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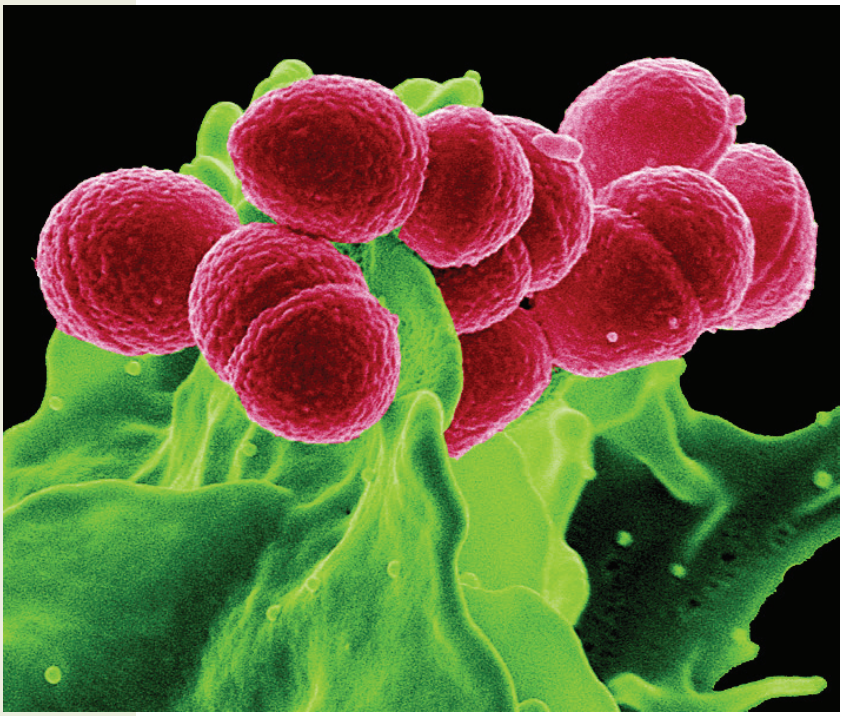


Cytoplasmic Membrane Systems: Structure, Function, and Membrane Trafficking

Under the light microscope, the cytoplasm of living cells appears relatively devoid of structure. Yet, even before the beginning of the twentieth century, examination of stained sections of animal tissues hinted at the existence of an extensive membrane network within the cytoplasm. It wasn't until the development of the electron microscope in the 1940s, however, that biologists began to appreciate the diverse array of membrane-bound structures present in the cytoplasm of most eukaryotic cells. These early electron microscopists saw membrane-bound vesicles of varying diameter containing material of different electron density; long channels bounded by membranes that radiate through the cytoplasm to form an interconnected network of canals; and stacks of flattened membrane-bound sacs, called *cisternae*.

It became evident from these early electron microscopic studies and the biochemical investigations that followed that the cytoplasm of eukaryotic cells was subdivided into a variety of distinct compartments bounded by membrane barriers. As more types of cells were examined, it became apparent that these membranous compartments in the cytoplasm formed different organelles that could be identified in diverse cells from yeast to multicellular plants and animals. The extent to which the

Colorized scanning electron micrograph of a human neutrophil, a type of white blood cell, ingesting a number of bacteria by the process of phagocytosis. Neutrophils are essential components of our innate immune response against pathogens. (From Scott D. Kobayashi et al., cover of PNAS, vol. 100, #19, 2003, © 2003, National Academy of Sciences, U.S.A., courtesy of Frank R. DeLeo.)

cytoplasm of a eukaryotic cell is occupied by membranous structures is illustrated in the electron micrograph of a maize root cell shown in Figure 8.1. As we will see in the following pages, each of these organelles contains a particular complement of proteins and is specialized for particular types of activities. Thus, just as a house or restaurant is divided into specialized rooms where different activities can take place independent of one another, the cytoplasm of a cell is divided into specialized membranous compartments for analogous reasons. Keep in mind, as you examine the micrographs in this chapter, that these cytoplasmic organelles may appear as stable structures, like the rooms of a house or restaurant, but in fact they are dynamic compartments that are in continual flux.

In the present chapter, we will examine the structure and functions of the endoplasmic reticulum, Golgi complex, endosomes, lysosomes, and vacuoles. Taken together, these organelles form an **endomembrane system** in which the individual components function as part of a coordinated unit. (Mitochondria and chloroplasts are not part of this interconnected system and were the subjects of Chapters 5 and 6. Current evidence suggests that peroxisomes, which were also discussed in Chapter 6, have a dual origin. The basic elements of the boundary membrane are thought to arise from the endoplasmic reticulum, but many of the membrane proteins and the soluble internal proteins are taken up from the cytosol, as described in Section 8.9.)

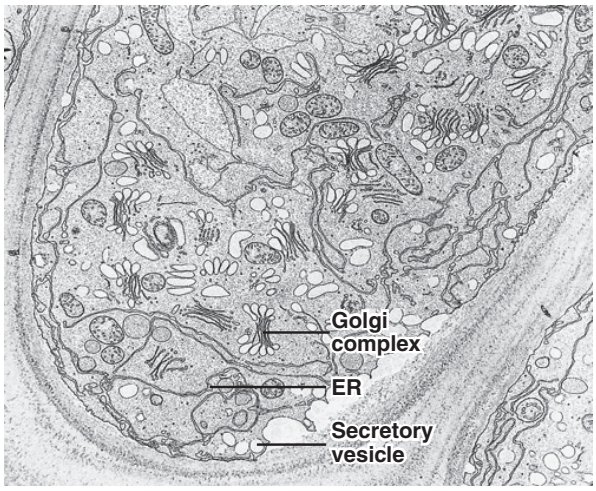


Figure 8.1 **Membrane-bound compartments of the cytoplasm.** The cytoplasm of this root cap cell of a maize plant contains an array of membrane-bound organelles whose structure and function will be examined in this chapter. As is evident in this micrograph, the combined surface area of the cytoplasmic membranes is many times greater than that of the surrounding plasma membrane. (COURTESY OF HILTON H. MOLLENHAUER.)

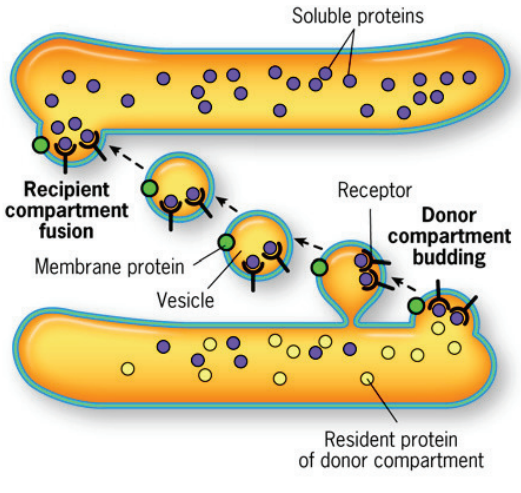
8.1 | An Overview of the Endomembrane System

The organelles of the endomembrane system are part of a dynamic, integrated network in which materials are shuttled back and forth from one part of the cell to another. For the most part, materials are shuttled between organelles—from the Golgi complex to the plasma membrane, for example—in small, membrane-bounded **transport vesicles** that bud from a donor membrane compartment (Figure 8.2*a*).[1] Transport vesicles move through the cytoplasm in a directed manner, often pulled by motor proteins that operate on tracks formed by microtubules and microfilaments of the cytoskeleton (see Figure 9.1*a*). When they reach their destination, the vesicles fuse with the membrane of the acceptor compartment, which receives the vesicle's soluble cargo as well as its membranous wrapper (Figure 8.2*a*). Repeated cycles of budding and fusion shuttle a diverse array of materials along numerous pathways that traverse the cell.

Several distinct pathways through the cytoplasm have been identified and are illustrated in the overview shown in Figure 8.2*b*. A **biosynthetic pathway** can be discerned in which proteins are synthesized in the endoplasmic reticulum, modified during passage through the Golgi complex, and transported from the Golgi complex to various destinations, such as the plasma membrane, a lysosome, or the large vacuole of a plant cell. This route is also referred to as the **secretory pathway**, as many of the proteins synthesized in the endoplasmic reticulum (as well as complex polysaccharides synthesized in the Golgi complex, Figure 7.37*c*) are destined to be discharged (**secreted**) from the cell. Secretory activities of cells can be divided into two types: constitutive and regulated (Figure 8.2*b*). During **constitutive secretion**, materials are transported in secretory vesicles from their sites of synthesis and discharged into the extracellular space in a continual manner. Most cells engage in constitutive secretion, a process that contributes not only to the formation of the extracellular matrix (Section 7.1), but to the formation of the plasma membrane itself. During **regulated secretion**, materials are stored as membrane-bound packages and discharged only in response to an appropriate stimulus. Regulated secretion occurs, for example, in endocrine cells that release hormones, in pancreatic acinar cells that release digestive enzymes, and in nerve cells that release neurotransmitters. In some of these cells, materials to be secreted are stored in large, densely packed, membrane-bound **secretory granules** (see Figure 8.3).

Proteins, lipids, and complex polysaccharides are transported through the cell along the biosynthetic or secretory pathway. We will focus in the first part of the chapter on the

[1]The term *vesicle* implies a spherical-shaped carrier. Cargo may also be transported in irregular or tubular-shaped membrane-bound carriers. For the sake of simplicity, we will generally refer to carriers as "vesicles," while keeping in mind they are not always spherical.



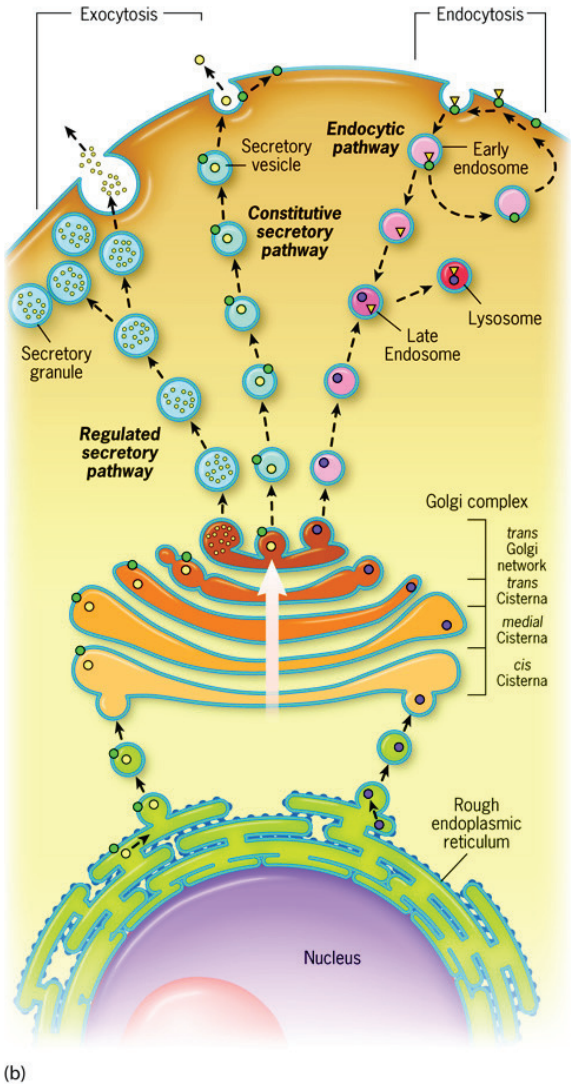


Figure 8.2 **An overview of the biosynthetic/secretory and endocytic pathways that unite endomembranes into a dynamic, interconnected network.** (*a*) Schematic diagram illustrating the process of vesicle transport by which materials are transported from a donor compartment to a recipient compartment. Vesicles form by membrane budding, during which specific membrane proteins (green spheres) of the donor membrane are incorporated into the vesicle membrane and specific soluble proteins (purple spheres) in the donor compartment are bound to specific receptors. When the transport vesicle subsequently fuses to another membrane, the proteins of the vesicle membrane become part of the recipient membrane, and the soluble proteins become sequestered within the lumen of the recipient compartment. (*b*) Materials follow the biosynthetic (or secretory) pathway from the endoplasmic reticulum, through the Golgi complex, and out to various locations including lysosomes, endosomes, secretory vesicles, secretory granules, vacuoles, and the plasma membrane. Materials follow the endocytic pathway from the cell surface to the interior by way of endosomes and lysosomes, where they are generally degraded by lysosomal enzymes.

synthesis and transport of proteins, as summarized in Figure 8.2*b*. During the discussion, we will consider several distinct classes of proteins. These include soluble proteins that are discharged from the cell, integral proteins of the various membranes depicted in Figure 8.2*b*, and soluble proteins that reside within the various compartments enclosed by the endomembranes (e.g., lysosomal enzymes). Whereas materials move out of the cell by the secretory pathway, the endocytic pathway operates in the opposite direction. By following the **endocytic pathway**, materials move from the outer surface of the cell to compartments, such as endosomes and lysosomes, located within the cytoplasm (Figure 8.2*b*).

The movement of vesicles and their contents along the various pathways of a cell is analogous to the movement of trucks carrying different types of cargo along the various highways of a city. Both types of transport require defined *traffic patterns* to ensure that materials are accurately delivered to the appropriate sites. For example, protein trafficking within a salivary gland cell requires that the proteins of salivary mucus, which are synthesized in the endoplasmic reticulum, are specifically *targeted* to secretory granules, while lysosomal enzymes, which are also manufactured in the endoplasmic reticulum, are specifically targeted to a lysosome. Different organelles also contain different integral membrane proteins. Consequently, membrane proteins must also be targeted to particular organelles, such as a lysosome or Golgi cisterna. These various types of cargo—secreted proteins, lysosomal enzymes, and membrane proteins—are routed to their appropriate cellular destinations by virtue of specific "addresses" or *sorting signals* that are encoded in the amino acid sequence of the proteins or in the attached oligosaccharides. The sorting signals are recognized by specific receptors that reside in the membranes or surface coats of budding vesicles, ensuring that the protein is transported to the appropriate destination. For the most part, the machinery responsible for driving this complex distribution system consists of soluble proteins that are *recruited* to specific membrane surfaces. During the course of this chapter we will try to understand why one protein is recruited, for example, to the endoplasmic

reticulum whereas another protein might be recruited to a particular region of the Golgi complex.

Great advances have been made over the past three decades in mapping the traffic patterns that exist in eukaryotic cells, identifying the specific addresses and receptors that govern the flow of traffic, and dissecting the machinery that ensures that materials are delivered to the appropriate sites in the cell. These subjects will be discussed in detail in the following pages. Motor proteins and cytoskeletal elements, which play key roles in the movements of transport vesicles and other endomembranes, will be described in the following chapter. We will begin the study of endomembranes by discussing a few of the most important experimental approaches that have led to our current understanding of the subject.

REVIEW

1. Compare and contrast the biosynthetic pathway with the endocytic pathway.
2. How are particular proteins targeted to particular subcellular compartments?

8.2 | A Few Approaches to the Study of Endomembranes

Early studies with the electron microscope provided biologists with a detailed portrait of the structure of cells but gave them little insight into the functions of the components they were observing. Determining the functions of cytoplasmic organelles required the development of new techniques and the execution of innovative experiments. The experimental approaches described in the following sections have proven particularly useful in providing the foundation of knowledge on which current research on cytoplasmic organelles is based.

Insights Gained from Autoradiography

Among the many cells in the body, the acinar cells of the pancreas have a particularly extensive endomembrane system. These cells function primarily in the synthesis and secretion of digestive enzymes. After secretion from the pancreas, these enzymes are shipped through ducts to the small intestine, where they degrade ingested food matter. Where within the pancreatic acinar cells are the secretory proteins synthesized, and how do they reach the surface of the cells where they are discharged? These questions are inherently difficult to answer because all of the steps in the process of secretion occur simultaneously within the cell. To follow the steps of a single cycle from start to finish, that is, from the synthesis of a secretory protein to its discharge from the cell, James Jamieson and George Palade of Rockefeller University utilized the technique of **autoradiography** (Section 18.4).

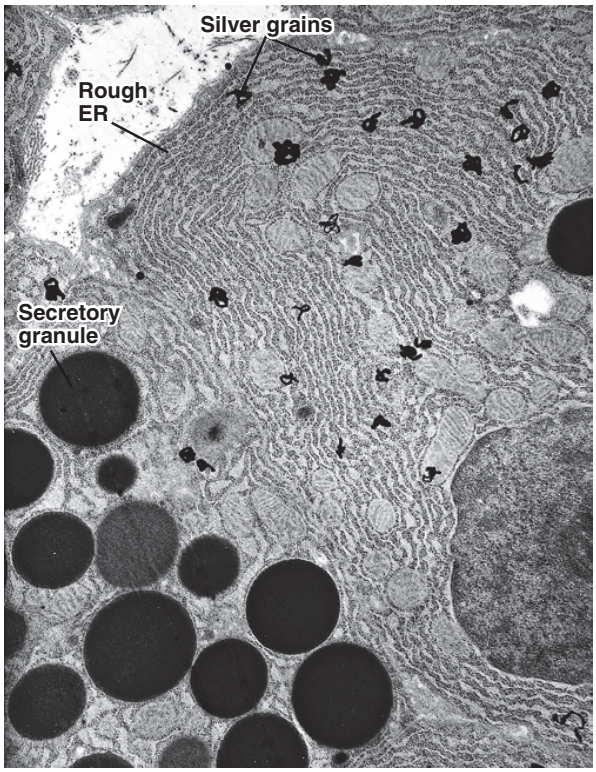
Autoradiography provides a means to visualize biochemical processes by allowing an investigator to determine the location of radioactively labeled materials within a cell. In this technique, tissue sections containing radioactive isotopes are covered with a thin layer of photographic emulsion, which is exposed by radiation emanating from radioisotopes within the tissue. Sites in the cells containing radioactivity are revealed under the microscope by silver grains in the overlying emulsion (Figure 8.3).

To determine the sites where secretory proteins are synthesized, Palade and Jamieson incubated slices of pancreatic tissue in a solution containing radioactive amino acids for a brief period of time. During this period, labeled amino acids were taken up by the living cells and incorporated into the digestive enzymes as they were being synthesized on ribosomes. The tissues were quickly fixed, and the locations of proteins that had been synthesized during the brief incubation with labeled amino acids were determined autoradiographically. Using this approach, the endoplasmic reticulum was discovered to be the site of synthesis of secretory proteins (Figure 8.3*a*).

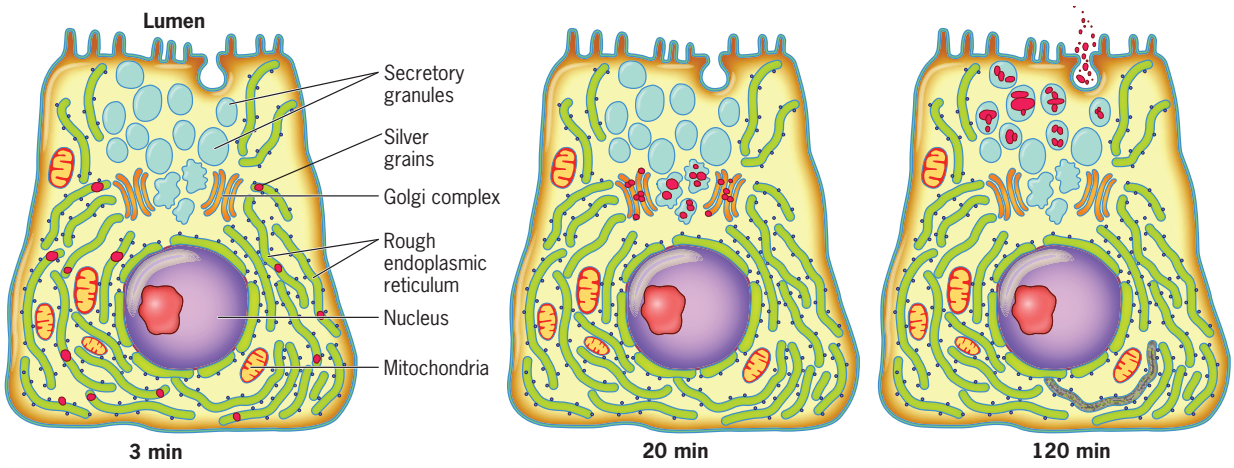
To determine the intracellular path followed by secretory proteins from their site of synthesis to their site of discharge, Palade and Jamieson carried out an additional experiment. After incubating the tissue for a brief period in radioactive amino acids, they washed the tissue free of excess isotope and transferred the tissue to a medium containing only unlabeled amino acids. An experiment of this type is called a *"pulse-chase."* The *pulse* refers to the brief incubation with radioactivity during which labeled amino acids are incorporated into protein. The *chase* refers to the period when the tissue is exposed to the unlabeled medium, a period during which additional proteins are synthesized using nonradioactive amino acids. The longer the chase, the farther the radioactive proteins manufactured during the pulse will have traveled from their site of synthesis within the cell. Using this approach, one can ideally follow the movements of newly synthesized molecules by observing a wave of radioactive material moving through the cytoplasmic organelles of cells from one location to the next until the process is complete. The results of these experiments—which first defined the biosynthetic (or secretory) pathway and tied a number of seemingly separate membranous compartments into an integrated functional unit—are summarized in Figure 8.3*b–d*.

Insights Gained from the Use of the Green Fluorescent Protein

The autoradiographic experiments described in the previous section require investigators to examine thin sections of different cells that have been fixed at various times after introduction of a radioactive label. Techniques involving the use of radioactive isotopes have largely been abandoned by modern cell biologists. An alternative technology allows researchers to follow the dynamic movements of specific proteins with their own eyes as they occur within a single living cell. This technology utilizes a gene isolated from a jellyfish that encodes a small protein, called the **green fluorescent protein (GFP)**, which emits a green fluorescent light. In this approach, DNA encoding GFP is fused to DNA encoding the protein to be studied, and the resulting chimeric (i.e., composite) DNA is introduced into cells that can be observed under the microscope. Once inside a cell, the chimeric DNA expresses a



(a)



(b) (c) (d)

Figure 8.3 **Autoradiography reveals the sites of synthesis and subsequent transport of secretory proteins.** (*a*) Electron micrograph of a section of a pancreatic acinar cell that had been incubated for 3 minutes in radioactive amino acids and then immediately fixed and prepared for autoradiography. The black silver grains that appear in the emulsion following development are localized over the endoplasmic reticulum. (*b–d*) Diagrams of a sequence of autoradiographs showing the movement of labeled secretory proteins (represented by the silver grains in red) through a pancreatic acinar cell. When the cell is pulse-labeled for 3 minutes and immediately fixed (as shown in *a*), radioactivity is localized in the endoplasmic reticulum (*b*). After a 3-minute pulse and 17-minute chase, radioactive label is concentrated in the Golgi complex and adjacent vesicles (*c*). After a 3-minute pulse and 117-minute chase, radioactivity is concentrated in the secretory granules and is beginning to be released into the pancreatic ducts (*d*). (A: Courtesy of James D. Jamieson and George Palade.)

chimeric protein consisting of GFP fused to the end of the protein to be studied. In most cases, the presence of GFP joined to the end of a protein has little or no effect on the movement or function of that protein.

Figure 8.4 shows a pair of micrographs depicting cells that contain a GFP fusion protein. In this case, the cells were infected with a strain of the vesicular stomatitis virus (VSV) in which one of the viral genes (*VSVG*) is fused to the *GFP* gene. Viruses are useful in these types of studies because they turn infected cells into factories for the production of viral proteins, which are carried like any other protein cargo through the biosynthetic pathway. When a cell is infected with VSV, massive amounts of the VSVG protein are produced in the endoplasmic reticulum (ER). The VSVG molecules then traffic through the Golgi complex and are transported to the plasma membrane of the infected cell where they are incorporated into viral envelopes. As in a radioactive pulse-chase experiment, the use of a virus allows investigators to follow a relatively synchronous wave of protein movement, in this case represented by a wave of green fluorescence that begins soon after infection. Synchrony can be enhanced, as was done in the experiment depicted in Figure 8.4, by use of a virus with a mutant VSVG protein that is unable to leave the ER of infected cells that are grown at an

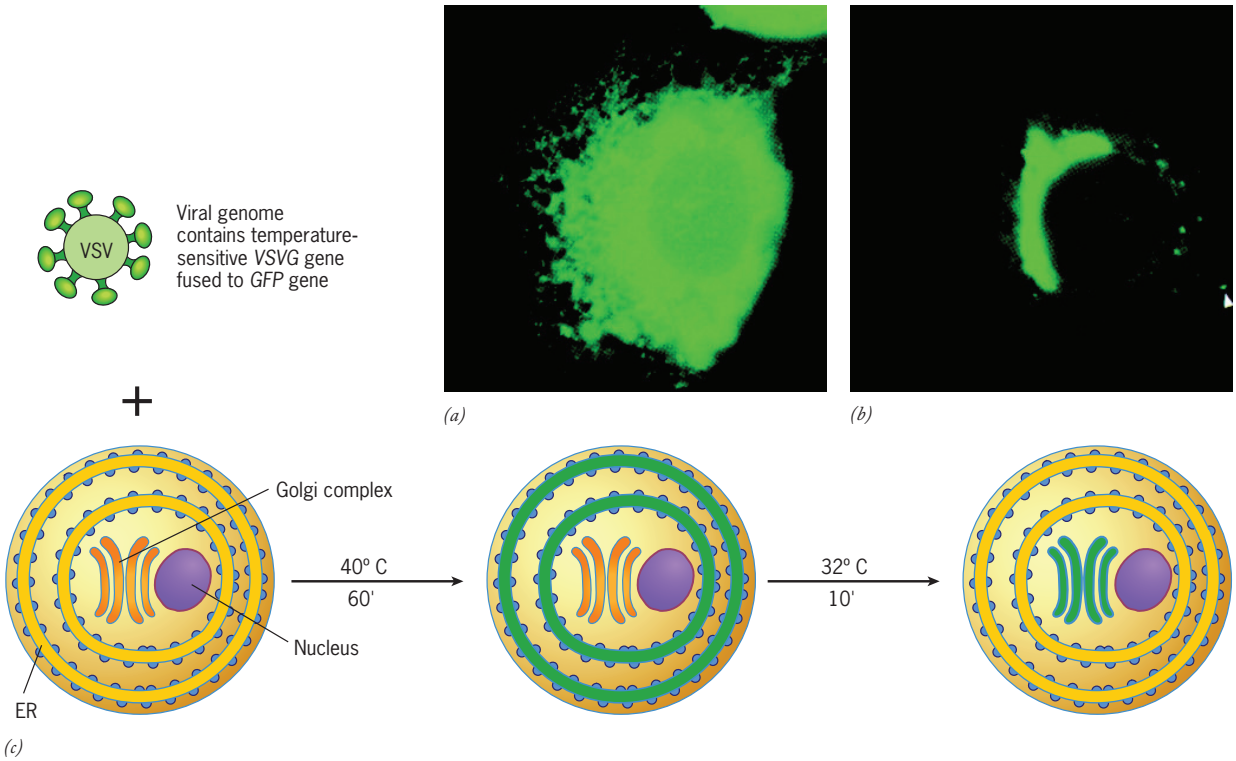


Figure 8.4 **The use of green fluorescent protein (GFP) reveals the movement of proteins within a living cell.** (*a*) Fluorescence micrograph of a live cultured mammalian cell that had been infected with the VSV virus at 40°C. This particular strain of the VSV virus contained a *VSVG* gene that (1) was fused to a gene encoding the fluorescent protein GFP and (2) contained a temperature-sensitive mutation that prevented the newly synthesized VSVG protein from leaving the ER when kept at 40°C. The green fluorescence in this micrograph is restricted to the ER. (*b*) Fluorescence micrograph of a live infected cell that was held at 40°C to allow the VSVG protein to accumulate in the ER and then incubated at 32°C for 10 minutes. The fluorescent VSVG protein has moved on to the Golgi complex. (*c*) Schematic drawing showing the retention of the mutant VSVG protein in the ER at 40°C and its synchronous movement to the Golgi complex within 10 minutes of incubation at the lower temperature. (A,B: From Daniel S. Chao et al., courtesy of Richard H. Scheller, J. Cell Biol. 144:873, 1999; reproduced with permission of The Rockefeller University Press.)

elevated temperature (e.g., 40°C). When the temperature is lowered to 32°C, the fluorescent GFP-VSVG protein that had accumulated in the ER (Figure 8.4*a*,*c*) moves synchronously to the Golgi complex (Figure 8.4*b*,*c*), where various processing events occur, and then on to the plasma membrane. Mutants of this type that function normally at a reduced (permissive) temperature, but not at an elevated (restrictive) temperature, are described as *temperature-sensitive mutants*. An experiment utilizing two different fluorescent probes is described on page 321.

Insights Gained from the Biochemical Analysis of Subcellular Fractions

Electron microscopy, autoradiography, and the use of GFP provide information on the structure and function of cellular organelles but fail to provide much insight into the molecular composition of these structures. Techniques to break up (**homogenize**) cells and isolate particular types of organelles were pioneered in the 1950s and 1960s by Albert Claude and Christian De Duve. When a cell is ruptured by homogenization, the cytoplasmic membranes become fragmented and the fractured edges of the membrane fragments fuse to form spherical vesicles less than 100 nm in diameter. Vesicles derived from different organelles (nucleus, mitochondrion, plasma membrane, endoplasmic reticulum, and so forth) have different properties, which allow them to be separated from one another, an approach that is called **subcellular fractionation**.

Membranous vesicles derived from the endomembrane system (primarily the endoplasmic reticulum and Golgi complex) form a heterogeneous collection of similar sized vesicles referred to as **microsomes**. A rapid (and crude) preparation of the microsomal fraction of a cell is depicted in Figure 8.5*a*. The microsomal fraction can be further fractionated into smooth and rough membrane fractions (Figure 8.5*b*,*c*) by the gradient techniques discussed in Section 18.6. Once isolated, the biochemical composition of various fractions can be determined. In recent years, the identification of the proteins present in cell fractions has been carried out using sophisticated proteomic technology. Once a particular organelle has been isolated, the proteins can be extracted, separated, and identified by mass spectrometry as discussed on page 72. Hundreds of

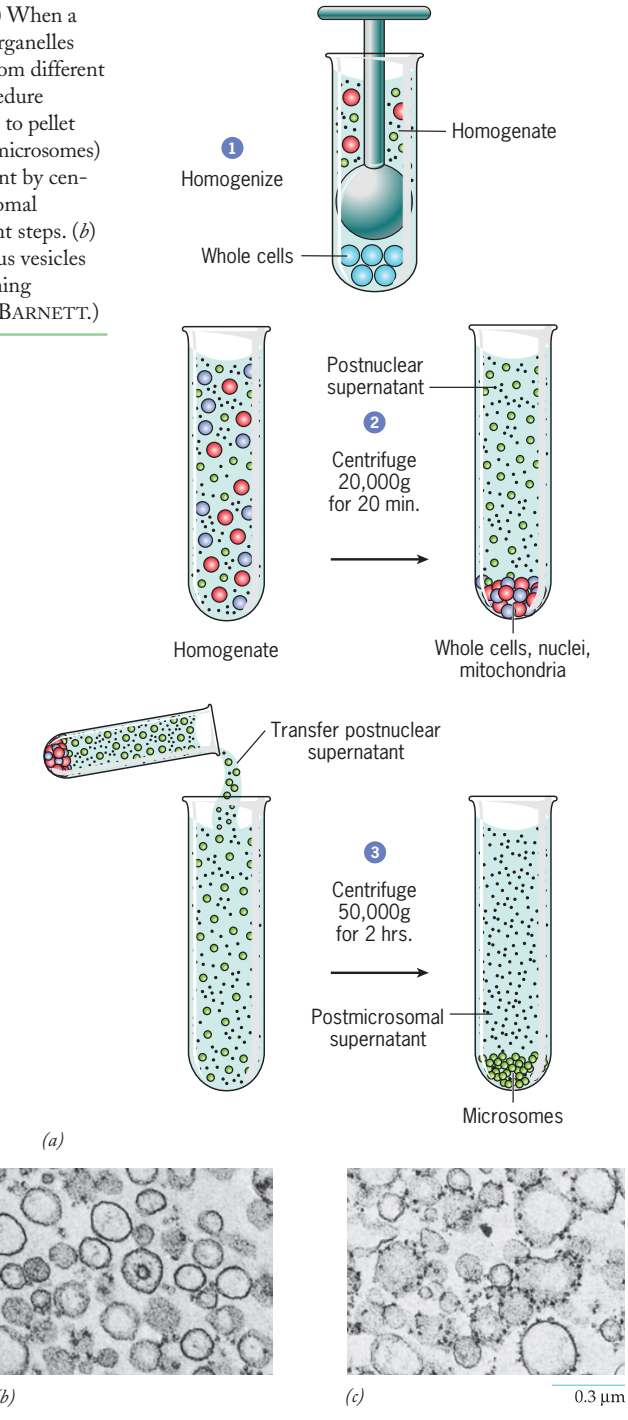


Figure 8.5 **Isolation of a microsomal fraction by differential centrifugation.** (*a*) When a cell is broken by mechanical homogenization (step 1), the various membranous organelles become fragmented and form spherical membranous vesicles. Vesicles derived from different organelles can be separated by various techniques of centrifugation. In the procedure depicted here, the cell homogenate is first subjected to low-speed centrifugation to pellet the larger particles, such as nuclei and mitochondria, leaving the smaller vesicles (microsomes) in the supernatant (step 2). The microsomes can be removed from the supernatant by centrifugation at higher speeds for longer periods of time (step 3). A crude microsomal fraction of this type can be fractionated into different vesicle types in subsequent steps. (*b*) Electron micrograph of a smooth microsomal fraction in which the membranous vesicles lack ribosomes. (*c*) Electron micrograph of a rough microsomal fraction containing ribosome-studded membranes. (B,C: COURTESY OF J. A. HIGGINS AND R. J. BARNETT.)

proteins can be identified simultaneously, providing a comprehensive molecular portrait of any organelle that can be prepared in a relatively pure state. In one example of this technology, it was found that a simple phagosome (page 315) containing an ingested latex bead comprised more than 160 different proteins, many of which had never previously been identified or were not known to be involved in phagocytosis.

