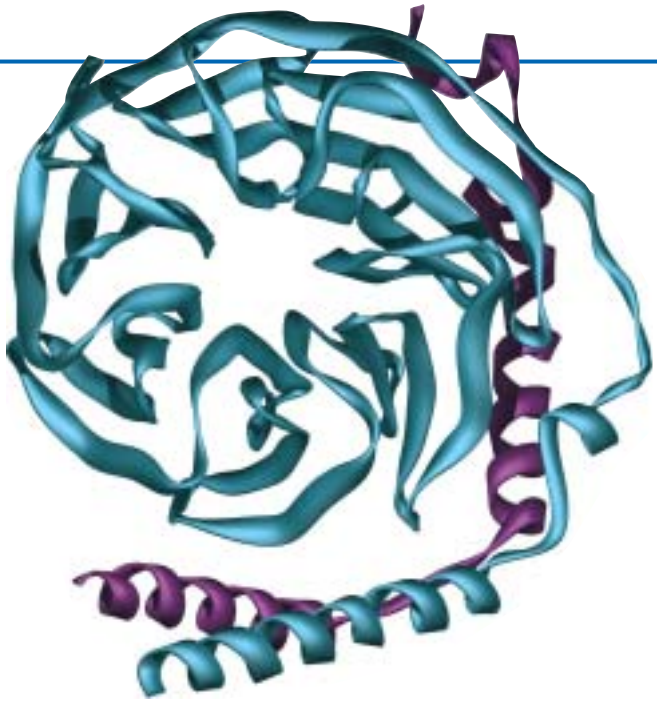
13

SIGNALING AT THE CELL SURFACE



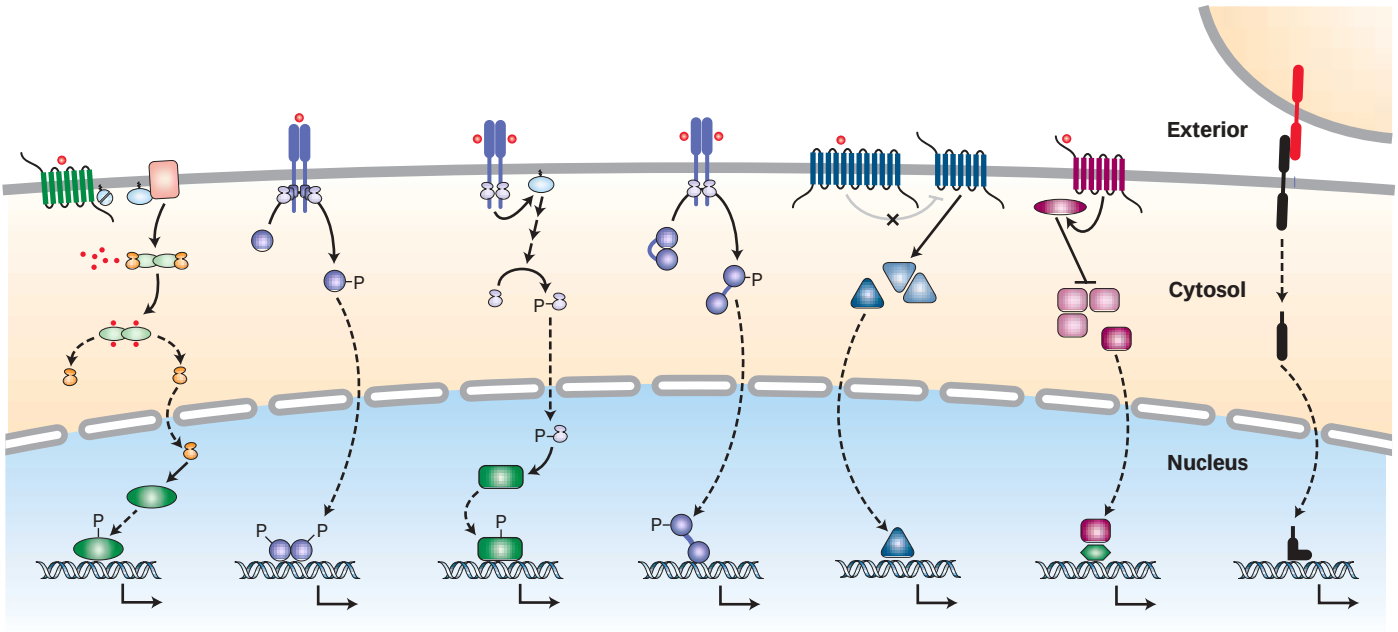
Three-dimensional structure of the G protein β (blue) and γ (purple) complex as obtained by x-ray crystallography.

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



G protein-coupled receptors	Cytokine receptors	Receptor tyrosine kinases	TGFβ receptors	Hedgehog (Hh) receptors	Wnt receptors	Notch receptor
Linked to a trimeric G protein that controls the activity of an effector protein (here adenylyl cyclase)	Associated with cytosolic JAK kinases	Cytosolic domain with tyrosine kinase activity	Cytosolic domain with serine/threonine kinase activity	Hh ligand tethered to membrane of signaling cell by cholesterol anchor	Palmitoylated Wnt ligand binds seven transmembrane protein receptor complex	Ligand, Delta, is a transmembrane protein on signaling cell
Activate cytosolic or nuclear transcription factors via several pathways (here one involving protein kinase A)	Activate cytosolic STAT transcription factors by phosphorylation	Activate cytosolic kinases (here MAP kinase) that translocate to the nucleus and activate nuclear transcription factors by phosphorylation	Activate Smad transcription factors in the cytosol by phosphorylation	Control processing of transcription factor by proteolysis; Hh binding causes release from cytosolic complex	Release an activated transcription factor from a multiprotein complex in the cytosol	Cytosolic domain of Notch released by proteolysis acts in association with nuclear transcription factors

▲ 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., TGFβ, Wnt, Hedgehog), or a key intracellular signal transduction component (e.g., NF-κB). 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., TGFβ 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-κB 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.

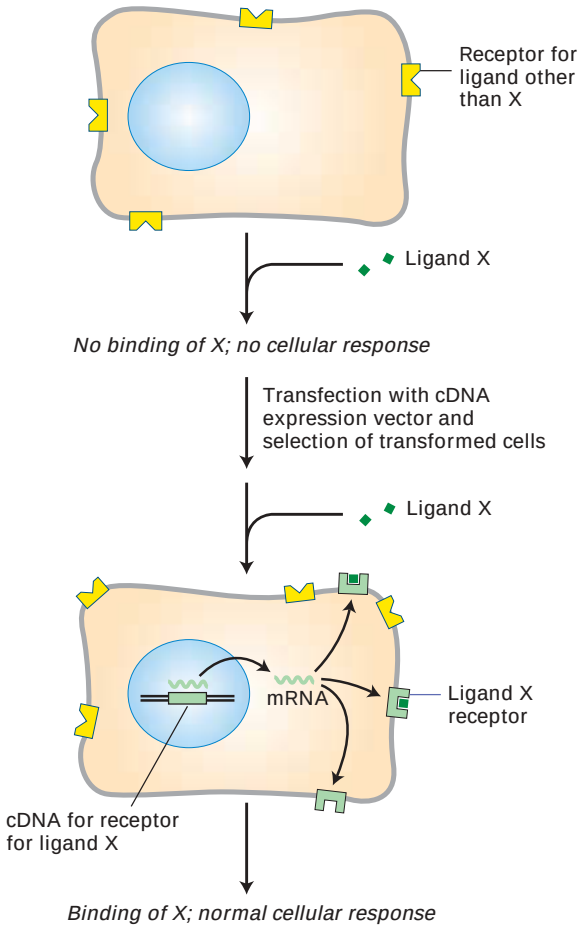
that are used to solubilize receptor proteins from the cell membrane. The labeled ligand provides a means for detecting the receptor during purification procedures.

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 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).

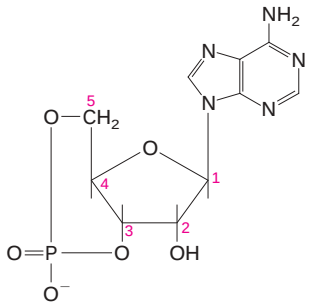
13.2 Intracellular Signal Transduction

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

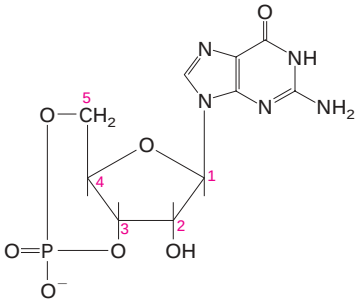
The binding of ligands ("first messengers") to many cell-surface receptors leads to a short-lived increase (or decrease) in the concentration of certain low-molecular-weight intracellular signaling molecules termed **second messengers.** These molecules include 3′,5′-cyclic AMP (cAMP), 3′,5′-cyclic GMP (cGMP), 1,2-diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (IP_3), whose structures are shown in Figure 13-7. Other important second messengers are Ca^{2+} 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



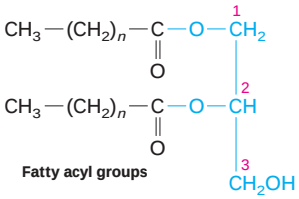
3′,5′-Cyclic AMP (cAMP)

Activates protein kinase A (PKA)



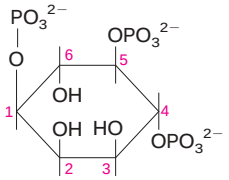
3′,5′-Cyclic GMP (cGMP)

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



1,2-Diacylglycerol (DAG)

Activates protein kinase C (PKC)



Inositol 1,4,5-trisphosphate (IP_3)

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.

MEDIA CONNECTIONS
Focus Animation: Second Messengers in Signaling Pathways

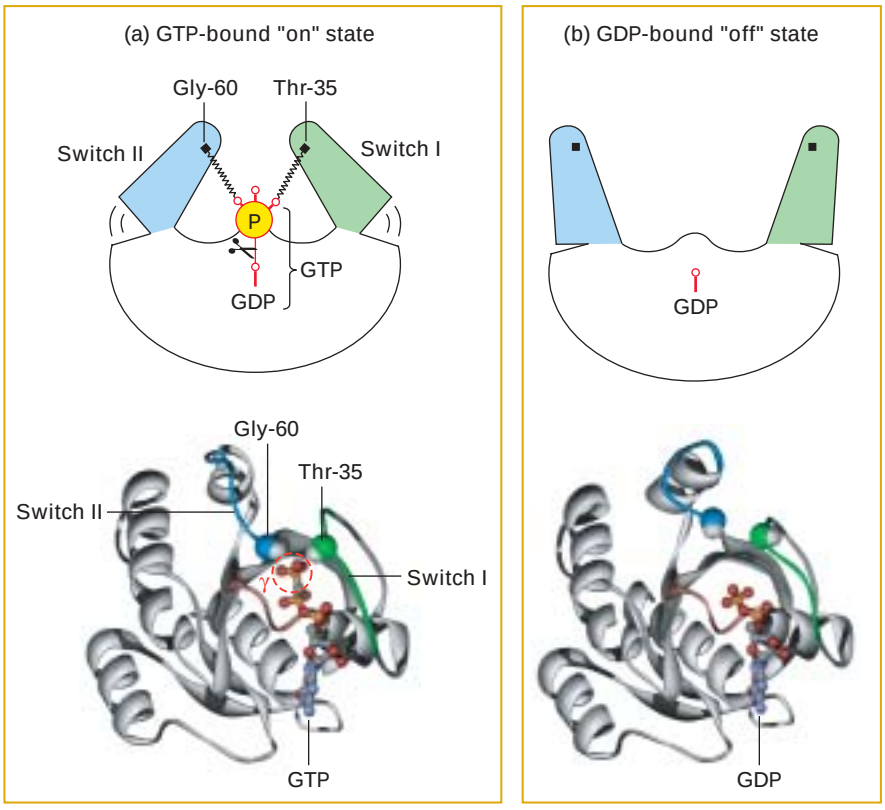
mode of action of cAMP and other second messengers is discussed in later sections.

