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 are mixed together in a test tube, the result is chemical chaos, 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—which can form large biochemical subcompartments with distinct functions—are involved in many important cell processes, including the synthesis of DNA and RNA, and the assembly of ribosomes (as discussed in Chapter 4, pp. 155–158). 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

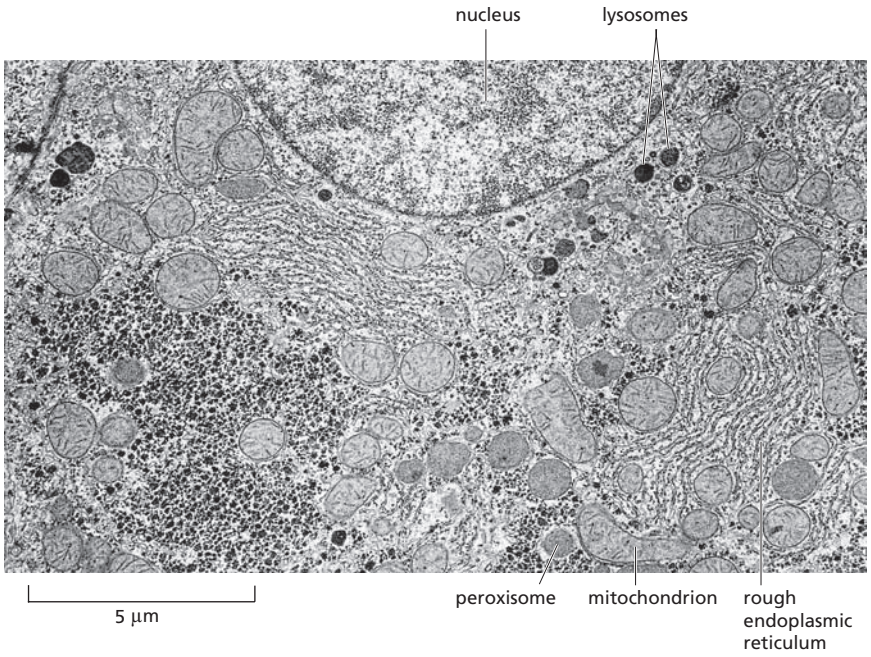


Figure 15–1 In eukaryotic cells, internal membranes create enclosed compartments that segregate different metabolic processes. Examples of many of the major membrane-enclosed organelles can be identified in this electron micrograph of part of a liver cell, seen in cross section. The small, black granules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

briefly consider their main functions. In the second section, we discuss how the protein composition of the different compartments is set up and 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 will be 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**). Most of these structures are **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,

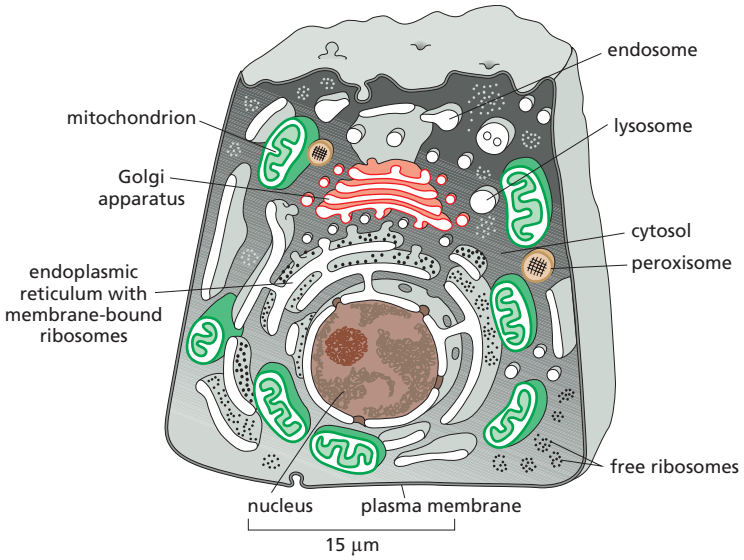


Figure 15–2 A cell from the lining of the intestine contains the basic set of membrane-enclosed organelles found in most animal cells. The nucleus, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, endosomes, mitochondria, and peroxisomes are distinct compartments separated from the cytosol by 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.

known as the *nuclear envelope*, and communicates with the cytosol via *nuclear pores* that perforate the envelope. The outer nuclear membrane is continuous with the membrane of the *endoplasmic reticulum* (*ER*), a system of interconnected membranous sacs and tubes 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 inserted into the ER membrane or delivered to 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 muscle contraction and other responses to extracellular signals (discussed in Chapters 16 and 17).

TABLE 15–1 THE MAIN FUNCTIONS OF MEMBRANE-ENCLOSED ORGANELLES 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	oxidative breakdown of toxic molecules (this chapter)

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 that break down lipids and destroy toxic molecules, producing hydrogen peroxide. *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 positioned 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

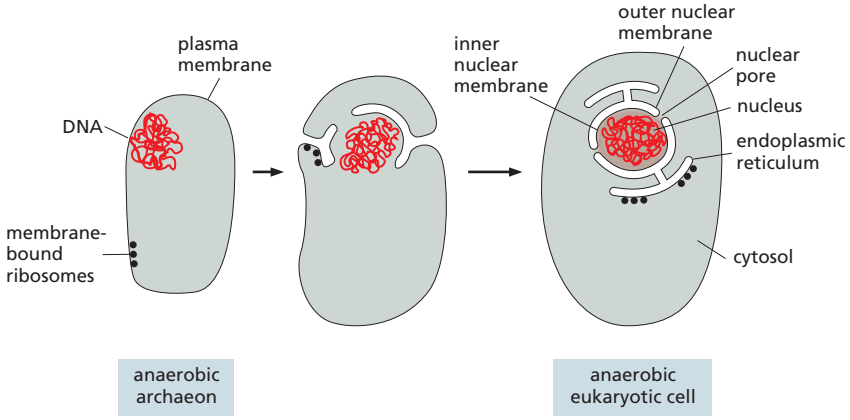


Figure 15–3 Nuclear membranes and the ER may have evolved through invagination of the plasma membrane. In modern bacteria and archaea, a single DNA molecule is typically attached to the plasma membrane. It is possible that, in a very ancient anaerobic archaeon, the plasma membrane, with its attached 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 precursors of the first eukaryotic cells are thought to have been simple microorganisms, resembling present-day archaea, 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 prokaryotes. Archaea and 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 that are 1000 to 10,000 times greater. 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 stages. 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 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

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?

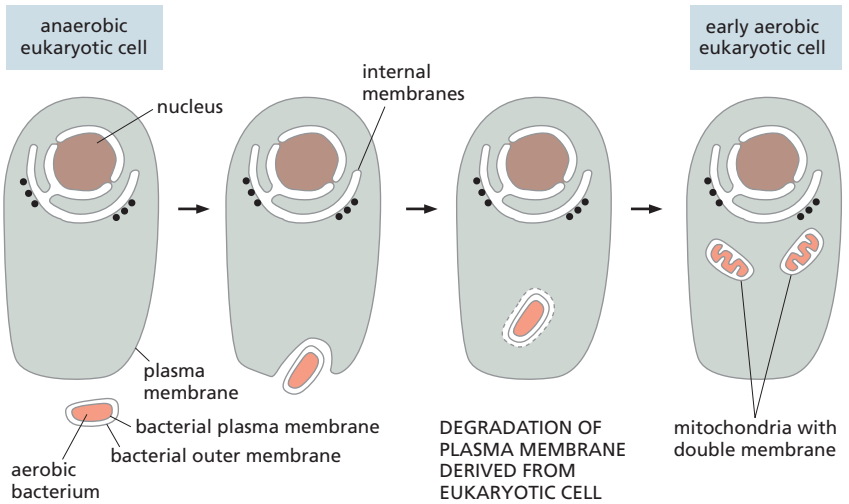


Figure 15–4 Mitochondria are thought to have originated when an aerobic bacterium was engulfed by a larger anaerobic eukaryotic cell. Chloroplasts are thought to have originated later in a similar way, when a eukaryotic cell with mitochondria engulfed a photosynthetic bacterium. This theory would explain 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.

traffic that connects the interiors of most of the other membrane-enclosed organelles to one another and to the outside of the cell.

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, 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 to other organelles. Peroxisomes make use of both pathways. Although these organelles acquire some of their membrane proteins from the ER, the bulk of their digestive enzymes enter directly from the cytosol.

In this section, we discuss the mechanisms by which proteins 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 of 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: the protein must be transported across its membrane (or membranes), which is normally impermeable to hydrophilic macromolecules. How this task is accomplished depends on the organelle.

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 the transport through nuclear pores, the transported protein must usually unfold for the translocator to guide it across the hydrophic interior of the membrane (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 than the ones just

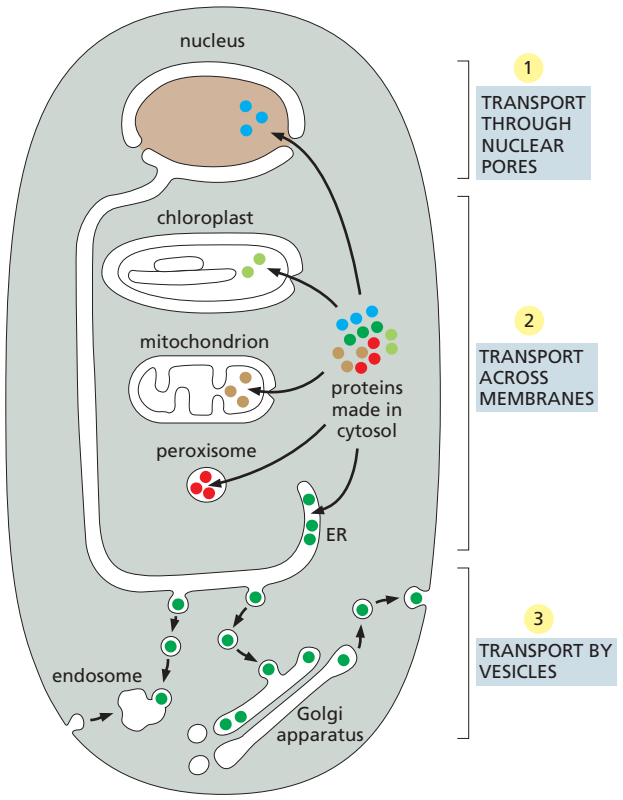


Figure 15–5 Membrane-enclosed organelles import proteins by one of three mechanisms. All of these processes require energy. The protein remains folded during transport in mechanisms 1 and 3 but usually has to be unfolded during mechanism 2.

