CHAPTER **FIFTEEN** 15

Intracellular Compartments and Protein Transport

At any one time, a typical eukaryotic cell carries out thousands of different chemical reactions, many of which are mutually incompatible. One series of reactions makes glucose, for example, while another breaks it down; some enzymes synthesize peptide bonds, whereas others hydrolyze them, and so on. Indeed, if the cells of an organ such as the liver are broken apart and their contents mixed together in a test tube, chemical chaos results, and the cells' enzymes and other proteins are quickly degraded by their own proteolytic enzymes. For a cell to operate effectively, the different intracellular processes that occur simultaneously must somehow be segregated.

Cells have evolved several strategies for isolating and organizing their chemical reactions. One strategy used by both prokaryotic and eukaryotic cells is to aggregate the different enzymes required to catalyze a particular sequence of reactions into large, multicomponent complexes. Such complexes are used, for example, in the synthesis of DNA, RNA, and proteins. A second strategy, which is most highly developed in eukaryotic cells, is to confine different metabolic processes—and the proteins required to perform them—within different membrane-enclosed compartments. As discussed in Chapters 11 and 12, cell membranes provide selectively permeable barriers through which the transport of most molecules can be controlled. In this chapter, we consider this strategy of membrane-dependent compartmentalization.

In the first section, we describe the principal membrane-enclosed compartments, or *membrane-enclosed organelles*, of eukaryotic cells and briefly consider their main functions. In the second section, we discuss how the protein composition of the different compartments is set up and

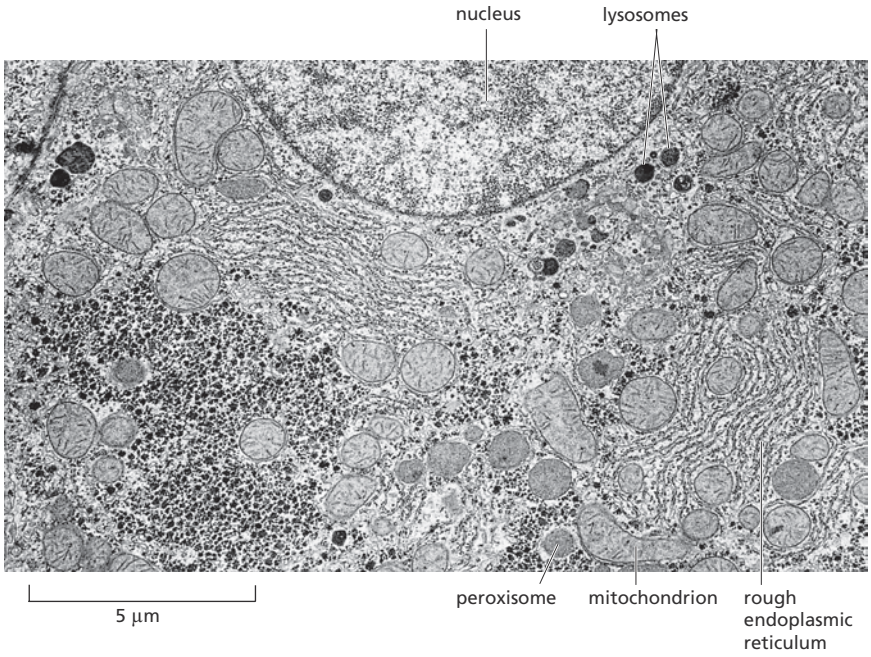


Figure 15–1 In eukaryotic cells, internal membranes create enclosed compartments that segregate different metabolic processes. Examples of many of the major membrane-enclosed organelles can be identified in this electron micrograph of part of a liver cell, seen in cross section. The small black granules between the compartments are aggregates of glycogen and the enzymes that control its synthesis and breakdown. (Courtesy of Daniel S. Friend.)

maintained. Each compartment contains a unique set of proteins that have to be transferred selectively from the cytosol, where they are made, to the compartment where they are used. This transfer process, called *protein sorting*, depends on signals built into the amino acid sequence of the proteins. In the third section, we describe how certain membrane-enclosed compartments in a eukaryotic cell communicate with one another by forming small, membrane-enclosed sacs, or *vesicles*. These vesicles pinch off from one compartment, move through the cytosol, and fuse with another compartment in a process called *vesicular transport*. In the last two sections, we discuss how this constant vesicular traffic also provides the main routes for releasing proteins from the cell by the process of *exocytosis* and for importing them by the process of *endocytosis*.

MEMBRANE-ENCLOSED ORGANELLES

Whereas a prokaryotic cell usually consists of a single compartment enclosed by the plasma membrane, eukaryotic cells are elaborately subdivided by internal membranes. When a cross section through a plant or an animal cell is examined in the electron microscope, numerous small, membrane-enclosed sacs, tubes, spheres, and irregularly shaped structures can be seen, often arranged without much apparent order (**Figure 15–1**). These structures are all distinct, membrane-enclosed organelles, or parts of such organelles, each of which contains a unique set of large and small molecules and carries out a specialized function. In this section, we review these functions and discuss how different membrane-enclosed organelles may have evolved.

Eukaryotic Cells Contain a Basic Set of Membrane-enclosed Organelles

The major **membrane-enclosed organelles** of an animal cell are illustrated in **Figure 15–2**, and their functions are summarized in **Table 15–1**. These organelles are surrounded by the *cytosol*, which is enclosed by the plasma membrane. The *nucleus* is generally the most prominent organelle in eukaryotic cells. It is surrounded by a double membrane, known as the *nuclear envelope*, and communicates with the cytosol via *nuclear pores* that perforate the envelope. The outer nuclear membrane

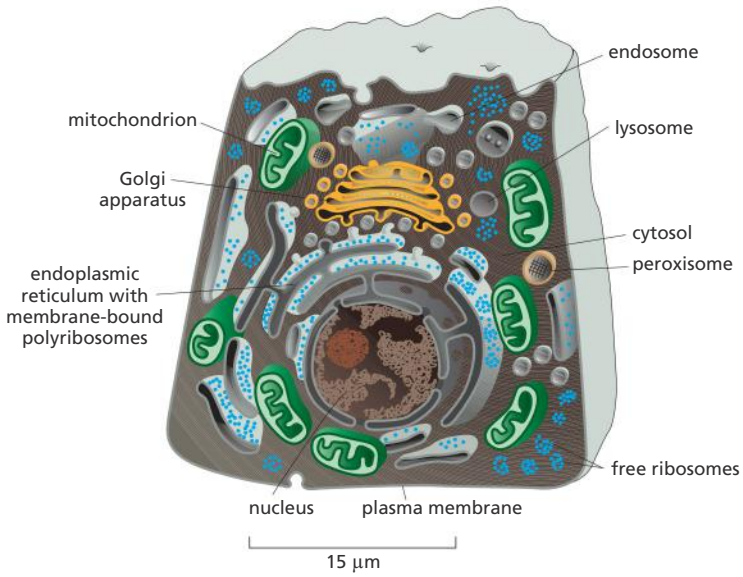


Figure 15–2 A cell from the lining of the intestine contains the basic set of membrane-enclosed organelles found in most animal cells. The nucleus, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, endosomes, mitochondria, and peroxisomes are distinct compartments separated from the cytosol (*gray*) by at least one selectively permeable membrane. Ribosomes are shown bound to the cytosolic surface of portions of the ER, called the rough ER; the ER that lacks ribosomes is called smooth ER. Additional ribosomes can be found free in the cytosol.

is continuous with the membrane of the *endoplasmic reticulum* (*ER*), a system of interconnected sacs and tubes of membrane that often extends throughout most of the cell. The ER is the major site of synthesis of new membranes in the cell. Large areas of the ER have ribosomes attached to the cytosolic surface and are designated *rough endoplasmic reticulum* (*rough ER*). The ribosomes are actively synthesizing proteins that are delivered into the ER membrane or into the ER interior, a space called the *lumen*. The *smooth endoplasmic reticulum* (*smooth ER*) lacks ribosomes. It is scanty in most cells but is highly developed for performing particular functions in others: for example, it is the site of steroid hormone synthesis in some endocrine cells of the adrenal gland and the site where a variety of organic molecules, including alcohol, are detoxified in liver cells. In many eukaryotic cells, the smooth ER also sequesters Ca^{2+} from the cytosol; the release and reuptake of Ca^{2+} from the ER is involved in the rapid response to many extracellular signals, as discussed in Chapters 12 and 16.

TABLE 15–1 THE MAIN FUNCTIONS OF MEMBRANE-ENCLOSED COMPARTMENTS OF A EUKARYOTIC CELL

Compartment	Main Function
Cytosol	contains many metabolic pathways (Chapters 3 and 13); protein synthesis (Chapter 7); the cytoskeleton (Chapter 17)
Nucleus	contains main genome (Chapter 5); DNA and RNA synthesis (Chapters 6 and 7)
Endoplasmic reticulum (ER)	synthesis of most lipids (Chapter 11); synthesis of proteins for distribution to many organelles and to the plasma membrane (this chapter)
Golgi apparatus	modification, sorting, and packaging of proteins and lipids for either secretion or delivery to another organelle (this chapter)
Lysosomes	intracellular degradation (this chapter)
Endosomes	sorting of endocytosed material (this chapter)
Mitochondria	ATP synthesis by oxidative phosphorylation (Chapter 14)
Chloroplasts (in plant cells)	ATP synthesis and carbon fixation by photosynthesis (Chapter 14)
Peroxisomes	oxidation of toxic molecules

The *Golgi apparatus*, which is usually situated near the nucleus, receives proteins and lipids from the ER, modifies them, and then dispatches them to other destinations in the cell. Small sacs of digestive enzymes called *lysosomes* degrade worn-out organelles, as well as macromolecules and particles taken into the cell by endocytosis. On their way to lysosomes, endocytosed materials must first pass through a series of compartments called *endosomes*, which sort the ingested molecules and recycle some of them back to the plasma membrane. *Peroxisomes* are small organelles that contain enzymes used in a variety of oxidative reactions that break down lipids and destroy toxic molecules. *Mitochondria* and (in plant cells) *chloroplasts* are each surrounded by a double membrane and are the sites of oxidative phosphorylation and photosynthesis, respectively (discussed in Chapter 14); both contain internal membranes that are highly specialized for the production of ATP.

Many of the membrane-enclosed organelles, including the ER, Golgi apparatus, mitochondria, and chloroplasts, are held in their relative locations in the cell by attachment to the cytoskeleton, especially to microtubules. Cytoskeletal filaments provide tracks for moving the organelles around and for directing the traffic of vesicles between one organelle and another. These movements are driven by motor proteins that use the energy of ATP hydrolysis to propel the organelles and vesicles along the filaments, as discussed in Chapter 17.

On average, the membrane-enclosed organelles together occupy nearly half the volume of a eukaryotic cell (**Table 15–2**), and the total amount of membrane associated with them is enormous. In a typical mammalian cell, for example, the area of the endoplasmic reticulum membrane is 20–30 times greater than that of the plasma membrane. In terms of its area and mass, the plasma membrane is only a minor membrane in most eukaryotic cells.

Much can be learned about the composition and function of an organelle once it has been isolated from other cell structures. For the most part, organelles are far too small to be isolated by hand, but it is possible to separate one type of organelle from another by differential centrifugation (described in Panel 4–3, pp. 164–165). Once a purified sample of one type of organelle has been obtained, the organelle's proteins can be identified. In many cases, the organelle itself can be incubated in a test tube under conditions that allow its functions to be studied. Isolated mitochondria, for example, can produce ATP from the oxidation of pyruvate to CO_2 and water, provided they are adequately supplied with ADP, inorganic phosphate, and O_2.

TABLE 15–2 THE RELATIVE VOLUMES AND NUMBERS OF THE MAJOR MEMBRANE-ENCLOSED ORGANELLES IN A LIVER CELL (HEPATOCYTE)

Intracellular Compartment	Percentage of Total Cell Volume	Approximate Number per Cell
Cytosol	54	1
Mitochondria	22	1700
Endoplasmic reticulum	12	1
Nucleus	6	1
Golgi apparatus	3	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

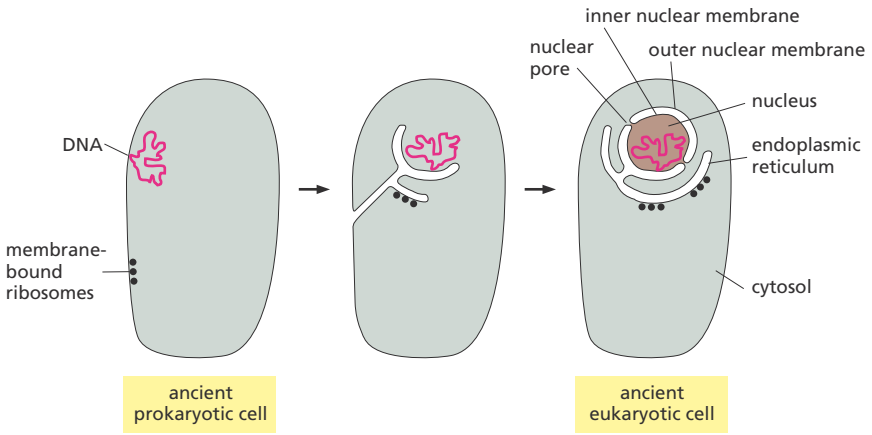


Figure 15–3 Nuclear membranes and the ER may have evolved through invagination of the plasma membrane. In bacteria, the single DNA molecule is typically attached to the plasma membrane. It is possible that in a very ancient prokaryotic cell, the plasma membrane, with its attached DNA, could have invaginated and, in subsequent generations, formed a two-layered envelope of membrane completely surrounding the DNA. This envelope is presumed to have eventually pinched off completely from the plasma membrane, ultimately producing a nuclear compartment penetrated by channels called nuclear pores, which enable communication with the cytosol. Other portions of the invaginated membrane may have formed the ER, which would explain why the space between the inner and outer nuclear membranes is continuous with the ER lumen.

Membrane-enclosed Organelles Evolved in Different Ways

In trying to understand the relationships between the different compartments of a modern eukaryotic cell, it is helpful to consider how they evolved. The compartments probably evolved in stages. The precursors of the first eukaryotic cells are thought to have been simple microorganisms, resembling bacteria, which had a plasma membrane but no internal membranes. The plasma membrane in such cells would have provided all membrane-dependent functions, including ATP synthesis and lipid synthesis, as does the plasma membrane in most modern bacteria. Bacteria can get by with this arrangement because of their small size, which gives them a high surface-to-volume ratio: their plasma membrane area is thus sufficient to sustain all the vital functions for which membranes are required. Present-day eukaryotic cells, by contrast, have volumes 1000 to 10,000 times greater than that of a typical bacterium such as *E. coli.* Such a large cell has a small surface-to-volume ratio and presumably could not survive with a plasma membrane as its only membrane. Thus, the increase in size typical of eukaryotic cells probably could not have occurred without the development of internal membranes.

Membrane-enclosed organelles are thought to have arisen in evolution in at least two ways. The nuclear membranes and the membranes of the ER, Golgi apparatus, endosomes, and lysosomes most likely originated by invagination of the plasma membrane, as illustrated for the nuclear and ER membranes in **Figure 15–3**. The ER, Golgi apparatus, peroxisomes, endosomes, and lysosomes are all part of what is collectively called the **endomembrane system**. As we discuss later, the interiors of these organelles communicate extensively with one another and with the outside of the cell by means of small vesicles that bud off from one of these organelles and fuse with another. Consistent with this proposed evolutionary origin, the interiors of these organelles are treated by the cell in many ways as "extracellular," as we will see. The hypothetical scheme shown in Figure 15–3 also explains why the nucleus is surrounded by two membranes.

Mitochondria and chloroplasts are thought to have originated in a different way. They differ from all other organelles in that they possess their own small genomes and can make some of their own proteins, as discussed in Chapter 14. The similarity of their genomes to those of bacteria and the close resemblance of some of their proteins to bacterial proteins strongly suggest that both these organelles evolved from bacteria that were engulfed by primitive pre-eukaryotic cells with which they initially lived in symbiosis (**Figure 15–4**). As might be expected from their origins, mitochondria and chloroplasts remain isolated from the extensive vesicular traffic that connects the interiors of most of the other membrane-enclosed organelles to one another and to the outside of the cell.

QUESTION 15–1

As shown in the drawings in Figure 15–3, the lipid bilayer of the inner and outer nuclear membranes forms a continuous sheet, joined around the nuclear pores. As membranes are two-dimensional fluids, this would imply that membrane proteins can diffuse freely between the two nuclear membranes. Yet each of these two nuclear membranes has a different protein composition, reflecting different functions. How could you reconcile this apparent contradiction?

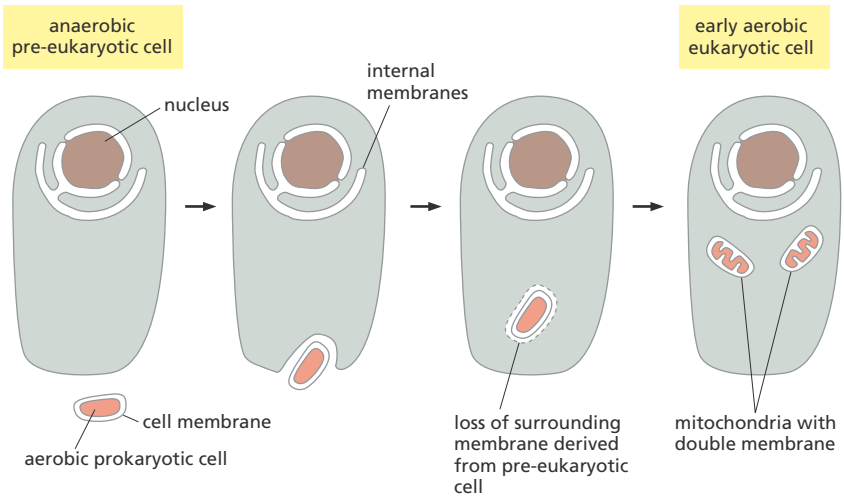


Figure 15–4 Mitochondria are thought to have originated when an aerobic prokaryote was engulfed by a larger pre-eukaryotic cell. Chloroplasts are thought to have originated later in a similar way, when a eukaryotic cell with mitochondria engulfed a photosynthetic prokaryote. This theory would explain why these organelles have two membranes, possess their own genomes, and do not participate in the vesicular traffic that connects the compartments of the endomembrane system.

PROTEIN SORTING

Before a eukaryotic cell divides, it must duplicate its membrane-enclosed organelles. As cells grow, membrane-enclosed organelles enlarge by incorporation of new molecules; the organelles then divide and, during cell division, are distributed between the two daughter cells. Organelle growth requires a supply of new lipids to make more membrane and a supply of the appropriate proteins—both membrane proteins and the soluble proteins that will occupy the interior of the organelle. Even in cells that are not dividing, proteins are being produced continually. These newly synthesized proteins must be accurately delivered to their appropriate organelle—some for eventual secretion from the cell and some to replace organelle proteins that have been degraded. Directing newly made proteins to their correct organelle is therefore necessary for any cell to grow and divide, or just to function properly.

For some organelles, including mitochondria, chloroplasts, peroxisomes, and the interior of the nucleus, proteins are delivered directly from the cytosol. For others, including the Golgi apparatus, lysosomes, endosomes, and the inner nuclear membrane, proteins and lipids are delivered indirectly via the ER, which is itself a major site of lipid and protein synthesis. Proteins enter the ER directly from the cytosol: some are retained there, but most are transported by vesicles to the Golgi apparatus and then onward to the plasma membrane or other organelles. Peroxisomes acquire some of their membrane proteins from the ER, but the bulk of their enzymes enter directly from the cytosol.

In this section, we discuss the mechanisms by which proteins directly enter membrane-enclosed organelles from the cytosol. Proteins made in the cytosol are dispatched to different locations in the cell according to specific address labels contained in their amino acid sequence. Once at the correct address, the protein enters either the membrane or the interior lumen of its designated organelle.

Proteins Are Transported into Organelles by Three Mechanisms

The synthesis of virtually all proteins in the cell begins on ribosomes in the cytosol. The exceptions are the few mitochondrial and chloroplast proteins that are synthesized on ribosomes inside these organelles; most mitochondrial and chloroplast proteins, however, are made in the cytosol and subsequently imported. The fate of any protein molecule synthesized in the cytosol depends on its amino acid sequence, which

can contain a *sorting signal* that directs the protein to the organelle in which it is required. Proteins that lack such signals remain as permanent residents in the cytosol; those that possess a sorting signal move from the cytosol to the appropriate organelle. Different sorting signals direct proteins into the nucleus, mitochondria, chloroplasts (in plants), peroxisomes, and the ER.

When a membrane-enclosed organelle imports a water-soluble protein to its interior—either from the cytosol or from another organelle—it faces a problem: how can it transport the protein across its membrane (or membranes), which are normally impermeable to hydrophilic macromolecules? This task is accomplished in different ways by different organelles.

1. Proteins moving from the cytosol into the nucleus are transported through the nuclear pores, which penetrate both the inner and outer nuclear membranes. The pores function as selective gates that actively transport specific macromolecules but also allow free diffusion of smaller molecules (mechanism 1 in **Figure 15–5**).
2. Proteins moving from the cytosol into the ER, mitochondria, or chloroplasts are transported across the organelle membrane by *protein translocators* located in the membrane. Unlike transport through nuclear pores, the transported protein must usually unfold in order to snake across the membrane through the translocator (mechanism 2 in Figure 15–5). Bacteria have similar protein translocators in their plasma membrane, which they use to export proteins from the cytosol to the cell exterior.
3. Proteins moving onward from the ER—and from one compartment of the endomembrane system to another—are transported by a mechanism that is fundamentally different. These proteins are ferried by *transport vesicles*, which pinch off from the membrane of one compartment and then fuse with the membrane of a second

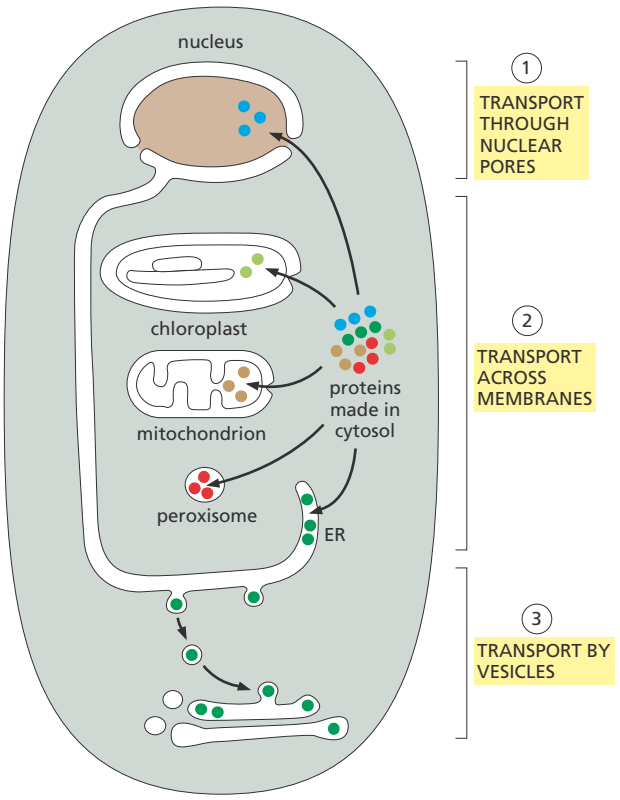


Figure 15–5 Membrane-enclosed organelles import proteins by one of three mechanisms. All of these processes require energy. The protein remains folded during transport in mechanisms 1 and 3 but usually has to be unfolded during mechanism 2.

TABLE 15–3 SOME TYPICAL SIGNAL SEQUENCES

Function of Signal	Example of Signal Sequence
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids are shown in *red* and negatively charged amino acids in *blue*. Important hydrophobic amino acids are shown in *green*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

compartment (mechanism 3 in Figure 15–5). In this process, transport vesicles deliver soluble cargo proteins, as well as the proteins and lipids that are part of the vesicle membrane.

Signal Sequences Direct Proteins to the Correct Compartment

The typical sorting signal on a protein is a continuous stretch of amino acid sequence, typically 15–60 amino acids long. This **signal sequence** is often (but not always) removed from the finished protein once it has been sorted. Some of the signal sequences used to specify different destinations in the cell are shown in **Table 15–3**.

Signal sequences are both necessary and sufficient to direct a protein to a particular destination. This has been shown by experiments in which the sequence is either deleted or transferred from one protein to another by genetic engineering techniques (discussed in Chapter 10). Deleting a signal sequence from an ER protein, for example, converts it into a cytosolic protein, while placing an ER signal sequence at the beginning of a cytosolic protein redirects the protein to the ER (**Figure 15–6**). The signal sequences specifying the same destination can vary greatly even though they have the same function: physical properties such as hydrophobicity

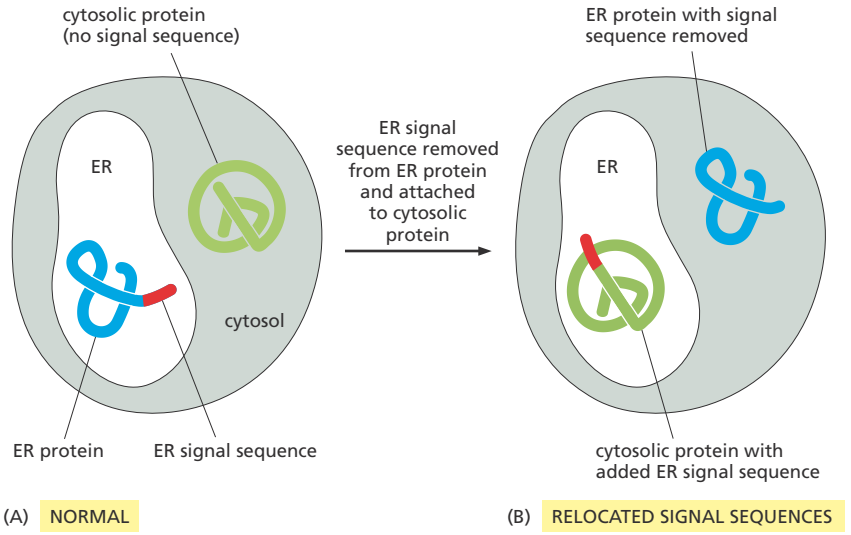


Figure 15–6 Signal sequences direct proteins to the correct destination. (A) Proteins destined for the ER possess an N-terminal signal sequence that directs them to that organelle, whereas those destined to remain in the cytosol lack any such signal sequence. (B) Recombinant DNA techniques can be used to change the destination of the two proteins: if the signal sequence is removed from an ER protein and attached to a cytosolic protein, both proteins are reassigned to the expected, inappropriate location.

or the placement of charged amino acids often appear to be more important for the function of these signals than the exact amino acid sequence.

Proteins Enter the Nucleus Through Nuclear Pores

The **nuclear envelope**, which encloses the nuclear DNA and defines the nuclear compartment, is formed from two concentric membranes. The *inner nuclear membrane* contains some proteins that act as binding sites for the chromosomes (discussed in Chapter 5) and others that provide anchorage for the *nuclear lamina*, a finely woven meshwork of protein filaments that lines the inner face of this membrane and provides structural support for the nuclear envelope (discussed in Chapter 17). The composition of the *outer nuclear membrane* closely resembles the membrane of the ER, with which it is continuous (**Figure 15–7**).

The nuclear envelope in all eukaryotic cells is perforated by **nuclear pores** that form the gates through which molecules enter or leave the nucleus. A nuclear pore is a large, elaborate structure composed of a complex of about 30 different proteins (**Figure 15–8**). Many of the proteins that line the nuclear pore contain extensive, unstructured regions in which the polypeptide chains are largely disordered. These disordered segments form a soft, tangled meshwork—like a kelp forest—that fills the center of the channel, preventing the passage of large molecules but allowing small, water-soluble molecules to pass freely and nonselectively between the nucleus and the cytosol.

Selected larger molecules and macromolecular complexes also need to pass through nuclear pores. RNA molecules, which are synthesized in the nucleus, and ribosomal subunits, which are assembled there, must be exported to the cytosol (discussed in Chapter 7). And newly made proteins that are destined for the nucleus must be imported from the cytosol (**Movie 15.1**). To gain entry to a pore, these large molecules and

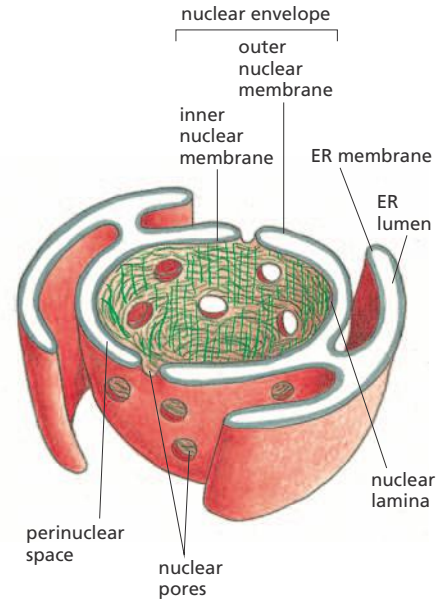
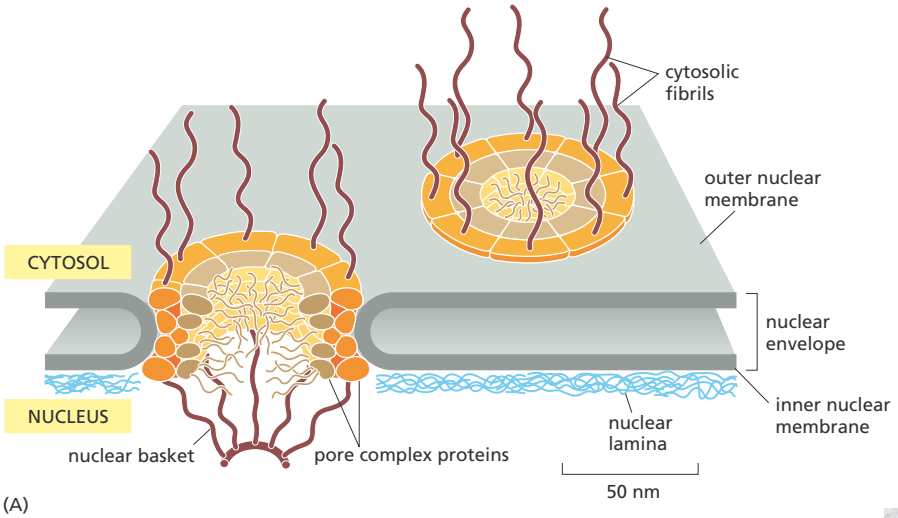


Figure 15–7 The outer nuclear membrane is continuous with the ER membrane. The double membrane of the nuclear envelope is penetrated by nuclear pores. The ribosomes that are normally bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown.



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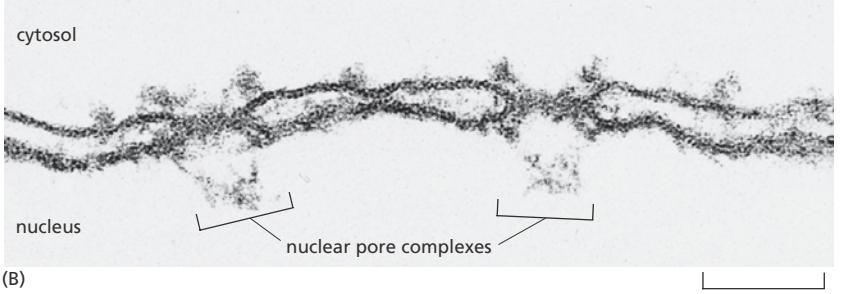

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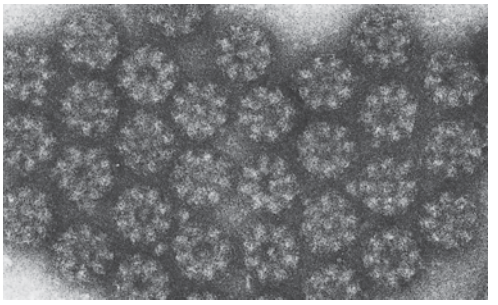


(C)

Figure 15–8 The nuclear pore complex forms a gate through which selected macromolecules and larger complexes enter or exit the nucleus. (A) Drawing of a small region of the nuclear envelope showing two pores. Protein fibrils protrude from both sides of the pore complex; on the nuclear side, they converge to form a basketlike structure. The spacing between the fibrils is wide enough that the fibrils do not obstruct access to the pores. (B) Electron micrograph of a region of nuclear envelope showing a side view of two nuclear pores (brackets). (C) Electron micrograph showing a face-on view of nuclear pore protein complexes; the membranes have been extracted with detergent. (B, courtesy of Werner W. Franke; C, courtesy of Ron Milligan.)

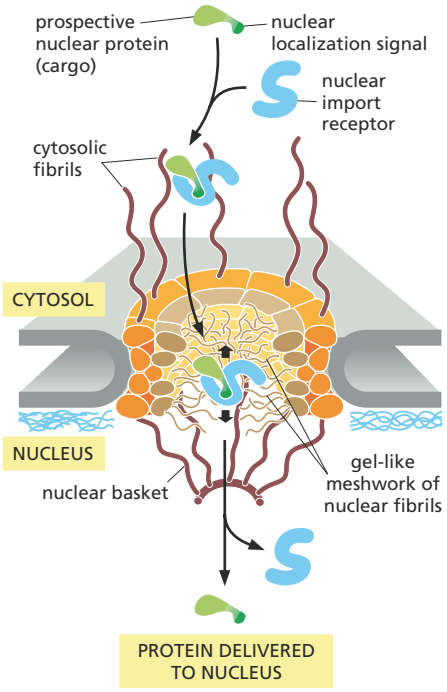


Figure 15–9 Prospective nuclear proteins are imported from the cytosol through nuclear pores. The proteins contain a nuclear localization signal that is recognized by nuclear import receptors, which interact with the cytosolic fibrils that extend from the rim of the pore. As indicated by the short *black* arrows, after being captured, the receptors move randomly with their cargo through the gel-like meshwork of nuclear fibrils, until nuclear entry triggers cargo release. After cargo delivery, the receptors return to the cytosol via nuclear pores for reuse. Similar types of transport receptors, operating in the reverse direction, export mRNAs from the nucleus (see Figure 7–23). These sets of import and export receptors have a similar basic structure.

macromolecular complexes must display an appropriate sorting signal. The signal sequence that directs a protein from the cytosol into the nucleus, called a *nuclear localization signal*, typically consists of one or two short sequences containing several positively charged lysines or arginines (see Table 15–3).

The nuclear localization signal on proteins destined for the nucleus is recognized by cytosolic proteins called *nuclear import receptors*. These receptors help direct a newly synthesized protein to a nuclear pore by interacting with the tentacle-like fibrils that extend from the rim of the pore into the cytosol (**Figure 15–9**). Once there, the nuclear import receptor penetrates the pore by grabbing onto short, repeated amino acid sequences within the tangle of nuclear pore proteins that fill the center of the pore. When the nuclear pore is empty, these repeated sequences bind to one another, forming a loosely packed gel. Nuclear import receptors interrupt these interactions, and they open a local passageway through the meshwork. The import receptors simply bump along from one repeat sequence to the next, until they enter the nucleus and deliver their cargo. The empty receptor then returns to the cytosol via the nuclear pore for reuse (see Figure 15–9).

Like any process that creates order, the import of nuclear proteins requires energy. In this case, the energy is provided by the hydrolysis of GTP, mediated by a monomeric GTPase named Ran. This GTP hydrolysis drives nuclear transport in the appropriate direction, as shown in **Figure 15–10**. Nuclear pore proteins operate this molecular gate at an amazing speed, rapidly pumping macromolecules in both directions through each pore.

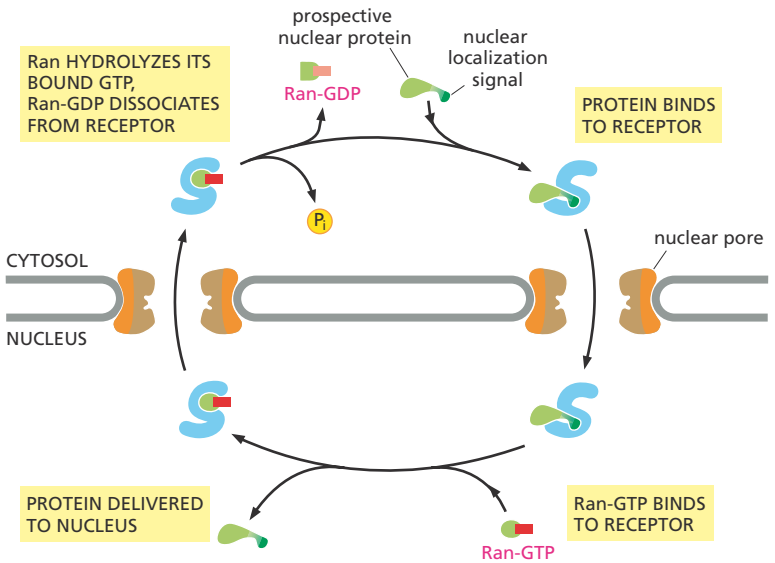


Figure 15–10 Energy supplied by GTP hydrolysis drives nuclear transport. A nuclear import receptor picks up a prospective nuclear protein in the cytosol and enters the nucleus. There it encounters a small monomeric GTPase called Ran, which carries a molecule of GTP. This Ran-GTP binds to the import receptor, causing it to release the nuclear protein. Having discharged its cargo in the nucleus, the receptor—still carrying Ran-GTP—is transported back through the pore to the cytosol. There, an accessory protein (not shown) triggers Ran to hydrolyze its bound GTP. Ran-GDP falls off the import receptor, which is then free to bind another protein destined for the nucleus. A similar cycle operates to export mRNAs and ribosomal subunits from the nucleus into the cytosol, using nuclear export receptors that recognize nuclear export signals (see Table 15–3).

Nuclear pores transport proteins in their fully folded conformation and ribosomal components as assembled particles. This feature distinguishes the nuclear transport mechanism from the mechanisms that transport proteins into most other organelles. Proteins have to unfold to cross the membranes of mitochondria and chloroplasts, as we discuss next.

Proteins Unfold to Enter Mitochondria and Chloroplasts

Both mitochondria and chloroplasts are surrounded by inner and outer membranes, and both organelles specialize in the synthesis of ATP. Chloroplasts also contain a third membrane system, the thylakoid membrane (discussed in Chapter 14). Although both organelles contain their own genomes and make some of their own proteins, most mitochondrial and chloroplast proteins are encoded by genes in the nucleus and are imported from the cytosol. These proteins usually have a signal sequence at their N-terminus that allows them to enter their specific organelle. Proteins destined for either organelle are translocated simultaneously across both the inner and outer membranes at specialized sites where the two membranes contact each other. Each protein is unfolded as it is transported, and its signal sequence is removed after translocation is complete (**Figure 15–11**).

Chaperone proteins (discussed in Chapter 4) inside the organelles help to pull the protein across the two membranes and to fold it once it is inside. Subsequent transport to a particular site within the organelle, such as the inner or outer membrane or the thylakoid membrane in chloroplasts, usually requires further sorting signals in the protein, which are often only exposed after the first signal sequence has been removed. The insertion of transmembrane proteins into the inner membrane, for example, is guided by signal sequences in the protein that start and stop the transfer process across the membrane, as we describe later for the insertion of transmembrane proteins in the ER membrane.

The growth and maintenance of mitochondria and chloroplasts require not only the import of new proteins but also the incorporation of new lipids into the organelle membranes. Most of their membrane phospholipids are thought to be imported from the ER, which is the main site of lipid synthesis in the cell. Phospholipids are transported to these organelles

QUESTION 15–2

Why do eukaryotic cells require a nucleus as a separate compartment when prokaryotic cells can manage perfectly well without?