Insights Gained from the Use of Cell-Free Systems

Once techniques to fractionate membranous organelles were developed, researchers began to probe the capabilities of these crude subcellular preparations. They found that the isolated parts of a cell were capable of remarkable activities. These early **cell-free systems**—so called because they did not contain whole cells—provided a wealth of information about biological processes that were impossible to study within the complex environment of intact cells. During the 1960s, for example, George Palade, Philip Siekevitz, and their colleagues at Rockefeller University set out to learn more about the properties of the rough microsomal fraction (shown in Figure 8.5*c*), whose membrane vesicles are derived from the rough ER (page 280). They found that they could strip a rough microsomal preparation of its attached particles, and the isolated particles (i.e., ribosomes) were capable of synthesizing proteins when provided with required ingredients from the cytosol. Under these conditions, the newly synthesized proteins were simply released by the ribosomes into the aqueous fluid of the test tube. When the same experiment was carried out using intact rough microsomes, the newly synthesized proteins were no longer released into the incubation medium but were trapped within the lumen of the membranous vesicles. It was concluded from these studies that the microsomal membrane was not required for the incorporation of amino acids into proteins but for sequestering newly synthesized secretory proteins within the ER cisternal space.

Over the past few decades, researchers have used cell-free systems to identify the roles of many of the proteins involved in membrane trafficking. Figure 8.6 shows a liposome with vesicles budding from its surface (arrows). As discussed on page 128, liposomes are vesicles whose surface consists of an artificial bilayer that is created in the laboratory from purified phospholipids. The buds and vesicles seen in Figure 8.6 were produced after the preparation of liposomes was incubated with purified proteins that normally comprise coats on the cytosolic surface of transport vesicles within the cell. Without the added coat proteins, vesicle budding could not occur. Using this strategy in which cellular processes are *reconstituted* in vitro from purified components, researchers have been able to study the proteins that bind to the membrane to initiate vesicle formation, the proteins responsible for cargo selection, and the proteins that sever the vesicle from the donor membrane.

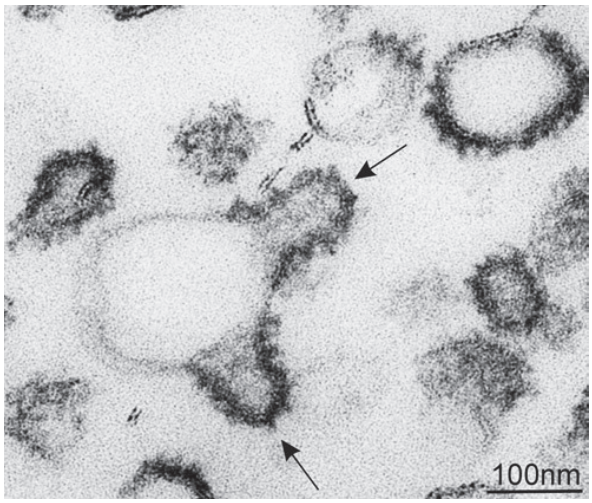
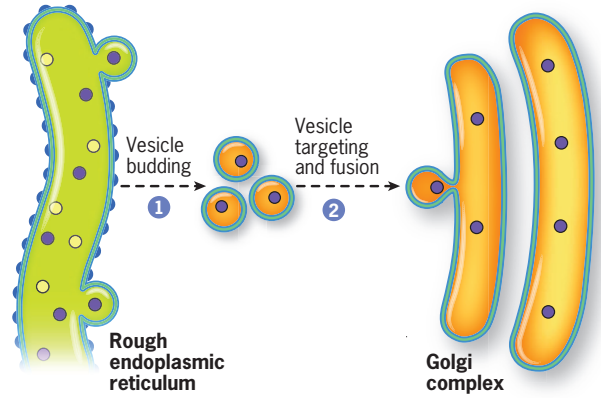


Figure 8.6 **Formation of coated vesicles in a cell-free system.** Electron micrograph of a liposome preparation that had been incubated with the components required to promote vesicle budding within the cell. The proteins in the medium have become attached to the surface of the liposomes and have induced the formation of protein-coated buds (arrows). (COURTESY OF LELIO ORCI AND RANDY SCHEKMAN.)

Insights Gained from the Study of Mutant Phenotypes

A mutant is an organism (or cultured cell) whose chromosomes contain one or more genes that encode abnormal proteins. When a protein encoded by a mutant gene is unable to carry out its normal function, the cell carrying the mutation exhibits a characteristic deficiency. Determining the precise nature of the deficiency provides information on the function of the normal protein. Study of the genetic basis of secretion has been carried out largely on yeast cells, most notably by Randy Schekman and colleagues at the University of California, Berkeley. Yeasts are particularly amenable to genetic studies because they have a small number of genes compared to other types of eukaryotes; they are small, single-celled organisms easily grown in culture; and they can be grown as haploids for the majority of their life cycle. A mutation in a single gene in a haploid cell produces an observable effect because the cells lack a second copy of the gene that would mask the presence of the abnormal one.

In yeast, as in all eukaryotic cells, vesicles bud from the ER and travel to the Golgi complex, where they fuse with the Golgi cisternae (Figure 8.7*a*). To identify genes whose encoded protein is involved in this portion of the secretory pathway (i.e., *SEC* genes), researchers screen for mutant cells that exhibit an abnormal distribution of cytoplasmic membranes. An electron micrograph of a wild-type yeast cell is shown in Figure 8.7*b*. The cell depicted in Figure 8.7*c* has a mutation in a gene that



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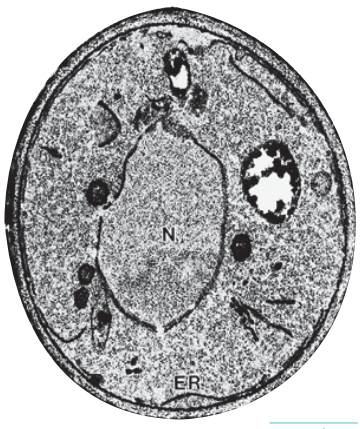

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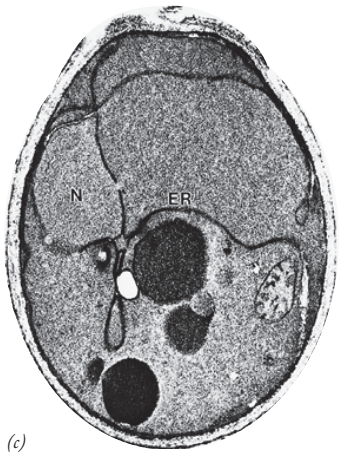

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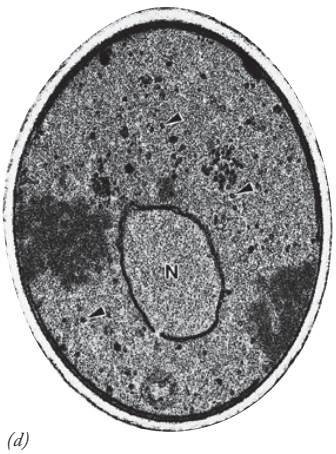


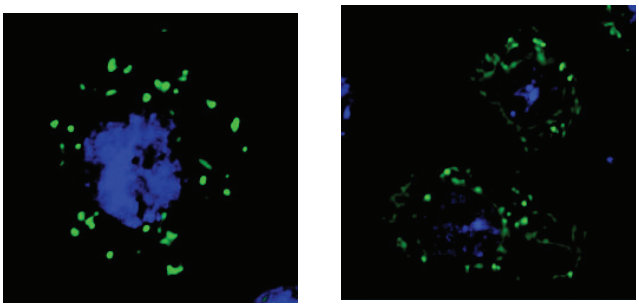
(*d*)

Figure 8.7 **The use of genetic mutants in the study of secretion.** (*a*) The first leg of the biosynthetic secretory pathway in budding yeast. The steps are described below. (*b*) Electron micrograph of a section through a wild-type yeast cell. (*c*) A yeast cell bearing a mutation in the *sec12* gene whose product is involved in the formation of vesicles at the ER membrane (step 1, part *a*). Because vesicles cannot form, expanded ER cisternae accumulate in the cell. (*d*) A yeast cell bearing a mutation in the *sec17* gene, whose product is involved in vesicle fusion (step 2, part *a*). Because they cannot fuse with Golgi membranes, the vesicles (indicated by the arrowheads) accumulate in the cell. [The mutants depicted in *c* and *d* are temperature-sensitive mutants. When kept at the lower (permissive) temperature, they are capable of normal growth and division.] (FROM CHRIS A. KAISER AND RANDY SCHEKMAN, CELL 61:724, 1990; © 1990, REPRINTED WITH PERMISSION FROM ELSEVIER.)

encodes a protein involved in the formation of vesicles at the ER membrane (step 1, Figure 8.7*a*). In the absence of vesicle formation, mutant cells accumulate an expanded endoplasmic reticulum. In contrast, the cell depicted in Figure 8.7*d* carries a mutation in a gene that encodes a protein involved in vesicle fusion (step 2, Figure 8.7*a*). When this gene product is defective, the mutant cells accumulate an excess number of unfused vesicles. Researchers have isolated dozens of different mutants which, taken as a group, exhibit disruptions in virtually every step of the secretory pathway. The genes responsible for these defects have been cloned and sequenced, and the proteins they encode have been isolated. Isolation of proteins from yeast has launched successful searches for homologous proteins (i.e., proteins with related sequence) in mammals.

One of the most important lessons learned from the use of all of these techniques is that the dynamic activities of the endomembrane system are highly conserved. Not only do yeast, plant, insect, and human cells carry out similar processes, but they do so with remarkably similar proteins. It is evident that the structural diversity of cells belies their underlying molecular similarities. In many cases, proteins from widely divergent species are interchangeable. For example, cell-free systems derived from mammalian cells can often utilize yeast proteins to facilitate vesicle transport. Conversely, yeast cells with genetic deficiencies that disrupt some phase of the biosynthetic pathway can often be "cured" by genetically engineering them to carry mammalian genes.

Over the past decade or so, researchers interested in searching for genes that affect a particular cellular process in plant or animal cells have taken advantage of a cellular phenomenon called *RNA interference* (*RNAi*). RNAi is a process in which cells produce small RNAs (called *siRNAs*) that bind to specific mRNAs and inhibit the translation of these mRNAs into proteins. This phenomenon and its use are discussed in detail in Sections 11.5 and 18.17. For the present purpose we will simply note that researchers can synthesize a collection (library) of siRNAs that are capable of inhibiting the translation of virtually any mRNA that is produced by a genome. Each mRNA represents the expression of a specific gene; therefore, one can find which genes are involved in a particular process by determining which siRNAs interfere with that process. In the experiment depicted in Figure 8.8, researchers set out to identify genes that were involved in various steps of the secretory pathway, a goal similar to that of the investigators studying the yeast mutants shown in Figure 8.7. In this case, researchers used a strain of cultured *Drosophila* cells and attempted to identify genes that affected the localization of mannosidase II, an enzyme that is synthesized in the endoplasmic reticulum and moves via transport vesicles to the Golgi complex, where it takes up residence. Figure 8.8*a* shows a control cell that is synthesizing a GFP-labeled version of mannosidase II; the fluorescence becomes localized in the numerous Golgi complexes of the cell as would be expected. Figure 8.8*b* shows a cell that contains siRNA molecules that have caused a relocation of the GFP-mannosidase into the ER, which is seen to be fused with the Golgi complexes. This type of phenotype is typically caused by the absence of one of the proteins involved in the transport of the enzyme from the ER to the Golgi complex. Of the 130 different siRNAs that were found to interfere in some way with the secretory pathway in this study, 31 of them generated a phenotype similar to that shown in Figure 8.8*b*. Included among these 31 siRNAs were numerous species that inhibited the expression of genes that were already known to be involved in the secretory pathway. In addition, the study identified other genes whose function had been unknown and are now presumed to be involved in these processes as well. Because it is easier to synthesize a small RNA than to generate an organism with a mutant gene, RNAi has become a common strategy to investigate the effect of a missing protein.



(a) *(b)*

Figure 8.8 **Inhibition of gene expression with RNA interference.** (*a*) A control *Drosophila* S2 cultured cell expressing GFP-labeled mannosidase II. The fluorescent enzyme becomes localized in the Golgi complex after its synthesis in the ER. (*b*) A cell that has been genetically engineered to express a specific siRNA, which binds to a complementary mRNA and inhibits translation of the encoded protein. In this case, the siRNA has caused the fluorescent enzyme to remain in the ER, which has fused with the Golgi membranes. This phenotype suggests that the mRNA being affected by the siRNA encodes a protein involved in an early step of the secretory pathway during which the enzyme is synthesized in the ER and traffics to the Golgi complex. Among the genes that exhibit this phenotype when targeted by siRNAs are those encoding proteins of the COPI coat, Sar1, and Sec23. The functions of these proteins are discussed later in the chapter. (FROM FREDERIC BARD ET AL., COURTESY OF VIVEK MALHOTRA, NATURE 439:604, 2006; © 2006, REPRINTED BY PERMISSION OF MACMILLAN PUBLISHERS LTD.)

REVIEW

1. Describe the differences between an autoradiograph of a pancreas cell that had been incubated in labeled amino acids for 3 minutes and immediately fixed and one of a cell that had been labeled for 3 minutes, chased for 40 minutes, and then fixed.
2. What techniques or approaches might you use to learn which proteins are normally present in the endoplasmic reticulum?
3. How does the isolation of a mutant yeast that accumulates vesicles provide information on the process of protein trafficking?
4. How can GFP be used to study membrane dynamics?

8.3 | The Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** comprises a network of membranes that penetrates much of the cytoplasm. The ER probably evolved from invaginations of the plasma membrane as depicted in Figure 1, page 27. Enclosed within the ER is an extensive space, or lumen, that is separated from the surrounding cytosol by the ER membrane. As will be evident in the following discussion, the composition of the **luminal** (or **cisternal**) **space** inside the ER membranes is quite different from that of the surrounding **cytosolic space**. Like other subcellular organelles, the ER is a highly dynamic structure undergoing continual turnover and reorganization.

The endoplasmic reticulum is divided into two subcompartments, the **rough endoplasmic reticulum (RER)** and the **smooth endoplasmic reticulum (SER)**. The rough ER is defined by the presence of ribosomes bound to its cytosolic surface, whereas the smooth ER lacks associated ribosomes. The RER is typically composed of a network of flattened sacs (**cisternae**), as shown in Figure 8.9*a*. The RER is continuous with the outer membrane of the nuclear envelope, which also bears ribosomes on its cytosolic surface (Figure 8.2*b*). In contrast, the membranes of the SER are highly curved and tubular, forming an interconnecting system of pipelines traversing the cytoplasm. When cells are homogenized, the SER tubules fragment into smooth-surfaced vesicles, whereas the RER sheets fragment into rough-surfaced vesicles (Figure 8.5*b*,*c*).

Fluorescently labeled proteins and lipids are capable of diffusing from one type of ER into the other, indicating that their membranes are continuous. In fact, the two types of ER share many of the same proteins and engage in certain common activities, such as the synthesis of certain lipids and cholesterol. At the same time, numerous proteins are found only in one or the other type of ER. For example, the high degree

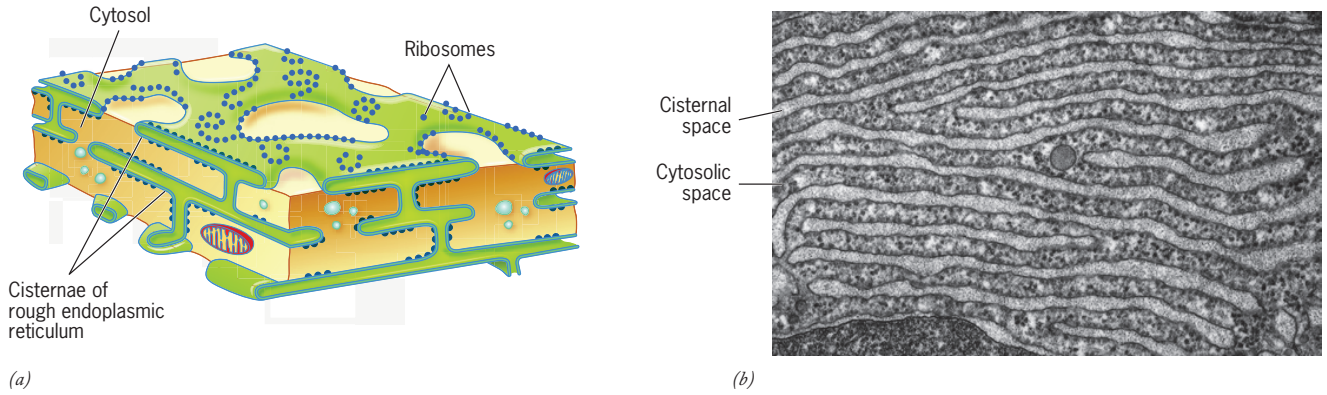


Figure 8.9 **The rough endoplasmic reticulum (RER).** (*a*) Schematic diagram showing the stacks of flattened cisternae that make up the rough ER. The cytosolic surface of the ER membrane contains bound ribosomes, which gives the cisternae their rough appearance. (*b*) Colorized transmission electron micrograph of a portion of the rough ER of a pancreatic acinar cell. The division of the rough ER into a cisternal space (which is devoid of ribosomes) and a cytosolic space is evident. (*c*) Scanning electron micrograph of the rough ER in a pancreatic acinar cell. (*d*) Visualization of the rough ER in a whole cultured cell as revealed by immunofluorescence staining for the enzyme protein disulfide isomerase (PDI), an ER resident protein. (B: MEDIMAGE/PHOTO RESEARCHERS, INC.; C: FROM K. TANAKA, INT. REV. CYTOL. 68:101, COURTESY OF K. TANAKA; 1980; D: FROM BRIAN STORRIE, RAINER PEPPERKOK, AND TOMMY NILSSON, TRENDS CELL BIOL. 10:388, © 2000, WITH PERMISSION FROM ELSEVIER SCIENCE.)

of curvature of the SER tubules is induced and maintained by the presence of membrane-bending proteins, called reticulons, which are largely absent from the flattened RER sheets. The RER, in contrast, contains proteins involved in the movement of nascent proteins into the ER lumen.

Different types of cells contain markedly different ratios of the two types of ER, depending on the activities of the cell. For example, cells that secrete large amounts of proteins, such as the cells of the pancreas or salivary glands, have extensive regions of RER (Figure 8.9*b–d*). We will return to the function of the RER shortly, but first we will describe the activities of the SER.

The Smooth Endoplasmic Reticulum

The SER is extensively developed in a number of cell types, including those of skeletal muscle, kidney tubules, and steroid-producing endocrine glands (Figure 8.10). SER functions include:

- Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex.
- Detoxification in the liver of a wide variety of organic compounds, including barbiturates and ethanol, whose chronic use can lead to proliferation of the SER in liver cells. Detoxification is carried out by a collection of oxygen-transferring enzymes (oxygenases), including the *cytochrome P450* family. These enzymes are noteworthy for their lack of substrate specificity, being able to oxidize thousands of different hydrophobic compounds and convert them into more hydrophilic, more readily excreted derivatives. The results are not always positive. For example, the relatively harmless compound benzo[*a*]pyrene formed when meat is charred on a grill is converted into a potent carcinogen by the "detoxifying" enzymes of the SER. Cytochrome P450s metabolize many prescribed medications, and genetic variation in these enzymes among humans may explain differences from one person to the next in the effectiveness and side effects of many drugs.

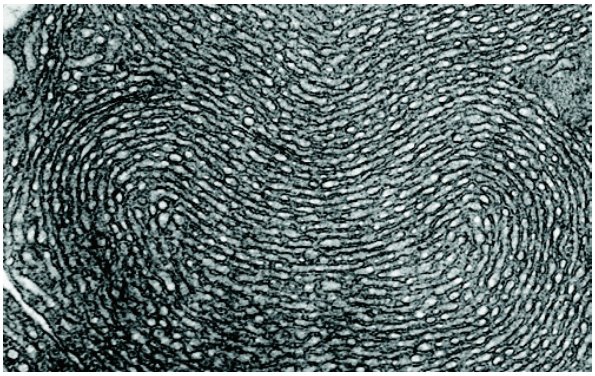


Figure 8.10 **The smooth ER (SER).** Electron micrograph of a Leydig cell from the testis showing the extensive smooth ER where steroid hormones are synthesized. DON FAWCETT/PHOTO RESEARCHERS, INC.

- Sequestering calcium ions within the cytoplasm of cells. The regulated release of Ca^{2+} from the SER of skeletal and cardiac muscle cells (known as the *sarcoplasmic reticulum* in muscle cells) triggers contraction.

Functions of the Rough Endoplasmic Reticulum

Early investigations into the functions of the RER were carried out on cells that secrete large quantities of proteins, such as the acinar cells of the pancreas (Figure 8.3) or the mucus-secreting cells of the lining of the digestive tract (Figure 8.11). It is evident from the drawing and micrograph of Figure 8.11 that the organelles of these epithelial secretory cells are positioned in the cell in such a way as to produce a distinct polarity from one end to the other. The nucleus and an extensive array of RER cisternae are located near the basal surface of the cell, which faces the blood supply. The Golgi complex is located in the central region of the cell. The apical surface of the cell faces a duct that carries the secreted proteins out of the organ. The cytoplasm at the apical end of the cell is filled with secretory granules whose contents are ready to be released into the duct upon arrival of the appropriate signal. The polarity of these glandular epithelial cells reflects the movement of secretory proteins through the cell from their site of synthesis to their site of discharge. The rough ER is the starting point of the biosynthetic pathway: it is the site of synthesis of the proteins, carbohydrate chains, and phospholipids that journey through the membranous compartments of the cell.

Synthesis of Proteins on Membrane-Bound versus Free Ribosomes The discovery of the rough endoplasmic reticulum as the site of synthesis of secretory proteins in pancreatic acinar cells was described earlier (Figure 8.3). Similar results were found for other types of secretory cells, including intestinal goblet cells that secrete mucoproteins, endocrine cells that secrete polypeptide hormones, plasma cells that secrete antibodies, and liver cells that secrete blood serum proteins.

Further experiments revealed that polypeptides are synthesized at two distinct locales within the cell.

1. Approximately one-third of the proteins encoded by a mammalian genome are synthesized on ribosomes attached to the cytosolic surface of the RER membranes. These include (a) secreted proteins, (b) integral membrane proteins, and (c) soluble proteins that reside within compartments of the endomembrane system, including the ER, Golgi complex, lysosomes, endosomes, vesicles, and plant vacuoles.
2. Other polypeptides are synthesized on "free" ribosomes, that is, on ribosomes that are not attached to the RER, and are subsequently released into the cytosol. This class includes (a) proteins destined to remain in the cytosol (such as the enzymes of glycolysis and the proteins of the cytoskeleton), (b) peripheral proteins of the cytosolic surface of membranes (such as spectrins and ankyrins that are only weakly associated with the plasma membrane's cytosolic surface), (c) proteins that are transported to the nucleus (Section 12.2), and (d) proteins to be incorporated

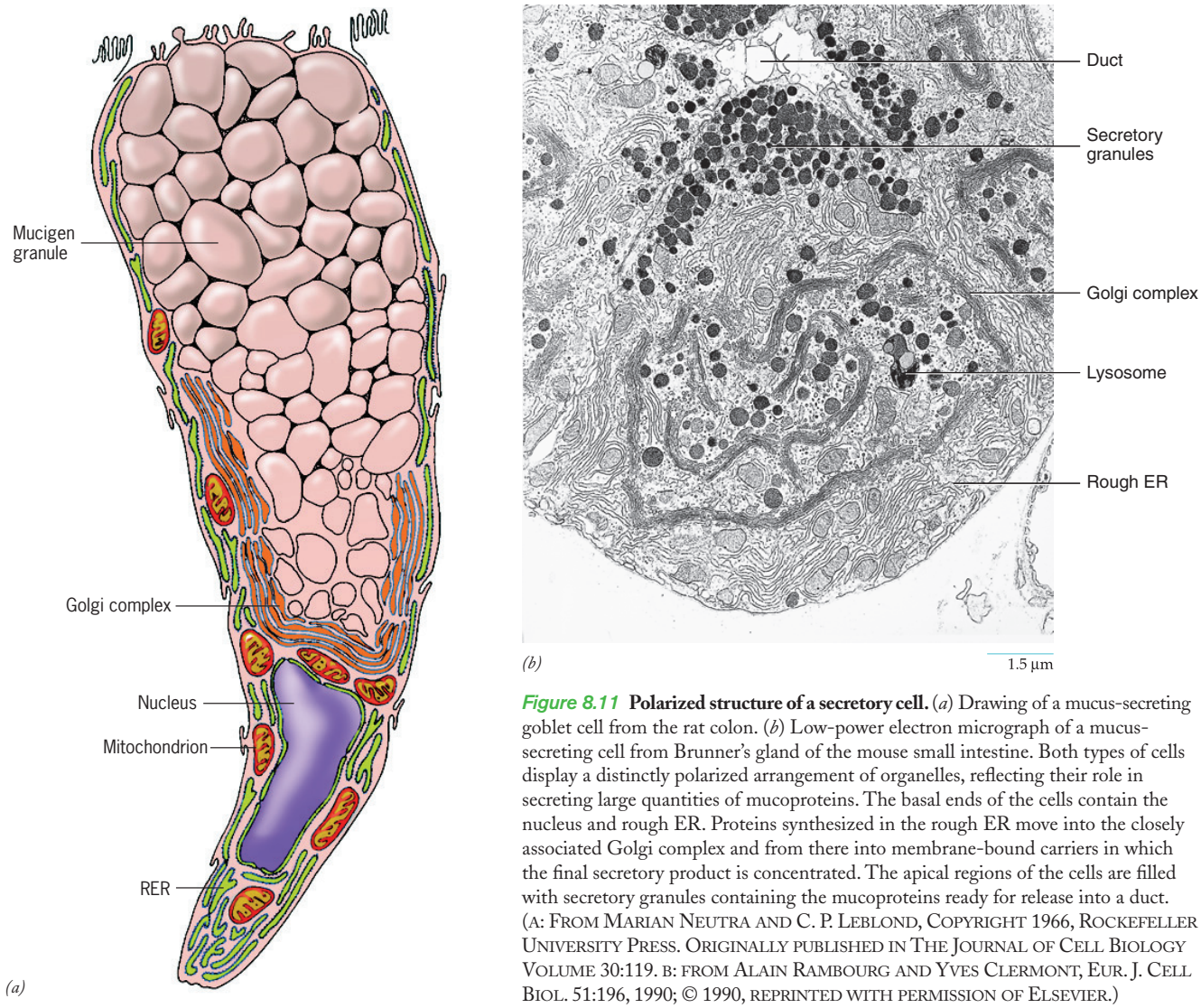


Figure 8.11 **Polarized structure of a secretory cell.** (*a*) Drawing of a mucus-secreting goblet cell from the rat colon. (*b*) Low-power electron micrograph of a mucus-secreting cell from Brunner's gland of the mouse small intestine. Both types of cells display a distinctly polarized arrangement of organelles, reflecting their role in secreting large quantities of mucoproteins. The basal ends of the cells contain the nucleus and rough ER. Proteins synthesized in the rough ER move into the closely associated Golgi complex and from there into membrane-bound carriers in which the final secretory product is concentrated. The apical regions of the cells are filled with secretory granules containing the mucoproteins ready for release into a duct. (A: From Marian Neutra and C. P. Leblond, Copyright 1966, Rockefeller University Press. Originally published in The Journal of Cell Biology Volume 30:119. B: from Alain Rambourg and Yves Clermont, Eur. J. Cell Biol. 51:196, 1990; © 1990, reprinted with permission of Elsevier.)

into peroxisomes, chloroplasts, and mitochondria. Proteins in the latter two groups are synthesized to completion in the cytosol and then imported *posttranslationally* into the appropriate organelle across its boundary membrane(s) (page 316).

What determines the location in a cell where a protein is synthesized? In the early 1970s, Günter Blobel, in collaboration with David Sabatini and Bernhard Dobberstein of Rockefeller University, first proposed, and then demonstrated, that the site of synthesis of a protein was determined by the sequence of amino acids in the N-terminal portion of the polypeptide, which is the first part to emerge from the ribosome during protein synthesis. They suggested the following:

1. Secretory proteins contain a **signal sequence** at their N-terminus that directs the emerging polypeptide and ribosome to the ER membrane.
2. The polypeptide moves into the cisternal space of the ER through a protein-lined, aqueous channel in the ER membrane. It was proposed that the polypeptide moves through the membrane as it is being synthesized, that is, *cotranslationally*.[2]

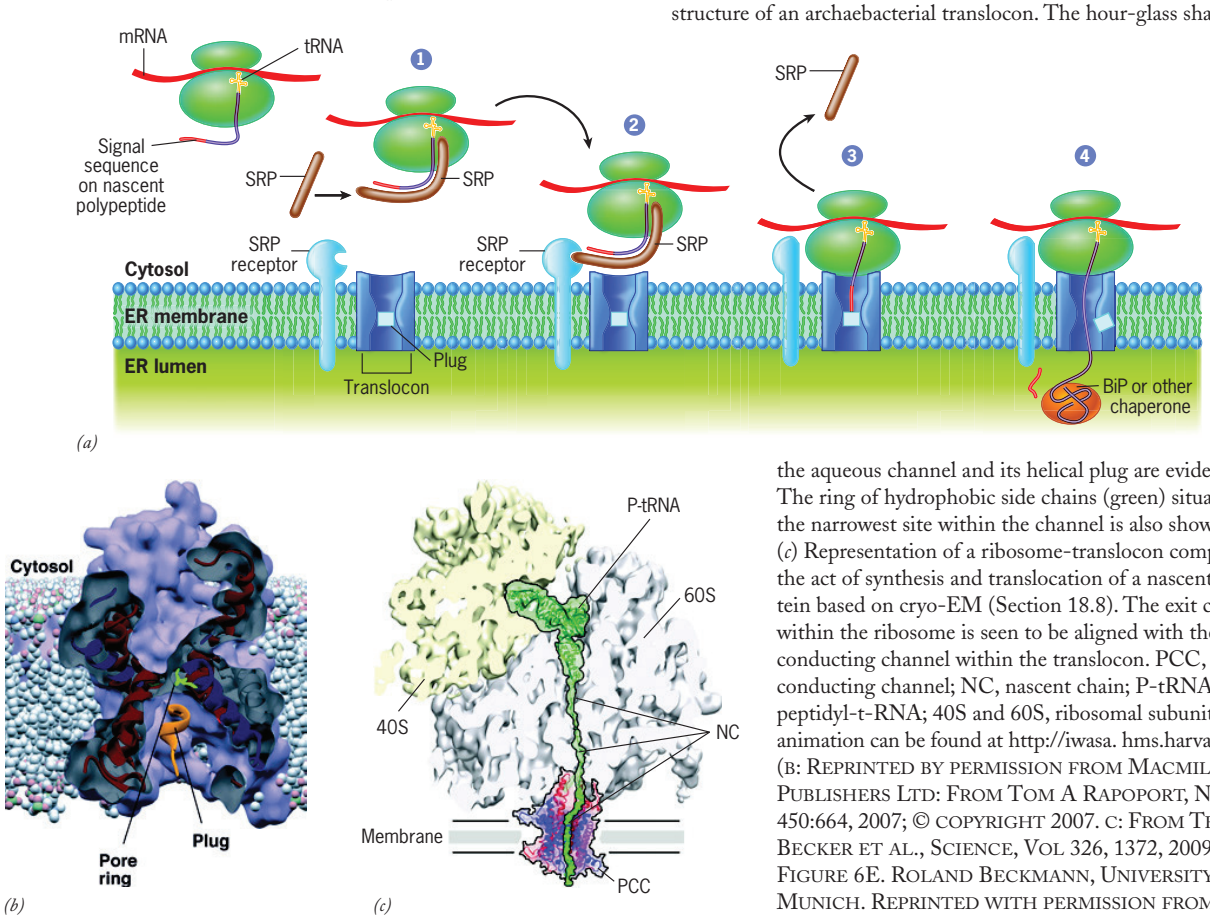
[2]It should be noted that protein transport across the ER membrane can also occur posttranslationally (i.e., after synthesis). In this process, the polypeptide is synthesized totally in the cytosol and then imported into the ER lumen through the same protein-conducting channels used in the cotranslational pathway. The posttranslational pathway is utilized much more heavily in yeast than in mammalian cells for import into the ER. In fact, yeast cells that are unable to engage in cotranslational transport into the ER are still able to survive, even though they grow more slowly than normal cells. It can also be noted that a substantial number of integral membrane proteins (including the SNAREs disscussed on page 302) have a single transmembrane domain located near the C-terminus of the polypeptide chain. Because of its position within the polypeptide, the transmembrane domain is the last segment to leave the ribosome and cannot be recognized by the SRP. These *tail-anchored proteins* must also be inserted into the membrane posttranslationally (see *Nature Revs. Mol. Cell Biol.* 12:787, 2011).

This proposal, known as the *signal hypothesis*, has been substantiated by a large body of experimental evidence. Even more importantly, Blobel's original concept that proteins contain built-in "address codes" has been shown to apply in principle to virtually all types of protein trafficking pathways throughout the cell.

