13 Intracellular Vesicular Traffic

Every cell must eat, communicate with the world around it, and quickly respond to changes in its environment. To help accomplish these tasks, cells continually adjust the composition of their plasma membrane in rapid response to need. They use an elaborate internal membrane system to add and remove cell-surface proteins embedded in the membrane, such as receptors, ion channels, and transporters. Through the process of *exocytosis*, the *biosynthetic–secretory pathway* delivers newly synthesized proteins, carbohydrates, and lipids to either the plasma membrane or the extracellular space. By the converse process of *endocytosis* (**Figure 13–1**) cells remove plasma membrane components and deliver them to internal compartments called *endosomes*, from where they can be recycled to the same or different regions of the plasma membrane or can be delivered to lysosomes for degradation. Cells also use endocytosis to capture important nutrients, such as vitamins, lipids, cholesterol, and iron; these are taken up together with the macromolecules to which they bind and are then released in endosomes or lysosomes and transported into the cytosol, where they are used in various biosynthetic processes.

The interior space, or *lumen*, of each membrane-enclosed compartment along the biosynthetic–secretory and endocytic pathways is topologically equivalent to the lumen of most other membrane-enclosed compartments and to the cell exterior. Proteins can travel in this space without having to cross a membrane, being passed from one compartment to another by means of numerous membrane-enclosed transport containers. Some of these containers are small spherical *vesicles*, while others are larger irregular vesicles or tubules formed from the donor compartment. We shall use the term **transport vesicle** to apply to all forms of these containers.

Within a eucaryotic cell, transport vesicles continually bud off from one membrane and fuse with another, carrying membrane components and soluble molecules, which are referred to as **cargo** (**Figure 13–2**). This membrane traffic flows along highly organized, directional routes, which allow the cell to secrete, eat, and remodel its plasma membrane. The biosynthetic–secretory pathway leads outward from the endoplasmic reticulum (ER) toward the Golgi apparatus and cell surface, with a side route leading to lysosomes, while the endocytic pathway leads inward from the plasma membrane. In each case, the flow of membrane between compartments is balanced, with retrieval pathways balancing the flow in the opposite direction, bringing membrane and selected proteins back to the compartment of origin (**Figure 13–3**).

To perform its function, each transport vesicle that buds from a compartment must be selective. It must take up only the appropriate molecules and must fuse only with the appropriate target membrane. A vesicle carrying cargo from the Golgi apparatus to the plasma membrane, for example, must exclude proteins that are to stay in the Golgi apparatus, and it must fuse only with the plasma membrane and not with any other organelle.

We begin this chapter by considering the molecular mechanisms of budding and fusion that underlie all vesicular transport. We then discuss the fundamental problem of how, in the face of this transport, the cell maintains the differences between the compartments. Finally, we consider the function of the Golgi apparatus, lysosomes, secretory vesicles, and endosomes, as we trace the pathways that connect these organelles.

In This Chapter

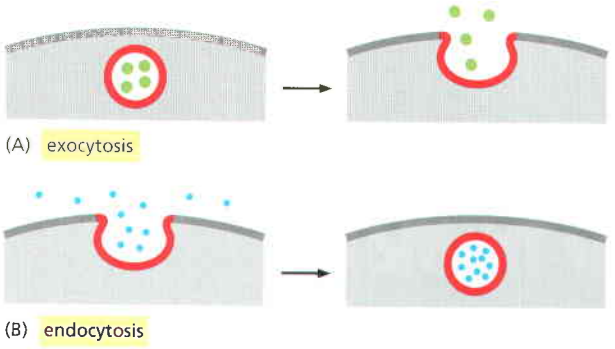


Figure 13–1 Exocytosis and endocytosis. (A) In exocytosis, a transport vesicle fuses with the plasma membrane. Its content is released into the extracellular space, while the vesicle membrane (*red*) becomes continuous with the plasma membrane. (B) In endocytosis, a plasma membrane patch (*red*) is internalized forming a transport vesicle. Its content derives from the extracellular space.

THE MOLECULAR MECHANISMS OF MEMBRANE TRANSPORT AND THE MAINTENANCE OF COMPARTMENTAL DIVERSITY

Vesicular transport mediates a continuous exchange of components between the ten or more chemically distinct, membrane-enclosed compartments that collectively comprise the biosynthetic-secretory and endocytic pathways. With this massive exchange, how can each compartment maintain its special identity? To answer this question, we must first consider what defines the character of a compartment. Above all, it is the composition of the enclosing membrane: molecular markers displayed on the cytosolic surface of the membrane serve as guidance cues for incoming traffic to ensure that transport vesicles fuse only with the correct compartment. Many of these membrane markers, however, are found on more than one compartment, and it is the specific combination of marker molecules that gives each compartment its unique molecular address.

How are these membrane markers kept at high concentration on one compartment and at low concentration on another? To answer this question, we need to consider how patches of membrane, enriched or depleted in specific membrane components, bud off from one compartment and transfer to another. **Panel 13–1** outlines some of the genetic and biochemical strategies that have been used to study the molecular machinery involved in vesicular transport.

We begin by discussing how cells segregate proteins into separate membrane domains by assembling a special protein coat on the membrane's cytosolic face. We consider how coats form, what they are made of, and how they are

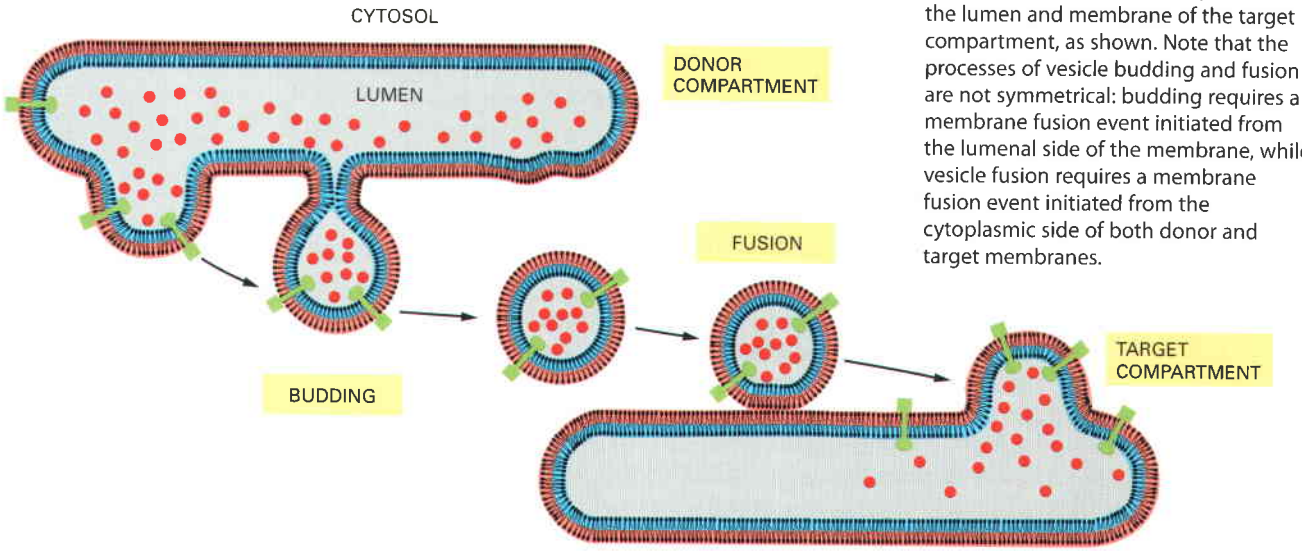


Figure 13–2 Vesicular transport. Transport vesicles bud off from one compartment and fuse with another. As they do so, they carry material as cargo from the *lumen* (the space within a membrane-enclosed compartment) and membrane of the donor compartment to the lumen and membrane of the target compartment, as shown. Note that the processes of vesicle budding and fusion are not symmetrical: budding requires a membrane fusion event initiated from the lumenal side of the membrane, while vesicle fusion requires a membrane fusion event initiated from the cytoplasmic side of both donor and target membranes.

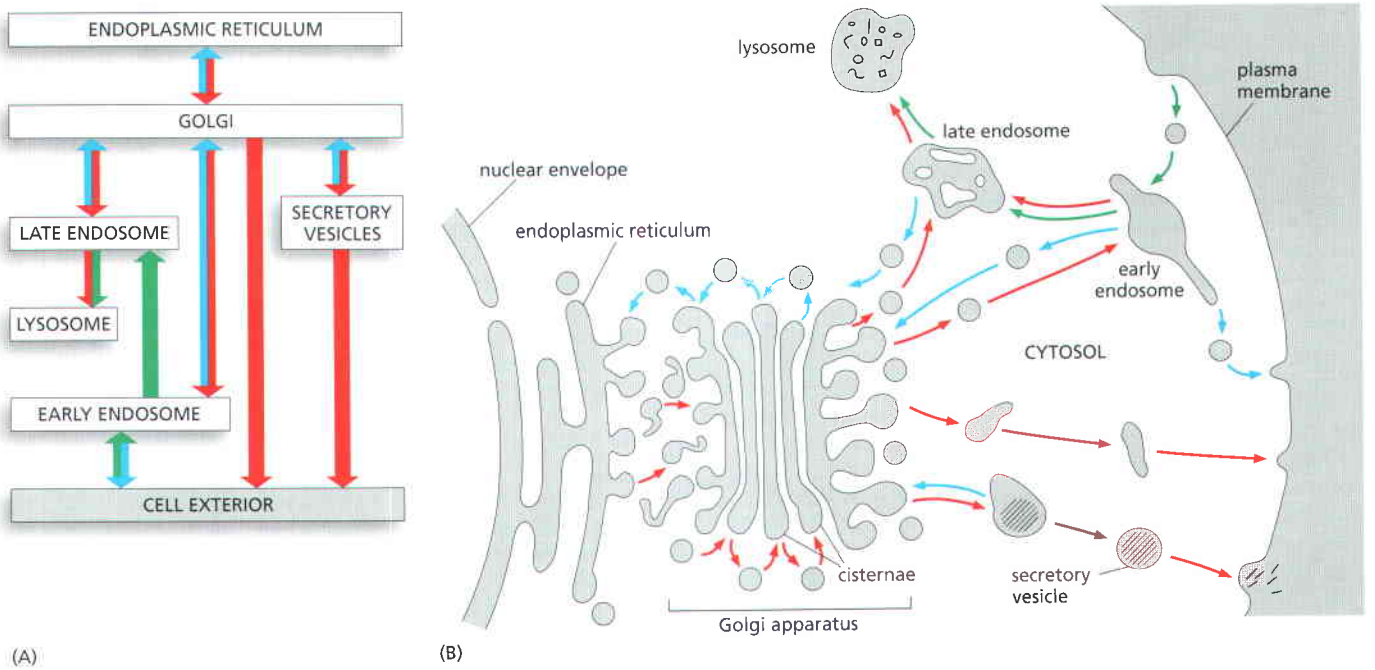


Figure 13–3 A "road-map" of the biosynthetic–secretory and endocytic pathways. (A) In this diagram, which was introduced in Chapter 12, the endocytic and biosynthetic–secretory pathways are illustrated with *green* and *red* arrows, respectively. In addition, *blue* arrows denote retrieval pathways for the backflow of selected components. (B) The compartments of the eucaryotic cell involved in vesicular transport. The lumen of each membrane-enclosed compartment is topologically equivalent to the outside of the cell. All compartments shown communicate with one another and the outside of the cell by means of transport vesicles. In the biosynthetic–secretory pathway *(red arrows),* protein molecules are transported from the ER to the plasma membrane or (via endosomes) to lysosomes. In the endocytic pathway *(green arrows),* molecules are ingested in vesicles derived from the plasma membrane and delivered to early endosomes and then (via late endosomes) to lysosomes. Many endocytosed molecules are retrieved from early endosomes and returned to the cell surface for reuse; similarly, some molecules are retrieved from the early and late endosomes and returned to the Golgi apparatus, and some are retrieved from the Golgi apparatus and returned to the ER. All of these retrieval pathways are shown with *blue arrows,* as in part (A).

used to extract specific components from a membrane for delivery to another compartment. Finally, we discuss how transport vesicles dock at the appropriate target membrane and fuse with it to deliver their cargo.

There Are Various Types of Coated Vesicles

Most transport vesicles form from specialized, coated regions of membranes. They bud off as **coated vesicles**, which have a distinctive cage of proteins covering their cytosolic surface. Before the vesicles fuse with a target membrane, they discard their coat, as is required for the two cytosolic membrane surfaces to interact directly and fuse.

The coat performs two main functions. First, it concentrates specific membrane proteins in a specialized patch, which then gives rise to the vesicle membrane. In this way, it selects the appropriate molecules for transport. Second, the coat molds the forming vesicle. Coat proteins assemble into a curved, basketlike lattice that deforms the membrane patch and thereby shapes the vesicle. This may explain why vesicles with the same type of coat often have a relatively uniform size and shape.

There are three well-characterized types of coated vesicles, distinguished by their coat proteins: *clathrin-coated, COPI-coated,* and *COPII-coated* (**Figure 13–4**). Each type is used for different transport steps. Clathrin-coated vesicles, for example, mediate transport from the Golgi apparatus and from the plasma membrane, whereas COPI- and COPII-coated vesicles most commonly mediate transport from the ER and from the Golgi cisternae (**Figure 13–5**). There is, however, much more variety in coated vesicles and their functions than this short list suggests. As we discuss below, there are several types of clathrin-coated vesicles,

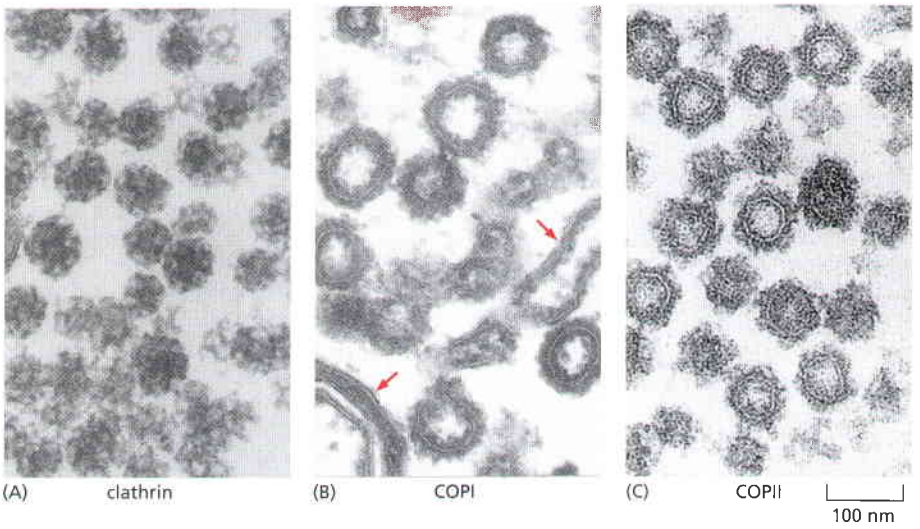


Figure 13–4 Electron micrograph of clathrin-coated, COPI-coated, and COPII-coated vesicles. All are shown in electron micrographs at the same scale. (A) Clathrin-coated vesicles. (B) Golgi cisternae (*arrows*) from a cell-free system in which COPI-coated vesicles bud in the test tube. (C) COPII-coated vesicles. (A and B, from L. Orci, B. Glick and J. Rothman, *Cell* 46:171–184, 1986. With permission from Elsevier; C, courtesy of Charles Barlowe and Lelio Orci.)

each specialized for a different transport step, and the COPI- and COPII-coated vesicles may be similarly diverse.

The Assembly of a Clathrin Coat Drives Vesicle Formation

Clathrin-coated vesicles, the first coated vesicles to be identified, transport material from the plasma membrane and between endosomal and Golgi compartments. **COPI-coated vesicles** and **COPII-coated vesicles** transport material early in the secretory pathway: COPII-coated vesicles bud from the ER, and COPI-coated vesicles bud from Golgi compartments (see Figure 13–5). We discuss clathrin-coated vesicles first, as they provide a good example of how vesicles form.

The major protein component of clathrin-coated vesicles is **clathrin** itself. Each clathrin subunit consists of three large and three small polypeptide chains that together form a three-legged structure called a *triskelion*. Clathrin triskelions assemble into a basketlike convex framework of hexagons and pentagons to form coated pits on the cytosolic surface of membranes (**Figure 13–6**). Under appropriate conditions, isolated triskelions spontaneously self-assemble into typical polyhedral cages in a test tube, even in the absence of the membrane vesicles that these baskets normally enclose (**Figure 13–7**). Thus, the clathrin triskelions determine the geometry of the clathrin cage.

Adaptor proteins, another major coat component in clathrin-coated vesicles, form a discrete second layer of the coat, positioned between the clathrin cage and the membrane. They bind the clathrin coat to the membrane and trap

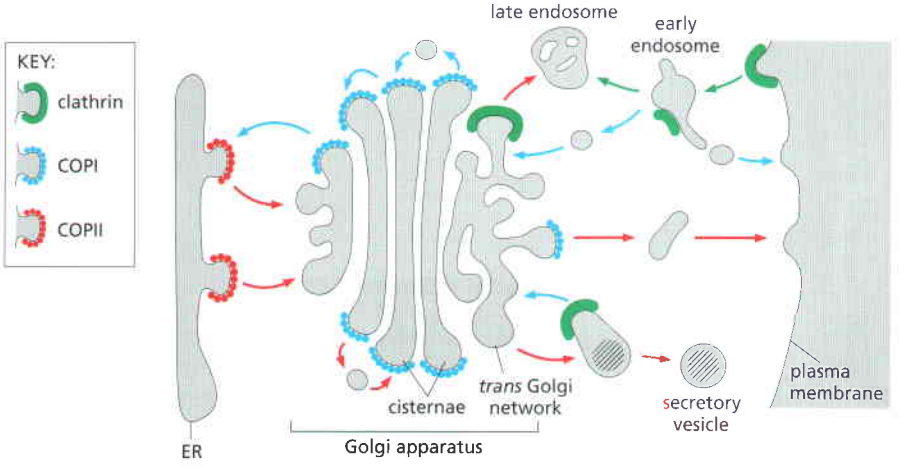


Figure 13–5 Use of different coats in vesicular traffic. Different coat proteins select different cargo and shape the transport vesicles that mediate the various steps in the biosynthetic– secretory and endocytic pathways. When the same coats function in different places in the cell, they usually incorporate different coat protein subunits that modify their properties (not shown). Many differentiated cells have additional pathways beside those shown here, including a sorting pathway from the *trans* Golgi network to the apical surface of epithelial cells and a specialized recycling pathway for proteins of synaptic vesicles in the synapses of neurons.

