

# Signal Transduction

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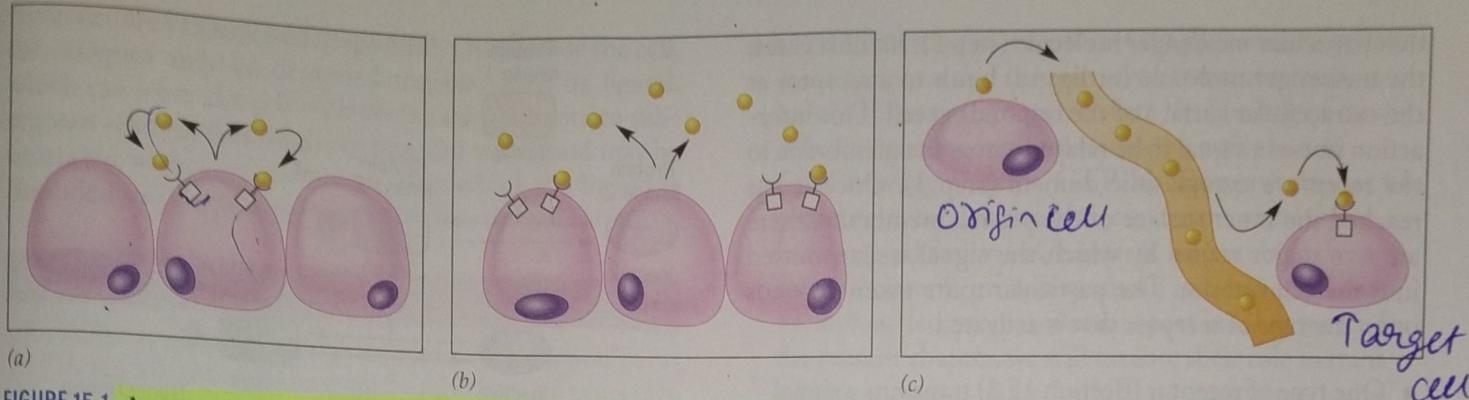
### THE HUMAN PERSPECTIVE:

#### Disorders Associated with G Protein-Coupled Receptors

The English poet John Donne expressed his belief in the interdependence of humans in the following phrase “No man is an island.” The same can be said of the cells that make up a complex multicellular organism. Most cells in a plant or animal are specialized to carry out one or more specific functions. Many biological processes require various cells to work together and to coordinate their activities. To make this possible, cells have to communicate with each other, which is accomplished by a process called cell signaling. Cell signaling makes it possible for cells to talk to each other and for an organism to function as a coherent system.

Cell signaling affects virtually every aspect of cell structure and function, which is one of the primary reasons that this chapter appears near the end of the book. On one hand, an understanding of cell signaling requires knowledge

*Three-dimensional structure of rhodopsin, the light-sensitive protein found in the rods of the retina. Rhodopsin is a member of a huge family of receptors characterized by seven membrane-spanning  $\alpha$  helices (shown as cylinders) that respond to extracellular stimuli and transmit the signal into the cytoplasm. Rhodopsin is activated when its light-absorbing retinal group (shown in red) absorbs a photon of light, which leads to a conformational change in the protein that is transmitted to a G protein at the inner surface of the membrane. (COURTESY OF CRAIG A. BEHNKE, EMERALD BIOSTRUCTURES.)*



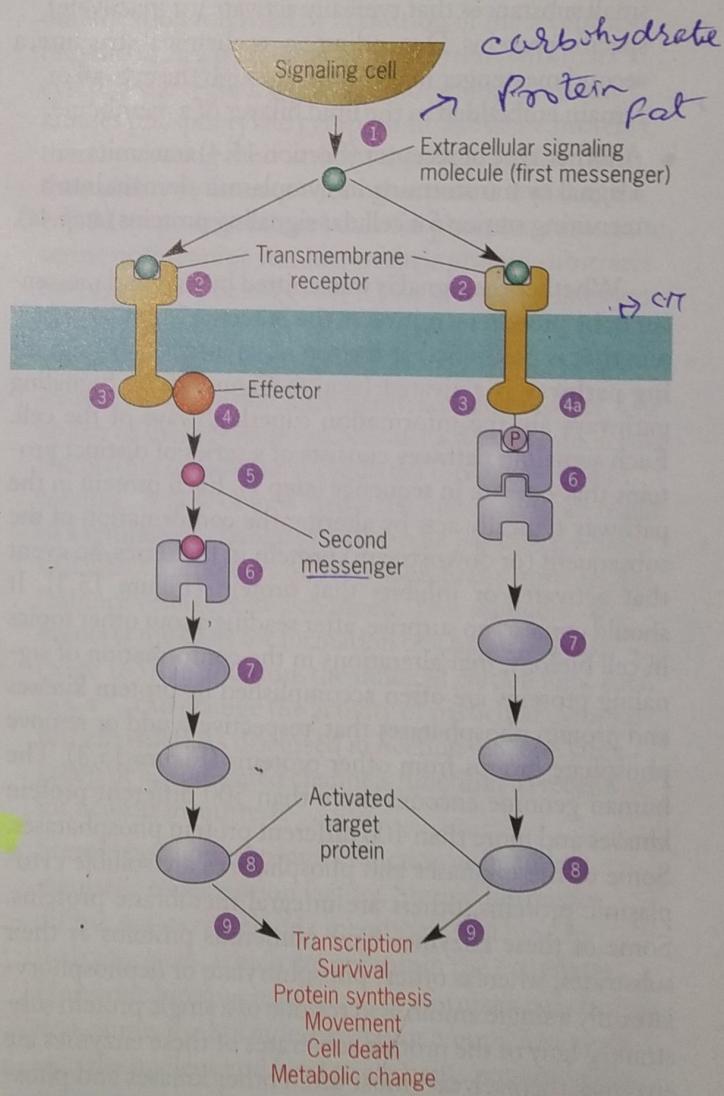
**FIGURE 15.1** Autocrine (a), paracrine (b), and endocrine (c) types of intercellular signaling.

about other types of cellular activity. On the other hand, insights into cell signaling can tie together a variety of seemingly independent cellular processes. Cell signaling is also intimately involved in the regulation of cell growth and division. This makes the study of cell signaling crucially important for understanding how a cell can lose the ability to control cell division and develop into a malignant tumor.

