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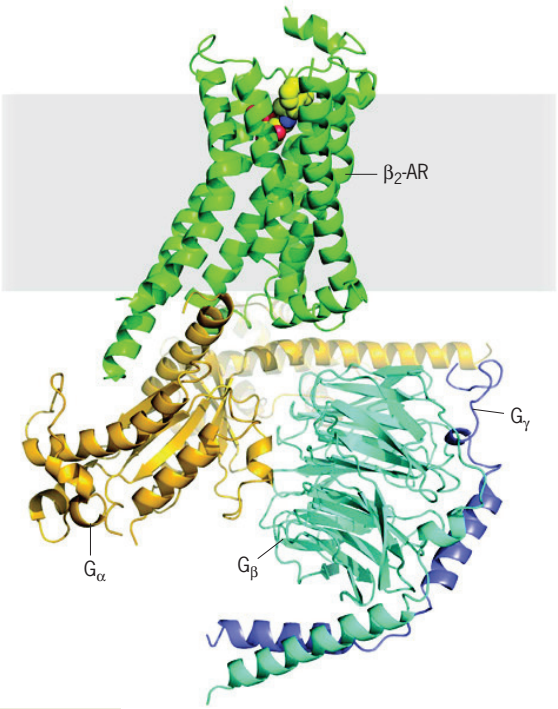

Cell Signaling and Signal Transduction: Communication Between Cells

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THE HUMAN PERSPECTIVE: Signaling Pathways and Human Longevity

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

Cell signaling affects virtually every aspect of cell structure and function, which is one of the primary reasons that this chapter appears near the end of the book. On one hand, an understanding of cell signaling requires knowledge about other types of cellular activity. On the other hand, insights into cell signaling can tie together a variety of seemingly independent cellular processes. Cell signaling is also intimately involved in the

Three-dimensional, X-ray crystallographic structure of a signaling complex between a β_2-adrenergic receptor (β_2-AR), which is a representative member of the G protein-coupled receptor (GPCR) superfamily, and a heterotrimeric G protein. The β_2-AR is shown in green and the three subunits of the G protein are shown in orange, cyan, and purple. The plasma membrane is shown as a grey shadow. GPCRs are integral membrane proteins characterized by their seven transmembrane helices. As a group, these proteins bind an astonishing array of biological messengers, which constitutes the first step in eliciting many of the body's most basic responses. [CONTINUED ON NEXT PAGE]

Binding of an agonist (e.g., epinephrine) to the binding pocket in the extracellular domain of the β_2AR causes a conformational change in the receptor that causes it to bind the G protein, which transmits the signal into the cell, inducing responses such as increased heart rate and relaxation of smooth muscle cells. Beta-adrenergic receptors are the targets of a number of important drugs, including β-blockers, which are widely prescribed for the treatment of high blood pressure and heart arrhythmias. GPCRs had been very difficult to crystallize so that high-resolution structures of these important proteins had been lacking. This situation is now changing as the result of recent advances in crystallization technology. In this case, crystallization of the signaling complex was accomplished using two additional small proteins (not shown in the image) that bound to the β_2 AR and the G protein to stabilize the complex during the crystallization process. (Courtesy of Søren G. F. Rasmussen, Andrew C. Kruse, and Brian K. Kobilka.)

regulation of cell growth and division. This makes the study of cell signaling crucially important for understanding how a cell can lose the ability to control cell division and develop into a malignant tumor.

15.1 | The Basic Elements of Cell Signaling Systems

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

An overview of cellular signaling pathways is depicted in Figure 15.2. Cell signaling is initiated with the release of a messenger molecule by a cell that is engaged in sending messages to other cells in the body (step 1, Figure 15.2). The extracellular environments of cells contain hundreds of different informational molecules, ranging from small compounds (e.g., steroids and neurotransmitters) to small, soluble protein hormones (e.g., glucagon and insulin) to huge glycoproteins bound to the surfaces of other cells. Cells can only respond to a particular extracellular message if they express **receptors** that specifically recognize and bind that messenger molecule (step 2). The molecule that binds to the receptor is called a **ligand**. Different types of cells possess different complements of receptors, which allow them to respond to different extracellular messengers. Even cells that share a specific receptor may respond very differently to the same extracellular messenger. Liver cells and smooth muscle cells both possess the β_2-adrenergic receptor shown in the chapter-opening image on page 617. Activation of this receptor by circulating adrenaline leads to glycogen breakdown in a liver cell and relaxation in a smooth muscle cell. These different outcomes following interaction with the same initial stimulus can be traced to different intracellular proteins that become engaged in the response in these two types of cells. Thus the type of activities in which a cell engages depends both on the stimuli that it receives and the intracellular machinery that it possesses at that particular time in its life.

In most cases, the extracellular messenger molecule binds to a receptor at the outer surface of the responding cell. This interaction induces a conformational change in the receptor that causes the signal to be relayed across the membrane to the receptor's cytoplasmic domain (step 3, Figure 15.2). Once it has reached the inner surface of the plasma membrane, there are two major routes by which the signal is transmitted into the cell interior, where it elicits the appropriate response. The particular route taken depends on the type of receptor that is activated. In the following discussion, we will focus on these two major routes of signal transduction, but keep in mind

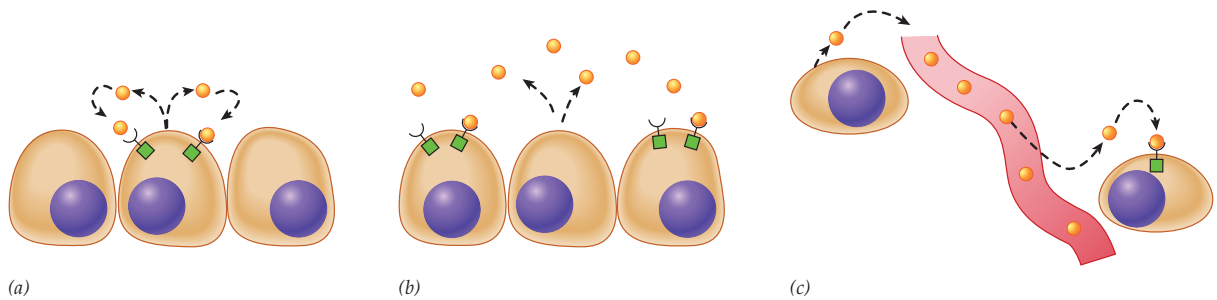


Figure 15.1 **Autocrine (*a*), paracrine (*b*), and endocrine (*c*) types of intercellular signaling.**

there are other ways that extracellular signals can have an impact on a cell. For example, we saw on page 169 how neurotransmitters act by opening plasma membrane ion channels and on page 525 how steroid hormones diffuse through the plasma membrane and bind to intracellular receptors. In the two major routes discussed in this chapter:

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

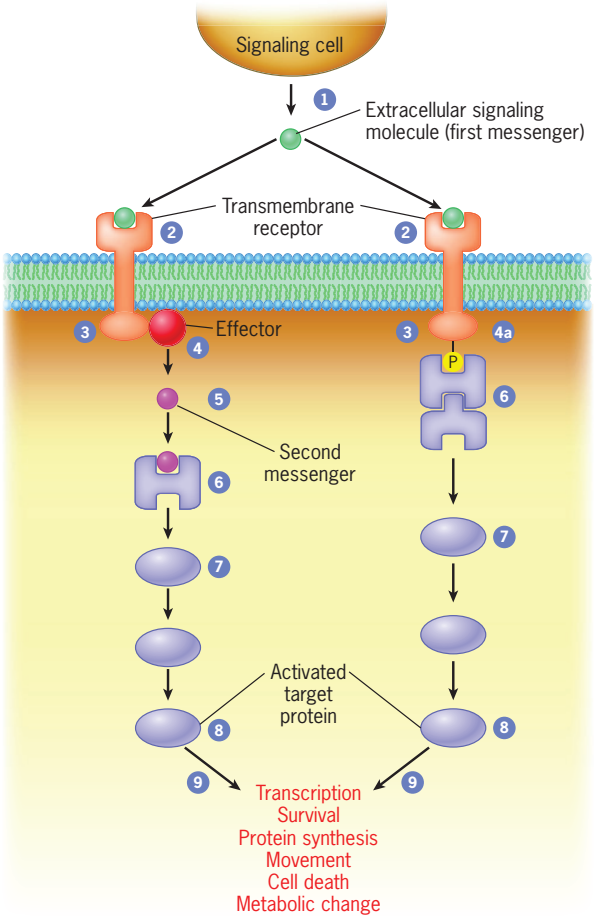


Figure 15.2 **An overview of the major signaling pathways by which extracellular messenger molecules can elicit intracellular responses.** Two different types of signal transduction pathways are depicted, one in which a signaling pathway is activated by a diffusible second messenger and another in which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

- Another type of receptor (Section 15.4) transmits a signal by transforming its cytoplasmic domain into a recruiting station for cellular signaling proteins (step 4a). Proteins interact with one another, or with components of a cellular membrane, by means of specific types of interaction domains, such as the SH3 domain discussed on page 62.

Whether the signal is transmitted by a second messenger or by protein recruitment, the outcome is similar; a protein that is positioned at the top of an intracellular **signaling pathway** is activated (step 6, Figure 15.2). Signaling pathways are the information superhighways of the cell. Each signaling pathway consists of a series of distinct proteins that operate in sequence (step 7). Most "signaling proteins" are constructed of multiple domains, which allows them to interact in a dynamic way with a number of different partners, either simultaneously or sequentially. This type of *modular* construction is illustrated by the Grb2 and IRS-1 proteins depicted in Figures 15.20 and 15.25*a*, respectively. Unlike Grb2 and IRS-1, which function exclusively in mediating protein-protein interactions, many signaling proteins also contain catalytic and/or regulatory domains that give them a more active role in a signaling pathway.

Each protein in a signaling pathway typically acts by altering the conformation of the subsequent (or downstream) protein in the series, an event that activates or inhibits that protein (Figure 15.3). It should come as no surprise, after reading about other topics in cell biology, that alterations in the conformation of signaling proteins are often accomplished by protein

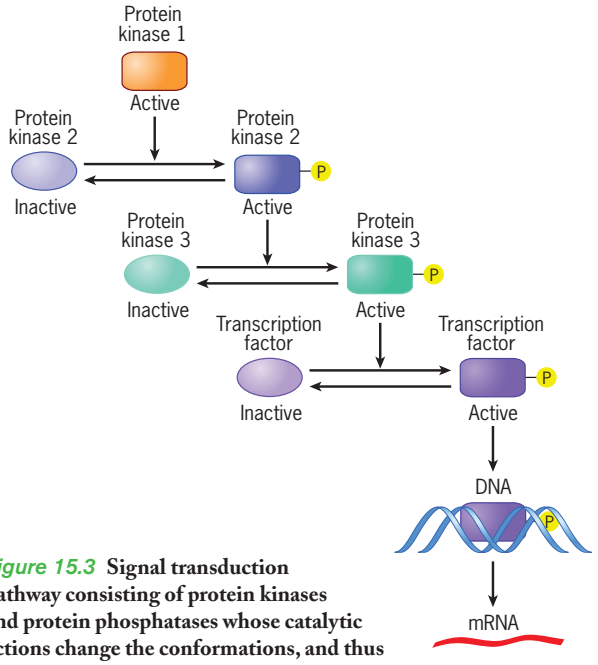


Figure 15.3 **Signal transduction pathway consisting of protein kinases and protein phosphatases whose catalytic actions change the conformations, and thus the activities, of the proteins they modify.** In the example depicted here, protein kinase 2 is activated by protein kinase 1. Once activated, protein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

kinases and protein phosphatases that, respectively, add or remove phosphate groups from other proteins (Figure 15.3). The human genome encodes more than 500 different protein kinases and approximately 150 different protein phosphatases. Whereas protein kinases typically work as a single subunit, many protein phosphatases contain a key regulatory subunit that determines substrate specificity. As a result, a single phosphatase catalytic subunit can form a host of different enzymes that remove phosphate groups from different protein substrates.

Most protein kinases transfer phosphate groups to serine or threonine residues of their protein substrates, but a very important group of kinases (roughly 90 in humous) phosphorylates tyrosine residues. Some protein kinases and phosphatases are soluble cytoplasmic proteins, others are integral membrane proteins. Many kinases are present in the cell in a self-inhibited state. Depending on the particular kinase, these enzymes can be activated in the cell by covalent modification or by interactions with other proteins, small molecules, or membrane lipids. It is remarkable that, even though thousands of proteins in a cell contain amino acid residues with the potential of being phosphorylated, each protein kinase or phosphatase is able to recognize only its specific substrates and ignore all of the others. Some protein kinases and phosphatases have numerous proteins as their substrates, whereas others phosphorylate or dephosphorylate only a single amino acid residue of a single protein substrate. Many of the protein substrates of these enzymes are enzymes themselves—most often other kinases and phosphatases—but the substrates also include ion channels, transcription factors, and various types of regulatory proteins. It is thought that at least 50 percent of transmembrane and cytoplasmic proteins are phosphorylated at one or more sites. Protein phosphorylation can change protein behavior in several different ways. Phosphorylation can activate or inactivate an enzyme, it can increase or decrease protein–protein interactions, it can induce a protein to move from one subcellular compartment to another, or it can act as a signal that initiates protein degradation. Large-scale proteomic approaches have been employed (page 70) to identify the substrates of various protein kinases and the specific residues that are phosphorylated in various tissues. One recent study of 9 different mouse tissues identified more that 6,000 phosphoproteins harboring nearly 36,000 sites of phosphorylation. The widespread occurrence of protein phosphorylation and its presumed importance is seen in Figure 15.4, which shows the marked difference in the frequency of tyrosine phosphorylation in certain proteins from two different types of cancer cells. The primary challenge is to understand the roles of these diverse posttranslational modifications in the activities of different cell types.

Signals transmitted along such signaling pathways ultimately reach *target proteins* (step 8, Figure 15.2) involved in basic cellular processes (step 9). Depending on the type of cell and message, the response initiated by the target protein may involve a change in gene expression, an alteration of the activity of metabolic enzymes, a reconfiguration of the cytoskeleton, an increase or decrease in cell mobility, a change in ion permeability, activation of DNA synthesis, or even the death of the cell. Virtually every activity in which a cell is engaged is regulated by signals originating at the cell surface. This

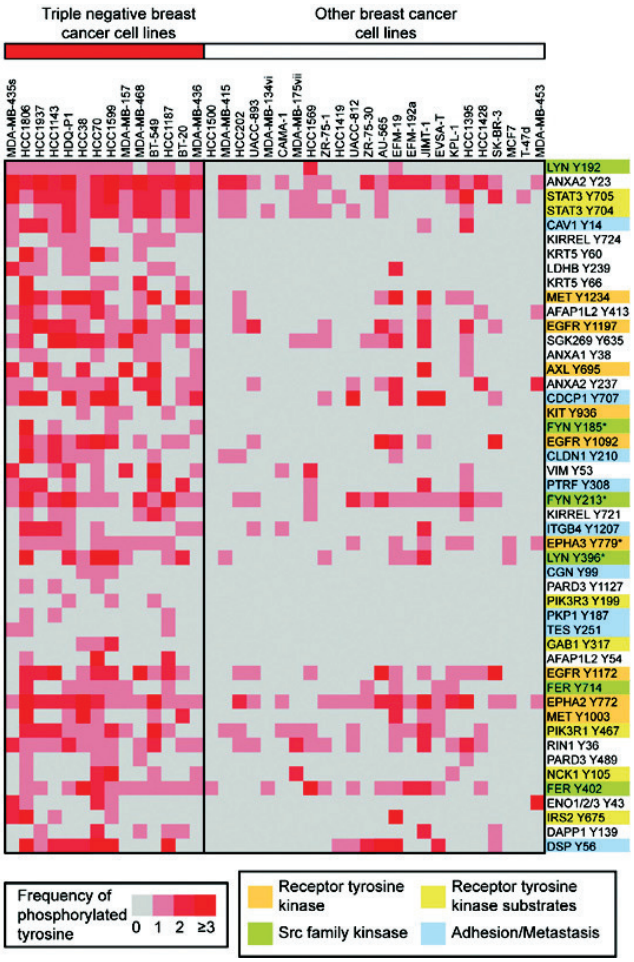


Figure 15.4 **A comparison in the frequency of tyrosine phosphorylation in two different types of breast cancer cells.** The panels on the left side of the figure show the frequency of phosphotyrosine (pTyr) residues in certain proteins (named on the right side of the figure) in triple-negative breast cancer cell lines. Triple-negative cells do not express three major molecular signatures of many breast cancer cells–estrogen receptor, progesterone receptor, and the growth factor receptor HER2. The frequency of pTyr residues in breast cancer cells that express these three signature proteins is shown in the panels on the right. The frequency of pTyr residues in a given protein in a given cell line is indicated by the intensity of the red shading of the box (see the key at the bottom of the figure). The names of the cell lines tested are given along the top of the figure. It is evident that the triple-negative cells have a much greater level of tyrosine phosphorylation than the other breast cancer cells. This may correlate with the loss of a particular protein tyrosine phosphatase activity (PTPN12) in many of the triple-negative cancers. (From J. G. Albeck and J. S. Brugge, from data by Ting-Lei Gu of Cell Signaling Technology, Cell 144:639, 2011. Reprinted with permission from Elsevier.)

overall process in which information carried by extracellular messenger molecules is translated into changes that occur inside a cell is referred to as **signal transduction**.

Finally, signaling has to be terminated. This is important because cells have to be responsive to additional messages that

they may receive. The first order of business is to eliminate the extracellular messenger molecule. To do this, certain cells produce extracellular enzymes that destroy specific extracellular messengers. In other cases, activated receptors are internalized (page 624). Once inside the cell, the receptor may be degraded together with its ligand, which can leave the cell with decreased sensitivity to subsequent stimuli.

REVIEW

1. What is meant by the term *signal transduction*? What are some of the steps by which signal transduction can occur?
2. What is a second messenger? Why do you suppose it is called this?

15.2 | A Survey of Extracellular Messengers and Their Receptors

A large variety of molecules can function as extracellular carriers of information. These include

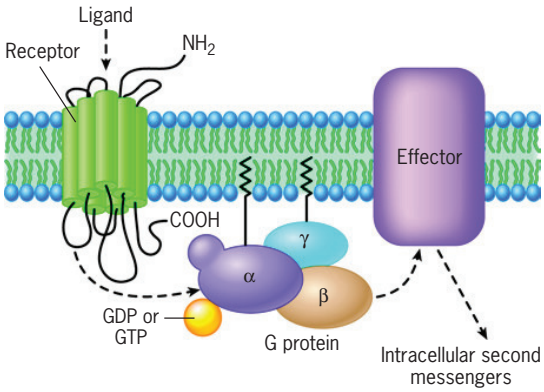
- Amino acids and amino acid derivatives. Examples include glutamate, glycine, acetylcholine, epinephrine, dopamine, and thyroid hormone. These molecules act as neurotransmitters and hormones.
- Gases, such as NO and CO.
- Steroids, which are derived from cholesterol. Steroid hormones regulate sexual differentiation, pregnancy, carbohydrate metabolism, and excretion of sodium and potassium ions.
- Eicosanoids, which are nonpolar molecules containing 20 carbons that are derived from a fatty acid named arachidonic acid. Eicosanoids regulate a variety of processes including pain, inflammation, blood pressure, and blood clotting. Several over-the-counter drugs that are used to treat headaches and inflammation inhibit eicosanoid synthesis.
- A wide variety of polypeptides and proteins. Some of these are present as transmembrane proteins on the surface of an interacting cell (page 251). Others are part of, or associate with, the extracellular matrix. Finally, a large number of proteins are excreted into the extracellular environment where they are involved in regulating processes such as cell division, differentiation, the immune response, or cell death and cell survival.

Extracellular signaling molecules are usually, but not always, recognized by specific receptors that are present on the surface of the responding cell. As illustrated in Figure 15.2, receptors bind their signaling molecules with high affinity and translate this interaction at the outer surface of the cell into changes that take place on the inside of the cell. The receptors that have evolved to mediate signal transduction are indicated below.

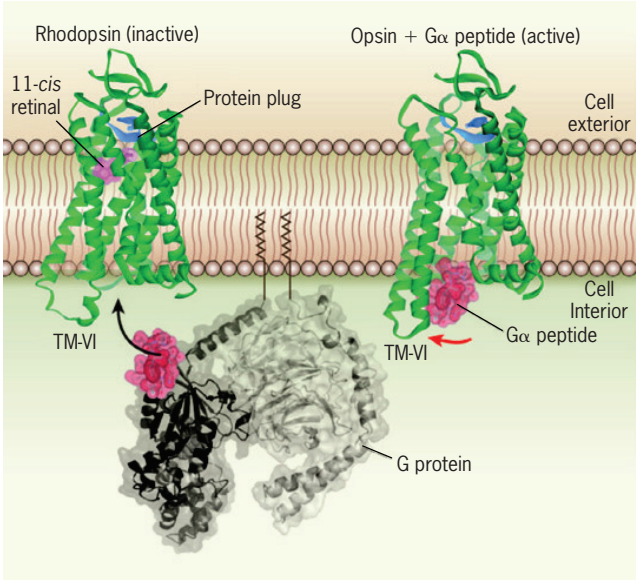
- G protein-coupled receptors (GPCRs) are a huge family of receptors that contain seven transmembrane α helices. These receptors translate the binding of extracellular signaling molecules into the activation of GTP-binding proteins. **GTP-binding proteins** (or **G proteins**) were discussed in connection with vesicle budding and fusion in Chapter 8, microtubule dynamics in Chapter 9, protein synthesis in Chapters 8 and 11, and nucleocytoplasmic transport in Chapter 12. In the present chapter, we will explore their role in transmitting messages along "cellular information circuits."
- Receptor protein-tyrosine kinases (RTKs) represent a second class of receptors that have evolved to translate the presence of extracellular messenger molecules into changes inside the cell. Binding of a specific extracellular ligand to an RTK usually results in receptor dimerization followed by activation of the receptor's protein-kinase domain, which is present within its cytoplasmic region. Upon activation, these protein kinases phosphorylate specific tyrosine residues of cytoplasmic substrate proteins, thereby altering their activity, their localization, or their ability to interact with other proteins within the cell.
- Ligand-gated channels represent a third class of cell-surface receptors that bind to extracellular ligands. The ability of these proteins to conduct a flow of ions across the plasma membrane is regulated directly by ligand binding. A flow of ions across the membrane can result in a temporary change in membrane potential, which will affect the activity of other membrane proteins, for instance, voltage-gated channels. This sequence of events is the basis for formation of a nerve impulse (page 166). In addition, the influx of certain ions, such as Ca^{2+}, can change the activity of particular cytoplasmic enzymes. As discussed in Section 4.8, one large group of ligand-gated channels functions as receptors for neurotransmitters.
- Steroid hormone receptors function as ligand-regulated transcription factors. Steroid hormones diffuse across the plasma membrane and bind to their receptors, which are present in the cytoplasm. Hormone binding results in a conformational change that causes the hormone–receptor complex to move into the nucleus and bind to elements present in the promoters or enhancers of hormone-responsive genes (see Figure 12.47). This interaction gives rise to an increase or decrease in the rate of gene transcription.
- Finally, there are a number of other types of receptors that act by unique mechanisms. Some of these receptors, for example, the B- and T-cell receptors that are involved in the response to foreign antigens, associate with known signaling molecules such as cytoplasmic protein-tyrosine kinases. We will concentrate in this chapter on the GPCRs and RTKs.

15.3 | G Protein-Coupled Receptors and Their Second Messengers

G protein-coupled receptors (**GPCRs**) are so named because they interact with G proteins, as discussed below. Members of the GPCR superfamily are also referred to as seven-



(a)



(b)

Figure 15.5 **The membrane-bound machinery for transducing signals by means of a seven transmembrane receptor and a heterotrimeric G protein.** (*a*) Receptors of this type, including those that bind epinephrine and glucagon, contain seven membrane-spanning helices. When bound to their ligand, the receptor interacts with a trimeric G protein, which activates an effector, such as adenylyl cyclase. As indicated in the figure, the α and γ subunits of the G protein are linked to the membrane by lipid groups that are embedded in the lipid bilayer. (*Note:* Many GPCRs may be active as complexes of two or more receptor molecules.) (*b*) A model depicting the activation of the GPCR rhodopsin based on X-ray crystallographic structures. On the left, rhodopsin is shown in its inactive (dark-adapted) conformation together with an unbound heterotrimeric G protein (called transducin). When the retinal cofactor (shown in red on the left rhodopsin molecule) absorbs a photon, it undergoes an isomerization reaction (from a *cis* to a *trans* form), which leads to the disruption of an ionic linkage between residues on the third and sixth transmembrane helix of the protein. This event in turn leads to a change in conformation of the protein, including the outward tilt and rotation of the sixth transmembrane helix (red curved arrow), which exposes a binding site for the G_α subunit of the G protein. The rhodopsin molecule on the right is shown in the proposed active conformation with a portion of the G_α subunit (in red) bound to the receptor's cytoplasmic face. (B: From Thue W. Schwartz and Wayne L. Hubbell, Nature 455, 473, 2008. Reprinted by permission from Macmillan Publishers Limited.)

transmembrane (7TM) receptors because they contain seven transmembrane helices (Figure 15.5). Thousands of different GPCRs have been identified in organisms ranging from yeast to flowering plants and mammals that together regulate an extraordinary spectrum of cellular processes. In fact, GPCRs constitute the single largest superfamily of proteins encoded by animal genomes. Included among the natural ligands that bind to GPCRs are a diverse array of hormones (both plant and animal), neurotransmitters, opium derivatives, chemoattractants (e.g., molecules that attract phagocytic cells of the immune system), odorants and tastants (molecules detected by olfactory and gustatory receptors eliciting the senses of smell and taste), and photons. A list of some of the ligands that operate by means of this pathway and the effectors through which they act is provided in Table 15.1.

Signal Transduction by G Protein-Coupled Receptors

Receptors G protein-coupled receptors normally have the following topology. Their amino-terminus is present on the outside of the cell, the seven α helices that traverse the plasma membrane are connected by loops of varying length, and the carboxyl-terminus is present on the inside of the cell (Figure 15.5). There are three loops present on the outside of the cell

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

Stimulus	Receptor	Effector	Physiologic response
Epinephrine	β-Adrenergic receptor	Adenylyl cyclase	Glycogen breakdown
Serotonin	Serotonin receptor	Adenylyl cyclase	Behavioral sensitization and learning in *Aplysia*
Light	Rhodopsin	cGMP phosphodiesterase	Visual excitation
IgE–antigen complexes	Mast cell IgE receptor	Phospholipase C	Secretion
f-Met Peptide	Chemotactic receptor	Phospholipase C	Chemotaxis
Acetylcholine	Muscarinic receptor	Potassium channel	Slowing of pacemaker activity

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that, together, form the ligand-binding pocket, whose structure varies among different GPCRs. There are also three loops present on the cytoplasmic side of the plasma membrane that provide binding sites for intracellular signaling proteins.

It is very difficult, for a number of technical reasons, to prepare crystals of unmodified GPCRs that are suitable for X-ray crystallographic analysis. For a number of years, rhodopsin was the only member of the superfamily to have its X-ray crystal structure determined. Rhodopsin has an unusually stable structure for a GPCR, owing to the fact that its ligand (a retinal group) is permanently bound to the protein and the protein molecule can only exist in a single inactive conformation in the absence of a stimulus (i.e., in the dark). Beginning in 2007, as a result of years of effort by a number of research groups, a flurry of GPCR crystal structures appeared in the literature. For the most part, these structures revealed the GPCR in the inactive state, but more recent reports have described the structures of modified versions of several GPCRs in the active state. There is also a body of structural and spectroscopic data (of the type described on page 135) that provide insights into some of the conformational changes that occur as a GPCR is activated and becomes bound to a G protein.

