

chapter 12

BIOSIGNALING

12.1 Molecular Mechanisms of Signal Transduction *422*

12.2 Gated Ion Channels *425*

12.3 Receptor Enzymes *429*

12.4 G Protein–Coupled Receptors and Second Messengers *435*

12.5 Multivalent Scaffold Proteins and Membrane Rafts *443*

12.6 Signaling in Microorganisms and Plants *452*

12.7 Sensory Transduction in Vision, Olfaction, and Gustation *456*

12.8 Regulation of Transcription by Steroid Hormones *465*

12.9 Regulation of the Cell Cycle by Protein Kinases *466*

12.10 Oncogenes, Tumor Suppressor Genes, and Programmed Cell Death *471*

When I first entered the study of hormone action, some 25 years ago, there was a widespread feeling among biologists that hormone action could not be studied meaningfully in the absence of organized cell structure. However, as I reflected on the history of biochemistry, it seemed to me there was a real possibility that hormones might act at the molecular level.

—Earl W. Sutherland, Nobel Address, 1971

The ability of cells to receive and act on signals from beyond the plasma membrane is fundamental to life. Bacterial cells receive constant input from membrane proteins that act as information receptors, sampling the surrounding medium for pH, osmotic strength, the availability of food, oxygen, and light, and the presence of noxious chemicals, predators, or competitors for food. These signals elicit appropriate responses, such as motion toward food or away from toxic substances or the formation of dormant spores in a nutrient-depleted medium. In multicellular organisms, cells with different functions exchange a wide variety of signals. Plant cells respond to growth hormones and to variations in sunlight. Animal cells exchange information about the concentrations of ions and glucose in extracellular fluids, the interdependent metabolic activities taking place in different tissues, and, in an embryo, the correct placement of cells during development. In all these cases, the signal represents *information* that is detected by specific receptors and converted to a cellular response, which always involves a *chemical* process. This conversion of information into a chemical change, **signal transduction,** is a universal property of living cells.

The number of different biological signals is large (Table 12–1), as is the variety of biological responses to these signals, but organisms use just a few evolutionarily conserved mechanisms to detect extracellular signals and *transduce* them into intracellular changes. In this chapter we examine some examples of the major classes of signaling mechanisms, looking at how they are integrated in specific biological functions such as the transmission of nerve signals; responses to hormones and growth factors; the senses of sight, smell, and taste; and

TABLE 12–1
Some Signals to Which Cells Respond

Antigens	Light
Cell surface glycoproteins/ oligosaccharides	Mechanical touch
	Neurotransmitters
Developmental signals	Nutrients
Extracellular matrix components	Odorants
Growth factors	Pheromones
Hormones	Tastants

control of the cell cycle. Often, the end result of a signaling pathway is the phosphorylation of a few specific target-cell proteins, which changes their activities and thus the activities of the cell. Throughout our discussion we emphasize the conservation of fundamental mechanisms for the transduction of biological signals and the adaptation of these basic mechanisms to a wide range of signaling pathways.

12.1 Molecular Mechanisms of Signal Transduction

Signal transductions are remarkably specific and exquisitely sensitive. **Specificity** is achieved by precise molecular complementarity between the signal and receptor molecules (Fig. 12–1a), mediated by the same kinds of weak (noncovalent) forces that mediate enzyme-substrate and antigen-antibody interactions. Multicellular organisms have an additional level of specificity, because the receptors for a given signal, or the intracellular targets of a given signal pathway, are present only in certain cell types. Thyrotropin-releasing hormone, for example, triggers responses in the cells of the anterior pituitary but not in hepatocytes, which lack receptors for this hormone. Epinephrine alters glycogen metabolism in hepatocytes but not in erythrocytes; in this case, both cell types have receptors for the hormone, but whereas hepatocytes contain glycogen and the glycogen-metabolizing enzyme that is stimulated by epinephrine, erythrocytes contain neither.

Three factors account for the extraordinary sensitivity of signal transducers: the high affinity of receptors for signal molecules, cooperativity (often but not always) in the ligand-receptor interaction, and amplification of the signal by enzyme cascades. The **affinity** between signal (ligand) and receptor can be expressed as the dissociation constant K_d, usually 10^{-10} M or less—meaning that the receptor detects picomolar concentrations of a signal molecule. Receptor-ligand interactions are quantified by Scatchard analysis, which yields a quantitative measure of affinity (K_d) and the number of ligand-binding sites in a receptor sample (Box 12–1).

Cooperativity in receptor-ligand interactions results in large changes in receptor activation with small changes in ligand concentration (recall the effect of cooperativity on oxygen binding to hemoglobin; see Fig. 5–12). **Amplification** by **enzyme cascades** results when an enzyme associated with a signal receptor is activated and, in turn, catalyzes the activation of many molecules of a second enzyme, each of which activates many molecules of a third enzyme, and so on (Fig. 12–1b). Such cascades can produce amplifications of several orders of magnitude within milliseconds.

The sensitivity of receptor systems is subject to modification. When a signal is present continuously, **desensitization** of the receptor system results (Fig. 12–1c); when the stimulus falls below a certain threshold, the system again becomes sensitive. Think of what happens to your visual transduction system when you walk from bright sunlight into a darkened room or from darkness into the light.

A final noteworthy feature of signal-transducing systems is **integration** (Fig. 12–1d), the ability of the system to receive multiple signals and produce a unified response appropriate to the needs of the cell or organism. Different signaling pathways converse with

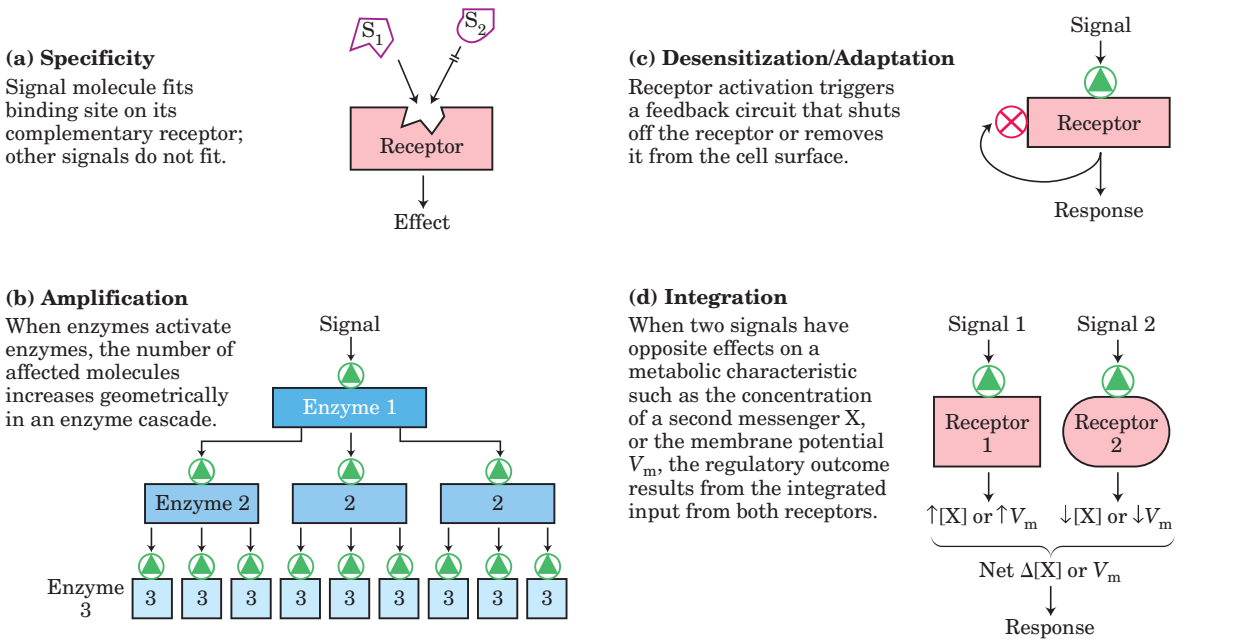


FIGURE 12–1 Four features of signal-transducing systems.

Scatchard Analysis Quantifies the Receptor-Ligand Interaction

The cellular actions of a hormone begin when the hormone (ligand, L) binds specifically and tightly to its protein receptor (R) on or in the target cell. Binding is mediated by noncovalent interactions (hydrogen-bonding, hydrophobic, and electrostatic) between the complementary surfaces of ligand and receptor. Receptor-ligand interaction brings about a conformational change that alters the biological activity of the receptor, which may be an enzyme, an enzyme regulator, an ion channel, or a regulator of gene expression.

Receptor-ligand binding is described by the equation

$$\underset{\text{Receptor}}{\text{R}} + \underset{\text{Ligand}}{\text{L}} \rightleftharpoons \underset{\text{Receptor-ligand complex}}{\text{RL}}$$

This binding, like that of an enzyme to its substrate, depends on the concentrations of the interacting components and can be described by an equilibrium constant:

$$\underset{\text{Receptor}}{\text{R}} + \underset{\text{Ligand}}{\text{L}} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \underset{\text{Receptor-ligand complex}}{\text{RL}}$$

$$K_a = \frac{[\text{RL}]}{[\text{R}][\text{L}]} = \frac{k_{+1}}{k_{-1}} = 1/K_d$$

where K_a is the association constant and K_d is the dissociation constant.

Like enzyme-substrate binding, receptor-ligand binding is saturable. As more ligand is added to a fixed amount of receptor, an increasing fraction of receptor molecules is occupied by ligand (Fig. 1a). A rough measure of receptor-ligand affinity is given by the concentration of ligand needed to give half-saturation of the receptor. Using **Scatchard analysis** of receptor-ligand binding, we can estimate both the dissociation constant K_d and the number of receptor-binding sites in a given preparation. When binding has reached equilibrium, the total number of possible binding sites, B_{max}, equals the number of unoccupied sites, represented by [R], plus the number of occupied or ligand-bound sites, [RL]; that is, $B_{max} = [\text{R}] + [\text{RL}]$. The number of unbound sites can be expressed in terms of total sites minus occupied sites: $[\text{R}] = B_{max} - [\text{RL}]$. The equilibrium expression can now be written

$$K_a = \frac{[\text{RL}]}{[\text{L}](B_{max} - [\text{RL}])}$$

Rearranging to obtain the ratio of receptor-bound ligand to free (unbound) ligand, we get

$$\frac{[\text{Bound}]}{[\text{Free}]} = \frac{[\text{RL}]}{[\text{L}]} = K_a(B_{max} - [\text{RL}])$$

$$= \frac{1}{K_d}(B_{max} - [\text{RL}])$$

From this slope-intercept form of the equation, we can see that a plot of [bound ligand]/[free ligand] versus [bound ligand] should give a straight line with a slope of $-K_a$ ($-1/K_d$) and an intercept on the abscissa of B_{max}, the total number of binding sites (Fig. 1b). Hormone-ligand interactions typically have K_d values of 10^{-9} to 10^{-11} M, corresponding to very tight binding.

Scatchard analysis is reliable for the simplest cases, but as with Lineweaver-Burk plots for enzymes, when the receptor is an allosteric protein, the plots deviate from linearity.

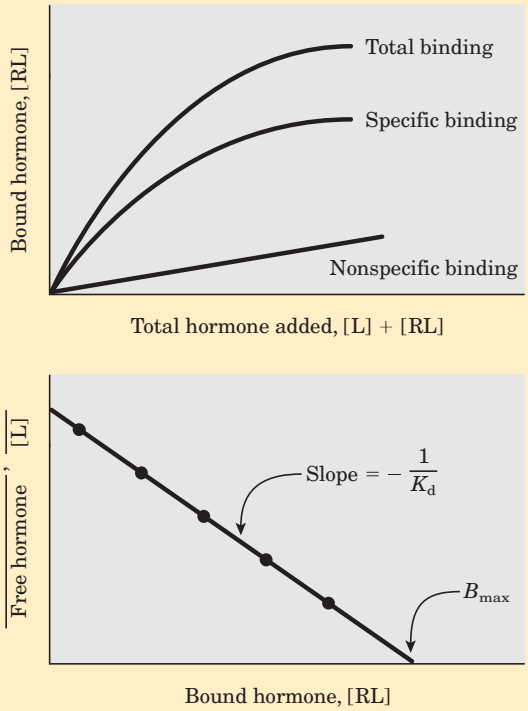


FIGURE 1 Scatchard analysis of a receptor-ligand interaction. A radiolabeled ligand (L)—a hormone, for example—is added at several concentrations to a fixed amount of receptor (R), and the fraction of the hormone bound to receptor is determined by separating the receptor-hormone complex (RL) from free hormone. **(a)** A plot of [RL] versus [L] + [RL] (total hormone added) is hyperbolic, rising toward a maximum for [RL] as the receptor sites become saturated. To control for nonsaturable, nonspecific binding sites (eicosanoid hormones bind nonspecifically to the lipid bilayer, for example), a separate series of binding experiments is also necessary. A large excess of unlabeled hormone is added along with the dilute solution of labeled hormone. The unlabeled molecules compete with the labeled molecules for specific binding to the saturable site on the receptor, but not for the nonspecific binding. The true value for specific binding is obtained by subtracting nonspecific binding from total binding. **(b)** A linear plot of [RL]/[L] versus [RL] gives K_d and B_{max} for the receptor-hormone complex. Compare these plots with those of V_0 versus [S] and $1/V_0$ versus 1/[S] for an enzyme-substrate complex (see Fig. 6–12, Box 6–1).

each other at several levels, generating a wealth of interactions that maintain homeostasis in the cell and the organism.

We consider here the molecular details of several representative signal-transduction systems. The trigger for each system is different, but the general features of signal transduction are common to all: a signal interacts with a receptor; the activated receptor interacts with cellular machinery, producing a second signal or a change in the activity of a cellular protein; the metabolic activity (broadly defined to include metabolism of RNA, DNA, and protein) of the target cell undergoes a change; and finally, the transduction event ends and the cell returns to its prestimulus state. To illustrate these general features of signaling systems, we provide examples of each of six basic signaling mechanisms (Fig. 12–2).

1. Gated ion channels of the plasma membrane that open and close (hence the term "gating") in response to the binding of chemical ligands or changes in transmembrane potential. These are the simplest signal transducers. The acetylcholine receptor ion channel is an example of this mechanism (Section 12.2).
2. Receptor enzymes, plasma membrane receptors that are also enzymes. When one of these receptors is activated by its extracellular ligand, it catalyzes the production of an intracellular second messenger. An example is the insulin receptor (Section 12.3).
3. Receptor proteins (serpentine receptors) that *indirectly* activate (through GTP-binding proteins, or G proteins) enzymes that generate intracellular second messengers. This is illustrated by the β-adrenergic receptor system that detects epinephrine (adrenaline) (Section 12.4).
4. Nuclear receptors (steroid receptors) that, when bound to their specific ligand (such as the hormone estrogen), alter the rate at which specific genes are transcribed and translated into cellular proteins. Because steroid hormones function through mechanisms intimately related to the regulation of gene expression, we consider them here only briefly (Section 12.8) and defer a detailed discussion of their action until Chapter 28.
5. Receptors that lack enzymatic activity but attract and activate cytoplasmic enzymes that act on downstream proteins, either by directly converting them to gene-regulating proteins or by activating a cascade of enzymes that finally activates a gene regulator. The JAK-STAT system exemplifies the first mechanism (Section 12.3); and the TLR4 (Toll) signaling system in humans, the second (Section 12.6).

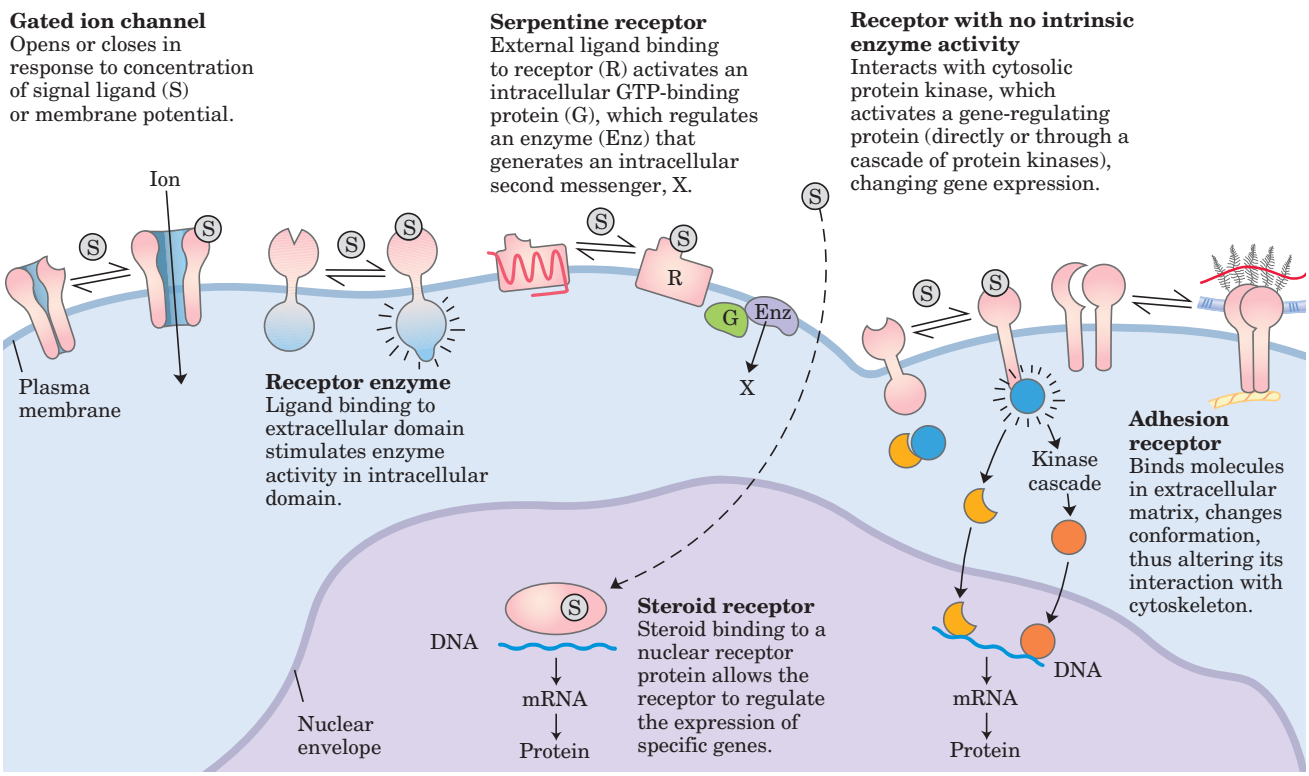


FIGURE 12–2 Six general types of signal transducers.

6. Receptors (adhesion receptors) that interact with macromolecular components of the extracellular matrix (such as collagen) and convey to the cytoskeletal system instructions on cell migration or adherence to the matrix. Integrins (discussed in Chapter 10) illustrate this general type of transduction mechanism.

As we shall see, transductions of all six types commonly require the activation of protein kinases, enzymes that transfer a phosphoryl group from ATP to a protein side chain.

SUMMARY 12.1 Molecular Mechanisms of Signal Transduction

- All cells have specific and highly sensitive signal-transducing mechanisms, which have been conserved during evolution.
- A wide variety of stimuli, including hormones, neurotransmitters, and growth factors, act through specific protein receptors in the plasma membrane.
- The receptors bind the signal molecule, amplify the signal, integrate it with input from other receptors, and transmit it into the cell. If the signal persists, receptor desensitization reduces or ends the response.
- Eukaryotic cells have six general types of signaling mechanisms: gated ion channels; receptor enzymes; membrane proteins that act through G proteins; nuclear proteins that bind steroids and act as transcription factors; membrane proteins that attract and activate soluble protein kinases; and adhesion receptors that carry information between the extracellular matrix and the cytoskeleton.

12.2 Gated Ion Channels

Ion Channels Underlie Electrical Signaling in Excitable Cells

The excitability of sensory cells, neurons, and myocytes depends on ion channels, signal transducers that provide a regulated path for the movement of inorganic ions such as Na^+, K^+, Ca^{2+}, and Cl^- across the plasma membrane in response to various stimuli. Recall from Chapter 11 that these ion channels are "gated"; they may be open or closed, depending on whether the associated receptor has been activated by the binding of its specific ligand (a neurotransmitter, for example) or by a change in the transmembrane electrical potential, V_m. The Na^+K^+ ATPase creates a charge imbalance across the plasma membrane by carrying 3 Na^+ out of the cell for every 2 K^+ carried in (Fig. 12–3a), making the inside negative relative to the outside. The membrane is said to be polarized. By convention, V_m is negative when the inside of the cell is negative relative to the outside. For a typical animal cell, $V_m = -60$ to -70 mV.

Because ion channels generally allow passage of either anions or cations but not both, ion flux through a channel causes a redistribution of charge on the two sides of the membrane, changing V_m. Influx of a positively charged ion such as Na^+, or efflux of a negatively charged ion such as Cl^-, depolarizes the membrane and brings V_m closer to zero. Conversely, efflux of K^+ hyperpolarizes the membrane and V_m becomes more negative. These ion fluxes through channels are passive, in contrast to active transport by the Na^+K^+ ATPase.

The direction of spontaneous ion flow across a polarized membrane is dictated by the electrochemical

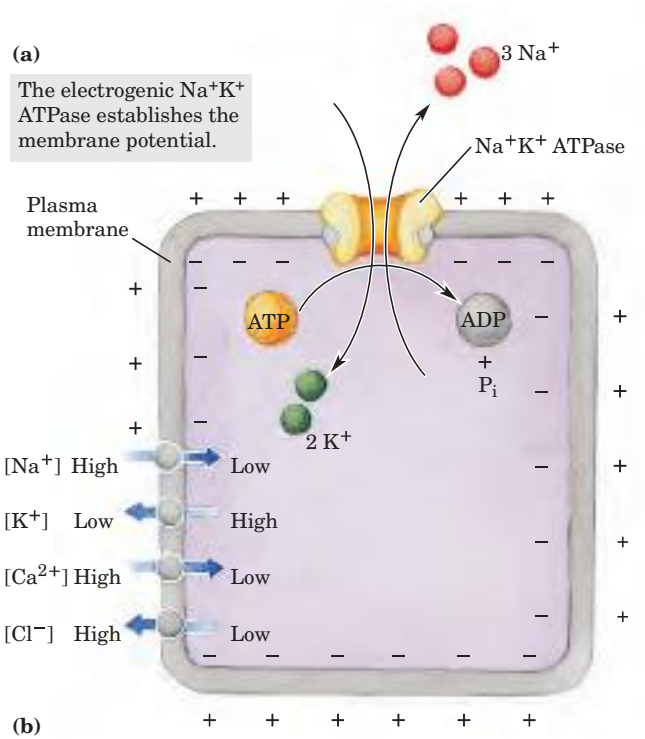


FIGURE 12–3 Transmembrane electrical potential. (a) The electrogenic Na^+K^+ ATPase produces a transmembrane electrical potential of −60 mV (inside negative). **(b)** Blue arrows show the direction in which ions tend to move spontaneously across the plasma membrane in an animal cell, driven by the combination of chemical and electrical gradients. The chemical gradient drives Na^+ and Ca^{2+} inward (producing depolarization) and K^+ outward (producing hyperpolarization). The electrical gradient drives Cl^- outward, against its concentration gradient (producing depolarization).

TABLE 12–2 Ion Concentrations in Cells and Extracellular Fluids (mM)

	K^+		Na^+		Ca^{2+}		Cl^-	
Cell type	*In*	*Out*	*In*	*Out*	*In*	*Out*	*In*	*Out*
Squid axon	400	20	50	440	≤0.4	10	40–150	560
Frog muscle	124	2.3	10.4	109	<0.1	2.1	1.5	78

potential of that ion across the membrane. The force (ΔG) that causes a cation (say, Na^+) to pass spontaneously inward through an ion channel is a function of the ratio of its concentrations on the two sides of the membrane (C_{in}/C_{out}) and of the difference in electrical potential ($\Delta\psi$ or V_m):

$$\Delta G = RT \ln \left(\frac{C_{in}}{C_{out}} \right) + Z \mathcal{F} V_m \qquad (12\text{–}1)$$

where R is the gas constant, T the absolute temperature, Z the charge on the ion, and $\mathcal{F}$ the Faraday constant. In a typical neuron or myocyte, the concentrations of Na^+, K^+, Ca^{2+}, and Cl^- in the cytosol are very different from those in the extracellular fluid (Table 12–2). Given these concentration differences, the resting V_m of −60 mV, and the relationship shown in Equation 12–1, opening of a Na^+ or Ca^{2+} channel will result in a spontaneous inward flow of Na^+ or Ca^{2+} (and depolarization), whereas opening of a K^+ channel will result in a spontaneous outward flux of K^+ (and hyperpolarization) (Fig. 12–3b).

A given ionic species continues to flow through a channel only as long as the combination of concentration gradient and electrical potential provides a driving force, according to Equation 12–1. For example, as Na^+ flows down its concentration gradient it depolarizes the membrane. When the membrane potential reaches +70 mV, the effect of this membrane potential (to resist further entry of Na^+) exactly equals the effect of the Na^+ concentration gradient (to cause more Na^+ to flow inward). At this equilibrium potential (E), the driving force (ΔG) tending to move an ion is zero. The equilibrium potential is different for each ionic species because the concentration gradients differ for each ion.

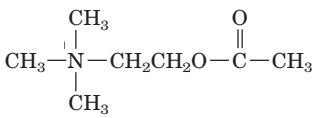
The number of ions that must flow to change the membrane potential significantly is negligible relative to the concentrations of Na^+, K^+, and Cl^- in cells and extracellular fluid, so the ion fluxes that occur during signaling in excitable cells have essentially no effect on the concentrations of those ions. However, because the intracellular concentration of Ca^{2+} is generally very low (~10^{-7} M), inward flow of Ca^{2+} can significantly alter the cytosolic [Ca^{2+}].

The membrane potential of a cell at a given time is the result of the types and numbers of ion channels open at that instant. In most cells at rest, more K^+ channels than Na^+, Cl^-, or Ca^{2+} channels are open and thus the resting potential is closer to the E for K^+ (−98 mV) than that for any other ion. When channels for Na^+, Ca^{2+}, or Cl^- open, the membrane potential moves toward the E for that ion. The precisely timed opening and closing of ion channels and the resulting transient changes in membrane potential underlie the electrical signaling by which the nervous system stimulates the skeletal muscles to contract, the heart to beat, or secretory cells to release their contents. Moreover, many hormones exert their effects by altering the membrane potentials of their target cells. These mechanisms are not limited to complex animals; ion channels play important roles in the responses of bacteria, protists, and plants to environmental signals.

To illustrate the action of ion channels in cell-to-cell signaling, we describe the mechanisms by which a neuron passes a signal along its length and across a synapse to the next neuron (or to a myocyte) in a cellular circuit, using acetylcholine as the neurotransmitter.

The Nicotinic Acetylcholine Receptor Is a Ligand-Gated Ion Channel

One of the best-understood examples of a **ligand-gated receptor channel** is the **nicotinic acetylcholine receptor** (see Fig. 11–51). The receptor channel opens in response to the neurotransmitter acetylcholine (and to nicotine, hence the name). This receptor is found in the postsynaptic membrane of neurons at certain synapses and in muscle fibers (myocytes) at neuromuscular junctions.



Acetylcholine (Ach)

Acetylcholine released by an excited neuron diffuses a few micrometers across the synaptic cleft or neuromuscular junction to the postsynaptic neuron or myocyte, where it interacts with the acetylcholine receptor and triggers electrical excitation (depolarization) of the receiving cell. The acetylcholine receptor is an allosteric protein with two high-affinity binding sites for acetylcholine, about 3.0 nm from the ion gate, on the two α

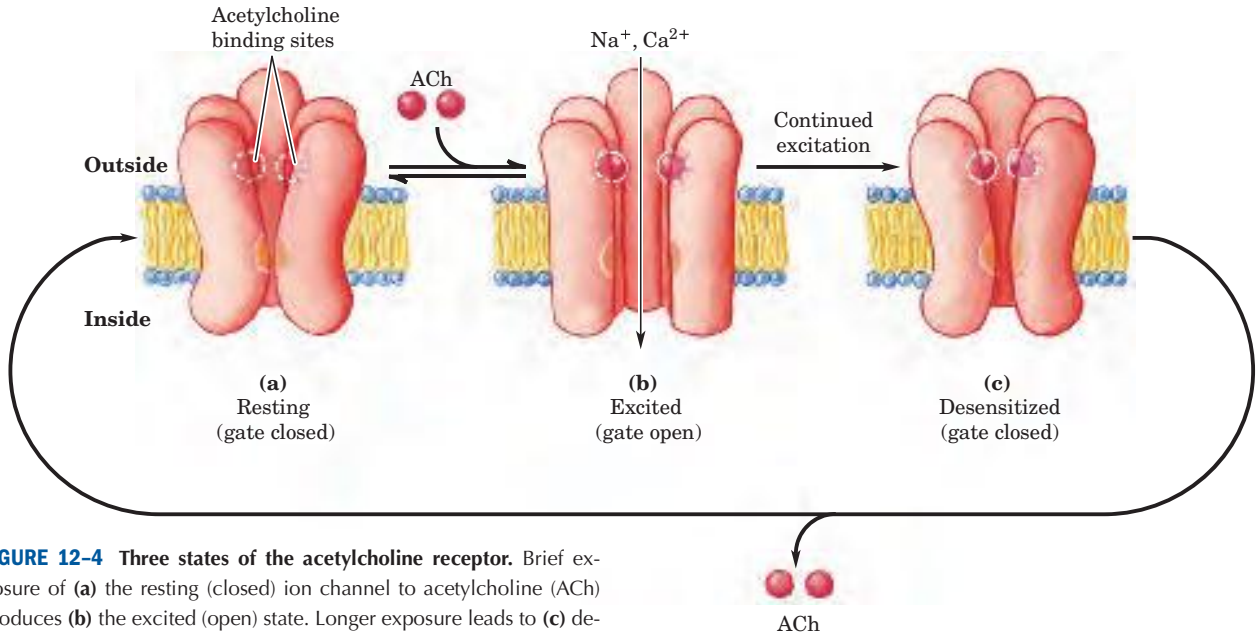


FIGURE 12–4 Three states of the acetylcholine receptor. Brief exposure of **(a)** the resting (closed) ion channel to acetylcholine (ACh) produces **(b)** the excited (open) state. Longer exposure leads to **(c)** desensitization and channel closure.

subunits. The binding of acetylcholine causes a change from the closed to the open conformation. The process is positively cooperative: binding of acetylcholine to the first site increases the acetylcholine-binding affinity of the second site. When the presynaptic cell releases a brief pulse of acetylcholine, both sites on the postsynaptic cell receptor are occupied briefly and the channel opens (Fig. 12–4). Either Na^+ or Ca^{2+} can now pass, and the inward flux of these ions depolarizes the plasma membrane, initiating subsequent events that vary with the type of tissue. In a postsynaptic neuron, depolarization initiates an action potential (see below); at a neuromuscular junction, depolarization of the muscle fiber triggers muscle contraction.

Normally, the acetylcholine concentration in the synaptic cleft is quickly lowered by the enzyme acetylcholinesterase, present in the cleft. When acetylcholine levels remain high for more than a few milliseconds, the receptor is desensitized (Fig. 12–1c). The receptor channel is converted to a third conformation (Fig. 12–4c) in which the channel is closed and the acetylcholine is very tightly bound. The slow release (in tens of milliseconds) of acetylcholine from its binding sites eventually allows the receptor to return to its resting state—closed and resensitized to acetylcholine levels.

Voltage-Gated Ion Channels Produce Neuronal Action Potentials

Signaling in the nervous system is accomplished by networks of neurons, specialized cells that carry an electrical impulse (action potential) from one end of the cell (the cell body) through an elongated cytoplasmic extension (the axon). The electrical signal triggers release of neurotransmitter molecules at the synapse, carrying the signal to the next cell in the circuit. Three types of **voltage-gated ion channels** are essential to this signaling mechanism. Along the entire length of the axon are **voltage-gated Na^+ channels** (Fig. 12–5; see also Fig. 11–50), which are closed when the membrane is at rest ($V_m = -60$ mV) but open briefly when the membrane is depolarized locally in response to acetylcholine (or some other neurotransmitter). The depolarization induced by the opening of Na^+ channels causes **voltage-gated K^+ channels** to open, and the resulting efflux of K^+ repolarizes the membrane locally. A brief pulse of depolarization traverses the axon as local depolarization triggers the brief opening of neighboring Na^+ channels, then K^+ channels. After each opening of a Na^+ channel, a short refractory period follows during which that channel cannot open again, and thus a unidirectional wave of depolarization sweeps from the nerve cell body toward the end of the axon. The voltage sensitivity of ion channels is due to the presence at critical positions in the channel protein of charged amino acid side chains that interact with the electric field across the membrane. Changes in transmembrane potential produce subtle conformational changes in the channel protein (see Fig. 11–50).

At the distal tip of the axon are **voltage-gated Ca^{2+} channels.** When the wave of depolarization reaches these channels, they open, and Ca^{2+} enters from the extracellular space. The rise in cytoplasmic $[Ca^{2+}]$ then triggers release of acetylcholine by exocytosis into the synaptic cleft (step ③ in Fig. 12–5). Acetylcholine diffuses to the postsynaptic cell (another

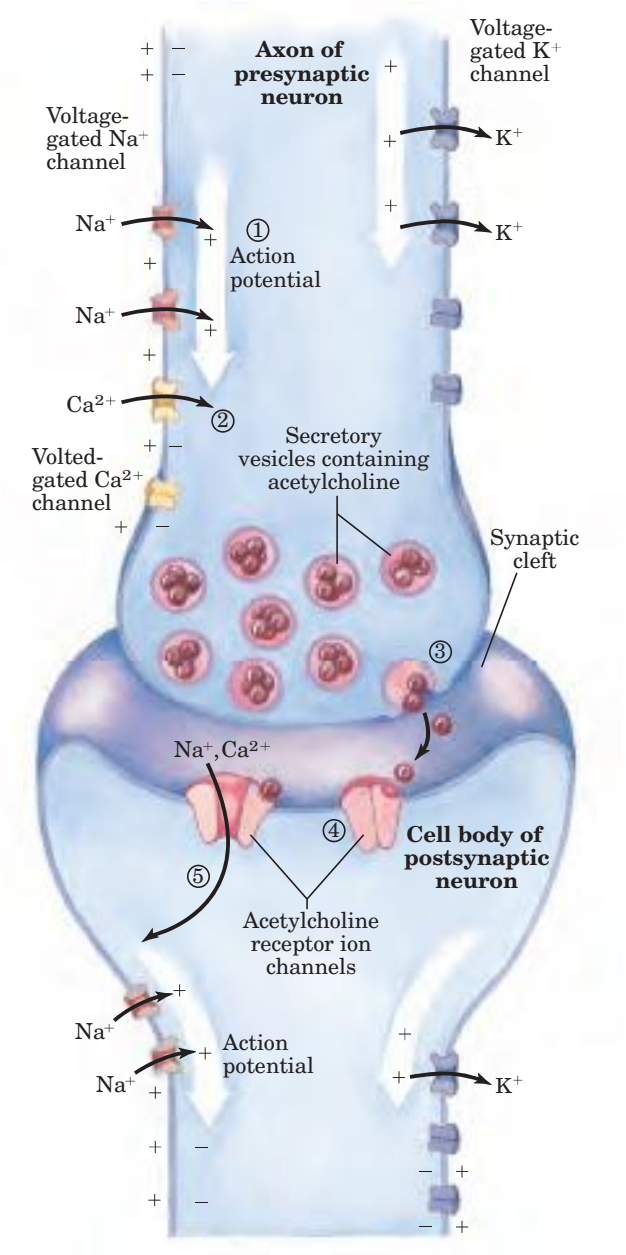


FIGURE 12–5 Role of voltage-gated and ligand-gated ion channels in neural transmission. Initially, the plasma membrane of the presynaptic neuron is polarized (inside negative) through the action of the electrogenic Na^+K^+ ATPase, which pumps 3 Na^+ out for every 2 K^+ pumped into the neuron (see Fig. 12–3). ① A stimulus to this neuron causes an action potential to move along the axon (white arrow), away from the cell body. The opening of one voltage-gated Na^+ channel allows Na^+ entry, and the resulting local depolarization causes the adjacent Na^+ channel to open, and so on. The directionality of movement of the action potential is ensured by the brief refractory period that follows the opening of each voltage-gated Na^+ channel. ② When the wave of depolarization reaches the axon tip, voltage-gated Ca^{2+} channels open, allowing Ca^{2+} entry into the presynaptic neuron. ③ The resulting increase in internal $[Ca^{2+}]$ triggers exocytic release of the neurotransmitter acetylcholine into the synaptic cleft. ④ Acetylcholine binds to a receptor on the postsynaptic neuron, causing its ligand-gated ion channel to open. ⑤ Extracellular Na^+ and Ca^{2+} enter through this channel, depolarizing the postsynaptic cell. The electrical signal has thus passed to the cell body of the postsynaptic neuron and will move along its axon to a third neuron by this same sequence of events.