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

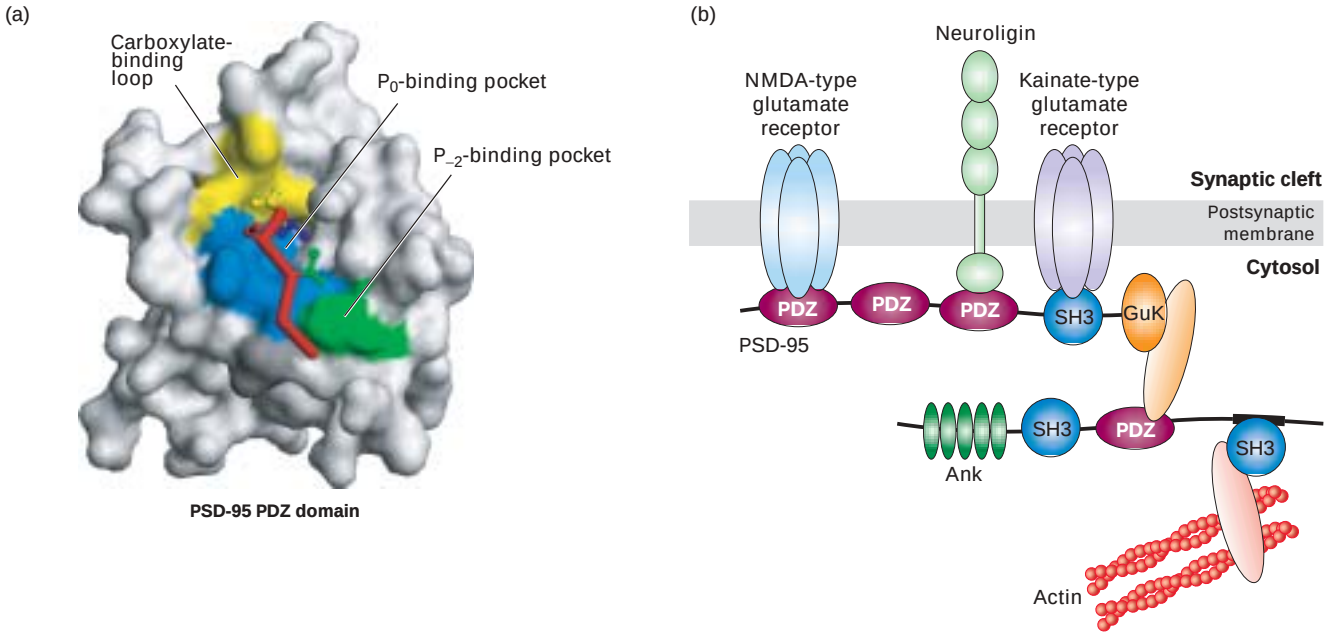
Although the epinephrine receptors expressed by adipose (fat-storage) cells appear to be uniformly distributed on the surface of these spherical cells, such a uniform distribution probably is rare. More common is the clustering of receptors and other membrane-associated signaling proteins to a particular 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 receptors 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 assuring 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

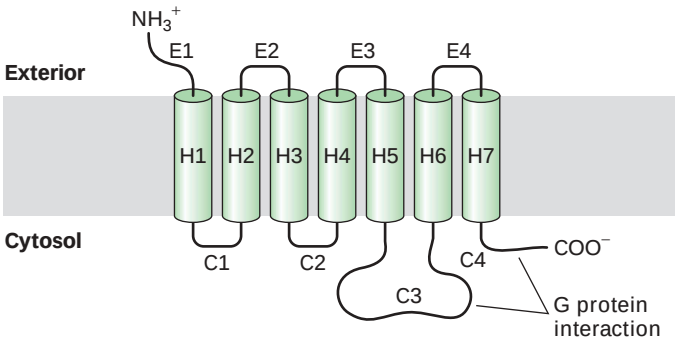
■ The level of second messengers, such as Ca^{2+}, cAMP, and IP_3, increases or occasionally decreases in response to binding of ligand to cell-surface receptors (see Figure 13-7). These nonprotein intracellular signaling molecules,

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 postsynaptic 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_α 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_α 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_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$. IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.

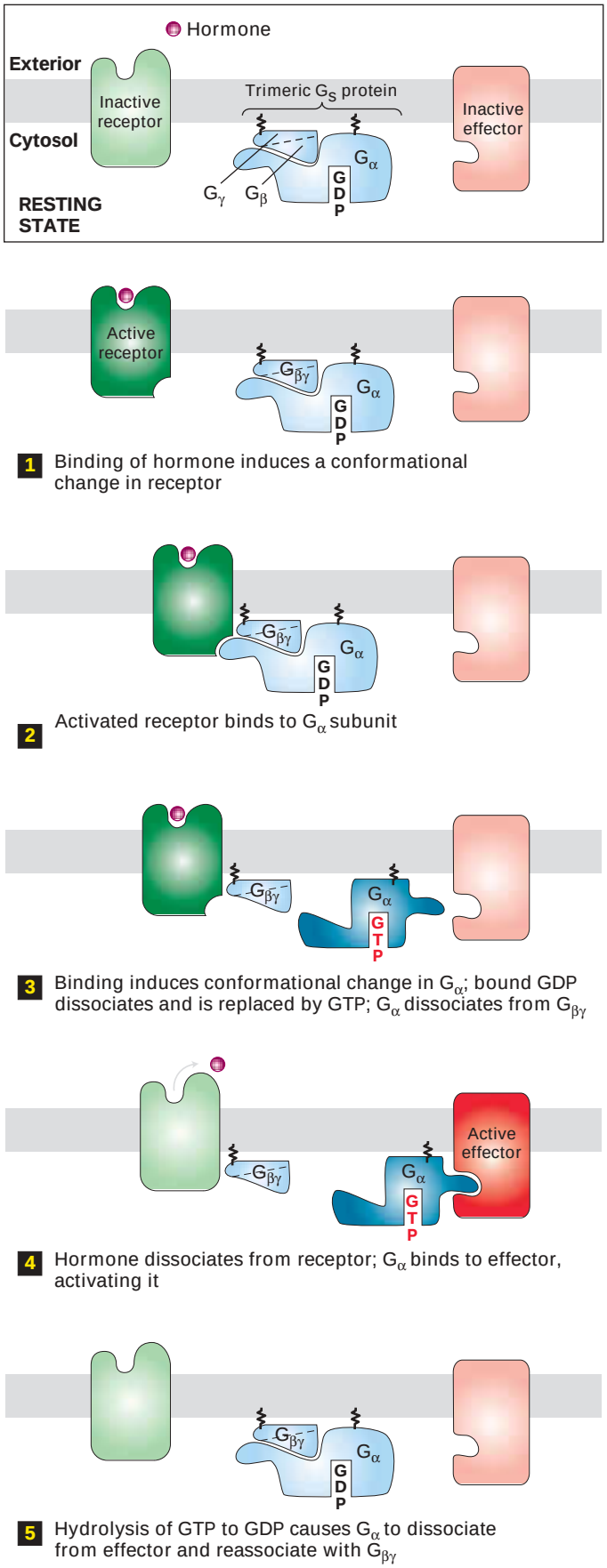
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

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_α·GTP, in some cases it is inhibited. Moreover, depending on the cell and ligand, the $G_{\beta\gamma}$ subunit, rather than G_α·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.

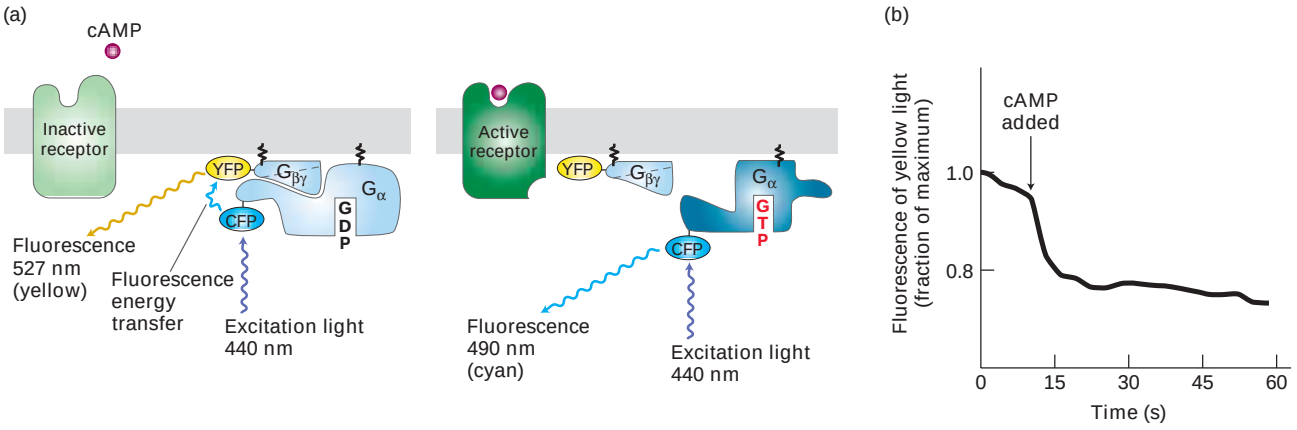
In this section, we first discuss how GPCR signals are transduced to an effector protein, a process that is similar for all receptors of this type. Then we focus on pathways in which cAMP is the second messenger, using the epinephrine-stimulated degradation of glycogen as an example.

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_α·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 $G_\alpha \cdot G_{\beta\gamma}$ 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, characteristic 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.]

MEDIA CONNECTIONS
Video: Chemotaxis of a Single *Dictyostelium* Cell to the Chemoattractant cAMP

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).

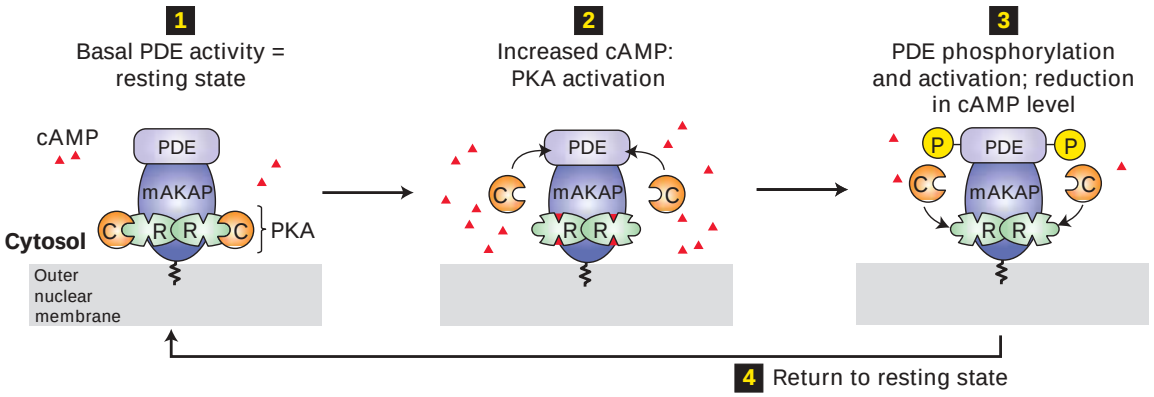
Once the exchange of nucleotides has occurred, the $G_\alpha \cdot$GTP complex dissociates from the $G_{\beta\gamma}$ subunit, but both remain anchored in the membrane. In most cases, $G_\alpha \cdot$GTP then interacts with and activates an associated effector protein, as depicted in Figure 13-11. This 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 $G_\alpha \cdot$GDP quickly reassociates with $G_{\beta\gamma}$, thus terminating effector activation. In many cases, a protein termed *RGS* (*r*egulator of *G* protein *s*ignaling) 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–CH_2–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 $G_\alpha \cdot$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 $G_\alpha \cdot G_{\beta\gamma}$ 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).*



▲ 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

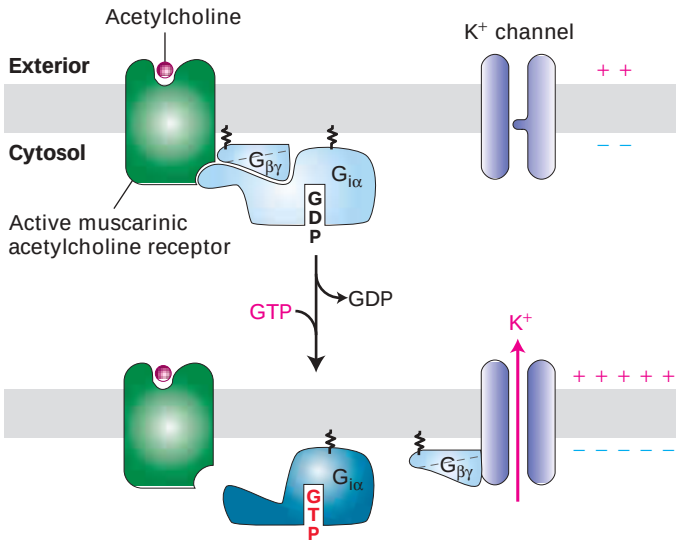
As we learned in Chapter 7, many neurotransmitter receptors are ligand-gated ion channels. These include some types of glutamate and serotonin receptors, as well as the nicotinic acetylcholine receptor found at nerve-muscle synapses. Many

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_t-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 $G_{\beta\gamma}$ subunit rather than by G_α·GTP. That $G_{\beta\gamma}$ 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 $G_{\beta\gamma}$ 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.