The first X-ray crystal structure of an active GPCR with its bound G protein was solved by Brian Kobilka and colleagues at Stanford University and is shown in the chapter-opening image on page 617. The inactive conformation of the GPCR is stabilized by noncovalent interactions between specific residues in the transmembrane α helices. Ligand binding disturbs these interactions, thereby causing the receptor to assume an active conformation. This requires rotations and shifts of the transmembrane α helices relative to each other. Because they are attached to the cytoplasmic loops, rotation or movement of these transmembrane α helices causes changes in the conformation of the cytoplasmic loops. This in turn leads to an increase in the affinity of the receptor for a G protein that is present on the cytoplasmic surface of the plasma membrane (Figure 15.5*b*). As a consequence, the ligand-bound receptor forms a receptor–G protein complex (Figure 15.6, step 1). The interaction with the receptor induces a conformational change in the α subunit of a G protein, causing the release of GDP, which is followed by binding of GTP (step 2). While in the activated state, a single receptor can

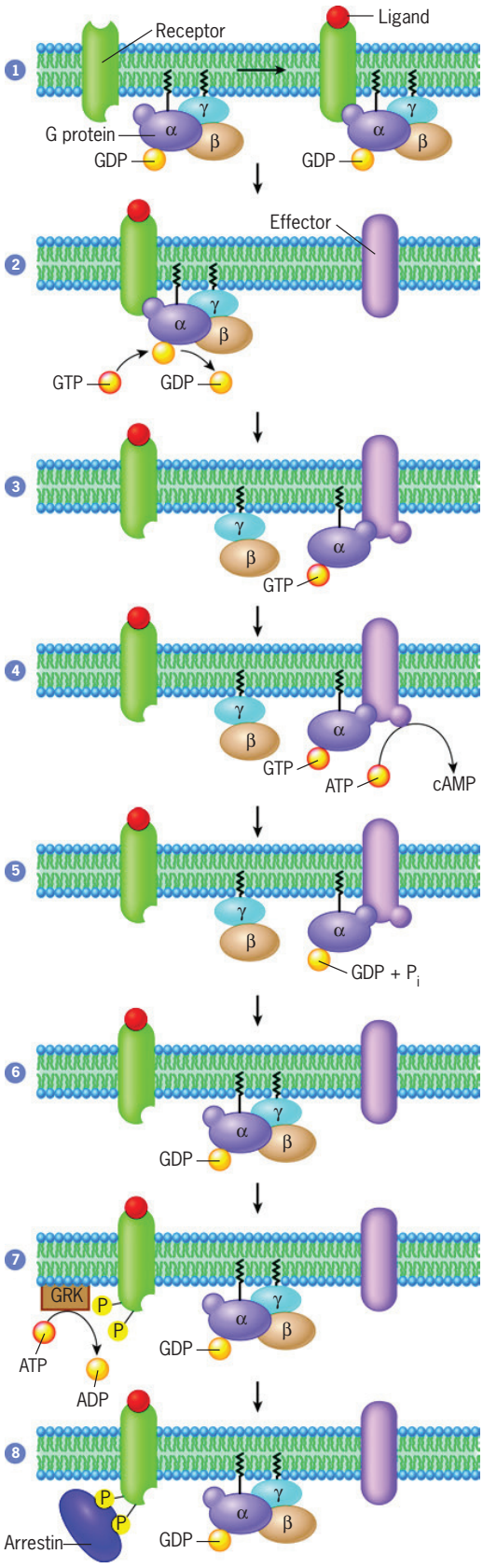

Figure 15.6 **The mechanism of receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins.** In step 1, the ligand binds to the receptor, altering its conformation and increasing its affinity for the G protein to which it binds. In step 2, the G_α subunit releases its GDP, which is replaced by GTP. In step 3, the G_α subunit dissociates from the $G_{\beta\gamma}$ complex and binds to an effector (in this case adenylyl cyclase), activating the effector. The $G_{\beta\gamma}$ dimer may also bind to an effector (not shown), such as an ion channel or an enzyme. In step 4, activated adenylyl cyclase produces cAMP. In step 5, the GTPase activity of G_α hydrolyzes the bound GTP, deactivating G_α. In step 6, G_α reassociates with $G_{\beta\gamma}$, reforming the trimeric G protein, and the effector ceases its activity. In step 7, the receptor has been phosphorylated by a GRK and in step 8 the phosphorylated receptor has been bound by an arrestin molecule, which inhibits the ligand-bound receptor from activating additional G proteins. The receptor bound to arrestin is likely to be taken up by endocytosis.

activate a number of G protein molecules, providing a means of signal amplification (discussed further on page 632).

G Proteins Heterotrimeric G proteins were discovered, purified, and characterized by Martin Rodbell and his colleagues at the National Institutes of Health and Alfred Gilman and colleagues at the University of Virginia. Their studies are discussed in the Experimental Pathways, which can be found on the Web at www.wiley.com/college/karp. These proteins are referred to as G proteins because they bind guanine nucleotides, either GDP or GTP. They are described as heterotrimeric because all of them consist of three different polypeptide subunits, called α, β, and γ. This property distinguishes them from small, monomeric G proteins, such as Ras, which are discussed later in this chapter. Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the α and γ subunits (Figure 15.5*a*).

The guanine nucleotide-binding site is present on the G_α subunit. Replacement of GDP by GTP, following interaction with an activated GPCR, results in a conformational change in the G_α subunit. In its GTP-bound conformation, the G_α subunit has a low affinity for $G_{\beta\gamma}$, leading to dissociation of the trimeric complex. Each dissociated G_α subunit (with GTP attached) is free to activate an effector protein, such as adenylyl cyclase (Figure 15.6, step 3). In this case, activation of the effector leads to the production of the second messenger cAMP (step 4). Other effectors include phospholipase C-β and cyclic GMP phosphodiesterase (see below). Second messengers, in turn, activate one or more cellular signaling proteins.

A G protein is said to be "on" when its α subunit is bound to GTP. G_α subunits can turn themselves off by hydrolysis of GTP to GDP and inorganic phosphate (Pi) (Figure 15.6, step 5). This results in a conformational change causing a decrease in the affinity for the effector and an increase in the affinity for the βγ subunit. Thus, following hydrolysis of GTP, the G_α subunit will dissociate from the effector and reassociate with the βγ subunit to reform the inactive heterotrimeric G protein (step 6). In a sense, heterotrimeric G proteins function as molecular timers. They are turned on by the interaction with an activated receptor and turn themselves off by hydrolysis of bound GTP after a certain amount of time has passed. While they are active, G_α subunits can turn on downstream effectors.

Heterotrimeric G proteins come in four flavors, G_s, G_q, G_i, and $G_{12/13}$. This classification is based on the G_α subunits and the effectors to which they couple. The particular response elicited by an activated GPCR depends on the type of G protein with which it interacts, although some GPCRs can interact with different G proteins and trigger more than one physiologic response. G_s family members couple receptors to adenylyl cyclase. Adenylyl cyclase is activated by GTP-bound G_s subunits. G_q family members contain G_α subunits that activate PLCβ. PLCβ hydrolyzes phosphatidylinositol bisphosphate, producing inositol trisphosphate and diacylglycerol (page 630). Activated G_i subunits function by inhibiting adenylyl cyclase. $G_{12/13}$ members are less well characterized than the other G protein families although their inappropriate activation has been associated with excessive cell proliferation and malignant transformation. Following its dissociation from the G_α subunit, the βγ complex also has a signaling function and it can couple to a number of different types of effectors, including PLCβ, K^+ and Ca^{2+} ion channels, and adenylyl cyclase.

Termination of the Response We have seen that ligand binding results in receptor activation. The activated receptors turn on G proteins, and G proteins turn on effectors. To prevent overstimulation, receptors have to be blocked from continuing to activate G proteins. To regain sensitivity to future stimuli, the receptor, the G protein, and the effector must all be returned to their inactive state. *Desensitization*, the process that blocks active receptors from turning on additional G proteins, takes place in two steps. In the first step, the cytoplasmic domain of the activated GPCR is phosphorylated by a specific type of kinase, called *G protein–coupled receptor kinase* (*GRK*) (Figure 15.6, step 7). GRKs form a small family of serine-threonine protein kinases that specifically recognize activated GPCRs.

Phosphorylation of the GPCR sets the stage for the second step, which is the binding of proteins, called *arrestins* (Figure 15.6, step 8). Arrestins form a small family of proteins that bind to GPCRs and compete for binding with heterotrimeric G proteins. As a consequence, arrestin binding prevents the further activation of additional G proteins. This action is termed desensitization because the cell stops responding to the stimulus, while that stimulus is still acting on the outer surface of the cell. Desensitization is one of the mechanisms that allows a cell to respond to a change in its environment, rather than continuing to "fire" endlessly in the presence of an unchanging environment. The importance of desensitization is illustrated by the observation that mutations that interfere with phosphorylation of rhodopsin by a GRK lead to the death of the photoreceptor cells in the retina. This type of retinal cell death is thought to be one of the causes of blindness resulting from the disease retinitis pigmentosa.

Arrestins can be described as protein hubs (page 62), in that they are capable of binding to a variety of different proteins involved in different intracellular processes. While they are bound to phosphorylated GPCRs (step 1, Figure 15.7), arrestin molecules are also capable of binding to AP2 adaptor molecules that are situated in clathrin-coated pits (page 310). The interaction between bound arrestin and clathrin-coated pits (step 2) promotes the uptake of phosphorylated GPCRs into the cell by endocytosis. Depending on the circumstances, receptors that have been removed from the surface by endocytosis can participate in several alternative outcomes. In some cases, receptors travel along the endocytic pathway into endosomes (page 312), where the associated arrestin molecules serve as a scaffold for the assembly of various cytoplasmic signaling complexes. The MAPK pathway, which is discussed at length later in the chapter, is thought to be activated by arrestin-bound GPCRs localized within endosomes (step 3). The discovery of "signaling endosomes" came as a surprise to researchers in the field of cell signaling who had been working under the assumption that GPCRs (and RTKs as well) were only capable of signal transduction when they resided at the cell surface. Now it appears that the signals transmitted from endosomes have different properties and physiological roles from those that arise from the plasma membrane. In a second outcome, internalized receptors may traffic from endosomes to lysosomes where they are degraded (step 4). If receptors are

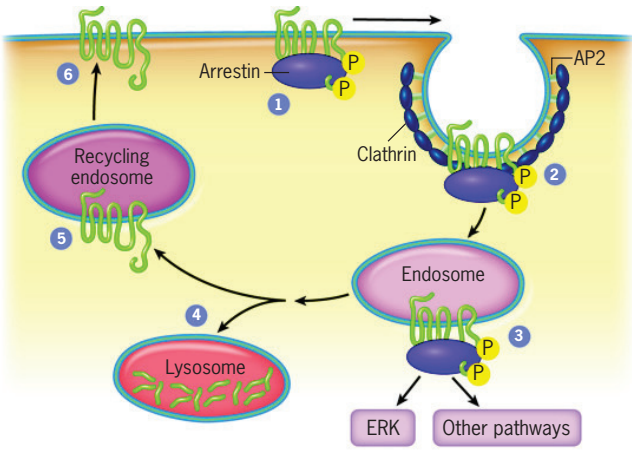

Figure 15.7 **Arrestin-mediated internalization of GPCRs**. Arrestin-bound GPCRs (step 1) are internalized when they are trapped in clathrin-coated pits which bud into the cytoplasm (step 2). As discussed in Section 8.8, clathrin-coated buds are transformed into clathrin-coated vesicles which deliver their contents, including the GPCRs, to endosomes. When present in the endosomes, arrestins can serve as scaffolds for the assembly of signaling complexes, including those that activate the MAPK cascade and the transcription factor ERK (step 3). Alternatively, the GPCRs can be delivered to lysosomes, where they are degraded (step 4), or they can be returned to the plasma membrane in a recycling endosome (step 5), where they can then interact with new extracellular ligands (step 6). (FROM S. L. RITTER AND R. A. HALL, NATURE REVIEWS MCB 10:820, 2009, BOX 1B. NATURE REVIEWS MOLECULAR CELL BIOLOGY BY NATURE PUBLISHING GROUP. REPRODUCED WITH PERMISSION OF NATURE PUBLISHING GROUP IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

degraded, the cells lose, at least temporarily, sensitivity for the ligand in question. Finally, according to a third scheme, the arrestin-bound GPCRs may be dephosphorylated and returned to the plasma membrane (step 5). If receptors are returned to the cell surface, the cells remain sensitive to the ligand (they are said to be *resensitized*).

Signaling by the activated G_α subunit is terminated by a less complex mechanism: the bound GTP molecule is simply hydrolyzed to GDP (step 5, Figure 15.6). Thus, the strength and duration of the signal are determined in part by the rate of GTP hydrolysis by the G_α subunit. G_α subunits possess a weak GTPase activity, which allows them to slowly hydrolyze the bound GTP and inactivate themselves. Termination of the response is accelerated by *regulators of G protein signaling* (*RGSs*). The interaction with an RGS protein increases the rate of GTP hydrolysis by the G_α subunit. Once the GTP is hydrolyzed, the G_α-GDP reassociates with the $G_{\beta\gamma}$ subunits to reform the inactive trimeric complex (step 6) as discussed above. This returns the system to the resting state.

The mechanism for transmitting signals across the plasma membrane by G proteins is of ancient evolutionary origin and is highly conserved. This is illustrated by an experiment in which yeast cells were genetically engineered to express a receptor for the mammalian hormone somatostatin. When these yeast cells were treated with somatostatin, the mammalian receptors at the cell surface interacted with the yeast heterotrimeric G proteins at the inner surface of the membrane and triggered a response leading to proliferation of the yeast cells.

The effects of certain mutations on the function of G protein-coupled receptors are discussed in the accompanying Human Perspective.

THE HUMAN PERSPECTIVE

Disorders Associated with G Protein-Coupled Receptors

GPCRs represent the largest family of genes encoded by the human genome. Their importance in human biology is reflected by the fact that over one-third of all prescription drugs act as ligands that bind to this huge superfamily of receptors. A number of inherited disorders have been traced to defects in both GPCRs (Figure 1) and heterotrimeric G proteins (Table 1). Retinitis pigmentosa (RP) is an inherited disease characterized by progressive degeneration of the retina and eventual blindness. RP can be caused by mutations in the gene that encodes rhodopsin, the visual pigment of the rods. Many of these mutations lead to premature termination or improper folding of the rhodopsin protein and its elimination from the cell before it reaches the plasma membrane (page 288). Other mutations may lead

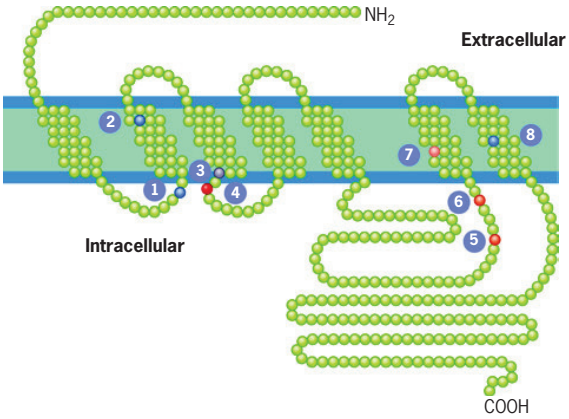

Figure 1 **Two-dimensional representation of a "composite" transmembrane receptor showing the approximate sites of a number of mutations responsible for causing human diseases.** Most of the mutations (numbers 1, 2, 5, 6, 7, and 8) result in constitutive stimulation of the effector, but others (3 and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

Table 1 Human Diseases Linked to the G Protein Pathway

Disease	Defective G protein*
Albright's hereditary osteodystrophy and pseudohypoparathyroidisms	$G_{s\alpha}$
McCune–Albright syndrome	$G_{s\alpha}$
Pituitary, thyroid tumors (*gsp* oncogene)	$G_{s\alpha}$
Adrenocortical, ovarian tumors (*gip* oncogene)	$G_{i\alpha}$
Combined precocious puberty and peudohypoparathyroidism	$G_{s\alpha}$

Disease	Defective G protein-coupled receptor
Familial hypocalciuric hypercalcemia	Human analogue of BoPCAR1 receptor
Neonatal severe hyperparathyroidism	Human analogue of BoPCAR1 receptor (homozygous)
Hyperthyroidism (thyroid adenomas)	Thyrotropin receptor
Familial male precocious puberty	Luteinizing hormone receptor
X-linked nephrogenic diabetes insipidus	V2 vasopressin receptor
Retinitis pigmentosa	Rhodopsin receptor
Color blindness, spectral sensitivity variations	Cone opsin receptor
Familial glucocorticoid deficiency and isolated glucocorticoid deficiency	Adrenocorticoptropic hormone (ACTH) receptor

*As described in the text, a G protein with a $G_{s\alpha}$ acts to stimulate the effector, whereas a G protein with a $G_{i\alpha}$ inhibits the effector.
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to the synthesis of a rhodopsin molecule that cannot activate its G protein and thus cannot pass the signal downstream to the effector.

RP results from a mutation that leads to a loss of function of the encoded receptor. Many mutations that alter the structure of signaling proteins can have an opposite effect, leading to what is described as a "gain of function." In one such case, mutations have been found to cause a type of benign thyroid tumor, called an adenoma. Unlike normal thyroid cells that secrete thyroid hormone only in response to stimulation by the pituitary hormone TSH, the cells of these thyroid adenomas secrete large quantities of thyroid hormone without having to be stimulated by TSH (the receptor is said to act *constitutively*). The TSH receptor in these cells contains an amino acid substitution that affects the structure of the third intracellular loop of the protein (Figure 1, mutations at sites 5 or 6). As a result of the mutation, the TSH receptor constitutively activates a G protein on its inner surface, sending a continual signal through the pathway that leads not only to excessive thyroid hormone secretion but to the excessive cell proliferation that causes the tumor. This conclusion was verified by introducing the mutant gene into cultured cells that normally lack this receptor and demonstrating that the synthesis of the mutant protein and its incorporation into the plasma membrane led to the continuous production of cAMP in the genetically engineered cells.

The mutation that causes thyroid adenomas is not found in the normal portion of a patient's thyroid but only in the tumor tissue, indicating that the mutation was not inherited but arose in one of the cells of the thyroid, which then proliferated to give rise to the tumor. A mutation in a cell of the body, such as a thyroid cell, is called a *somatic mutation* to distinguish it from an inherited mutation that would be present in all of the individual's cells. As will be evident in the following chapter, somatic mutations are a primary cause of human cancer. At least one cancer-causing virus has been shown to encode a protein that acts as a constitutively active GPCR. The virus is a type of herpes virus that is responsible for Kaposi's sarcoma, which causes purplish skin lesions and is prevalent in AIDS patients. The virus genome encodes a constitutively active receptor for interleukin-8, which stimulates signaling pathways that control cell proliferation.

As noted in Table 1, mutations in genes that encode the subunits of heterotrimeric G proteins can also lead to inherited disorders. This is illustrated by a report on two male patients suffering from a rare combination of endocrine disorders: precocious puberty and hypoparathyroidism. Both patients were found to contain a single amino acid substitution in one of the G_α isoforms. The alteration in amino acid sequence caused two effects on the mutant G protein. At temperatures below normal body temperature, the mutant G protein remained in the active state, even in the absence of a bound ligand. In contrast, at normal body temperatures, the mutant G protein was inactive, both in the presence and absence of bound ligand. The testes, which are housed outside of the body's core, have a lower temperature than the body's visceral organs (33°C vs. 37°C). Normally, the endocrine cells of the testes initiate testosterone production at the time of puberty in response to the pituitary hormone LH, which begins to be produced at that time. The circulating LH binds to LH receptors on the surface of the testicular cells, inducing the synthesis of cAMP and subsequent production of the male sex hormone. The testicular cells of the patients bearing the G protein mutation were stimulated to synthesize cAMP in the absence of the LH ligand, leading to premature synthesis of testosterone and precocious puberty. In contrast, the mutation in this same G_α subunit in the cells of the parathyroid glands, which function at a temperature of 37°C, caused the G protein to remain inactive. As a result, the cells of the parathyroid gland could not respond to stimuli that would normally cause them to secrete parathyroid hormone, leading to the condition of hypoparathyroidism. The fact that most of the bodily organs functioned in a normal manner in these patients suggests that this particular G_α isoform is not essential in the activities of most other cells.

Mutations are thought of as rare and disabling changes in the nucleotide sequence of a gene. Genetic polymorphisms, in contrast, are thought of as common, "normal" variations within the population (page 416). Yet it has become clear in recent years that genetic polymorphisms may have considerable impact on human disease, causing certain individuals to be more or less susceptible to particular disorders than other individuals. This has been well documented in the case of GPCRs. For example, certain alleles of the gene encoding the β_2 adrenergic receptor have been associated with an increased likelihood of developing asthma or high blood pressure; certain alleles of a dopamine receptor are associated with increased risk of substance abuse or schizophrenia; and certain alleles of a chemokine receptor (*CCR5*) are associated with prolonged survival in HIV-infected individuals. As discussed on page 417, identifying associations between disease susceptibility and genetic polymorphisms is a current focus of clinical research.

Bacterial Toxins Because G proteins are so important to the normal physiology of multicellular organisms, they provide excellent targets for bacterial pathogens. For example, cholera toxin (produced by the bacterium *Vibrio cholerae*) exerts its effect by modifying G_α subunits and inhibiting their GTPase activity in the cells of the intestinal epithelium. As a result, adenylyl cyclase molecules remain in an activated mode, churning out cAMP, which causes the epithelial cells to secrete large volumes of fluid into the intestinal lumen. The loss of water associated with this inappropriate response often leads to death due to dehydration.

Pertussis toxin is one of several virulence factors produced by *Bordetella pertussis*, a microorganism that causes whooping cough. Whooping cough is a debilitating respiratory tract infection seen in 50 million people worldwide each year, causing death in about 350,000 of these cases annually. Pertussis toxin also inactivates G_α subunits, thereby interfering with the signaling pathway that leads the host to mount a defensive response against the bacterial infection.

Second Messengers

The Discovery of Cyclic AMP, a Prototypical Second Messenger How does the binding of a hormone to the plasma membrane change the activity of cytoplasmic enzymes, such as glycogen phosphorylase, an enzyme involved in glycogen metabolism? The answer to this question was provided by studies that began in the mid-1950s in the laboratories of Earl Sutherland and his colleagues at Case Western Reserve University. Sutherland's goal was to develop an in vitro system to study the physiologic responses to hormones. After considerable effort, he was able to activate glycogen phosphorylase in a preparation of *broken* cells that had been incubated with glucagon or epinephrine. This broken-cell preparation could be divided by centrifugation into a particulate fraction consisting primarily of cell membranes and a soluble supernatant fraction. Even though glycogen phosphorylase was present only in the supernatant fraction, the particulate material was required to obtain the hormone response. Subsequent experiments indicated that the response occurred in at least two distinct steps. If the particulate fraction of a liver homogenate was isolated and incubated with the hormone, some substance was released that, when added to the supernatant fraction, activated the soluble glycogen phosphorylase molecules. Sutherland identified the substance released by the membranes of the particulate fraction as cyclic adenosine monophosphate (*cyclic AMP*, or simply *cAMP*). This discovery is heralded as the beginning of the study of signal transduction. As will be discussed below, cAMP stimulates glucose mobilization by activating a protein kinase that adds a phosphate group onto a specific serine residue of the glycogen phosphorylase polypeptide.

Cyclic AMP is a **second messenger** that is capable of diffusing to other sites within the cell. The synthesis of cyclic AMP follows the binding of a first messenger—a hormone or other ligand—to a receptor at the outer surface of the cell. Figure 15.8 shows the diffusion of cyclic AMP within the cytoplasm of a neuron following stimulation by an extracellular messenger molecule. Whereas the first messenger binds exclusively to a single receptor species, the second messenger often stimulates a variety of cellular activities. As a result, second messengers enable cells to mount a large-scale, coordinated response following stimulation by a single extracellular ligand. Other second messengers include Ca^{2+}, phosphoinositides, inositol trisphosphate, diacylglycerol, cGMP, and nitric oxide.

Phosphatidylinositol-Derived Second Messengers It wasn't very long ago that the phospholipids of cell membranes were considered strictly as structural molecules that made membranes cohesive and impermeable to aqueous solutes. Our appreciation of phospholipids has increased with the realization that these molecules form the precursors of a number of second messengers. Phospholipids of cell membranes are

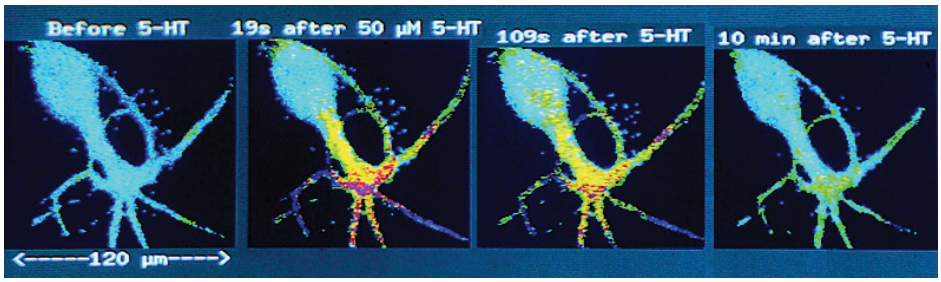
Before 5-HT
19s after 50 μM 5-HT
109s after 5-HT
10 min after 5-HT
<-----120 μm---->

Figure 15.8 **The localized formation of cAMP in a live cell in response to the addition of an extracellular messenger molecule.** This series of photographs shows a sensory nerve cell from the sea hare *Aplysia*. The concentration of free cAMP is indicated by the color: blue represents a low cAMP concentration, yellow an intermediate concentration, and red a high concentration. The left image shows the intracellular cAMP level in the unstimulated neuron, and the next three images show the effects of stimulation by the neurotransmitter serotonin (5-hydroxytryptamine) at the times indicated. Notice that the cAMP levels drop by 109s despite the continued presence of the neurotransmitter. (The cAMP level was determined indirectly in this experiment by microinjection of a fluorescently labeled cAMP-dependent protein kinase labeled with both fluorescein and rhodamine on different subunits. Energy transfer between the subunits (see page 738) provides a measure of cAMP concentration.) (From Brian J. Bacskai et al., Science 260:223, 1993. Reprinted with permission from AAAS.)

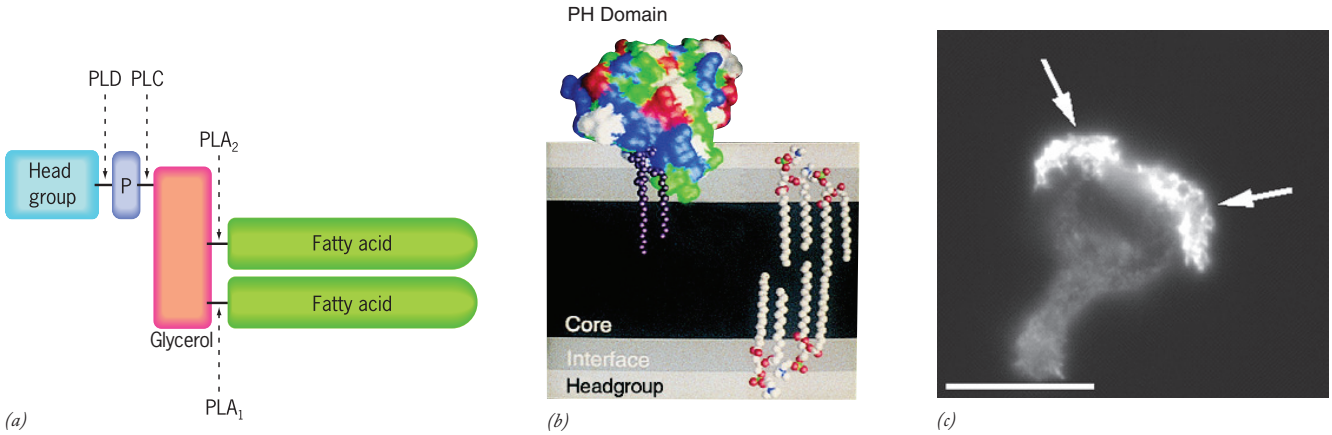

Figure 15.9 **Phospholipid-based second messengers.** (*a*) The structure of a generalized phospholipid (see Figure 2.22). Phospholipids are subject to attack by four types of phospholipases that cleave the molecule at the indicated sites. Of these enzymes, we will focus on PLC, which splits the phosphorylated head group from the diacylglycerol (see Figure 15.10). (*b*) A model showing the interaction between a portion of a PLC enzyme molecule containing a PH domain that binds to the phosphorylated inositol ring of a phosphoinositide. This interaction holds the enzyme to the inner surface of the plasma membrane and may alter its enzymatic activity. (*c*) Fluorescence micrograph of a cell that has been stimulated to move toward a chemoattractant (i.e., a chemical to which the cell is attracted). This cell has been stained with an antibody that binds specifically to PI 3,4,5-trisphosphate (PIP_3), which is seen to be localized at the leading edge of the migrating cell (arrows). Bar equals 15 μm. (B: From James H. Hurley and Jay A. Grobler, Curr. Opin. Struct. Biol. 7:559, 1997; C: from Paula Rickert et al., courtesy of Henry R. Bourne, University of California, San Francesco, Trends Cell Biol. 10:470, 2000. Both images reprinted with permission from Elsevier.)

converted into second messengers by a variety of enzymes that are regulated in response to extracellular signals. These enzymes include phospholipases (lipid-splitting enzymes), phospholipid kinases (lipid-phosphorylating enzymes), and phospholipid phosphatases (lipid-dephosphorylating enzymes). Phospholipases are enzymes that hydrolyze specific ester bonds that connect the different building blocks that make up a phospholipid molecule. Figure 15.9*a* shows the cleavage sites within a generalized phospholipid that are attacked by the main classes of phospholipases. All four of the enzyme classes depicted in Figure 15.9*a* can be activated in response to extracellular signals and the products they produce function as second messengers. In this section, we will focus on the best-studied lipid second messengers, which are derived from phosphatidylinositol, and are generated following the transmission of signals by G protein-coupled receptors and receptor protein-tyrosine kinases. Another group of lipid second messengers derived from sphingomyelin is not discussed.

Phosphatidylinositol Phosphorylation When the neurotransmitter acetylcholine binds to the surface of a smooth muscle cell within the wall of the stomach, the muscle cell is stimulated to contract. When a foreign antigen binds to the surface of a mast cell, the cell is stimulated to secrete histamine, a substance that can trigger the symptoms of an allergy attack. Both of these responses, one leading to contraction and the other to secretion, are triggered by the same second messenger, a substance derived from the compound phosphatidyl–inositol, a minor component of most cellular membranes (see Figure 4.10).

The first indication that phospholipids might be involved in cellular responses to extracellular signals emerged from studies carried out in the early 1950s by Lowell and Mabel Hokin of Montreal General Hospital and McGill University. These investigators had set out to study the effects of acetylcholine on RNA synthesis in the pancreas. To carry out these studies, they incubated slices of pigeon pancreas in [^{32}P]orthophosphate. The idea was that [^{32}P]orthophosphate would be incorporated into nucleoside triphosphates, which are used as precursors during the synthesis of RNA. Interestingly, they found that treatment of the tissue with acetylcholine led to the incorporation of radioactivity into the phospholipid fraction of the cell. Further analysis revealed that the isotope was incorporated primarily into phosphatidylinositol (PI), which was rapidly converted to other phosphorylated derivatives, which are collectively referred to as **phosphoinositides**. This suggested that inositol-containing lipids can be phosphorylated by specific lipid kinases that are activated in response to extracellular messenger molecules, such as acetylcholine. It is now well established that lipid kinases are activated in response to a large variety of extracellular signals.