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.

described. 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 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

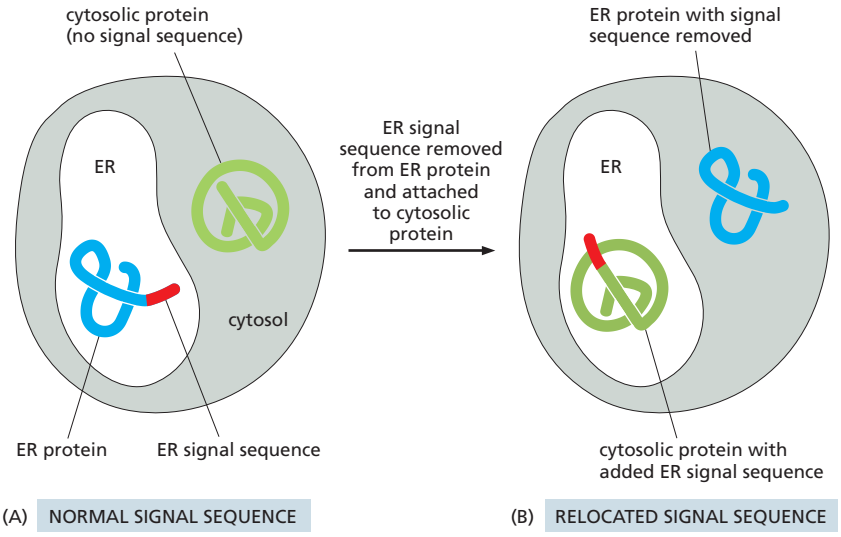


Figure 15–6 Signal sequences direct proteins to the correct destination. (A) Proteins destined for the ER possess an N-terminal signal sequence that directs them to that organelle, whereas those destined to remain in the cytosol lack any such signal sequence. (B) Recombinant DNA techniques can be used 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.

they have the same function: physical properties such as hydrophobicity 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, each present in multiple copies (**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). Newly made proteins

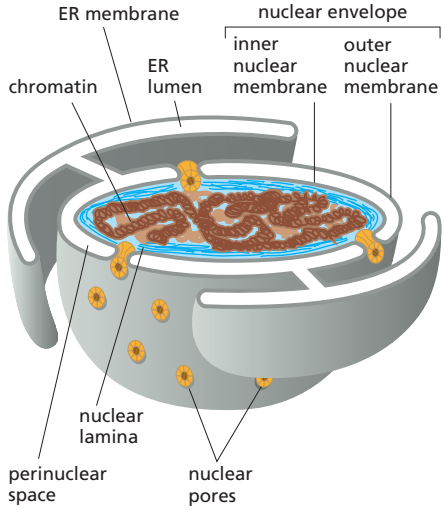
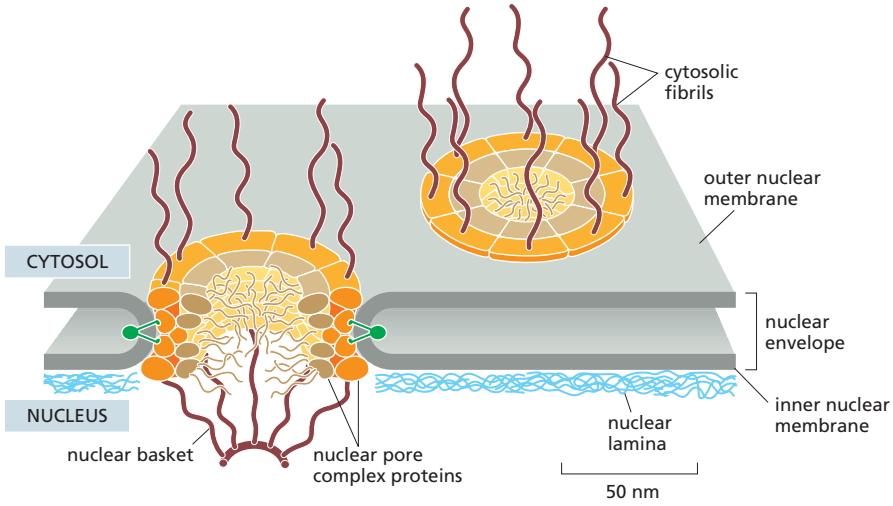


Figure 15–7 The outer nuclear membrane is continuous with the ER membrane. The double membrane of the nuclear envelope is penetrated by nuclear pores. The ribosomes that are normally bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown.



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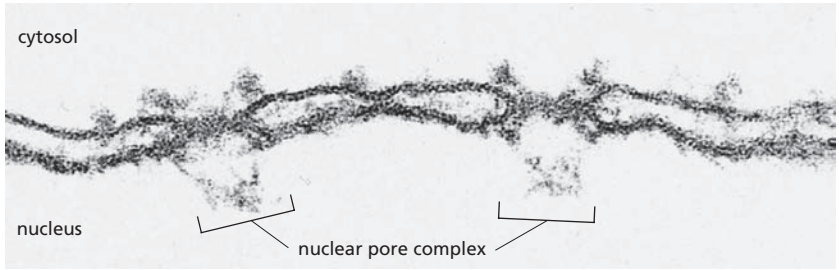

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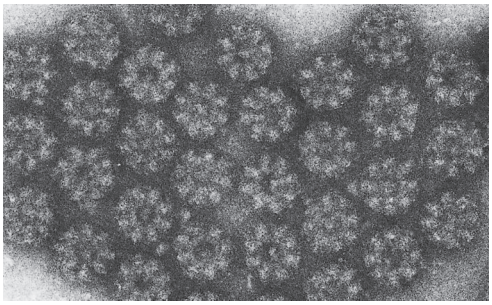

(C)

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.)

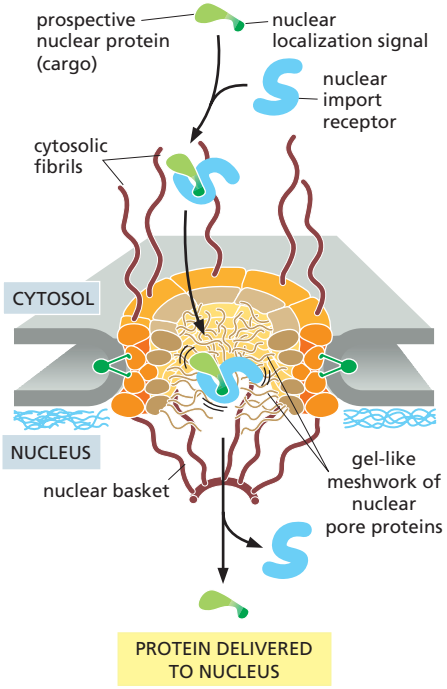

Figure 15–9 Prospective nuclear proteins are imported from the cytosol through nuclear pores. The proteins contain a nuclear localization signal that is recognized by nuclear import receptors, which interact with the cytosolic fibrils that extend from the rim of the pore. After being captured, the receptors with their cargo jostle their way through the gel-like meshwork formed from the unstructured regions of the nuclear pore proteins 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 7–25). These sets of import and export receptors have a similar basic structure.

that are destined for the nucleus must also be imported from the cytosol (**Movie 15.1**). To gain entry to a pore, these large molecules and 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 then 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).

The import of nuclear proteins is powered by energy provided by the hydrolysis of GTP. This hydrolysis is mediated by a monomeric GTPase named Ran. Like other GTPases, Ran exists in two conformations: one bearing a molecule of GTP, the other GDP. These forms, however, are differently localized: Ran-GTP is present in high concentrations in the nucleus, whereas Ran-GDP is produced in the cytosol (**Figure 15–10A**). In the nucleus, Ran-GTP displaces the prospective nuclear protein from its receptor, allowing the imported protein to be released. The import receptor—now bearing Ran–GTP—returns to the cytosol, where hydrolysis of GTP allows Ran-GDP to dissociate, leaving the receptor free to pick up another protein destined for the nucleus. In this way, GTP hydrolysis drives nuclear transport in the appropriate direction (**Figure 15–10B**).

Nuclear export receptors work in a similar way, driving protein and RNA traffic from the nucleus to the cytosol. They recognize nuclear export signals, which are different from those specifying import (see Table 15–3), and they also use Ran to couple the transport to an energy source.

Nuclear pore proteins operate this molecular gate at an amazing speed, rapidly pumping macromolecules in both directions through each pore. Proteins are transported into the nucleus 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.

QUESTION 15–2

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

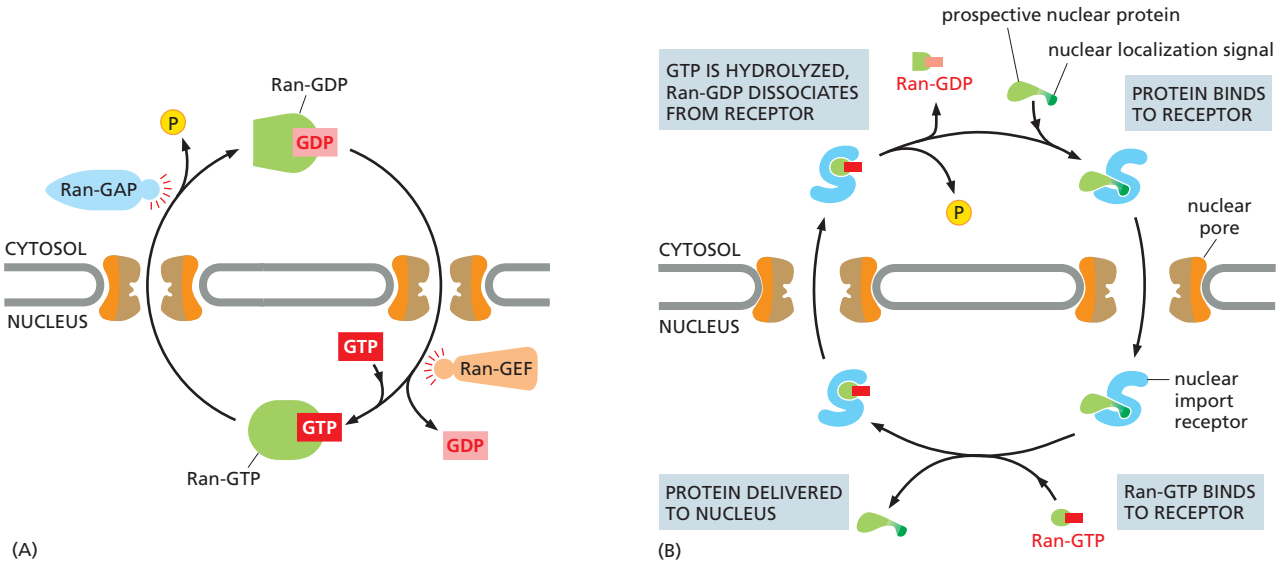

Figure 15–10 Energy supplied by GTP hydrolysis drives nuclear transport. (A) The small monomeric GTPase, Ran, exists in two conformations—one carrying GTP, the other GDP (see Figure 4–48 or 16–12). Ran is converted from one conformation to the other with the help of accessory proteins that are differently localized. The accessory protein that triggers GTP hydrolysis, called Ran-GAP (GTPase-activating protein), is found exclusively in the cytosol, where it converts Ran-GTP to Ran-GDP. The accessory protein that causes Ran-GDP to release its GDP and take up GTP, called Ran-GEF (guanine nucleotide exchange factor), is found exclusively in the nucleus. The localization of these accessory proteins guarantees that the concentration of Ran-GTP is higher in the nucleus, thus driving the nuclear import cycle in the desired direction. (B) A nuclear import receptor picks up a prospective nuclear protein in the cytosol and enters the nucleus. There it encounters Ran-GTP, which 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, where Ran hydrolyzes its bound GTP. Ran-GDP falls off the import receptor, which is then free to bind another protein destined for the nucleus. Ran-GDP is carried into the nucleus by its own unique import receptor (not shown).