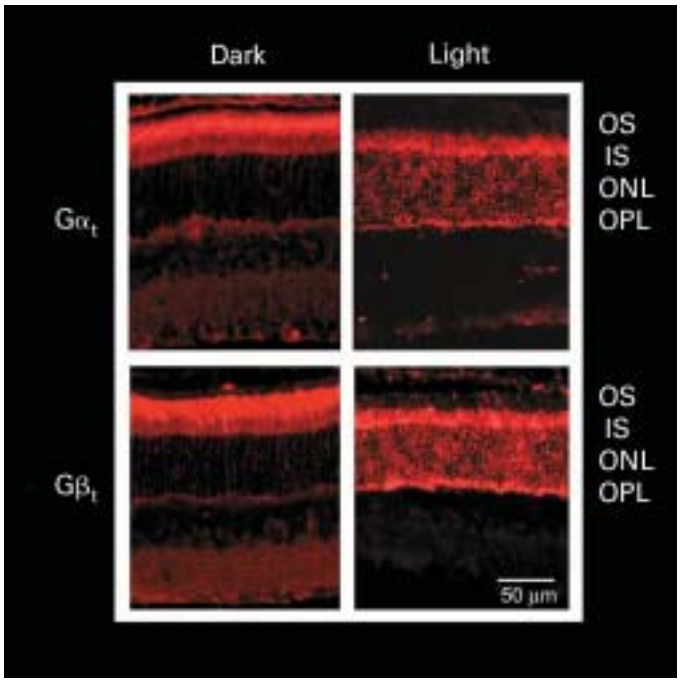
▲ FIGURE 13-21 **Operational model of muscarinic acetylcholine receptor in the heart muscle plasma membrane.** These receptors are linked via a trimeric G protein to K^+ channels. Binding of acetylcholine triggers activation of the $G_{i\alpha}$ subunit and its dissociation from the $G_{\beta\gamma}$ subunit in the usual way (see Figure 13-11). In this case, the released $G_{\beta\gamma}$ subunit (rather than $G_{i\alpha}$·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 $G_{i\alpha}$ is hydrolyzed to GDP and $G_{i\alpha}$·GDP recombines with $G_{\beta\gamma}$. [See K. Ho et al., 1993, *Nature* **362**:31, and Y. Kubo et al., 1993, *Nature* **362**:127.]

G_t-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 *metarhodopsin 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



▲ 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

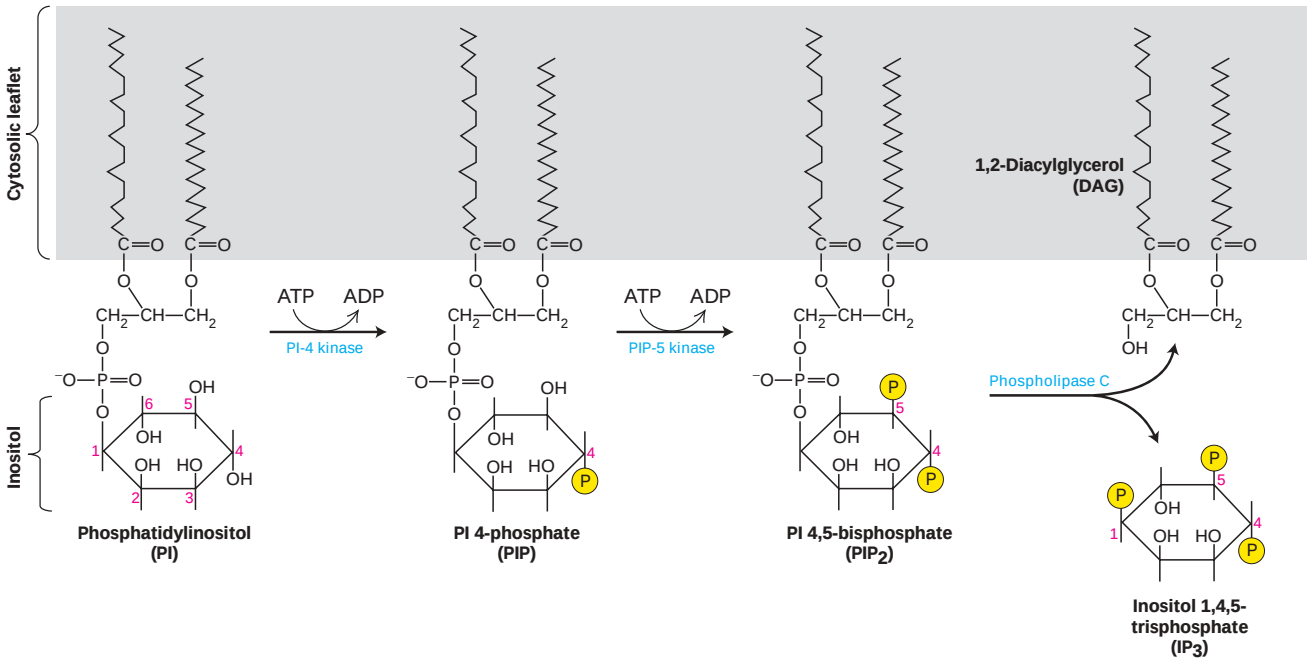
- The cardiac muscarinic acetylcholine receptor is a GPCR whose effector protein is a K^+ channel. Receptor activation causes release of the $G_{\beta\gamma}$ subunit, which opens K^+ channels (see Figure 13-21). The resulting hyperpolarization of the cell membrane slows the rate of heart muscle contraction.
- 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 $G_{t\alpha}$ subunit.
- The effector protein activated by $G_{t\alpha}$·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 $G_{t\alpha}$ causes conformational changes in the protein that disrupt its molecular interactions with $G_{\beta\gamma}$ and enable $G_{t\alpha}$·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 (PLCβ) by the general mechanism outlined in Figure 13-11.



▲ FIGURE 13-28 **Synthesis of DAG and IP_3 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 PIP_2. Cleavage of PIP_2 by phospholipase C (PLC) yields the two important second messengers DAG and IP_3. [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 (IP_3) Triggers Release of Ca^{2+} from the Endoplasmic Reticulum

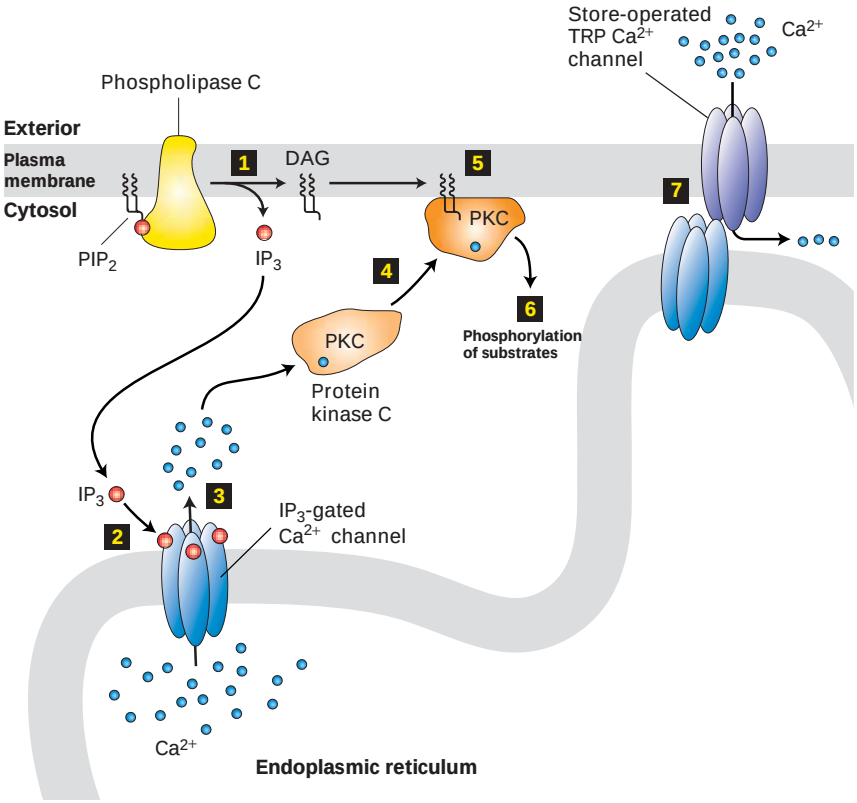
Most intracellular Ca^{2+} ions are sequestered in the mitochondria and in the lumen of the endoplasmic reticulum (ER) and other vesicles. Cells employ various mechanisms for regulating the concentration of Ca^{2+} ions in the cytosol, which usually is kept below 0.2 μM. For instance, Ca^{2+} ATPases pump cytosolic Ca^{2+} ions across the plasma membrane to the cell exterior or into the lumens of intracellular Ca^{2+}-storing compartments (see Figure 7-7). As we discuss below, a small rise in cytosolic Ca^{2+} induces a variety of cellular responses, and thus the cytosolic concentration of Ca^{2+} is carefully controlled.

Binding of many hormones to their cell-surface receptors on liver, fat, and other cells induces an elevation in cytosolic Ca^{2+} even when Ca^{2+} ions are absent from the surrounding extracellular fluid. In this situation, Ca^{2+} is released into the cytosol from the ER lumen through operation of the *IP_3-gated Ca^{2+} channel* in the ER membrane. This large protein is composed of four identical subunits, each containing an IP_3-binding site in the N-terminal cytosolic domain. IP_3 binding induces opening of the channel, allowing Ca^{2+} 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 IP_3 causes release of Ca^{2+} ions from the vesicles. This simple experiment demonstrates the specificity of the IP_3 effect.

The IP_3-mediated rise in the cytosolic Ca^{2+} level is only transient because Ca^{2+} ATPases located in the plasma membrane and ER membrane actively pump Ca^{2+} from the cytosol to the cell exterior and ER lumen, respectively. Furthermore, within a second of its generation, one specific phosphate on IP_3 is hydrolyzed, yielding inositol 1,4-bisphosphate, which does not stimulate Ca^{2+} release from the ER.

Without some means for replenishing depleted stores of intracellular Ca^{2+}, a cell would soon be unable to increase the cytosolic Ca^{2+} level in response to hormone-induced IP_3. Patch-clamping studies have revealed that a plasma-membrane Ca^{2+} channel, called the TRP channel or the store-operated channel, opens in response to depletion of ER Ca^{2+} stores (see Figure 13-29). In a way that is not understood, depletion of Ca^{2+} in the ER lumen leads to a conformational change in the IP_3-gated Ca^{2+} channel that allows it to bind to the TRP Ca^{2+} channel in the plasma membrane, causing the latter to open. Indeed, expression in cells of a specific fragment of the ER membrane IP_3-gated Ca^{2+} channel prevents opening of the TRP channel upon depletion of ER Ca^{2+} stores, implicating an interaction between the two Ca^{2+} channels in opening the TRP channel.

Opening of IP_3-gated Ca^{2+} channels is potentiated by cytosolic Ca^{2+} ions, which increase the affinity of these channel receptors for IP_3, resulting in greater release of stored Ca^{2+}. Higher concentrations of cytosolic Ca^{2+}, however, inhibit IP_3-induced release of Ca^{2+} from intracellular stores by decreasing the affinity of the receptor for IP_3. This complex regulation of IP_3-gated Ca^{2+} channels in ER membranes by cytosolic Ca^{2+} can lead to rapid oscillations in the cytosolic Ca^{2+} level when the IP_3 pathway in cells is stimu-



◀ FIGURE 13-29 **IP_3/DAG pathway and the elevation of cytosolic Ca^{2+}.** 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 PIP_2 by phospholipase C yields IP_3 and DAG (step 1). After diffusing through the cytosol, IP_3 interacts with and opens Ca^{2+} channels in the membrane of the endoplasmic reticulum (step 2), causing release of stored Ca^{2+} ions into the cytosol (step 3). One of various cellular responses induced by a rise in cytosolic Ca^{2+} 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 Ca^{2+} stores are depleted, the IP_3-gated Ca^{2+} channels bind to and open store-operated TRP Ca^{2+} channels in the plasma membrane, allowing influx of extracellular Ca^{2+} (step 7). [Adapted from J. W. Putney, 1999, *Proc. Nat'l. Acad. Sci. USA* **96**:14669.]