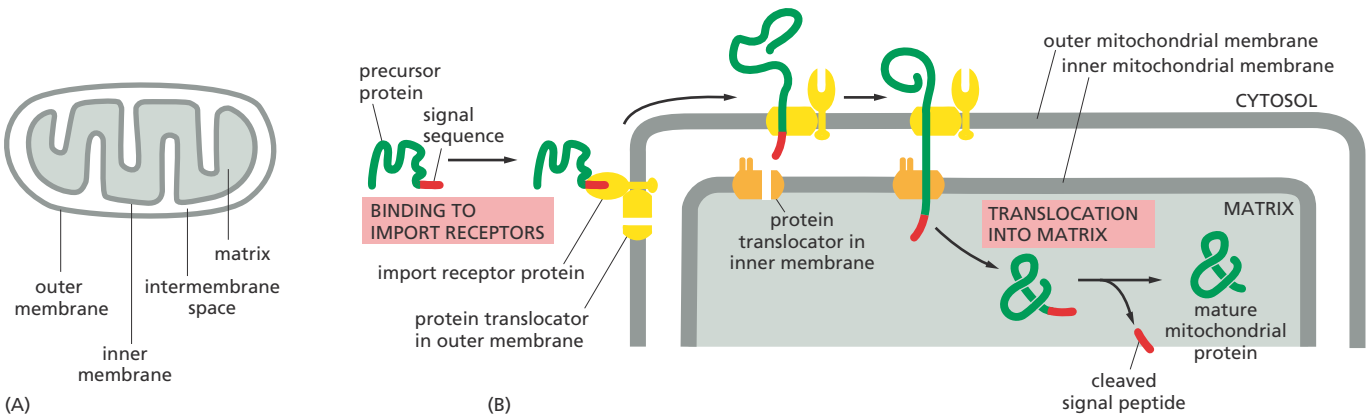


Figure 15–11 Mitochondrial precursor proteins are unfolded during import. (A) A mitochondrion has an outer and inner membrane, both of which must be crossed for a mitochondrial precursor protein to enter the organelle. (B) To initiate transport, the mitochondrial signal sequence on a mitochondrial precursor protein is recognized by a receptor in the outer mitochondrial membrane. This receptor is associated with a protein translocator. The complex of receptor, precursor protein, and translocator diffuses laterally in the outer membrane until it encounters a second translocator in the inner membrane. The two translocators then transport the protein across both the outer and inner membranes, unfolding the protein in the process (**Movie 15.2**). The signal sequence is finally cleaved off by a signal peptidase in the mitochondrial matrix. Proteins are imported into chloroplasts by a similar mechanism. The chaperone proteins that help pull the protein across the membranes and help it to refold are not shown.

by lipid-carrying proteins that extract a phospholipid molecule from one membrane and deliver it into another. Such transport may occur at specific junctions where mitochondrial and ER membranes are held in close proximity. Thanks to these lipid-carrying proteins, the different cell membranes are able to maintain different lipid compositions.

Proteins Enter Peroxisomes from Both the Cytosol and the Endoplasmic Reticulum

Peroxisomes generally contain one or more enzymes that produce hydrogen peroxide, hence their name. These organelles are present in all eukaryotic cells, where they break down a variety of molecules, including toxins, alcohol, and fatty acids. They also synthesize certain phospholipids, including those that are abundant in the myelin sheath that insulates nerve cell axons.

Peroxisomes acquire the bulk of their proteins via selective transport from the cytosol. A short sequence of only three amino acids serves as an import signal for many peroxisomal proteins. This sequence is recognized by receptor proteins in the cytosol, at least one of which escorts its cargo protein all the way into the peroxisome before returning to the cytosol. Like the membranes of mitochondria and chloroplasts, the peroxisomal membrane contains a protein translocator that aids in the transport. Unlike the mechanism that operates in mitochondria and chloroplasts, however, proteins do not need to unfold to enter the peroxisome—and the transport mechanism is still mysterious.

Although most peroxisomal proteins—including those embedded in the peroxisomal membrane—come from the cytosol, a few membrane proteins arrive via vesicles that bud from the ER membrane. The vesicles either fuse with preexisting peroxisomes or import peroxisomal proteins from the cytosol to grow into mature peroxisomes.

The most severe peroxisomal disease, called Zellweger syndrome, is caused by mutations that block peroxisomal protein import. Individuals with this disorder are born with severe abnormalities in their brain, liver, and kidneys. Most do not survive past the first six months of life—a grim reminder of the crucial importance of these underappreciated organelles for proper cell function and for the health of the organism.

Proteins Enter the Endoplasmic Reticulum While Being Synthesized

The endoplasmic reticulum is the most extensive membrane system in a eukaryotic cell (**Figure 15–12A**). Unlike the organelles discussed so far, it serves as an entry point for proteins destined for other organelles, as well as for the ER itself. Proteins destined for the Golgi apparatus, endosomes, and lysosomes, as well as proteins destined for the cell surface, all first enter the ER from the cytosol. Once inside the ER lumen, or embedded in the ER membrane, individual proteins will not re-enter the cytosol during their onward journey. They will instead be ferried by transport vesicles from organelle to organelle within the endomembrane system, or to the plasma membrane.

Two kinds of proteins are transferred from the cytosol to the ER: (1) water-soluble proteins are completely translocated across the ER membrane and are released into the ER lumen; (2) prospective transmembrane proteins are only partly translocated across the ER membrane and become embedded in it. The water-soluble proteins are destined either for secretion (by release at the cell surface) or for the lumen of an organelle of the endomembrane system. The transmembrane proteins are destined

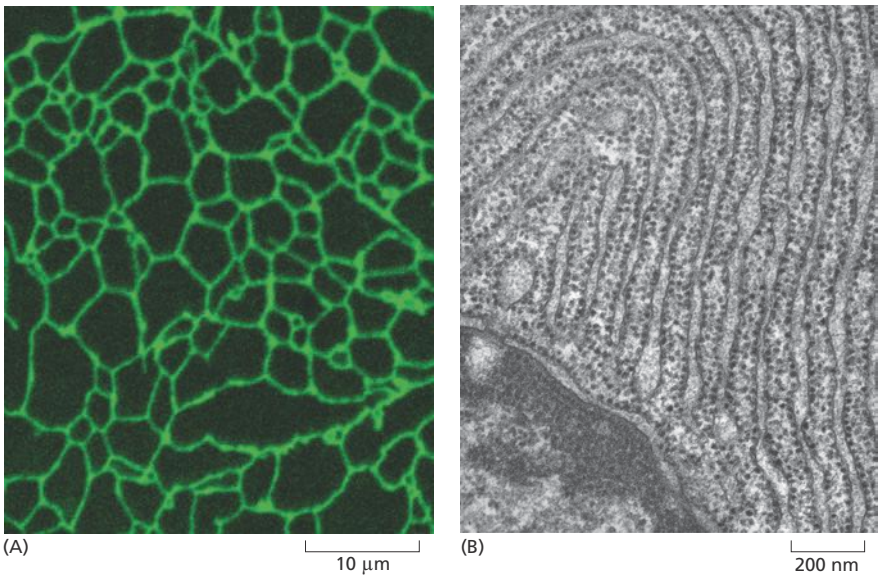


Figure 15–12 The endoplasmic reticulum is the most extensive membrane network in eukaryotic cells. (A) Fluorescence micrograph of a living plant cell showing the ER as a complex network of tubes. The cell shown here has been genetically engineered so that it contains a fluorescent protein in the ER lumen. Only part of the ER network in the cell is shown. (B) An electron micrograph showing the rough ER in a cell from a dog's pancreas, which makes and secretes large amounts of digestive enzymes. The cytosol is filled with closely packed sheets of ER, studded with ribosomes. A portion of the nucleus and its nuclear envelope can be seen at the bottom left; note that the outer nuclear membrane, which is continuous with the ER, is also studded with ribosomes. For a dynamic view of the ER network, watch **Movie 15.3**. (A, courtesy of Petra Boevink and Chris Hawes; B, courtesy of Lelio Orci.)

to reside in the membrane of one of these organelles or in the plasma membrane. All of these proteins are initially directed to the ER by an *ER signal sequence*, a segment of eight or more hydrophobic amino acids (see Table 15–3, p. 494), which is also involved in the process of translocation across the membrane.

Unlike the proteins that enter the nucleus, mitochondria, chloroplasts, or peroxisomes, most of the proteins that enter the ER begin to be threaded across the ER membrane before the polypeptide chain has been completely synthesized. This requires that the ribosome synthesizing the protein be attached to the ER membrane. These membrane-bound ribosomes coat the surface of the ER, creating regions termed **rough endoplasmic reticulum** because of its characteristic beaded appearance when viewed in an electron microscope (**Figure 15–12B**).

There are, therefore, two separate populations of ribosomes in the cytosol. *Membrane-bound ribosomes* are attached to the cytosolic side of the ER membrane (and outer nuclear membrane) and are making proteins that are being translocated into the ER. *Free ribosomes* are unattached to any membrane and are making all of the other proteins encoded by the nuclear DNA. Membrane-bound ribosomes and free ribosomes are structurally and functionally identical; they differ only in the proteins they are making at any given time. When a ribosome happens to be making a protein with an ER signal sequence, the signal sequence directs the ribosome to the ER membrane. Because proteins with an ER signal sequence are translocated as they are being made, no additional energy is required for their transport; the elongation of each polypeptide provides the thrust needed to push the growing chain through the ER membrane.

As an mRNA molecule is translated, many ribosomes bind to it, forming a *polyribosome* (discussed in Chapter 7). In the case of an mRNA molecule encoding a protein with an ER signal sequence, the polyribosome becomes riveted to the ER membrane by the growing polypeptide chains, which have become inserted into the ER membrane (**Figure 15–13**).

Soluble Proteins Made on the ER Are Released into the ER Lumen

Two protein components help guide ER signal sequences to the ER membrane: (1) a *signal-recognition particle* (*SRP*), present in the cytosol, binds

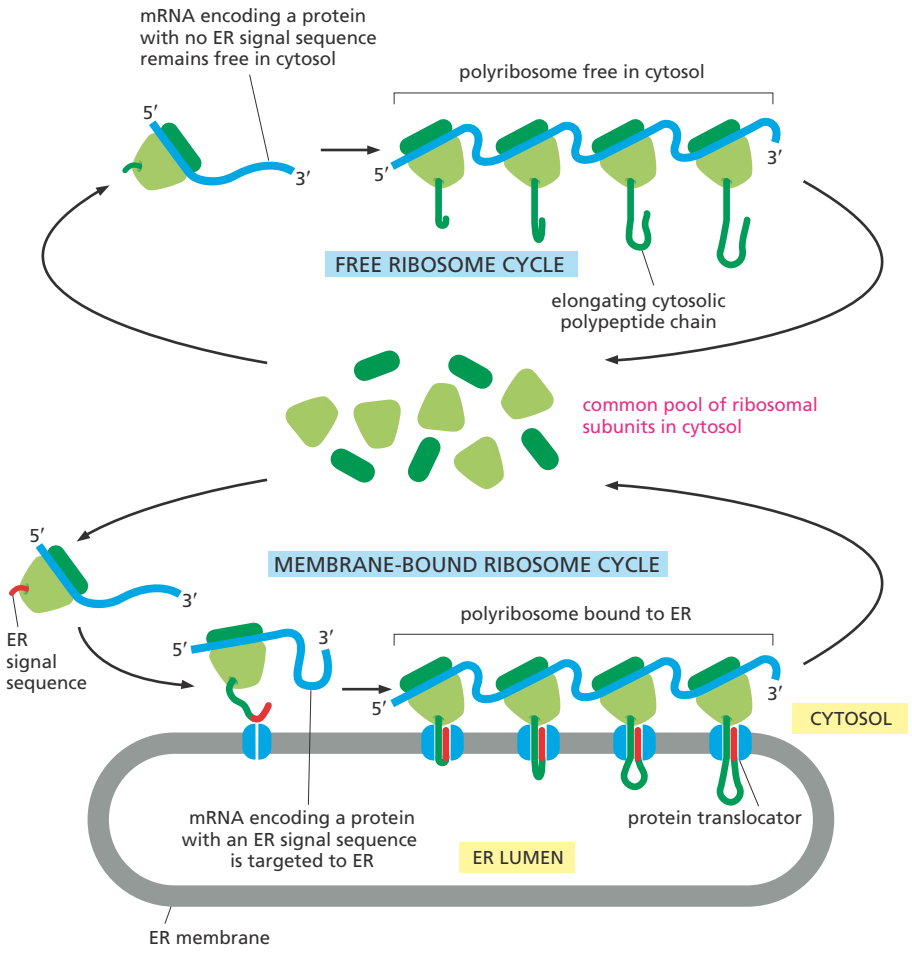


Figure 15–13 A common pool of ribosomes is used to synthesize all the proteins encoded by the nuclear genome. Ribosomes that are translating proteins with no ER signal sequence remain free in the cytosol. Ribosomes that are translating proteins containing an ER signal sequence (*red*) on the growing polypeptide chain will be directed to the ER membrane. Many ribosomes bind to each mRNA molecule, forming a polyribosome. At the end of each round of protein synthesis, the ribosomal subunits are released and rejoin the common pool in the cytosol. As we see shortly, how the ribosome and signal sequence bind to the ER and translocation channel is more complicated than illustrated here.

to both the ribosome and the ER signal sequence when it emerges from the ribosome, and (2) an *SRP receptor*, embedded in the ER membrane, recognizes the SRP. Binding of an SRP to a ribosome that displays an ER signal sequence slows protein synthesis by that ribosome until the SRP engages with an SRP receptor on the ER. Once bound, the SRP is released, the receptor passes the ribosome to a protein translocator in the ER membrane, and protein synthesis recommences. The polypeptide is then threaded across the ER membrane through a *channel* in the translocator (**Figure 15–14**). Thus the SRP and SRP receptor function as molecular matchmakers, uniting ribosomes that are synthesizing proteins with an ER signal sequence and available translocation channels in the ER membrane.

In addition to directing proteins to the ER, the signal sequence—which for soluble proteins is almost always at the N-terminus, the end synthesized first—functions to open the channel in the protein translocator. This sequence remains bound to the channel, while the rest of the polypeptide chain is threaded through the membrane as a large loop. It is removed by a transmembrane signal peptidase, which has an active site facing the lumenal side of the ER membrane. The cleaved signal sequence is then released from the translocation channel into the lipid bilayer and rapidly degraded.

Once the C-terminus of a soluble protein has passed through the translocation channel, the protein will be released into the ER lumen (**Figure 15–15**).

QUESTION 15–3

Explain how an mRNA molecule can remain attached to the ER membrane while individual ribosomes translating it are released and rejoin the cytosolic pool of ribosomes after each round of translation.

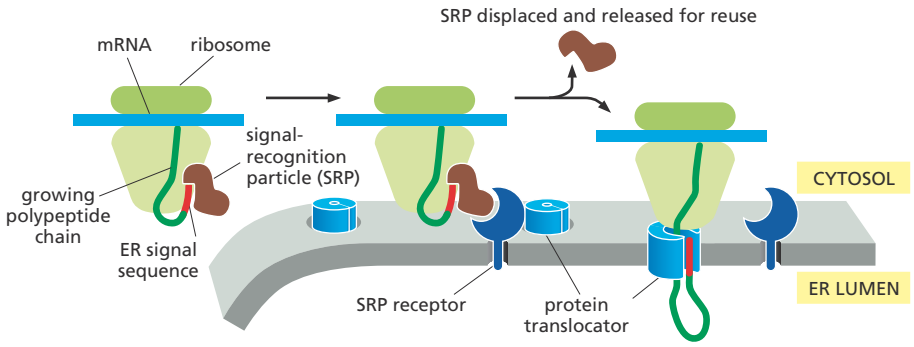


Figure 15–14 An ER signal sequence and an SRP direct a ribosome to the ER membrane. The SRP binds to both the exposed ER signal sequence and the ribosome, thereby slowing protein synthesis by the ribosome. The SRP–ribosome complex then binds to an SRP receptor in the ER membrane. The SRP is released, passing the ribosome from the SRP receptor to a protein translocator in the ER membrane. Protein synthesis resumes, and the translocator starts to transfer the growing polypeptide across the lipid bilayer.

Start and Stop Signals Determine the Arrangement of a Transmembrane Protein in the Lipid Bilayer

Not all proteins made by ER-bound ribosomes are released into the ER lumen. Some remain embedded in the ER membrane as transmembrane proteins. The translocation process for such proteins is more complicated than it is for soluble proteins, as some parts of the polypeptide chain must be translocated completely across the lipid bilayer, whereas other parts remain fixed in the membrane.

In the simplest case, that of a transmembrane protein with a single membrane-spanning segment, the N-terminal signal sequence initiates translocation—as it does for a soluble protein. But the transfer process is halted by an additional sequence of hydrophobic amino acids, a *stop-transfer sequence*, further along the polypeptide chain. At this point, the translocation channel releases the growing polypeptide chain sideways into the lipid bilayer. The N-terminal signal sequence is cleaved off, whereas the stop-transfer sequence remains in the bilayer, where it forms an α-helical membrane-spanning segment that anchors the protein in the membrane. As a result, the protein ends up as a single-pass transmembrane protein inserted in the membrane with a defined orientation—the N-terminus on the lumenal side of the lipid bilayer and the C-terminus on the cytosolic side (**Figure 15–16**). Once inserted into the membrane, a transmembrane protein does not change its orientation, which is retained throughout any subsequent vesicle budding and fusion events.

In some transmembrane proteins, an internal, rather than an N-terminal, signal sequence is used to start the protein transfer; this internal signal sequence, called a *start-transfer sequence,* is never removed from

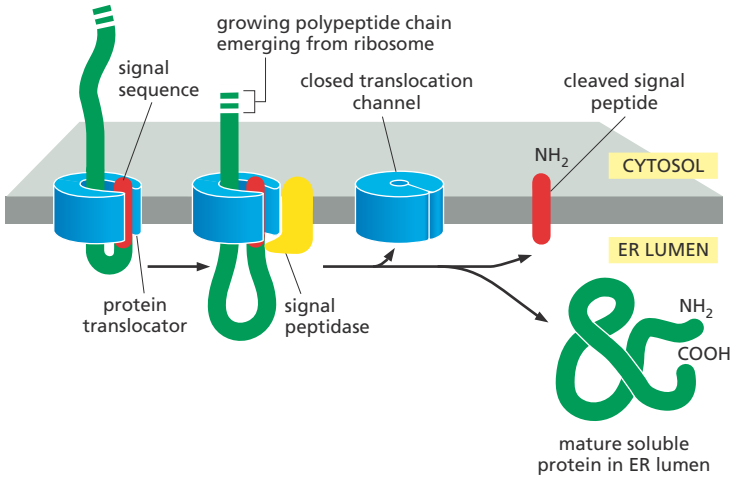


Figure 15–15 A soluble protein crosses the ER membrane and enters the lumen. The protein translocator binds the signal sequence and threads the rest of the polypeptide across the lipid bilayer as a loop. At some point during the translocation process, the signal peptide is cleaved from the growing protein by a signal peptidase. This cleaved signal sequence is ejected into the bilayer, where it is degraded. Once protein synthesis is complete, the translocated polypeptide is released as a soluble protein into the ER lumen, and the pore of the translocation channel closes. The membrane-bound ribosome is omitted from this and the following two figures for clarity.

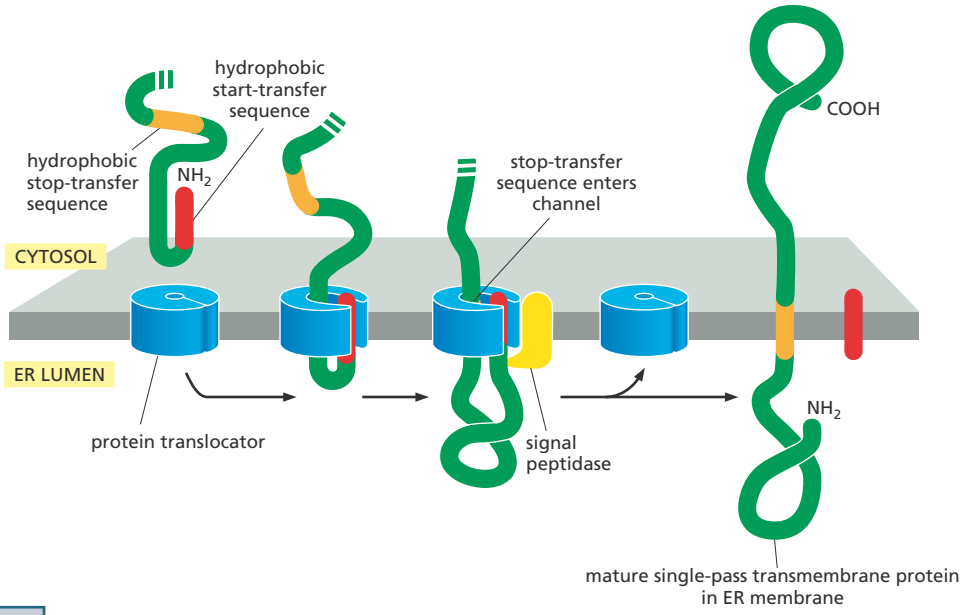


Figure 15–16 A single-pass transmembrane protein is retained in the lipid bilayer. An N-terminal ER signal sequence (*red*) initiates transfer as in Figure 15–15. In addition, the protein also contains a second hydrophobic sequence, which acts as a stop-transfer sequence (*orange*). When this sequence enters the translocation channel, the channel discharges the growing polypeptide chain sideways into the lipid bilayer. The N-terminal signal sequence is cleaved off, leaving the transmembrane protein anchored in the membrane (**Movie 15.4**). Protein synthesis on the cytosolic side then continues to completion.

QUESTION 15–4

A. Predict the membrane orientation of a protein that is synthesized with an uncleaved, internal signal sequence (shown as the *red* start-transfer sequence in Figure 15–17) but does not contain a stop-transfer sequence.
B. Similarly, predict the membrane orientation of a protein that is synthesized with an N-terminal cleaved signal sequence followed by a stop-transfer sequence, followed by a start-transfer sequence.
C. What arrangement of signal sequences would enable the insertion of a multipass protein with an odd number of transmembrane segments?

the polypeptide. This arrangement occurs in some transmembrane proteins in which the polypeptide chain passes back and forth across the lipid bilayer. In these cases, hydrophobic signal sequences are thought to work in pairs: an internal start-transfer sequence serves to initiate translocation, which continues until a stop-transfer sequence is reached; the two hydrophobic sequences are then released into the bilayer, where they remain as membrane-spanning α helices (**Figure 15–17**). In complex multipass proteins, in which many hydrophobic α helices span the bilayer, additional pairs of start- and stop-transfer sequences come into play: one sequence reinitiates translocation further down the polypeptide chain, and the other stops translocation and causes polypeptide release, and so on for subsequent starts and stops. Thus, multipass membrane proteins are stitched into the lipid bilayer as they are being synthesized, by a mechanism resembling the workings of a sewing machine.

Having considered how proteins enter the ER lumen or become embedded in the ER membrane, we now discuss how they are carried onward by vesicular transport.

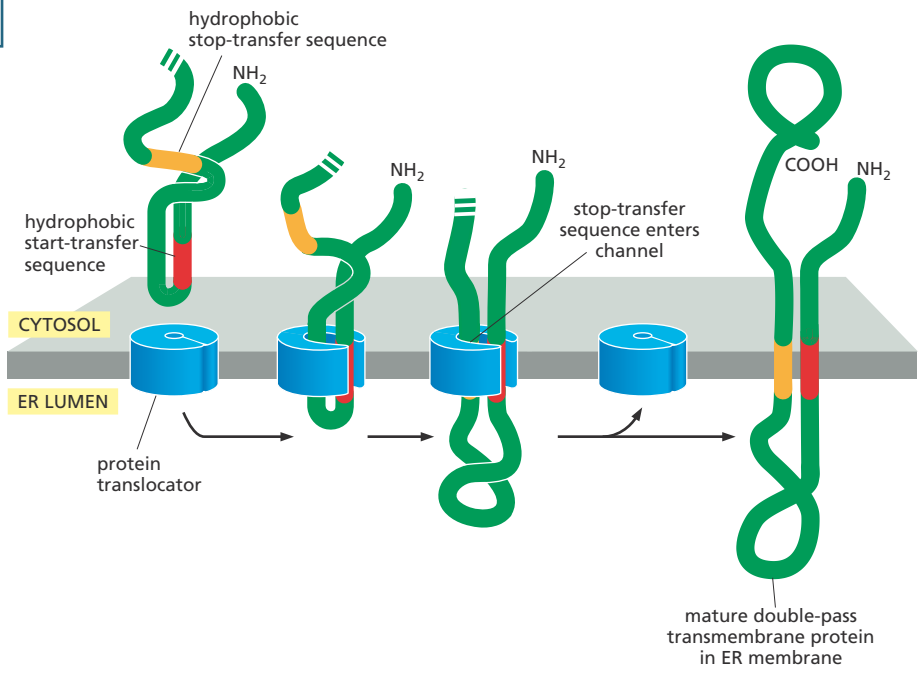


Figure 15–17 A double-pass transmembrane protein has an internal ER signal sequence. This internal sequence (*red*) not only acts as a start-transfer signal, it also helps to anchor the final protein in the membrane. Like the N-terminal ER signal sequence, the internal signal sequence is recognized by an SRP, which brings the ribosome to the ER membrane (not shown). When a stop-transfer sequence (*orange*) enters the translocation channel, the channel discharges both sequences into the lipid bilayer. Neither the start-transfer nor the stop-transfer sequence is cleaved off, and the entire polypeptide chain remains anchored in the membrane as a double-pass transmembrane protein. Proteins that span the membrane more times contain further pairs of start- and stop-transfer sequences, and the same process is repeated for each pair.

VESICULAR TRANSPORT

Entry into the ER lumen or membrane is usually only the first step on a pathway to another destination. That destination, initially at least, is generally the Golgi apparatus; there, proteins and lipids are modified and sorted for shipment to other sites. Transport from the ER to the Golgi apparatus—and from the Golgi apparatus to other compartments of the endomembrane system—is carried out by the continual budding and fusion of transport vesicles. This **vesicular transport** extends outward from the ER to the plasma membrane, and inward from the plasma membrane to lysosomes, and thus provides routes of communication between the interior of the cell and its surroundings. As proteins and lipids are transported outward along these pathways, many of them undergo various types of chemical modification, such as the addition of carbohydrate side chains.

In this section, we discuss how vesicles shuttle proteins and membranes between intracellular compartments, allowing cells to eat, drink, and secrete. We also consider how these transport vesicles are directed to their proper destination, be it an organelle of the endomembrane system or the plasma membrane.

Transport Vesicles Carry Soluble Proteins and Membrane Between Compartments

Vesicular transport between membrane-enclosed compartments of the endomembrane system is highly organized. A major outward *secretory pathway* starts with the synthesis of proteins on the ER membrane and their entry into the ER, and it leads through the Golgi apparatus to the cell surface; at the Golgi apparatus, a side branch leads off through endosomes to lysosomes. A major inward *endocytic pathway*, which is responsible for the ingestion and degradation of extracellular molecules, moves materials from the plasma membrane, through endosomes, to lysosomes (**Figure 15–18**).

To function optimally, each transport vesicle that buds off from a compartment must take with it only the proteins appropriate to its destination and must fuse only with the appropriate target membrane. A vesicle carrying cargo from the Golgi apparatus to the plasma membrane, for example, must exclude proteins that are to stay in the Golgi apparatus,

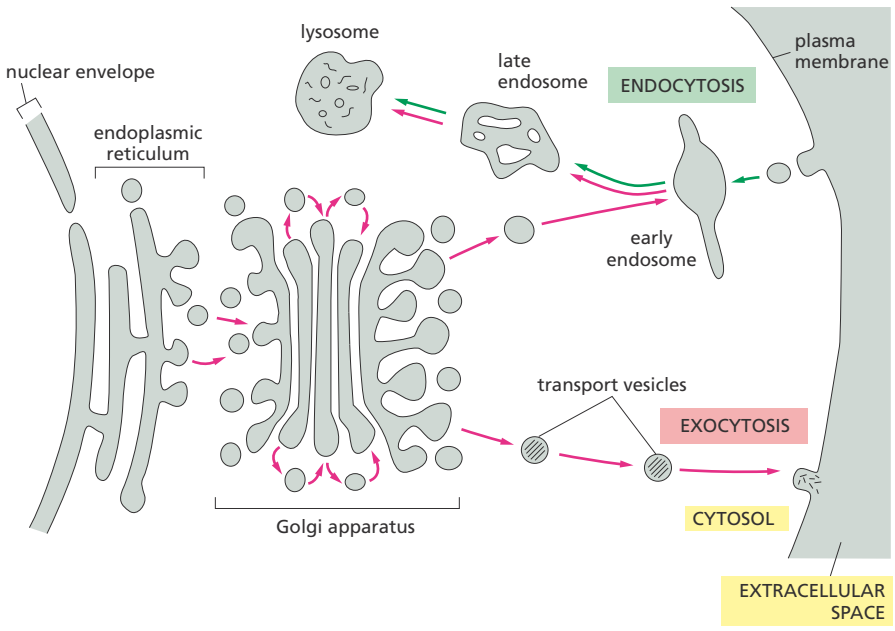


Figure 15–18 Transport vesicles bud from one membrane and fuse with another, carrying membrane components and soluble proteins between compartments of the endomembrane system and the plasma membrane. The membrane of each compartment or vesicle maintains its orientation, so the cytosolic side always faces the cytosol and the noncytosolic side faces the lumen of the compartment or the outside of the cell (see Figure 11–18). The extracellular space and each of the membrane-enclosed compartments (shaded *gray*) communicate with one another by means of transport vesicles, as shown. In the outward secretory pathway (*red* arrows), protein molecules are transported from the ER, through the Golgi apparatus, to the plasma membrane or (via early and late endosomes) to lysosomes. In the inward endocytic pathway (*green* arrows), extracellular molecules are ingested (endocytosed) in vesicles derived from the plasma membrane and are delivered to early endosomes and, usually, on to lysosomes via late endosomes.

and it must fuse only with the plasma membrane and not with any other organelle. While participating in this constant flow of membrane components, each organelle must maintain its own distinct identity, that is, its own distinctive protein and lipid composition. All of these recognition events depend on proteins displayed on the surface of the transport vesicle. As we will see, different types of transport vesicles shuttle between the various organelles, each carrying a distinct set of molecules.

Vesicle Budding Is Driven by the Assembly of a Protein Coat

Vesicles that bud from membranes usually have a distinctive protein coat on their cytosolic surface and are therefore called **coated vesicles**. After budding from its parent organelle, the vesicle sheds its coat, allowing its membrane to interact directly with the membrane to which it will fuse. Cells produce several kinds of coated vesicles, each with a distinctive protein coat. The coat serves at least two functions: it helps shape the membrane into a bud and captures molecules for onward transport.

The best-studied vesicles are those that have an outer coat made of the protein **clathrin**. These *clathrin-coated vesicles* bud from both the Golgi apparatus on the outward secretory pathway and from the plasma membrane on the inward endocytic pathway. At the plasma membrane, for example, each vesicle starts off as a *clathrin-coated pit*. Clathrin molecules assemble into a basketlike network on the cytosolic surface of the membrane, and it is this assembly process that starts shaping the membrane into a vesicle (**Figure 15–19**). A small GTP-binding protein called

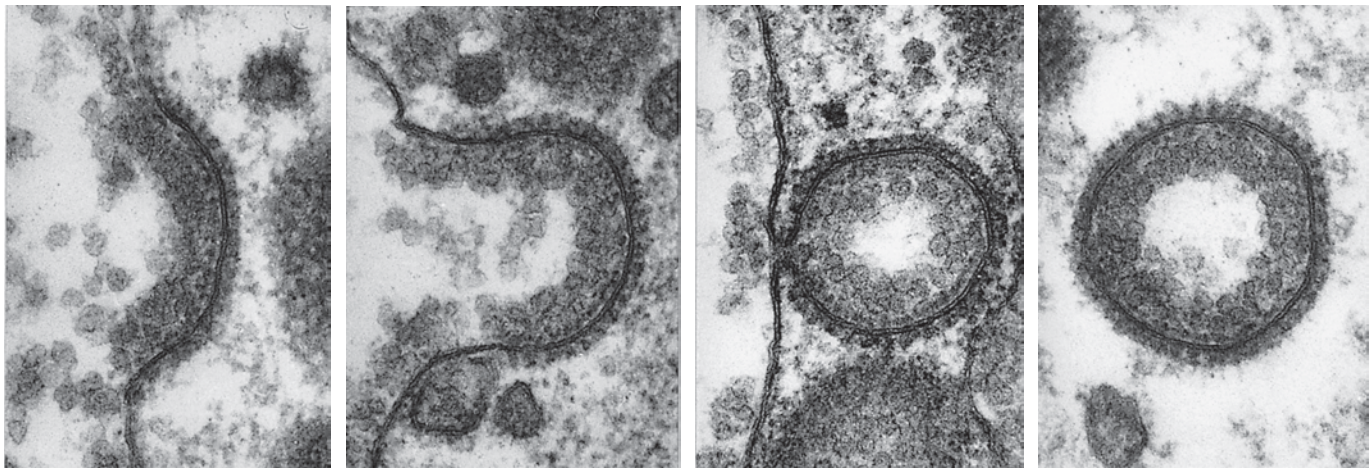
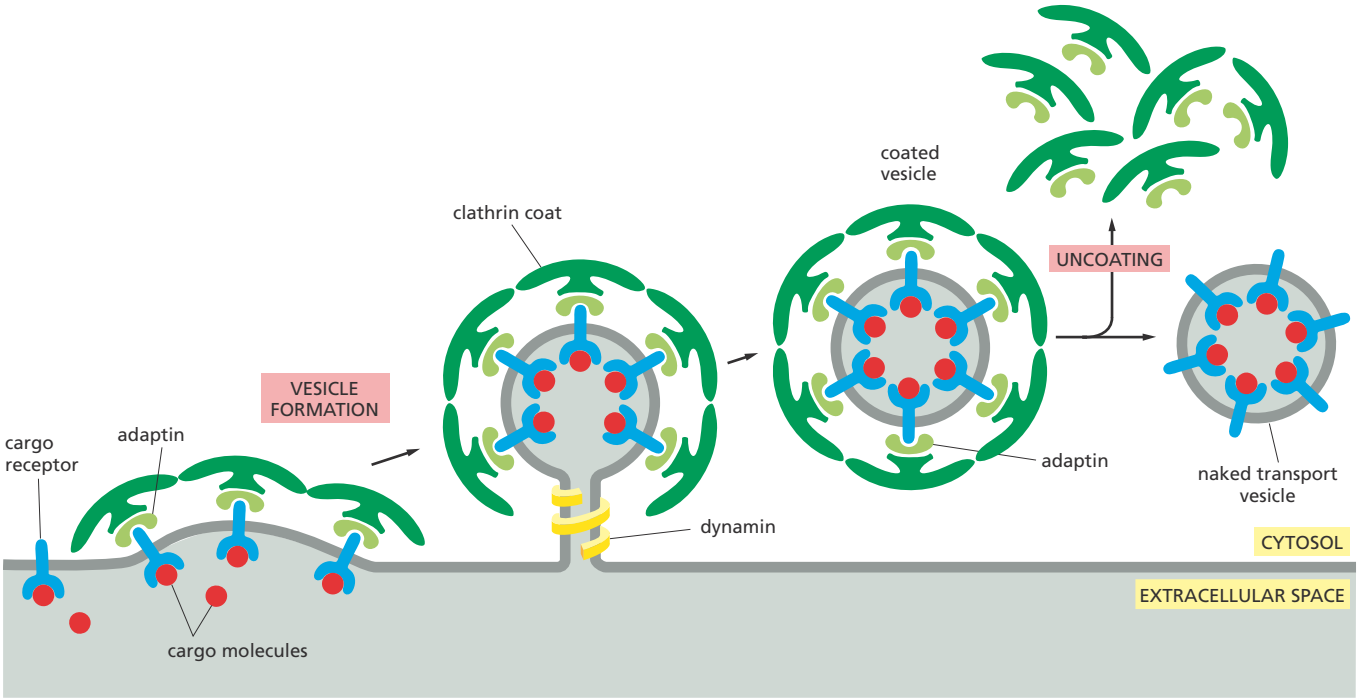


Figure 15–19 Clathrin molecules form basketlike cages that help shape membranes into vesicles. (A) Electron micrographs showing the sequence of events in the formation of a clathrin-coated vesicle from a clathrin-coated pit. The clathrin-coated pits and vesicles shown here are unusually large and are being formed at the plasma membrane of a hen oocyte. They are involved in taking up particles made of lipid and protein into the oocyte to form yolk. (B) Electron micrograph showing numerous clathrin-coated pits and vesicles budding from the inner surface of the plasma membrane of cultured skin cells. (A, courtesy of M.M. Perry and A.B. Gilbert, *J. Cell Sci.* 39:257–272, 1979. With permission from The Company of Biologists Ltd; B, from J. Heuser, *J. Cell Biol.* 84:560–583, 1980. With permission from Rockefeller University Press.)



dynamin assembles as a ring around the neck of each deeply invaginated coated pit. Together with other proteins recruited to the neck of the vesicle, the dynamin causes the ring to constrict, thereby pinching off the vesicle from its parent membrane. Other kinds of transport vesicles, with different coat proteins, are also involved in vesicular transport. They form in a similar way and carry their own characteristic sets of molecules between the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane. But how does a transport vesicle select its particular cargo? The mechanism is best understood for clathrin-coated vesicles.

Clathrin itself plays no part in choosing specific molecules for transport. This is the function of a second class of coat proteins called *adaptins,* which both secure the clathrin coat to the vesicle membrane and help select cargo molecules for transport. Molecules for onward transport carry specific *transport signals* that are recognized by *cargo receptors* in the Golgi or plasma membrane. Adaptins help capture specific cargo molecules by trapping the cargo receptors that bind them. In this way, a selected set of cargo molecules, bound to their specific receptors, is incorporated into the lumen of each newly formed clathrin-coated vesicle (**Figure 15–20**). There are different types of adaptins: the adaptins that bind cargo receptors in the plasma membrane, for example, are not the same as those that bind cargo receptors in the Golgi apparatus, reflecting the differences in the cargo molecules to be transported from each of these sources.

Another class of coated vesicles, called *COP-coated vesicles* (COP being shorthand for "coat protein"), is involved in transporting molecules between the ER and the Golgi apparatus and from one part of the Golgi apparatus to another (**Table 15–4**).

Vesicle Docking Depends on Tethers and SNAREs

After a transport vesicle buds from a membrane, it must find its way to its correct destination to deliver its contents. Often, the vesicle is actively transported by motor proteins that move along cytoskeletal fibers, as discussed in Chapter 17.

Figure 15–20 Clathrin-coated vesicles transport selected cargo molecules. Here, as in Figure 15–19, the vesicles are shown budding from the plasma membrane. Cargo receptors, with their bound cargo molecules, are captured by adaptins, which also bind clathrin molecules to the cytosolic surface of the budding vesicle (**Movie 15.5**). Dynamin proteins assemble around the neck of budding vesicles; once assembled, the dynamin molecules—which are monomeric GTPases (discussed in Chapter 16)—hydrolyze their bound GTP and, with the help of other proteins recruited to the neck (not shown), pinch off the vesicle. After budding is complete, the coat proteins are removed, and the naked vesicle can fuse with its target membrane. Functionally similar coat proteins are found in other types of coated vesicles.

TABLE 15–4 SOME TYPES OF COATED VESICLES

Type of Coated Vesicle	Coat Proteins	Origin	Destination
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COP-coated	COP proteins	ER Golgi cisterna Golgi apparatus	Golgi apparatus Golgi cisterna ER

Once a transport vesicle has reached its target, it must recognize and dock with its specific organelle. Only then can the vesicle membrane fuse with the target membrane and unload the vesicle's cargo. The impressive specificity of vesicular transport suggests that each type of transport vesicle in the cell displays molecular markers on its surface that identify the vesicle according to its origin and cargo. These markers must be recognized by complementary receptors on the appropriate target membrane, including the plasma membrane.

The identification process depends on a diverse family of monomeric GTPases called **Rab proteins**. Specific Rab proteins on the surface of each type of vesicle are recognized by corresponding *tethering proteins* on the cytosolic surface of the target membrane. Each organelle and each type of transport vesicle carries a unique combination of Rab proteins, which serve as molecular markers for each membrane type. The coding system of matching Rab and tethering proteins helps to ensure that transport vesicles fuse only with the correct membrane.

Additional recognition is provided by a family of transmembrane proteins called **SNAREs**. Once the tethering protein has captured a vesicle by grabbing hold of its Rab protein, SNAREs on the vesicle (called v-SNAREs) interact with complementary SNAREs on the target membrane (called t-SNAREs), firmly docking the vesicle in place (**Figure 15–21**).