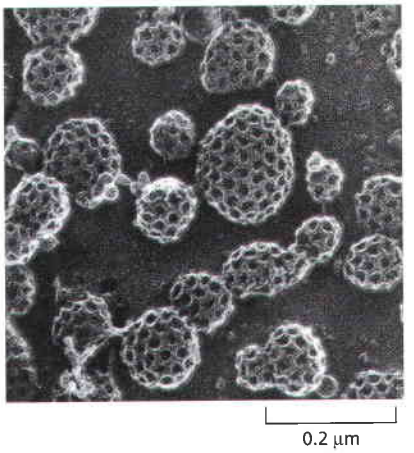


Figure 13–6 Clathrin-coated pits and vesicles. This rapid-freeze, deep-etch electron micrograph shows numerous clathrin-coated pits and vesicles on the inner surface of the plasma membrane of cultured fibroblasts. The cells were rapidly frozen in liquid helium, fractured, and deep-etched to expose the cytoplasmic surface of the plasma membrane. (From J. Heuser, *J. Cell Biol.* 84:560–583, 1980. With permission from The Rockefeller University Press.)

various transmembrane proteins, including transmembrane receptors that capture soluble cargo molecules inside the vesicle—so-called *cargo receptors*. In this way, a selected set of transmembrane proteins, together with the soluble proteins that interact with them, are packaged into each newly formed clathrin-coated transport vesicle (**Figure 13–8**).

There are several types of adaptor proteins. The best characterized have four different protein subunits; others are single-chain proteins. Each type of adaptor protein is specific for a different set of cargo receptors and its use leads to the formation of distinct clathrin-coated vesicles. Clathrin-coated vesicles budding from different membranes use different adaptor proteins and thus package different receptors and cargo molecules.

The sequential assembly of adaptor complexes and the clathrin coat on the cytosolic surface of the membrane generate forces that result in the formation of a clathrin-coated vesicle. Lateral interactions between adaptor complexes and between clathrin molecules aid in forming the vesicle.

Not All Coats Form Basketlike Structures

Not all coats are as regular and universal as the examples of clathrin or COP coats suggest. Some coats may be better described as specialized protein assemblies that form patches dedicated to specific cargo proteins. An example is a coat called **retromer**, which assembles on endosomes and forms vesicles that return *acid hydrolase receptors*, such as the *mannose-6-phosphate receptor*, to the Golgi apparatus (**Figure 13–9**). We discuss later the important role of these receptors in delivering enzymes into new lysosomes.

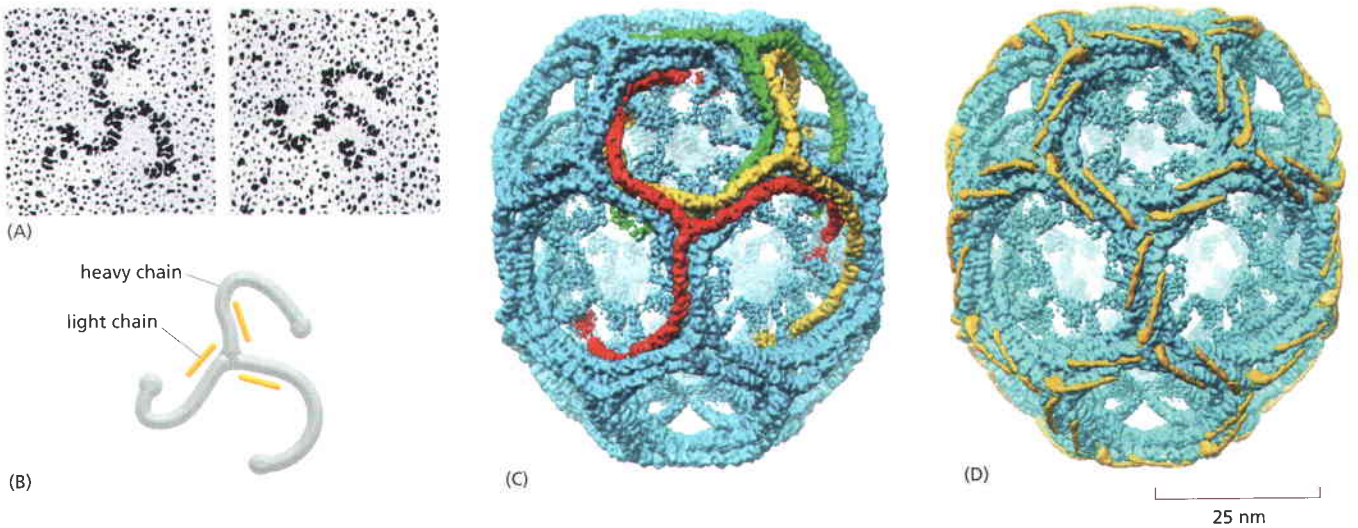


Figure 13–7 The structure of a clathrin coat. <TATT> (A) Electron micrographs of clathrin triskelions shadowed with platinum. Each triskelion is composed of three clathrin heavy chains and three clathrin light chains as shown in (B). (C and D) A cryoelectron micrograph taken of a clathrin coat composed of 36 triskelions organized in a network of 12 pentagons and 6 hexagons, with heavy chains (C) and light chains (D) highlighted. The interwoven legs of the clathrin triskelions form an outer shell into which the N-terminal domains of the triskelions protrude to form an inner layer visible through the openings. It is this inner layer that contacts the adaptor proteins shown in Figure 13–8. Although the coat shown is too small to enclose a membrane vesicle, the clathrin coats on vesicles are constructed in a similar way, from 12 pentagons and a larger number of hexagons, resembling the architecture of a soccer ball. (A, from E. Ungewickell and D. Branton, *Nature* 289:420–422, 1981; C and D, from A. Fotin et al., *Nature* 432:573–579, 2004. All with permission from Macmillan Publishers Ltd.)

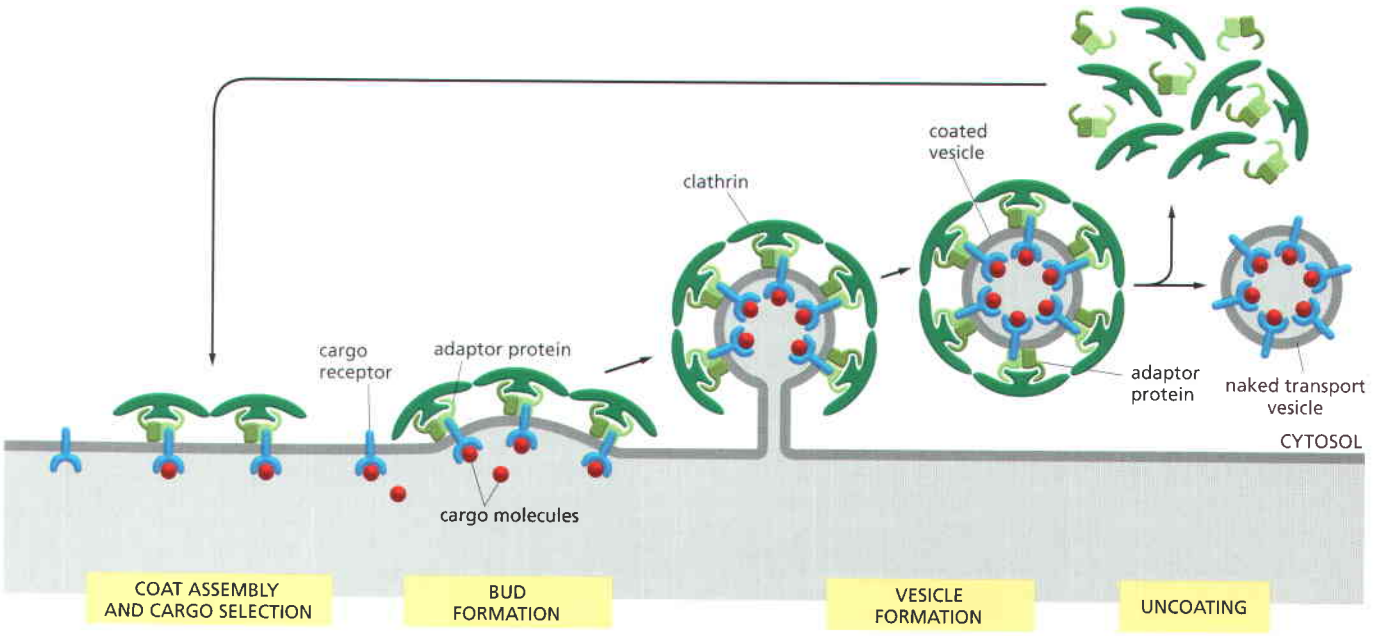


Figure 13–8 The assembly and disassembly of a clathrin coat. The assembly of the coat introduces curvature into the membrane, which leads in turn to the formation of uniformly sized coated buds. The adaptor proteins bind clathrin triskelions and membrane-bound cargo receptors, thereby mediating the selective recruitment of both membrane and cargo molecules into the vesicle. The clathrin coat is rapidly lost shortly after the vesicle forms.

Retromer is a multiprotein complex that assembles into a coat on endosomal membranes only when:

1. it can bind to the cytoplasmic tails of the cargo receptors,
2. it can interact directly with a curved phospholipid bilayer, and
3. it can bind to a specific phosphorylated phosphatidylinositol lipid (a *phosphoinositide*), which acts as an endosomal marker, as we discuss next.

Because these three requirements must be met simultaneously, retromer is thought to act as a *coincidence detector* and only assemble at the right time and place. Upon binding as a dimer, it stabilizes the membrane curvature, which makes the binding of additional retromers in its proximity more likely. The cooperative assembly of retromer then leads to the formation and budding of a transport vesicle, which delivers its cargo to the Golgi apparatus.

The adaptor proteins found in clathrin coats also bind to phosphoinositides, which not only have a major role in directing when and where coats assemble in the cell, but are used much more widely as molecular markers of compartment identity. This helps to control membrane trafficking events, as we discuss next.

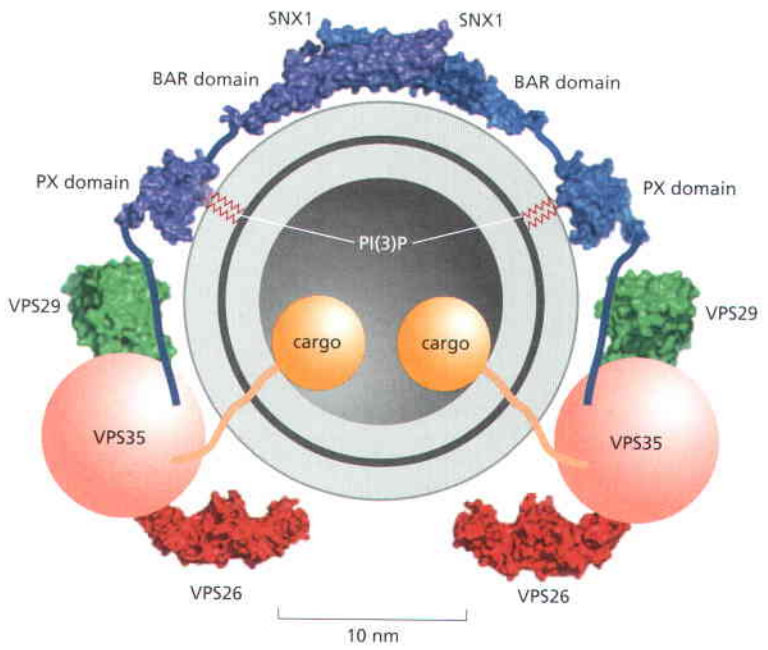


Figure 13–9 A model for retromer assembly on endosomal membranes. The four retromer subunits, SNX1, VPS29, VPS35, and VPS26, form coated domains on endosome membranes that capture cargo molecules, including transmembrane proteins such as acid hydrolase receptors, into vesicles that return to the *trans* Golgi network. VPS35 binds to the cytoplasmic tails of the transmembrane cargo proteins. SNX1 protein contains different protein modules: a *PX domain* that binds to the phosphorylated phosphatidylinositol PI(3)P, and a *BAR domain* that mediates dimerization and attachment to highly curved membranes. Both PX and BAR domains are protein modules that are found in many proteins, where they carry out similar functions. With the exception of the PI(3)P, which is enlarged for visibility, the membrane and other components are drawn approximately to scale. (Adapted from J.S. Bonifacino and R. Rojas, *Nat. Rev. Mol. Cell Biol.* 7:568–579, 2006. With permission from Macmillan Publishers Ltd.)

proteins they recruit into the bud. Together, these protein–lipid and protein–protein and interactions tightly bind the coat to the membrane, causing the membrane to deform into a bud, which then pinches off as a coated vesicle.

The coat-recruitment GTPases also have a role in coat disassembly. The hydrolysis of bound GTP to GDP causes the GTPase to change its conformation so that its hydrophobic tail pops out of the membrane, causing the vesicle's coat to disassemble. Although it is not known what triggers the GTP hydrolysis process, it has been proposed that the GTPases work like timers, which hydrolyze GTP at a slow but predictable rate. COPII coats, for example, accelerate GTP hydrolysis by Sar1, thereby triggering coat disassembly at a certain time after coat assembly has begun. Thus, a fully formed vesicle will be produced only when bud formation occurs faster than the timed disassembly process; otherwise, disassembly will be triggered before a vesicle pinches off, and the process will have to start again at a more appropriate time and place.

Not All Transport Vesicles Are Spherical

Although vesicle budding at various locations in the cell has many similarities, each cell membrane poses its own special challenges. The plasma membrane, for example, is comparatively flat and stiff, owing to its cholesterol-rich lipid composition and underlying cortical cytoskeleton. Thus, clathrin coats have to produce considerable force to introduce curvature, especially at the neck of the bud where dynamin and its associated proteins facilitate the sharp bends required for the pinching-off process. In contrast, vesicle budding from many intracellular membranes occurs preferentially at regions where the membranes are already curved, such as the rims of the Golgi cisternae or ends of membrane tubules. In these places, the primary function of the coats is to capture the appropriate cargo proteins rather than to deform the membrane.

Transport vesicles occur in various sizes and shapes. When living cells are genetically engineered to express fluorescent membrane components, the endosomes and *trans* Golgi network are seen in a fluorescence microscope to continually send out long tubules. Coat proteins assemble onto the tubules and help recruit specific cargo. The tubules then either regress or pinch off with the help of dynamin-like proteins to form transport vesicles. Depending on the relative efficiencies of tubule formation and the pinching-off process, vesicles of different sizes and shapes are produced. Thus, vesicular transport does not necessarily occur only through uniformly sized spherical vesicles, but can involve larger portions of a donor organelle.

Tubules have a much higher surface-to-volume ratio than the organelles from which they form. They are therefore relatively enriched in membrane proteins compared with soluble cargo proteins. As we discuss later, this property of tubules is used for sorting proteins in endosomes.

Rab Proteins Guide Vesicle Targeting

To ensure an orderly flow of vesicular traffic, transport vesicles must be highly selective in recognizing the correct target membrane with which to fuse. Because of the diversity and crowding of membrane systems in the cytoplasm, a vesicle is likely to encounter many potential target membranes before it finds the correct one. Specificity in targeting is ensured because all transport vesicles display surface markers that identify them according to their origin and type of cargo, and target membranes display complementary receptors that recognize the appropriate markers. This crucial process depends on two types of proteins: *Rab proteins* direct the vesicle to specific spots on the correct target membrane, and then *SNARE* proteins mediate the fusion of the lipid bilayers.

Rab proteins play a central part in the specificity of vesicular transport. Like the coat-recruitment GTPases discussed earlier (see Figure 13–13), they also are monomeric GTPases. With over 60 known members, they are the largest subfamily of such GTPases. Each Rab protein is associated with one or more membrane-

Summary

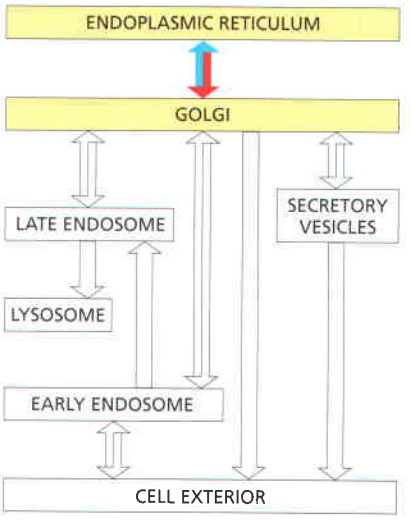
Directed and selective transport of particular membrane components from one membrane-enclosed compartment of a eucaryotic cell to another maintains the differences between those compartments. Transport vesicles, which can be spherical, tubular, or irregularly shaped, bud from specialized coated regions of the donor membrane. The assembly of the coat helps to collect specific membrane and soluble cargo molecules for transport and to drive the formation of the vesicle.

There are various types of coated vesicles. The best characterized are clathrin-coated vesicles, which mediate transport from the plasma membrane and the trans *Golgi network, and COPI- and COPII-coated vesicles, which mediate transport between Golgi cisternae and between the ER and the Golgi apparatus, respectively. In clathrin-coated vesicles, adaptor proteins link the clathrin to the vesicle membrane and also trap specific cargo molecules for packaging into the vesicle. The coat is shed rapidly after budding, enabling the vesicle to fuse with its appropriate target membrane.*

Local synthesis of phosphoinositides creates binding sites that trigger coat assembly and vesicle budding. In addition, monomeric GTPases help regulate various steps in vesicular transport, including both vesicle budding and docking. The coat-recruitment GTPases, including Sar1 and the Arf proteins, regulate coat assembly and disassembly. A large family of Rab proteins functions as vesicle targeting GTPases. Rab proteins are recruited to transport vesicles and target membranes. The assembly and disassembly of Rab proteins and their effectors in specialized membrane domains are dynamically controlled by GTP binding and hydrolysis. Active Rab proteins recruit Rab effectors, such as motor proteins, which transport vesicles on actin filaments or microtubules, and filamentous tethering proteins, which help ensure that the vesicles deliver their contents only to the appropriate membrane-enclosed compartment. Complementary v-SNARE proteins on transport vesicles and t-SNARE proteins on the target membrane form stable trans-SNARE complexes, which force the two membranes into close apposition so that their lipid bilayers can fuse.