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins on Membrane-Bound Ribosomes The steps that take place during the synthesis of a secretory, lysosomal, or plant vacuolar protein are shown in Figure 8.12*a*. The synthesis of the polypeptide begins after a messenger RNA binds to a free ribosome, that is, one that is *not* attached to a cytoplasmic membrane. In fact, all ribosomes are thought to be identical; those employed in the synthesis of secretory, lysosomal, or plant vacuolar proteins are taken from the same population (*pool*) as those used for production of proteins that remain in the cytosol. Polypeptides synthesized on membrane-bound ribosomes contain a signal sequence—which typically includes a stretch of 6–15 hydrophobic amino acid residues—that targets the *nascent* polypeptide to the ER membrane and leads to the compartmentalization of the

FIGURE IN FOCUS

Figure 8.12 **A schematic model of the synthesis of a secretory protein (or a lysosomal enzyme) on a membrane-bound ribosome of the RER.** (*a*) Synthesis of the polypeptide begins on a free ribosome. As the signal sequence (shown in red) emerges from the ribosome, it binds to the SRP (step 1), which stops further translation until the SRP-ribosome-nascent chain complex can make contact with the ER membrane. The SRP-ribosome complex then collides with and binds to an SRP receptor (SR) situated within the ER membrane (step 2). Attachment of this complex to the SRP receptor is followed by release of the SRP and the association of the ribosome with a translocon of the ER membrane (step 3). These latter events are accompanied by the reciprocal hydrolysis of GTP molecules (not shown) bound to both the SRP and its receptor. In the model depicted here, the signal peptide then binds to the interior of the translocon, displacing the plug from the channel and allowing the remainder of the polypeptide to translocate through the membrane cotranslationally (step 4). After the nascent polypeptide passes into the lumen of the ER, the signal peptide is cleaved by a membrane protein (the signal peptidase, not shown), and the protein undergoes folding with the aid of ER chaperones, such as BiP. Studies suggest that translocons are organized into groups of two or four units rather than singly as shown here. (*b*) Cross-sectional view of the translocon channel from the side based on the X-ray crystal structure of an archaebacterial translocon. The hour-glass shape of the aqueous channel and its helical plug are evident. The ring of hydrophobic side chains (green) situated at the narrowest site within the channel is also shown. (*c*) Representation of a ribosome-translocon complex in the act of synthesis and translocation of a nascent protein based on cryo-EM (Section 18.8). The exit channel within the ribosome is seen to be aligned with the conducting channel within the translocon. PCC, protein conducting channel; NC, nascent chain; P-tRNA, peptidyl-t-RNA; 40S and 60S, ribosomal subunits. (An animation can be found at http://iwasa. hms.harvard.edu) (B: REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD: FROM TOM A RAPOPORT, NATURE 450:664, 2007; © COPYRIGHT 2007. C: FROM THOMAS BECKER ET AL., SCIENCE, VOL 326, 1372, 2009, FIGURE 6E. ROLAND BECKMANN, UNIVERSITY OF MUNICH. REPRINTED WITH PERMISSION FROM AAAS.)



polypeptide within the ER lumen. (A *nascent* polypeptide is one in the process of being synthesized.) Although the signal sequence is usually located at or near the N-terminus, it occupies an internal position in some polypeptides.

As it emerges from the ribosome, the hydrophobic signal sequence is recognized by a **signal recognition particle (SRP)**, which consists in mammalian cells of six distinct polypeptides and a small RNA molecule, called the 7SL RNA. The SRP binds to both the signal sequence on the nascent polypeptide and the ribosome (step 1, Figure 8.12*a*), temporarily arresting further synthesis of the polypeptide. The bound SRP serves as a tag that enables the entire complex (SRP-ribosome-nascent polypeptide) to bind specifically to the cytosolic surface of the ER membrane. Binding to the ER occurs through two sequential interactions: one between the SRP and the **SRP receptor** (step 2), and the other between the ribosome and the **translocon** (step 3). The translocon is a protein-lined channel embedded in the ER membrane through which the nascent polypeptide is able to move in its passage from the ribosome to the ER lumen.

One of the major feats in the field of membrane trafficking over the past few years has been the determination of the three-dimensional structure of a prokaryotic version of the translocon by X-ray crystallography (Figure 8.12*b*). This effort revealed the presence within the translocon of a pore in the shape of an hourglass with a ring of six hydrophobic amino acids situated at its narrowest diameter. In the inactive (i.e., nontranslocating) state, which was the state in which the translocon was crystallized, the opening in the pore ring is plugged by a short α helix. This plug was originally proposed to seal the channel, preventing the unwanted passage of calcium and other ions between the cytosol and the ER lumen, but this model is now debated.

Once the SRP-ribosome-nascent chain complex binds to the ER membrane (step 2, Figure 8.12*a*), the SRP is released from its ER receptor, the ribosome becomes attached to the cytosolic end of the translocon, and the signal sequence on the nascent polypeptide is inserted into the narrow aqueous channel of the translocon (step 3). It is proposed that contact of the signal sequence with the interior of the translocon leads to displacement of the plug and opening of the passageway. The growing polypeptide is then translocated through the hydrophobic pore ring and into the ER lumen (step 4 and Figure 8.12*c*). Because the pore ring observed in the crystal structure has a diameter (5-8Å) that is considerably smaller than that of a helical polypeptide chain, it is presumed that the pore expands as the nascent chain traverses the channel. (Expansion is possible because the residues that make up the ring are situated on different helices of the translocon protein.) Upon termination of translation and passage of the completed polypeptide through the translocon, the membrane-bound ribosome is released from the ER membrane and the helical plug is reinserted into the translocon channel.

Several of the steps involved in the synthesis and trafficking of secretory proteins are regulated by the binding or hydrolysis of GTP. As will be discussed at length in Chapter 15 and elsewhere in this chapter, **GTP-binding proteins** (or **G proteins**) play key regulatory roles in many different cellular processes.[3] G proteins can be present in at least two alternate conformations, one containing a bound GTP molecule and the other a bound GDP molecule. GTP-bound and GDP-bound versions of a G protein have different conformations and thus have different abilities to bind other proteins. Because of this difference in binding properties, G proteins act like "molecular switches"; the GTP-bound protein typically turns the process on, and hydrolysis of the bound GTP turns it off. Among the components depicted in Figure 8.12, both the SRP and the SRP receptor are G proteins that interact with one another in their GTP-bound states. The hydrolysis of GTP bound to these two proteins occurs between steps 2 and 3 and triggers the release of the signal sequence by the SRP and its insertion into the translocon.

Processing of Newly Synthesized Proteins in the Endoplasmic Reticulum As it enters the RER cisterna, a nascent polypeptide is acted on by a variety of enzymes located within either the membrane or the lumen of the RER. The N-terminal portion containing the signal peptide is removed from most nascent polypeptides by a proteolytic enzyme, the **signal peptidase**. Carbohydrates are added to the nascent protein by the enzyme *oligosaccharyltransferase* (discussed on page 286). Both the signal peptidase and oligosaccharyltransferase are integral membrane proteins associated with the translocon that act on nascent proteins as they enter the ER lumen.

The RER is a major protein processing plant. To meet its obligations, the RER lumen is packed with molecular chaperones that recognize and bind to unfolded or misfolded proteins and give them the opportunity to attain their correct (native) three-dimensional structure (page 288). The ER lumen also contains a number of protein-processing enzymes, such as *protein disulfide isomerase* (*PDI*). Proteins enter the ER lumen with their cysteine residues in the reduced (—SH) state, but they leave the compartment with many of these residues joined to one another as oxidized disulfides (—SS—) (page 53). The formation (and rearrangement) of disulfide bonds is catalyzed by PDI. Disulfide bonds play an important role in maintaining the stability of proteins that are present at the extracellular surface of the plasma membrane or secreted into the extracellular space.

The endoplasmic reticulum is ideally constructed for its role as a port of entry for the biosynthetic pathway of the cell. Its membrane provides a large surface area to which many ribosomes can attach (an estimated 13 million per liver cell). The lumen of the ER cisternae provides a specialized local environment that favors the modification, folding, and assembly of a selected subset of the cell's proteins. The segregation of these newly synthesized proteins in the ER cisternae removes them from the cytosol and allows them to be modified and dispatched toward their ultimate destination, whether outside the cell or within one of the cytoplasm's membranous organelles.

Synthesis of Integral Membrane Proteins on Membrane-Bound Ribosomes Integral membrane proteins—other than those of mitochondria and chloroplasts—are also

[3]GTP proteins generally require accessory proteins to carry out their function. The roles of these proteins are discussed in Chapter 15 and illustrated in Figure 15.21. They will not be considered in the present chapter, even though they are involved in these activities.

synthesized on membrane-bound ribosomes of the ER. These membrane proteins are translocated into the ER membrane as they are synthesized (i.e., cotranslationally) using the same machinery described for the synthesis of secretory and lysosomal proteins (Figure 8.12). Unlike soluble secretory and lysosomal proteins, however, which pass entirely through the ER membrane during translocation, integral proteins contain one or more hydrophobic transmembrane segments (page 134) that are shunted directly from the channel of the translocon into the lipid bilayer. How can such a transfer take place?

The X-ray crystallographic studies of the translocon described above showed the translocon to have a clam-shaped conformation with a groove or seam along one side of the wall where the channel might open and close. As a polypeptide passes through the translocon, it is proposed that this lateral "gate" in the channel continually opens and closes, which gives each segment of the nascent polypeptide an opportunity to partition itself according to its solubility properties into either the aqueous compartment within the translocon channel or the surrounding hydrophobic core of the lipid bilayer. Those segments of the nascent polypeptide that are sufficiently hydrophobic will spontaneously "dissolve" into the lipid bilayer and ultimately become transmembrane segments of an integral membrane protein. This concept has received strong support from an in vitro study in which translocons were given the opportunity to translocate custom-designed nascent proteins that contained test segments of varying hydrophobicity. The more hydrophobic the test segment, the greater the likelihood it would pass through the wall of the translocon and become integrated as a transmembrane segment of the bilayer.

Figure 8.13 shows the synthesis of a pair of integral membrane proteins containing a single transmembrane segment. Single-spanning membrane proteins can have an orientation with their N-terminus facing either the cytosol or the lumen of the ER (and eventually the extracellular space). As noted on page 135, the most common determinant of membrane protein alignment is the presence of positively charged amino acid residues flanking the cytosolic end of a transmembrane segment (see Figure 4.18). During the synthesis of membrane proteins, the inner lining of the translocon is thought to orient the nascent polypeptide, as indicated in Figure 8.13, so that the more positive end faces the cytosol. In multispanning proteins (as shown in Figure 4.32*d*), sequential transmembrane segments typically have opposite orientations. For these proteins, their arrangement within the membrane is determined by the direction in which the first transmembrane segment is inserted. Once that has been determined, every other transmembrane segment has to be rotated 180° before it can exit the translocon. Studies performed with purified components in cell-free systems suggest that a translocon, by itself, is capable of properly orienting transmembrane segments. It would appear that the translocon is more than a simple passageway through the ER membrane; it is a complex machine capable of recognizing various signal sequences and performing complex mechanical activities.

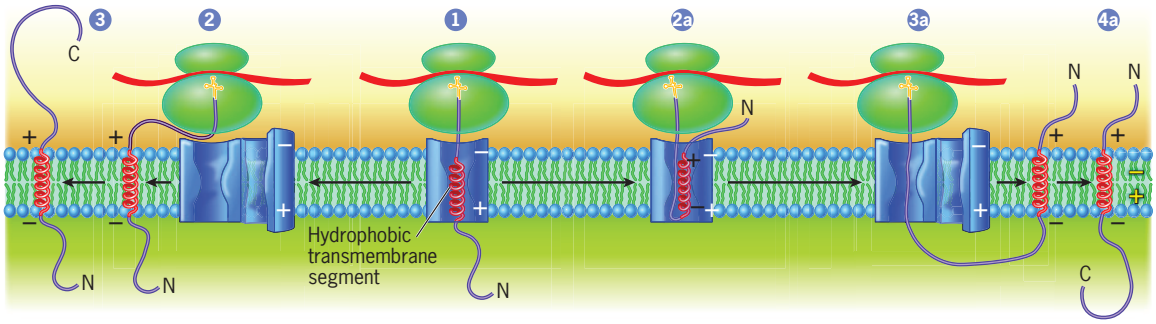


Figure 8.13 **A schematic model for the synthesis of an integral membrane protein** that contains a single transmembrane segment near the N-terminus of the nascent polypeptide. The SRP and the various components of the membrane that were shown in Figure 8.12 are also involved in the synthesis of integral proteins, but they have been omitted for simplicity. The nascent polypeptide enters the translocon just as if it were a secretory protein (step 1). However, the entry of the hydrophobic transmembrane sequence into the pore blocks further translocation of the nascent polypeptide through the channel. Steps 2–3 show the synthesis of a transmembrane protein whose N-terminus is in the lumen of the ER and C-terminus is in the cytosol. In step 2, the lateral gate of the translocon has opened and expelled the transmembrane segment into the bilayer. Step 3 shows the final disposition of the protein. Steps 2a–4a show the synthesis of a transmembrane protein whose C-terminus is in the lumen and N-terminus is in the cystosol. In step 2a, the translocon has reoriented the transmembrane segment, in keeping with its reversed positively and negatively charged flanks. In step 3a, the translocon has opened laterally and expelled the transmembrane segment into the bilayer. Step 4a shows the final disposition of the protein. White-colored + and − signs indicate the proposed charge displayed by the inner lining of the translocon. The difference in charge between the phospholipids of the cytosolic and luminal leaflets of the bilayer (indicated by the yellow + and − signs) is also thought to play a role in determining membrane protein topology. The transmembrane segments are shown as helices based on studies indicating that these regions adopt a helical secondary structure within the ribosomal exit tunnel before they enter the translocon.

Membrane Biosynthesis in the ER Membranes do not arise *de novo*, that is, as new entities from pools of protein and lipid that mix together. Instead, membranes arise from preexisting membranes. Membranes grow as newly synthesized proteins and lipids are inserted into existing membranes in the ER. As will be apparent in the following discussion, membrane components move from the ER to virtually every other compartment in the cell. As the membrane moves from one compartment to the next, its proteins and lipids are modified by enzymes that reside in the cell's various organelles. These modifications contribute to giving each membrane compartment a unique composition and distinct identity.

Keep in mind that cellular membranes are asymmetric: the two phospholipid layers (leaflets) of a membrane have different compositions (page 128). This asymmetry is established initially in the endoplasmic reticulum. Asymmetry is maintained as membrane carriers bud from one compartment and fuse to the next. As a result, domains situated at the cytosolic surface of the ER membrane can be identified on the cytosolic surface of transport vesicles, the cytosolic surface of Golgi cisternae, and the internal (cytoplasmic) surface of the plasma membrane (Figure 8.14). Similarly, domains situated at the luminal surface of the ER membrane maintain their orientation and are found at the external (exoplasmic) surface of the plasma membrane. In fact, in many ways, including its high calcium concentration and abundance of proteins with disulfide bonds and carbohydrate chains, the lumen of the ER (as well as other compartments of the secretory pathway) is a lot like the extracellular space.

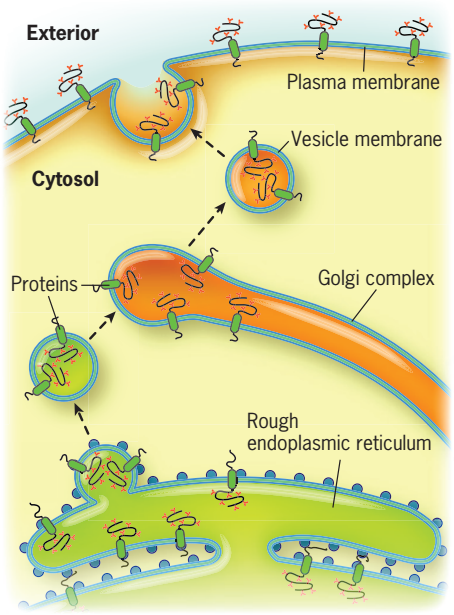


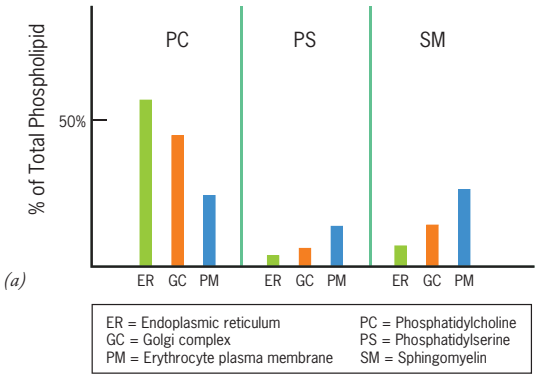
Figure 8.14 **Maintenance of membrane asymmetry.** As each protein is synthesized in the rough ER, it becomes inserted into the lipid bilayer in a predictable orientation determined by its amino acid sequence. This orientation is maintained throughout its travels in the endomembrane system, as illustrated in this figure. The carbohydrate chains, which are first added in the ER, provide a convenient way to assess membrane sidedness because they are always present on the cisternal side of the cytoplasmic membranes, which becomes the exoplasmic side of the plasma membrane following the fusion of vesicles with the plasma membrane.

Synthesis of Membrane Lipids Most membrane lipids are synthesized entirely within the endoplasmic reticulum. The major exceptions are (1) sphingomyelin and glycolipids, whose synthesis begins in the ER and is completed in the Golgi complex, and (2) some of the unique lipids of the mitochondrial and chloroplast membranes, which are synthesized by enzymes that reside in those membranes. The enzymes involved in the synthesis of phospholipids are themselves integral proteins of the ER membrane with their active sites facing the cytosol. Newly synthesized phospholipids are inserted into the half of the bilayer facing the cytosol. Some of these lipid molecules are later flipped into the opposite leaflet through the action of enzymes called *flippases*. Lipids are carried from the ER to the Golgi complex and plasma membrane as part of the bilayer that makes up the walls of transport vesicles.

The membranes of different organelles have markedly different lipid composition (Figure 8.15*a*), which indicates that changes take place as membrane flows through the cell. Several factors may contribute to such changes (Figure 8.15*b*):

1. Most membranous organelles contain enzymes that modify lipids already present within a membrane, converting one type of phospholipid (e.g., phosphatidylserine) to another (e.g., phosphatidylcholine) (step 1, Figure 8.15*b*).
2. When vesicles bud from a compartment (as in Figure 8.2*a*), some types of phospholipids may be preferentially included within the membrane of the forming vesicle, while other types may be left behind (step 2, Figure 8.15*b*).
3. Cells contain **lipid-transfer proteins** that can bind and transport lipids *through the aqueous cytosol* from one membrane compartment to another (step 3, Figure 8.15*b*). These proteins facilitate the movement of specific lipids from the ER to other organelles without the involvement of transport vesicles. Lipid transfer is thought to occur at sites where the ER comes into very close proximity to the outer membrane of other organelles.

Glycosylation in the Rough Endoplasmic Reticulum Nearly all of the proteins produced on membrane-bound ribosomes—whether integral components of a membrane, soluble lysosomal or vacuolar enzymes, or parts of the extracellular matrix—become glycoproteins. Carbohydrate groups have key roles in the function of many glycoproteins, particularly as binding sites in their interactions with other macromolecules, as occurs during many cellular processes. They also aid in the proper folding and stabilization of the protein to which they are attached. The sequences of sugars that comprise the oligosaccharides of glycoproteins are highly specific; if the oligosaccharides are isolated from a purified protein of a



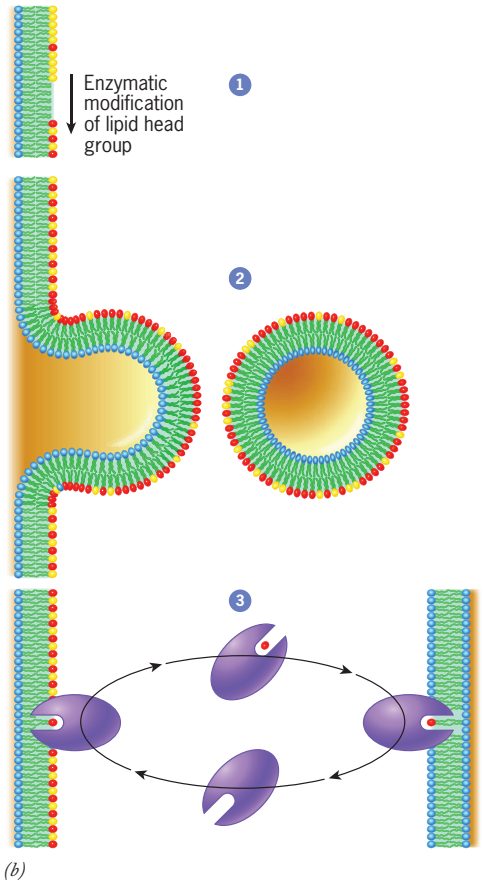


Figure 8.15 **Modifying the lipid composition of membranes.** (*a*) Histogram indicating percentage of each of three phospholipids (phosphatidylcholine, phosphatidylserine, and sphingomyelin) in three different cellular membranes (ER, Golgi complex, and plasma membrane). The percentage of each lipid changes gradually as membrane flows from the ER to the Golgi to the plasma membrane. (*b*) Schematic diagram showing three distinct mechanisms that might explain how the phospholipid composition of one membrane in the endomembrane system can be different from another membrane in the system, even though the membranous compartments are spatially and temporally continuous. (1) The head groups of phospholipids of the bilayer are modified enzymatically; (2) the membrane of a forming vesicle contains a different phospholipid composition from the membrane it buds from; (3) lipids can be removed from one membrane and inserted into another membrane by lipid-transfer proteins.

given type of cell, their sequence is consistent and predictable. How is the order of sugars in oligosaccharides achieved?

The addition of sugars to an oligosaccharide chain is catalyzed by a large family of membrane-bound enzymes called **glycosyltransferases**. Each of these enzymes transfers a specific monosaccharide from a nucleotide sugar, such as GDP-mannose or UDP-*N*-acetylglucosamine (Figure 8.16), to the growing end of the carbohydrate chain. The sequence in which sugars are transferred during assembly of an oligosaccharide depends on the sequence of action of glycosyltransferases that participate in the process. This in turn depends on the location of specific enzymes within the various membranes of the secretory pathway. Thus the arrangement of sugars in the oligosaccharide chains of a glycoprotein depends on the spatial localization of particular enzymes in the assembly line.

The initial steps in the assembly of *N*-linked oligosaccharides (as opposed to *O*-linked oligosaccharides; see Figure 4.11) of both soluble proteins and integral membrane proteins are shown in Figure 8.16. The basal, or core, segment of each carbohydrate chain is not assembled on the protein itself but put together independently on a lipid carrier and then transferred, as a block, to specific asparagine residues of the polypeptide. This lipid carrier, which is named **dolichol phosphate**, is embedded in the ER membrane. Sugars are added to the dolichol phosphate molecule one at a time by membrane-bound glycosyltransferases, beginning with step 1 of Figure 8.16. This part of the glycosylation process is essentially invariant; in mammalian cells, it begins with the transfer of *N*-acetylglucosamine 1-phosphate, followed by the transfer of another *N*-acetylglucosamine, then nine mannose and three glucose units in the precise pattern indicated in Figure 8.16. This preassembled block of 14 sugars is then transferred by the ER enzyme oligosaccharyltransferase from dolichol phosphate to certain asparagines in the nascent polypeptide (step 10, Figure 8.16) as the polypeptide is being translocated into the ER lumen.

Mutations that lead to the total absence of *N*-glycosylation cause the death of embryos prior to implantation. However, mutations that lead to partial disruption of the glycosylation pathway in the ER are responsible for serious inherited disorders affecting nearly every organ system. These diseases are called Congenital Diseases of Glycosylation, or CDGs and they are usually identified through blood tests that detect abnormal glycosylation of serum proteins. One of these diseases, CDG1b, can be managed through a remarkably simple

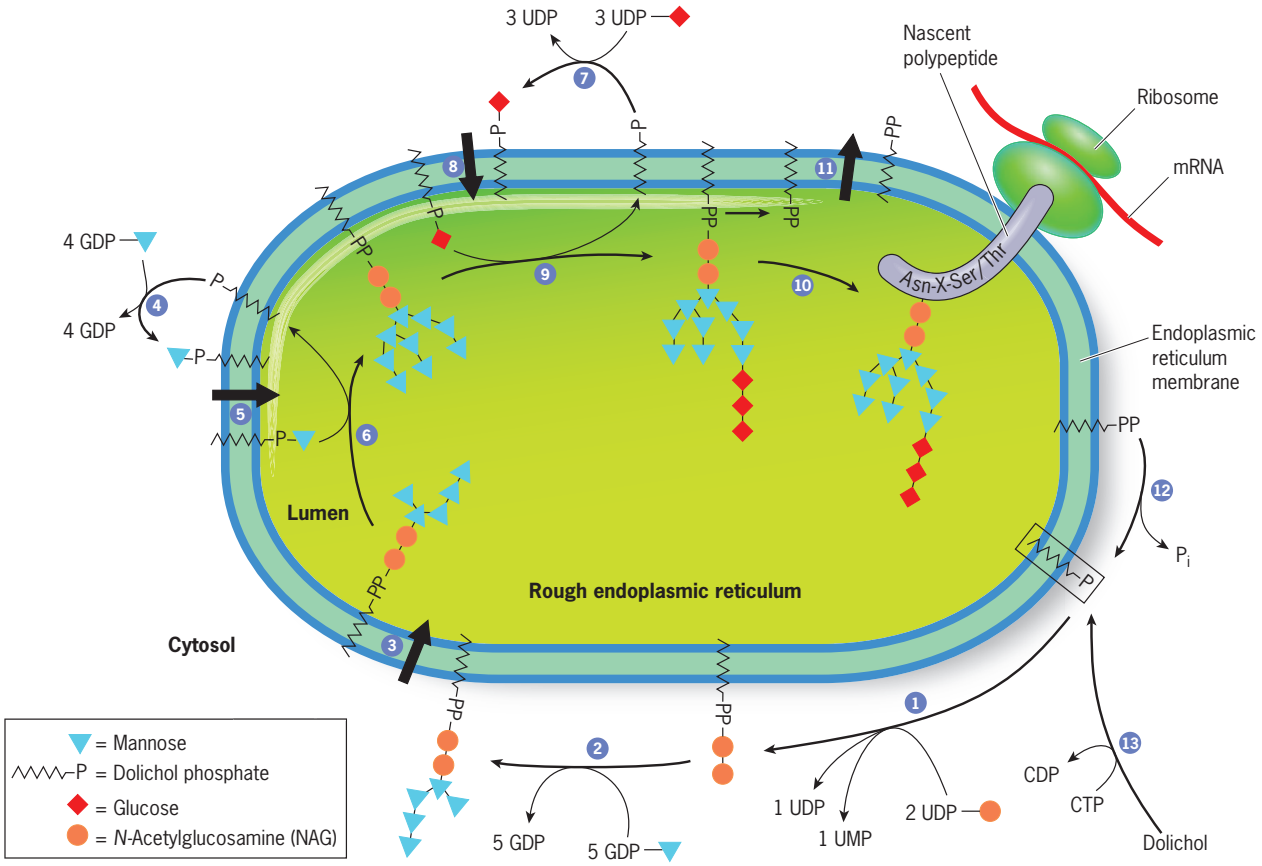


Figure 8.16 **Steps in the synthesis of the core portion of an *N*-linked oligosaccharide in the rough ER.** The first seven sugars (five mannose and two NAG residues) are transferred one at a time to the dolichol-PP on the cytosolic side of the ER membrane (steps 1 and 2). At this stage, the dolichol with its attached oligosaccharide is then flipped across the membrane (step 3), and the remaining sugars (four mannose and three glucose residues) are attached on the luminal side of the membrane. These latter sugars are attached one at a time on the cytosolic side of the membrane to the end of a dolichol phosphate molecule (as in steps 4 and 7), which then flips across the membrane (steps 5 and 8) and donates its sugar to the growing end of the oligosaccharide chain (steps 6 and 9). Once the oligosaccharide is completely assembled, it is transferred enzymatically to an asparagine residue (within the sequence N-X-S/T) of the nascent polypeptide (step 10). The dolichol-PP is flipped back across the membrane (step 11) and is ready to begin accepting sugars again (steps 12 and 13). (From D. Voet and J. G. Voet, Biochemistry, 2e, copyright 1995; John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

treatment. CDG1b results from the deficiency of the enzyme phosphomannose isomerase, which catalyzes the conversion of fructose 6-phosphate to mannose 6-phosphate, a crucial reaction in the pathway that makes mannose available for incorporation into oligosaccharides. The disease can be managed by providing patients with oral supplements of mannose. The treatment was first tested in a boy who was dying from uncontrolled gastrointestinal bleeding, one of the usual complications of the disease. Within months of taking mannose supplements the child was living a normal life.

Shortly after it is transferred to the nascent polypeptide, the oligosaccharide chain undergoes a process of gradual modification. This modification begins in the ER with the enzymatic removal of two of the three terminal glucose residues (step 1, Figure 8.17). This sets the stage for an important event in the life of a newly synthesized glycoprotein in which it is screened by a system of **quality control** that determines whether or not it is fit to move on to the next compartment of the biosynthetic pathway. To begin this screening process, each glycoprotein, which at this stage contains a single remaining glucose, binds to an ER chaperone (calnexin or calreticulin) (step 2). Removal of the remaining glucose by glucosidase II leads to release of the glycoprotein from the chaperone (step 3). If a glycoprotein at this stage has not completed its folding or has misfolded, it is recognized by a conformation-sensing enzyme (called UGGT) that adds a single glucose residue back to one of the mannose residues at the exposed end of the recently trimmed oligosaccharide (step 4). UGGT recognizes incompletely folded or misfolded proteins because they display exposed hydrophobic residues

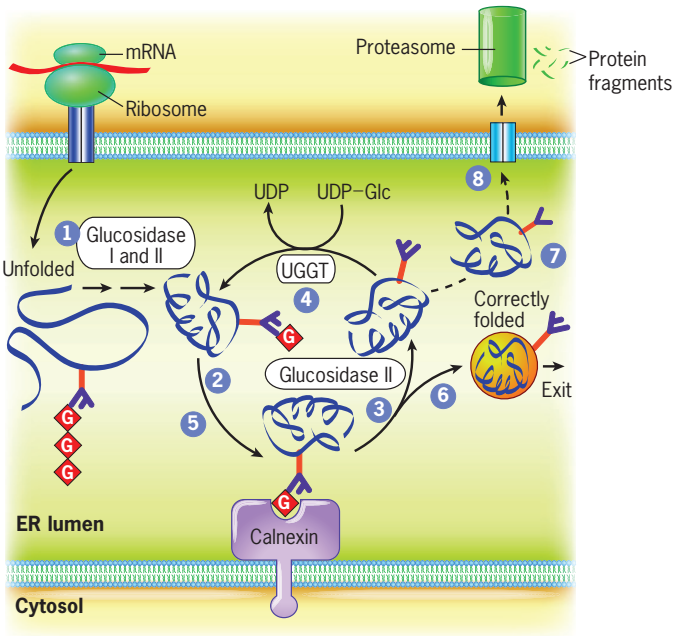


Figure 8.17 **Quality control: ensuring that misfolded proteins do not proceed forward.** Based on this proposed mechanism, misfolded proteins are recognized by a glucosyltransferase (UGGT) which adds a glucose to the end of the oligosaccharide chains. Glycoproteins containing monoglucosylated oligosaccharides are recognized by the membrane-bound chaperone calnexin and given an opportunity to achieve their correctly folded (native) state. If that does not occur after repeated attempts, the protein is dislocated to the cytosol and destroyed. The steps are described in the text. A soluble chaperone (calreticulin) participates in this same quality-control pathway. (L. Ellgaard et al., Science 286:984, 1999; copyright 1999, Reprinted with permission from AAAS.)

that are absent from properly folded proteins. Once the glucose residue has been added, the "tagged" glycoprotein is recognized by the same ER chaperones, which give the protein another chance to fold properly (step 5). After a period of time with the chaperone, the added glucose residue is removed and the conformation-sensing enzyme checks it again to see if it has achieved its proper three-dimensional structure. If it is still partially unfolded or misfolded, another glucose residue is added and the process is repeated until, eventually, the glycoprotein has either folded correctly and continues on its way (step 6), or remains misfolded and is destroyed. Studies suggest that the "decision" to destroy the defective protein begins with the activity of a slow-acting enzyme in the ER that trims a mannose residue from an exposed end of the oligosaccharide of a protein that has been in the ER for an extended period. Once one or more of these mannose residues have been removed (step 7), the protein can no longer be recycled and, instead, is sentenced to degradation (step 8).

We will pick up the story of protein glycosylation again on page 292, where the oligosaccharide that is assembled in the ER is enlarged as it passes through the Golgi complex on its journey through the biosynthetic pathway.