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 are closely apposed. 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

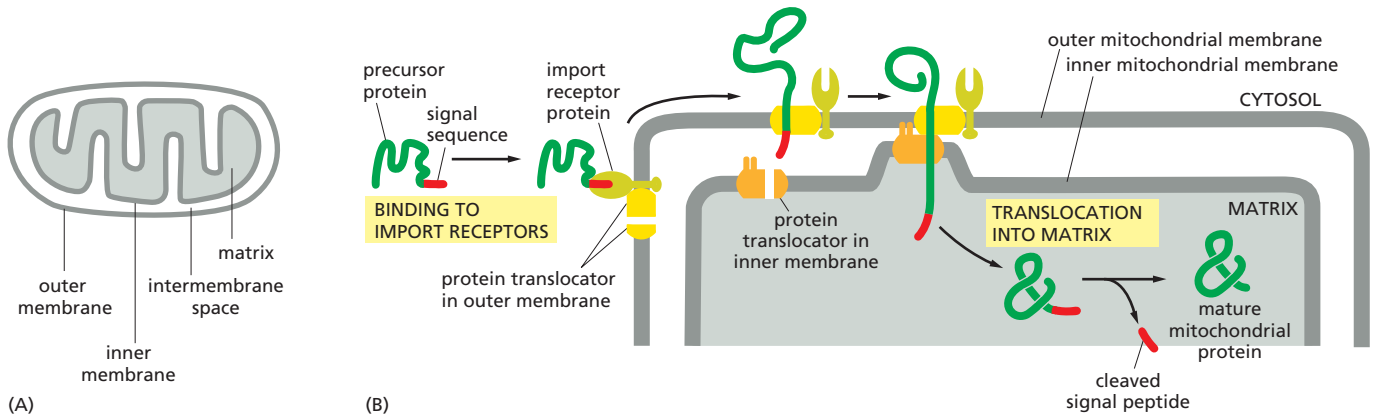

Figure 15–11 Mitochondrial precursor proteins are unfolded during import. (A) A mitochondrion has an outer and inner membrane, both of which must be crossed for a mitochondrial precursor protein to enter the organelle. (B) To initiate transport, the mitochondrial signal sequence on a mitochondrial precursor protein is recognized by a receptor in the outer mitochondrial membrane. This receptor is associated with a protein translocator, which transports the signal sequence across the outer mitochondrial membrane to the intermembrane space. The complex of receptor, precursor protein, and translocator then diffuses laterally in the outer membrane until the signal sequence is recognized by a second translocator in the inner membrane. Together, the two translocators 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. Some of the energy needed for this protein translocation comes from the hydrolysis of ATP, which allows the chaperones to function.

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 by lipid-carrying proteins that extract a phospholipid molecule from one membrane and deliver it into another. Such transport frequently occurs at specific junctions where the membranes of different organelles are held in close proximity. By controlling which lipids are transported, the different cell membranes are able to maintain different lipid compositions.

Proteins Enter Peroxisomes from both the Cytosol and the Endoplasmic Reticulum

Peroxisomes are packed with enzymes that digest toxins and synthesize certain phospholipids, including those present in the myelin sheath surrounding nerve cell axons. These organelles 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 (see Table 15–3, p. 502). 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 translocator that aids in protein 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 come from the cytosol, a few of the proteins embedded in the peroxisomal membrane arrive via vesicles that bud from the ER. The vesicles either fuse with preexisting peroxisomes or import additional peroxisomal proteins from the cytosol to grow into mature peroxisomes.

Mutations that block peroxisomal protein import can cause severe illness. Individuals with Zellweger syndrome, for example, 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 peroxisomes, and peroxisomal protein transport, 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 (see Figure 15–5).

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 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. 502), 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**).

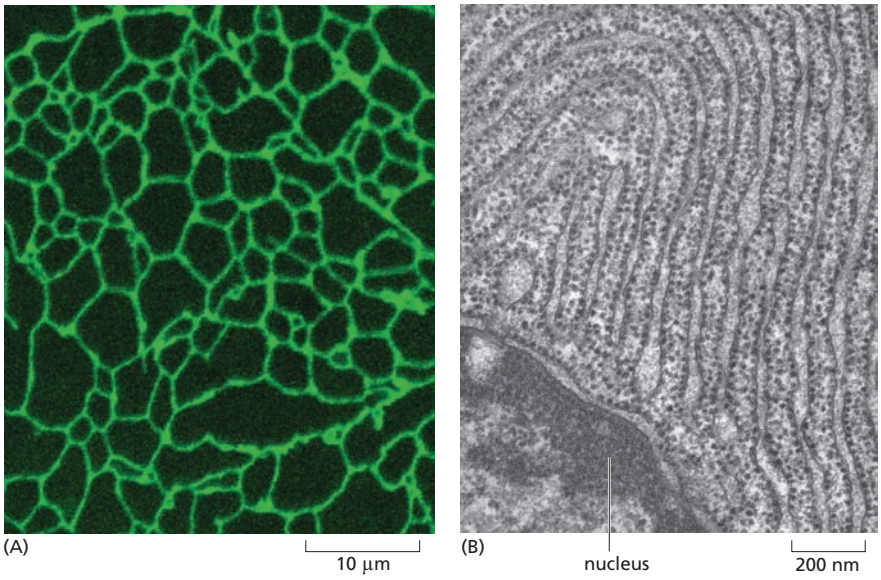

Figure 15–12 The endoplasmic reticulum is the most extensive membrane network in eukaryotic cells. (A) Fluorescence micrograph of a living plant cell showing the ER as a complex network of tubes. The cell shown here has been genetically engineered so that it contains a fluorescent protein in the ER lumen. 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, from P. Boevink et al., *The Plant Journal* 15:441–447, 1998. With permission from John Wiley & Sons; B, courtesy of Lelio Orci.)

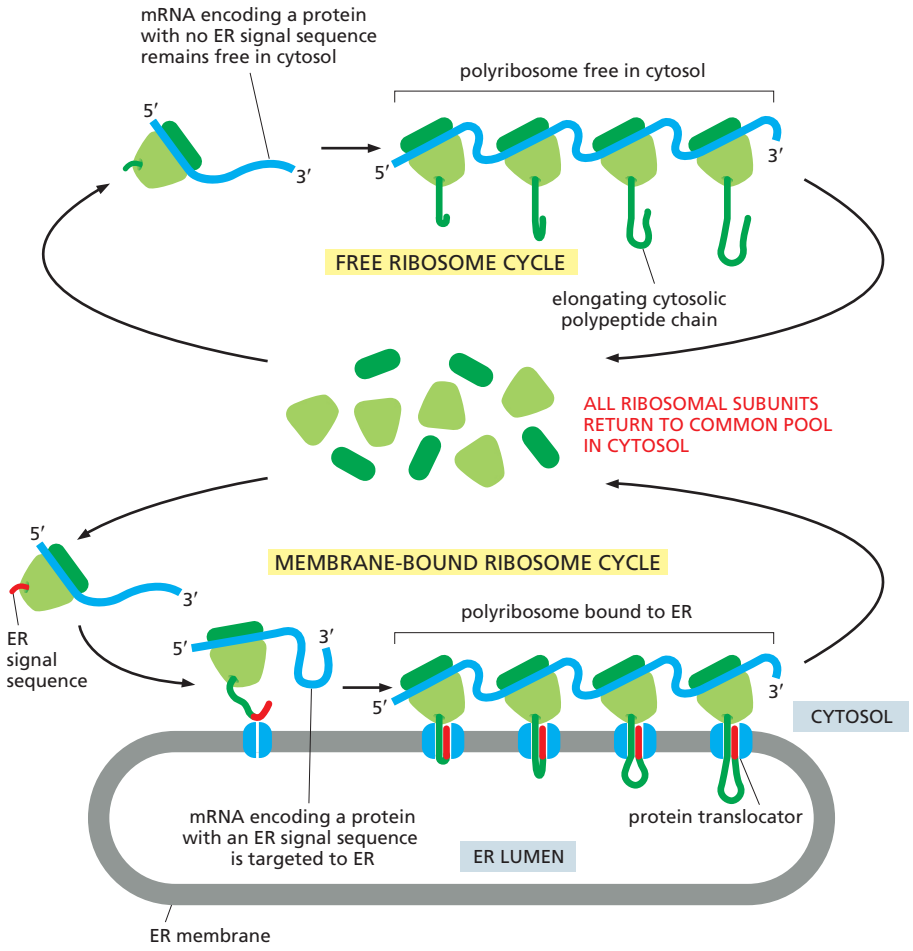

Figure 15–13 A common pool of ribosomes is used to synthesize all the proteins encoded by the nuclear genome. Ribosomes that are translating proteins with no ER signal sequence remain free in the cytosol. Ribosomes that are translating proteins containing an ER signal sequence (*red*) on the growing 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.

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 directing synthesis of 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 to both the ribosome and the ER signal sequence as it emerges from

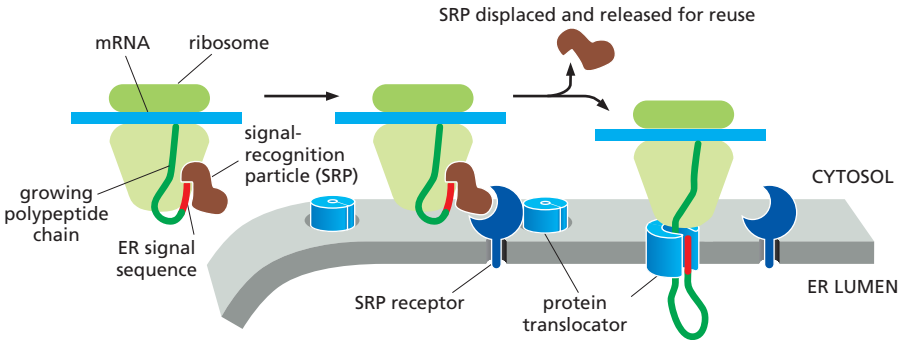

Figure 15–14 An ER signal sequence and an SRP direct a ribosome to the ER membrane. The SRP (*brown*) binds to both the exposed ER signal sequence and the ribosome, thereby slowing protein synthesis by the ribosome. The SRP–ribosome complex then binds to an SRP receptor (*dark blue*) in the ER membrane.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

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**). The SRP and SRP receptor thus function as molecular matchmakers, bringing together ribosomes that are synthesizing proteins with an ER signal sequence and protein translocators within 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 protein translocator. This sequence remains bound to the translocator, while the rest of the polypeptide chain is threaded through the membrane as a large loop. The signal sequence 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 protein translocator into the lipid bilayer and rapidly degraded.