## 15.1 THE BASIC ELEMENTS OF CELL SIGNALING SYSTEMS

It may be helpful to begin the discussion of this complex subject by describing a few of the general features that are shared by most signaling pathways. Cells usually communicate with each other through **extracellular messenger molecules**. Extracellular messengers can travel a short distance and stimulate cells that are in close proximity to the origin of the message, or they can travel throughout the body, potentially stimulating cells that are far away from the source. In the case of *autocrine* signaling, the cell that is producing the messenger expresses receptors on its surface that can respond to that messenger (Figure 15.1a). Consequently, cells releasing the message will stimulate (or inhibit) themselves. During *paracrine* stimulation (Figure 15.1b), messenger molecules travel only short distances through the extracellular space to cells that are in close proximity to the cell that is generating the message. Paracrine messenger molecules are usually limited in their ability to travel around the body because they are inherently unstable, or they are degraded by enzymes, or they bind to the extracellular matrix. Finally, during *endocrine* signaling, messenger molecules reach their target cells via passage through the bloodstream (Figure 15.1c). Endocrine messengers are also called *hormones*, and they typically act on target cells located at distant sites in the body.

An overview of cellular signaling pathways is depicted in Figure 15.2. Cell signaling is initiated with the release of a messenger molecule by a cell that is engaged in sending messages to other cells in the body (step 1, Figure 15.2). Cells can only respond to an extracellular message if they express **receptors** that specifically recognize and bind

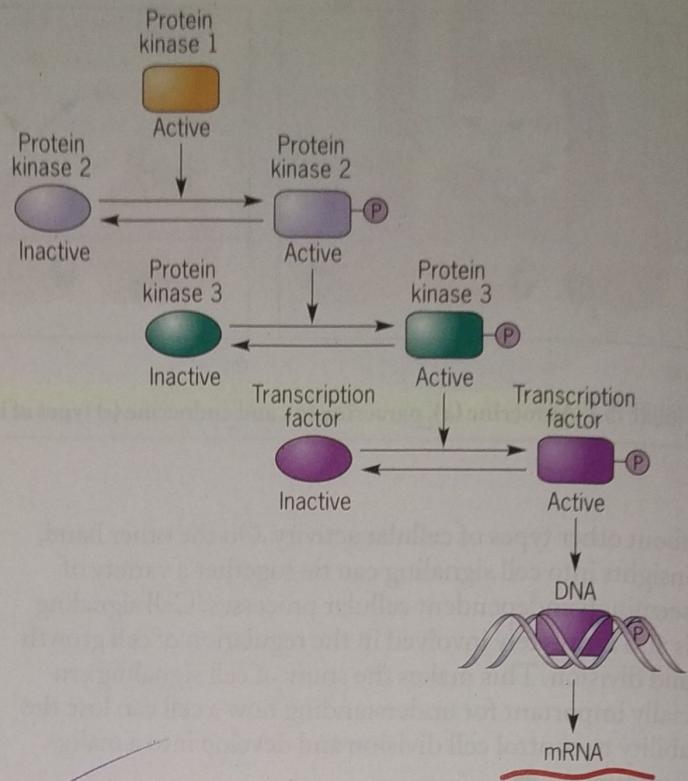


**FIGURE 15.2** An overview of the signaling pathways by which extracellular messenger molecules can elicit intracellular responses. Two different types of signal transduction pathways are depicted, one in which a signaling pathway is activated by a diffusible second messenger and another in which a signaling pathway is activated by recruitment of proteins to the plasma membrane. Most signal transduction pathways involve a combination of these mechanisms. It should also be noted that signaling pathways are not typically linear tracks as depicted here, but are branched and interconnected to form a complex web. The steps are described in the text.

that particular messenger molecule (step 2). In most cases, the messenger molecule (or ligand) binds to a receptor at the extracellular surface of the responding cell. This interaction causes a signal to be relayed across the membrane to the receptor's cytoplasmic domain (step 3). Once it has reached the inner surface of the plasma membrane, there are two major routes by which the signal is transmitted into the cell interior. The particular route taken depends upon the type of receptor that is activated.

- One type of receptor (Section 15.3) transmits a signal from its cytoplasmic domain to a nearby enzyme (step 4), which generates a **second messenger** (step 5). Because it brings about (effects) the cellular response by generating a second messenger, the enzyme responsible is referred to as an **effector**. Second messengers are small substances that typically activate (or inactivate) specific proteins. Depending on its chemical structure, a second messenger may diffuse through the cytosol or remain embedded in the lipid bilayer of a membrane.
- Another type of receptor (Section 15.4) transmits a signal by transforming its cytoplasmic domain into a recruiting station for cellular signaling proteins (step 4a).

Whether the signal is transmitted by a second messenger or by protein recruitment, the outcome is similar; a protein that is positioned at the top of an intracellular **signaling pathway** is activated (step 6, Figure 15.2). Signaling pathways are the information superhighways of the cell. Each signaling pathway consists of a series of distinct proteins that operate in sequence (step 7). Each protein in the pathway typically acts by altering the conformation of the subsequent (or downstream) protein in the series, an event that activates or inhibits that protein (Figure 15.3). It should come as no surprise, after reading about other topics in cell biology, that alterations in the conformation of signaling proteins are often accomplished by protein kinases and protein phosphatases that, respectively, add or remove phosphate groups from other proteins (Figure 15.3). The human genome encodes more than 500 different protein kinases and more than 100 different protein phosphatases. Some of these kinases and phosphatases are soluble cytoplasmic proteins, others are integral membrane proteins. Some of these enzymes have numerous proteins as their substrates, whereas others phosphorylate or dephosphorylate only a single amino acid residue of a single protein substrate. Many of the protein substrates of these enzymes are enzymes themselves—most often other kinases and phosphatases—but the substrates also include ion channels, transcription factors, and various types of regulatory proteins. It is thought that at least 50 percent of transmembrane and cytoplasmic proteins are phosphorylated at one or more sites. Protein phosphorylation can change protein behavior in several different ways. Phosphorylation can activate or inactivate an enzyme, it can increase or decrease protein–protein interactions, it can induce a protein to move from one subcellular compartment to another, or it can act as a signal that initiates protein degradation. Many



**FIGURE 15.3** Signal transduction pathway consisting of protein kinases and protein phosphatases whose catalytic actions change the conformations, and thus the activities, of the proteins they modify. In the example depicted here, protein kinase 2 is activated by protein kinase 1. Once activated, protein kinase 2 phosphorylates protein kinase 3, activating the enzyme. Protein kinase 3 then phosphorylates a transcription factor, increasing its affinity for a site on the DNA. Binding of a transcription factor to the DNA affects the transcription of the gene in question. Each of these activation steps in the pathway is reversed by a phosphatase.