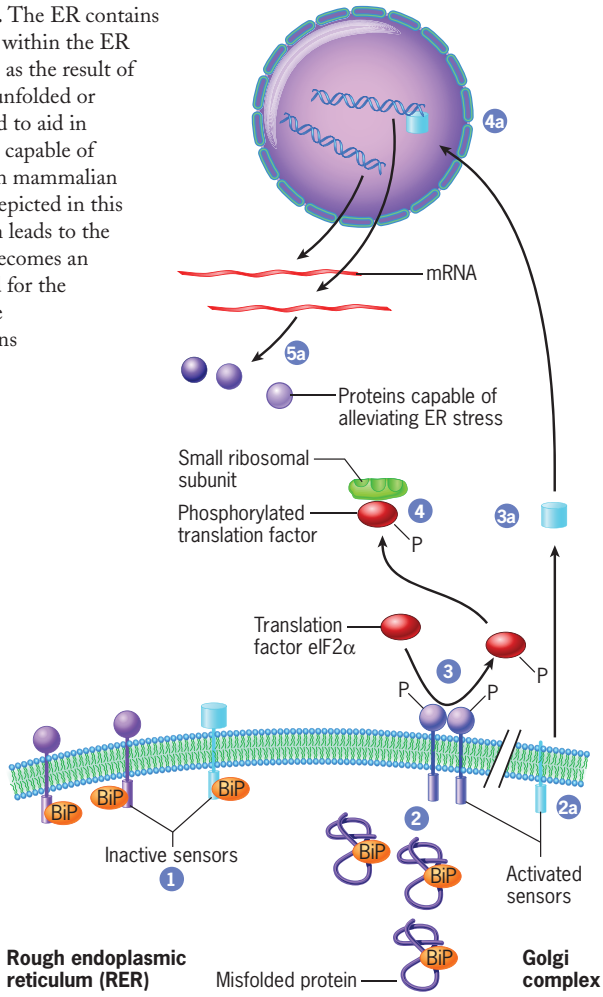
Mechanisms that Ensure the Destruction of Misfolded Proteins We have just seen how proteins that fail to fold properly are detected by ER enzymes. It was a surprise to discover that misfolded proteins are not destroyed in the ER, but instead are transported into the cytosol by a process of *dislocation*. The precise mechanism of dislocation remains a matter of debate. Once in the cytosol, the oligosaccharide chains are removed, and the misfolded proteins are destroyed in proteasomes, which are protein-degrading machines whose structure and function are discussed in Section 12.7. This process, known as *ER-associated degradation* (*ERAD*), ensures that aberrant proteins are not transported to other parts of the cell, but it can have negative consequences. In most patients with cystic fibrosis, the plasma membrane of epithelial cells is lacking the abnormal protein encoded by the cystic fibrosis gene (page 162). In these cases, the mutant protein is destroyed by the quality-control process of the ER and thus fails to reach the cell surface.

Under certain circumstances, misfolded proteins can be generated in the ER at a rate faster than they can be exported to the cytoplasm. The accumulation of misfolded proteins, which is potentially lethal to cells, triggers a comprehensive "plan of action" within the cell known as the **unfolded protein response (UPR)**. The ER contains protein sensors that monitor the concentration of unfolded or misfolded proteins in the ER lumen. According to the prevailing model outlined in Figure 8.18, the sensors are normally kept in an inactive state by molecular chaperones, particularly BiP. If circumstances should lead to an accumulation of misfolded proteins, the BiP molecules in the ER lumen are called into service as chaperones for the misfolded proteins, rendering them incapable of inhibiting the sensors. Activation of the sensors leads to a multitude of signals that are transmitted into both the nucleus and cytosol and result in

- the expression of hundreds of different genes whose encoded proteins have the potential to alleviate stressful conditions within the ER. These include genes that encode (1) ER-based molecular chaperones that can help misfolded proteins reach the native state, (2) proteins involved in the transport of the proteins out of the ER, and (3) proteins involved in the selective destruction of abnormal proteins as discussed above.
- phosphorylation of a key protein (eIFα) required for protein synthesis. This modification inhibits protein synthesis and decreases the flow of newly synthesized proteins into the ER. This gives the cell an opportunity to remove those proteins that are already present in the ER lumen.

Interestingly, the UPR is more than a cell-survival mechanism; it also includes the activation of a pathway that leads to the death of the cell. It is presumed that the UPR provides a mechanism to relieve itself of the stressful conditions. If these corrective measures are not successful, the cell-death pathway is triggered and the cell is destroyed.

Figure 8.18 **A model of the mammalian unfolded protein response (UPR).** The ER contains transmembrane proteins that function as sensors of stressful events that occur within the ER lumen. Under normal conditions, these sensors are present in an inactive state as the result of their association with chaperones, particularly BiP (step 1). If the number of unfolded or misfolded proteins should increase to a high level, the chaperones are recruited to aid in protein folding, which leaves the sensors in their unbound, activated state and capable of initiating a UPR. At least three distinct UPR pathways have been identified in mammalian cells, each activated by a different protein sensor. Two of these pathways are depicted in this illustration. In one of these pathways, the release of the inhibitory BiP protein leads to the dimerization of a sensor (called PERK) (step 2). In its dimeric state, PERK becomes an activated protein kinase that phosphorylates a protein (eIF2α) that is required for the initiation of protein synthesis (step 3). This translation factor is inactive in the phosphorylated state, which stops the cell from synthesizing additional proteins in the ER (step 4), giving the cell more time to process those proteins already present in the ER lumen. In the second pathway depicted here, release of the inhibitory BiP protein allows the sensor (called ATF6) to move on to the Golgi complex where the cytosolic domain of the protein is cleaved away from its transmembrane domain (step 2a). The cytosolic portion of the sensor diffuses through the cytosol (step 3a) and into the nucleus (step 4a), where it stimulates the expression of genes whose encoded proteins can alleviate the stress in the ER (step 5a). These include chaperones, coat proteins that form on transport vesicles, and proteins of the quality-control machinery. (Discussion of the third protein sensor (IRE1) can be found in *Science* 334:1081, 2011.)



From the ER to the Golgi Complex: The First Step in Vesicular Transport

The RER cisternae contain specialized exit sites that are devoid of ribosomes and serve as places where the first transport vesicles in the biosynthetic pathway are formed. The trip from the ER toward the Golgi complex can be followed visually in living cells by tagging secretory proteins with the green fluorescent protein (GFP) as described on page 273. Using this technique it is found that soon after they bud from the ER membrane, transport vesicles fuse with one another to form larger vesicles and interconnected tubules in the region between the ER and Golgi complex. This region has been named the *ERGIC* (*e*ndoplasmic *r*eticulum *G*olgi *i*ntermediate *c*ompartment), and the vesicular-tubular carriers that form there are called *VTCs* (see Figure 8.25*a*). Once formed, the VTCs move farther away from the ER toward the Golgi complex. The movement of two of these vesicular-tubular membranous carriers from the ERGIC to the Golgi complex is shown in Figure 8.19. Movement of VTCs occurs along tracks composed of microtubules.

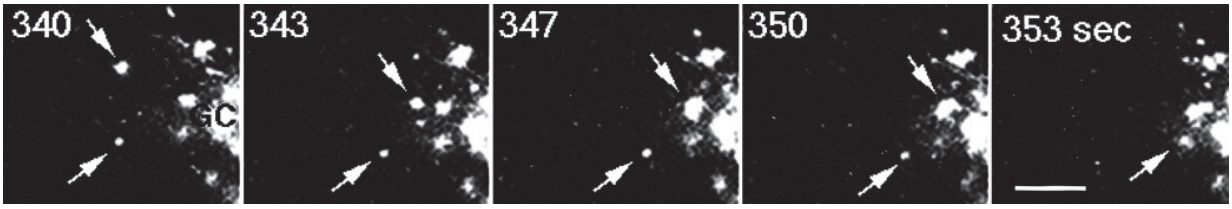


Figure 8.19 **Visualizing membrane traffic with the use of a fluorescent tag.** This series of photographs shows a small portion of a living mammalian cell that has been infected with the vesicular stomatitis virus (VSV) containing a *VSVG-GFP* chimeric gene (page 274). Once it is synthesized in the RER, the fusion protein emits a green fluorescence, which can be followed as the protein moves through the cell. In the series of photographs shown here, two vesicular-tubular carriers (VTCs) (arrows) containing the fluorescent protein have budded from the ER and are moving toward the Golgi complex (GC). The series of events depicted here took place over a period of 13 seconds. Bar represents 6 μm. (From John F. Presley et al., Nature 389:82, 1997; © 1997, reprinted by permission of Macmillan Publishers Ltd.)

REVIEW

1. What are the major morphological differences between the RER and SER? What are the major differences in their functions?
2. Describe the steps that occur between the time a ribosome attaches to a messenger RNA encoding a secretory protein and the time the protein leaves the RER.
3. How are newly synthesized integral proteins inserted into a membrane?
4. Describe some of the ways that membranous organelles can maintain their unique compositions despite the continuous traffic of membranes and materials moving through them.
5. Describe how membrane asymmetry is maintained as membrane moves from the ER to the plasma membrane.
6. Describe the mechanisms by which the cell ensures that misfolded proteins (1) will not go unrecognized within the ER and (2) will not accumulate to excessive levels within the ER lumen.

8.4 | The Golgi Complex

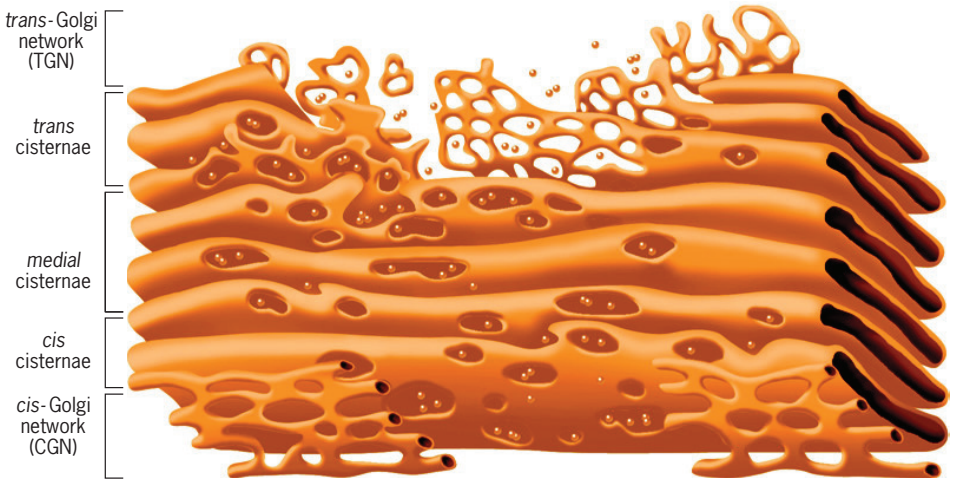
In the latter years of the nineteenth century, an Italian biologist, Camillo Golgi, was inventing new types of staining procedures that might reveal the organization of nerve cells within the central nervous system. In 1898, Golgi applied a metallic stain to nerve cells from the cerebellum and discovered a darkly stained reticular network located near the cell nucleus. This network, which was later identified in other cell types and named the **Golgi complex**, helped earn its discoverer the Nobel Prize in 1906. The Golgi complex remained a center of controversy for decades between those who believed that the organelle existed in living cells and those who believed it was an *artifact*, that is, an artificial structure formed during preparation for microscopy. It wasn't until the Golgi complex was clearly identified in unfixed, freeze-fractured cells (see Figure 18.17) that its existence was verified beyond reasonable doubt.

The Golgi complex has a characteristic morphology consisting primarily of flattened, disklike, membranous cisternae with dilated rims and associated vesicles and tubules (Figure 8.20*a*). The cisternae, whose diameters are typically 0.5 to 1.0 μm, are arranged in an orderly stack, much like a stack of pancakes, and are curved so as to resemble a shallow bowl (Figure 8.20*b*).[4] Typically, a Golgi stack contains fewer than eight cisternae. An individual cell may contain from a few to several thousand distinct stacks, depending on the cell type. The Golgi stacks in mammalian cells are interconnected to form a single, large ribbon-like complex (Figure 8.20*c*) situated adjacent to the cell's nucleus (Figure 8.20*d*). A closer look at an individual cisterna suggests that vesicles bud from a peripheral tubular domain of each cisterna (Figure 8.20*e*). As discussed later, many of these vesicles contain a distinct protein coat that is visible in Figure 8.20*e*.

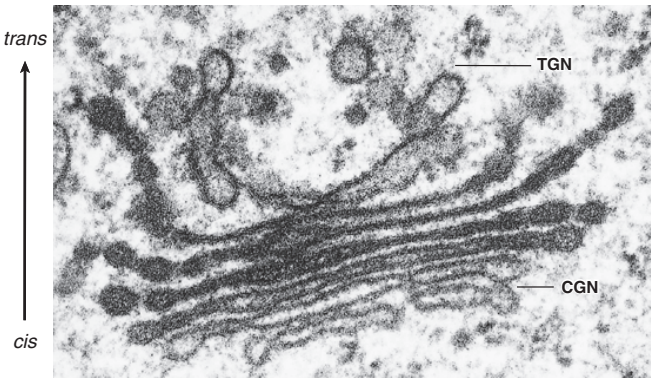
The Golgi complex is divided into several functionally distinct compartments arranged along an axis from the *cis* or entry face closest to the ER to the *trans* or exit face at the opposite end of the stack (Figures 8.20*a*,*b*). The *cis*-most face of the organelle is composed of an interconnected network of tubules referred to as the ***cis* Golgi network (CGN)**. The CGN is thought to function primarily as a sorting station that distinguishes between proteins to be shipped back to the ER (page 298) and those that are allowed to proceed to the next Golgi station. The bulk of the Golgi complex consists of a series of large, flattened cisternae, which are divided into ***cis***, ***medial***, and ***trans* cisternae** (Figure 8.20*a*). The *trans*-most face of the organelle contains a distinct network of tubules and vesicles called the ***trans* Golgi network (TGN)**. The TGN is a sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or to various intracellular destinations. The membranous elements of the Golgi complex are thought to be supported mechanically by a peripheral membrane skeleton or scaffold composed of a variety of proteins, including members of the spectrin, ankyrin, and actin families—proteins that are also present as part of the plasma membrane skeleton (page 147). The Golgi scaffold may be physically linked with motor proteins that direct the movement of vesicles and tubules entering and exiting the Golgi complex. A separate group of fibrous proteins are thought to form a Golgi "matrix," that plays a key role in the disassembly and reassembly of the Golgi complex during mitosis.

[4]A single Golgi stack in plant cells is sometimes called a *dictyosome*.

Figure 8.20 **The Golgi complex.** (*opposite*) (*a*) Schematic model of a portion of a Golgi complex from an epithelial cell of the male rat reproductive tract. The elements of the *cis* and *trans* compartments are often discontinuous and appear as tubular networks. (*b*) Electron micrograph of a portion of a tobacco root cap cell showing the *cis* to *trans* polarity of the Golgi stack. (*c*) Electron tomographic image of a slice from a mouse pancreatic beta cell that synthesizes and secretes the protein insulin. The individual Golgi stacks are seen to be interconnected to form a continuous ribbon. The *trans* face (or TGN) of each Golgi stack has been colored red and the *cis* face has been colored light blue. Cellular tomography is discussed in Section 18.2. (*d*) Fluorescence micrograph of a cultured mammalian cell. The position of the Golgi complex is revealed by the red fluorescence, which marks the localization of antibodies to a COPI coat protein. (*e*) Electron micrograph of a single isolated Golgi cisterna showing two distinct domains, a concave central domain and an irregular peripheral domain. The peripheral domain consists of a tubular network from which protein-coated buds are being pinched off. (A: From A. Rambourg and Y. Clermont, Copyright 1990, Rockefeller University Press. Originally published in The Journal of Cell Biology, Volume 51:195. B: Courtesy of Thomas H. Giddings and Andrew Staehelin; C: Reproduced courtesy of Brad Marsh, Institute for Molecular Bioscience, The University of Queensland, Australia. Reproduced with permission from Rockefeller University Press. Image originally published in J. Cell Biol. 187:449, 2009; D: from Andrei V. Nikonov et al., J. Cell Biol. 158:500, 2002; Courtesy of Gert Kreibich, reproduced with permission of The Rockefeller University Press; E: from Peggy J. Weidman and John Heuser, Trends Cell Biol. 5:303, 1995; © 1995, with permission from Elsevier Science.)



(a)



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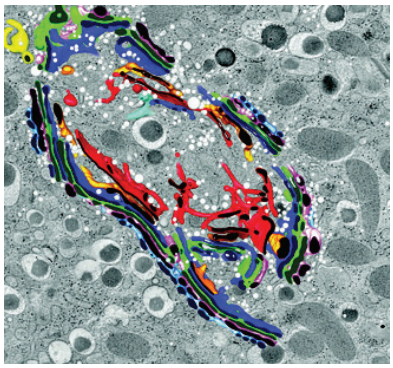

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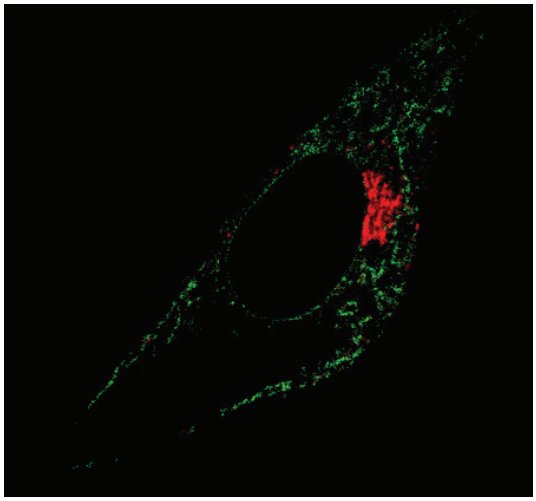

(d)

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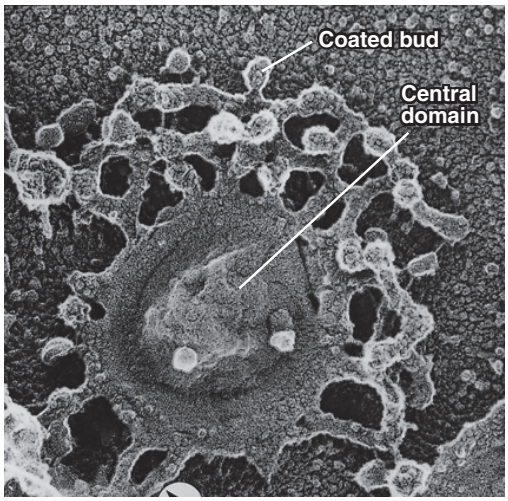


(e)

0.1 µm

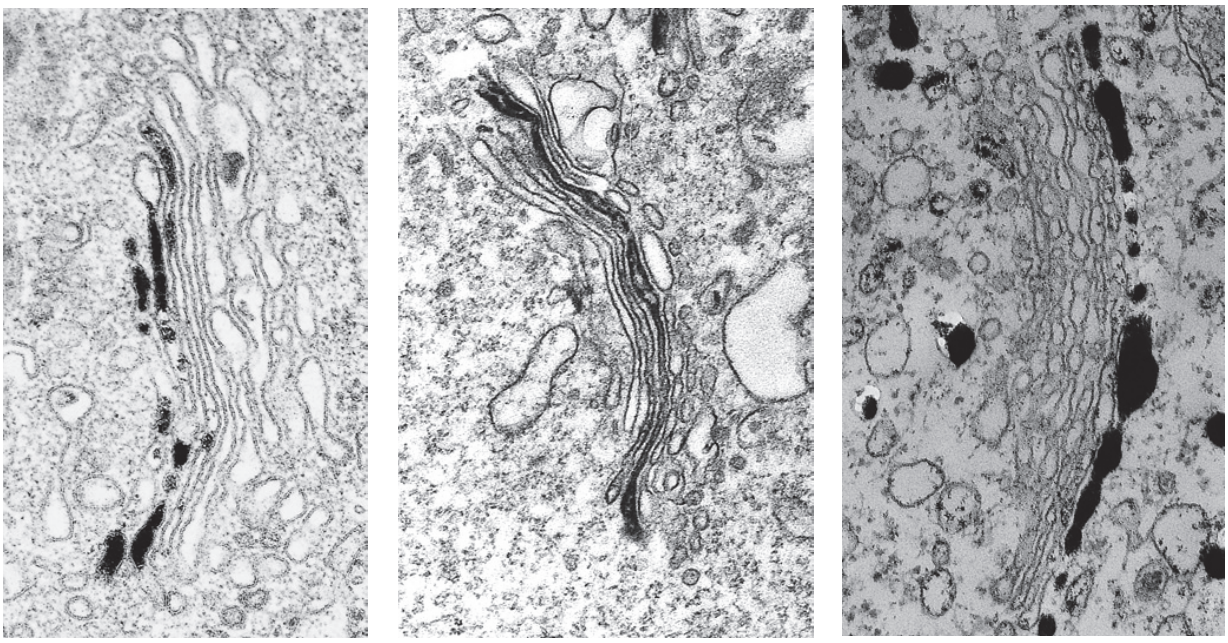


Figure 8.21 **Regional differences in membrane composition across the Golgi stack.** (*a*) Reduced osmium tetroxide preferentially impregnates the *cis* cisternae of the Golgi complex. (*b*) The enzyme mannosidase II, which is involved in trimming the mannose residues from the core oligosaccharide as described in the text, is preferentially localized in the *medial* cisternae. (*c*) The enzyme nucleoside diphosphatase, which splits dinucleotides (e.g., UDP) after they have donated their sugar, is preferentially localized in the *trans* cisternae. (A,C: From Robert S. Decker, J. Cell Biol. 61:603, 1974; B: From Angel Velasco et al., J. Cell Biol. 122:41, 1993; all reproduced with permission of The Rockefeller University Press.)

Figure 8.21 provides visual evidence that the Golgi complex is not uniform in composition from one end to the other. Differences in composition of the membrane compartments from the *cis* to the *trans* face reflect the fact that the Golgi complex is primarily a "processing plant." Newly synthesized membrane proteins, as well as secretory and lysosomal proteins, leave the ER and enter the Golgi complex at its *cis* face and then pass across the stack to the *trans* face. As they progress along the stack, proteins that were originally synthesized in the rough ER are sequentially modified in specific ways. In the best-studied Golgi activity, a protein's carbohydrates are modified by a series of stepwise enzymatic reactions, as discussed in the following section.

Glycosylation in the Golgi Complex

The Golgi complex plays a key role in the assembly of the carbohydrate component of glycoproteins and glycolipids. When we left the topic of synthesis of *N*-linked carbohydrate chains on page 288, the glucose residues had just been removed from the ends of the core oligosaccharide. As newly synthesized soluble and membrane glycoproteins pass through the *cis* and *medial* cisternae of the Golgi stack, most of the mannose residues are also removed from the core oligosaccharides, and other sugars are added sequentially by various glycosyltransferases.

In the Golgi complex, as in the RER, the sequence in which sugars are incorporated into oligosaccharides is determined by the spatial arrangement of the specific glycosyltransferases that come into contact with the newly synthesized protein as it moves through the Golgi stack. The enzyme sialyltransferase, for example, which places a sialic acid at the terminal position of the chain in animal cells, is localized in the *trans* face of the Golgi stack, as would be expected if newly synthesized glycoproteins were continually moving toward this part of the organelle. In contrast to the glycosylation events that occur in the ER, which assemble a single core oligosaccharide, the glycosylation steps in the Golgi complex can be quite varied, producing carbohydrate domains of remarkable sequence diversity. One of many possible glycosylation pathways is shown in Figure 8.22. Unlike the *N*-linked oligosaccharides, whose synthesis begins in the ER, those attached to proteins by *O*-linkages (Figure 4.11) are assembled entirely within the Golgi complex.

The Golgi complex is also the site of synthesis of most of a cell's complex polysaccharides, including the glycosaminoglycan chains of the proteoglycan shown in Figure 7.9*a* and the pectins and hemicellulose found in the cell walls of plants (see Figure 7.36*c*).

The Movement of Materials through the Golgi Complex

That materials move through the various compartments of the Golgi complex has long been established; however, two contrasting views of the way this occurs have dominated the field for years. Up until the mid-1980s, it was generally accepted that Golgi cisternae were transient structures. It was supposed that Golgi cisternae formed at the *cis* face of the

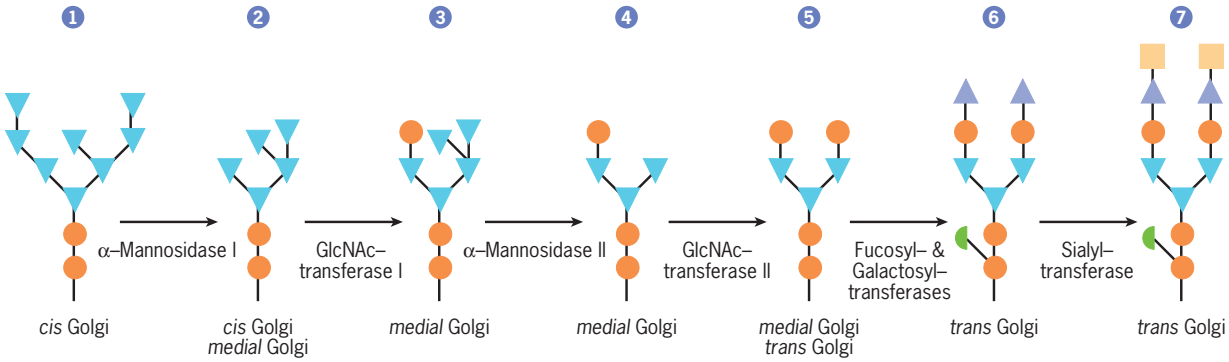


Figure 8.22 **Steps in the glycosylation of a typical mammalian *N*-linked oligosaccharide in the Golgi complex.** Following the removal of the three glucose residues, various mannose residues are subsequently removed, while a variety of sugars (*N*-acetylglucosamine, galactose, fucose, and sialic acid) are added to the oligosaccharide by specific glycosyltransferases. These enzymes are integral membrane proteins whose active sites face the lumen of the Golgi cisternae. This is only one of numerous glycosylation pathways.

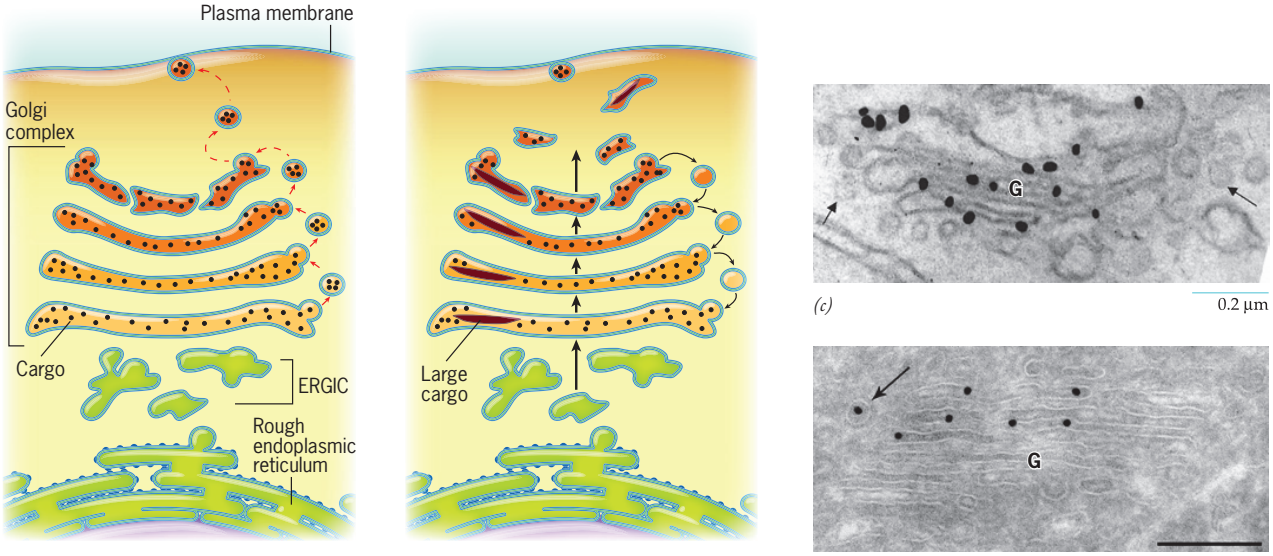
stack by fusion of membranous carriers from the ER and ERGIC and that each cisterna physically moved from the *cis* to the *trans* end of the stack, changing in composition as it progressed. This is known as the *cisternal maturation model* because, according to the model, each cisterna "matures" into the next cisterna along the stack.

From the mid-1980s to the mid-1990s, the maturation model of Golgi movement was largely abandoned and replaced by an alternate model, which proposed that the cisternae of a Golgi stack remain in place as stable compartments. In this latter model, which is known as the *vesicular transport model*, cargo (i.e., secretory, lysosomal, and membrane proteins) is shuttled through the Golgi stack, from the CGN to the TGN, in vesicles that bud from one membrane compartment and fuse with a neighboring compartment farther along the stack. The vesicular transport model is illustrated in Figure 8.23*a*, and its acceptance was based largely on the following observations:

1. Each of the various Golgi cisternae of a stack has a distinct population of resident enzymes (Figure 8.21). How could the various cisternae have such different properties if each cisterna was giving rise to the next one in line, as suggested by the cisternal maturation model?
2. Large numbers of vesicles can be seen in electron micrographs to bud from the rims of Golgi cisternae. In 1983, James Rothman and his colleagues at Stanford University demonstrated, using cell-free preparations of Golgi membranes (page 276), that transport vesicles were capable of budding from one Golgi cisterna and fusing with another Golgi cisterna in vitro. This landmark experiment formed the basis for a hypothesis suggesting that inside the cell, cargo-bearing vesicles budded from *cis* cisternae and fused with cisternae situated at a more *trans* position in the stack.

Although both models of Golgi function continue to have their proponents, the consensus of opinion has shifted back to the cisternal maturation model. Several of the major reasons for this shift can be noted:

- The cisternal maturation model envisions a highly dynamic Golgi complex in which the major elements of the organelle, the cisternae, are continually being formed at the *cis* face and dispersed at the *trans* face. According to this view, the very existence of the Golgi complex itself depends on the continual influx of transport carriers from the ER and ERGIC. As predicted by the cisternal maturation model, when the formation of transport carriers from the ER is blocked either by treatment of cells with specific drugs or the use of temperature-sensitive mutants (page 275), the Golgi complex simply disappears. When the drugs are removed or the mutant cells are returned to the permissive temperature, the Golgi complex rapidly reassembles as ER–to–Golgi transport is renewed.
- Certain materials that are produced in the endoplasmic reticulum and travel through the Golgi complex can be shown to remain within the Golgi cisternae and never appear within Golgi-associated transport vesicles. For example, studies on fibroblasts indicate that large complexes of procollagen molecules (the precursors of extracellular collagen) (illustrated by the red objects in Figure 8.23*b*) move from the *cis* cisternae to the *trans* cisternae without ever leaving the cisternal lumen.
- It was assumed until the mid-1990s that transport vesicles always moved in a "forward" (*anterograde*) direction, that is, from a *cis* origin to a more *trans* destination. But a large body of evidence has indicated that vesicles can move in a "backward" (*retrograde*) direction, that is, from a *trans* donor membrane to a *cis* acceptor membrane.
- Studies on live budding yeast cells containing fluorescently labeled Golgi proteins have shown directly that the composition of an individual Golgi cisterna can change over time—from one that contains early (*cis*) Golgi resident proteins to one that contains late (*trans*) Golgi resident proteins. The results of this experiment are shown in the opening micrograph of Chapter 18, and they are not



(a) Vesicular transport model *(b)* Cisternal maturation model *(c)* *(d)*

Figure 8.23 **The dynamics of transport through the Golgi complex.** (*a*) In the vesicular transport model, cargo (black dots) is carried in an anterograde direction by transport vesicles, while the cisternae themselves remain as stable elements. (*b*) In the cisternal maturation model, the cisternae progress gradually from a *cis* to a *trans* position and then disperse at the TGN. Transport vesicles carry resident Golgi enzymes (indicated by the colored vesicles) in a retrograde direction. The red lens-shaped objects represent large cargo materials, such as procollagen complexes of fibroblasts. (*c*) Electron micrograph of an area of Golgi complex in a thin frozen section of a cell that had been infected with vesicular stomatitis virus (VSV). The black dots are nanosized gold particles bound by means of antibodies to VSVG protein, an anterograde cargo molecule. The cargo is restricted to the cisternae and does not appear in nearby vesicles (arrows). (*d*) Electron micrograph of similar nature to that of *c* but, in this case, the gold particles are not bound to cargo, but to mannosidase II, a resident enzyme of the *medial* Golgi cisternae. The enzyme appears in both a vesicle (arrow) and cisternae. The labeled vesicle is presumably carrying the enzyme in a retrograde direction, which compensates for the anterograde movement of the enzyme as the result of cisternal maturation. Bar, 0.2 μm. (A third model for intra-Golgi transport is discussed in *Cell* 133:951, 2008.) (C: From Alexander A. Mironov et al., courtesy of Alberto Luini, J. Cell Biol. 155:1234, 2001; D: from Jose A. Martinez-Menárguez et al., courtesy of Judith Klumperman, J. Cell Biol. 155:1214, 2001; Both reproduced with permission of The Rockefeller University Press.)

compatible with the vesicular transport model. Whether or not these results on yeast can be extrapolated to a mammalian Golgi complex, which has a more complex, stacked structure, remains to be determined.

A current version of the cisternal maturation model is depicted in Figure 8.23*b*. Unlike the original versions of the cisternal maturation model, the version shown in Figure 8.23*b* acknowledges a role for transport vesicles, which have been clearly shown to bud from Golgi membranes. In this model, however, these transport vesicles do not shuttle cargo in an anterograde direction, but instead carry resident Golgi enzymes in a retrograde direction. Instead, it is the Golgi cisternae themselves that serve as the primary anterograde Golgi carriers. This model of intra-Golgi transport is supported by electron micrographs of the type illustrated in Figure 8.23*c*,*d*. These micrographs depict ultrathin sections of cultured mammalian cells that were cut from a frozen block. In both cases, the frozen sections were treated with antibodies that were linked to gold particles prior to examination in the electron microscope. Figure 8.23*c* shows a section through a Golgi complex after treatment with gold-labeled antibodies that bind to a cargo protein, in this case the viral protein VSVG (page 274). The VSVG molecules are present within the cisternae, but are absent from the nearby vesicles (arrows), indicating that cargo is carried in an anterograde direction within maturing cisternae but not within small transport vesicles. Figure 8.23*d* shows a section through a Golgi complex after treatment with gold-labeled antibodies that bind to a Golgi resident protein, in this case the processing enzyme mannosidase II. Unlike the VSVG cargo protein, mannosidase II molecules are found in both the cisternae and associated vesicles (arrow), which strongly supports the proposal that these vesicles are utilized to carry Golgi resident enzymes in a retrograde direction. Golgi resident proteins may also move in a retrograde direction through tubules that have been seen to connect different Golgi cisternae.