TRANSPORT FROM THE ER THROUGH THE GOLGI APPARATUS

As discussed in Chapter 12, newly synthesized proteins cross the ER membrane from the cytosol to enter the biosynthetic–secretory pathway. During their subsequent transport, from the ER to the Golgi apparatus and from the Golgi apparatus to the cell surface and elsewhere, these proteins are successively modified as they pass through a series of compartments. Transfer from one compartment to the next involves a delicate balance between forward and backward (retrieval) transport pathways. Some transport vesicles select cargo molecules and move them to the next compartment in the pathway, while others retrieve escaped proteins and return them to a previous compartment where they normally function. Thus, the pathway from the ER to the cell surface consists of many sorting steps, which continuously select membrane and soluble lumenal proteins for packaging and transport—in vesicles or organelle fragments that bud from the ER and Golgi apparatus.



In this section we focus mainly on the **Golgi apparatus** (also called the **Golgi complex**). It is a major site of carbohydrate synthesis, as well as a sorting and dispatching station for products of the ER. The cell makes many polysaccharides in the Golgi apparatus, including the pectin and hemicellulose of the cell wall in plants and most of the glycosaminoglycans of the extracellular matrix in animals (discussed in Chapter 19). The Golgi apparatus also lies on the exit route from the ER, and a large proportion of the carbohydrates that it makes are attached as oligosaccharide side chains to the many proteins and lipids that the ER sends to it. A subset of these oligosaccharide groups serve as tags to direct specific proteins into vesicles that then transport them to lysosomes. But most proteins and lipids, once they have acquired their appropriate oligosaccharides in the Golgi apparatus, are recognized in other ways for targeting into the transport vesicles going to other destinations.

Proteins Leave the ER in COPII-Coated Transport Vesicles

To initiate their journey along the biosynthetic–secretory pathway, proteins that have entered the ER and are destined for the Golgi apparatus or beyond are first packaged into small COPII-coated transport vesicles. These vesicles bud from specialized regions of the ER called *ER exit sites*, whose membrane lacks bound ribosomes. Most animal cells have ER exit sites dispersed throughout the ER network.

Originally, it was thought that all proteins that are not tethered in the ER enter transport vesicles by default. It is now clear, however, that entry into vesicles that leave the ER is usually a selective process. Many membrane proteins are actively recruited into such vesicles, where they become concentrated. It is thought that these cargo proteins display exit (transport) signals on their cytosolic surface that components of the COPII coat recognize (**Figure 13–20**); these coat components act as cargo receptors and are recycled back to the ER after they have delivered their cargo to the Golgi apparatus. Soluble cargo proteins in the ER lumen, by contrast, have exit signals that attach them to transmembrane cargo receptors, which in turn bind through exit signals in their cytoplasmic tails to components of the COPII coat. At a lower rate, proteins without exit signals can also enter transport vesicles, so that even proteins that normally function in the ER (so-called *ER resident proteins)* slowly leak out of the ER and are delivered to the Golgi apparatus. Similarly, secretory proteins that are made in high concentrations may leave the ER without the help of exit signals or cargo receptors.

The exit signals that direct soluble proteins out of the ER for transport to the Golgi apparatus and beyond are not well understood. Some transmembrane proteins that serve as cargo receptors for packaging some secretory proteins into COPII-coated vesicles are lectins that bind to oligosaccharides. The ERGIC53 lectin, for example, binds to mannose and is thought to recognize this sugar on two secreted blood-clotting factors (Factor V and Factor VIII), thereby packaging the proteins into transport vesicles in the ER. ERGIC53's role in protein transport was identified because humans who lack it owing to an inherited mutation have lowered serum levels of Factors V and VIII, and they therefore bleed excessively.

Only Proteins That Are Properly Folded and Assembled Can Leave the ER

To exit from the ER, proteins must be properly folded and, if they are subunits of multimeric protein complexes, they may need to be completely assembled. Those that are misfolded or incompletely assembled remain in the ER, where they are bound to chaperone proteins (discussed in Chapter 6), such as *BiP* or *calnexin.* The chaperones may cover up the exit signals or somehow anchor the proteins in

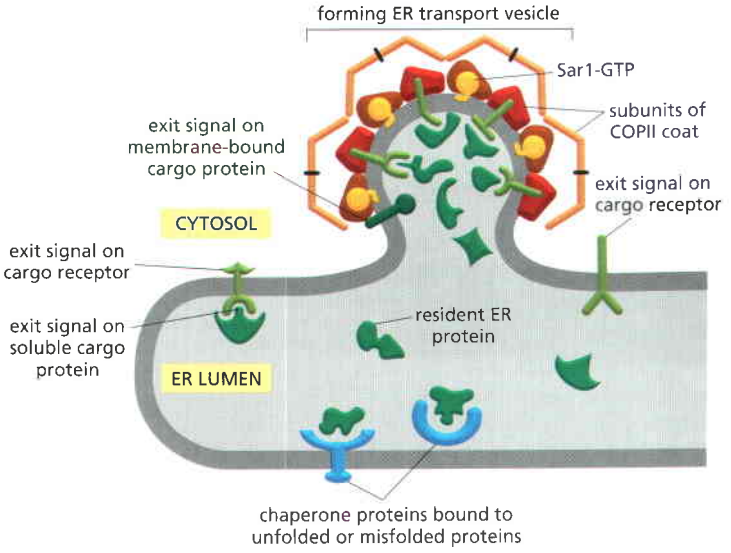


Figure 13–20 The recruitment of cargo molecules into ER transport vesicles. By binding directly or indirectly to the COPII coat, membrane and soluble cargo proteins, respectively, become concentrated in the transport vesicles as they leave the ER. Membrane proteins are packaged into budding transport vesicles through interactions of exit signals on their cytosolic tails with the COPII coat. Some of the membrane proteins that the coat traps function as cargo receptors, binding soluble proteins in the lumen and helping to package them into vesicles. A typical 50-nm transport vesicle contains about 200 membrane proteins, which can be of many different types. As indicated, unfolded or incompletely assembled proteins are bound to chaperones and retained in the ER compartment.

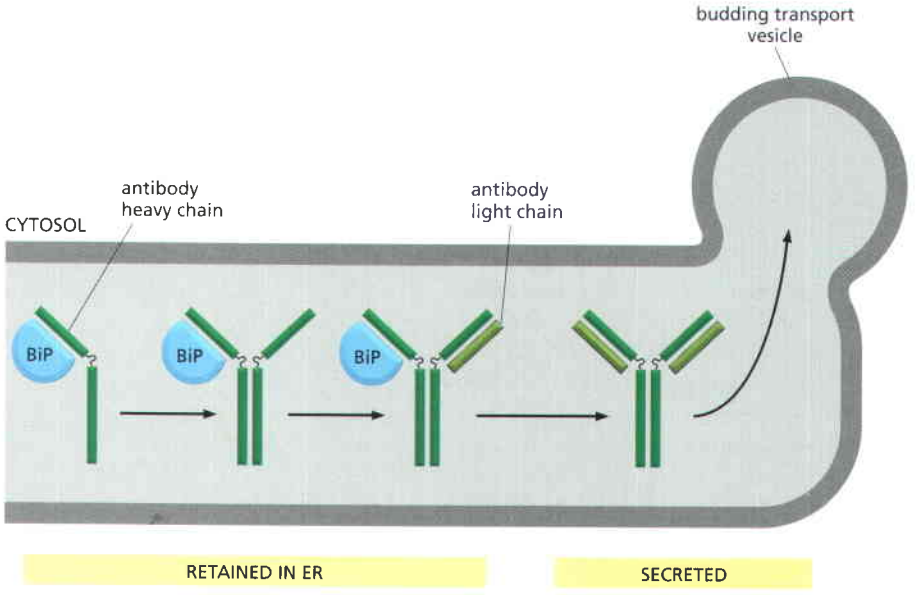


Figure 13–21 Retention of incompletely assembled antibody molecules in the ER. Antibodies are made up of two heavy and two light chains (discussed in Chapter 25), which assemble in the ER. The chaperone BiP is thought to bind to all incompletely assembled antibody molecules and to cover up an exit signal. Thus, only completely assembled antibodies leave the ER and are secreted.

the ER (**Figure 13–21**). Such failed proteins are eventually transported back into the cytosol, where they are degraded by proteasomes (discussed in Chapters 6 and 12). This quality-control step prevents the onward transport of misfolded or misassembled proteins that could potentially interfere with the functions of normal proteins. There is a surprising amount of corrective action. More than 90% of the newly synthesized subunits of the T cell receptor (discussed in Chapter 25) and of the acetylcholine receptor (discussed in Chapter 11), for example, are normally degraded without ever reaching the cell surface where they function. Thus, cells must make a large excess of many protein molecules to produce a select few that fold, assemble, and function properly.

The process of continual degradation of a portion of ER proteins also provides an early warning system to alert the immune system when a virus infects cells. Using specialized ABC-type transporters, the ER imports peptide fragments of viral proteins produced by proteases in the proteasome. The foreign peptides are loaded onto class I MHC proteins in the ER lumen and then transported to the cell surface. T lymphocytes then recognize the peptides as non-self antigens and kill the infected cells (discussed in Chapter 25).

Sometimes, however, there are drawbacks to the stringent quality-control mechanism. The predominant mutations that cause cystic fibrosis, a common inherited disease, result in the production of a slightly misfolded form of a plasma membrane protein important for Cl^- transport. Although the mutant protein would function perfectly normally if it reached the plasma membrane, it remains in the ER. This devastating disease thus results not because the mutation inactivates the protein but because the active protein is discarded before it reaches the plasma membrane.

Vesicular Tubular Clusters Mediate Transport from the ER to the Golgi Apparatus

After transport vesicles have budded from ER exit sites and have shed their coat, they begin to fuse with one another. This fusion of membranes from the same compartment is called *homotypic fusion,* to distinguish it from *heterotypic fusion,* in which a membrane from one compartment fuses with the membrane of a different compartment. As with heterotypic fusion, homotypic fusion requires a set of matching SNAREs. In this case, however, the interaction is symmetrical, with both membranes contributing v-SNAREs and t-SNAREs (**Figure 13–22**).

The structures formed when ER-derived vesicles fuse with one another are called *vesicular tubular clusters,* because they have a convoluted appearance in

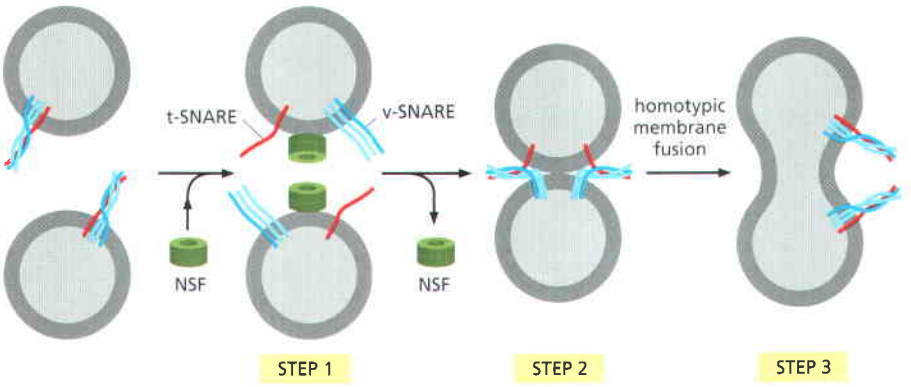


Figure 13–22 Homotypic membrane fusion. <AAAA> In step 1, NSF pries apart identical pairs of v-SNAREs and t-SNAREs in both membranes (see Figure 13–18). In steps 2 and 3, the separated matching SNAREs on adjacent identical membranes interact, which leads to membrane fusion and the formation of one continuous compartment called a vesicular tubular cluster. Subsequently, the compartment grows by further homotypic fusion with vesicles from the same kind of membrane, displaying matching SNAREs. Homotypic fusion is not restricted to the formation of vesicular tubular clusters; in a similar process, endosomes fuse to generate larger endosomes. Rab proteins help regulate the extent of homotypic fusion and hence the size of the compartments in a cell (not shown).

the electron microscope (**Figure 13–23**A). These clusters constitute a new compartment that is separate from the ER and lacks many of the proteins that function in the ER. They are generated continually and function as transport containers that bring material from the ER to the Golgi apparatus. The clusters are relatively short-lived because they move quickly along microtubules to the Golgi apparatus, with which they fuse to deliver their contents (Figure 13–23B).

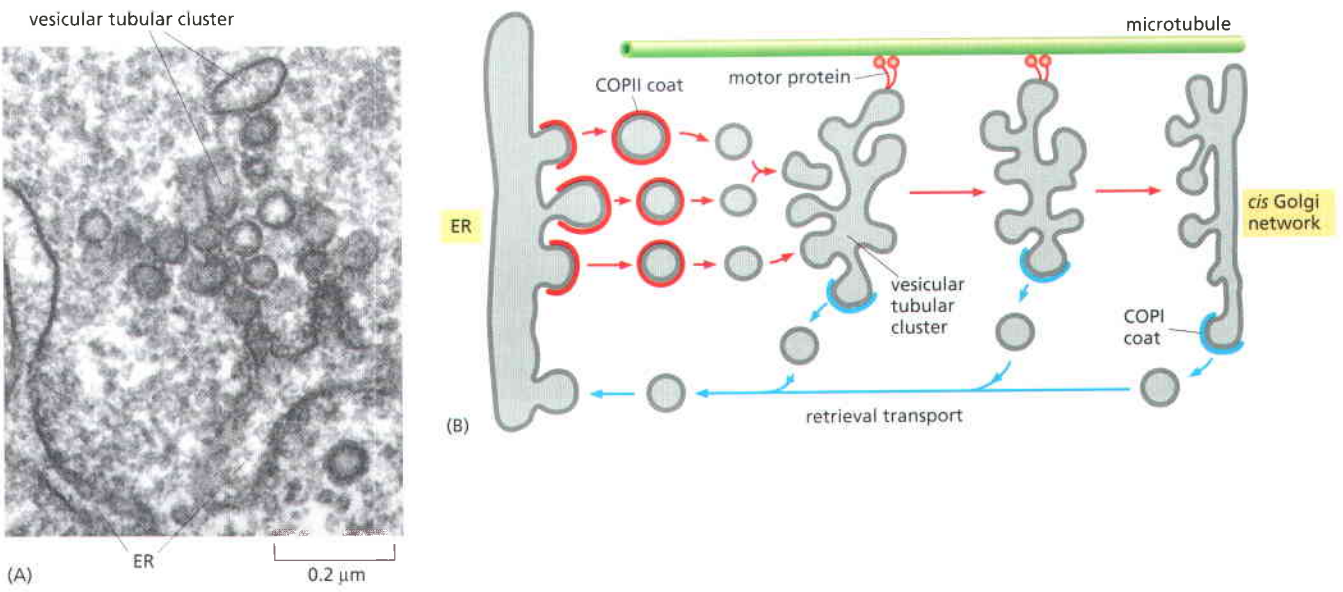
As soon as vesicular tubular clusters form, they begin to bud off transport vesicles of their own. Unlike the COPII-coated vesicles that bud from the ER, these vesicles are COPI-coated. They carry back to the ER resident proteins that have escaped, as well as proteins such as cargo receptors that participated in the ER budding reaction and are being returned. This retrieval process demonstrates the exquisite control mechanisms that regulate coat assembly reactions. The COPI coat assembly begins only seconds after the COPII coats have been shed. It remains a mystery how this switch in coat assembly is controlled.

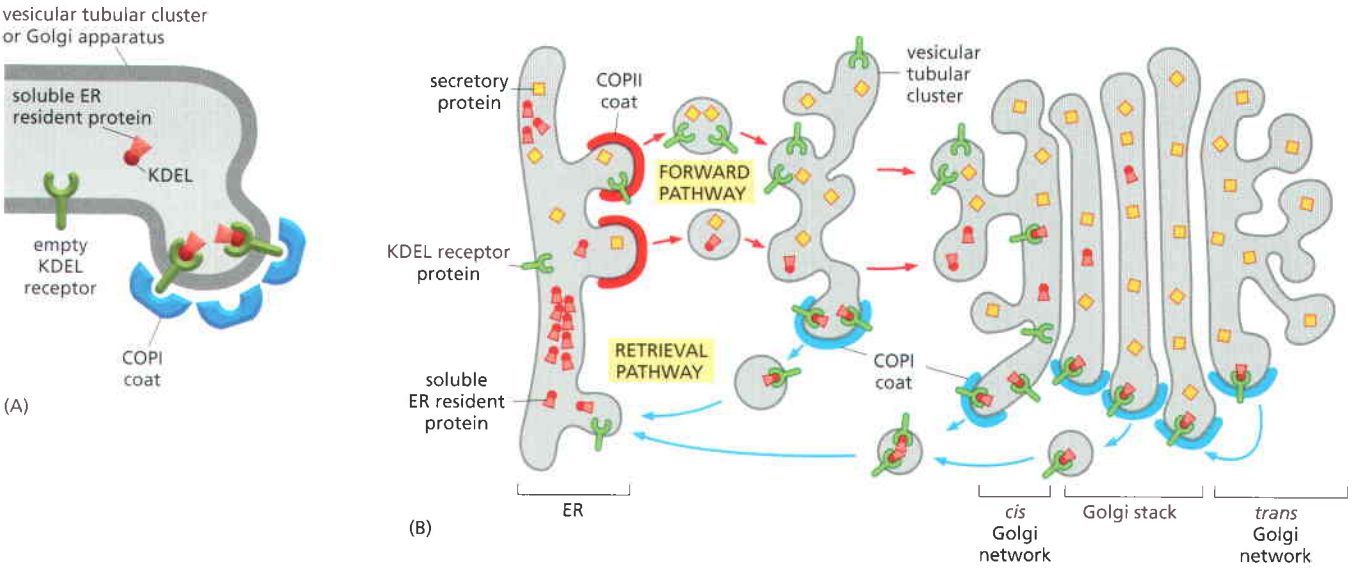
The *retrieval* (or *retrograde*) *transport* continues as the vesicular tubular clusters move towards the Golgi apparatus. Thus, the clusters continuously mature, gradually changing their composition as selected proteins are returned to the ER. A similar retrieval process continues from the Golgi apparatus, after the vesicular tubular clusters have delivered their cargo.