Once the C-terminus of a soluble protein has passed through the translocator, the protein is 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.

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

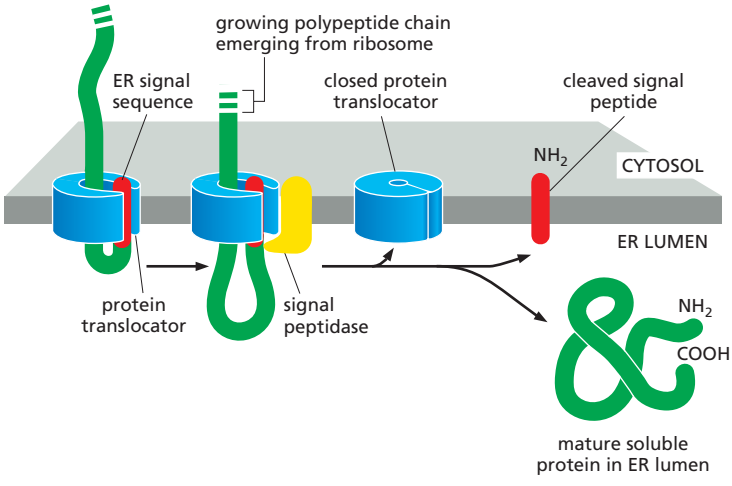

Figure 15–15 A soluble protein crosses the ER membrane and enters the lumen. The protein translocator binds the signal sequence and threads the rest of the polypeptide across the lipid bilayer as a loop. At some point during the translocation process, the signal peptide is cleaved from the growing protein by a signal peptidase (*yellow*). 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 protein translocator closes. The membrane-bound ribosome is omitted from this and the following two figures for clarity.

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 within 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 then halted by an additional sequence of hydrophobic amino acids, a *stop-transfer sequence*, further along the polypeptide chain. At this point, the protein translocator releases the growing polypeptide chain sideways into the lipid bilayer. The N-terminal signal sequence is cleaved off, and 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 will never change its orientation; its cytosolic portion will always remain in the cytosol, even if the protein is subsequently transported to another organelle via vesicle budding and fusion (see Figure 11–18).

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 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. In this way, multipass membrane proteins are stitched into the lipid bilayer as they are being synthesized, by a mechanism resembling the workings of a sewing machine.

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?

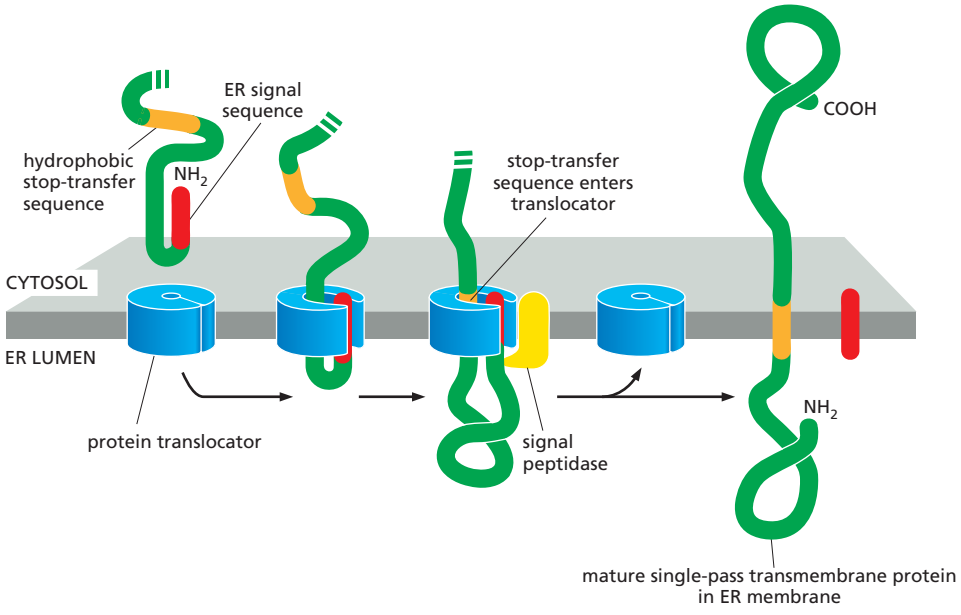

Figure 15–16 A single-pass transmembrane protein is retained in the lipid bilayer. An N-terminal ER signal sequence (*red*) initiates transfer as in Figure 15–15. In addition to this sequence, the protein also contains a second hydrophobic sequence, which acts as a stop-transfer sequence (*orange*). When this sequence enters the protein translocator, the growing polypeptide chain is discharged 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.

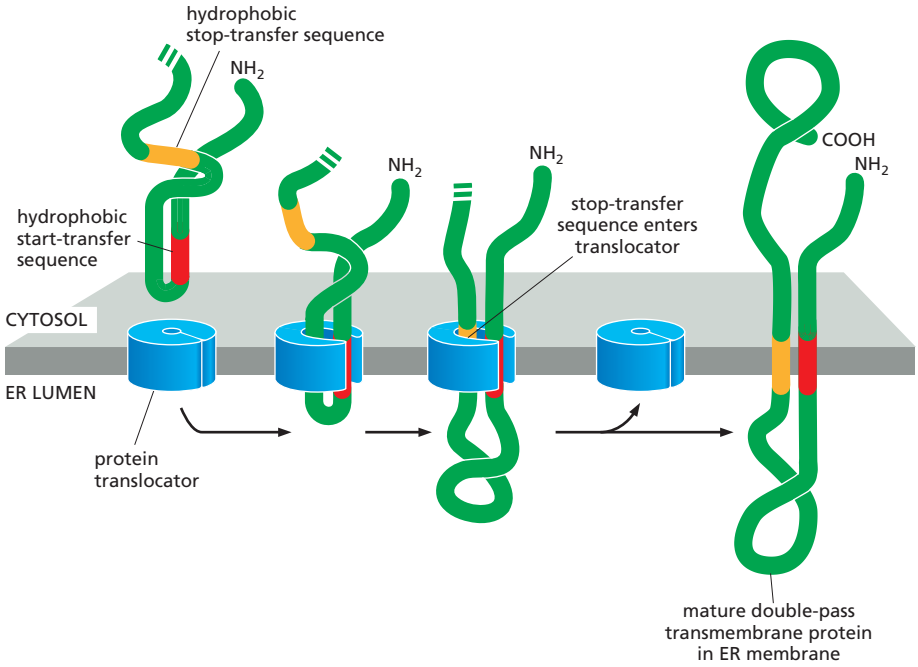

Figure 15–17 A double-pass transmembrane protein has an internal ER signal sequence. This internal sequence (*red*) not only acts as a start-transfer signal, it also helps to anchor the final protein in the membrane. Like the N-terminal ER signal sequence, the internal signal sequence is recognized by an SRP, which brings the ribosome to the ER membrane (not shown). When a stop-transfer sequence (*orange*) enters the protein translocator, the translocator 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 than twice contain additional pairs of start- and stop-transfer sequences, and the same process is repeated for each pair.

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.

VESICULAR TRANSPORT

Entry into the ER lumen or membrane is usually only the first step on the 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, where it allows proteins and other molecules to be secreted by exocytosis, and it reaches inward from the plasma membrane to lysosomes, allowing extracellular molecules to be imported by endocytosis (**Figure 15–18**). Together, these pathways thus provide routes of communication between the interior of the cell and its surroundings.

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 15–19**).

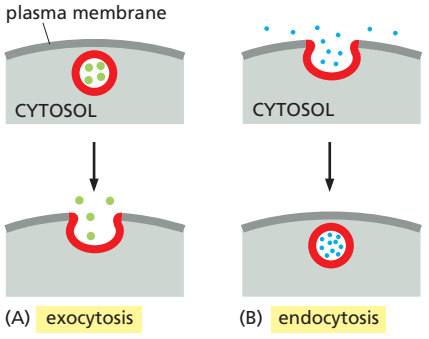

Figure 15–18 Vesicular transport allows materials to exit or enter the cell. (A) During exocytosis, a vesicle fuses with the plasma membrane, releasing its content to the cell's surroundings. (B) During endocytosis, extracellular materials are captured by vesicles that bud inward from the plasma membrane and are carried into the cell.

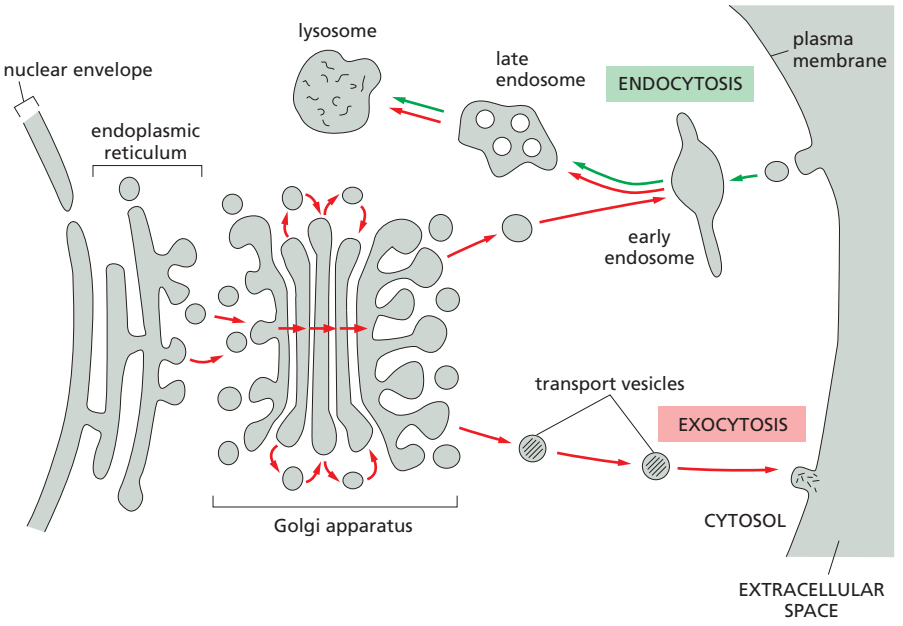

Figure 15–19 Transport vesicles bud from one membrane and fuse with another, carrying membrane components and soluble proteins between compartments of the endomembrane system and the plasma membrane. The membrane of each compartment or vesicle maintains its orientation, so the cytosolic side always faces 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 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. 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. Note that movement through the Golgi apparatus occurs by vesicles that shuttle between its individual cisternae and by a process of maturation, whereby the cisternae themselves move through the stack (central *red* arrows).