The cisternal maturation model depicted in Figure 8.23*b* explains how different Golgi cisternae in a stack can have a unique identity. An enzyme such as mannosidase II, for example, which removes mannose residues from oligosaccharides and is largely restricted to the *medial* cisternae (Figure 8.21), can be recycled backward in transport vesicles as each cisterna moves toward the *trans* end of the stack. It should be noted that a number of prominent researchers continue to argue, based on other experimental results, that cargo can be carried

by transport vesicles between Golgi cisternae in an anterograde direction. Thus, the matter remains to be settled.

REVIEW

1. Describe the steps that occur as a soluble secretory protein, such as a digestive enzyme in a pancreas cell, moves from the RER to the *cis* Golgi cisterna; from a *cis* cisterna to the TGN.
2. What is the role of dolichol phosphate in the synthesis of membrane glycoproteins? How is the sequence of sugars attached to the protein determined?
3. How does the process of glycosylation in the Golgi complex compare to that in the RER?
4. How can the cisternal maturation model of Golgi activity be reconciled with the presence of transport vesicles in the Golgi region?

8.5 | Types of Vesicle Transport and Their Functions

The biosynthetic pathway of a eukaryotic cell consists of a series of distinct membrane-bound organelles that function in the synthesis, modification, and delivery of soluble and membrane proteins to their appropriate destination in the cell. As was illustrated in Figure 8.2*a*, materials are carried between compartments by vesicles (or other types of membrane-bound carriers) that bud from donor membranes and fuse with acceptor membranes. If one scrutinizes electron micrographs for vesicles caught in the act of budding, one finds that most of these membranous buds are covered on their cytosolic surface by a "fuzzy," electron-dense layer. Further analysis reveals that the dark-staining layer consists of a protein coat formed from soluble proteins that assemble on the cytosolic surface of the donor membrane at sites where budding takes place. Each coated bud pinches off to form a **coated vesicle** such as those shown in Figure 8.24. Vesicles of similar size and structure can be formed in cell-free systems as illustrated in Figure 8.6. (The discovery of coated vesicles is discussed in the Experimental Pathways on page 319.)

Protein coats have at least two distinct functions: (1) they act as a mechanical device that causes the membrane to curve and form a budding vesicle, and (2) they provide a mechanism for selecting the components to be carried by the vesicle. Selected components include (*a*) cargo consisting of secretory, lysosomal, and membrane proteins to be transported and (*b*) the machinery required to target and dock the vesicle to the correct acceptor membrane (page 300). In the best understood cases (see Figures 8.26 and 8.40), the vesicle coat is composed of two distinct protein layers: an outer cage or scaffolding that forms the framework for the coat and an inner layer of adaptors that binds both to the outer surface of the lipid bilayer and the membrane's cargo. As discussed below, the adaptors are able to select specific cargo molecules by virtue of their specific affinity for the cytosolic "tails" of integral proteins that reside in the donor membrane (see Figure 8.25*b*).

Several distinct classes of coated vesicles have been identified; they are distinguished by the proteins that make up their coat, their appearance in the electron microscope, and their role in cell trafficking. The three best studied coated vesicles are the following:

1. **COPII-coated vesicles** (Figure 8.24*a*) move materials from the ER "forward" to the ERGIC and Golgi complex. (Recall from page 289 that the ERGIC is the intermediate compartment situated between the ER and Golgi complex.) (COP is an acronym for coat proteins.)
2. **COPI-coated vesicles** (Figure 8.24*b*) move materials in a retrograde direction (1) from the ERGIC and Golgi stack "backward" toward the ER and (2) from *trans* Golgi cisternae "backward" to *cis* Golgi cisternae (see Figure 8.25*a*). Additional roles for COPI vesicles have been debated.
3. **Clathrin-coated vesicles** move materials from the TGN to endosomes, lysosomes, and plant vacuoles. They also move materials from the plasma membrane to cytoplasmic compartments along the endocytic pathway. They have also been implicated in trafficking from endosomes and lysosomes.

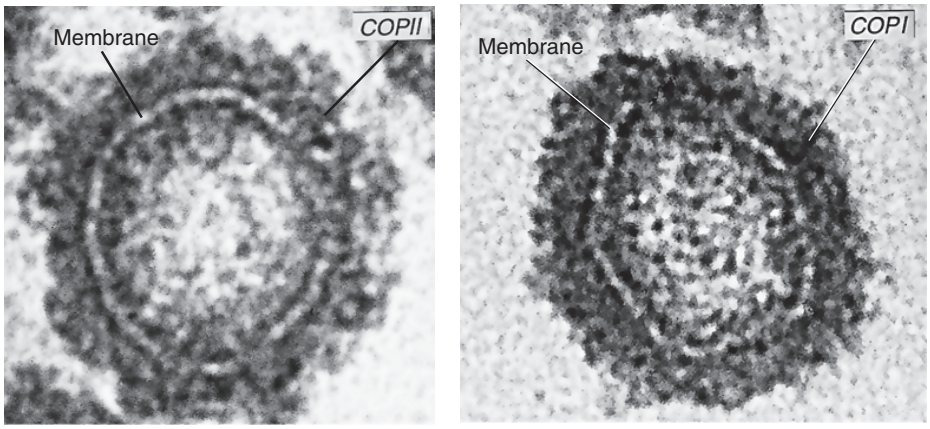


Figure 8.24 **Coated vesicles.** These electron micrographs show the membranes of these vesicles to be covered on their outer (cytosolic) surface by a distinct protein coat. The micrograph on the left (*a*) shows a COPII-coated vesicle, whereas the micrograph on the right (*b*) shows a COPI-coated vesicle. (COURTESY OF RANDY SCHEKMAN AND LELIO ORCI.)

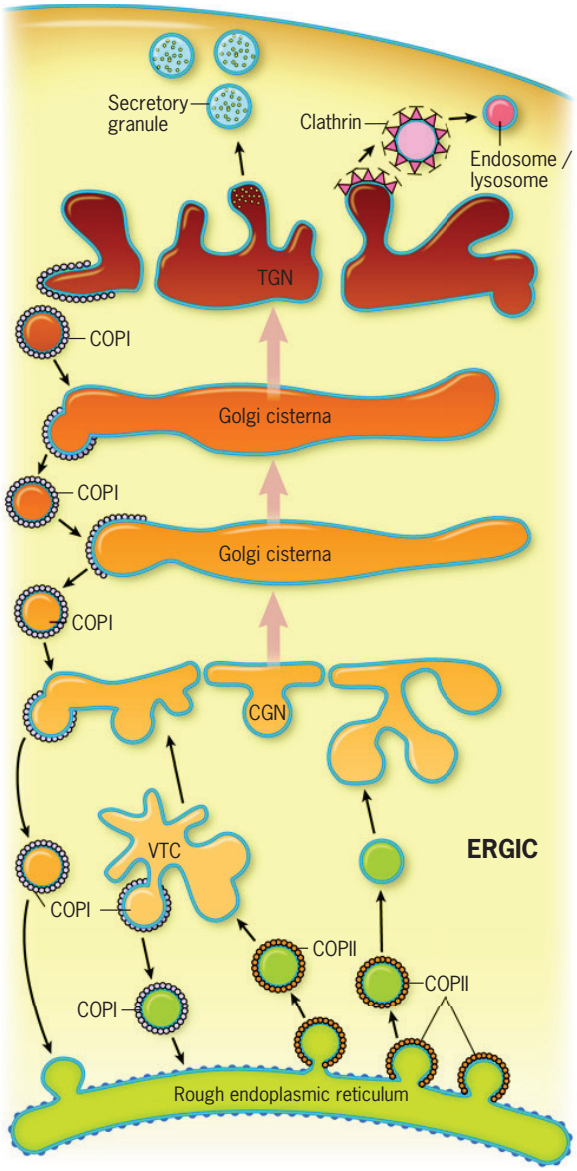
We will consider each type of coated vesicle in the following sections. A summary of the various transport steps along the biosynthetic or secretory pathway that is mediated by each of these coated vesicles is indicated in Figure 8.25*a*.

COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex

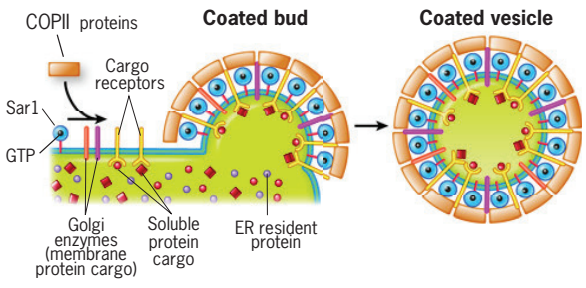
COPII-coated vesicles mediate the first leg of the journey through the biosynthetic pathway—from the ER to the ERGIC and Golgi complex (Figure 8.25*a*,*b*). The COPII coat contains a number of proteins that were first identified in mutant yeast cells that were unable to carry out transport from the ER to the Golgi complex. Homologues of the yeast proteins were subsequently found in the coats of vesicles budding from the ER in mammalian cells. Antibodies to COPII-coat proteins block budding of vesicles from ER membranes but have no effect on movement of cargo at other stages in the secretory pathway.

COPII coats select and concentrate certain components for transport in vesicles. Certain integral membrane proteins of the ER are selectively captured because they contain "ER export" signals as part of their cytosolic tail. These signals interact specifically with COPII proteins of the vesicle coat (Figure 8.25*b*). Proteins selected by COPII-coated vesicles include (1) enzymes that act at later stages in the biosynthetic pathway, such as the glycosyltransferases of the Golgi complex (indicated as orange membrane proteins in Figure 8.25*b*), (2) membrane proteins involved in the docking and fusion of the vesicle with the target compartment, and (3) membrane proteins that are able to bind soluble cargo (such as the secretory proteins, indicated by the red spheres in Figure 8.25*b*). Cells lacking a specific cargo receptor typically fail to transport a specific subset of proteins from the ER to the Golgi complex. For example, mutations in one cargo receptor (ERGIC-53) have been linked to an inherited bleeding disorder. Persons with this disorder fail to secrete certain coagulation factors that promote blood clotting.

Among the COPII coat proteins is a small G protein called Sar1, which is recruited specifically to the ER membrane. Like other G proteins, Sar1 plays a regulatory role, in this case by initiating vesicle formation and by regulating the



(a)



(b)

Figure 8.25 **Proposed movement of materials by vesicular transport between membranous compartments of the biosynthetic/secretory pathway.** (*a*) The three different types of coated vesicles indicated in this schematic drawing are thought to have distinct transport roles. COPII-coated vesicles mediate transport from the ER to the ERGIC and Golgi complex. COPI-coated vesicles return proteins from the ERGIC and Golgi complex to the ER. COPI-coated vesicles also transport Golgi enzymes between cisternae in a retrograde direction. Clathrin-coated vesicles mediate transport from the TGN to endosomes and lysosomes. Transport of materials along the endocytic pathway is not shown in this drawing. (*b*) Schematic drawing of the assembly of a COPII-coated vesicle at the ER. Assembly begins when Sar1 is recruited to the ER membrane and activated by exchange of its bound GDP with a bound GTP. These steps are shown in Figure 8.26. Cargo proteins of the ER lumen (red spheres and diamonds) bind to the luminal ends of transmembrane cargo receptors. These receptors are then concentrated within the coated vesicle through interaction of their cytosolic tails with components of the COPII coat. ER resident proteins (e.g., BiP) are generally excluded from the coated vesicles. Those that do happen to become included in a coated vesicle are returned to the ER as described later in the text.

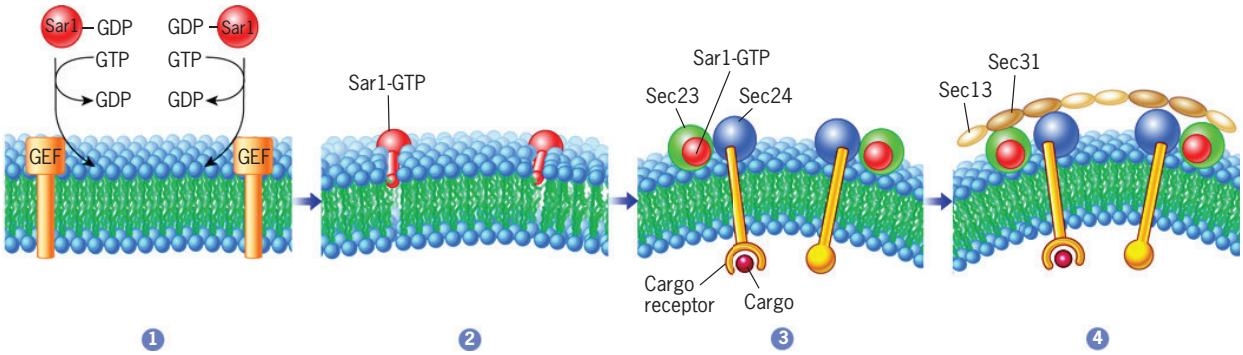


Figure 8.26 **Proposed roles of the COPII coat proteins in generating membrane curvature, assembling the protein coat, and capturing cargo.** In step 1, Sar1-GDP molecules have been recruited to the ER membrane by a protein called a GEF (guanine-exchange factor) that catalyzes the exchange of the bound GDP with a bound GTP. In step 2, each Sar1-GTP molecule has extended a finger-like α helix along the membrane within the cytosolic leaflet. This event expands the leaflet and induces the curvature of the lipid bilayer at that site. In step 3, a dimer composed of two COPII polypeptides (Sec23 and Sec24) has been recruited by the bound Sar1-GTP. The Sec23-Sec24 heterodimer is thought to further induce the curvature of the membrane in the formation of a vesicle. Both Sar1 and Sec23-Sec24 can bring about membrane curvature when incubated with synthetic liposomes in vitro. Transmembrane cargo accumulates within the forming COPII vesicle as their cytosolic tails bind to the Sec24 polypeptide of the COPII coat. Sec24 can exist in at least four different isoforms. It is likely that different isoforms of this protein recognize and bind membrane proteins with different sorting signals, thus broadening the specificity in types of materials that can be transported by COPII vesicles. In step 4, the remaining COPII polypeptides (Sec13 and Sec31) have joined the complex to form an outer structural scaffold of the coat. (Reprinted from Stephan Fath et al., courtesy of Jonathan Goldberg, Cell 129:1333, 2007, with permission from Elsevier.)

assembly of the vesicle coat. These activities are illustrated in Figure 8.26. In step 1 of Figure 8.26, Sar1 is recruited to the ER membrane in the GDP-bound form and is induced to exchange its GDP for a molecule of GTP. Upon binding of GTP, Sar1 undergoes a conformational change that causes its N-terminal α helix to insert itself into the cytosolic leaflet of the ER bilayer (step 2). This event has been demonstrated to bend the lipid bilayer, which is an important step in the conversion of a flattened membrane into a spherical vesicle. Membrane bending is probably aided by a change in packing of the lipids that make up the two leaflets of the bilayer. In step 3, Sar1-GTP has recruited two additional polypeptides of the COPII coat, Sec23 and Sec24, which bind as a "banana-shaped" dimer. Because of its curved shape (Figure 8.27), the Sec23-Sec24 dimer provides additional pressure on the membrane surface to help it further bend into a curved bud. Sec24 also functions as the primary adaptor protein of the COPII coat that interacts specifically with the ER export signals in the cytosolic tails of membrane proteins that are destined to traffic on to the Golgi complex. In Figure 8.26, step 4, the remaining subunits of the COPII coat, Sec13 and Sec31, bind to the membrane to form the outer structural cage of the protein coat. Figure 8.27 shows a representation of a 40-nm vesicle with the COPII coat bound to its surface. The Sec13-Sec31 cage assembles into a relatively simple lattice in which each vertex is formed by the convergence of four Sec13-Sec31 legs (Figure 8.27). A certain degree of flexibility is built into the hinge between the Sec13-Sec31 subunits that allow them to form cages of varying diameter, thus accommodating vesicles of varying size.

Once the entire COPII coat has assembled, the bud is separated from the ER membrane in the form of a COPII-coated vesicle. Before the coated vesicle can fuse with a target membrane, the protein coat must be disassembled and its components released into the cytosol. Disassembly is triggered by hydrolysis of the bound GTP to produce a Sar1-GDP subunit, which has decreased affinity for the vesicle

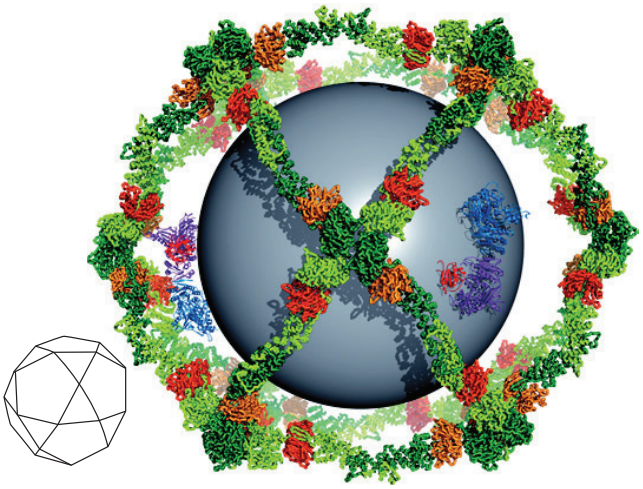


Figure 8.27 **A molecular model of the outer Sec13-Sec31 cage of the COPII coat** as it would assemble around the surface of a 40-nm "vesicle." Each edge or leg of the lattice that makes up the cage consists of a heterotetramer (two Sec31 subunits seen as dark green and light green and two Sec13 subunits seen as orange and red). Four such legs converge to form each vertex of the lattice. Two copies of the Sar1-Sec23-Sec24 complex (shown in red, magenta, and blue, respectively) that would form the inner layer of the COPII coat are also shown in this model. It can be seen how the inner surface of the Sec23-Sec24 complex conforms to the curvature of the vesicle. Inset shows a COPII lattice, which is comprised of triangular and square, pentagonal, and/or hexagonal faces. (From Stephan Fath et al., courtesy of Jonathan Goldberg, Cell 129:1333, 2007, by permission of Elsevier.)

membrane. Dissociation of Sar1-GDP from the membrane is followed by the release of the other COPII subunits.

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

COPI-coated vesicles were first identified in experiments in which cells were treated with molecules similar in structure to GTP (GTP analogues) but, unlike GTP, cannot be hydrolyzed. Under these conditions, COPI-coated vesicles accumulated within the cell and could be isolated from homogenized cells by density gradient centrifugation (Section 18.6). COPI-coated vesicles accumulate in the presence of a nonhydrolyzable GTP analogue because, similar to their COPII counterparts, the coat contains a small membrane-bending GTP-binding protein, called Arf1, whose bound GTP must be hydrolyzed before the coat can disassemble. COPI-coated vesicles have been most clearly implicated in the retrograde transport of proteins, including the movement of (1) Golgi resident enzymes in a *trans*-to-*cis* direction (as indicated in Figure 8.23*d*, which shows a gold-labeled mannosidase II molecule in a COPI vesicle) and (2) ER resident enzymes from the ERGIC and the Golgi complex back to the ER (Figure 8.25*a*). To understand the role of COPI-coated vesicles in retrograde transport, we have to consider a more general subject.

Retaining and Retrieving Resident ER Proteins If vesicles continually bud from membrane compartments, how does each compartment retain its unique composition? What determines, for example, whether a particular protein in the membrane of the ER remains in the ER or proceeds on to the Golgi complex? Studies suggest that proteins are maintained in an organelle by a combination of two mechanisms:

1. *Retention* of resident molecules that are excluded from transport vesicles. Retention may be based primarily on the physical properties of the protein. For example, soluble proteins that are part of large complexes or membrane proteins with short transmembrane domains are not likely to enter a transport vesicle.
2. *Retrieval* of "escaped" molecules back to the compartment in which they normally reside.

Proteins that normally reside in the ER, those both in the lumen and in the membrane, contain short amino acid sequences at their C-terminus that serve as *retrieval signals*, ensuring their return to the ER if they should be accidentally carried forward to the ERGIC or Golgi complex. The retrieval of "escaped" ER proteins from these compartments is accomplished by specific receptors that capture the molecules and return them to the ER in COPI-coated vesicles (Figures 8.25*a*, 8.28). Soluble resident proteins of the ER lumen (such as protein disulfide isomerase and the molecular chaperones that facilitate folding) typically possess the retrieval signal "lys-asp-glu-leu" (or KDEL in single-letter nomenclature). As shown in Figure 8.28, these proteins are recognized and returned to the ER by the *KDEL receptor*, an integral membrane protein that shuttles between the *cis* Golgi and the ER compartments. If the KDEL sequence is deleted from an ER protein, the escaped proteins are not returned to the ER

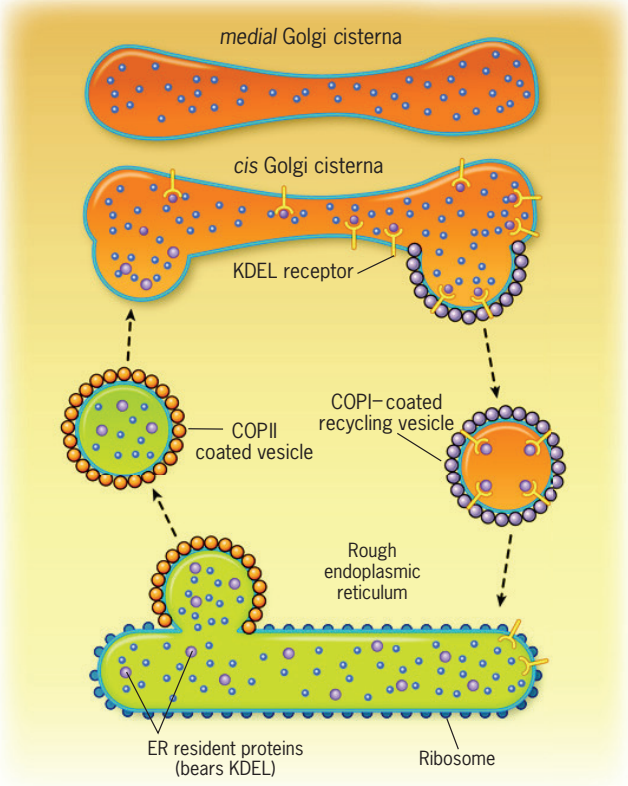


Figure 8.28 **Retrieving ER proteins.** Resident proteins of the ER contain amino acid sequences that lead to their retrieval from the Golgi complex if they are accidentally incorporated into a Golgi-bound transport vesicle. Soluble ER proteins bear the retrieval signal KDEL. Retrieval is accomplished as soluble ER proteins bind to KDEL receptors residing in the membranous wall of *cis* Golgi compartments. The KDEL receptors, in turn, bind to proteins of the COPI coat, which allows the entire complex to be recycled back to the ER.

but instead are carried forward through the Golgi complex. Conversely, when a cell is genetically engineered to express a lysosomal or secretory protein that contains an added KDEL C-terminus, that protein is returned to the ER rather than being sent on to its proper destination. Membrane proteins that reside in the ER also have a retrieval signal that binds to the COPI coat, facilitating their return to the ER. The most common retrieval sequences for ER membrane proteins involve two closely linked basic residues, most commonly KKXX (where K is lysine and X is any residue). Each membrane compartment in the biosynthetic pathway may have its own retrieval signals, which helps explain how each compartment can maintain its unique complement of proteins despite the constant movement of vesicles in and out of that compartment.

Beyond the Golgi Complex: Sorting Proteins at the TGN

Despite all the discussion of transport vesicles, we have yet to examine how a particular protein that has been synthesized in the ER is targeted toward a particular cellular destination. It is

important that a cell be able to distinguish among the various proteins that it manufactures. A pancreatic cell, for example, has to segregate newly synthesized digestive enzymes that will be secreted into a duct, from newly synthesized cell-adhesion molecules that will ultimately reside in the plasma membrane, from lysosomal enzymes that are destined for lysosomes. This is accomplished as the cell sorts proteins destined for different sites into different membrane-bound carriers. The *trans* Golgi network (TGN), which is the last stop in the Golgi complex, functions as a major sorting station, directing proteins to various destinations. The best understood of the post-Golgi pathways is one that carries lysosomal enzymes.

Sorting and Transport of Lysosomal Enzymes Lysosomal proteins are synthesized on membrane-bound ribosomes of the ER and carried to the Golgi complex along with other types of proteins. Once in the Golgi cisternae, soluble lysosomal enzymes are specifically recognized by enzymes that catalyze the two-step addition of a phosphate group to certain mannose sugars of the *N*-linked carbohydrate chains (Figure 8.29*a*). Thus, unlike other glycoproteins sorted at the TGN, lysosomal enzymes possess phosphorylated mannose residues, which act as sorting signals. This mechanism of protein sorting was discovered through studies on cells from humans that lacked one of the enzymes involved in phosphate addition (discussed in the Human Perspective on page 306). Lysosomal enzymes carrying a mannose 6-phosphate signal are recognized and captured by *mannose 6-phosphate receptors* (*MPRs*), which are integral membrane proteins that span the TGN membranes (Figure 8.29*b*).

Lysosomal enzymes are transported from the TGN in clathrin-coated vesicles (which are the third and last type of coated vesicles to be discussed). The structure of clathrin-coated vesicles is described in detail on page 308 in connection with endocytosis, a process that is better understood than budding at the TGN. It will suffice at this point to note that the coats of these vesicles contain (1) an outer honeycomb-like lattice composed of the protein clathrin, which forms a structural scaffold, and (2) an inner shell composed of protein adaptors, which covers the surface of the vesicle membrane that faces the cytosol (Figure 8.30). The term *adaptor* describes a molecule that physically links two or more components. Lysosomal enzymes are escorted from the TGN by a family of adaptor proteins called **GGAs**.

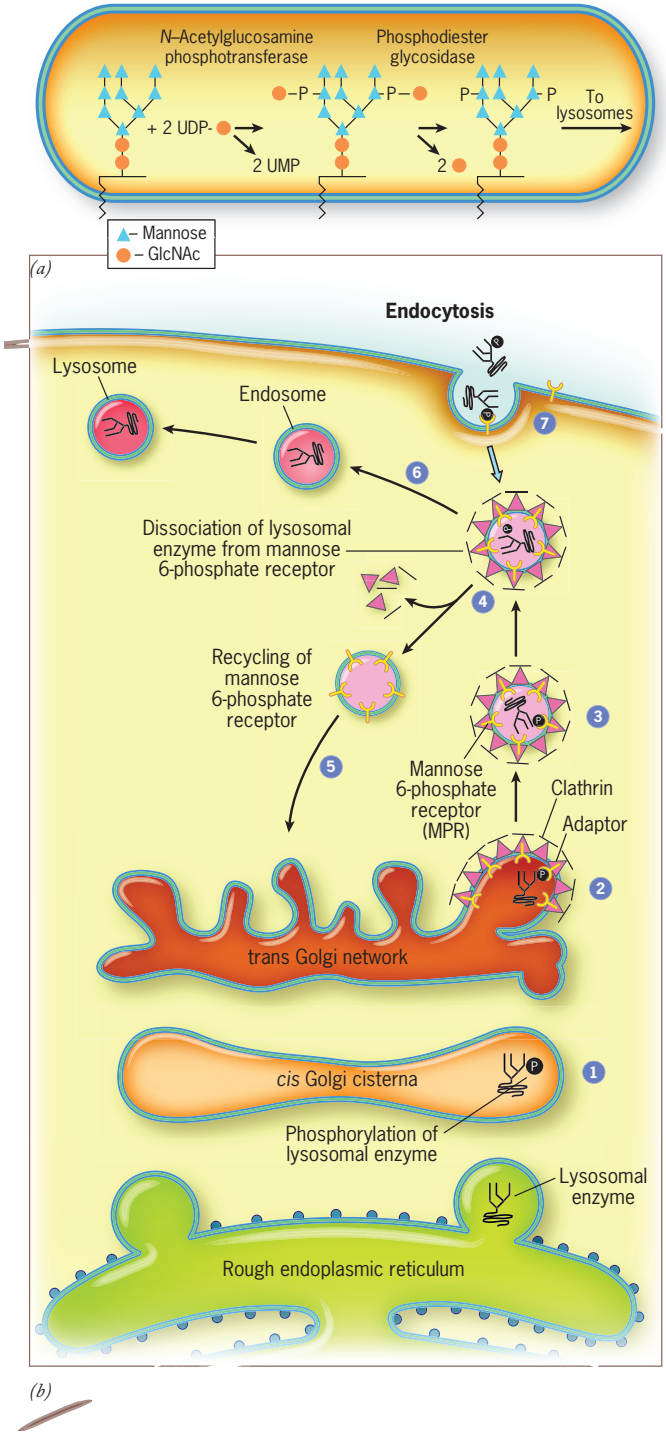


Figure 8.29 **Targeting lysosomal enzymes to lysosomes.** (*a*) Lysosomal enzymes are recognized by an enzyme in the *cis* cisternae that transfers a phosphorylated *N*-acetylglucosamine from a nucleotide sugar donor to one or more mannose residues of *N*-linked oligosaccharides. The glucosamine moiety is then removed in a second step by a second enzyme, leaving mannose 6-phosphate residues as part of the oligosaccharide chain. (*b*) Schematic diagram showing the pathways followed by a lysosomal enzyme (black) from its site of synthesis in the ER to its delivery to a lysosome. The mannose residues of the lysosomal enzyme are phosphorylated in the Golgi cisternae (step 1) and then selectively incorporated into a clathrin-coated vesicle at the TGN (step 2). The mannose 6-phosphate receptors are thought to have a dual role (step 3): they interact specifically with the lysosomal enzymes on the luminal side of the vesicle, and they interact specifically with adaptors on the cytosolic surface of the vesicle (shown in Figure 8.30). The mannose 6-phosphate receptors separate from the enzymes (step 4) and are returned to the Golgi complex (step 5). The lysosomal enzymes are delivered to an endosome (step 6) and eventually to a lysosome. Mannose 6-phosphate receptors are also present in the plasma membrane, where they capture lysosomal enzymes that are secreted into the extracellular space and return the enzymes to a pathway that directs them to a lysosome (step 7).

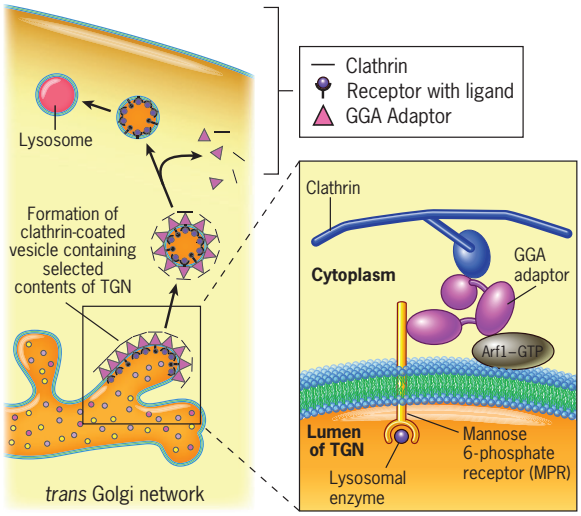


Figure 8.30 **The formation of clathrin-coated vesicles at the TGN.** Clathrin-coated vesicles that bud from the TGN contain GGA, an adaptor protein consisting of several distinct domains. One of the GGA domains binds to the cytosolic domains of membrane proteins, including those that will ultimately reside in the boundary membrane of the lysosome and also the MPR that transports lysosomal enzymes. Other GGA domains bind to Arf1 and to the surrounding cytosolic network of clathrin molecules.

As indicated in the inset of Figure 8.30, a GGA molecule has several domains, each capable of grasping a different protein involved in vesicle formation. The outer ends of the GGA adaptors bind to clathrin molecules, holding the clathrin scaffolding onto the surface of the vesicle. On their inner surface, the GGA adaptors bind to a sorting signal in the cytosolic tails of the mannose 6-phosphate receptors. The MPRs, in turn, bind to soluble lysosomal enzymes within the vesicle lumen (Figure 8.30, inset). As a result of these interactions with GGA adaptors, the MPRs in the TGN membrane and lysosomal enzymes within the TGN lumen become concentrated into clathrin-coated vesicles. As in the formation of COPI and COPII vesicles, production of clathrin-coated vesicles at the TGN begins with the recruitment to the membrane of a small GTP-binding protein, in this case Arf1, which sets the stage for the binding of the other coat proteins. Like Sar1 (Figure 8.26), Arf1 extends a membrane-bending α helix that acts in conjunction with the clathrin coat to initiate formation of the budding vesicle. After the vesicle has budded from the TGN, the clathrin coat is lost and the uncoated vesicle proceeds to its destination, which may be an early endosome, late endosome, or plant vacuole. Before they reach one of these organelles, the MPRs dissociate from the lysosomal enzymes and return to the TGN (Figure 8.29*b*) for another round of lysosomal enzyme transport.

Sorting and Transport of Nonlysosomal Proteins Lysosomal proteins are not the only materials that are exported from the TGN. As indicated in Figure 8.2, membrane proteins destined for the plasma membrane and secretory materials destined for export from the cell are also transported from the TGN, but the mechanisms are poorly understood. According to one model, membranous carriers are produced as the TGN fragments into vesicles and tubules of various size and shape. This concept fits with the cisternal maturation model, which proposes that the cisternae of the Golgi complex move continually toward the TGN, where they would have to disperse to allow continued maturation of the Golgi stack. Proteins that are discharged from the cell by a process of regulated secretion, such as digestive enzymes and hormones, are thought to form selective aggregates that eventually become contained in large, densely packed secretory granules. These aggregates are apparently trapped as immature secretory granules bud from the rims of the *trans* Golgi cisternae and TGN. In some cells, long tubules are seen to be pulled out of the TGN by motor proteins that operate along microtubular tracks. These tubules are then split into a number of vesicles or granules by membrane fission. Once they have departed from the TGN, the contents of the secretory granules become more concentrated. Eventually, the mature granules are stored in the cytoplasm until their contents are released following stimulation of the cell by a hormone or nerve impulse.