MEDIA CONNECTIONS
Focus Animation: Second Messengers in Signaling Pathways

lated. For example, stimulation of hormone-secreting cells in the pituitary by luteinizing hormone–releasing hormone (LHRH) causes rapid, repeated spikes in the cytosolic Ca^{2+} level; each spike is associated with a burst in secretion of luteinizing hormone (LH). The purpose of the fluctuations of Ca^{2+}, rather than a sustained rise in cytosolic Ca^{2+}, is not understood. One possibility is that a sustained rise in Ca^{2+} may be toxic to cells.

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

After its formation by hydrolysis of PIP_2 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 Ca^{2+} 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 Ca^{2+} ions and DAG, suggesting an interaction between the two branches of the IP_3/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.

Ca^{2+}/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 IP_3-mediated increase in the cytosolic level of free Ca^{2+}. Such localized increases in cytosolic Ca^{2+} 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 IP_3-mediated rise in Ca^{2+} 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 Ca^{2+} 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 Ca^{2+}, although the increase in Ca^{2+} 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 Ca^{2+}

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.

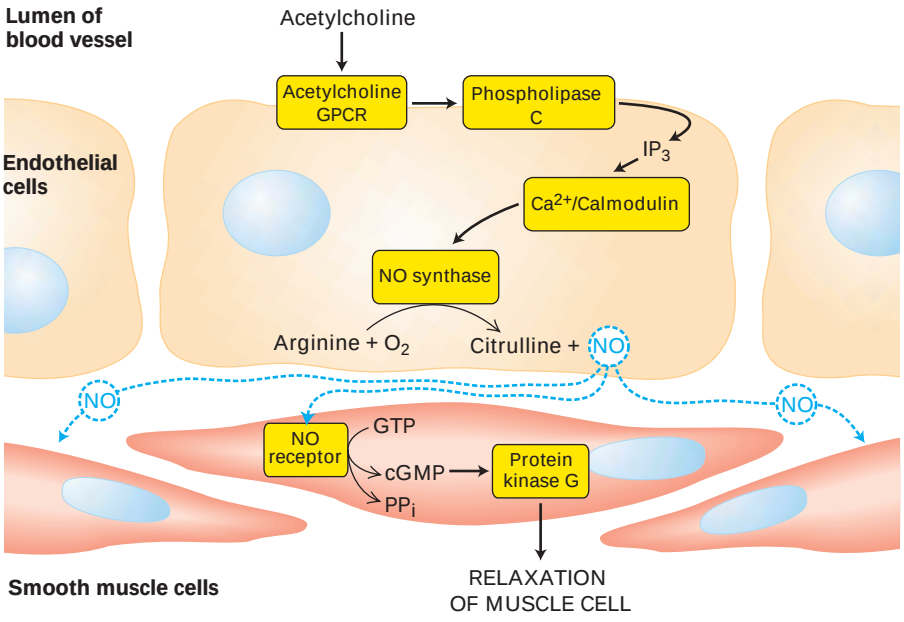
The Ca^{2+}/calmodulin complex also plays a key role in controlling the diameter of blood vessels and thus their ability to deliver oxygen to tissues. This pathway involves a novel signaling molecule and provides another example of cGMP functioning as a second messenger.

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

- Simulation 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).

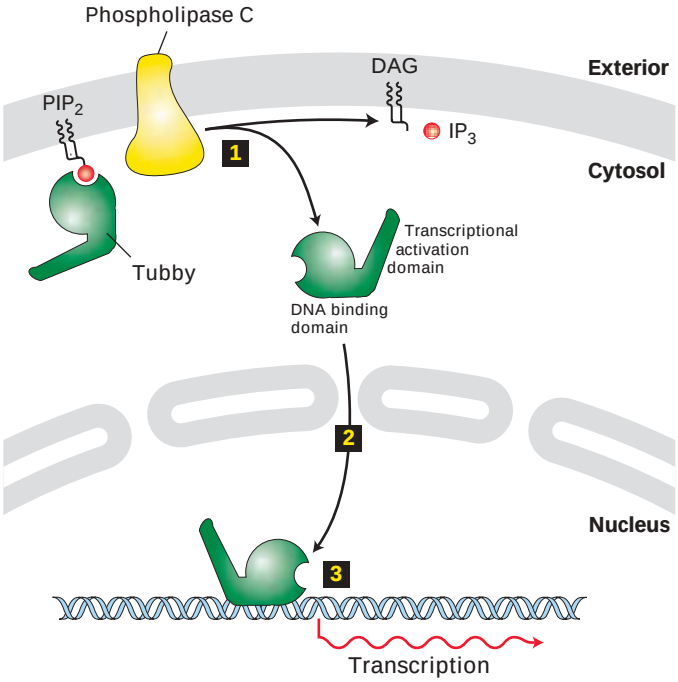
13.6 Activation of Gene Transcription by G Protein–Coupled Receptors

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.

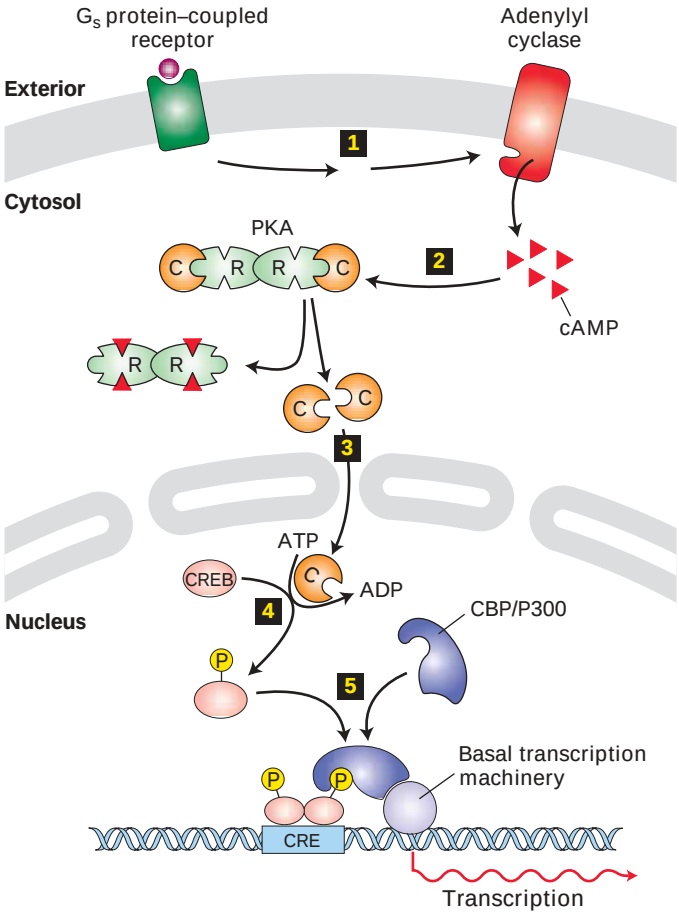
Membrane-Localized Tubby Transcription Factor Is Released by Activation of Phospholipase C

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_o- 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 PIP_2 in the plasma membrane. Receptor stimulation (not shown) leads to activation of phospholipase C, hydrolysis of PIP_2, 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 IP_3 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.]

MEDIA CONNECTIONS
Overview Animation: Extracellular Signaling

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

for controlling cell proliferation, as these examples show, but it also causes some cells to differentiate along specific pathways, as discussed in Chapter 15.

KEY CONCEPTS OF SECTION 14.1

TGFβ Receptors and the Direct Activation of Smads

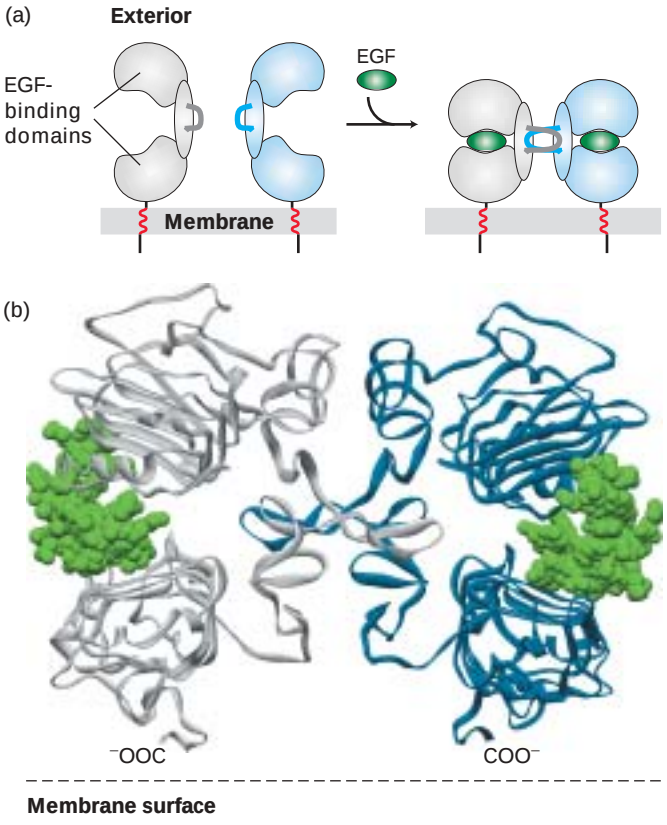
- TGFβ is produced as an inactive precursor that is stored in the extracellular matrix. Several mechanisms can release the active, mature dimeric growth factor (see Figure 14-1).
- Stimulation by TGFβ leads to activation of the intrinsic serine/threonine kinase activity in the cytosolic domain of the type I (RI) receptor, which then phosphorylates an R-Smad, exposing a nuclear-localization signal.
- After phosphorylated R-Smad binds a co-Smad, the resulting complex translocates into the nucleus, where it interacts with various transcription factors to induce expression of target genes (see Figure 14-2).
- Oncoproteins (e.g., Ski and SnoN) and I-Smads (e.g., Smad7) act as negative regulators of TGFβ signaling.
- TGFβ signaling generally inhibits cell proliferation. Loss of various components of the signaling pathway contributes to abnormal cell proliferation and malignancy.