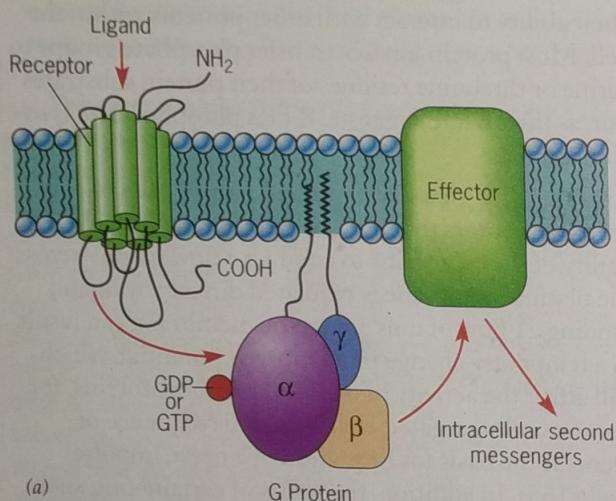
of the protein kinases and their target proteins have been identified: the primary challenge is to understand the roles of these diverse posttranslational modifications in the activities of different cell types.

Signals transmitted along such signaling pathways ultimately reach **target proteins** (step 8, Figure 15.2) involved in basic cellular processes (step 9). Depending on the type of cell and message, the response initiated by the target protein may involve a change in gene expression, an alteration of the activity of metabolic enzymes, a reconfiguration of the cytoskeleton, an increase or decrease in cell motility, a change in ion permeability, activation of DNA synthesis, or even the death of the cell. Virtually every activity in which a cell is engaged is regulated by signals originating at the cell surface. This overall process in which information carried by extracellular messenger molecules is translated into changes that occur inside a cell is referred to as **signal transduction**.

Finally, signaling has to be terminated. This is important because cells have to be responsive to additional messages that they may receive. The first order of business is to eliminate the extracellular messenger molecule. To do this, certain cells produce extracellular enzymes that destroy specific extracellular messengers. In other cases, activated

## 15.3 G PROTEIN-COUPLED RECEPTORS AND THEIR SECOND MESSENGERS

**G protein-coupled receptors (GPCRs)** are so named because they interact with G proteins, as discussed below. Members of the GPCR family are also referred to as seven-transmembrane (7TM) receptors because they contain seven transmembrane helices (Figure 15.4). Thousands of different GPCRs have been identified in organisms ranging from yeast to flowering plants and mammals that together regulate an extraordinary spectrum of cellular processes. In fact, GPCRs constitute the single largest superfamily of proteins encoded by animal genomes. Included among the natural ligands that bind to GPCRs are a diverse array of hormones, neurotransmitters, opium derivatives, chemoattractants (e.g., molecules that attract phagocytic cells of the immune system), odorants and tastants (molecules detected by olfactory and gustatory receptors eliciting the senses of smell and taste),



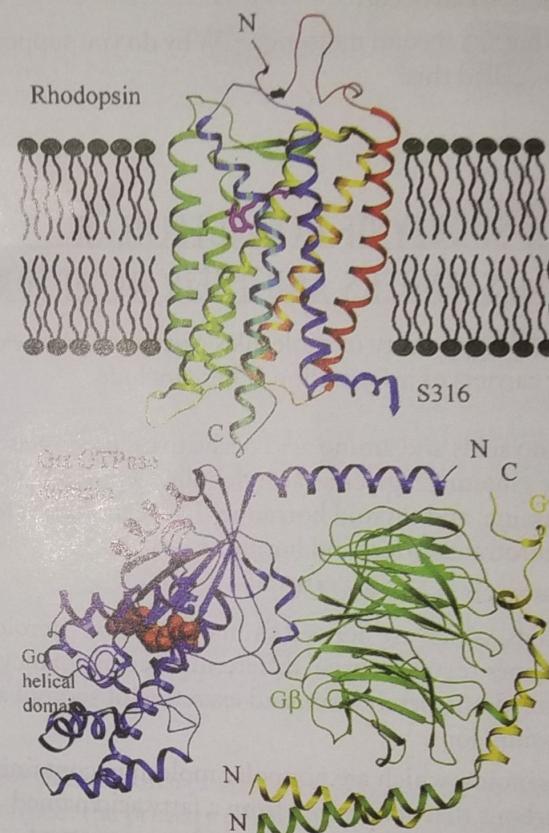
(a)

**FIGURE 15.4** The membrane-bound machinery for transducing signals by means of a seven transmembrane receptor and a heterotrimeric G protein. (a) Receptors of this type, including those that bind epinephrine and glucagon, contain seven membrane-spanning helices. When bound to their ligand, the receptor interacts with a trimeric G protein, which activates an effector, such as adenylyl cyclase. As indicated in the figure, the  $\alpha$  and  $\gamma$  subunits of the G protein are linked to the membrane by lipid groups that are

and photons. A list of some of the ligands that operate by means of this pathway and the effectors through which they act is provided in Table 15.1.

### Signal Transduction by G Protein-Coupled Receptors

**Receptors** G protein-coupled receptors normally have the following topology. Their amino-terminus is present on the outside of the cell, the seven  $\alpha$  helices that traverse



(b)

embedded in the lipid bilayer. (Note: Many GPCRs may be active as complexes of two or more receptor molecules.) (b) Ribbon model showing the proposed orientation of a GPCR (rhodopsin) and a heterotrimeric G protein (transducin) with respect to the membrane. The three subunits of the G protein are color coded. The bound GTP is shown in red. The C-terminal portion of rhodopsin (after S316) is not shown. (B: FROM HEIDI E. HAMM, PROC. NAT'L ACAD. SCI. U.S.A. 98:4819, 2001.)