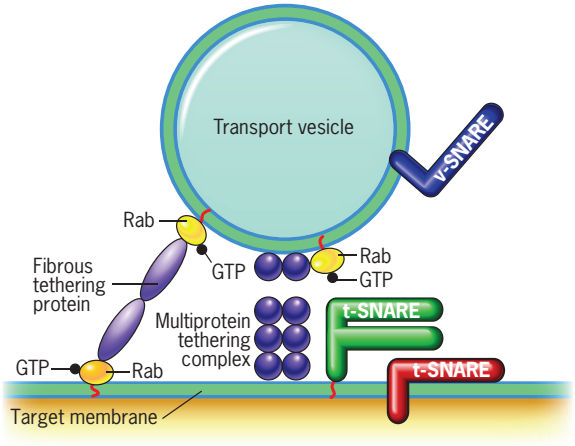
The targeted delivery of integral proteins to the plasma membrane appears to be based largely on sorting signals in the cytoplasmic domains of the membrane proteins. Considerable research has focused on polarized cells, such as those depicted in Figure 8.11. In these cells, membrane proteins destined to reside in the apical portion of the plasma membrane contain different sorting signals from those destined for the lateral or basal portion. These two groups of plasma membrane proteins are clustered into different TGN membrane domains and transported to the cell surface in separate carriers. Plasma membrane proteins of nonpolarized cells, such as fibroblasts and white blood cells, may not require special sorting signals. Such proteins may simply be carried from the TGN to the cell surface in vesicles of the constitutive secretory pathway (Figure 8.2*b*).

Targeting Vesicles to a Particular Compartment

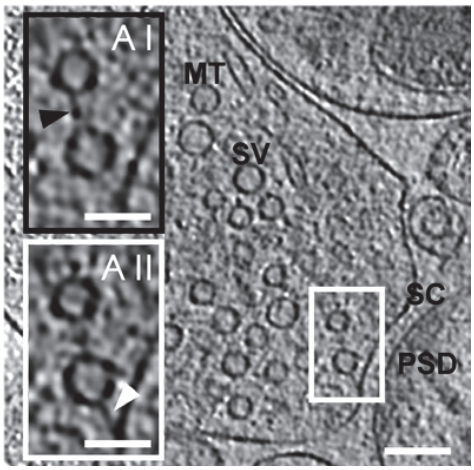
Vesicle fusion requires specific interactions between different membranes. Vesicles from the ER, for example, fuse with the ERGIC or *cis* Golgi network and not with a *trans* cisterna. Selective fusion is one of the factors that ensures a directed flow through the membranous compartments of the cell. Despite a major research effort, we still do not fully understand the mechanisms by which cells target vesicles to particular compartments. It is thought that a vesicle contains specific proteins associated with its membrane that govern the movements and fusion potential of that vesicle. To understand the nature of these proteins, we will consider the steps that occur between the stages of vesicle budding and vesicle fusion.

1. ***Movement of the vesicle toward the specific target compartment.*** In many cases, membranous vesicles must move considerable distances through the cytoplasm before reaching their eventual target. These types of movement are mediated largely by microtubules, which act like railroad tracks carrying cargo containers along a defined pathway to a predetermined destination. The membranous carriers seen in Figure 8.19, for example, were observed to move from the ERGIC to the Golgi complex over microtubules.

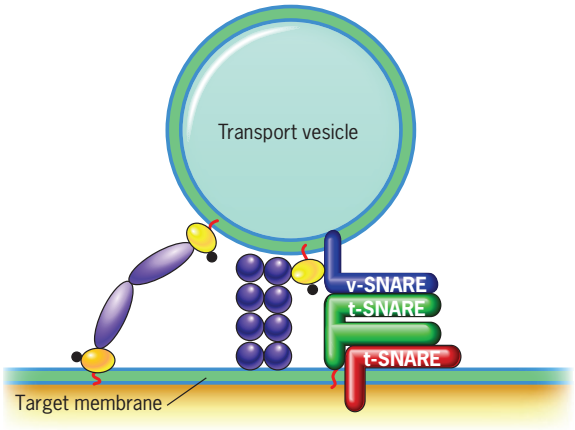
2. ***Tethering vesicles to the target compartment.*** The initial contacts between a transport vesicle and its target membrane, such as a Golgi cisterna, are thought to be mediated by a diverse collection of "tethering" proteins. Two groups of tethering proteins have been described (Figure 8.31*a*): rod-shaped, fibrous proteins that are capable of forming a molecular bridge between the two membranes over a considerable distance (50–200 nm) and large multiprotein complexes that appear to hold the two membranes in closer proximity. Different tethering proteins initiate fusion between different types of membranes. For example, one class of fibrous tethering proteins, called golgins, act in and around the Golgi complex. Among various proposed functions, golgins may serve as



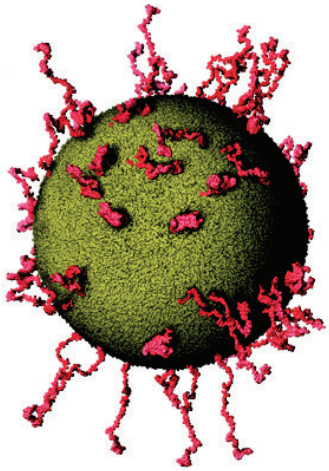
(a) Tethering



(b)



(c) Docking



(d)

Figure 8.31 **Proposed steps in the targeting of transport vesicles to target membranes.** (*a*) According to this model, Rab proteins on the vesicle and target membrane are involved in recruiting tethering proteins that mediate initial contact between the two membranes. Two types of tethering proteins are depicted: highly elongated fibrous proteins (e.g., golgins and EEA1) and multiprotein complexes (e.g., the exocyst and TRAPPI). (*b*) Electron tomographic image of a slice through a mammalian nerve terminus showing the network of synaptic vesicles that are present in close association with the presynaptic plasma membrane (page 169). The left insets (corresponding to the white box on the right) show the presence of filamentous connectors between a pair of synaptic vesicles (upper inset) and between one of the synaptic vesicles and the adjacent plasma membrane (lower inset). Bars: main panel, 100 nm; insets, 50 nm. (*c*) During the docking stage leading up to membrane fusion, a v-SNARE in the vesicle membrane interacts with the t-SNAREs in the target membrane to form a four-stranded α-helical bundle that brings the two membranes into intimate contact (see next figure). In the cases described in the text, SNAP-25, one of the t-SNAREs, is a peripheral membrane protein that is bound to the lipid bilayer by a lipid anchor rather than a transmembrane domain. SNAP-25 contributes two helices to the four-helix SNARE bundle. (*d*) A model of a synaptic vesicle showing the distribution of only one of its constitutent proteins, the SNARE synaptobrevin. The surface density and structures of the synaptobrevin molecules are based on calculations of the number of these proteins per vesicle and the known structure of the molecule. A complete portrait of the proteins on the surface of a synaptic vesicle is shown in the image in Figure 4.4*d*. (B: From Rubén Fernández-Busnadiego, et al., courtesy of Wolfgang Baumeister, J. Cell Biol. 188, 147, 2010, reproduced with permission of The Rockefeller University Press; D: From Shigeo Takamori et al., Cell 127:841, 2006, courtesy of Reinhard Jahn, with permission from Elsevier.)

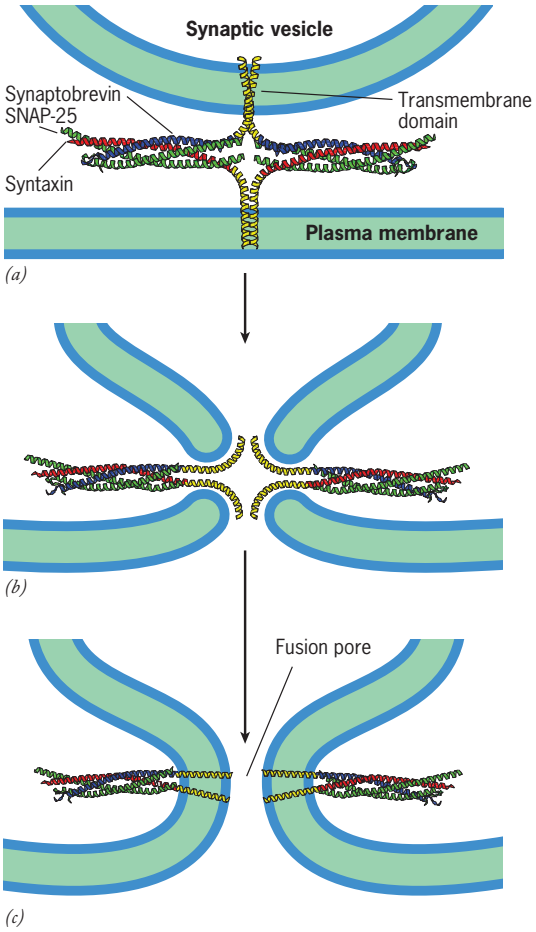
"tentacles" to reach out and capture transport vesicles carrying Golgi-bound cargo. Several different tethering complexes have been visualized with the electron microscope. Figure 8.31*b* shows tomographic images of the presynaptic zone of a mammalian nerve terminal. These 3D images reveal the presence of filamentous structures—presumably constructed of tethering proteins—connecting synaptic vesicles both to one another and to the adjacent plasma membrane with which these vesicles would have fused. Much of the specificity between vesicle and target may be conferred by a family of small G proteins called **Rabs**, which cycle between an active GTP-bound state and an inactive GDP-bound state. GTP-bound Rabs associate with membranes by a lipid anchor. With over 60 different Rab genes identified in humans, these proteins constitute the most diverse group of proteins involved in membrane trafficking. More importantly, different Rabs become associated with different membrane compartments. This preferential localization gives each compartment a unique surface identity, which is required to recruit the proteins involved in targeting specificity. In their GTP-bound state, Rabs play a key role in vesicle targeting by recruiting specific cytosolic tethering proteins to specific membrane surfaces (Figure 8.31*a*). Rabs also play a key role in recruiting numerous proteins involved in other aspects of membrane trafficking, including the motor proteins that move membranous vesicles through the cytoplasm (shown in Figure 9.52*c*).

3. ***Docking vesicles to the target compartment.*** At some point during the process leading to vesicle fusion, the membranes of the vesicle and target compartment come into close contact with one another as the result of an interaction between the cytosolic regions of integral proteins of the two membranes. The key proteins that engage in these interactions are called **SNAREs**, and they constitute a family of more than 35 membrane proteins whose members are localized to specific subcellular compartments. Although SNAREs vary considerably in structure and size, all contain a segment in their cytosolic domain called a *SNARE motif* that consists of 60–70 amino acids capable of forming a complex with another SNARE motif. SNAREs can be divided functionally into two categories, **v-SNAREs**, which become incorporated into the membranes of transport vesicles during budding, and **t-SNAREs**, which are located in the membranes of target compartments (Figure 8.31*c*). The best studied SNAREs are those that mediate docking of synaptic vesicles with the presynaptic membrane during the regulated release of neurotransmitters (page 169). In this case, the plasma membrane of the nerve cell contains two t-SNAREs, syntaxin and SNAP-25, whereas the synaptic vesicle membrane contains a single v-SNARE, synaptobrevin. The distribution of synaptobrevin molecules on a representative synaptic vesicle is shown in Figure 8.31*d*. As the synaptic vesicle and presynaptic membrane approach one another, the SNARE motifs of t- and v-SNARE molecules from apposing membranes interact to form four-stranded bundles as shown in Figure 8.32*a*. Each bundle consists of four α helices, two donated by SNAP-25 and one each donated by syntaxin and synaptobrevin. These parallel α helices zip together to form a tightly interwoven complex that pulls the two apposing lipid bilayers into very close association (Figures 8.31*c* and 8.32*a*). The formation of similar four-stranded helical bundles occurs among other SNAREs at other sites throughout the cell, wherever membranes are destined to fuse. It is interesting to note that the SNAREs of the synaptic vesicle and presynaptic membrane are the targets of two of the most potent bacterial toxins; those responsible for botulism and tetanus. These deadly toxins act as proteases whose only known substrates are SNAREs. Cleavage of the neuronal SNAREs by the toxins blocks the release of neurotransmitters, which causes paralysis.

4. ***Fusion between vesicle and target membranes.*** When artificial lipid vesicles (liposomes) containing purified t-SNAREs are mixed with liposomes containing purified v-SNAREs, the two types of vesicles fuse with one another, but not with themselves. This finding indicates that interactions between t- and v-SNAREs are capable of pulling two lipid bilayers together with sufficient force to cause them to fuse (Figure 8.32*b*,*c*). However, a large body of evidence suggests that, although interaction between v- and t-SNAREs is required for membrane fusion, it is not sufficient by itself to bring about fusion *within a cell*. According to the prevailing view regarding the regulated secretion of neurotransmitter molecules, the four-stranded SNARE bundle remains locked in an arrested state by interaction with accessory proteins. Vesicles at this stage remain docked at the membrane and ready to discharge their contents almost instantaneously once they receive an activating signal, in the form of a rise in Ca^{2+} concentration (as discussed below). Regardless of how it is regulated, once the lipid bilayers of the two membranes merge, the SNAREs that previously projected from separate membranes now reside in the same membrane (Figure 8.32*c*). Dissociation of the four-stranded SNARE complex is achieved by a doughnut-shaped, cytosolic protein called NSF that attaches to the SNARE bundle and, using energy from ATP hydrolysis, twists it apart.

Now that we have described the events that occur during the fusion of a vesicle with a target membrane, we can return to the question: How is the specificity of this interaction determined? According to current consensus, the ability of a particular vesicle and target membrane to fuse is determined by the specific combination of interacting proteins, including tethering proteins, Rabs, and SNAREs that can be assembled at that site in the cell. Taken together, these multiple interactions between several types of proteins provide a high level of specificity, ensuring that each membrane compartment can be selectively recognized.

Exocytosis The fusion of a secretory vesicle or secretory granule with the plasma membrane and subsequent discharge of its contents is called **exocytosis**. Exocytosis probably occurs on a rather continual basis in most cells, as proteins and other



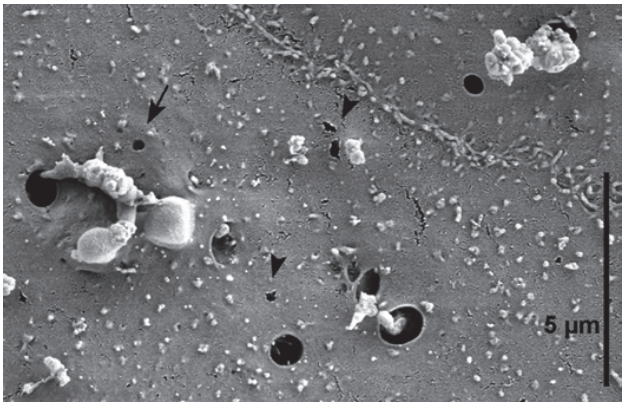


Figure 8.32 **A model of the interactions between v- and t-SNAREs leading to membrane fusion and exocytosis.** (*a*) The synaptic vesicle has become docked to the plasma membrane through the formation of four-stranded bundles comprising α helices donated by syntaxin (red), synaptobrevin (blue), and SNAP-25 (green). SNAP-25 contributes two helices and lacks a transmembrane domain (yellow). (*b*) A speculative transition state in the fusion of the two membranes. A small water-filled cavity is shown at the center of the transmembrane helix bundle. (*c*) The transmembrane helices that previously resided in the two separate membranes are now present in the same bilayer, and a fusion pore has opened between the vesicle and target membrane. The neurotransmitter contents of the vesicle can now be discharged by exocytosis. (*d*) Scanning electron micrograph of the extracellular surface of a pair of cultured alveolar (lung) cells stimulated to discharge proteins that had been stored in secretory granules. Material is seen being expelled from the cell through smooth, circular openings that are presumed to be dilated fusion pores. The arrow shows a fusion pore that has not dilated, which is readily distinguished from holes (arrowhead) accidentally formed during specimen preparation. (A–C: Reprinted from Pehr A B Harbury, Structure 6:1490, 1998, with permission from Elsevier. D: From Thomas Haller et al., J. Cell Biol. 155:286, 2001; reproduced with permission of The Rockefeller University Press.)

materials are delivered to both the plasma membrane and extracellular space. However, the best studied examples of exocytosis are those that occur during regulated secretion, most notably the release of neurotransmitters into the synaptic cleft. In these cases, membrane fusion produces an opening through which the contents of the vesicle or granule are released into the extracellular space. It was noted on page 169 that the arrival of a nerve impulse at the terminal knob of a neuron leads to an increase in the influx of Ca^{2+} and the subsequent discharge of neurotransmitter molecules by exocytosis. In this case, fusion is regulated by a calcium-binding protein (synaptotagmin) present in the membrane of the synaptic vesicle. In other types of cells, exocytosis is generally triggered by release of Ca^{2+} from cytoplasmic stores. Contact between the vesicle and plasma membranes is thought to lead to the formation of a small, protein-lined "fusion pore" (Figure 8.32*c*). Some fusion pores may simply re-close, but in most cases, the pore rapidly dilates to form an opening for discharge of the vesicle contents (Figure 8.32*d*). Regardless of the mechanism, when a cytoplasmic vesicle fuses with the plasma membrane, the luminal surface of the vesicle membrane becomes part of the outer surface of the plasma membrane, whereas the cytosolic surface of the vesicle membrane becomes part of the inner (cytosolic) surface of the plasma membrane (Figure 8.14).

REVIEW

1. What determines the specificity of interaction between a transport vesicle and the membrane compartment with which it will fuse? How are the SNARE proteins involved in the process of membrane fusion?
2. Describe the steps that ensure that a lysosomal enzyme will be targeted to a lysosome rather than a secretory vesicle. What is the role of GGA proteins?
3. Contrast the roles of COPI- and COPII-coated vesicles in protein trafficking.
4. How do retrieval signals ensure that proteins are kept as residents of a particular membrane compartment?

8.6 | Lysosomes

Lysosomes are an animal cell's digestive organelles. A typical lysosome contains at least 50 different hydrolytic enzymes (Table 8.1) produced in the rough ER and targeted to these organelles. Taken together, lysosomal enzymes can hydrolyze virtually every type of biological macromolecule. The enzymes of a lysosome share an important property: all have their

Table 8.1 **A Sampling of Lysosomal Enzymes**

Enzyme	Substrate
Phosphatases	
Acid phosphatase	Phosphomonoesters
Acid phosphodiesterase	Phosphodiesters
Nucleases	
Acid ribonuclease	RNA
Acid deoxyribonuclease	DNA
Proteases	
Cathepsin	Proteins
Collagenase	Collagen
GAG-hydrolyzing enzymes	
Iduronate sulfatase	Dermatan sulfate
β-Galactosidase	Keratan sulfate
Heparan *N*-sulfatase	Heparan sulfate
α-*N*-Acetylglucosaminidase	Heparan sulfate
Polysaccharidases and oligosaccharidases	
α-Glucosidase	Glycogen
Fucosidase	Fucosyloligosaccharides
α-Mannosidase	Mannosyloligosaccharides
Sialidase	Sialyloligosaccharides
Sphingolipid hydrolyzing enzymes	
Ceramidase	Ceramide
Glucocerebrosidase	Glucosylceramide
β-Hexosaminidase	G_{M2} ganglioside
Arylsulfatase A	Galactosylsulfatide
Lipid hydrolyzing enzymes	
Acid lipase	Triacylglycerols
Phospholipase	Phospholipids

optimal activity at an acid pH and thus are **acid hydrolases**. The pH optimum of these enzymes is suited to the low pH of the lysosomal compartment, which is approximately 4.6. The high internal proton concentration is maintained by a proton pump (a V-type H^+-ATPase, page 160) present in the organelle's boundary membrane. Lysosomal membranes also contain a variety of highly glycosylated integral proteins whose carbohydrate chains are thought to form a protective lining that shields the membrane from attack by the enclosed enzymes.

Although lysosomes house a predictable collection of enzymes, their appearance in electron micrographs is neither distinctive nor uniform. Figure 8.33 shows a small portion of a Kupffer cell, a phagocytic cell in the liver that engulfs aging red blood cells. The lysosomes of a Kupffer cell exhibit an irregular shape and variable electron density, illustrating how difficult it is to identify these organelles on the basis of morphology alone.

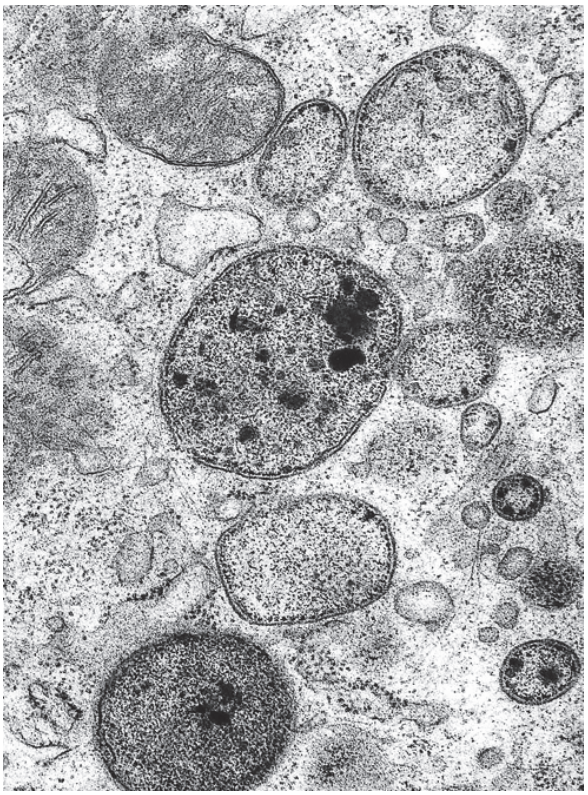


Figure 8.33 **Lysosomes.** Portion of a phagocytic Kupffer cell of the liver showing at least 10 lysosomes of highly variable size. (From Hans Glaumann et al., J. Cell Biol. 67:887, 1975; Reproduced with permission of The Rockefeller University Press.)

The presence within a cell of what is, in essence, a bag of destructive enzymes suggests a number of possible functions. The best studied role of lysosomes is the breakdown of materials brought into the cell from the extracellular environment. Many single-celled organisms ingest food particles, which are then enzymatically disassembled in a lysosome. The resulting nutrients pass through the lysosomal membrane into the cytosol. In mammals, phagocytic cells, such as macrophages and neutrophils, function as scavengers that ingest debris and potentially dangerous microorganisms (page 315). Ingested bacteria are generally inactivated by the low pH of the lysosome and then digested enzymatically. As shown in Figure 17.24, peptides produced by this digestive process are "posted" on the cell surface where they alert the immune system to the presence of a foreign agent.

Autophagy

Lysosomes also play a key role in organelle **turnover**, that is, the regulated destruction of the cell's own organelles and their replacement. During this process, which is called **autophagy**, an organelle, such as the mitochondrion shown in Figure 8.34,

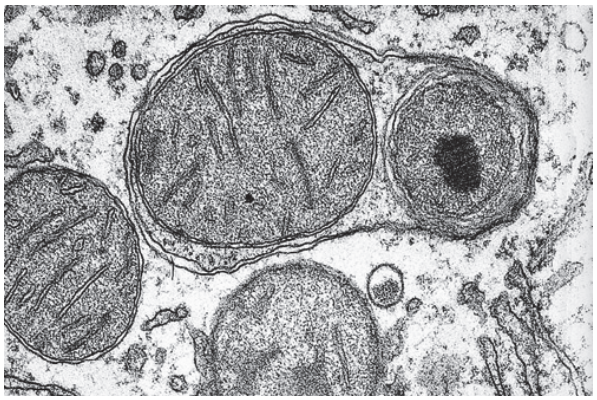


Figure 8.34 **Autophagy.** Electron micrograph of a mitochondrion and peroxisome enclosed in a double membrane wrapper. This autophagic vacuole (or autophagosome) would have fused with a lysosome and its contents digested. (Don Fawcett and Daniel Friend/Photo Researchers Inc.)

is surrounded by a double membrane structure (or *phagophore*) to produce a double-membrane sequestering vesicle called an *autophagosome* (Figure 8.35). The origin of the double membrane in mammalian cells is unclear, with possibilities ranging from the ER to the outer mitochondrial membrane. Once formed, the outer membrane of the autophagosome fuses with a lysosome, generating a structure called an *autolysosome*, in which both the inner membrane of the autophagosome and the enclosed contents are degraded. The products of these degradative reactions are made available to the cell. It is estimated that 1 to 1.5 percent of the proteins within a healthy liver cell are degraded via autophagy per hour as part of a normal process of cellular renovation. Enclosure within autophagosomal vesicles is thought to be relatively selective rather than simply a random process. Autophagy probably evolved in early eukaryotic organisms as a response to nutrient deprivation. If a population of cells—whether derived from yeast, plants or animals—is placed under starvation conditions, a marked increase in autophagy is observed. Under these conditions, cells acquire energy to maintain their life by cannibalizing their own organelles. The same autophagic process is induced in the cells of a mammalian embryo before it has implanted in the uterus and thus before it can take advantage of nutrients delivered via the mother's bloodstream.

Our understanding of the importance of autophagy in animals has coincided with the discovery and analysis of a host of genes (known as *Atg* genes) that are required for various steps in the autophagic pathway. Deletion of certain of these autophagy genes can have serious consequences for embryonic development or adult physiology of model organisms, whether it is a worm or a mouse. It has been shown, for example, that autophagy helps protect an organism against intracellular threats ranging from abnormal protein aggregates to invading bacteria and parasites. Autophagy has also been implicated in the prevention of neurodegeneration. If autophagy is blocked in a particular portion of the brain of a laboratory

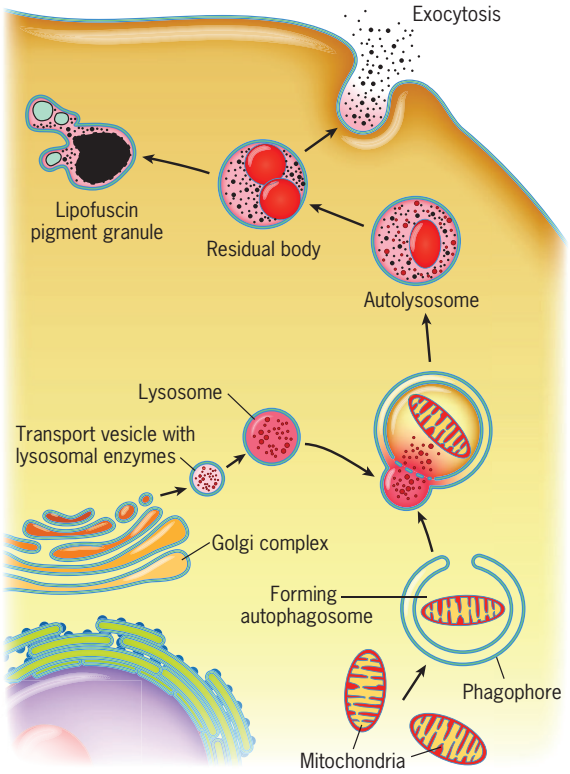


Figure 8.35 **A summary of the autophagic pathway.** The steps are described in the text.

animal, that region of the nervous system experiences massive loss of nerve cells. These findings reveal the importance of autophagy in protecting brain cells from the continuous damage to proteins and organelles that is experienced by these long-lived cells. Autophagy may even play a role in the prevention of certain types of cancers and slowing the body's aging process.

The role of lysosomes in autophagy is summarized in Figure 8.35. Once the digestive process in the autolysosome has been completed, the organelle is termed a *residual body*. Depending on the type of cell, the contents of the residual body may be eliminated from the cell by exocytosis, or they may be retained within the cytoplasm indefinitely as a *lipofuscin granule*. Lipofuscin granules increase in number as an individual becomes older; accumulation is particularly evident in long-lived cells such as neurons, where these granules are considered a major characteristic of the aging process. The role of lysosomes in various diseases is discussed in the accompanying Human Perspective.

REVIEW

1. Describe three distinct roles of lysosomes.
2. Describe the events that occur during the autophagic destruction of a mitochondrion.

Disorders Resulting from Defects in Lysosomal Function

Our understanding of the mechanisms by which proteins are targeted to particular organelles began with the discovery that the mannose 6-phosphate residues in lysosomal enzymes act as an "address" for delivery of these proteins to lysosomes. The discovery of the lysosome address was made in studies of patients with a rare and fatal inherited condition known as *I-cell disease*. Many cells in these patients contain lysosomes that are bloated with undegraded materials. Materials accumulate in the lysosomes because of the absence of hydrolytic enzymes. When fibroblasts from these patients were studied in culture, it was found that lysosomal enzymes are synthesized at normal levels but are secreted into the medium and not targeted to lysosomes. Further analysis revealed that the secreted enzymes lacked the mannose phosphate residues that are present on the lysosomal enzymes of cells from normal individuals. The I-cell defect was soon traced to the deficiency of an enzyme (*N*-acetylglucosamine phosphotransferase) required for mannose phosphorylation in the Golgi complex (see Figure 8.29*a*).

In 1965, H. G. Hers of the University of Louvain in Belgium offered an explanation as to how the absence of a seemingly unimportant lysosomal enzyme, α-glucosidase, could lead to the development of a fatal inherited condition known as Pompe disease. Hers suggested that, in the absence of α-glucosidase, undigested glycogen accumulated in lysosomes, causing swelling of the organelles and irreversible damage to the cells and tissues. Diseases of this type, characterized by the deficiency of a single lysosomal enzyme and the corresponding accumulation of undegraded substrate (Figure 1), are called **lysosomal storage disorders**. Over 40 such diseases have been described, affecting approximately 1 in 8000 infants. Those diseases resulting from an accumulation of undegraded sphingolipids are listed in Table 1. The symptoms of lysosomal storage diseases can range from very severe to barely detectable, depending primarily on the degree of enzyme dysfunction. Several diseases have also been traced to mutations in lysosomal membrane proteins that impair the transport of substances from the lumen of the lysosome to the cytosol.

Among the best studied lysosomal storage diseases is Tay-Sachs disease, which results from a deficiency of the enzyme β-*N*-hexosaminidase A, an enzyme that degrades the ganglioside G_{M2} (Figure 4.6). G_{M2} is a major component of the membranes of brain cells, and in the absence of the hydrolytic enzyme, the ganglioside accumulates in the bloated lysosomes of brain cells (Figure 1), causing dysfunction. In its severe form, which strikes during infancy, the disease is characterized by progressive mental and motor retardation, as well as skeletal, cardiac, and respiratory abnormalities. The disease is very rare in the general population but reached an

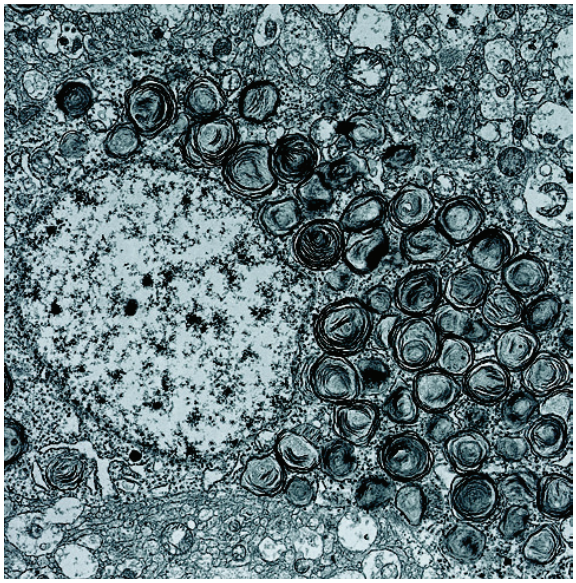


Figure 1 **Lysosomal storage disorders.** Electron micrograph of a section through a portion of a neuron of a person with a lysosomal storage disease characterized by an inability to degrade G_{M2} gangliosides. These cytoplasmic vacuoles stain for both lysosomal enzymes and the ganglioside, indicating they are lysosomes in which undigested glycolipids have accumulated. (COURTESY OF KINUKO SUZUKI.)

Table 1 ***Sphingolipid Storage Diseases***

Disease	Enzyme deficiency	Principal storage substance	Consequences
G_{M1} Gangliosidosis	G_{M1} β-Galactosidase	Ganglioside G_{M1}	Mental retardation, liver enlargement, skeletal involvement, death by age 2
Tay-Sachs disease	Hexosaminidase A	Ganglioside G_{M2}	Mental retardation, blindness, death by age 3
Fabry's disease	α-Galactosidase A	Trihexosylceramide	Skin rash, kidney failure, pain in lower extremities
Sandhoff's disease	Hexosaminidases A and B	Ganglioside G_{M2} and globoside	Similar to Tay-Sachs disease but more rapidly progressing
Gaucher's disease	Glucocerebrosidase	Glucocerebroside	Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Liver and spleen enlargement, mental retardation
Farber's lipogranulomatosis	Ceramidase	Ceramide	Painful and progressively deformed joints, skin nodules, death within a few years
Krabbe's disease	Galactocerebrosidase	Galactocerebroside	Loss of myelin, mental retardation, death by age 2
Sulfatide lipidosis	Arylsulfatase A	Sulfatide	Mental retardation, death in first decade

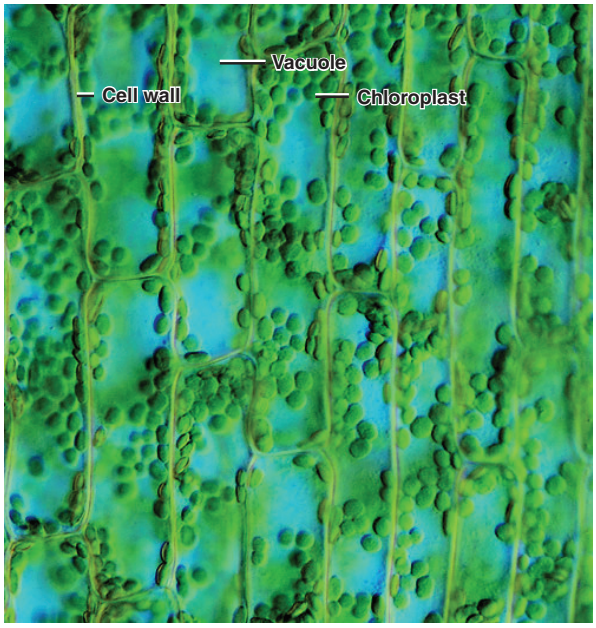
incidence up to 1 in 3600 newborns among Jews of eastern European ancestry. The incidence of the disease has dropped dramatically in this ethnic population in recent years as the result of identification of carriers, genetic counseling of parents at risk, and prenatal diagnosis by amniocentesis. In fact, all of the known lysosomal storage diseases can be diagnosed prenatally.