14.2 Cytokine Receptors and the JAK-STAT Pathway

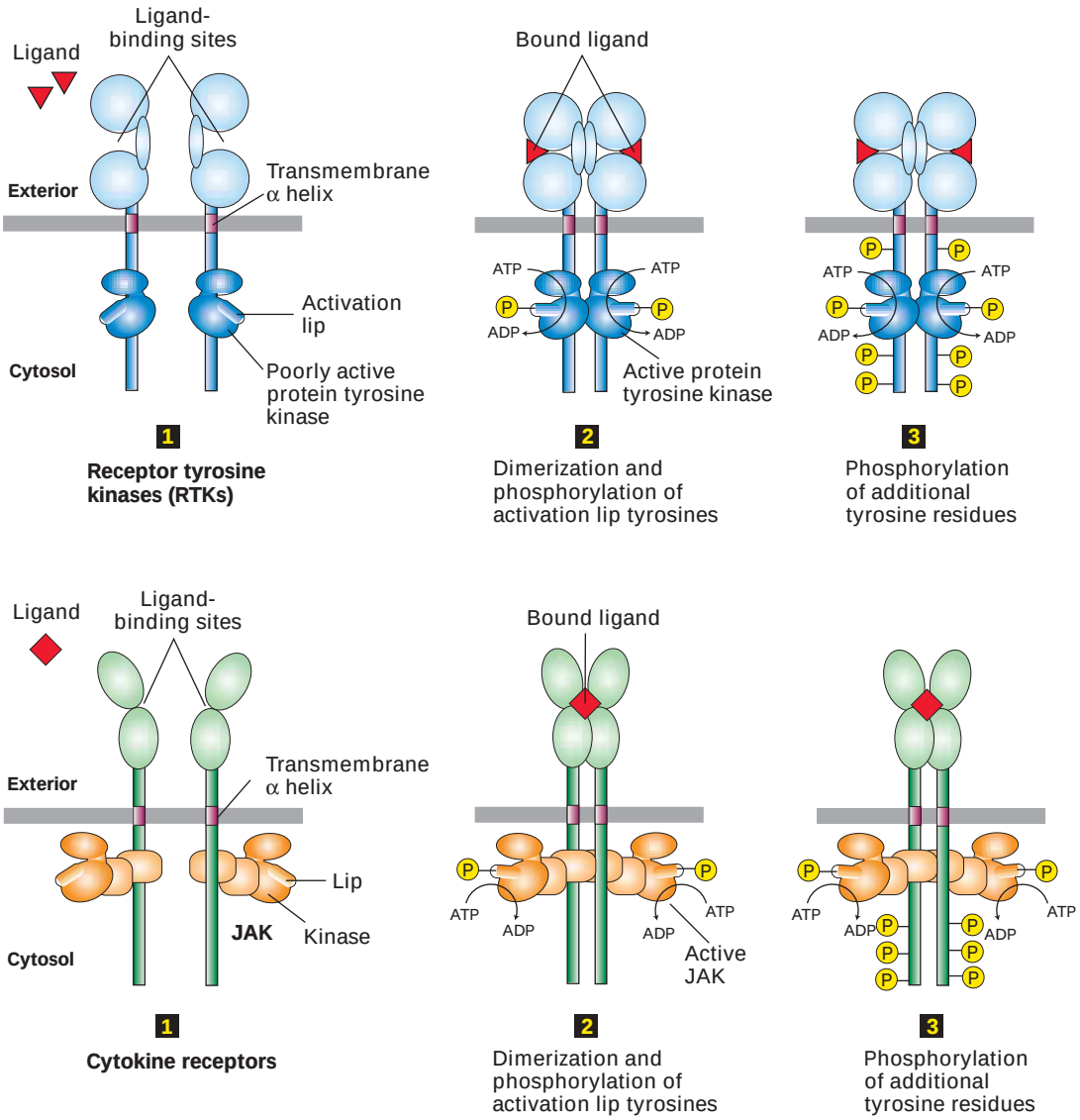
We turn now to a second important class of cell-surface receptors, the **cytokine receptors,** whose cytosolic domains are closely associated with a member of a family of cytosolic protein tyrosine kinases, the *JAK kinases*. A third class of receptors, the **receptor tyrosine kinases (RTKs),** contain intrinsic protein tyrosine kinase activity in their cytosolic domains. The mechanisms by which cytokine receptors and receptor tyrosine kinases become activated by ligands are very similar, and there is considerable overlap in the intracellular signal-transduction pathways triggered by activation of receptors in both classes. In this section, we first describe some similarities in signaling from these two receptor classes. We then discuss the JAK-STAT pathway, which is initiated mainly by activation of cytokine receptors.

Cytokine Receptors and Receptor Tyrosine Kinases Share Many Signaling Features

Ligand binding to both cytokine receptors and RTKs triggers formation of functional dimeric receptors. In some cases, the ligand induces association of two monomeric receptor subunits diffusing in the plane of the plasma membrane (Figure 14-4). In others, the receptor is a dimer in the absence of ligand, and ligand binding alters the conformation of the extracellular domains of the two subunits. In either case, formation of a functional dimeric receptor causes one of the poorly active cytosolic kinases to phosphorylate a particular tyrosine residue in the *activation lip* of the second kinase. This phosphorylation activates kinase activity and leads to phosphorylation of the second kinase in the dimer, as well as several tyrosine



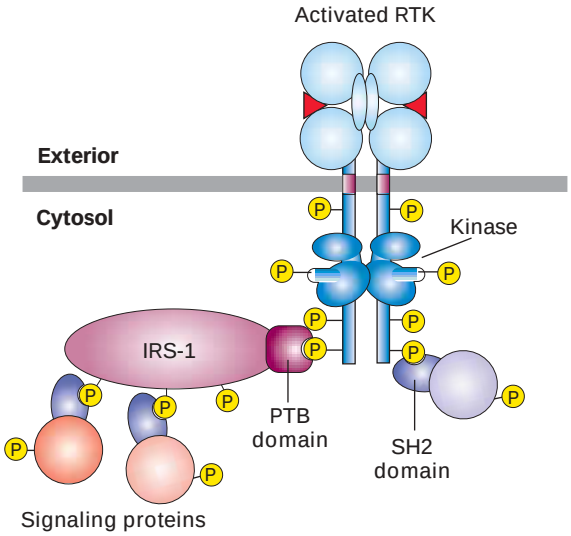
▲ FIGURE 14-4 **Dimerization of the receptor for epidermal growth factor (EGF), a receptor tyrosine kinase.** (a) Schematic depiction of the extracellular and transmembrane domains of the EGF receptor. Binding of one EGF molecule to a monomeric receptor causes an alteration in the structure of a loop located between the two EGF-binding domains. Dimerization of two identical ligand-bound receptor monomers in the plane of the membrane occurs primarily through interactions between the two "activated" loop segments. (b) Structure of the dimeric EGF receptor's extracellular domain bound to transforming growth factor α (TGFα), a homolog of EGF. The EGF receptor extracellular domains are shown in white *(left)* and blue *(right).* The two smaller TGFα molecules are colored green. Note the interaction between the "activated" loop segments in the two receptors. [Part (a) adapted from J. Schlessinger, 2002, *Cell* **110**:669; part (b) from T. Garrett et al., 2002, *Cell* **110**:763.]



▲ FIGURE 14-5 **General structure and ligand-induced activation of receptor tyrosine kinases (RTKs) and cytokine receptors.** The cytosolic domain of RTKs contains a protein tyrosine kinase catalytic site, whereas the cytosolic domain of cytokine receptors associates with a separate JAK kinase (step 1). In both types of receptor, ligand binding causes a conformational change that promotes formation of a functional dimeric receptor, bringing together two intrinsic or associated kinases, which then phosphorylate each other on a tyrosine residue in the activation lip (step 2). Phosphorylation causes the lip to move out of the kinase catalytic site, thus allowing ATP or a protein substrate to bind. The activated kinase then phosphorylates other tyrosine residues in the receptor's cytosolic domain (step 3). The resulting phosphotyrosines function as docking sites for various signal-transduction proteins (see Figure 14-6).

residues in the cytosolic domain of the receptor (Figure 14-5). As we see later, phosphorylation of residues in the activation loop is a general mechanism by which many kinases are activated.

Certain phosphotyrosine residues formed in activated cytokine receptors and RTKs serve as binding, or "docking," sites for *SH2 domains* or *PTB domains*, which are present in a large array of intracellular signal-transduction proteins. Once they are bound to an activated receptor, some signal-transduction proteins are phosphorylated by the receptor's intrinsic or associated kinase to achieve their active form. Binding of other signal-transduction proteins, present in the cytosol in unstimulated cells, positions them near their substrates localized in the plasma membrane. Both mechanisms can trigger downstream signaling. Several cytokine receptors (e.g., the IL-4 receptor) and RTKs (e.g., the insulin receptor)

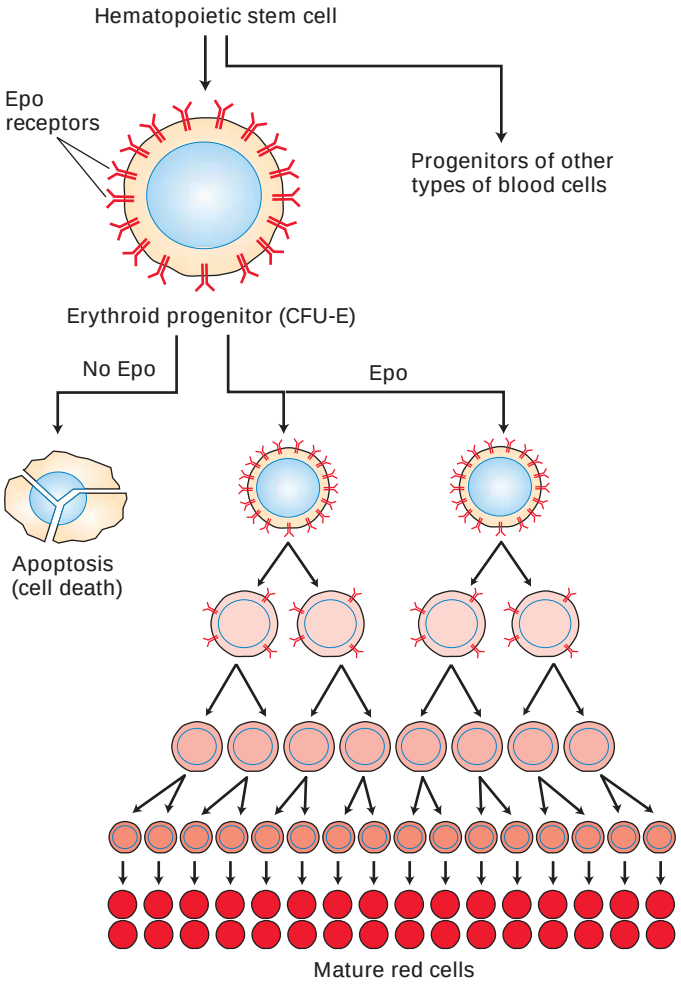


▲ FIGURE 14-6 **Recruitment of signal-transduction proteins to the cell membrane by binding to phosphotyrosine residues in activated receptors.** Cytosolic proteins with SH2 (purple) or PTB (maroon) domains can bind to specific phosphotyrosine residues in activated RTKs (shown here) or cytokine receptors. In some cases, these signal-transduction proteins then are phosphorylated by the receptor's intrinsic or associated protein tyrosine kinase, enhancing their activity. Certain RTKs and cytokine receptors utilize multidocking proteins such as IRS-1 to increase the number of signaling proteins that are recruited and activated. Subsequent phosphorylation of the IRS-1 by receptor kinase activity creates additional docking sites for SH2-containing signaling proteins.

bind IRS1 or other multidocking proteins via a PTB domain in the docking protein (Figure 14-6).The activated receptor then phosphorylates the bound docking protein, forming many phosphotyrosines that in turn serve as docking sites for SH2-containing signaling proteins. Some of these proteins in turn may also be phosphorylated by the activated receptor.

Cytokines Influence Development of Many Cell Types

The **cytokines** form a family of relatively small, secreted proteins (generally containing about 160 amino acids) that control many aspects of growth and differentiation of specific types of cells. During pregnancy prolactin, for example, induces epithelial cells lining the immature ductules of the mammary gland to differentiate into the acinar cells that produce milk proteins and secrete them into the ducts. Another cytokine, interleukin 2 (IL-2), is essential for proliferation and functioning of the T cells of the immune system; its close relative IL-4 is essential for formation of functional antibody-producing B cells. Some cytokines, such as interferon α, are produced and secreted by many types of cells fol-



▶ FIGURE 14-7 **Role of erythropoietin in formation of red blood cells (erythrocytes).** Erythroid progenitor cells, called *colony-forming units erythroid (CFU-E)*, are derived from hematopoietic stem cells, which also give rise to progenitors of other blood cell types. In the absence of erythropoietin (Epo), CFU-E cells undergo apoptosis. Binding of erythropoietin to its receptors on a CFU-E induces transcription of several genes whose encoded proteins prevent programmed cell death (apoptosis), allowing the cell to survive and undergo a program of three to five terminal cell divisions. Epo stimulation also induces expression of erythrocyte-specific proteins such as the globins, which form hemoglobin, and the membrane proteins glycophorin and anion-exchange protein. The Epo receptor and other membrane proteins are lost from these cells as they undergo differentiation. If CFU-E cells are cultured with erythropoietin in a semisolid medium (e.g., containing methylcellulose), daughter cells cannot move away, and thus each CFU-E produces a colony of 30–100 erythroid cells, hence its name.
[See M. Socolovsky et al., 2001, *Blood* **98**:3261]

lowing virus infection. The secreted interferons act on nearby cells to induce enzymes that render these cells more resistant to virus infection.