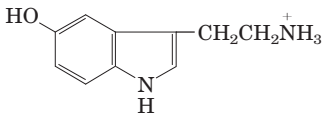
neuron or a myocyte), where it binds to acetylcholine receptors and triggers depolarization. Thus the message is passed to the next cell in the circuit.

We see, then, that gated ion channels convey signals in either of two ways: by changing the cytosolic concentration of an ion (such as Ca^{2+}), which then serves as an intracellular **second messenger** (the hormone or neurotransmitter is the first messenger), or by changing V_m and affecting other membrane proteins that are sensitive to V_m. The passage of an electrical signal through one neuron and on to the next illustrates both types of mechanism.

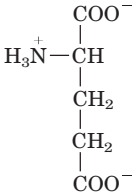
Neurons Have Receptor Channels That Respond to Different Neurotransmitters

Animal cells, especially those of the nervous system, contain a variety of ion channels gated by ligands, voltage, or both. The neurotransmitters 5-hydroxytryptamine (serotonin), glutamate, and glycine can all act through receptor channels that are structurally related to the acetylcholine receptor. Serotonin and glutamate trigger the opening of cation (K^+, Na^+, Ca^{2+}) channels, whereas glycine opens Cl^--specific channels. Cation and anion channels are distinguished by subtle differences in the amino acid residues that line the hydrophilic channel. Cation channels have negatively charged Glu and Asp side chains at crucial positions. When a few of these acidic residues are experimentally replaced with basic residues, the cation channel is converted to an anion channel.

Depending on which ion passes through a channel, the ligand (neurotransmitter) for that channel either depolarizes or hyperpolarizes the target cell. A single neuron normally receives input from several (or many) other neurons, each releasing its own characteristic neurotransmitter with its characteristic depolarizing or hyperpolarizing effect. The target cell's V_m therefore reflects the *integrated* input (Fig. 12–1d) from multi-



Serotonin
(5-hydroxytryptamine)



Glutamate

ple neurons. The cell responds with an action potential only if the integrated input adds up to a net depolarization of sufficient size.

The receptor channels for acetylcholine, glycine, glutamate, and γ-aminobutyric acid (GABA) are gated by *extracellular* ligands. *Intracellular* second messengers—such as cAMP, cGMP (3′,5′-cyclic GMP, a close analog of cAMP), IP_3 (inositol 1,4,5-trisphosphate), Ca^{2+}, and ATP—regulate ion channels of another class, which, as we shall see in Section 12.7, participate in the sensory transductions of vision, olfaction, and gustation.

SUMMARY 12.2 Gated Ion Channels

- Ion channels gated by ligands or membrane potential are central to signaling in neurons and other cells.
- The acetylcholine receptor of neurons and myocytes is a ligand-gated ion channel.
- The voltage-gated Na^+ and K^+ channels of neuronal membranes carry the action potential along the axon as a wave of depolarization (Na^+ influx) followed by repolarization (K^+ efflux).
- The arrival of an action potential triggers neurotransmitter release from the presynaptic cell. The neurotransmitter (acetylcholine, for example) diffuses to the postsynaptic cell, binds to specific receptors in the plasma membrane, and triggers a change in V_m.

12.3 Receptor Enzymes

A fundamentally different mechanism of signal transduction is carried out by the receptor enzymes. These proteins have a ligand-binding domain on the extracellular surface of the plasma membrane and an enzyme active site on the cytosolic side, with the two domains connected by a single transmembrane segment. Commonly, the receptor enzyme is a protein kinase that phosphorylates Tyr residues in specific target proteins; the insulin receptor is the prototype for this group. In plants, the protein kinase of receptors is specific for Ser or Thr residues. Other receptor enzymes synthesize the intracellular second messenger cGMP in response to extracellular signals. The receptor for atrial natriuretic factor is typical of this type.

The Insulin Receptor Is a Tyrosine-Specific Protein Kinase

Insulin regulates both metabolism and gene expression: the insulin signal passes from the plasma membrane receptor to insulin-sensitive metabolic enzymes and to the nucleus, where it stimulates the transcription of specific genes. The active insulin receptor consists of two identical α chains protruding from the outer face of the plasma membrane and two transmembrane β subunits with their carboxyl termini protruding into the cytosol (Fig. 12–6, step ①). The α chains contain the insulin-binding domain, and the intracellular domains of the β chains contain the protein kinase activity that transfers a phosphoryl group from ATP to the hydroxyl group of Tyr residues in specific target proteins. Signaling through the insulin receptor begins (step ①) when binding of insulin to the α chains activates the Tyr kinase activity of the β chains, and each αβ monomer phosphorylates three critical Tyr residues near the carboxyl terminus of the β chain of its partner in the dimer. This **autophosphorylation** opens up the active site so that the enzyme can phosphorylate Tyr residues of other target proteins (Fig. 12–7).

One of these target proteins (Fig. 12–6, step ②) is insulin receptor substrate-1 (IRS-1). Once phosphorylated on its Tyr residues, IRS-1 becomes the point of nucleation for a complex of proteins (step ③) that carry the message from the insulin receptor to end targets in the cytosol and nucleus, through a long series of intermediate proteins. First, a Ⓟ–Tyr residue in IRS-1 is bound by the **SH2 domain** of the protein Grb2. (SH2 is an abbreviation of *S*rc *h*omology *2*; the sequences of SH2 domains are similar to a domain in another protein Tyr kinase, Src (pronounced sark).) A number of signaling proteins contain SH2 domains, all of which bind Ⓟ–Tyr residues in a protein partner. Grb2 also contains a second protein-binding domain, SH3, that binds to regions rich in Pro residues. Grb2 binds to a proline-rich region of Sos, recruiting Sos to the growing receptor complex. When bound to Grb2, Sos catalyzes the replacement of bound GDP by GTP on Ras, one of a family of guanosine nucleotide–binding proteins **(G proteins)** that mediate a wide variety of signal transductions (Section 12.4). When GTP is bound, Ras can activate a protein kinase, Raf-1 (step ④), the first of three protein kinases—Raf-1, MEK, and ERK—that form a cascade in which each kinase activates the next by phosphorylation (step ⑤). The protein kinase ERK is activated by phosphorylation of both a Thr and a Tyr residue. When activated, it mediates some of the biological effects of insulin by entering the nucleus and phosphorylating proteins such as Elk1, which modulates the transcription of about 100 insulin-regulated genes (step ⑥).

The proteins Raf-1, MEK, and ERK are members of three larger families, for which several nomenclatures are employed. ERK is a member of the **MAPK** family (*m*itogen-*a*ctivated *p*rotein *k*inases; mitogens are signals that act from outside the cell to induce mitosis and cell division). Soon after discovery of the first MAPK, that enzyme was found to be activated by another protein kinase, which came to be called MAP kinase kinase (MEK

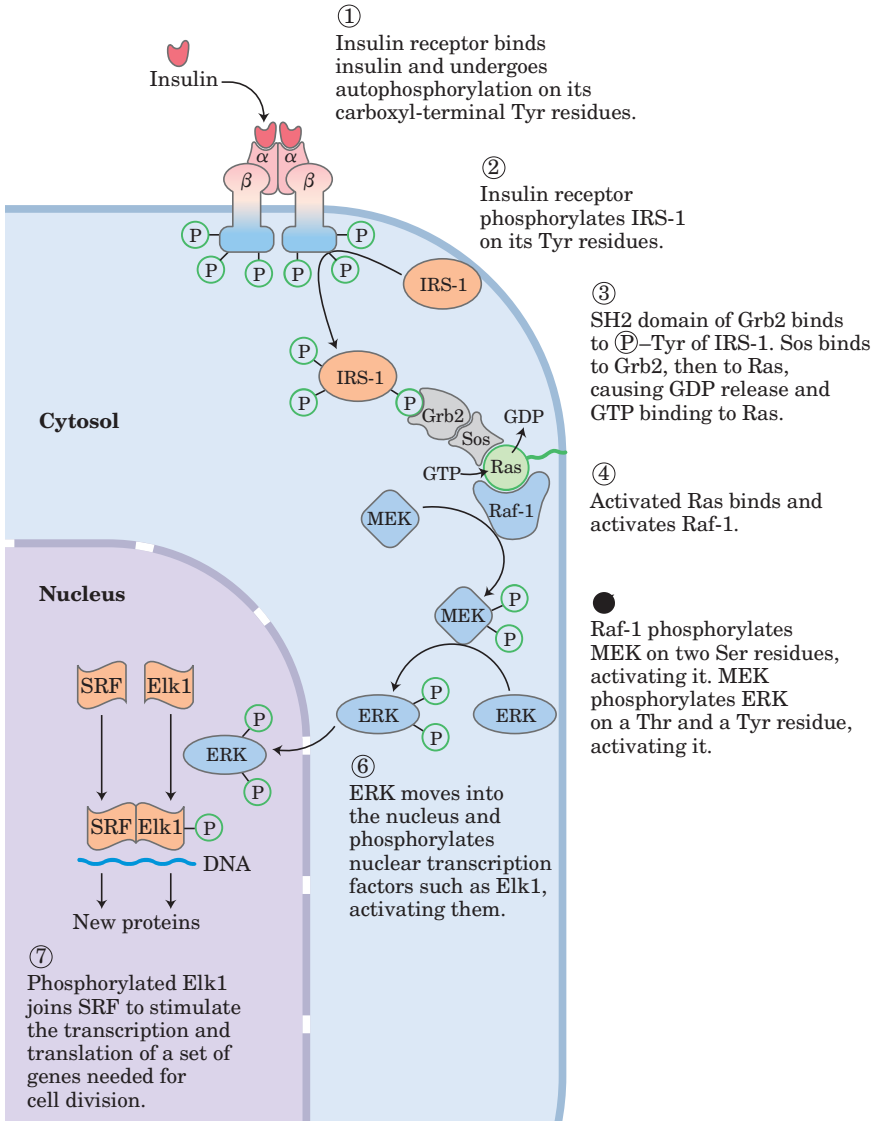


FIGURE 12–6 Regulation of gene expression by insulin. The insulin receptor consists of two α chains on the outer face of the plasma membrane and two β chains that traverse the membrane and protrude from the cytoplasmic face. Binding of insulin to the α chains triggers a conformational change that allows the autophosphorylation of Tyr residues in the carboxyl-terminal domain of the β subunits. Autophosphorylation further activates the Tyr kinase domain, which then catalyzes phosphorylation of other target proteins. The signaling pathway by which insulin regulates the expression of specific genes consists of a cascade of protein kinases, each of which activates the next. The insulin receptor is a Tyr-specific kinase; the other kinases (all shown in blue) phosphorylate Ser or Thr residues. MEK is a dual-specificity kinase, which phosphorylates both a Thr and a Tyr residue in ERK (extracellular *r*egulated *k*inase); MEK is mitogen-activated, ERK-activating kinase; SRF is serum response factor. Abbreviations for other components are explained in the text.

belongs to this family); and when a third kinase that activated MAP kinase kinase was discovered, it was given the slightly ludicrous family name MAP kinase kinase kinase (Raf-1 is a member of this family; Fig. 12–6). Slightly less cumbersome are the acronyms for these three families, MAPK, MAPKK, and MAPKKK. Kinases in the MAPK and MAPKKK families are specific for Ser or Thr residues, but MAPKKs (here, MEK) phosphorylate both a Ser and a Tyr residue in their substrate, a MAPK (here, ERK).

Biochemists now recognize the insulin pathway as but one instance of a more general theme in which hormone signals, via pathways similar to that shown in Figure 12–6, result in phosphorylation of target enzymes by protein kinases. The target of phosphorylation is often another protein kinase, which then phosphorylates a third protein kinase, and so on. The result is a cascade of reactions that amplifies the initial signal by many orders of magnitude (see Fig. 12–1b). Cascades such as that shown in Figure 12–6 are called **MAPK cascades.**

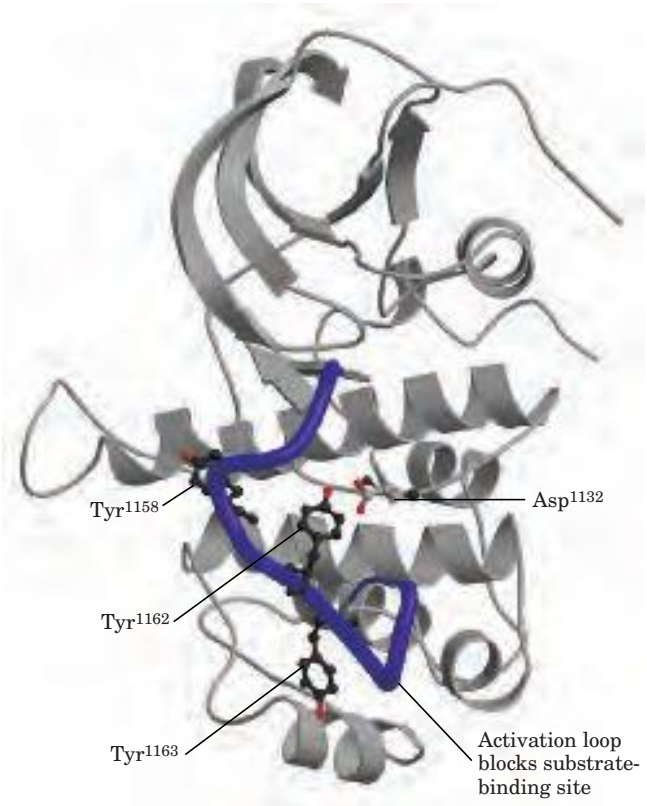
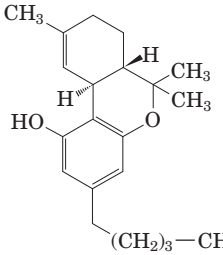


FIGURE 12–7 Activation of the insulin-receptor Tyr kinase by autophosphorylation. (a) In the inactive form of the Tyr kinase domain (PDB ID 1IRK), the activation loop (blue) sits in the active site, and none of the critical Tyr residues (black and red ball-and-stick structures) are phosphorylated. This conformation is stabilized by hydrogen bonding between Tyr^{1162} and Asp^{1132}. **(b)** When insulin binds to the α chains of insulin receptors, the Tyr kinase of each β subunit of the dimer phosphorylates three Tyr residues (Tyr^{1158}, Tyr^{1162}, and Tyr^{1163}) on the other β subunit (shown here; PDB ID 1IR3). (Phosphoryl groups are depicted here as an orange space-filling phosphorus atom and red ball-and-stick oxygen atoms.) The effect of introducing three highly charged Ⓟ–Tyr residues is to force a 30 Å change in the position of the activation loop, away from the substrate-binding site, which becomes available to bind to and phosphorylate a target protein, shown here as a red arrow.

Grb2 is not the only protein that associates with phosphorylated IRS-1. The enzyme phosphoinositide 3-kinase (PI-3K) binds IRS-1 through the former's SH2 domain (Fig. 12–8). Thus activated, PI-3K converts the membrane lipid phosphatidylinositol 4,5-bisphosphate (see Fig. 10–15), also called PIP_2, to phosphatidylinositol 3,4,5-trisphosphate (PIP_3). When bound to PIP_3, protein kinase B (PKB) is phosphorylated and activated by yet another protein kinase, PDK1. The activated PKB then phosphorylates Ser or Thr residues on its target proteins, one of which is glycogen synthase kinase 3 (GSK3). In its active, nonphosphorylated form, GSK3 phosphorylates glycogen synthase, inactivating it and thereby contributing to the slowing of glycogen synthesis. (This mechanism is believed to be only part of the explanation for the effects of insulin on glycogen metabolism.) When phosphorylated by PKB, GSK3 is inactivated. By thus preventing inactivation of glycogen synthase in liver and muscle, the cascade of protein phosphorylations initiated by insulin stimulates glycogen synthesis (Fig. 12–8). In muscle, PKB triggers the movement of glucose transporters (GLUT4) from internal vesicles to the plasma membrane, stimulating glucose uptake from the blood (Fig. 12–8; see also Box 11–2). PKB also functions in several other signaling pathways, including that triggered by Δ^9-tetrahydrocannabinol (THC), the active ingredient of marijuana



Δ^9-Tetrahydrocannabinol (THC)

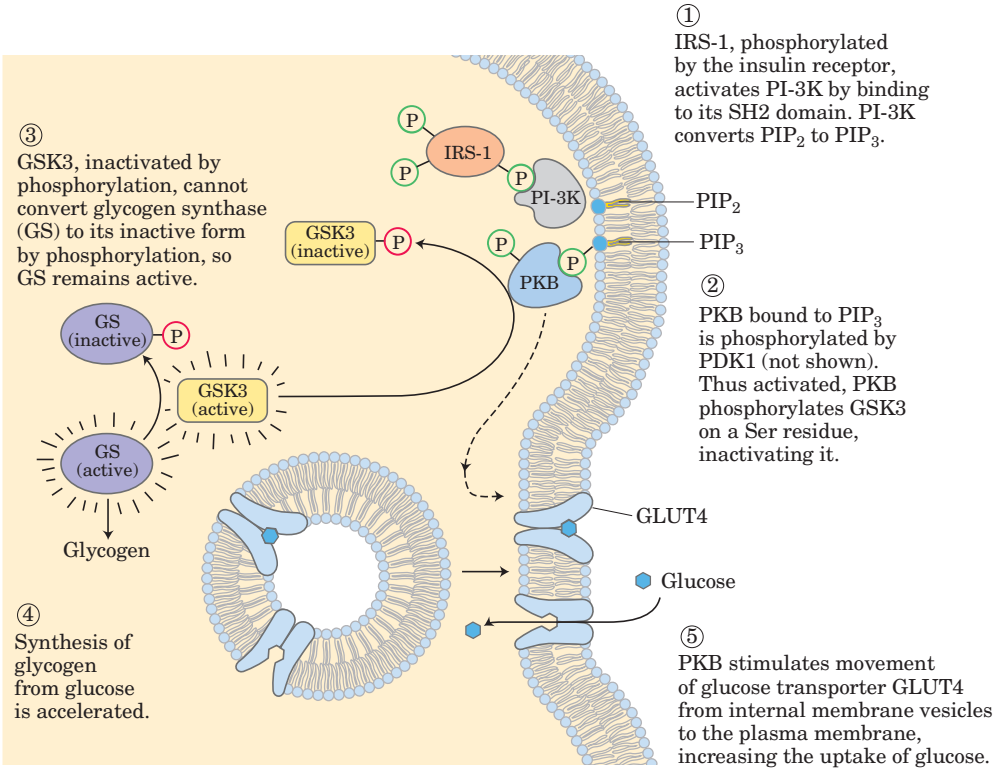
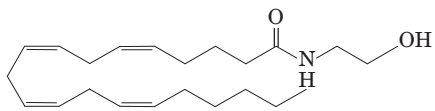


FIGURE 12–8 Activation of glycogen synthase by insulin. Transmission of the signal is mediated by PI-3 kinase (PI-3K) and protein kinase B (PKB).

and hashish. THC activates the CB_1 receptor in the plasma membrane of neurons in the brain, triggering a signaling cascade that involves MAPKs. One consequence of CB_1 activation is the stimulation of appetite, one of the well-established effects of marijuana use. The normal ligands for the CB_1 receptor are endocannabinoids such as anandamide, which serve to protect the brain from the toxicity of excessive neuronal activity—as in an epileptic seizure, for example. Hashish has for centuries been used in the treatment of epilepsy.



Anandamide (arachidonylethanolamide, an endogenous cannabinoid)

As in all signaling pathways, there is a mechanism for terminating signaling through the PI-3K–PKB pathway. A PIP_3-specific phosphatase (PTEN in humans) removes the phosphate at the 3 position of PIP_3 to produce PIP_2, which no longer serves as a binding site for PKB, and the signaling chain is broken. In various types of advanced cancer, tumor cells often have a defect in the PTEN gene and thus have abnormally high levels of PIP_3 and of PKB activity. The result seems to be a continuing signal for cell division and thus tumor growth. ■

What spurred the evolution of such complicated regulatory machinery? This system allows one activated receptor to activate several IRS-1 molecules, amplifying the insulin signal, and it provides for the integration of signals from several receptors, each of which can phosphorylate IRS-1. Furthermore, because IRS-1 can activate any of several proteins that contain SH2 domains, a single receptor acting through IRS-1 can trigger two or more signaling pathways; insulin affects gene expression through the Grb2-Sos-Ras-MAPK pathway and glycogen metabolism through the PI-3K–PKB pathway.

The insulin receptor is the prototype for a number of receptor enzymes with a similar structure and **receptor Tyr kinase** activity. The receptors for epidermal growth factor and platelet-derived growth factor, for example, have structural and sequence similarities to the insulin receptor, and both have a protein Tyr kinase activity that phosphorylates IRS-1. Many of these receptors dimerize after binding ligand; the insulin receptor is already a dimer before insulin binds. The binding of adaptor proteins such as Grb2 to Ⓟ–Tyr residues is a common mechanism for promoting protein-protein interactions, a subject to which we return in Section 12.5.

In addition to the many receptors that act as protein Tyr kinases, a number of receptorlike plasma membrane proteins have protein Tyr phosphatase activity. Based on the structures of these proteins, we can surmise that their ligands are components of the extracellular matrix or the

surfaces of other cells. Although their signaling roles are not yet as well understood as those of the receptor Tyr kinases, they clearly have the potential to reverse the actions of signals that stimulate these kinases.

A variation on the basic theme of receptor Tyr kinases is seen in receptors that have no intrinsic protein kinase activity but, when occupied by their ligand, bind a *soluble* Tyr kinase. One example is the system that regulates the formation of erythrocytes in mammals. The **cytokine** (developmental signal) for this system is erythropoietin (EPO), a 165 amino acid protein produced in the kidneys. When EPO binds to its plasma membrane receptor (Fig. 12–9), the receptor dimerizes and can now bind the soluble protein kinase JAK (*Ja*nus *k*inase). This binding activates JAK, which phosphorylates several Tyr residues in the cytoplasmic domain of the EPO receptor. A family of transcription factors, collectively called STATs (*s*ignal *t*ransducers and *a*ctivators of *t*ranscription), are also targets of the JAK kinase activity. An SH2 domain in STAT5 binds Ⓟ–Tyr residues in the EPO receptor, positioning it for this phosphorylation by JAK. When STAT5 is phosphorylated in response to EPO, it forms dimers, exposing a signal for its transport into the nucleus. There, STAT5 causes the expression (transcription) of specific genes essential for erythrocyte maturation. This JAK-STAT system operates in a number of other signaling pathways, including that for the hormone leptin, described in detail in Chapter 23 (see Fig. 23–34). Activated JAK can also trigger, through Grb2, the MAPK cascade (Fig. 12–6), which leads to altered expression of specific genes.

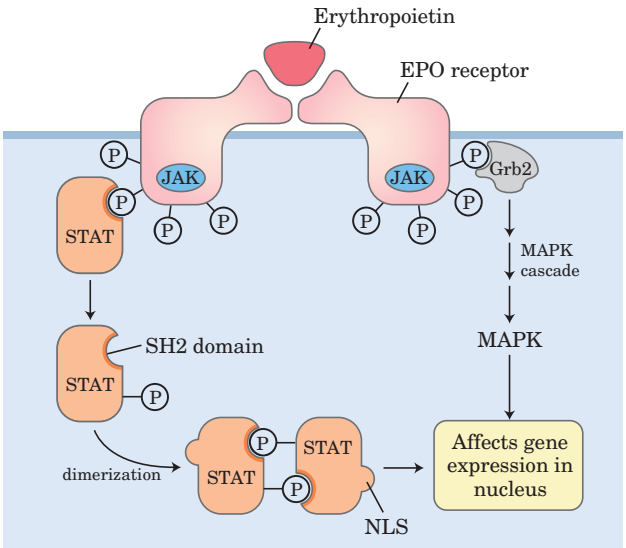
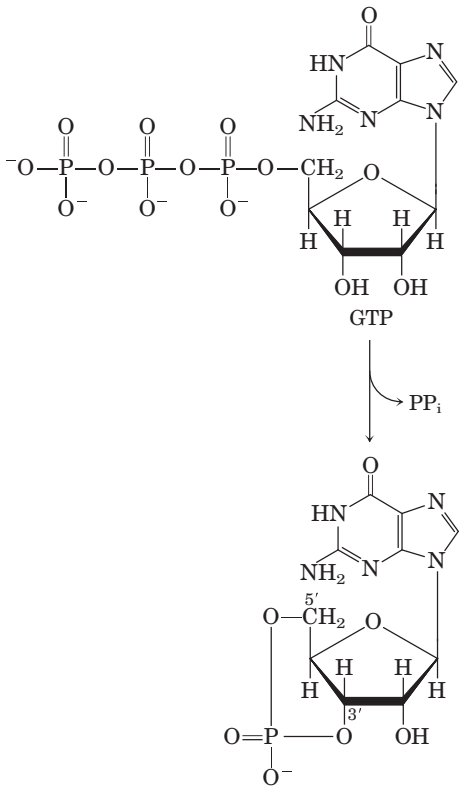


FIGURE 12–9 The JAK-STAT transduction mechanism for the erythropoietin receptor. Binding of erythropoietin (EPO) causes dimerization of the EPO receptor, which allows the soluble Tyr kinase JAK to bind to the internal domain of the receptor and phosphorylate it on several Tyr residues. The STAT protein STAT5 contains an SH2 domain and binds to the Ⓟ–Tyr residues on the receptor, bringing the receptor into proximity with JAK. Phosphorylation of STAT5 by JAK allows two STAT molecules to dimerize, each binding the other's Ⓟ–Tyr residue. Dimerization of STAT5 exposes a nuclear localization sequence (NLS) that targets STAT5 for transport into the nucleus. In the nucleus, STAT causes the expression of genes controlled by EPO. A second signaling pathway is also triggered by autophosphorylation of JAK that is associated with EPO binding to its receptor. The adaptor protein Grb2 binds Ⓟ–Tyr in JAK and triggers the MAPK cascade, as in the insulin system (see Fig. 12–6).

Src is another soluble protein Tyr kinase that associates with certain receptors when they bind their ligands. Src was the first protein found to have the characteristic Ⓟ–Tyr-binding domain that was subsequently named the Src homology (SH2) domain. Yet another example of a receptor's association with a soluble protein kinase is the Toll-like receptor (TLR4) system through which mammals detect the bacterial lipopolysaccharide (LPS), a potent toxin. We return to the Toll-like receptor system in Section 12.6, in the context of the evolution of signaling proteins.

Receptor Guanylyl Cyclases Generate the Second Messenger cGMP

Guanylyl cyclases (Fig. 12–10) are another type of receptor enzyme. When activated, a guanylyl cyclase produces **guanosine 3′,5′-cyclic monophosphate (cyclic GMP, cGMP)** from GTP:



Guanosine 3′,5′-cyclic monophosphate (cGMP)

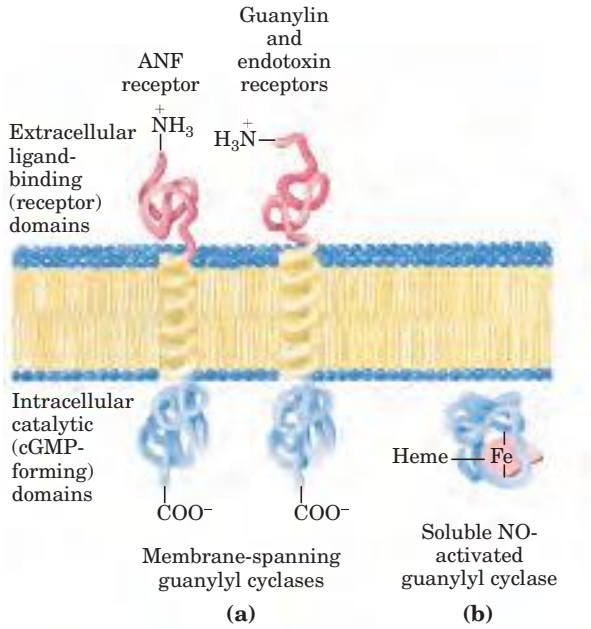
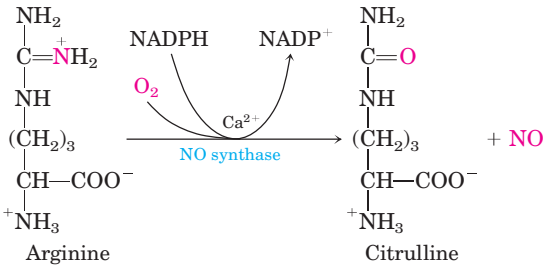


FIGURE 12–10 Two types (isozymes) of guanylyl cyclase that participate in signal transduction. (a) One isozyme exists in two similar membrane-spanning forms that are activated by their extracellular ligands: atrial natriuretic factor, ANF (receptors in cells of the renal collecting ducts and the smooth muscle of blood vessels), and guanylin (receptors in intestinal epithelial cells). The guanylin receptor is also the target of a type of bacterial endotoxin that triggers severe diarrhea. **(b)** The other isozyme is a soluble enzyme that is activated by intracellular nitric oxide (NO); this form is found in many tissues, including smooth muscle of the heart and blood vessels.

Cyclic GMP is a second messenger that carries different messages in different tissues. In the kidney and intestine it triggers changes in ion transport and water retention; in cardiac muscle (a type of smooth muscle) it signals relaxation; in the brain it may be involved both in development and in adult brain function. Guanylyl cyclase in the kidney is activated by the hormone **atrial natriuretic factor (ANF),** which is released by cells in the atrium of the heart when the heart is stretched by increased blood volume. Carried in the blood to the kidney, ANF activates guanylyl cyclase in cells of the collecting ducts (Fig. 12–10a). The resulting rise in [cGMP] triggers increased renal excretion of Na^+ and, consequently, of water, driven by the change in osmotic pressure. Water loss reduces the blood volume, countering the stimulus that initially led to ANF secretion. Vascular smooth muscle also has an ANF receptor—guanylyl cyclase; on binding to this receptor, ANF causes relaxation (vasodilation) of the blood vessel, which increases blood flow while decreasing blood pressure.

A similar receptor guanylyl cyclase in the plasma membrane of intestinal epithelial cells is activated by an intestinal peptide, **guanylin,** which regulates Cl^- secretion in the intestine. This receptor is also the target of a heat-stable peptide endotoxin produced by *Escherichia coli* and other gram-negative bacteria. The elevation in [cGMP] caused by the endotoxin increases Cl^- secretion and consequently decreases reabsorption of water by the intestinal epithelium, producing diarrhea.

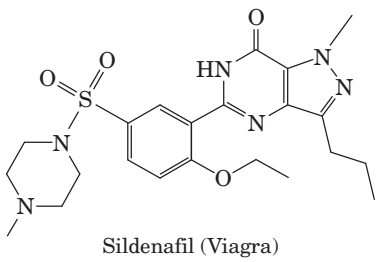
A distinctly different type of guanylyl cyclase is a cytosolic protein with a tightly associated heme group (Fig. 12–10b), an enzyme activated by nitric oxide (NO). Nitric oxide is produced from arginine by Ca^{2+}-dependent **NO synthase,** present in many mammalian tissues, and diffuses from its cell of origin into nearby cells. NO is sufficiently nonpolar to cross plasma membranes without a carrier. In the target cell, it binds to the heme group of guanylyl cyclase and activates cGMP production. In the heart, cGMP reduces the forcefulness of contractions by stimulating the ion pump(s) that expel Ca^{2+} from the cytosol.



This NO-induced relaxation of cardiac muscle is the same response brought about by nitroglycerin tablets and other nitrovasodilators taken to relieve angina, the pain caused by contraction of a heart deprived of O_2 because of blocked coronary arteries. Nitric oxide is unstable and its action is brief; within seconds of its formation, it undergoes oxidation to nitrite or nitrate. Nitrovasodilators produce long-lasting relaxation of cardiac muscle because they break down over several hours, yielding a steady stream of NO. The value of nitroglycerin as a treatment for angina was discovered serendipitously in factories producing nitroglycerin as an explosive in the 1860s. Workers with angina reported that their condition was much improved during the work week but returned on weekends. The physicians treating these workers heard this story so often that they made the connection, and a drug was born.

The effects of increased cGMP synthesis diminish after the stimulus ceases, because a specific phosphodiesterase (cGMP PDE) converts cGMP to the inactive 5′-GMP. Humans have several isoforms of cGMP PDE, with different tissue distributions. The isoform in the blood vessels of the penis is inhibited by the drug sildenafil (Viagra), which therefore causes cGMP levels to remain elevated once raised by an appropriate stimulus, accounting for the usefulness of this drug in the treatment of erectile dysfunction. ■

Most of the actions of cGMP in animals are believed to be mediated by **cGMP-dependent protein kinase,** also called **protein kinase G** or **PKG,** which, when ac-



Sildenafil (Viagra)

tivated by cGMP, phosphorylates Ser and Thr residues in target proteins. The catalytic and regulatory domains of this enzyme are in a single polypeptide (M_r ~80,000). Part of the regulatory domain fits snugly in the substrate-binding site. Binding of cGMP forces this part of the regulatory domain out of the binding site, activating the catalytic domain.

Cyclic GMP has a second mode of action in the vertebrate eye: it causes ion-specific channels to open in the retinal rod and cone cells. We return to this role of cGMP in the discussion of vision in Section 12.7.

SUMMARY 12.3 Receptor Enzymes

- The insulin receptor is the prototype of receptor enzymes with Tyr kinase activity. When insulin binds to its receptor, each $\alpha\beta$ monomer of the receptor phosphorylates the β chain of its partner, activating the receptor's Tyr kinase activity. The kinase catalyzes the phosphorylation of Tyr residues on other proteins such as IRS-1.
- Ⓟ–Tyr residues in IRS-1 serve as binding sites for proteins with SH2 domains. Some of these proteins, such as Grb2, have two or more protein-binding domains and can serve as adaptors that bring two proteins into proximity.
- Further protein-protein interactions result in GTP binding to and activation of the Ras protein, which in turn activates a protein kinase cascade that ends with the phosphorylation of target proteins in the cytosol and nucleus. The result is specific metabolic changes and altered gene expression.
- Several signals, including atrial natriuretic factor and the intestinal peptide guanylin, act through receptor enzymes with guanylyl cyclase activity. The cGMP produced acts as a second messenger, activating cGMP-dependent protein kinase (PKG). This enzyme alters metabolism by phosphorylating specific enzyme targets.
- Nitric oxide (NO) is a short-lived messenger that acts by stimulating a soluble guanylyl cyclase, raising [cGMP] and stimulating PKG.

12.4 G Protein–Coupled Receptors and Second Messengers

A third mechanism of signal transduction, distinct from gated ion channels and receptor enzymes, is defined by three essential components: a plasma membrane receptor with seven transmembrane helical segments, an enzyme in the plasma membrane that generates an intracellular second messenger, and a **guanosine nucleotide–binding protein (G protein).** The G protein, stimulated by the activated receptor, exchanges bound GDP for GTP; the GTP-protein dissociates from the occupied receptor and binds to a nearby enzyme, altering its activity. The human genome encodes more than 1,000 members of this family of receptors, specialized for transducing messages as diverse as light, smells, tastes, and hormones. The β-adrenergic receptor, which mediates the effects of epinephrine on many tissues, is the prototype for this type of transducing system.

The β-Adrenergic Receptor System Acts through the Second Messenger cAMP

Epinephrine action begins when the hormone binds to a protein receptor in the plasma membrane of a hormone-sensitive cell. **Adrenergic receptors** ("adrenergic" reflects the alternative name for epinephrine, adrenaline) are of four general types, α_1, α_2, β_1, and β_2, defined by subtle differences in their affinities and responses to a group of agonists and antagonists. **Agonists** are structural analogs that bind to a receptor and mimic the effects of its natural ligand; **antagonists** are analogs that bind without triggering the normal effect and thereby block the effects of agonists. In some cases, the affinity of the synthetic agonist or antagonist for the receptor is greater than that of the natural agonist (Fig. 12–11). The four types of adrenergic receptors are found in different target tissues and mediate different responses to epinephrine. Here we focus on the ***β*-adrenergic receptors** of muscle, liver, and adipose tissue. These receptors mediate changes in fuel metabolism, as described in Chapter 23, including the increased breakdown of glycogen and fat. Adrenergic receptors of the β_1 and β_2 subtypes act through the same mechanism, so in our discussion, "β-adrenergic" applies to both types.