Several of the reactions of phosphoinositide metabolism are shown in Figure 15.10. As indicated on the left side of this figure, the inositol ring, which resides at the cytoplasmic surface of the bilayer, has six carbon atoms. Carbon number 1 is involved in the linkage between inositol and diacylglycerol. The 3, 4, or 5 carbons can be phosphorylated by specific phosphoinositide kinases present in cells to generate 7 distinct phosphoinositides. For example, transfer of a single phosphate group to the 4-position of the inositol sugar of PI by PI 4-kinase (PI4K) generates PI 4-phosphate (PI(4)P), which can

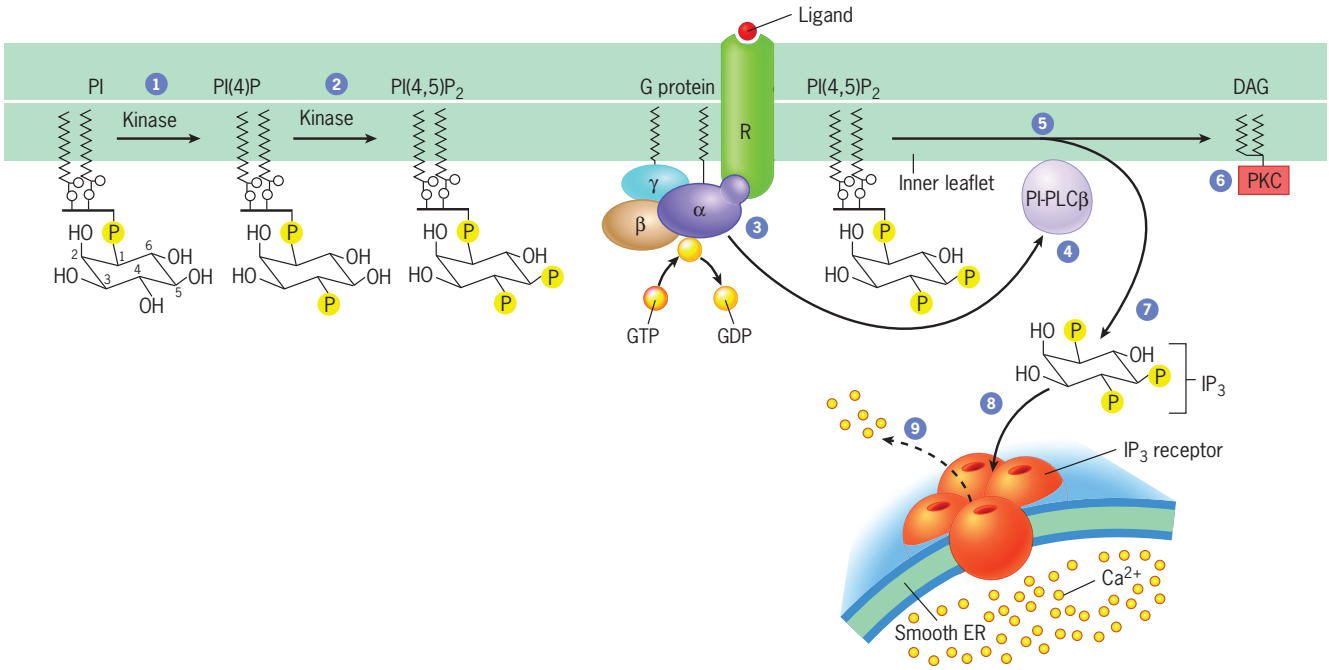

Figure 15.10 **The generation of second messengers as a result of ligand-induced breakdown of phosphoinositides (PI) in the lipid bilayer.** In steps 1 and 2, phosphate groups are added by lipid kinases to phosphatidylinositol (PI) to form PIP_2. When a stimulus is received by a receptor, the ligand-bound receptor activates a heterotrimeric G protein containing a $G_{\alpha q}$ subunit (step 3), which activates the enzyme PI-specific phospholipase C-β(step 4), which catalyzes the reaction in which PI(4, 5)P_2 is split into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (step 5). DAG recruits the protein kinase PKC to the membrane and activates the enzyme (step 6). IP_3 diffuses into the cytosol (step 7), where it binds to an IP_3 receptor and Ca^{2+} channel in the membrane of the SER (step 8). Binding of IP_3 to its receptor causes release of calcium ions into the cytosol (step 9).

be phosphorylated by PIP 5-kinase (PIP5K) to form PI 4,5-bisphosphate (PI(4,5)P_2; Figure 15.10, steps 1 and 2). PI(4,5)P_2 can be phosphorylated by PI 3-kinase (PI3K) to form PI(3,4,5)P_3 (PIP_3) (shown in Figure 15.25*c*). The phosphorylation of PI(4,5)P_2 to form PIP_3 is of particular interest because the PI3K enzymes involved in this process can be controlled by a large variety of extracellular molecules and PI3K overactivity has been associated with human cancers. The formation of PIP_3 during the response to insulin is discussed on page 645.

All of the phospholipid species discussed above remain in the cytoplasmic leaflet of the plasma membrane; they are membrane-bound second messengers. Just as there are lipid kinases to add phosphate groups to phosphoinositides, there are lipid phosphatases (e.g., PTEN) to remove them. The activity of these kinases and phosphatases are coordinated so that specific phosphoinositides appear at specific membrane compartments at specific times after a signal has been received. The role of specific phosphoinositides in membrane trafficking was discussed on page 311.

The phosphorylated inositol rings of phosphoinositides form binding sites for several lipid-binding domains (PH, PX, and FYVE) found in proteins. Best known is the **PH domain** (Figure 15.9*b*), which has been identified in over 150 different proteins. Binding of a protein by its PH domain to PI(3, 4)P_2 or PIP_3 typically recruits the protein to the cytoplasmic face of the plasma membrane where it can interact with other membrane-bound proteins, including activators, inhibitors, or substrates. Figure 15.9*c* shows an example where PIP_3 is specifically localized to a particular portion of the plasma membrane of a cell. PIP_3 is produced at the front of the cell by a localized lipid kinase and is subsequently degraded at the rear and sides of the cell by a localized lipid phosphatase. The cell shown in Figure 15.9*c* is engaged in chemotaxis, which is to say that it is moving toward an increasing concentration of a particular chemical in the medium that serves as the chemoattractant. This is the mechanism that causes phagocytic cells such as macrophages to move toward bacteria or other targets that they engulf. Chemotaxis depends on the localized production of phosphoinositide messengers, which bind to certain actin-binding proteins (page 372) to influence the formation of actin filaments and lamellipodia that are required to move the cell in the direction of the target.

Phospholipase C Not all inositol-containing second messengers remain in the lipid bilayer of a membrane. When acetylcholine binds to a smooth muscle cell, or an antigen binds to a mast cell, the bound receptor activates a heterotrimeric G protein (Figure 15.10, step 3), which, in turn, activates the effector *phosphatidylinositol-specific phospholipase*

C-β (PLCβ) (step 4). Like the protein depicted in Figure 15.9*b*, PLCβ is situated at the inner surface of the membrane (Figure 15.10), bound there by the interaction between its PH domain and a phosphoinositide embedded in the bilayer. PLCβ catalyzes a reaction that splits $PI(4,5)P_2$ into two molecules, *inositol 1,4,5-trisphosphate* (*IP_3*) and *diacylglycerol* (*DAG*) (step 5, Figure 15.10), both of which play important roles as second messengers in cell signaling. We will examine each of these second messengers separately.

Diacylglycerol Diacylglycerol (Figure 15.10) is a lipid molecule that remains in the plasma membrane following its formation by PLCβ. There it recruits and activates effector proteins that bear a DAG-binding C1 domain. The best-studied of these effectors is a family of proteins called *protein kinase C* (*PKC*) (step 6, Figure 15.10), which phosphorylate serine and threonine residues on a wide variety of target proteins.

Protein kinase C isoforms have a number of important roles in cellular growth and differentiation, cellular metabolism, cell death, and immune responses. The apparent importance of protein kinase C in growth control is seen in studies with a group of powerful plant compounds, called *phorbol esters*, that resemble DAG. These compounds activate protein kinase C isoforms in a variety of cultured cells, causing them to lose growth control and behave temporarily as malignant cells. When the phorbol ester is removed from the medium, the cells recover their normal growth properties. In contrast, cells that have been genetically engineered to constitutively express protein kinase C exhibit a permanent malignant phenotype in cell culture and can cause tumors in susceptible mice. The importance of PKC in the development of immune responses is seen in studies of a specific PKC inhibitor called AEB071, which is being tested in clinical trials as an immunosuppressant to prevent rejection of transplanted kidneys and to treat psoriasis, an autoimmune skin disease.

Inositol 1,4,5-trisphosphate (*IP_3*) Inositol 1,4,5-trisphosphate (IP_3) is a sugar phosphate—a small, water-soluble molecule capable of rapid diffusion throughout the interior of the cell. IP_3 molecules formed at the membrane diffuse into the cytosol (step 7, Figure 15.10) and bind to a specific IP_3 receptor located at the surface of the smooth endoplasmic reticulum (step 8). It was noted on page 280, that the smooth endoplasmic reticulum is a site of calcium storage in a variety of cells. The IP_3 receptor also functions as a tetrameric Ca^{2+} channel. Binding of IP_3 opens the channel, allowing Ca^{2+} ions to diffuse into the cytoplasm (step 9). Calcium ions can also be considered as intracellular or second messengers because they bind to various target molecules, triggering specific responses. In the two examples used above, contraction of a smooth muscle cell and exocytosis of histamine-containing secretory granules in a mast cell, both are triggered by elevated calcium levels. So, too, is the response of a liver cell to the hormone vasopressin (the same hormone that has antidiuretic activity in the kidney, page 151). Vasopressin binds to its receptor at the liver cell surface and causes a series of IP_3-mediated bursts of Ca^{2+} release, which appear as oscillations

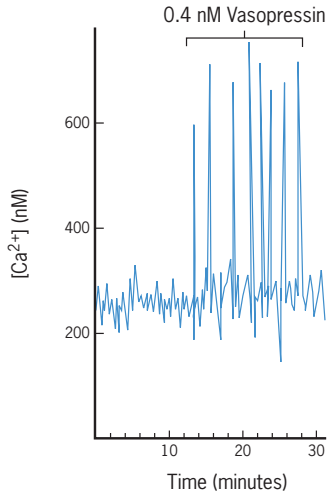

Figure 15.11 **Experimental demonstration of changes in free calcium concentration in response to hormone stimulation.** A single liver cell was injected with aequorin, a protein extracted from certain jellyfish that luminesces when it binds calcium ions. The intensity of the luminescence is proportional to the concentration of free calcium ions. Exposure of the cell to vasopressin leads to controlled spikes in the concentration of free calcium at periodic intervals. Higher concentrations of hormone do not increase the height (amplitude) of the spikes, but they do increase their frequency. (Reprinted with permission from N. M. Woods, K. S. Cuthbertson, and P. H. Cobbold, Nature 319:601, 1986; copyright 1986. Nature by Nature Publishing Group. Reproduced with permission of Nature Publishing Group in the format reuse in a book/textbook via Copyright Clearance Center.)

of free cytosolic calcium in the recording shown in Figure 15.11. The frequency and intensity of such oscillations may encode information that governs the cell's specific response. A list of some of the responses mediated by IP_3 is indicated in Table 15.2. We will have more discussion about Ca^{2+} ions in Section 15.5.

The Specificity of G Protein-Coupled Responses

A wide variety of agents, including hormones, neurotransmitters, and sensory stimuli, act by way of GPCRs and heterotrimeric G proteins to transmit information across the plasma membrane, triggering a wide variety of cellular responses. Thus as a group, GPCRs are capable of binding a diverse array of ligands. In addition, the receptor for a given ligand can exist in several different versions (isoforms). For example, researchers have identified 9 different isoforms of the adrenergic receptor, which binds epinephrine, and 15 different isoforms of the receptor for serotonin, a powerful neurotransmitter released by nerve cells in parts of the brain. Different isoforms can have different affinities for the ligand or may interact with different types of G proteins. Different isoforms of a receptor may coexist in the same plasma membrane, or they may occur in the membranes of different types of target cells. The heterotrimeric G proteins that transmit signals from receptor to effector can also exist in multiple forms, as can many of the effectors. The human genome encodes at least 16 different G_α

Table 15.2 Summary of Cellular Responses Elicited by Adding IP_3 to Either Permeabilized or Intact Cells

Cell type	Response
Vascular smooth muscle	Contraction
Stomach smooth muscle	Contraction
Slime mold	Cyclic GMP formation, actin polymerization
Blood platelets	Shape change, aggregation
Salamander rods	Modulation of light response
Xenopus oocytes	Calcium mobilization, membrane depolarization
Sea urchin eggs	Membrane depolarization, cortical reaction
Lacrimal gland	Increased potassium current

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subunits, 5 different G_β subunits, and 11 different G_γ subunits, along with 9 isoforms of the effector adenylyl cyclase. Different combinations of specific subunits construct G proteins having different capabilities of reacting with specific isoforms of both receptors and effectors.

As mentioned on page 624, some G proteins act by inhibiting their effectors. The same stimulus can activate a stimulatory G protein (one with a $G_{\alpha s}$ subunit) in one cell and an inhibitory G protein (one with a $G_{\alpha i}$ subunit) in a different cell. For example, when epinephrine binds to a β-adrenergic receptor on a cardiac muscle cell, a G protein with a $G_{\alpha s}$ subunit is activated, which stimulates cAMP production, leading to an increase in the rate and force of contraction. In contrast, when epinephrine binds to an α-adrenergic receptor on a smooth muscle cell in the intestine, a G protein with a $G_{\alpha i}$ subunit is activated, which inhibits cAMP production, producing muscle relaxation. Finally, some adrenergic receptors turn on G proteins with $G_{\alpha q}$ subunits, leading to activation of PLCβ. Clearly, the same extracellular messenger can activate a variety of pathways in different cells.

Regulation of Blood Glucose Levels

Glucose can be utilized as a source of energy by nearly all cell types present in the body. It is oxidized to CO_2 and H_2O by glycolysis and the TCA cycle, providing cells with ATP that can be used to drive energy-requiring reactions. The body maintains glucose levels in the bloodstream within a narrow range. As discussed in Chapter 3, excess glucose is stored in animal cells as glycogen, a large branched polymer composed of glucose monomers that are linked through glycosidic bonds. The hormone glucagon is produced by the alpha cells of the pancreas in response to low blood glucose levels. Glucagon stimulates breakdown of glycogen and release of glucose into the bloodstream, thereby causing glucose levels to rise. The hormone insulin is produced by the beta cells of the pancreas in response to high glucose levels and stimulates glucose uptake and storage as glycogen. Finally, epinephrine—

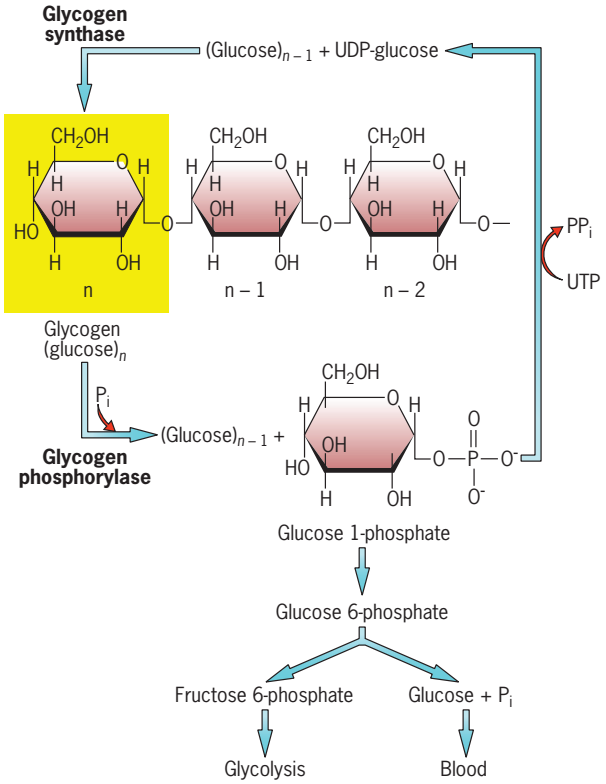


Figure 15.12 **The reactions that lead to glucose storage or mobilization.** The activities of two of the key enzymes in these reactions, glycogen phosphorylase and glycogen synthase, are controlled by hormones that act through signal transduction pathways. Glycogen phosphorylase is activated in response to glucagon and epinephrine, whereas glycogen synthase is activated in response to insulin (page 646).

which is sometimes called the "fight-or-flight" hormone—is produced by the adrenal gland in stressful situations. Epinephrine causes an increase in blood glucose levels to provide the body with the extra energy resources needed to deal with the stressful situation at hand.

Insulin acts through a receptor protein-tyrosine kinase and its signal transduction is discussed on page 644. In contrast, both glucagon and epinephrine act by binding to GPCRs. Glucagon is a small protein that is composed of 29 amino acids, whereas epinephrine is a small molecule that is derived from the amino acid tyrosine. Structurally speaking, these two molecules have nothing in common, yet both of them bind to GPCRs and stimulate the breakdown of glycogen into glucose 1-phosphate (Figure 15.12). In addition, the binding of either of these hormones leads to the inhibition of the enzyme glycogen synthase, which catalyzes the opposing reaction in which glucose units are added to growing glycogen molecules. Thus two different stimuli (glucagon and epinephrine), recognized by different receptors, induce the same response in a single target cell. The two receptors differ from one another primarily in the structure of the ligand-binding pocket on the extracellular surface of the cell, which is specific for one or the other hormone.

Following activation by their respective ligands, both receptors activate the same type of heterotrimeric G proteins that cause an increase in the levels of cAMP.

Glucose Mobilization: An Example of a Response Induced by cAMP cAMP is synthesized by adenylyl cyclase, an integral membrane protein whose catalytic domain resides at the inner surface of the plasma membrane (Figure 15.13). cAMP evokes a response that leads to glucose mobilization by initiating a chain of reactions, as illustrated in Figure 15.14. The first step in this *reaction cascade* occurs as the hormone binds to its receptor, activating a $G_{\alpha s}$ subunit, which activates an adenylyl cyclase effector. The activated enzyme catalyzes the formation of cAMP (steps 1 and 2, Figure 15.14).

Once formed, cAMP molecules diffuse into the cytoplasm where they bind to an allosteric site on a regulatory subunit of a cAMP-dependent protein kinase (*protein kinase A*, *PKA*) (step 3, Figure 15.14). In its inactive form, PKA is a heterotetramer composed of two regulatory (R) and two catalytic (C) subunits. The regulatory subunits normally inhibit the catalytic activity of the enzyme. cAMP binding causes the dissociation of the regulatory subunits, thereby releasing the active catalytic subunits of PKA. The target substrates of PKA in a liver cell include two enzymes that play a pivotal role in glucose metabolism, glycogen synthase and phosphorylase kinase (steps 4 and 5). Phosphorylation of glycogen synthase inhibits its catalytic activity and thus prevents the conversion of glucose to glycogen. In contrast, phosphorylation of phosphorylase kinase activates the enzyme to catalyze the transfer of phosphate groups to glycogen phosphorylase molecules. As discovered by Krebs and Fischer (page 115), the addition of a single phosphate group to a specific serine residue in the glycogen phosphorylase polypeptide activates this enzyme (step 6), stimulating the breakdown of glycogen (step 7). The glucose 1-phosphate formed in the reaction is converted to glucose, which diffuses into the bloodstream and so reaches the other tissues of the body (step 8).

As one might expect, a mechanism must exist to reverse the steps discussed above; otherwise the cell would remain in the activated state indefinitely. Liver cells contain phosphatases that remove the phosphate groups added by the kinases. A particular member of this family of enzymes, protein phosphatase-1, can remove phosphates from all of the phosphorylated enzymes of Figure 15.14: phosphorylase kinase, glycogen synthase, and glycogen phosphorylase. The destruction of cAMP molecules present in the cell is accomplished by the enzyme cAMP phosphodiesterase, which helps terminate the response.

Signal Amplification The binding of a single hormone molecule at the cell surface can activate a number of G proteins, each of which can activate an adenyl cyclase effector, each of which can produce a large number of cAMP messengers in a short period of time. Thus, the production of a second messenger provides a mechanism to greatly amplify the signal generated from the original message. Many of the steps in the reaction cascade illustrated in Figure 15.14 result in amplification of the signal (these steps are indicated by the blue arrows). cAMP molecules activate PKA. Each PKA catalytic subunit phosphorylates a large number of phosphorylase kinase molecules, which in turn phosphorylate an even larger number of glycogen phosphorylase molecules, which in turn can catalyze the formation of a much larger number of glucose phosphates. Thus, what begins as a barely perceptible stimulus at the cell surface is rapidly transformed into a major mobilization of glucose within the cell.

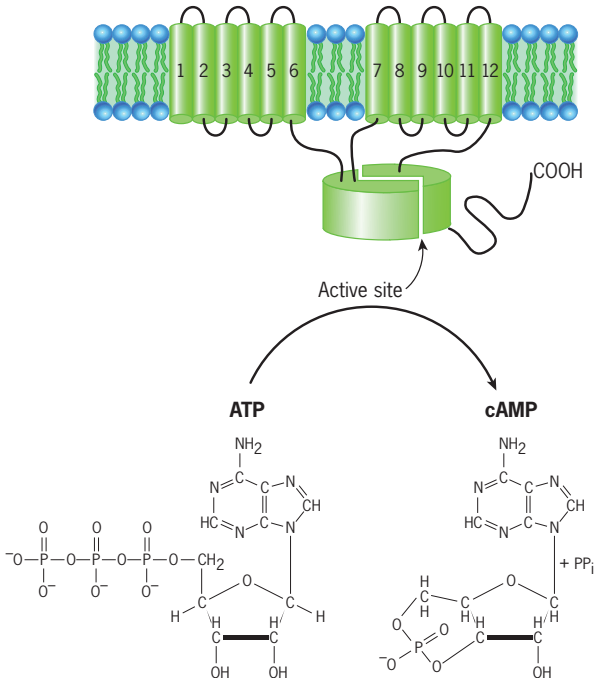


Figure 15.13 **Formation of cyclic AMP from ATP is catalyzed by adenylyl cyclase,** an integral membrane protein that consists of two parts, each containing six transmembrane helices (shown here in two dimensions). The enzyme's active site is located on the inner surface of the membrane in a cleft situated between two similar cytoplasmic domains. The breakdown of cAMP (not shown) is accomplished by a phosphodiesterase, which converts the cyclic nucleotide to a 5′ monophosphate.

Other Aspects of cAMP Signal Transduction Pathways Although the most rapid and best-studied effects of cAMP are produced in the cytoplasm, the nucleus and its genes also participate in the response. A fraction of the activated PKA molecules translocate into the nucleus where they phosphorylate key nuclear proteins (step 9, Figure 15.14), most notably a transcription factor called *CREB* (*cAMP response element-binding protein*). The phosphorylated version of CREB binds as a dimer to sites on the DNA (Figure 15.14, step 10) containing a particular nucleotide sequence (TGACGTCA), known as the *cAMP response element* (*CRE*). Recall from page 525 that response elements are sites in the DNA where transcription factors bind and increase the rate of initiation of transcription. CREs are located in the regulatory regions of genes that play a role in the response to cAMP. In liver cells,

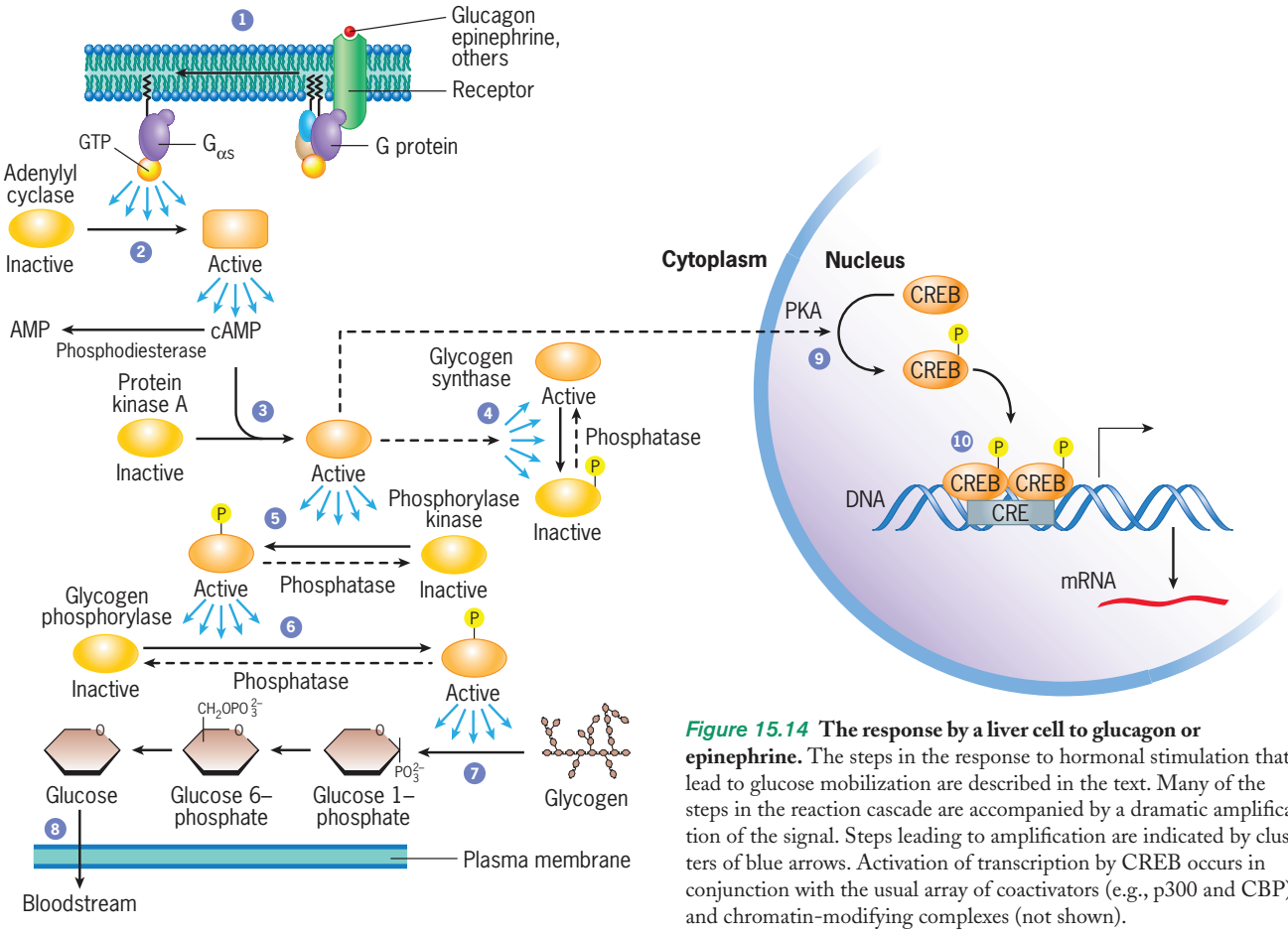


Figure 15.14 **The response by a liver cell to glucagon or epinephrine.** The steps in the response to hormonal stimulation that lead to glucose mobilization are described in the text. Many of the steps in the reaction cascade are accompanied by a dramatic amplification of the signal. Steps leading to amplification are indicated by clusters of blue arrows. Activation of transcription by CREB occurs in conjunction with the usual array of coactivators (e.g., p300 and CBP) and chromatin-modifying complexes (not shown).

for example, several of the enzymes involved in gluconeogenesis, a pathway by which glucose is formed from the intermediates of glycolysis (see Figure 3.31), are encoded by genes that contain nearby CREs. Thus, epinephrine and glucagon not only activate catabolic enzymes involved in glycogen breakdown, they promote the synthesis of anabolic enzymes required to synthesize glucose from smaller precursors.

cAMP is produced in many different cells in response to a wide variety of different ligands (i.e., first messengers). Several of the hormonal responses mediated by cAMP in mammalian cells are listed in Table 15.3. Cyclic AMP pathways have also been implicated in processes occurring in the nervous system, including learning, memory, and drug addiction. Chronic use of opiates, for example, leads to elevated levels of adenylyl cyclase and PKA, which may be partially responsible for the physiologic responses that occur during drug withdrawal. Another cyclic nucleotide, cyclic GMP, also acts as a second messenger in certain cells as illustrated by the induced

Table 15.3 ***Examples of Hormone-Induced Responses Mediated by cAMP***

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

relaxation of smooth muscle cells discussed on page 656. As discussed in the next section, cyclic GMP also plays a key role in the signaling pathway involved in vision.

Because cAMP exerts most of its effects by activating PKA, the response of a given cell to cAMP is typically determined by the specific proteins phosphorylated by this kinase (Figure 15.15). Although activation of PKA in a liver cell in response to epinephrine leads to the breakdown of glycogen, activation of the same enzyme in a kidney tubule cell in response to vasopressin causes an increase in the permeability of the membrane to water, and activation of the enzyme in a thyroid cell in response to TSH leads to the secretion of thyroid hormone. Clearly, PKA must phosphorylate different substrates in each of these cell types, thereby linking the increase in cAMP levels induced by epinephrine, vasopressin, and TSH to different physiological responses.

