In extreme cases, such *cardiac hypertrophy* causes failure of the heart muscle, a major cause of heart disease. This epinephrine-induced cell proliferation results

in part from activation of the MAP kinase cascade. As just described, the GPCR-arrestin complex can trigger this cascade.

Another, perhaps more important, way that activation of β -adrenergic receptors promotes cardiac hypertrophy involves another type of receptor. The G_s protein activated by β -adrenergic receptors can somehow lead to activation of a specific extracellular metal-containing protease that, in turn, cleaves the transmembrane precursor of epidermal growth factor (EGF). The soluble EGF released into the extracellular space binds to and activates EGF receptors on the same cell in an autocrine fashion. As we learn in the next chapter, the EGF receptor belongs to the receptor tyrosine kinase (RTK) class of receptors, which commonly trigger the MAP kinase cascade leading to cell proliferation. Similar cross-talk between two types of receptors occurs in many other signaling systems. Just as no cell lives in isolation, no receptor and no signal-transduction pathway function by themselves. ■

KEY CONCEPTS OF SECTION 13.6

Activation of Gene Transcription by G Protein-Coupled Receptors

- Activation of phospholipase C by receptors coupled to G_o or G_q proteins releases the Tubby transcription factor, which is bound to PIP_2 embedded in the plasma membrane of resting cells (see Figure 13-31).
- Signal-induced activation of protein kinase A (PKA) often leads to phosphorylation of CREB protein, which together with the CBP/300 co-activator stimulates transcription of many target genes (see Figure 13-32).
- The GPCR-arrestin complex activates several cytosolic kinases, initiating cascades that lead to transcriptional activation of many genes controlling cell growth.

PERSPECTIVES FOR THE FUTURE

Very soon we will know the identity of all the pieces in many signal-transduction pathways, but putting the puzzle together to predict cellular responses remains elusive. For instance, we can enumerate the G proteins, kinases, phosphatases, arrestins, and other proteins that participate in signaling from β -adrenergic receptors in liver cells, but we are still far from being able to predict, quantitatively, how liver cells react over time to a given dose of adrenaline. In part this is because complex feedback (and in some cases feed-forward) loops regulate the activity of multiple enzymes and other components in the pathway. Although biochemical and cell biological experiments tell us how these interactions occur, we cannot describe quantitatively the rates or extent of these reactions in living cells.

The emerging field of biological systems analysis attempts to develop an integrated view of a cell's response to external

signals. Mathematical equations are formulated that incorporate rate constants for enzyme catalysis, formation of protein-protein complexes, and concentrations and diffusion rates of all the various signal-transduction proteins. These models incorporate information about changes in the subcellular localization of proteins with time (e.g., movement of transcription factors into the nucleus or endocytosis of surface receptors) and the effect on the activity of any given protein (e.g., glycogen phosphorylase) of the local Ca^{2+} concentration and the presence of multiple kinases and phosphatases. By comparing the results of such calculations with actual experimental results (say, by increasing or decreasing selectively the concentration of one component of the pathway) we can determine, in principle, whether we have accounted for all of the components of the pathway. Such mathematical modeling will also help the pharmaceutical industry develop new drugs that might activate or inhibit specific pathways. Modeling can enable one to extrapolate the results of experiments on drugs on proteins in test tubes or on cultured cells in order to predict their efficacy and side effects in living organisms.

In this chapter we focused primarily on signal-transduction pathways activated by individual G protein-coupled receptors. However, even these relatively simple pathways presage the more complex situation within living cells. As we've seen, activation of a single type of receptor often leads to production of multiple second messengers or activation of several types of downstream transducing proteins. Moreover, the same cellular response (e.g., glycogen breakdown) is affected by multiple signaling pathways activated by multiple types of receptors. Interaction of different signaling pathways permits the fine-tuning of cellular activities required to carry out complex developmental and physiological processes, and the ability of cells to respond appropriately to extracellular signals also depends on regulation of signaling pathways themselves.