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, 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 it 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 15–20**). A GTP-binding protein called *dynamin* assembles as a ring around the neck of each deeply invaginated clathrin-coated pit. Together with other proteins recruited to the neck of the vesicle, the dynamin causes the neck 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

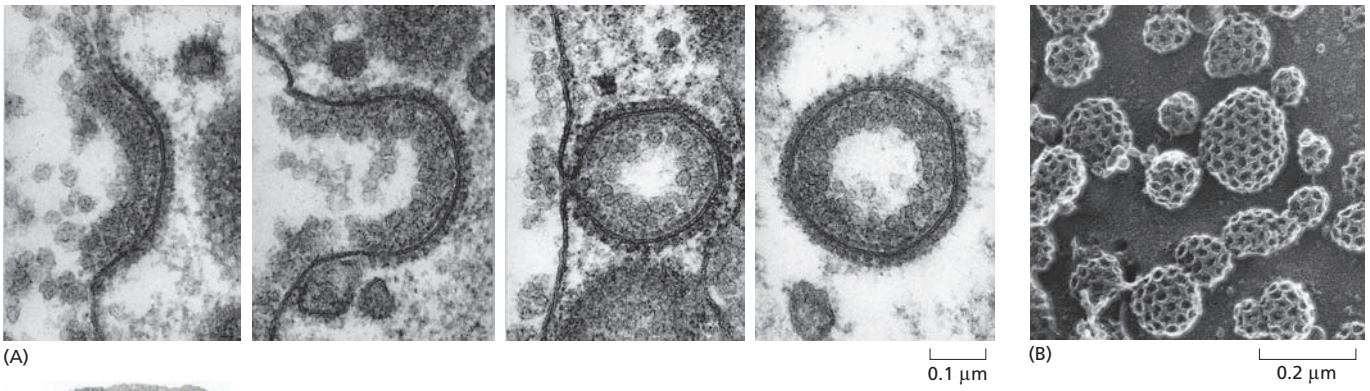

Figure 15–20 Clathrin molecules form basketlike cages that help shape membranes into vesicles. (A) Electron micrographs showing the sequence of events in the formation of a clathrin-coated vesicle from a clathrin-coated pit. The clathrin-coated pits and vesicles shown here are unusually large and are 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. (C) In a test tube, clathrin molecules sometimes self-assemble into basketlike cages. The structure of one such clathrin cage was determined by cryoelectron microscopy. The positions of three of the constituent clathrin molecules, each of which has a characteristic three-armed shape, are highlighted in *red*, *green*, and *brown*. (A, from 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; C, from A. Fotin et al., *Nature* 432:573–579, 2004. With permission from Macmillan Publishers Ltd.)

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 15–21**). 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.

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

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
COPII-coated	COPII proteins	ER	Golgi cisterna
COPI-coated	COPI proteins	Golgi cisterna	ER

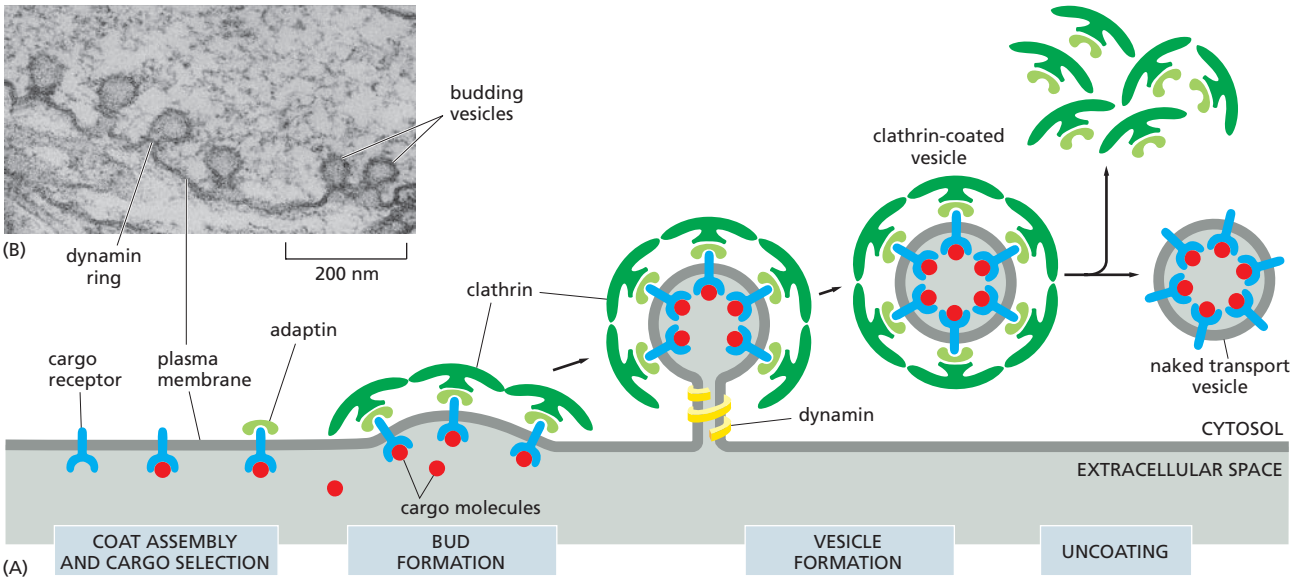

Figure 15–21 Clathrin-coated vesicles transport selected cargo molecules. Here, as in Figure 15–20, the vesicles are shown budding from the plasma membrane. (A) Cargo receptors, with their bound cargo molecules, are captured by adaptins, which also bind clathrin molecules to the cytosolic surface of the budding vesicle (Movie 15.5). Dynamin proteins assemble around the neck of budding vesicles; once assembled, the dynamin molecules 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. (B) In flies that produce a mutant dynamin protein, clathrin-coated pits assemble and dynamin is recruited around the neck of budding vesicles but fail to pinch them off, as can be seen in this electron micrograph of the plasma membrane in a fly's nerve ending. Flies with this mutation become paralyzed, because clathrin-mediated endocytosis grinds to a halt, preventing the recycling of vesicles needed to release neurotransmitters (see Figure 12–40). (B, from J.H. Koenig and K. Ikeda, *J. Neurosci.* 9:3844–3860, 1989. With permission from the Society for Neuroscience.)

Vesicle Docking Depends on Tethers and SNAREs

After a transport vesicle buds from a membrane, it must find its way to the 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 (see Figure 17–20).

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 15–22).

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 or to the extracellular space, but it also adds the vesicle membrane to the membrane of the organelle (see Figure 15–22). After vesicle docking, 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

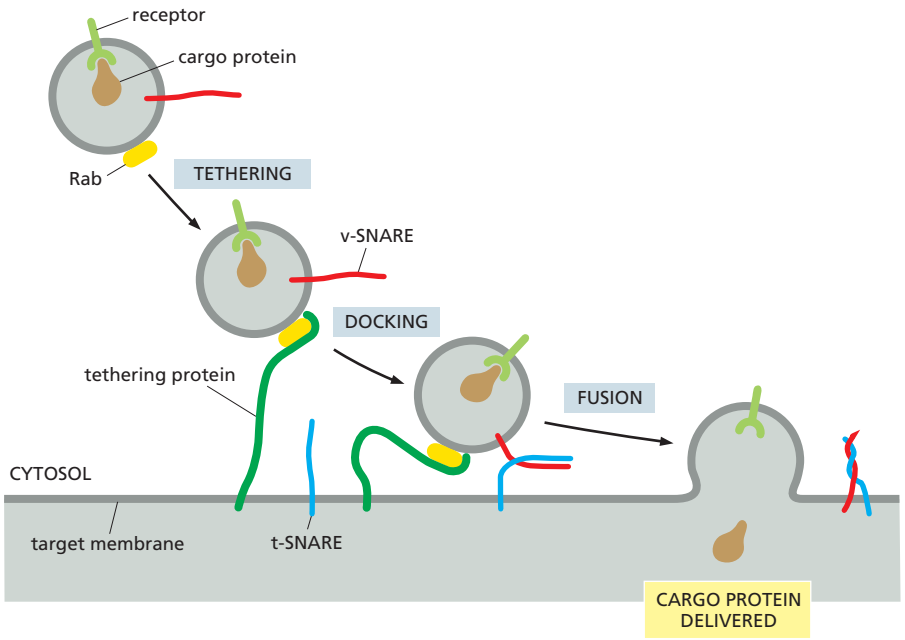

Figure 15–22 Rab proteins, tethering proteins, and SNAREs help direct transport vesicles to their target membranes. A filamentous tethering protein (*green*) on a membrane binds to a Rab protein (*yellow*) on the surface of a vesicle. This interaction allows the vesicle to dock on its particular target membrane. A v-SNARE (*red*) on the vesicle then binds to a complementary t-SNARE (*blue*) 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 15–23).

interact, fusion requires a much closer approach: the two bilayers must come within 1.5 nanometers (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 that provides the means to cross this energy barrier. For vesicle fusion, the SNARE proteins themselves catalyze the process: when fusion is triggered, the v-SNAREs and t-SNAREs wrap around each other tightly, thereby acting like a winch that pulls the two lipid bilayers into close proximity (**Figure 15–23**).

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?

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 15–19). Each molecule that travels along this secretory pathway passes through a fixed sequence of membrane-enclosed compartments and is often chemically modified en route.

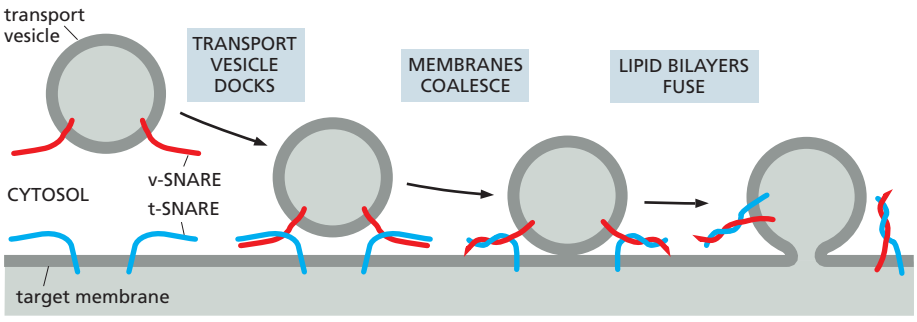

Figure 15–23 Following vesicle docking, SNARE proteins can catalyze the fusion of the vesicle and target membranes. Once appropriately triggered, the tight pairing of v-SNAREs and t-SNAREs draws the two lipid bilayers into close apposition. The force of the SNAREs winding together squeezes out any water 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.