The same SNAREs involved in docking also play a central role in catalyzing the membrane fusion required for a transport vesicle to deliver its cargo. Fusion not only delivers the soluble contents of the vesicle into the interior of the target organelle, but it also adds the vesicle membrane to the membrane of the organelle (see Figure 15–21). After vesicle docking,

QUESTION 15–5

The budding of clathrin-coated vesicles from eukaryotic plasma membrane fragments can be observed when adaptins, clathrin, and dynamin-GTP are added to the membrane preparation. What would you observe if you omitted (A) adaptins, (B) clathrin, or (C) dynamin? (D) What would you observe if the plasma membrane fragments were from a prokaryotic cell?

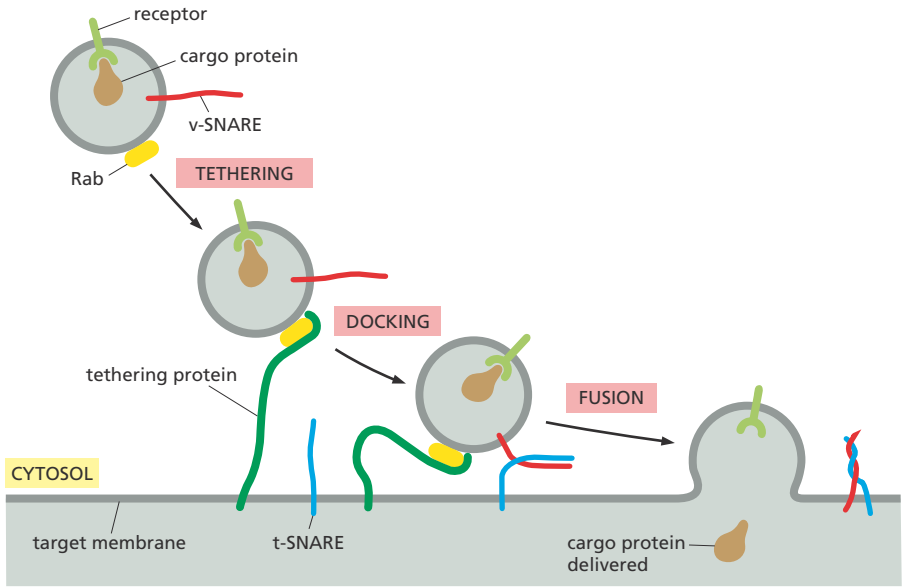


Figure 15–21 Rab proteins, tethering proteins, and SNAREs help direct transport vesicles to their target membranes. A filamentous tethering protein on a membrane binds to a Rab protein on the surface of a vesicle. This interaction allows the vesicle to dock on its particular target membrane. A v-SNARE on the vesicle then binds to a complementary t-SNARE on the target membrane. Whereas Rab and tethering proteins provide the initial recognition between a vesicle and its target membrane, complementary SNARE proteins ensure that transport vesicles dock at their appropriate target membranes. These SNARE proteins also catalyze the final fusion of the two membranes (see Figure 15–22).

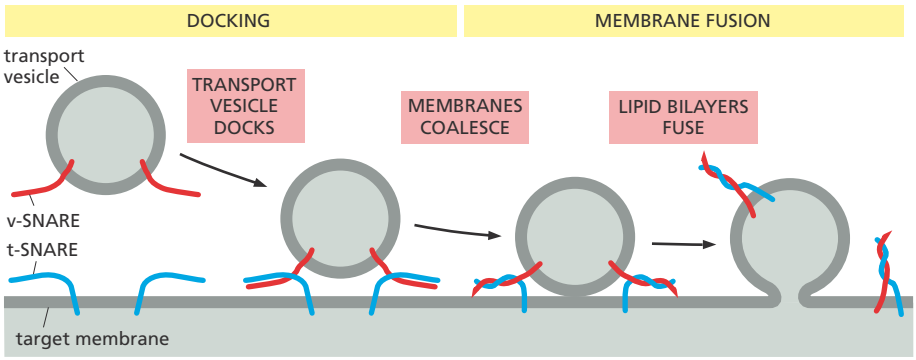


Figure 15–22 Following vesicle docking, SNARE proteins can catalyze the fusion of the vesicle and target membranes. Once appropriately triggered, the tight pairing of v-SNAREs and t-SNAREs draws the two lipid bilayers into close apposition. The force of the SNAREs winding together squeezes out any water molecules that remain trapped between the two membranes, allowing their lipids to flow together to form a continuous bilayer. In a cell, other proteins recruited to the fusion site help to complete the fusion process. After fusion, the SNAREs are pried apart so that they can be used again.

the fusion of a vesicle with its target membrane sometimes requires a special stimulatory signal. Whereas docking requires only that the two membranes come close enough for the SNAREs protruding from the two lipid bilayers to interact, fusion requires a much closer approach: the two bilayers must come within 1.5 nm of each other so that their lipids can intermix. For this close approach, water must be displaced from the hydrophilic surfaces of the membranes—a process that is energetically highly unfavorable and thus prevents membranes from fusing randomly. All membrane fusions in cells must therefore be catalyzed by specialized proteins that assemble to form a fusion complex, which provides the means to cross this energy barrier. The SNARE proteins themselves catalyze the fusion process: once fusion is triggered, the v-SNAREs and t-SNAREs wrap around each other, thereby acting like a winch that pulls the two lipid bilayers into close proximity (**Figure 15–22**).

SECRETORY PATHWAYS

Vesicular traffic is not confined to the interior of the cell. It extends to and from the plasma membrane. Newly made proteins, lipids, and carbohydrates are delivered from the ER, via the Golgi apparatus, to the cell surface by transport vesicles that fuse with the plasma membrane in the process of *exocytosis* (see Figure 15–18). Each molecule that travels along this route passes through a fixed sequence of membrane-enclosed compartments and is often chemically modified en route.

In this section, we follow the outward path of proteins as they travel from the ER, where they are made and modified, through the Golgi apparatus, where they are further modified and sorted, to the plasma membrane. As a protein passes from one compartment to another, it is monitored to check that it has folded properly and assembled with its appropriate partners, so that only correctly built proteins make it to the cell surface. Incorrect assemblies, which are often in the majority, are degraded inside the cell. Quality, it seems, is more important than economy when it comes to the production and transport of proteins via this pathway.

Most Proteins Are Covalently Modified in the ER

Most proteins that enter the ER are chemically modified there. *Disulfide bonds* are formed by the oxidation of pairs of cysteine side chains (see Figure 4–30), a reaction catalyzed by an enzyme that resides in the ER lumen. The disulfide bonds help to stabilize the structure of proteins that will encounter degradative enzymes and changes in pH outside the cell—either after they are secreted or after they are incorporated into the plasma membrane. Disulfide bonds do not form in the cytosol because the environment there is reducing.

Many of the proteins that enter the ER lumen or ER membrane are converted to glycoproteins in the ER by the covalent attachment of short

branched oligosaccharide side chains composed of multiple sugars. This process of *glycosylation* is carried out by glycosylating enzymes present in the ER but not in the cytosol. Very few proteins in the cytosol are glycosylated, and those that are have only a single sugar attached to them. The oligosaccharides on proteins can serve various functions. They can protect a protein from degradation, hold it in the ER until it is properly folded, or help guide it to the appropriate organelle by serving as a transport signal for packaging the protein into appropriate transport vesicles. When displayed on the cell surface, oligosaccharides form part of the cell's outer carbohydrate layer or *glycocalyx* (see Figure 11–33) and can function in the recognition of one cell by another.

In the ER, individual sugars are not added one-by-one to the protein to create the oligosaccharide side chain. Instead, a preformed, branched oligosaccharide containing a total of 14 sugars is attached *en bloc* to all proteins that carry the appropriate site for glycosylation. The oligosaccharide is originally attached to a specialized lipid, called *dolichol*, in the ER membrane; it is then transferred to the amino (NH_2) group of an asparagine side chain on the protein, immediately after a target asparagine emerges in the ER lumen during protein translocation (**Figure 15–23**). The addition takes place in a single enzymatic step that is catalyzed by a membrane-bound enzyme (an oligosaccharyl transferase) that has its active site exposed on the lumenal side of the ER membrane—which explains why cytosolic proteins are not glycosylated in this way. A simple sequence of three amino acids, of which the asparagine is one, defines which asparagines in a protein receive the oligosaccharide. Oligosaccharide side chains linked to an asparagine NH_2 group in a protein are said to be *N-linked* and are by far the most common type of linkage found on glycoproteins.

The addition of the 14-sugar oligosaccharide in the ER is only the first step in a series of further modifications before the mature glycoprotein reaches the cell surface. Despite their initial similarity, the *N*-linked

QUESTION 15–6

Why might it be advantageous to add a preassembled block of 14 sugar residues to a protein in the ER, rather than building the sugar chains step-by-step on the surface of the protein by the sequential addition of sugars by individual enzymes?

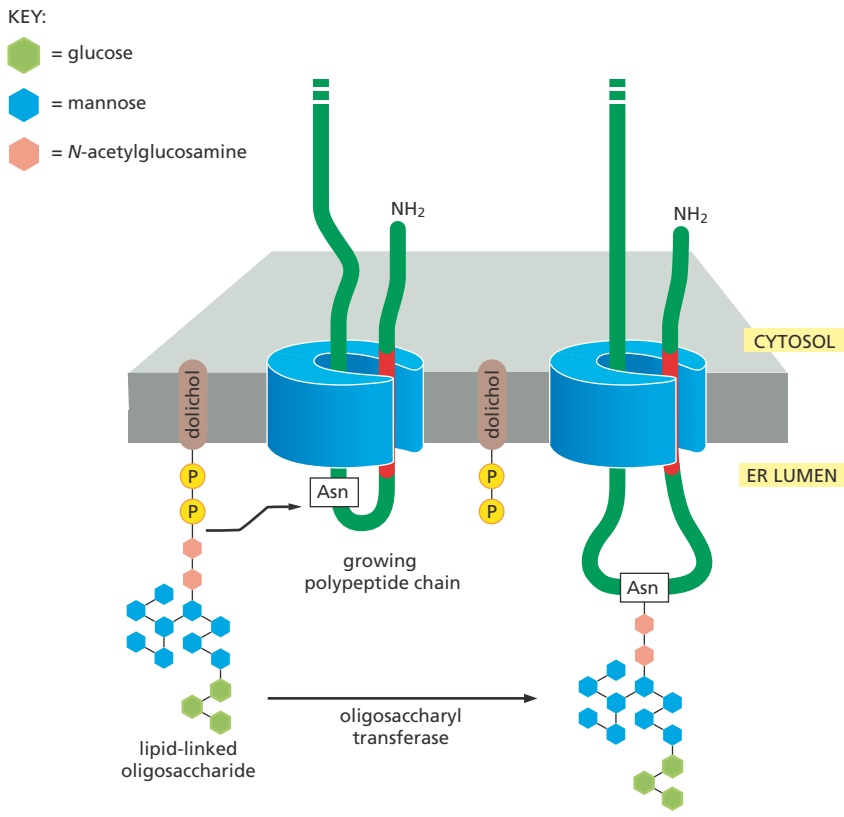


Figure 15–23 Many proteins are glycosylated on asparagines in the ER. When an appropriate asparagine enters the ER lumen, it is glycosylated by addition of a branched oligosaccharide side chain. Each oligosaccharide chain is transferred as an intact unit to the asparagine from a lipid called dolichol, catalyzed by the enzyme oligosaccharyl transferase. Asparagines that are glycosylated are always present in the tripeptide sequences asparagine-X-serine or asparagine-X-threonine, where X can be almost any amino acid.

oligosaccharides on mature glycoproteins are remarkably diverse. All of the diversity results from extensive modification of the original precursor structure shown in Figure 15–23. This oligosaccharide processing begins in the ER and continues in the Golgi apparatus.

Exit from the ER Is Controlled to Ensure Protein Quality

Some proteins made in the ER are destined to function there. They are retained in the ER (and are returned to the ER whenever they escape to the Golgi apparatus) by a C-terminal sequence of four amino acids called an *ER retention signal* (see Table 15–3, p. 494). This retention signal is recognized by a membrane-bound receptor protein in the ER and Golgi apparatus. Most proteins that enter the ER, however, are destined for other locations; they are packaged into transport vesicles that bud from the ER and fuse with the Golgi apparatus.

Exit from the ER is highly selective. Proteins that fail to fold correctly, and dimeric or multimeric proteins that do not assemble properly, are actively retained in the ER by binding to *chaperone proteins* that reside there. The chaperones hold these proteins in the ER until proper folding or assembly occurs. Chaperones prevent misfolded proteins from aggregating, which helps steer proteins along a path toward proper folding (**Figure 15–24** and see Figures 4–9 and 4–10); if proper folding and assembly still fail, the proteins are exported to the cytosol, where they are degraded. Antibody molecules, for example, are composed of four polypeptide chains (see Figure 4–33) that assemble into the complete antibody molecule in the ER. Partially assembled antibodies are retained in the ER until all four polypeptide chains have assembled; any antibody molecule that fails to assemble properly is degraded. In this way, the ER controls the quality of the proteins that it exports to the Golgi apparatus.

Sometimes, however, this quality control mechanism can be detrimental to the organism. For example, the predominant mutation that causes the common genetic disease *cystic fibrosis*, which leads to severe lung damage, produces a plasma-membrane transport protein that is slightly misfolded; even though the mutant protein could function normally as a chloride channel if it reached the plasma membrane, it is retained in the ER, with dire consequences. Thus this devastating disease comes about not because the mutation inactivates an important protein but because the active protein is discarded by the cells before it is given an opportunity to function.

The Size of the ER Is Controlled by the Demand for Protein

Although chaperones help proteins in the ER fold properly and retain those that do not, this quality control system can become overwhelmed. When that happens, misfolded proteins accumulate in the ER. If the buildup is large enough, it triggers a complex program called the **unfolded protein response** (**UPR**). This program prompts the cell to produce more

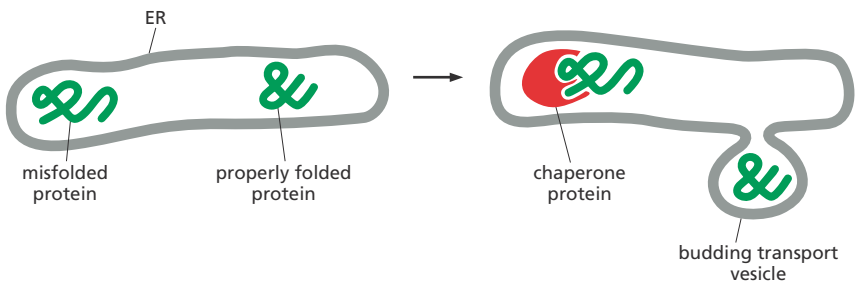


Figure 15–24 Chaperones prevent misfolded or partially assembled proteins from leaving the ER. Misfolded proteins bind to chaperone proteins in the ER lumen and are thus retained there, whereas normally folded proteins are transported in transport vesicles to the Golgi apparatus. If the misfolded proteins fail to refold normally, they are transported back into the cytosol, where they are degraded (not shown).

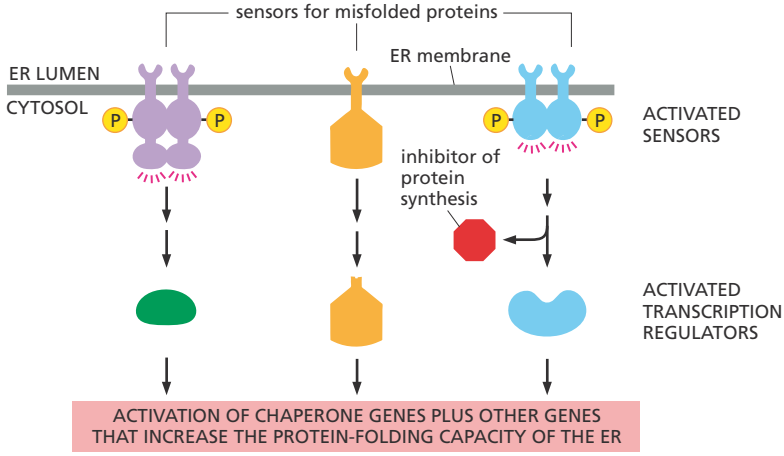


Figure 15–25 Accumulation of misfolded proteins in the ER lumen triggers an unfolded protein response (UPR). The misfolded proteins are recognized by several types of transmembrane sensor proteins in the ER membrane, each of which activates a different part of the UPR. Some sensors stimulate the production of transcription regulators that activate genes encoding chaperones or other proteins of the ER quality control system. Another sensor also inhibits protein synthesis, reducing the flow of proteins through the ER.

ER, including more chaperones and other proteins concerned with quality control (**Figure 15–25**).

The UPR allows a cell to adjust the size of its ER according to the load of proteins entering the secretory pathway. In some cases, however, even an expanded ER cannot cope, and the UPR directs the cell to self-destruct by undergoing apoptosis. Such a situation may occur in adult-onset diabetes, where tissues gradually become resistant to the effects of insulin. To compensate for this resistance, the insulin-secreting cells in the pancreas produce more and more insulin. Eventually, their ER reaches a maximum capacity, at which point the UPR can trigger cell death. As more insulin-secreting cells are eliminated, the demand on the surviving cells increases, making it more likely that they will die as well, further exacerbating the disease.

Proteins Are Further Modified and Sorted in the Golgi Apparatus

The **Golgi apparatus** is usually located near the cell nucleus, and in animal cells it is often close to the centrosome, a small cytoskeletal structure near the cell center (see Figure 17–12). The Golgi apparatus consists of a collection of flattened, membrane-enclosed sacs called cisternae, which are piled like stacks of pita bread. Each stack contains 3–20 cisternae (**Figure 15–26**). The number of Golgi stacks per cell varies greatly depending on the cell type: some cells contain one large stack, while others contain hundreds of very small ones.

Each Golgi stack has two distinct faces: an entry, or *cis*, face and an exit, or *trans,* face. The *cis* face is adjacent to the ER, while the *trans* face points toward the plasma membrane. The outermost cisterna at each face is connected to a network of interconnected membranous tubes and vesicles (see Figure 15–26A). Soluble proteins and membrane enter the *cis Golgi network* via transport vesicles derived from the ER. The proteins travel through the cisternae in sequence by means of transport vesicles that bud from one cisterna and fuse with the next. Proteins exit from the *trans Golgi network* in transport vesicles destined for either the cell surface or another organelle of the endomembrane system (see Figure 15–18).

Both the *cis* and *trans* Golgi networks are thought to be important for protein sorting: proteins entering the *cis* Golgi network can either move onward through the Golgi stack or, if they contain an ER retention signal, be returned to the ER; proteins exiting from the *trans* Golgi network are sorted according to whether they are destined for lysosomes (via endosomes) or for the cell surface. We discuss some examples of sorting by

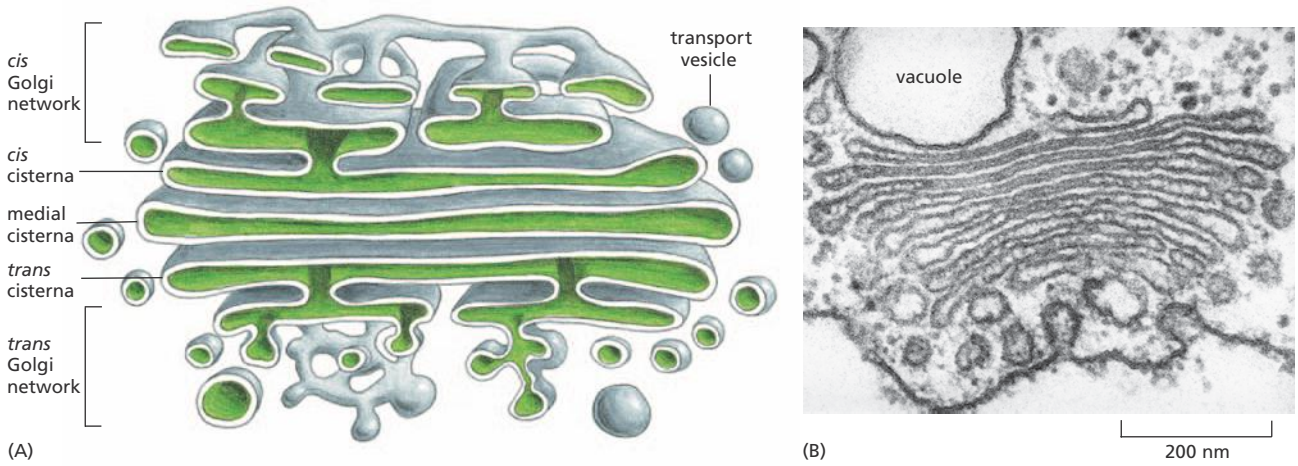


Figure 15–26 The Golgi apparatus consists of a stack of flattened, membrane-enclosed sacs. (A) A three-dimensional model of a Golgi stack reconstructed from a sequential series of electron micrographs of the Golgi apparatus in a secretory animal cell. To see how such models are assembled, watch **Movie 15.6**. (B) Electron micrograph of a Golgi stack from a plant cell, where the Golgi apparatus is especially distinct; the stack is oriented as in (A). (C) A pita-bread model of the Golgi apparatus. (A, redrawn from A. Rambourg and Y. Clermont, *Eur. J. Cell Biol.* 51:189–200, 1990, with permission from Elsevier; B, courtesy of George Palade.)

the *trans* Golgi network later, and we present some of the methods for tracking proteins through the secretory pathways of the cell in **How We Know**, pp. 512–513.

Many of the oligosaccharide chains that are added to proteins in the ER (see Figure 15–23) undergo further modifications in the Golgi apparatus. On some proteins, for example, more complex oligosaccharide chains are created by a highly ordered process in which sugars are added and removed by a series of enzymes that act in a rigidly determined sequence as the protein passes through the Golgi stack. As would be expected, the enzymes that act early in the chain of processing events are located in cisternae close to the *cis* face, while enzymes that act late are located in cisternae near the *trans* face.

Secretory Proteins Are Released from the Cell by Exocytosis

In all eukaryotic cells, a steady stream of vesicles buds from the *trans* Golgi network and fuses with the plasma membrane in the process of **exocytosis**. This *constitutive exocytosis pathway* supplies the plasma membrane with newly made lipids and proteins (**Movie 15.7**), enabling the plasma membrane to expand prior to cell division and refreshing old lipids and proteins in nonproliferating cells. The constitutive pathway also carries soluble proteins to the cell surface to be released to the outside, a process called **secretion**. Some of these proteins remain attached to the cell surface; some are incorporated into the extracellular matrix; still others diffuse into the extracellular fluid to nourish or signal other cells. Entry into the constitutive pathway does not require a particular signal sequence like those that direct proteins to endosomes or back to the ER.

In addition to the constitutive exocytosis pathway, which operates continually in all eukaryotic cells, there is a *regulated exocytosis pathway*, which operates only in cells that are specialized for secretion. Each specialized *secretory cell* produces large quantities of a particular product—such as a hormone, mucus, or digestive enzymes—which is stored in

TRACKING PROTEIN AND VESICLE TRANSPORT

Over the years, biologists have taken advantage of a variety of techniques to untangle the pathways and mechanisms by which proteins are sorted and transported into and out of the cell and its resident organelles. Biochemical, genetic, molecular biological, and microscopic techniques all provide ways to monitor how proteins shuttle from one cell compartment to another. Some can even track the migration of proteins and transport vesicles in real time in living cells.

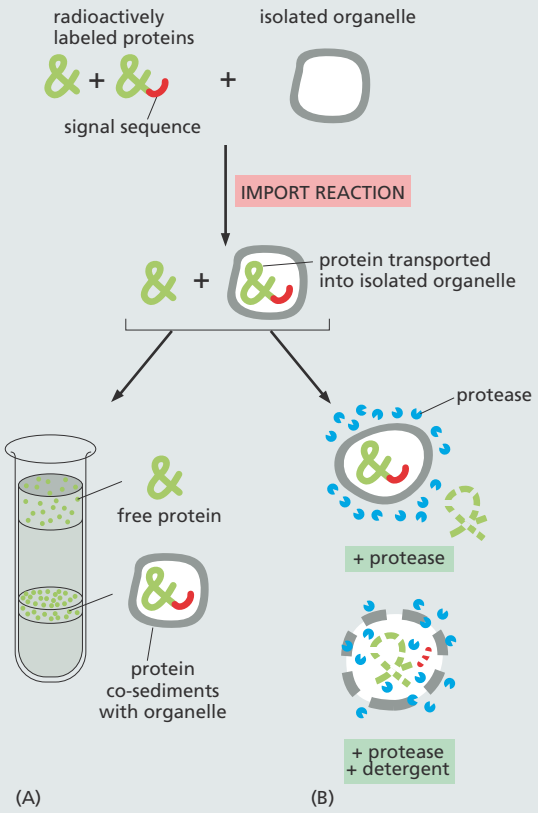


Figure 15–27 Several methods can be used to determine whether a labeled protein bearing a particular signal sequence is transported into a preparation of isolated organelles. (A) The labeled protein with or without a signal sequence is incubated with the organelles, and the preparation is centrifuged. Only those labeled proteins that contained a signal sequence will be transported and therefore will co-fractionate with the organelle. (B) The labeled proteins are incubated with the organelle, and a protease is added to the preparation. A transported protein will be selectively protected from digestion by the organelle membrane; adding a detergent that disrupts the organelle membrane will eliminate that protection, and the transported protein will also be degraded.

In a tube

A protein bearing a signal sequence can be introduced to a preparation of isolated organelles in a test tube. This mixture can then be tested to see whether the protein is taken up by the organelle. The protein is usually produced *in vitro* by cell-free translation of a purified mRNA encoding the polypeptide; in the process, radioactive amino acids can be used to label the protein so that it will be easy to isolate and to follow. The labeled protein is incubated with a selected organelle and its translocation is monitored by one of several methods (**Figure 15–27**).

Ask a yeast

Movement of proteins between different cell compartments via transport vesicles has been studied extensively using genetic techniques. Studies of mutant yeast cells that are defective for secretion at high temperatures have identified numerous genes involved in carrying proteins from the ER to the cell surface. Many of these mutant genes encode temperature-sensitive proteins (discussed in Chapter 19). These mutant proteins may function normally at 25°C, but, when the yeast cells are shifted to 35°C, the proteins are inactivated. As a result, when researchers raise the temperature, the various proteins destined for secretion instead accumulate inappropriately in the ER, Golgi apparatus, or transport vesicles—depending on the particular mutation (**Figure 15–28**).

At the movies

The most commonly used method for tracking a protein as it moves throughout the cell involves tagging the polypeptide with a fluorescent protein, such as green fluorescent protein (GFP). Using the genetic engineering techniques discussed in Chapter 10, this small protein can be fused to other cell proteins. Fortunately, for many proteins studied, the addition of GFP to one or other end does not perturb the protein's normal function or transport. The movement of a GFP-tagged protein can then be monitored in a living cell with a fluorescence microscope. In 2008, the Nobel Prize in Chemistry was awarded to Martin Chalfie and Roger Tsien for the development and refinement of this technology.

Such GFP fusion proteins are widely used to study the location and movement of proteins in cells (**Figure**

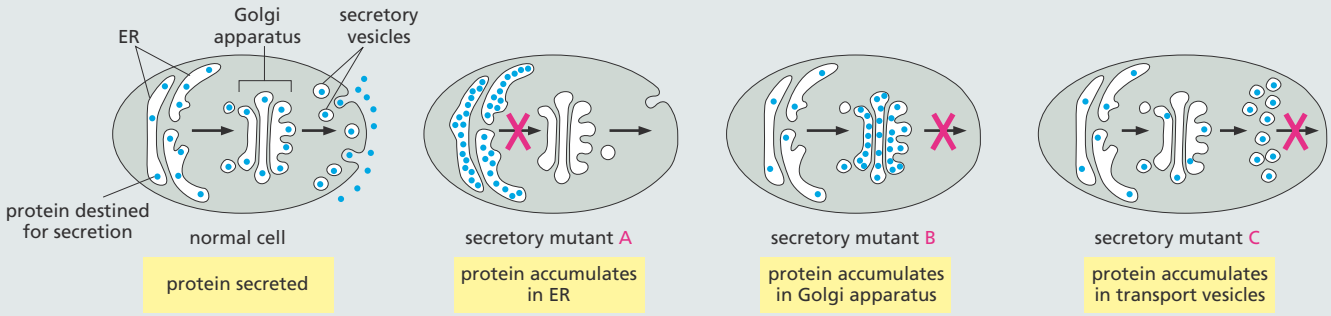


Figure 15–28 Temperature-sensitive mutants have been used to dissect the protein secretory pathway in yeast. Mutations in genes involved at different stages of the transport process, as indicated by the *red* X, result in the accumulation of proteins in the ER, the Golgi apparatus, or transport vesicles.

15–29). GFP fused to a protein that shuttles in and out of the nucleus, for example, can be used to study nuclear transport events. GFP fused to a plasma membrane protein can be used to measure the kinetics of its movement through the secretory pathway. Movies demonstrating the power and beauty of this technique are included on the DVD that accompanies this book (Movie 15.1, Movie 15.7, Movie 15.8, and Movie 15.11).

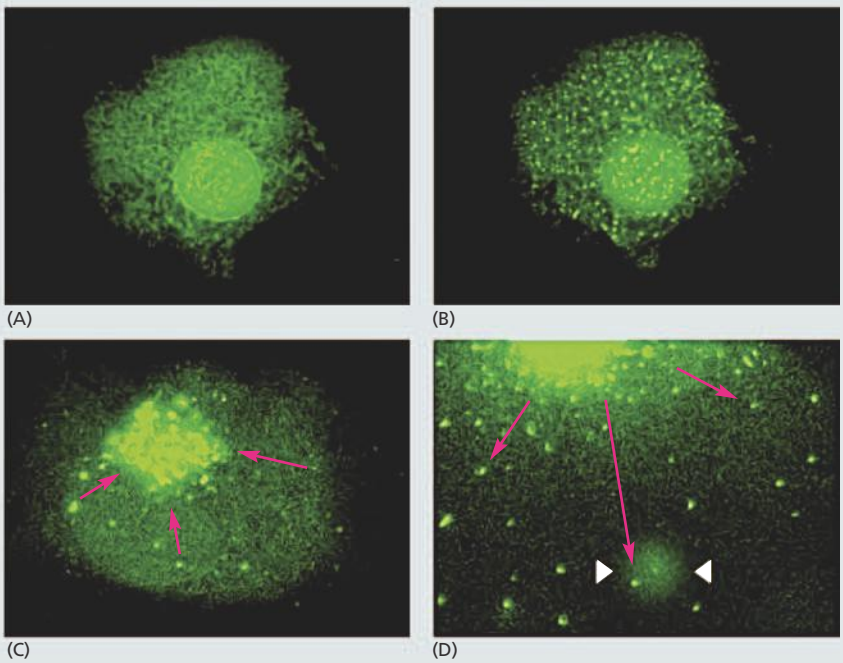


Figure 15–29 Tagging a protein with GFP allows the resulting fusion protein to be tracked throughout the cell. In this experiment, GFP is fused to a viral coat protein and expressed in cultured animal cells. In an infected cell, the viral protein moves through the secretory pathway from the ER to the cell surface, where the virus particles are assembled. *Red arrows* indicate the direction of protein movement. The viral coat protein used in this experiment contains a mutation that allows export from the ER only at a low temperature. (A) At high temperatures, the fusion protein labels the ER. (B) As the temperature is lowered, the GFP fusion protein rapidly accumulates at ER exit sites. (C) The fusion protein then moves to the Golgi apparatus. (D) Finally, the fusion protein is delivered to the plasma membrane, shown here in a more close-up view. The halo between the two *white arrowheads* marks the spot where a single vesicle has fused, allowing the fusion protein to incorporate into the plasma membrane. These images are stills taken from Movie 15.7. (A–D, courtesy of Jennifer Lippincott-Schwartz.)

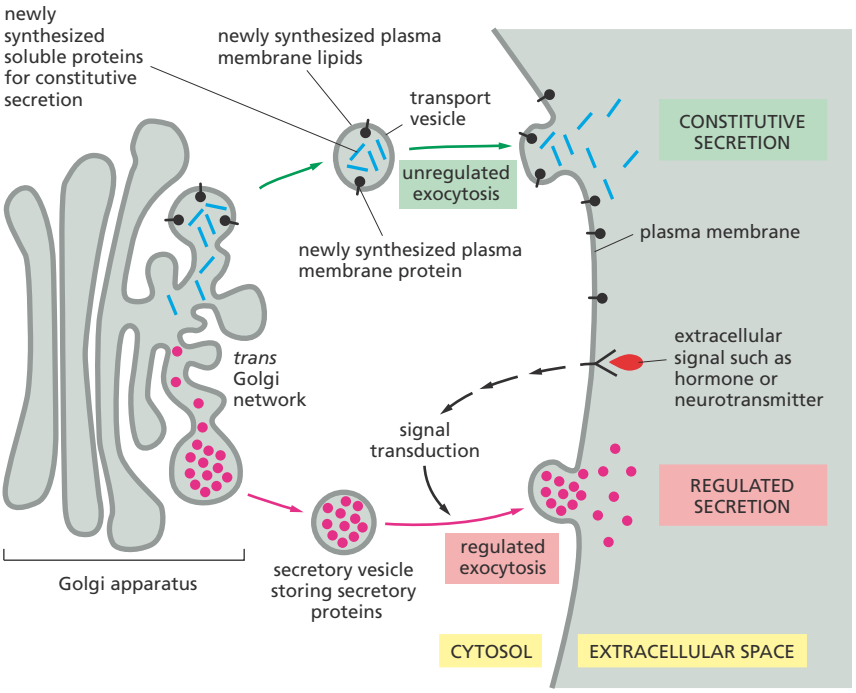


Figure 15–30 In secretory cells, the regulated and constitutive pathways of exocytosis diverge in the *trans* Golgi network. Many soluble proteins are continually secreted from the cell by the constitutive secretory pathway, which operates in all eukaryotic cells (Movie 15.8). This pathway also continually supplies the plasma membrane with newly synthesized lipids and proteins. Specialized secretory cells have, in addition, a regulated exocytosis pathway by which selected proteins in the *trans* Golgi network are diverted into secretory vesicles, where the proteins are concentrated and stored until an extracellular signal stimulates their secretion. It is unclear how these special aggregates of secretory proteins (*red*) are segregated into secretory vesicles. Secretory vesicles have unique proteins in their membranes; perhaps some of these proteins act as receptors for secretory protein aggregates in the *trans* Golgi network.

secretory vesicles for later release. These vesicles, which are part of the endomembrane system, bud off from the *trans* Golgi network and accumulate near the plasma membrane. There they wait for the extracellular signal that will stimulate them to fuse with the plasma membrane and release their contents to the cell exterior by exocytosis (**Figure 15–30**). An increase in blood glucose, for example, signals insulin-producing endocrine cells in the pancreas to secrete the hormone (**Figure 15–31**).

Proteins destined for regulated secretion are sorted and packaged in the *trans* Golgi network. Proteins that travel by this pathway have special surface properties that cause them to aggregate with one another under the ionic conditions (acidic pH and high Ca^{2+}) that prevail in the *trans* Golgi network. The aggregated proteins are packaged into secretory vesicles, which pinch off from the network and await a signal instructing them to fuse with the plasma membrane. Proteins secreted by the constitutive pathway, on the other hand, do not aggregate and are therefore carried automatically to the plasma membrane by the transport vesicles of the constitutive pathway. Selective aggregation has another function: it allows secretory proteins to be packaged into secretory vesicles at concentrations much higher than the concentration of the unaggregated protein in the Golgi lumen. This increase in concentration can reach

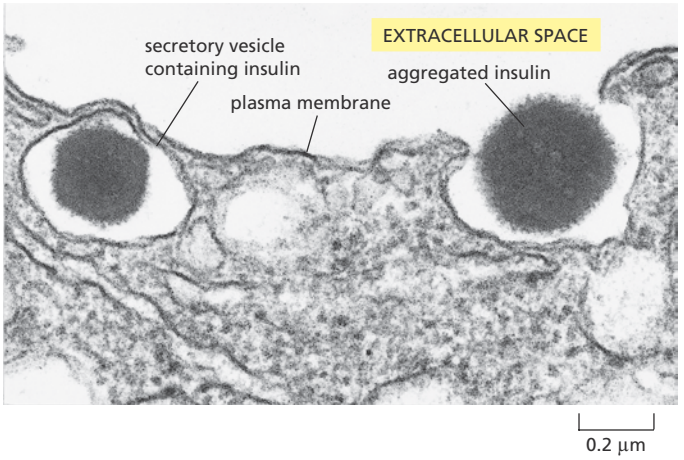


Figure 15–31 Secretory vesicles store insulin in a pancreatic β cell. The electron micrograph shows the release of insulin into the extracellular space in response to an increase in glucose levels in the blood. The insulin in each secretory vesicle is stored in a highly concentrated, aggregated form. After secretion, the insulin aggregates dissolve rapidly in the blood. (Courtesy of Lelio Orci, from L. Orci, J.D. Vassali, and A. Perrelet, *Sci. Am.* 259:85–94, 1988. With permission from Scientific American.)

200-fold, enabling secretory cells to release large amounts of the protein promptly when triggered to do so (see Figure 15–30).

When a secretory vesicle or transport vesicle fuses with the plasma membrane and discharges its contents by exocytosis, its membrane becomes part of the plasma membrane. Although this should greatly increase the surface area of the plasma membrane, it does so only transiently because membrane components are removed from other regions of the surface by endocytosis almost as fast as they are added by exocytosis. This removal returns both the lipids and the proteins of the vesicle membrane to the Golgi network, where they can be used again.

QUESTION 15–7

What would you expect to happen in cells that secrete large amounts of protein through the regulated secretory pathway if the ionic conditions in the ER lumen could be changed to resemble those in the lumen of the *trans* Golgi network?

ENDOCYTIC PATHWAYS

Eukaryotic cells are continually taking up fluid, as well as large and small molecules, by the process of **endocytosis**. Specialized cells are also able to internalize large particles and even other cells. The material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first buds inward and then pinches off to form an intracellular *endocytic vesicle*. The ingested materials, including the membrane components, are delivered to *endosomes*, from which they can be recycled to the plasma membrane or sent to lysosomes for digestion. The metabolites generated by digestion are transferred directly out of the lysosome into the cytosol, where they can be used by the cell.

Two main types of endocytosis are distinguished on the basis of the size of the endocytic vesicles formed. *Pinocytosis* ("cellular drinking") involves the ingestion of fluid and molecules via small pinocytic vesicles (<150 nm in diameter). *Phagocytosis* ("cellular eating") involves the ingestion of large particles, such as microorganisms and cell debris, via large vesicles called *phagosomes* (generally >250 nm in diameter). Whereas all eukaryotic cells are continually ingesting fluid and molecules by pinocytosis, large particles are ingested mainly by specialized *phagocytic cells*.

In this final section, we trace the endocytic pathway from the plasma membrane to lysosomes. We start by considering the uptake of large particles by phagocytosis.

Specialized Phagocytic Cells Ingest Large Particles

The most dramatic form of endocytosis, **phagocytosis**, was first observed more than a hundred years ago. In protozoa, phagocytosis is a form of feeding: these unicellular eukaryotes ingest large particles such as bacteria by taking them up into phagosomes (**Movie 15.9**). The phagosomes then fuse with lysosomes, where the food particles are digested. Few cells in multicellular organisms are able to ingest large particles efficiently. In the animal gut, for example, large particles of food have to be broken down to individual molecules by extracellular enzymes before they can be taken up by the absorptive cells lining the gut.