Over one hundred PKA substrates have been described. Most of these carry out different functions, which brings up the question as to how PKA phosphorylates the appropriate substrates in response to a particular stimulus, in a particular cell type. This question was answered in part by the observation that different cells express different PKA substrates and in part by the discovery of PKA-anchoring proteins or *AKAPs* that function as signaling hubs. The first AKAPs were discovered as proteins that co-purified with PKA. At least 50 different AKAPs have been discovered since, several of which are shown in Figure 15.16. As indicated in this figure, AKAPs provide a structural framework or *scaffold* for coordinating protein–protein interactions by sequestering PKA to specific locations within the cell. As a consequence, PKA accumulates in close proximity to one or more substrates. When cAMP levels rise and PKA is activated, the relevant substrates are present close by and they are the first ones to become phosphorylated. Substrate selection thus is partly a consequence of the subcellular localization of PKA in the presence of particular substrates. Different cells express different AKAPs, resulting in localization of PKA in the presence of different substrates and consequently phosphorylation of different substrates following an increase in cAMP levels. It is interesting to note that, unlike most proteins with a similar function, AKAPs have a diverse structure, suggesting that evolution has co-opted a variety of different types of proteins to carry out a similar role in cell signaling.

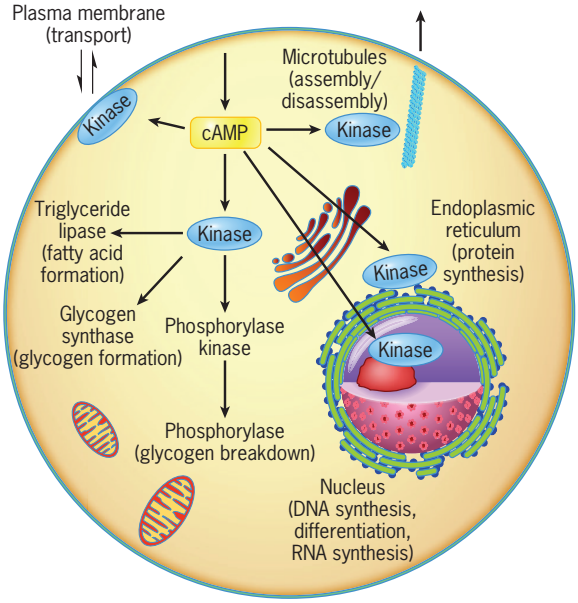


Figure 15.15 **Schematic illustration of the variety of processes that can be affected by changes in cAMP concentration.** All of these effects are thought to be mediated by activation of the same enzyme, protein kinase A. In fact, the same hormone can elicit very different responses in different cells, even when it binds to the same receptor. Epinephrine, for example, binds to a similar β-adrenergic receptor in liver cells, fat cells, and smooth muscle cells of the intestine, causing the production of cAMP in all three cell types. The responses, however, are quite different: glycogen is broken down in the liver cell, triacylglycerols are broken down in the fat cell, and the smooth muscle cells undergo relaxation. In addition to PKA, cAMP is known to interact with ion channels, phosphodiesterases, and GEFs (page 641).

The Role of GPCRs in Sensory Perception

Our ability to see, taste, and smell depends largely on GPCRs. It was mentioned above that rhodopsin, whose structure and activation is depicted in Figure 15.5*b*, is a GPCR. Rhodopsin is the light-sensitive protein present in the rods of our retina, which are the photoreceptor cells that respond to low light intensity and provide us with a black-and-white picture of our environment at night or in a darkened room. Several closely related GPCRs are present in the cones of the retina, which provide us with color vision under conditions of brighter light. Absorption of a single photon of light induces a conformational change in the rhodopsin molecule, which transmits a signal to a heterotrimeric G protein (called *transducin*), which activates a coupled effector. The effector in this case is the enzyme cGMP phosphodiesterase, which hydrolyzes the cyclic nucleotide cGMP, a second messenger similar in structure to cAMP (Figure 15.13). cGMP plays an important role in visual excitation in the rod cells of the retina. In the dark, cGMP levels remain high and thus capable of binding to cGMP-gated sodium channels in the plasma membrane, keeping the channels in an open configuration, leading to a continued inward ionic current (a "dark current"). Activation of cGMP phosphodiesterase results in lowered cGMP levels, leading to the closure of the sodium channels. This response, which is unusual in that it is triggered by a decrease in the concentration of a second messenger, may lead to the generation of action potentials along the optic nerve.

Our sense of smell depends on nerve impulses transmitted along olfactory neurons that extend from the epithelium that lines our upper nasal cavity to the olfactory bulb that is located in our brain stem. The distal tips of these neurons, which are located in the nasal epithelium, contain *odorant receptors*, which are GPCRs capable of binding various chemicals that enter our nose. Mammalian odorant receptors were first identified in 1991 by Linda Buck and Richard Axel of Columbia University. It is estimated that humans express roughly 400 different odorant receptors that, taken together, are able to combine with a large variety of different chemical

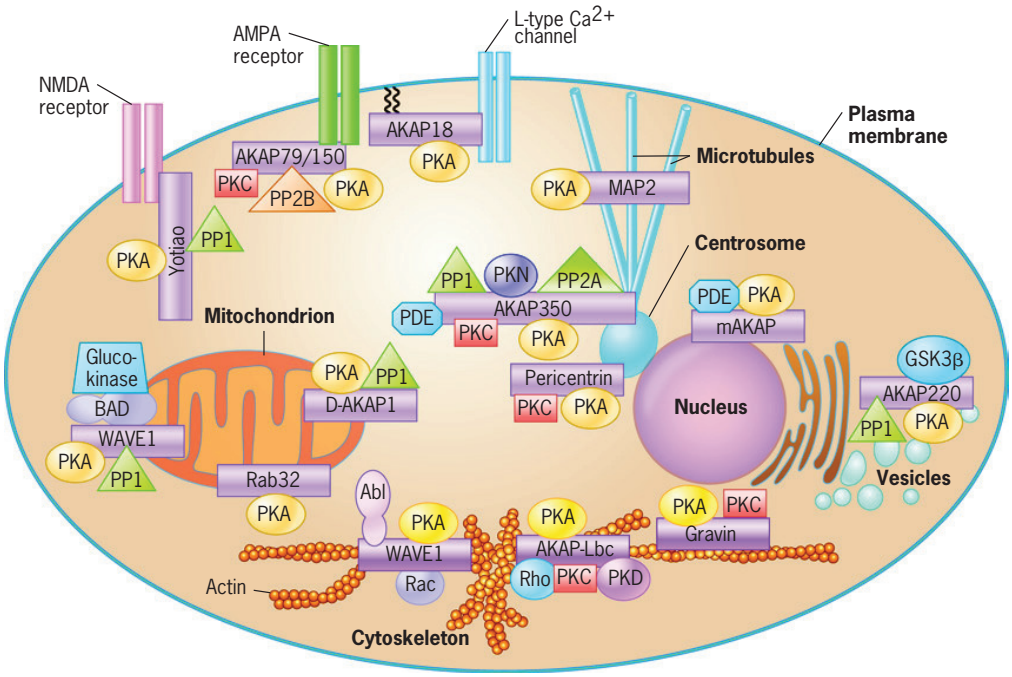


Figure 15.16 **A schematic representation of AKAP signaling complexes operating in different subcellular compartments.** The AKAP in each of these protein complexes is represented by the purple bar. In each case, the AKAP forms a scaffold that brings together a PKA molecule with potential substrates and other proteins involved in the signaling pathway, including phosphatases (green triangles) that can remove the added phosphate groups and phosphodiesterases that can terminate continued signaling. The AKAPs shown here target PKA to a number of different compartments, including the plasma membrane, mitochondrion, cytoskeleton, centrosome, and nucleus. (Reprinted with permission from W. Wong and J. D. Scott, Nature Reviews Mol Cell Biol 5:961, 2004; copyright 2004. Nature Reviews Molecular Cell Biology by Nature Publishing Group. Reproduced with permission of Nature Publishing Group in the format reuse in a book/textbook via Copyright Clearance Center.)

structures (odorants).[1] Each olfactory receptor neuron expresses only one allele of one of the hundreds of different odorant receptor genes. Consequently, each of these sensory neurons contains only one specific odorant receptor and is only capable of responding to one or a few related chemicals. As a result, activation of different neurons containing different odorant receptors provides us with the perception of different aromas. That does not mean that a single chemical cannot interact with more than one olfactory receptor, but rather that the specific combination of receptors that are activated by that compound may play a key role in producing a particular smell. Mutations in a specific gene encoding a particular odorant receptor can leave a person with the inability to detect a particular chemical in their environment that most other members of the population can perceive. When activated by bound ligands, odorant receptors signal through heterotrimeric G proteins to adenylyl cyclase, resulting in the synthesis of cAMP and the opening of a cAMP-gated cation channel. This response leads to the generation of action potentials that are transmitted to the brain.

[1]The human genome contains roughly 1000 genes that encode odorant receptors but the majority are present as nonfunctional pseudogenes (page 408). Mice, which depend more heavily than humans on their sense of smell, have more than 1000 of these genes in their genome, and 95 percent of them encode functional receptors.

Our perception of taste is much less discriminating than our perception of smell. Each taste receptor cell in the tongue transmits a sense of one of only five basic taste qualities, namely: salty, sour, sweet, bitter, or umami (from the Japanese word meaning "flavorful"). Taste receptor cells that elicit the taste of umami respond to the amino acids aspartate and glutamate and to purine nucleotides, generating a perception that a food is "savory." This is the reason that monosodium glutamate and disodium guanylate are commonly added to processed foods to enhance flavor. The pleasurable umami taste is thought to have evolved as a mechanism to drive mammals to seek high-protein foods. The perception that a food or beverage is salty or sour is elicited directly by sodium ions or protons in the food. These ions pass through the plasma membrane of receptor cells via Na^+ or H^+ channels, respectively, eventually leading to a depolarization of the cell's plasma membrane (page 166). In contrast, the perception that a food is bitter, sweet, or savory depends on a compound interacting with a GPCR at the surface of the receptor cell. Humans encode a family of about 30 bitter-taste receptors called T2Rs, which are coupled to the same heterotrimeric G protein. As a group, these taste receptors bind a diverse array of different compounds, including plant alkaloids or cyanides, that evoke a bitter taste in our mouths. For the most part, substances that evoke this perception are toxic compounds that elicit a distasteful, protective response that causes us to expel

the food matter from our mouth. Unlike olfactory cells that contain a single receptor protein, a single taste-bud cell that evokes a bitter sensation contains a variety of different T2R receptors that respond to unrelated noxious substances. As a result, many diverse substances evoke the same basic taste, which is simply that the food we have eaten is bitter and disagreeable. In contrast, a food that elicits a sweet taste is likely to be one that contains energy-rich carbohydrates. Humans possess only one high affinity sweet-taste receptor (a T1R2-T1R3 heterodimer) and it responds to sugars, certain sweet tasting peptides and proteins (e.g., monellin), and artificial sweeteners. Umami receptors consist of a TR1-TR3 heterodimer. Fortunately, food that is chewed releases odorants that travel via the throat to olfactory neurons in our nasal mucosa, allowing the brain to learn much more about the food we have eaten than the relatively simple messages provided by taste receptors. It is this merged input from both olfactory and taste (gustatory) receptors that provides us with our rich sense of taste. The importance of olfactory neurons in our perception of taste becomes more evident when we have a cold that causes us to lose some of our appreciation for the taste of food.

REVIEW

1. What is the role of G proteins in a signaling pathway?
2. Describe Sutherland's experiment that led to the concept of the second messenger.
3. What is meant by the term *amplification* in regard to signal transduction? How does the use of a reaction cascade result in amplification of a signal? How does it increase the possibilities for metabolic regulation?
4. How is it possible that the same first messenger, such as epinephrine, can evoke different responses in different target cells? That the same second messenger, such as cAMP, can also evoke different responses in different target cells? That the same response, such as glycogen breakdown, can be initiated by different stimuli?
5. Describe the steps that lead from the synthesis of cAMP at the inner surface of the plasma membrane of a liver cell to the release of glucose into the bloodstream. How is this process controlled by GRKs and arrestin? By protein phosphatases? By cAMP phosphodiesterase?
6. Describe the steps between the binding of a ligand such as glucagon to a seven transmembrane receptor and the activation of an effector, such as adenylyl cyclase. How is the response normally attenuated?
7. What is the mechanism of formation of the second messenger IP_3? What is the relationship between the formation of IP_3 and an elevation of intracellular $[Ca^{2+}]$?
8. Describe the relationship between phosphatidylinositol, diacylglycerol, calcium ions, and protein kinase C. How do phorbol esters interfere with signal pathways involving DAG?

15.4 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Protein-tyrosine kinases are enzymes that phosphorylate specific tyrosine residues on protein substrates. Protein-tyrosine phosphorylation is a mechanism for signal transduction that appeared with the evolution of multicellular organisms. Over 90 different protein-tyrosine kinases are encoded by the human genome. These kinases are involved in the regulation of growth, division, differentiation, survival, attachment to the extracellular matrix, and migration of cells. Expression of mutant protein-tyrosine kinases that cannot be regulated and are continually active can lead to uncontrolled cell division and the development of cancer. One type of leukemia, for example, occurs in cells that contain an unregulated version of the protein-tyrosine kinase ABL.

Protein-tyrosine kinases can be divided in two groups: **Receptor protein-tyrosine kinases** (**RTKs**), which are integral membrane proteins that contain a single transmembrane helix and an extracellular ligand binding domain, and **non-receptor** (or *cytoplasmic*) **protein-tyrosine kinases**. The human genome encodes nearly 60 RTKs and 32 non-receptor TKs. The first RTK to be studied, EGFR, was identified in 1978 by Stanley Cohen of Vanderbilt University. The discovery of the first non-receptor TK is discussed on page 696. RTKs are activated directly by extracellular growth and differentiation factors such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) or by metabolic regulators such as insulin. Non-receptor protein-tyrosine kinases are regulated indirectly by extracellular signals and they control processes as diverse as the immune response, cell adhesion, and neuronal cell migration. This section of the chapter is focused on signal transduction by RTKs.

Receptor Dimerization An obvious question comes to mind when considering the mechanics of signal transduction: How is the presence of a growth factor on the outside of the cell translated into biochemical changes inside the cell? Although structural biologists have yet to solve the three-dimensional structure of an entire RTK, it is widely accepted on the basis of other techniques that ligand binding results in the dimerization of the extracellular ligand-binding domains of a pair of receptors. Two mechanisms for receptor dimerization have been recognized: ligand-mediated dimerization and receptor-mediated dimerization (Figure 15.17). Early work suggested that ligands of RTKs contain two receptor-binding sites. This made it possible for a single growth or differentiation factor molecule to bind to two receptors at the same time, thereby causing ligand-mediated receptor dimerization (Figure 15.17*a*). This model was supported by the observation that growth and differentiation factors such as platelet-derived growth factor (PDGF) or colony-stimulating factor-1 (CSF-1) are composed of two similar or identical disulfide-linked subunits, in which each subunit contains a receptor-binding site. However, not all growth factors were found to conform to this model. Some growth factors (e.g., EGF or

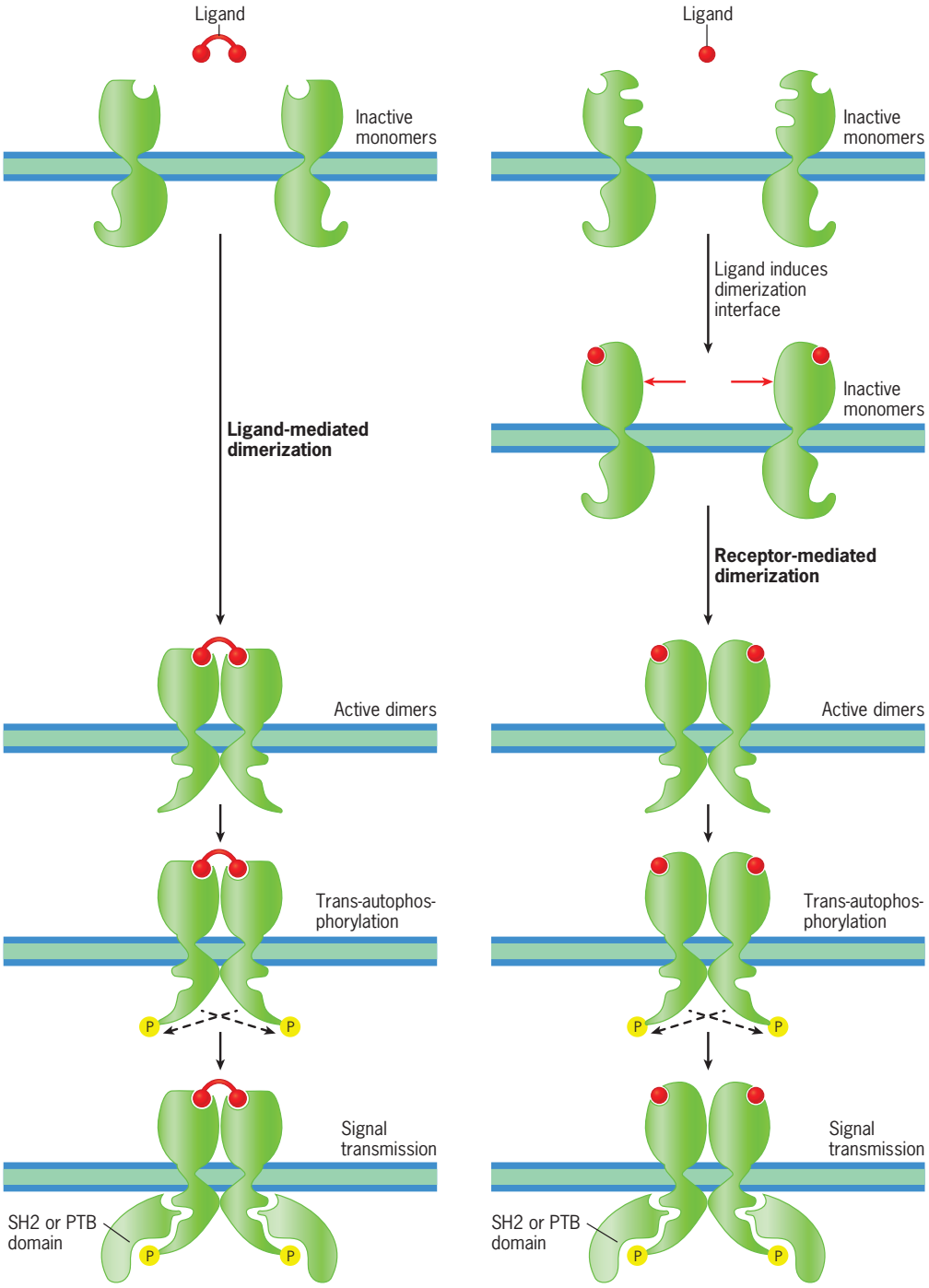


Figure 15.17 **Steps in the activation of a receptor protein-tyrosine kinase (RTK).** (*a*) Ligand-mediated dimerization. In the nonactivated state, the receptors are present in the membrane as monomers. Binding of a bivalent ligand leads directly to dimerization of the receptor and activation of its kinase activity, causing it to add phosphate groups to the cytoplasmic domain of the other receptor subunit. The newly formed phosphotyrosine residues of the receptor serve as binding sites for target proteins containing either SH2 or PTB domains. The target proteins become activated as a result of their interaction with the receptor. (*b*) Receptor-mediated dimerization. The sequence of events are similar to those in part *a*, except that the ligand is monovalent and, consequently, a separate ligand molecule binds to each of the inactive monomers. Binding of each ligand induces a conformational change in the receptor that creates a dimerization interface (red arrows). The ligand-bound monomers interact through this interface to become an active dimer. (Based on a drawing by J. Schlessinger and A. Ullrich, Neuron 9:384, 1992; by permission of Cell Press. Neuron by Cell Press. Reproduced with permission of Cell Press in the format reuse in a book/textbook via Copyright Clearance Center.)

TGFα) contain only a single receptor-binding site. Structural work now supports a second mechanism (Figure 15.17*b*) in which ligand binding induces a conformational change in the extracellular domain of a receptor, leading to the formation or exposure of a receptor dimerization interface. With this mechanism, ligands act as allosteric regulators that turn on the ability of their receptors to form dimers. A small number of RTKs, including the insulin and IGF-1 receptors, are present as inactive dimers in the absence of ligand (see Figure 15.24). For most RTKs, receptor dimerization results in the juxtapositioning of two protein-tyrosine kinase domains on the cytoplasmic side of the plasma membrane. Bringing two kinase domains in close contact allows for *trans-autophosphorylation*, in which the protein kinase activity of one receptor of the dimer phosphorylates tyrosine residues in the cytoplasmic domain of the other receptor of the dimer, and vice versa (Figure 15.17*a*,*b*).

Protein Kinase Activation Autophosphorylation sites on RTKs can carry out two different functions: they can regulate the receptor's kinase activity or serve as binding sites for cytoplasmic signaling molecules. Kinase activity is usually controlled by autophosphorylation on tyrosine residues that are present in the *activation loop* of the kinase domain. The activation loop, when unphosphorylated, obstructs the substrate-binding site, thereby preventing ATP from entering. Following its phosphorylation, the activation loop is stabilized in a position away from the substrate-binding site, resulting in activation of the kinase domain. Once their kinase domain has been activated, the receptor subunits proceed to phosphorylate each other on tyrosine residues that are present in regions adjacent to the kinase domain. It is these autophosphorylation sites that act as binding sites for cellular signaling proteins.

Phosphotyrosine-Dependent Protein–Protein Interactions Signaling pathways consist of a chain of signaling proteins that interact with one another in a sequential manner (see Figure 15.3). Signaling proteins are able to associate with activated protein-tyrosine kinase receptors, because such proteins contain domains that bind specifically to phosphorylated tyrosine residues (as in Figure 15.17). The best-studied pTyr-binding domains are the *Src-homology 2* (*SH2*) *domain* and the *phosphotyrosine-binding* (*PTB*) *domain*. SH2 domains were initially identified as part of proteins encoded by the genome of tumor-causing (oncogenic) viruses. They are composed of approximately 100 amino acids and contain a conserved binding-pocket that accommodates a phosphorylated tyrosine residue (Figure 15.18). More than 110 SH2 domains are encoded by the human genome. They mediate a large number of phosphorylation-dependent protein–protein interactions. These interactions occur following phosphorylation of specific tyrosine residues. The specificity of the interactions is determined by the amino acid sequence immediately adjacent to the phosphorylated tyrosine residues. For example, the SH2 domain of the Src protein-tyrosine kinase recognizes pTyr-Glu-Glu-Ile, whereas the SH2 domains of PI 3-kinase bind to pTyr-Met-X-Met (in which X can be any residue). It is interesting to note that the budding-yeast genome encodes only one SH2-domain-containing protein, which correlates with the overall lack of tyrosine-kinase signaling activity in these lower single-celled eukaryotes.

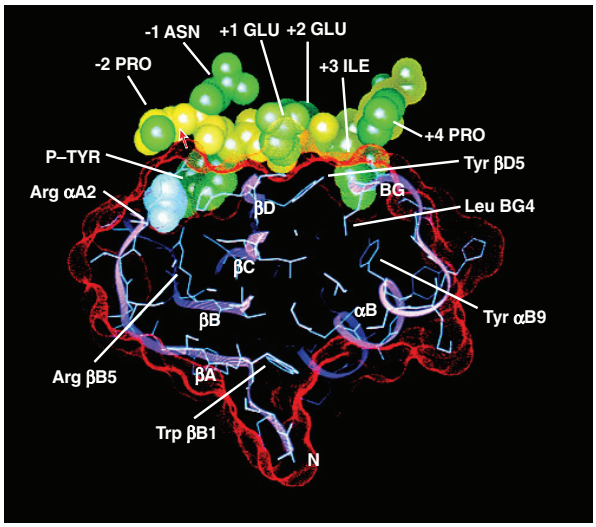


Figure 15.18 **The interaction between an SH2 domain of a protein and a peptide containing a phosphotyrosine residue.** The SH2 domain of the protein is shown in a cutaway view with the accessible surface area represented by red dots and the polypeptide backbone as a purple ribbon. The phosphotyrosine-containing heptapeptide (Pro-Asn-pTyr-Glu-Glu-Ile-Pro) is shown as a space-filling model whose side chains are colored green and backbone is colored yellow. The phosphate group is shown in light blue. The phosphorylated tyrosine residue and the isoleucine residue (+3) are seen to project into pockets on the surface of the SH2 domain, creating a tightly fitting interaction, but only when the key tyrosine residue is phosphorylated. (From Gabriel Waksman et al., courtesy of John Kuriyan, Cell 72:783, 1993. Reprinted with permission from Elsevier.)

PTB domains were discovered more recently. They can bind to phosphorylated tyrosine residues that are usually present as part of an asparagine-proline-X-tyrosine (Asn-Pro-X-Tyr) motif. The story is more complicated, however, because some PTB domains appear to bind specifically to an unphosphorylated Asn-Pro-X-Tyr motif, whereas others bind specifically to the phosphorylated motif. PTB domains are poorly conserved and different PTB domains possess different residues that interact with their ligands.

Activation of Downstream Signaling Pathways We have seen that receptor protein-tyrosine kinases (RTKs) are autophosphorylated on one or more tyrosine residues. A variety of signaling proteins with SH2 or PTB domains are present in the cytoplasm. Receptor activation therefore results in formation of signaling complexes, in which SH2- or PTB-containing signaling proteins bind to specific autophosphorylation sites present on the receptor (as in Figure 15.17). We can distinguish several groups of signaling proteins that can interact with activated RTKs, including adaptor proteins, docking proteins, transcription factors, and enzymes (Figure 15.19).

- Adaptor proteins function as linkers that enable two or more signaling proteins to become joined together as part of a signaling complex (Figure 15.19*a*). Adaptor proteins

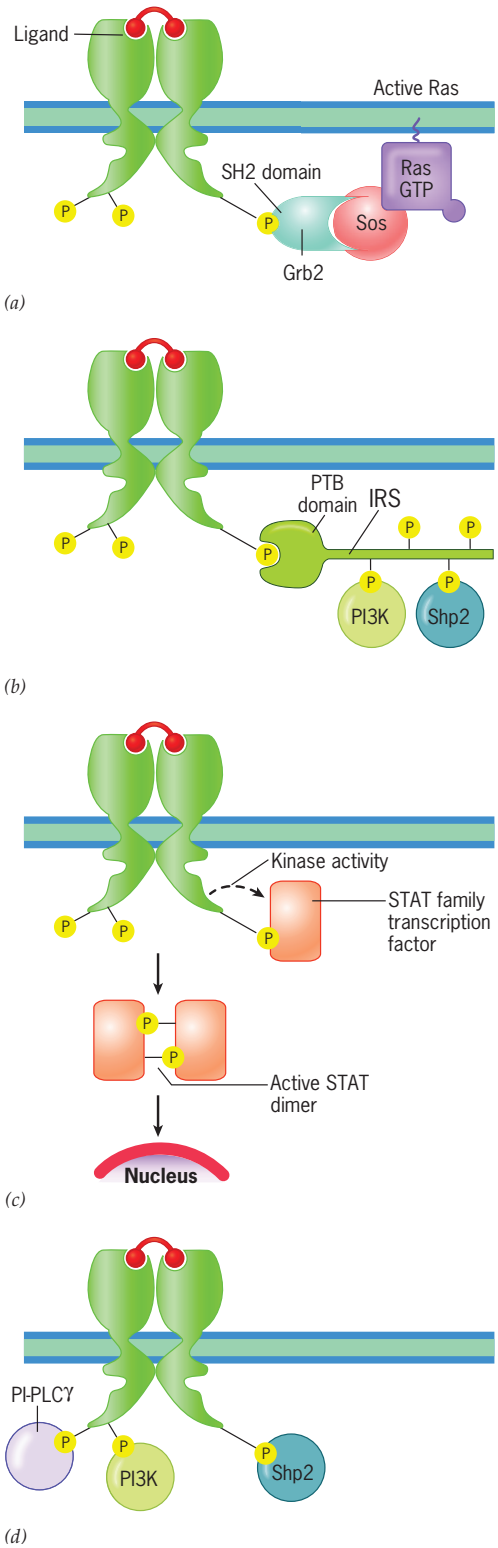


Figure 15.19 **A diversity of signaling proteins.** Cells contain numerous proteins with SH2 or PTB domains that bind to phosphorylated tyrosine residues. (*a*) Adaptor proteins, such as Grb2, function as a link between other proteins. As shown here, Grb2 can serve as a link between an activated growth factor RTK and Sos, an activator of a downstream protein named Ras. The function of Ras is discussed later. (*b*) The docking protein IRS contains a PTB domain that allows it to bind to the activated receptor. Once bound, tyrosine residues on the docking protein are phosphorylated by the receptor. These phosphorylated residues function as binding sites for other signaling proteins. (*c*) Certain transcription factors bind to activated RTKs, an event that leads to the phosphorylation and activation of the transcription factor and its translocation to the nucleus. Members of the STAT family of transcription factors become activated in this manner. (*d*) A wide array of signaling enzymes are activated following binding to an activated RTK. In the case depicted here, a phospholipase (PLC-γ), a lipid kinase (PI3K), and a protein-tyrosine phosphatase (Shp2) have all bound to phosphotyrosine sites on the receptor.