The Retrieval Pathway to the ER Uses Sorting Signals

The retrieval pathway for returning escaped proteins back to the ER depends on *ER retrieval signals*. Resident ER membrane proteins, for example, contain signals that bind directly to COPI coats and are thus packaged into COPI-coated

Figure 13–23 Vesicular tubular clusters. (A) An electron micrograph section of vesicular tubular clusters forming from the ER membrane. Many of the vesicle-like structures seen in the micrograph are cross sections of tubules that extend above and below the plane of this thin section and are interconnected. (B) Vesicular tubular clusters move along microtubules to carry proteins from the ER to the Golgi apparatus. COPI coats mediate the budding of vesicles that return to the ER from these clusters. As indicated, the coats quickly disassemble after the vesicles have formed. (A, courtesy of William Balch.)





transport vesicles for retrograde delivery to the ER. The best-characterized retrieval signal of this type consists of two lysines, followed by any two other amino acids, at the extreme C-terminal end of the ER membrane protein. It is called a *KKXX sequence*, based on the single-letter amino acid code.

Soluble ER resident proteins, such as BiP, also contain a short retrieval signal at their C-terminal end, but it is different: it consists of a Lys-Asp-Glu-Leu or a similar sequence. If this signal (called the *KDEL sequence*) is removed from BiP by genetic engineering, the protein is slowly secreted from the cell. If the signal is transferred to a protein that is normally secreted, the protein is now efficiently returned to the ER, where it accumulates.

Unlike the retrieval signals on ER membrane proteins, which can interact directly with the COPI coat, soluble ER resident proteins must bind to specialized receptor proteins such as the *KDEL receptor*—a multipass transmembrane protein that binds to the KDEL sequence and packages any protein displaying it into COPI-coated retrograde transport vesicles (**Figure 13–24**). To accomplish this task, the KDEL receptor itself must cycle between the ER and the Golgi apparatus, and its affinity for the KDEL sequence must differ in these two compartments. The receptor must have a high affinity for the KDEL sequence in vesicular tubular clusters and the Golgi apparatus, so as to capture escaped, soluble ER resident proteins that are present there at low concentration. It must have a low affinity for the KDEL sequence in the ER, however, to unload its cargo in spite of the very high concentration of KDEL-containing resident proteins in the ER.

How does the affinity of the KDEL receptor change depending on the compartment in which it resides? The answer is not known, but it may be related to the different ionic conditions and pH in the different compartments, which are regulated by ion transporters in the compartment membrane. As we discuss later, pH-sensitive protein–protein interactions form the basis for many of the sorting steps in the cell.

Most membrane proteins that function at the interface between the ER and Golgi apparatus, including v- and t-SNAREs and some cargo receptors, enter the retrieval pathway back to the ER. Whereas the recycling of some of these proteins is mediated by signals, as just described, for others no specific signal seems to be required. Thus, while retrieval signals increase the efficiency of the retrieval process, some proteins randomly enter budding vesicles destined for the ER and are returned to the ER at a slower rate. Many Golgi enzymes cycle constantly between the ER and the Golgi, but their rate of return to the ER is slow enough for most of the protein to be found in the Golgi apparatus.

Figure 13–24 A model for the retrieval of soluble ER resident proteins. ER resident proteins that escape from the ER are returned by vesicular transport. (A) The KDEL receptor present in vesicular tubular clusters and the Golgi apparatus captures the soluble ER resident proteins and carries them in COPI-coated transport vesicles back to the ER. Upon binding its ligands in this environment, the KDEL receptor may change conformation, so as to facilitate its recruitment into budding COPI-coated vesicles. (B) The retrieval of ER proteins begins in vesicular tubular clusters and continues from all parts of the Golgi apparatus. In the environment of the ER, the ER resident proteins dissociate from the KDEL receptor, which is then returned to the Golgi apparatus for reuse.

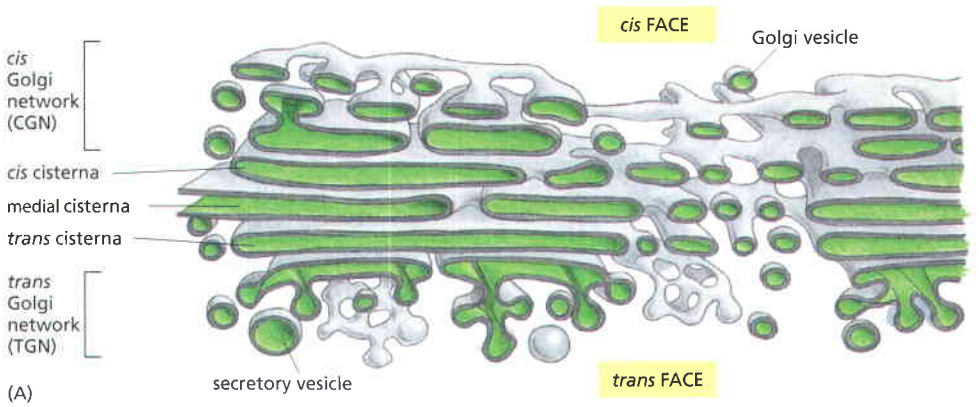
Many Proteins Are Selectively Retained in the Compartments in Which They Function

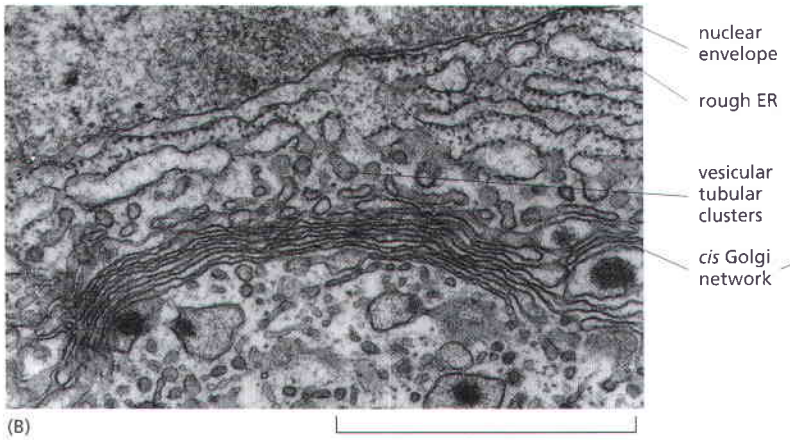
The KDEL retrieval pathway only partly explains how ER resident proteins are maintained in the ER. As expected, cells that express genetically modified ER resident proteins, from which the KDEL sequence has been experimentally removed, secrete these proteins. But the rate of secretion is much slower than for a normal secretory protein. It seems that a mechanism that is independent of their KDEL signal anchors ER resident proteins and that only those proteins that escape this retention mechanism are captured and returned via the KDEL receptor. A suggested retention mechanism is that ER resident proteins bind to one another, thus forming complexes that are too big to enter transport vesicles efficiently. Because ER resident proteins are present in the ER at very high concentrations (estimated to be millimolar), relatively low-affinity interactions would suffice to tie up most of the proteins in such complexes.

Aggregation of proteins that function in the same compartment—called *kin recognition*—is a general mechanism that compartments use to organize and retain their resident proteins. Golgi enzymes that function together, for example, also bind to each other and are thereby restrained from entering transport vesicles leaving the Golgi apparatus.

The Golgi Apparatus Consists of an Ordered Series of Compartments

Because of its large and regular structure, the Golgi apparatus was one of the first organelles described by early light microscopists. It consists of a collection of flattened, membrane-enclosed compartments, called *cisternae*, that somewhat resemble a stack of pita breads. Each Golgi stack usually consists of four to six cisternae (**Figure 13–25**), although some unicellular flagellates can have up to





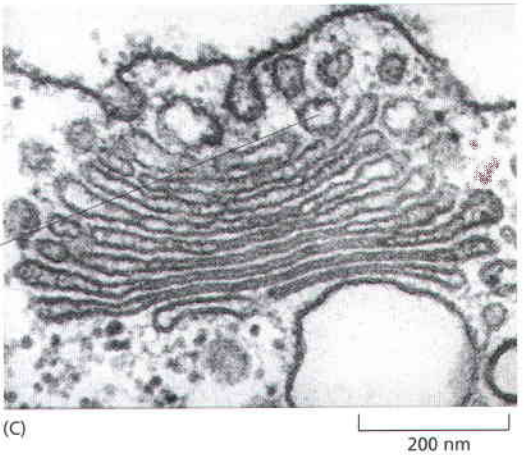


Figure 13–25 The Golgi apparatus. (A) Three-dimensional reconstruction from electron micrographs of the Golgi apparatus in a secretory animal cell. The *cis* face of the Golgi stack is that closest to the ER. (B) A thin-section electron micrograph emphasizing the transitional zone between the ER and the Golgi apparatus in an animal cell. (C) An electron micrograph of a Golgi apparatus in a plant cell (the green alga *Chlamydomonas*) seen in cross section. In plant cells, the Golgi apparatus is generally more distinct and more clearly separated from other intracellular membranes than in animal cells. (A, redrawn from A. Rambourg and Y. Clermont, *Eur. J. Cell Biol.* 51:189–200, 1990. With permission from Wissenschaftliche Verlagsgesellschaft; B, courtesy of Brij J. Gupta; C, courtesy of George Palade.)

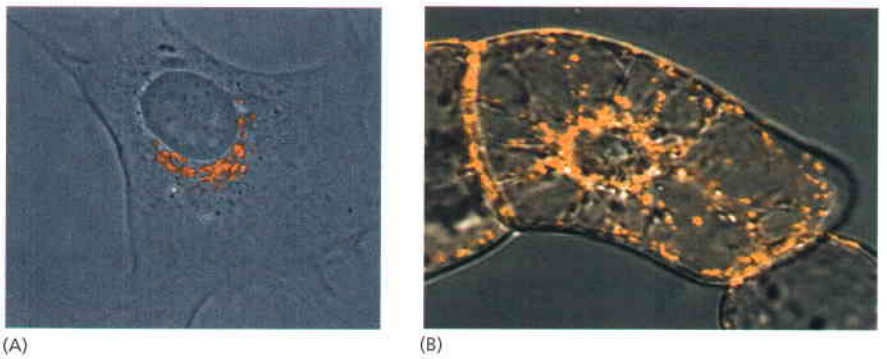


Figure 13–26 Localization of the Golgi apparatus in animal and plant cells. (A) The Golgi apparatus in a cultured fibroblast stained with a fluorescent antibody that recognizes a Golgi resident protein (*red*). The Golgi apparatus is polarized, facing the direction in which the cell was crawling before fixation. (B) The Golgi apparatus in a plant cell that is expressing a fusion protein consisting of a resident Golgi enzyme fused to green fluorescent protein. The bright *orange* spots (false color) are Golgi stacks. (A, courtesy of John Henley and Mark McNiven; B, courtesy of Chris Hawes.)

60. In animal cells, tubular connections between corresponding cisternae link many stacks, thus forming a single complex, which is usually located near the cell nucleus and close to the centrosome (**Figure 13–26**A). This localization depends on microtubules. If microtubules are experimentally depolymerized, the Golgi apparatus reorganizes into individual stacks that are found throughout the cytoplasm, adjacent to ER exit sites. Some cells, including most plant cells, have hundreds of individual Golgi stacks dispersed throughout the cytoplasm (Figure 13–26B).

During their passage through the Golgi apparatus, transported molecules undergo an ordered series of covalent modifications. Each Golgi stack has two distinct faces: a ***cis* face** (or entry face) and a ***trans* face** (or exit face). Both *cis* and *trans* faces are closely associated with special compartments, each composed of a network of interconnected tubular and cisternal structures: the ***cis* Golgi network** (**CGN**) and the ***trans* Golgi network** (**TGN**), respectively. The CGN is a collection of fused vesicular tubular clusters arriving from the ER. Proteins and lipids enter the *cis* Golgi network and exit from the *trans* Golgi network bound for the cell surface or another compartment. Both networks are important for protein sorting: proteins entering the CGN can either move onward in the Golgi apparatus or be returned to the ER. Similarly, proteins exiting from the TGN move onward and are sorted according to their next destination: lysosomes, secretory vesicles, or the cell surface. They also can be returned to an earlier compartment.

As described in Chapter 12, a single species of *N-linked oligosaccharide* is attached *en bloc* to many proteins in the ER and then trimmed while the protein is still in the ER. The oligosaccharide intermediates created by the trimming reactions serve to help proteins fold and to help transport misfolded proteins to the cytosol for degradation. Thus, they play an important role in controlling the quality of proteins exiting from the ER. Once these ER functions have been fulfilled, the cell is free to redesign the oligosaccharides for new functions. This happens in the Golgi apparatus, which generates the heterogeneous oligosaccharide structures seen in mature proteins. Upon arrival in the CGN, proteins pass through the *cis* Golgi network, before entering the first of the Golgi processing compartments (the *cis* Golgi cisternae). They then move to the next compartment (the medial cisternae) and finally to the *trans* cisternae, where glycosylation is completed. The lumen of the *trans* cisternae is thought to be continuous with the TGN, the place where proteins are segregated into different transport packages and dispatched to their final destinations.

The oligosaccharide processing steps occur in an organized sequence in the Golgi stack, with each cisterna containing a characteristic abundance of processing enzymes. Proteins are modified in successive stages as they move from cisterna to cisterna across the stack, so that the stack forms a multistage processing unit. This compartmentalization might seem unnecessary, since each oligosaccharide processing enzyme can accept a glycoprotein as a substrate only after it has been properly processed by the preceding enzyme. Nonetheless, it is clear that processing occurs in a spatial as well as a biochemical sequence: enzymes catalyzing early processing steps are concentrated in the cisternae toward the *cis* face of the Golgi stack, whereas enzymes catalyzing later processing steps are concentrated in the cisternae toward the *trans* face.

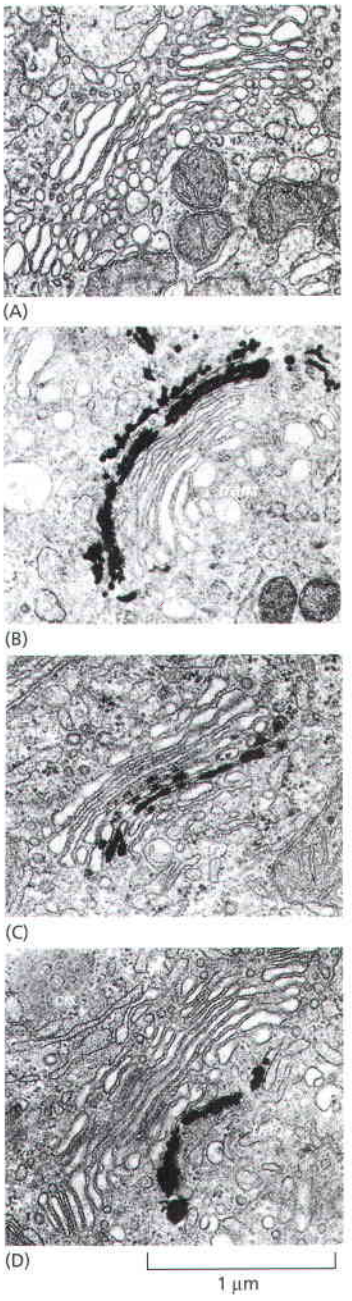


Figure 13–27 Molecular compartmentalization of the Golgi apparatus. A series of electron micrographs shows the Golgi apparatus (A) unstained, (B) stained with osmium, which is preferentially reduced by the cisternae of the *cis* compartment, and (C and D) stained to reveal the location of specific enzymes. Nucleoside diphosphatase is found in the *trans* Golgi cisternae (C), while acid phosphatase is found in the *trans* Golgi network (D). Note that usually more than one cisterna is stained. The enzymes are therefore thought to be highly enriched rather than precisely localized to a specific cisterna. (Courtesy of Daniel S. Friend.)

Investigators discovered the functional differences between the *cis*, medial, and *trans* subdivisions of the Golgi apparatus by localizing the enzymes involved in processing *N*-linked oligosaccharides in distinct regions of the organelle, both by physical fractionation of the organelle and by labeling the enzymes in electron microscope sections with antibodies. The removal of mannose residues and the addition of *N*-acetylglucosamine, for example, were shown to occur in the medial compartment, while the addition of galactose and sialic acid was found to occur in the *trans* compartment and the *trans* Golgi network (**Figure 13–27**). **Figure 13–28** summarizes the functional compartmentalization of the Golgi apparatus.

The Golgi apparatus is especially prominent in cells that are specialized for secretion of glycoproteins, such as the goblet cells of the intestinal epithelium, which secrete large amounts of polysaccharide-rich mucus into the gut (**Figure 13–29**). In such cells, unusually large vesicles are found on the *trans* side of the Golgi apparatus, which faces the plasma membrane domain where secretion occurs.

Oligosaccharide Chains Are Processed in the Golgi Apparatus

Whereas the ER lumen is full of soluble lumenal resident proteins and enzymes, the resident proteins in the Golgi apparatus are all membrane bound. The enzymatic reactions in the Golgi apparatus seem to be carried out entirely on its membrane surface. All of the Golgi glycosidases and glycosyl transferases are single-pass transmembrane proteins, many of which are organized in multienzyme complexes. Thus, the two synthetic organelles in the biosynthetic–secretory pathway, the ER and the Golgi apparatus, are organized in fundamentally different ways.

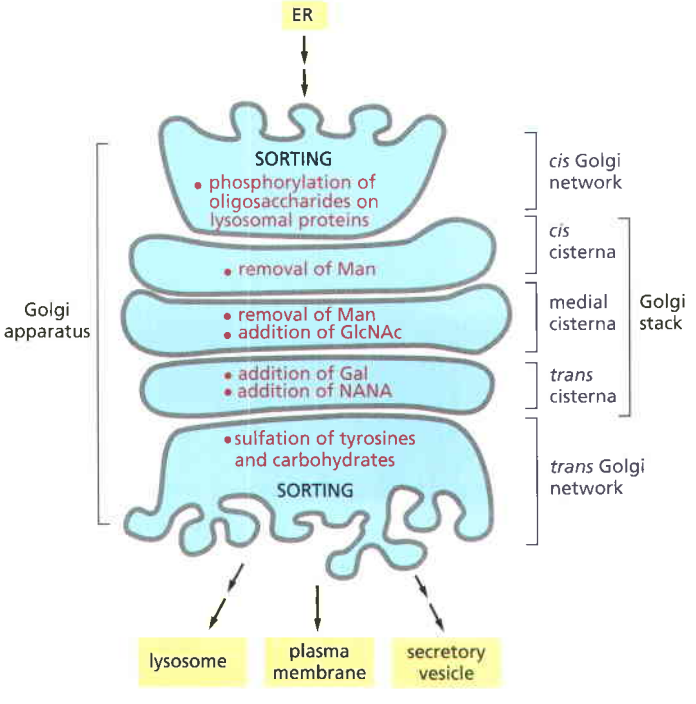


Figure 13–28 Oligosaccharide processing in Golgi compartments. The localization of each processing step shown was determined by a combination of techniques, including biochemical subfractionation of the Golgi apparatus membranes and electron microscopy after staining with antibodies specific for some of the processing enzymes. Processing enzymes are not restricted to a particular cisterna; instead, their distribution is graded across the stack—such that early-acting enzymes are present mostly in the *cis* Golgi cisternae and later-acting enzymes are mostly in the *trans* Golgi cisternae.