Nevertheless, phagocytosis is important in most animals for purposes other than nutrition. **Phagocytic cells**—including *macrophages*, which are widely distributed in tissues, and other white blood cells, such as *neutrophils*—defend us against infection by ingesting invading microorganisms. To be taken up by macrophages or neutrophils, particles must first bind to the phagocytic cell surface and activate one of a variety of surface receptors. Some of these receptors recognize antibodies, the proteins that help protect us against infection by binding to the surface of microorganisms. Binding of antibody-coated bacteria to these receptors induces the phagocytic cell to extend sheetlike projections of the plasma membrane, called *pseudopods*, that engulf the bacterium

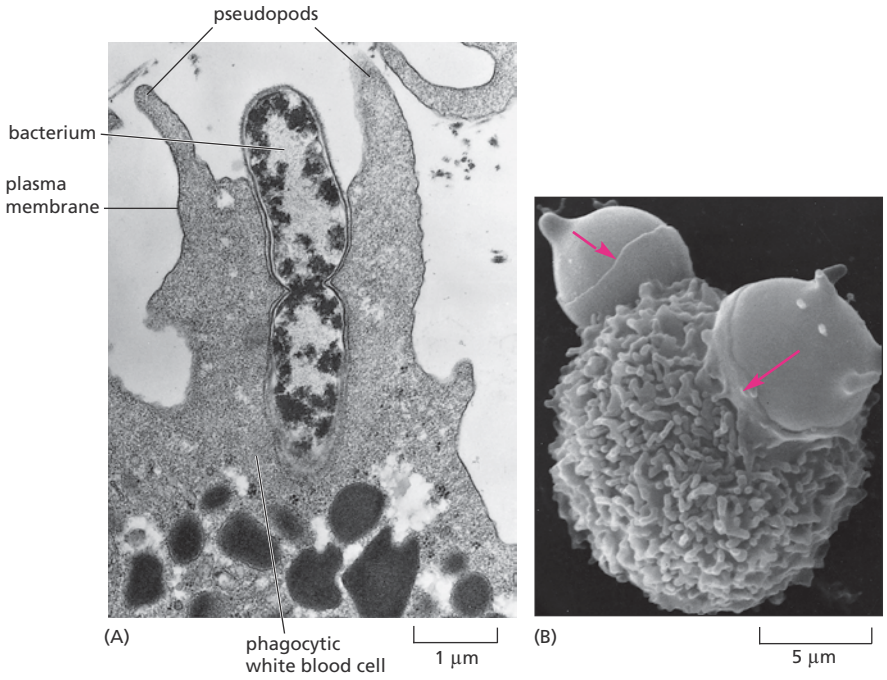


Figure 15–32 Specialized phagocytic cells can ingest other cells. (A) Electron micrograph of a phagocytic white blood cell (a neutrophil) ingesting a bacterium, which is in the process of dividing. (B) Scanning electron micrograph showing a macrophage engulfing a pair of red blood cells. The *red* arrows point to the edges of the pseudopods that the phagocytic cells are extending like collars to envelop their prey. (A, courtesy of Dorothy F. Bainton; B, courtesy of Jean Paul Revel.)

(**Figure 15–32A**) and fuse at their tips to form a phagosome. The phagosome then fuses with a lysosome, and the microbe is destroyed. Some pathogenic bacteria have evolved tricks for subverting the system: for example, *Mycobacterium tuberculosis*, the agent responsible for tuberculosis, can inhibit the membrane fusion that unites the phagosome with a lysosome. Instead of being destroyed, the engulfed organism survives and multiplies within the macrophage. Although the mechanism is not completely understood, identifying the proteins involved will provide therapeutic targets for drugs that could restore the macrophages' ability to eliminate the infection.

Phagocytic cells also play an important part in scavenging dead and damaged cells and cell debris. Macrophages, for example, ingest more than 10^{11} of your worn-out red blood cells each day (**Figure 15–32B**).

Fluid and Macromolecules Are Taken Up by Pinocytosis

Eukaryotic cells continually ingest bits of their plasma membrane, along with small amounts of extracellular fluid, in the process of **pinocytosis.** The rate at which plasma membrane is internalized in **pinocytic vesicles** varies from cell type to cell type, but it is usually surprisingly large. A macrophage, for example, swallows 25% of its own volume of fluid each hour. This means that it removes 3% of its plasma membrane each minute, or 100% in about half an hour. Pinocytosis occurs more slowly in fibroblasts, but more rapidly in some phagocytic amoebae. Because a cell's total surface area and volume remain unchanged during this process, as much membrane is being added to the cell surface by exocytosis as is being removed by endocytosis (see Figure 15–18). It is not known how eukaryotic cells maintain this remarkable balance.

Pinocytosis is carried out mainly by the clathrin-coated pits and vesicles that we discussed earlier (see Figures 15–19 and 15–20). After they pinch off from the plasma membrane, clathrin-coated vesicles rapidly shed their coat and fuse with an endosome. Extracellular fluid is trapped in the coated pit as it invaginates to form a coated vesicle, and so substances dissolved in the extracellular fluid are internalized and delivered to endosomes. This fluid intake by clathrin-coated and other types of pinocytic vesicles is generally balanced by fluid loss during exocytosis.

Receptor-mediated Endocytosis Provides a Specific Route into Animal Cells

Pinocytosis, as just described, is indiscriminate. The endocytic vesicles simply trap any molecules that happen to be present in the extracellular fluid and carry them into the cell. In most animal cells, however, pinocytosis via clathrin-coated vesicles also provides an efficient pathway for taking up specific macromolecules from the extracellular fluid. These macromolecules bind to complementary receptors on the cell surface and enter the cell as receptor–macromolecule complexes in clathrin-coated vesicles. This process, called **receptor-mediated endocytosis**, provides a selective concentrating mechanism that increases the efficiency of internalization of particular macromolecules more than 1000-fold compared with ordinary pinocytosis, so that even minor components of the extracellular fluid can be taken up in large amounts without taking in a correspondingly large volume of extracellular fluid. An important example of receptor-mediated endocytosis is the ability of animal cells to take up the cholesterol they need to make new membrane.

Cholesterol is a lipid that is extremely insoluble in water (see Figure 11–7). It is transported in the bloodstream bound to protein in the form of particles called low-density lipoproteins, or LDL. Cholesterol-containing LDLs, which are secreted by the liver, bind to receptors located on cell surfaces causing the receptor–LDL complexes to be ingested by receptor-mediated endocytosis and delivered to endosomes. The interior of endosomes is more acidic than the surrounding cytosol or the extracellular fluid, and in this acidic environment the LDL dissociates from its receptor: the receptors are returned in transport vesicles to the plasma membrane for reuse, while the LDL is delivered to lysosomes. In the lysosomes, the LDL is broken down by hydrolytic enzymes. The cholesterol is released and escapes into the cytosol, where it is available for new membrane synthesis (**Figure 15–33**).

This pathway for cholesterol uptake is disrupted in individuals who inherit a defective gene encoding the LDL receptor protein. In some cases, the receptors are missing; in others, they are present but nonfunctional. In either case, because the cells are deficient in taking up LDL, cholesterol accumulates in the blood and predisposes the individuals to develop atherosclerosis. Unless they take drugs (statins) to reduce their

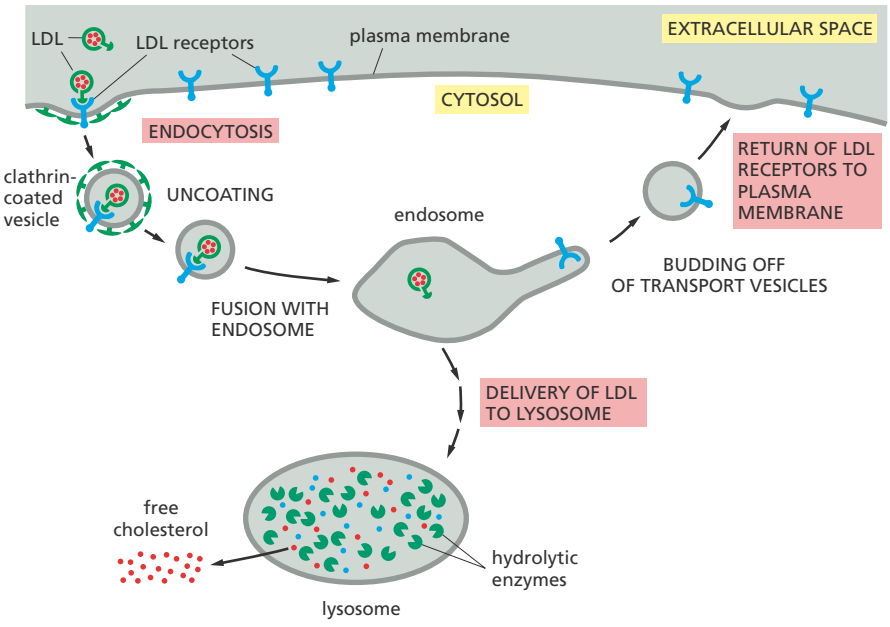


Figure 15–33 LDL enters cells via receptor-mediated endocytosis. LDL binds to LDL receptors on the cell surface and is internalized in clathrin-coated vesicles. The vesicles lose their coat and then fuse with endosomes. In the acidic environment of the endosome, LDL dissociates from its receptors. The LDL ends up in lysosomes, where it is degraded to release free cholesterol (*red dots*), but the LDL receptors are returned to the plasma membrane via transport vesicles to be used again (**Movie 15.10**). For simplicity, only one LDL receptor is shown entering the cell and returning to the plasma membrane. Whether it is occupied or not, an LDL receptor typically makes one round trip into the cell and back every 10 minutes, making a total of several hundred trips in its 20-hour life-span.

blood cholesterol, they will likely die at an early age of heart attacks, which result from cholesterol clogging the coronary arteries that supply the heart muscle.

Receptor-mediated endocytosis is also used to take up many other essential metabolites, such as vitamin B_{12} and iron, that cells cannot take up by the processes of transmembrane transport discussed in Chapter 12. Vitamin B_{12} and iron are both required, for example, for the synthesis of hemoglobin, which is the major protein in red blood cells; they enter immature red blood cells as part of a complex with their respective receptor proteins. Many cell-surface receptors that bind extracellular signal molecules are also ingested by this pathway: some are recycled to the plasma membrane for reuse, whereas others are degraded in lysosomes. Unfortunately, receptor-mediated endocytosis can also be exploited by viruses: the influenza virus, which causes the flu, gains entry into cells in this way.

QUESTION 15–8

Iron (Fe) is an essential trace metal that is needed by all cells. It is required, for example, for synthesis of the heme groups and iron-sulfur centers that are part of the active site of many proteins involved in electron-transfer reactions; it is also required in hemoglobin, the main protein in red blood cells. Iron is taken up by cells by receptor-mediated endocytosis. The iron-uptake system has two components: a soluble protein called transferrin, which circulates in the bloodstream; and a transferrin receptor—a transmembrane protein that, like the LDL receptor in Figure 15–33, is continually endocytosed and recycled to the plasma membrane. Fe ions bind to transferrin at neutral pH but not at acidic pH. Transferrin binds to the transferrin receptor at neutral pH only when it has an Fe ion bound, but it binds to the receptor at acidic pH even in the absence of bound iron. From these properties, describe how iron is taken up, and discuss the advantages of this elaborate scheme.

Endocytosed Macromolecules Are Sorted in Endosomes

Because most extracellular material taken up by pinocytosis is rapidly delivered to **endosomes**, it is possible to visualize the endosomal compartment by incubating living cells in fluid containing an electron-dense marker that will show up when viewed in an electron microscope. When examined in this way, the endosomal compartment reveals itself to be a complex set of connected membrane tubes and larger vesicles. Two sets of endosomes can be distinguished in such loading experiments: the marker molecules appear first in *early endosomes*, just beneath the plasma membrane; 5–15 minutes later, they show up in *late endosomes,* closer to the nucleus (see Figure 15–18). Early endosomes mature gradually into late endosomes as they fuse with each other or with a preexisting late endosome (**Movie 15.11**). The interior of the endosome compartment is kept acidic (pH 5–6) by an ATP-driven H^+ (proton) pump in the endosomal membrane that pumps H^+ into the endosome lumen from the cytosol.

The endosomal compartment acts as the main sorting station in the inward endocytic pathway, just as the *trans* Golgi network serves this function in the outward secretory pathway. The acidic environment of the endosome plays a crucial part in the sorting process by causing many (but not all) receptors to release their bound cargo. The routes taken by receptors once they have entered an endosome differ according to the type of receptor: (1) most are returned to the same plasma membrane domain from which they came, as is the case for the LDL receptor discussed earlier; (2) some travel to lysosomes, where they are degraded; and (3) some proceed to a different domain of the plasma membrane, thereby transferring their bound cargo molecules across the cell from one extracellular space to another, a process called *transcytosis* (**Figure 15–34**).

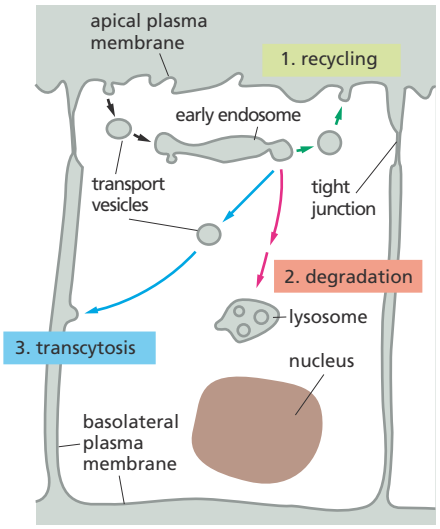


Figure 15–34 The fate of receptor proteins following their endocytosis depends on the type of receptor. Three pathways from the endosomal compartment in an epithelial cell are shown. Receptors that are not specifically retrieved from early endosomes follow the pathway from the endosomal compartment to lysosomes, where they are degraded. Retrieved receptors are returned either to the same plasma membrane domain from which they came (*recycling*) or to a different domain of the plasma membrane (*transcytosis*). Tight junctions separate the apical and basolateral plasma membranes preventing their resident receptor proteins from diffusing from one domain to another. If the ligand that is endocytosed with its receptor stays bound to the receptor in the acidic environment of the endosome, it will follow the same pathway as the receptor; otherwise it will be delivered to lysosomes for degradation.

Cargo proteins that remain bound to their receptors share the fate of their receptors. Cargo that dissociates from receptors in the endosome is doomed to destruction in lysosomes, along with most of the contents of the endosome lumen. Late endosomes contain some lysosomal enzymes, so digestion of cargo proteins and other macromolecules begins in the endosome and continues as the endosome gradually matures into a lysosome: once it has digested most of its ingested contents, the endosome takes on the dense, rounded appearance characteristic of a mature, "classical" lysosome.

Lysosomes Are the Principal Sites of Intracellular Digestion

Many extracellular particles and molecules ingested by cells end up in **lysosomes**, which are membranous sacs of hydrolytic enzymes that carry out the controlled intracellular digestion of both extracellular materials and worn-out organelles. They contain about 40 types of hydrolytic enzymes, including those that degrade proteins, nucleic acids, oligosaccharides, and lipids. All of these enzymes are optimally active in the acidic conditions (pH ~5) maintained within lysosomes. The membrane of the lysosome normally keeps these destructive enzymes out of the cytosol (whose pH is about 7.2), but the enzymes' acid dependence protects the contents of the cytosol against damage even if some of them should escape.

Like all other intracellular organelles, the lysosome not only contains a unique collection of enzymes but also has a unique surrounding membrane. The lysosomal membrane contains transporters that allow the final products of the digestion of macromolecules, such as amino acids, sugars, and nucleotides, to be transferred to the cytosol; from there, they can be either excreted or utilized by the cell. The membrane also contains an ATP-driven H^+ pump, which, like the ATPase in the endosome membrane, pumps H^+ into the lysosome, thereby maintaining its contents at an acidic pH (**Figure 15–35**). Most of the lysosomal membrane proteins are unusually highly glycosylated; the sugars, which cover much of the protein surfaces facing the lumen, protect the proteins from digestion by the lysosomal proteases.

The specialized digestive enzymes and membrane proteins of the lysosome are synthesized in the ER and transported through the Golgi apparatus to the *trans* Golgi network. While in the ER and the *cis* Golgi network, the enzymes are tagged with a specific phosphorylated sugar group (mannose 6-phosphate), so that when they arrive in the *trans* Golgi network they can be recognized by an appropriate receptor, the mannose 6-phosphate receptor. This tagging permits the lysosomal enzymes to be sorted and packaged into transport vesicles, which bud off and deliver their contents to lysosomes via endosomes (see Figure 15–18).

Depending on their source, materials follow different paths to lysosomes. We have seen that extracellular particles are taken up into phagosomes, which fuse with lysosomes, and that extracellular fluid and macromolecules are taken up into smaller endocytic vesicles, which deliver their contents to lysosomes via endosomes.

Cells have an additional pathway that supplies materials to lysosomes; this pathway, called **autophagy**, is used to degrade obsolete parts of the cell—the cell literally eats itself. In electron micrographs of liver cells, for example, one often sees lysosomes digesting mitochondria, as well as other organelles. The process begins with the enclosure of the organelle by a double membrane, creating an *autophagosome*, which then fuses with a lysosome (**Figure 15–36**). It is still debated where these membrane fragments originate, or how specific cell components are marked

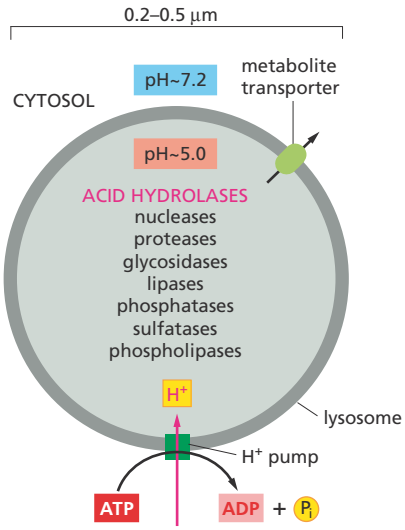


Figure 15–35 A lysosome contains a large variety of hydrolytic enzymes, which are only active under acidic conditions. The lumen of the lysosome is maintained at an acidic pH by an ATP-driven H^+ pump in the membrane that hydrolyzes ATP to pump H^+ into the lumen.

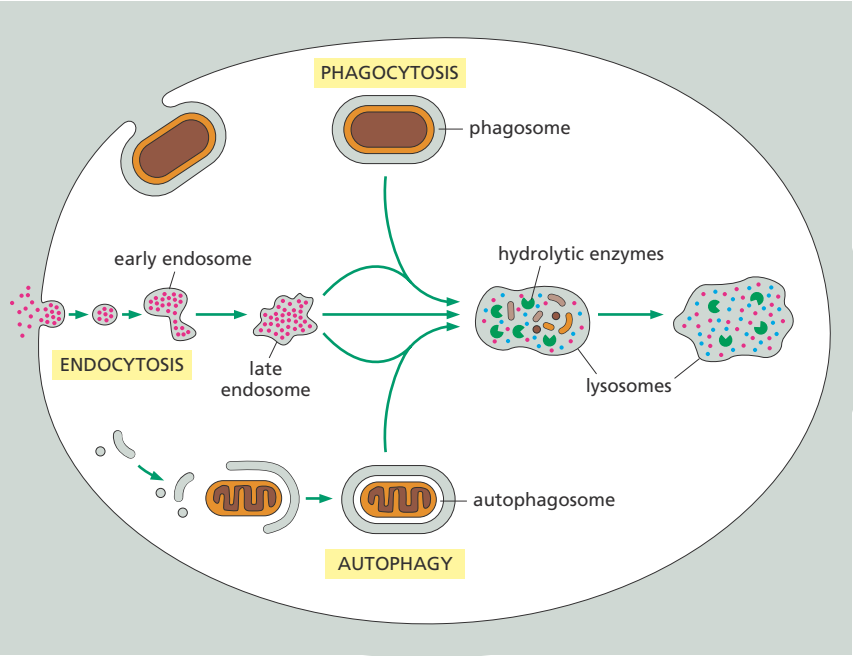


Figure 15–36 Materials destined for degradation in lysosomes follow different pathways to the lysosome. Each pathway leads to the intracellular digestion of materials derived from a different source. Early endosomes, phagosomes, and autophagosomes can fuse with either lysosomes or late endosomes, both of which contain acid-dependent hydrolytic enzymes.

for such destruction, but autophagy of organelles and cytosolic proteins increases when eukaryotic cells are starved or when they remodel themselves extensively during development. The amino acids generated by this cannibalistic form of digestion can then be recycled to allow continued protein synthesis.

ESSENTIAL CONCEPTS

- Eukaryotic cells contain many membrane-enclosed organelles, including a nucleus, an endoplasmic reticulum (ER), a Golgi apparatus, lysosomes, endosomes, mitochondria, chloroplasts (in plant cells), and peroxisomes. The ER, Golgi apparatus, peroxisomes, endosomes, and lysosomes are all part of the *endomembrane system*.
- Most organelle proteins are made in the cytosol and transported into the organelle where they function. Sorting signals in the amino acid sequence guide the proteins to the correct organelle; proteins that function in the cytosol have no such signals and remain where they are made.
- Nuclear proteins contain nuclear localization signals that help direct their active transport from the cytosol into the nucleus through nuclear pores, which penetrate the double-membrane nuclear envelope. The proteins are transported in their fully folded conformation.
- Most mitochondrial and chloroplast proteins are made in the cytosol and are then transported into the organelles by protein translocators in their membranes. The proteins are unfolded during the transport process.
- The ER makes most of the cell's lipids and many of its proteins. The proteins are made by ribosomes that are directed to the ER by a signal-recognition particle (SRP) in the cytosol that recognizes an ER signal sequence on the growing polypeptide chain. The ribosome–SRP complex binds to a receptor on the ER membrane, which passes the

ribosome to a protein translocator that threads the growing polypeptide across the ER membrane through a translocation channel.

- Water-soluble proteins destined for secretion or for the lumen of an organelle of the endomembrane system pass completely into the ER lumen, while transmembrane proteins destined for either the membrane of these organelles or for the plasma membrane remain anchored in the lipid bilayer by one or more membrane-spanning α helices.
- In the ER lumen, proteins fold up, assemble with their protein partners, form disulfide bonds, and become decorated with oligosaccharide chains.
- Exit from the ER is an important quality-control step; proteins that either fail to fold properly or fail to assemble with their normal partners are retained in the ER by chaperone proteins, which prevent their aggregation and help them fold; proteins that still fail to fold or assemble are transported to the cytosol, where they are degraded.
- Excessive accumulation of misfolded proteins triggers an unfolded protein response that expands the ER, increases its capacity to fold new proteins properly, and reduces protein synthesis.
- Protein transport from the ER to the Golgi apparatus and from the Golgi apparatus to other destinations is mediated by transport vesicles that continually bud off from one membrane and fuse with another, a process called vesicular transport.
- Budding transport vesicles have distinctive coat proteins on their cytosolic surface; the assembly of the coat helps drive both the budding process and the incorporation of cargo receptors, with their bound cargo molecules, into the forming vesicle.
- Coated vesicles rapidly lose their protein coat, enabling them to dock and then fuse with a particular target membrane; docking and fusion are mediated by proteins on the surface of the vesicle and target membrane, including Rab and SNARE proteins.
- The Golgi apparatus receives newly made proteins from the ER; it modifies their oligosaccharides, sorts the proteins, and dispatches them from the *trans* Golgi network to the plasma membrane, lysosomes (via endosomes), or secretory vesicles.
- In all eukaryotic cells, transport vesicles continually bud from the *trans* Golgi network and fuse with the plasma membrane; this process of constitutive exocytosis delivers proteins to the cell surface for secretion and incorporates lipids and proteins into the plasma membrane.
- Specialized secretory cells also have a regulated exocytosis pathway, in which molecules concentrated and stored in secretory vesicles are released from the cell by exocytosis when the cell is signaled to secrete.
- Cells ingest fluid, molecules, and sometimes even particles by endocytosis, in which regions of plasma membrane invaginate and pinch off to form endocytic vesicles.
- Much of the material that is endocytosed is delivered to endosomes, which mature into lysosomes, in which the material is degraded by hydrolytic enzymes; most of the components of the endocytic vesicle membrane, however, are recycled in transport vesicles back to the plasma membrane for reuse.

KEY TERMS

autophagy
chaperone protein
clathrin
coated vesicle
endocytosis
endomembrane system
endoplasmic reticulum (ER)
endosome
exocytosis
Golgi apparatus
lysosome
membrane-enclosed organelle
nuclear envelope
nuclear pore
peroxisome
phagocytic cell
phagocytosis
pinocytosis
Rab protein
receptor-mediated endocytosis
rough endoplasmic reticulum
secretion
secretory vesicle
signal sequence
SNARE
transport vesicle
unfolded protein response (UPR)
vesicular transport

QUESTIONS

QUESTION 15–9

Which of the following statements are correct? Explain your answers.

A. Ribosomes are cytoplasmic structures that, during protein synthesis, become linked by an mRNA molecule to form polyribosomes.

B. The amino acid sequence Leu-His-Arg-Leu-Asp-Ala-Gln-Ser-Lys-Leu-Ser-Ser is a signal sequence that directs proteins to the ER.

C. All transport vesicles in the cell must have a v-SNARE protein in their membrane.

D. Transport vesicles deliver proteins and lipids to the cell surface.

E. If the delivery of prospective lysosomal proteins from the *trans* Golgi network to the late endosomes were blocked, lysosomal proteins would be secreted by the constitutive secretion pathways shown in Figure 15–30.

F. Lysosomes digest only substances that have been taken up by cells by endocytosis.

G. *N*-linked sugar chains are found on glycoproteins that face the cell surface, as well as on glycoproteins that face the lumen of the ER, *trans* Golgi network, and mitochondria.

QUESTION 15–10

Some proteins shuttle back and forth between the nucleus and the cytosol. They need a nuclear export signal to get out of the nucleus. How do you suppose they get into the nucleus?

QUESTION 15–11

Influenza viruses are surrounded by a membrane that contains a fusion protein, which is activated by acidic pH. Upon activation, the protein causes the viral membrane to fuse with cell membranes. An old folk remedy against flu recommends that one should spend a night in a horse's stable. Odd as it may sound, there is a rational explanation for this advice. Air in stables contains ammonia (NH_3) generated by bacteria in the horse's urine. Sketch a diagram showing the pathway (in detail) by which flu virus enters cells, and speculate how NH_3 may protect cells from virus infection. (Hint: NH_3 can neutralize acidic solutions by the reaction $NH_3 + H^+ \rightarrow NH_4^+$.)

QUESTION 15–12

Consider the v-SNAREs that direct transport vesicles from the *trans* Golgi network to the plasma membrane. They, like all other v-SNAREs, are membrane proteins that are integrated into the membrane of the ER during their biosynthesis and are then carried by transport vesicles to their destination. Thus, transport vesicles budding from the ER contain at least two kinds of v-SNAREs—those that target the vesicles to the *cis* Golgi cisternae, and those that are in transit to the *trans* Golgi network to be packaged in different transport vesicles destined for the plasma membrane. (A) Why might this be a problem? (B) Suggest possible ways in which the cell might solve it.

QUESTION 15–13

A particular type of *Drosophila* mutant becomes paralyzed when the temperature is raised. The mutation affects the structure of dynamin, causing it to be inactivated at the higher temperature. Indeed, the function of dynamin was discovered by analyzing the defect in these mutant fruit flies. The complete paralysis at the elevated temperature suggests that synaptic transmission between nerve and muscle cells (discussed in Chapter 12) is blocked. Suggest why signal transmission at a synapse might require dynamin. On the basis of your hypothesis, what would you expect to see in electron micrographs of synapses of flies that were exposed to the elevated temperature?

QUESTION 15–14

Edit each of the following statements, if required, to make them true: "Because nuclear localization sequences are not cleaved off by proteases following protein import into the nucleus, they can be reused to import nuclear proteins after mitosis, when cytosolic and nuclear proteins have become intermixed. This is in contrast to ER signal sequences, which are cleaved off by a signal peptidase once they reach the lumen of the ER. ER signal sequences cannot therefore be reused to import ER proteins after mitosis, when cytosolic and ER proteins have become intermixed; these ER proteins must therefore be degraded and resynthesized."

QUESTION 15–15

Consider a protein that contains an ER signal sequence at its N-terminus and a nuclear localization sequence in its middle. What do you think the fate of this protein would be? Explain your answer.

QUESTION 15–16

Compare and contrast protein import into the ER and into the nucleus. List at least two major differences in the mechanisms, and speculate why the ER mechanism might not work for nuclear import and vice versa.

QUESTION 15–17

During mitosis, the nuclear envelope breaks down and intranuclear proteins completely intermix with cytosolic proteins. Is this consistent with the evolutionary scheme proposed in Figure 15–3?

QUESTION 15–18

A protein that inhibits certain proteolytic enzymes (proteases) is normally secreted into the bloodstream by liver cells. This inhibitor protein, antitrypsin, is absent from the bloodstream of patients who carry a mutation that results in a single amino acid change in the protein. Antitrypsin deficiency causes a variety of severe problems, particularly in lung tissue, because of the uncontrolled activity of proteases. Surprisingly, when the mutant antitrypsin is synthesized in the laboratory, it is as active as the normal antitrypsin at inhibiting proteases. Why, then, does the mutation cause the disease? Think of more than one possibility, and suggest ways in which you could distinguish between them.

QUESTION 15–19

Dr. Outonalimb's claim to fame is her discovery of forgettin, a protein predominantly made by the pineal gland in human teenagers. The protein causes selective short-term unresponsiveness and memory loss when the auditory system receives statements like "Please take out the garbage!" Her hypothesis is that forgettin has a hydrophobic ER signal sequence at its C-terminus that is recognized by an SRP and causes it to be translocated across the ER membrane by the mechanism shown in Figure 15–14. She predicts that the protein is secreted from pineal cells into the bloodstream, from where it exerts its devastating systemic effects. You are a member of the committee deciding whether she should receive a grant for further work on her hypothesis. Critique her proposal, and remember that grant reviews should be polite and constructive.

QUESTION 15–20

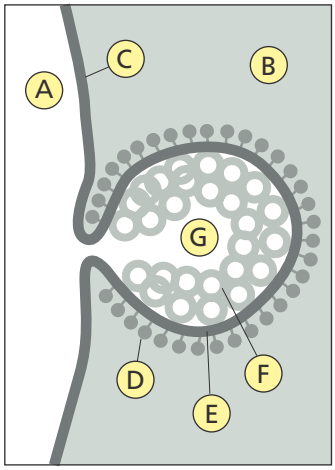
Taking the evolutionary scheme in Figure 15–3 one step further, suggest how the Golgi apparatus could have evolved. Sketch a simple diagram to illustrate your ideas. For the Golgi apparatus to be functional, what else would be needed?

QUESTION 15–21

If membrane proteins are integrated into the ER membrane by means of the ER protein translocator (which is itself composed of membrane proteins), how do the first protein translocation channels become incorporated into the ER membrane?

QUESTION 15–22

The sketch in **Figure Q15–22** is a schematic drawing of the electron micrograph shown in the third panel of Figure 15–19A. Name the structures that are labeled in the sketch.



Q15–22

QUESTION 15–23

What would happen to proteins bound for the nucleus if there were insufficient energy to transport them?

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CHAPTER **SIXTEEN** 16

Cell Signaling

Individual cells, like multicellular organisms, need to sense and respond to their environment. A free-living cell—even a humble bacterium—must be able to track down nutrients, tell the difference between light and dark, and avoid poisons and predators. And if such a cell is to have any kind of "social life," it must be able to communicate with other cells. When a yeast cell is ready to mate, for example, it secretes a small protein called a mating factor. Yeast cells of the opposite "sex" detect this chemical mating call and respond by halting their progress through the cell-division cycle and reaching out toward the cell that emitted the signal (**Figure 16–1**).

In a multicellular organism, things are much more complicated. Cells must interpret the multitude of signals they receive from other cells to help coordinate their behaviors. During animal development, for example, cells in the embryo exchange signals to determine which specialized role each cell will adopt, what position it will occupy in the animal, and whether it will survive, divide, or die. Later in life, a large variety of signals coordinates the animal's growth and its day-to-day physiology and behavior. In plants as well, cells are in constant communication with one another. These cell–cell interactions allow the plant to coordinate what happens in its roots, stems, and leaves.

In this chapter, we examine some of the most important mechanisms by which cells send signals and interpret the signals they receive. First, we present an overview of the general principles of cell signaling. We then consider two of the main systems animal cells use to receive and interpret signals, followed by a brief discussion of cell signaling mechanisms in plants. Finally, we consider how extensive and intricate signaling networks interact to control complex behaviors.

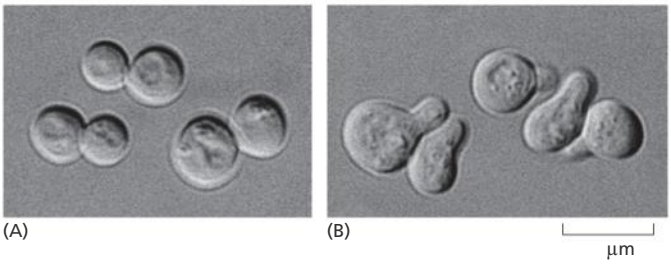


Figure 16–1 Yeast cells respond to mating factor. Budding yeast (*Saccharomyces cerevisiae*) cells are normally spherical (A), but when they are exposed to an appropriate mating factor produced by neighboring yeast cells (B), they extend a protrusion toward the source of the factor. (Courtesy of Michael Snyder.)

GENERAL PRINCIPLES OF CELL SIGNALING

Information can come in a variety of forms, and communication frequently involves converting the signals that carry that information from one form to another. When you receive a call from a friend on your mobile phone, for instance, the phone converts the radio signals, which travel through the air, into sound waves, which you hear. This process of conversion is called **signal transduction** (**Figure 16–2**).

The signals that pass between cells are simpler than the sorts of messages that humans ordinarily exchange. In a typical communication between cells, the *signaling cell* produces a particular type of *extracellular signal molecule* that is detected by the *target cell*. As in human conversation, most animal cells both send and receive signals, and they can therefore act as both signaling cells and target cells.

Target cells possess proteins called *receptors* that recognize and respond specifically to the signal molecule. Signal transduction begins when the receptor on a target cell receives an incoming extracellular signal and converts it to the *intracellular signaling molecules* that alter cell behavior. Most of this chapter is concerned with signal reception and transduction—the events that cell biologists have in mind when they refer to **cell signaling**. First, however, we look briefly at the different types of extracellular signals that cells send to one another.

Signals Can Act over a Long or Short Range

Cells in multicellular organisms use hundreds of kinds of *extracellular signal molecules* to communicate with one another. The signal molecules can be proteins, peptides, amino acids, nucleotides, steroids, fatty acid derivatives, or even dissolved gases—but they all rely on only a handful of basic styles of communication for getting the message across.

In multicellular organisms, the most "public" style of cell-to-cell communication involves broadcasting the signal throughout the whole body by secreting it into an animal's bloodstream or a plant's sap. Extracellular signal molecules used in this way are called **hormones**, and, in animals, the cells that produce hormones are called *endocrine* cells (**Figure 16–3A**). Part of the pancreas, for example, is an endocrine gland that produces several hormones—including insulin, which regulates glucose uptake in cells all over the body.

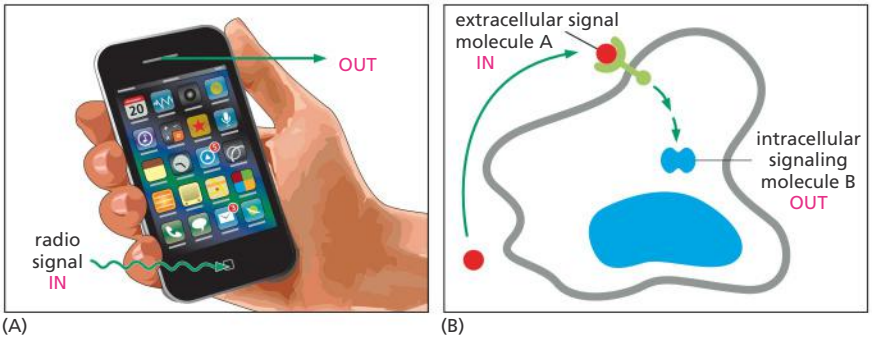


Figure 16–2 Signal transduction is the process whereby one type of signal is converted to another. (A) When a mobile telephone receives a radio signal, it converts it into a sound signal; when transmitting a signal, it does the reverse. (B) A target cell converts an extracellular signal molecule (molecule A) into an intracellular signaling molecule (molecule B).

Somewhat less public is the process known as *paracrine signaling*. In this case, rather than entering the bloodstream, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighborhood of the cell that secretes them. Thus, they act as **local mediators** on nearby cells (**Figure 16–3B**). Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound function in this way. In some cases, cells can respond to the local mediators that they themselves produce, a form of paracrine communication called *autocrine signaling*; cancer cells sometimes promote their own survival and proliferation in this way.

Neuronal signaling is a third form of cell communication. Like endocrine cells, nerve cells (neurons) can deliver messages over long distances. In the case of neuronal signaling, however, a message is not broadcast widely but is instead delivered quickly and specifically to individual target cells through private lines. As described in Chapter 12, the axon of a neuron terminates at specialized junctions (*synapses*) on target cells that can lie far from the neuronal cell body (**Figure 16–3C**). The axons that extend from the spinal cord to the big toe in an adult human, for example, can be more than a meter in length. When activated by signals from the environment or from other nerve cells, a neuron sends electrical impulses racing along its axon at speeds of up to 100 m/sec. On reaching the axon terminal, these electrical signals are converted into a chemical form: each electrical impulse stimulates the nerve terminal to release a pulse of an extracellular signal molecule called a **neurotransmitter**. The neurotransmitter then diffuses across the narrow (<100 nm) gap that separates the membrane of the axon terminal from that of the target cell, reaching its destination in less than 1 msec.

A fourth style of signal-mediated cell-to-cell communication—the most intimate and short-range of all—does not require the release of a secreted molecule. Instead, the cells make direct physical contact through signal

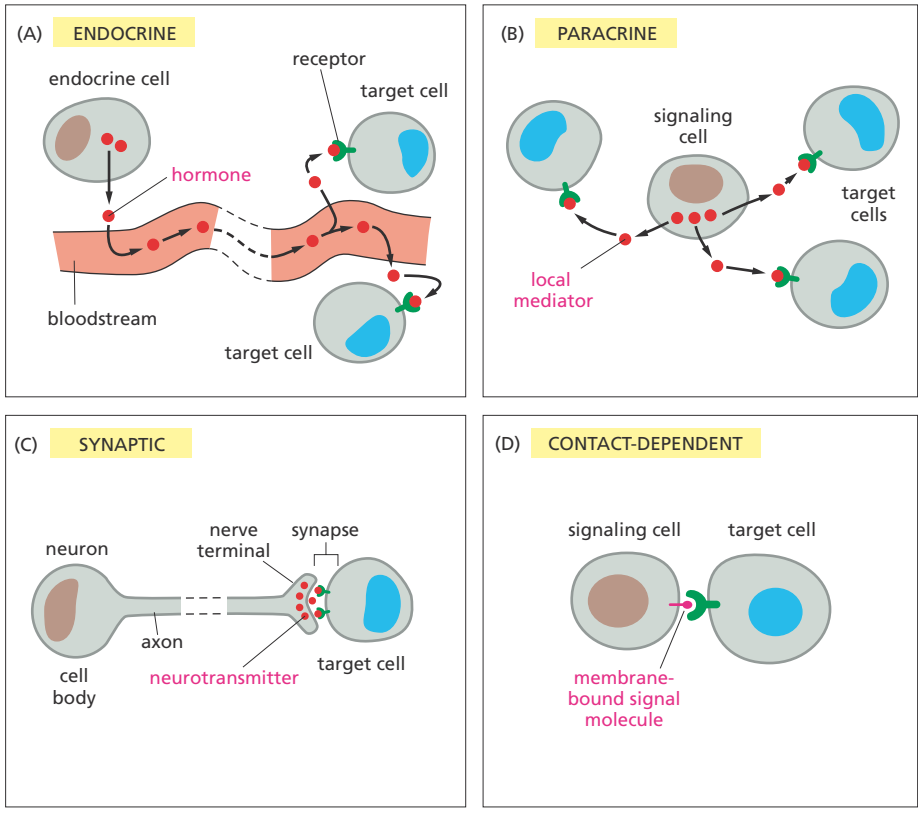


Figure 16–3 Animal cells use extracellular signal molecules to communicate with one another in various ways. (A) Hormones produced in endocrine glands are secreted into the bloodstream and are distributed widely throughout the body. (B) Paracrine signals are released by cells into the extracellular fluid in their neighborhood and act locally. (C) Neuronal signals are transmitted electrically along a nerve cell axon. When this electrical signal reaches the nerve terminal, it causes the release of neurotransmitters onto adjacent target cells. (D) In contact-dependent signaling, a cell-surface-bound signal molecule binds to a receptor protein on an adjacent cell. Many of the same types of signal molecules are used for endocrine, paracrine, and neuronal signaling. The crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

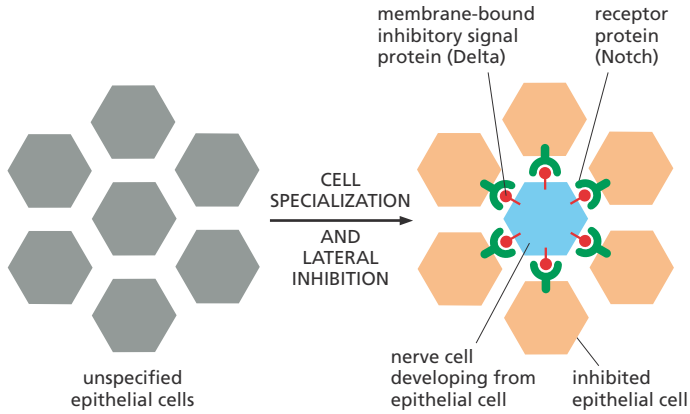


Figure 16–4 Contact-dependent signaling controls nerve-cell production in the fruit fly *Drosophila*. The fly nervous system originates in the embryo from a sheet of epithelial cells. Isolated cells in this sheet begin to specialize as neurons, while their neighbors remain non-neuronal and maintain the structure of the epithelial sheet. The signals that control this process are transmitted via direct cell–cell contacts: each future neuron delivers an inhibitory signal to the cells next to it, deterring them from specializing as neurons too—a process called lateral inhibition. Both the signal molecule (in this case, Delta) and the receptor molecule (called Notch) are transmembrane proteins.

molecules lodged in the plasma membrane of the signaling cell and receptor proteins embedded in the plasma membrane of the target cell (**Figure 16–3D**). During embryonic development, for example, such *contact-dependent signaling* allows adjacent cells that are initially similar to become specialized to form different cell types (**Figure 16–4**).