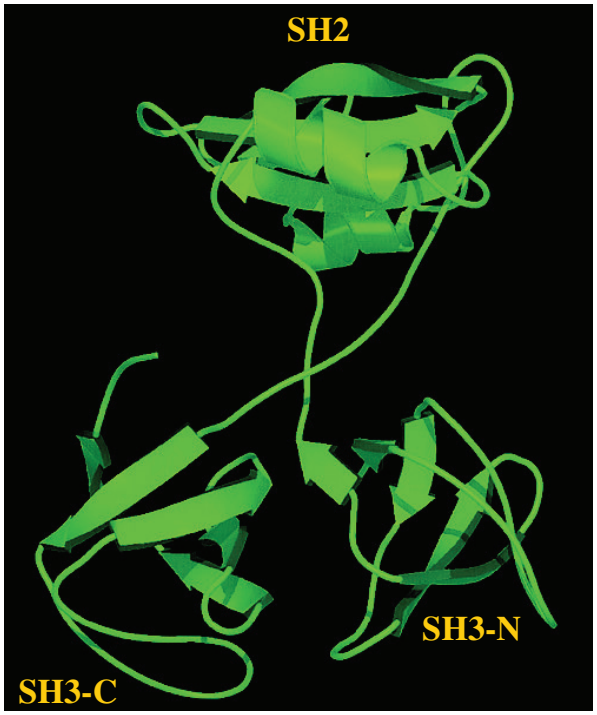


Figure 15.20 **Tertiary structure of an adaptor protein, Grb2.** Grb2 consists of three parts: two SH3 domains and one SH2 domain. SH2 domains bind to a protein (e.g., the activated EGF receptor) containing a particular motif that includes a phosphotyrosine residue. SH3 domains bind to a protein (e.g., Sos) that contains a particular motif that is rich in proline residues. Dozens of proteins that bear these domains have been identified. Interactions involving SH3 and SH2 domains are shown in Figures 2.40 and 15.18, respectively. Other adaptor proteins include Nck, Shc, and Crk. (From Sébastien Maignan et al. Science 268:291, 1995. Reprinted with permission from AAAS. Image provided courtesy of Arnaud Ducruix.)

contain an SH2 domain and one or more additional protein–protein interaction domains. For instance, the adaptor protein Grb2 contains one SH2 and two SH3 (Src-homology 3) domains (Figure 15.20). As shown on page 62, SH3 domains bind to proline-rich sequence motifs. The SH3 domains of Grb2 bind constitutively to other proteins, including Sos and Gab. The SH2 domain

binds to phosphorylated tyrosine residues within a Tyr-X-Asn motif. Consequently, tyrosine phosphorylation of the Tyr-X-Asn motif on an RTK results in translocation of Grb2-Sos or Grb2-Gab from the cytosol to a receptor, which is present at the plasma membrane (Figure 15.19*a*).

- Docking proteins, such as IRS, supply certain receptors with additional tyrosine phosphorylation sites (Figure 15.19*b*). Docking proteins contain either a PTB domain or an SH2 domain and a number of tyrosine phosphorylation sites. Binding of an extracellular ligand to a receptor leads to autophosphorylation of the receptor, which provides a binding site for the PTB or SH2 domain of the docking protein. Once bound together, the receptor phosphorylates tyrosine residues present on the docking protein. These phosphorylation sites then act as binding sites for additional signaling molecules. Docking proteins provide versatility to the signaling process, because the ability of the receptor to turn on signaling molecules can vary with the docking proteins that are expressed in a particular cell.
- Transcription factors were discussed at length in Chapter 12. Transcription factors that belong to the STAT family play an important role in the function of the immune system. STATs contain an SH2 domain together with a tyrosine phosphorylation site that can act as a binding site for the SH2 domain of another STAT molecule (Figure 15.19*c*). Tyrosine phosphorylation of STAT SH2 binding sites situated within a dimerized receptor leads to the recruitment of STAT proteins (Figure 15.19*c*). Upon association with the receptor complex, tyrosine residues in these STAT proteins are phosphorylated. As a result of the interaction between the phosphorylated tyrosine residue on one STAT protein and the SH2 domain on a second STAT protein, and vice versa, these transcription factors will form dimers. Dimers, but not monomers, move to the nucleus where they stimulate the transcription of specific genes involved in an immune response. The role of STATs in signaling an immune response is discussed in Section 17.4.
- Signaling enzymes include protein kinases, protein phosphatases, lipid kinases, phospholipases, and GTPase activating proteins. When equipped with SH2 domains, these enzymes associate with activated RTKs and are turned on directly or indirectly as a consequence of this association (Figure 15.19*d*). Three general mechanisms have been identified by which these enzymes are activated following their association with a receptor. Enzymes can be activated simply as a result of translocation to the membrane, which places them in close proximity to their substrates. Enzymes can also be activated through an allosteric mechanism (page 115), in which binding to phosphotyrosine results in a conformational change in the SH2 domain that causes a conformational change in the catalytic domain, resulting in a change in catalytic activity. Finally, enzymes can be regulated directly by phosphorylation. As will be described below, signaling proteins that associate with activated RTKs initiate cascades of events that lead to the biochemical changes required to respond to the presence of extracellular messenger molecules.

Ending the Response Signal transduction by RTKs is usually terminated by internalization of the receptor. Exactly what causes receptor internalization remains an area of active research. One mechanism involves a receptor-binding protein named Cbl. When RTKs are activated by ligands, they autophosphorylate tyrosine residues, which can act as a binding site for Cbl, which possesses an SH2 domain. Cbl then associates with the receptor and catalyzes the attachment of a ubiquitin molecule to the receptor. Ubiquitin is a small protein that is linked covalently to other proteins, thereby marking those proteins for internalization (page 312) or degradation (page 542). Binding of the Cbl complex to activated receptors is followed by receptor ubiquitination and internalization. As in the case of GPCRs (Figure 15.7), internalized RTKs can have several alternate fates; they can be degraded in lysosomes, returned to the plasma membrane, or become part of endosomal signaling complexes and engage in continued intracellular signaling.

Now that we have discussed some of the general mechanisms by which RTKs are able to activate signaling pathways, we can look more closely at a couple of important pathways that are activated downstream of RTKs. First we will discuss the Ras-MAP kinase pathway, which is probably the best characterized signaling cascade that is turned on by activated protein-tyrosine kinases. A different cascade will be described in the context of the insulin receptor.

The Ras-MAP Kinase Pathway

Retroviruses are small viruses that carry their genetic information in the form of RNA. Some of these viruses contain genes, called oncogenes, that enable them to transform normal cells into tumor cells. Ras was originally described as the product of a retroviral oncogene and, only later, determined to be derived from its mammalian host. It was subsequently discovered that approximately 30 percent of all human cancers contain mutant versions of *RAS* genes. At this point it is important to note that Ras proteins are part of a superfamily of more than 150 small (monomeric) G proteins including the Rabs (page 302), Sar1 (page 296), and Ran (page 492). These proteins are involved in the regulation of numerous processes, including cell division, differentiation, gene expression, cytoskeletal organization, vesicle trafficking, and nucleocytoplasmic transport. The principles discussed in connection with Ras apply to many members of the small G-protein superfamily.

Ras is a small GTPase that is anchored at the inner surface of the plasma membrane by a covalently attached lipid group that is embedded in the inner leaflet of the bilayer (Figure 15.19*a*). Ras is functionally similar to the heterotrimeric G proteins that were discussed earlier and, like those proteins, Ras also acts as both a switch and a molecular timer. Unlike heterotrimeric G proteins, however, Ras consists of only a single small subunit. Ras proteins are present in two different forms: an active GTP-bound form and an inactive GDP-bound form (Figure 15.21*a*). Ras-GTP binds and activates downstream signaling proteins. Ras is turned off by hydrolysis of its bound GTP to GDP. Mutations in one of the human *RAS* genes that lead to tumor formation prevent the protein

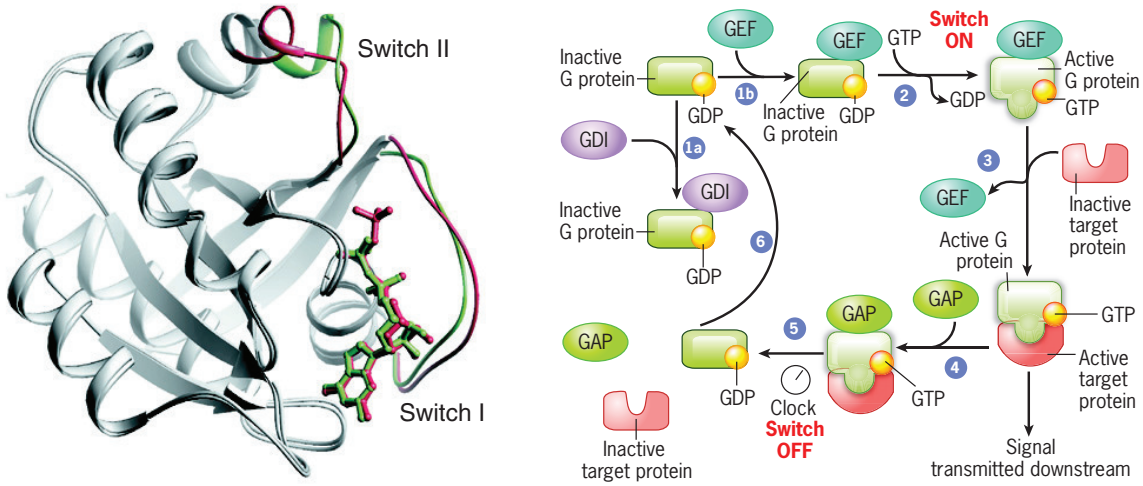


Figure 15.21 **The structure of a G protein and the G protein cycle.** (*a*) Comparison of the tertiary structure of the active GTP-bound state (red) and inactive GDP-bound state (green) of the small G protein Ras. A bound guanine nucleotide is depicted in the ball-and-stick form. The differences in conformation occur in two flexible regions of the molecule known as switch I and switch II. The difference in conformation shown here affects the molecule's ability to bind to other proteins. (*b*) The G protein cycle. G proteins are in their inactive state when they are bound by a molecule of GDP. If the inactive G protein interacts with a guanine nucleotide dissociation inhibitor (GDI), release of the GDP is inhibited and the protein remains in the inactive state (step 1a). If the inactive G protein interacts with a guanine nucleotide exchange factor (GEF; step 1b), the G protein exchanges its GDP for a GTP (step 2), which activates the G protein so that it can bind to a downstream target protein (step 3). Binding to the GTP-bound G protein activates the target protein, which is typically an enzyme such as a protein kinase or a protein phosphatase. This has the effect of transmitting the signal farther downstream along the signaling pathway. G proteins have a weak intrinsic GTPase activity that is stimulated by interaction with a GTPase-activating protein (GAP) (step 4). The degree of GTPase stimulation by a GAP determines the length of time that the G protein is active. Consequently, the GAP serves as a type of clock that regulates the duration of the response (step 5). Once the GTP has been hydrolyzed, the complex dissociates, and the inactive G protein is ready to begin a new cycle (step 6). (A: From Steven J. Gamblin and Stephen J. Smerdon, Struct. 7:R200, 1999. Reprinted with permission from Elsevier.)

from hydrolyzing the bound GTP back to the GDP form. As a result, the mutant version of Ras remains in the "on" position, sending a continuous message downstream along the signaling pathway, keeping the cell in the proliferative mode.

The cycling of monomeric G proteins, such as Ras, between active and inactive states is aided by accessory proteins that bind to the G protein and regulate its activity (Figure 15.21*b*). These accessory proteins include

1. ***GTPase-activating proteins* (*GAPs*).** Most monomeric G proteins possess some capability to hydrolyze a bound GTP, but this capability is greatly accelerated by interaction with specific GAPs. Because they stimulate hydrolysis of the bound GTP, which inactivates the G protein, GAPs dramatically shorten the duration of a G protein-mediated response. Mutations in one of the Ras-GAP genes (*NF1*) cause neurofibromatosis 1, a disease in which patients develop large numbers of benign tumors (neurofibromas) along the sheaths that line the nerve trunks.
2. ***Guanine nucleotide-exchange factors* (*GEFs*).** An inactive G protein is converted to the active form when the bound GDP is replaced with a GTP. GEFs are proteins that bind to an inactive monomeric G protein and stimulate dissociation of the bound GDP. Once the GDP is released, the G protein rapidly binds a GTP, which is present at relatively high concentration in the cell, thereby activating the G protein.
3. ***Guanine nucleotide-dissociation inhibitors* (*GDIs*).** GDIs are proteins that inhibit the release of a bound GDP from a monomeric G protein, thus maintaining the protein in the inactive, GDP-bound state.

The activity and localization of these various accessory proteins are tightly regulated by other proteins, which thus regulate the state of the G protein.

Ras-GTP can be thought of as a signaling hub because it can interact directly with several downstream targets. Here we will discuss Ras as an element of the **Ras-MAP kinase cascade**. The Ras-MAP kinase cascade is turned on in response to a wide variety of extracellular signals and plays a key role in regulating vital activities such as cell proliferation and differentiation. The pathway relays extracellular signals from the plasma membrane through the cytoplasm and into the nucleus. The overall outline of the pathway is depicted in Figure 15.22. This pathway is activated when a growth factor, such as EGF or PDGF, binds to the extracellular domain of its RTK. Many activated RTKs possess phosphorylated tyrosine residues that act as docking sites for the adaptor protein Grb2.

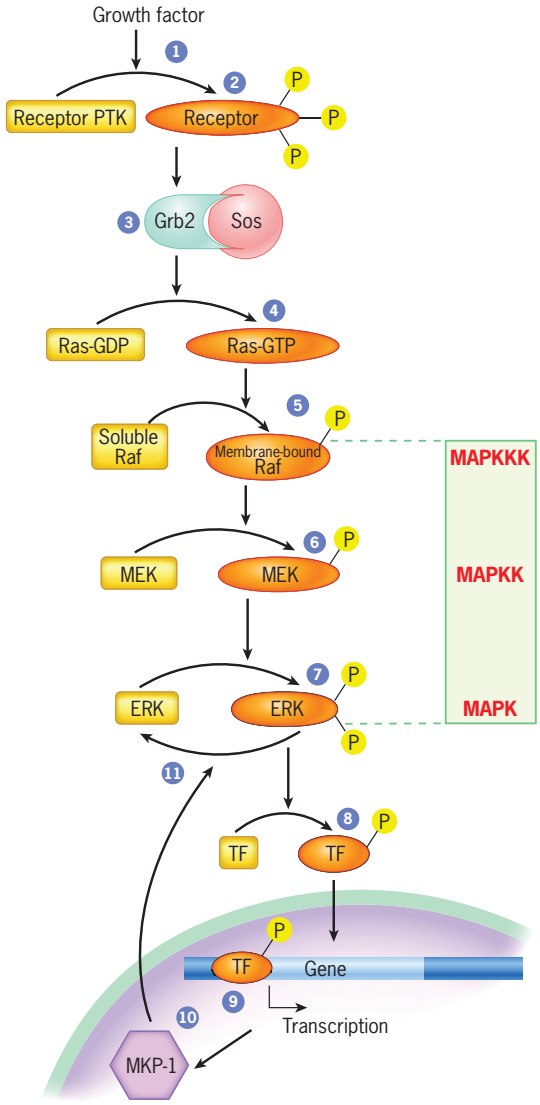


Figure 15.22 **The steps of a generalized MAP kinase cascade.** Binding of growth factor to its receptor (step 1) leads to the autophosphorylation of tyrosine residues of the receptor (step 2) and the subsequent recruitment of the Grb2-Sos proteins (step 3). This complex causes the GTP-GDP exchange of Ras (step 4), which recruits the protein Raf to the membrane, where it is phosphorylated and thus activated (step 5). In the pathway depicted here, Raf phosphorylates and activates another kinase named MEK (step 6), which in turn phosphorylates and activates still another kinase termed ERK (step 7). This three-step phosphorylation scheme shown in steps 5–7 is characteristic of all MAP kinase cascades. Because of their sequential kinase activity, Raf is known as a MAPKKK (MAP kinase kinase kinase), MEK as a MAPKK (MAP kinase kinase), and ERK as a MAPK (MAP kinase). MAPKKs are dual-specificity kinases, a term denoting that they can phosphorylate tyrosine as well as serine and threonine residues. All MAPKs have a tripeptide near their catalytic site with the sequence Thr-X-Tyr. MAPKK phosphorylates MAPK on both the threonine and tyrosine residue of this sequence, thereby activating the enzyme (step 7). Once activated, MAPK translocates into the nucleus where it phosphorylates transcription factors (TF, step 8), such as Elk-1. Phosphorylation of the transcription factors increases their affinity for regulatory sites on the DNA (step 9), leading to an increase in the transcription of specific genes (e.g., *Fos* and *Jun*) involved in the growth response. One of the genes whose expression is stimulated encodes a MAPK phosphatase (MKP-1; step 10). Members of the MKP family can remove phosphate groups from both tyrosine and threonine residues of MAPK (step 11), which inactivates MAPK and stops further signaling activity along the pathway. (H. Sun and N. K. Tonks, Trends Biochem Science 19:484, 1994. Trends in Biochemical Sciences by International Union of Biochemistry Reproduced with permission of Elsevier Ltd. in the format reuse in a book/textbook via Copyright Clearance Center.)

Grb2, in turn, binds to Sos, which is a guanine nucleotide exchange factor (a GEF) for Ras. Creation of a Grb2-binding site on an activated receptor promotes the translocation of Grb2-Sos from the cytoplasm to the cytoplasmic surface of the plasma membrane, placing Sos in close proximity to Ras (as in Figure 15.19*a*).

Simply bringing Sos to the plasma membrane is sufficient to cause Ras activation. This was illustrated by an experiment with a mutant version of Sos that is permanently tethered to the inner surface of the plasma membrane. Expression of this membrane-bound Sos mutant results in constitutive activation of Ras and transformation of the cell to a malignant phenotype. Interaction with Sos opens the Ras nucleotide-binding site. As a result, GDP is released and is replaced by GTP. Exchange of GDP for GTP in the nucleotide-binding site of Ras results in a conformational change and the creation of a binding interface for a number of proteins, including an important signaling protein called Raf. Raf is then recruited to the inner surface of the plasma membrane where it is activated by a combination of phosphorylation and dephosphorylation reactions.

Raf is a serine-threonine protein kinase. One of its substrates is the protein kinase MEK (Figure 15.22). MEK, which is activated as a consequence of phosphorylation by Raf, goes on to phosphorylate and activate two MAP kinases named ERK1 and ERK2. Over 160 proteins that can be phosphorylated by these kinases have been identified, including transcription factors, protein kinases, cytoskeletal proteins, apoptotic regulators, receptors, and other signaling proteins. Once activated, the MAP kinase is able to move into the nucleus where it phosphorylates and activates a number of transcription factors and other nuclear proteins. Eventually, the pathway leads to the activation of genes involved in cell proliferation, including cyclin D1, which plays a key role in driving a cell from G1 into S phase (Figure 14.8).

As discussed in the following chapter, oncogenes are identified by their ability to cause cells to become cancerous. Oncogenes are derived from normal cellular genes that have either become mutated or are overexpressed. Many of the proteins that play a part in the Ras signaling pathway were discovered because they were encoded by cancer-causing oncogenes. This includes the genes for Ras, Raf, and a number of the transcriptional factors generated at the end of the pathway (e.g., Fos and Jun). Genes for several of the RTKs situated at the beginning of the pathway, including the receptors for both EGF and PDGF, have also been identified

among the many known oncogenes. The fact that so many proteins in this pathway are encoded by genes that can cause cancer when mutated emphasizes the importance of the pathway in the control of cell growth and proliferation.

Adapting the MAP Kinase to Transmit Different Types of Information The same basic pathway from RTKs through Ras to the activation of transcription factors, as illustrated in Figure 15.22, is found in all eukaryotes investigated, from yeast through flies and nematodes to mammals. Evolution has adapted the pathway to meet many different ends. In yeast, for example, the MAP kinase cascade is required for cells to respond to mating factors; in fruit flies, the pathway is utilized during the differentiation of the photoreceptors in the compound eye; and in flowering plants, the pathway transmits signals that initiate a defense against pathogens. In each case, the core of the pathway contains a trio of enzymes that act sequentially: a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase (MAPK) (Figure 15.22). Each of these components is represented in a particular organism by a small family of proteins. To date, 14 different MAPKKKs, 7 different MAPKKs, and 13 different MAPKs have been identified in mammals. By utilizing different members of these protein families, mammals are able to assemble a number of different MAP kinase pathways that transmit different types of extracellular signals. We have already described how mitogenic stimuli are transmitted along one type of MAP kinase pathway that leads to cell proliferation. In contrast, when cells are exposed to stressful stimuli, such as X-rays or damaging chemicals, signals are transmitted along different MAP kinase pathways that cause the cell to withdraw from the cell cycle, rather than progressing through it as indicated in Figure 15.22. Withdrawal from the cell cycle gives the cell time to repair the damage resulting from the adverse conditions.

Recent studies have focused on the signaling specificity of MAP kinase cascades in an attempt to understand how cells are able to utilize similar proteins as components of pathways that elicit different cellular responses. Studies of amino acid sequences and protein structures suggest that part of the answer lies in selective interactions between enzymes and substrates. For example, certain members of the MAPKKK family phosphorylate specific members of the MAPKK family, which in turn phosphorylate specific members of the MAPK family. But many members of these families can participate in more than one MAPK signaling pathway.

Specificity in MAP kinase pathways is also achieved by spatial localization of the component proteins. Spatial localization is accomplished by structural (i.e., nonenzymatic) proteins referred to as *scaffolding proteins*, whose apparent function is to tether the appropriate members of a signaling pathway in a specific spatial orientation that enhances their mutual interactions. The AKAPs depicted in Figure 15.16 are examples of scaffolding proteins involved in cAMP-driven pathways. Another group of scaffolding proteins, such as the yeast proteins shown in Figure 15.23*a, b,* play a role in routing signals through one of various MAP kinase pathways. In some cases, scaffolding proteins can take an active role in signaling

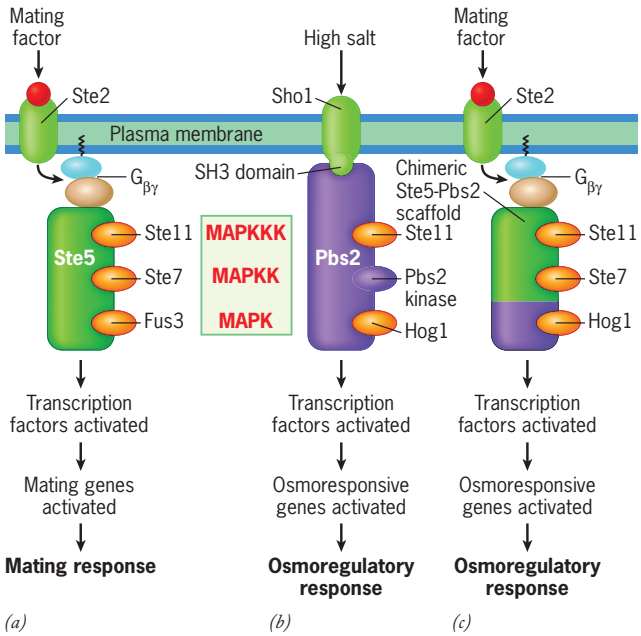


Figure 15.23 **The roles of scaffolding proteins in mediating two yeast MAPK pathways.** *(a)* The MAPK pathway that regulates mating in these cells is elicited by a mating factor that binds to a GPCR, Ste2, leading to the activation of a $G_{\beta\gamma}$ which binds to the scaffolding protein Ste5, which in turn binds the MAPKKK, MAPKK, and MAPK proteins of the pathway, *(b)* The MAPK pathway that regulates the yeast osmoregulatory response in cells exposed to high salt. The activated receptor (Sho1) binds to the Pbs2 scaffolding protein by its SH3 domain. The MAPKKK Ste11 is shared in these two pathways but is recruited into one or the other response by virtue of its interaction with the appropriate protein scaffold. The scaffold Pbs2 does not recruit a separate MAPKK, but has its own MAPKK enzymatic activity. *(c)* When cells are genetically engineered to express a chimeric Ste5-Pbs2 scaffold, they respond to a mating factor by exhibiting the osmoregulatory response. (See *Science* 332:680, 2011, for a discussion of scaffold proteins and this experiment.)

events. For example, they can induce a change in conformation of bound signaling proteins, leading to their activation or inhibition. A few scaffolding proteins are known to have an enzymatic role, as illustrated by the MAPKK activity of the yeast Pbs2 scaffold shown in Figure 15.23*b*. In addition to facilitating a particular series of reactions, scaffolding proteins may prevent proteins involved in one signaling pathway from participating in other pathways. As a result, several pathways can share the same limited set of signaling proteins without compromising specificity. This is the case for the yeast MAPKKK protein Ste11 shown in Figure 15.23*a,b*, which participates in both the mating and osmoregulatory response depending upon which scaffolding protein it has interacted with. The importance of scaffolding proteins is well illustrated by an experiment in which parts of two different yeast MAPK-cascade scaffolding proteins (Ste5 and Pbs2) were genetically combined to form a chimeric protein (Ste5-Pbs2) (Figure 15.23*c*). Normally these two scaffolds mediate two

different MAPK signaling pathways (Figure 15.23*a,b*). When yeast cells containing the chimeric protein were exposed to a mating factor that normally stimulates the mating response, the cells responded by displaying the osmoregulatory response.

Signaling by the Insulin Receptor

Our bodies spend considerable effort maintaining blood glucose levels within a narrow range. A decrease in blood glucose levels can lead to loss of consciousness and coma, as the central nervous system depends largely on glucose for its energy metabolism. A persistent elevation in blood glucose levels results in a loss of glucose, fluids, and electrolytes in the urine and serious health problems. The levels of glucose in the circulation are monitored by the pancreas. When blood glucose levels fall below a certain level, the alpha cells of the pancreas secrete glucagon. As discussed earlier, glucagon acts through GPCRs and stimulates the breakdown of glycogen resulting in an increase in blood glucose levels. When glucose levels rise, as occurs after a carbohydrate-rich meal, the beta cells of the pancreas respond by secreting insulin. Insulin functions as an extracellular messenger molecule, informing cells that glucose levels are high. Cells that express insulin receptors on their surface, such as cells in the liver, respond to this message by increasing glucose uptake, increasing glycogen and triglyceride synthesis, and/or decreasing gluconeogenesis.

The Insulin Receptor Is a Protein-Tyrosine Kinase

Each insulin receptor is composed of an α and a β chain, which are derived from a single precursor protein by proteolytic processing. The α chain is entirely extracellular and contains the insulin-binding site. The β chain is composed of an extracellular region, a single transmembrane region, and a cytoplasmic region (Figure 15.24). The α and β chains are linked together by disulfide bonds (Figure 15.24). Two of these αβ heterodimers are held together by disulfide bonds between the α chains. Thus, while most RTKs are thought to be present on the cell surface as monomers, insulin receptors are present as stable dimers. Like other RTKs, insulin receptors are inactive in the absence of ligand (Figure 15.24*a*). Recent work suggests that the insulin receptor dimer binds a single insulin molecule. This causes repositioning of the ligand-binding domains on the outside of the cell, which causes the tyrosine kinase domains on the inside of the cell to come into close physical proximity (Figure 15.24*b*). Juxtaposition of the kinase domains leads to trans-autophosphorylation and receptor activation (Figure 15.24*c*).

Several tyrosine phosphorylation sites have been identified in the cytoplasmic region of the insulin receptor. Three of these phosphorylation sites are present in the activation loop. In the unphosphorylated state, the activation loop assumes a conformation in which it occupies the active site. Upon phosphorylation of the three tyrosine residues, the activation loop assumes a new conformation away from the catalytic cleft. This new conformation requires a rotation of the small and large lobes of the kinase domain with respect to each other, thereby bringing residues that are essential for catalysis closer together. In addition, the activation loop now leaves the catalytic cleft open so that it can bind substrates. Following activation of the kinase domain, the receptor phosphorylates itself on tyrosine residues that are present adjacent to the membrane and in the carboxyl-terminal tail (Figure 15.24*c*).

Insulin Receptor Substrates 1 and 2 Most RTKs possess autophosphorylation sites that directly recruit SH2 domain-containing signaling proteins (as in Figure 15.19*a*, *c*, and *d*). The insulin receptor is an exception to this general rule, because it associates instead with a small family of docking proteins (Figure 15.19*b*), called **insulin-receptor substrates** (**IRSs**). The IRSs, in turn, provide the binding sites for SH2 domain-containing signaling proteins. Some of the events that occur during insulin signaling are shown in Figure

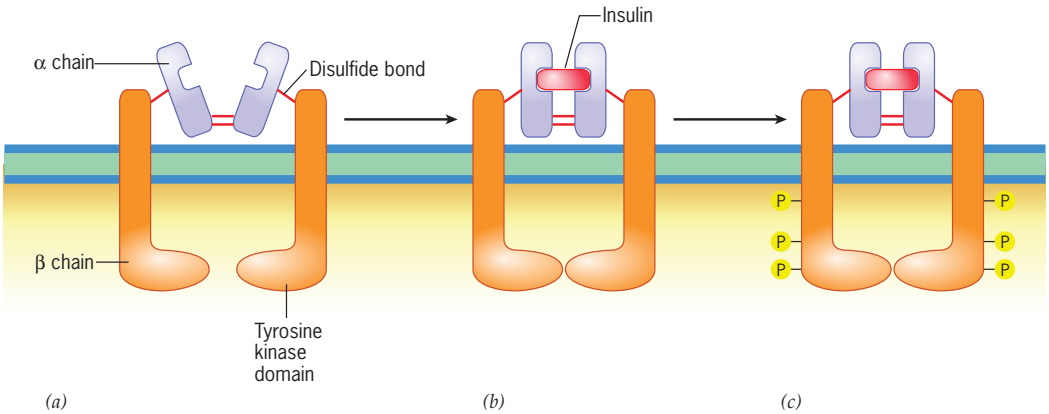


Figure 15.24 **The response of the insulin receptor to ligand binding.** (*a*) The insulin receptor, shown here in schematic form in the inactive state, is a tetramer consisting of two α and two β subunits. (*b*) In this model, binding of a single insulin molecule to the α subunits causes a conformational change in the β subunits, which activates the tyrosine kinase activity of the β subunits. (*c*) The activated β subunits phosphorylate tyrosine residues located on the cytoplasmic domain of the receptor as well as tyrosine residues on several insulin receptor substrates (IRSs) that are discussed below.