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 the secretory 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 once they have been 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 an 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 15–24**). 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 target asparagine is one, defines which sites 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 this is 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 oligosaccharides on mature glycoproteins are remarkably diverse. All of the diversity results from extensive modification of the original precursor structure shown in Figure 15–24. This *oligosaccharide processing* begins in the ER and continues in the Golgi apparatus.

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?

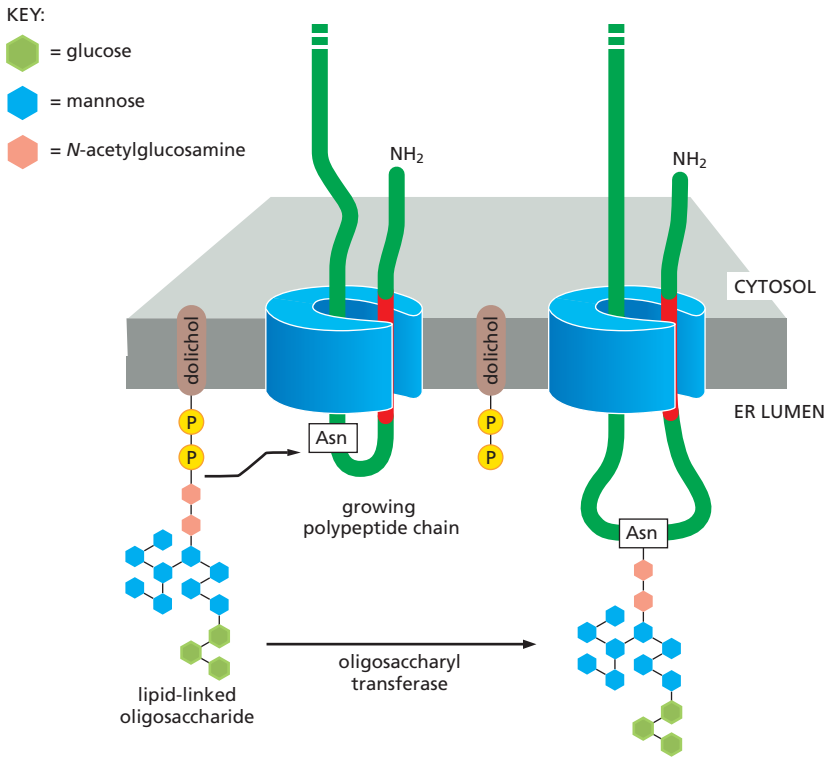

Figure 15–24 Many proteins are glycosylated on asparagines in the ER. When an appropriate asparagine in a growing polypeptide chain enters the ER lumen, it is glycosylated by addition of a branched oligosaccharide side chain. Each oligosaccharide chain is transferred as an intact unit to the asparagine 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.

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 should they manage to escape to the Golgi apparatus) by a C-terminal sequence of four amino acids called an *ER retention signal* (see Table 15–3, p. 502). 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 (see Figures 4–8 and 4–9); if proper folding and assembly still fail, the proteins are exported to the cytosol, where they are degraded by the proteasome (see Figure 7–43). 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 and then degraded, with dire consequences. 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.

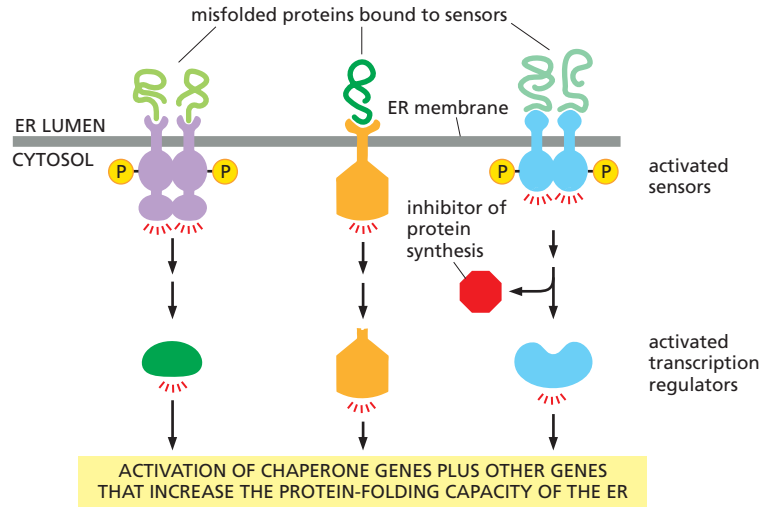

Figure 15–25 Accumulation of misfolded proteins in the ER lumen triggers an unfolded protein response (UPR). The misfolded proteins are recognized by several types of transmembrane sensor proteins in the ER membrane, each of which activates a different component of the UPR. Some sensors stimulate the production of transcription regulators that activate genes encoding chaperones or other proteins involved in ER quality control. Another sensor can also inhibit protein synthesis, reducing the flow of proteins through the ER (Movie 15.6 and Movie 15.7).

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

Although chaperones help proteins in the ER fold properly and retain those that do not, this quality control system can become overwhelmed. When this 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 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 to properly handle the volume of proteins entering the secretory pathway. In some cases, however, even an expanded ER cannot keep up with the demand, 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 17–13). The Golgi apparatus consists of a collection of flattened, membrane-enclosed sacs called *cisternae*, which are piled like stacks of pita bread (Figure 15–26). Each stack contains 3–20 cisternae, and 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 pieces of membrane enter the *cis Golgi network* via transport vesicles derived from the ER.

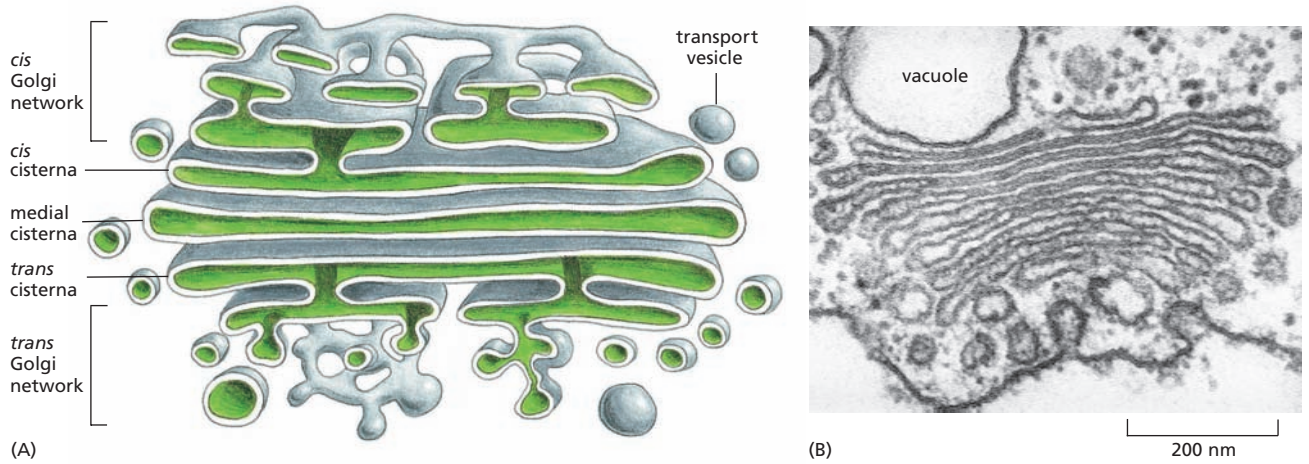

Figure 15–26 The Golgi apparatus consists of a stack of flattened, membrane-enclosed sacs. (A) A three-dimensional model of a Golgi stack reconstructed from a sequential series of electron micrographs of the Golgi apparatus in a secretory animal cell. To see how such models are assembled, watch **Movie 15.8**. (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. Vesicles are shown as olives. (B, courtesy of George Palade.)

The proteins travel through the cisternae in sequence in two ways: (1) by means of transport vesicles that bud from one cisterna and fuse with the next; and (2) by a maturation process in which the Golgi cisternae themselves migrate through the Golgi stack. Proteins finally exit from the *trans Golgi network* in transport vesicles destined for either the cell surface or another organelle of the endomembrane system (see Figure 15–19).

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 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. 520–521.

Many of the oligosaccharide chains that are added to proteins in the ER (see Figure 15–24) 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

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.

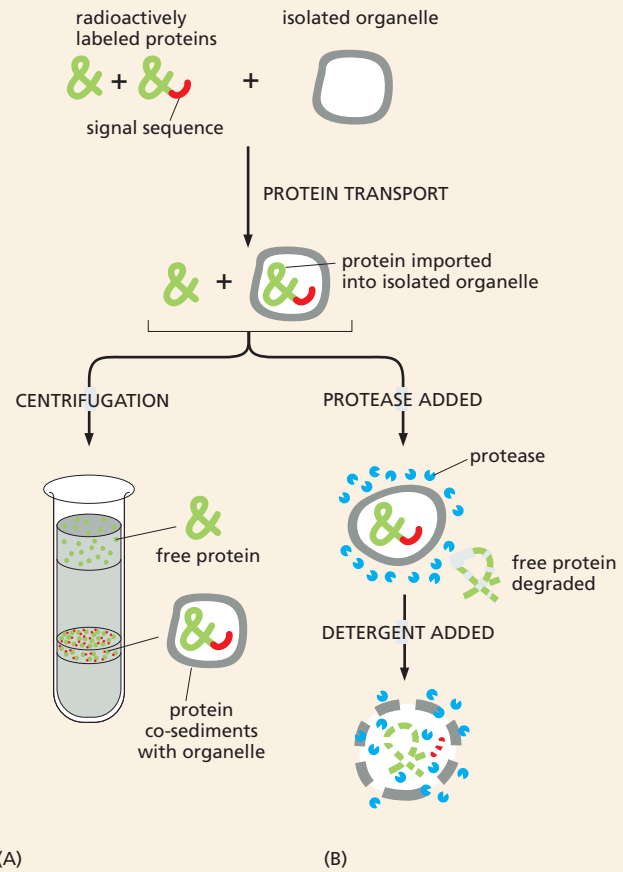

Figure 15–27 Several methods can be used to determine whether a labeled protein bearing a particular signal sequence is transported into a preparation of isolated organelles. (A) The labeled protein with or without a signal sequence is incubated with the organelles, and the preparation is centrifuged. 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 Osamu Shimomura, Martin Chalfie, and Roger Tsien for the development and refinement of this technology.