To contrast these different signaling styles, imagine trying to advertise a potentially stimulating lecture—or a concert or football game. An endocrine signal would be akin to broadcasting the information over a radio station. A localized paracrine signal would be the equivalent of posting a flyer on selected notice boards in your neighborhood. Neuronal signals—long-distance but personal—would be similar to a phone call, a text message, or an e-mail, and contact-dependent signaling would be like a good old-fashioned, face-to-face conversation. In autocrine signaling, you might write a note to remind yourself to attend.

Table 16–1 lists some examples of hormones, local mediators, neurotransmitters, and contact-dependent signal molecules. The action of several of these is discussed in more detail later in the chapter.

Each Cell Responds to a Limited Set of Extracellular Signals, Depending on Its History and Its Current State

A typical cell in a multicellular organism is exposed to hundreds of different signal molecules in its environment. These may be free in the extracellular fluid, embedded in the extracellular matrix in which most cells reside, or bound to the surface of neighboring cells. Each cell must respond very selectively to this mixture of signals, disregarding some and reacting to others, according to the cell's specialized function.

Whether a cell responds to a signal molecule depends first of all on whether it possesses a **receptor** for that signal. Each receptor is usually activated by only one type of signal. Without the appropriate receptor, a cell will be deaf to the signal and will not respond to it. By producing only a limited set of receptors out of the thousands that are possible, a cell restricts the types of signals that can affect it.

Of course, even this restricted set of extracellular signal molecules could change the behavior of a target cell in a large variety of ways. They could alter the cell's shape, movement, metabolism, or gene expression, or some combination of these. As we will see, the signal from a cell-surface receptor is generally conveyed into the target cell interior via a set of intracellular signaling molecules. These molecules act in sequence and ultimately alter the activity of *effector proteins*, those that have some direct effect on the behavior of the target cell. This intracellular relay

QUESTION 16–1

To remain a local stimulus, paracrine signal molecules must be prevented from straying too far from their points of origin. Suggest different ways by which this could be accomplished. Explain your answers.

TABLE 16–1 SOME EXAMPLES OF SIGNAL MOLECULES

Signal Molecule	Site of Origin	Chemical Nature	Some Actions
Hormones			
Adrenaline (epinephrine)	adrenal gland	derivative of the amino acid tyrosine	increases blood pressure, heart rate, and metabolism
Cortisol	adrenal gland	steroid (derivative of cholesterol)	affects metabolism of proteins, carbohydrates, and lipids in most tissues
Estradiol	ovary	steroid (derivative of cholesterol)	induces and maintains secondary female sexual characteristics
Insulin	β cells of pancreas	protein	stimulates glucose uptake, protein synthesis, and lipid synthesis in various cell types
Testosterone	testis	steroid (derivative of cholesterol)	induces and maintains secondary male sexual characteristics
Thyroid hormone (thyroxine)	thyroid gland	derivative of the amino acid tyrosine	stimulates metabolism in many cell types
Local Mediators			
Epidermal growth factor (EGF)	various cells	protein	stimulates epidermal and many other cell types to proliferate
Platelet-derived growth factor (PDGF)	various cells, including blood platelets	protein	stimulates many cell types to proliferate
Nerve growth factor (NGF)	various innervated tissues	protein	promotes survival of certain classes of neurons; promotes their survival and growth of their axons
Histamine	mast cells	derivative of the amino acid histidine	causes blood vessels to dilate and become leaky, helping to cause inflammation
Nitric oxide (NO)	nerve cells; endothelial cells lining blood vessels	dissolved gas	causes smooth muscle cells to relax; regulates nerve-cell activity
Neurotransmitters			
Acetylcholine	nerve terminals	derivative of choline	excitatory neurotransmitter at many nerve–muscle synapses and in central nervous system
γ-Aminobutyric acid (GABA)	nerve terminals	derivative of the amino acid glutamic acid	inhibitory neurotransmitter in central nervous system
Contact-dependent Signal Molecules			
Delta	prospective neurons; various other developing cell types	transmembrane protein	inhibits neighboring cells from becoming specialized in same way as the signaling cell

system and the intracellular effector proteins on which it acts vary from one type of specialized cell to another, so that different types of cells respond to the same signal in different ways. For example, when a heart pacemaker cell is exposed to the neurotransmitter *acetylcholine*, its rate of firing decreases. When a salivary gland is exposed to the same signal, it secretes components of saliva, even though the receptors are the same on both cell types. In skeletal muscle, acetylcholine binds to a different receptor protein, causing the cell to contract (**Figure 16–5**). Thus, the extracellular signal molecule alone is not the message: the information conveyed by the signal depends on how the target cell receives and interprets the signal.

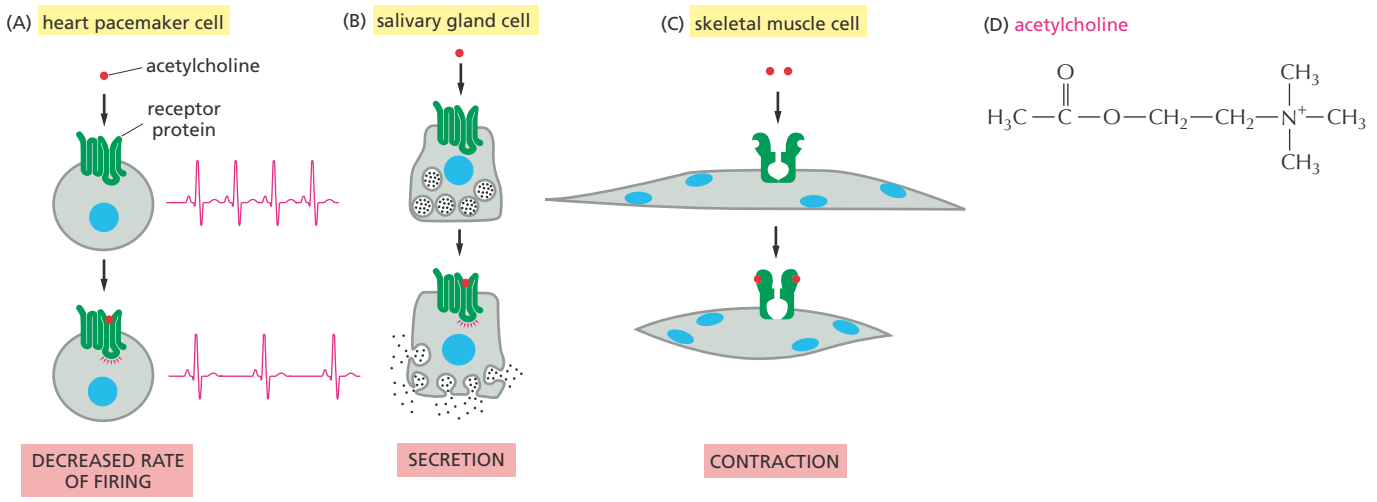


Figure 16–5 The same signal molecule can induce different responses in different target cells. Different cell types are configured to respond to the neurotransmitter acetylcholine in different ways. Acetylcholine binds to similar receptor proteins on heart pacemaker cells (A) and salivary gland cells (B), but it evokes different responses in each cell type. Skeletal muscle cells (C) produce a different type of receptor protein for the same signal. (D) For such a versatile molecule, acetylcholine has a fairly simple chemical structure.

A typical cell possesses many sorts of receptors—each present in tens to hundreds of thousands of copies. Such variety makes the cell simultaneously sensitive to many different extracellular signals and allows a relatively small number of signal molecules, used in different combinations, to exert subtle and complex control over cell behavior. A combination of signals can evoke a response that is different from the sum of the effects that each signal would trigger on its own. As we discuss later, this "tailoring" of a cell's response occurs, in part, because the intracellular relay systems activated by the different signals interact. Thus the presence of one signal will often modify the effects of another. One combination of signals might enable a cell to survive; another might drive it to differentiate in some specialized way; and another might cause it to divide. In the absence of any signals, most animal cells are programmed to kill themselves (**Figure 16–6**).

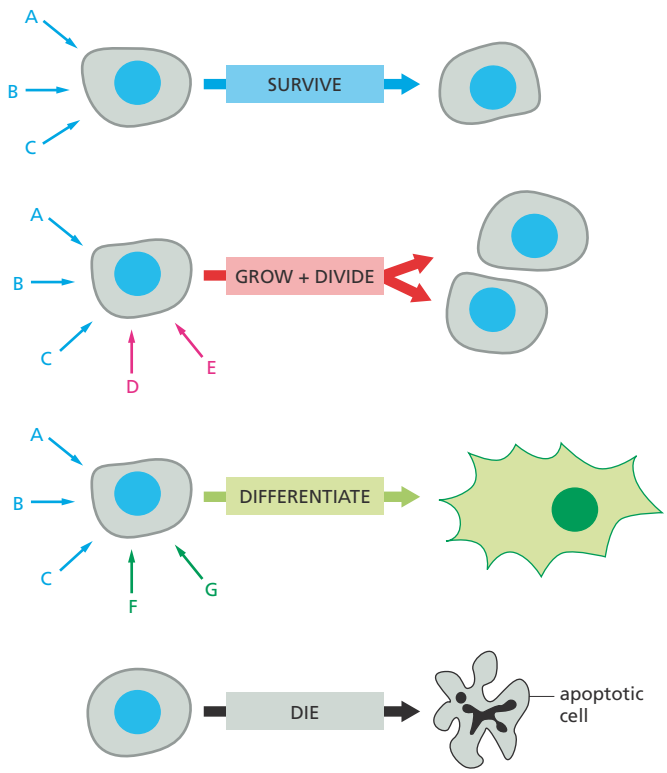


Figure 16–6 An animal cell depends on multiple extracellular signals. Every cell type displays a set of receptor proteins that enables it to respond to a specific set of extracellular signal molecules produced by other cells. These signal molecules work in combinations to regulate the behavior of the cell. As shown here, cells may require multiple signals (*blue* arrows) to survive, additional signals (*red* arrows) to grow and divide, and still other signals (*green* arrows) to differentiate. If deprived of survival signals, most cells undergo a form of cell suicide known as apoptosis (discussed in Chapter 18).

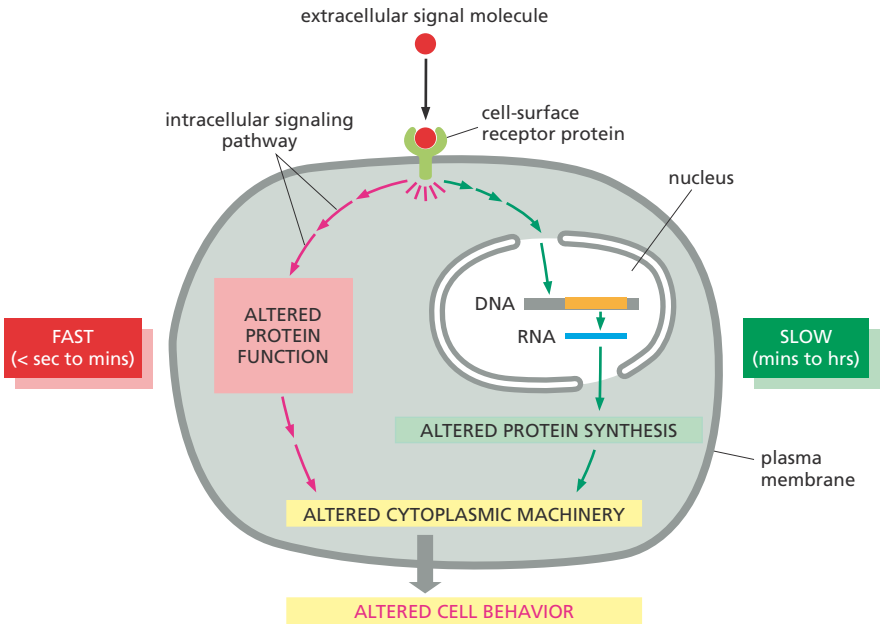


Figure 16–7 Extracellular signals can act slowly or rapidly. Certain types of cell responses—such as cell differentiation or increased cell growth and division (see Figure 16–6)—involve changes in gene expression and the synthesis of new proteins; they therefore occur relatively slowly. Other responses—such as changes in cell movement, secretion, or metabolism—need not involve changes in gene expression and therefore occur more quickly (see Figure 16–5).

A Cell's Response to a Signal Can Be Fast or Slow

The length of time a cell takes to respond to an extracellular signal can vary greatly, depending on what needs to happen once the message has been received. Some extracellular signals act swiftly: acetylcholine can stimulate a skeletal muscle cell to contract within milliseconds and a salivary gland cell to secrete within a minute or so. Such rapid responses are possible because, in each case, the signal affects the activity of proteins that are already present inside the target cell, awaiting their marching orders.

Other responses take more time. Cell growth and cell division, when triggered by the appropriate signal molecules, can take many hours to execute. This is because the response to these extracellular signals requires changes in gene expression and the production of new proteins (**Figure 16–7**). We will encounter additional examples of both fast and slow responses—and the signal molecules that stimulate them—later in the chapter.

Some Hormones Cross the Plasma Membrane and Bind to Intracellular Receptors

Extracellular signal molecules generally fall into two classes. The first and largest class consists of molecules that are too large or too hydrophilic to cross the plasma membrane of the target cell. They rely on receptors on the surface of the target cell to relay their message across the membrane (**Figure 16–8A**). The second, and smaller, class of signals

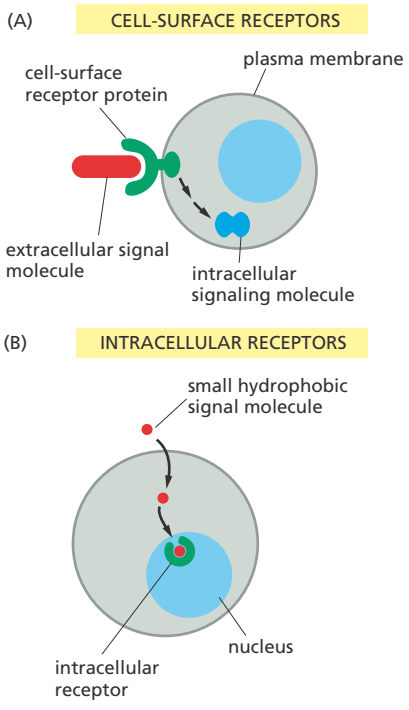


Figure 16–8 Extracellular signal molecules bind either to cell-surface receptors or to intracellular enzymes or receptors. (A) Most extracellular signal molecules are large and hydrophilic and are therefore unable to cross the plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate one or more intracellular signaling molecules in the target cell. (B) Some small, hydrophobic, extracellular signal molecules, by contrast, pass through the target cell's plasma membrane and either activate intracellular enzymes directly or bind to intracellular receptors—in the cytosol or in the nucleus (as shown here)—that then regulate gene transcription or other functions.

consists of molecules that are small enough or hydrophobic enough to pass through the plasma membrane and into the cytosol. Once inside, these signal molecules usually activate intracellular enzymes or bind to intracellular receptor proteins that regulate gene expression (**Figure 16–8B**).

One important category of signal molecules that rely on intracellular receptor proteins is the family of **steroid hormones**—including *cortisol*, *estradiol*, and *testosterone*—and the *thyroid hormones* such as *thyroxine* (**Figure 16–9**). All of these hydrophobic molecules pass through the plasma membrane of the target cell and bind to receptor proteins located in either the cytosol or the nucleus. Both the cytosolic and nuclear receptors are referred to as **nuclear receptors**, because, when activated by hormone binding, they act as transcription regulators in the nucleus (discussed in Chapter 8). In unstimulated cells, nuclear receptors are typically present in an inactive form. When a hormone binds, the receptor undergoes a large conformational change that activates the protein, allowing it to promote or inhibit the transcription of specific target genes (**Figure 16–10**). Each hormone binds to a different nuclear receptor, and each receptor acts at a different set of regulatory sites in DNA (discussed in Chapter 8). Moreover, a given hormone usually regulates different sets of genes in different cell types, thereby evoking different physiological responses in different target cells.

Nuclear receptors and the hormones that activate them have essential roles in human physiology (see Table 16–1, p. 529). Loss of these signaling systems can have dramatic consequences, as illustrated by what happens in individuals who lack the receptor for the male sex hormone testosterone. Testosterone in humans shapes the formation of the external genitalia and influences brain development in the fetus; at puberty, the hormone triggers the development of male secondary sexual characteristics. Some very rare individuals are genetically male—that is, they have both an X and a Y chromosome—but lack the testosterone receptor as a result of a mutation in the corresponding gene; thus, they make testosterone, but their cells cannot respond to it. As a result, these individuals develop as females, which is the path that sexual and brain development would take if no male or female hormones were produced. Such a sex reversal demonstrates the crucial role of the testosterone receptor in sexual development, and it also shows that the receptor is required not just in one cell type to mediate one effect of testosterone, but in many cell types to help produce the whole range of features that distinguish men from women.

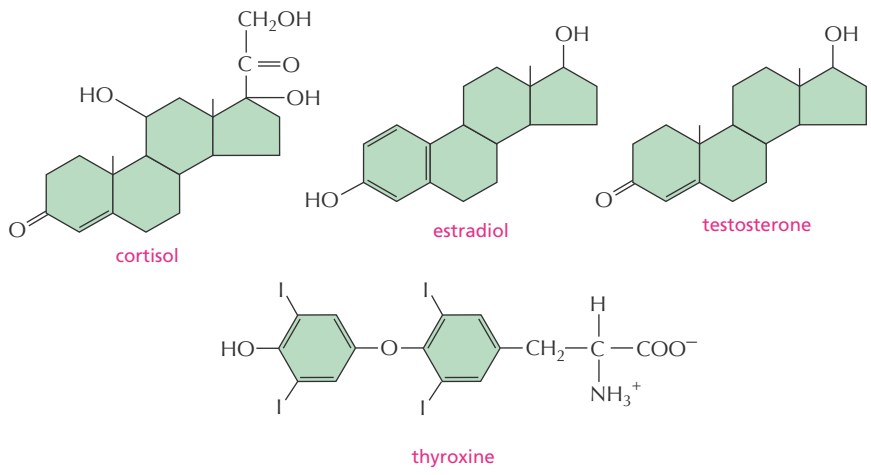


Figure 16–9 Some small hydrophobic hormones bind to intracellular receptors that act as transcription regulators. Although these signal molecules differ in their chemical structures and functions, they all act by binding to intracellular receptor proteins that act as transcription regulators. Their receptors are not identical, but they are evolutionarily related, belonging to the *nuclear receptor superfamily*. The sites of origin and functions of these hormones are given in Table 16–1 (p. 529).

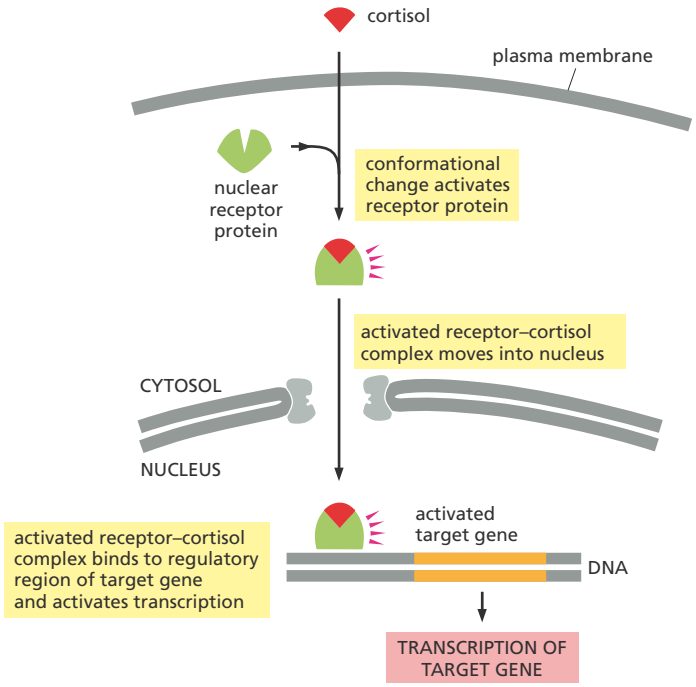


Figure 16–10 The steroid hormone cortisol acts by activating a transcription regulator. Cortisol is one of the hormones produced by the adrenal glands in response to stress. It crosses the plasma membrane and binds to its receptor protein, which is located in the cytosol. The receptor–hormone complex is then transported into the nucleus via the nuclear pores. Cortisol binding activates the receptor protein, which is then able to bind to specific regulatory sequences in DNA and activate (or repress, not shown) the transcription of specific target genes. Whereas the receptors for cortisol and some other steroid hormones are located in the cytosol, those for other steroid hormones and for thyroid hormones are already bound to DNA in the nucleus even in the absence of hormone.

Some Dissolved Gases Cross the Plasma Membrane and Activate Intracellular Enzymes Directly

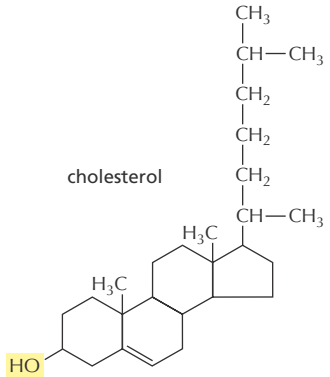
Steroid hormones and thyroid hormones are not the only extracellular signal molecules that can pass through the plasma membrane. Some dissolved gases can diffuse across the membrane to the cell interior and directly regulate the activity of specific intracellular proteins. This direct approach allows such signals to alter a target cell within a few seconds or minutes. The gas **nitric oxide** (**NO**) acts in this way. NO is synthesized from the amino acid arginine and diffuses readily from its site of synthesis into neighboring cells. The gas acts only locally because it is quickly converted to nitrates and nitrites (with a half-life of about 5–10 seconds) by reacting with oxygen and water outside cells.

Endothelial cells—the flattened cells that line every blood vessel—release NO in response to neurotransmitters secreted by nearby nerve endings. This NO signal causes smooth muscle cells in the adjacent vessel wall to relax, allowing the vessel to dilate, so that blood flows through it more freely (**Figure 16–11**). The effect of NO on blood vessels accounts for the action of nitroglycerine, which has been used for almost 100 years to treat patients with angina—pain caused by inadequate blood flow to the heart muscle. In the body, nitroglycerine is converted to NO, which rapidly relaxes blood vessels, thereby reducing the workload on the heart and decreasing the muscle's need for oxygen-rich blood. Many nerve cells also use NO to signal neighboring cells: NO released by nerve terminals in the penis, for instance, acts as a local mediator to trigger the blood-vessel dilation responsible for penile erection.

Inside many target cells, NO binds to and activates the enzyme *guanylyl cyclase*, stimulating the formation of *cyclic GMP* from the nucleotide GTP (see Figure 16–11B). Cyclic GMP is itself a small intracellular signaling molecule that forms the next link in the NO signaling chain that leads to the cell's ultimate response. The impotence drug Viagra enhances penile erection by blocking the enzyme that degrades cyclic GMP, prolonging the NO signal. Cyclic GMP is very similar in its structure and mechanism

QUESTION 16–2

Consider the structure of cholesterol, a small hydrophobic molecule with a sterol backbone similar to that of three of the hormones shown in Figure 16–9, but possessing fewer polar groups such as –OH, =O, and $–COO^-$. If cholesterol were not normally found in cell membranes, could it be used effectively as a hormone if an appropriate intracellular receptor evolved?



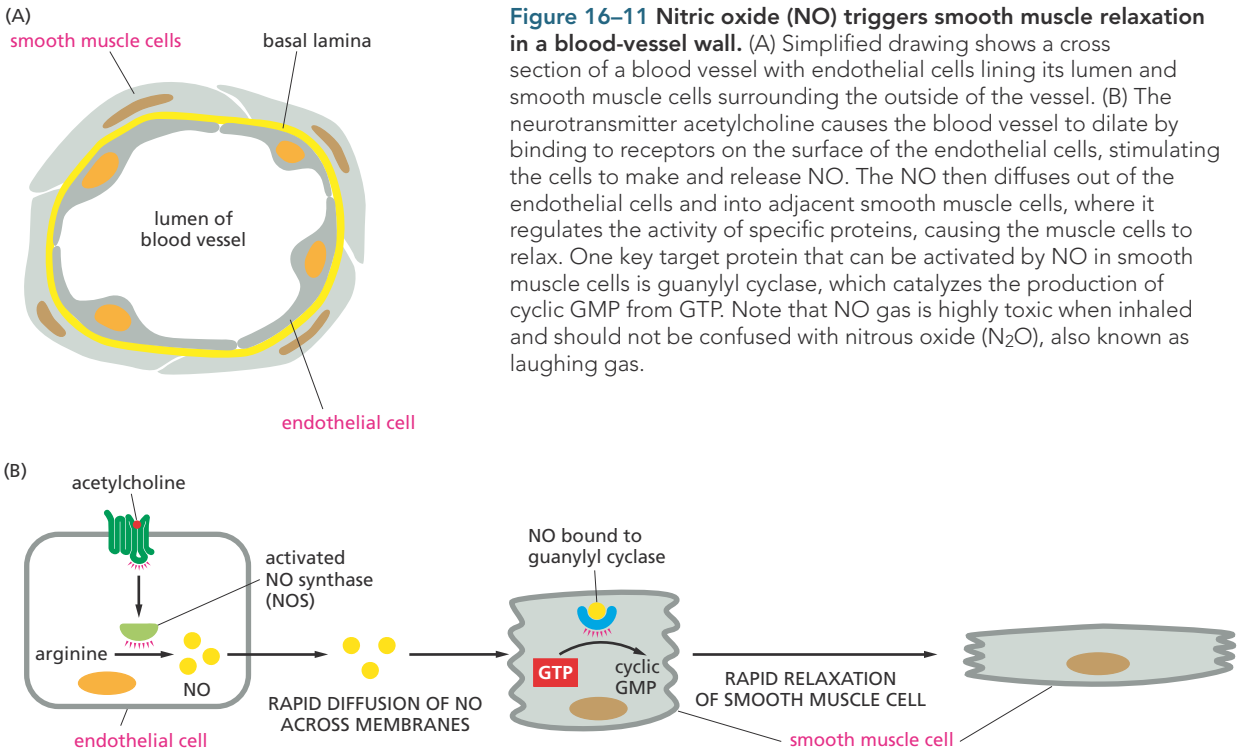


Figure 16–11 Nitric oxide (NO) triggers smooth muscle relaxation in a blood-vessel wall. (A) Simplified drawing shows a cross section of a blood vessel with endothelial cells lining its lumen and smooth muscle cells surrounding the outside of the vessel. (B) The neurotransmitter acetylcholine causes the blood vessel to dilate by binding to receptors on the surface of the endothelial cells, stimulating the cells to make and release NO. The NO then diffuses out of the endothelial cells and into adjacent smooth muscle cells, where it regulates the activity of specific proteins, causing the muscle cells to relax. One key target protein that can be activated by NO in smooth muscle cells is guanylyl cyclase, which catalyzes the production of cyclic GMP from GTP. Note that NO gas is highly toxic when inhaled and should not be confused with nitrous oxide (N_2O), also known as laughing gas.

of action to *cyclic AMP*, a much more commonly used intracellular signaling molecule, which we discuss later.

Cell-Surface Receptors Relay Extracellular Signals via Intracellular Signaling Pathways

In contrast to NO and the steroid and thyroid hormones, the vast majority of signal molecules are too large or hydrophilic to cross the plasma membrane of the target cell. These proteins, peptides, and small hydrophilic molecules bind to cell-surface receptor proteins that span the plasma membrane (see Figure 16–8A). Transmembrane receptors detect a signal on the outside and relay the message, in a new form, across the membrane into the interior of the cell.

The receptor protein performs the primary step in signal transduction: it recognizes the extracellular signal and generates new intracellular signals in response (see Figure 16–2B). The resulting intracellular signaling process usually works like a molecular relay race, in which the message is passed "downstream" from one **intracellular signaling molecule** to another, each activating or generating the next signaling molecule in the pathway, until a metabolic enzyme is kicked into action, the cytoskeleton is tweaked into a new configuration, or a gene is switched on or off. This final outcome is called the response of the cell (**Figure 16–12**).

The components of these **intracellular signaling pathways** perform one or more crucial functions (**Figure 16–13**):

1. They can simply *relay* the signal onward and thereby help spread it through the cell.
2. They can *amplify* the signal received, making it stronger, so that a few extracellular signal molecules are enough to evoke a large intracellular response.
3. They can detect signals from more than one intracellular signaling pathway and *integrate* them before relaying a signal onward.

QUESTION 16–3

In principle, how might an intracellular signaling protein amplify a signal as it relays it onward?

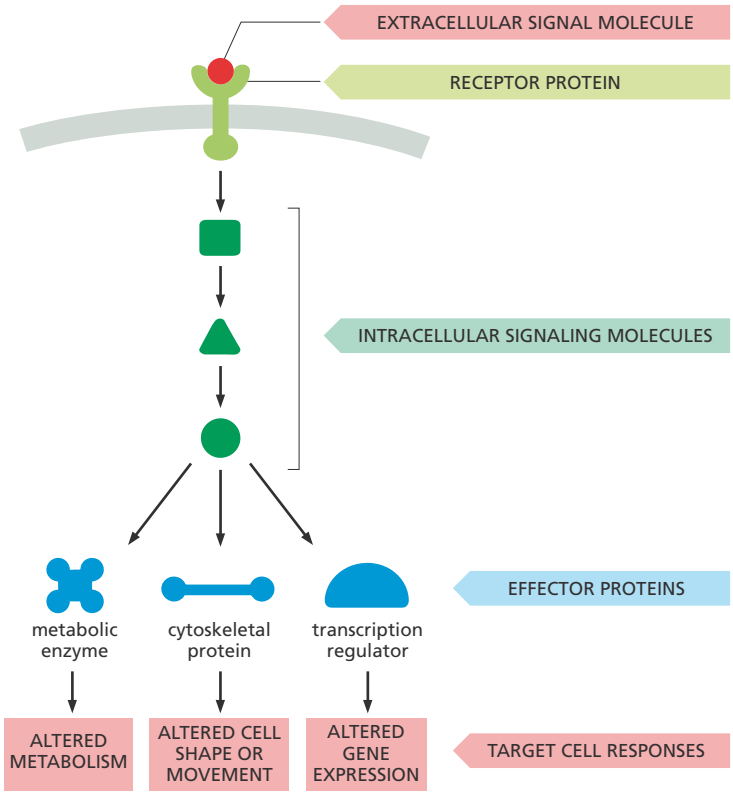


Figure 16–12 Many extracellular signals act via cell-surface receptors to change the behavior of the target cell. The receptor protein activates one or more intracellular signaling pathways, each mediated by a series of intracellular signaling molecules, which can be proteins or small messenger molecules; only one pathway is shown. Signaling molecules eventually interact with specific *effector proteins*, altering them to change the behavior of the cell in various ways.

4. They can *distribute* the signal to more than one effector protein, creating branches in the information flow diagram and evoking a complex response.

The steps in a signaling pathway are generally subject to modulation by *feedback regulation*. In positive feedback, a component that lies downstream in the pathway acts on an earlier component in the same pathway to enhance the response to the initial signal; in negative feedback, a downstream component acts to inhibit an earlier component in the pathway to diminish the response to the initial signal (**Figure 16–14**). Such feedback regulation is very common in biological systems and can lead to complex responses: positive feedback can generate all-or-none, switchlike responses, for example, whereas negative feedback can generate responses that oscillate on and off.

Some Intracellular Signaling Proteins Act as Molecular Switches

Many of the key intracellular signaling proteins behave as **molecular switches**: receipt of a signal causes them to toggle from an inactive to an active state. Once activated, these proteins can stimulate—or in other cases suppress—other proteins in the signaling pathway. They then persist in an active state until some other process switches them off again.

The importance of the switching-off process is often underappreciated: imagine the consequences if a signaling pathway that boosts your heart rate were to remain active indefinitely. If a signaling pathway is to recover after transmitting a signal and make itself ready to transmit another, every activated protein in the pathway must be reset to its original, unstimulated state. Thus, for every activation step along the pathway, there has to be an inactivation mechanism. The two are equally important for a signaling pathway to be useful.

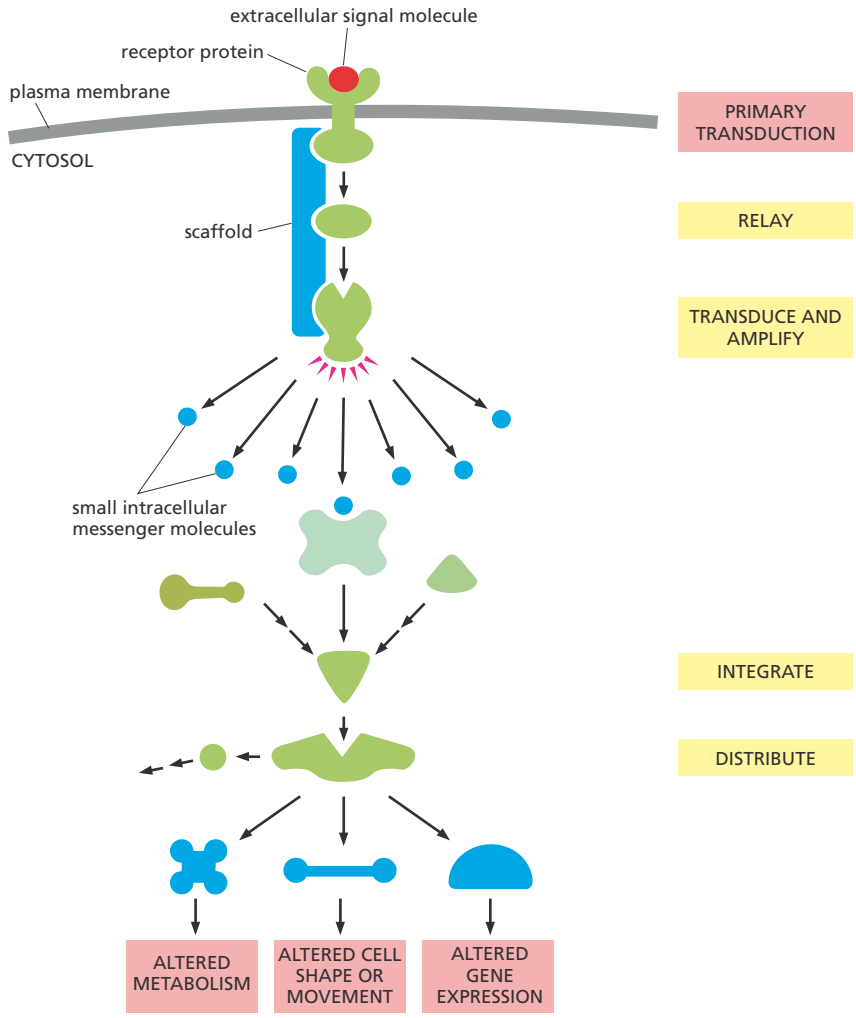


Figure 16–13 Intracellular signaling proteins can relay, amplify, integrate, and distribute the incoming signal. In this example, a receptor protein located on the cell surface transduces an extracellular signal into an intracellular signal, which initiates one or more intracellular signaling pathways that relay the signal into the cell interior. Each pathway includes intracellular signaling proteins that can function in one of the various ways shown; some, for example, integrate signals from other intracellular signaling pathways. Many of the steps in the process can be modulated by other molecules or events in the cell (not shown). Note that some proteins in the pathway may be held in close proximity by a scaffold protein, which allows them to be activated at a specific location in the cell and with greater speed, efficiency, and selectivity. We discuss the production and function of small intracellular messenger molecules later in the chapter.

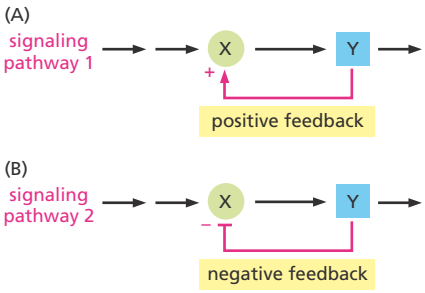


Figure 16–14 Feedback regulation within an intracellular signaling pathway can adjust the response to an extracellular signal. In these simple examples, a downstream protein in two signaling pathways, protein Y, acts to (A) increase via positive feedback or (B) decrease via negative feedback the activity of the protein that activated it.

Proteins that act as molecular switches fall mostly into one of two classes. The first—and by far the largest—class consists of proteins that are activated or inactivated by phosphorylation, a chemical modification discussed in Chapter 4 (see Figure 4–42). For these molecules, the switch is thrown in one direction by a **protein kinase**, which covalently attaches a phosphate group onto the switch protein, and in the other direction by a **protein phosphatase**, which takes the phosphate off again (**Figure 16–15A**). The activity of any protein that is regulated by phosphorylation depends—moment by moment—on the balance between the activities of the protein kinases that phosphorylate it and the protein phosphatases that dephosphorylate it.

Many of the switch proteins controlled by phosphorylation are themselves protein kinases, and these are often organized into *phosphorylation cascades*: one protein kinase, activated by phosphorylation, phosphorylates the next protein kinase in the sequence, and so on, transmitting the signal onward and, in the process, amplifying, distributing, and regulating it. Two main types of protein kinases operate in intracellular signaling pathways: the most common are **serine/threonine kinases**, which—as the name implies—phosphorylate proteins on serines or threonines; others are **tyrosine kinases**, which phosphorylate proteins on tyrosines.