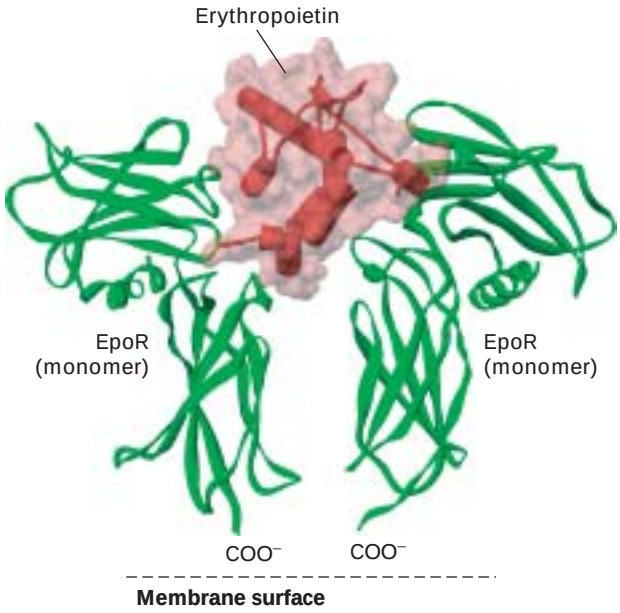
MEDICINE

Many cytokines induce formation of important types of blood cells. For instance, granulocyte colony stimulating factor (G-CSF) induces a particular type of progenitor cell in the bone marrow to divide several times and then differentiate into granulocytes, the type of white blood cell that inactivates bacteria and other pathogens. Because many cancer therapies reduce granulocyte formation by the body, G-CSF often is administered to patients to stimulate proliferation and differentiation of granulocyte progenitor cells, thus restoring the normal level of granulocytes in the blood. Thrombopoietin, a "cousin" of G-CSF, similarly acts on megakaryocyte progenitors to divide and differentiate into megakaryocytes. These then fragment into the cell pieces called *platelets,* which are critical for blood clotting. ▮

Another related cytokine, **erythropoietin (Epo),** triggers production of red blood cells by inducing the proliferation and differentiation of erythroid progenitor cells in the bone marrow (Figure 14-7). Erythropoietin is synthesized by kidney cells that monitor the concentration of oxygen in the blood. A drop in blood oxygen signifies a lower than optimal level of erythrocytes (red blood cells), whose major function is to transport oxygen complexed to hemoglobin. By means of the oxygen-sensitive transcription factor HIF-1α, the kidney cells respond to low oxygen by synthesizing more erythropoietin and secreting it into the blood (see Figure 15-9). As the level of erythropoietin rises, more and more erythroid progenitors are saved from death, allowing each to produce ≈50 or so red blood cells in a period of only two days. In this way, the body can respond to the loss of blood by accelerating the production of red blood cells.

All Cytokines and Their Receptors Have Similar Structures and Activate Similar Signaling Pathways

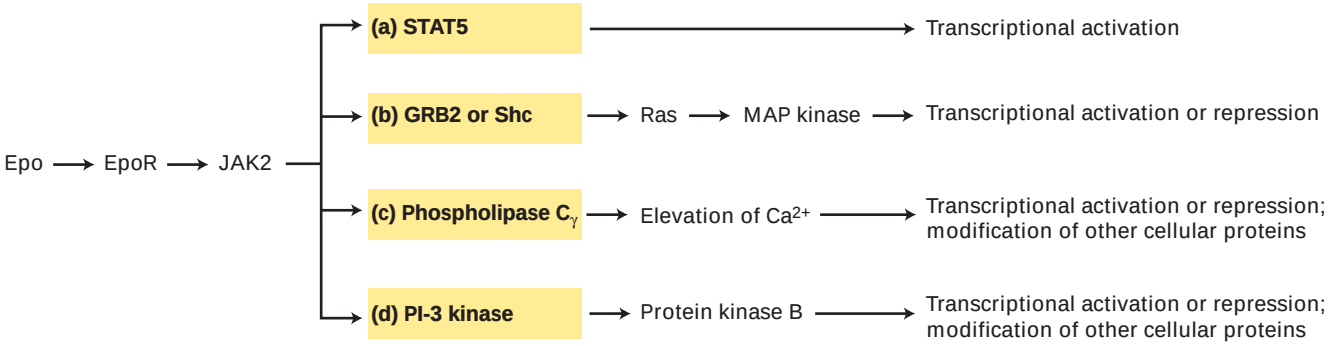
Strikingly, all cytokines have a similar tertiary structure, consisting of four long conserved α helices folded together in a specific orientation. Similarly, the structures of all cytokine receptors are quite similar, with their extracellular domains constructed of two subdomains, each of which contains seven conserved β strands folded together in a characteristic fashion. The interaction of erythropoietin with the dimeric erythropoietin receptor (EpoR), depicted in Figure 14-8, exemplifies the binding of a cytokine to its receptor. The structural homology among cytokines is evidence that they all evolved from a common ancestral protein. Likewise, the various receptors undoubtedly evolved from a single common ancestor.



▲ FIGURE 14-8 **Structure of erythropoietin bound to the extracellular domains of a dimeric erythropoietin receptor (EpoR).** Erythropoietin contains four conserved long α helices that are folded in a particular arrangement. The extracellular domain of an EpoR monomer is constructed of two subdomains, each of which contains seven conserved β strands folded in a characteristic fashion. Side chains of residues on two of the helices in erythropoietin contact loops on one EpoR monomer, while residues on the two other Epo helices bind to the same loop segments in a second receptor monomer, thereby stabilizing the dimeric receptor. The structures of other cytokines and their receptors are similar to erythropoietin and EpoR. [Adapted from R. S. Syed et al., 1998, *Nature* **395**:511.]

Whether or not a cell responds to a particular cytokine depends simply on whether or not it expresses the corresponding (cognate) receptor. Although all cytokine receptors activate similar intracellular signaling pathways, the response of any particular cell to a cytokine signal depends on the cell's constellation of transcription factors, chromatin structures, and other proteins relating to the developmental history of the cell. If receptors for prolactin or thrombopoietin, for example, are expressed experimentally in an erythroid progenitor cell, the cell will respond to these cytokines by dividing and differentiating into red blood cells, not into mammary cells or megakaryocytes.

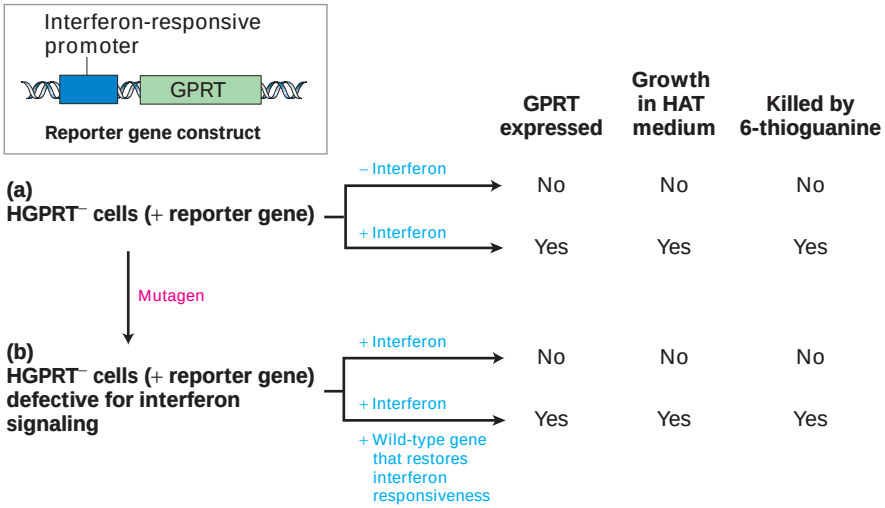
Figure 14-9 summarizes the intracellular signaling pathways activated when the EpoR binds erythropoietin. Stimulation of other cytokine receptors by their specific ligands activates similar pathways. All these pathways eventually lead to activation of transcription factors, causing an increase or decrease in expression of particular target genes. Here we focus on the *JAK-STAT pathway;* the other pathways are discussed in later sections.



▲ FIGURE 14-9 **Overview of signal-transduction pathways triggered by ligand binding to the erythropoietin receptor (EpoR), a typical cytokine receptor.** Four major pathways can transduce a signal from the activated, phosphorylated EpoR-JAK complex (see Figure 14-5, *bottom*). Each pathway ultimately regulates transcription of different sets of genes. (a) In the most direct pathway, the transcription factor STAT5 is phosphorylated and activated directly in the cytosol. (b) Binding of linker proteins (GRB2 or Shc) to an activated EpoR leads to activation of the Ras–MAP kinase pathway. (c, d) Two phosphoinositide pathways are triggered by recruitment of phospholipase C_γ and PI-3 kinase to the membrane following activation of EpoR. Elevated levels of Ca^{2+} and activated protein kinase B also modulate the activity of cytosolic proteins that are not involved in control of transcription.

Somatic Cell Genetics Revealed JAKs and STATs as Essential Signal-Transduction Proteins

Soon after the discovery and cloning of cytokines, most of their receptors were isolated by expression cloning or other strategies. Elucidation of the essential components of their intracellular signaling pathways, however, awaited development of new types of genetic approaches using cultured mammalian cells. In these studies, a bacterial **reporter gene** encoding guanine phosphoribosyl transferase (GPRT) was linked to an upstream interferon-responsive promoter. The resulting construct was introduced into cultured mammalian cells that were genetically deficient in the human homolog HGPRT. GPRT or HGPRT is necessary for incorporation of purines



▲ EXPERIMENTAL FIGURE 14-10 **Mutagenized cells carrying an interferon-responsive reporter gene were used to identify JAKs and STATs as essential signal-transduction proteins.** A reporter gene was constructed consisting of an interferon-responsive promoter upstream of the bacterial gene encoding GPRT, a key enzyme in the purine salvage pathway (see Figure 6-39). (a) Introduction of this construct into mammalian cells lacking the mammalian homolog HGPRT yielded reporter cells that grew in HAT medium and were killed by 6-thioguanine in the presence but not the absence of interferon. (b) Following treatment of reporter cells with a mutagen, cells with defects in the signaling pathway initiated by interferon do not induce GPRT in response to interferon and thus cannot incorporate the toxic purine 6-thioguanine. Restoration of interferon responsiveness by functional complementation with wild-type DNA clones identified genes encoding JAKs and STATs. See the text for details. [See R. McKendry et al., 1991, *Proc. Nat'l. Acad. Sci. USA* **88**:11455; D. Watling et al., 1993, *Nature* **366**:166; and G. Stark and A. Gudkov, 1999, *Human Mol. Genet.* **8**:1925.]

in the culture medium into ribonucleotides and then into DNA or RNA. As shown in Figure 14-10a, HGPRT-negative cells carrying the reporter gene responded to interferon treatment by expressing GPRT and thus acquiring the ability to grow in HAT medium. This medium does not allow growth of cells lacking GPRT or HGPRT, since synthesis of purines by the cells is blocked by aminopterin (the A in HAT), and thus DNA synthesis is dependent on incorporation of purines from the culture medium (see Figure 6-39). Simultaneously the cells acquired sensitivity to killing by the purine analog 6-thioguanine, which is converted into the corresponding ribonucleotide by GPRT; incorporation of this purine into DNA in place of guanosine eventually causes cell death.

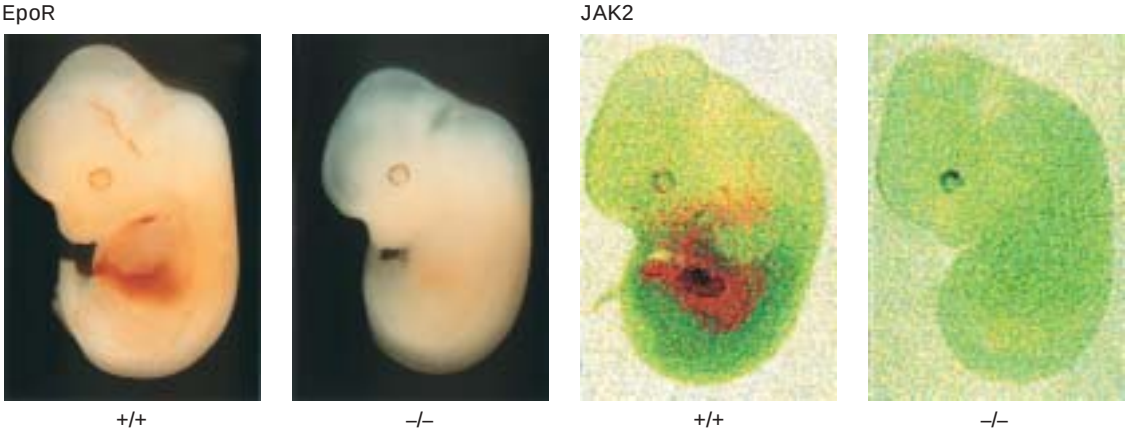
The reporter cells were then heavily treated with mutagens in an attempt to inactivate both alleles of the genes encoding critical signal-transduction proteins in the interferon signaling pathway. Researchers looked for mutant cells that expressed the interferon receptor (as evidenced by the cell's ability to bind radioactive interferon) but did not express GPRT in response to interferon and thus survived killing by 6-thioguanine when cells were cultured in the presence of interferon (Figure 14-10b). After many such interferon-nonresponding mutant cell lines were obtained, they were used to screen a genomic or cDNA library for the wild-type genes that complemented the mutated genes in nonresponding cells, a technique called **functional complementation** (see Figure 9-20). In this case, mutant cells expressing the corresponding recombinant wild-type gene grew on HAT medium and were sensitive to killing by 6-thioguanine in the presence of interferon. That is, they acted like wild-type cells.