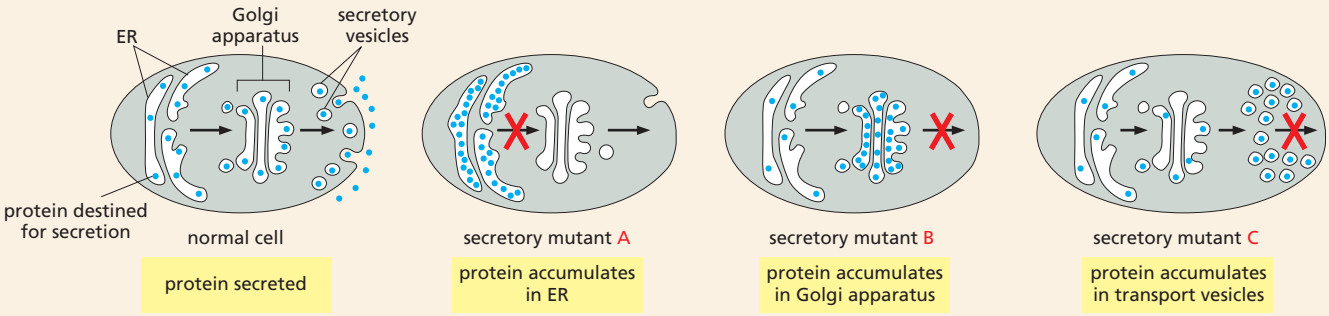

Figure 15–28 Temperature-sensitive mutants have been used to dissect the protein secretory pathway in yeast. Mutations in genes involved at different stages of the transport process, as indicated by the *red* X, result in the accumulation of proteins in the ER, the Golgi apparatus, or transport vesicles.

Such GFP fusion proteins are widely used to study the location and movement of proteins in cells (**Figure 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 15.1**, **15.9**, **15.10**, and **15.13** demonstrate the power and beauty of this technique.

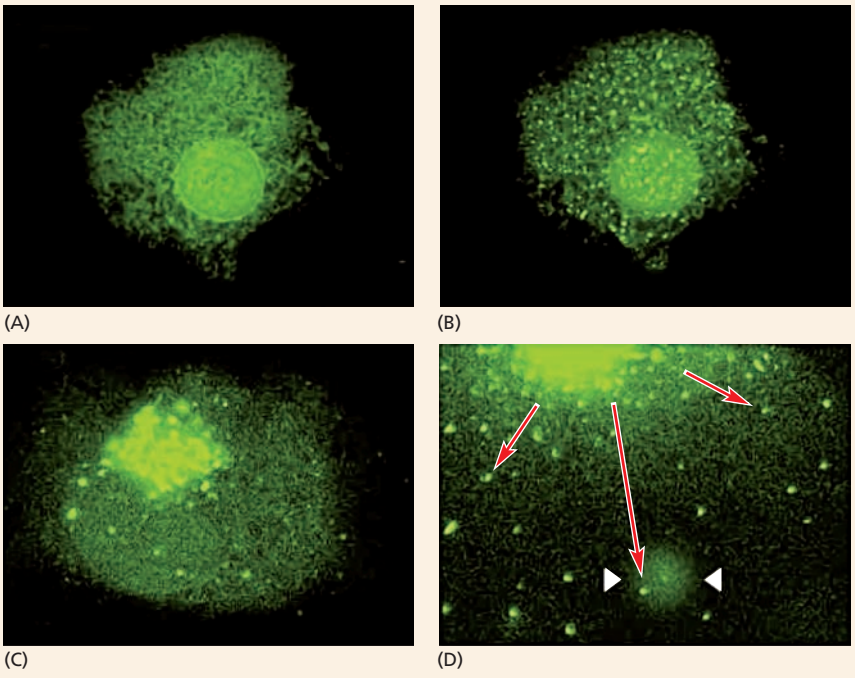

Figure 15–29 Tagging a protein with GFP allows the resulting fusion protein to be tracked throughout the cell. In this experiment, GFP is fused to a viral coat protein and expressed in cultured animal cells. In an infected cell, the viral protein moves through the secretory pathway from the ER to the 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 15.9**. (A–D, courtesy of Jennifer Lippincott-Schwartz.)

membrane with newly made lipids and proteins (Movie 15.9), 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 **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 an 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

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?

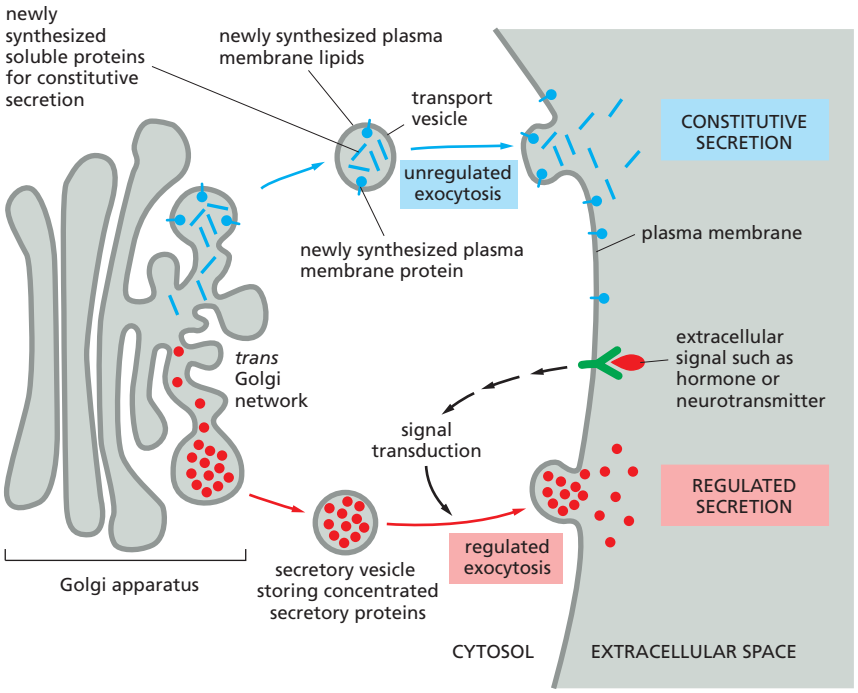

Figure 15–30 In secretory cells, the regulated and constitutive pathways of exocytosis diverge in the *trans* Golgi network. Many soluble proteins are continually secreted from the cell by the constitutive secretory pathway (*blue* arrows), which operates in all eukaryotic cells (Movie 15.10). This pathway also continually supplies the plasma membrane with newly synthesized lipids and proteins. Specialized secretory cells have, in addition, a regulated exocytosis pathway (*red* arrows) 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.

protein in the Golgi lumen. This increase in concentration can reach 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. Similar membrane retrieval pathways also operate in the Golgi apparatus to return lipids and selected proteins to the endoplasmic reticulum.

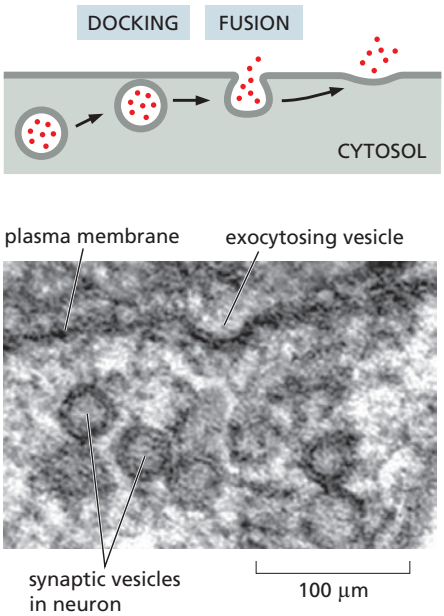

Figure 15–31 Secretory vesicles store and release concentrated proteins. The process, which takes place through vesicle docking and fusion (see Figure 15–23), requires a signal to initiate. The cryoelectron micrograph shows the release of concentrated neurotransmitter from a cultured mouse hippocampal neuron. The sample was rapidly frozen just 5 ms after the neuron was stimulated to fire. (From S. Watanabe, *Front. Synaptic. Neurosci.* 8:1–10, 2016.)

ENDOCYTIC PATHWAYS

Eukaryotic cells are continually taking up fluid, along with large and small molecules, by the process of **endocytosis**. Certain 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 15.11**). 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

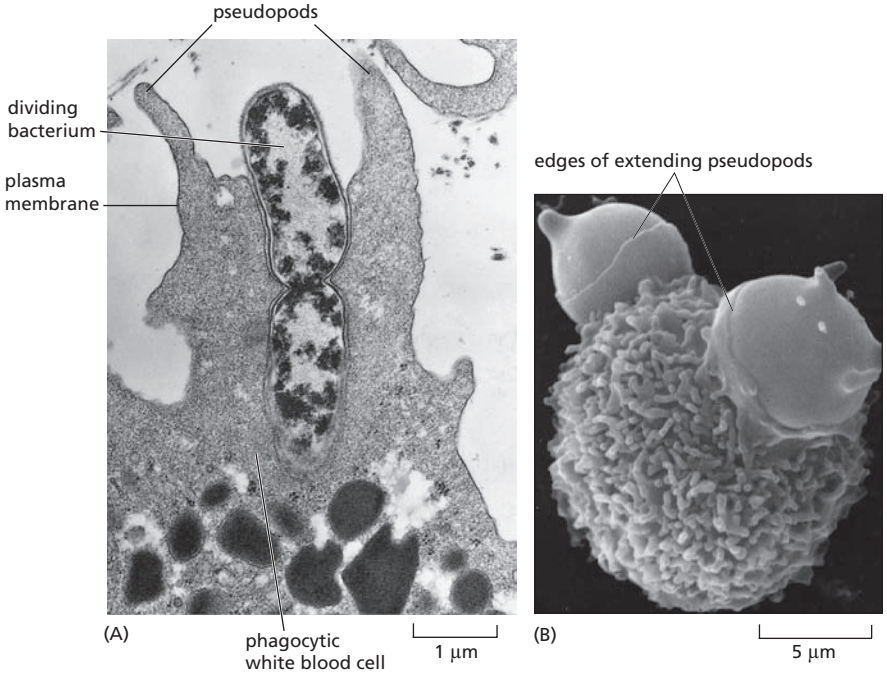

Figure 15–32 Specialized phagocytic cells can ingest other cells. (A) Electron micrograph of a phagocytic white blood cell (a neutrophil) ingesting a bacterium, which is in the process of dividing. (B) Scanning electron micrograph showing a macrophage engulfing a pair of red blood cells. The lines point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

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 15–32A**). These pseudopods fuse at their tips to form a phagosome, which then fuses with a lysosome, where 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} worn-out red blood cells in the human body 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 even 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 15–19). 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 15–20 and 15–21). 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. However, pinocytosis can sometimes be more selective. In most animal cells, specific macromolecules can be taken up from the extracellular fluid via clathrin-coated vesicles. The macromolecules bind to complementary receptors on the cell surface and enter the cell as receptor–macromolecule complexes in clathrin-coated vesicles (see Figure 15–21). 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. Such is the case when animal cells import 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 proteins 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 the surface of cells. The resulting receptor–LDL complexes can then 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 empty receptors are returned, via 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. Freed from the bulky LDLs, cholesterol escapes into the cytosol, where it can be used to synthesize new membrane (**Figure 15–33**).