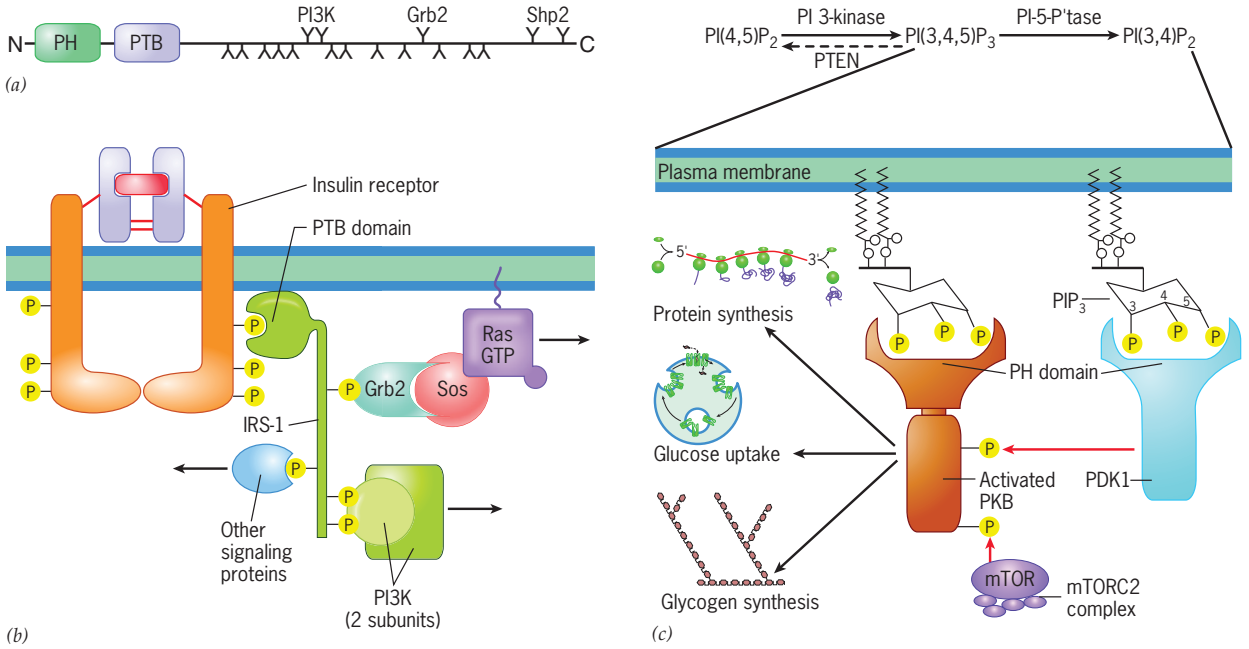


Figure 15.25 **The role of tyrosine-phosphorylated IRS in activating a variety of signaling pathways.** (*a*) Schematic representation of an IRS polypeptide. The N-terminal portion of the molecule contains a PH domain that allows it to bind to phosphoinositides of the membrane and a PTB domain that allows it to bind to a specific phosphorylated tyrosine residue (#960) on the cytoplasmic domain of an activated insulin receptor. Once bound to the insulin receptor, a number of tyrosine residues in the IRS may be phosphorylated (indicated as Y). These phosphorylated tyrosines can serve as binding sites for other proteins, including a lipid kinase (PI3K), an adaptor protein (Grb2), and a protein-tyrosine phosphatase (Shp2). (*b*) Phosphorylation of IRSs by the activated insulin receptor is known to activate PI3K and Ras pathways, both of which are discussed in the chapter. Other pathways that are less well defined are also activated by IRSs. (The IRS is drawn as an extended, two-dimensional molecule for purposes of illustration.) (*c*) Activation of PI3K leads to the formation of membrane-bound phosphoinositides, including PIP_3. One of the key kinases in numerous signaling pathways is PKB (AKT), which interacts at the plasma membrane with PIP_3 by means of a PH domain. This interaction changes the conformation of PKB, making it a substrate for another PIP_3-bound kinase (PDK1), which phosphorylates PKB. The second phosphate shown linked to PKB is added by a second kinase, mostly likely mTOR. Once activated, PKB dissociates from the plasma membrane and moves into the cytosol and nucleus. PKB is a major component of a number of separate signaling pathways that mediate the insulin response. These pathways lead to translocation of glucose transporters to the plasma membrane, synthesis of glycogen, and the synthesis of new proteins in the cell. PKB also plays a key role in promoting cell survival by inhibiting the proapoptotic protein Bad (page 659) and/or activating the transcription factor NF-κB (page 660).

15.25. Following ligand binding and kinase activation, the insulin receptor autophosphorylates tyrosine #960, which then forms a binding site for the phosphotyrosine binding (PTB) domains of insulin receptor substrates. As indicated in Figure 15.25*a*, IRSs are characterized by the presence of an N-terminal PH domain, a PTB domain, and a long tail containing tyrosine phosphorylation sites. The PH domain may interact with phospholipids present at the inside leaflet of the plasma membrane, the PTB domain binds to tyrosine phosphorylation sites on the activated receptor, and the tyrosine phosphorylation sites provide docking sites for SH2 domain-containing signaling proteins. At least four members of the IRS family have been identified. Based on the results obtained in knockout experiments in mice, it is thought that IRS-1 and IRS-2 are most relevant to insulin-receptor signaling.

Autophosphorylation of the activated insulin receptor at Tyr960 provides a binding site for IRS-1 or IRS-2. Only after stable association with either IRS-1 or IRS-2 is the activated insulin receptor able to phosphorylate tyrosine residues present on these docking proteins (Figure 15.25*b*). Both IRS-1 and IRS-2 contain a large number of potential tyrosine phosphorylation sites that include binding sites for the SH2 domains of PI 3-kinase, Grb2, and Shp2 (Figure 15.25*a*,*b*). These proteins associate with the receptor-bound IRS-1 or IRS-2 and activate downstream signaling pathways.

PI 3-kinase (*PI3K*) is composed of two subunits, one containing two SH2 domains and the other containing the catalytic domain (Figure 15.25*b*). PI 3-kinase, which is activated directly as a consequence of binding of its two SH2 domains to tyrosine phosphorylation sites, phosphorylates phosphoinositides at the 3 position of the inositol ring (Figure 15.25*c*). The products of this enzyme, which include PI 3,4-bisphosphate $PI(3,4)P_2$ and PI 3,4,5-trisphosphate (PIP_3), remain in the cytosolic leaflet of the plasma membrane where they provide binding sites for PH domain-containing signaling proteins such as the serine-threonine kinases PKB and PDK1. As indicated in Figure 15.25*c*, PKB (more commonly known as AKT) plays a role in mediating the response to

insulin, as well as to other extracellular signals. Recruitment of PDK1 to the plasma membrane, in close proximity to PKB, provides a setting in which PDK1 can phosphorylate and activate the Ser/Thr kinase activity of PKB (Figure 15.25*c*). While phosphorylation by PDK1 is essential, it is not sufficient for activation of PKB. Activation of PKB also depends on phosphorylation by a second kinase, mTOR, which has a crucial role in regulating numerous cellular activities. PI3K signaling is terminated by removal of the phosphate at the 3-position on the inositol ring by the lipid phosphatase PTEN (Figure 15.25*c*).

Glucose Transport PKB is directly involved in regulating glucose transport and glycogen synthesis. The glucose transporter GLUT4 carries out insulin-dependent glucose transport from the blood (page 157). In the absence of insulin, GLUT4 is present in membrane vesicles in the cytoplasm of insulin responsive cells (Figure 15.26). These vesicles fuse with the plasma membrane in response to insulin, a process that is referred to as GLUT4 translocation. The increase in numbers of glucose transporters in the plasma membrane leads to increased glucose uptake (Figure 15.26). GLUT4 translocation depends on activation of PI 3-kinase and PKB. This conclusion is based on experiments showing that inhibitors of PI3K block GLUT4 translocation. In addition, overexpression of PI3K or PKB stimulates GLUT4 translocation. It is well known that many receptors activate PI3K, whereas it is only the insulin receptor that stimulates GLUT4 translocation. This suggests that there is a second pathway downstream of the insulin receptor that is essential for GLUT4 translocation to occur. Detailed understanding of how the two pathways work together to stimulate GLUT4 translocation is still lacking.

Excess glucose that is taken up by muscle and liver cells is stored in the form of glycogen. Glycogen synthesis is carried out by glycogen synthase, an enzyme that is turned off by phosphorylation on serine and threonine residues. Glycogen synthase kinase-3 (GSK-3) has been identified as a negative regulator of glycogen synthase. GSK-3, in turn, is inactivated following phosphorylation by PKB. Thus, activation of the PI 3-kinase-PKB pathway in response to insulin leads to a decrease in GSK-3 kinase activity, resulting in an increase in glycogen synthase activity (Figure 15.25*c*). Activation of protein phosphatase 1, an enzyme known to dephosphorylate glycogen synthase, contributes further to glycogen synthase activation (Figure 15.14).

Diabetes Mellitus One of the most common human diseases, diabetes mellitus, is caused by defects in insulin signaling. Diabetes occurs in two varieties: type 1, which accounts for 5–10 percent of the cases, and type 2, which accounts for the remaining 90–95 percent. Type 1 diabetes is caused by an inability to produce insulin and is discussed in the Human Perspective of Chapter 17. Type 2 diabetes is a more complex disease whose incidence is increasing around the world at an alarming rate. The rising incidence of the disease is most likely a result of changing lifestyle and eating habits. A high-calorie diet combined with a sedentary lifestyle is thought to lead to a chronic increase in insulin secretion. Elevated levels of insulin overstimulate target cells in the liver and elsewhere in the body, which leads to a condition referred to as *insulin resistance*, in which these target cells stop responding to the presence of the hormone. This is turn leads to a chronic elevation in blood glucose levels, which stimulates the pancreas to secrete even more insulin, setting up a viscious cycle that can ultimately lead to the death of the insulin-secreting beta cells of the pancreas. Most of the health risks that result from diabetes—cardiovascular disease, blindness, kidney disease, and reduced circulation in the limbs leading to amputations—are thought to be due to damage to the body's blood vessels, but the molecular mechanism by which insulin resistance and its consequent metabolic effects lead to this condition remain the subject of debate. The relationship between insulin signaling pathways and lifespan is discussed in the accompanying Human Perspective.

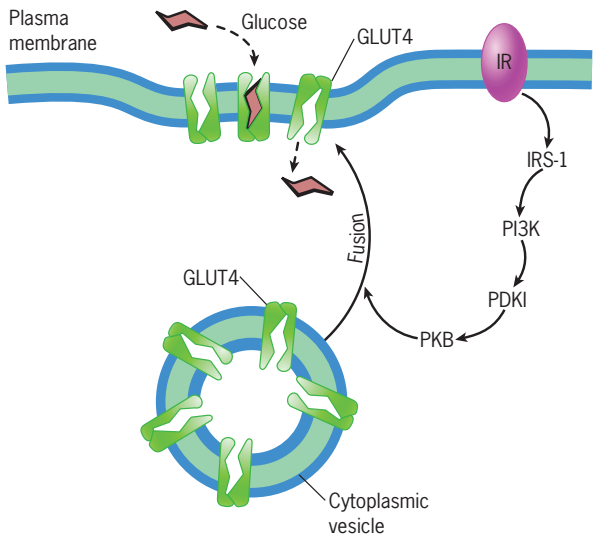


Figure 15.26 **Regulation of glucose uptake in muscle and fat cells by insulin.** Glucose transporters are stored in the walls of cytoplasmic vesicles that form by budding from the plasma membrane (endocytosis). When the insulin level increases, a signal is transmitted through the IRS-PI3K-PKB pathway, which triggers the translocation of cytoplasmic vesicles to the cell periphery. The vesicles fuse with the plasma membrane (exocytosis), delivering the transporters to the cell surface where they can mediate glucose uptake. A second pathway leading from the insulin receptor to GLUT4 translocation is not shown (see *Trends Biochem. Sci.* 31:215, 2006). (D. Voet and J. G. Voet, Biochemistry, 2e; copyright 1995, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

THE HUMAN PERSPECTIVE

Signaling Pathways and Human Longevity

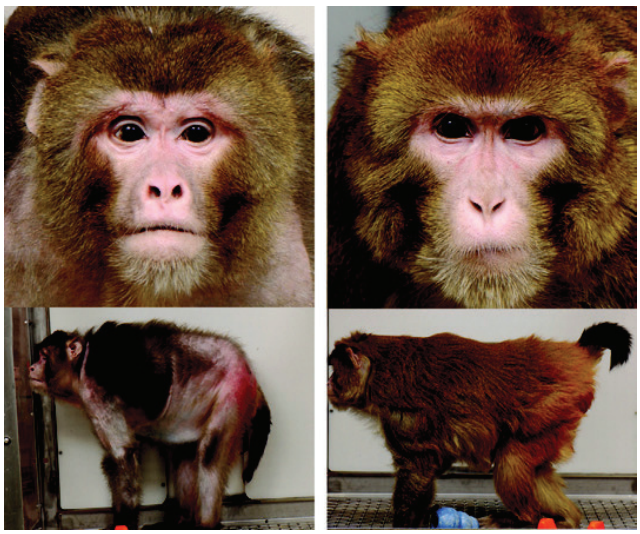
Many factors are known to contribute to the aging process, some genetic and others nongenetic. Discussions of aging have appeared in several places in the text: in the Human Perspective on free radicals (page 35), in the Human Perspective on mitochondrial diseases (page 208); in the Human Perspective on DNA repair deficiencies (page 569); in the section on the nuclear lamina (page 490); and in the discussion of telomeres (page 508). In recent years, a new contributor to the aging process has received attention: the activity of a signaling pathway involving insulin and a related protein IGF-1, which is the focus of the present Human Perspective.

The lifespans of animals can be increased by restricting the calories present in the diet. As first shown in the 1930s, mice that are maintained on very strict diets typically live 30 to 40 percent longer than their littermates who are fed diets of normal caloric content. Two separate long-term studies are currently in progress on rhesus monkeys to see if they too live longer and healthier lives when maintained on calorie-restricted diets. Significant differences in the published data between these two groups have made it difficult to draw firm conclusions on the value of calorie restriction (CR) in primates. One team of researchers at the Wisconsin National Primate Research Center reported in 2009 that animals in their CR group have lower blood levels of glucose, insulin, and triglycerides and were less prone to age-related disorders such as diabetes and coronary artery disease. The effect of calorie restriction on the external appearance of one of these animals is seen in the photographs of Figure 1. The Wisconsin group also reported that 37 percent of the control group (i.e., animals that had enjoyed unrestricted diets) had died during the 20 years of the study compared to only 13 percent of the CR group. In contrast, the other team of researchers at the National Institute of Aging reported in late 2012 that calorie restriction did not improve survival outcome. In fact, individuals in their CR group did not exhibit the reduced cardiovascular disease that characterized the CR individuals in the Wisconsin study. One important difference between the CR and control groups in the NIA study was noted: none of the animals in the CR group had died from cancer as compared to five deaths from cancer from those in the control group. It has been suggested that differences in animal survival between the two studies may be explained by the fact that individuals of the control group in the Wisconsin study were allowed to eat unlimited amounts of high-sucrose-containing foods, which may have made them less healthy than the control animals in the NIA study. Neither of these studies has been conducted long enough to determine if the animals' maximum life span (more than 40 years) is increased as a result of CR.

As reported in numerous television news shows, a growing number of humans are hoping to extend their life span by practicing calorie restriction, which in essence means that they are willing to subject themselves to an extremely limited, but balanced, diet. The National Institutes of Aging has also begun a study (named CALERIE) on human subjects who are overweight (but not obese) that are kept on diets containing about 25 percent fewer calories than would be required to maintain their initial body weight. After a period of six months of calorie restriction, these individuals show remarkable metabolic changes; they have a lower body temperature, their blood insulin and LDL-cholesterol levels are lower, they have lost weight as would be expected, and their energy expenditure is reduced beyond that expected due simply to their lower body mass. In addition, the level of DNA damage experienced by the cells of these individuals is reduced, which suggests a decrease in production of reactive oxygen species (page 35).

A number of early studies demonstrated that the lifespan of a worm or fruit fly can also be dramatically increased by reducing the activity of insulin-like growth factors and their receptors. Studies of humans support this relationship; humans that live exceptionally long lives often exhibit unusually high insulin sensitivity—that is, their tissues respond fully to relatively low circulating insulin levels. Low insulin levels are also linked to reduced incidence of cancer. Thus, just as high insulin levels and increased insulin resistance are associated with poor health, low insulin levels and increased insulin sensitivity appear to be associated with good health. It is interesting to note that calorie restriction in laboratory animals leads to decreased insulin levels and increased insulin sensitivity, so that these two paths to increased longevity may be acting by the same mechanism.

Although the medical community has typically focused on insulin as the primary metabolic hormone, many basic researchers in the field of aging have focused on the related hormone insulin-growth factor 1 (IGF-1). In one study, it was found that mutations in the gene encoding the IGF-1 receptor are especially frequent in a group of centenarians (humans living over the age of 100). Both insulin and IGF-1 share the downstream effector, mTOR. mTOR became a central focus of the field of mammalian aging when it was



(a) *(b)*

Figure 1 **The effects of calorie restriction on Rhesus macaques.** Photographs of *(a)* a typical control animal at 27.6 years of age (about the average life span) and *(b)* an age-matched animal on a calorie-restricted diet. (Contasting results from the NIA study can be found in an advanced online publication in the 8/30/2012 issue of Nature.) (From R. J. Colman, et al., Science 325:201, 2009. Reprinted with permission from AAAS. Courtesy Ricki Colman, Wisconsin National Primate Research Center, University of Wisconsin.)

discovered that rapamycin, an inhibitor of the mTOR kinase, significantly extended the lifespan of mice and decreased the incidence of age-related disorders. This is the first compound that has been shown to increase life span in mammals, and it has also has this capability when given to yeast, worms, and flies. Unfortunately, rapamycin is also a potent suppressor of the immune system so it is not itself considered to be a viable anti-aging drug. Calorie restriction has also been shown to reduce signaling through the mTOR pathway. These findings support the notion that mTOR plays an important role in the aging process. mTOR is a nutrient sensor and a primary regulator of cellular metabolism. mTOR is a protein kinase that exists as a component of two distinct complexes mTORC1 and mTORC2. mTORC1, which is especially sensitive to rapamycin, is activated by the availability of nutrients, especially amino acids, and can stimulate lipid and protein synthesis, inhibit autophagy, and promote cell growth and proliferation. Studies suggest that reduced nutrient availability, as occurs during calorie restriction, reduces mTORC1 signaling. A number of proteins both upstream and downstream of mTOR have been implicated in regulating lifespan, including S6K1 (which phosphorylates numerous proteins involved in protein synthesis, thereby enhancing mRNA translation), the protein deacetylase Sir2 (which removes acetyl groups from histones and nonhistone proteins), Atg proteins (which regulate autophagy), and the transcription factor FOXO (which activates expression of genes whose encoded proteins include molecular chaperones and proteins that play a role in defense against oxidative stress). Untangling the roles of these various components has proven to be very difficult and there is considerable debate as to how mTOR inhibition increases lifespan.

Signaling Pathways in Plants

Plants and animals share certain basic signaling mechanisms, including the use of Ca^{2+} and phosphoinositide messengers, but other pathways are unique to each major kingdom. For example, cyclic nucleotides, which may be the most ubiquitous animal cell messengers, appear to play little, if any, role in plant cell signaling. Receptor tyrosine kinases are also lacking in plant cells. On the other hand, plants contain a type of protein kinase that is absent from animal cells.

It has long been known that bacterial cells have a protein kinase that phosphorylates histidine residues and mediates the cell's response to a variety of environmental signals. Until 1993, these enzymes were thought to be restricted to bacterial cells but were then discovered in both yeast and flowering plants. In both types of eukaryotes, the enzymes are transmembrane proteins with an extracellular domain that acts as a receptor for external stimuli and a cytoplasmic, histidine kinase domain that transmits the signal to the cytoplasm. One of the best studied of these plant proteins is encoded by the *Etr1* gene. The product of the *Etr1* gene encodes a receptor for the gas ethylene (C_2H_4), a plant hormone that regulates a diverse array of developmental processes, including seed germination, flowering, and fruit ripening. Binding of ethylene to its receptor leads to transmission of signals along a pathway that is very similar to the MAP kinase cascade found in yeast and animal cells. As in other eukaryotes, the downstream targets of the MAP kinase pathway in plants are transcription factors that activate expression of specific genes encoding proteins required for the hormone response. As researchers analyze the massive amount of data obtained from sequencing *Arabidopsis* and other plant genomes, the similarities and differences between plant and animal signaling pathways should become more apparent.

REVIEW

1. Describe the steps between the binding of an insulin molecule at the surface of a target cell and the activation of the effector PI3K. How does the action of insulin differ from other ligands that act by means of receptor tyrosine kinases?
2. What is the role of Ras in signaling pathways? How is this affected by the activity of a Ras-GAP? How does Ras differ from a heterotrimeric G protein?
3. What is an SH2 domain, and what role does it play in signaling pathways?
4. How does the MAP kinase cascade alter the transcriptional activity of a cell?
5. What is the relationship between type 2 diabetes and insulin production? How is it that a drug that increases insulin sensitivity might help treat this disease?

15.5 | The Role of Calcium as an Intracellular Messenger

Calcium ions play a significant role in a remarkable variety of cellular activities, including muscle contraction, immune responses, cell division, secretion, fertilization, synaptic transmission, metabolism, transcription, cell movement, and cell death. In each of these cases, an extracellular message is received at the cell surface and leads to a dramatic increase in concentration of calcium ions within the cytosol. The concentration of calcium ions in a particular cellular compartment is controlled by the regulated activity of Ca^{2+} pumps, Ca^{2+} exchangers, and/or Ca^{2+} ion channels located within the membranes that surround the compartment (as in Figure 15.28). The concentration of Ca^{2+} ions in the cytosol of a resting cell is maintained at very low levels, typically about 10^{-7} M. In contrast, the concentration of this ion in the extracellular space or within the lumen of the ER or a plant cell vacuole is typically 10,000 times higher than the cytosol. The cytosolic calcium level is kept very low because (1) Ca^{2+} ion channels in both the plasma and ER membranes are normally kept closed, making these membranes highly impermeable to this ion, and (2) energy-driven Ca^{2+} transport systems of the plasma and ER membranes pump calcium out of the cytosol.[2] Abnormal elevation of cytosolic Ca^{2+} concentration, as can occur in brain cells following a stroke, can lead to massive cell death.

[2]Mitochondria also play an important role in sequestering and releasing Ca^{2+} ions, but their role was considered in Chapter 5 and will not be discussed here.

IP_3 and Voltage-Gated Ca^{2+} Channels We have described in previous pages two major types of signaling receptors, GPCRs and RTKs. It was noted on page 630 that interaction of an extracellular messenger molecule with a GPCR can lead to the activation of the enzyme phospholipase C-β, which splits the phosphoinositide PIP_2, to release the molecule IP_3, which opens calcium channels in the ER membrane, leading to a rise in cytosolic [Ca^{2+}]. Extracellular messengers that signal through RTKs can trigger a similar response. The primary difference is that RTKs activate members of the phospholipase C-γ subfamily, which possess an SH2 domain that allows them to bind to the activated, phosphorylated RTK. There are numerous other PLC isoforms. For example, PLCδ is actived by Ca^{2+} ions, and PLCϵ is activated by Ras-GTP. All PLC isoforms carry out the same reaction, producing IP_3 and linking a multitude of cell surface receptors to an increase in cytoplasmic Ca^{2+}. There is another major route leading to elevation of cytosolic [Ca^{2+}], which was encountered in our discussion of synaptic transmission on page 169. In this case, a nerve impulse leads to a depolarization of the plasma membrane, which triggers the opening of voltage-gated calcium channels in the plasma membrane, allowing the influx of Ca^{2+} ions from the extracellular medium.

Visualizing Cytoplasmic Ca^{2+} Concentration in Real Time Our understanding of the role of Ca^{2+} ions in cellular responses has been greatly advanced by the development of indicator molecules that emit light in the presence of free calcium. In the mid-1980s, new types of highly sensitive, fluorescent, calcium-binding compounds (e.g., *fura*-2) were developed in the laboratory of Roger Tsien at the University of California, San Diego. These compounds are synthesized in a form that can enter a cell by diffusing across its plasma membrane. Once inside a cell, the compound is modified to a form that is unable to leave the cell. Using these probes, the concentration of free calcium ions in different parts of a living cell can be determined over time by monitoring the light emitted using a fluorescence microscope and computerized imaging techniques. Use of calcium-sensitive, light-emitting molecules has provided dramatic portraits of the complex spatial and temporal changes in free cytosolic calcium concentration that occur in a single cell in response to various types of stimuli. This is one of the advantages of studying calcium-mediated responses compared to responses mediated by other types of messengers whose location in a cell cannot be readily visualized.

Depending on the type of responding cell, a particular stimulus may induce repetitive oscillations in the concentration of free calcium ions, as seen in Figure 15.11; cause a wave of Ca^{2+} release that spreads from one end of the cell to the other (see Figure 15.29); or trigger a localized and transient release of Ca^{2+} in one part of the cell. Figure 15.27 shows a Purkinje cell, a type of neuron in the mammalian cerebellum that maintains synaptic contact with thousands of other cells through an elaborate network of postsynaptic dendrites. The micrograph in Figure 15.27 shows the release of free calcium in a localized region of the "dendritic tree" of the cell following synaptic activation. The burst of calcium release remains restricted to this region of the cell.

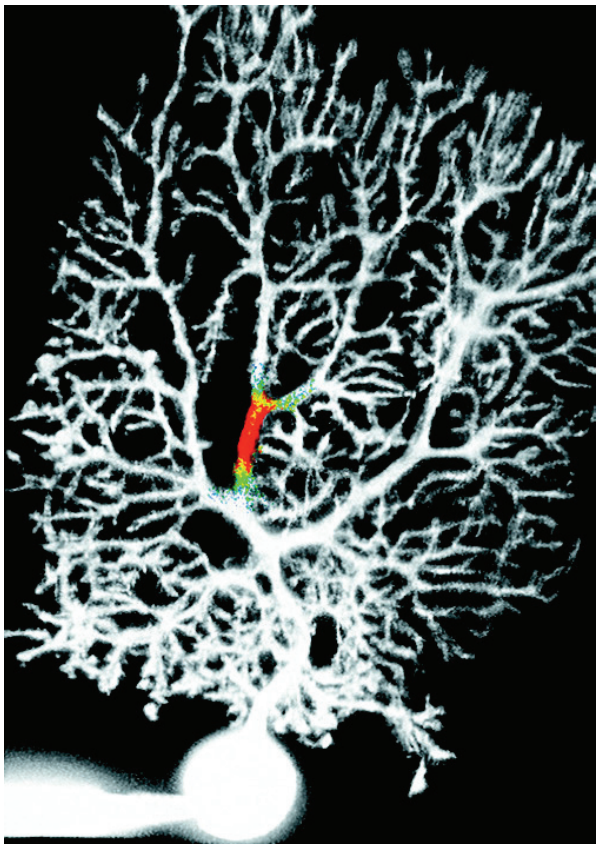


Figure 15.27 **Experimental demonstration of localized release of intracellular Ca^{2+} within a single dendrite of a neuron.** The mechanism of IP_3-mediated release of Ca^{2+} from intracellular stores was described on page 630. In the micrograph shown here, which pictures an enormously complex Purkinje cell (neuron) of the cerebellum, calcium ions have been released locally within a small portion of the complex "dendritic tree." Calcium release from the ER (shown in red) was induced in the dendrite following the local production of IP_3, which followed repetitive activation of a nearby synapse. The sites of release of cytosolic Ca^{2+} ions are revealed by fluorescence from a fluorescent calcium indicator that was loaded into the cell prior to stimulation of the cell. (From Elizabeth A. Finch and George J. Augustine, Nature, vol. 396, cover of 12/24/98. Reprinted by permission from Macmillan Publishers Limited.)