Cloning of the genes identified by this procedure led to recognition of two key signal-transduction proteins: a JAK tyrosine kinase and a STAT transcription factor. Subsequent work showed that one (sometimes two) of the four human JAK proteins and at least one of several STAT proteins are involved in signaling downstream from all cytokine receptors. To understand how JAK and STAT proteins function, we examine one of the best-understood cytokine signaling pathways, that downstream of the erythropoietin receptor.

Receptor-Associated JAK Kinases Activate STAT Transcription Factors Bound to a Cytokine Receptor

The JAK2 kinase is tightly bound to the cytosolic domain of the erythropoietin receptor (EpoR). Like the three other members of the JAK family of kinases, JAK2 contains an N-terminal receptor-binding domain, a C-terminal kinase domain that is normally poorly active catalytically, and a middle domain of unknown function. JAK2, erythropoietin, and the EpoR are all required for formation of adult-type erythrocytes, which normally begins at day 12 of embryonic development in mice. As Figure 14-11 shows, embryonic mice lacking functional genes encoding either the EpoR or JAK2 cannot form adult-type erythrocytes and eventually die owing to the inability to transport oxygen to the fetal organs.

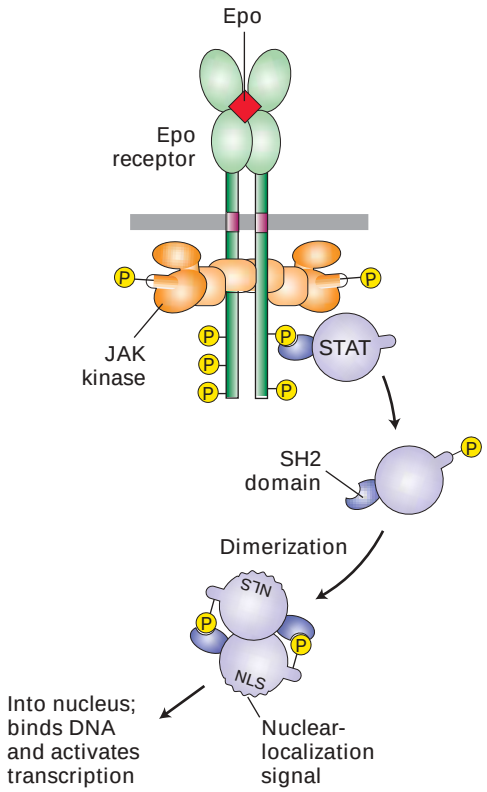
As already noted, erythropoietin binds simultaneously to the extracellular domains of two EpoR monomers on the cell surface (see Figure 14-8). As a result, the associated JAKs are brought close enough together that one can phosphorylate the other on a critical tyrosine in the activation lip. As with other kinases, phosphorylation of the activation lip leads to a conformational change that reduces the K_m for ATP or the substrate to be phosphorylated, thus increasing the kinase activity. One piece of evidence for this activation mechanism comes from study of a mutant JAK2 in which the critical tyrosine is mutated to phenylalanine. The mutant JAK2 binds normally to the EpoR but cannot be phosphorylated.



▲ EXPERIMENTAL FIGURE 14-11 **Studies with mutant mice reveal that both the erythropoietin receptor (EpoR) and JAK2 are essential for development of erythrocytes**. Mice in which both alleles of the EpoR or JAK2 gene are "knocked out" develop normally until embryonic day 13, at which time they begin to die of anemia due to the lack of erythrocyte-mediated transport of oxygen to the fetal organs. The red organ in the wild-type embryos (+/+) is the fetal liver, the major site of erythrocyte production at this developmental stage. The absence of color in the mutant embryos (−/−) indicates the absence of erythrocytes containing hemoglobin. Otherwise the mutant embryos appear normal, indicating that the main function of the EpoR and JAK2 in early mouse development is to support production of erythrocytes. [EpoR images from H. Wu et al., 1995, *Cell* **83**:59; JAK2 images from H. Neubauer et al., 1998, *Cell* **93**:307.]

Expression of this mutant JAK2 in erythroid cells in greater than normal amounts totally blocks EpoR signaling, as the mutant JAK2 blocks the function of the wild-type protein. This type of mutation, referred to as a *dominant negative*, causes loss of function even in cells that carry copies of the wild-type gene (Chapter 9).

Once the JAK kinases become activated, they phosphorylate several tyrosine residues on the cytosolic domain of the receptor. Certain of these phosphotyrosine residues then serve as binding sites for a group of transcription factors collectively termed *STATs*. All STAT proteins contain an N-terminal SH2 domain that binds to a phosphotyrosine in the receptor's cytosolic domain, a central DNA-binding domain, and a C-terminal domain with a critical tyrosine residue. Once a STAT is bound to the receptor, the C-terminal tyrosine is phosphorylated by an associated JAK kinase (Figure 14-12). This arrangement ensures that in a particular cell only those STAT proteins with an SH2 domain that can bind to a particular receptor protein will be activated. A phosphorylated STAT dissociates spontaneously from the receptor, and two phosphorylated STAT proteins form a dimer in which the SH2 domain on each binds to the phosphotyrosine in the other. Because dimerization exposes the nuclear-localization signal (NLS), STAT dimers move into the nucleus, where they bind to specific **enhancer** sequences controlling target genes.



▲ FIGURE 14-12 **JAK-STAT signaling pathway.** Following ligand binding to a cytokine receptor and activation of an associated JAK kinase, JAK phosphorylates several tyrosine residues on the receptor's cytosolic domain (see Figure 14-5, *bottom*). After an inactive monomeric STAT transcription factor binds to a phosphotyrosine in the receptor, it is phosphorylated by active JAK. Phosphorylated STATs spontaneously dissociate from the receptor and spontaneously dimerize. Because the STAT homodimer has two phosphotyrosine–SH2 domain interactions, whereas the receptor-STAT complex is stabilized by only one such interaction, phosphorylated STATs tend not to rebind to the receptor. The STAT dimer, which has two exposed nuclear-localization signals (NLS), moves into the nucleus, where it can bind to promoter sequences and activate transcription of target genes.

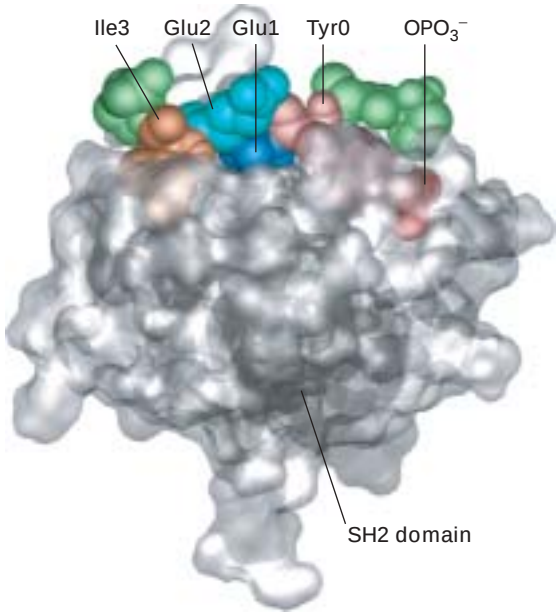
Different STATs activate different genes in different cells. In erythroid progenitors, for instance, stimulation by erythropoietin leads to activation of STAT5. The major protein induced by active STAT5 is Bcl-x_L, which prevents the programmed cell death, or **apoptosis,** of these progenitors, allowing them to proliferate and differentiate into erythroid cells (see Figure 14-7). Indeed, mice lacking STAT5 are highly anemic because many of the erythroid progenitors undergo apoptosis even in the presence of high erythropoietin levels. Such mutant mice produce *some* erythrocytes and thus survive, because the erythropoietin receptor is linked to other anti-apoptotic pathways that do not involve STAT proteins (see Figure 14-9).

SH2 and PTB Domains Bind to Specific Sequences Surrounding Phosphotyrosine Residues

As noted earlier, many intracellular signal-transduction proteins contain an SH2 or PTB domain by which they bind to an activated receptor or other component of a signaling pathway containing a phosphotyrosine residue (see Figure 14-6). The SH2 domain derived its full name, the *Src homology 2 domain,* from its homology with a region in the prototypical cytosolic tyrosine kinase encoded by the *src* gene. The three-dimensional structures of SH2 domains in different proteins are very similar, but each binds to a distinct sequence of amino acids surrounding a phosphotyrosine residue. The unique amino acid sequence of each SH2 domain determines the specific phosphotyrosine residues it binds.

The SH2 domain of the Src tyrosine kinase, for example, binds strongly to any peptide containing a critical four-residue core sequence: phosphotyrosine–glutamic acid–glutamic acid–isoleucine (Figure 14-13). These four amino acids make intimate contact with the peptide-binding site in the Src SH2 domain. Binding resembles the insertion of a two-pronged "plug"—the phosphotyrosine and isoleucine side chains of the peptide—into a two-pronged "socket" in the SH2 domain. The two glutamic acids fit snugly onto the surface of the SH2 domain between the phosphotyrosine socket and the hydrophobic socket that accepts the isoleucine residue.

Variations in the hydrophobic socket in the SH2 domains of different STATs and other signal-transduction proteins allow them to bind to phosphotyrosines adjacent to different sequences, accounting for differences in their binding partners.



▲ FIGURE 14-13 **Surface model of the SH2 domain from Src kinase bound to a phosphotyrosine-containing peptide.** The peptide bound by this SH2 domain (gray) is shown in spacefill. The phosphotyrosine (Tyr0 and OPO_3^-, orange) and isoleucine (Ile3, orange) residues fit into a two-pronged socket on the surface of the SH2 domain; the two glutamate residues (Glu1, dark blue; Glu2, light blue) are bound to sites on the surface of the SH2 domain between the two sockets. Nonbinding residues on the target peptide are colored green. [See G. Waksman et al., 1993, *Cell* **72**:779.]

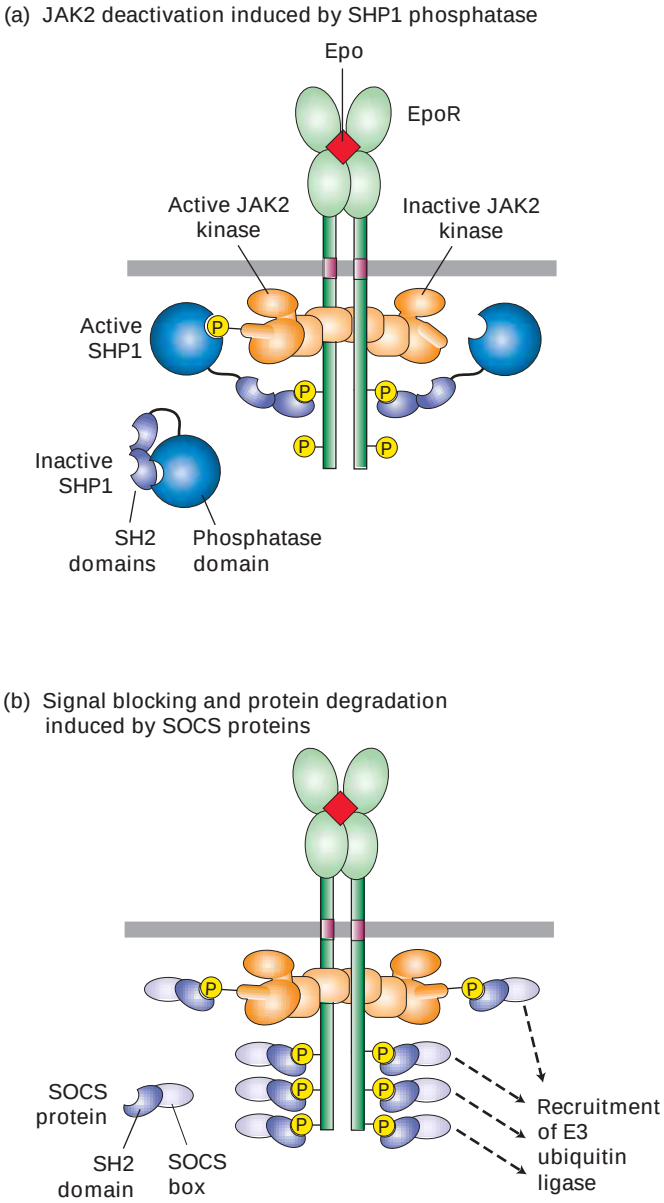
The binding specificity of SH2 domains is largely determined by residues C-terminal to the phosphotyrosine in a target peptide. In contrast, the binding specificity of PTB domains is determined by specific residues five to eight residues N-terminal to a phosphotyrosine residue. Sometimes a PTB domain binds to a target peptide even if the tyrosine is not phosphorylated.