**Table 15.1 Examples of Physiologic Processes Mediated by GPCRs and Heterotrimeric G Proteins**

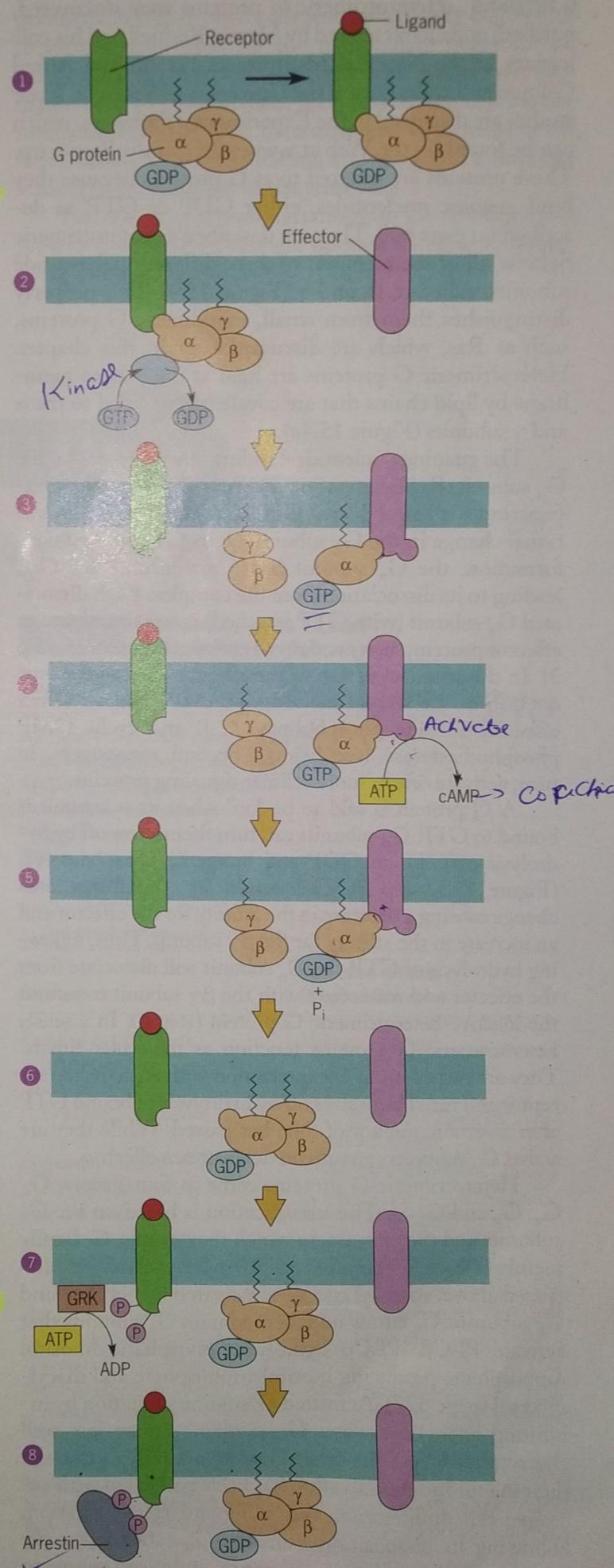
Stimulus	Receptor	Effector	Physiologic Response
Epinephrine	$\beta$ -Adrenergic receptor	Adenyl cyclase	Glycogen breakdown
Serotonin	Serotonin receptor	Adenyl cyclase	Behavioral sensitization and learning in <i>Aplysia</i>
Light	Rhodopsin	cGMP phosphodiesterase	Visual excitation
IgE-antigen complexes	Mast cell IgE receptor	Phospholipase C	Secretion
f-Met Peptide	Chemotactic receptor	Phospholipase C	Chemotaxis
Acetylcholine	Muscarinic receptor	Potassium channel	Slowing of pacemaker activity

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the plasma membrane are connected by loops of varying length, and the carboxyl-terminus is present on the inside of the cell (Figure 15.4). There are three loops present on the outside of the cell that, together, form the ligand-binding site. There are also three loops present on the cytoplasmic side of the plasma membrane that provide binding sites for intracellular signaling proteins. G proteins bind to the third intracellular loop. Arrestins, whose function is described on page 622, also bind to the third intracellular loop and compete with G proteins for binding to the receptor. Finally, there is a growing number of proteins that bind to the carboxyl-termini of GPCRs. Many of these proteins act as molecular scaffolds that link receptors to various signaling proteins and effectors present in the cell.

There is not much structural information that can help us understand how the change in conformation caused by binding of a hormone or neurotransmitter to the extracellular domain of a GPCR is transferred across the plasma membrane. The allosteric model holds that GPCRs can exist in an active and an inactive conformation. The inactive conformation is stabilized by non-covalent interactions between the transmembrane  $\alpha$  helices. Ligand binding disturbs these interactions, thereby causing the receptor to assume an active conformation. This requires rotations and shifts of the transmembrane  $\alpha$  helices relative to each other. Because they are attached to the cytoplasmic loops, rotation or movement of these transmembrane  $\alpha$  helices relative to each other causes changes in the conformation of the cytoplasmic loops. This in turn leads to an increase in the affinity of the receptor for a G protein that is present on the cytoplasmic surface of the plasma membrane (Figure 15.4b). As a consequence, the ligand-bound receptor forms a receptor-G protein complex (Figure 15.5, step 1). The interaction with the receptor induces a conformational change in the  $\alpha$  subunit of a G protein, causing the release of GDP, which is followed by binding of GTP (step 2). While in the activated state, a single receptor can activate a number of G protein molecules, providing a means of signal amplification (discussed further on page 631).

**FIGURE 15.5** The mechanism of receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins. In step 1, the ligand binds to the receptor, altering its conformation and increasing its affinity for the G protein to which it binds. In step 2, the  $G_{\alpha}$  subunit releases its GDP, which is replaced by GTP. In step 3, the  $G_{\alpha}$  subunit dissociates from the  $G_{\beta\gamma}$  complex and binds to an effector (in this case adenylyl cyclase), activating the effector. The  $G_{\beta\gamma}$  dimer may also bind to an effector (not shown), such as an ion channel or an enzyme. In step 4, activated adenylyl cyclase produces cAMP. In step 5, the GTPase activity of  $G_{\alpha}$  hydrolyzes the bound GTP, deactivating  $G_{\alpha}$ . In step 6,  $G_{\alpha}$  reassociates with  $G_{\beta\gamma}$ , reforming the trimeric G protein, and the effector ceases its activity. In step 7, the receptor has been phosphorylated by a GRK and in step 8 the phosphorylated receptor has been bound by an arrestin molecule, which inhibits the ligand-bound receptor from activating additional G proteins. The receptor bound to arrestin is likely to be taken up by endocytosis.



**G Proteins** Heterotrimeric G proteins were discovered, purified, and characterized by Martin Rodbell and his colleagues at the National Institutes of Health and Alfred Gilman and colleagues at the University of Virginia. Their studies are discussed in the Experimental Pathways, which can be found on the Web at [www.wiley.com/college/karp](http://www.wiley.com/college/karp). These proteins are referred to as G proteins because they bind guanine nucleotides, either GDP or GTP, as described on page 639. They are described as heterotrimeric because all of them consist of three different polypeptide subunits, called  $\alpha$ ,  $\beta$ , and  $\gamma$  (Figure 15.4). This property distinguishes them from small, monomeric G proteins, such as Ras, which are discussed later in this chapter. Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the  $\alpha$  and  $\gamma$  subunits (Figure 15.4a).

The guanine nucleotide-binding site is present on the  $G_\alpha$  subunit. Replacement of GDP by GTP, following interaction with an activated GPCR, results in a conformational change in the  $G_\alpha$  subunit. In its GTP-bound conformation, the  $G_\alpha$  subunit has a low affinity for  $G_{\beta\gamma}$ , leading to its dissociation from the complex. Each dissociated  $G_\alpha$  subunit (with GTP attached) is free to activate an effector protein, such as adenylyl cyclase (Figure 15.5, step 3). In this case, activation of the effector leads to the production of the second messenger cAMP (step 4). Other effectors include phospholipase C- $\beta$  and cyclic GMP phosphodiesterase (see below). Second messengers, in turn, activate one or more cellular signaling proteins.