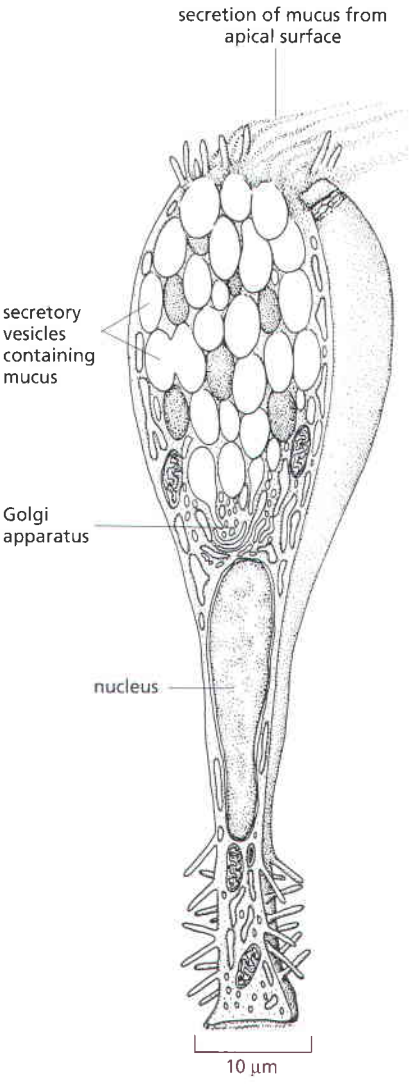


Figure 13–29 A goblet cell of the small intestine. This cell is specialized for secreting mucus, a mixture of glycoproteins and proteoglycans synthesized in the ER and Golgi apparatus. Like all epithelial cells, goblet cells are highly polarized, with the apical domain of their plasma membrane facing the lumen of the gut and the basolateral domain facing the basal lamina. The Golgi apparatus is also highly polarized, which facilitates the discharge of mucus by exocytosis at the apical domain of the plasma membrane. (After R.V. Krstic, Illustrated Encyclopedia of Human Histology. New York: Springer-Verlag, 1984. With permission from Springer-Verlag.)

Two broad classes of *N*-linked oligosaccharides, the **complex oligosaccharides** and the **high-mannose oligosaccharides**, are attached to mammalian glycoproteins (Figure 13–30). Sometimes, both types are attached (in different places) to the same polypeptide chain.

Complex oligosaccharides are generated when the original *N*-linked oligosaccharide added in the ER is trimmed and further sugars are added. By contrast, high-mannose oligosaccharides are trimmed but have no new sugars added to them in the Golgi apparatus. They contain just two *N*-acetylglucosamines and many mannose residues, often approaching the number originally present in the lipid-linked oligosaccharide precursor added in the ER. Complex oligosaccharides can contain more than the original two *N*-acetylglucosamines, as well as a variable number of galactose and sialic acids and, in some cases, fucose. Sialic acid is of special importance because it is the only sugar in glycoproteins that bears a negative charge. Whether a given oligosaccharide remains high-mannose or is processed depends largely on its position in the protein. If the oligosaccharide is

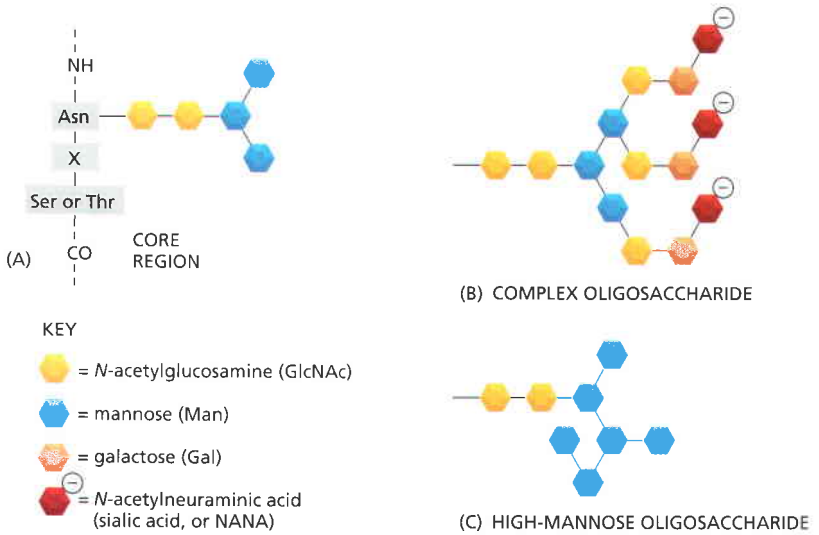


Figure 13–30 The two main classes of asparagine-linked (*N*-linked) oligosaccharides found in mature mammalian glycoproteins. (A) Both complex oligosaccharides and high-mannose oligosaccharides share a common *core region* derived from the original *N*-linked oligosaccharide added in the ER (see Figure 12–50) and typically containing two *N*-acetylglucosamines (GlcNAc) and three mannoses (Man). (B) Each complex oligosaccharide consists of a *core region*, together with a *terminal region* that contains a variable number of copies of a special trisaccharide unit *(N-acetylglucosamine–galactose–sialic acid)* linked to the core mannoses. Frequently, the terminal region is truncated and contains only GlcNAc and galactose (Gal) or just GlcNAc. In addition, a fucose residue may be added, usually to the core GlcNAc attached to the asparagine (Asn). Thus, although the steps of processing and subsequent sugar addition are rigidly ordered, complex oligosaccharides can be heterogeneous. Moreover, although the complex oligosaccharide shown has three terminal branches, two and four branches are also common, depending on the glycoprotein and the cell in which it is made. (C) High-mannose oligosaccharides are not trimmed back all the way to the core region and contain additional mannose residues. Hybrid oligosaccharides with one Man branch and one GlcNAc and Gal branch are also found (not shown).

The three amino acids indicated in (A) constitute the sequence recognized by the oligosaccharyl transferase enzyme that adds the initial oligosaccharide to the protein. Ser = serine; Thr = threonine; X = any amino acid, except proline.

accessible to the processing enzymes in the Golgi apparatus, it is likely to be converted to a complex form; if it is inaccessible because its sugars are tightly held to the protein's surface, it is likely to remain in a high-mannose form. The processing that generates complex oligosaccharide chains follows the highly ordered pathway shown in **Figure 13–31**.

Beyond these commonalities in oligosaccharide processing that are shared among most cells, the products of the carbohydrate modifications carried out in the Golgi apparatus are highly complex and have given rise to a new field called glycobiology. The human genome, for example, encodes hundreds of different Golgi glycosyl transferases, which are expressed differently from one cell type to another, resulting in a variety of glycosylated forms of a given protein or lipid in different cell types and at varying stages of differentiation, depending on the spectrum of enzymes expressed by the cell. The complexity of modifications is not limited to *N*-linked oligosaccharides but also occurs on *O-linked sugars*, as we discuss next.

Proteoglycans Are Assembled in the Golgi Apparatus

In addition to the *N*-linked oligosaccharide alterations made to proteins as they pass through the Golgi cisternae *en route* from the ER to their final destinations, many proteins are also modified in other ways. Some proteins have sugars added to the hydroxyl groups of selected serine or threonine side chains. This ***O*-linked glycosylation** (**Figure 13–32**), like the extension of *N*-linked oligosaccharide chains, is catalyzed by a series of glycosyl transferase enzymes that use the sugar nucleotides in the lumen of the Golgi apparatus to

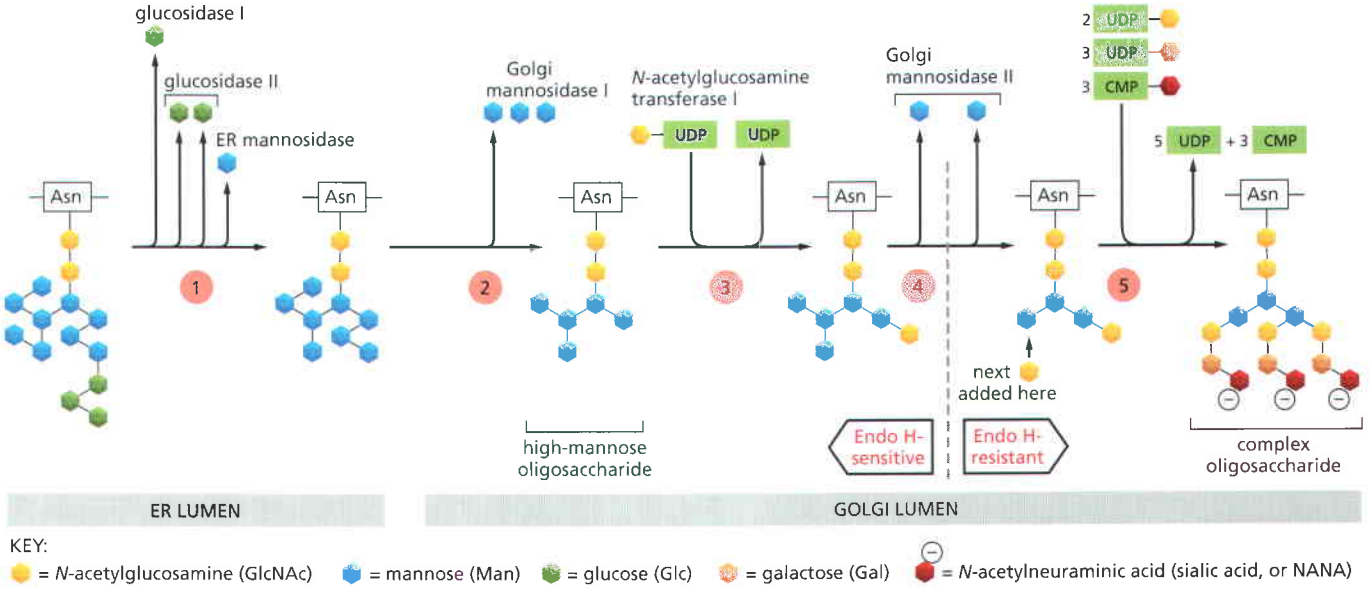


Figure 13–31 Oligosaccharide processing in the ER and the Golgi apparatus. The processing pathway is highly ordered, so that each step shown depends on the previous one. Step 1: Processing begins in the ER with the removal of the glucoses from the oligosaccharide initially transferred to the protein. Then a mannosidase in the ER membrane removes a specific mannose. Step 2: The remaining steps occur in the Golgi stack, where Golgi mannosidase I first removes three more mannoses. Step 3: *N*-acetylglucosamine transferase I then adds an *N*-acetylglucosamine. Step 4: Mannosidase II then removes two additional mannoses. This yields the final core of three mannoses that is present in a complex oligosaccharide. At this stage, the bond between the two *N*-acetylglucosamines in the core becomes resistant to attack by a highly specific endoglycosidase *(Endo H)*. Since all later structures in the pathway are also Endo H-resistant, treatment with this enzyme is widely used to distinguish complex from high-mannose oligosaccharides. Step 5: Finally, as shown in Figure 13–30, additional *N*-acetylglucosamines, galactoses, and sialic acids are added. These final steps in the synthesis of a complex oligosaccharide occur in the cisternal compartments of the Golgi apparatus. Three types of glycosyl transferase enzymes act sequentially, using sugar substrates that have been activated by linkage to the indicated nucleotide. The membranes of the Golgi cisternae contain specific carrier proteins that allow each sugar nucleotide to enter in exchange for the nucleoside phosphates that are released after the sugar is attached to the protein on the lumenal face.

Note that as a biosynthetic organelle, the Golgi apparatus differs fundamentally from the ER: all sugars are assembled inside the lumen from sugar nucleotides. By contrast, in the ER, the *N*-linked precursor oligosaccharide is assembled partly in the cytosol and partly in the lumen, and all lumenal reactions use dolichol-linked sugars as their substrates (see Figure 12–52).

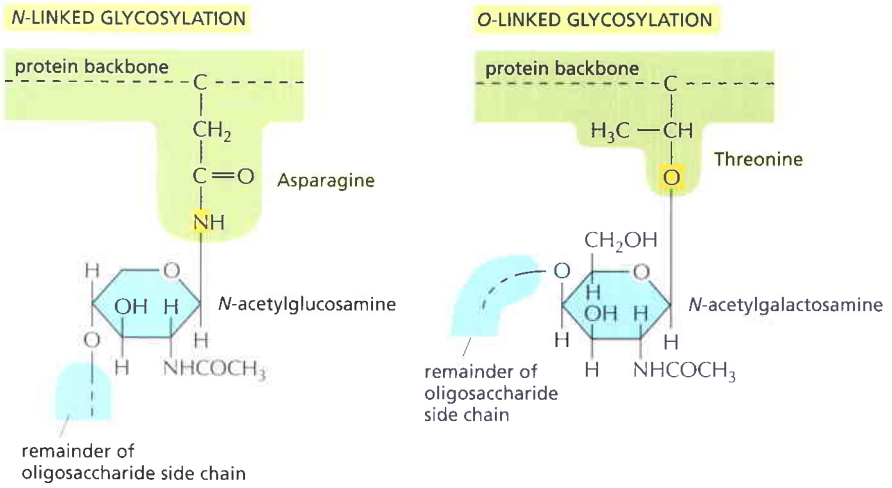


Figure 13–32 ***N*- and *O*-linked glycosylation.** In each case, only the single sugar group that is directly attached to the protein chain is shown.

add sugar residues to a protein one at a time. Usually, *N*-acetylgalactosamine is added first, followed by a variable number of additional sugar residues, ranging from just a few to 10 or more.

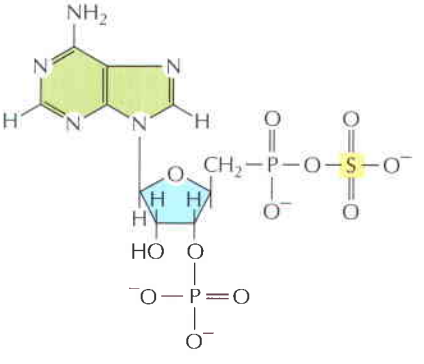
The Golgi apparatus confers the heaviest *O*-linked glycosylation of all on *mucins*, the glycoproteins in mucus secretions (see Figure 13–29), and on *proteoglycan core proteins*, which it modifies to produce **proteoglycans.** As discussed in Chapter 19, this process involves the polymerization of one or more *glycosaminoglycan chains* (long unbranched polymers composed of repeating disaccharide units; see Figure 19–58) via a xylose link onto serines on a core protein. Many proteoglycans are secreted and become components of the extracellular matrix, while others remain anchored to the extracellular face of the plasma membrane. Still others form a major component of slimy materials, such as the mucus that is secreted to form a protective coating on the surface of many epithelia.

The sugars incorporated into glycosaminoglycans are heavily sulfated in the Golgi apparatus immediately after these polymers are made, thus adding a significant portion of their characteristically large negative charge. Some tyrosines in proteins also become sulfated shortly before they exit from the Golgi apparatus. In both cases, the sulfation depends on the sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) (**Figure 13–33**), which is transported from the cytosol into the lumen of the *trans* Golgi network.

What Is the Purpose of Glycosylation?

There is an important difference between the construction of an oligosaccharide and the synthesis of other macromolecules such as DNA, RNA, and protein. Whereas nucleic acids and proteins are copied from a template in a repeated series of identical steps using the same enzyme or set of enzymes, complex carbohydrates require a different enzyme at each step, each product being recognized as the exclusive substrate for the next enzyme in the series. The vast abundance of glycoproteins and the complicated pathways that have evolved to synthesize them suggest that the oligosaccharides on glycoproteins and glycosphingolipids have very important functions.

N-linked glycosylation, for example, is prevalent in all eucaryotes, including yeasts. *N*-linked oligosaccharides also occur in a very similar form in archaeal cell wall proteins, suggesting that the whole machinery required for their synthesis is evolutionarily ancient. *N*-linked glycosylation promotes protein folding in two ways. First, it has a direct role in making folding intermediates more soluble, thereby preventing their aggregation. Second, the sequential modifications of the *N*-linked oligosaccharide establish a "glyco-code" that marks the progression of protein folding and mediates the binding of the protein to chaperones (discussed in Chapter 12) and lectins—for example, in guiding ER-to-Golgi transport. As we discuss later, lectins also participate in protein sorting in the *trans* Golgi network.



3′-phosphoadenosine-5′-phosphosulfate (PAPS)

Figure 13–33 The structure of PAPS.

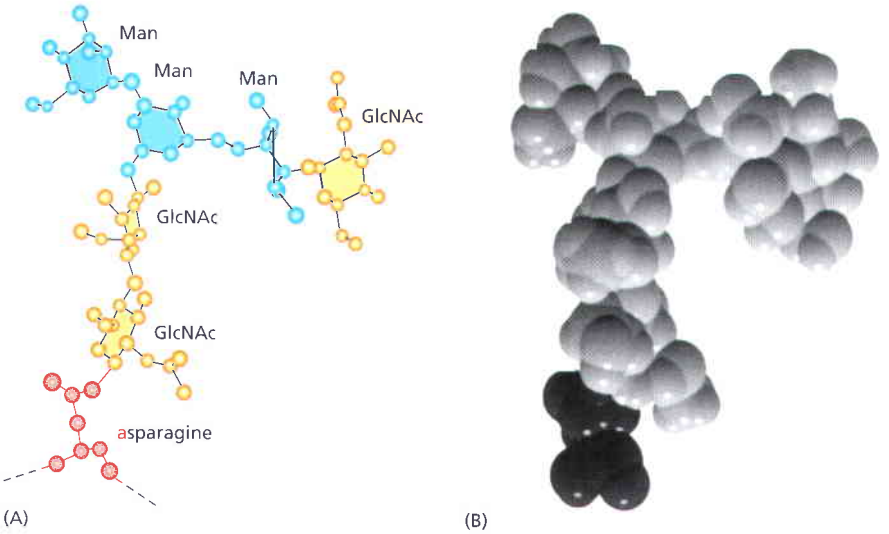


Figure 13–34 The three-dimensional structure of a small *N*-linked oligosaccharide. The structure was determined by x-ray crystallographic analysis of a glycoprotein. This oligosaccharide contains only 6 sugars, whereas there are 14 sugars in the *N*-linked oligosaccharide that is initially transferred to proteins in the ER (see Figures 12–50 and 12–51). (A) A backbone model showing all atoms except hydrogens. (B) A space-filling model, with the asparagine indicated by dark atoms. (B, courtesy of Richard Feldmann.)