The β-adrenergic receptor is an integral protein with seven hydrophobic regions of 20 to 28 amino acid residues that "snake" back and forth across the plasma membrane seven times. This protein is a member of a very large family of receptors, all with seven transmembrane helices, that are commonly called **serpentine receptors, G protein–coupled receptors (GPCR),** or **7 transmembrane segment (7tm) receptors.** The binding of epinephrine to a site on the

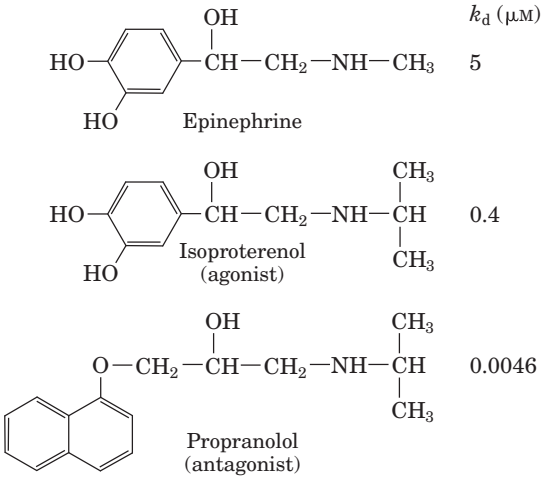


FIGURE 12–11 Epinephrine and its synthetic analogs. Epinephrine, also called adrenaline, is released from the adrenal gland and regulates energy-yielding metabolism in muscle, liver, and adipose tissue. It also serves as a neurotransmitter in adrenergic neurons. Its affinity for its receptor is expressed as a dissociation constant for the receptor-ligand complex. Isoproterenol and propranolol are synthetic analogs, one an agonist with an affinity for the receptor that is higher than that of epinephrine, and the other an antagonist with extremely high affinity.

receptor deep within the membrane (Fig. 12–12, step ①) promotes a conformational change in the receptor's intracellular domain that affects its interaction with the second protein in the signal-transduction pathway, a heterotrimeric GTP-binding **stimulatory G protein,** or **G_s,** on the cytosolic side of the plasma membrane. Alfred G. Gilman and Martin Rodbell discovered that when GTP is bound to G_s, G_s stimulates the production of cAMP by adenylyl cyclase (see below) in the plasma membrane. The function of G_s as a molecular switch resembles that of another class of G proteins typified by Ras, discussed in Section 12.3 in the context of the insulin receptor. Structurally, G_s and Ras are quite distinct; G proteins of the Ras type are monomers (M_r ~20,000), whereas the G proteins that interact with serpentine


Alfred G. Gilman


Martin Rodbell, 1925–1998

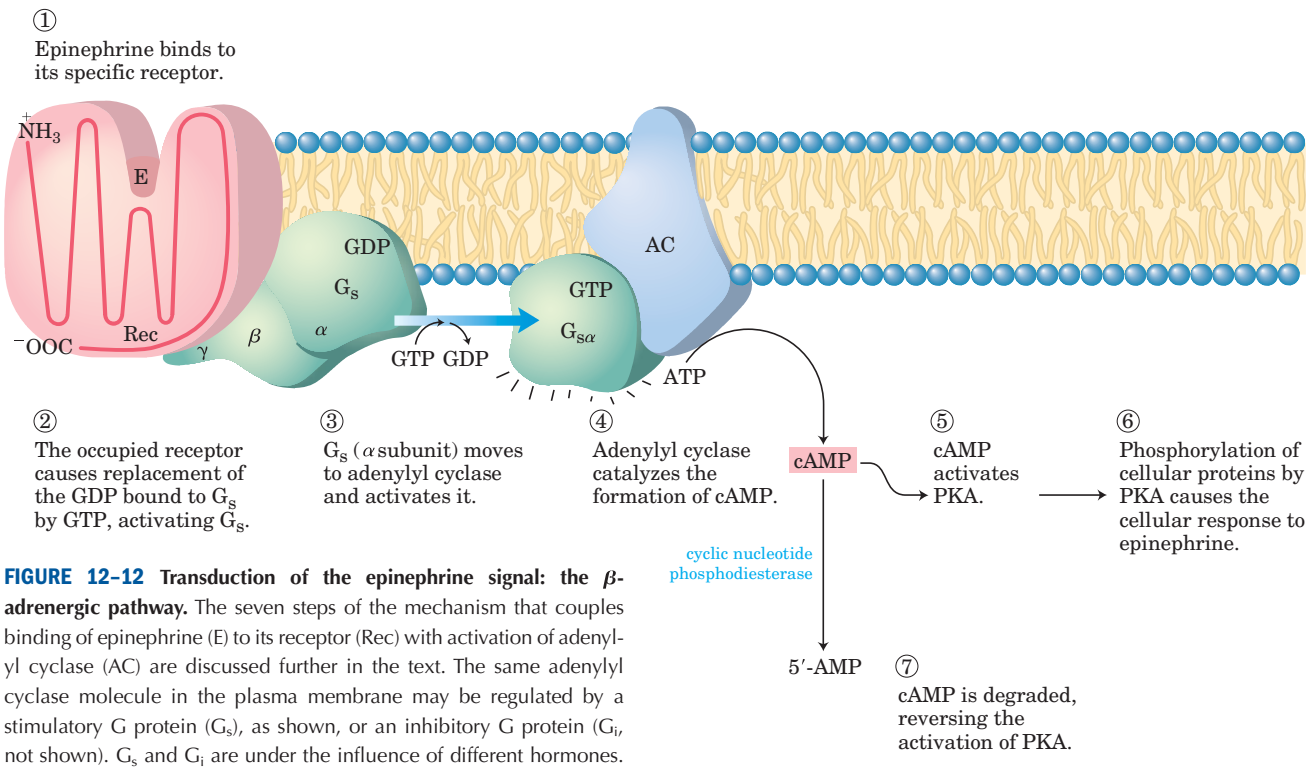
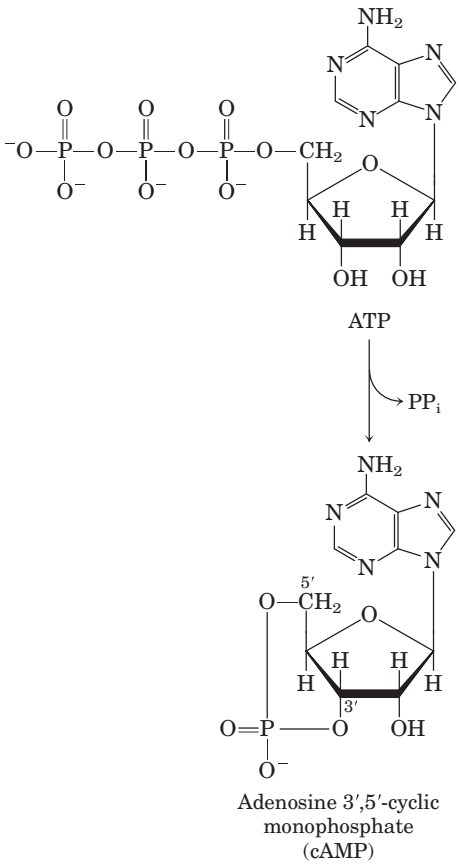


FIGURE 12–12 Transduction of the epinephrine signal: the β-adrenergic pathway. The seven steps of the mechanism that couples binding of epinephrine (E) to its receptor (Rec) with activation of adenylyl cyclase (AC) are discussed further in the text. The same adenylyl cyclase molecule in the plasma membrane may be regulated by a stimulatory G protein (G_s), as shown, or an inhibitory G protein (G_i, not shown). G_s and G_i are under the influence of different hormones. Hormones that induce GTP binding to G_i cause *inhibition* of adenylyl cyclase, resulting in lower cellular [cAMP].

receptors are trimers of three different subunits, α (M_r 43,000), β (M_r 37,000), and γ (M_r 7,500 to 10,000).

When the nucleotide-binding site of G_s (on the α subunit) is occupied by GTP, G_s is active and can activate adenylyl cyclase (AC in Fig. 12–12); with GDP bound to the site, G_s is inactive. Binding of epinephrine enables the receptor to catalyze displacement of bound GDP by GTP, converting G_s to its active form (step ②). As this occurs, the β and γ subunits of G_s dissociate from the α subunit, and $G_{s\alpha}$, with its bound GTP, moves in the plane of the membrane from the receptor to a nearby molecule of adenylyl cyclase (step ③). The $G_{s\alpha}$ is held to the membrane by a covalently attached palmitoyl group (see Fig. 11–14).

Adenylyl cyclase (Fig. 12–13) is an integral protein of the plasma membrane, with its active site on the cytosolic face. It catalyzes the synthesis of cAMP from ATP:



The association of active $G_{s\alpha}$ with adenylyl cyclase stimulates the cyclase to catalyze cAMP synthesis (Fig. 12–12, step ④), raising the cytosolic [cAMP]. This stimulation by $G_{s\alpha}$ is self-limiting; $G_{s\alpha}$ is a GTPase that turns itself off by converting its bound GTP to GDP (Fig. 12–14). The now inactive $G_{s\alpha}$ dissociates from adenylyl cyclase, rendering the cyclase inactive. After $G_{s\alpha}$ reassociates with the β and γ subunits ($G_{s\beta\gamma}$), G_s is again available to interact with a hormone-bound receptor.

Trimeric G Proteins: Molecular On/Off Switches

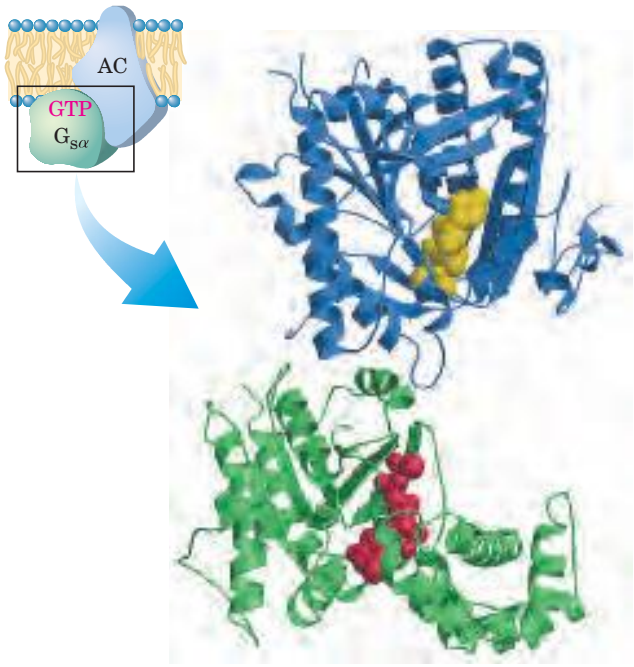


FIGURE 12–13 Interaction of $G_{s\alpha}$ with adenylyl cyclase. (PDB ID 1AZS) The soluble catalytic core of the adenylyl cyclase (AC, blue), severed from its membrane anchor, was cocrystallized with $G_{s\alpha}$ (green) to give this crystal structure. The plant terpene forskolin (yellow) is a drug that strongly stimulates the enzyme, and GTP (red) bound to $G_{s\alpha}$ triggers interaction of $G_{s\alpha}$ with adenylyl cyclase.

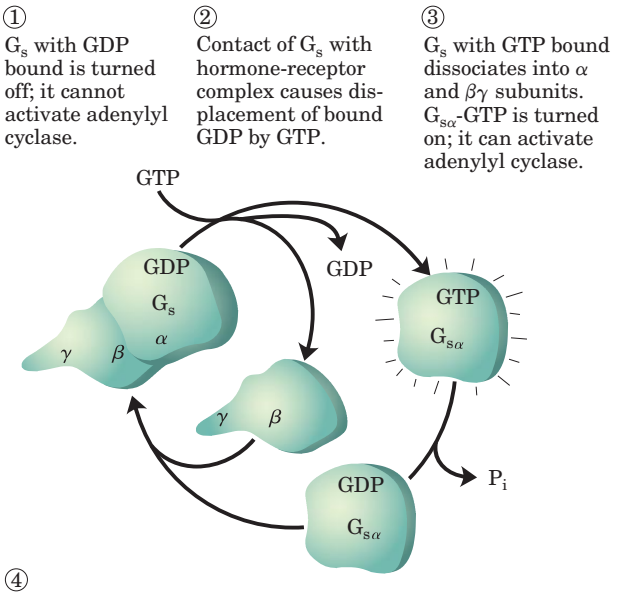


FIGURE 12–14 Self-inactivation of G_s. The steps are further described in the text. The protein's intrinsic GTPase activity, in many cases stimulated by RGS proteins (*r*egulators of *G* protein *s*ignaling), determines how quickly bound GTP is hydrolyzed to GDP and thus how long the G protein remains active.

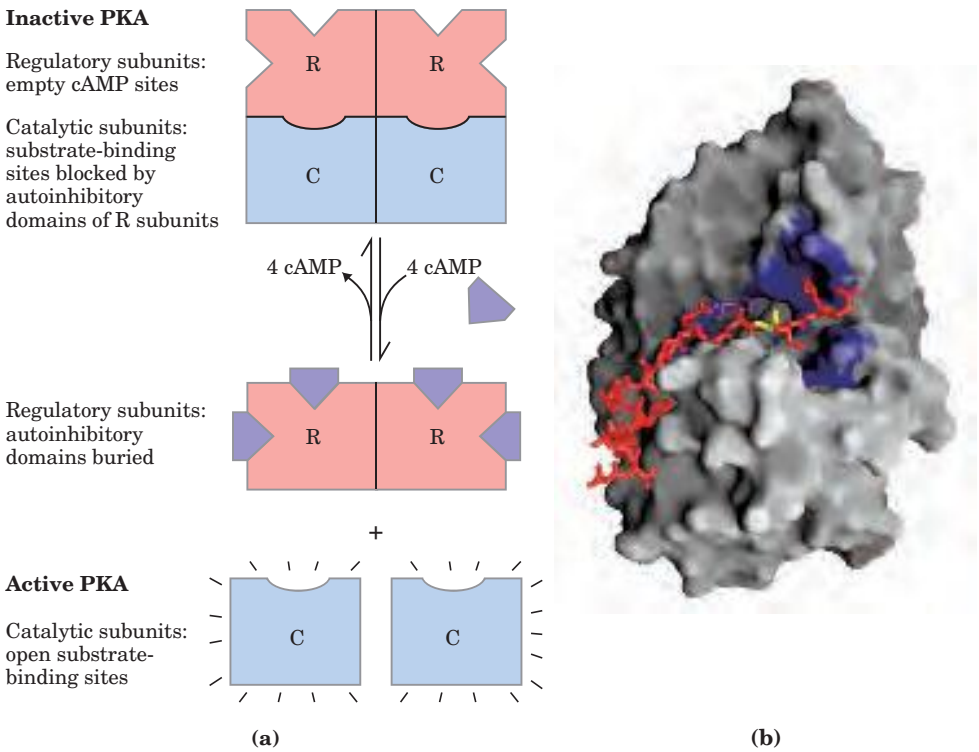


FIGURE 12–15 **Activation of cAMP-dependent protein kinase, PKA.** **(a)** A schematic representation of the inactive R_2C_2 tetramer, in which the autoinhibitory domain of a regulatory (R) subunit occupies the substrate-binding site, inhibiting the activity of the catalytic (C) subunit. Cyclic AMP activates PKA by causing dissociation of the C subunits from the inhibitory R subunits. Activated PKA can phosphorylate a variety of protein substrates (Table 12–3) that contain the PKA consensus sequence (X–Arg–(Arg/Lys)–X–(Ser/Thr)–B, where X is any residue and B is any hydrophobic residue), including phosphorylase *b* kinase. **(b)** The substrate-binding region of a catalytic subunit revealed by x-ray crystallography (derived from PDB ID 1JBP). Enzyme side chains known to be critical in substrate binding and specificity are in blue. The peptide substrate (red) lies in a groove in the enzyme surface, with its Ser residue (yellow) positioned in the catalytic site. In the inactive R_2C_2 tetramer, the autoinhibitory domain of R lies in this groove, blocking access to the substrate.

One downstream effect of epinephrine is to activate glycogen phosphorylase *b*. This conversion is promoted by the enzyme phosphorylase *b* kinase, which catalyzes the phosphorylation of two specific Ser residues in phosphorylase *b*, converting it to phosphorylase *a* (see Fig. 6–31). Cyclic AMP does not affect phosphorylase *b* kinase directly. Rather, **cAMP-dependent protein kinase,** also called **protein kinase A** or **PKA,** which is allosterically activated by cAMP (Fig. 12–12, step ⑤), catalyzes the phosphorylation of inactive phosphorylase *b* kinase to yield the active form.

The inactive form of PKA contains two catalytic subunits (C) and two regulatory subunits (R) (Fig. 12–15a), which are similar in sequence to the catalytic and regulatory domains of PKG (cGMP-dependent protein kinase). The tetrameric R_2C_2 complex is catalytically inactive, because an autoinhibitory domain of each R subunit occupies the substrate-binding site of each C subunit. When cAMP binds to two sites on each R subunit, the R subunits undergo a conformational change and the R_2C_2 complex dissociates to yield two free, catalytically active C subunits. This same basic mechanism—displacement of an autoinhibitory domain—mediates the allosteric activation of many types of protein kinases by their second messengers (as in Figs 12–7 and 12–23, for example).

As indicated in Figure 12–12 (step ⑥), PKA regulates a number of enzymes (Table 12–3). Although the proteins regulated by cAMP-dependent phosphorylation have diverse functions, they share a region of sequence similarity around the Ser or Thr residue that undergoes phosphorylation, a sequence that marks them for regulation by PKA. The catalytic site of PKA (Fig. 12–15b) interacts with several residues near the Thr or Ser residue in the target protein, and these interactions define the substrate specificity. Comparison of the sequences of a number of protein substrates for PKA has yielded the consensus sequence—the specific neighboring residues needed to mark a Ser or Thr residue for phosphorylation (see Table 12–3).

Signal transduction by adenylyl cyclase entails several steps that amplify the original hormone signal (Fig.

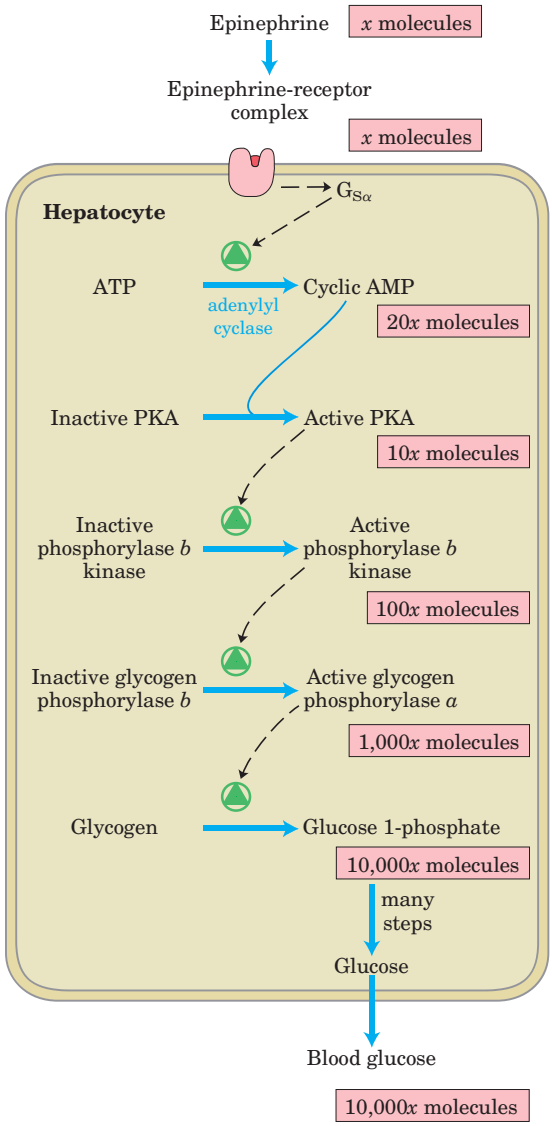
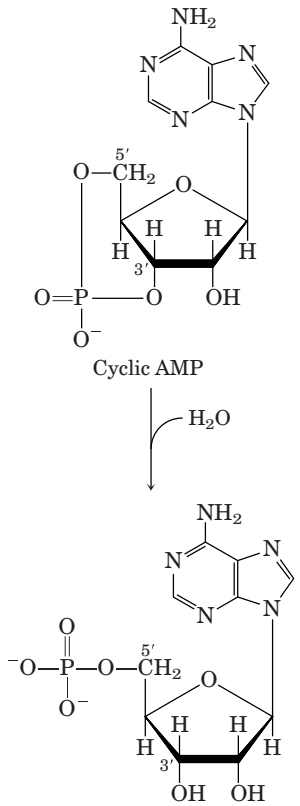


FIGURE 12–16 Epinephrine cascade. Epinephrine triggers a series of reactions in hepatocytes in which catalysts activate catalysts, resulting in great amplification of the signal. Binding of a small number of molecules of epinephrine to specific β-adrenergic receptors on the cell surface activates adenylyl cyclase. To illustrate amplification, we show 20 molecules of cAMP produced by each molecule of adenylyl cyclase, the 20 cAMP molecules activating 10 molecules of PKA, each PKA molecule activating 10 molecules of the next enzyme (a total of 100), and so forth. These amplifications are probably gross underestimates.

12–16). First, the binding of one hormone molecule to one receptor catalytically activates several G_s molecules. Next, by activating a molecule of adenylyl cyclase, each active $G_{s\alpha}$ molecule stimulates the catalytic synthesis of many molecules of cAMP. The second messenger cAMP now activates PKA, each molecule of which catalyzes the phosphorylation of many molecules of the target protein—phosphorylase *b* kinase in Figure 12–16. This kinase activates glycogen phosphorylase *b*, which leads to the rapid mobilization of glucose from glycogen. The net effect of the cascade is amplification of the hormonal signal by several orders of magnitude, which accounts for the very low concentration of epinephrine (or any other hormone) required for hormone activity.

Cyclic AMP, the intracellular second messenger in this system, is short-lived. It is quickly degraded by **cyclic nucleotide phosphodiesterase** to 5′-AMP (Fig. 12–12, step ⑦), which is not active as a second messenger:



The intracellular signal therefore persists only as long as the hormone receptor remains occupied by epinephrine. Methyl xanthines such as caffeine and theophylline (a component of tea) inhibit the phosphodiesterase, increasing the half-life of cAMP and thereby potentiating agents that act by stimulating adenylyl cyclase.

The β-Adrenergic Receptor Is Desensitized by Phosphorylation

As noted earlier, signal-transducing systems undergo desensitization when the signal persists. Desensitization of the β-adrenergic receptor is mediated by a protein kinase that phosphorylates the receptor on the intracellular domain that normally interacts with G_s (Fig. 12–17). When the receptor is occupied by epinephrine,

TABLE 12–3 Some Enzymes and Other Proteins Regulated by cAMP-Dependent Phosphorylation (by PKA)

Enzyme/protein	*Sequence phosphorylated**	*Pathway/process regulated*
Glycogen synthase	RASCTSSS	Glycogen synthesis
Phosphorylase *b* kinase		
α subunit	VEFRRLSI	Glycogen breakdown
β subunit	RTKRSGSV	
Pyruvate kinase (rat liver)	GVLRRASVAZL	Glycolysis
Pyruvate dehydrogenase complex (type L)	GYLRRASV	Pyruvate to acetyl-CoA
Hormone-sensitive lipase	PMRRSV	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRGSSIPQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FIGRRQSL	Synthesis of L-DOPA, dopamine, norepinephrine, and epinephrine
Histone H1	AKRKASGPPVS	DNA condensation
Histone H2B	KKAKASRKESYSVYVYK	DNA condensation
Cardiac phospholamban (cardiac pump regulator)	AIRRAST	Intracellular [Ca^{2+}]
Protein phosphatase-1 inhibitor-1	IRRRRPTP	Protein dephosphorylation
PKA consensus sequence†	XR(R/K)X(S/T)B	Many

*The phosphorylated S or T residue is shown in red. All residues are given as their one-letter abbreviations (see Table 3–1).
†X is any amino acid; B is any hydrophobic amino acid.

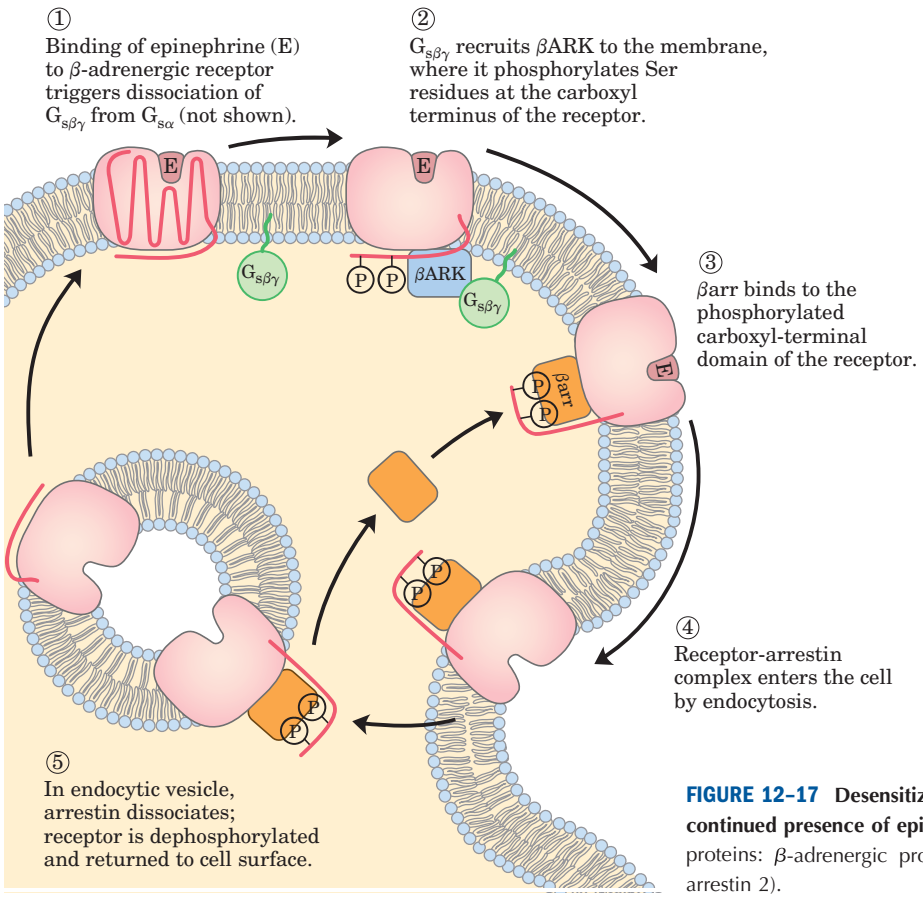


FIGURE 12–17 Desensitization of the β-adrenergic receptor in the continued presence of epinephrine. This process is mediated by two proteins: β-adrenergic protein kinase (βARK) and β-arrestin (βarr; arrestin 2).

β-adrenergic receptor kinase (βARK) phosphorylates Ser residues near the carboxyl terminus of the receptor. Normally located in the cytosol, βARK is drawn to the plasma membrane by its association with th $G_{s\beta\gamma}$ subunits and is thus positioned to phosphorylate the receptor. The phosphorylation creates a binding site for the protein **β-arrestin (βarr),** also called arrestin 2, and binding of β-arrestin effectively prevents interaction between the receptor and the G protein. The binding of β-arrestin also facilitates receptor sequestration, the removal of receptors from the plasma membrane by endocytosis into small intracellular vesicles. Receptors in the endocytic vesicles are dephosphorylated, then returned to the plasma membrane, completing the circuit and resensitizing the system to epinephrine. β-Adrenergic receptor kinase is a member of a family of **G protein–coupled receptor kinases (GRKs),** all of which phosphorylate serpentine receptors on their carboxyl-terminal cytosolic domains and play roles similar to that of βARK in desensitization and resensitization of their receptors. At least five different GRKs and four different arrestins are encoded in the human genome; each GRK is capable of desensitizing a subset of the serpentine receptors, and each arrestin can interact with many different types of phosphorylated receptors.

While preventing the signal from a serpentine receptor from reaching its associated G protein, arrestins can also initiate a second signaling cascade, by acting as **scaffold proteins** that bring together several protein kinases that function in a cascade. For example, the β-arrestin associated with the serpentine receptor for angiotensin, a potent regulator of blood pressure, binds the three protein kinases Raf-1, MEK1, and ERK (Fig. 12–18), serving as a scaffold that facilitates any signaling process, such as insulin signaling (Fig. 12–6), that requires these three protein kinases to interact. This is one of many known examples of cross-talk between systems triggered by different ligands (angiotensin and insulin, in this case).

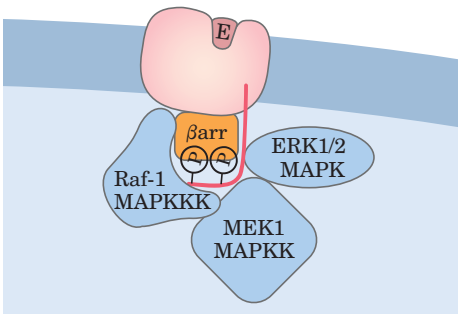


FIGURE 12–18 β-Arrestin uncouples the serpentine receptor from its G protein and brings together the three enzymes of the MAPK cascade. The effect is that one stimulus triggers two distinct response pathways: the path activated by the G protein and the MAPK cascade.

Cyclic AMP Acts as a Second Messenger for a Number of Regulatory Molecules

Epinephrine is only one of many hormones, growth factors, and other regulatory molecules that act by changing the intracellular [cAMP] and thus the activity of PKA (Table 12–4). For example, glucagon binds to its receptors in the plasma membrane of adipocytes, activating (via a G_s protein) adenylyl cyclase. PKA, stimulated by the resulting rise in [cAMP], phosphorylates and activates two proteins critical to the conversion of stored fat to fatty acids (perilipin and hormone-sensitive triacylglycerol lipase; see Fig. 17–3), leading to the mobilization of fatty acids. Similarly, the peptide hormone ACTH (adrenocorticotropic hormone, also called corticotropin), produced by the anterior pituitary, binds to specific receptors in the adrenal cortex, activating adenylyl cyclase and raising the intracellular [cAMP]. PKA then phosphorylates and activates several of the enzymes required for the synthesis of cortisol and other steroid hormones. The catalytic subunit of PKA can also move into the nucleus, where it phosphorylates a protein that alters the expression of specific genes.

Some hormones act by *inhibiting* adenylyl cyclase, *lowering* cAMP levels, and *suppressing* protein phosphorylation. For example, the binding of somatostatin to its receptor leads to activation of an **inhibitory G protein,** or **G_i,** structurally homologous to G_s, that inhibits adenylyl cyclase and lowers [cAMP]. Somatostatin therefore counterbalances the effects of glucagon. In adipose tissue, prostaglandin E_1 (PGE_1; see Fig. 10–18b) inhibits adenylyl cyclase, thus lowering [cAMP] and slowing the

TABLE 12–4 Some Signals That Use cAMP as Second Messenger

Corticotropin (ACTH)
Corticotropin-releasing hormone (CRH)
Dopamine [D_1, D_2]*
Epinephrine (β-adrenergic)
Follicle-stimulating hormone (FSH)
Glucagon
Histamine [H_2]*
Luteinizing hormone (LH)
Melanocyte-stimulating hormone (MSH)
Odorants (many)
Parathyroid hormone
Prostaglandins E_1, E_2 (PGE_1, PGE_2)
Serotonin [5-HT-1a, 5-HT-2]*
Somatostatin
Tastants (sweet, bitter)
Thyroid-stimulating hormone (TSH)

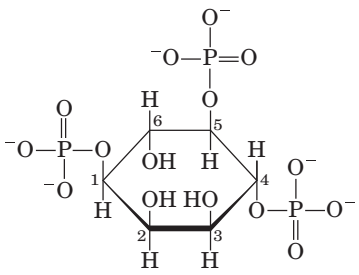
*Receptor subtypes in square brackets. Subtypes may have different transduction mechanisms. For example, serotonin is detected in some tissues by receptor subtypes 5-HT-1a and 5-HT-1b, which act through adenylyl cyclase and cAMP, and in other tissues by receptor subtype 5-HT-1c, acting through the phospholipase C–IP_3 mechanism (see Table 12–5).

mobilization of lipid reserves triggered by epinephrine and glucagon. In certain other tissues PGE_1 stimulates cAMP synthesis, because its receptors are coupled to adenylyl cyclase through a stimulatory G protein, G_s. In tissues with α_2-adrenergic receptors, epinephrine lowers [cAMP], because the α_2 receptors are coupled to adenylyl cyclase through an inhibitory G protein, G_i. In short, an extracellular signal such as epinephrine or PGE_1 can have quite different effects on different tissues or cell types, depending on three factors: the type of receptor in each tissue, the type of G protein (G_s or G_i) with which the receptor is coupled, and the set of PKA target enzymes in the cells.

A fourth factor that explains how so many signals can be mediated by a single second messenger (cAMP) is the confinement of the signaling process to a specific region of the cell by scaffold proteins. AKAPs (*A kinase anchoring proteins*) are bivalent; one part binds to the R subunit of PKA, and another to a specific structure within the cell, confining the PKA to the vicinity of that structure. For example, specific AKAPs bind PKA to microtubules, actin filaments, Ca^{2+} channels, mitochondria, and the nucleus. Different types of cells have different AKAPs, so cAMP might stimulate phosphorylation of mitochondrial proteins in one cell and phosphorylation of actin filaments in another. In studies of the intracellular localization of biochemical changes, biochemistry meets cell biology, and techniques that cross this boundary become invaluable (Box 12–2).

Two Second Messengers Are Derived from Phosphatidylinositols

A second class of serpentine receptors are coupled through a G protein to a plasma membrane **phospholipase C (PLC)** that is specific for the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate (see Fig. 10–15). This hormone-sensitive enzyme catalyzes the formation of two potent second messengers: **diacylglycerol** and **inositol 1,4,5-trisphosphate,** or **IP_3** (not to be confused with PIP_3, p. 431).

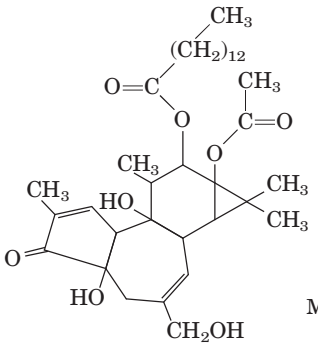


Inositol 1,4,5-trisphosphate (IP_3)

When a hormone of this class (Table 12–5) binds its specific receptor in the plasma membrane (Fig. 12–19, step ①), the receptor-hormone complex catalyzes GTP-GDP exchange on an associated G protein, $\mathbf{G_q}$ (step ②), activating it exactly as the β-adrenergic receptor activates G_s (Fig. 12–12). The activated G_q in turn activates a specific membrane-bound PLC (step ③), which catalyzes the production of the two second messengers diacylglycerol and IP_3 by hydrolysis of phosphatidylinositol 4,5-bisphosphate in the plasma membrane (step ④).

Inositol trisphosphate, a water-soluble compound, diffuses from the plasma membrane to the endoplasmic reticulum, where it binds to specific IP_3 receptors and causes Ca^{2+} channels within the ER to open. Sequestered Ca^{2+} is thus released into the cytosol (step ⑤), and the cytosolic [Ca^{2+}] rises sharply to about 10^{-6} M. One effect of elevated [Ca^{2+}] is the activation of **protein kinase C (PKC).** Diacylglycerol cooperates with Ca^{2+} in activating PKC, thus also acting as a second messenger (step ⑥). PKC phosphorylates Ser or Thr residues of specific target proteins, changing their catalytic activities (step ⑦). There are a number of isozymes of PKC, each with a characteristic tissue distribution, target protein specificity, and role.