IP_3 receptors described earlier are one of two main types of Ca^{2+} ion channels present in the ER membrane; the other type are called *ryanodine receptors* (*RyRs*) because they bind the toxic plant alkaloid ryanodine. Ryanodine receptors are found primarily in excitable cells and are best studied in cardiac and skeletal muscle cells, where they mediate the rise in Ca^{2+} levels following the arrival of an action potential. Mutations in the cardiac RyR isoform have been linked to occurrences of sudden death during periods of exercise. Depending on the type of cell in which they are found, RyRs can be opened by a variety of agents, including calcium itself. The influx of a limited amount of calcium through open channels in the plasma membrane induces the opening of ryanodine

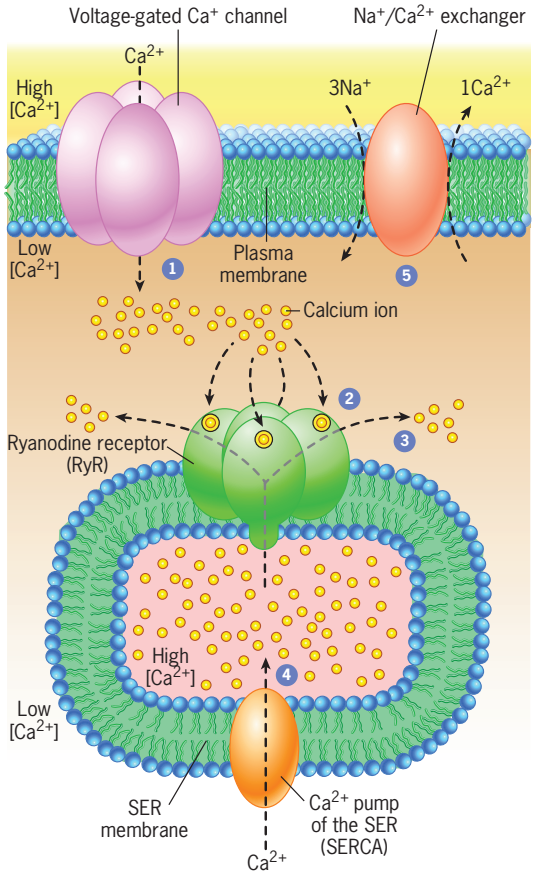


Figure 15.28 **Calcium-induced calcium release,** as it occurs in a cardiac muscle cell. A depolarization in membrane voltage causes the opening of voltage-gated calcium channels in the plasma membrane, allowing entry of a small amount of Ca^{2+} into the cytosol (step 1). The calcium ions bind to ryanodine receptors in the SER membrane (step 2), leading to release of stored Ca^{2+} into the cytosol (step 3), which triggers the cell's contraction. The calcium ions are subsequently removed from the cytosol by the action of Ca^{2+} pumps located in the membrane of the SER (step 4) and a Na^+/Ca^{2+} secondary transport system in the plasma membrane (step 5), which leads to relaxation. This cycle is repeated after each heart beat. (Reprinted with permission after M. J. Berridge, Nature 361:317, 1993; copyright 1993. Nature by Nature Publishing Group. Reproduced with permission of Nature Publishing Group in the format reuse in a book/textbook via Copyright Clearance Center.)

receptors in the ER, causing the release of Ca^{2+} into the cytosol (Figure 15.28). This phenomenon is called *calcium-induced calcium release* (*CICR*).

Extracellular signals that are transmitted by Ca^{2+} ions typically act by opening a small number of Ca^{2+} ion channels at the cell surface at the site of the stimulus. As Ca^{2+} ions rush through these channels and enter the cytosol, they act on nearby Ca^{2+} ion channels in the ER, causing these channels to open and release additional calcium into adjacent regions of the cytosol. In some responses, the elevation of Ca^{2+} levels remains localized to a small region of the cytosol (as in Figure 15.27). In other cases, a propagated wave of calcium release spreads through the entire cytoplasmic compartment. One of the most dramatic Ca^{2+} waves occurs within the first minute or so following fertilization and is induced by the sperm's contact with the plasma membrane of the egg (Figure 15.29). The sudden rise in cytoplasmic calcium concentration following fertilization triggers a number of events, including activation of cyclin-dependent kinases (page 575) that drive the zygote toward its first mitotic division. Calcium waves are transient because the ions are rapidly pumped out of the cytosol and back into the ER and/or the extracellular space.

Recent research in the field of calcium signaling has focused on a phenomenon known as *store-operated calcium entry* (or *SOCE*), in which the "store" refers to the calcium ions stored in the ER. During periods of repeated cellular responses, the stockpile of intracellular calcium ions can become depleted. During SOCE the depletion of calcium levels in the ER triggers a response leading to the opening of calcium channels in the plasma membrane as depicted in Figure 15.30. Once these channels have opened, Ca^{2+} ions can enter the cytosol from where they can be pumped back into the ER, thereby replenishing the ER's calcium stores. The mechanism responsible for SOCE had been an unsolved mystery for many years until it was discovered recently that these events are orchestrated by a signaling system operating between the ER and plasma membrane. In this system, the depletion of Ca^{2+} in the ER leads to the clustering within the ER membrane of a Ca^{2+}-sensing protein called STIM1 into regions where the ER and plasma membranes come into close proximity (25–50 nm) to one another. Following their rearrangement in the ER membrane, the STIM1 clusters act to recruit

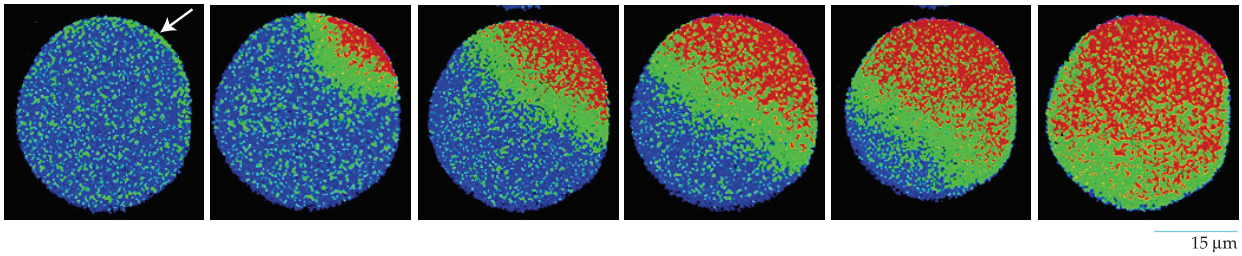


Figure 15.29 **Calcium wave in a starfish egg induced by a fertilizing sperm.** The unfertilized egg was injected with a calcium-sensitive fluorescent dye, fertilized, and photographed at 10-second intervals. The rise in Ca^{2+} concentration is seen to spread from the point of sperm entry (arrow) throughout the entire egg. The blue color indicates low free $[Ca^{2+}]$, whereas the red color indicates high free $[Ca^{2+}]$. A similar Ca^{2+} wave in mammalian eggs is triggered by the formation of IP_3 by a phospholipase C that is brought into the egg by the fertilizing sperm. (Courtesy of Stephen A. Stricker.)

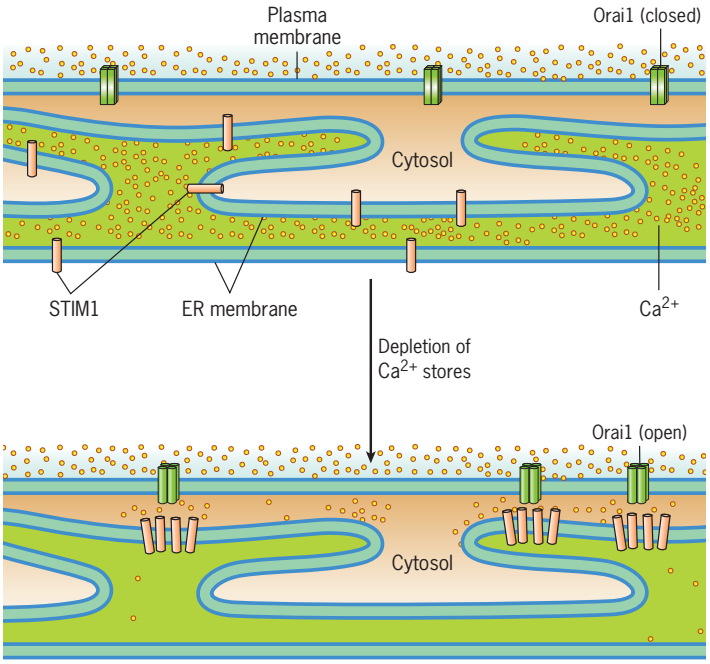


Figure 15.30 **A model for store-operated calcium entry.** When the ER lumen contains abundant Ca^{2+} ions, the STIM1 proteins of the ER membrane and the Orai1 proteins of the plasma membrane are situated diffusely in their respective membranes, and the Orai1 calcium channel is closed. If the ER stores are depleted, a signaling system operates between the two membranes, causing the two proteins to become clustered within their respective membranes in close proximity to one another. Apparent interaction between the two membrane proteins leads to opening of the Orai1 channel and the influx of Ca^{2+} ions into the cytosol from where they can be pumped into the ER lumen.

subunits of a plasma membrane protein called Orail into adjacent regions of the plasma membrane (Figure 15.30). Orai1 is a tetrameric Ca^{2+} ion channel that had been identified as being involved in a particular type of inherited human immune deficiency that results from a lack of Ca^{2+} stores in T lymphocytes. Contact between the cytosolic surfaces of the STIM1 and Orai1 proteins in these ER-plasma membrane junctions leads to the opening of the Orai1 channels, the influx of Ca^{2+} into microdomains of the cytosol near the STIM1 clusters, and the refilling of the cell's ER stores.

Ca^{2+}-Binding Proteins Unlike cAMP, whose action is usually mediated by stimulation of a protein kinase, calcium can affect a number of different types of cellular effectors, including protein kinases (Table 15.4). Depending on the cell type, calcium ions can activate or inhibit various enzyme and transport systems, change the ionic permeability of membranes, induce membrane fusion, or alter cytoskeletal structure and function. Calcium does not bring about these responses by itself but acts in conjunction with a number of **calcium-binding proteins** (examples are discussed on pages 303 and 370). The best-studied calcium-binding protein is **calmodulin**, which participates in many signaling pathways.

Calmodulin is found universally in plants, animals, and eukaryotic microorganisms, and it has virtually the same amino acid sequence from one end of the eukaryotic spectrum to the other. Each molecule of calmodulin (Figure 15.31) contains four binding sites for calcium. Calmodulin does not have sufficient affinity for Ca^{2+} to bind the ion in a nonstimulated cell. If, however, the Ca^{2+} concentration rises in response to a stimulus, the ions bind to calmodulin, changing the conformation of the protein and increasing its affinity for a variety of effectors. Depending on the cell type, the calcium–calmodulin (Ca^{2+}–CaM) complex may bind to a protein kinase, a cyclic nucleotide phosphodiesterase, ion channels, or even to the calcium-transport system of the plasma membrane. In the latter instance, rising levels of calcium activate the system re-

Table 15.4 ***Examples of Mammalian Proteins Activated by Ca^{2+}***

Protein	Protein function
Troponin C	Modulator of muscle contraction
Calmodulin	Ubiquitous modulator of protein kinases and other enzymes (MLCK, CaM kinase II, adenylyl cyclase I)
Calretinin, retinin	Activator of guanylyl cylase
Calcineurin B	Phosphatase
Calpain	Protease
PI-specific PLC	Generator of IP_3 and diacylglycerol
α-Actinin	Actin-bundling protein
Annexin	Implicated in endo- and exocytosis, inhibition of PLA_2
Phospholipase A_2	Producer of arachidonic acid
Protein kinase C	Ubiquitous protein kinase
Gelsolin	Actin-severing protein
IP_3 receptor	Effector of intracellular Ca^{2+} release
Ryanodine receptor	Effector of intracellular Ca^{2+} release
Na^+/Ca^{2+} exchanger	Effector of the exchange of Ca^{2+} for Na^+ across the plasma membrane
Ca^{2+}-ATPase	Pumps Ca^{2+} across membranes
Ca^{2+} antiporters	Exchanger of Ca^{2+} for monovalent ions
Caldesmon	Regulator of muscle contraction
Villin	Actin organizer
Arrestin	Terminator of photoreceptor response
Calsequestrin	Ca^{2+} buffer

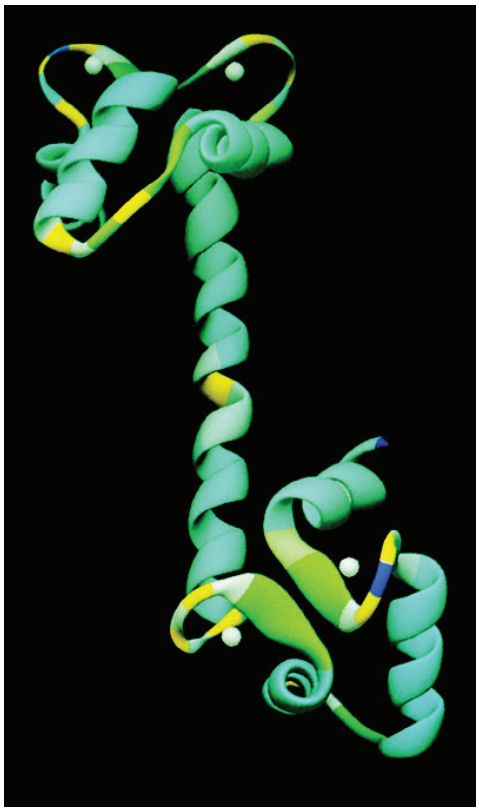


Figure 15.31 **Calmodulin.** A ribbon diagram of calmodulin (CaM) with four bound calcium ions (white spheres). Binding of these Ca^{2+} ions changes the conformation of calmodulin, exposing a hydrophobic surface that promotes interaction of Ca^{2+}–CaM with a large number of target proteins. (COURTESY MICHAEL CARSON, UNIVERSITY OF ALABAMA AT BIRMINGHAM.)

sponsible for ridding the cell of excess quantities of the ion, thus constituting a self-regulatory mechanism for maintaining low intracellular calcium concentrations. The Ca^{2+}–CaM complex can also stimulate gene transcription through activation of various protein kinases (CaMKs) that phosphorylate transcription factors. In the best-studied case, one of these protein kinases phosphorylates CREB on the same serine residue as PKA (Figure 15.14).

Regulating Calcium Concentrations in Plant Cells

Calcium ions (acting in conjunction with calmodulin) are important intracellular messengers in plant cells. The levels of cytosolic calcium change dramatically within certain plant cells in response to a variety of stimuli, including changes in light, pressure, gravity, and the concentration of plant hormones such as abscisic acid. The concentration of Ca^{2+} in the cytosol of a resting plant cell is kept very low by the action of transport proteins situated in the plasma membrane and vacuolar membrane (tonoplast).

The role of Ca^{2+} in plant cell signaling is illustrated by guard cells that regulate the diameter of the microscopic pores

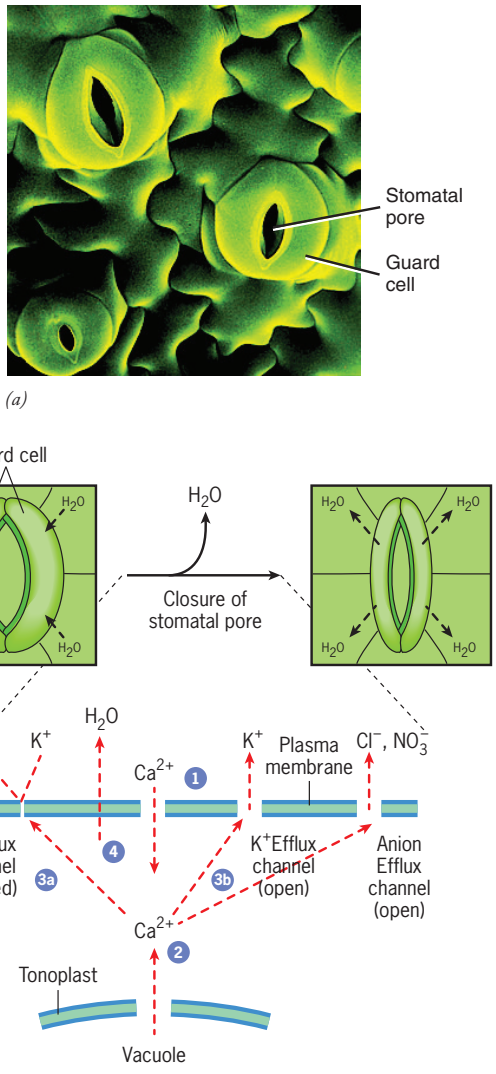


Figure 15.32 **A simplified model of the role of Ca^{2+} in guard cell closure.** (*a*) Photograph of stomatal pores, each flanked by a pair of guard cells. The stomata are kept open as turgor pressure is kept high within the guard cells, causing them to bulge outward as seen here. (*b*) One of the factors controlling stomatal pore size is the hormone abscisic acid (ABA). When ABA levels rise, calcium ion channels in the plasma membrane are opened, allowing the influx of Ca^{2+} (step 1), which triggers the release of Ca^{2+} from internal stores (step 2). The subsequent elevation of intracellular [Ca^{2+}] closes K^+ influx channels (step 3a) and opens K^+ and anion efflux channels (step 3b). These ion movements lead to a drop in internal solute concentration and the osmotic loss of water (step 4). (Phosphorylation by protein kinases also plays a role in these events.) (A: DR. JEREMY BURGESS/PHOTO RESEARCHERS, INC.)

(stomata) of a leaf (Figure 15.32*a*). Stomata are a major site of water loss in plants, and the diameter of their aperture is tightly controlled, which prevents dehydration. The diameter of the stomatal pore decreases as the fluid (turgor) pressure in the guard cell decreases. The drop in turgor pressure is caused

in turn by a decrease in the ionic concentration (osmolarity) of the guard cell. Adverse conditions, such as high temperatures and low humidity, stimulate the release of the plant stress hormone abscisic acid. Studies suggest that abscisic acid binds to a GPCR in the plasma membrane of guard cells, triggering the opening of Ca^{2+} ion channels in the same membrane (Figure 15.32*b*). The resulting influx of Ca^{2+} into the cytosol triggers the release of additional Ca^{2+} from intracellular stores. The elevated cytosolic Ca^{2+} concentration leads to closure of K^+ influx channels in the plasma membrane and opening of both K^+ and anion efflux channels. These changes produce a net outflow of K^+ ions and anions (NO_3^- and Cl^-) and a resulting decrease in turgor pressure.

REVIEW

1. How is the $[Ca^{2+}]$ of the cytosol maintained at such a low level? How does the concentration change in response to stimuli?
2. What is the role of calcium-binding proteins such as calmodulin in eliciting a response?
3. Describe the role of calcium in mediating the diameter of stomata in guard cells.

15.6 | Convergence, Divergence, and Cross-Talk Among Different Signaling Pathways

The signaling pathways described above and illustrated schematically in the various figures depict linear pathways leading directly from a receptor at the cell surface to an end target. In actual fact, signaling pathways in the cell are much more complex. For example:

- Signals from a variety of unrelated receptors, each binding to its own ligand, can *converge* to activate a common effector, such as Ras or Raf.
- Signals from the same ligand, such as EGF or insulin, can *diverge* to activate a variety of different effectors and pathways, leading to diverse cellular responses.
- Signals can be passed back and forth between different pathways, a phenomenon known as *cross-talk*.

These characteristics of cell-signaling pathways are illustrated schematically in Figure 15.33.

Signaling pathways provide a mechanism for routing information through a cell, not unlike the way the central nervous system routes information to and from the various organs of the body. Just as the central nervous system collects information about the environment from various sense organs, the cell receives information about its environment through the activation of various surface receptors, which act like sensors to detect extracellular stimuli. Like sense organs that are sensitive to specific forms of stimuli (e.g., light, pressure, or sound waves), cell-surface receptors can bind only to specific ligands and are unaffected by the presence of a large variety of unrelated molecules. A single cell may have dozens of different receptors sending signals to the cell interior simultaneously. Once they have been transmitted into the cell, signals from these receptors can be selectively routed along a number of different signaling pathways that may cause a cell to divide, change shape, activate a particular metabolic pathway, or even commit suicide (discussed in a following section). In this way, the cell integrates information arriving from different sources and mounts an appropriate and comprehensive response.

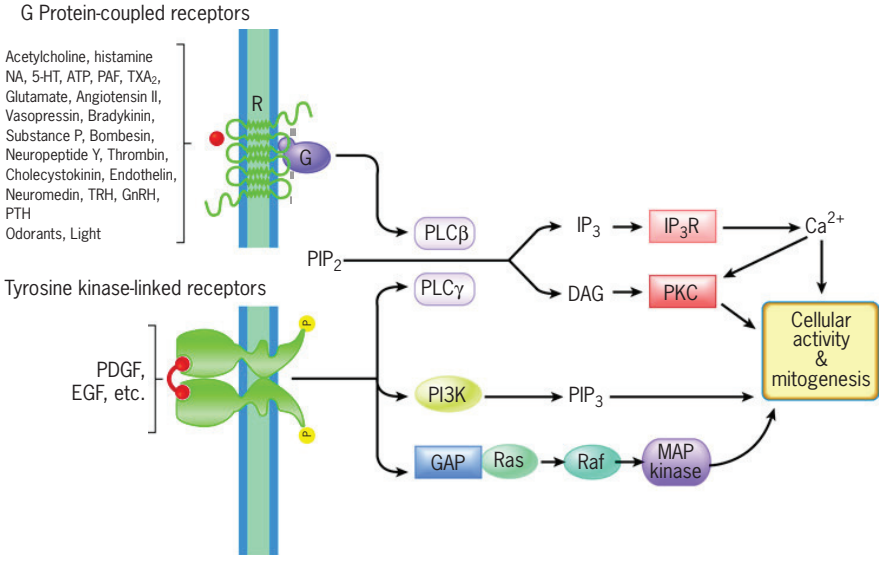


Figure 15.33 **Examples of convergence, divergence, and cross-talk among various signal-transduction pathways.** This drawing shows the outlines of signal-transduction pathways initiated by receptors that act by means of both heterotrimeric G proteins and receptor protein-tyrosine kinases. The two are seen to converge by the activation of different phospholipase C isoforms, both of which lead to the production of the same second messengers (IP_3 and DAG). Activation of the RTK by either PDGF or EGF leads to the transmission of signals along three different pathways, an example of divergence. Cross-talk between the two types of pathways is illustrated by calcium ions, which are released from the SER by action of IP_3 and can then act on various proteins, including protein kinase C (PKC), whose activity is also stimulated by DAG. (M. J. Berridge, reprinted with permission from Nature Vol. 361, p. 315, 1993, copyright 1993. Nature by Nature Publishing Group. Reproduced with permission of Nature Publishing Group in the format reuse in a book/textbook via Copyright Clearance Center.)

Examples of Convergence, Divergence, and Cross-Talk Among Signaling Pathways

1. ***Convergence.*** We have discussed two distinct types of cell-surface receptors in this chapter: G protein-coupled receptors and receptor tyrosine kinases. Another type of cell-surface receptor that is capable of signal transduction was discussed in Chapter 7, namely, integrins. Although these three types of receptors may bind to very different ligands, all of them can lead to the formation of phosphotyrosine docking sites for the SH2 domain of the adaptor protein Grb2 in close proximity to the plasma membrane (Figure 15.34). The recruitment of the Grb2-Sos complex results in the activation of Ras and transmission of signals down the MAP kinase pathway. As a result of this convergence, signals from diverse receptors can lead to the transcription and translation of a similar set of growth-promoting genes in each target cell.
2. ***Divergence.*** Evidence of signal divergence has been evident in virtually all of the examples of signal transduction that have been described in this chapter. A quick look at Figure 15.15 or 15.25*b*, *c* illustrates how a single stimulus—a ligand binding to a GPCR or an insulin receptor—sends signals out along a variety of different pathways.
3. ***Cross-talk.*** In previous sections, we examined a number of signaling pathways as if each operated as an independent, linear chain of events. In fact, the information circuits that operate in cells are more likely to resemble an interconnected web in which components produced in one pathway can participate in events occurring in other pathways. The more that is learned about information signaling in cells, the more cross-talk between signaling pathways that is discovered. Rather than attempting to catalog the ways that information can be passed back and forth within a cell, we will look at an example involving cAMP that illustrates the importance of this type of cross-talk.

Cyclic AMP was depicted earlier as an initiator of a reaction cascade leading to glucose mobilization. However, cAMP can also inhibit the growth of a variety of cells, including fibroblasts and fat cells, by blocking signals transmitted through the MAP kinase cascade. Cyclic AMP is thought to accomplish this by activating PKA, the cAMP-dependent kinase, which can phosphorylate and inhibit Raf, the protein that heads the MAP kinase cascade (Figure 15.35). These two pathways also intersect at another important signaling effector, the transcription factor CREB. CREB was described on page 632 as a terminal effector of cAMP-mediated pathways. It was assumed for years that CREB could only be phosphorylated by the cAMP-dependent kinase, PKA. It has since become apparent that CREB is a substrate of a much wider range

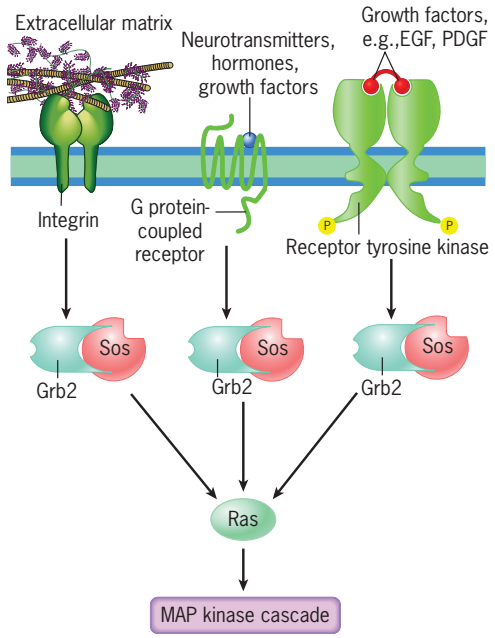


Figure 15.34 **Signals transmitted from a G protein-coupled receptor, an integrin, and a receptor tyrosine kinase all converge on Ras and are then transmitted along the MAP kinase cascade.**

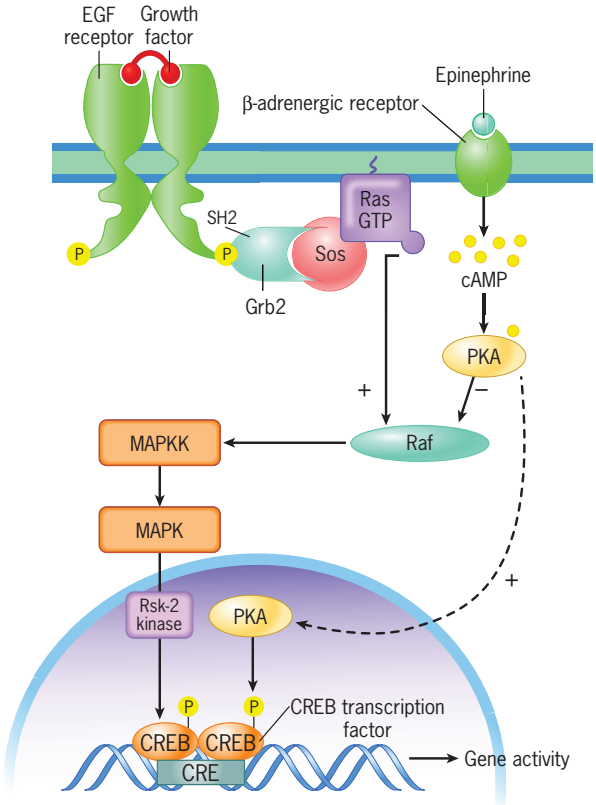


Figure 15.35 **An example of cross-talk between two major signaling pathways.** Cyclic AMP acts in some cells, by means of the cAMP-dependent kinase PKA, to block the transmission of signals from Ras to Raf, which inhibits the activation of the MAP kinase cascade. In addition, both PKA and the kinases of the MAP kinase cascade phosphorylate the transcription factor CREB on the same serine residue, activating the transcription factor and allowing it to bind to specific sites on the DNA.

of kinases. For example, one of the kinases that phosphorylates CREB is Rsk-2, which is activated as a result of phosphorylation by MAPK (Figure 15.35). In fact, both PKA and Rsk-2 phosphorylate CREB on precisely the same amino acid residue, Ser133, which should endow the transcription factor with the same potential in both pathways.

A major unanswered question is raised by these examples of convergence, divergence, and cross-talk: How are different stimuli able to evoke distinct responses, even though they utilize similar pathways? PI3K, for example, is an enzyme that is activated by a remarkable variety of stimuli, including cell adhesion to the ECM, insulin, and EGF. How is it that activation of PI3K in an insulin-stimulated liver cell promotes GLUT4 translocation and protein synthesis, whereas activation of PI3K in an adherent epithelial cell promotes cell survival? Ultimately, these contrasting cellular responses must be due to differences in the protein composition of different cell types. Part of the answer probably lies in the fact that different cells have different isoforms of these various proteins, including PI3K. Some of these isoforms are encoded by different, but related genes, whereas others are generated by alternative splicing (page 534), or other mechanisms. Different isoforms of PI3K, PKB, or PLC, for example, may bind to different sets of upstream and downstream components, which could allow similar pathways to evoke distinct responses. The variation in responses elicited by different cells possessing similar signaling proteins may also be partly explained by the presence of different protein scaffolds in each of the cell types. As shown in Figure 15.23, the specificity of a response can be orchestrated by the scaffolds with which the signaling proteins can interact. But it isn't likely that variations in isoforms and scaffolds can fully explain the extraordinary diversity of cellular responses any more than differences in the structures of neurons can explain the range of responses evoked by the nervous system. Hopefully, as the signaling pathways of more and more cells are described, we will gain a better understanding of the specificity that can be achieved through the use of similar signaling molecules.

15.7 | The Role of NO as an Intercellular Messenger

During the 1980s, a new type of messenger was discovered that was neither an organic compound, such as cAMP, nor an ion, such as Ca^{2+}; it was an inorganic gas—nitric oxide (NO). NO is unusual because it acts both as an extracellular messenger, mediating intercellular communication, and as a second messenger, acting within the cell in which it is generated. NO is formed from the amino acid L-arginine in a reaction that requires oxygen and NADPH and that is catalyzed by the enzyme nitric oxide synthase (NOS). Since its discovery, it has become evident that NO is involved in a myriad of biological processes including anticoagulation, neurotransmission, smooth muscle relaxation, and visual perception.