The other class of switch proteins involved in intracellular signaling pathways are **GTP-binding proteins**. These toggle between an active and an inactive state depending on whether they have GTP or GDP bound to

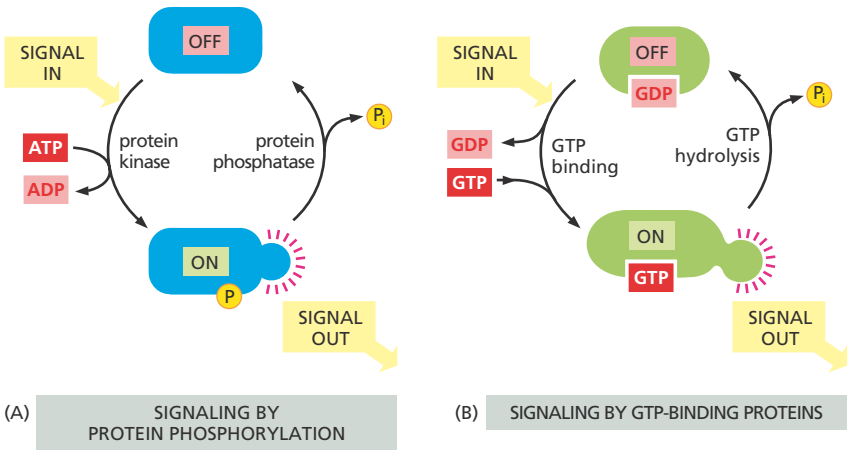


Figure 16–15 Many intracellular signaling proteins act as molecular switches. These proteins can be activated—or in some cases inhibited—by the addition or removal of a phosphate group. (A) In one class of switch protein, the phosphate is added covalently by a protein kinase, which transfers the terminal phosphate group from ATP to the signaling protein; the phosphate is then removed by a protein phosphatase. (B) In the other class of switch protein, a GTP-binding protein is activated when it exchanges its bound GDP for GTP (which, in a sense, adds a phosphate to the protein); the protein then switches itself off by hydrolyzing its bound GTP to GDP.

them, respectively (**Figure 16–15B**). Once activated by GTP binding, these proteins have intrinsic GTP-hydrolyzing (*GTPase*) activity, and they shut themselves off by hydrolyzing their bound GTP to GDP.

Two main types of GTP-binding proteins participate in intracellular signaling pathways. Large, *trimeric GTP-binding proteins* (also called *G proteins*) relay messages from *G-protein-coupled receptors*; we discuss this major class of GTP-binding proteins in detail shortly. Other cell-surface receptors rely on small, *monomeric GTPases* to help relay their signals. These monomeric GTP-binding proteins are aided by two sets of regulatory proteins. *Guanine nucleotide exchange factors* (*GEFs*) activate the switch proteins by promoting the exchange of GDP for GTP, and *GTPase-activating proteins* (*GAPs*) turn them off by promoting GTP hydrolysis (**Figure 16–16**).

Cell-Surface Receptors Fall into Three Main Classes

All cell-surface receptor proteins bind to an extracellular signal molecule and transduce its message into one or more intracellular signaling molecules that alter the cell's behavior. Most of these receptors belong to one of three large classes, which differ in the transduction mechanism they use.

1. *Ion-channel-coupled receptors* change the permeability of the plasma membrane to selected ions, thereby altering the membrane potential and, if the conditions are right, producing an electrical current (**Figure 16–17A**).
2. *G-protein-coupled receptors* activate membrane-bound, trimeric GTP-binding proteins (G proteins), which then activate (or inhibit) an enzyme or an ion channel in the plasma membrane, initiating an intracellular signaling cascade (**Figure 16–17B**).
3. *Enzyme-coupled receptors* either act as enzymes or associate with enzymes inside the cell (**Figure 16–17C**); when stimulated, the enzymes can activate a wide variety of intracellular signaling pathways.

The number of different types of receptors in each of these three classes is even greater than the number of extracellular signals that act on them. This is because for many extracellular signal molecules there is more than one type of receptor, and these may belong to different receptor classes. The neurotransmitter acetylcholine, for example, acts on skeletal muscle cells via an ion-channel-coupled receptor, whereas in heart cells it acts through a G-protein-coupled receptor. These two types of

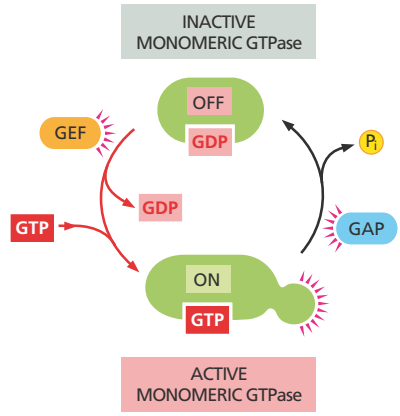


Figure 16–16 The activity of monomeric GTP-binding proteins is controlled by two types of regulatory proteins. Guanine nucleotide exchange factors (GEFs) promote the exchange of GDP for GTP, thereby switching the GTP-binding protein on. GTPase-activating proteins (GAPs) stimulate the hydrolysis of GTP to GDP, thereby switching the GTP-binding protein off.

receptors generate different intracellular signals and thus enable the two types of cells to react to acetylcholine in different ways, increasing contraction in skeletal muscle and decreasing the rate of contractions in heart (see Figure 16–5A and C).

This plethora of cell-surface receptors also provides targets for many foreign substances that interfere with our physiology, from heroin and nicotine to tranquilizers and chili peppers. These substances either block or overstimulate the receptor's natural activity. Many drugs and poisons act in this way (**Table 16–2**), and a large part of the pharmaceutical industry is devoted to producing drugs that will exert a precisely defined effect by binding to a specific type of cell-surface receptor.

Ion-channel–coupled Receptors Convert Chemical Signals into Electrical Ones

Of all the types of cell-surface receptors, **ion-channel-coupled receptors** (also known as transmitter-gated ion channels) function in the simplest and most direct way. As we discuss in detail in Chapter 12, these receptors are responsible for the rapid transmission of signals across synapses in the nervous system. They transduce a chemical signal, in the form of a pulse of secreted neurotransmitter molecules delivered to the outside of the target cell, directly into an electrical signal, in the

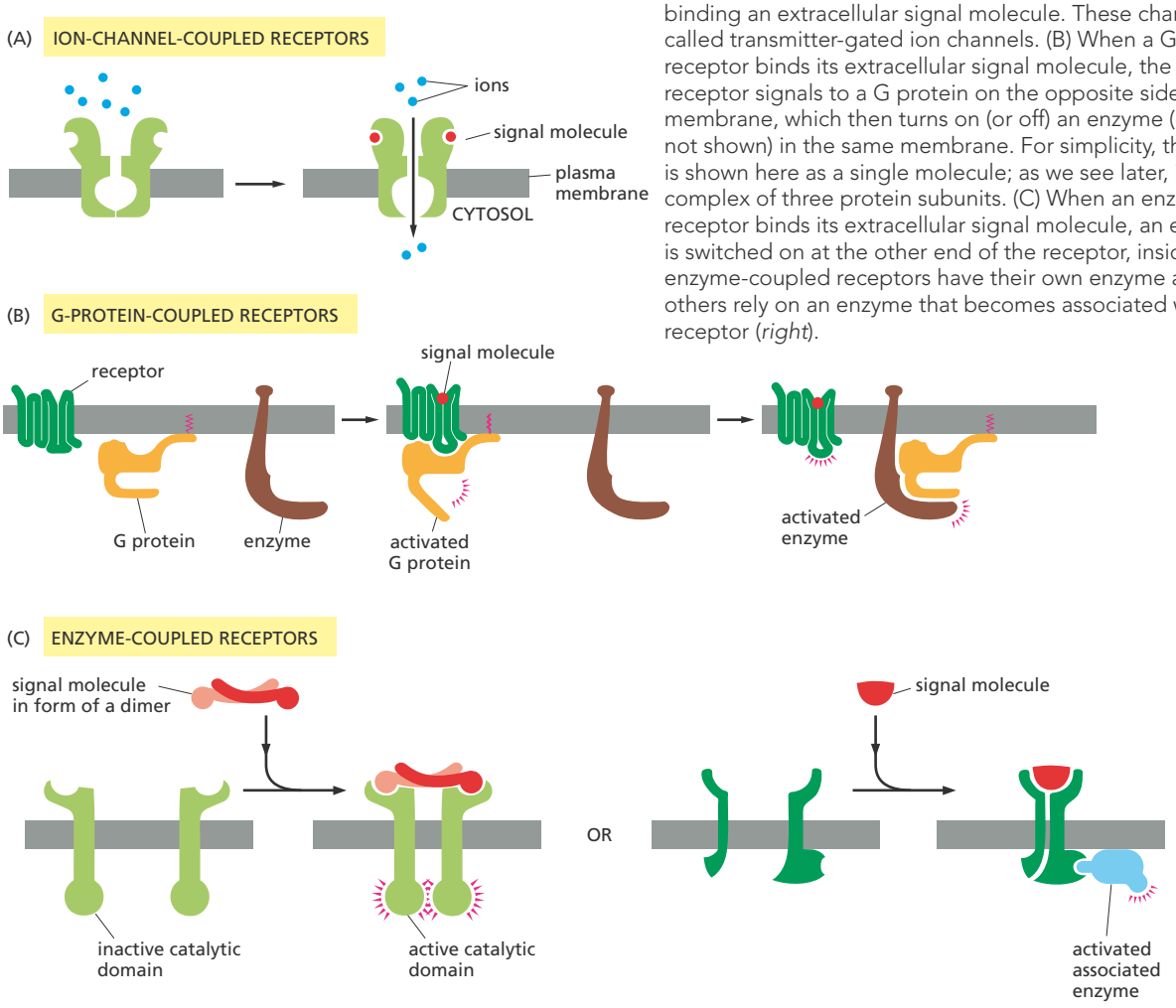


Figure 16–17 Cell-surface receptors fall into one of three main classes. (A) An ion-channel-coupled receptor opens in response to binding an extracellular signal molecule. These channels are also called transmitter-gated ion channels. (B) When a G-protein-coupled receptor binds its extracellular signal molecule, the activated receptor signals to a G protein on the opposite side of the plasma membrane, which then turns on (or off) an enzyme (or an ion channel; not shown) in the same membrane. For simplicity, the G protein is shown here as a single molecule; as we see later, it is actually a complex of three protein subunits. (C) When an enzyme-coupled receptor binds its extracellular signal molecule, an enzyme activity is switched on at the other end of the receptor, inside the cell. Many enzyme-coupled receptors have their own enzyme activity (*left*), while others rely on an enzyme that becomes associated with the activated receptor (*right*).

TABLE 16–2 SOME FOREIGN SUBSTANCES THAT ACT ON CELL-SURFACE RECEPTORS

Substance	Normal Signal	Receptor Action	Effect
Barbiturates and benzodiazepines (Valium and Ambien)	γ-aminobutyric acid (GABA)	stimulate GABA-activated ion-channel-coupled receptors	relief of anxiety; sedation
Nicotine	acetylcholine	stimulates acetylcholine-activated ion-channel-coupled receptors	constriction of blood vessels; elevation of blood pressure
Morphine and heroin	endorphins and enkephalins	stimulate G-protein-coupled opiate receptors	analgesia (relief of pain); euphoria
Curare	acetylcholine	blocks acetylcholine-activated ion-channel-coupled receptors	blockage of neuromuscular transmission, resulting in paralysis
Strychnine	glycine	blocks glycine-activated ion-channel-coupled receptors	blockage of inhibitory synapses in spinal cord and brain, resulting in seizures and muscle spasm
Capsaicin	heat	stimulates temperature-sensitive ion-channel-coupled receptors	induces painful, burning sensation; prolonged exposure paradoxically leads to analgesia
Menthol	cold	stimulates temperature-sensitive ion-channel-coupled receptors	in moderate amounts, induces a cool sensation; in higher doses, can cause burning pain

form of a change in voltage across the target cell's plasma membrane (see Figure 12–40). When the neurotransmitter binds, this type of receptor alters its conformation so as to open an ion channel in the plasma membrane, allowing the flow of specific types of ions, such as Na^+, K^+, or Ca^{2+} (see Figure 16–17A and **Movie 16.1**). Driven by their electrochemical gradients, the ions rush into or out of the cell, creating a change in the membrane potential within milliseconds. This change in potential may trigger a nerve impulse or make it easier (or harder) for other neurotransmitters to do so. As we discuss later, the opening of Ca^{2+} channels has additional important effects, as changes in the Ca^{2+} concentration in the target cell cytosol can profoundly alter the activities of many Ca^{2+}-responsive proteins.

Whereas ion-channel-coupled receptors are especially important in nerve cells and other electrically excitable cells such as muscle cells, G-protein-coupled receptors and enzyme-coupled receptors are important for practically every cell type in the body. Most of the remainder of this chapter deals with these two receptor families and with the signal transduction processes that they use.

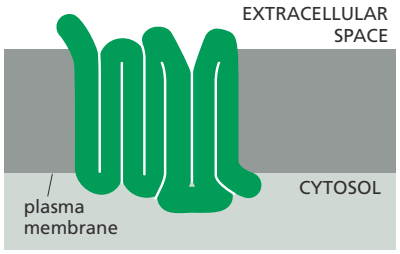
QUESTION 16–4

The signaling mechanisms used by a steroid-hormone-type nuclear receptor and by an ion-channel-coupled receptor are relatively simple as they have few components. Can they lead to an amplification of the initial signal, and, if so, how?

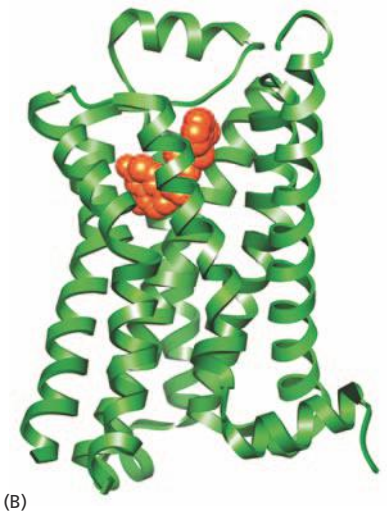
G-PROTEIN-COUPLED RECEPTORS

G-protein-coupled receptors (**GPCRs**) form the largest family of cell-surface receptors. There are more than 700 GPCRs in humans, and mice have about 1000 involved in the sense of smell alone. These receptors mediate responses to an enormous diversity of extracellular signal molecules, including hormones, local mediators, and neurotransmitters. The signal molecules are as varied in structure as they are in function: they can be proteins, small peptides, or derivatives of amino acids or fatty acids, and for each one of them there is a different receptor or set of receptors. Because GPCRs are involved in such a large variety of cell processes, they are an attractive target for the development of drugs to treat many disorders. About one-third of all drugs used today work through GPCRs.

Despite the diversity of the signal molecules that bind to them, all GPCRs that have been analyzed have a similar structure: each is made of a single



(A)



(B)

Figure 16–18 All GPCRs possess a similar structure. The polypeptide chain traverses the membrane as seven α helices. The cytoplasmic portions of the receptor bind to a G protein inside the cell. (A) For receptors that recognize small signal molecules, such as adrenaline or acetylcholine, the ligand usually binds deep within the plane of the membrane to a pocket that is formed by amino acids from several transmembrane segments. (B) Shown here is the structure of a GPCR that binds to adrenaline (*red*). Stimulation of this receptor by adrenaline makes the heart beat faster. Receptors that recognize signal molecules that are proteins usually have a large extracellular domain that, together with some of the transmembrane segments, binds the protein ligand (not shown).

polypeptide chain that threads back and forth across the lipid bilayer seven times (**Figure 16–18**). This superfamily of *seven-pass transmembrane receptor proteins* includes rhodopsin (the light-activated photoreceptor protein in the vertebrate eye), the olfactory (smell) receptors in the vertebrate nose, and the receptors that participate in the mating rituals of single-celled yeasts (see Figure 16–1). Evolutionarily speaking, GPCRs are ancient: even prokaryotes possess structurally similar membrane proteins—such as the bacteriorhodopsin that functions as a light-driven H^+ pump (see Figure 11–27). Although they resemble eukaryotic GPCRs, these prokaryotic proteins do not act through G proteins, but are coupled to other signal transduction systems.

We begin this section with a discussion of how G proteins are activated by GPCRs. We then consider how activated G proteins stimulate ion channels and how they regulate membrane-bound enzymes that control the concentrations of small intracellular messenger molecules, including cyclic AMP and Ca^{2+}—which in turn control the activity of important intracellular signaling proteins. We end with a discussion of how light-activated GPCRs in photoreceptors in our eyes enable us to see.

Stimulation of GPCRs Activates G-Protein Subunits

When an extracellular signal molecule binds to a GPCR, the receptor protein undergoes a conformational change that enables it to activate a **G protein** located on the other side of the plasma membrane. To explain how this activation leads to the transmission of a signal, we must first consider how G proteins are constructed and how they function.

There are several varieties of G proteins. Each is specific for a particular set of receptors and for a particular set of target enzymes or ion channels in the plasma membrane. All of these G proteins, however, have a similar general structure and operate in a similar way. They are composed of three protein subunits—α, β, and γ—two of which are tethered to the plasma membrane by short lipid tails. In the unstimulated state, the α subunit has GDP bound to it, and the G protein is idle (**Figure 16–19A**). When an extracellular signal molecule binds to its receptor, the altered receptor activates a G protein by causing the α subunit to decrease its affinity for GDP, which is then exchanged for a molecule of GTP. In some cases, this activation breaks up the G-protein subunits, so that the activated α subunit, clutching its GTP, detaches from the βγ complex, which is also activated (**Figure 16–19B**). The two activated parts of the G protein—the α subunit and the βγ complex—can then each interact directly with target proteins in the plasma membrane, which in turn may relay the signal to other destinations in the cell. The longer these target proteins remain bound to an α or a βγ subunit, the more prolonged the relayed signal will be.

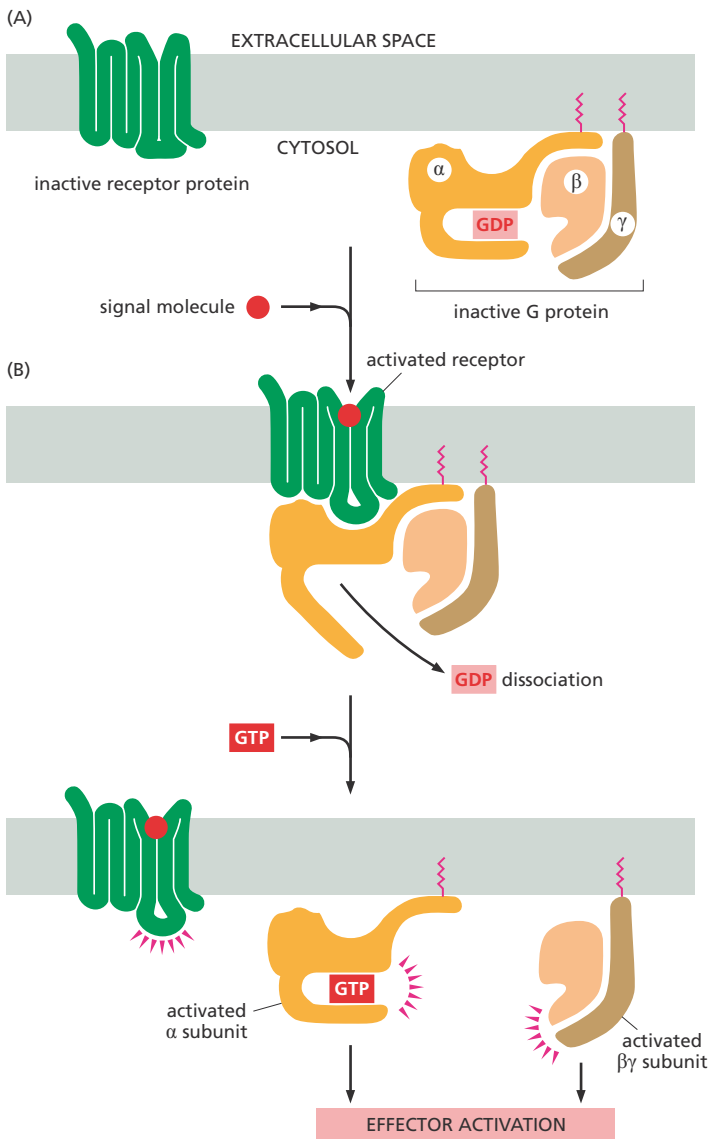


Figure 16–19 An activated GPCR activates G proteins by encouraging the α subunit to expel its GDP and pick up GTP. (A) In the unstimulated state, the receptor and the G protein are both inactive. Although they are shown here as separate entities in the plasma membrane, in some cases at least, they are associated in a preformed complex. (B) Binding of an extracellular signal molecule to the receptor changes the conformation of the receptor, which in turn alters the conformation of the bound G protein. The alteration of the α subunit of the G protein allows it to exchange its GDP for GTP. This exchange triggers an additional conformational change that activates both the α subunit and a βγ complex, which dissociate to interact with their preferred target proteins in the plasma membrane (Movie 16.2). The receptor stays active as long as the external signal molecule is bound to it, and it can therefore catalyze the activation of many molecules of G protein. Note that both the α and γ subunits of the G protein have covalently attached lipid molecules (*red*) that help anchor the subunits to the plasma membrane.

The amount of time that the α and βγ subunits remain "switched on"—and hence available to relay signals—also determines how long a response lasts. This timing is controlled by the behavior of the α subunit. The α subunit has an intrinsic GTPase activity, and it eventually hydrolyzes its bound GTP to GDP, returning the whole G protein to its original, inactive conformation (**Figure 16–20**). GTP hydrolysis and inactivation usually occur within seconds after the G protein has been activated. The inactive G protein is now ready to be reactivated by another activated receptor.

Some Bacterial Toxins Cause Disease by Altering the Activity of G Proteins

G proteins demonstrate a general principle of cell signaling mentioned earlier: the mechanisms that shut a signal off are as important as the mechanisms that turn it on (see Figure 16–15B). The shut-off mechanisms also offer as many opportunities for control—and as many dangers for mishap. Consider cholera, for example. The disease is caused by a bacterium that multiplies in the human intestine, where it produces a protein called *cholera toxin*. This protein enters the cells that line the intestine and modifies the α subunit of a G protein called G_s—so named because

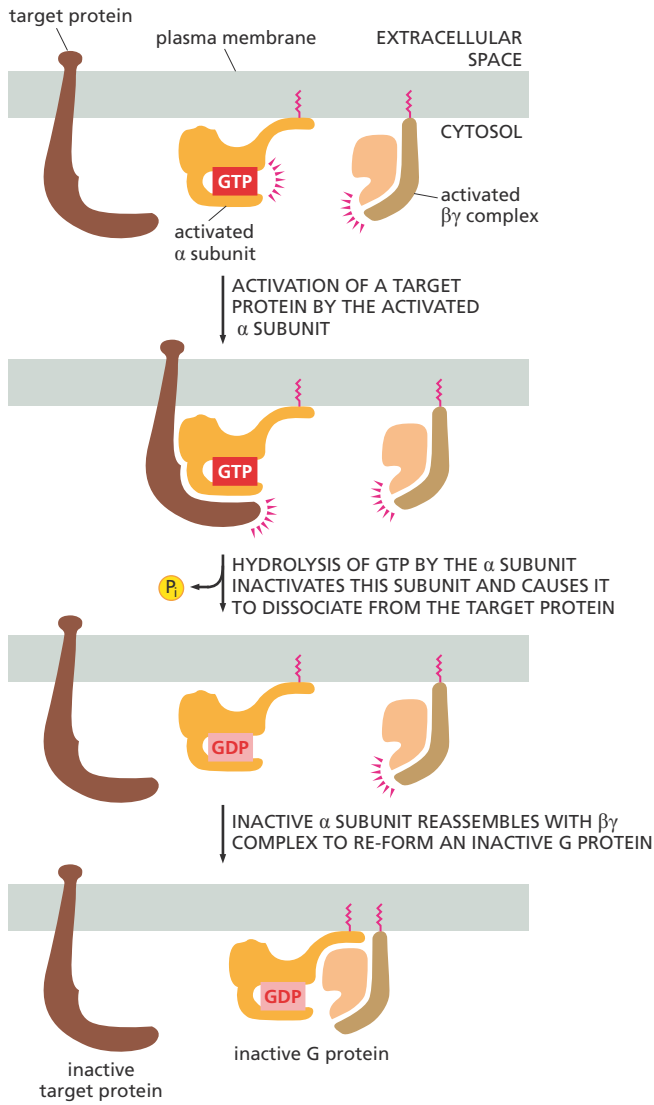


Figure 16–20 The G-protein α subunit switches itself off by hydrolyzing its bound GTP to GDP. When an activated α subunit interacts with its target protein, it activates that target protein (or in some cases inactivates it; not shown) for as long as the two remain in contact. Normally the α subunit hydrolyzes its bound GTP to GDP within seconds. This loss of GTP inactivates the α subunit, which dissociates from its target protein and—if the α subunit had separated from the βγ complex (as shown)—reassociates with a βγ complex to re-form an inactive G protein. The G protein is now ready to couple to another activated receptor, as in Figure 16–19B. Both the activated α subunit and the activated βγ complex can interact with target proteins in the plasma membrane. See also Movie 16.2.

QUESTION 16–5

GPCRs activate G proteins by reducing the strength of GDP binding to the G protein. This results in rapid dissociation of bound GDP, which is then replaced by GTP, because GTP is present in the cytosol in much higher concentrations than GDP. What consequences would result from a mutation in the α subunit of a G protein that caused its affinity for GDP to be reduced without significantly changing its affinity for GTP? Compare the effects of this mutation with the effects of cholera toxin.

it *stimulates* the enzyme adenylyl cyclase, which we discuss shortly. The modification prevents G_s from hydrolyzing its bound GTP, thus locking the G protein in the active state, in which it continuously stimulates adenylyl cyclase. In intestinal cells, this stimulation causes a prolonged and excessive outflow of Cl^- and water into the gut, resulting in catastrophic diarrhea and dehydration. The condition often leads to death unless urgent steps are taken to replace the lost water and ions.

A similar situation occurs in whooping cough (pertussis), a common respiratory infection against which infants are now routinely vaccinated. In this case, the disease-causing bacterium colonizes the lung, where it produces a protein called *pertussis toxin*. This protein alters the α subunit of a different type of G protein, called G_i, because it *inhibits* adenylyl cyclase. In this case, however, modification by the toxin disables the G protein by locking it into its inactive GDP-bound state. Inhibiting G_i, like activating G_s, results in the prolonged and inappropriate activation of adenylyl cyclase, which, in this case, stimulates coughing. Both the diarrhea-producing effects of cholera toxin and the cough-provoking effects of pertussis toxin help the disease-causing bacteria move from host to host.

Some G Proteins Directly Regulate Ion Channels

The target proteins recognized by G-protein subunits are either enzymes or ion channels in the plasma membrane. There are about 20 different

types of mammalian G proteins, each activated by a particular set of cell-surface receptors and dedicated to activating a particular set of target proteins. Consequently, the binding of an extracellular signal molecule to a GPCR leads to changes in the activities of a specific subset of the possible target proteins in the plasma membrane, leading to a response that is appropriate for that signal and that type of cell.

We look first at an example of direct G-protein regulation of ion channels. The heartbeat in animals is controlled by two sets of nerves: one speeds the heart up, the other slows it down. The nerves that signal a slow-down in heartbeat do so by releasing acetylcholine (see Figure 16–5A), which binds to a GPCR on the surface of the heart pacemaker cells. This GPCR activates the G protein, G_i. In this case, the βγ complex binds to the intracellular face of a K^+ channel in the plasma membrane of the pacemaker cell, forcing the ion channel into an open conformation (**Figure 16–21A and B**). This channel opening slows the heart rate by increasing the plasma membrane's permeability to K^+, making it more difficult to electrically activate, as explained in Chapter 12. The original signal is terminated—and the K^+ channel recloses—when the α subunit inactivates itself by hydrolyzing its bound GTP, returning the G protein to its inactive state (**Figure 16–21C**).

Many G Proteins Activate Membrane-bound Enzymes that Produce Small Messenger Molecules

When G proteins interact with ion channels, they cause an immediate change in the state and behavior of the cell. Their interactions with enzymes, in contrast, have consequences that are less rapid and more complex, as they lead to the production of additional intracellular signaling molecules. The two most frequent target enzymes for G proteins are *adenylyl cyclase*, which produces the **small intracellular signaling molecule** *cyclic AMP*, and *phospholipase C*, which generates the small

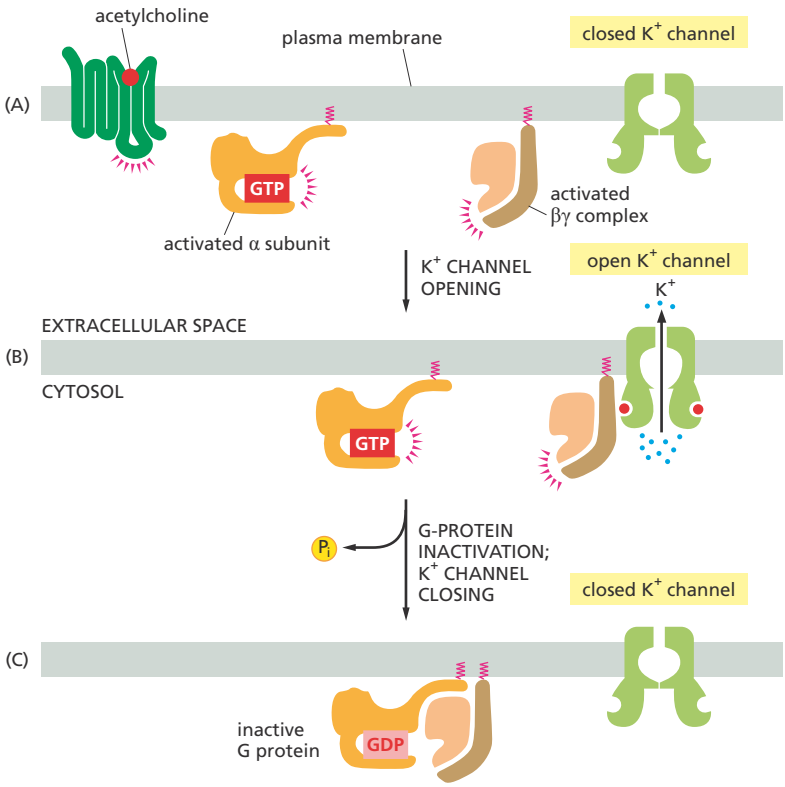


Figure 16–21 A G_i protein directly couples receptor activation to the opening of K^+ channels in the plasma membrane of heart pacemaker cells. (A) Binding of the neurotransmitter acetylcholine to its GPCR on the heart cells results in the activation of the G protein, G_i. (B) The activated βγ complex directly opens a K^+ channel in the plasma membrane, increasing its permeability to K^+ and thereby making the membrane harder to activate and slowing the heart rate. (C) Inactivation of the α subunit by hydrolysis of its bound GTP returns the G protein to its inactive state, allowing the K^+ channel to close.

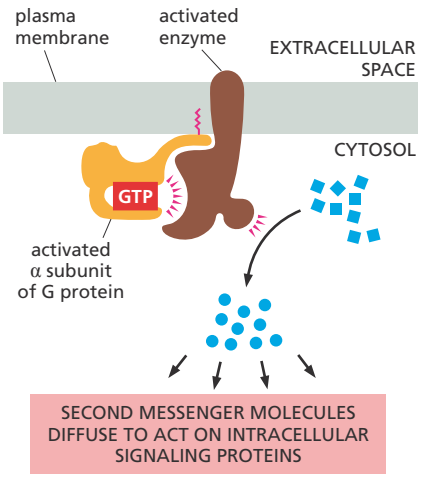


Figure 16–22 Enzymes activated by G proteins increase the concentrations of small intracellular signaling molecules. Because each activated enzyme generates many molecules of these second messengers, the signal is greatly amplified at this step in the pathway (see Figure 16–31). The signal is relayed onward by the second messenger molecules, which bind to specific signaling proteins in the cell and influence their activity.

intracellular signaling molecules *inositol trisphosphate* and *diacylglycerol*. Inositol trisphosphate, in turn, promotes the accumulation of cytosolic Ca^{2+}—yet another small intracellular signaling molecule.

Adenylyl cyclase and phospholipase C are activated by different types of G proteins, allowing cells to couple the production of these small intracellular signaling molecules to different extracellular signals. Although the coupling may be either stimulatory or inhibitory—as we saw in our discussion of the actions of cholera toxin and pertussis toxin—we concentrate here on G proteins that stimulate enzyme activity.

The small intracellular signaling molecules generated by these enzymes are often called *small messengers*, or *second messengers*—the "first messengers" being the extracellular signals that activated the enzymes in the first place. Once activated, the enzymes generate large quantities of small messengers, which rapidly diffuse away from their source, thereby amplifying and spreading the intracellular signal (**Figure 16–22**).

Different small messenger molecules produce different responses. We first examine the consequences of an increase in the cytosolic concentration of cyclic AMP. This will take us along one of the main types of signaling pathways that lead from the activation of GPCRs. We then discuss the actions of three other small messenger molecules—inositol trisphosphate, diacylglycerol, and Ca^{2+}—which will lead us along a different signaling route.

The Cyclic AMP Signaling Pathway Can Activate Enzymes and Turn On Genes

Many extracellular signals acting via GPCRs affect the activity of the enzyme **adenylyl cyclase** and thus alter the intracellular concentration of the small messenger molecule **cyclic AMP**. Most commonly, the activated G-protein α subunit switches on the adenylyl cyclase, causing a dramatic and sudden increase in the synthesis of cyclic AMP from ATP (which is always present in the cell). Because it stimulates the cyclase, this G protein is called G_s. To help terminate the signal, a second enzyme, called *cyclic AMP phosphodiesterase*, rapidly converts cyclic AMP to ordinary AMP (**Figure 16–23**). One way that caffeine acts as a stimulant is by inhibiting this phosphodiesterase in the nervous system, blocking cyclic AMP degradation and thereby keeping the concentration of this small messenger high.

Cyclic AMP phosphodiesterase is continuously active inside the cell. Because it eliminates cyclic AMP so quickly, the cytosolic concentration of this small messenger can change rapidly in response to extracellular

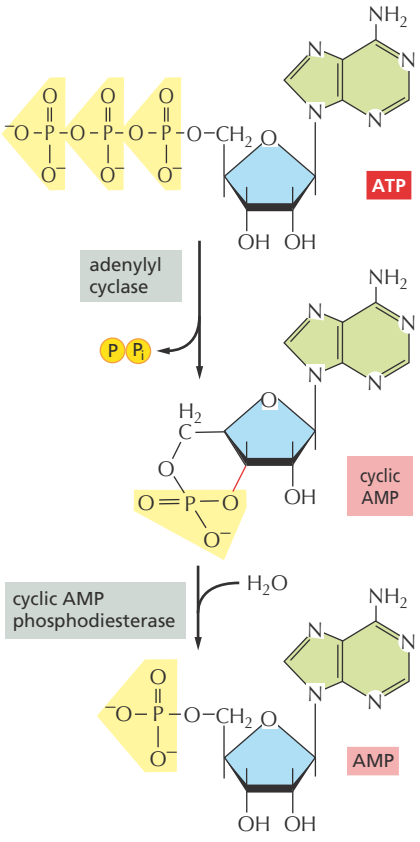


Figure 16–23 Cyclic AMP is synthesized by adenylyl cyclase and degraded by cyclic AMP phosphodiesterase. Cyclic AMP (abbreviated cAMP) is formed from ATP by a cyclization reaction that removes two phosphate groups from ATP and joins the "free" end of the remaining phosphate group to the sugar part of the AMP molecule (*red* bond). The degradation reaction breaks this new bond, forming AMP.

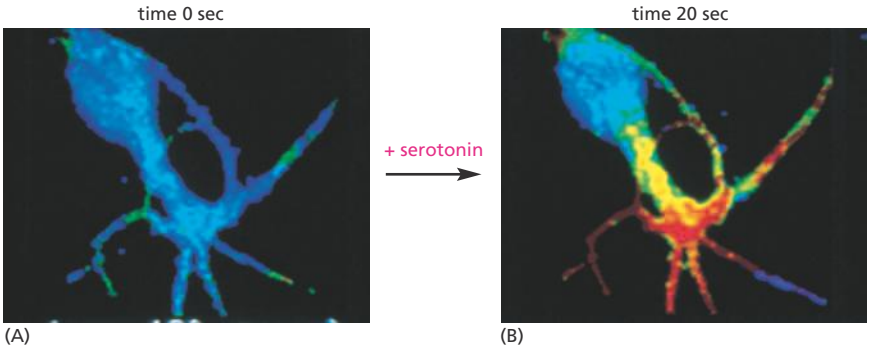


Figure 16–24 Cyclic AMP concentration rises rapidly in response to an extracellular signal. A nerve cell in culture responds to the binding of the neurotransmitter serotonin to a GPCR by synthesizing cyclic AMP. The concentration of intracellular cyclic AMP was monitored by injecting into the cell a fluorescent protein whose fluorescence changes when it binds cyclic AMP. *Blue* indicates a low level of cyclic AMP, *yellow* an intermediate level, and *red* a high level. (A) In the resting cell, the cyclic AMP concentration is about 5×10^{-8} M. (B) Twenty seconds after adding serotonin to the culture medium, the intracellular concentration of cyclic AMP has risen more than twentyfold (to $> 10^{-6}$ M) in the parts of the cell where the serotonin receptors are concentrated. (Courtesy of Roger Tsien.)

signals, rising or falling tenfold in a matter of seconds (**Figure 16–24**). Cyclic AMP is water-soluble, so it can, in some cases, carry the signal throughout the cell, traveling from the site on the membrane where it is synthesized to interact with proteins located in the cytosol, in the nucleus, or on other organelles.

Cyclic AMP exerts most of its effects by activating the enzyme **cyclic-AMP-dependent protein kinase** (**PKA**). This enzyme is normally held inactive in a complex with a regulatory protein. The binding of cyclic AMP to the regulatory protein forces a conformational change that releases the inhibition and unleashes the active kinase. Activated PKA then catalyzes the phosphorylation of particular serines or threonines on specific intracellular proteins, thus altering the activity of these target proteins. In different cell types, different sets of proteins are available to be phosphorylated, which largely explains why the effects of cyclic AMP vary with the type of target cell.

Many kinds of cell responses are mediated by cyclic AMP; a few are listed in **Table 16–3**. As the table shows, different target cells respond very differently to extracellular signals that change intracellular cyclic AMP concentrations. When we are frightened or excited, for example, the adrenal gland releases the hormone *adrenaline*, which circulates in the bloodstream and binds to a class of GPCRs called adrenergic receptors (see Figure 16–18B), which are present on many types of cells. The consequences vary from one cell type to another, but all the cell responses help prepare the body for sudden action. In skeletal muscle, for instance, adrenaline increases intracellular cyclic AMP, causing the breakdown of glycogen—the polymerized storage form of glucose. It does so by activating PKA, which leads to both the activation of an enzyme that promotes glycogen breakdown (**Figure 16–25**) and the inhibition of an enzyme that drives glycogen synthesis. By stimulating glycogen breakdown and

TABLE 16–3 SOME CELL RESPONSES MEDIATED BY CYCLIC AMP

Extracellular Signal Molecule*	Target Tissue	Major Response
Adrenaline	heart	increase in heart rate and force of contraction
Adrenaline	skeletal muscle	glycogen breakdown
Adrenaline, glucagon	fat	fat breakdown
Adrenocorticotropic hormone (ACTH)	adrenal gland	cortisol secretion

*Although all of the signal molecules listed here are hormones, some responses to local mediators and to neurotransmitters are also mediated by cyclic AMP.

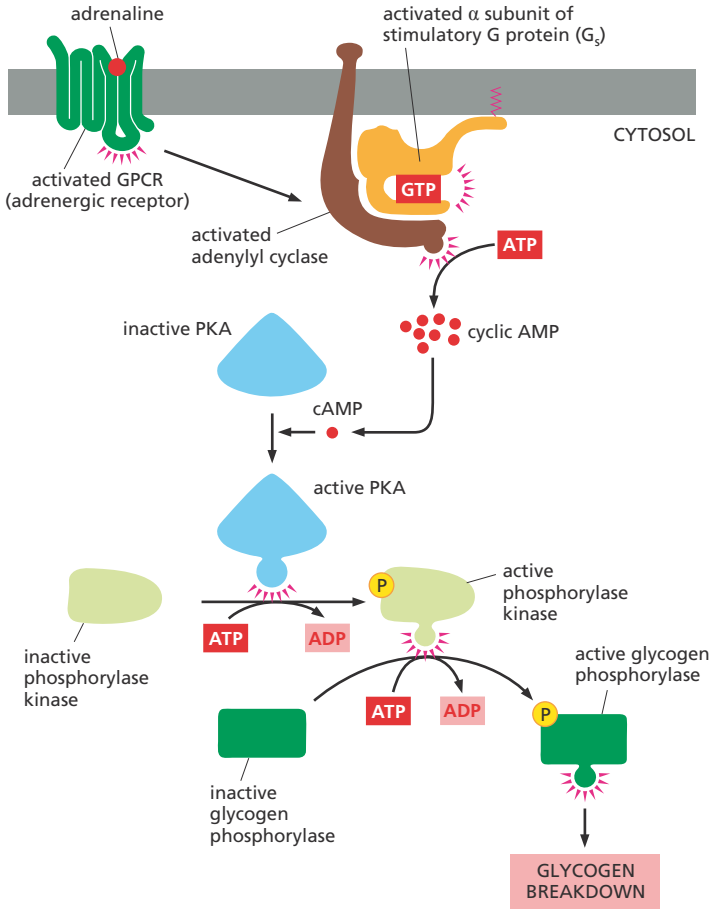


Figure 16–25 Adrenaline stimulates glycogen breakdown in skeletal muscle cells. The hormone activates a GPCR, which turns on a G protein (G_s) that activates adenylyl cyclase to boost the production of cyclic AMP. The increase in cyclic AMP activates PKA, which phosphorylates and activates an enzyme called phosphorylase kinase. This kinase activates glycogen phosphorylase, the enzyme that breaks down glycogen. Because these reactions do not involve changes in gene transcription or new protein synthesis, they occur rapidly.

inhibiting its synthesis, the increase in cyclic AMP maximizes the amount of glucose available as fuel for anticipated muscular activity. Adrenaline also acts on fat cells, stimulating the breakdown of fat to fatty acids. These fatty acids can then be exported to fuel ATP production in other cells.