In the past few years, the prospects for treatment of lysosomal storage diseases have improved with the demonstration that the symptoms of Gaucher's disease, a deficiency of the lysosomal enzyme glucocerebrosidase, can be alleviated by *enzyme replacement therapy*. Infants with Gaucher's disease accumulate large quantities of glucocerebroside lipids in the lysosomes of their macrophages, causing spleen enlargement and anemia. Initial attempts to correct the disease by infusing a solution of the normal human enzyme into the bloodstream were unsuccessful because the enzyme was taken up by liver cells, which are not seriously affected by the deficiency. To target macrophages, the enzyme was purified from human placental tissue and treated with three different glycosidases to remove terminal sugars on the enzyme's oligosaccharide chains, which exposed underlying mannose residues (see Figure 8.22). Following infusion into the bloodstream, this modified enzyme (marketed under the name Cerezyme) is recognized by mannose receptors on the surface of macrophages and rapidly taken up by receptor-mediated endocytosis (page 308). Because lysosomes are the natural target site of materials brought into the macrophage by endocytosis, the enzymes are efficiently delivered to the precise sites in the cell where the deficiency is manifested. Thousands of victims of this disease have been successfully treated in this way. Enzyme replacement therapy for the treatment of several other lysosomal storage diseases has either been approved or is being investigated in clinical trials. (The movie *Extraordinary Measures* is based on the search for a replacement enzyme to treat Pompe disease.) Unfortunately, many of these diseases affect the central nervous system, which is unable to take up circulating enzymes because of the blood–brain barrier (page 262).

An alternate approach that has shown some promise in preclinical trials is referred to as *substrate reduction therapy*, in which small-molecular-weight drugs (e.g., Zavesca) are administered to inhibit the synthesis of the substances that accumulate in the disease. Finally it can be noted that, although it is accompanied by considerable risk to the patient, bone marrow (or cord blood) transplantation has proven relatively successful in treating some of these diseases. It is thought that the foreign transplanted cells, which contain normal copies of the gene in question, secrete a limited amount of the normal lysosomal enzyme. Some of these enzyme molecules are then taken up by the patient's own cells, which lessens the impact of the enzyme deficiency.

8.7 | Plant Cell Vacuoles

As much as 90 percent of the volume of many plant cells is occupied by a single membrane-bound, fluid-filled central **vacuole** (Figure 8.36). While simple in structure, plant vacuoles carry out a wide spectrum of essential functions. Many of a cell's solutes and macromolecules, including ions, sugars, amino acids, proteins, and polysaccharides, are stored temporarily in the vacuole. Vacuoles may also store a host of toxic compounds. Some of these compounds (such



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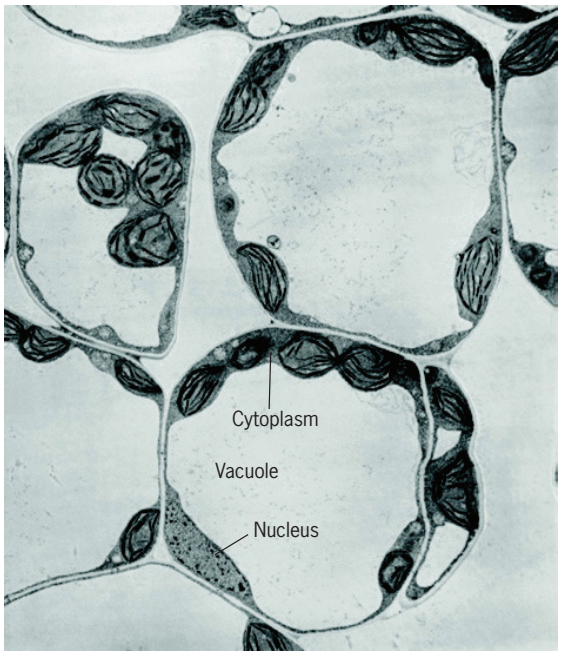


(b)

Figure 8.36 **Plant cell vacuoles.** (*a*) Each of the cylindrical leaf cells of the aquatic plant *Elodea* contains a large central vacuole surrounded by a layer of cytoplasm containing the chloroplasts that are visible in the micrograph. (*b*) Transmission electron micrograph of spinach leaf mesophyll cells showing the large central vacuole and thin layer of surrounding cytoplasm. (A: M. I. WALKER/PHOTO RESEARCHERS, INC.; B: BIOPHOTO ASSOCIATES/PHOTO RESEARCHERS, INC.)

as cyanide-containing glycosides and glucosinolates) are part of an arsenal of chemical weapons that are released when the cell is injured by an herbivore or fungus. Other toxic compounds are simply the by-products of metabolic reactions; because plants lack the type of excretory systems found in animals, they utilize their vacuoles to isolate these by-products from the rest of the cell. Some of these compounds, such as digitalis, have proven to have important clinical value.

The membrane that bounds the vacuole, the **tonoplast**, contains a number of active transport systems that pump ions into the vacuolar compartment to a concentration much higher than that in the cytoplasm or the extracellular fluid. Because of its high ion concentration, water enters the vacuole by osmosis. Hydrostatic (turgor) pressure exerted by the vacuole not only provides mechanical support for the soft tissues of a plant (page 150), it also stretches the cell wall during cell growth.

Plant vacuoles are also sites of intracellular digestion, not unlike lysosomes, which are absent in plants. In fact, plant vacuoles have some of the same acid hydrolases found in lysosomes. The pH of the vacuole is maintained at a low value by a V-type H^+-ATPase within the tonoplast that pumps protons into the vacuolar fluid. Like the proteins of the lysosome, many of the proteins of a plant vacuole are synthesized on membrane-bound ribosomes of the RER, transported through the Golgi complex, and sorted at the *trans* face of the Golgi before being targeted to the vacuole.

REVIEW

1. Describe three distinct roles of plant vacuoles.
2. In what ways is a plant vacuole similar to a lysosome? In what ways is it different?

8.8 | The Endocytic Pathway: Moving Membrane and Materials into the Cell Interior

We have described at length how a cell transports materials from the RER and Golgi to the plasma membrane and extracellular space. We can now turn to the movement of materials in the opposite direction. We considered in Chapter 4 how low-molecular-weight solutes pass through the plasma membrane. But how are cells able to take in materials that are too large to penetrate this membrane regardless of its permeability properties? And how are proteins that reside in the plasma membrane recycled to the cell's internal compartments? Both of these requirements are met by the endocytic pathway, in which segments of the plasma membrane invaginate to form cytoplasmic vesicles that are transported into the cell interior. We will consider two basic processes in this section of the chapter, endocytosis and phagocytosis, which occur by different mechanisms. **Endocytosis** is primarily a process by which the cell internalizes cell-surface receptors and bound extracellular ligands. **Phagocytosis** describes the uptake of particulate matter.

Endocytosis

Endocytosis can be divided broadly into two categories: bulk-phase endocytosis and receptor-mediated endocytosis. *Bulk-phase endocytosis* (also known as *pinocytosis*) is the nonspecific uptake of extracellular fluids. Any molecules, large or small, that happen to be present in the enclosed fluid also gain entry into the cell. Bulk-phase endocytosis can be visualized by adding a substance to the culture medium, such as the dye lucifer yellow or the enzyme horseradish peroxidase, that is taken up by cells nonspecifically. Bulk-phase endocytosis also removes portions of the plasma membrane and may function primarily in the recycling of membrane between the cell-surface and interior compartments. **Receptor-mediated endocytosis (RME)**, in contrast, brings about the uptake of specific extracellular macromolecules (ligands) following their binding to receptors on the external surface of the plasma membrane.[5]

Receptor-Mediated Endocytosis and the Role of Coated Pits Receptor-mediated endocytosis provides a means for the selective and efficient uptake of macromolecules that may be present at relatively low concentrations in the extracellular fluid. Cells have receptors for the uptake of many different types of ligands, including hormones, growth factors, enzymes, and blood-borne proteins carrying certain nutrients. Substances that enter a cell by means of clathrin-mediated RME become bound to receptors that collect in specialized domains of the plasma membrane, known as **coated pits**. Receptors are concentrated in coated pits at 10–20 times their level in the remainder of the plasma membrane. Coated pits (Figure 8.37*a*) are recognized in electron micrographs as sites where the surface is indented and the plasma membrane is covered on its cytoplasmic face by a bristly, electron-dense coat containing clathrin. Coated pits invaginate into the cytoplasm (Figure 8.37*b*) and then pinch free of the plasma membrane to form coated vesicles (Figure 8.37*c*,*d*). To understand the mechanism of coated vesicle formation, we need to examine the molecular structure of the clathrin coat.

Figure 8.38 shows a coated pit as seen from the extracellular and cytoplasmic surfaces of the plasma membranes of cells that had been engaged in receptor-mediated endocytosis. When viewed from its cytoplasmic surface (Figure 8.38*b*,*c*), the bristly coat appears as a network of polygons (hexagons and pentagons) resembling a honeycomb. The geometric construction of the coat is derived from the structure of its clathrin building blocks. Each clathrin molecule consists of three heavy chains and three light chains, joined together at the center to form a three-legged assembly called a *triskelion*

[5]Over the years, several distinct mechanisms of receptor-mediated endocytosis have been described, although some of these proposed mechanisms remain controversial. For our purpose, we will discuss only one mechanism of endocytosis—that which is accomplished by means of clathrin-containing coats, the same type of protein coat discussed on page 299 in connection with vesicles formed at the TGN. (Discussions of clathrin-independent endocytosis can be found in *Ann. Rev. Biochem.* 78, 857, 2009; *J. Cell Sci.* 122: 1713, 2009; and *Curr. Opin. Cell Biol.* 22, 519, 2010 & 23:413, 2011.)

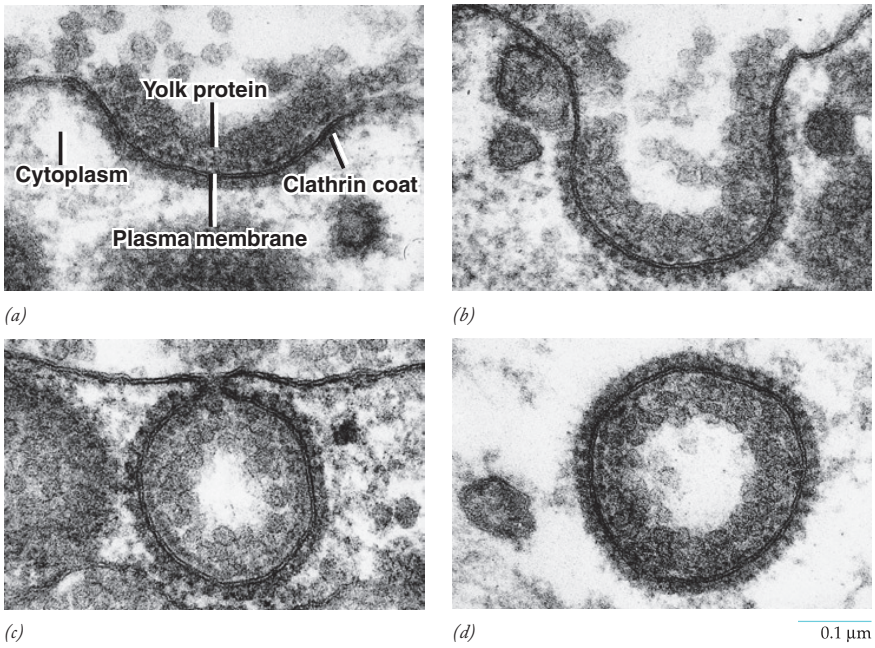


Figure 8.37 **Receptor-mediated endocytosis.** This sequence of micrographs shows the steps in the uptake of yolk lipoproteins by the hen oocyte. (*a*) The proteins to be taken into the cell are concentrated on the extracellular surface of an indented region of the plasma membrane, forming a coated pit. The cytosolic surface of the plasma membrane of the coated pit is covered with a layer of bristly, electron-dense material containing the protein clathrin. (*b*) The coated pit has sunk inward to form a coated bud. (*c*) The plasma membrane is about to pinch off as a vesicle containing the yolk protein on its luminal (previously extracellular) surface and clathrin on its cytosolic surface. (*d*) A coated vesicle that is no longer attached to the plasma membrane. The next step in the process is the release of the clathrin coat. (From M. M. Perry and A. B. Gilbert, J. Cell Science 39:257, 1979, by permission of the Company of Biologists, Ltd. http://jcs.biologists.org/content/39/1/257.full.pdf+html?sid=2493468d-1f02-467a-a192-c2c876bd98b9)

(Figure 8.39). The overlapping arrangement of the triskelions within the clathrin scaffold of a coated vesicle is shown in Figure 8.40. Each leg of a clathrin triskelion extends outward along two edges of a polygon. The clathrin molecules overlap in such a way that each vertex of a polygon contains a center of one of the component triskelions.

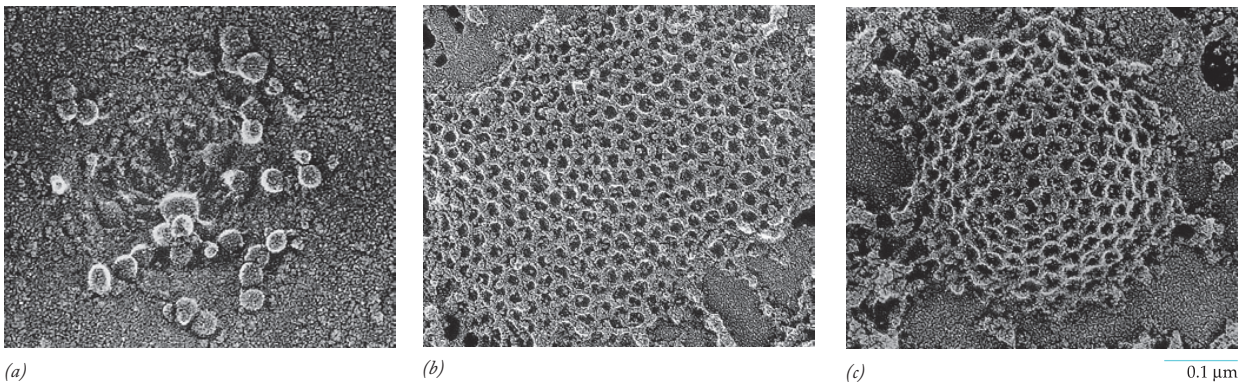


Figure 8.38 **Coated pits.** (*a*) Electron micrograph of a replica formed on the extracellular surface of a whole, freeze-dried fibroblast that had been incubated with LDL-cholesterol. Particles of LDL-cholesterol are visible as spherical structures located on the *extracellular surface* of the coated pit. (*b*) Electron micrograph of a replica formed on the *cytosolic surface* of a coated pit of a ruptured fibroblast. The coat is composed of a flattened network of clathrin-containing polygons associated with the inner surface of the plasma membrane. (*c*) Electron micrograph of a replica of the cytosolic surface of a coated bud showing the invaginated plasma membrane surrounded by a clathrin lattice that has assumed a hemispheric shape. (A–C: From John Heuser and Louise Evans, J. Cell Biol. 84:568, 1980; reproduced with permission of The Rockefeller University Press.)

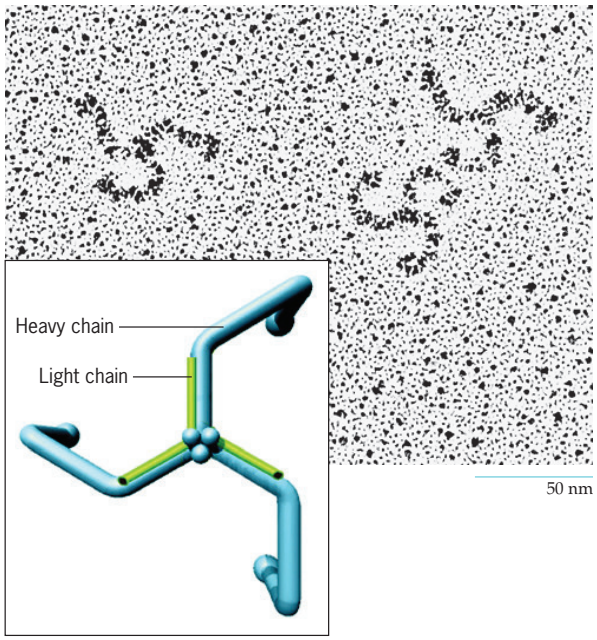
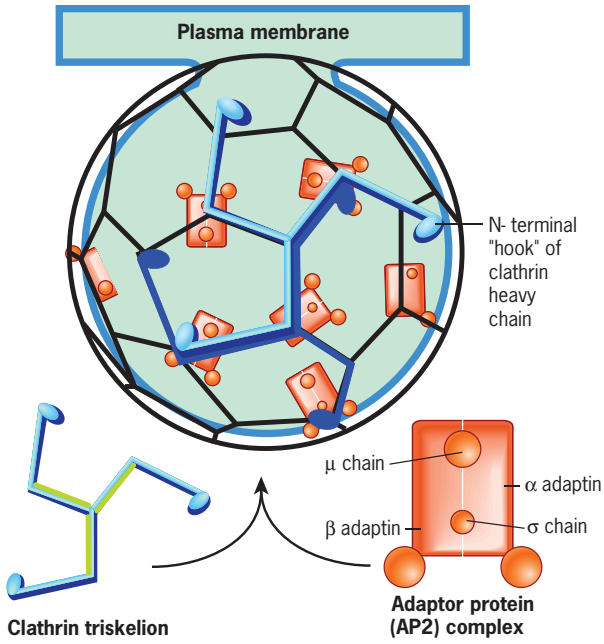


Figure 8.39 **Clathrin triskelions.** Electron micrograph of a metal-shadowed preparation of clathrin triskelions. Inset shows the triskelion is composed of three heavy chains. The inner portion of each heavy chain is linked to a smaller light chain. (REPRINTED WITH PERMISSION FROM MACMILLAN PUBLISHERS LTD: ERNST UNGEWICKELL AND DANIEL BRANTON, NATURE 289:421, 1981; © 1981.)

Figure 8.40 **Molecular organization of a coated vesicle.** (*a*) Schematic drawing of the surface of a coated vesicle showing the arrangement of triskelions and adaptors in the outer clathrin coat. The sides of the polygons are formed by parts of the legs of overlapping triskelions. The N-terminus of each clathrin heavy chain forms a "hook" that projects toward the surface of the membrane where it engages an adaptor. Each adaptor, which consists of four different polypeptide subunits, can bind a diverse array of accessory proteins that are not shown in this illustration. Both the hooks and adaptors are situated at the vertices of the polyhedrons. (*Note:* Not all of the triskelions of the lattice are shown in this figure; if they were, every vertex would have a clathrin hub, hook, and associated adaptor.) (*b*) Schematic drawing of a cross section through the surface of a coated vesicle showing the interactions of the AP2 adaptor complexes with both the clathrin coat and membrane receptors. Recruitment of AP2 adaptors to the plasma membrane is facilitated by the presence of $PI(4,5)P_2$ molecules in the inner (cytosolic) leaflet of the membrane as shown in Figure 8.42. Each receptor is bound to a ligand being internalized. (*c*) Reconstruction of a clathrin cage containing 36 triskelions showing the overlapping arrangement of several of these trimeric molecules (shown in different colors). (An animation can be found at http://iwasa.hms.harvard.edu) (A: ANNUAL REVIEW OF BIOCHEMISTRY. VOLUME 66 BY RICHARDSON, CHARLES C. ; ABELSON, JOHN N. ; RAETZ, CHRISTIAN R. H. COPYRIGHT 1997 REPRODUCED WITH PERMISSION OF ANNUAL REVIEWS, INC. IN THE FORMAT TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER. C: FROM ALEXANDER FOTIN ET AL., NATURE 432:574, 2004, COURTESY OF STEPHEN C. HARRISON; © 2004. REPRINTED WITH PERMISSION FROM MACMILLAN PUBLISHERS LTD.)

Like the clathrin-coated vesicles that bud from the TGN (page 299), the coated vesicles that form during endocytosis also contain a layer of adaptors situated between the clathrin lattice and the surface of the vesicle facing the cytosol. The best studied adaptor operating in connection with clathrin-mediated endocytosis is AP2. Unlike the GGA adaptors utilized at the TGN, which consist of a single subunit with several domains (Figure 8.30), the AP2 adaptors that become incorporated into the vesicles that bud from the plasma membrane contain multiple subunits having different functions (Figure 8.40). The μ subunit of AP2 adaptors engages the cytoplasmic tails of specific plasma membrane receptors, leading to the concentration of these selected receptors—and their bound cargo molecules—into the emerging coated vesicle (discussed further in the Experimental Pathways on page 321). In contrast, the β-adaptin subunit of AP2 adaptors binds and recruits the clathrin molecules of the overlying lattice. A comparison of Figures 8.26 and 8.40 shows both marked differences and similarities between the coats of COPII- and clathrin-coated vesicles. Both coats contain two



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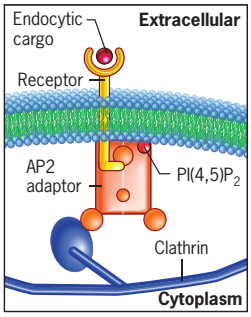

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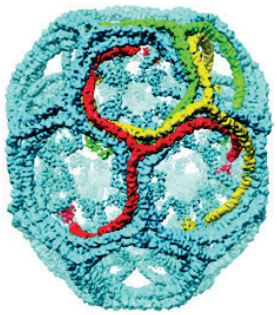
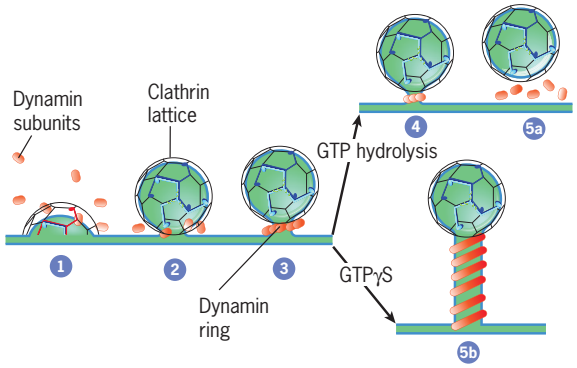


(c)

distinct layers: an outer geometric scaffold and an inner layer of adaptor proteins. The structure of the outer scaffolds, however, are very different; the subunits of the clathrin lattice (three-legged clathrin complexes) overlap extensively, whereas those of the COPII lattice (rod-like Sec13-Sec31 complexes) do not overlap at all. In addition, each vertex of a COPII coat is formed by four edges rather than three as in a clathrin coat. Whether there is a functional basis for these two different types of construction strategies is unclear.

The structure depicted in Figure 8.40 is a greatly simplified version of an actual coated vesicle, which may contain upwards of two dozen different accessory proteins that form a dynamic network of interacting molecules. These proteins have roles in cargo recruitment, coat assembly, membrane bending and invagination, interaction with cytoskeletal components, vesicle release, and membrane uncoating. The best studied of these accessory proteins is dynamin.

Dynamin is a large GTP-binding protein that is required for the release of a clathrin-coated vesicle from the membrane on which it forms. Dynamin self-assembles into a helical collar around the neck of an invaginated coated pit (Figure 8.41), just before it pinches off from the membrane. In the model depicted in Figure 8.41*a*, steps 3–4, hydrolysis of the bound GTP by the polymerized dynamin molecules induces a twisting motion in the dynamin helix that severs the coated vesicle from the plasma membrane. According to this mechanism, dynamin acts as an enzyme capable of utilizing the chemical energy of GTP to generate mechanical forces. Dissociation of the clathrin coat from the vesicle requires the help of an additional protein, the ATPase Hsc70, which is recruited to the clathrin coat by a cofactor, auxilin.



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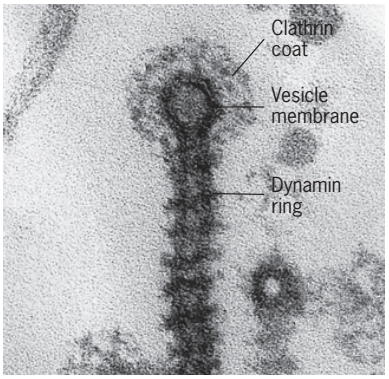


(b) 150 nm

Figure 8.41 **The role of dynamin in the formation of clathrin-coated vesicles.** (*a*) The clathrin lattice of the coated pit (step 1) undergoes rearrangement to form an invaginated vesicle connected to the overlying plasma membrane by a stalk (step 2). At this point, the dynamin subunits, which are concentrated in the region, undergo polymerization to form a ring around the stalk (step 3). Changes in the conformation of the ring, which are thought to be induced by GTP hydrolysis (step 4), lead to fission of the coated vesicle from the plasma membrane and disassembly of the dynamin ring (step 5a). If vesicle budding occurs in the presence of GTPγS, a nonhydrolyzable analogue of GTP, dynamin polymerization continues beyond formation of a simple collar, producing a narrow tubule constructed from several turns of the dynamin helix (step 5b). Structural models of dynamin action can be found in *Nature* 477:556, 561, 2011. (*b*) Electron micrograph showing a coated vesicle forming in the presence of GTPγS, which corresponds to the stage depicted in step 5b of part *a*. (A: Reprinted from P. De Camilli et al. Current Opin Neuro-Biol. Vol 5, p. 562, 1995, with permission from Elsevier. B: from Kohji Takei et al., Nature vol. 374, cover 3/9/95; © 1995, reprinted by permission of Macmillan Publishers Ltd.)

The Role of Phosphoinositides in the Formation of Coated Vesicles Although this discussion emphasizes the protein molecules of the coat and vesicle, the phospholipids of the vesicle membrane also play an important role. As discussed in Chapter 15, phosphate groups can be added to different positions of the sugar ring of the phospholipid phosphatidylinositol (PI), converting them into phosphoinositides (see Figure 15.10). Seven distinct phosphoinositides are identified: PI(3)P, PI(4)P, PI(5)P, PI(3,4)P_2, PI(4,5)P_2, PI(3,5)P_2, and PI(3,4,5)P_3. The phosphorylated rings of these phosphoinositides reside at the surface of the membrane where they can be recognized and bound by particular proteins. Different phosphoinositides are concentrated in different membrane compartments, which helps give each compartment a unique "surface identity." The inner leaflet of the plasma membrane, for example, tends to contain elevated levels of PI(4,5)P_2, which plays an important role in recruiting proteins involved in clathrin-mediated endocytosis, such as dynamin and AP2 (see Figure 8.40*b*). Not only can a phosphoinositide aid in recruiting specific proteins, it can also induce conformational changes in a bound protein that facilitate a molecular process. The clathrin adaptor AP2, for example, normally exists in the cytosol in a locked conformation. Binding of the AP2 complex to PI(4,5)P_2 sites in the plasma membrane induces a major conformational change in AP2 that opens a cargo binding site within the adaptor, allowing it to interact with the cytoplasmic tails of specific membrane receptors. A structural model depicting this series of events is shown in Figure 8.42.

A lipid species such as PI(4,5)P_2 can have a dynamic regulatory role because it can be rapidly formed and destroyed by enzymes that are localized at particular places and times within the cell. In the example of endocytosis, PI(4,5)P_2

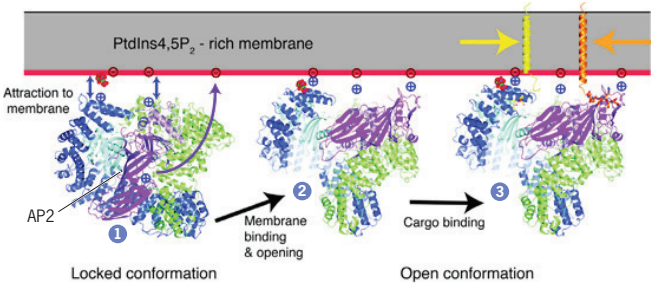


Figure 8.42 **A structural model depicting the changes in protein conformation that occur upon AP2 binding to the plasma membrane.** Binding of the AP2 adaptor is mediated by its interaction with PI(4,5)P_2 molecules in the inner leaflet of the plasma membrane (step 1). Binding of the adaptor to the membrane induces a large conformational change in the adaptor (step 2) that facilitates its interaction with specific motifs in the cytoplasmic tails of certain membrane receptors (shown in yellow and orange) (step 3). (FROM L. P. JACKSON, ET. AL., COURTESY OF DAVID J. OWEN, CELL 141;1228, 2010, FIG. 7. © 2010, REPRINTED BY PERMISSION FROM ELSEVIER.) (SEE SUPPLEMENTAL MOVIE S3.)

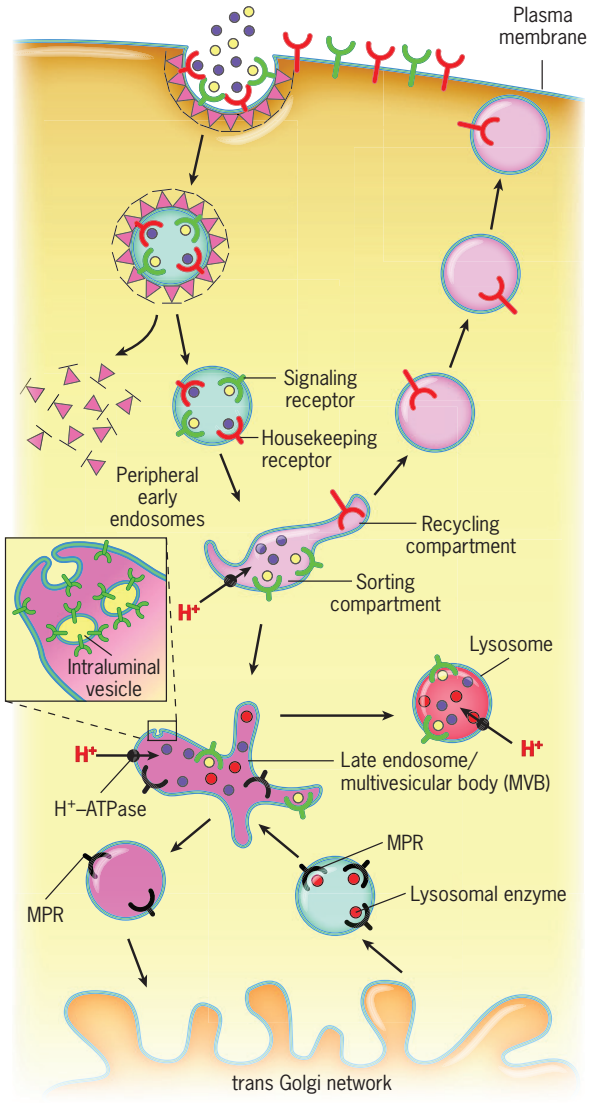
disappears from a site of endocytosis about the time the coated vesicle is pinched away from the plasma membrane. Other PIs that are thought to serve as "landmarks" within the secretory/endocytic pathways include PI(3)P localized at early endosomes and intraluminal vesicles of late endosomes; PI(4)P localized at the TGN, secretory granules, and synaptic vesicles; and PI(3,5)P_2 localized at the late endosome boundary membrane.

The Endocytic Pathway Molecules taken into a cell by endocytosis are routed through a well-defined **endocytic pathway** (Figure 8.43*a*). Before describing events that occur along the endocytic pathway, it is worth considering two different types of receptors that are subjected to endocytosis. One group of receptors, which we will refer to as "housekeeping receptors," is responsible for the uptake of materials that will be used by the cell. The best studied examples are the transferrin and LDL (low-density lipoprotein) receptors, which mediate the delivery to cells of iron and cholesterol, respectively. The LDL receptor is discussed in detail at the end of this section. The red-colored receptor of Figure 8.43*a* represents a housekeeping receptor. The second group of receptors, which we will refer to as "signaling receptors," is responsible for binding extracellular ligands that carry messages that change the activities of the cell. These ligands, which include hormones such as insulin and growth factors such as EGF, bind to the surface receptor (shown in green in Figure 8.43*a*) and signal a physiologic response inside the cell (discussed at length in Chapter 15). Endocytosis of the first group of receptors leads typically to the delivery of the bound materials, such as iron and cholesterol, to the cell and the return of the receptor to the cell surface for additional rounds of uptake. Endocytosis of the second group of receptors often leads to the destruction of the receptor, a process called *receptor down-regulation*, which has the effect of reducing the sensitivity of the cell to further stimulation by the hormone or growth factor. Receptor down-regulation is a mechanism by which cells regulate their ability to respond to extracellular messengers. "Signaling receptors" are typically marked for endocytosis and subsequent destruction by the covalent attachment of a "tag" to the cytoplasmic tail of the receptor while it resides at the cell surface. The tag is a small protein called ubiquitin, which is added enzymatically. Membrane proteins that are not normally subjected to endocytosis become internalized if they are made to carry an added ubiquitin.[6]

Following internalization, vesicle-bound materials are transported to a dynamic network of tubules and vesicles known collectively as **endosomes**, which represent distribution centers along the endocytic pathway. The fluid in the lumen of endosomes is acidified by a H^+-ATPase in the boundary membrane. Endosomes are divided into two classes: **early endosomes**, which are typically located near the peripheral region of the cell, and **late endosomes**, which are typically located closer to the nucleus. According to the prevailing model, early endosomes progressively mature into late endosomes. This transformation from an early to a late endosome is characterized by a decrease in pH, an exchange of Rab proteins (e.g., from Rab5 to Rab7), and a major change in the internal morphology of the structures.