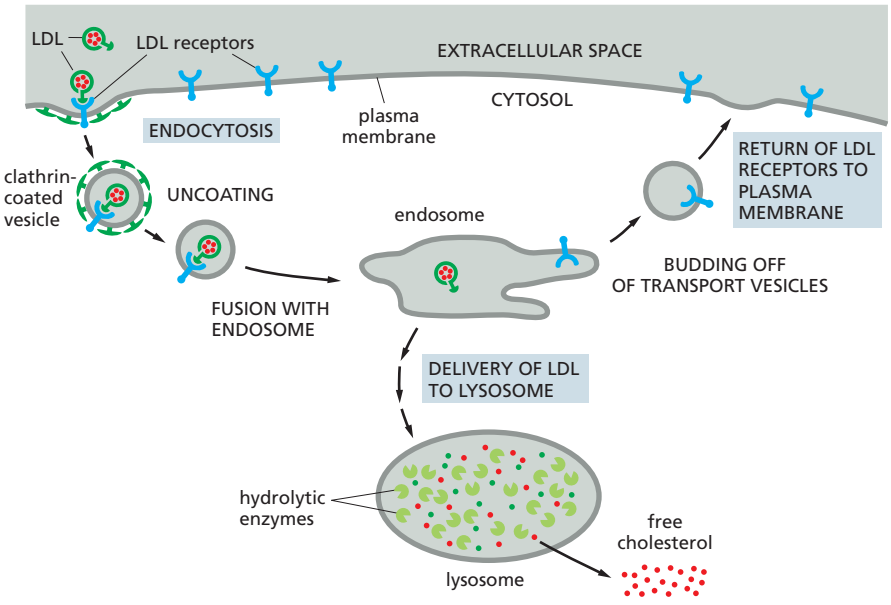

Figure 15–33 LDL enters cells via receptor-mediated endocytosis. LDL binds to LDL receptors on the cell surface and is internalized in clathrin-coated vesicles. The vesicles lose their coat and then fuse with endosomes. In the acidic environment of the endosome, LDL dissociates from its receptors. The LDL ends up in lysosomes, where it is degraded to release free cholesterol (*red* dots), while the LDL receptors are returned to the plasma membrane via transport vesicles to be used again (**Movie 15.12**). 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 over its 20-hour life-span.

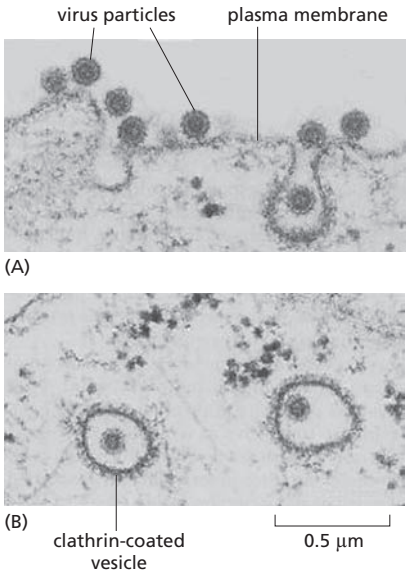

Figure 15–34 Viruses can enter cells via receptor-mediated endocytosis. (A) Electron micrograph showing viruses bound to receptors on the surface of a T cell; one virus particle is being internalized in a clathrin-coated vesicle. (B) A pair of viruses have been taken up by receptor-mediated endocytosis. These vesicles will fuse with lysosomes, where the low pH will allow the release of the viral genome into the cytoplasm—a necessary step in viral replication. (A, from E. Fries and A. Helenius, *Eur. J. Biochem.* 97:213–220, 1979. With permission from John Wiley & Sons; B, from K. Simons, H. Garoff, A. Helenius, *Sci. Am.* 246:58–66, 1982. With permission from the authors.)

This pathway for cholesterol uptake is disrupted in individuals who inherit a defective version of the 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 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, which cells cannot take up by the processes of transmembrane transport discussed in Chapter 12. Vitamin B_{12} and iron are both required, for example, to make hemoglobin, which is the major protein in red blood cells; these substances 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 (**Figure 15–34**). The influenza virus, which causes the flu, and HIV, which causes AIDS, gain 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 a fluorescent marker that will show up when viewed in a fluorescence 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 to 15 minutes later, they show up in *late endosomes*, located closer to the nucleus (see Figure 15–19). Early endosomes mature gradually into late endosomes as they fuse with each other or with a preexisting late endosome (**Movie 15.13**). 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.

Just as the Golgi network acts as the main sorting station in the outward secretory pathway, the endosomal compartment serves this function in the inward endocytic 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

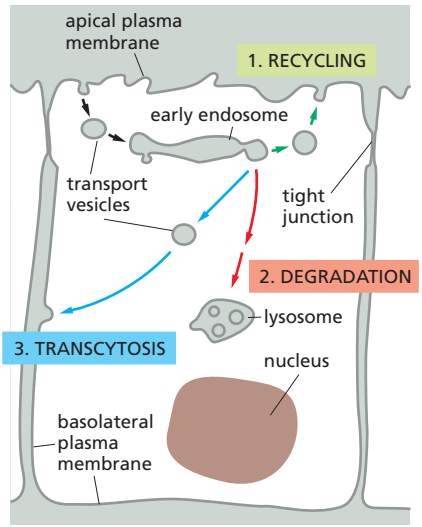

Figure 15–35 The fate of receptor proteins following their endocytosis depends on the type of receptor. Three pathways from the endosomal compartment in an epithelial cell are shown. Receptors that are not specifically retrieved from early endosomes follow the pathway from the endosomal compartment to 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 (see Figure 11–32). 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.

bound cargo molecules across the cell from one extracellular space to another, a process called *transcytosis* (**Figure 15–35**).

Cargo proteins that remain bound to their receptors share the fate of their receptors. Those that dissociate from their receptors in the endosome are 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 should some of them 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, these materials can be either exported 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 15–36**). Most of the lysosomal membrane proteins are unusually highly glycosylated; the sugars, which cover much of the protein surfaces facing the lysosome 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

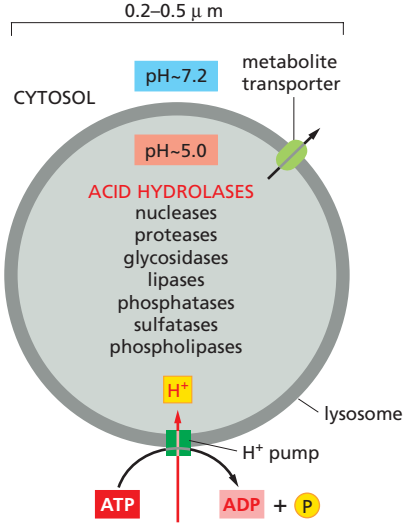

Figure 15–36 A lysosome contains a large variety of hydrolytic enzymes, which are only active under acidic conditions. The lumen of the lysosome is maintained at an acidic pH by an ATP-driven H^+ pump in the membrane that hydrolyzes ATP to pump H^+ into the lumen.

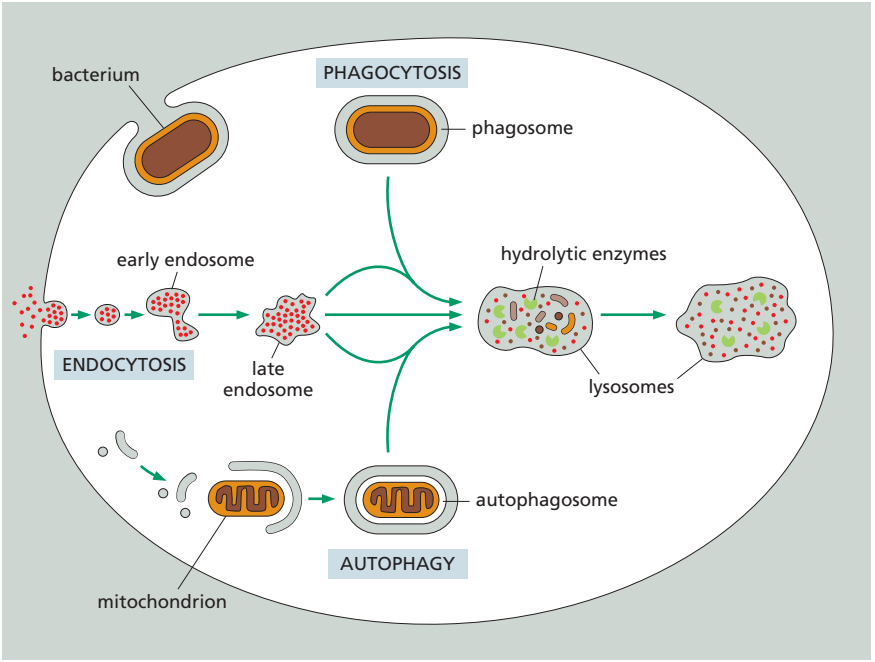

Figure 15–37 Materials destined for degradation in lysosomes follow different pathways to the lysosome. Each pathway leads to the intracellular digestion of materials derived from a different source. Early endosomes, phagosomes, and autophagosomes can fuse with either lysosomes or late endosomes, both of which contain acid-dependent hydrolytic enzymes. Where the membrane fragments that form the autophagosome originate is still actively investigated.

sorted and packaged into transport vesicles, which bud off and deliver their contents to lysosomes via endosomes (see Figure 15–19).

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: as the term suggests, 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 involves the enclosure of the organelle by a double membrane, creating an *autophagosome*, which then fuses with a lysosome (**Figure 15–37**). Autophagy of organelles and cytosolic proteins—some of which are marked for destruction by the attachment of ubiquitin tags (as discussed in Chapter 4)—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 of the 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.
- 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, tethering, 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
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
tethering protein
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.

H. Ribosomes bound to the outer nuclear membrane make proteins that are translocated co-translationally into the membrane.

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 enter the cell by receptor-mediated endocytosis. The viruses are surrounded by a membrane that contains a fusion protein, which is activated by the acidic pH in the endosome. 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.

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? Explain your answer.

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 15–20A. Name the structures that are labeled in the sketch.

QUESTION 15–23

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

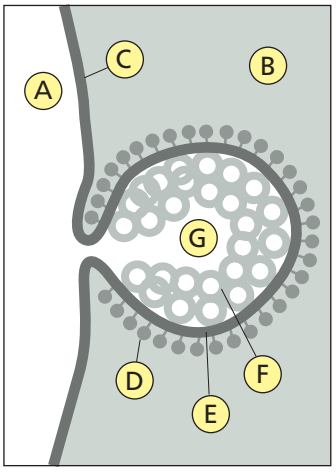

Figure Q15–22