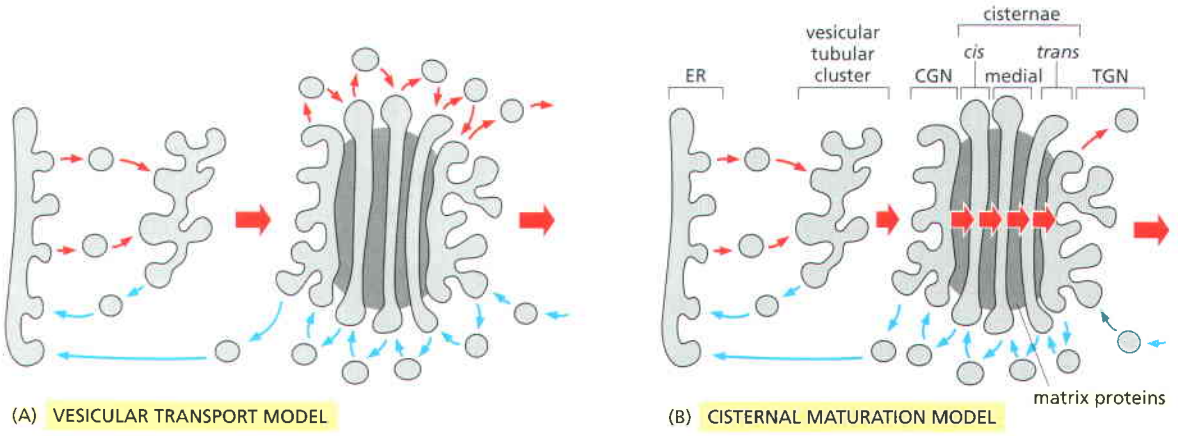
Because chains of sugars have limited flexibility, even a small *N*-linked oligosaccharide protruding from the surface of a glycoprotein (**Figure 13–34**) can limit the approach of other macromolecules to the protein surface. In this way, for example, the presence of oligosaccharides tends to make a glycoprotein more resistant to digestion by proteolytic enzymes. It may be that the oligosaccharides on cell-surface proteins originally provided an ancestral cell with a protective coat. Compared to the rigid bacterial cell wall, a mucus coat has the advantage that it leaves the cell with the freedom to change shape and move.

The sugar chains have since become modified to serve other purposes as well. The mucus coat of lung and intestinal cells, for example, protects against many pathogens. The recognition of sugar chains by *lectins* in the extracellular space is important in many developmental processes and in cell–cell recognition: *selectins*, for example, are lectins that function in cell–cell adhesion during lymphocyte migration, as discussed in Chapter 19. The presence of oligosaccharides may modify a protein's antigenic properties, making glycosylation an important factor in the production of proteins for pharmaceutical purposes.

Glycosylation can also have important regulatory roles. Signaling through the cell-surface signaling receptor Notch, for example, determines the cell's fate in development. Notch is a transmembrane protein that is *O*-glycosylated by addition of a single fucose to some serines, threonines, and hydroxylysines. Some cell types express an additional glycosyl transferase that adds an *N*-acetylglucosamine to each of these fucoses in the Golgi apparatus. This addition changes the specificity of the Notch receptor for the cell-surface signal proteins that activate the receptor.

Transport Through the Golgi Apparatus May Occur by Vesicular Transport or by Cisternal Maturation

It is still uncertain how the Golgi apparatus achieves and maintains its polarized structure and how molecules move from one cisterna to another. Functional evidence from *in vitro* transport assays, and the finding of abundant transport vesicles in the vicinity of Golgi cisternae, initially led to the view that these vesicles transport proteins between the cisternae, budding from one cisterna and fusing with the next. According to this **vesicular transport model**, the Golgi apparatus is a relatively static structure, with its enzymes held in place, while the molecules in transit move through the cisternae in sequence, carried by transport vesicles (**Figure 13–35**A). A retrograde flow of vesicles retrieves escaped ER and Golgi proteins and returns them to preceding compartments. Directional flow could be achieved because forward-moving cargo molecules are selectively allowed access to forward-moving vesicles. Although both forward-moving and retrograde types of vesicles are likely to be COPI-coated, the coats may contain different adaptor proteins that confer selectivity on the packaging of cargo



molecules. An alternative possibility is that the transport vesicles shuttling between Golgi cisternae are not directional at all, instead transporting cargo material randomly back and forth; directional flow would then occur because of the continual input at the *cis* cisterna and output at the *trans* cisterna.

A different hypothesis, called the **cisternal maturation model**, views the Golgi as a dynamic structure in which the cisternae themselves move. The vesicular tubular clusters that arrive from the ER fuse with one another to become a *cis* Golgi network. According to this model, this network then progressively matures to become a *cis* cisterna, then a medial cisterna, and so on. Thus, at the *cis* face of a Golgi stack, new *cis* cisternae would continually form and then migrate through the stack as they mature (Figure 13–35B). This model is supported by microscopic observations demonstrating that large structures such as collagen rods in fibroblasts and scales in certain algae—which are much too large to fit into classical transport vesicles—move progressively through the Golgi stack.

In the cisternal maturation model, retrograde flow explains the characteristic distribution of Golgi enzymes. Everything moves continuously forward with the maturing cisterna, including the processing enzymes that belong in the early Golgi apparatus. But budding COPI-coated vesicles continually collect the appropriate enzymes, almost all of which are membrane proteins, and carry them back to the earlier cisterna where they function. A newly formed *cis* cisterna would therefore receive its normal complement of resident enzymes primarily from the cisterna just ahead of it and would later pass those enzymes back to the next *cis* cisterna that forms.

As we discuss later, when a cisterna finally moves forward to become part of the *trans* Golgi network, various types of coated vesicles bud off it until this network disappears, to be replaced by a maturing cisterna just behind. At the same time, other transport vesicles are continually retrieving membrane from post-Golgi compartments and returning this membrane to the *trans* Golgi network.

The vesicular transport and the cisternal maturation models are not mutually exclusive. Indeed, evidence suggests that transport may occur by a combination of the two mechanisms, in which some cargo is moved forward rapidly in transport vesicles, whereas other cargo is moved forward more slowly as the Golgi apparatus constantly renews itself through cisternal maturation.

Figure 13–35 Two possible models explaining the organization of the Golgi apparatus and the transport of proteins from one cisterna to the next. It is likely that the transport through the Golgi apparatus in the forward direction *(red arrows)* involves elements of both models. (A) In the vesicular transport model, Golgi cisternae are static organelles, which contain a characteristic complement of resident enzymes. The passing of molecules from *cis* to *trans* through the Golgi is accomplished by forward-moving transport vesicles, which bud from one cisterna and fuse with the next in a *cis*-to-*trans* direction. (B) According to the alternative cisternal maturation model, each Golgi cisterna matures as it migrates outward through the stack. At each stage, the Golgi resident proteins that are carried forward in a cisterna are moved backward to an earlier compartment in COPI-coated vesicles. When a newly formed cisterna moves to a medial position, for example, "left-over" *cis* Golgi enzymes would be extracted and transported retrogradely to a new *cis* cisterna behind. Likewise, the medial enzymes would be received by retrograde transport from the cisternae just ahead. In this way, a *cis* cisterna would mature to a medial cisterna as it moves outward.

Golgi Matrix Proteins Help Organize the Stack

The unique architecture of the Golgi apparatus depends on both the microtubule cytoskeleton, as already mentioned, and cytoplasmic Golgi matrix proteins, which form a scaffold between adjacent cisternae and give the Golgi stack its structural integrity. Some of the matrix proteins form long, filamentous tethers, which are thought to help retain Golgi transport vesicles close to the organelle. When the cell prepares to divide, mitotic protein kinases phosphorylate the Golgi matrix proteins, causing the Golgi apparatus to fragment and disperse throughout the cytosol. The Golgi fragments are then distributed evenly to

the two daughter cells, where the matrix proteins are dephosphorylated, leading to the reassembly of the Golgi apparatus.

Remarkably, the Golgi matrix proteins can assemble into appropriately localized stacks near the centrosome even when Golgi membrane proteins are experimentally prevented from leaving the ER. This observation suggests that the matrix proteins are largely responsible for both the structure and location of the Golgi apparatus.

Summary

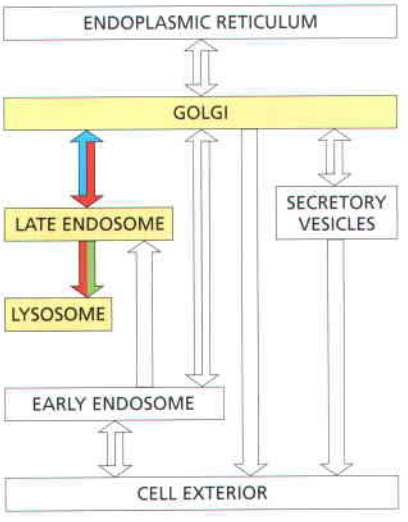
Correctly folded and assembled proteins in the ER are packaged into COPII-coated transport vesicles that pinch off from the ER membrane. Shortly thereafter, the vesicles shed their coat and fuse with one another to form vesicular tubular clusters. The clusters then move on microtubule tracks to the Golgi apparatus, where they fuse with one another to form the cis*-Golgi network. Any resident ER proteins that escape from the ER are returned there from the vesicular tubular clusters and Golgi apparatus by retrograde transport in COPI-coated vesicles.*

The Golgi apparatus, unlike the ER, contains many sugar nucleotides, which glycosyl transferase enzymes use to perform glycosylation reactions on lipid and protein molecules as they pass through the Golgi apparatus. The mannoses on the N-linked oligosaccharides that are added to proteins in the ER are often initially removed, and further sugars are added. Moreover, the Golgi apparatus is the site where O-linked glycosylation occurs and where glycosaminoglycan chains are added to core proteins to form proteoglycans. Sulfation of the sugars in proteoglycans and of selected tyrosines on proteins also occurs in a late Golgi compartment.

The Golgi apparatus modifies the many proteins and lipids that it receives from the ER and then distributes them to the plasma membrane, lysosomes, and secretory vesicles. The Golgi apparatus is a polarized organelle, consisting of one or more stacks of disc-shaped cisternae, each stack organized as a series of at least three functionally distinct compartments, termed cis, medial, *and* trans *cisternae. The* cis *and* trans *cisternae are both connected to special sorting stations, called the* cis *Golgi network and the* trans *Golgi network, respectively. Proteins and lipids move through the Golgi stack in the* cis*-to-*trans *direction. This movement may occur by vesicular transport, by progressive maturation of the* cis *cisternae as they migrate continuously through the stack, or, most likely, by a combination of these two mechanisms. Continual retrograde vesicular transport from more distal cisternae is thought to keep the enzymes concentrated in the cisternae where they are needed. The finished new proteins end up in the* trans *Golgi network, which packages them in transport vesicles and dispatches them to their specific destinations in the cell.*

TRANSPORT FROM THE *TRANS* GOLGI NETWORK TO LYSOSOMES

The *trans* Golgi network sorts all of the proteins that pass through the Golgi apparatus (except those that are retained there as permanent residents) according to their final destination. The sorting mechanism is especially well understood for those proteins destined for the lumen of lysosomes, and in this section we consider this selective transport process. We begin with a brief account of lysosome structure and function.



Lysosomes Are the Principal Sites of Intracellular Digestion

Lysosomes are membrane-enclosed compartments filled with soluble hydrolytic enzymes that control intracellular digestion of macromolecules. Lysosomes contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. All are **acid hydrolases**. For optimal activity, they need to be activated by

proteolytic cleavage and require an acid environment, which the lysosome provides by maintaining a pH of about 4.5–5.0 in its interior. By this arrangement, the contents of the cytosol are doubly protected against attack by the cell's own digestive system: the membrane of the lysosome keeps the digestive enzymes out of the cytosol, but even if they leak out, they can do little damage at the cytosolic pH of about 7.2.

Like all other intracellular organelles, the lysosome not only contains a unique collection of enzymes, but also has a unique surrounding membrane. Most of the lysosomal membrane proteins, for example, are unusually highly glycosylated, which helps to protect them from the lysosomal proteases in the lumen. Transport proteins in the lysosomal membrane carry the final products of the digestion of macromolecules—such as amino acids, sugars, and nucleotides—to the cytosol, where the cell can either reuse or excrete them.

A *vacuolar H^+ ATPase* in the lysosomal membrane uses the energy of ATP hydrolysis to pump H^+ into the lysosome, thereby maintaining the lumen at its acidic pH (**Figure 13–36**). The lysosomal H^+ pump belongs to the family of *V-type ATPases* and has a similar architecture to the mitochondrial and chloroplast ATP synthases (F-type ATPases), which convert the energy stored in H^+ gradients into ATP (see Figure 11–12). By contrast to these enzymes, however, the vacuolar H^+ ATPase exclusively works in reverse, pumping H^+ into the organelle. Similar or identical V-type ATPases acidify all endocytic and exocytic organelles, including lysosomes, endosomes, selected compartments of the Golgi apparatus, and many transport and secretory vesicles. In addition to providing a low-pH environment that is suitable for reactions occurring in the organelle lumen, the H^+ gradient provides a source of energy that drives the transport of small metabolites across the organelle membrane.

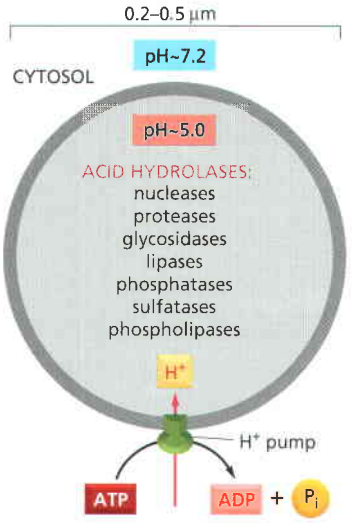


Figure 13–36 Lysosomes. The acid hydrolases are hydrolytic enzymes that are active under acidic conditions. A V-type ATPase in the membrane pumps H^+ into the lysosome, maintaining its lumen at an acidic pH.

Lysosomes Are Heterogeneous

Lysosomes were initially discovered by the biochemical fractionation of cell extracts; only later were they seen clearly in the electron microscope. Although extraordinarily diverse in shape and size, staining them with specific antibodies shows they are members of a single family of organelles. They can also be identified by histochemistry, using the precipitate produced by the action of an acid hydrolase on its substrate to indicate which organelles contain the hydrolase (**Figure 13–37**). By this criterion, lysosomes are found in all eucaryotic cells.

The heterogeneity of lysosomal morphology contrasts with the relatively uniform structures of most other cell organelles. The diversity reflects the wide variety of digestive functions that acid hydrolases mediate, including the breakdown of intra- and extracellular debris, the destruction of phagocytosed microorganisms, and the production of nutrients for the cell. The diversity of lysosomal morphology, however, also reflects the way lysosomes form: late endosomes contain material received from both the plasma membrane by endocytosis and newly synthesized lysosomal hydrolases, and they therefore already bear a resemblance to lysosomes. Late endosomes fuse with preexisting lysosomes to form structures that are sometimes referred to as *endolysosomes*, which then fuse with one another (**Figure 13–38**). When the majority of the endocytosed material within an endolysosome has been digested so that only resistant or slowly digestable residues remain, these organelles become "classical" lysosomes. These are relatively dense, round, and small, but they can enter

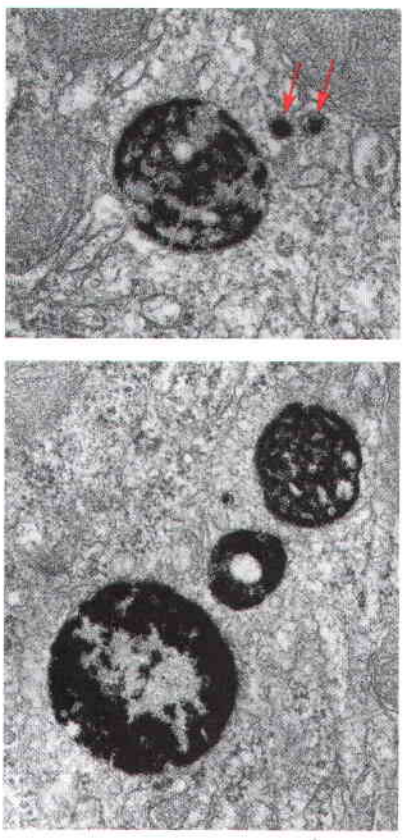


Figure 13–37 Histochemical visualization of lysosomes. These electron micrographs show two sections of a cell stained to reveal the location of acid phosphatase, a marker enzyme for lysosomes. The larger membrane-enclosed organelles, containing dense precipitates of lead phosphate, are lysosomes. Their diverse morphology reflects variations in the amount and nature of the material they are digesting. The precipitates are produced when tissue fixed with glutaraldehyde (to fix the enzyme in place) is incubated with a phosphatase substrate in the presence of lead ions. *Red arrows* in the top panel indicate two small vesicles thought to be carrying acid hydrolases from the Golgi apparatus. (Courtesy of Daniel S. Friend.)