Receptors that are taken up by endocytosis are transported in vesicles to an early endosome, which serves as a sorting station that directs different types of receptors and ligands along different pathways (Figure 8.43*a*). "Housekeeping receptors" typically dissociate from their bound ligands as a result of the high H^+ concentration of the early endosomes. The receptors are then concentrated into specialized tubular compartments of the early endosome, which represent recycling centers. Vesicles that bud from these tubules carry receptors back to the plasma membrane for additional rounds of endocytosis (Figure 8.43*a*). In contrast, released ligands (e.g., LDLs) become concentrated into a sorting compartment before being dispatched to a late endosome and ultimately to a lysosome, where final processing occurs. As noted above, "signaling receptors" with attached ubiquitin tags do not recycle back to the membrane. Instead, these ubiquitinated receptors are sequestered into a population of small, spherical vesicles that crowd the interior of the late endosome (Figure 8.43*b*). This vesiculation process (shown in the inset on the left side of Figure 8.43*a*) is orchestrated by a series of protein complexes (called ESCRT complexes). Four different ESCRT complexes act in sequence to (1) sort the ubiquitinated receptors into a cluster within the late endosomal membrane, (2) cause that patch of membrane to invaginate as a bud into the lumen of the late endosome, and (3) sever the neck of the invagination to release the newly formed intraluminal

[6]In its best-studied role, ubiquitin is attached to a protein to target that protein for degradation as discussed in Section 12.7. This type of degradation signal is composed of a number of ubiquitin molecules that are joined to one another (to form a polyubiquitin chain) by covalent linkages at a specific residue on each ubiquitin molecule, namely Lys48. In contrast, internalization of a membrane protein is thought to be triggered by attachment to that protein of either a single ubiquitin molecule or a short polyubiquitin chain in which the ubiquitin molecules are linked to one another at Lys63 rather than Lys48 (see *Trends Cell Biol.* 21:647.2011).



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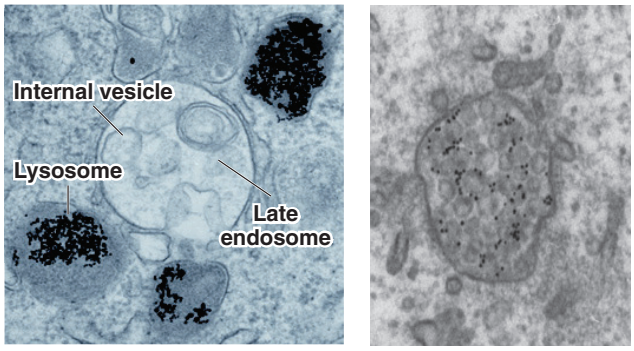


(b) (c)

Figure 8.43 **The endocytic pathway.** The movement of materials from the extracellular space to early endosomes where sorting occurs. Endocytosis of two types of receptor–ligand complexes is shown. Housekeeping receptors, such as the LDL receptor (shown in red), are typically sent back to the plasma membrane, whereas their ligands (purple spheres) are transferred to late endosomes. Signaling receptors, such as the EGF receptor (shown in green), are typically transported to late endosomes along with their ligands (yellow). Late endosomes also receive newly synthesized lysosomal enzymes (red spheres) from the TGN. These enzymes are carried by mannose 6-phosphate receptors (MPRs), which return to the TGN. The contents of late endosomes are transferred to lysosomes by a number of routes (not shown). The inset on the left shows an enlarged view of a portion of a late endosome with an intraluminal vesicle budding inward from the outer membrane. The membranes of these vesicles contain receptors to be degraded. (*b*) Electron micrograph showing the internal vesicles within the lumen of a late endosome. A number of lysosomes are present in the vicinity. (*c*) The gold particles seen in this electron micrograph are bound to EGF receptors that were internalized by endocytosis and have become localized within the membranes of the internal vesicles of this late endosome. (B: From J. Paul Luzio, Paul R. Pryor, and Nicholas A. Bright, Nature Revs. Mol. Cell Biol. 8:625, 2007; © 2007, reprinted by permission of Macmillan Publishers Ltd., C: Courtesy of Clare Futter.)

vesicle. The segregation of signaling receptors within the lumen of a late endosome is shown in Figure 8.43*c*. Because of these internal vesicles, late endosomes are also referred to as *multivesicular bodies* (*MVBs*).

LDLs and Cholesterol Metabolism Among many examples of receptor-mediated endocytosis, the first studied and best understood is one that provides animal cells with exogenous cholesterol. Animal cells use cholesterol as an essential part of their plasma membranes and as a precursor to steroid hormones. Cholesterol is a hydrophobic molecule that is transported in the blood as part of huge lipoprotein complexes, such as the *low-density lipoprotein* (*LDL*) shown in Figure 8.44. Each LDL particle contains a central core of about 1500 cholesterol molecules esterified to long-chain fatty acids. The core is surrounded by a single layer of phospholipids that contains a single copy of a large protein, called *apolipoprotein B-100*, which binds specifically to LDL receptors on the surfaces of cells.

LDL receptors are transported to the plasma membranes of cells, where they become concentrated in coated pits, even in the absence of LDL ligand. As a result, the receptors are on the cell surface ready to take up the blood-borne lipoproteins if they should become available. Once the LDL particles are bound to a coated pit, the pit invaginates to form a coated vesicle, the clathrin coat is disassembled, and the LDL receptors pass through the early endosomes and back to the plasma membrane, as depicted in Figure 8.43*a*. Meanwhile, the LDL particles are delivered to late endosomes and lysosomes, where the protein component is degraded and the cholesterol is deesterified and used by the cell in membrane assembly or other metabolic processes (e.g., steroid hormone formation). Persons with a rare inherited disorder called *Niemann-Pick type C disease* lack one of the proteins required to transfer

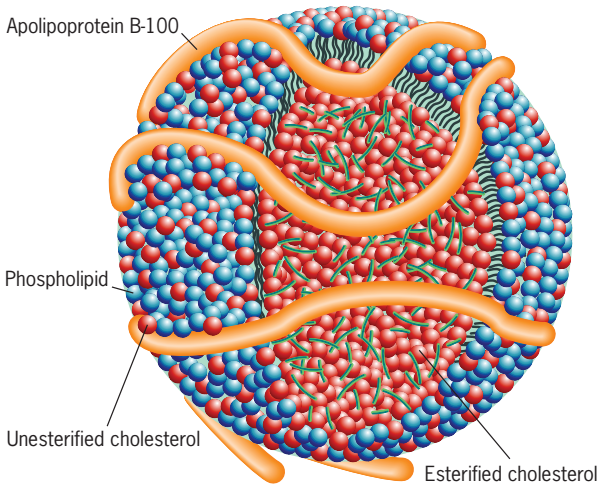


Figure 8.44 **LDL cholesterol.** Each particle consists of esterified cholesterol molecules, surrounded by a mixed monomolecular layer of phospholipids and cholesterol, and a single molecule of the protein apolipoprotein B-100, which interacts specifically with the LDL receptor projecting from the plasma membrane.

cholesterol out of lysosomes. The resulting accumulation of cholesterol in these organelles leads to nerve degeneration and death during early childhood. Studies of a different disease that led to the discovery of receptor-mediated endocytosis and LDL internalization are described in the accompanying Experimental Pathways.

The level of LDL in the blood has been related to the development of atherosclerosis, a condition characterized by formation of plaques in the inner lining of arteries that reduce the flow of blood through the vessel and act as sites for the formation of blood clots. Blood clots that block the coronary arteries are the leading cause of myocardial infarction (heart attack). Studies suggest that atherosclerosis results from a chronic inflammatory response that is initiated by the deposition of LDL within the inner walls of blood vessels, as indicated in Figure 8.45. Lowering blood LDL levels is most readily accomplished by administration of drugs called *statins* (e.g., lovastatin and Lipitor) that block HMG CoA reductase, a key enzyme in the synthesis of cholesterol (page 319). When blood cholesterol levels are lowered, the risk of heart attack is reduced.

LDLs are not the only cholesterol-transporting agents in the blood. *HDLs* (*high-density lipoproteins*) have a similar construction but contain a different protein (apolipoprotein A-I) and play a different physiologic role in the body. LDL serves primarily to carry cholesterol molecules from the liver, where they are synthesized and packaged, through the blood to the body's cells. HDL carries cholesterol in the opposite direction. Excess cholesterol is transported out of the plasma membrane of the body's cells directly to circulating HDL particles, which carry cholesterol to the liver for excretion. Just as high blood levels of LDL are associated with increased risk of heart disease, high blood levels of HDL are associated with decreased risk, which has led to HDL being called the "good cholesterol." There is little doubt that lowering LDL levels is beneficial, but the consequences of raising HDL levels is less clear-cut. For example, cholesterol molecules can be transferred from HDL to other lipoprotein particles by an enzyme called cholesteryl ester transfer protein (CETP), an activity that tends to lower HDL cholesterol levels. CETP has become a focus of research following the discovery of a population of Japanese families whose members routinely live more than 100 years and carry mutations in the *CETP* gene. Several small-molecular-weight CETP inhibitors have been tested in clinical trials and found to increase HDL levels in the blood. One of these drug candidates (torcetrapib) was dropped from further study despite the fact that it raised HDL blood levels. For reasons that are unclear, subjects taking the drug and a statin were considerably more likely to die than those in the control group who took only the statin. Preliminary study of

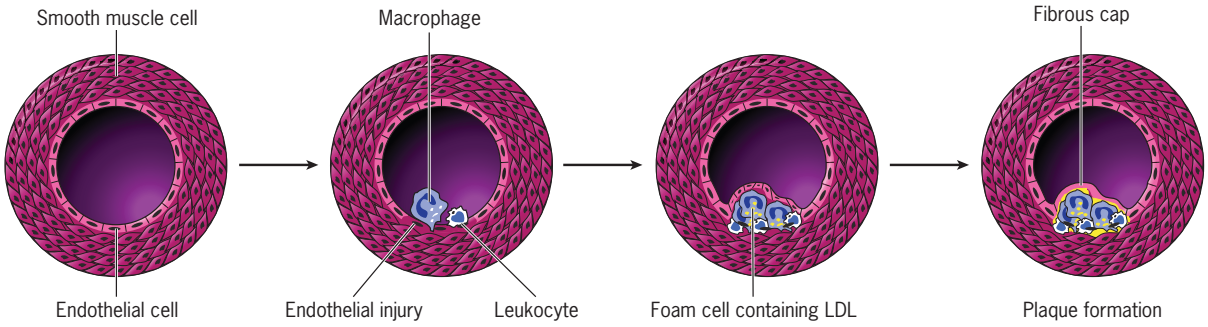


Figure 8.45 **A model of atherosclerotic plaque formation.** According to this model, plaque formation is initiated by various types of injury to the endothelial cells that line the vessel, including damage inflicted by oxygen free radicals that chemically alter the LDL-cholesterol particles. The injured endothelium acts as an attractant for white blood cells (leukocytes) and macrophages, which migrate beneath the endothelium and begin a process of chronic inflammation. The macrophages ingest the oxidized LDL, which becomes deposited in the cytoplasm as cholesterol-rich fatty droplets. These cells are referred to as macrophage foam cells and they are often already present in the blood vessels of adolescents and young adults. Substances released by the macrophages stimulate the proliferation of smooth muscle cells, which produce a dense, fibrous connective tissue matrix (fibrous cap) that bulges into the arterial lumen. Not only do these bulging lesions restrict blood flow, they are prone to rupture, which can trigger the formation of a blood clot and ensuing heart attack.

another CETP inhibitor (anacetrapib) did not show any evidence of harmful effects from the drug. Although anacetrapib raised HDL levels dramatically (by an average of more than 100 percent), it remains to be seen if this response will translate into increased long-term survival of patients at risk for heart disease. Another cholesterol-lowering drug target is PCSK9, a proteolytic enzyme that destroys LDL receptors in the liver. Persons that carry inactivating mutations in the gene encoding PCSK9 have reduced LDL cholesterol levels and decreased heart disease. At least two PCSK9 inhibitors (REGN727 and AMG145) are in clinical trials.

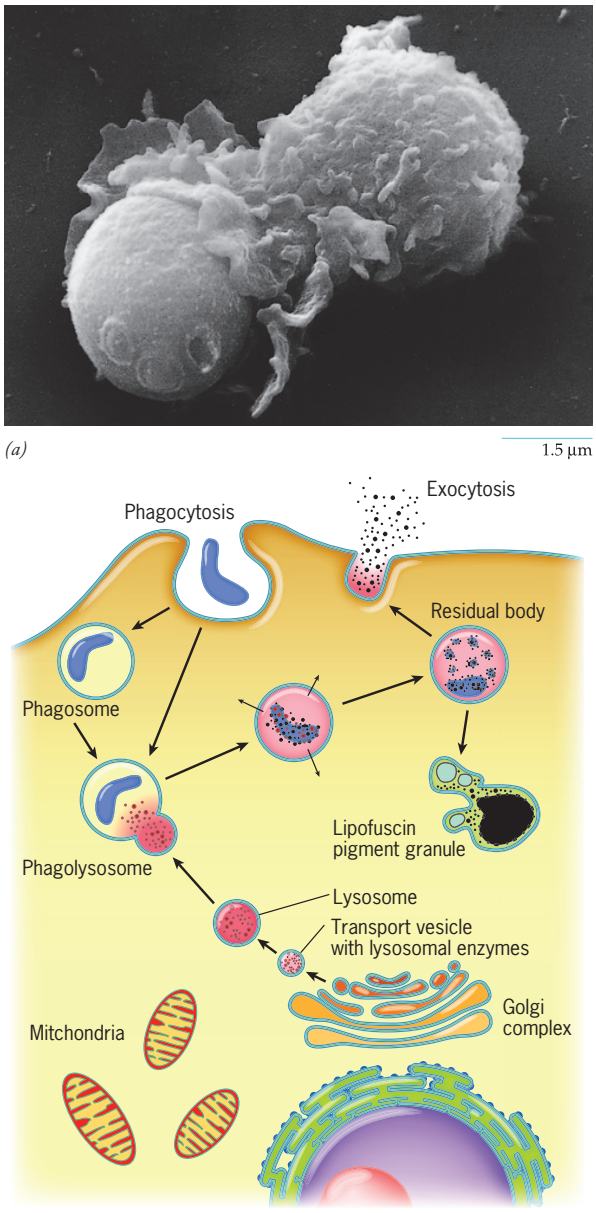


Figure 8.46 **Phagocytosis.** (*a*) The process of engulfment as illustrated by a polymorphonuclear leukocyte ingesting a yeast cell (lower left). (*b*) The steps that occur in the phagocytic pathway (see also page 305). (A: From Janet Boyles and Dorothy F. Bainton, Cell 24:906, 1981, reprinted by permission of Elsevier.)

Phagocytosis

Phagocytosis ("cell eating") is carried out extensively by a few types of cells specialized for the uptake of relatively large particles (>0.5 µm diameter) from the environment. Many single-celled protists, such as amoebas and ciliates, make their livelihood by trapping food particles and smaller organisms and enclosing them within folds of the plasma membrane. The folds fuse to produce a vacuole (or *phagosome*) that pinches off inwardly from the plasma membrane. The phagosome fuses with a lysosome, and the material is digested within the resulting *phagolysosome*.

In most animals, phagocytosis is a protective mechanism rather than a mode of feeding. Mammals possess a variety of "professional" phagocytes, including macrophages and neutrophils, that wander through the blood and tissues phagocytizing invading organisms, damaged and dead cells, and debris. These materials are recognized and bound by receptors on the surface of the phagocyte prior to uptake. Once inside the phagocyte, microorganisms may be killed by lysosomal enzymes or by oxygen free radicals generated within the lumen of the phagosome. The process of particle engulfment is pictured in the chapter-opening photograph and in Figure 8.46*a*. The steps in the digestion of engulfed materials are illustrated in Figure 8.46*b*. The engulfment of particulate material by phagocytosis is driven by contractile activities of the actin-containing microfilaments that underlie the plasma membrane.

Not all bacteria ingested by phagocytic cells are destroyed. In fact, some species hijack the phagocytic machinery to promote their own survival in the body. *Mycobacterium tuberculosis*, the agent responsible for tuberculosis, for example, is taken into the cytoplasm of a macrophage by phagocytosis, but the bacterium is able to inhibit the fusion of its phagosome with a lysosome. Even if the phagosome does become highly acidic, the bacterium appears able to maintain its own physiological pH despite the lowered pH of its surrounding medium. The bacterium responsible for Q fever, *Coxiella burnetii*, becomes enclosed in a phagosome that does fuse with a lysosome, but neither the acidic environment nor the lysosomal enzymes can destroy the pathogen. *Listeria monocytogenes*, a bacterium that causes meningitis, produces proteins that destroy the integrity of the lysosomal membrane, allowing the bacterium to escape into the cell's cytosol (see Figure 9.67).

REVIEW

1. Describe the steps that occur between the binding of an LDL particle to the plasma membrane of a cell and the entry of cholesterol molecules into the cytosol.
2. Describe the molecular structure of clathrin and the relationship between its structure and function.
3. How does the role of phagocytosis in the life of an amoeba compare to its role in a multicellular animal? What are the major steps that occur during phagocytosis?

8.9 | Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts

The division of the contents of a cell into large numbers of compartments presents many organizational challenges to the cell's protein-trafficking machinery. We have seen in this chapter that protein trafficking within a eukaryotic cell is governed by (1) sorting signals, such as the signal peptide of secreted proteins or mannose-phosphate groups of lysosomal enzymes, and (2) receptors that recognize these signals and deliver proteins containing them to the proper compartment. Four of the cell's major organelles—nucleus, mitochondria, chloroplasts, and peroxisomes—import proteins through one or more outer boundary membranes. As in the case of the rough ER, proteins that are imported by these organelles contain amino acid sequences that serve as addresses that are recognized by receptors at the organelle's outer membrane. Unlike the rough ER, which generally imports its proteins cotranslationally, the proteins of these other organelles are imported posttranslationally, that is, following their complete synthesis on free ribosomes in the cytosol.

The import of proteins into the nucleus is a specialized topic and will be discussed separately in Section 12.2. Import of proteins into peroxisomes, mitochondria, and chloroplasts will be discussed in the following sections.

Uptake of Proteins into Peroxisomes

Peroxisomes are very simple organelles having only two subcompartments in which an imported protein can be placed: the boundary membrane and the internal matrix (page 206). Proteins destined for a peroxisome possess a *peroxisomal targeting signal*, either a *PTS* for a peroxisomal matrix protein or an *mPTS* for a peroxisomal membrane protein. Several different PTSs, mPTSs, and PTS receptors have been identified. PTS receptors bind to peroxisome-destined proteins in the cytosol and shuttle them to the surface of the peroxisome, where they can enter the organelle. Some evidence suggests that at least one of the PTS receptors (PEX5) moves from the cytosol into the peroxisomal membrane where it forms a transient pore that facilitates the movement of proteins into the peroxisome. Unlike mitochondria and chloroplasts, whose imported proteins must assume an unfolded state, peroxisomes are somehow able to import peroxisomal matrix proteins in their native, folded conformation, even those that consist of several subunits. The mechanism by which peroxisomes are able to accomplish this daunting assignment remains a matter of speculation.

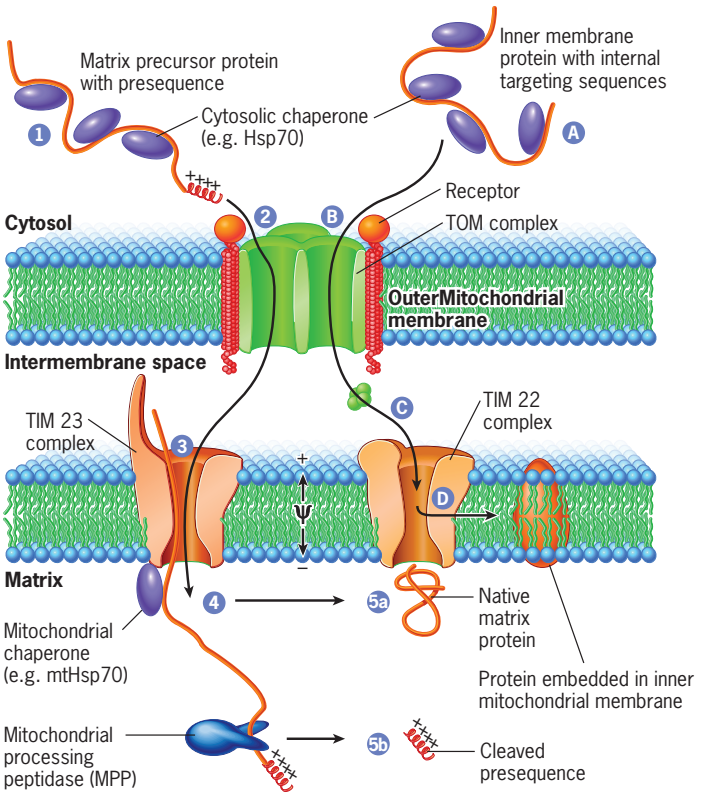
Uptake of Proteins into Mitochondria

Mitochondria have four subcompartments into which proteins can be delivered: an outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), intermembrane space, and matrix (see Figure 5.3*c*). Although mitochondria synthesize a few of their own integral membrane polypeptides (13 in mammals), roughly 99 percent of the organelle's proteins are encoded by the nuclear genome, synthesized in the cytosol, and imported posttranslationally. We will restrict the present discussion to proteins of the mitochondrial matrix and inner mitochondrial membrane, which together constitute the vast majority of the proteins targeted to this organelle. As with peroxisomal proteins, and proteins of other compartments, mitochondrial proteins contain signal sequences that target them to their home base. Most mitochondrial matrix proteins contain a removable targeting sequence (called the *presequence*) located at the N-terminus of the molecule (step 1, Figure 8.47*a*). A presequence typically includes a number of positively charged residues on one face of the helix and hydrophobic residues on the opposite face. In contrast, most proteins destined for the IMM contain internal targeting sequences that remain as part of the molecule.

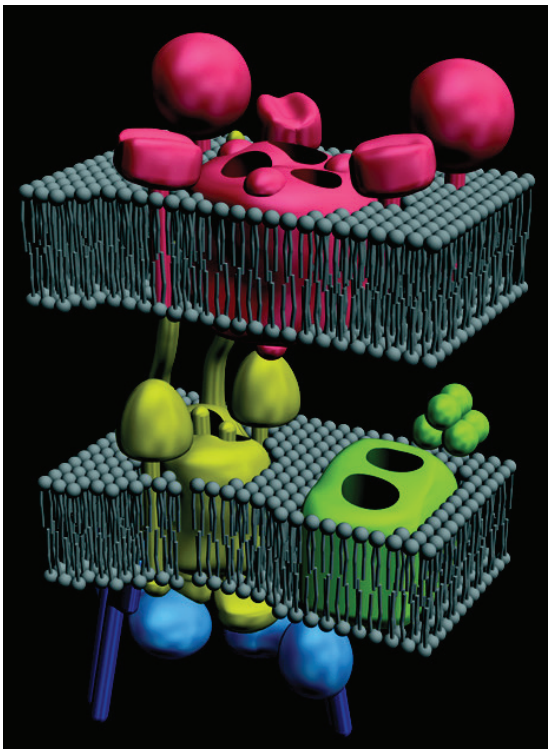
Before a protein can enter a mitochondrion, several events are thought to take place. First, the protein must be presented to the mitochondrion in a relatively extended, or unfolded, state (steps 1 and A, Figure 8.47*a*). Several different molecular chaperones (e.g., Hsp70 and Hsp90) have been implicated in preparing polypeptides for uptake into mitochondria, including ones that specifically direct mitochondrial proteins to the cytosolic surface of the OMM (Figure 8.47*a*). The OMM contains a protein-import complex, the *TOM complex*, which includes (1) receptors that recognize and bind mitochondrial proteins and (2) protein-lined channels through which unfolded polypeptides are translocated across the outer membrane (steps 2 and B).[7] Proteins that are destined for the IMM or matrix must pass through the intermembrane space and engage a second protein-import complex located in the IMM, called a *TIM complex*. The IMM contains two major TIM complexes: TIM22 and TIM23. TIM22 binds integral proteins of the IMM that contain an internal targeting sequence and inserts them into the lipid bilayer (steps C–D, Figure 8.47*a*). TIM23, in contrast, binds proteins with an N-terminal presequence, which includes all of the proteins of the matrix (as well as a number of proteins of the IMM that will not be discussed). TIM23 recognizes and translocates the matrix proteins completely through the IMM and into the inner aqueous compartment (step 3, Figure 8.47*a*). Translocation occurs at sites where the outer and inner mitochondrial membranes come into close proximity so that the imported protein can cross both membranes simultaneously. Movement into the matrix is powered by the electric potential across the IMM acting on the positively charged targeting signal; if the potential is dissipated by addition of a drug such as DNP (page 198), translocation ceases.

As it enters the matrix, a polypeptide interacts with mitochondrial chaperones, such as mtHsp70 (step 4, Figure 8.47*a*),

[7]It is interesting to note that, unlike the translocon of the ER or peroxisome, the pore-forming protein of the TOM complex (Tom 40) is a β-barrel protein, like the other integral proteins of the OMM (page 182), reflecting its evolution from the outer membrane of an ancestral bacterium. This has functional consequences, as the β-barrel protein cannot open laterally to allow integral proteins to insert into the OMM. As a result, OMM proteins have to pass into the intermembrane space before entering the OMM bilayer.



(a)



(b)

Figure 8.47 **Importing proteins into a mitochondrion.** (*a*) Proposed steps taken by proteins imported posttranslationally into either the mitochondrial matrix or inner mitochondrial membrane. The polypeptide is targeted to a mitochondrion by a targeting sequence, which is located at the N-terminus in the matrix protein (step 1) and is located internally in most inner membrane proteins (step A). Cytosolic Hsp70 molecules unfold the polypeptides prior to their entry into the mitochondrion. The proteins are recognized by membrane receptors (red transmembrane proteins) and translocated through the OMM by way of pores in the TOM complex of the OMM (step 2 or B). Most integral proteins of the IMM are directed to the TIM22 complex of the IMM (step C), which steers them into the lipid bilayer of the IMM (step D). Mitochondrial matrix proteins are translocated through the TIM23 complex of the IMM (step 3). Once the protein enters the matrix, it is bound by a mitochondrial chaperone (step 4), which may either pull the polypeptide into the matrix or act like a Brownian ratchet to ensure that it diffuses into the matrix (these alternate chaperone mechanisms are discussed in the text). Once in the matrix, the unfolded protein assumes its native conformation (step 5a) with the help of Hsp60 chaperones (not shown). The presequence is removed enzymatically (step 5b). (*b*) A three-dimensional model of the mitochondrial protein-import machinery, showing the number, relative size, and topology of the various proteins involved in this activity. The TOM complex is a reddish color, the TIM23 complex is yellow-green, the TIM22 complex is green, and the cooperating chaperones are blue. (B: From Toshiya Endo, Hayashi Yamamoto, and Masatoshi Esaki, J. Cell Science, cover of vol. 116, #16, 2003; reproduced by permission of The Company of Biologists, Ltd. http://jcs.biologists.org/content/116/16.cover-expansion)

that mediate entry into the aqueous compartment. Two mechanisms have been proposed to explain the general action of molecular chaperones involved in the movement of proteins across membranes, which is a widespread phenomenon. According to one view, the chaperones act as force-generating motors that use energy derived from ATP hydrolysis to actively "pull" the unfolded polypeptide through the translocation pore. According to the alternate view, the chaperones aid in the diffusion of the polypeptide across the membrane. Diffusion is a random process in which a molecule can move in any available direction. Consider what would happen if an unfolded polypeptide had entered a translocation pore in the mitochondrial membrane and had "poked its head" into the matrix. Then consider what would happen if a chaperone residing on the inner surface of the membrane were able to bind the protruding polypeptide in such a way that it blocked the diffusion of the polypeptide back through the pore and into the cytosol, but did not block its diffusion further into the matrix. As the polypeptide diffused further into the matrix, it would be bound repeatedly by the chaperone and at each stage prevented from diffusing backward. This mechanism of chaperone action is referred to as *biased diffusion*, and the chaperone is said to be acting as a "Brownian ratchet"; the term *Brownian* implies random diffusion, and a "ratchet" is a device that allows movement in only one direction. Recent studies suggest that both mechanisms of chaperone action are probably utilized and act cooperatively. Regardless of the mechanism of entry, once in the matrix the polypeptide achieves its

native conformation (step 5a, Figure 8.47*a*) following enzymatic removal of the presequence (step 5b).

Uptake of Proteins into Chloroplasts

Chloroplasts have six subcompartments into which proteins can be delivered: an inner and outer envelope membrane and intervening intermembrane space, as well as the stroma, thylakoid membrane, and thylakoid lumen (Figure 8.48). Chloroplast and mitochondrial import mechanisms exhibit many similarities, although their translocation machineries have evolved independently. As in the mitochondria,

1. the vast majority of chloroplast proteins (approximately 3000 in higher plants) are imported from the cytosol,
2. the outer and inner envelope membranes contain distinct translocation complexes (*Toc* and *Tic complexes*, respectively) that work together during import,
3. chaperones aid in the unfolding of the polypeptides in the cytosol and folding of the proteins in the chloroplast, and
4. most proteins destined for the chloroplast are synthesized with a removable N-terminal sequence (termed the *transit peptide*) that is highly variable in length and sequence.

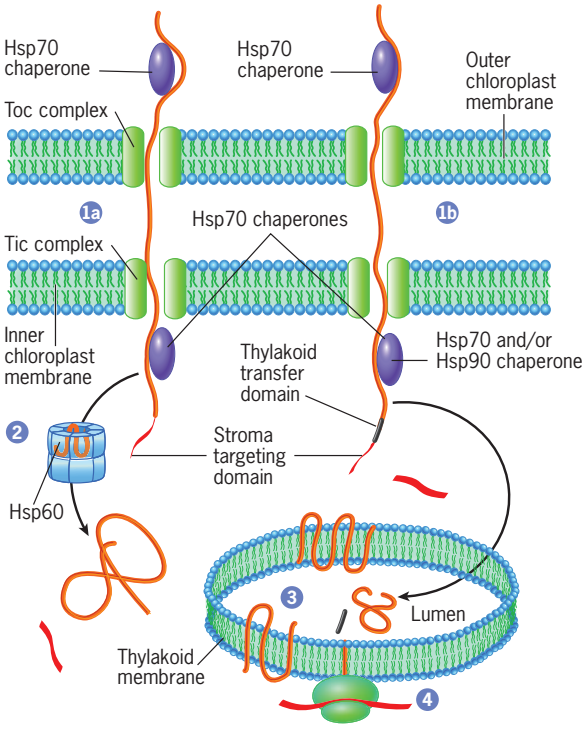


Figure 8.48 **Importing proteins into a chloroplast.** Proteins encoded by nuclear genes are synthesized in the cytosol and imported through protein-lined pores in both membranes of the outer chloroplast envelope (step 1). Proteins destined for the stroma (step 1a) contain a stroma-targeting domain at their N-terminus, whereas proteins destined for the thylakoid (step 1b) contain both a stroma-targeting domain and a thylakoid-transfer domain at their N-terminus. Stromal proteins remain in the stroma (step 2) following translocation through the outer envelope and removal of their single targeting sequence. The presence of the thylakoid transfer domain causes thylakoid proteins to be translocated either into or completely through the thylakoid membrane (step 3). A number of the proteins of the thylakoid membrane are encoded by chloroplast genes and synthesized by chloroplast ribosomes that are bound to the outer surface of the thylakoid membrane (step 4).

The transit peptide does more than simply target a polypeptide to a chloroplast: it provides an "address" that localizes the polypeptide to one of several possible subcompartments within the organelle (Figure 8.48). All proteins translocated through the chloroplast envelope contain a *stroma targeting domain* as part of their transit peptide, which guarantees that the polypeptide will enter the stroma. Once in the stroma, the stroma targeting domain is removed by a processing peptidase located in that compartment. Those polypeptides that belong in a thylakoid membrane or thylakoid lumen bear an additional segment in their transit peptide, called the *thylakoid transfer domain*, that dictates entry into the thylakoids. Several distinct pathways have been identified by which proteins are either inserted into the thylakoid membrane or translocated into the thylakoid lumen. These pathways exhibit striking similarities to transport systems in bacterial cells, the presumed ancestors of chloroplasts. Many of the proteins that reside within the thylakoid membrane are encoded by chloroplast genes and synthesized on membrane-bound ribosomes of the chloroplast, as illustrated in Figure 8.48, step 4.

REVIEW

1. How are proteins, such as the enzymes of the TCA cycle, able to arrive in the mitochondrial matrix?
2. What is the role of cytosolic and mitochondrial chaperones in the process of mitochondrial import?
3. Distinguish between two possible import mechanisms: biased diffusion and force-generating motors.
4. Describe the steps by which a polypeptide would move from the cytosol where it is synthesized to the thylakoid lumen.