The action of a group of compounds known as **tumor promoters** is attributable to their effects on PKC. The best understood of these are the phorbol esters, synthetic compounds that are potent activators of PKC. They apparently mimic cellular diacylglycerol as second messengers, but unlike naturally occurring diacylglycerols they are not rapidly metabolized. By continuously activating PKC, these synthetic tumor promoters interfere with the normal regulation of cell growth and division (discussed in Section 12.10). ■



Myristoylphorbol acetate (a phorbol ester)

Calcium Is a Second Messenger in Many Signal Transductions

In many cells that respond to extracellular signals, Ca^{2+} serves as a second messenger that triggers intracellular responses, such as exocytosis in neurons and endocrine cells, contraction in muscle, and cytoskeletal rearrangement during amoeboid movement. Normally, cytosolic [Ca^{2+}] is kept very low ($<10^{-7}$ M) by the action of Ca^{2+} pumps in the ER, mitochondria, and plasma membrane. Hormonal, neural, or other stimuli cause either an influx of Ca^{2+} into the cell through specific

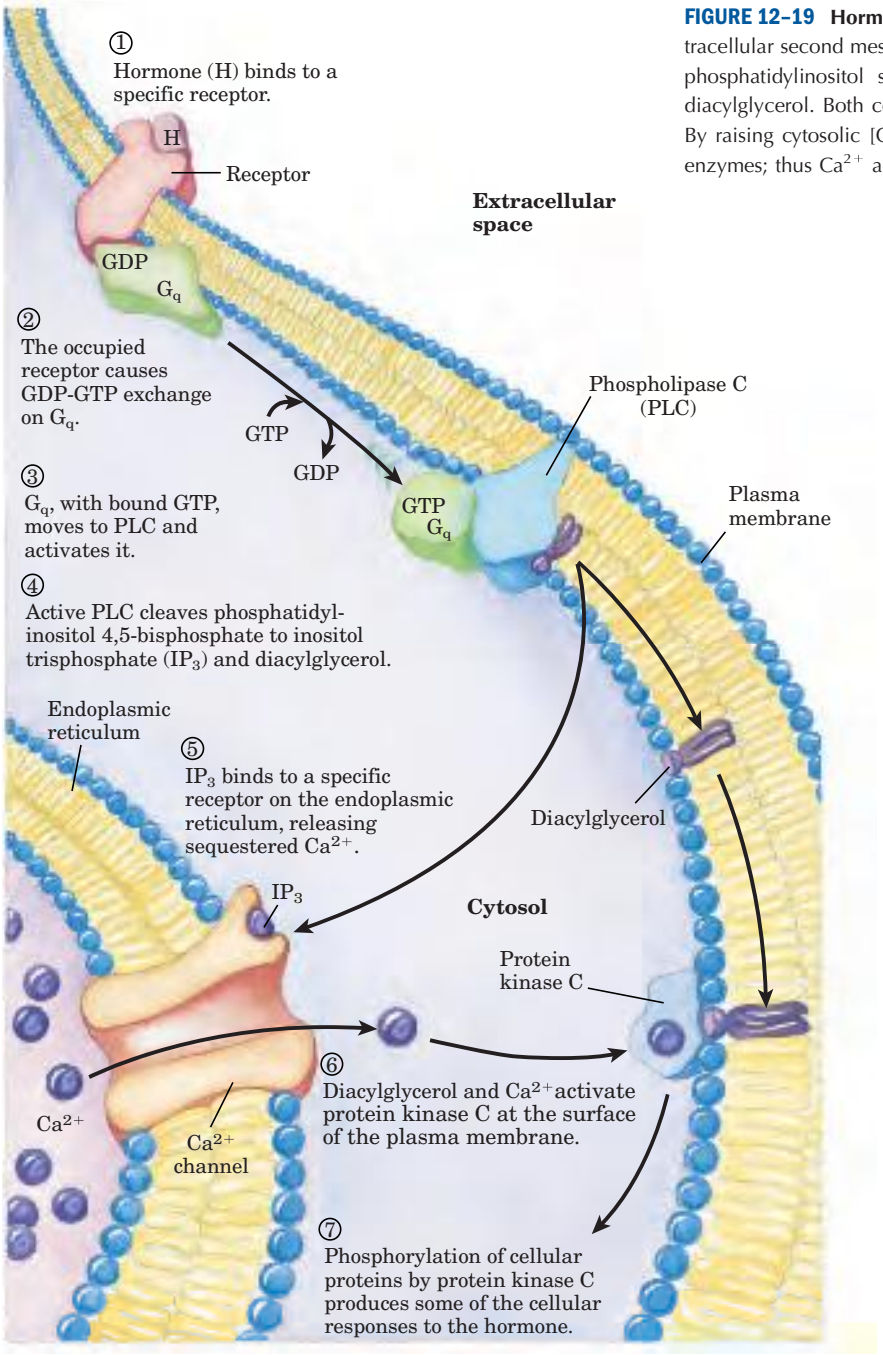


FIGURE 12–19 Hormone-activated phospholipase C and IP_3. Two intracellular second messengers are produced in the hormone-sensitive phosphatidylinositol system: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Both contribute to the activation of protein kinase C. By raising cytosolic [Ca^{2+}], IP_3 also activates other Ca^{2+}-dependent enzymes; thus Ca^{2+} also acts as a second messenger.

TABLE 12–5 Some Signals That Act through Phospholipase C and IP_3

Acetylcholine [muscarinic M_1]	Gastrin-releasing peptide	Platelet-derived growth factor (PDGF)
α_1-Adrenergic agonists	Glutamate	Serotonin [5-HT-1c]*
Angiogenin	Gonadotropin-releasing hormone (GRH)	Thyrotropin-releasing hormone (TRH)
Angiotensin II	Histamine [H_1]*	Vasopressin
ATP [P_{2x} and P_{2y}]*	Light (*Drosophila*)	
Auxin	Oxytocin	

*Receptor subtypes are in square brackets; see footnote to Table 12–4.

Ca^{2+} channels in the plasma membrane or the release of sequestered Ca^{2+} from the ER or mitochondria, in either case raising the cytosolic [Ca^{2+}] and triggering a cellular response.

Very commonly, [Ca^{2+}] does not simply rise and then decrease, but rather oscillates with a period of a few seconds (Fig. 12–20), even when the extracellular concentration of hormone remains constant. The mechanism underlying [Ca^{2+}] oscillations presumably entails feedback regulation by Ca^{2+} of either the phospholipase that generates IP_3 or the ion channel that regulates Ca^{2+} release from the ER, or both. Whatever the mechanism, the effect is that one kind of signal (hormone concentration, for example) is converted into another (frequency and amplitude of intracellular [Ca^{2+}] "spikes").

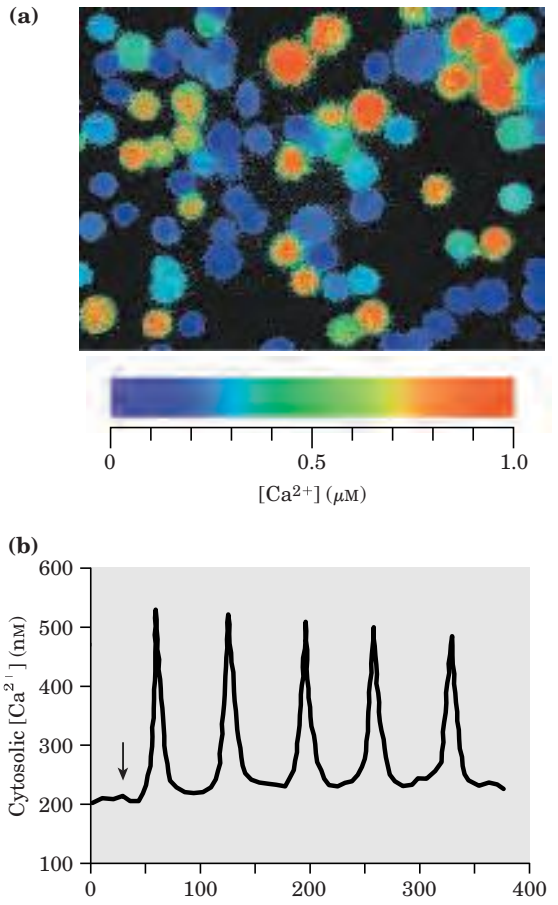


FIGURE 12–20 Triggering of oscillations in intracellular [Ca^{2+}] by extracellular signals. (a) A dye (fura) that undergoes fluorescence changes when it binds Ca^{2+} is allowed to diffuse into cells, and its instantaneous light output is measured by fluorescence microscopy. Fluorescence intensity is represented by color; the color scale relates intensity of color to [Ca^{2+}], allowing determination of the absolute [Ca^{2+}]. In this case, thymocytes (cells of the thymus) have been stimulated with extracellular ATP, which raises their internal [Ca^{2+}]. The cells are heterogeneous in their responses; some have high intracellular [Ca^{2+}] (red), others much lower (blue). **(b)** When such a probe is used to measure [Ca^{2+}] in a single hepatocyte, we observe that the agonist norepinephrine (added at the arrow) causes oscillations of [Ca^{2+}] from 200 to 500 nM. Similar oscillations are induced in other cell types by other extracellular signals.

Changes in intracellular [Ca^{2+}] are detected by Ca^{2+}-binding proteins that regulate a variety of Ca^{2+}-dependent enzymes. **Calmodulin (CaM)** (M_r 17,000) is an acidic protein with four high-affinity Ca^{2+}-binding sites. When intracellular [Ca^{2+}] rises to about 10^{-6} M (1 μM), the binding of Ca^{2+} to calmodulin drives a conformational change in the protein (Fig. 12–21). Calmodulin associates with a variety of proteins and, in its Ca^{2+}-bound state, modulates their activities. Calmodulin is a member of a family of Ca^{2+}-binding proteins that also includes troponin (p. 185), which triggers skeletal muscle contraction in response to increased [Ca^{2+}]. This family shares a characteristic Ca^{2+}-binding structure, the EF hand (Fig. 12–21c).

Calmodulin is also an integral subunit of a family of enzymes, the **Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I–IV).** When intracellular [Ca^{2+}] increases in response to some stimulus, calmodulin binds Ca^{2+}, undergoes a change in conformation, and activates the CaM kinase. The kinase then phosphorylates a number of target enzymes, regulating their activities. Calmodulin is also a regulatory subunit of phosphorylase *b* kinase of muscle, which is activated by Ca^{2+}. Thus Ca^{2+} triggers ATP-requiring muscle contractions while also activating glycogen breakdown, providing fuel for ATP synthesis. Many other enzymes are also known to be modulated by Ca^{2+} through calmodulin (Table 12–6).

TABLE 12–6 Some Proteins Regulated by Ca^{2+} and Calmodulin

Adenylyl cyclase (brain)
Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I to IV)
Ca^{2+}-dependent Na^+ channel (*Paramecium*)
Ca^{2+}-release channel of sarcoplasmic reticulum
Calcineurin (phosphoprotein phosphatase 2B)
cAMP phosphodiesterase
cAMP-gated olfactory channel
cGMP-gated Na^+, Ca^{2+} channels (rod and cone cells)
Glutamate decarboxylase
Myosin light chain kinases
NAD^+ kinase
Nitric oxide synthase
Phosphoinositide 3-kinase
Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)
RNA helicase (p68)



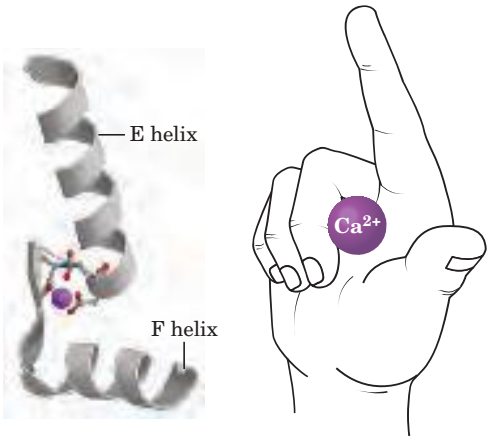


FIGURE 12–21 Calmodulin. This is the protein mediator of many Ca^{2+}-stimulated enzymatic reactions. Calmodulin has four high-affinity Ca^{2+}-binding sites ($K_d \approx 0.1$ to 1 μM). **(a)** A ribbon model of the crystal structure of calmodulin (PDB ID 1CLL). The four Ca^{2+}-binding sites are occupied by Ca^{2+} (purple). The amino-terminal domain is on the left; the carboxyl-terminal domain on the right. **(b)** Calmodulin associated with a helical domain (red) of one of the many enzymes it regulates, calmodulin-dependent protein kinase II (PDB ID 1CDL). Notice that the long central α helix visible in **(a)** has bent back on itself in binding to the helical substrate domain. The central helix is clearly more flexible in solution than in the crystal. **(c)** Each of the four Ca^{2+}-binding sites occurs in a helix-loop-helix motif called the EF hand, also found in many other Ca^{2+}-binding proteins.

SUMMARY 12.4 G Protein–Coupled Receptors and Second Messengers

- A large family of plasma membrane receptors with seven transmembrane segments act through heterotrimeric G proteins. On ligand binding, these receptors catalyze the exchange of GTP for GDP bound to an associated G protein, forcing dissociation of the α subunit of the G protein. This subunit stimulates or inhibits the activity of a nearby membrane-bound enzyme, changing the level of its second messenger product.
- The β-adrenergic receptor binds epinephrine, then through a stimulatory G protein, G_s, activates adenylyl cyclase in the plasma membrane. The cAMP produced by adenylyl cyclase is an intracellular second messenger that stimulates cAMP-dependent protein kinase, which mediates the effects of epinephrine by phosphorylating key proteins, changing their enzymatic activities or structural features.
- The cascade of events in which a single molecule of hormone activates a catalyst that in turn activates another catalyst, and so on, results in large signal amplification; this is characteristic of most hormone-activated systems.
- Some receptors stimulate adenylyl cyclase through G_s; others inhibit it through G_i. Thus cellular [cAMP] reflects the integrated input of two (or more) signals.
- Cyclic AMP is eventually eliminated by cAMP phosphodiesterase, and G_s turns itself off by hydrolysis of its bound GTP to GDP. When the epinephrine signal persists, β-adrenergic receptor–specific protein kinase and arrestin 2 temporarily desensitize the receptor and cause it to move into intracellular vesicles. In some cases, arrestin also acts as a scaffold protein, bringing together protein components of a signaling pathway such as the MAPK cascade.
- Some serpentine receptors are coupled to a plasma membrane phospholipase C that cleaves PIP_2 to diacylglycerol and IP_3. By opening Ca^{2+} channels in the endoplasmic reticulum, IP_3 raises cytosolic [Ca^{2+}]. Diacylglycerol and Ca^{2+} act together to activate protein kinase C, which phosphorylates and changes the activity of specific cellular proteins. Cellular [Ca^{2+}] also regulates a number of other enzymes, often through calmodulin.

BOX 12–2 WORKING IN BIOCHEMISTRY

FRET: Biochemistry Visualized in a Living Cell

Fluorescent probes are commonly used to detect rapid biochemical changes in single living cells. They can be designed to give an essentially instantaneous report (within nanoseconds) on the changes in intracellular concentration of a second messenger or in the activity of a protein kinase. Furthermore, fluorescence microscopy has sufficient resolution to reveal where in the cell such changes are occurring. In one widely used procedure, the fluorescent probes are derived from a naturally occurring fluorescent protein, the green fluorescent protein (GFP) of the jellyfish *Aequorea victoria* (Fig. 1).

When excited by absorption of a photon of light, GFP emits a photon (that is, it fluoresces) in the green region of the spectrum. GFP is an 11-stranded β barrel, and the light-absorbing/emitting center of the protein (its chromophore) comprises the tripeptide Ser^{65}–Tyr^{66}–Gly^{67}, located within the barrel (Fig. 2). Variants of this protein, with different fluorescence spectra, can be produced by genetic engineering of the GFP gene. For example, in the yellow fluorescent protein (YFP), Ala^{206} in GFP is replaced by a Lys residue, changing the wavelength of light absorption and fluorescence. Other variants of GFP fluoresce blue (BFP) or cyan (CFP) light, and a related protein (mRFP1) fluoresces red light (Fig. 3). GFP and its variants are compact structures that retain their ability to fold into their native β-barrel conformation even when fused with another protein. Investigators are using these fluorescent hybrid proteins as spectroscopic rulers to measure distances between interacting components within a cell.



FIGURE 1 *Aequorea victoria,* a jellyfish abundant in Puget Sound, Washington State.

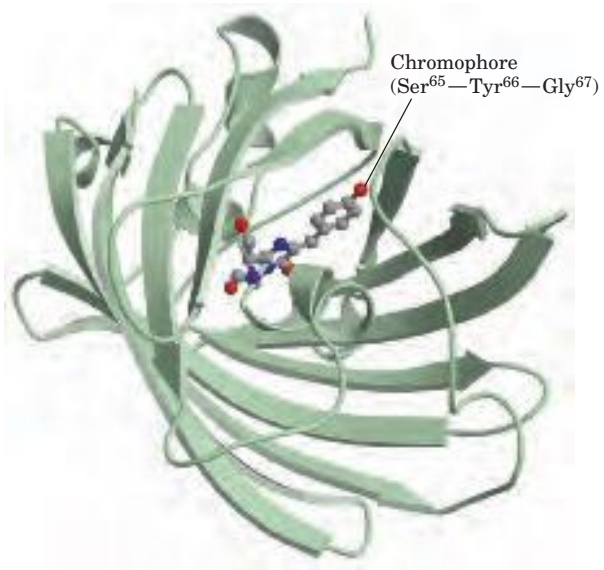


FIGURE 2 Green fluorescent protein (GFP), with the fluorescent chromophore shown in ball-and-stick form (derived from PDB ID 1GFL).

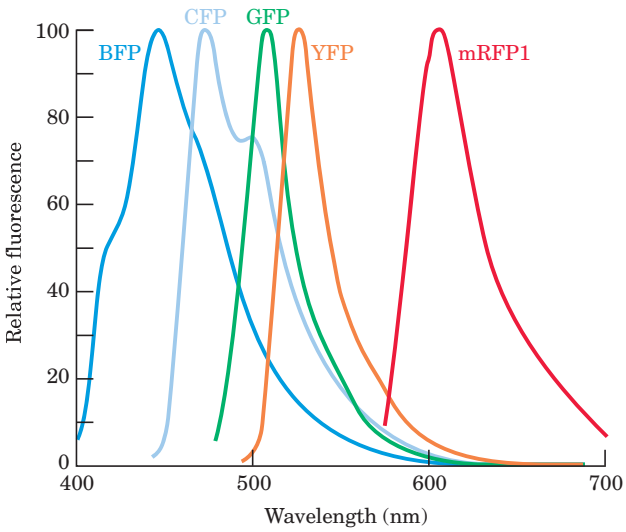


FIGURE 3 Emission spectra of GFP variants.

An excited fluorescent molecule such as GFP or YFP can dispose of the energy from the absorbed photon in either of two ways: (1) by fluorescence, emitting a photon of slightly longer wavelength (lower energy) than the exciting light, or (2) by nonradiative **fluorescence resonance energy transfer (FRET),** in which the energy of the excited molecule (the donor) passes directly to a nearby molecule (the acceptor) *without emission of a photon*, exciting the acceptor (Fig. 4). The acceptor can now decay to its ground state by fluorescence; the emitted photon has a longer wavelength (lower energy) than both the original exciting light and the fluorescence emission of the donor. This second mode of decay (FRET) is possible only when donor and acceptor are close to each other (within 1 to 50 Å); the efficiency of FRET is inversely proportional to the *sixth power* of the distance between donor and acceptor. Thus very small changes in the distance between donor and acceptor register as very large changes in FRET, measured as the fluorescence of the acceptor molecule when the donor is excited. With sufficiently sensitive light detectors, this fluorescence signal can be located to specific regions of a single, living cell.

FRET has been used to measure [cAMP] in living cells. The gene for GFP is fused with that for the regulatory subunit (R) of cAMP-dependent protein kinase, and the gene for BFP is fused with that for the catalytic subunit (C) (Fig. 5). When these two hybrid proteins are expressed in a cell, BFP (donor; excitation at 380 nm, emission at 460 nm) and GFP (acceptor; excitation at 475 nm, emission at 545 nm) in the inactive PKA (R_2C_2 tetramer) are close enough to undergo FRET. Wherever in the cell [cAMP] increases, the R_2C_2 complex dissociates into R_2 and 2C and the FRET signal is lost, because donor and acceptor are now too far apart for efficient FRET. Viewed in the fluorescence microscope, the region of higher [cAMP] has a minimal GFP signal and higher BFP signal. Measuring the ratio of emission at 460 nm and 545 nm gives a sensitive measure of the change in [cAMP]. By determining this ratio for all regions of the cell, the investigator can generate a false color image of the

(continued on next page)

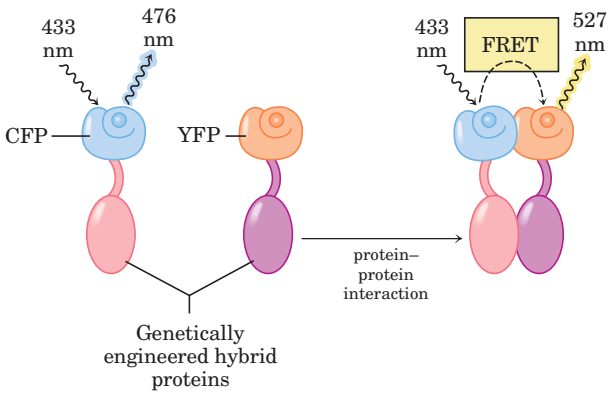


FIGURE 4 When the donor protein (CFP) is excited with monochromatic light of wavelength 433 nm, it emits fluorescent light at 476 nm (left). When the (red) protein fused with CFP interacts with the (purple) protein fused with YFP, that interaction brings CFP and YFP close enough to allow fluorescence resonance energy transfer (FRET) between them. Now, when CFP absorbs light of 433 nm, instead of fluorescing at 476 nm, it transfers energy directly to YFP, which then fluoresces at its characteristic emission wavelength, 527 nm. The ratio of light emission at 527 and 476 nm is therefore a measure of the interaction of the red and purple protein.

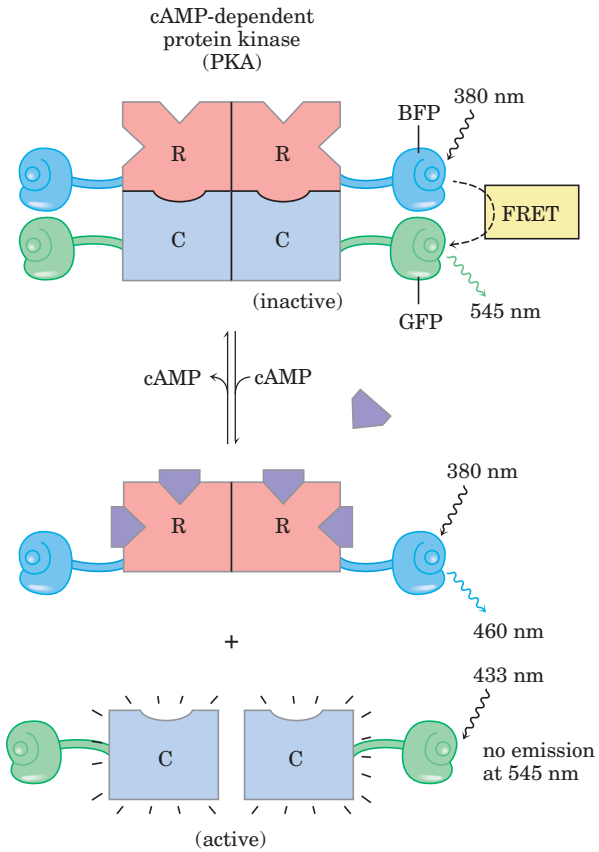


FIGURE 5 **Measuring [cAMP] with FRET.** Gene fusion creates hybrid proteins that exhibit FRET when the PKA regulatory and catalytic subunits are associated (low [cAMP]). When [cAMP] rises, the subunits dissociate, and FRET ceases. The ratio of emission at 460 nm (dissociated) and 545 nm (complexed) thus offers a sensitive measure of [cAMP].

BOX 12–2 WORKING IN BIOCHEMISTRY *(continued from previous page)*

cell in which the ratio, or relative [cAMP], is represented by the intensity of the color. Images recorded at timed intervals reveal changes in [cAMP] over time.

A variation of this technology has been used to measure the activity of PKA in a living cell (Fig. 6). Researchers create a phosphorylation target for PKA by producing a hybrid protein containing four elements: YFP (acceptor); a short peptide with a Ser residue surrounded by the consensus sequence for PKA; a Ⓟ–Ser-binding domain (called 14-3-3); and CFP (donor). When the Ser residue is not phosphorylated, 14-3-3 has no affinity for the Ser residue and the hybrid protein exists in an extended form, with the donor and acceptor too far apart to generate a FRET signal. Wherever PKA is active in the cell, it phosphorylates the Ser residue of the hybrid protein, and 14-3-3 binds to the Ⓟ–Ser. In doing so, it draws YFP and CFP together and a FRET signal is detected with the fluorescence microscope, revealing the presence of active PKA.

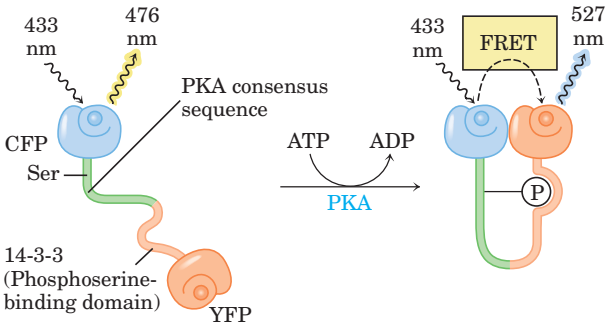


FIGURE 6 Measuring the activity of PKA with FRET. An engineered protein links YFP and CFP via a peptide that contains a Ser residue surrounded by the consensus sequence for phosphorylation by PKA, and the 14-3-3 phosphoserine binding domain. Active PKA phosphorylates the Ser residue, which docks with the 14-3-3 binding domain, bringing the fluorescence proteins close enough to allow FRET to occur, revealing the presence of active PKA.

12.5 Multivalent Scaffold Proteins and Membrane Rafts

About 10% of the 30,000 to 35,000 genes in the human genome encode signaling proteins—receptors, G proteins, enzymes that generate second messengers, protein kinases (>500), proteins involved in desensitization, and ion channels. Not every signaling protein is expressed in a given cell type, but most cells doubtless contain many such proteins. How does one protein find another in a signaling pathway, and how are their interactions regulated? As is becoming clear, the reversible phosphorylation of Tyr, Ser, and Thr residues in signaling proteins creates *docking sites* for other proteins, and many signaling proteins are *multivalent* in that they can interact with several different proteins simultaneously to form multiprotein signaling complexes. In this section we present a few examples to illustrate the general principles of protein interactions in signaling.

Protein Modules Bind Phosphorylated Tyr, Ser, or Thr Residues in Partner Proteins

We have seen that the protein Grb2 in the insulin signaling pathway (Fig. 12–6) binds through its SH2 domain to other proteins that contain exposed Ⓟ–Tyr residues. The human genome encodes at least 87 SH2-containing proteins, many already known to participate in signaling. The Ⓟ–Tyr residue is bound in a deep pocket in an SH2 domain, with each of its phosphate oxygens participating in hydrogen-bonding or electrostatic interactions; the positive charges on two Arg residues figure prominently in the binding. Subtle differences in the structure of SH2 domains in different proteins account for the specificities of their interactions with various Ⓟ–Tyr-containing proteins. The three to five residues on the carboxyl-terminal side of the Ⓟ–Tyr residue are critical in determining the specificity of interactions with SH2 domains (Fig. 12–22).

PTB domains (phosphotyrosine-binding domains) also bind Ⓟ–Tyr in partner proteins, but their critical sequences and three-dimensional structures distinguish them from SH2 domains. The human genome encodes 24 proteins that contain PTB domains, including IRS-1, which we have already met in its role as a scaffold protein in insulin-signal transduction (Fig. 12–6).

Many of the signaling protein kinases, including PKA, PKC, PKG, and members of the MAPK cascade, phosphorylate Ser or Thr residues in their target proteins, which in some cases acquire the ability to interact with partner proteins through the phosphorylated residue, triggering a downstream process. An alphabet soup of domains that bind Ⓟ–Ser or Ⓟ–Thr residues has been identified, and more are sure to be found. Each domain favors a certain sequence around the phosphorylated residue, so the domains represent families of highly specific recognition sites, able to bind to a specific subset of phosphorylated proteins.

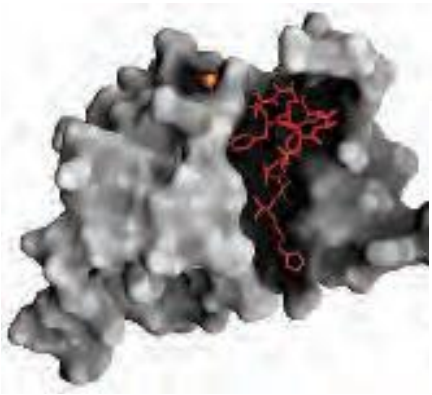


FIGURE 12–22 Structure of an SH2 domain and its interaction with a Ⓟ–Tyr residue in a partner protein. (PDB ID 1SHC) The SH2 domain is shown as a gray surface contour representation. The phosphorus of the phosphate group in the interacting Ⓟ–Tyr is visible as an orange sphere; most of the residue is obscured in this view. The next few residues toward the carboxyl end of the partner protein are shown in red. The SH2 domain interacts with Ⓟ–Tyr (which, as the phosphorylated residue, is assigned the index position 0) and also with the next three residues toward the carboxyl terminus (designated +1, +2, +3). The residues important in the Ⓟ–Tyr residue are conserved in all SH2 domains. Some SH2 domains (Src, Fyn, Hck, Nck) favor negatively charged residues in the +1 and +2 positions; others (PLC-γ1, SHP-2) have a long hydrophobic groove that selects for aliphatic residues in positions +1 to +5. These differences define subclasses of SH2 domains that have different partner specificities.

In some cases, the domain-binding partner is internal. Phosphorylation of some protein kinases inhibits their activity by favoring the interaction of an SH2 domain with a Ⓟ–Tyr in another domain of the same enzyme. For example, the soluble protein Tyr kinase Src, when phosphorylated on a critical Tyr residue, is rendered inactive as an SH2 domain needed to bind to the substrate protein instead binds to an internal Ⓟ–Tyr (Fig. 12–23). Glycogen synthase kinase 3 (GSK3) is inactive when phosphorylated on a Ser residue in its autoinhibitory domain (Fig. 12–23b). Dephosphorylation of that domain frees the enzyme to bind and phosphorylate its target proteins. Similarly, the polar head group of the phospholipid PIP_3, protruding from the inner leaflet of the plasma membrane, provides points of attachment for proteins that contain SH3 and other domains.

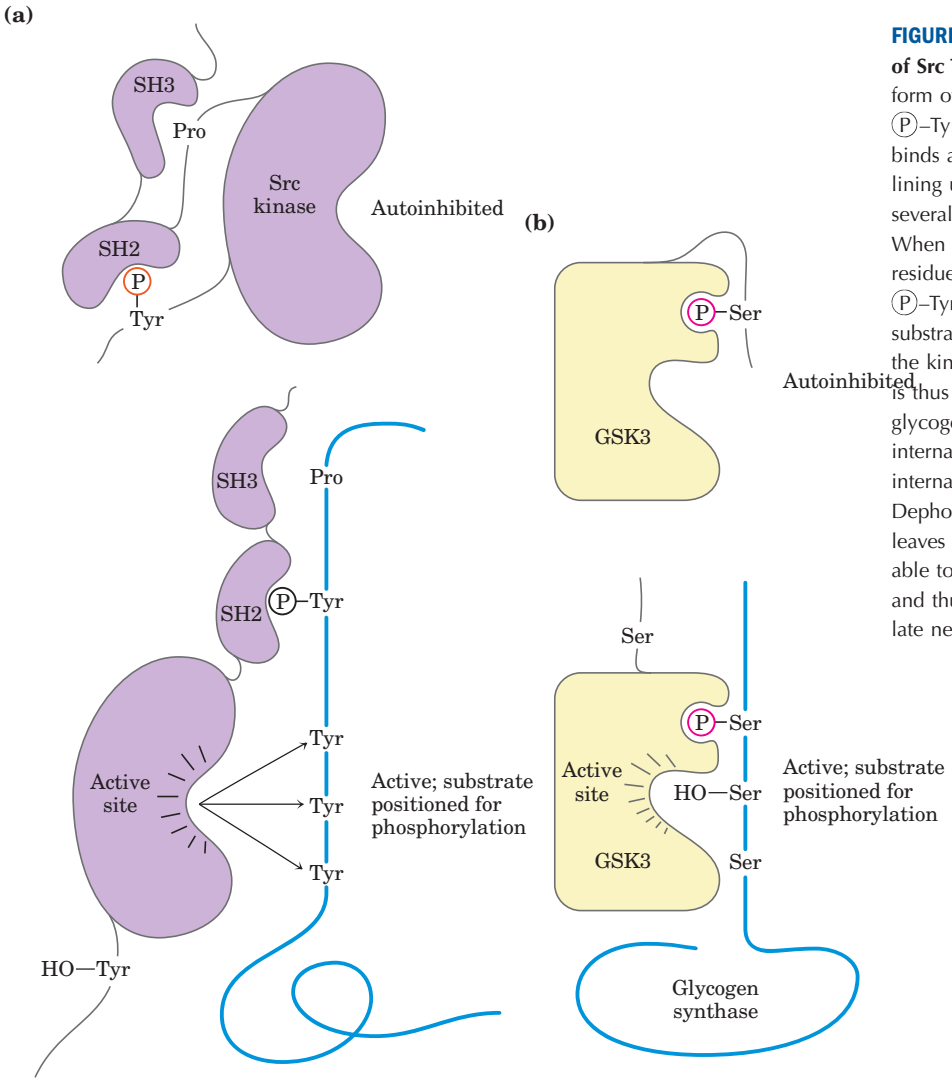


FIGURE 12–23 Mechanism of autoinhibition of Src Tyr kinase and GSK3. (a) In the active form of Src kinase, an SH2 domain binds a Ⓟ–Tyr in the substrate, and an SH3 domain binds a proline-rich region of the substrate, lining up the active site of the kinase with several target Tyr residues in the substrate. When Src is phosphorylated on a specific Tyr residue, the SH2 domain binds to the internal Ⓟ–Tyr instead of to the Ⓟ–Tyr of the substrate, preventing productive binding of the kinase to its protein substrate; the enzyme is thus autoinhibited. **(b)** In the autoinhibited glycogen synthase kinase 3 (GSK3), an internal Ⓟ–Ser residue is bound to an internal Ⓟ–Ser-binding domain (top). Dephosphorylation of this internal Ser residue leaves the Ⓟ–Ser-binding site of GSK3 available to bind Ⓟ–Ser in a protein substrate, and thus to position the kinase to phosphorylate neighboring Ser residues (bottom).