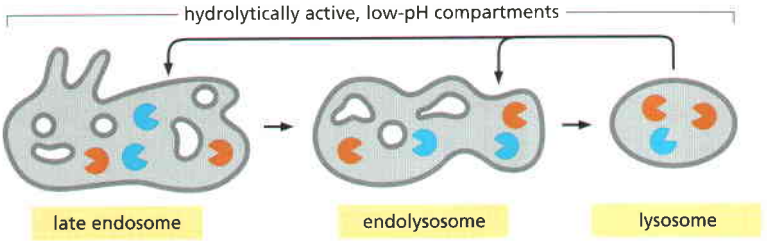


Figure 13–38 A model for lysosome maturation. The heterogeneity of lysosomal morphology reflects, in part, the different nature of the materials delivered to the organelle, as well as the different stages in the maturation cycle shown here.

the cycle again by fusing with late endosomes or endolysosomes. Thus, there is no real distinction between late endosomes and lysosomes: they are the same except that they are in different stages of a maturation cycle. For this reason, lysosomes are sometimes viewed as a heterogeneous collection of distinct organelles, the common feature of which is a high content of hydrolytic enzymes. It is especially hard to apply a narrower definition than this in plant cells, as we discuss next.

Plant and Fungal Vacuoles Are Remarkably Versatile Lysosomes

Most plant and fungal cells (including yeasts) contain one or several very large, fluid-filled vesicles called **vacuoles**. They typically occupy more than 30% of the cell volume, and as much as 90% in some cell types (**Figure 13–39**). Vacuoles are related to animal cell lysosomes and contain a variety of hydrolytic enzymes, but their functions are remarkably diverse. The plant vacuole can act as a storage organelle for both nutrients and waste products, as a degradative compartment, as an economical way of increasing cell size (**Figure 13–40**), and as a controller of *turgor pressure* (the osmotic pressure that pushes outward on the cell wall and keeps the plant from wilting). The same cell may have different vacuoles with distinct functions, such as digestion and storage.

The vacuole is important as a homeostatic device, enabling plant cells to withstand wide variations in their environment. When the pH in the environment drops, for example, the flux of H^+ into the cytosol is balanced, at least in part, by an increased transport of H^+ into the vacuole, which tends to keep the pH in the cytosol constant. Similarly, many plant cells maintain an almost constant turgor pressure despite large changes in the tonicity of the fluid in their

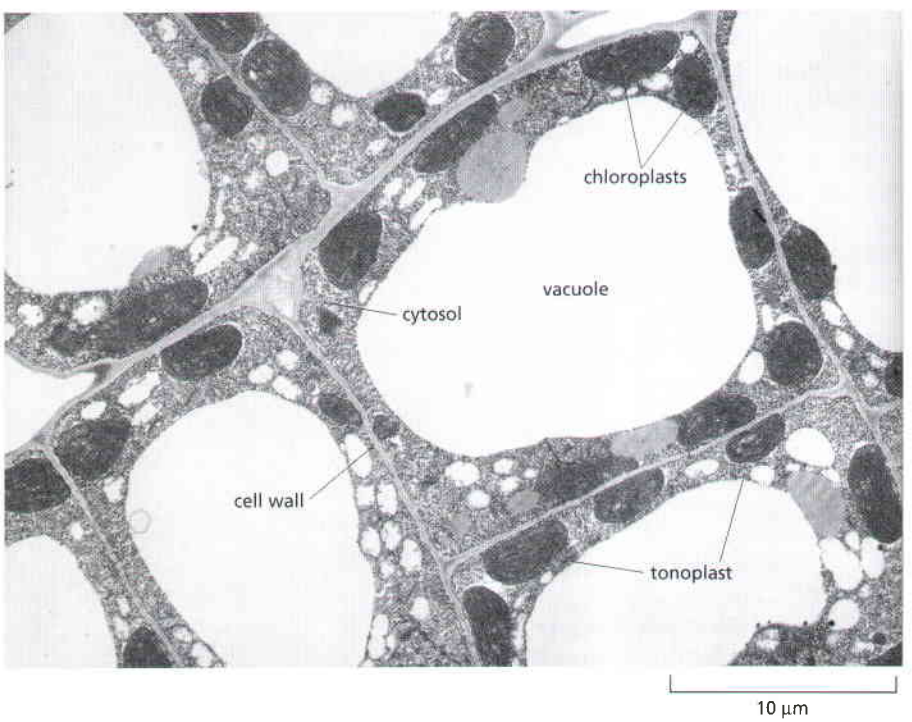


Figure 13–39 The plant cell vacuole. This electron micrograph of cells in a young tobacco leaf shows the cytosol as a thin layer, containing chloroplasts, pressed against the cell wall by the enormous vacuole. The vacuole membrane is called the tonoplast. (Courtesy of J. Burgess.)

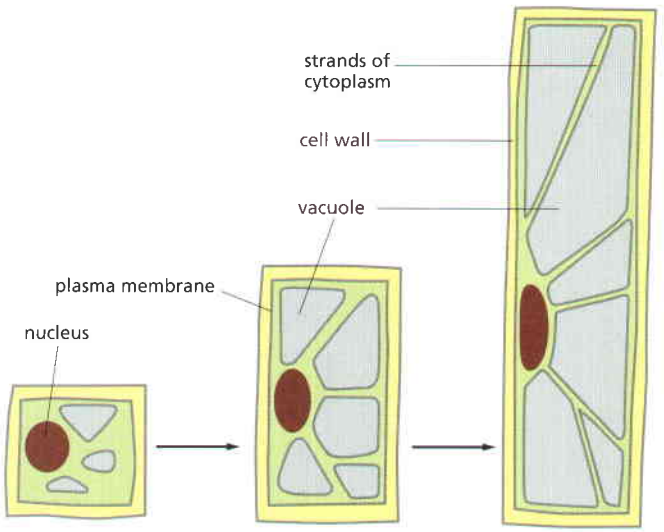


Figure 13–40 The role of the vacuole in controlling the size of plant cells. A plant cell can achieve a large increase in volume without increasing the volume of the cytosol. Localized weakening of the cell wall orients a turgor-driven cell enlargement that accompanies the uptake of water into an expanding vacuole. The cytosol is eventually confined to a thin peripheral layer, which is connected to the nuclear region by strands of cytoplasm stabilized by bundles of actin filaments (not shown).

immediate environment. They do so by changing the osmotic pressure of the cytosol and vacuole—in part by the controlled breakdown and resynthesis of polymers such as polyphosphate in the vacuole, and in part by altering the transport rates of sugars, amino acids, and other metabolites across the plasma membrane and the vacuolar membrane. The turgor pressure regulates the activities of distinct transporters in each membrane to control these fluxes.

Humans often harvest substances stored in plant vacuoles. In different species, these range from rubber to opium to the flavoring of garlic. Many stored products have a metabolic function. Proteins, for example, can be preserved for years in the vacuoles of the storage cells of many seeds, such as those of peas and beans. When the seeds germinate, these proteins are hydrolyzed and the resulting amino acids provide a food supply for the developing embryo. Anthocyanin pigments stored in vacuoles color the petals of many flowers so as to attract pollinating insects, while noxious molecules released from vacuoles when a plant is eaten or damaged provide a defense against predators.

Multiple Pathways Deliver Materials to Lysosomes

Lysosomes are usually meeting places where several streams of intracellular traffic converge. A route that leads outwards from the ER via the Golgi apparatus delivers most digestive enzymes, while at least three paths from different sources feed substances into lysosomes for digestion.

The best studied of these degradation paths in lysosomes is the one followed by macromolecules taken up from extracellular fluid by *endocytosis*. As discussed in detail later, endocytosed molecules are initially delivered in vesicles to small, irregularly shaped intracellular organelles called *early endosomes*. Here endocytosed materials first meet the lysosomal hydrolases, which are delivered to the endosome from the Golgi apparatus. Some of the ingested molecules are selectively retrieved and recycled to the plasma membrane, while others pass on into *late endosomes*. The interior of the late endosomes is mildly acidic (pH ~6), and it is the site where the hydrolytic digestion of the endocytosed molecules begins. As discussed above, mature lysosomes form by a maturation process from late endosomes, accompanied by a further decrease in internal pH. As lysosomes mature, endosomal membrane proteins are selectively retrieved from the developing lysosome by transport vesicles that deliver these proteins back to endosomes or the TGN.

All cell types use a second degradation pathway in lysosomes to dispose of obsolete parts of the cell itself—a process called **autophagy**. In a liver cell, for example, an average mitochondrion has a lifetime of about 10 days, and electron microscopic images of normal cells reveal lysosomes containing (and presumably digesting) mitochondria, as well as other organelles. The process seems to begin with the enclosure of an organelle by a double membrane of

unknown origin, creating an *autophagosome*, which then fuses with a lysosome (or a late endosome). The process is highly regulated, and selected cell components can somehow be marked for lysosomal destruction during cell remodeling. For example, the smooth ER that proliferates in a liver cell in a detoxification response to lipid-soluble drugs such as phenobarbital (discussed in Chapter 12) is selectively removed by autophagy after the drug is withdrawn.

Similarly, other obsolete organelles, including senescent peroxisomes or mitochondria, can be selectively targeted for degradation by autophagy. Under starvation conditions, large portions of the cytosol are nonselectively captured into autophagosomes. Metabolites derived from the digestion of the captured material help the cell survive when external nutrients are limiting.

In addition to maintaining basic cell functions in balance and helping to dispose of obsolete parts, autophagy also has a role in development and health. It helps restructure differentiating cells by disposing of no longer needed parts and helps defend against invading viruses and bacteria. Autophagy is uniquely suited as a mechanism that can remove whole organelles or large protein aggregates, which other mechanisms such as proteasomal degradation cannot handle.

We still know very little about the events that lead to the formation of autophagosomes, or how the process of autophagy is controlled and targeted at specific organelles. More than 25 different proteins have been identified in yeast and animal cells that participate in the process. Autophagy can be divided into four general steps: (1) nucleation and extension of a delimiting membrane into a crescent-shaped structure that engulfs a portion of the cytoplasm, (2) closure of the autophagosome into a sealed double-membrane-bounded compartment, (3) fusion of the new compartment with lysosomes, and (4) digestion of the inner membrane of the autophagosome and its contents (**Figure 13–41**). Many mysteries remain to be solved, including identifying the membrane system from which the vesicles that form the autophagosomal envelope derive, and how some target organelles can be enclosed so selectively.

As we discuss later, the third pathway that brings materials to lysosomes for degradation is found mainly in cells specialized for the *phagocytosis* of large particles and microorganisms. Such professional phagocytes (macrophages and neutrophils in vertebrates) engulf objects to form a *phagosome*, which is then converted to a lysosome in the manner described for the autophagosome. **Figure 13–42** summarizes the three pathways.

A Mannose 6-Phosphate Receptor Recognizes Lysosomal Proteins in the *Trans* Golgi Network

We now consider the pathway that delivers lysosomal hydrolases and membrane proteins to lysosomes. Both classes of proteins are co-translationally transported into the rough ER and then transported through the Golgi apparatus to the TGN. The transport vesicles that deliver these proteins to endosomes (from where the proteins are moved on to lysosomes) bud from the TGN. The vesicles incorporate the lysosomal proteins and exclude the many other proteins being packaged into different transport vesicles for delivery elsewhere.

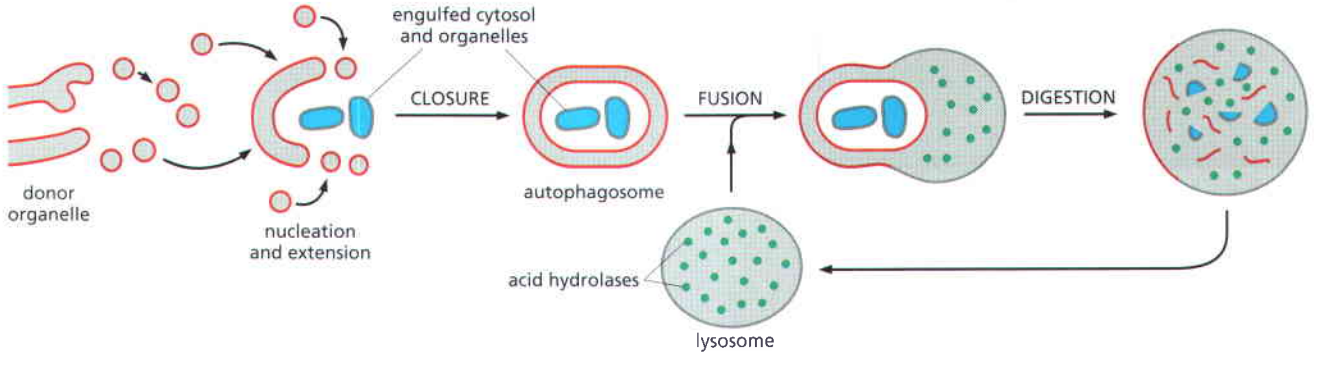
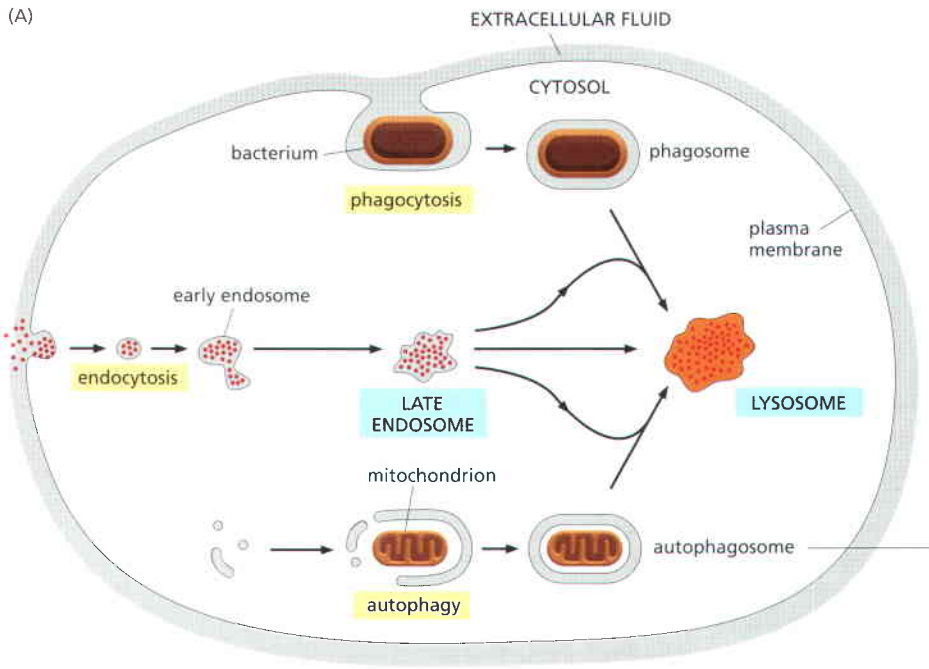


Figure 13–41 A model of autophagy. After a nucleation event in the cytoplasm, a crescent of autophagosomal membrane grows by fusion of vesicles of unknown origin that extend its edges. Eventually, a membrane fusion event closes the autophagosome, sequestering a portion of the cytoplasm of the cell in a double membrane. The autophagosome then fuses with lysosomes containing acid hydrolases that digest its content.



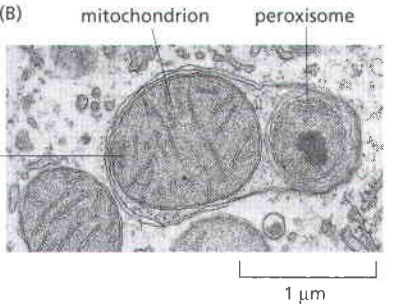


Figure 13–42 Three pathways to degradation in lysosomes. (A) Materials in each pathway are derived from a different source. Note that the autophagosome has a double membrane. (B) An electron micrograph of an autophagosome containing a mitochondrion and a peroxisome. (B, courtesy of Daniel S. Friend, from D.W. Fawcett, A Textbook of Histology, 12th ed. New York: Chapman and Hall, 1994. With permission from Kluwer.)

How are lysosomal proteins recognized and selected in the TGN with the required accuracy? We know the answer for the lysosomal hydrolases. They carry a unique marker in the form of *mannose 6-phosphate (M6P)* groups, which are added exclusively to the *N*-linked oligosaccharides of these soluble lysosomal enzymes as they pass through the lumen of the *cis* Golgi network (**Figure 13–43**). Transmembrane **M6P receptor proteins**, which are present in the TGN, recognize the M6P groups. The receptor proteins bind to lysosomal hydrolases on the lumenal side of the membrane and to adaptor proteins in assembling clathrin coats on the cytosolic side. In this way, they help package the hydrolases into clathrin-coated vesicles that bud from the TGN. The vesicles shed their coat and deliver their contents to early endosomes.

The M6P Receptor Shuttles Between Specific Membranes

The M6P receptor protein binds its specific oligosaccharide at pH 6.5–6.7 in the TGN and releases it at pH 6, which is the pH in the interior of late endosomes. Thus, as the pH drops during endosomal maturation, the lysosomal hydrolases dissociate from the M6P receptor and eventually begin to digest the material delivered by endocytosis. An acid phosphatase removes the phosphate group from the mannose, thereby destroying the sorting signal and contributing to the release of the lysosomal hydrolases from the M6P receptor. Having released their bound enzymes, the M6P receptors are retrieved into retromer-coated transport vesicles that bud from endosomes; the receptors are then returned to the membrane of the TGN for reuse (**Figure 13–44**). Transport in either direction requires signals in the cytoplasmic tail of the M6P receptor that direct this protein to the endosome or back to the Golgi apparatus. These signals are recognized by the retromer complex (see Figure 13–9) that recruits M6P receptors into vesicles in endosomes. The recycling of the M6P receptor resembles the recycling of the KDEL receptor discussed earlier, although it differs in the type of coated vesicles that mediate the transport.

Not all the hydrolase molecules that are tagged with M6P get to lysosomes. Some escape the normal packaging process in the *trans* Golgi network and are transported "by default" to the cell surface, where they are secreted into the extracellular fluid. Some M6P receptors, however, also take a detour to the plasma membrane, where they recapture the escaped lysosomal hydrolases and return them by *receptor-mediated endocytosis* to lysosomes via early and late endosomes. As lysosomal hydrolases require an acidic milieu to work, they can do little harm in the extracellular fluid, which usually has a neutral pH of 7.4.