Signaling from Cytokine Receptors Is Modulated by Negative Signals

Signal-induced transcription of target genes for too long a period can be as dangerous for the cell as too little induction. Thus cells must be able to turn off a signaling pathway quickly unless the extracellular signal remains continuously present. In various progenitor cells, two classes of proteins serve to dampen signaling from cytokine receptors, one over the short term (minutes) and the other over longer periods of time.

Short-Term Regulation by SHP1 Phosphatase Mutant mice lacking *SHP1 phosphatase* die because of excess production of erythrocytes and several other types of blood cells. Analysis of these mutant mice offered the first suggestion that SHP1, a phosphotyrosine **phosphatase,** negatively regulates signaling from several types of cytokine receptors in several types of progenitor cells.

How SHP1 dampens cytokine signaling is depicted in Figure 14-14a. In addition to a phosphatase catalytic domain, SHP1 has two SH2 domains. When cells are not stimulated



▲ FIGURE 14-14 **Two mechanisms for terminating signal transduction from the erythropoietin receptor (EpoR).** (a) SHP1, a protein tyrosine phosphatase, is present in an inactive form in unstimulated cells. Binding of an SH2 domain in SHP1 to a particular phosphotyrosine in the activated receptor unmasks its phosphatase catalytic site and positions it near the phosphorylated tyrosine in the lip region of JAK2. Removal of the phosphate from this tyrosine inactivates the JAK kinase. (b) SOCS proteins, whose expression is induced in erythropoietin-stimulated erythroid cells, inhibit or permanently terminate signaling over longer time periods. Binding of SOCS to phosphotyrosine residues on the EpoR or JAK2 blocks binding of other signaling proteins *(left).* The SOCS box can also target proteins such as JAK2 for degradation by the ubiquitin-proteasome pathway *(right).* Similar mechanisms regulate signaling from other cytokine receptors. [Part (a) adapted from S. Constantinescu et al., 1999, *Trends Endocrin. Metabol.* **10**:18; part (b) adapted from B. T. Kile and W. S. Alexander, 2001, *Cell. Mol. Life Sci.* **58**:1]

by a cytokine (are in the resting state), one of the SH2 domains physically binds to and inactivates the catalytic site in SHP1. In the stimulated state, however, this blocking SH2 domain binds to a specific phosphotyrosine residue in the activated receptor. The conformational change that accompanies this binding unmasks the SHP1 catalytic site and also brings it adjacent to the phosphotyrosine residue in the activation lip of the JAK associated with the receptor. By removing this phosphate, SHP1 inactivates the JAK, so that it can no longer phosphorylate the receptor or other substrates (e.g., STATs) unless additional cytokine molecules bind to cell-surface receptors, initiating a new round of signaling.

Long-Term Regulation by SOCS Proteins Among the genes whose transcription is induced by STAT proteins are those encoding a class of small proteins, termed *SOCS proteins*, that terminate signaling from cytokine receptors. These negative regulators, also known as *CIS proteins*, act in two ways (Figure 14-14b). First, the SH2 domain in several SOCS proteins binds to phosphotyrosines on an activated receptor, preventing binding of other SH2-containing signaling proteins (e.g., STATs) and thus inhibiting receptor signaling. One SOCS protein, SOCS-1, also binds to the critical phosphotyrosine in the activation lip of activated JAK2 kinase, thereby inhibiting its catalytic activity. Second, all SOCS proteins contain a domain, called the *SOCS box*, that recruits components of E3 ubiquitin ligases (see Figure 3-13). As a result of binding SOCS-1, for instance, JAK2 becomes polyubiquitinated and then degraded in **proteasomes,** thus permanently turning off all JAK2-mediated signaling pathways. The observation that proteasome inhibitors prolong JAK2 signal transduction supports this mechanism.

Studies with cultured mammalian cells have shown that the receptor for growth hormone, which belongs to the cytokine receptor superfamily, is down-regulated by another SOCS protein, SOCS-2. Strikingly, mice deficient in this SOCS protein grow significantly larger than their wild-type counterparts and have long bone lengths and proportionate enlargement of most organs. Thus SOCS proteins play an essential negative role in regulating intracellular signaling from the receptors for erythropoietin, growth hormone, and other cytokines.

Mutant Erythropoietin Receptor That Cannot Be Down-Regulated Leads to Increased Hematocrit

In normal adult men and women, the percentage of erythrocytes in the blood (the *hematocrit*) is maintained very close to 45–47 percent. A drop in the hematocrit results in increased production of erythropoietin by the kidney. The elevated erythropoietin level causes more erythroid progenitors to undergo terminal proliferation and differentiation into mature erythrocytes, soon restoring the hematocrit to its normal level. In endurance sports, such as cross-country skiing, where oxygen transport to the muscles may become limiting, an excess of red blood cells may confer a competitive advantage. For this reason, use of supplemental erythropoietin to increase the hematocrit above the normal level is banned in many athletic competitions, and athletes are regularly tested for the presence of commercial recombinant erythropoietin in their blood and urine.

Supplemental erythropoietin not only confers a possible competitive advantage but also can be dangerous. Too many red cells can cause the blood to become sluggish and clot in small blood vessels, especially in the brain. Several athletes who doped themselves with erythropoietin have died of a stroke while exercising.

Discovery of a mutant, unregulated erythropoietin receptor (EpoR) explained a suspicious situation in which a winner of three gold medals in Olympic cross-country skiing was found to have a hematocrit above 60 percent. Testing for erythropoietin in his blood and urine, however, revealed lower-than-normal amounts. Subsequent DNA analysis showed that the athlete was heterozygous for a mutation in the gene encoding the erythropoietin receptor. The mutant allele encoded a truncated receptor missing several of the tyrosines that normally become phosphorylated after stimulation by erythropoietin. As a consequence, the mutant receptor was able to activate STAT5 and other signaling proteins normally, but was unable to bind the negatively acting SHP1 phosphatase, which usually terminates signaling (see Figure 14-14a). Thus the very low level of erythropoietin produced by this athlete induced prolonged intracellular signaling in his erythroid progenitor cells, resulting in production of higher-than-normal numbers of erythrocytes. This example vividly illustrates the fine level of control over signaling from the erythropoietin receptor in the human body. ▮

KEY CONCEPTS OF SECTION 14.2

Cytokine Receptors and the JAK-STAT Pathway

- Two receptor classes, cytokine receptors and receptor tyrosine kinases, transduce signals via their associated or intrinsic protein tyrosine kinases. Ligand binding triggers formation of functional dimeric receptors and phosphorylation of the activation lip in the kinases, enhancing their catalytic activity (see Figure 14-5).
- All cytokines are constructed of four α helices that are folded in a characteristic arrangement.
- Erythropoietin, a cytokine secreted by kidney cells, prevents apoptosis and promotes proliferation and differentiation of erythroid progenitor cells in the bone marrow. An excess of erythropoietin or mutations in its receptor that prevent down-regulation result in production of elevated numbers of red blood cells.
- All cytokine receptors are closely associated with a JAK protein tyrosine kinase, which can activate several down-

stream signaling pathways leading to changes in transcription of target genes or in the activity of proteins that do not regulate transcription (see Figure 14-9).

- The JAK-STAT pathway operates downstream of all cytokine receptors. STAT monomers bound to receptors are phosphorylated by receptor-associated JAKs, then dimerize and move to the nucleus, where they activate transcription (see Figure 14-12).
- Short peptide sequences containing phosphotyrosine residues are bound by SH2 and PTB domains, which are found in many signal-transducing proteins. Such protein-protein interactions are important in many signaling pathways.
- Signaling from cytokine receptors is terminated by the phosphotyrosine phosphatase SHP1 and several SOCS proteins (see Figure 14-14).

14.3 Receptor Tyrosine Kinases and Activation of Ras

We return now to the receptor tyrosine kinases (RTKs), which have intrinsic protein tyrosine kinase activity in their cytosolic domains. The ligands for RTKs are soluble or membrane-bound peptide or protein hormones including nerve growth factor (NGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin. Ligand-induced activation of an RTK stimulates its tyrosine kinase activity, which subsequently stimulates the *Ras–MAP kinase pathway* and several other signal-transduction pathways. RTK signaling pathways have a wide spectrum of functions including regulation of cell proliferation and differentiation, promotion of cell survival, and modulation of cellular metabolism.

Some RTKs have been identified in studies on human cancers associated with mutant forms of growth-factor receptors, which send a proliferative signal to cells even in the absence of growth factor. For example, a constitutively active mutant form of Her2, a receptor for EGF-like proteins, enables uncontrolled proliferation of cancer cells even in the absence of EGF, which is required for proliferation of normal cells (see Figure 23-14). Alternatively, overproduction of the wild-type receptor for EGF in certain human breast cancers results in proliferation at low EGF levels that do not stimulate normal cells; monoclonal antibodies targeted to the EGF receptor have proved therapeutically useful in these patients. Other RTKs have been uncovered during analysis of developmental mutations that lead to blocks in differentiation of certain cell types in *C. elegans*, *Drosophila*, and the mouse. ▮

Here we discuss how ligand binding leads to activation of RTKs and how activated receptors transmit a signal to the **Ras protein,** the GTPase switch protein that functions in transducing signals from many different RTKs. The transduction of signals downstream from Ras to a common cascade of serine/threonine kinases, leading ultimately to activation of MAP kinase and certain transcription factors, is covered in the following section.

Ligand Binding Leads to Transphosphorylation of Receptor Tyrosine Kinases

All RTKs constitute an extracellular domain containing a ligand-binding site, a single hydrophobic transmembrane α helix, and a cytosolic domain that includes a region with protein tyrosine kinase activity. Most RTKs are monomeric, and ligand binding to the extracellular domain induces formation of receptor dimers, as depicted in Figure 14-4 for the EGF receptor. Some monomeric ligands, including FGF, bind tightly to heparan sulfate, a negatively charged polysaccharide component of the extracellular matrix (Chapter 6); this association enhances ligand binding to the monomeric receptor and formation of a dimeric receptor-ligand complex (Figure 14-15). The ligands for some RTKs are dimeric; their binding brings two receptor monomers together directly. Yet other RTKs, such as the insulin receptor, form disulfide-linked dimers in the absence of hormone; binding of ligand to this type of RTK alters its conformation in such a way that the receptor becomes activated.

Regardless of the mechanism by which ligand binds and locks an RTK into a functional dimeric state, the next step is universal. In the resting, unstimulated state, the intrinsic kinase activity of an RTK is very low. In the dimeric receptor, however, the kinase in one subunit can phosphorylate one or more tyrosine residues in the activation lip near the catalytic site in the other subunit. This leads to a conformational change that facilitates binding of ATP in some receptors (e.g., insulin receptor) and binding of protein substrates in other receptors (e.g., FGF receptor). The resulting enhanced kinase activity then phosphorylates other sites in the cytosolic domain of the receptor. This ligand-induced activation of RTK kinase activity is analogous to the activation of the JAK kinases associated with cytokine receptors (see Figure 14-5). The difference resides in the location of the kinase catalytic site, which is within the cytosolic domain of RTKs, but within a separate JAK kinase in the case of cytokine receptors.

As in signaling by cytokine receptors, phosphotyrosine residues in activated RTKs serve as docking sites for proteins involved in downstream signal transduction. Many phosphotyrosine residues in activated RTKs interact with *adapter proteins*, small proteins that contain SH2, PTB, or SH3 domains but have no intrinsic enzymatic or signaling activities (see Figure 14-6). These proteins couple activated RTKs to other components of signal-transduction pathways such as the one involving Ras activation.