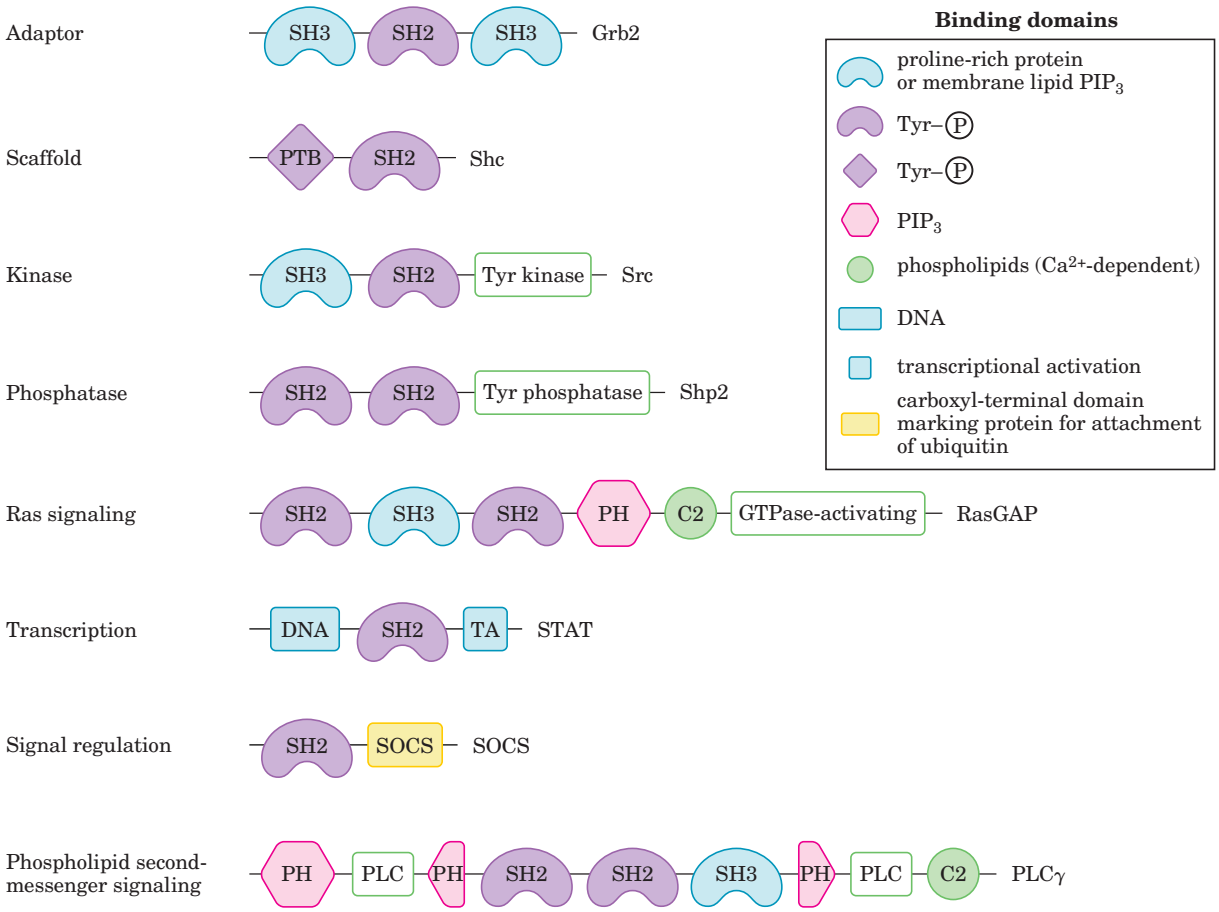


FIGURE 12–24 Some binding modules of signaling proteins. Each protein is represented by a line (with the amino terminus to the left); symbols indicate the location of conserved binding domains (with specificities as listed in the key; PH denotes plextrin homology; other abbreviations explained in the text); green boxes indicate catalytic activities. The name of each protein is given at its carboxyl-terminal end. These signaling proteins interact with phosphorylated proteins or phospholipids in many permutations and combinations to form integrated signaling complexes.

Most of the proteins involved in signaling at the plasma membrane have one or more protein- or phospholipid-binding domains; many have three or more, and thus are multivalent in their interactions with other signaling proteins. Figure 12–24 shows a few of the many multivalent proteins known to participate in signaling.

A remarkable picture of signaling pathways has emerged from studies of many signaling proteins and the multiple binding domains they contain (Fig. 12–25). An initial signal results in phosphorylation of the receptor or a target protein, triggering the assembly of large multiprotein complexes, held together on scaffolds made from adaptor proteins with multivalent binding capacities. Some of these complexes have several protein kinases that activate each other in turn, producing a cascade of phosphorylation and a great amplification of the initial signal. Animal cells also have phosphotyrosine phosphatases (PTPases), which remove the phosphate from Ⓟ–Tyr residues, reversing the effect of phosphorylation. Some of these phosphatases are receptorlike membrane proteins, presumably controlled by extracellular factors not yet identified; other PTPases are soluble and contain SH2 domains. In addition, animal cells have protein phosphoserine and phosphothreonine phosphatases, which reverse the effects of Ser- and Thr-specific protein kinases. We can see, then, that signaling occurs in protein circuits, effectively hard-wired from signal receptor to response effector and able to be switched off instantly by the hydrolysis of a single phosphate ester bond.

The multivalency of signaling proteins allows for the assembly of many different combinations of signaling modules, each combination presumably suited to particular signals, cell types, and metabolic circumstances. The large variety of protein kinases and of phosphoprotein-binding domains, each with its own specificity (the consensus sequence required in its substrate), provides for many permutations and combinations and many different signaling circuits of extraordinary complexity. And given the variety of specific phosphatases that reverse

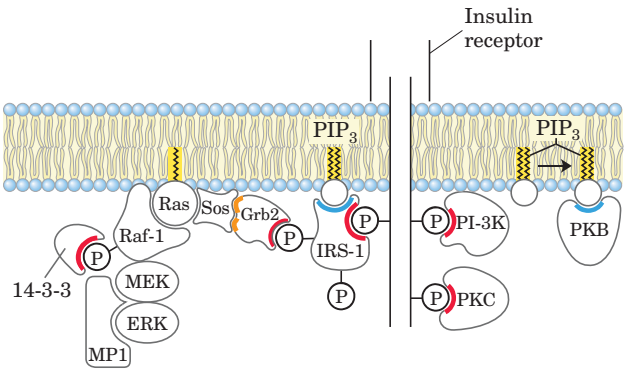


FIGURE 12–25 Insulin-induced formation of supramolecular signaling complexes. The binding of insulin to its receptor sets off a series of events that lead eventually to the formation of membrane-associated complexes involving the 12 signaling proteins shown here, as well as others. Phosphorylation of Tyr residues in the insulin receptor initiates complex formation, and dephosphorylation of any of the phosphoproteins breaks the circuit. Four general types of interaction hold the complex together: the binding of a protein to a second phosphoprotein through SH2 or PTB domains in the first (red); the binding of SH3 domains in the first with proline-rich domains in the second (orange); the binding of PH domains in one protein to the phospholipid PIP_3 in the plasma membrane (blue); or the association of a protein (RAS) with the plasma membrane through a lipid covalently bound to the protein (yellow). Two proteins shown here are not described in the text: 14-3-3, which binds a Ⓟ–Ser in Raf and mediates its interaction with MEK; and MP1, a scaffold protein that cements the links between Raf, MEK, and ERK.

the action of protein kinases, some under specific types of external control, a cell can quickly "disconnect" the entire protein circuitry of a signaling pathway. Together, these mechanisms confer a huge capacity for cellular regulation in response to signals of many types.

Membrane Rafts and Caveolae May Segregate Signaling Proteins

Membrane rafts are regions of the membrane bilayer enriched in sphingolipids, sterols, and certain proteins, including many attached to the bilayer by GPI anchors (Chapter 11). Some receptor Tyr kinases, such as the receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), appear to be localized in rafts; other signaling proteins, such as the small G protein Ras (which is prenylated) and the heterotrimeric G protein G_s (also prenylated, on the α and γ subunits), are not. Growing evidence suggests that this sequestration of signaling proteins is functionally significant. When cholesterol is removed from rafts by treatment with cyclodextrin (which binds cholesterol and removes it from membranes), the rafts are disrupted and a number of signaling pathways become defective.

How might localization in rafts influence signaling through a receptor? There are several possibilities. If a receptor Tyr kinase in a raft is phosphorylated, and the phosphotyrosine phosphatase that reverses this phosphorylation is in another raft, then dephosphorylation of the Tyr kinase will be slowed or prevented. If two signaling proteins must interact during transduction of a signal, the probability of encounters between these proteins is greatly enhanced if both are in the same raft. Interactions between scaffold proteins might be strong enough to pull into a raft a signaling protein not normally located there, or strong enough to pull receptors out of a raft. For example, the EGF receptor in isolated fibroblasts is normally concentrated in the specialized rafts called caveolae (see Fig. 11–21), but treatment with EGF causes the receptor to leave the raft. This migration depends on the receptor's protein kinase activity; mutant receptors lacking this activity remain in the rafts during treatment with EGF. Caveolin, an integral membrane protein localized in caveolae, is phosphorylated on Tyr residues in response to insulin, and phosphorylation may allow the now-activated EGF receptor to draw its binding partners into the raft. Finally, another example of the clustering of signaling proteins in rafts is the β-adrenergic receptor. This receptor is segregated in membrane rafts that also contain the G proteins, adenylyl cyclase, PKA, and a specific protein phosphatase, PP2, providing a highly integrated signaling unit. Spatial segregation of signaling proteins in rafts adds yet another dimension to the already complex processes initiated by extracellular signals.

SUMMARY 12.5 Multivalent Scaffold Proteins and Membrane Rafts

- Many signaling proteins have domains that bind phosphorylated Tyr, Ser, or Thr residues in other proteins; the binding specificity for each domain is determined by sequences that adjoin the phosphorylated residue.
- SH2 and PTB domains bind to proteins containing Ⓟ–Tyr residues; other domains bind Ⓟ–Ser and Ⓟ–Thr residues in various contexts.
- Plextrin homology domains bind the membrane phospholipid PIP_3.
- Many signaling proteins are multivalent, with several different binding modules. By combining the substrate specificities of various protein kinases with the specificities of domains that bind phosphorylated Ser, Thr, or Tyr residues, and with phosphatases that can rapidly inactivate a pathway, cells create a large number of multiprotein signaling complexes.
- Membrane rafts and caveolae sequester groups of signaling proteins in small regions of the plasma membrane, enhancing their interactions and making signaling more efficient.

12.6 Signaling in Microorganisms and Plants

Much of what we have said here about signaling relates to mammalian tissues or cultured cells from such tissues. Bacteria, eukaryotic microorganisms, and vascular plants must also respond to a variety of external signals, such as O_2, nutrients, light, noxious chemicals, and so on. We turn here to a brief consideration of the kinds of signaling machinery used by microorganisms and plants.

Bacterial Signaling Entails Phosphorylation in a Two-Component System

E. coli responds to a number of nutrients in its environment, including sugars and amino acids, by swimming toward them, propelled by one or a few flagella. A family of membrane proteins have binding domains on the outside of the plasma membrane to which specific **attractants** (sugars or amino acids) bind (Fig. 12–26). Ligand binding causes another domain on the inside of the plasma membrane to phosphorylate itself on a His residue. This first component of the **two-component system,** the **receptor His kinase,** then catalyzes the transfer of the phosphoryl group from the His residue to an Asp residue on a second, soluble protein, the **response regulator;** this phosphoprotein moves to the base of the flagellum, carrying the signal from the membrane receptor. The flagellum is driven by a rotary motor that can propel the cell through its medium or cause it to stall, depending on the direction of the motor's rotation. Information from the receptor allows the cell to determine whether it is moving toward or away from the source of the attractant. If its motion is toward the attractant, the response regulator signals the cell to continue in a straight line; if away from it, the cell tumbles momentarily, acquiring a new direction. Repetition of this behavior results in a random path, biased toward movement in the direction of increasing attractant concentration.

E. coli detects not only sugars and amino acids but also O_2, extremes of temperature, and other environmental factors, using this basic two-component system. Two-component systems have been detected in many other bacteria, including gram-positive and gram-negative eubacteria and archaebacteria, as well as in protists and fungi. Clearly this signaling mechanism developed early in the course of cellular evolution and has been conserved.

Various signaling systems used by animal cells also have analogs in the prokaryotes. As the full genomic sequences of more, and more diverse, bacteria become known, researchers have discovered genes that encode proteins similar to protein Ser or Thr kinases, Ras-like proteins regulated by GTP binding, and proteins with SH3 domains. Receptor Tyr kinases have not been detected in bacteria, but Ⓟ–Tyr residues do occur in some bacterial proteins, so there must be an enzyme that phosphorylates Tyr residues.

Signaling Systems of Plants Have Some of the Same Components Used by Microbes and Mammals

Like animals, vascular plants must have a means of communication between tissues to coordinate and direct growth and development; to adapt to conditions of O_2, nutrients, light, and temperature; and to warn of the presence of noxious chemicals and damaging pathogens (Fig. 12–27). At least a billion years of evolution have passed since the plant and animal branches of the eukaryotes diverged, which is reflected in the differences in signaling mechanisms: some plant mechanisms are conserved—that is, are similar to those in animals (protein kinases, scaffold proteins, cyclic nucleotides, electrogenic ion pumps, and gated ion channels); some are similar to bacterial two-component systems; and some are unique to plants (light-sensing mechanisms, for ex-

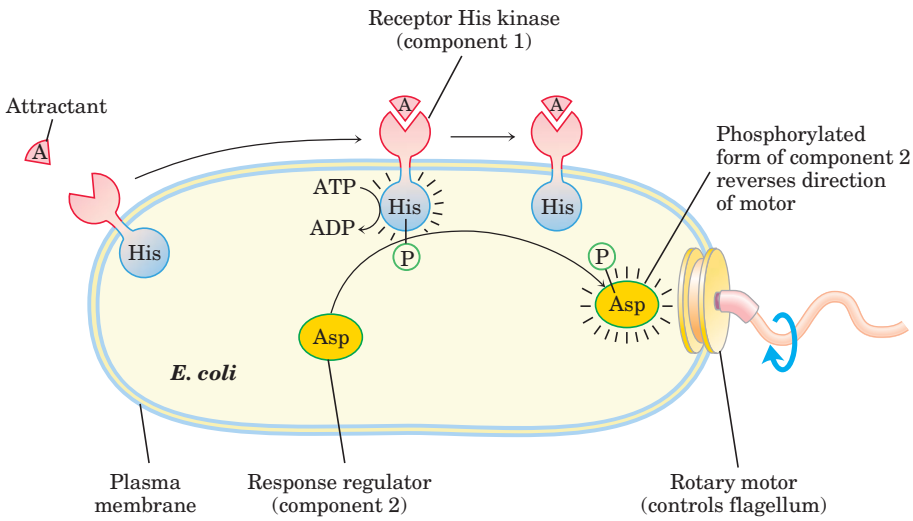


FIGURE 12–26 The two-component signaling mechanism in bacterial chemotaxis. When an attractant ligand (A) binds to the receptor domain of the membrane-bound receptor, a protein His kinase in the cytosolic domain (component 1) is activated and autophosphorylates on a His residue. This phosphoryl group is then transferred to an Asp residue on component 2 (in some cases a separate protein; in others, another domain of the receptor protein). After phosphorylation on Asp, component 2 moves to the base of the flagellum, where it determines the direction of rotation of the flagellar motor.

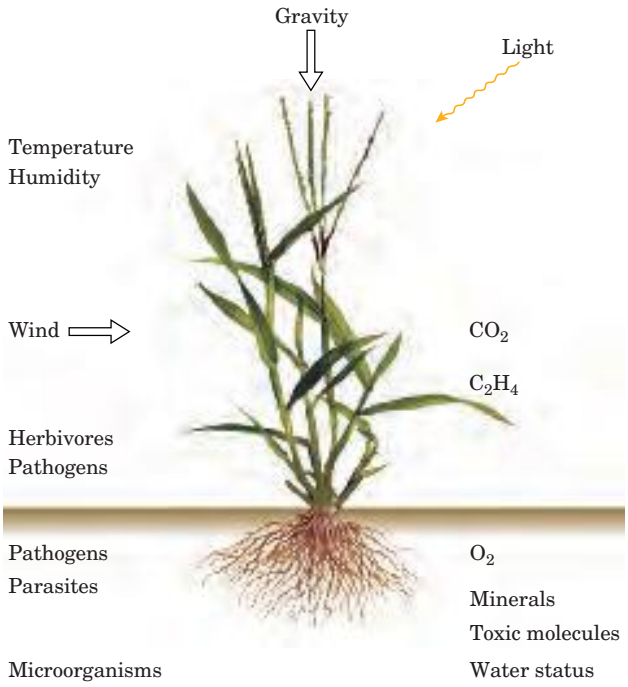


FIGURE 12–27 Some stimuli that produce responses in plants.

ample) (Table 12–7). The genome of the widely studied plant *Arabidopsis thaliana*, for example, encodes about 1,000 protein Ser/Thr kinases, including about 60 MAPKs and nearly 400 membrane-associated receptor kinases that phosphorylate Ser or Thr residues; a variety of protein phosphatases; scaffold proteins that bring other proteins together in signaling complexes; enzymes for the synthesis and degradation of cyclic nucleotides; and 100 or more ion channels, including about 20 gated by cyclic nucleotides. Inositol phospholipids are present, as are kinases that interconvert them by phosphorylation of inositol head groups.

However, some types of signaling proteins common in animal tissues are not present in plants, or are represented by only a few genes. Cyclic nucleotide–dependent protein kinases (PKA and PKG) appear to be absent, for example. Heterotrimeric G proteins and protein Tyr kinase genes are much less prominent in the plant genome, and serpentine (G protein–coupled) receptors, the largest gene family in the human genome (>1,000 genes), are very sparsely represented in the plant genome. DNA-binding nuclear steroid receptors are certainly not prominent, and may be absent from plants. Although plants lack the most widely conserved light-sensing mechanism present in animals (rhodopsin, with retinal as pigment), they have a rich collection of other light-detecting mechanisms not found in animal tissues—phytochromes and cryptochromes, for example (Chapter 19).

The kinds of compounds that elicit signals in plants are similar to certain signaling molecules in mammals (Fig. 12–28). Instead of prostaglandins, plants have jasmonate; instead of steroid hormones, brassinosteroids.

TABLE 12–7 Signaling Components Present in Mammals, Plants, or Bacteria

Signaling protein	*Mammals*	*Plants*	*Bacteria*
Ion channels	+	+	+
Electrogenic ion pumps	+	+	+
Two-component His kinases	+	+	+
Adenylyl cyclase	+	+	+
Guanylyl cyclase	+	+	?
Receptor protein kinases (Ser/Thr)	+	+	?
Ca^{2+} as second messenger	+	+	?
Ca^{2+} channels	+	+	?
Calmodulin, CaM-binding protein	+	+	–
MAPK cascade	+	+	–
Cyclic nucleotide–gated channels	+	+	–
IP_3-gated Ca^{2+} channels	+	+	–
Phosphatidylinositol kinases	+	+	–
Serpentine receptors	+	+/–	+
Trimeric G proteins	+	+/–	–
PI-specific phospholipase C	+	?	–
Tyrosine kinase receptors	+	?	–
SH2 domains	+	?	?
Nuclear steroid receptors	+	–	–
Protein kinase A	+	–	–
Protein kinase G	+	–	–

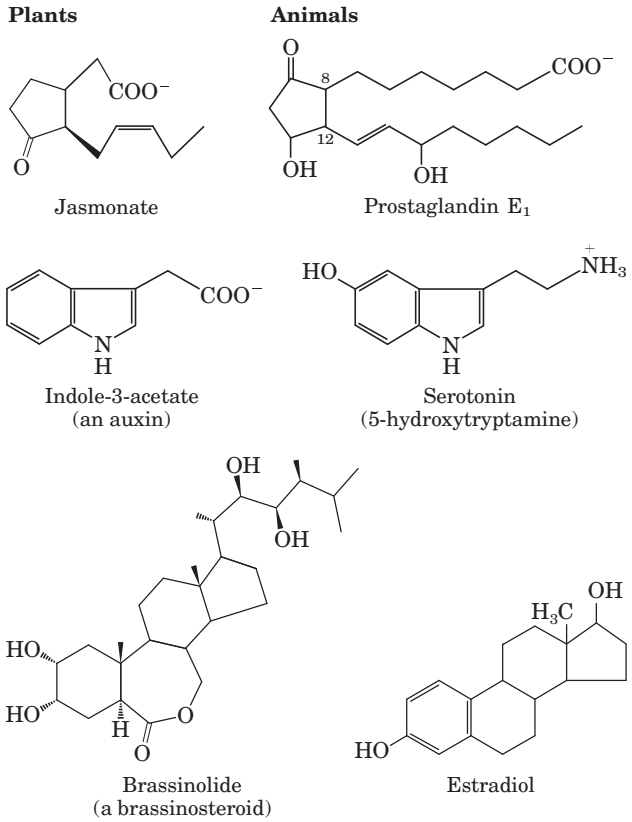


FIGURE 12–28 Structural similarities between plant and animal signals. The plant signals jasmonate, indole-3-acetate, and brassinolide resemble the mammalian signals prostaglandin E_1, serotonin, and estradiol.

About 100 different small peptides serve as plant signals, and both plants and animals use compounds derived from aromatic amino acids as signals.

Plants Detect Ethylene through a Two-Component System and a MAPK Cascade

The receptors for the plant hormone ethylene ($CH_2{=}CH_2$) are related in primary sequence to the receptor His kinases of the bacterial two-component systems and probably evolved from them; the cyanobacterial origin of chloroplasts (see Fig. 1–36) may have brought the bacterial signaling genes into the plant cell nucleus. In *Arabidopsis*, the two-component signaling system is contained within a single protein. The first downstream component affected by ethylene signaling is a protein Ser/Thr kinase (CTR-1; Fig. 12–29) with sequence homology to Raf, the protein kinase that begins the MAPK cascade in the mammalian response to insulin (see the comparison in Fig. 12–30). In plants, in the absence of ethylene, the CTR-1 kinase is active and *inhibits* the MAPK cascade, preventing transcription of ethylene-responsive genes. Exposure to ethylene *inactivates* the CTR-1 kinase, thereby activating the MAPK cascade that leads to activation of the transcription factor EIN3. Active EIN3 stimulates the synthesis of a second transcription factor (ERF1), which in turn activates transcription of a number of ethylene-responsive genes; the gene products affect processes ranging from seedling development to fruit ripening.

Although apparently derived from the bacterial two-component signaling system, the ethylene system in *Arabidopsis* is different in that the His kinase activity that defines component 1 in bacteria is not essential to the transduction in *Arabidopsis*. The genome of the cyanobacterium *Anabaena* encodes proteins with both an ethylene-binding domain and an active His kinase domain. It seems likely that in the course of evolution, the ethylene receptor of vascular plants was derived from that of a cyanobacterial endosymbiont, and that the bacterial His kinase became a Ser/Thr kinase in the plant.

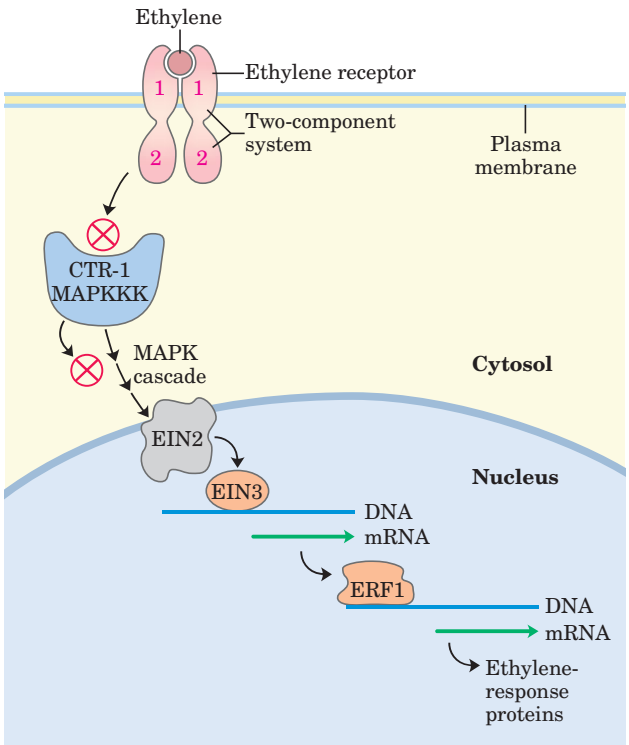


FIGURE 12–29 Transduction mechanism for detection of ethylene by plants. The ethylene receptor in the plasma membrane (red) is a two-component system contained within a single protein, which has both a receptor domain (component 1) and a response regulator domain (component 2). The receptor controls (in ways we do not yet understand) the activity of CTR1, a protein kinase similar to MAPKKKs and therefore presumed to be part of a MAPK cascade. CTR1 is a negative regulator of the ethylene response; when CTR1 is *inactive*, the ethylene signal passes through the gene product EIN2 (thought to be a nuclear envelope protein), which somehow causes increased synthesis of ERF1, a transcription factor; ERF1 in turn stimulates expression of proteins specific to the ethylene response.

Receptorlike Protein Kinases Transduce Signals from Peptides and Brassinosteroids

One common motif in plant signaling involves **receptorlike kinases (RLKs)** with a single helical segment in the plasma membrane that connects a receptor domain on the outside of the membrane with a protein Ser/Thr kinase on the cytoplasmic side. This type of receptor participates in the defense mechanism triggered by infection with a bacterial pathogen (Fig. 12–30a). The signal to turn on the genes needed for defense against infection is a peptide (flg22) released by breakdown of flagellin, the major protein of the bacterial flagellum. Binding of flg22 to the FLS2 receptor of *Arabidopsis* induces receptor dimerization and autophosphorylation on Ser and Thr residues, and the downstream effect is activation of a MAPK cascade like that described above for insulin action (Fig. 12–6). The final kinase in this cascade activates a specific transcription factor, triggering synthesis of the proteins that defend against the bacterial infection. The steps between receptor phosphorylation and the MAPK cascade are not yet known. A phosphoprotein phosphatase (KAPP) associates with the active receptor protein and inactivates it by dephosphorylation to end the response.

The MAPK cascade in the plant's defense against bacterial pathogens is remarkably similar to the innate immune response triggered by bacterial lipopolysaccharide and mediated by the Toll-like receptors in mammals (Fig. 12–30b). Other membrane receptors use similar mechanisms to activate a MAPK cascade, ultimately activating transcription factors and turning on the genes essential to the defense response.

Most of the several hundred RLKs in plants are presumed to act in similar ways: ligand binding induces dimerization and autophosphorylation, and the

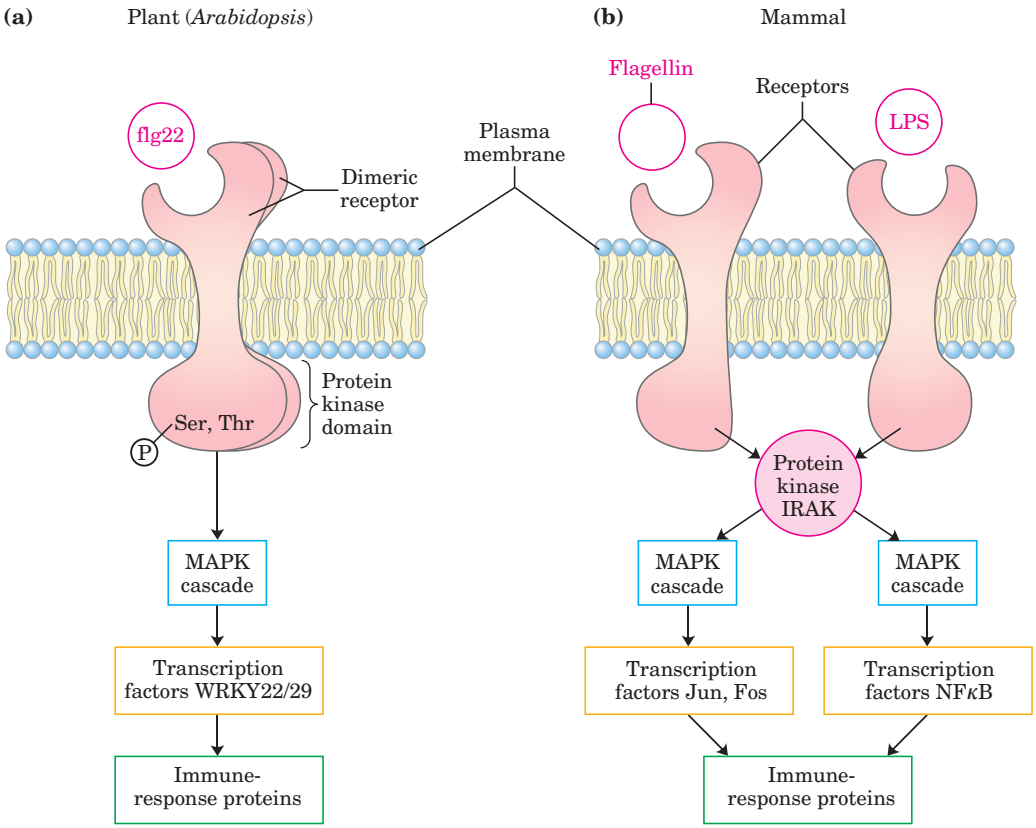


FIGURE 12–30 Similarities between the signaling pathways that trigger immune responses in plants and animals. (a) In the plant *Arabidopsis thaliana*, the peptide flg22, derived from the flagella of a bacterial pathogen, binds to its receptor in the plasma membrane, causing the receptors to form dimers and triggering autophosphorylation of the cytosolic protein kinase domain on a Ser or Thr residue (*not* a Tyr). Autophosphorylation activates the receptor protein kinase, which then phosphorylates downstream proteins. The activated receptor also activates (by means unknown) a MAPKKK. The resulting kinase cascade leads to phosphorylation of a nuclear protein that normally inhibits the transcription factors WRKY22 and 29, triggering proteolytic degradation of the inhibitor and freeing the transcription factors to stimulate gene expression related to the immune response. **(b)** In mammals, the toxic bacterial lipopolysaccharide (LPS; see Fig. 7–32) is detected by plasma membrane receptors that associate with and activate a soluble protein kinase (IRAK). The major flagellar protein of pathogenic bacteria acts through a similar receptor to activate IRAK. Then IRAK initiates two distinct MAPK cascades that end in the nucleus, causing the synthesis of proteins needed in the immune response. Jun, Fos, and NFκB are transcription factors.

activated receptor kinase triggers downstream responses by phosphorylating key proteins at Ser or Thr residues. The ligands for these kinases have been identified in only a few cases: brassinosteroids, the peptide trigger for the self-incompatibility response that prevents self-pollination, and CLV1 peptide, a factor involved in regulating the fate of stem cells (undifferentiated cells) in plant development.

SUMMARY 12.6 Signaling in Microorganisms and Plants

- Bacteria and unicellular eukaryotes have a variety of sensory systems that allow them to sample and respond to their environment. In the two-component system, a receptor His kinase senses the signal and autophosphorylates a His residue, then phosphorylates the response regulator on an Asp residue.
- Plants respond to many environmental stimuli, and employ hormones and growth factors to coordinate the development and metabolic activities of their tissues. Plant genomes encode hundreds of signaling proteins, including some very similar to those used in signal transductions in mammalian cells.
- Two-component signaling mechanisms common in bacteria have been acquired in altered forms by plants. Cyanobacteria use typical two-component systems in the detection of chemical signals and light; plants use related proteins—which autophosphorylate on Ser/Thr, not His, residues—to detect ethylene.
- Plant receptorlike kinases (RLKs), with an extracellular ligand-binding domain, a single transmembrane segment, and a cytosolic protein kinase domain, participate in detecting a wide variety of stimuli, including peptides that originate from pathogens, brassinosteroid hormones, self-incompatible pollen, and developmental signals. RLKs autophosphorylate Ser/Thr residues, then activate downstream proteins that in some cases are MAPK cascades. The end result of many such signals is increased transcription of specific genes.

12.7 Sensory Transduction in Vision, Olfaction, and Gustation

The detection of light, smells, and tastes (vision, olfaction, and gustation, respectively) in animals is accomplished by specialized sensory neurons that use signal-transduction mechanisms fundamentally similar to those that detect hormones, neurotransmitters, and growth factors. An initial sensory signal is amplified greatly by mechanisms that include gated ion channels and intracellular second messengers; the system adapts to continued stimulation by changing its sensitivity to the stimulus (desensitization); and sensory input from several receptors is integrated before the final signal goes to the brain.

Light Hyperpolarizes Rod and Cone Cells of the Vertebrate Eye

In the vertebrate eye, light entering through the pupil is focused on a highly organized collection of light-sensitive neurons (Fig. 12–31). The light-sensing cells are of two types: **rods** (about 10^9 per retina), which sense low levels of light but cannot discriminate colors, and **cones** (about 3×10^6 per retina), which are less sensitive to light but can discriminate colors. Both cell types are long, narrow, specialized sensory neurons with two distinct cellular compartments: the **outer segment** contains dozens of membranous disks loaded with the membrane protein rhodopsin, and the **inner segment** contains the nucleus and many mitochondria, which produce the ATP essential to phototransduction.

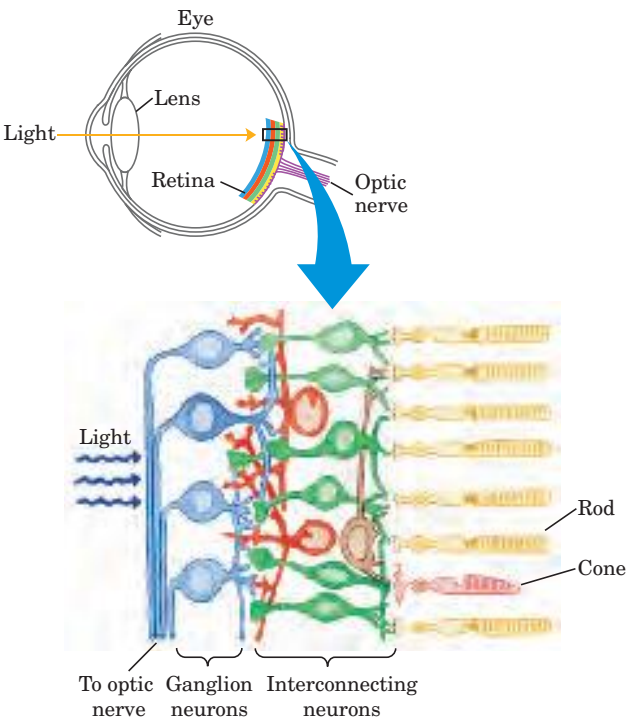


FIGURE 12–31 Light reception in the vertebrate eye. The lens of the eye focuses light on the retina, which is composed of layers of neurons. The primary photosensory neurons are rod cells (yellow), which are responsible for high-resolution and night vision, and cone cells of three subtypes (pink), which initiate color vision. The rods and cones form synapses with several ranks of interconnecting neurons that convey and integrate the electrical signals. The signals eventually pass from ganglion neurons through the optic nerve to the brain.

Like other neurons, rods and cones have a transmembrane electrical potential (V_m), produced by the electrogenic pumping of the Na^+K^+ ATPase in the plasma membrane of the inner segment (Fig. 12–32). Also contributing to the membrane potential is an ion channel in the outer segment that permits passage of either Na^+ or Ca^{2+} and is gated (opened) by cGMP. In the dark, rod cells contain enough cGMP to keep this channel open. The membrane potential is therefore determined by the net difference between the Na^+ and K^+ pumped by the inner segment (which polarizes the membrane) and the influx of Na^+ through the ion channels of the outer segment (which tends to depolarize the membrane).

The essence of signaling in the retinal rod or cone cell is a light-induced decrease in the concentration of cGMP, which causes the cGMP-gated ion channel to close. The plasma membrane then becomes hyperpolarized by the Na^+K^+ ATPase. Rod and cone cells synapse with interconnecting neurons (Fig. 12–31) that carry information about the electrical activity to the ganglion neurons near the inner surface of the retina. The ganglion neurons integrate the output from many rod or cone cells and send the resulting signal through the optic nerve to the visual cortex of the brain.

Light Triggers Conformational Changes in the Receptor Rhodopsin

Visual transduction begins when light falls on rhodopsin, many thousands of molecules of which are present in each disk of the outer segments of rod and cone cells. **Rhodopsin** (M_r 40,000) is an integral protein with seven membrane-spanning α helices (Fig. 12–33), the characteristic serpentine architecture. The amino-terminal domain projects into the disk, and the carboxyl-terminal domain faces the cytosol of the outer segment. The light-absorbing pigment (chromophore) **11-*cis*-retinal** is covalently attached to **opsin,** the protein component of rhodopsin, through a Schiff base to a Lys residue. The retinal lies near the middle of the bilayer (Fig. 12–33), oriented with its long axis approximately in the plane of the membrane. When a photon is absorbed by the retinal component of rhodopsin, the energy causes a photochemical change; 11-*cis*-retinal is converted to **all-*trans*-retinal** (see Figs 1–18b, 10–21). This change in the structure of the chromophore causes conformational changes in the rhodopsin molecule—the first stage in visual transduction.