A G protein is said to be "on" when its  $\alpha$  subunit is bound to GTP.  $G_\alpha$  subunits can turn themselves off by hydrolysis of GTP to GDP and inorganic phosphate (Pi) (Figure 15.5, step 5). This results in a conformational change causing a decrease in the affinity for the effector and an increase in the affinity for the  $\beta\gamma$  subunit. Thus, following hydrolysis of GTP, the  $G_\alpha$  subunit will dissociate from the effector and reassociate with the  $\beta\gamma$  subunit to reform the inactive heterotrimeric G protein (step 6). In a sense, heterotrimeric G proteins function as molecular timers. They are turned on by the interaction with an activated receptor and turn themselves off by hydrolysis of bound GTP after a certain amount of time has passed. While they are active,  $G_\alpha$  subunits can turn on downstream effectors.

Heterotrimeric G proteins come in four flavors,  $G_s$ ,  $G_q$ ,  $G_i$ , and  $G_{12/13}$ . This classification is based on the  $G_\alpha$  subunits and the effectors to which they couple.  $G_s$  family members couple receptors to adenylyl cyclase. As discussed above, adenylyl cyclase is activated by GTP-bound  $G_\alpha$  subunits.  $G_q$  family members contain  $G_\alpha$  subunits that activate PLC $\beta$ . PLC $\beta$  hydrolyzes phosphatidylinositol diphosphate, producing inositol triphosphate and diacylglycerol (page 627). Activated  $G_i$  subunits function by inhibiting adenylyl cyclase.  $G_{12/13}$  members are less well characterized than the other G protein families although their inappropriate activation has been associated with excessive cell proliferation and malignant transformation. Following its dissociation from the  $G_\alpha$  subunit, the  $\beta\gamma$  complex also has a signaling function and it can couple to

at least four different types of effectors: PLC $\beta$ , K $^+$  ion channels, adenylyl cyclase, and PI 3-kinase.

**Termination of the Response** We have seen that ligand binding results in receptor activation. The activated receptors turn on G proteins, and G proteins turn on effectors. To prevent overstimulation, receptors have to be blocked from continuing to activate G proteins. To regain sensitivity to future stimuli, the receptor, the G protein, and the effector must all be returned to their inactive state. *Desensitization*, the process that blocks active receptors from turning on additional G proteins, takes place in two steps. In the first step, the cytoplasmic domain of the activated GPCR is phosphorylated by a specific type of kinase, called *G protein-coupled receptor kinase (GRK)* (Figure 15.5, step 7). GRKs form a small family of serine-threonine protein kinases. The changes in conformation that make it possible for GPCRs to activate G proteins also make them good substrates for GRKs. As a result, GRKs specifically recognize activated GPCRs.

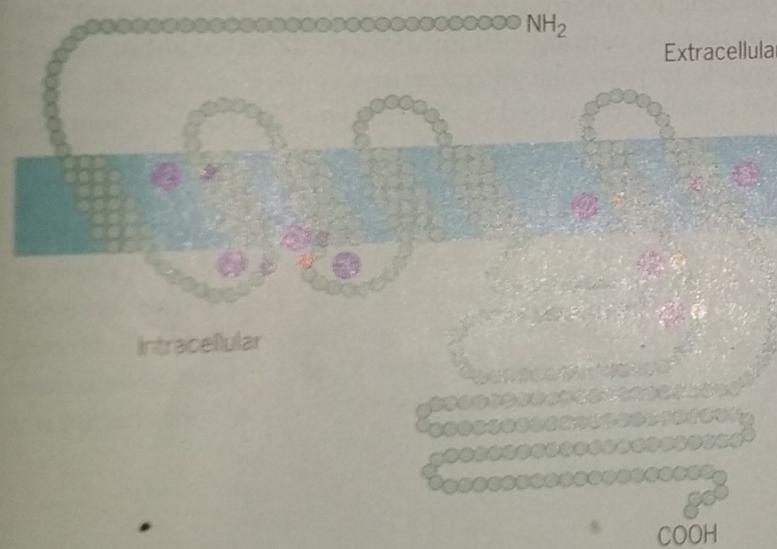
Phosphorylation of the GPCR sets the stage for the second step, which is the binding of proteins, called *arrestins* (Figure 15.5, step 8). Arrestins form a small group of proteins that bind to GPCRs and compete for binding with heterotrimeric G proteins. As a consequence, arrestin binding prevents the further activation of additional G proteins. This action is termed desensitization because the cell stops responding to the stimulus, while that stimulus is still acting on the outer surface of the cell. Desensitization is one of the mechanisms that allows a cell to respond to a change in its environment, rather than continuing to "fire" endlessly in the presence of an unchanging environment. The importance of desensitization is illustrated by the observation that mutations that interfere with phosphorylation of rhodopsin by a GRK lead to the death of the photoreceptor cells in the retina. This type of retinal cell death is thought to be one of the causes of blindness resulting from the disease retinitis pigmentosa.

While they are bound to phosphorylated GPCRs, arrestin molecules are also capable of binding to clathrin molecules that are situated in clathrin-coated pits (page 311). The interaction between bound arrestin and clathrin promotes the uptake of phosphorylated GPCRs into the cell by endocytosis. Depending upon the circumstances, receptors that have been removed from the surface by endocytosis may be dephosphorylated and returned to the plasma membrane. Alternatively, internalized receptors are degraded in the lysosomes (see Figure 8.42). If receptors are degraded, the cells lose, at least temporarily, sensitivity for the ligand in question. If receptors are returned to the cell surface, the cells remain sensitive to the ligand.

Signaling by the activated  $G_\alpha$  subunit is terminated by a less complex mechanism: the bound GTP molecule is simply hydrolyzed to GDP (step 5, Figure 15.5). Thus, the strength and duration of the signal are determined in part by the rate of GTP hydrolysis by the  $G_\alpha$  subunit.  $G_\alpha$  subunits possess a weak GTPase activity, which allows them to slowly hydrolyze the bound GTP and inactivate

# Disorders Associated with G Protein-Coupled Receptors

The human genome may encode as many as 2000 different GPCRs. Their importance in human biology is reflected by the fact that over one-third of all prescription drugs act as ligands that bind to this huge superfamily of receptors. A number of inherited disorders have been traced to defects in both GPCRs (Figure 1) and heterotrimeric G proteins (Table 1). Retinitis pigmentosa (RP) is an inherited disease characterized by progressive degeneration of the retina and eventual blindness. RP



**FIGURE 1** Two-dimensional representation of a “composite” transmembrane receptor showing the approximate sites of a number of mutations responsible for causing human diseases. Most of the mutations (numbers 1, 2, 5, 6, 7, and 8) result in constitutive stimulation of the effector, but others (3 and 4) result in blockage of the receptor’s ability to stimulate the effector. Mutations at sites 1 and 2 are found in the MSH (melanocyte-stimulating hormone) receptor; 3 in the ACTH (adrenocorticotropic hormone) receptor; 4 in the vasopressin receptor; 5 and 6 in the TSH (thyroid-stimulating hormone) receptor; 7 in the LH (luteinizing hormone) receptor; and 8 in rhodopsin, the light-sensitive pigment of the retina.