KEY TERMS

adenylyl cyclase 548	paracrine signaling 535
adrenergic receptors 548	PDZ domains 543
agonist 539	phospholipase C (β isoform) 561
autocrine signaling 535	protein kinase A 550
calmodulin 563	protein kinase C 563
cAMP 537	rhodopsin 556
competition assay 539	second messengers 541
desensitization 544	signal amplification 552
endocrine signaling 535	signal transduction 534
functional expression assay 540	stimulatory G protein 548
G protein-coupled receptors 534	transducin 556
IP_3/DAG pathway 561	trimeric G proteins 534
muscarinic acetylcholine receptors 556	visual adaptation 560

REVIEW THE CONCEPTS

1. Signaling by soluble extracellular molecules can be classified into three types: endocrine, paracrine, and autocrine. Describe how these three methods of cellular signaling differ. Growth hormone is secreted from the pituitary, which is located at the base of the brain, and acts through growth hormone receptors located on the liver. Is this an example of endocrine, paracrine, or autocrine signaling? Why?

2. A ligand binds two different receptors with a K_d value of 10^{-7} M for receptor 1 and a K_d value of 10^{-9} M for receptor 2. For which receptor does the ligand show the greater affinity? Calculate the fraction of receptors that have a bound ligand ($[\text{RL}]/R_T$) for ligand with receptor 1 and for ligand with receptor 2, if the concentration of free ligand is 10^{-8} M.

3. A study of the properties of cell-surface receptors can be greatly enhanced by isolation or cloning of the cell-surface receptor. Describe how a cell-surface receptor can be isolated by affinity chromatography. How can you clone a cell surface receptor using a functional-expression assay?

4. Signal-transducing trimeric G proteins consist of three subunits designated α , β , γ . The G_α subunit is a GTPase switch protein that cycles between active and inactive states depending upon whether it is bound to GTP or to GDP. Review the steps for ligand-induced activation of effector proteins mediated by the trimeric-G-protein complex. Suppose that you have isolated a mutant G_α subunit that has an increased GTPase activity. What effect would this mutation have on the G protein and the effector protein?

5. Membrane proteins are often found clustered. Describe how protein clustering can be mediated by adapter proteins or by specialized lipid rafts termed caveolae. What advantage might there be to having a cluster of membrane proteins involved in a signaling pathway rather than spread out in the membrane?

6. Epinephrine binds to both β -adrenergic and α -adrenergic receptors. Describe the opposite actions on the effector protein, adenylyl cyclase, elicited by the binding of epinephrine to these two types of receptors. Describe the effect of adding an agonist or antagonist to a β -adrenergic receptor on the activity of adenylyl cyclase.

7. In liver and muscle cells, epinephrine stimulates the release of glucose from glycogen by inhibiting glycogen synthesis and stimulating glycogen breakdown. Outline the molecular events that occur after epinephrine binds to its receptor and the resultant increase in the concentration of intracellular cAMP. How are the cAMP levels returned to normal? Describe the events that occur after cAMP levels decline.

8. Continuous exposure of a G_s protein–coupled receptor to its ligand leads to a phenomenon known as desensitization. Describe several molecular mechanisms for receptor desensitization. How can a receptor be reset to its original sensitized state? What effect would a mutant receptor lacking serine or threonine phosphorylation sites have on a cell?

9. A number of different molecules act as second messengers. Activation of rhodopsin by light induces the closing of gated cation channels with cyclic GMP as a second messenger. Describe the effect of light on rhodopsin. On what effector protein does the trimeric G protein act? What type of trimeric G protein is involved in this event?

10. Visual adaptation and receptor desensitization involve similar phosphorylation mechanisms. Describe how the β -adrenergic receptor kinase (BARK) and rhodopsin kinase play important roles in these processes. What role does dephosphorylation play in these reactions?

11. Inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) are second messenger molecules derived from the cleavage of the phosphoinositide PIP_2 (phosphatidylinositol 4,5-bisphosphate) by activated phospholipase C. Describe the role of IP_3 in the release of Ca^{2+} from the endoplasmic reticulum. How do cells replenish the endoplasmic reticulum stores of Ca^{2+} ? What is the principal function of DAG?

12. In 1992, the journal *Science* named nitric oxide the Molecule of the Year. Describe how this important second messenger is synthesized. How does nitric oxide cause relaxation of smooth muscle cells?

13. Ligand binding to G protein–coupled receptors can result in activation of gene transcription. Describe how the second messengers PIP_2 and cAMP can activate transcription of genes.

ANALYZE THE DATA

Mutations in the trimeric G proteins can cause many diseases in humans. Patients with acromegaly often have pituitary tumors that oversecrete the pituitary hormone called growth hormone (GH). A subset of these growth hormone (GH)–secreting pituitary tumors result from mutations in G proteins. GH-releasing hormone (GHRH) stimulates GH release from the pituitary by binding to GHRH receptors and stimulating adenylyl cyclase. Cloning and sequencing of the wild-type and mutant $G_{s\alpha}$ gene from normal individuals and patients with the pituitary tumors revealed a missense mutation in the $G_{s\alpha}$ gene sequence.

a. To investigate the effect of the mutation on $G_{s\alpha}$ activity, wild-type and mutant $G_{s\alpha}$ cDNA were transfected into cells that lack the $G_{s\alpha}$ gene. These cells express a β_2 -adrenergic receptor, which can be activated by isoproterenol, a β_2 -adrenergic receptor agonist. Membranes were isolated from transfected cells and assayed for adenylyl cyclase activity in the presence of GTP or the hydrolysis-resistant GTP analog, GTP- γ S. From the

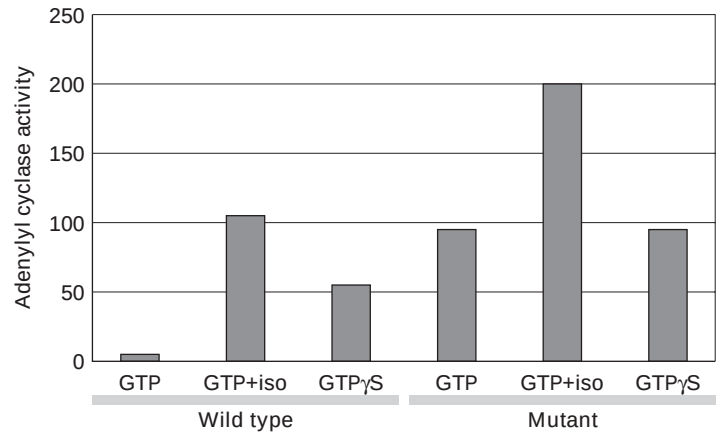


figure above, what do you conclude about the effect of the mutation on $G_{s\alpha}$ activity in the presence of GTP alone compared with GTP- γ S alone or GTP plus isoproterenol (iso)?

b. In the transfected cells described in part a, what would you predict would be the cAMP levels in cells transfected with the wild-type $G_{s\alpha}$ and the mutant $G_{s\alpha}$? What effect might this have on the cells?

c. To further characterize the molecular defect caused by this mutation, the intrinsic GTPase activity present in both wild-type and mutant $G_{s\alpha}$ was assayed. Assays for GTPase activity showed that the mutation reduced the $K_{cat-GTP}$ (catalysis rate constant for GTP hydrolysis) from a wild-type value of 4.1 min^{-1} to the mutant value of 0.1 min^{-1} . What do you conclude about the effect of the mutation on the GTPase activity present in the mutant $G_{s\alpha}$ subunit? How do these GTPase results explain the adenylyl cyclase results shown in part a?

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