Excited Rhodopsin Acts through the G Protein Transducin to Reduce the cGMP Concentration

In its excited conformation, rhodopsin interacts with a second protein, **transducin,** which hovers nearby on the cytoplasmic face of the disk membrane (Fig. 12–33). Transducin (T) belongs to the same family of heterotrimeric GTP-binding proteins as G_s and G_i. Although

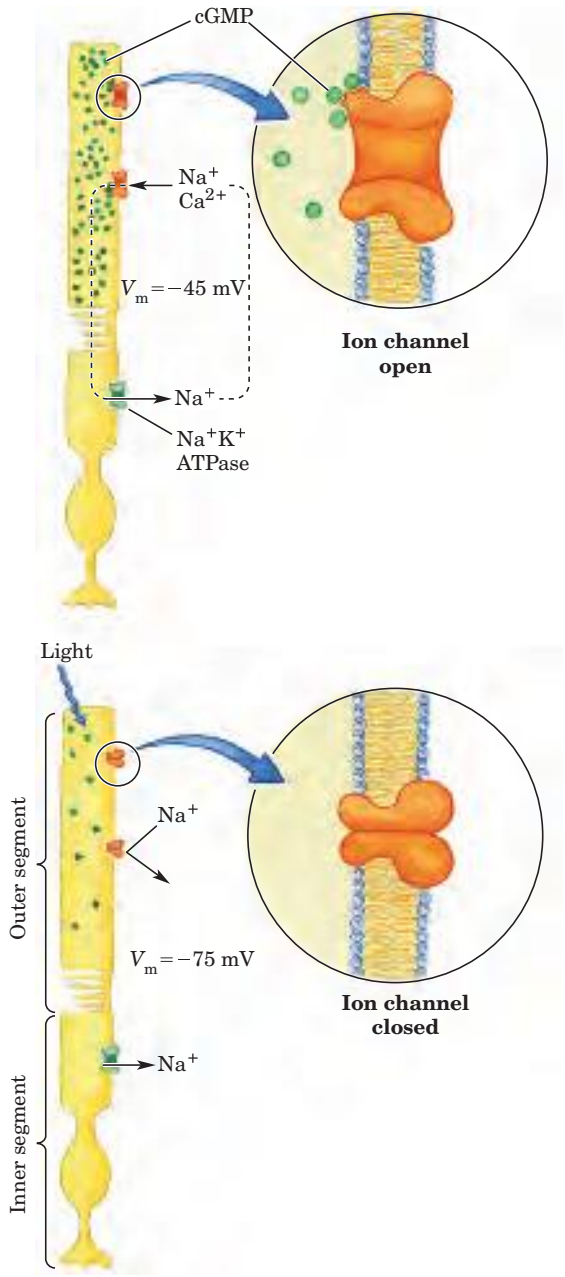


FIGURE 12–32 Light-induced hyperpolarization of rod cells. The rod cell consists of an outer segment that is filled with stacks of membranous disks (not shown) containing the photoreceptor rhodopsin and an inner segment that contains the nucleus and other organelles. Cones have a similar structure. ATP in the inner segment powers the Na^+K^+ ATPase, which creates a transmembrane electrical potential by pumping 3 Na^+ out for every 2 K^+ pumped in. The membrane potential is reduced by the flow of Na^+ and Ca^{2+} into the cell through cGMP-gated cation channels in the plasma membrane of the outer segment. When rhodopsin absorbs light, it triggers degradation of cGMP (green dots) in the outer segment, causing closure of the cation channel. Without cation influx through this channel, the cell becomes hyperpolarized. This electrical signal is passed to the brain through the ranks of neurons shown in Figure 12–31.

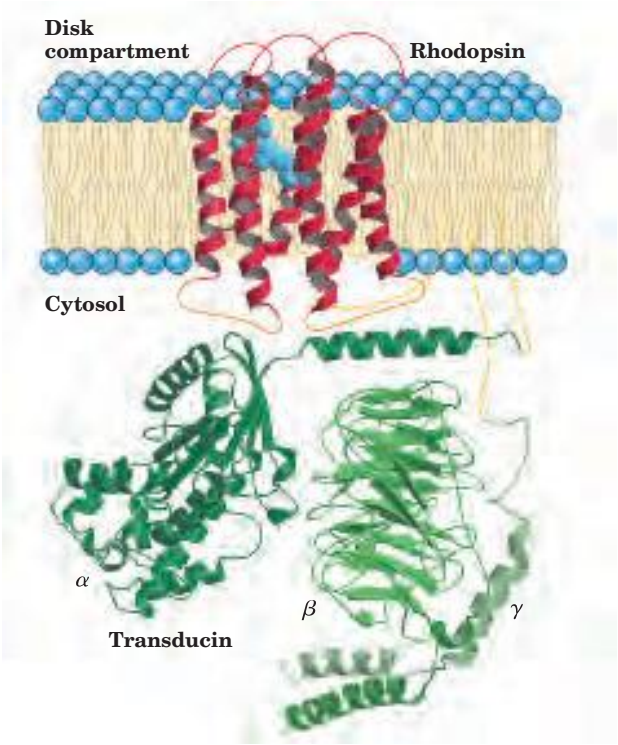


FIGURE 12–33 Likely structure of rhodopsin complexed with the G protein transducin. (PDB ID 1BAC) Rhodopsin (red) has seven transmembrane helices embedded in the disk membranes of rod outer segments and is oriented with its carboxyl terminus on the cytosolic side and its amino terminus inside the disk. The chromophore 11-*cis* retinal (blue), attached through a Schiff base linkage to Lys^{256} of the seventh helix, lies near the center of the bilayer. (This location is similar to that of the epinephrine-binding site in the β-adrenergic receptor.) Several Ser and Thr residues near the carboxyl terminus are substrates for phosphorylations that are part of the desensitization mechanism for rhodopsin. Cytosolic loops that interact with the G protein transducin are shown in orange; their exact positions are not yet known. The three subunits of transducin (green) are shown in their likely arrangement. Rhodopsin is palmitoylated at its carboxyl terminus, and both the α and γ subunits of transducin have attached lipids (yellow) that assist in anchoring them to the membrane.

specialized for visual transduction, transducin shares many functional features with G_s and G_i. It can bind either GDP or GTP. In the dark, GDP is bound, all three subunits of the protein (T_α, T_β, and T_γ) remain together, and no signal is sent. When rhodopsin is excited by light, it interacts with transducin, catalyzing the replacement of bound GDP by GTP from the cytosol (Fig. 12–34, steps ① and ②). Transducin then dissociates into T_α and $T_{\beta\gamma}$, and the T_α-GTP carries the signal from the excited receptor to the next element in the transduction pathway, cGMP phosphodiesterase (PDE); this enzyme converts cGMP to 5′-GMP (steps ③ and ④). Note that this is not the same cyclic nucleotide phosphodiesterase that hydrolyzes cAMP to terminate the β-adrenergic response. The cGMP-specific PDE is unique to the visual cells of the retina.

PDE is an integral protein with its active site on the cytoplasmic side of the disk membrane. In the dark, a tightly bound inhibitory subunit very effectively suppresses PDE activity. When T_α-GTP encounters PDE, the inhibitory subunit is released, and the enzyme's activity immediately increases by several orders of magnitude. Each molecule of active PDE degrades many molecules of cGMP to the biologically inactive 5′-GMP, lowering [cGMP] in the outer segment within a fraction of a second. At the new, lower [cGMP], the cGMP-gated ion channels close, blocking reentry of Na^+ and Ca^{2+} into the outer segment and hyperpolarizing the membrane of the rod or cone cell (step ⑤). Through this process, the initial stimulus—a photon—changes the V_m of the cell.

Amplification of the Visual Signal Occurs in the Rod and Cone Cells

Several steps in the visual-transduction process result in great amplification of the signal. Each excited rhodopsin molecule activates at least 500 molecules of transducin, each of which can activate a molecule of PDE. This phosphodiesterase has a remarkably high turnover number, each activated molecule hydrolyzing 4,200 molecules of cGMP per second. The binding of cGMP to cGMP-gated ion channels is cooperative (at least three cGMP molecules must be bound to open one channel), and a relatively small change in [cGMP] therefore registers as a large change in ion conductance. The result of these amplifications is exquisite sensitivity to light. Absorption of a single photon closes 1,000 or more ion channels and changes the cell's membrane potential by about 1 mV.

The Visual Signal Is Quickly Terminated

As your eyes move across this line, the images of the first words disappear rapidly—before you see the next series of words. In that short interval, a great deal of biochemistry has taken place. Very shortly after illumination of the rod or cone cells stops, the photosensory system shuts off. The α subunit of transducin (with bound GTP) has intrinsic GTPase activity. Within milliseconds after the decrease in light intensity, GTP is hydrolyzed and T_α reassociates with $T_{\beta\gamma}$. The inhibitory subunit of PDE, which had been bound to T_α-GTP, is released and reassociates with PDE, strongly inhibiting that enzyme. To return [cGMP] to its "dark" level, the enzyme guanylyl cyclase converts GTP to cGMP (step ⑦ in Fig. 12–34) in a reaction that is inhibited by high [Ca^{2+}] (>100 nM). Calcium levels drop during illumination, because the steady-state [Ca^{2+}] in the outer segment is the result of outward pumping of Ca^{2+} through the Na^+-Ca^{2+} exchanger of the plasma membrane and inward movement of Ca^{2+} through open cGMP-gated channels. In the dark, this produces a [Ca^{2+}] of about 500 nM—enough to inhibit cGMP synthesis. After brief illumination, Ca^{2+} entry slows and [Ca^{2+}] declines (step

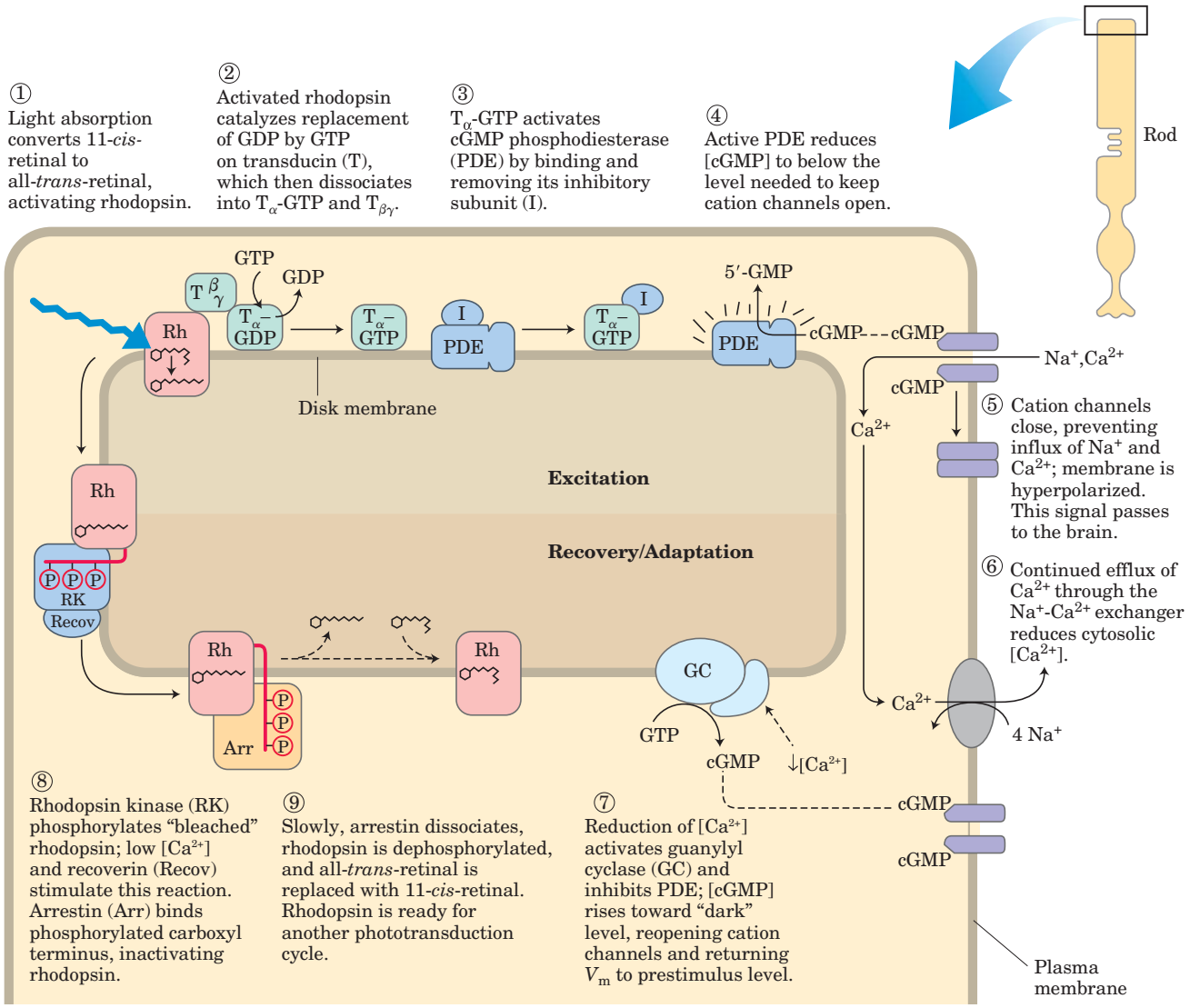


FIGURE 12–34 Molecular consequences of photon absorption by rhodopsin in the rod outer segment. The top half of the figure (steps ① to ⑤) describes excitation; the bottom (steps ⑥ to ⑨), recovery and adaptation after illumination.

⑥). The inhibition of guanylyl cyclase by Ca^{2+} is relieved, and the cyclase converts GTP to cGMP to return the system to its prestimulus state (step ⑦).

Rhodopsin Is Desensitized by Phosphorylation

Rhodopsin itself also undergoes changes in response to prolonged illumination. The conformational change induced by light absorption exposes several Thr and Ser residues in the carboxyl-terminal domain. These residues are quickly phosphorylated by **rhodopsin kinase** (step ⑧ in Fig. 12–34), which is functionally and structurally homologous to the β-adrenergic kinase (βARK) that desensitizes the β-adrenergic receptor (Fig. 12–17). The Ca^{2+}-binding protein **recoverin** inhibits rhodopsin kinase at high $[Ca^{2+}]$, but the inhibition is relieved when $[Ca^{2+}]$ drops after illumination, as described above. The phosphorylated carboxyl-terminal domain of rhodopsin is bound by the protein **arrestin 1,** preventing further interaction between activated rhodopsin and transducin. Arrestin 1 is a close homolog of arrestin 2 (βarr; Fig. 12–17). On a relatively long time scale (seconds to minutes), the all-*trans*-retinal of an excited rhodopsin molecule is removed and replaced by 11-*cis*-retinal, to produce rhodopsin that is ready for another round of excitation (step ⑨ in Fig. 12–34).

Humans cannot synthesize retinal from simpler precursors and must obtain it in the diet in the form of vitamin A (see Fig. 10–21). Given the role of retinal in the process of vision, it is not surprising that dietary deficiency of vitamin A causes night blindness (poor vision at night or in dim light). ■

Cone Cells Specialize in Color Vision

Color vision in cone cells involves a path of sensory transduction essentially identical to that described above, but triggered by slightly different light receptors. Three types of cone cells are specialized to detect light from different regions of the spectrum, using three related photoreceptor proteins (opsins). Each cone cell expresses only one kind of opsin, but each type is closely related to rhodopsin in size, amino acid sequence, and presumably three-dimensional structure. The differences among the opsins, however, are great enough to place the chromophore, 11-*cis*-retinal, in three slightly different environments, with the result that the three photoreceptors have different absorption spectra (Fig. 12–35). We discriminate colors and hues by integrating the output from the three types of cone cells, each containing one of the three photoreceptors.

Color blindness, such as the inability to distinguish red from green, is a fairly common, genetically inherited trait in humans. The various types of color blindness result from different opsin mutations. One form is due to loss of the red photoreceptor; affected individuals are **red$^-$ dichromats** (they see only two primary colors). Others lack the green pigment and are **green$^-$ dichromats.** In some cases, the red and green photoreceptors are present but have a changed amino acid sequence that causes a change in their absorption spectra, resulting in abnormal color vision. Depending on which pigment is altered, such individuals are **red-anomalous trichromats** or **green-anomalous trichromats.** Examination of the genes for the visual receptors has allowed the diagnosis of color blindness in a famous "patient" more than a century after his death (Box 12–3)! ■

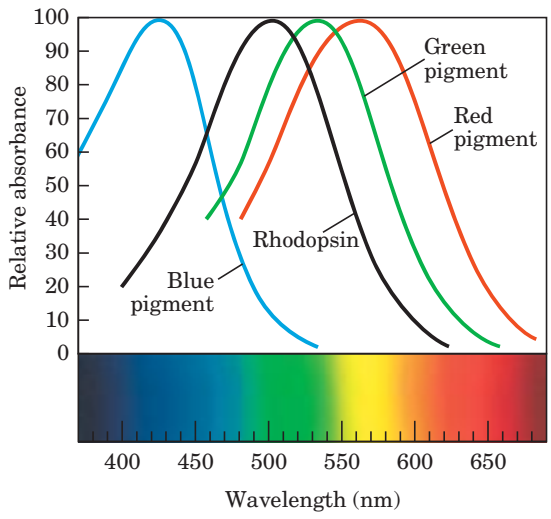


FIGURE 12–35 Absorption spectra of purified rhodopsin and the red, green, and blue receptors of cone cells. The spectra, obtained from individual cone cells isolated from cadavers, peak at about 420, 530, and 560 nm, and the maximum absorption for rhodopsin is at about 500 nm. For reference, the visible spectrum for humans is about 380 to 750 nm.

Vertebrate Olfaction and Gustation Use Mechanisms Similar to the Visual System

The sensory cells used to detect odors and tastes have much in common with the rod and cone cells that detect light. Olfactory neurons have a number of long thin cilia extending from one end of the cell into a mucous layer that overlays the cell. These cilia present a large surface area for interaction with olfactory signals. The receptors for olfactory stimuli are ciliary membrane proteins with the familiar serpentine structure of seven transmembrane α helices. The olfactory signal can be any one of the many volatile compounds for which there are specific receptor proteins. Our ability to discriminate odors stems from hundreds of different olfactory receptors in the tongue and nasal passages and from the brain's ability to integrate input from different types of olfactory receptors to recognize a "hybrid" pattern, extending our range of discrimination far beyond the number of receptors.

The olfactory stimulus arrives at the sensory cells by diffusion through the air. In the mucous layer overlaying the olfactory neurons, the odorant molecule binds directly to an olfactory receptor or to a specific binding protein that carries the odorant to a receptor (Fig. 12–36). Interaction between odorant and receptor triggers a change in receptor conformation that results in the replacement of bound GDP by GTP on a G protein, G_{olf}, analogous to transducin and to G_s of the β-adrenergic system. The activated G_{olf} then activates adenylyl cyclase of the ciliary membrane, which synthesizes cAMP from ATP, raising the local [cAMP]. The cAMP-gated Na^+ and Ca^{2+} channels of the ciliary membrane open, and the influx of Na^+ and Ca^{2+} produces a small depolarization called the **receptor potential.** If a sufficient number of odorant molecules encounter receptors, the receptor potential is strong enough to cause the neuron to fire an action potential. This is relayed to the brain in several stages and registers as a specific smell. All these events occur within 100 to 200 ms.

Some olfactory neurons may use a second transduction mechanism. They have receptors coupled through G proteins to PLC rather than to adenylyl cyclase. Signal reception in these cells triggers production of IP_3 (Fig. 12–19), which opens IP_3-gated Ca^{2+} channels in the ciliary membrane. Influx of Ca^{2+} then depolarizes the ciliary membrane and generates a receptor potential or regulates Ca^{2+}-dependent enzymes in the olfactory pathway.

In either type of olfactory neuron, when the stimulus is no longer present, the transducing machinery shuts

BOX 12–3 BIOCHEMISTRY IN MEDICINE

Color Blindness: John Dalton's Experiment from the Grave

The chemist John Dalton (of atomic theory fame) was color-blind. He thought it probable that the vitreous humor of his eyes (the fluid that fills the eyeball behind the lens) was tinted blue, unlike the colorless fluid of normal eyes. He proposed that after his death, his eyes should be dissected and the color of the vitreous humor determined. His wish was honored. The day after Dalton's death in July 1844, Joseph Ransome dissected his eyes and found the vitreous humor to be perfectly colorless. Ransome, like many scientists, was reluctant to throw samples away. He placed Dalton's eyes in a jar of preservative (Fig. 1), where they stayed for a century and a half.

Then, in the mid-1990s, molecular biologists in England took small samples of Dalton's retinas and extracted DNA. Using the known gene sequences for the opsins of the red and green photopigments, they amplified the relevant sequences (using techniques described in Chapter 9) and determined that Dalton had the opsin gene for the red photopigment but lacked the opsin gene for the green photopigment. Dalton was a green$^-$ dichromat. So, 150 years after his death, the experiment Dalton started—by hypothesizing about the cause of his color blindness—was finally finished.



FIGURE 1 Dalton's eyes.

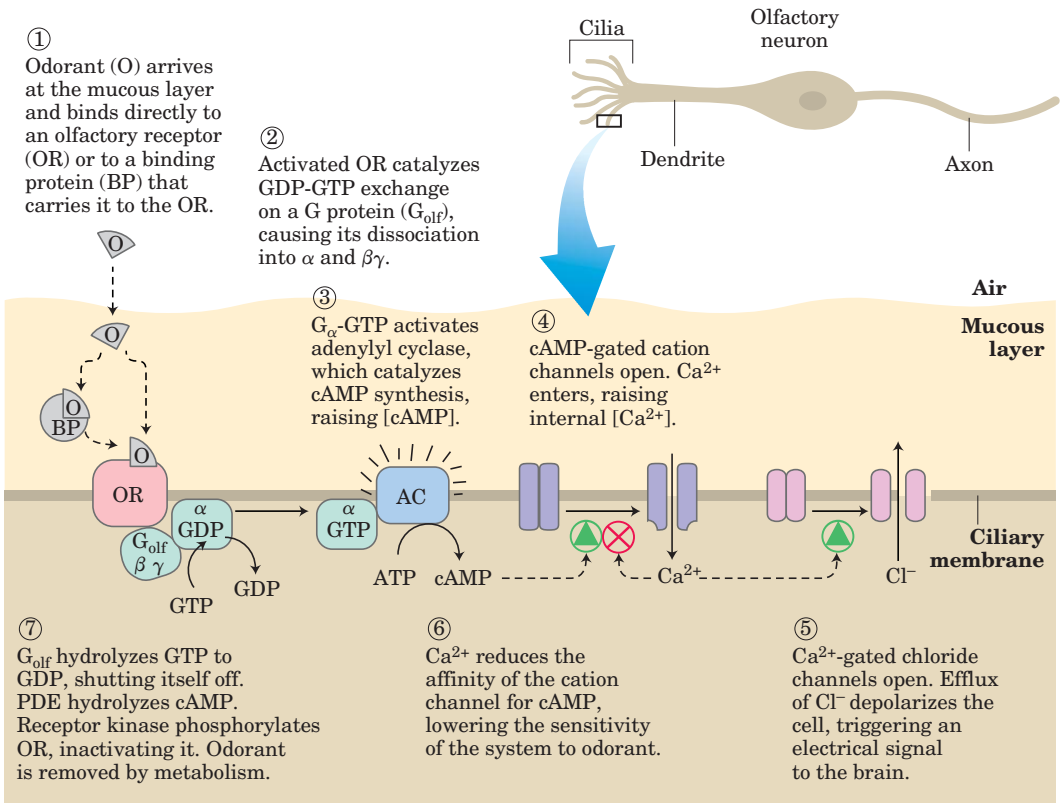


FIGURE 12–36 Molecular events of olfaction. These interactions occur in the cilia of olfactory receptor cells.

itself off in several ways. A cAMP phosphodiesterase returns [cAMP] to the prestimulus level. G_{olf} hydrolyzes its bound GTP to GDP, thereby inactivating itself. Phosphorylation of the receptor by a specific kinase prevents its interaction with G_{olf}, by a mechanism analogous to that used to desensitize the β-adrenergic receptor and rhodopsin. And lastly, some odorants are enzymatically destroyed by oxidases.

The sense of taste in vertebrates reflects the activity of gustatory neurons clustered in taste buds on the surface of the tongue. In these sensory neurons, serpentine receptors are coupled to the heterotrimeric G protein **gustducin** (very similar to the transducin of rod and cone cells). Sweet-tasting molecules are those that bind receptors in "sweet" taste buds. When the molecule (tastant) binds, gustducin is activated by replacement of bound GDP with GTP and then stimulates cAMP production by adenylyl cyclase. The resulting elevation of [cAMP] activates PKA, which phosphorylates K^+ channels in the plasma membrane, causing them to close. Reduced efflux of K^+ depolarizes the cell (Fig. 12–37). Other taste buds specialize in detecting bitter, sour, or salty tastants, using various combinations of second messengers and ion channels in the transduction mechanisms.

G Protein–Coupled Serpentine Receptor Systems Share Several Features

We have now looked at four systems (hormone signaling, vision, olfaction, and gustation) in which membrane receptors are coupled to second messenger–generating enzymes through G proteins. It is clear that signaling mechanisms arose early in evolution; serpentine receptors, heterotrimeric G proteins, and adenylyl cyclase are found in virtually all eukaryotic organisms. Even the common brewer's yeast *Saccharomyces* uses serpentine receptors and G proteins to detect the opposite mating type. Overall patterns have been conserved, and the introduction of variety has given modern organisms the ability to respond to a wide range of stimuli (Table 12–8). Of the 35,000 or so genes in the human genome, as many as 1,000 encode serpentine receptors, including hundreds for olfactory stimuli and a number of "orphan receptors" for which the natural ligand is not yet known.

All well-studied transducing systems that act through heterotrimeric G proteins share certain common features (Fig. 12–38). The receptors have seven transmembrane segments, a domain (generally the loop between transmembrane helices 6 and 7) that interacts with a G protein, and a carboxyl-terminal cytoplasmic domain that undergoes reversible phosphorylation on several Ser or Thr residues. The ligand-binding site (or, in the case of light reception, the light receptor) is buried deep in the membrane and includes residues from several of the transmembrane segments. Ligand binding (or light) induces a conformational change in the receptor, exposing a domain that can interact with a G protein. Heterotrimeric G proteins activate or inhibit effector enzymes (adenylyl cyclase, PDE, or PLC), which change the concentration of a second messenger (cAMP, cGMP, IP_3, or Ca^{2+}). In the hormone-detecting

TABLE 12–8 Some Signals Transduced by G Protein–Coupled Serpentine Receptors

Acetylcholine (muscarinic)	Leukotrienes
Adenosine	Light
Angiotensin	Luteinizing hormone (LH)
ATP (extracellular)	Melatonin
Bradykinin	Odorants
Calcitonin	Opioids
Cannabinoids	Oxytocin
Catecholamines	Platelet-activating factor
Cholecystokinin	Prostaglandins
Corticotropin-releasing factor (CRF)	Secretin
Cyclic AMP (*Dictyostelium discoideum*)	Serotonin
Dopamine	Somatostatin
Follicle-stimulating hormone (FSH)	Tastants
γ-Aminobutyric acid (GABA)	Thyrotropin
Glucagon	Thyrotropin-releasing hormone (TRH)
Glutamate	Vasoactive intestinal peptide
Growth hormone–releasing hormone (GHRH)	Vasopressin
Histamine	Yeast mating factors

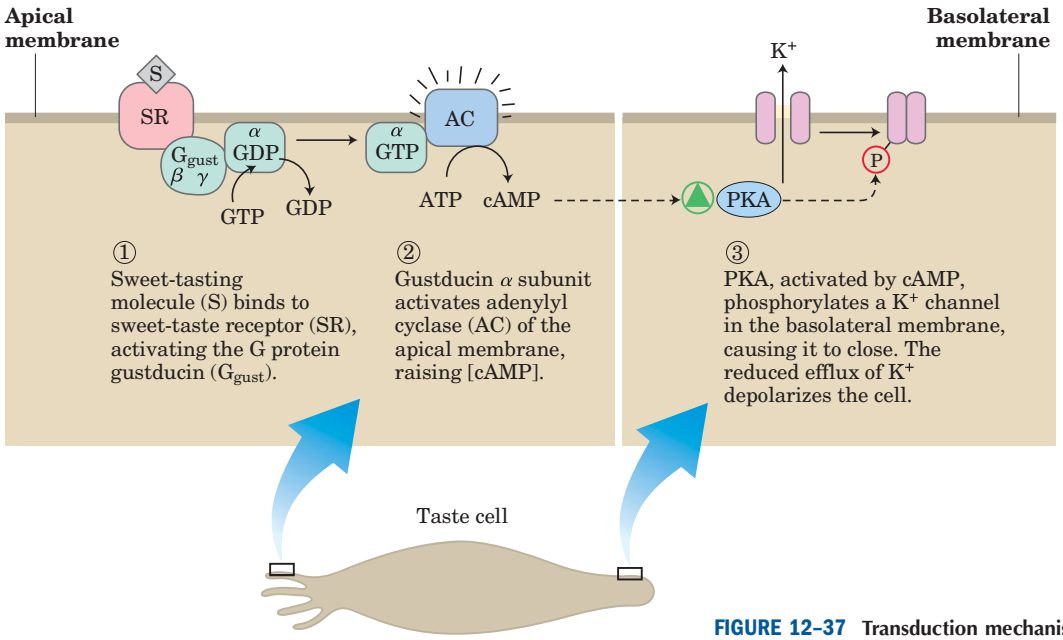


FIGURE 12–37 Transduction mechanism for sweet tastants.

systems, the final output is an activated protein kinase that regulates some cellular process by phosphorylating a protein critical to that process. In sensory neurons, the output is a change in membrane potential and a consequent electrical signal that passes to another neuron in the pathway connecting the sensory cell to the brain.

All these systems self-inactivate. Bound GTP is converted to GDP by the intrinsic GTPase activity of G proteins, often augmented by GTPase-activating proteins (GAPs) or RGS proteins (*r*egulators of *G*-protein *s*ignaling). In some cases, the effector enzymes that are the targets of G protein modulation also serve as GAPs.

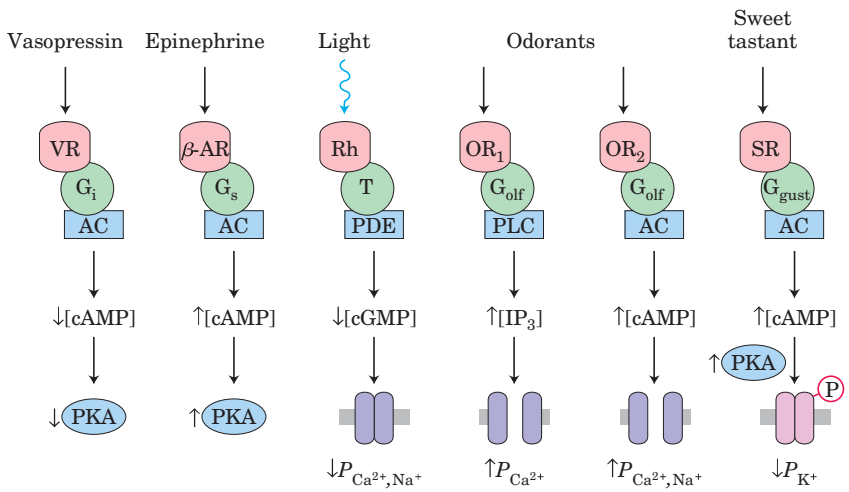


FIGURE 12–38 **Common features of signaling systems that detect hormones, light, smells, and tastes.** Serpentine receptors provide signal specificity, and their interaction with G proteins provides signal amplification. Heterotrimeric G proteins activate effector enzymes: adenylyl cyclase (AC), phospholipase C (PLC), and phosphodiesterases (PDE) that degrade cAMP or cGMP. Changes in concentration of the second messengers (cAMP, cGMP, IP_3) result in alterations of enzymatic activities by phosphorylation or alterations in the permeability (*P*) of surface membranes to Ca^{2+}, Na^+, and K^+. The resulting depolarization or hyperpolarization of the sensory cell (the signal) is passed through relay neurons to sensory centers in the brain. In the best-studied cases, desensitization includes phosphorylation of the receptor and binding of a protein (arrestin) that interrupts receptor–G protein interactions. VR is the vasopressin receptor; other receptor and G protein abbreviations are as used in earlier illustrations.

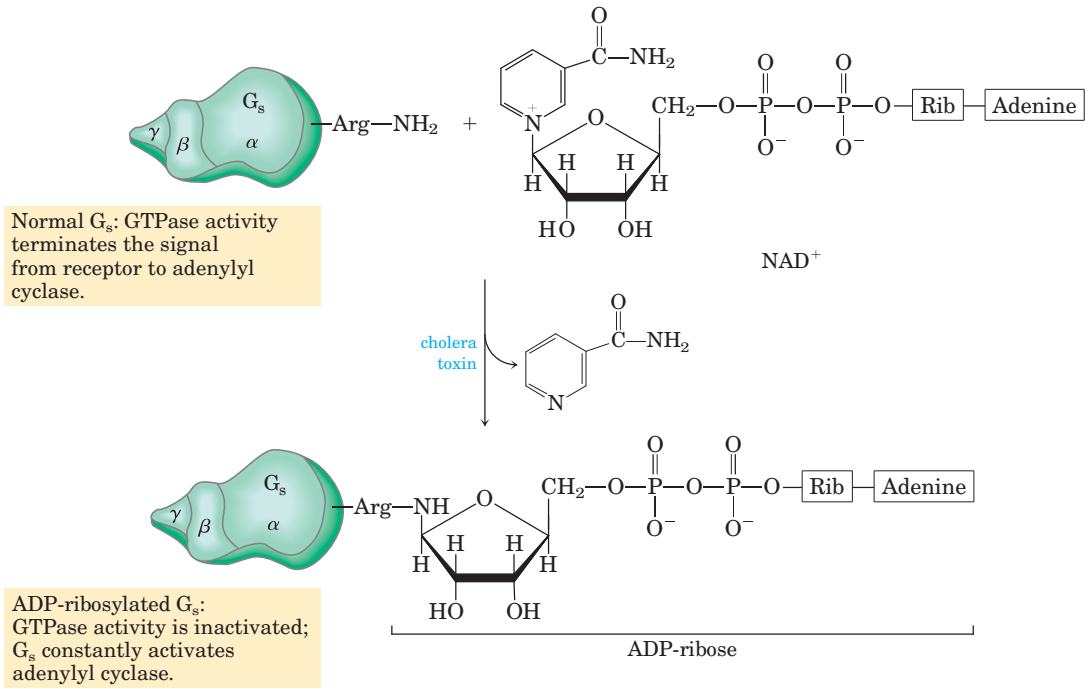


FIGURE 12–39 Toxins produced by bacteria that cause cholera and whooping cough (pertussis). These toxins are enzymes that catalyze transfer of the ADP-ribose moiety of NAD^+ to an Arg residue (cholera toxin) or a Cys residue (pertussis toxin) of G proteins: G_s in the case of cholera (as shown here) and G_i in whooping cough. The G proteins thus modified fail to respond to normal hormonal stimuli. The pathology of both diseases results from defective regulation of adenylyl cyclase and overproduction of cAMP.

Disruption of G-Protein Signaling Causes Disease

Biochemical studies of signal transductions have led to an improved understanding of the pathological effects of toxins produced by the bacteria that cause cholera and pertussis (whooping cough). Both toxins are enzymes that interfere with normal signal transductions in the host animal. **Cholera toxin,** secreted by *Vibrio cholerae* found in contaminated drinking water, catalyzes the transfer of ADP-ribose from NAD^+ to the α subunit of G_s (Fig. 12–39), blocking its GTPase activity and thereby rendering G_s permanently activated. This results in continuous activation of the adenylyl cyclase of intestinal epithelial cells and chronically high [cAMP], which triggers constant secretion of Cl^-, HCO_3^-, and water into the intestinal lumen. The resulting dehydration and electrolyte loss are the major pathologies in cholera. The **pertussis toxin,** produced by *Bordetella pertussis,* catalyzes ADP-ribosylation of G_i, preventing displacement of GDP by GTP and blocking inhibition of adenylyl cyclase by G_i. ■

SUMMARY 12.7 Sensory Transduction in Vision, Olfaction, and Gustation

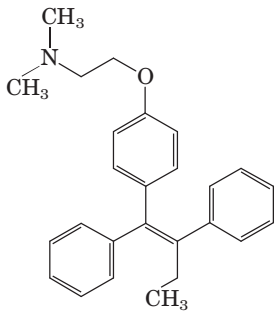
- Vision, olfaction, and gustation in vertebrates employ serpentine receptors, which act through heterotrimeric G proteins to change the V_m of the sensory neuron.
- In rod and cone cells of the retina, light activates rhodopsin, which stimulates replacement of GDP by GTP on the G protein transducin. The freed α subunit of transducin activates cGMP phosphodiesterase, which lowers [cGMP] and thus closes cGMP-dependent ion channels in the outer segment of the neuron. The resulting hyperpolarization of the rod or cone cell carries the signal to the next neuron in the pathway, and eventually to the brain.
- In olfactory neurons, olfactory stimuli, acting through serpentine receptors and G proteins, trigger either an increase in [cAMP] (by activating adenylyl cyclase) or an increase in $[Ca^{2+}]$ (by activating PLC). These second messengers affect ion channels and thus the V_m.
- Gustatory neurons have serpentine receptors that respond to tastants by altering [cAMP], which in turn changes V_m by gating ion channels.
- There is a high degree of conservation of signaling proteins and transduction mechanisms across species.