In some cases, the effects of increasing cyclic AMP are rapid; in skeletal muscle, for example, glycogen breakdown occurs within seconds of adrenaline binding to its receptor (see Figure 16–25). In other cases, cyclic AMP responses involve changes in gene expression that take minutes or hours to develop. In these slow responses, PKA typically phosphorylates transcription regulators, which then activate the transcription of selected genes. Thus an increase in cyclic AMP in certain neurons in the brain controls the production of proteins involved in some forms of learning. **Figure 16–26** illustrates a typical cyclic-AMP-mediated pathway from the plasma membrane to the nucleus.

We now turn to the other enzyme-mediated signaling pathway that leads from GPCRs—the pathway that begins with the activation of the membrane-bound enzyme *phospholipase C* and leads to an increase in the small messengers *diacylglycerol*, *inositol trisphosphate*, and Ca^{2+}.

QUESTION 16–6

Explain why cyclic AMP must be broken down rapidly in a cell to allow rapid signaling.

The Inositol Phospholipid Pathway Triggers a Rise in Intracellular Ca^{2+}

Some GPCRs exert their effects via a G protein called G_q, which activates the membrane-bound enzyme **phospholipase C** instead of adenylyl cyclase. Examples of signal molecules that act through phospholipase C are given in **Table 16–4**.

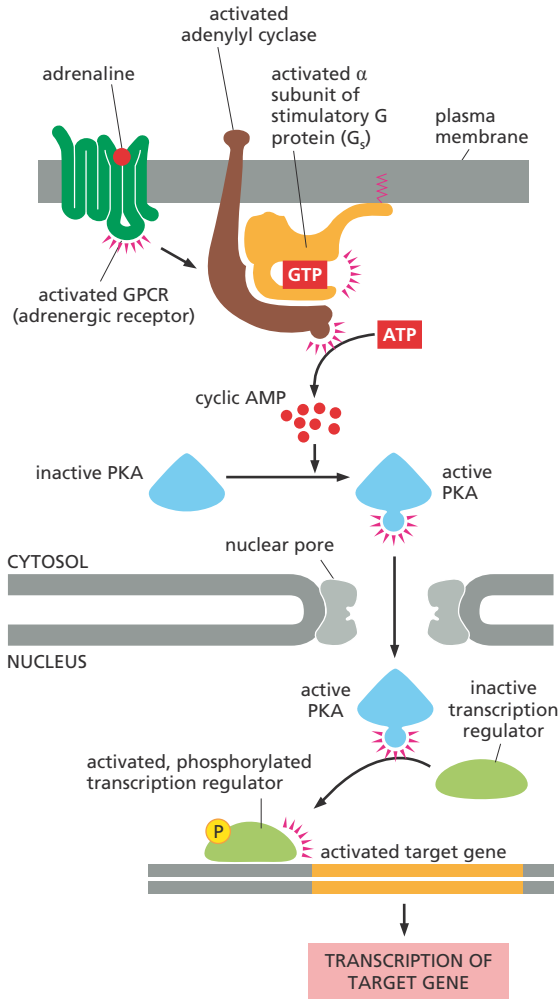


Figure 16–26 A rise in intracellular cyclic AMP can activate gene transcription. Binding of a signal molecule to its GPCR can lead to the activation of adenylyl cyclase and a rise in the concentration of cytosolic cyclic AMP. The increase in cyclic AMP activates PKA, which then moves into the nucleus and phosphorylates specific transcription regulators. Once phosphorylated, these proteins stimulate the transcription of a whole set of target genes (Movie 16.3). This type of signaling pathway controls many processes in cells, ranging from hormone synthesis in endocrine cells to the production of proteins involved in long-term memory in the brain. Activated PKA can also phosphorylate and thereby regulate other proteins and enzymes in the cytosol (as shown in Figure 16–25).

Once activated, phospholipase C propagates the signal by cleaving a lipid molecule that is a component of the plasma membrane. The molecule is an **inositol phospholipid** (a phospholipid with the sugar inositol attached to its head) that is present in small quantities in the cytosolic leaflet of the membrane lipid bilayer (see Figure 11–18). Because of the involvement of this phospholipid, the signaling pathway that begins with the activation of phospholipase C is often referred to as the *inositol phospholipid pathway*. It operates in almost all eukaryotic cells and can regulate a host of different effector proteins.

The action of phospholipase C generates two small messenger molecules: **inositol 1,4,5-trisphosphate** (**IP_3**) and **diacylglycerol** (**DAG**). Both molecules play a crucial part in relaying the signal (**Figure 16–27**).

TABLE 16–4 SOME CELL RESPONSES MEDIATED BY PHOSPHOLIPASE C ACTIVATION

Signal Molecule	Target Tissue	Major Response
Vasopressin (a peptide hormone)	liver	glycogen breakdown
Acetylcholine	pancreas	secretion of amylase (a digestive enzyme)
Acetylcholine	smooth muscle	contraction
Thrombin (a proteolytic enzyme)	blood platelets	aggregation

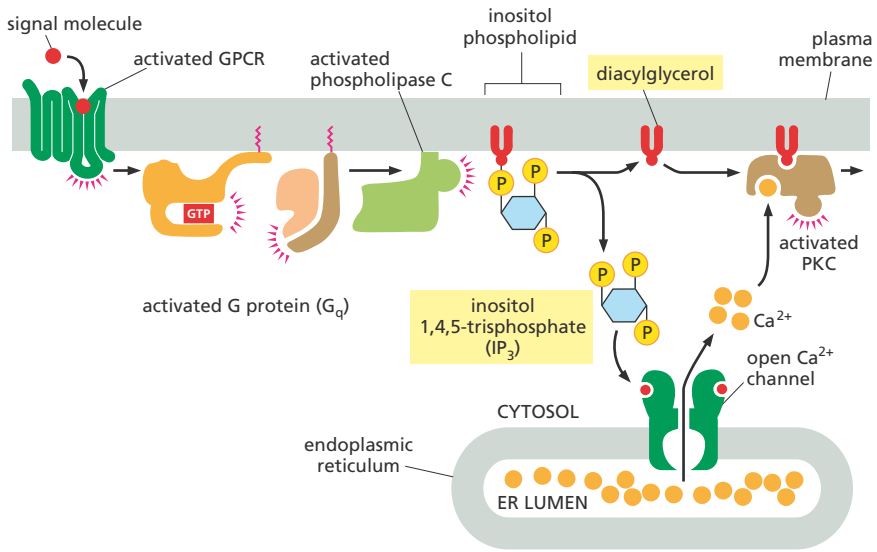


Figure 16–27 Phospholipase C activates two signaling pathways. Two small messenger molecules are produced when a membrane inositol phospholipid is hydrolyzed by activated phospholipase C. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and triggers the release of Ca^{2+} from the ER by binding to and opening special Ca^{2+} channels in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to rush out of the ER and into the cytosol. Diacylglycerol remains in the plasma membrane and, together with Ca^{2+}, helps activate the enzyme protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane. PKC then phosphorylates its own set of intracellular proteins, further propagating the signal. At the start of the pathway, both the α subunit and the βγ subunit of the G protein G_q are involved in activating phospholipase C.

IP_3 is a water-soluble sugar phosphate that is released into the cytosol; there it binds to and opens Ca^{2+} channels that are embedded in the endoplasmic reticulum (ER) membrane. Ca^{2+} stored inside the ER rushes out into the cytosol through these open channels, causing a sharp rise in the cytosolic concentration of free Ca^{2+}, which is normally kept very low. This Ca^{2+} in turn signals to other proteins, as discussed below.

Diacylglycerol is a lipid that remains embedded in the plasma membrane after it is produced by phospholipase C; there, it helps recruit and activate a protein kinase, which translocates from the cytosol to the plasma membrane. This enzyme is called **protein kinase C** (**PKC**) because it also needs to bind Ca^{2+} to become active (see Figure 16–27). Once activated, PKC phosphorylates a set of intracellular proteins that varies depending on the cell type. PKC operates on the same principle as PKA, although the proteins it phosphorylates are different.

A Ca^{2+} Signal Triggers Many Biological Processes

Ca^{2+} has such an important and widespread role as an intracellular messenger that we will digress to consider its functions more generally. A surge in the cytosolic concentration of free Ca^{2+} is triggered by many kinds of cell stimuli, not only those that act through GPCRs. When a sperm fertilizes an egg cell, for example, Ca^{2+} channels open, and the resulting rise in cytosolic Ca^{2+} triggers the egg to start development (**Figure 16–28**); for muscle cells, a signal from a nerve triggers a rise in cytosolic Ca^{2+} that initiates muscle contraction; and in many secretory cells, including nerve cells, Ca^{2+} triggers secretion. Ca^{2+} stimulates all these responses by binding to and influencing the activity of various Ca^{2+}-responsive proteins.

The concentration of free Ca^{2+} in the cytosol of an unstimulated cell is extremely low (10^{-7} M) compared with its concentration in the extracellular fluid (about 10^{-3} M) and in the ER. These differences are maintained by membrane-embedded Ca^{2+} pumps that actively remove Ca^{2+} from the cytosol—sending it either into the ER or across the plasma membrane and out of the cell. As a result, a steep electrochemical gradient of Ca^{2+} exists across both the ER membrane and the plasma membrane (discussed in Chapter 12). When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes down its electrochemical gradient into the cytosol, where it triggers changes in Ca^{2+}-responsive proteins in the

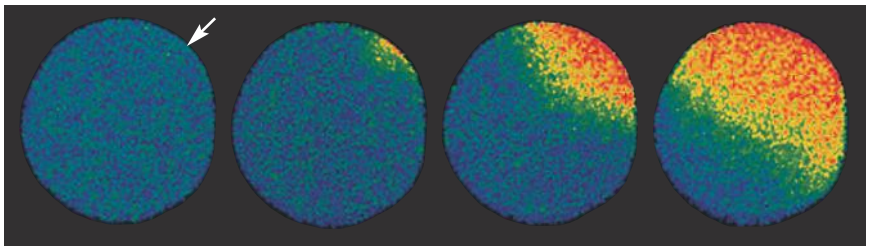


Figure 16–28 Fertilization of an egg by a sperm triggers an increase in cytosolic Ca^{2+} in the egg. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. When a sperm enters the egg, a wave of cytosolic Ca^{2+} (*red*)—released from the ER—sweeps across the egg from the site of sperm entry (*arrow*). This Ca^{2+} wave provokes a change in the egg surface, preventing entry of other sperm, and it also initiates embryonic development. To catch this Ca^{2+} wave, go to Movie 16.4. (Courtesy of Stephen A. Stricker.)

cytosol. The same Ca^{2+} pumps that normally operate to keep cytosolic Ca^{2+} concentrations low also help to terminate the Ca^{2+} signal.

The effects of Ca^{2+} in the cytosol are largely indirect, in that they are mediated through the interaction of Ca^{2+} with various kinds of Ca^{2+}-responsive proteins. The most widespread and common of these is **calmodulin**, which is present in the cytosol of all eukaryotic cells that have been examined, including those of plants, fungi, and protozoa. When Ca^{2+} binds to calmodulin, the protein undergoes a conformational change that enables it to interact with a wide range of target proteins in the cell, altering their activities (**Figure 16–29**). One particularly important class of targets for calmodulin is the **Ca^{2+}/calmodulin-dependent protein kinases** (**CaM-kinases**). When these kinases are activated by binding to calmodulin complexed with Ca^{2+}, they influence other processes in the cell by phosphorylating selected proteins. In the mammalian brain, for example, a neuron-specific CaM-kinase is abundant at synapses, where it is thought to play an important part in some forms of learning and memory. This CaM-kinase is activated by the pulses of Ca^{2+} signals that occur during neural activity, and mutant mice that lack the kinase show a marked inability to remember where things are.

QUESTION 16–7

Why do you suppose cells have evolved intracellular Ca^{2+} stores for signaling even though there is abundant extracellular Ca^{2+}?

GPCR-Triggered Intracellular Signaling Cascades Can Achieve Astonishing Speed, Sensitivity, and Adaptability

The steps in the *signaling cascades* associated with GPCRs take a long time to describe, but they often take only seconds to execute. Consider how quickly a thrill can make your heart race (when adrenaline stimulates the GPCRs in your cardiac pacemaker cells), or how fast the smell of food can make your mouth water (through the GPCRs for odors in your

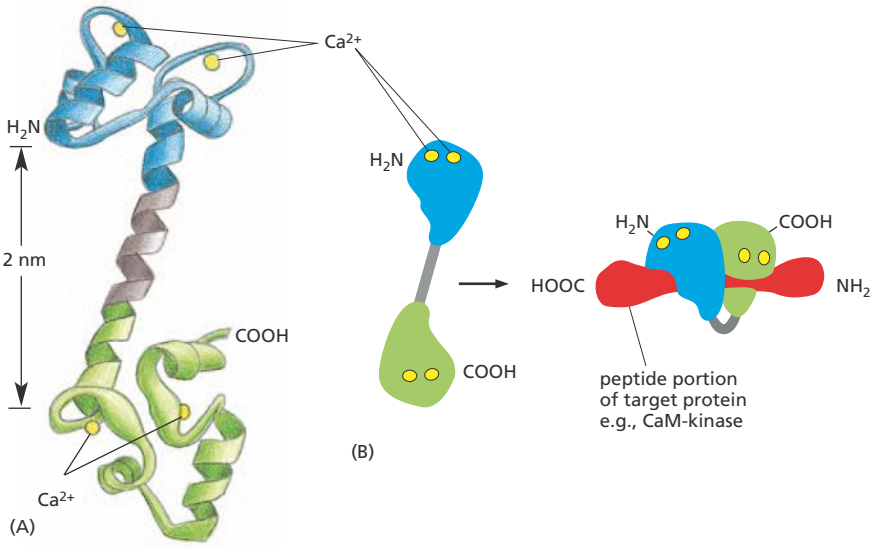


Figure 16–29 Calcium binding changes the shape of the calmodulin protein. (A) Calmodulin has a dumbbell shape, with two globular ends connected by a long α helix. Each end has two Ca^{2+}-binding domains. (B) Simplified representation of the structure, showing the conformational changes in Ca^{2+}/calmodulin that occur when it binds to an isolated segment of a target protein. In this conformation, the α helix jackknifes to surround the target (Movie 16.5). (A, from Y.S. Babu et al., *Nature* 315:37–40, 1985. With permission from Macmillan Publishers Ltd; B, from W.E. Meador, A.R. Means, and F.A. Quiocho, *Science* 257:1251–1255, 1992, and M. Ikura et al., *Science* 256:632–638, 1992. With permission from the AAAS.)

nose and the GPCRs for acetylcholine in salivary cells, which stimulate secretion). Among the fastest of all responses mediated by a GPCR, however, is the response of the eye to light: it takes only 20 msec for the most quickly responding photoreceptor cells of the retina (the cone photoreceptors, which are responsible for color vision in bright light) to produce their electrical response to a sudden flash of light.

This exceptional speed is achieved in spite of the necessity to relay the signal over multiple steps of an intracellular signaling cascade. But photoreceptors also provide a beautiful illustration of the positive advantages of intracellular signaling cascades: in particular, such cascades allow spectacular amplification of the incoming signal and also allow cells to adapt so as to be able to detect signals of widely varying intensity. The quantitative details have been most thoroughly analyzed for the rod photoreceptor cells in the eye, which are responsible for noncolor vision in dim light (**Figure 16–30**). In this photoreceptor cell, light is sensed by rhodopsin, a G-protein-coupled light receptor. Light-activated rhodopsin activates a G protein called transducin. The activated α subunit of transducin then activates an intracellular signaling cascade that causes cation channels to close in the plasma membrane of the photoreceptor cell. This produces a change in the voltage across the cell membrane, which alters neurotransmitter release and ultimately leads to a nerve impulse being sent to the brain.

The signal is repeatedly amplified as it is relayed along this intracellular signaling pathway (**Figure 16–31**). When lighting conditions are dim, as on a moonless night, the amplification is enormous: as few as a dozen photons absorbed in the entire retina will cause a perceptible signal to be delivered to the brain. In bright sunlight, when photons flood through each photoreceptor cell at a rate of billions per second, the signaling cascade undergoes a form of *adaptation*, stepping down the amplification more than 10,000-fold, so that the photoreceptor cells are not overwhelmed and can still register increases and decreases in the strong light. The adaptation depends on negative feedback: an intense response in the photoreceptor cell decreases the cytosolic Ca^{2+} concentration, inhibiting the enzymes responsible for signal amplification.

Adaptation frequently occurs in intracellular signaling pathways that respond to extracellular signal molecules, allowing cells to respond to fluctuations in the concentration of such molecules regardless of whether they are present in small or large amounts. By taking advantage of positive and negative feedback mechanisms (see Figure 16–14), adaptation thus allows a cell to respond both to messages that are whispered and to those that are shouted.

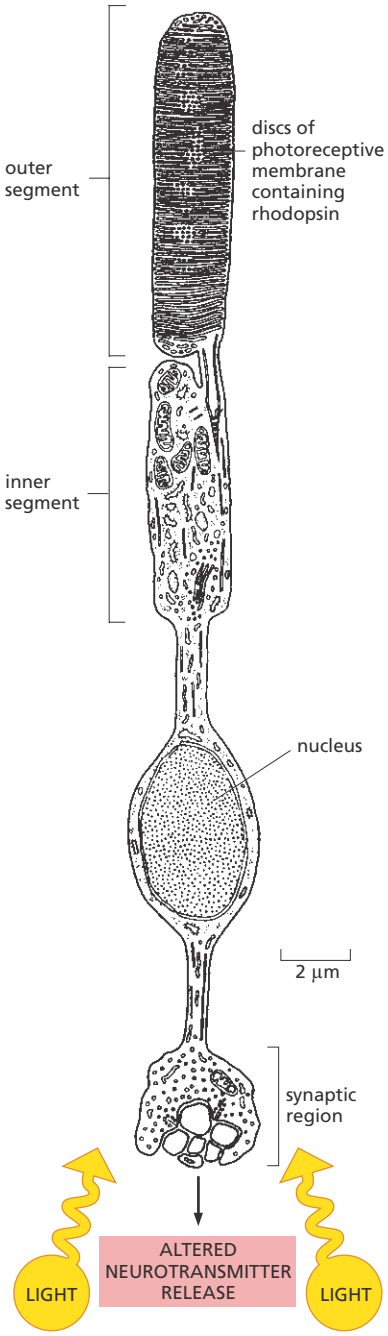


Figure 16–30 A rod photoreceptor cell from the retina is exquisitely sensitive to light. Drawing of a rod photoreceptor. The light-absorbing rhodopsin proteins are embedded in many pancake-shaped vesicles (*discs*) of membrane inside the outer segment of the cell. Neurotransmitter is released from the opposite end of the cell to control firing of the retinal nerve cells that pass on the signal to the nerve cells in the retina that connect to the brain. When the rod cell is stimulated by light, a signal is relayed from the rhodopsin molecules in the discs, through the cytosol of the outer segment, to ion channels that allow positive ions to flow through the plasma membrane of the outer segment. These cation channels close in response to the cytosolic signal, producing a change in the membrane potential of the rod cell. By mechanisms similar to those that control neurotransmitter release in ordinary nerve cells, the change in membrane potential alters the rate of neurotransmitter release from the synaptic region of the cell. (Adapted from T.L. Lentz, Cell Fine Structure. Philadelphia: Saunders, 1971. With permission from Elsevier.)

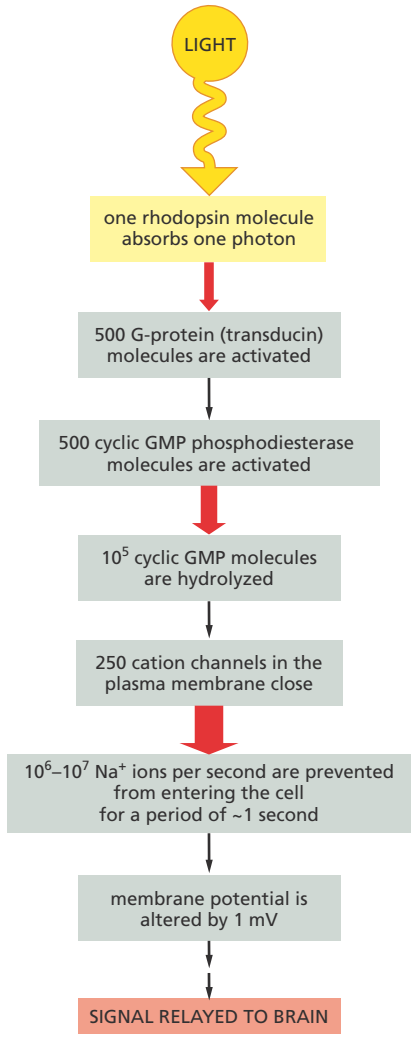


Figure 16–31 The light-induced signaling cascade in rod photoreceptor cells greatly amplifies the light signal. When rod photoreceptors are adapted for dim light, signal amplification is enormous. The intracellular signaling pathway from the G protein transducin uses components that differ from the ones in previous figures. The cascade functions as follows. In the absence of a light signal, the small messenger molecule cyclic GMP is continuously produced by an enzyme in the cytosol of the photoreceptor cell. The cyclic GMP then binds to cation channels in the photoreceptor cell plasma membrane, keeping them open. Activation of rhodopsin by light results in the activation of transducin α subunits. These turn on an enzyme called cyclic GMP phosphodiesterase, which breaks down cyclic GMP to GMP (much as cyclic AMP phosphodiesterase breaks down cyclic AMP; see Figure 16–23). The sharp fall in the cytosolic concentration of cyclic GMP causes the bound cyclic GMP to dissociate from the cation channels, which therefore close. Closing these channels decreases the influx of Na^+, thereby altering the voltage gradient (membrane potential) across the plasma membrane and, ultimately, the rate of neurotransmitter release, as described in Chapter 12. The *red* arrows indicate the steps at which amplification occurs, with the thickness of the arrow roughly indicating the magnitude of the amplification.

Taste and smell also depend on GPCRs. It seems likely that this mechanism of signal reception, invented early in the evolution of the eukaryotes, has its origins in the basic and universal need of cells to sense and respond to their environment. Of course, GPCRs are not the only receptors that activate intracellular signaling cascades. We now turn to another major class of cell-surface receptors—enzyme-coupled receptors—which play a key part in controlling cell numbers, cell differentiation, and cell movement in multicellular animals, especially during development.

ENZYME-COUPLED RECEPTORS

Like GPCRs, **enzyme-coupled receptors** are transmembrane proteins that display their ligand-binding domains on the outer surface of the plasma membrane (see Figure 16–17C). Instead of associating with a G protein, however, the cytoplasmic domain of the receptor either acts as an enzyme itself or forms a complex with another protein that acts as an enzyme. Enzyme-coupled receptors were discovered through their role in responses to extracellular signal proteins ("growth factors") that regulate the growth, proliferation, differentiation, and survival of cells in animal tissues (see Table 16–1, p. 529, for examples). Most of these signal proteins function as local mediators and can act at very low concentrations (about 10^{-9} to 10^{-11} M). Responses to them are typically slow (on the order of hours), and their effects may require many intracellular transduction steps that usually lead to a change in gene expression.

Enzyme-coupled receptors, however, can also mediate direct, rapid reconfigurations of the cytoskeleton, changing the cell's shape and the way that it moves. The extracellular signals that induce such changes are often not diffusible signal proteins, but proteins attached to the surfaces over which a cell is crawling.

The largest class of enzyme-coupled receptors consists of receptors with a cytoplasmic domain that functions as a tyrosine protein kinase, which phosphorylates particular tyrosines on specific intracellular signaling proteins. These receptors, called **receptor tyrosine kinases** (**RTKs**), will be our main focus in this section.

We begin with a discussion of how RTKs are activated in response to extracellular signals. We then consider how activated RTKs transmit the

QUESTION 16–8

One important feature of any intracellular signaling pathway is its ability to be turned off. Consider the pathway shown in Figure 16–31. Where would off switches be required? Which ones do you suppose would be the most important?

signal along two major intracellular signaling pathways that terminate at various effector proteins in the target cell. Finally, we describe how some enzyme-coupled receptors bypass such intracellular signaling cascades and use a more direct mechanism to regulate gene transcription.

Abnormal cell growth, proliferation, differentiation, survival, and migration are fundamental features of a cancer cell, and abnormalities in signaling via RTKs and other enzyme-coupled receptors have a major role in the development of most cancers.

Activated RTKs Recruit a Complex of Intracellular Signaling Proteins

To do its job as a signal transducer, an enzyme-coupled receptor has to switch on the enzyme activity of its intracellular domain (or of an associated enzyme) when an external signal molecule binds to its extracellular domain. Unlike the seven-pass transmembrane GPCRs, enzyme-coupled receptor proteins usually have only one transmembrane segment, which spans the lipid bilayer as a single α helix. Because a single α helix is poorly suited to transmit a conformational change across the bilayer, enzyme-coupled receptors have a different strategy for transducing the extracellular signal. In many cases, the binding of an extracellular signal molecule causes two receptor molecules to come together in the plasma membrane, forming a dimer. This pairing brings the two intracellular tails of the receptors together, activating their kinase domains so that each receptor tail phosphorylates the other. In the case of RTKs, the phosphorylations occur on specific tyrosines.

This tyrosine phosphorylation then triggers the assembly of a transient but elaborate intracellular signaling complex on the cytosolic tails of the receptor. The newly phosphorylated tyrosines serve as docking sites for a whole zoo of intracellular signaling proteins—perhaps as many as 10 or 20 different molecules (**Figure 16–32**). Some of these proteins become phosphorylated and activated on binding to the receptor, and they then propagate the signal; others function solely as scaffolds, which couple the receptor to other signaling proteins, thereby helping to build the active signaling complex (see Figure 16–13). All of these docked

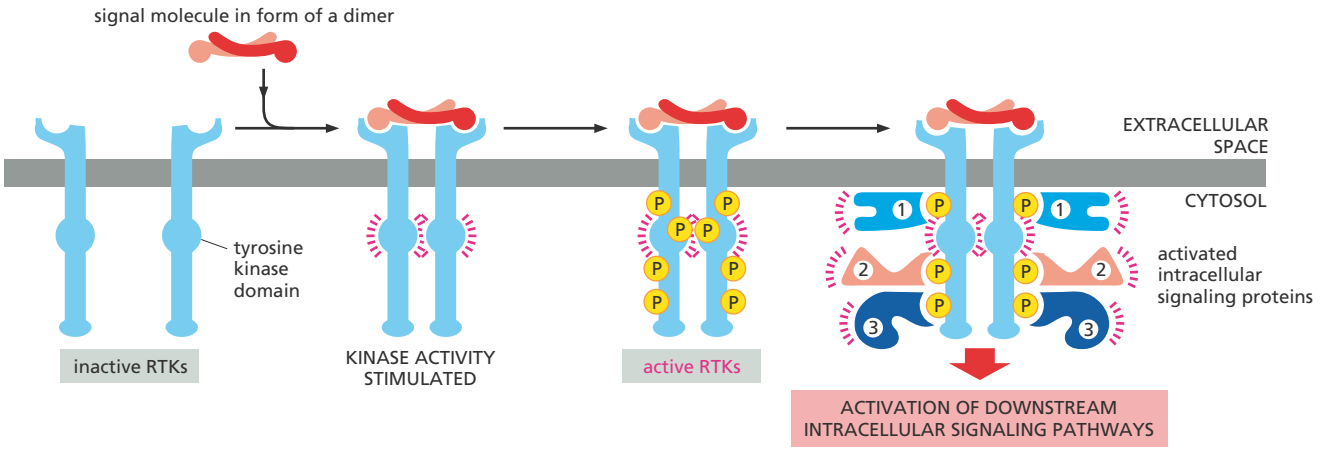


Figure 16–32 Activation of an RTK stimulates the assembly of an intracellular signaling complex. Typically, the binding of a signal molecule to the extracellular domain of an RTK causes two receptor molecules to associate into a dimer. The signal molecule shown here is itself a dimer and thus can physically cross-link two receptor molecules; other signal molecules induce a conformational change in the RTKs, causing the receptors to dimerize (not shown). In either case, dimer formation brings the kinase domains of each cytosolic receptor tail into contact with the other; this activates the kinases to phosphorylate the adjacent tail on several tyrosines. Each phosphorylated tyrosine serves as a specific docking site for a different intracellular signaling protein, which then helps relay the signal to the cell's interior; these proteins contain a specialized interaction domain—in this case, a module called an SH2 domain—that recognizes and binds to specific phosphorylated tyrosines on the cytosolic tail of an activated RTK or on another intracellular signaling protein.

intracellular signaling proteins possess a specialized *interaction domain*, which recognizes specific phosphorylated tyrosines on the receptor tails. Other interaction domains allow intracellular signaling proteins to recognize phosphorylated lipids that are produced on the cytosolic side of the plasma membrane in response to certain signals, as we discuss later.

While they last, the signaling protein complexes assembled on the cytosolic tails of the RTKs can transmit a signal along several routes simultaneously to many destinations in the cell, thus activating and coordinating the numerous biochemical changes that are required to trigger a complex response, such as cell proliferation or differentiation. To help terminate the response, the tyrosine phosphorylations are reversed by protein tyrosine phosphatases, which remove the phosphates that were added to the tyrosines of both the RTKs and other intracellular signaling proteins in response to the extracellular signal. In some cases, activated RTKs (as well as some GPCRs) are inactivated in a more brutal way: they are dragged into the interior of the cell by endocytosis and then destroyed by digestion in lysosomes.

Different RTKs recruit different collections of intracellular signaling proteins, producing different effects; however, certain components are used by most RTKs. These include, for example, a phospholipase C that functions in the same way as the phospholipase C activated by GPCRs to trigger the inositol phospholipid signaling pathway discussed earlier (see Figure 16–27). Another intracellular signaling protein that is activated by almost all RTKs is a small GTP-binding protein called Ras, as we discuss next.

Most RTKs Activate the Monomeric GTPase Ras

As we have seen, activated RTKs recruit and activate many kinds of intracellular signaling proteins, leading to the formation of large signaling complexes on the cytosolic tail of the RTK. One of the key members of these signaling complexes is **Ras**—a small GTP-binding protein that is bound by a lipid tail to the cytoplasmic face of the plasma membrane. Virtually all RTKs activate Ras, including platelet-derived growth factor (PDGF) receptors, which mediate cell proliferation in wound healing, and nerve growth factor (NGF) receptors, which play an important part in the development of certain vertebrate neurons.

The Ras protein is a member of a large family of small GTP-binding proteins, often called **monomeric GTPases** to distinguish them from the trimeric G proteins that we encountered earlier. Ras resembles the α subunit of a G protein and functions as a molecular switch in much the same way. It cycles between two distinct conformational states—active when GTP is bound and inactive when GDP is bound. Interaction with an activating protein called Ras-GEF encourages Ras to exchange its GDP for GTP, thus switching Ras to its activated state (**Figure 16–33**); after a delay, Ras is switched off by a GAP protein called Ras-GAP (see Figure 16–16), which promotes the hydrolysis of its bound GTP to GDP (**Movie 16.6**).

In its active state, Ras initiates a phosphorylation cascade in which a series of serine/threonine protein kinases phosphorylate and activate one another in sequence, like an intracellular game of dominoes. This relay system, which carries the signal from the plasma membrane to the nucleus, includes a three-protein-kinase module called the **MAP-kinase signaling module**, in honor of the final kinase in the chain, the mitogen-activated protein kinase, or **MAP kinase**. (As we discuss in Chapter 18, *mitogens* are extracellular signal molecules that stimulate cell proliferation.) In this pathway, outlined in **Figure 16–34**, MAP kinase

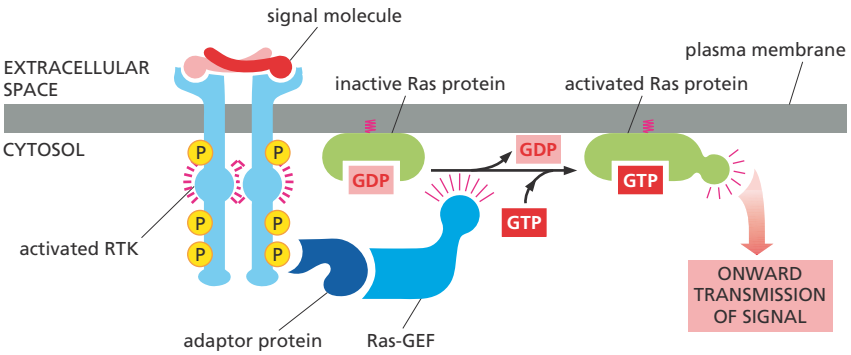


Figure 16–33 RTKs activate Ras. An adaptor protein docks on a particular phosphotyrosine on the activated receptor (the other signaling proteins that are shown bound to the receptor in Figure 16–32 are omitted for simplicity). The adaptor recruits a Ras guanine nucleotide exchange factor (Ras-GEF) that stimulates Ras to exchange its bound GDP for GTP. The activated Ras protein can now stimulate several downstream signaling pathways, one of which is shown in Figure 16–34. Note that the Ras protein contains a covalently attached lipid group (*red*) that helps anchor the protein to the inside of the plasma membrane.

is phosphorylated and activated by an enzyme called, logically enough, MAP kinase kinase. And this protein is itself switched on by a MAP kinase kinase kinase (which is activated by Ras). At the end of the MAP-kinase cascade, MAP kinase phosphorylates various effector proteins, including certain transcription regulators, altering their ability to control gene transcription. This change in the pattern of gene expression may stimulate cell proliferation, promote cell survival, or induce cell differentiation: the precise outcome will depend on which other genes are active in the cell and what other signals the cell receives. How biologists unravel such complex signaling pathways is discussed in **How We Know**, pp. 556–557.

Before Ras was discovered in normal cells, a mutant form of it was found in human cancer cells; the mutation inactivated the GTPase activity of Ras, so that the protein could not shut itself off, promoting uncontrolled cell proliferation and the development of cancer. About 30% of human cancers contain such activating mutations in a *Ras* gene; of the cancers that do not, many have mutations in genes that encode proteins that function in the same signaling pathway as Ras. Many of the genes that encode normal intracellular signaling proteins were initially identified in the hunt for cancer-promoting *oncogenes* (discussed in Chapter 20).

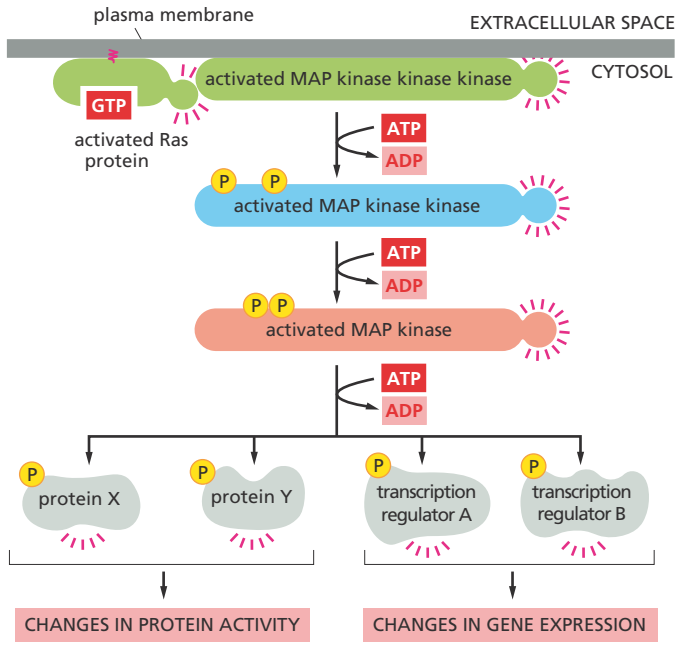


Figure 16–34 Ras activates a MAP-kinase signaling module. The Ras protein activated by the process shown in Figure 16–33 activates a three-kinase signaling module, which relays the signal. The final kinase in the module, MAP kinase, phosphorylates various downstream signaling or effector proteins.

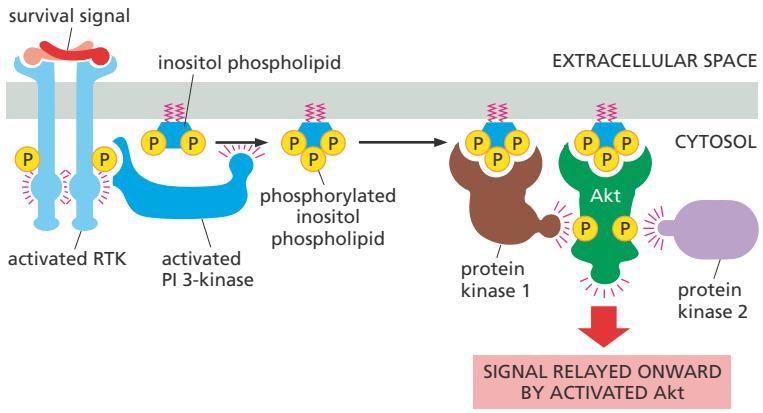


Figure 16–35 RTKs activate the PI-3-kinase–Akt signaling pathway. An extracellular survival signal, such as IGF, activates an RTK, which recruits and activates PI 3-kinase. PI 3-kinase then phosphorylates an inositol phospholipid that is embedded in the cytosolic side of the plasma membrane. The resulting phosphorylated inositol phospholipid then attracts intracellular signaling proteins that have a special domain that recognizes it. One of these signaling proteins, Akt, is a protein kinase that is activated at the membrane by phosphorylation mediated by two other protein kinases (here called protein kinases 1 and 2); protein kinase 1 is also recruited by the phosphorylated lipid docking sites. Once activated, Akt is released from the plasma membrane and phosphorylates various downstream proteins on specific serines and threonines (not shown).

RTKs Activate PI 3-Kinase to Produce Lipid Docking Sites in the Plasma Membrane

Many of the extracellular signal proteins that stimulate animal cells to survive and grow act through RTKs. These include signal proteins belonging to the insulin-like growth factor (IGF) family. One crucially important signaling pathway that these RTKs activate to promote cell growth and survival relies on the enzyme **phosphoinositide 3-kinase** (**PI 3-kinase**), which phosphorylates inositol phospholipids in the plasma membrane. These phosphorylated lipids then serve as docking sites for specific intracellular signaling proteins, which relocate from the cytosol to the plasma membrane, where they can activate one another. One of the most important of these relocated signaling proteins is the serine/threonine protein kinase *Akt* (**Figure 16–35**).

Akt, also called protein kinase B (PKB), promotes the growth and survival of many cell types, often by inactivating the signaling proteins it phosphorylates. For example, Akt phosphorylates and inactivates a cytosolic protein called Bad. In its active state, Bad encourages the cell to kill itself by indirectly activating a cell-suicide program called apoptosis (discussed in Chapter 18). Phosphorylation by Akt thus promotes cell survival by inactivating a protein that otherwise promotes cell death (**Figure 16–36**).