**Table 1** Human Diseases Linked to the G Protein Pathway

Disease	Defective G Protein*
Albright’s hereditary osteodystrophy and pseudohypoparathyroidisms	$G_{\alpha i}$
McCune-Albright syndrome	$G_{\alpha i}$
Pituitary, thyroid tumors ( <i>gsp</i> oncogene)	$G_{\alpha i}$
Adrenocortical, ovarian tumors ( <i>gip</i> oncogene)	$G_{\alpha i}$
Combined precocious puberty and pseudohypoparathyroidism	$G_{\alpha i}$
Disease	Defective G Protein-Coupled Receptor
Familial hypocalciuric hypercalcemia	Human analogue of BoPCAR1 receptor
Neonatal severe hyperparathyroidism	Human analogue of BoPCAR1 receptor (homozygous)
Hyperthyroidism (thyroid adenomas)	Thyrotropin receptor
Familial male precocious puberty	Luteinizing hormone receptor
X-linked nephrogenic diabetes insipidus	V2 vasopressin receptor
Retinitis pigmentosa	Rhodopsin receptor
Color blindness, spectral sensitivity variations	Cone opsin receptor
Familial glucocorticoid deficiency and isolated glucocorticoid deficiency	Adrenocorticotropic hormone (ACTH) receptor

\*As described in the text, a G protein with a  $G_{\alpha i}$  acts to stimulate the effector, whereas a G protein with a  $G_{\alpha o}$  inhibits the effector.  
Source: D. E. Clapham, reprinted with permission from *Nature*, vol. 371, p. 109, 1994. © Copyright 1994, by Macmillan Magazines Ltd.

**Table 15.2**

## Examples of Responses Mediated by Protein Kinase C

Tissue	Response
Blood platelets	Serotonin release
Mast cells	Histamine release
Adrenal medulla	Secretion of epinephrine
Pancreas	Secretion of insulin
Pituitary cells	Secretion of GH and LH
Thyroid	Secretion of calcitonin
Testes	Testosterone synthesis
Neurons	Dopamine release
Smooth muscle	Increased contractility
Liver	Glycogen hydrolysis
Adipose tissue	Fat synthesis

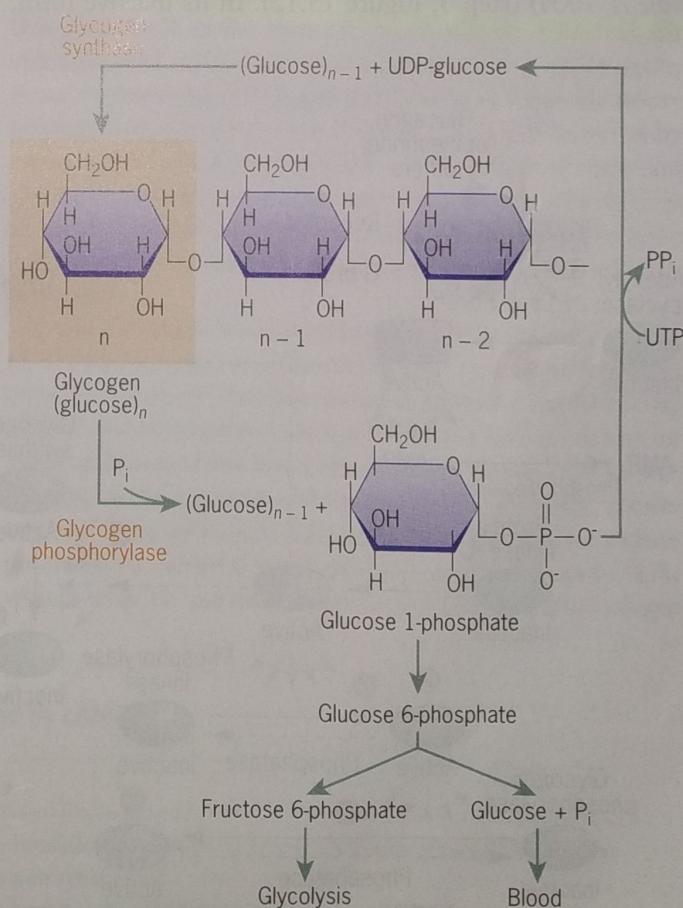
the adrenergic receptor, which binds epinephrine, and 15 different isoforms of the receptor for serotonin, a powerful neurotransmitter released by nerve cells in parts of the brain governing emotions. Different isoforms can have different affinities for the ligand or may interact with different types of G proteins. Different isoforms of a receptor may coexist in the same plasma membrane, or they may occur in the membranes of different types of target cells. The heterotrimeric G proteins that transmit signals from receptor to effector can also exist in multiple forms, as can many of the effectors. The human genome encodes at least 16 different  $G_{\alpha}$  subunits, 5 different  $G_{\beta}$  subunits, and 11 different  $G_{\gamma}$  subunits, along with 9 isoforms of the effector adenylyl cyclase. Different combinations of specific subunits construct G proteins having different capabilities of reacting with specific isoforms of both receptors and effectors.

As mentioned on page 622, some G proteins act by inhibiting their effectors. The same stimulus can activate a stimulatory G protein (one with a  $G_{\alpha s}$  subunit) in one cell and an inhibitory G protein (one with a  $G_{\alpha i}$  subunit) in a different cell. For example, when epinephrine binds to a  $\beta$ -adrenergic receptor on a cardiac muscle cell, a G protein with a  $G_{\alpha s}$  subunit is activated, which stimulates cAMP production, leading to an increase in the rate and force of contraction. In contrast, when epinephrine binds to an  $\alpha$ -adrenergic receptor on a smooth muscle cell in the intestine, a G protein with a  $G_{\alpha i}$  subunit is activated, which inhibits cAMP production, producing muscle relaxation. Finally, some adrenergic receptors turn on G proteins with  $G_{\alpha q}$  subunits, leading to activation of PLC $\beta$ . Clearly, the same extracellular messenger can activate a variety of pathways in different cells.