12.8 Regulation of Transcription by Steroid Hormones

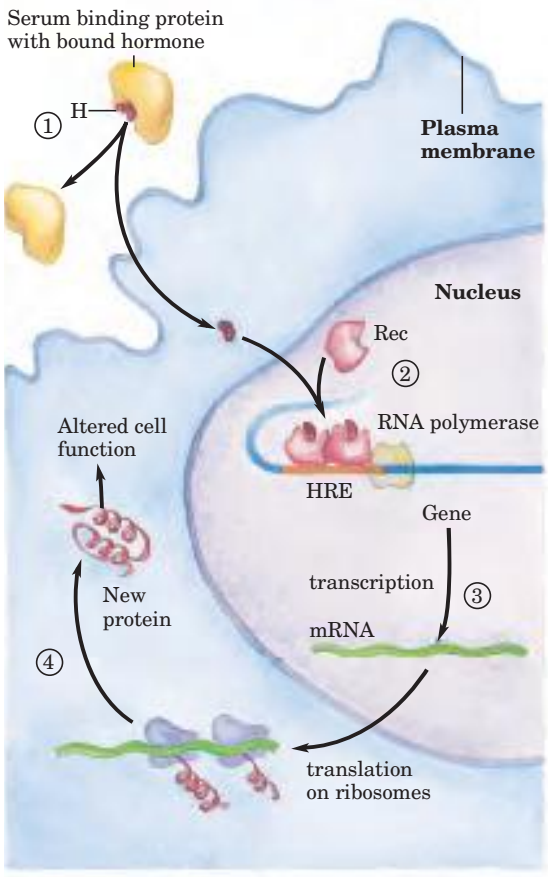
The large group of steroid, retinoic acid (retinoid), and thyroid hormones exert at least part of their effects by a mechanism fundamentally different from that of other hormones: they act in the nucleus to alter gene expression. We therefore discuss their mode of action in detail in Chapter 28, along with other mechanisms for regulating gene expression. Here we give a brief overview.

Steroid hormones (estrogen, progesterone, and cortisol, for example), too hydrophobic to dissolve readily in the blood, are carried on specific carrier proteins from their point of release to their target tissues. In target cells, these hormones pass through the plasma membranes by simple diffusion and bind to specific receptor proteins in the nucleus (Fig. 12–40). Hormone binding triggers changes in the conformation of the receptor proteins so that they become capable of interacting with specific regulatory sequences in DNA called **hormone response elements (HREs),** thus altering gene expression (see Fig. 28–31). The bound receptor-hormone complex can either enhance or suppress the expression of specific genes adjacent to HREs. Hours or days are required for these regulators to have their full effect—the time required for the changes in RNA synthesis and subsequent protein synthesis to become evident in altered metabolism.

The specificity of the steroid-receptor interaction is exploited in the use of the drug **tamoxifen** to treat breast cancer. In some types of breast cancer, division of the cancerous cells depends on the continued presence of the hormone estrogen. Tamoxifen competes with estrogen for binding to the estrogen receptor, but the tamoxifen-receptor complex has little or no effect on gene expression; tamoxifen is an antagonist of estrogen. Consequently, tamoxifen administered after surgery or during chemotherapy for hormone-dependent breast cancer slows or stops the growth of remaining cancerous cells.



Tamoxifen



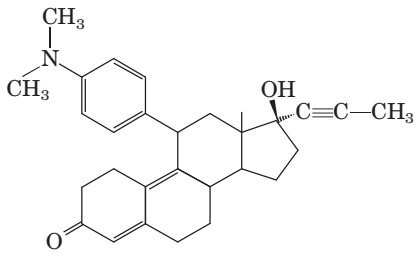
1 Hormone (H), carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein (Rec) in the nucleus.

2 Hormone binding changes the conformation of Rec; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3 Binding regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4 Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

FIGURE 12–40 General mechanism by which steroid and thyroid hormones, retinoids, and vitamin D regulate gene expression. The details of transcription and protein synthesis are discussed in Chapters 26 and 27. At least some steroids also act through plasma membrane receptors by a completely different mechanism.



RU486
(mifepristone)

Another steroid analog, the drug **RU486,** is used to terminate early (preimplantation) pregnancies. An antagonist of the hormone progesterone, RU486 binds to the progesterone receptor and blocks hormone actions essential to implantation of the fertilized ovum in the uterus. ■

The classic mechanism for steroid hormone action through nuclear receptors does not explain certain effects of steroids that are too fast to be the result of altered protein synthesis. For example, the estrogen-mediated dilation of blood vessels is known to be independent of gene transcription or protein synthesis, as is the steroid-induced decrease in cellular [cAMP]. Another transduction mechanism is probably responsible for some of these effects. A plasma membrane protein predicted to have seven transmembrane helical segments binds progesterone with very high affinity and mediates the inhibition of adenylyl cyclase by that hormone, accounting for the decrease in [cAMP]. A second nonclassical mechanism involves the rapid activation of the MAPK cascade by progesterone, acting through the soluble progesterone receptor. This is the same receptor that, in the nucleus, causes the much slower changes in gene expression that constitute the classic mechanism of progesterone action. How the MAPK cascade is activated is not yet clear.

SUMMARY 12.8 Regulation of Transcription by Steroid Hormones

- Steroid hormones enter cells and bind to specific receptor proteins.
- The hormone-receptor complex binds specific regions of DNA, the hormone response elements, and regulates the expression of nearby genes by interacting with transcription factors.
- Two other, faster-acting mechanisms produce some of the effects of steroids. Progesterone triggers a rapid drop in [cAMP], mediated by a plasma membrane receptor, and binding of progesterone to the classic soluble steroid receptor activates a MAPK cascade.

12.9 Regulation of the Cell Cycle by Protein Kinases

One of the most dramatic roles for protein phosphorylation is the regulation of the eukaryotic cell cycle. During embryonic growth and later development, cell division occurs in virtually every tissue. In the adult organism most tissues become quiescent. A cell's "decision" to divide or not is of crucial importance to the organism. When the regulatory mechanisms that limit cell division are defective and cells undergo unregulated division, the result is catastrophic—cancer. Proper cell division requires a precisely ordered sequence of biochemical events that assures every daughter cell a full complement of the molecules required for life. Investigations into the control of cell division in diverse eukaryotic cells have revealed universal regulatory mechanisms. Protein kinases and protein phosphorylation are central to the timing mechanism that determines entry into cell division and ensures orderly passage through these events.

The Cell Cycle Has Four Stages

Cell division in eukaryotes occurs in four well-defined stages (Fig. 12–41). In the S (synthesis) phase, the DNA is replicated to produce copies for both daughter

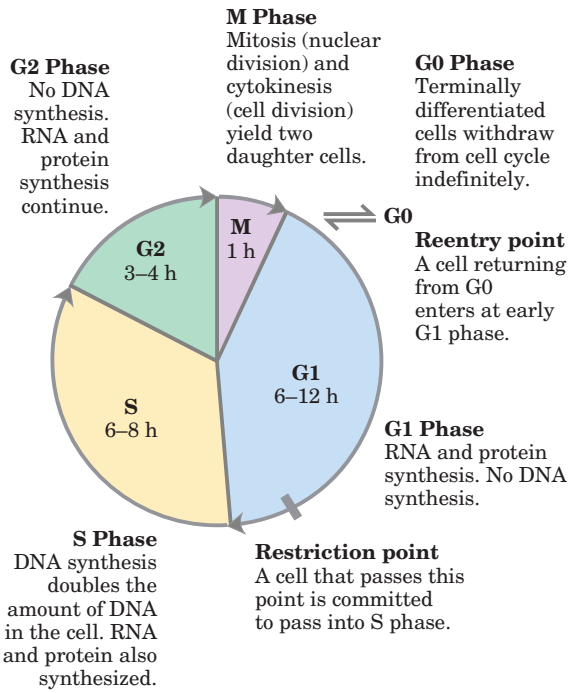


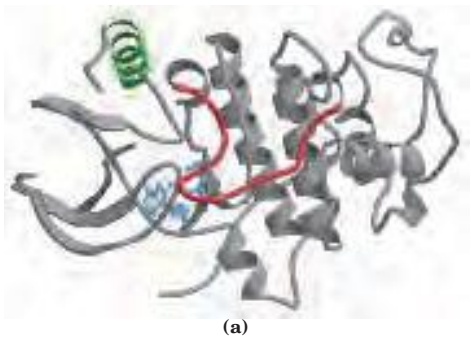
FIGURE 12–41 Eukaryotic cell cycle. The durations (in hours) of the four stages vary, but those shown are typical.

cells. In the G2 phase (G indicates the gap between divisions), new proteins are synthesized and the cell approximately doubles in size. In the M phase (mitosis), the maternal nuclear envelope breaks down, matching chromosomes are pulled to opposite poles of the cell, each set of daughter chromosomes is surrounded by a newly formed nuclear envelope, and cytokinesis pinches the cell in half, producing two daughter cells. In embryonic or rapidly proliferating tissue, each daughter cell divides again, but only after a waiting period (G1). In cultured animal cells the entire process takes about 24 hours.

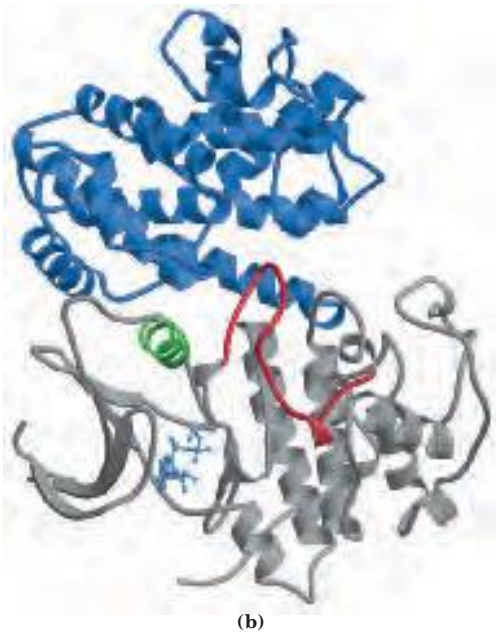
After passing through mitosis and into G1, a cell either continues through another division or ceases to divide, entering a quiescent phase (G0) that may last hours, days, or the lifetime of the cell. When a cell in G0 begins to divide again, it reenters the division cycle through the G1 phase. Differentiated cells such as hepatocytes or adipocytes have acquired their specialized function and form; they remain in the G0 phase.

Levels of Cyclin-Dependent Protein Kinases Oscillate

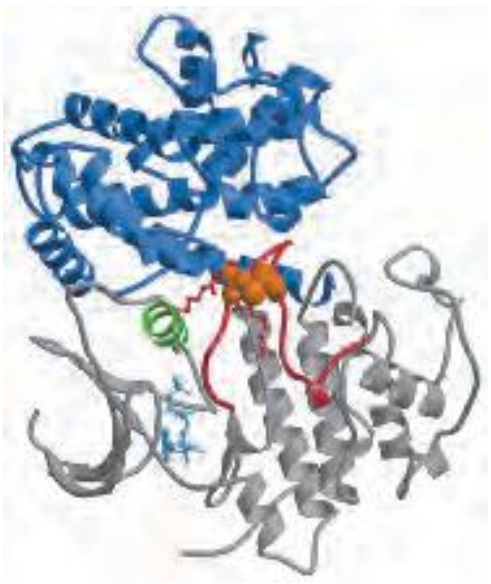
The timing of the cell cycle is controlled by a family of protein kinases with activities that change in response to cellular signals. By phosphorylating specific proteins at precisely timed intervals, these protein kinases orchestrate the metabolic activities of the cell to produce orderly cell division. The kinases are heterodimers with a regulatory subunit, **cyclin,** and a catalytic subunit, **cyclin-dependent protein kinase (CDK).** In the absence of cyclin, the catalytic subunit is virtually inactive. When cyclin binds, the catalytic site opens up, a residue essential to catalysis becomes accessible (Fig. 12–42), and the activity of the catalytic subunit increases 10,000-fold. Animal cells have at least ten different cyclins (designated A, B, and so forth) and at least eight cyclin-dependent kinases (CDK1 through CDK8), which act in various combinations at specific points in the cell cycle. Plants also use a family of CDKs to regulate their cell division.



(a)



(b)



(c)

FIGURE 12–42 Activation of cyclin-dependent protein kinases (CDKs) by cyclin and phosphorylation. CDKs, a family of related enzymes, are active only when associated with cyclins, another protein family. The crystal structure of CDK2 with and without cyclin reveals the basis for this activation. **(a)** Without cyclin (PDB ID 1HCK), CDK2 folds so that one segment, the T loop (red), obstructs the binding site for protein substrates and thus inhibits protein kinase activity. The binding site for ATP (blue) is also near the T loop. **(b)** When cyclin binds (PDB ID 1FIN), it forces conformational changes that move the T loop away from the active site and reorient an amino-terminal helix (green), bringing a residue critical to catalysis (Glu^{51}) into the active site. **(c)** Phosphorylation of a Thr residue (dark orange space-filling structure) in the T loop produces a negatively charged residue that is stabilized by interaction with three Arg residues (red ball-and-stick structures), holding CDK in its active conformation (PDB ID 1JST).

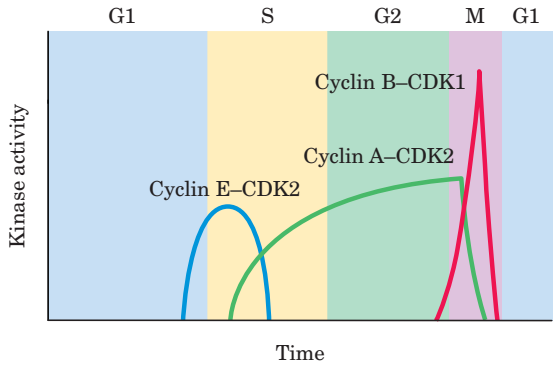


FIGURE 12–43 Variations in the activities of specific CDKs during the cell cycle in animals. Cyclin E–CDK2 activity peaks near the G1 phase–S phase boundary, when the active enzyme triggers synthesis of enzymes required for DNA synthesis (see Fig. 12–46). Cyclin A–CDK2 activity rises during the S and G2 phases, then drops sharply in the M phase, as cyclin B–CDK1 peaks.

In a population of animal cells undergoing synchronous division, some CDK activities show striking oscillations (Fig. 12–43). These oscillations are the result of four mechanisms for regulating CDK activity: phosphorylation or dephosphorylation of the CDK, controlled degradation of the cyclin subunit, periodic synthesis of CDKs and cyclins, and the action of specific CDK-inhibiting proteins.

Regulation of CDKs by Phosphorylation The activity of a CDK is strikingly affected by phosphorylation and dephosphorylation of two critical residues in the protein (Fig. 12–44a). Phosphorylation of Tyr^{15} near the amino terminus renders CDK2 inactive; the Ⓟ–Tyr residue is in the ATP-binding site of the kinase, and the negatively charged phosphate group blocks the entry of ATP. A specific phosphatase dephosphorylates this Ⓟ–Tyr residue, permitting the binding of ATP. Phosphorylation

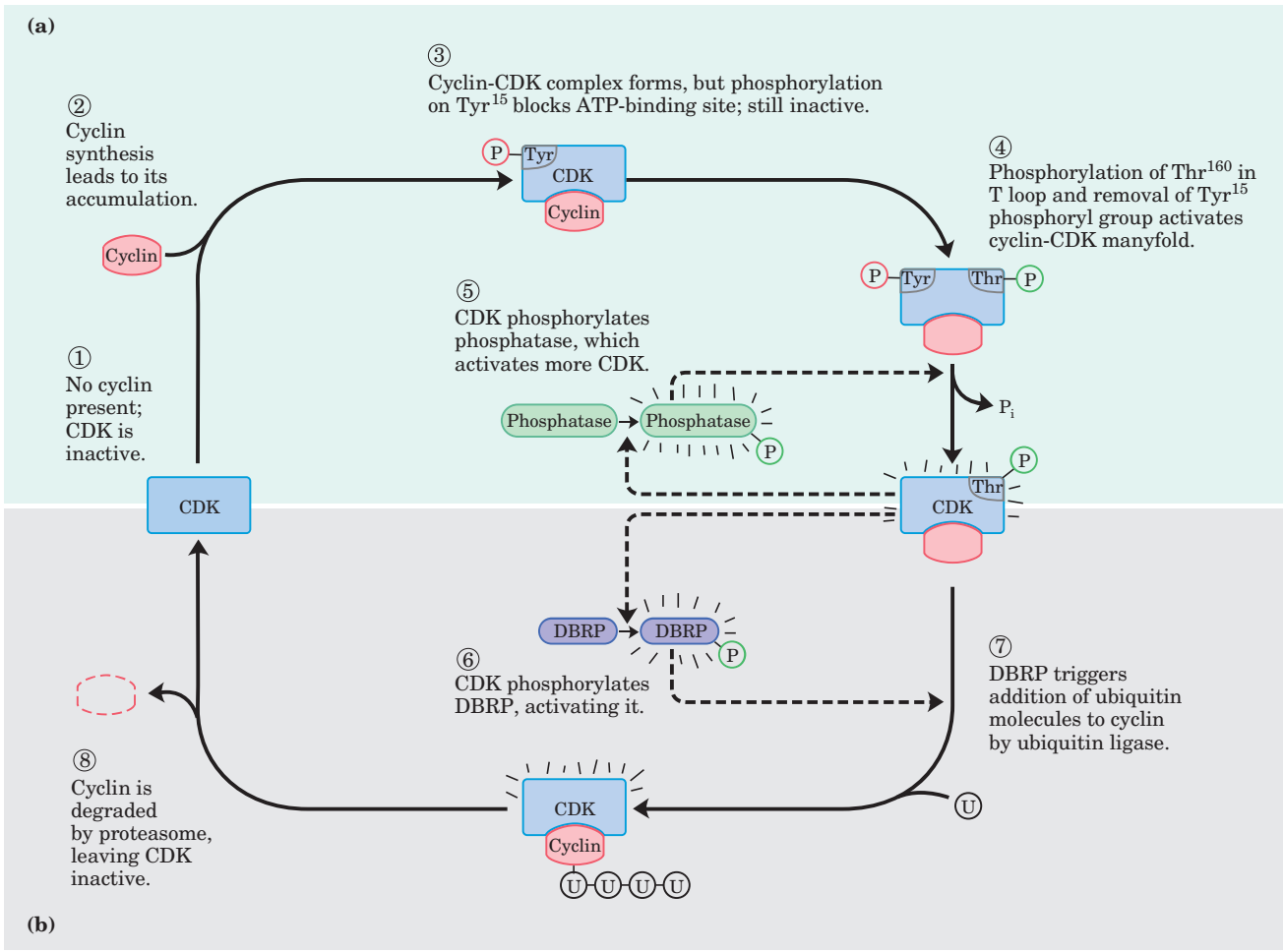


FIGURE 12–44 Regulation of CDK by phosphorylation and proteolysis. (a) The cyclin-dependent protein kinase activated at the time of mitosis (the M phase CDK) has a "T loop" that can fold into the substrate-binding site. When Thr^{160} in the T loop is phosphorylated, the loop moves out of the substrate-binding site, activating the CDK manyfold. **(b)** The active cyclin-CDK complex triggers its own inactivation by phosphorylation of DBRP (destruction box recognizing protein). DBRP and ubiquitin ligase then attach several molecules of ubiquitin (U) to cyclin, targeting it for destruction by proteasomes, proteolytic enzyme complexes.

of Thr^{160} in the "T loop" of CDK, catalyzed by the CDK-activating kinase, forces the T loop out of the substrate-binding cleft, permitting substrate binding and catalytic activity.

One circumstance that triggers this control mechanism is the presence of single-strand breaks in DNA, which leads to arrest of the cell cycle in G2. A specific protein kinase (called Rad3 in yeast), which is activated by single-strand breaks, triggers a cascade leading to the inactivation of the phosphatase that dephosphorylates Tyr^{15} of CDK. The CDK remains inactive and the cell is arrested in G2. The cell will not divide until the DNA is repaired and the effects of the cascade are reversed.

Controlled Degradation of Cyclin Highly specific and precisely timed proteolytic breakdown of mitotic cyclins regulates CDK activity throughout the cell cycle. Progress through mitosis requires first the activation then the destruction of cyclins A and B, which activate the catalytic subunit of the M-phase CDK. These cyclins contain near their amino terminus the sequence Arg–Thr–Ala–Leu–Gly–Asp–Ile–Gly–Asn, the "destruction box," which targets them for degradation. (This usage of "box" derives from the common practice, in diagramming the sequence of a nucleic acid or protein, of enclosing within a box a short sequence of nucleotide or amino acid residues with some specific function. It does not imply any three-dimensional structure.) The protein DBRP (destruction box recognizing protein) recognizes this sequence and initiates the process of cyclin degradation by bringing together the cyclin and another protein, **ubiquitin.** Cyclin and activated ubiquitin are covalently joined by the enzyme ubiquitin ligase (Fig. 12–44b). Several more ubiquitin molecules are then appended, providing the signal for a proteolytic enzyme complex, or **proteasome,** to degrade cyclin.

What controls the timing of cyclin breakdown? A feedback loop occurs in the overall process shown in Figure 12–44. Increased CDK activity activates cyclin proteolysis. Newly synthesized cyclin associates with and activates CDK, which phosphorylates and activates DBRP. Active DBRP then causes proteolysis of cyclin. Lowered [cyclin] causes a decline in CDK activity, and the activity of DBRP also drops through slow, constant dephosphorylation and inactivation by a DBRP phosphatase. The cyclin level is ultimately restored by synthesis of new cyclin molecules.

The role of ubiquitin and proteasomes is not limited to the regulation of cyclin; as we shall see in Chapter 27, both also take part in the turnover of cellular proteins, a process fundamental to cellular housekeeping.

Regulated Synthesis of CDKs and Cyclins The third mechanism for changing CDK activity is regulation of the rate of synthesis of cyclin or CDK or both. For example, cyclin D, cyclin E, CDK2, and CDK4 are synthesized only when a specific transcription factor, E2F, is present in the nucleus to activate transcription of their genes. Synthesis of E2F is in turn regulated by extracellular signals such as **growth factors** and **cytokines** (inducers of cell division), compounds found to be essential for the division of mammalian cells in culture. These growth factors induce the synthesis of specific nuclear transcription factors essential to the production of the enzymes of DNA synthesis. Growth factors trigger phosphorylation of the nuclear proteins Jun and Fos, transcription factors that promote the synthesis of a variety of gene products, including cyclins, CDKs, and E2F. In turn, E2F controls production of several enzymes essential for the synthesis of deoxynucleotides and DNA, enabling cells to enter the S phase (Fig. 12–45).

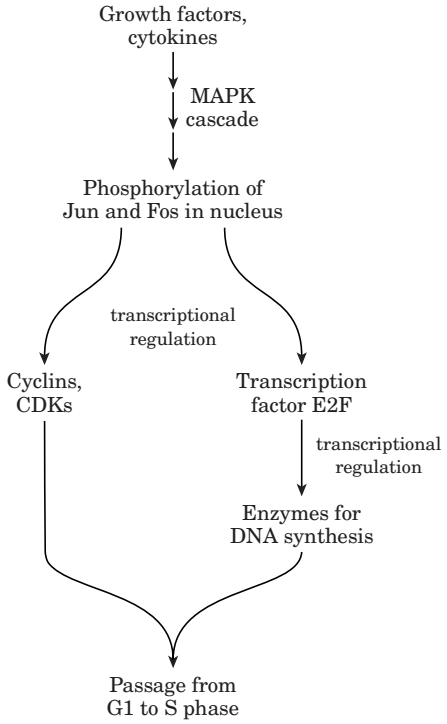


FIGURE 12–45 Regulation of cell division by growth factors. The path from growth factors to cell division leads through the enzyme cascade that activates MAPK; phosphorylation of the nuclear transcription factors Jun and Fos; and the activity of the transcription factor E2F, which promotes synthesis of several enzymes essential for DNA synthesis.

Inhibition of CDKs Finally, specific protein inhibitors bind to and inactivate specific CDKs. One such protein is p21, which we discuss below.

These four control mechanisms modulate the activity of specific CDKs that, in turn, control whether a cell will divide, differentiate, become permanently quiescent, or begin a new cycle of division after a period of quiescence. The details of cell cycle regulation, such as the number of different cyclins and kinases and the

combinations in which they act, differ from species to species, but the basic mechanism has been conserved in the evolution of all eukaryotic cells.

CDKs Regulate Cell Division by Phosphorylating Critical Proteins

We have examined how cells maintain close control of CDK activity, but how does the activity of CDK control the cell cycle? The list of target proteins that CDKs are known to act upon continues to grow, and much remains to be learned. But we can see a general pattern behind CDK regulation by inspecting the effect of CDKs on the structures of laminin and myosin and on the activity of retinoblastoma protein.

The structure of the nuclear envelope is maintained in part by highly organized meshworks of intermediate filaments composed of the protein laminin. Breakdown of the nuclear envelope before segregation of the sister chromatids in mitosis is partly due to the phosphorylation of laminin by a CDK, which causes laminin filaments to depolymerize.

A second kinase target is the ATP-driven actin-myosin contractile machinery that pinches a dividing cell into two equal parts during cytokinesis. After the division, CDK phosphorylates a small regulatory subunit of myosin, causing dissociation of myosin from actin filaments and inactivating the contractile machinery. Subsequent dephosphorylation allows reassembly of the contractile apparatus for the next round of cytokinesis.

A third and very important CDK substrate is the **retinoblastoma protein, pRb;** when DNA damage is detected, this protein participates in a mechanism that arrests cell division in G1 (Fig. 12–46). Named for the retinal tumor cell line in which it was discovered, pRb functions in most, perhaps all, cell types to regulate cell division in response to a variety of stimuli. Unphosphorylated pRb binds the transcription factor E2F; while bound to pRb, E2F cannot promote transcription of a group of genes necessary for DNA synthesis (the genes for DNA polymerase α, ribonucleotide reductase, and other proteins; Chapter 25). In this state, the cell cycle cannot proceed from the G1 to the S phase, the step that commits a cell to mitosis and cell division. The pRb-E2F blocking mechanism is relieved when pRb is phosphorylated by cyclin E–CDK2, which occurs in response to a signal for cell division to proceed.

When the protein kinases ATM and ATR detect damage to DNA, such as a single-strand break, they activate p53 to serve as a transcription factor that stimulates the synthesis of the protein p21 (Fig. 12–46). This protein inhibits the protein kinase activity of cyclin E–CDK2. In the presence of p21, pRb remains unphosphorylated and bound to E2F, blocking the activity of this transcription factor, and the cell cycle is arrested in G1. This gives the cell time to repair its DNA before entering the S phase, thereby avoiding the potentially disastrous transfer of a defective genome to one or both daughter cells.

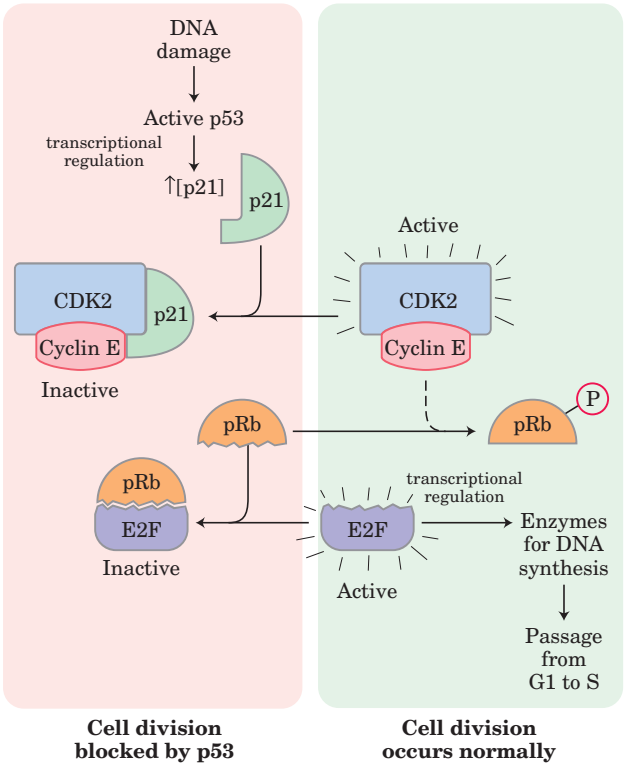


FIGURE 12–46 Regulation of passage from G1 to S by phosphorylation of pRb. When the retinoblastoma protein, pRb, is phosphorylated, it cannot bind and inactivate EF2, a transcription factor that promotes synthesis of enzymes essential to DNA synthesis. If the regulatory protein p53 is activated by ATM and ATR, protein kinases that detect damaged DNA, it stimulates the synthesis of p21, which can bind to and inhibit cyclin E–CDK2 and thus prevent phosphorylation of pRb. Unphosphorylated pRb binds and inactivates E2F, blocking passage from G1 to S until the DNA has been repaired.

SUMMARY 12.9 Regulation of the Cell Cycle by Protein Kinases

- Progression through the cell cycle is regulated by the cyclin-dependent protein kinases (CDKs), which act at specific points in the cycle, phosphorylating key proteins and modulating their activities. The catalytic subunit of CDKs is inactive unless associated with the regulatory cyclin subunit.
- The activity of a cyclin-CDK complex changes during the cell cycle through differential synthesis of CDKs, specific degradation of cyclin, phosphorylation and dephosphorylation of critical residues in CDKs, and binding of inhibitory proteins to specific cyclin-CDKs.

12.10 Oncogenes, Tumor Suppressor Genes, and Programmed Cell Death

Tumors and cancer are the result of uncontrolled cell division. Normally, cell division is regulated by a family of extracellular growth factors, proteins that cause resting cells to divide and, in some cases, differentiate. Defects in the synthesis, regulation, or recognition of growth factors can lead to cancer.

Oncogenes Are Mutant Forms of the Genes for Proteins That Regulate the Cell Cycle

Oncogenes were originally discovered in tumor-causing viruses, then later found to be closely similar to or derived from genes in the animal host cells, proto-oncogenes, which encode growth-regulating proteins. During viral infections, the DNA sequence of a proto-oncogene is sometimes copied by the virus and incorporated into its genome (Fig. 12–47). At some point during the viral infection cycle, the gene can become defective by truncation or mutation. When this viral oncogene is expressed in its host cell during a subsequent infection, the abnormal protein product interferes with normal regulation of cell growth, sometimes resulting in a tumor.

Proto-oncogenes can become oncogenes without a viral intermediary. Chromosomal rearrangements, chemical agents, and radiation are among the factors that can cause oncogenic mutations. The mutations that produce oncogenes are genetically dominant; if either of a pair of chromosomes contains a defective gene, that gene product sends the signal "divide" and a tumor will result. The oncogenic defect can be in any of the proteins involved in communicating the "divide" signal. We know of oncogenes that encode secreted proteins, growth factors, transmembrane proteins (receptors), cytoplasmic proteins (G proteins and protein kinases), and the nuclear transcription factors that control the expression of genes essential for cell division (Jun, Fos).

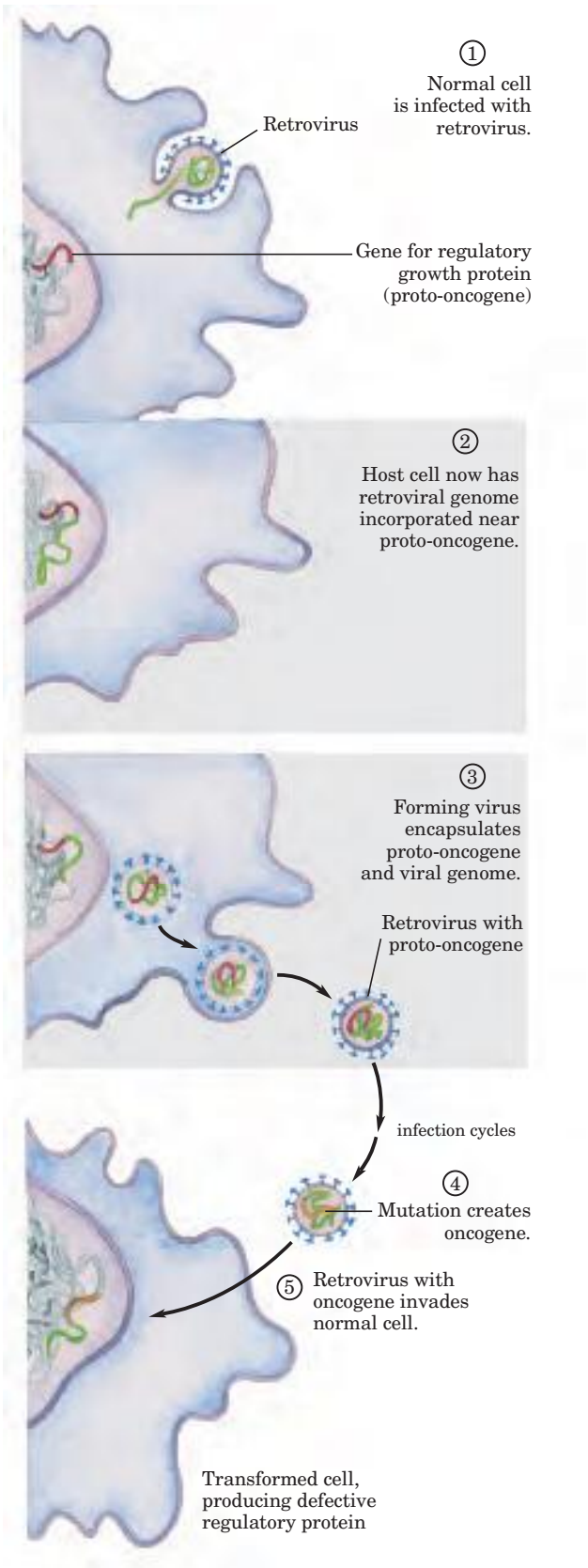


FIGURE 12–47 **Conversion of a regulatory gene to a viral oncogene.** ① A normal cell is infected by a retrovirus (Chapter 26), which ② inserts its own genome into the chromosome of the host cell, near the gene for a regulatory protein (the proto-oncogene). ③ Viral particles released from the infected cell sometimes "capture" a host gene, in this case a proto-oncogene. ④ During several cycles of infection, a mutation occurs in the viral proto-oncogene, converting it to an oncogene. ⑤ When the virus subsequently infects a cell, it introduces the oncogene into the cell's DNA. Transcription of the oncogene leads to the production of a defective regulatory protein that continuously gives the signal for cell division, overriding normal regulatory mechanisms. Host cells infected with oncogene-carrying viruses undergo unregulated cell division—they form tumors. Proto-oncogenes can also undergo mutation to oncogenes without the intervention of a retrovirus, as described in the text.

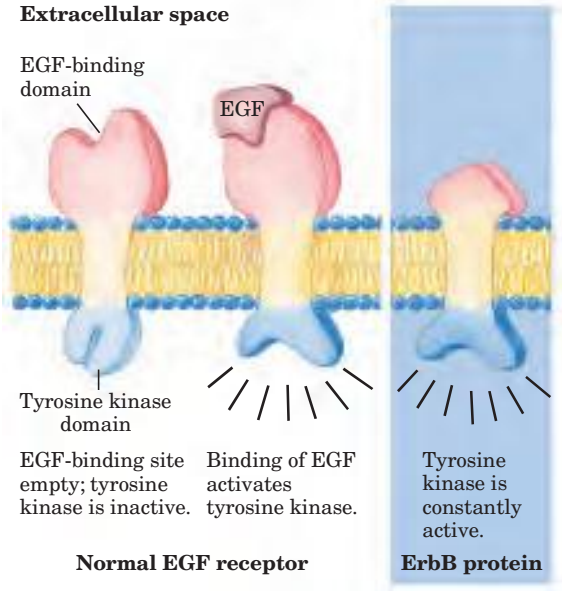


FIGURE 12–48 Oncogene-encoded defective EGF receptor. The product of the *erbB* oncogene (the ErbB protein) is a truncated version of the normal receptor for epidermal growth factor (EGF). Its intracellular domain has the structure normally induced by EGF binding, but the protein lacks the extracellular binding site for EGF. Unregulated by EGF, ErbB continuously signals cell division.