As with many other biological phenomena, the discovery that NO functions as a messenger molecule began with an accidental observation. It had been known for many years that acetylcholine acts in the body to relax the smooth muscle cells of blood vessels, but the response could not be duplicated in vitro. When portions of a major blood vessel such as the aorta were incubated in physiologic concentrations of acetylcholine in vitro, the preparation usually showed little or no response. In the late 1970s, Robert Furchgott, a pharmacologist at a New York State medical center, was studying the in vitro response of pieces of rabbit aorta to various agents. In his earlier studies, Furchgott used strips of aorta that had been dissected from the organ. For technical reasons, Furchgott switched from strips of aortic tissue to aortic rings and discovered that the new preparations responded to acetylcholine by undergoing relaxation. Further investigation revealed that the strips had failed to display the relaxation response because the delicate endothelial layer that lines the aorta had been rubbed away during the dissection. This surprising finding suggested that the endothelial cells were somehow involved in the response by the adjacent muscle cells. In subsequent studies, it was found that acetylcholine binds to receptors on the surface of endothelial cells, leading to the production and release of an agent that diffuses through the cell's plasma membrane and causes the muscle cells to relax. The diffusible relaxing agent was identified in 1986 as nitric oxide by Louis Ignarro at UCLA and Salvador Moncada at the Wellcome Research Labs in England. The steps in the acetylcholine-induced relaxation response are illustrated in Figure 15.36.

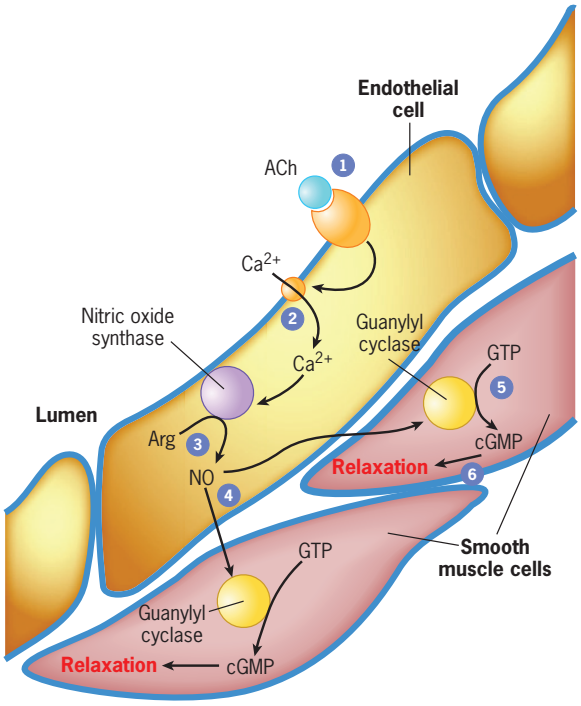


Figure 15.36 **A signal transduction pathway that operates by means of NO and cyclic GMP that leads to the dilation of blood vessels.** The steps illustrated in the figure are described in the text. (From R. G. Knowles and S. Moncada, Trends Biochem Science 17:401, 1992. Trends in biochemical sciences by International Union of Biochemistry Reproduced with permission of Elsevier Ltd. in the format reuse in a book/textbook via Copyright Clearance Center.)

The binding of acetylcholine to the outer surface of an endothelial cell (step 1, Figure 15.36) signals a rise in cytosolic Ca^{2+} concentration (step 2) that activates nitric oxide synthase (step 3). The NO formed in the endothelial cell diffuses across the plasma membrane and into the adjacent smooth muscle cells (step 4), where it binds and stimulates guanylyl cyclase (step 5), the enzyme that synthesizes cyclic GMP (cGMP), which is an important second messenger similar in structure to cAMP. Cyclic GMP binds to a cGMP-dependent protein kinase (a PKG), which phosphorylates specific substrates causing relaxation of the muscle cell (step 6) and dilation of the blood vessel.

NO as an Activator of Guanylyl Cyclase The discovery that NO acts as an activator of guanylyl cyclase was made in the late 1970s by Ferid Murad and colleagues at the University of Virginia. Murad was working with azide (N_3), a potent inhibitor of electron transport, and chanced to discover that the molecule stimulated cGMP production in cellular extracts. Murad and colleagues ultimately demonstrated that azide was being converted enzymatically into nitric oxide, which served as the actual guanylyl cyclase activator. These studies also explained the action of nitroglycerine, which had been used since the 1860s to treat the pain of angina that results from an inadequate flow of blood to the heart. Nitroglycerine is metabolized to nitric oxide, which stimulates the relaxation of the smooth muscles lining the blood vessels of the heart, increasing blood flow to the organ. The therapeutic benefits of nitroglycerine were discovered through an interesting observation. Persons with heart disease who worked with nitroglycerine in Alfred Nobel's dynamite factory were found to suffer more from the pain of angina on days they weren't at work. It is only fitting that the Nobel Prize, which is funded by a donation from Alfred Nobel's estate, was awarded in 1998 for the discovery of NO as a signaling agent.

Inhibiting Phosphodiesterase The discovery of NO as a second messenger has also led to the development of Viagra (sildenafil). During sexual arousal, nerve endings in the penis release NO, which causes relaxation of smooth muscle cells in the lining of penile blood vessels and engorgement of the organ with blood. As described above, NO mediates this response in smooth muscle cells by activation of the enzyme guanylyl cyclase and subsequent production of cGMP. Viagra (and related drugs) has no effect on the release of NO or the activation of guanylyl cyclase, but instead acts as an inhibitor of cGMP phosphodiesterase, the enzyme that destroys cGMP. Inhibition of this enzyme leads to maintained, elevated levels of cGMP, which promotes the development and maintenance of an erection. Viagra is quite specific for one particular isoform of cGMP phosphodiesterase, PDE5, which is the version that acts in the penis. Another isoform of the enzyme, PDE3, plays a key role in the regulation of heart muscle contraction, but fortunately is not inhibited by the drug. Viagra was discovered when a potential angina medication had unexpected side effects.

Recent investigations have revealed that NO has a variety of actions within the body that do not involve production of cGMP. For example, NO is added to the —SH group of certain cysteine residues in well over a hundred proteins, including hemoglobin, Ras, ryanodine channels, and caspases. This posttranslational modification, which is called *S-nitrosylation*, alters the activity, turnover, or interactions of the protein.

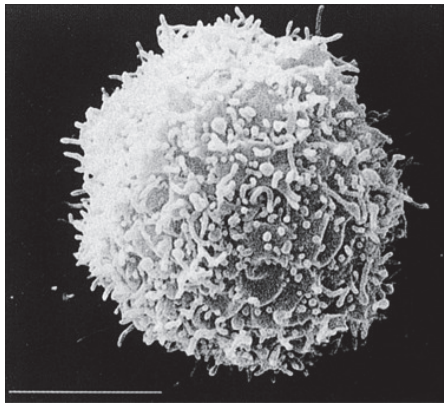
REVIEW

1. Describe the steps in the signaling pathway by which nitric oxide mediates dilation of blood vessels.

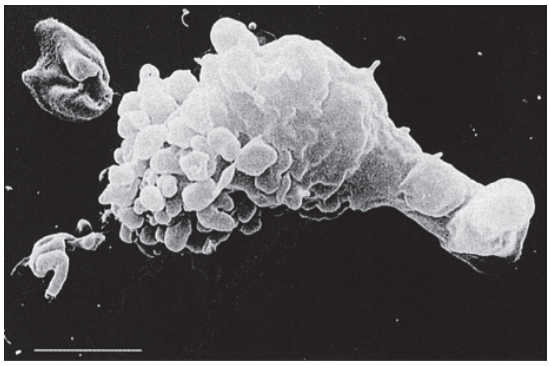
15.8 | Apoptosis (Programmed Cell Death)

Apoptosis, or programmed cell death, is a normal process that is unique to animal cells. Apoptosis occurs through an orchestrated sequence of events that leads to the death of a cell. Death by apoptosis is a neat, orderly process (Figure 15.37) characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighboring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments, and the rapid engulfment of the "corpse" by phagocytosis. Apoptosis is often contrasted with a different type of cell death called *necrosis,* which generally follows some type of physical trauma or biochemical insult. Like apoptosis, necrosis is generally considered to be a regulated and programmed process, although much less orderly in nature. Necrosis is characterized by the swelling of both the cell and its internal membranous organelles, membrane breakdown, leakage of cell contents into the medium, and the resulting induction of inflammation. Because it is a safe and orderly process, apoptosis might be compared to the controlled implosion of a building using carefully placed explosives as compared to simply blowing up the structure without concern for what happens to the flying debris.

Why do our bodies have unwanted cells, and where do we find cells that become targeted for elimination? The short answer is: almost anywhere you look. During embryonic development, the earliest form of the human hand resembles a paddle without any space between the tissues that will become the fingers. The fingers are essentially carved out of the paddle via the elimination of the excess cells by apoptosis. Three stages of this process as it occurs in mice are shown in Figure 15.38. T lymphocytes are cells of the immune system that recognize and kill abnormal or pathogen-infected target cells. These target cells are recognized by specific receptors that are present on the surfaces of T lymphocytes. During embryonic development, T lymphocytes are produced that possess receptors capable of binding tightly to proteins present on the surfaces of *normal* cells within the body. T lymphocytes that have this dangerous capability are eliminated by apoptosis (see Figure 17.25). Apoptosis does not stop with the end of embryonic development. It has been estimated that 10^{10}–10^{11} cells in the adult body die every day by apoptosis. For example, apoptosis is involved in the elimination of cells that have sustained irreparable genomic damage. This is important because damage to the genetic blueprint can result in unregulated cell division and the



(a)



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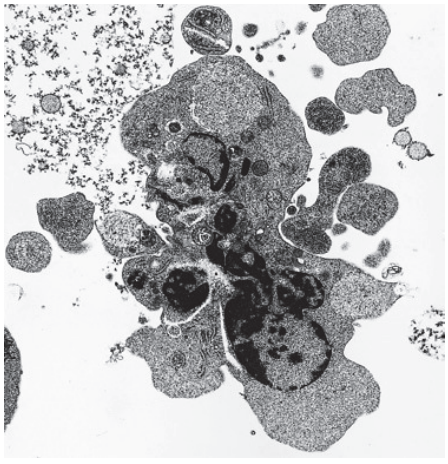

(c) 2 µm

Figure 15.37 **A comparison of normal and apoptotic cells.** (*a*,*b*) Scanning electron micrographs of a normal (*a*) and apoptotic (*b*) T-cell hybridoma. The apoptotic cell exhibits many surface blebs that are budded off in the cell. Bar equals 4 mm. (*c*) Transmission electron micrograph of an apoptotic cell treated with an inhibitor that arrests apoptosis at the membrane blebbing stage. (A,B: From S. J. Martin et al., Trends Biochem. Sci. 19:28, 1994. Reprinted with permission from Elsevier; C: courtesy of Nicola J. McCarthy.)

development of cancer. Apoptosis is also responsible for the death of cells that are no longer required, such as activated T cells that have responded to an infectious agent that has been eliminated. Finally, apoptosis appears to be involved in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Elimination of essential neurons during disease progression gives rise to loss of memory or decrease in motor coordination. These examples show that apoptosis is important in shaping tissues and organs during embryonic development and in maintaining homeostasis within the bodies of adult animals. Serious diseases can result from both the failure to carry out apoptosis when the elimination of cells (e.g., cancer cells) is appropriate and from the overactive induction of apoptosis when the elimination of cells is not appropriate (e.g., type 1 diabetes).

The term *apoptosis* was coined in 1972 by John Kerr, Andrew Wylie, and A. R. Currie of the University of Aberdeen, Scotland, in a landmark paper that described for the first time the coordinated events that occurred during the programmed death of a wide range of cells. Insight into the molecular basis of apoptosis was first revealed in studies on the nematode worm *C. elegans*, whose cells can be followed with absolute precision during embryonic development. Of the 1090 cells produced during the development of this worm, 131 cells are normally destined to die by apoptosis. In 1986, Robert Horvitz and his colleagues at the Massachusetts Institute of Technology discovered that worms carrying a mutation in the *CED-3* gene proceed through development without losing any of their cells to apoptosis. This finding suggested that the product of the *CED-3* gene played a crucial role in the process of apoptosis in this organism. Once a gene has been identified in one organism, such as a nematode, researchers can search for homologous genes in other organisms, such as humans or other mammals. The identification of the *CED-3* gene in nematodes led to the discovery of a homologous family of proteins in mammals, which are now called **caspases**. Caspases are a distinctive group of cysteine proteases (i.e., proteases with a key cysteine residue in their

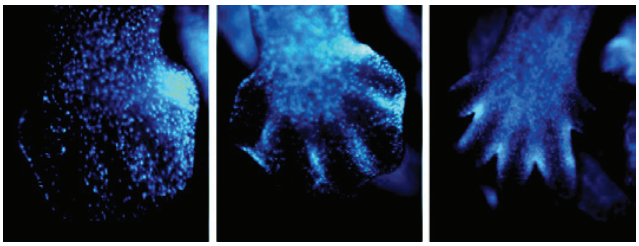

Figure 15.38 **Apoptosis carves out the structure of the mammalian digits.** Three stages in this process in a mouse embryo. In this particular mouse, which is called a MacBlue mouse, all of the embryonic macrophages express the cyan fluorescent protein. Fluorescent macrophages have infiltrated the regions of the footpad where apoptosis has occurred and are clearing the space between the digits. (From David A. Hume, Nature Immunol. 9:13, 2008; © 2008, Reprinted by permission from Macmillan Publishers Limited.)

catalytic site) that are activated at an early stage of apoptosis and are responsible for triggering most, if not all, of the changes observed during cell death. Caspases accomplish this feat by cleaving a select group of essential proteins. Among the targets of caspases are the following:

- *More than a dozen protein kinases, including focal adhesion kinase* (*FAK*), *PKB, PKC, and Raf1.* Inactivation of FAK, for example, is presumed to disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbors. Inactivation of certain other kinases, such as PKB, serves to disrupt prosurvival signaling pathways. Caspases also disrupt the generation of survival signals by inactivating the NF-κB pathway (page 660).
- *Lamins*, which make up the inner lining of the nuclear envelope. Cleavage of lamins leads to the disassembly of the nuclear lamina and shrinkage of the nucleus.
- *Proteins of the cytoskeleton*, such as those of intermediate filaments, actin, tubulin, and gelsolin. Cleavage and consequent inactivation of these proteins lead to changes in cell shape.
- *An endonuclease called caspase activated DNase* (*CAD*), which is activated following caspase cleavage of an inhibitory protein. Once activated, CAD translocates from the cytoplasm to the nucleus where it attacks DNA, severing it into fragments.

Recent studies have focused on the events that lead to the activation of a cell's suicide program. Apoptosis can be triggered by both internal stimuli, such as abnormalities in the DNA, and external stimuli, such as certain cytokines (proteins secreted by cells of the immune system). For example, epithelial cells of the prostate become apoptotic when deprived of the male sex hormone testosterone. This is the reason why prostate cancer that has spread to other tissues is often treated with drugs that interfere with testosterone production. Studies indicate that external stimuli activate apoptosis by a signaling pathway, called the *extrinsic pathway*, that is distinct from that utilized by internal stimuli, which is called the *intrinsic pathway*. Here we will discuss the extrinsic and intrinsic pathways separately. However, it should be noted that there is cross-talk between these pathways and that extracellular apoptotic signals can cause activation of the intrinsic pathway.

The Extrinsic Pathway of Apoptosis

The steps in the extrinsic pathway are illustrated in Figure 15.39. In the case depicted in the figure, the stimulus for apoptosis is carried by an extracellular messenger protein called tumor necrosis factor (TNF), which was named for its ability to kill tumor cells. TNF is a trimeric protein produced by certain cells of the immune system in response to adverse conditions, such as exposure to ionizing radiation, elevated temperature, viral infection, or toxic chemical agents such as those used in cancer chemotherapy. Like other types of first messengers discussed in this chapter, TNF evokes its response by binding to a transmembrane receptor, TNFR1. The trimeric TNFR1 protein is a member of a family of related "death receptors" that turns on the apoptotic process. The cytoplasmic domain of each TNF receptor subunit contains a segment of about 70 amino acids called a "death domain" (each green segment in Figure 15.39) that mediates protein–protein interactions. Binding of TNF to the trimeric receptor produces a change in conformation of the receptor's death domain, which leads to the recruitment of a number of proteins, as indicated in Figure 15.39.

The last proteins to join the complex that assembles at the inner surface of the plasma membrane are two procaspase-8

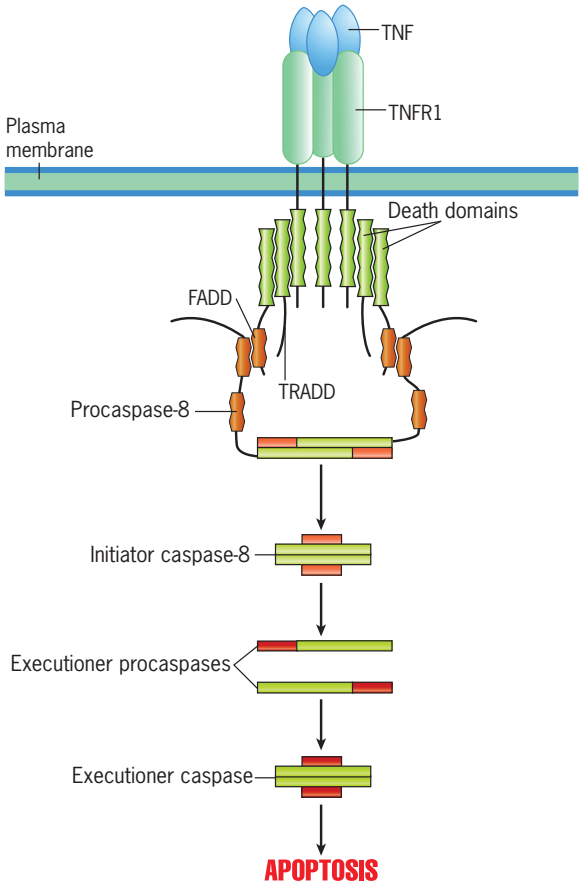

Figure 15.39 **A simplified model of the extrinsic (receptor-mediated) pathway of apoptosis.** When TNF binds to a TNF receptor (TNFR1), the activated receptor binds two different cytoplasmic adaptor proteins (TRADD and FADD) and procaspase-8 to form a multiprotein complex at the inner surface of the plasma membrane. The cytoplasmic domains of the TNF receptor, FADD, and TRADD interact with one another by homologous regions called death domains that are present in each protein (indicated as green boxes). Procaspase-8 and FADD interact by means of homologous regions called death effector domains (indicated as brown boxes). Once assembled in the complex, the two procaspase molecules cleave one another to generate an active caspase-8 molecule containing four polypeptide segments. Caspase-8 is an initiator complex that activates downstream (executioner) caspases that carry out the death sentence. It can be noted that the interaction between TNF and TNFR1 also activates other signaling pathways, one of which leads to cell survival rather than self-destruction (page 660).

molecules (Figure 15.39). These proteins are called "procaspases" because each is a precursor of a caspase; it contains an extra portion that must be removed by proteolytic processing to activate the enzyme. The synthesis of caspases as proenzymes protects the cell from accidental proteolytic damage. Unlike most proenzymes, procaspases exhibit a low level of proteolytic activity. According to one model, when two or more procaspases are held in close association with one another, as they are in Figure 15.39, they are capable of cleaving one another's polypeptide chain and converting the other molecule to the fully active caspase. The final mature enzyme (i.e., caspase-8) contains four polypeptide chains, derived from two procaspase precursors as illustrated in the figure.

Activation of caspase-8 is similar in principle to the activation of effectors by a hormone or growth factor. In all of these signaling pathways, the binding of an extracellular ligand causes a change in conformation of a receptor that leads to the binding and activation of proteins situated downstream in the pathway. Caspase-8 is described as an *initiator* caspase because it initiates apoptosis by cleaving and activating downstream, or *executioner*, caspases, that carry out the controlled self-destruction of the cell as described above.

The Intrinsic Pathway of Apoptosis

Internal stimuli, such as irreparable genetic damage, lack of oxygen (hypoxia), extremely high concentrations of cytosolic Ca^{2+}, viral infection, ER stress, or severe oxidative stress (i.e., the production of large numbers of destructive free radicals), trigger apoptosis by the intrinsic pathway illustrated in Figure 15.40. Activation of the intrinsic pathway is regulated by members of the Bcl-2 family of proteins, which are characterized by the presence of one or more small BH domains. Bcl-2 family members can be subdivided into three groups: (1) proapoptotic members (containing several BH domains) that promote apoptosis (Bax and Bak), (2) antiapoptotic members (containing several BH domains) that protect cells from apoptosis (e.g., Bcl-x_L, Bcl-w, and Bcl-2),[3] and (3) BH3-only proteins (so-named because they contain only one BH domain), which promote apoptosis by an indirect mechanism. According to the prevailing view, BH3-only proteins (e.g., Bid, Bad, Puma, and Bim) can exert their proapoptotic effect in two different ways, depending on the particular proteins involved. In some cases they promote apoptosis by inhibiting antiapoptotic Bcl-2 members, whereas in other cases they promote apoptosis by activating proapoptotic Bax or Bak. In either case, the BH3-only proteins are the likely determinants as to whether a cell follows a pathway of survival or death. In a healthy cell, the BH3-only proteins are either absent or strongly inhibited, and the antiapoptotic Bcl-2 proteins are able to restrain proapoptotic members. The mechanism by which this occurs is debated. It is only in the face of certain types of stress that the BH3-only proteins are expressed or activated, thereby shifting the balance in the direction of apoptosis. In these circumstances, the restraining effects of the antiapoptotic Bcl-2 proteins are overridden, and the proapop-

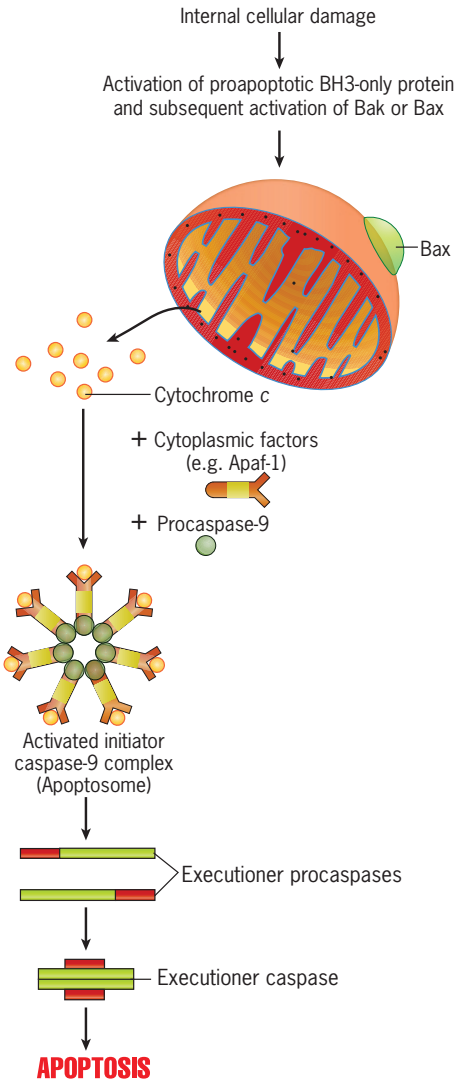

Figure 15.40 **The intrinsic (mitochondria-mediated) pathway of apoptosis.** Various types of cellular stress cause proapoptotic members of the Bcl-2 family of proteins–either Bax or Bak–to oligomerize within the outer mitochondrial membrane, forming channels that facilitate the release of cytochrome *c* molecules from the intermembrane space. Once in the cytosol, the cytochrome *c* molecules form a multisubunit complex with a cytosolic protein called Apaf-1 and procaspase-9 molecules. Procaspase-9 molecules are apparently activated to their full proteolytic capacity as the result of a conformational change induced by association with Apaf-1. Caspase-9 molecules cleave and activate executioner caspases, which carry out the apoptotic response. The intrinsic pathway can be triggered in some cells (e.g., hepatocytes) by extracellular signals. This occurs as the initiator caspase of the extrinsic pathway, caspase 8, cleaves a BH3-only protein called Bid, generating a protein fragment (tBid) that binds to Bax, inducing insertion of Bax into the OMM and release of cytochrome c from mitochondria.

[3]The first member of the family, Bcl-2 itself, was originally identified in 1985 as a cancer-causing oncogene in human lymphomas. The gene encoding Bcl-2 was overexpressed in these malignant cells as the result of a translocation. We now understand that Bcl-2 acts as an oncogene by promoting survival of potential cancer cells that would otherwise die by apoptosis.

totic protein Bax is free to translocate from the cytosol to the outer mitochondrial membrane (OMM). Although the mechanism is not entirely clear, it is thought that Bax molecules (and/or Bak molecules, which are permanent residents of the OMM) undergo a change in conformation that causes them to assemble into a multisubunit, protein-lined channel within the OMM. Once formed, this channel dramatically increases the permeability of the OMM and promotes the release of certain mitochondrial proteins, most notably cytochrome *c*, which resides in the intermembrane space (see Figure 5.17). Nearly all of the cytochrome *c* molecules present in all of a cell's mitochondria can be released from an apoptotic cell in a period as short as five minutes. Cells lacking *both* Bax and Bak are protected from apoptosis, revealing the essential roles of these proapoptotic proteins in this process.

Release of proapoptotic mitochondrial proteins such as cytochrome *c* is apparently the "point of no return," that is, an event that irreversibly commits the cell to apoptosis. Once in the cytosol, cytochrome *c* forms part of a wheel-shaped multiprotein complex called the *apoptosome*, that also includes several molecules of procaspase-9. Procaspase-9 molecules are thought to become activated by simply joining the multiprotein complex and do not require proteolytic cleavage (Figure 15.40). Like caspase-8, which is activated by the receptor-mediated pathway described above, caspase-9 is an initiator caspase that activates downstream executioner caspases, which bring about apoptosis.[4] The extrinsic (receptor-mediated) and intrinsic (mitochondria-mediated) pathways ultimately converge by activating the same executioner caspases, which cleave the same cellular targets.

You might be wondering why cytochrome *c*, a component of the electron transport chain, and the mitochondrion, an organelle that functions as the cell's power plant, would be involved in initiating apoptosis. There is no obvious answer to this question at the present time. The key role of mitochondria in apoptosis is even more perplexing when one considers that these organelles have evolved from prokaryotic endosymbionts and that prokaryotes do not undergo apoptosis.

As cells execute the apoptotic program, they lose contact with their neighbors and start to shrink. Finally, the cell disintegrates into a condensed, membrane-enclosed apoptotic body. This entire apoptotic program can be executed in less than an hour. The apoptotic bodies are recognized by the presence of phosphatidylserine on their surface. Phosphatidylserine is a phospholipid that is normally present only on the inner leaflet of the plasma membrane. During apoptosis, a phospholipid "scramblase" moves phosphatidylserine molecules to the outer leaflet of the plasma membrane where they are recognized as an "eat me" signal by specialized macrophages. Apoptotic cell death thus occurs without spilling cellular content into the extracellular environment (Figure 15.41). This is important because the release of cellular debris can trigger inflammation, which can cause a significant amount of tissue damage.

[4]Other intrinsic pathways that are independent of Apaf-1 and caspase-9, and possibly independent of cytochrome *c*, have also been described.

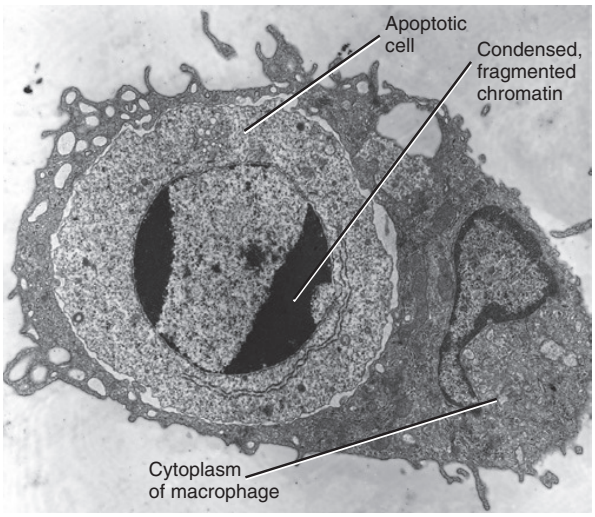

Figure 15.41 **Clearance of apoptotic cells is accomplished by phagocytosis.** This electron micrograph shows an apoptotic cell "corpse" within the cytoplasm of a phagocyte. Note the compact nature of the engulfed cell and the dense state of its chromatin. (From Peter M. Henson, Donna L. Bratton, and Valerie A. Fadok, Curr. Biol. 11:R796, 2001, Fig. 1A, © 2001, with permission from Elsevier.)

Signaling Cell Survival Just as there are signals that commit a cell to self-destruction, there are opposing signals that maintain cell survival. In fact, interaction of TNF with a TNF receptor often transmits two distinct and opposing signals into the cell interior: one stimulating apoptosis and another stimulating cell survival. As a result, most cells that possess TNF receptors do not undergo apoptosis when treated with TNF. This was a disappointing finding because it was originally hoped that TNF could be used as an agent to kill tumor cells. Cell survival is typically mediated through the activation of a key transcription factor called NF-κB, which activates the expression of genes encoding cell-survival proteins. It would appear that the fate of a cell—whether survival or death—depends on a delicate balance between proapoptotic and antiapoptotic signals.

REVIEW

1. What are some of the functions of apoptosis in vertebrate biology? Describe the steps that occur between (a) the time that a TNF molecule binds to its receptor and the eventual death of the cell and (b) the time a proapoptotic Bcl-2 member binds to the outer mitochondrial membrane and the death of the cell.
2. What is the role of the formation of caspase-containing complexes in the process of apoptosis?