## Regulation of Blood Glucose Levels

Glucose can be utilized as a source of energy by all cell types present in the body. It is oxidized to  $CO_2$  and  $H_2O$  by glycolysis and the TCA cycle, providing cells with ATP that can be used to drive energy-requiring reactions. Because it is such an important resource, the body maintains glucose levels in the bloodstream within a narrow range. As discussed in Chapter 3, excess glucose is stored in animal cells as glycogen, a large branched polymer composed of glucose monomers that are linked through glycosidic bonds. The hormone glucagon is produced by the alpha cells of the pancreas in response to low blood glucose levels. Glucagon stimulates breakdown of glycogen and release of glucose into the bloodstream, thereby causing glucose levels to rise. The hormone insulin is produced by the beta cells of the pancreas in response to high glucose levels and stimulates glucose uptake and storage as glycogen. Finally, epinephrine—which is sometimes called the “fight or flight” hormone—is produced by the adrenal gland in stressful situations. Epinephrine causes an increase in blood glucose levels to provide the body with the extra energy resources needed to deal with the stressful situation at hand.

Insulin acts through a receptor protein-tyrosine kinase and its signal transduction is discussed on page 641. In contrast, both glucagon and epinephrine act by binding to GPCRs. Glucagon is a small protein that is composed of 29 amino acids, whereas epinephrine is a small molecule that is derived from the amino acid tyrosine. Structurally speaking, these two molecules have nothing in common, yet both of them bind to GPCRs and stimulate the breakdown of glycogen into glucose 1-phosphate (Figure 15.10). In addition, the binding of either of these hormones leads to the inhibition of the enzyme glycogen synthase, which catalyzes the opposing reaction in which glucose units are added to growing glycogen molecules. Thus two different stimuli (glucagon and epinephrine), recognized by different receptors, induce the same response in a single target cell. The two receptors differ from one another primarily in the structure of the ligand-binding pocket on the extracellular surface of the cell, which is specific for one or the other hormone. Following activation by their respective ligands, both receptors activate the same type of heterotrimeric G proteins that cause an increase in the levels of cAMP.

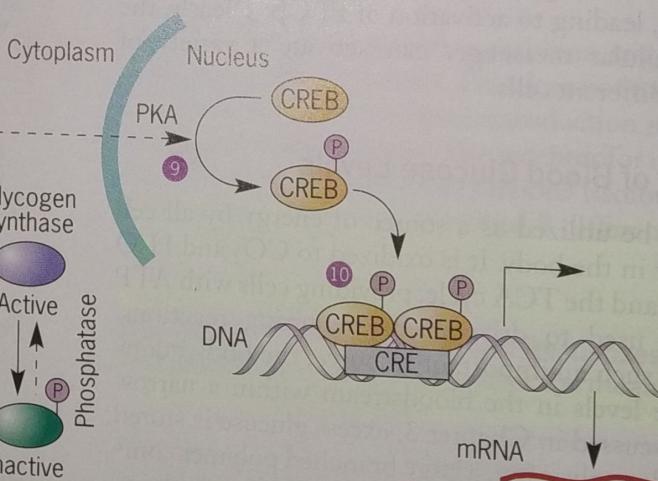
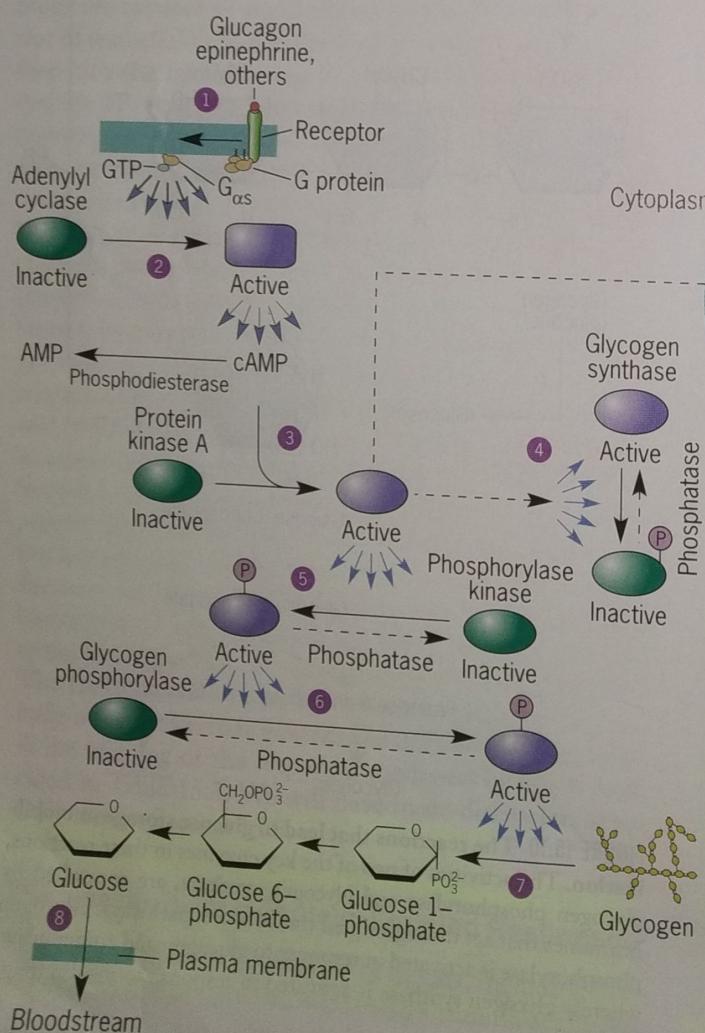
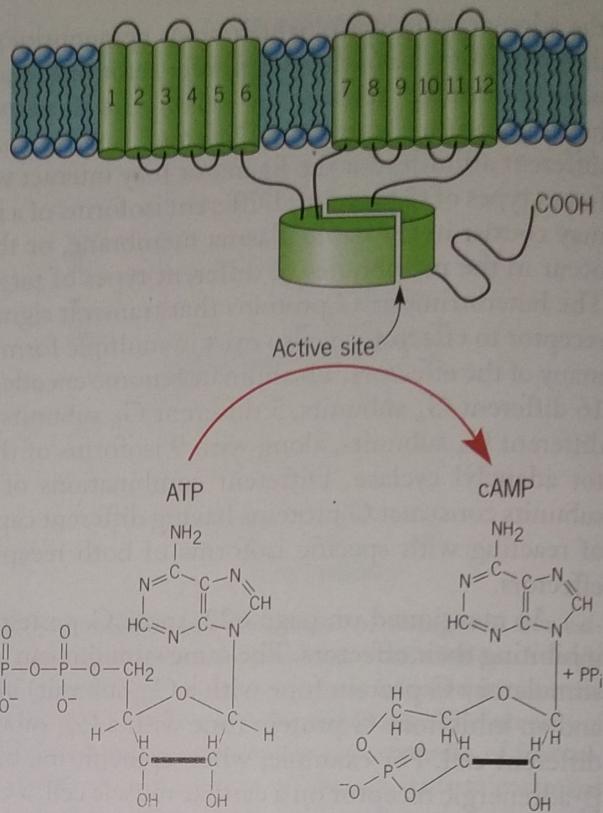


**FIGURE 15.10** The reactions that lead to glucose storage or mobilization. The activities of two of the key enzymes in these reactions, glycogen phosphorylase and glycogen synthase, are controlled by hormones that act through signal transduction pathways. Glycogen phosphorylase is activated in response to glucagon and epinephrine, whereas glycogen synthase is activated in response to insulin (page 644).