In addition to promoting cell survival, the *PI-3-kinase–Akt signaling pathway* also stimulates cells to grow in size. It does so by indirectly activating a large serine/threonine kinase called *Tor*. Tor stimulates cells to grow both by enhancing protein synthesis and by inhibiting protein

QUESTION 16–9

Would you expect to activate RTKs by exposing the exterior of cells to antibodies that bind to the respective proteins? Would your answer be different for GPCRs? (Hint: review Panel 4–3, on pp. 164–165, regarding the properties of antibody molecules.)

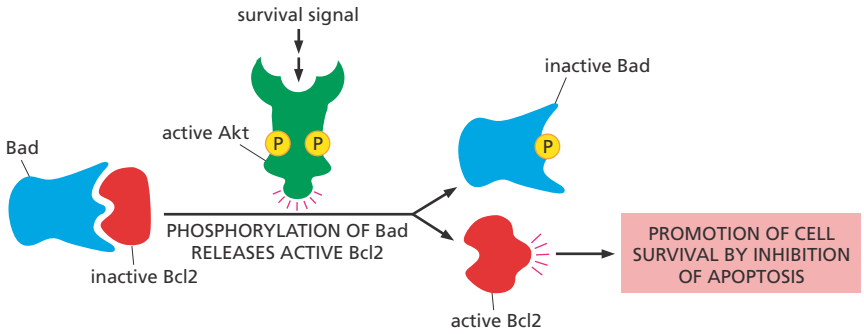


Figure 16–36 Activated Akt promotes cell survival. One way it does so is by phosphorylating and inactivating a protein called Bad. In its unphosphorylated state, Bad promotes apoptosis (a form of cell death) by binding to and inhibiting a protein, called Bcl2, which otherwise suppresses apoptosis. When Bad is phosphorylated by Akt, Bad releases Bcl2, which now blocks apoptosis, thereby promoting cell survival.

UNTANGLING CELL SIGNALING PATHWAYS

Intracellular signaling pathways are never mapped out in a single experiment. Although insulin was first isolated from dog pancreas in the early 1920s, the molecular chain of events that links the binding of insulin to its receptor with the activation of the transporter proteins that take up glucose has taken decades to untangle—and is still not completely understood.

Instead, investigators figure out, piece by piece, how all the links in the chain fit together—and how each contributes to the cell's response to an extracellular signal molecule such as the hormone insulin. Here, we discuss the kinds of experiments that allow scientists to identify individual links and, ultimately, to piece together complex signaling pathways.

Close encounters

Most signaling pathways depend on proteins that physically interact with one another. There are several ways to detect such direct contact. One involves using a protein as "bait." For example, to isolate the receptor that binds to insulin, one could attach insulin to a chromatography column. Cells that respond to the hormone are broken open and their contents poured over the column. Proteins that bind to insulin will stick to this column and can later be eluted and identified (see Figure 4–48).

Protein–protein interactions in a signaling pathway can also be identified by *co-immunoprecipitation*. For example, cells exposed to an extracellular signal molecule can be broken open, and antibodies can be used to grab the receptor protein known to recognize the signal molecule (see Panel 4–2, pp. 146–147, and Panel 4–3, pp. 164–165). If the receptor is strongly associated with other proteins, these will be captured as well. In this way, researchers can identify which proteins interact when an extracellular signal molecule stimulates cells.

Once two proteins are known to bind to each other, the experimenter can use recombinant DNA technology to pinpoint which parts of the proteins are required for the interaction. For example, to determine which phosphorylated tyrosine on a receptor tyrosine kinase (RTK) a certain intracellular signaling protein binds, a series of mutant receptors is constructed, each missing a different tyrosine from its cytoplasmic domain (**Figure 16–37**). In this way, the specific tyrosines required for binding can be determined. Similarly, one can determine whether this phosphotyrosine docking site is required for the receptor to transmit a signal to the cell.

Jamming the pathway

Ultimately, one wants to assess what part a particular protein plays in a signaling pathway. A first test may involve using recombinant DNA technology to introduce into cells a gene encoding a constantly active form of the protein, to see if this mimics the effect of the extracellular signal molecule. Consider Ras, for example. The mutant form of Ras involved in human

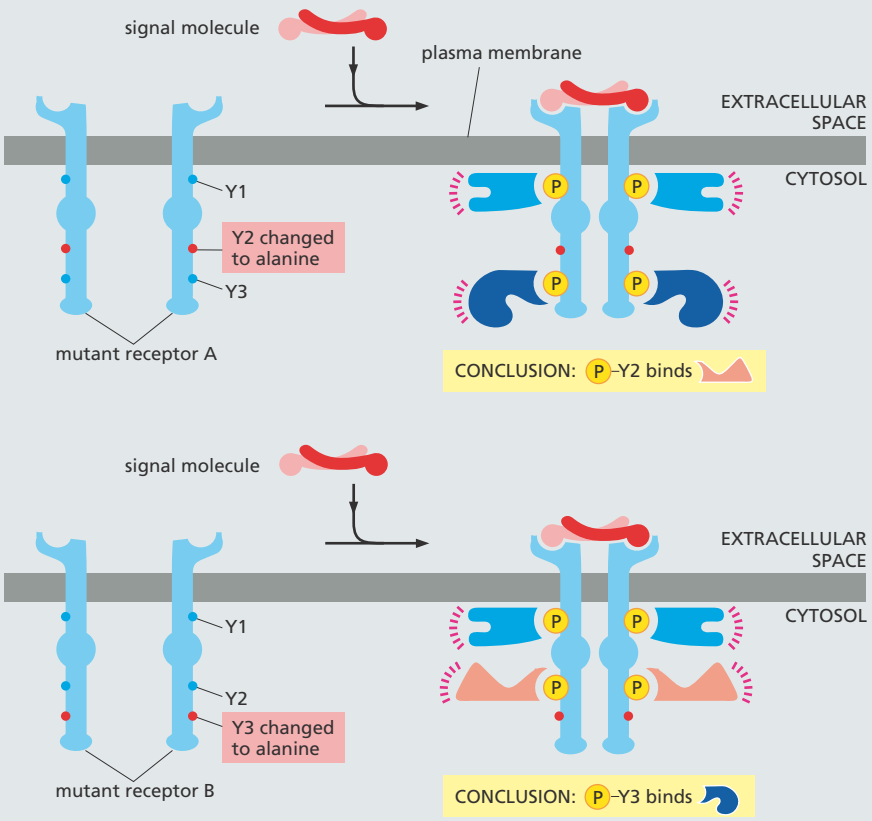


Figure 16–37 Mutant proteins can help to determine exactly where an intracellular signaling molecule binds. As shown in Figure 16–32, on binding their extracellular signal molecule, a pair of RTKs come together and phosphorylate specific tyrosines on each other's cytoplasmic tails. These phosphorylated tyrosines attract different intracellular signaling proteins, which then become activated and pass on the signal. To determine which tyrosine binds to a specific intracellular signaling protein, a series of mutant receptors is constructed. In the mutants shown, tyrosines Y2 and Y3 have been replaced, one at a time, by an alanine (*red*). As a result, the mutant receptors no longer bind to one of the intracellular signaling proteins shown in Figure 16–32. The effect on the cell's response to the signal can then be determined. It is important that the mutant receptor be tested in a cell that does not have its own normal receptors for the signal molecule.

cancers is constantly active because it has lost its ability to hydrolyze the bound GTP that keeps the Ras protein switched on. This continuously active form of Ras can stimulate some cells to proliferate, even in the absence of a proliferation signal.

Conversely, a specific signaling protein can be inactivated. In the case of Ras, for example, one could "knock down" the activity of the *Ras* gene in cells by RNA interference (see Figure 8–26). Such cells do not proliferate in response to extracellular mitogens, indicating the importance of normal Ras signaling in the proliferative response.

Making mutants

One powerful strategy that scientists use to identify proteins that participate in cell signaling involves screening tens of thousands of animals—fruit flies or nematode worms, for example (discussed in Chapter 19)—to search for mutants in which a signaling pathway is not functioning properly. By examining enough mutant animals, many of the genes that encode the proteins involved in a signaling pathway can be identified.

Such classical genetic screens can also help determine the order in which intracellular signaling proteins act in a pathway. Suppose that a genetic screen uncovers a pair of new proteins, X and Y, involved in the Ras signaling pathway. To determine whether these proteins lie upstream or downstream of Ras, one could create cells that express an inactive, mutant form of each, and then ask whether these mutant cells can be "rescued" by the addition of a continuously active form of Ras. If the constantly active Ras overcomes the blockage created by the mutant protein, the protein must operate upstream of Ras in the pathway (**Figure 16–38A**). However, if Ras operates upstream of the protein, a constantly active Ras would be unable to transmit a signal past the obstruction caused by the disabled protein (**Figure 16–38B**). Through such experiments, even the most complex intracellular signaling pathways can be mapped out, one step at a time (**Figure 16–38C**).

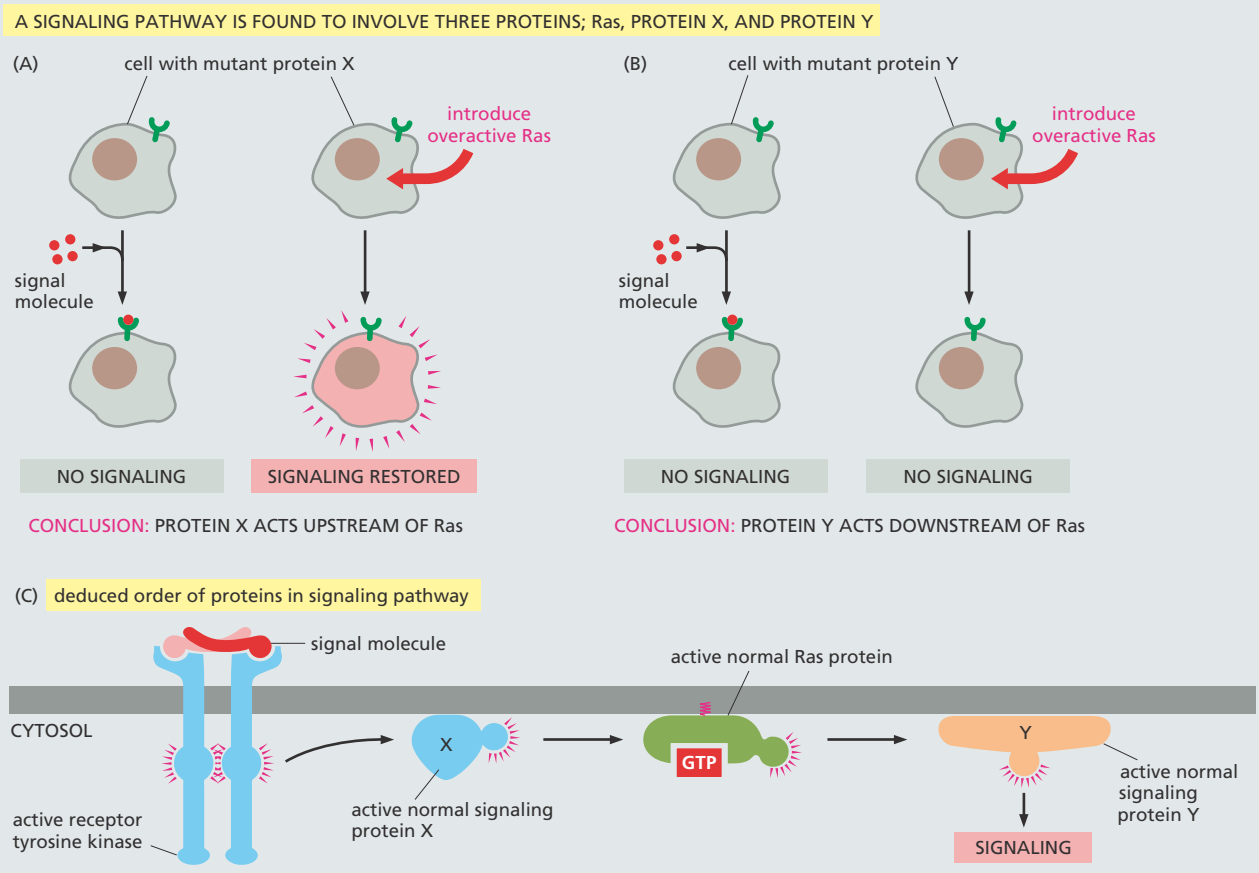


Figure 16–38 The use of mutant cell lines and an overactive form of Ras can help dissect an intracellular signaling pathway. In this hypothetical pathway, Ras, protein X, and protein Y are required for proper signaling. (A) In cells in which protein X has been inactivated, signaling does not occur. However, this signaling blockage can be overcome by the addition of an overactive form of Ras, such that the pathway is active even in the absence of the extracellular signal molecule. This result indicates that protein X acts upstream of Ras in the pathway. (B) Signaling is also disrupted in cells in which protein Y has been inactivated. In this case, introduction of an overactive Ras does not restore normal signaling, indicating that protein Y operates downstream of Ras. (C) Based on these results, the deduced order of the signaling pathway is shown.

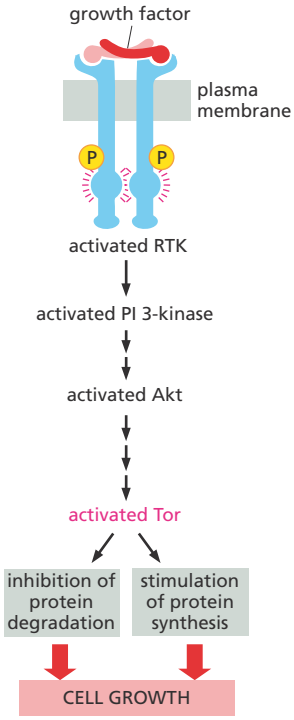


Figure 16–39 Akt stimulates cells to grow in size by activating the serine/threonine kinase Tor. The binding of a growth factor to an RTK activates the PI-3-kinase–Akt signaling pathway (as shown in Figure 16–35). Akt then indirectly activates Tor by phosphorylating and inhibiting a protein that helps to keep Tor shut down (not shown). Tor stimulates protein synthesis and inhibits protein degradation by phosphorylating key proteins in these processes (not shown). The anticancer drug rapamycin slows cell growth by inhibiting Tor. In fact, the Tor protein derives its name from the fact that it is a *t*arget *o*f *r*apamycin.

degradation (**Figure 16–39**). The anticancer drug rapamycin works by inactivating Tor, indicating the importance of this signaling pathway in regulating cell growth and survival—and the consequences of its disregulation in cancer.

Figure 16–40 summarizes the main intracellular signaling cascades activated by GPCRs and RTKs.

Some Receptors Activate a Fast Track to the Nucleus

Not all receptors trigger complex signaling cascades to carry a message to the nucleus. Some use a more direct route to control gene expression. One such receptor is the protein Notch.

Notch is a crucially important receptor in all animals, both during development and in adults. Among other things, it controls the development of neural cells in *Drosophila*, as mentioned earlier (see Figure 16–4). In this simple signaling pathway, the receptor itself acts as a transcription regulator. When activated by the binding of Delta, which is a transmembrane signal protein on the surface of a neighboring cell, the Notch receptor is cleaved. This cleavage releases the cytosolic tail of the receptor, which is then free to move to the nucleus where it helps to activate the appropriate set of Notch-responsive genes (**Figure 16–41**).

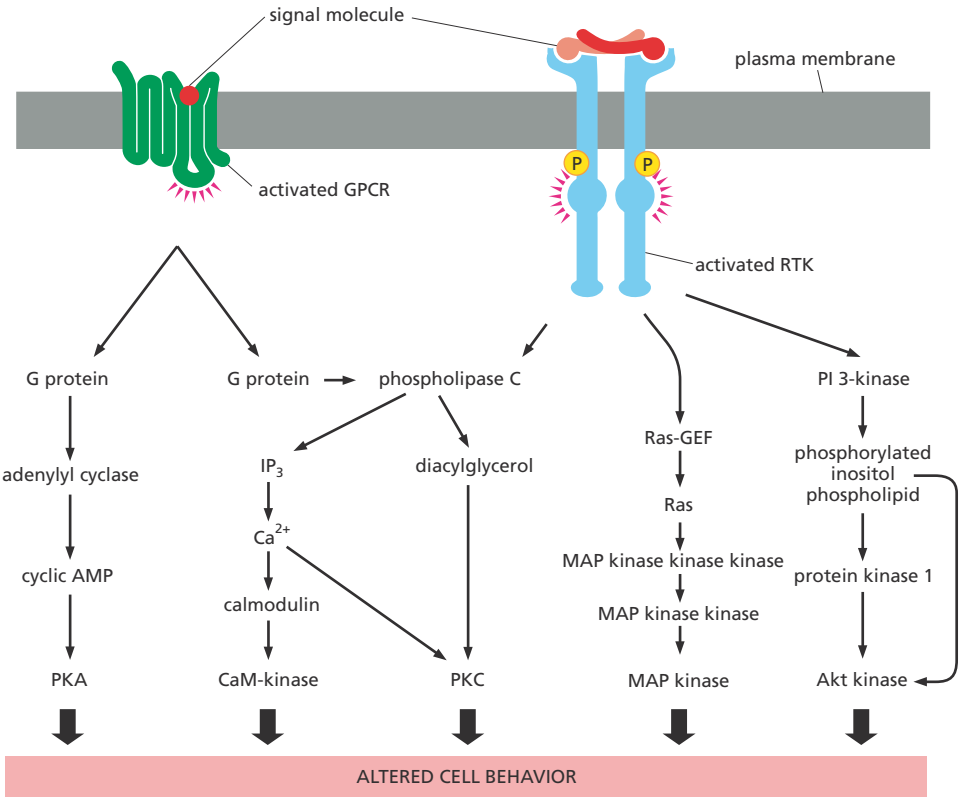


Figure 16–40 Both GPCRs and RTKs activate multiple intracellular signaling pathways. The figure reviews five of these pathways: two leading from GPCRs—through adenylyl cyclase and through phospholipase C—and three leading from RTKs—through phospholipase C, Ras, and PI 3-kinase. Each pathway differs from the others, yet they use some common components to transmit their signals. Because all five eventually activate protein kinases, it seems that each is capable in principle of regulating practically any process in the cell.

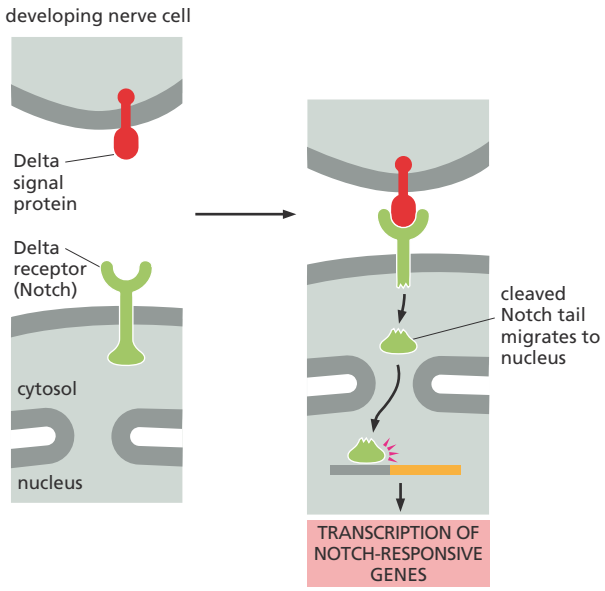


Figure 16–41 The Notch receptor itself is a transcription regulator. When the membrane-bound signal protein Delta binds to its receptor, Notch, on a neighboring cell, the receptor is cleaved. The released part of the cytosolic tail of Notch migrates to the nucleus, where it activates Notch-responsive genes. One consequence of this signaling process is shown in Figure 16–4.

Cell–Cell Communication Evolved Independently in Plants and Animals

Plants and animals have been evolving independently for more than a billion years, the last common ancestor being a single-celled eukaryote that most likely lived on its own. Because these kingdoms diverged so long ago—when it was still "every cell for itself"—each has evolved its own molecular solutions to the complex problem of becoming multicellular. Thus the mechanisms for cell–cell communication in plants and animals are in some ways quite different. At the same time, however, plants and animals started with a common set of eukaryotic genes—including some used by single-celled organisms to communicate among themselves—so their signaling systems also show some similarities.

Like animals, plants make extensive use of transmembrane cell-surface receptors—especially enzyme-coupled receptors. The spindly weed *Arabidopsis thaliana* (see Figure 1–32) has hundreds of genes encoding *receptor serine/threonine kinases*. These are, however, structurally distinct from the receptor serine/threonine kinases found in animal cells (which we do not discuss in this chapter). The plant receptors are thought to play an important part in a large variety of cell signaling processes, including those governing plant growth, development, and disease resistance. In contrast to animal cells, plant cells seem not to use RTKs, steroid-hormone-type nuclear receptors, or cyclic AMP, and they seem to use few GPCRs.

One of the best-studied signaling systems in plants mediates the response of cells to ethylene—a gaseous hormone that regulates a diverse array of developmental processes, including seed germination and fruit ripening. Tomato growers use ethylene to ripen their fruit, even after it has been picked. Although ethylene receptors are not evolutionarily related to any of the classes of receptor proteins that we have discussed so far, they function as enzyme-coupled receptors. Surprisingly, it is the empty receptor that is active: in the absence of ethylene, the empty receptor activates an associated protein kinase that ultimately shuts off the ethylene-responsive genes in the nucleus; when ethylene is present, the receptor and kinase are inactive, and the ethylene-responsive genes are transcribed (**Figure 16–42**). This strategy, whereby signals act to relieve transcriptional inhibition, is commonly used in plants.

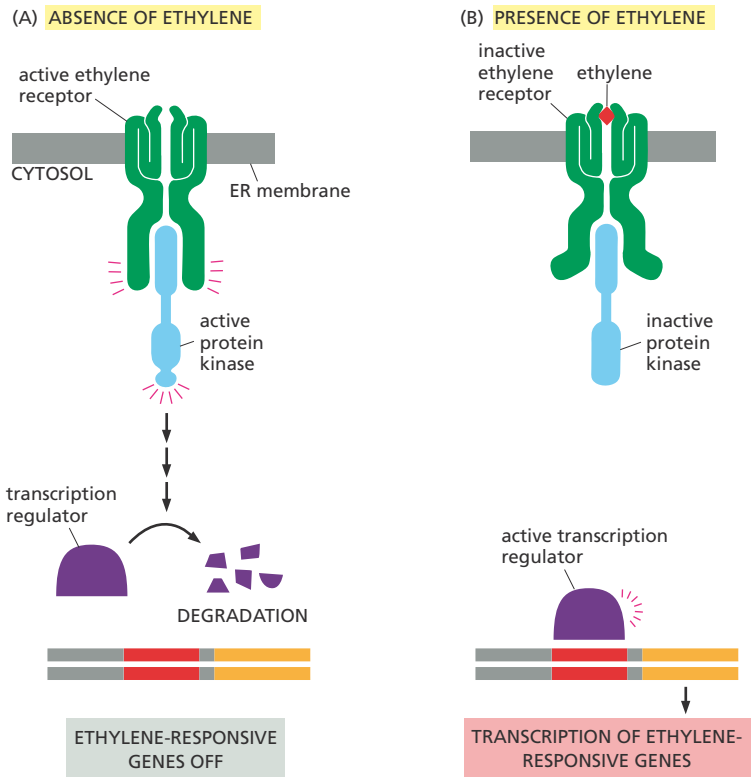


Figure 16–42 The ethylene signaling pathway turns on genes by relieving inhibition. (A) In the absence of ethylene, the receptor directly activates an associated protein kinase, which then indirectly promotes the destruction of the transcription regulator that switches on ethylene-responsive genes. As a result, the genes remain turned off. (B) In the presence of ethylene, the receptor and kinase are both inactive, and the transcription regulator remains intact and stimulates the transcription of the ethylene-responsive genes. The kinase that ethylene receptors interact with is a serine/threonine kinase that is closely related to the MAP kinase kinase kinase found in animal cells (see Figure 16–34).

Protein Kinase Networks Integrate Information to Control Complex Cell Behaviors

Although the signaling pathways we have described thus far may seem dauntingly complex, the complexity of cell signaling is actually much greater than we have let on. First, we have not discussed all of the intracellular signaling pathways that operate in cells, even though many of these are critical for normal development. Second, although we depict these signaling pathways as being linear and self-contained, they do not work independently of one another. Instead, they are connected by interactions of many sorts. The most extensive links are those mediated by the protein kinases present in each pathway. These kinases often phosphorylate, and hence regulate, components in other signaling pathways, in addition to components in their own pathway. Thus a certain amount of cross-talk occurs between the different pathways. To give an idea of the scale of the complexity, genome sequencing studies suggest that about 2% of our ~21,000 protein-coding genes code for protein kinases; moreover, hundreds of distinct types of protein kinases are thought to be present in a single mammalian cell. How can we make sense of this tangled web of interacting signaling pathways, and what is the function of such complexity?

A cell receives messages from many sources, and it must integrate this information to generate an appropriate response: to live or die, to divide, to differentiate, to change shape, to move, to send out a chemical message of its own, and so on (see Figure 16–6 and **Movies 16.7 and 16.8**). Through the cross-talk between signaling pathways, the cell is able to bring together multiple bits of information and react to the combination. Thus some intracellular signaling proteins act as integrating devices, usually by having several potential phosphorylation sites, each of which can be phosphorylated by a different protein kinase. Information received from different sources can converge on such proteins, which then convert the input to a single outgoing signal (**Figure 16–43**, and see Figure 16–13). The integrating proteins in turn can deliver a signal to many downstream

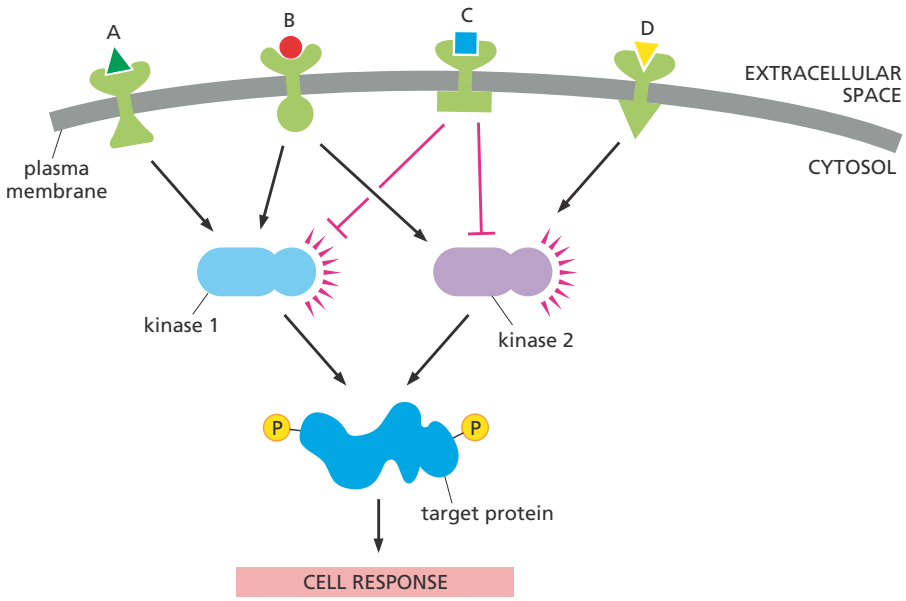


Figure 16–43 Intracellular signaling proteins serve to integrate incoming signals. Extracellular signals A, B, C, and D activate different receptors in the plasma membrane. The receptors act upon two protein kinases, which they either activate (arrowhead) or inhibit (crossbar). The kinases phosphorylate a same target protein and when it is fully phosphorylated, the target protein triggers a cellular response.

It can be seen that signal molecule B activates both protein kinases and therefore produces a strong output response. Signals A and D each activate a different kinase and therefore produce a response only if they are simultaneously present. Signal molecule C inhibits the cell response and will compete with the other signal molecules. The net outcome will depend both on the numbers of signaling molecules and the strengths of their connections. In a real cell these parameters would be determined by evolution.

targets. In this way, the intracellular signaling system may act like a network of nerve cells in the brain—or like a collection of microprocessors in a computer—interpreting complex information and generating complex responses.

Our understanding of these intricate networks is still evolving: we are still discovering new links in the chains, new signaling partners, new connections, and even new pathways. Unraveling the intracellular signaling pathways—in both animals and plants—is one of the most active areas of research in cell biology, and new discoveries are being made every day. Genome sequencing projects continue to provide long lists of components involved in signal transduction in a large variety of organisms. Even when we have identified all the components, however, it will remain a major challenge to figure out exactly how they work together to allow cells to integrate the diverse array of signals in their environment and respond in the appropriate manner.

In a way, learning how cells "think" is a problem akin to learning how we, as humans, think. Although we know, for example, how neurotransmitters activate certain neurons and how one neuron communicates with another, we are nowhere near understanding how all these components operate together to enable us to reason, converse, laugh, love, and attempt to uncover the fundamental features of life on Earth.

ESSENTIAL CONCEPTS

- **Cells in multicellular organisms communicate through a large variety of extracellular chemical signals.**
- **In animals, hormones are carried in the blood to distant target cells, but most other extracellular signal molecules act over only a short distance. Neighboring cells often communicate through direct cell–cell contact.**
- **For an extracellular signal molecule to influence a target cell it must interact with a receptor protein on or in a target cell. Each receptor protein recognizes a particular signal molecule.**
- **Small, hydrophobic, extracellular signal molecules, such as steroid hormones and nitric oxide, can cross the plasma membrane and activate intracellular proteins, which are usually either transcription regulators or enzymes.**

- Most extracellular signal molecules cannot pass through the plasma membrane; they bind to cell-surface receptor proteins that convert (transduce) the extracellular signal into different intracellular signals, which are usually organized into signaling pathways.
- There are three main classes of cell-surface receptors: (1) ion-channel-coupled receptors, (2) G-protein-coupled receptors (GPCRs), and (3) enzyme-coupled receptors.
- GPCRs and enzyme-coupled receptors respond to extracellular signals by activating one or more intracellular signaling pathways, which, in turn, activate effector proteins that alter the behavior of the cell.
- Turning off signaling pathways is as important as turning them on. Each activated component in a signaling pathway must be subsequently inactivated or removed for the pathway to function again.
- GPCRs activate trimeric GTP-binding proteins called G proteins; these act as molecular switches, transmitting the signal onward for a short period before switching themselves off by hydrolyzing their bound GTP to GDP.
- G proteins directly regulate ion channels or enzymes in the plasma membrane. Some directly activate (or inactivate) the enzyme adenylyl cyclase, which increases (or decreases) the intracellular concentration of the small messenger molecule cyclic AMP; others directly activate the enzyme phospholipase C, which generates the small messenger molecules inositol trisphosphate (IP_3) and diacylglycerol.
- IP_3 opens Ca^{2+} channels in the membrane of the endoplasmic reticulum, releasing a flood of free Ca^{2+} ions into the cytosol. The Ca^{2+} itself acts as a second messenger, altering the activity of a wide range of Ca^{2+}-responsive proteins. These include calmodulin, which activates various target proteins such as Ca^{2+}/calmodulin-dependent protein kinases (CaM-kinases) .
- A rise in cyclic AMP activates protein kinase A (PKA), while Ca^{2+} and diacylglycerol in combination activate protein kinase C (PKC).
- PKA, PKC, and CaM-kinases phosphorylate selected signaling and effector proteins on serines and threonines, thereby altering their activity. Different cell types contain different sets of signaling and effector proteins and are therefore affected in different ways.
- Enzyme-coupled receptors have intracellular protein domains that function as enzymes or are associated with intracellular enzymes. Many enzyme-coupled receptors are receptor tyrosine kinases (RTKs), which phosphorylate themselves and selected intracellular signaling proteins on tyrosines. The phosphotyrosines on RTKs then serve as docking sites for various intracellular signaling proteins.
- Most RTKs activate the monomeric GTPase Ras, which, in turn, activates a three-protein MAP-kinase signaling module that helps relay the signal from the plasma membrane to the nucleus.
- Ras mutations stimulate cell proliferation by keeping Ras (and, consequently, the Ras–MAP kinase signaling pathway) constantly active and are a common feature of many human cancers.
- Some RTKs stimulate cell growth and cell survival by activating PI 3-kinase, which phosphorylates specific inositol phospholipids in the cytosolic leaflet of the plasma membrane lipid bilayer. This inositol phosphorylation creates lipid docking sites that attract specific signaling proteins from the cytosol, including the protein kinase Akt, which becomes active and relays the signal onward.
- Other receptors, such as Notch, have a direct pathway to the nucleus. When activated, part of the receptor migrates from the plasma membrane to the nucleus, where it regulates the transcription of specific genes.

- Plants, like animals, use enzyme-coupled cell-surface receptors to recognize the extracellular signal molecules that control their growth and development; these receptors often act by relieving the transcriptional repression of specific genes.
- Different intracellular signaling pathways interact, enabling each cell type to produce the appropriate response to a combination of extracellular signals. In the absence of such signals, most animal cells have been programmed to kill themselves by undergoing apoptosis.
- We are far from understanding how a cell integrates all of the many extracellular signals that bombard it to generate an appropriate response.

KEY TERMS

adaptation
adenylyl cyclase
Ca^{2+}/calmodulin-dependent protein kinase (CaM-kinase)
calmodulin
cell signaling
cyclic AMP
cyclic-AMP-dependent protein kinase (PKA)
diacylglycerol (DAG)
enzyme-coupled receptor
extracellular signal molecule
G protein
G-protein-coupled receptor (GPCR)
GTP-binding protein
hormone
inositol phospholipid
inositol 1,4,5-trisphosphate (IP_3)
intracellular signaling pathway
ion-channel-coupled receptor
local mediator
MAP kinase
MAP-kinase signaling module
molecular switch
monomeric GTPase
neurotransmitter
nitric oxide (NO)
nuclear receptor
phosphoinositide 3-kinase (PI 3-kinase)
phospholipase C
protein kinase
protein kinase C (PKC)
protein phosphatase
Ras
receptor
receptor serine/threonine kinase
receptor tyrosine kinase (RTK)
serine/threonine kinase
signal transduction
small intracellular signaling molecule
steroid hormone
tyrosine kinase

QUESTIONS

QUESTION 16–10

If some cell-surface receptors, including Notch, can rapidly signal to the nucleus by activating latent transcription regulators at the plasma membrane, why do most cell-surface receptors use long, indirect signaling cascades to influence gene transcription in the nucleus?

QUESTION 16–11

Which of the following statements are correct? Explain your answers.

A. The extracellular signal molecule acetylcholine has different effects on different cell types in an animal and often binds to different cell-surface receptor molecules on different cell types.

B. After acetylcholine is secreted from cells, it is long-lived, because it has to reach target cells all over the body.

C. Both the GTP-bound α subunits and nucleotide-free $\beta\gamma$ complexes—but not GDP-bound, fully assembled G proteins—can activate other molecules downstream of GPCRs.

D. IP_3 is produced directly by cleavage of an inositol phospholipid without incorporation of an additional phosphate group.

E. Calmodulin regulates the intracellular Ca^{2+} concentration.

F. Different signals originating from the plasma membrane can be integrated by cross-talk between different signaling pathways inside the cell.

G. Tyrosine phosphorylation serves to build binding sites for other proteins to bind to RTKs.

QUESTION 16–12

The Ras protein functions as a molecular switch that is set to its "on" state by other proteins that cause it to expel its bound GDP and bind GTP. A GTPase-activating protein helps reset the switch to the "off" state by inducing Ras to hydrolyze its bound GTP to GDP much more rapidly than it would without this encouragement. Thus, Ras works like a light switch that one person turns on and another turns off. You are given a mutant cell that lacks the GTPase-activating protein. What abnormalities would you expect to find in the way in which Ras activity responds to extracellular signals?

QUESTION 16–13

A. Compare and contrast signaling by neurons, which secrete neurotransmitters at synapses, with signaling carried out by endocrine cells, which secrete hormones into the blood.

B. Discuss the relative advantages of the two mechanisms.

QUESTION 16–14

Two intracellular molecules, X and Y, are both normally synthesized at a constant rate of 1000 molecules per second per cell. Molecule X is broken down slowly: each molecule of X survives on average for 100 seconds. Molecule Y is broken down 10 times faster: each molecule of Y survives on average for 10 seconds.

A. Calculate how many molecules of X and Y the cell contains at any time.

B. If the rates of synthesis of both X and Y are suddenly increased tenfold to 10,000 molecules per second per cell—without any change in their degradation rates—how many molecules of X and Y will there be after one second?

C. Which molecule would be preferred for rapid signaling?

QUESTION 16–15

"One of the great kings of the past ruled an enormous kingdom that was more beautiful than anywhere else in the world. Every plant glistened as brilliantly as polished jade, and the softly rolling hills were as sleek as the waves of the summer sea. The wisdom of all of his decisions relied on a constant flow of information brought to him daily by messengers who told him about every detail of his kingdom so that he could take quick, appropriate actions when needed. Despite the beauty and efficiency, his people felt doomed living under his rule, for he had an adviser who had studied cell signal transduction and accordingly administered the king's Department of Information. The adviser had implemented the policy that all messengers will be immediately beheaded whenever spotted by the Royal Guard, because for rapid signaling the lifetime of messengers ought to be short. Their plea "Don't hurt me, I'm only the messenger!" was to no avail, and the people of the kingdom suffered terribly because of the rapid loss of their sons and daughters." Why is the analogy on which the king's adviser based his policies inappropriate? Briefly discuss the features that set cell signaling pathways apart from the human communication pathway described in the story.

QUESTION 16–16

In a series of experiments, genes that code for mutant forms of an RTK are introduced into cells. The cells also express their own normal form of the receptor from their normal gene, although the mutant genes are constructed so that the mutant RTK is expressed at considerably higher concentration than the normal RTK. What would be the consequences of introducing a mutant gene that codes for an RTK (A) lacking its extracellular domain, or (B) lacking its intracellular domain?

QUESTION 16–17

Discuss the following statement: "Membrane proteins that span the membrane many times can undergo a conformational change upon ligand binding that can be sensed on the other side of the membrane. Thus, individual protein molecules can transmit a signal across a membrane. In contrast, individual single-span membrane proteins cannot transmit a conformational change across the membrane but require oligomerization."

QUESTION 16–18

What are the similarities and differences between the reactions that lead to the activation of G proteins and the reactions that lead to the activation of Ras?

QUESTION 16–19

Why do you suppose cells use Ca^{2+} (which is kept by Ca^{2+} pumps at a cytosolic concentration of 10^{-7} M) for intracellular signaling and not another ion such as Na^{+} (which is kept by the Na^{+} pump at a cytosolic concentration of 10^{-3} M)?

QUESTION 16–20

It seems counterintuitive that a cell, having a perfectly abundant supply of nutrients available, would commit suicide if not constantly stimulated by signals from other cells (see Figure 16–6). What do you suppose might be the advantages of such regulation?

QUESTION 16–21

The contraction of the myosin–actin system in muscle cells is triggered by a rise in intracellular Ca^{2+}. Muscle cells have specialized Ca^{2+} channels—called ryanodine receptors because of their sensitivity to the drug ryanodine—that are embedded in the membrane of the sarcoplasmic reticulum, a specialized form of the endoplasmic reticulum. In contrast to the IP_3-gated Ca^{2+} channels in the endoplasmic reticulum shown in Figure 16–27, the signaling molecule that opens ryanodine receptors is Ca^{2+} itself. Discuss the consequences of ryanodine channels for muscle cell contraction.

QUESTION 16–22

Two protein kinases, K1 and K2, function sequentially in an intracellular signaling pathway. If either kinase contains a mutation that permanently inactivates its function, no response is seen in cells when an extracellular signal is received. A different mutation in K1 makes it permanently active, so that in cells containing that mutation a response is observed even in the absence of an extracellular signal. You characterize a double-mutant cell that contains K2 with the inactivating mutation and K1 with the activating mutation. You observe that the response is seen even in the absence of an extracellular signal. In the normal signaling pathway, does K1 activate K2 or does K2 activate K1? Explain your answer.

QUESTION 16–23

A. Trace the steps of a long and indirect signaling pathway from a cell-surface receptor to a change in gene expression in the nucleus.

B. Compare this pathway with two short and direct pathways from the cell surface to the nucleus.

QUESTION 16–24

How does PI 3-kinase activate the Akt kinase after activation of RTK?

QUESTION 16–25

Animal cells and plant cells have some very different intracellular signaling mechanisms but also share some common mechanisms. Why do you think this is so?