Some oncogenes encode surface receptors with defective or missing signal-binding sites such that their intrinsic Tyr kinase activity is unregulated. For example, the protein ErbB is essentially identical to the normal receptor for epidermal growth factor, except that ErbB lacks the amino-terminal domain that normally binds EGF (Fig. 12–48) and as a result sends the "divide" signal whether EGF is present or not. Mutations in *erbB2*, the gene for a receptor Tyr kinase related to ErbB, are commonly associated with cancers of the glandular epithelium in breast, stomach, and ovary. (For an explanation of the use of abbreviations in naming genes and their products, see Chapter 25.)

Mutant forms of the G protein Ras are common in tumor cells. The *ras* oncogene encodes a protein with normal GTP binding but no GTPase activity. The mutant Ras protein is therefore always in its activated (GTP-bound) form, regardless of the signals arriving through normal receptors. The result can be unregulated growth. Mutations in *ras* are associated with 30% to 50% of lung and colon carcinomas and more than 90% of pancreatic carcinomas.

Defects in Tumor Suppressor Genes Remove Normal Restraints on Cell Division

Tumor suppressor genes encode proteins that normally restrain cell division. Mutation in one or more of these genes can lead to tumor formation. Unregulated growth due to defective tumor suppressor genes, unlike that due to oncogenes, is genetically recessive; tumors form only if *both* chromosomes of a pair contain a defective gene. In a person who inherits one correct copy and one defective copy, every cell has one defective copy of the gene. If any one of those 10^{12} somatic cells undergoes mutation in the one good copy, a tumor may grow from that doubly mutant cell. Mutations in both copies of the genes for pRb, p53, or p21 yield cells in which the normal restraint on cell division is lost and a tumor forms.

Retinoblastoma is a cancer of the retina that occurs in children who have two defective *Rb* alleles. Very young children who develop retinoblastoma commonly have multiple tumors in both eyes. Each tumor is derived from a single retinal cell that has undergone a mutation in its one good copy of the *Rb* gene. (A fetus with two mutant alleles in every cell is nonviable.) Retinoblastoma patients also have a high incidence of cancers of the lung, prostate, and breast.

A far less likely event is that a person born with two good copies of a gene will have two independent mutations in the *same* gene in the *same* cell, but this does occur. Some individuals develop retinoblastomas later in childhood, usually with only one tumor in only one eye. These individuals were presumably born with two good copies of *Rb* in every cell, but both *Rb* genes in a single retinal cell have undergone mutation, leading to a tumor.

Mutations in the gene for p53 also cause tumors; in more than 90% of human cutaneous squamous cell carcinomas (skin cancers) and about 50% of all other human cancers, *p53* is defective. Those very rare individuals who *inherit* one defective copy of *p53* commonly have the Li-Fraumeni cancer syndrome, in which multiple cancers (of the breast, brain, bone, blood, lung, and skin) occur at high frequency and at an early age. The explanation for multiple tumors in this case is the same as that for *Rb* mutations: an individual born with one defective copy of *p53* in every somatic cell is likely to suffer a second *p53* mutation in more than one cell in his or her lifetime.

Mutations in oncogenes and tumor suppressor genes do not have an all-or-none effect. In some cancers, perhaps in all, the progression from a normal cell to a malignant tumor requires an accumulation of mutations (sometimes over several decades), none of which, alone, is responsible for the end effect. For example, the development of colorectal cancer has several recognizable stages, each associated with a mutation (Fig. 12–49). If a normal epithelial cell in the colon undergoes mutation of both copies of the tumor suppressor gene *APC* (adenomatous polyposis coli), it begins to divide faster than normal and produces a clone of itself, a benign polyp (early adenoma). For reasons not yet known, the *APC* mutation results in chromosomal instability; whole regions of a chromosome are lost or re-

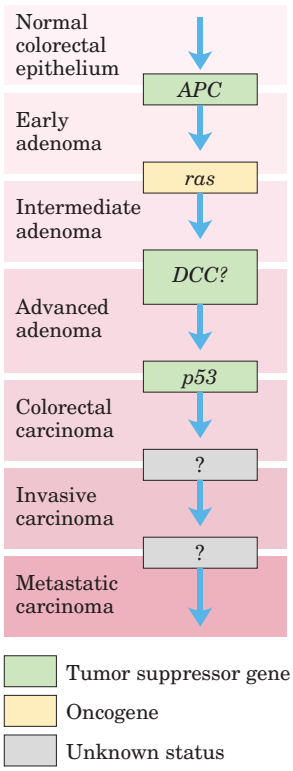


FIGURE 12–49 From normal epithelial cell to colorectal cancer. In the colon, mutations in both copies of the tumor suppressor gene *APC* lead to benign clusters of epithelial cells that multiply too rapidly (early adenoma). If a cell already defective in *APC* suffers a second mutation in the proto-oncogene *ras,* the doubly mutant cell gives rise to an intermediate adenoma, forming a benign polyp of the colon. When one of these cells undergoes further mutations in the tumor suppressor genes *DCC* (probably) and *p53,* increasingly aggressive tumors form. Finally, mutations in genes not yet characterized lead to a malignant tumor and finally to a metastatic tumor that can spread to other tissues. Most malignant tumors probably result from a series of mutations such as this.

arranged during cell division. This instability can lead to another mutation, commonly in *ras*, that converts the clone into an intermediate adenoma. A third mutation (probably in the tumor suppressor gene *DCC*) leads to a late adenoma. Only when both copies of *p53* become defective does this cell mass become a carcinoma, a malignant, life-threatening cancer. The full sequence therefore requires at least seven genetic "hits": two on each of three tumor suppressor genes (*APC, DCC,* and *p53*) and one on the protooncogene *ras*. There are probably several other routes to colorectal cancer as well, but the principle that full malignancy results only from multiple mutations is likely to hold. When a polyp is detected in the early adenoma stage and the cells containing the first mutations are removed surgically, late adenomas and carcinomas will not develop; hence the importance of early detection. ■

Apoptosis Is Programmed Cell Suicide

Many cells can precisely control the time of their own death by the process of **programmed cell death,** or **apoptosis** (app′-a-toe′-sis; from the Greek for "dropping off," as in leaves dropping in the fall). In the development of an embryo, for example, some cells must die. Carving fingers from stubby limb buds requires the precisely timed death of cells between developing finger bones. During development of the nematode *Caenorhabditis elegans* from a fertilized egg, exactly 131 cells (of a total of 1,090 somatic cells in the embryo) must undergo programmed death in order to construct the adult body.

Apoptosis also has roles in processes other than development. When an antibody-producing cell begins to make antibodies against an antigen normally present in the body, that cell undergoes programmed death in the thymus gland—an essential mechanism for eliminating anti-self antibodies. The monthly sloughing of cells of the uterine wall (menstruation) is another case of apoptosis mediating normal cell death. Sometimes cell suicide is not programmed but occurs in response to biological circumstances that threaten the rest of the organism. For example, a virus-infected cell that dies before completion of the infection cycle prevents spread of the virus to nearby cells. Severe stresses such as heat, hyperosmolarity, UV light, and gamma irradiation also trigger cell suicide; presumably the organism is better off with aberrant cells dead.

The regulatory mechanisms that trigger apoptosis involve some of the same proteins that regulate the cell cycle. The signal for suicide often comes from outside, through a surface receptor. Tumor necrosis factor (TNF), produced by cells of the immune system, interacts with cells through specific TNF receptors. These receptors have TNF-binding sites on the outer face of the plasma membrane and a "death domain" of about 80 amino acid residues that passes the self-destruct signal through the membrane to cytosolic proteins such as TRADD (*T*NF *r*eceptor-*a*ssociated *d*eath *d*omain) (Fig. 12–50). Another receptor, Fas, has a similar death domain that allows it to interact with the cytosolic protein FADD (*F*as-*a*ssociated *d*eath *d*omain), which activates a cytosolic protease called caspase 8. This enzyme belongs to a family of proteases that participate in apoptosis; all are synthesized as inactive proenzymes, all have a critical *C*ys residue at the active site, and all hydrolyze their target proteins on the carboxyl-terminal side of specific *Asp* residues (hence the name caspase).

When caspase 8, an "initiator" caspase, is activated by an apoptotic signal carried through FADD, it further self-activates by cleaving its own proenzyme form. Mitochondria are one target of active caspase 8. The protease causes the release of certain proteins contained between the inner and outer mitochondrial membranes:

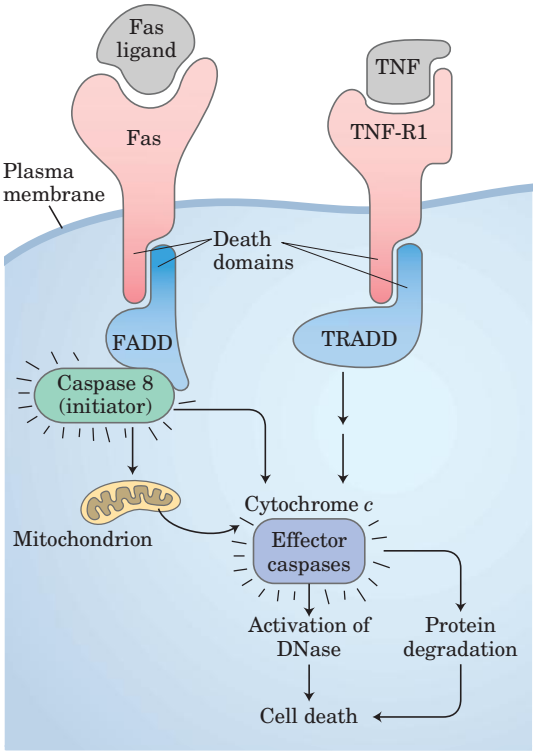


FIGURE 12–50 Initial events of apoptosis. Receptors in the plasma membrane (Fas, TNF-R1) receive signals from outside the cell (the Fas ligand or tumor necrosis factor (TNF), respectively). Activated receptors foster interaction between the "death domain" (an 80 amino acid sequence) in Fas or TNF-R1 and a similar death domain in the cytosolic proteins FADD or TRADD. FADD activates a cytosolic protease, caspase 8, that proteolytically activates other cellular proteases. TRADD also activates proteases. The resulting proteolysis is a primary factor in cell death.

cytochrome *c* (Chapter 19) and several "effector" caspases. Cytochrome *c* binds to the proenzyme form of the effector enzyme caspase 9 and stimulates its proteolytic activation. The activated caspase 9 in turn catalyzes wholesale destruction of cellular proteins—a major cause of apoptotic cell death. One specific target of caspase action is a caspase-activated deoxyribonuclease.

In apoptosis, the monomeric products of protein and DNA degradation (amino acids and nucleotides) are released in a controlled process that allows them to be taken up and reused by neighboring cells. Apoptosis thus allows the organism to eliminate a cell without wasting its components.

SUMMARY 12.10 Oncogenes, Tumor Suppressor Genes, and Programmed Cell Death

- Oncogenes encode defective signaling proteins. By continually giving the signal for cell division, they lead to tumor formation. Oncogenes are genetically dominant and may encode defective growth factors, receptors, G proteins, protein kinases, or nuclear regulators of transcription.
- Tumor suppressor genes encode regulatory proteins that normally inhibit cell division; mutations in these genes are genetically recessive but can lead to tumor formation.
- Cancer is generally the result of an accumulation of mutations in oncogenes and tumor suppressor genes.
- Apoptosis can be triggered by extracellular signals such as TNF through plasma membrane receptors.

Key Terms

Terms in bold are defined in the glossary.

signal transduction 421
enzyme cascade 422
desensitization 422
ligand-gated receptor channel 426
voltage-gated ion channel 427
second messenger 428
autophosphorylation 429
SH2 domain 429
G proteins 429
MAPK cascade 430
receptor Tyr kinase 432
serpentine receptors 435
G protein–coupled receptors (GPCR) 435
7 transmembrane segment (7tm) receptors 435
stimulatory G protein (G_s) 436
β-adrenergic receptor kinase (βARK) 441
β-arrestin (βarr; arrestin 2) 441
G protein–coupled receptor kinases (GRKs) 441
scaffold proteins 441
inhibitory G protein (G_i) 441
calmodulin (CaM) 444
Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I–IV) 444
two-component signaling systems 452
receptor His kinase 452
response regulator 452
receptorlike kinase (RLK) 455
hormone response element (HRE) 465
tamoxifen 465
RU486 466
cyclin 467
cyclin-dependent protein kinase (CDK) 467
ubiquitin 469
proteasome 469
growth factors 469
cytokine 469
retinoblastoma protein (pRb) 470
oncogene 471
tumor suppressor genes 472
programmed cell death 473
apoptosis 473

Further Reading

General

Cohen, P. (2001) The role of protein phosphorylation in human health and disease: the Sir Hans Krebs Medal Lecture. *Eur. J. Biochem.* **268,** 5001–5010.
An intermediate-level review of the role of protein kinases, their alteration in disease, and the drugs that affect their activity.

Cohen, P. (2000) The regulation of protein function by multisite phosphorylation—a 25 year update. *Trends Biochem. Sci.* **25,** 596–601.
Historical account of the developments in protein phosphorylation.

Heilmeyer, L. & Friedrich, P. (eds) (2001) *Protein Modules in Cellular Signalling,* IOS Press, Washington, DC.

Receptor Ion Channels

See also Chapter 11, Further Reading, Ion Channels.

Aidley, D.J. & Stanfield, P.R. (1996) *Ion Channels: Molecules in Action,* Cambridge University Press, Cambridge.
Clear, concise introduction to the physics, chemistry, and molecular biology used in research on ion channels; emphasis is on molecular approaches.

Lehmann-Horn, F. & Jurkat-Rott, K. (1999) Voltage-gated ion channels and hereditary disease. *Physiol. Rev.* **79,** 1317–1372.
Advanced review of the structure and function of ion channels, with special emphasis on cases in which ion-channel defects produce human diseases.

Receptor Enzymes

Foster, D.C., Wedel, B.J., Robinson, S.W., & Garbers, D.L. (1999) Mechanisms of regulation and functions of guanylyl cyclases. *Rev. Physiol. Biochem. Pharmacol.* **135,** 1–39.
Advanced review of the structure and function of signal-transducing guanylyl cyclases.

Saltiel, A.R. & Pessin, J.E. (2002) Insulin signaling pathways in time and space. *Trends Biochem Sci.* **12,** 65–71.
Short, intermediate-level review.

Schaeffer, H.J. & Weber, M.J. (1999) Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol. Cell. Biol.* **19,** 2435–2444.
Intermediate-level review of MAPKs and the basis for specific signaling through these general signaling proteins.

Schlessinger, J. (2000) Cell signaling by receptor tyrosine kinases. *Cell* **103,** 211–225.

Shepherd, P.R., Withers, D.J., & Siddle, K. (1998) Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem. J.* **333,** 471–490.
Intermediate-level review of the importance of PKB and PI-3K in metabolic regulation by insulin.

Shields, J.M., Pruitt, K., McFall, A., Shaub, A., & Der, C.J. (2000) Understanding Ras: it ain't over 'til it's over. *Trends Cell Biol.* **10,** 147–154.
Intermediate-level review of the monomeric G protein Ras.

Widmann, C., Gibson, S., Jarpe, M.B., & Johnson, G.L. (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol. Rev.* **79,** 143–180.
Advanced review of the roles of MAPKs in diverse organisms, from yeast, slime mold, and nematode to vertebrates and plants.

Zajchowski, L.D. & Robbins, S.M. (2002) Lipid rafts and little caves: compartmentalized signalling in membrane microdomains. *Eur. J. Biochem.* **269,** 737–752.

Serpentine Receptors

Hahn, K. & Toutchkine, A. (2002) Live-cell fluorescent biosensors for activated signaling proteins. *Curr. Opin. Cell Biol.* **14,** 167–172.
Brief, intermediate-level review.

Hamm, H.E. (1998) The many faces of G protein signaling. *J. Biol. Chem.* **273,** 669–672.
Introduction to a series of short reviews on G proteins.

Lodowski, D.T., Pitcher, J.A., Capel, W.D., Lefkowitz, R.J., & Tesmer, J.J.G. (2003) Keeping G proteins at bay: a complex between G protein–coupled receptor kinase 2 and $G_{\beta\gamma}$. *Science* **300,** 1256–1262.

Martin, T.F.J. (1998) Phosphoinositide lipids as signaling molecules. *Annu. Rev. Cell Dev. Biol.* **14,** 231–264.
Discussion of the roles of phosphatidylinositol derivatives in signal transduction, cytoskeletal regulation, and membrane trafficking.

Perry, S.J. & Lefkowitz, R.J. (2002) Arresting developments in heptahelical receptor signaling and regulation. *Trends Cell Biol.* **12,** 130–138.
Role of arrestins in serpentine receptor adaptation.

Pinna, L.A. & Ruzzene, M. (1996) How do protein kinases recognize their substrates? *Biochim. Biophys. Acta* **1314,** 191–225.
Advanced review of the factors, including consensus sequences, that give protein kinases their specificity.

Roach, P.J. (1991) Multisite and hierarchal protein phosphorylation. *J. Biol. Chem.* **266,** 14,139–14,142.
Report on the importance of multiple phosphorylation sites in the fine regulation of protein function.

Skiba, N.P. & Hamm, H.E. (1998) How $G_{s\alpha}$ activates adenylyl cyclase. *Nat. Struct. Biol.* **5,** 88–92.
Intermediate-level review of the mechanism of G-protein action, based on structural studies.

Zaccolo, M., De Giorgi, F., Cho, C.Y., Feng, L., Knapp, T., Negulescu, P.A., Taylor, S.S., Tsien, R.Y., & Pozzan, T. (2000) A genetically encoded, fluorescent indicator for cyclic AMP in living cells. *Nat. Cell Biol.* **2,** 25–29.
Describes the technique shown in Figure 5 of Box 12–2.

Zhang, J., Campbell, R.E., Ting, A.Y., & Tsien, R.Y. (2002) Creating new fluorescent probes for cell biology. *Nat. Rev. Molec. Cell Biol.* **3,** 906–918.
The basis for techniques such as those described in Box 12–2.

Scaffold Proteins and Membrane Rafts

See also Chapter 11, Further Reading, Membrane Dynamics.

Filipek, S., Stenkamp, R.E., Teller, D.C., & Palczewski, K. (2003) G protein-coupled receptor rhodopsin: A prospectus. *Annu. Rev. Physiol.* **65,** 851–879.
Advanced review.

Lim, W.A. (2002) The modular logic of signaling proteins: building allosteric switches from simple binding domains. *Curr. Opin. Struct. Biol.* **12,** 61–68.

Michel, J.J.C. & Scott, J.D. (2002) AKAP mediated signal transduction. *Annu. Rev. Pharmacol. Toxicol.* **42,** 235–257.
Advanced review of the proteins that target PKA to subcellular compartments.

Neel, B.G., Gu, H., & Pao, L. (2003) The "Shp"ing news: SH2 domain–containing tyrosine phosphatases in cell signaling. *Trends Biochem. Sci.* **28,** 284–293.

Pawson, T. & Nash, P. (2003) Assembly of cell regulatory systems through protein interaction domains. *Science* **300,** 445–452.
Intermediate-level review of multivalent proteins and the complexes they form.

Saltiel, A.R. & Pessin, J.E. (2002) Insulin signaling pathways in time and space. *Trends Cell Biol.* **12,** 65–71.

Siegal, G. (1999) The surprisingly flexible PTB domain. *Nat. Struct. Biol.* **6,** 7–10.

Tsui-Pierchala, B.A., Encinas, M., Milbrandt, J., & Johnson, E.M., Jr. (2002) Lipid rafts in neuronal signaling and function. *Trends Neurosci.* **25,** 412–417.

Yaffe, M.D. & Elia, A.E.H. (2001) Phosphoserine/threonine-binding domains. *Curr. Opin. Cell Biol.* **13,** 131–138.

Calcium Ions in Signaling

Berridge, M.J. (1993) Inositol triphosphate and calcium signalling. *Nature* **361,** 315–325.
Classic description of the IP_3 signaling system.

Berridge, M.J., Lipp, P., & Bootman, M.D. (2000) The versatility and universality of calcium signaling. *Nat. Rev. Molec. Cell Biol.* **1,** 11–21.
Intermediate review.

Chin, D. & Means, A.R. (2000) Calmodulin: a prototypical calcium receptor. *Trends Cell Biol.* **10,** 322–328.

Nowycky, M.C. & Thomas, A.P. (2002) Intracellular calcium signaling. *J. Cell Sci.* **115,** 3715–3716.
A comprehensive poster of all elements of Ca^{2+} signaling.

Takahashi, A., Camacho, P., Lechleiter, J.D., & Herman, B. (1999) Measurement of intracellular calcium. *Physiol. Rev.* **79,** 1089–1125.
Advanced review of methods for estimating intracellular Ca^{2+} levels in real time.

Thomas, A.P., Bird, G.S.J., Hajnoczky, G., Robb-Gaspers, L.D., & Putney, J.W., Jr. (1996) Spatial and temporal aspects of cellular calcium signaling. *FASEB J.* **10,** 1505–1517.

Signaling in Plants and Bacteria

Bakal, C.J. & Davies, J.E. (2000) No longer an exclusive club: eukaryotic signaling domains in bacteria. *Trends Cell Biol.* **10,** 32–38.
Intermediate review.

Becraft, P.W. (2002) Receptor kinase signaling in plant development. *Annu. Rev. Cell Dev. Biol.* **18,** 163–192.
Advanced review.

Chang, C. & Stadler, R. (2001) Ethylene hormone receptor action in *Arabidopsis*. *Bioessays* **23,** 619–627.

Cock, J.M., Vanoosthuyse, V., & Gaude, T. (2002) Receptor kinase signalling in plants and animals: distinct molecular systems with mechanistic similarities. *Curr. Opin. Cell Biol.* **14,** 230–236.

Hwang, I., Chen, H.-C., & Sheen, J. (2002) Two-component circuitry in *Arabidopsis* cytokinin signal transduction. *Nature* **413,** 383–389.

Jones, A.M. (2002) G-protein–coupled signaling in *Arabidopsis*. *Curr. Opin. Plant Biol.* **5,** 402–407.

Matsubayashi, Y., Yang, H., & Sakagami, Y. (2001) Peptide signals and their receptors in higher plants. *Trends Plant Sci.* **6,** 573–577.

McCarty, D.R. & Chory, J. (2000) Conservation and innovation in plant signaling pathways. *Cell* **103,** 201–209.

Meijer, H.J.G. & Munnik, T. (2003) Phospholipid-based signaling in plants. *Annu. Rev. Plant Biol.* **54,** 265–306.
Advanced review.

Ouaked, F., Rozhon, W., Lecourieus, D., & Hirt, H. (2003) A MAPK pathway mediates ethylene signaling in plants. *EMBO J.* **22,** 1282–1288.

Talke, I.N., Blaudez, D., Maathuis, F.J.M., & Sanders, D. (2003) CNGCs: prime targets of plant cyclic nucleotide signalling? *Trends Plant Sci.* **8,** 286–293.

Tichtinsky, G., Vanoosthuyse, V., Cock, J.M., & Gaude, T. (2003) Making inroads into plant receptor kinase signalling pathways. *Trends Plant Sci.* **8,** 231–237.

Vision, Olfaction, and Gustation

Baylor, D. (1996) How photons start vision. *Proc. Natl. Acad. Sci. USA* **93,** 560–565.
One of six short reviews on vision in this journal issue.

Margolskee, R.F. (2002) Molecular mechanisms of bitter and sweet taste transduction. *J. Biol. Chem.* **277,** 1–4.

Menon, S.T., Han, M., & Sakmar, T.P. (2001) Rhodopsin: structural basis of molecular physiology. *Physiol. Rev.* **81,** 1659–1688.
Advanced review.

Mombaerts, P. (2001) The human repertoire of odorant receptor genes and pseudogenes. *Annu. Rev. Genomics Hum. Genet.* **2,** 493–510.
Advanced review.

Nathans, J. (1989) The genes for color vision. *Sci. Am.* **260** (February), 42–49.

Ronnett, G.V. & Moon, C. (2002) G proteins and olfactory signal transduction. *Annu. Rev. Physiol.* **64,** 189–222.
Advanced review.

Scott, K. & Zuker, C. (1997) Lights out: deactivation of the phototransduction cascade. *Trends Biochem. Sci.* **22,** 350–354.

Steroid Hormone Receptors and Action

Carson-Jurica, M.A., Schrader, W.T., & O'Malley, B.W. (1990) Steroid receptor family—structure and functions. *Endocr. Rev.* **11,** 201–220.
Advanced discussion of the structure of nuclear hormone receptors and the mechanisms of their action.

Hall, J.M., Couse, J.F., & Korach, K.S. (2001) The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J. Biol. Chem.* **276,** 36,869–36,872.
Brief, intermediate-level review.

Jordan, V.C. (1998) Designer estrogens. *Sci. Am.* **279** (October), 60–67.
Introductory review of the mechanism of estrogen action and the effects of estrogenlike compounds in medicine.

Lösel, R.M., Falkenstein, E., Feuring, M., Schultz, A., Tillmann, H.-C., Rossol-Haseroth, K., & Wehling, M. (2003) Nongenomic steroid action: controversies, questions, and answers. *Physiol. Rev.* **83,** 965–1016.
Detailed review of the evidence for steroid hormone action through plasma membrane receptors.

Cell Cycle and Cancer

Cavenee, W.K. & White, R.L. (1995) The genetic basis of cancer. *Sci. Am.* **272** (March), 72–79.

Chau, B.N. & Wang, J.Y.J. (2003) Coordinated regulation of life and death by RB. *Nat. Rev. Cancer* **3,** 130–138.

Fearon, E.R. (1997) Human cancer syndromes—clues to the origin and nature of cancer. *Science* **278,** 1043–1050.
Intermediate-level review of the role of inherited mutations in the development of cancer.

Herwig, S. & Strauss, M. (1997) The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis. *Eur. J. Biochem.* **246,** 581–601.

Hunt, M. & Hunt, T. (1993) *The Cell Cycle: An Introduction,* W. H. Freeman and Company/Oxford University Press, New York/Oxford.

Kinzler, K.W. & Vogelstein, B. (1996) Lessons from hereditary colorectal cancer. *Cell* **87,** 159–170.
Evidence for multistep processes in the development of cancer.

Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division. *Cell* **88,** 323–331.
Intermediate coverage of the function of protein p53 in the normal cell cycle and in cancer.

Morgan, D.O. (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* **13,** 261–291.
Advanced review.

Obaya, A.J. & Sedivy, J.M. (2002) Regulation of cyclin-Cdk activity in mammalian cells. *Cell. Mol. Life Sci.* **59,** 126–142.

Rajagopalan, H., Nowak, M.A., Vogelstein, B., & Lengauer, C. (2003) The significance of unstable chromosomes in colorectal cancer. *Nat. Rev. Cancer* **3,** 695–701.

Sherr, C.J. & McCormick, F. (2002) The RB and p53 pathways in cancer. *Cancer Cell* **2,** 102–112.

Weinberg, R.A. (1996) How cancer arises. *Sci. Am.* **275** (September), 62–70.

Yamasaki, L. (2003) Role of the RB tumor suppressor in cancer. *Cancer Treatment Res.* **115,** 209–239.
Advanced review.

Apoptosis

Anderson, P. (1997) Kinase cascades regulating entry into apoptosis. *Microbiol. Mol. Biol. Rev.* **61,** 33–46.

Ashkenazi, A. & Dixit, V.M. (1998) Death receptors: signaling and modulation. *Science* **281,** 1305–1308.
This and the papers by Green and Reed and by Thornberry and Lazebnik (below) are in an issue of *Science* devoted to apoptosis.

Duke, R.C., Ojcius, D.M., & Young, J.D.-E. (1996) Cell suicide in health and disease. *Sci. Am.* **275** (December), 80–87.

Green, D.R. & Reed, J.C. (1998) Mitochondria and apoptosis. *Science* **281,** 1309–1312.

Jacobson, M.D., Weil, M., & Raff, M.C. (1997) Programmed cell death in animal development. *Cell* **88,** 347–354.

Lawen, A. (2003) Apoptosis—an introduction. *Bioessays* **25,** 888–896.

Thornberry, N.A. & Lazebnik, Y. (1998) Caspases: enemies within. *Science* **281,** 1312.

Problems

1. Therapeutic Effects of Albuterol The respiratory symptoms of asthma result from constriction of the bronchi and bronchioles of the lungs due to contraction of the smooth muscle of their walls. This constriction can be reversed by raising the [cAMP] in the smooth muscle. Explain the therapeutic effects of albuterol, a β-adrenergic agonist taken (by inhalation) for asthma. Would you expect this drug to have any side effects? How might one design a better drug that did not have these effects?

2. Amplification of Hormonal Signals Describe all the sources of amplification in the insulin receptor system.

3. Termination of Hormonal Signals Signals carried by hormones must eventually be terminated. Describe several different mechanisms for signal termination.

4. Specificity of a Signal for a Single Cell Type Discuss the validity of the following proposition. A signaling molecule (hormone, growth factor, or neurotransmitter) elicits identical responses in different types of target cells if they contain identical receptors.

5. Resting Membrane Potential A variety of unusual invertebrates, including giant clams, mussels, and polychaete worms, live on the fringes of hydrothermal vents on the ocean bottom, where the temperature is 60 °C.

(a) The adductor muscle of a deep-sea giant clam has a resting membrane potential of −95 mV. Given the intracellular and extracellular ionic compositions shown below, would you have predicted this membrane potential? Why or why not?

	Concentration (mM)	
Ion	*Intracellular*	*Extracellular*
Na^+	50	440
K^+	400	20
Cl^-	21	560
Ca^{2+}	0.4	10

(b) Assume that the adductor muscle membrane is permeable to only one of the ions listed above. Which ion could determine the V_m?

6. Membrane Potentials in Frog Eggs Fertilization of a frog oocyte by a sperm cell triggers ionic changes similar to those observed in neurons (during movement of the action potential) and initiates the events that result in cell division and development of the embryo. Oocytes can be stimulated to divide without fertilization by suspending them in 80 mM KCl (normal pond water contains 9 mM KCl).

(a) Calculate how much the change in extracellular [KCl] changes the resting membrane potential of the oocyte. (Hint: Assume the oocyte contains 120 mM K^+ and is permeable *only* to K^+.) Assume a temperature of 20 °C.

(b) When the experiment is repeated in Ca^{2+}-free water, elevated [KCl] has no effect. What does this suggest about the mechanism of the KCl effect?

7. Excitation Triggered by Hyperpolarization In most neurons, membrane *depolarization* leads to the opening of voltage-dependent ion channels, generation of an action potential, and ultimately an influx of Ca^{2+}, which causes release of neurotransmitter at the axon terminus. Devise a cellular strategy by which *hyperpolarization* in rod cells could produce excitation of the visual pathway and passage of visual signals to the brain. (Hint: The neuronal signaling pathway in higher organisms consists of a *series* of neurons that relay information to the brain (see Fig. 12–31). The signal released by one neuron can be either excitatory or inhibitory to the following, postsynaptic neuron.)

8. Hormone Experiments in Cell-Free Systems In the 1950s, Earl W. Sutherland, Jr., and his colleagues carried out pioneering experiments to elucidate the mechanism of action of epinephrine and glucagon. Given what you have learned in this chapter about hormone action, interpret each of the experiments described below. Identify substance X and indicate the significance of the results.

(a) Addition of epinephrine to a homogenate of normal liver resulted in an increase in the activity of glycogen phosphorylase. However, if the homogenate was first centrifuged at a high speed and epinephrine or glucagon was added to the clear supernatant fraction that contains phosphorylase, no increase in the phosphorylase activity occurred.

(b) When the particulate fraction from the centrifugation in (a) was treated with epinephrine, substance X was produced. The substance was isolated and purified. Unlike epinephrine, substance X activated glycogen phosphorylase when added to the clear supernatant fraction of the centrifuged homogenate.

(c) Substance X was heat-stable; that is, heat treatment did not affect its capacity to activate phosphorylase. (Hint: Would this be the case if substance X were a protein?) Substance X was nearly identical to a compound obtained when pure ATP was treated with barium hydroxide. (Fig. 8–6 will be helpful.)

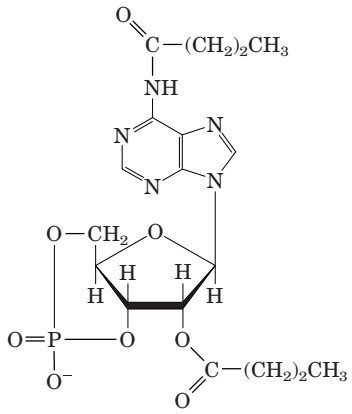
9. Effect of Cholera Toxin on Adenylyl Cyclase The gram-negative bacterium *Vibrio cholerae* produces a protein, cholera toxin (M_r 90,000), that is responsible for the characteristic symptoms of cholera: extensive loss of body water and Na^+ through continuous, debilitating diarrhea. If body fluids and Na^+ are not replaced, severe dehydration results; untreated, the disease is often fatal. When the cholera toxin gains access to the human intestinal tract it binds tightly to specific sites in the plasma membrane of the epithelial cells lining the small intestine, causing adenylyl cyclase to undergo prolonged activation (hours or days).

(a) What is the effect of cholera toxin on [cAMP] in the intestinal cells?

(b) Based on the information above, suggest how cAMP normally functions in intestinal epithelial cells.

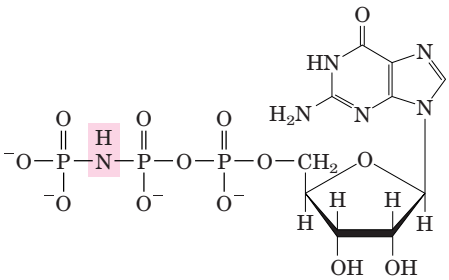
(c) Suggest a possible treatment for cholera.

10. Effect of Dibutyryl cAMP versus cAMP on Intact Cells The physiological effects of epinephrine should in principle be mimicked by addition of cAMP to the target cells. In practice, addition of cAMP to intact target cells elicits only a minimal physiological response. Why? When the structurally related derivative dibutyryl cAMP (shown below) is added to intact cells, the expected physiological response is readily apparent. Explain the basis for the difference in cellular response to these two substances. Dibutyryl cAMP is widely used in studies of cAMP function.



Dibutyryl cAMP
(N^6,$O^{2'}$-Dibutyryl adenosine 3′,5′-cyclic monophosphate)

11. Nonhydrolyzable GTP Analogs Many enzymes can hydrolyze GTP between the β and γ phosphates. The GTP analog β,γ-imidoguanosine 5′-triphosphate Gpp(NH)p, shown below, cannot be hydrolyzed between the β and γ phosphates. Predict the effect of microinjection of Gpp(NH)p into a myocyte on the cell's response to β-adrenergic stimulation.



Gpp(NH)p
(β,γ-imidoguanosine 5′-triphosphate)

12. G Protein Differences Compare the G proteins G_s, which acts in transducing the signal from β-adrenergic receptors, and Ras. What properties do they share? How do they differ? What is the functional difference between G_s and G_I?

13. EGTA Injection EGTA (ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid) is a chelating agent with high affinity and specificity for Ca^{2+}. By microinjecting a cell with an appropriate Ca^{2+}-EDTA solution, an experimenter can prevent cytosolic [Ca^{2+}] from rising above 10^{-7} M. How would EGTA microinjection affect a cell's response to vasopressin (see Table 12–5)? To glucagon?



14. Visual Desensitization Oguchi's disease is an inherited form of night blindness. Affected individuals are slow to recover vision after a flash of bright light against a dark background, such as the headlights of a car on the freeway. Suggest what the molecular defect(s) might be in Oguchi's disease. Explain in molecular terms how this defect accounts for the night blindness.

15. Mutations in PKA Explain how mutations in the R or C subunit of cAMP-dependent protein kinase (PKA) might lead to (a) a constantly active PKA or (b) a constantly inactive PKA.

16. Mechanisms for Regulating Protein Kinases Identify eight general types of protein kinases found in eukaryotic cells, and explain what factor is *directly* responsible for activating each type.

17. Mutations in Tumor Suppressor Genes and Oncogenes Explain why mutations in tumor suppressor genes are recessive (both copies of the gene must be defective for the regulation of cell division to be defective) whereas mutations in oncogenes are dominant.



18. Retinoblastoma in Children Explain why some children with retinoblastoma develop multiple tumors of the retina in both eyes, whereas others have a single tumor in only one eye.

19. Mutations in *ras* How does a mutation in the *ras* gene that leads to formation of a Ras protein with no GTPase activity affect a cell's response to insulin?