**FIGURE 15.11** Formation of cyclic AMP from ATP is catalyzed by adenylyl cyclase, an integral membrane protein that consists of two parts, each containing six transmembrane helices (shown here in two dimensions). The enzyme's active site is located on the inner surface of the membrane in a cleft situated between two similar cytoplasmic domains. The breakdown of cAMP (not shown) is accomplished by a phosphodiesterase, which converts the cyclic nucleotide to a 5' monophosphate.

**Glucose Mobilization: An Example of a Response Induced by cAMP** cAMP is synthesized by *adenylyl cyclase*, an integral membrane protein whose catalytic domain resides at the inner surface of the plasma membrane (Figure 15.11). cAMP evokes a response that leads to glucose mobilization by initiating a chain of reactions, as illustrated in Figure 15.12. The first step in this *reaction cascade* occurs as the hormone binds to its receptor, activating a  $G_{\alpha s}$  subunit, which activates an adenylyl cyclase effector. The activated enzyme catalyzes the formation of cAMP (steps 1 and 2, Figure 15.12).

Once formed, cAMP molecules diffuse into the cytoplasm where they bind to an allosteric site on a regulatory subunit of a cAMP-dependent protein kinase (*protein kinase A, PKA*) (step 3, Figure 15.12). In its inactive form,



**FIGURE 15.12** The response by a liver cell to glucagon or epinephrine. The steps in the response to hormonal stimulation that lead to glucose mobilization are described in the text. Many of the steps in the reaction cascade are accompanied by a dramatic amplification of the signal. Steps leading to amplification are indicated by clusters of blue arrows.

PKA is a heterotetramer composed of two regulatory (R) and two catalytic (C) subunits. The regulatory subunits normally inhibit the catalytic activity of the enzyme. cAMP binding causes the dissociation of the regulatory subunits, thereby releasing the active catalytic subunits of PKA. The target substrates of PKA in a liver cell include two enzymes that play a pivotal role in glucose metabolism, glycogen synthase and phosphorylase kinase (steps 4 and 5). Phosphorylation of glycogen synthase inhibits its catalytic activity and thus prevents the conversion of glucose to glycogen. In contrast, phosphorylation of phosphorylase kinase activates the enzyme to catalyze the transfer of phosphate groups to glycogen phosphorylase molecules. As discovered by Krebs and Fischer, the addition of a single phosphate group to a specific serine residue in the glycogen phosphorylase polypeptide activates this enzyme (step 6), stimulating the breakdown of glycogen (step 7). The glucose 1-phosphate formed in the reaction is converted to glucose, which diffuses into the bloodstream and so reaches the other tissues of the body (step 8).

As one might expect, a mechanism must exist to reverse the steps discussed above; otherwise the cell would remain in the activated state indefinitely. Liver cells contain phosphatases that remove the phosphate groups added by the kinases. A particular member of this family of enzymes, protein phosphatase-1, can remove phosphate from all of the phosphorylated enzymes of Figure 15.12: phosphorylase kinase, glycogen synthase, and glycogen phosphorylase. The destruction of cAMP molecules present in the cell is accomplished by the enzyme cAMP phosphodiesterase, which helps terminate the response.

**Signal Amplification** The binding of a single hormone molecule at the cell surface can activate a number of G proteins, each of which can activate an adenylyl cyclase effector, each of which can produce a large number of cAMP messengers in a short period of time. Thus, the production of a second messenger provides a mechanism to greatly amplify the signal generated from the original message. Many of the steps in the reaction cascade illustrated in Figure 15.12 result in amplification of the signal (these steps are indicated by the blue arrows). cAMP mol-

ecules activate PKA. Each PKA catalytic subunit phosphorylates a large number of phosphorylase kinase molecules, which in turn phosphorylate an even larger number of glycogen phosphorylase molecules, which in turn can catalyze the formation of a much larger number of glucose phosphates. Thus, what begins as a barely perceptible stimulus at the cell surface is rapidly transformed into a major mobilization of glucose within the cell.

#### Other Aspects of cAMP Signal Transduction Pathways

Although the most rapid and best-studied effects of cAMP are produced in the cytoplasm, the nucleus and its genes also participate in the response. A fraction of the activated PKA molecules translocate into the nucleus where they phosphorylate key nuclear proteins (step 9, Figure 15.12), most notably a transcription factor called *CREB* (*cAMP response element-binding protein*). The phosphorylated version of CREB binds as a dimer to sites on the DNA (Figure 15.12, step 10) containing a particular nucleotide sequence (TGACGTCA), known as the *cAMP response element (CRE)*. Recall from page 524 that response elements are sites in the DNA where transcription factors bind and increase the rate of initiation of transcription. CREs are located in the regulatory regions of genes that play a role in the response to cAMP. In liver cells, for example, several of the enzymes involved in gluconeogenesis, a pathway by which glucose is formed from the intermediates of glycolysis (see Figure 3.31), are encoded by genes that contain nearby CREs. Thus, epinephrine and glucagon not only activate catabolic enzymes involved in glycogen breakdown, they promote the synthesis of anabolic enzymes required to synthesize glucose from smaller precursors.

cAMP is produced in many different cells in response to a wide variety of different ligands (i.e., first messengers). Several of the hormonal responses mediated by cAMP in mammalian cells are listed in Table 15.4. Cyclic AMP pathways have also been implicated in processes occurring in the nervous system, including learning, memory, and drug addiction. Chronic use of opiates, for example, leads to elevated levels of adenylyl cyclase and PKA, which may be partially responsible for the physiologic

**Table 15.4** Examples of Hormone-Induced Responses Mediated by cAMP

Tissue	Hormone	Response
Liver	Epinephrine and glucagon	Glycogen breakdown, glucose synthesis (gluconeogenesis), inhibition of glycogen synthesis
Skeletal muscle	Epinephrine	Glycogen breakdown, inhibition of glycogen synthesis
Cardiac muscle	Epinephrine	Increased contractility
Adipose	Epinephrine, ACTH, and glucagon	Triacylglycerol catabolism
Kidney	Vasopressin (ADH)	Increased permeability of epithelial cells to water
Thyroid	TSH	Secretion of thyroid hormones
Bone	Parathyroid hormone	Increased calcium resorption
Ovary	LH	Increased secretion of steroid hormones
Adrenal cortex	ACTH	Increased secretion of glucocorticoids