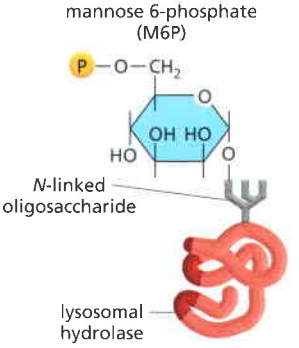
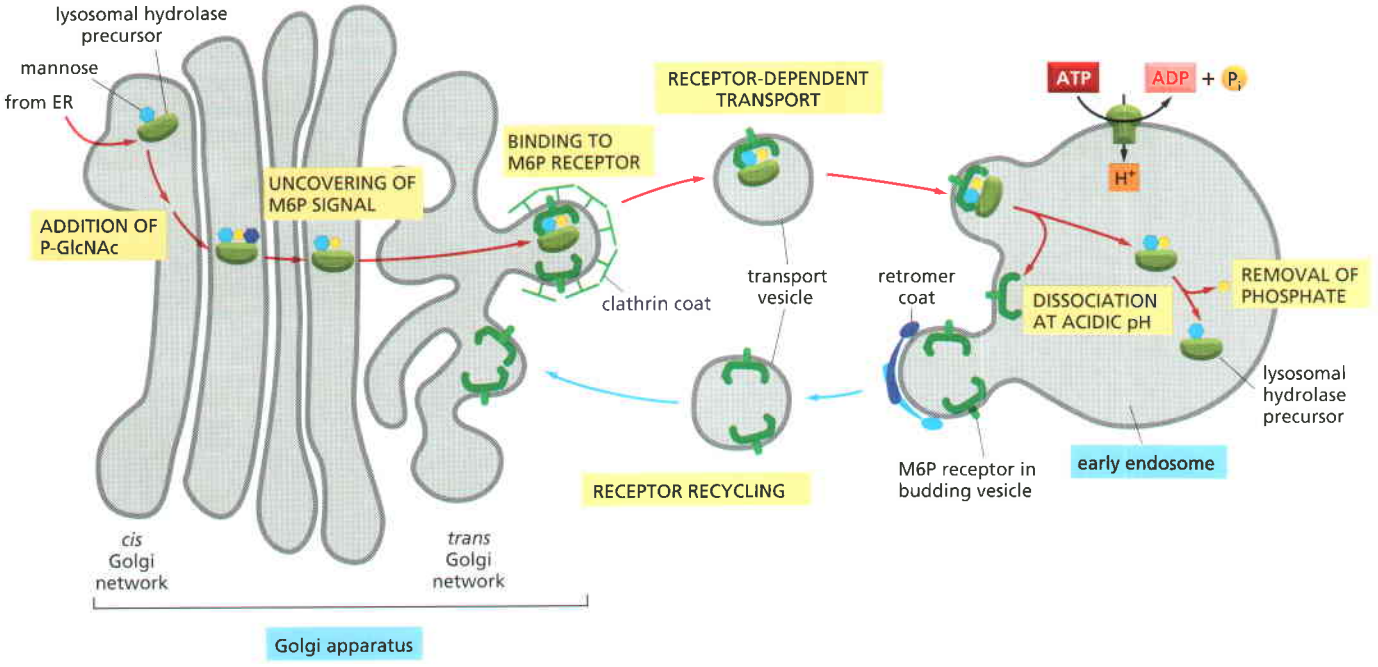


Figure 13–43 The structure of mannose 6-phosphate on a lysosomal hydrolase.



A Signal Patch in the Hydrolase Polypeptide Chain Provides the Cue for M6P Addition

The sorting system that segregates lysosomal hydrolases and dispatches them to endosomes works because M6P groups are added only to the appropriate glycoproteins in the Golgi apparatus. This requires specific recognition of the hydrolases by the Golgi enzymes responsible for adding M6P. Since all glycoproteins leave the ER with identical *N*-linked oligosaccharide chains, the signal for adding the M6P units to oligosaccharides must reside somewhere in the polypeptide chain of each hydrolase. Genetic engineering experiments have revealed that the recognition signal is a cluster of neighboring amino acids on each protein's surface, known as a *signal patch.*

Two enzymes act sequentially to catalyze the addition of M6P groups to lysosomal hydrolases. The first is a GlcNAc phosphotransferase in the *cis* Golgi that specifically binds the hydrolase and adds GlcNAc-phosphate to one or two of the mannose residues on each oligosaccharide chain (**Figure 13–45**). A second enzyme in the *trans* Golgi then cleaves off the GlcNAc residue, leaving behind a newly created M6P marker. Since most lysosomal hydrolases contain multiple oligosaccharides, they acquire many M6P residues, providing a high-affinity signal for the M6P receptor.

Defects in the GlcNAc Phosphotransferase Cause a Lysosomal Storage Disease in Humans

Genetic defects that affect one or more of the lysosomal hydrolases cause a number of human **lysosomal storage diseases**. The defects result in an accumulation of undigested substrates in lysosomes, with severe pathological consequences, most often in the nervous system. In most cases, there is a mutation in a structural gene that codes for an individual lysosomal hydrolase. This occurs in *Hurler's disease,* for example, in which the enzyme required for the breakdown of certain types of glycosaminoglycan chains is defective or missing. The most severe form of lysosomal storage disease, however, is a very rare disorder called *inclusion-cell disease* (*I-cell disease*). In this condition, almost all of the hydrolytic enzymes are missing from the lysosomes of fibroblasts, and their undigested substrates accumulate in lysosomes, which consequently form large *inclusions* in the patients' cells.

Figure 13–44 The transport of newly synthesized lysosomal hydrolases to lysosomes. The sequential action of two enzymes in the *cis* and *trans* Golgi network adds mannose 6-phosphate (M6P) groups to the precursors of lysosomal enzymes (see Figure 13–45). They then segregate from all other types of proteins in the TGN because monomeric adaptor proteins in the clathrin coat bind the M6P receptors, which, in turn, bind the modified lysosomal hydrolases. The clathrin-coated vesicles bud off from the TGN, shed their coat, and fuse with early endosomes. At the lower pH of the endosome, the hydrolases dissociate from the M6P receptors, and the empty receptors are recycled in retromer-coated vesicles to the Golgi apparatus for further rounds of transport. In the endosomes, the phosphate is removed from the mannose sugars attached to the hydrolases, further ensuring that the hydrolases do not return to the Golgi apparatus with the receptor.

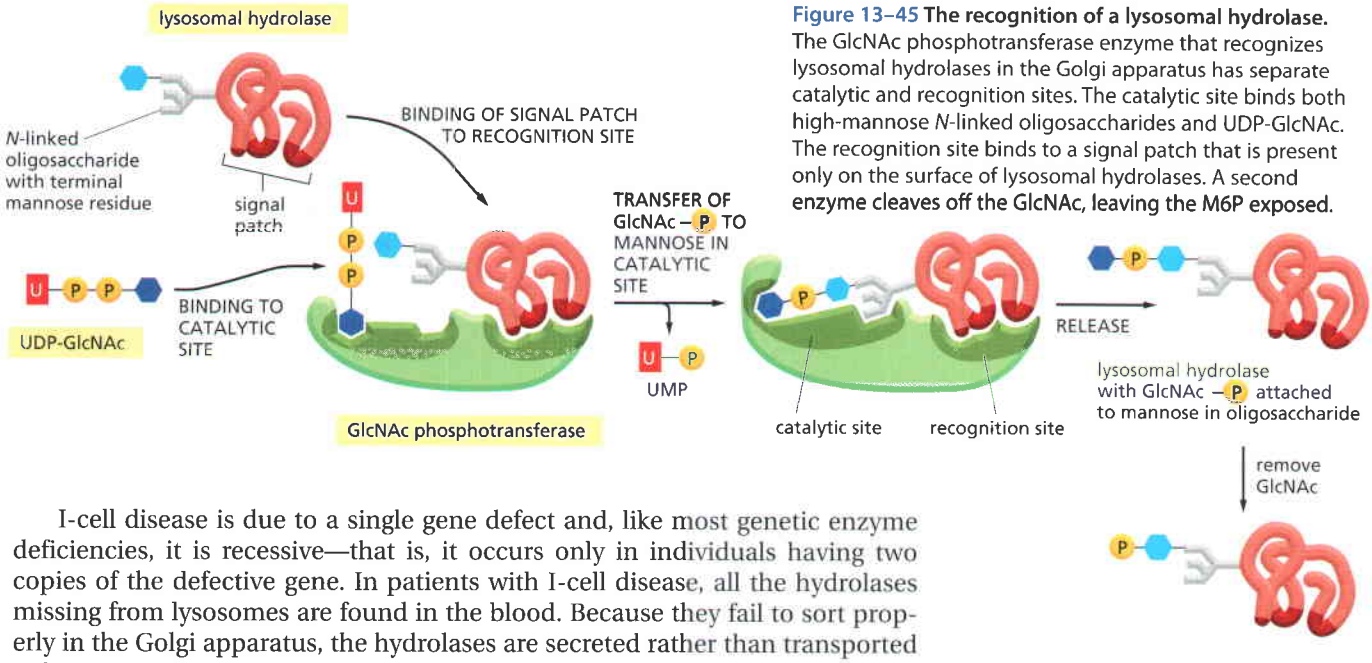


Figure 13–45 The recognition of a lysosomal hydrolase. The GlcNAc phosphotransferase enzyme that recognizes lysosomal hydrolases in the Golgi apparatus has separate catalytic and recognition sites. The catalytic site binds both high-mannose *N*-linked oligosaccharides and UDP-GlcNAc. The recognition site binds to a signal patch that is present only on the surface of lysosomal hydrolases. A second enzyme cleaves off the GlcNAc, leaving the M6P exposed.

I-cell disease is due to a single gene defect and, like most genetic enzyme deficiencies, it is recessive—that is, it occurs only in individuals having two copies of the defective gene. In patients with I-cell disease, all the hydrolases missing from lysosomes are found in the blood. Because they fail to sort properly in the Golgi apparatus, the hydrolases are secreted rather than transported to lysosomes. The missorting has been traced to a defective or missing GlcNAc-phosphotransferase. Because lysosomal enzymes are not phosphorylated in the *cis* Golgi network, the M6P receptors do not segregate them into the appropriate transport vesicles in the TGN. Instead, the lysosomal hydrolases are carried to the cell surface and secreted by a default pathway.

In I-cell disease, the lysosomes in some cell types, such as hepatocytes, contain a normal complement of lysosomal enzymes, implying that there is another pathway for directing hydrolases to lysosomes that is used by some cell types but not others. The nature of this M6P-independent pathway is unknown. Similarly, an M6P-independent pathway in all cells sorts the membrane proteins of lysosomes from the TGN for transport to late endosomes, and those proteins are therefore normal in I-cell disease. These membrane proteins exit from the TGN in clathrin-coated vesicles that are distinct from those that transport the M6P-tagged hydrolases and use different adaptor proteins.

It is unclear why cells need more than one sorting pathway to construct lysosomes, although it is perhaps not surprising that different mechanisms operate for soluble and membrane-bound lysosomal proteins, especially since—unlike M6P receptors—those membrane proteins are lysosomal residents and need not be returned to the TGN.

Some Lysosomes Undergo Exocytosis

Targeting of material to lysosomes is not necessarily the end of the pathway. *Lysosomal secretion* of undigested content enables all cells to eliminate undigestible debris. For most cells, this seems to be a minor pathway, used only when the cells are stressed. Some cell types, however, contain specialized lysosomes that have acquired the necessary machinery for fusion with the plasma membrane. *Melanocytes* in the skin, for example, produce and store pigments in their lysosomes. These pigment-containing *melanosomes* release their pigment into the extracellular space of the epidermis by exocytosis. The pigment is then taken up by keratinocytes, leading to normal skin pigmentation. In some genetic disorders, defects in melanosome exocytosis block this transfer process, leading to forms of hypopigmentation (albinism).

Summary

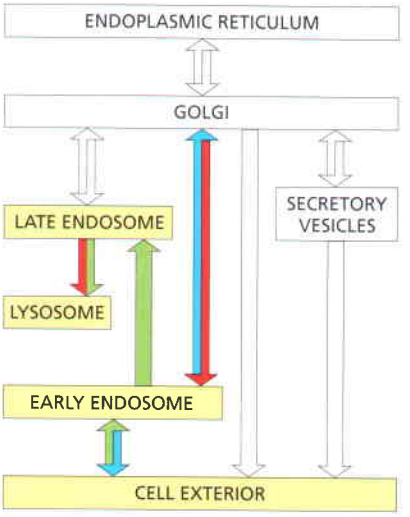
Lysosomes are specialized for the intracellular digestion of macromolecules. They contain unique membrane proteins and a wide variety of soluble hydrolytic enzymes that

operate best at pH 5, which is the internal pH of lysosomes. An ATP-driven H^+ pump in the lysosomal membrane maintains this low pH. Newly synthesized lysosomal proteins are transferred into the lumen of the ER, transported through the Golgi apparatus, and then carried from the trans *Golgi network to late endosomes by means of clathrin-coated transport vesicles.*

The lysosomal hydrolases contain N*-linked oligosaccharides that are covalently modified in a unique way in the* cis *Golgi network so that their mannose residues are phosphorylated. These mannose 6-phosphate (M6P) groups are recognized by an M6P receptor protein in the* trans *Golgi network that segregates the hydrolases and helps package them into budding transport vesicles that deliver their contents to endosomes. The M6P receptors shuttle back and forth between the* trans *Golgi network and these endosomes. The low pH in endosomes and removal of the phosphate from the M6P group cause the lysosomal hydrolases to dissociate from these receptors, making the transport of the hydrolases unidirectional. A separate transport system uses clathrin-coated vesicles to deliver resident lysosomal membrane proteins from the* trans *Golgi network.*

TRANSPORT INTO THE CELL FROM THE PLASMA MEMBRANE: ENDOCYTOSIS

The routes that lead inward from the cell surface to lysosomes start with the process of **endocytosis**, by which cells take up macromolecules, particulate substances, and, in specialized cases, even other cells. In this process, the material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an *endocytic vesicle* containing the ingested substance or particle. Two main types of endocytosis differ according to the size of the endocytic vesicles formed. In *phagocytosis* ("cell eating"), large particles are ingested via large vesicles called *phagosomes* (generally >250 nm in diameter). In *pinocytosis* ("cell drinking"), fluid and solutes are ingested via small *pinocytic vesicles* (about 100 nm in diameter). Most eucaryotic cells are continually ingesting fluid and solutes by pinocytosis; large particles are ingested most efficiently by specialized phagocytic cells.



Specialized Phagocytic Cells Can Ingest Large Particles

Phagocytosis is a special form of endocytosis in which a cell uses large endocytic vesicles called **phagosomes** to ingest large particles such as microorganisms and dead cells. <TCAT> In protozoa, phagocytosis is a form of feeding: large particles taken up into phagosomes end up in lysosomes, and the products of the subsequent digestive processes pass into the cytosol to be used as food. However, few cells in multicellular organisms are able to ingest such large particles efficiently. In the gut of animals, for example, extracellular processes break down food particles, and cells import the small hydrolysis products.

Phagocytosis is important in most animals for purposes other than nutrition, and it is carried out mainly by specialized cells—so-called *professional phagocytes.* In mammals, two classes of white blood cells act as professional phagocytes—**macrophages** and **neutrophils**. These cells develop from hemopoietic stem cells (discussed in Chapter 23), and they ingest invading microorganisms to defend us against infection. Macrophages also have an important role in scavenging senescent cells and cells that have died by apoptosis (discussed in Chapter 18). In quantitative terms, the clearance of senescent and dead cells is by far the most important: our macrophages phagocytose more than 10^{11} senescent red blood cells in each of us every day, for example.

Whereas the endocytic vesicles involved in pinocytosis are small and uniform, phagosomes have diameters that are determined by the size of the ingested particle, and they can be almost as large as the phagocytic cell itself (**Figure 13–46**). The phagosomes fuse with lysosomes inside the cell, and the ingested material is then degraded. Any indigestible substances will remain in

lysosomes, forming *residual bodies*, which can be excreted from cells by exocytosis, as we have previously discussed. Some of the internalized plasma membrane components never reach the lysosome, because they are retrieved from the phagosome in transport vesicles and returned to the plasma membrane.

To be phagocytosed, particles must first bind to the surface of the phagocyte. Not all particles that bind are ingested, however. Phagocytes have a variety of specialized surface receptors that are functionally linked to the phagocytic machinery of the cell. Phagocytosis is a triggered process. That is, it requires the activation of receptors that transmit signals to the cell interior and initiate the response. By contrast, pinocytosis is a constitutive process. It occurs continuously, regardless of the needs of the cell. The best-characterized triggers of phagocytosis are antibodies, which protect us by binding to the surface of infectious microorganisms to form a coat that exposes the tail region on the exterior of each antibody molecule. This tail region is called the Fc region (discussed in Chapter 25). The antibody coat is recognized by specific *Fc receptors* on the surface of macrophages and neutrophils, whose binding induces the phagocytic cell to extend pseudopods that engulf the particle and fuse at their tips to form a phagosome (**Figure 13–47**A). Localized actin polymerization, initiated by Rho-family GTPases and their activating Rho-GEFs (discussed in Chapters 15 and 16), shapes the pseudopods. An active Rho GTPase switches on the kinase activity of local PI kinases, and initial actin polymerization occurs in response to an accumulation of $PI(4,5)P_2$ in the membrane (see Figure 13–11). To seal off the phagosome and complete its engulfment, actin is depolymerized at its base as $PI(4,5)P_2$ is subjected to a PI 3-kinase, which converts it to $PI(3,4,5)P_3$. $PI(3,4,5)P_3$ is required for closure of the phagosome and may also contribute to reshaping the actin network to help drive the invagination of the forming phagosome (Figure 13–47B). In this way, the ordered generation and consumption of specific phosphoinositides guides sequential steps in phagosome formation.

Several other classes of receptors that promote phagocytosis have been characterized. Some recognize *complement* components, which collaborate with antibodies in targeting microbes for destruction (discussed in Chapter 24). Others directly recognize oligosaccharides on the surface of certain microorganisms. Still others recognize cells that have died by apoptosis. Apoptotic cells lose the asymmetric distribution of phospholipids in their plasma membrane. As a consequence, negatively charged phosphatidylserine, which is normally confined to the cytosolic leaflet of the lipid bilayer, is now exposed on the outside of the cell, where it helps to trigger the phagocytosis of the dead cell.

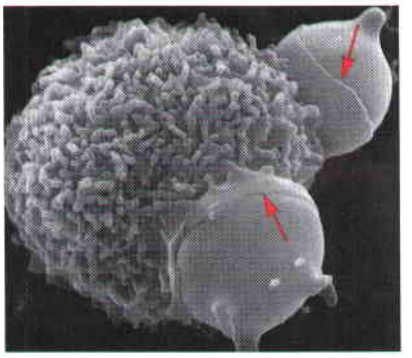
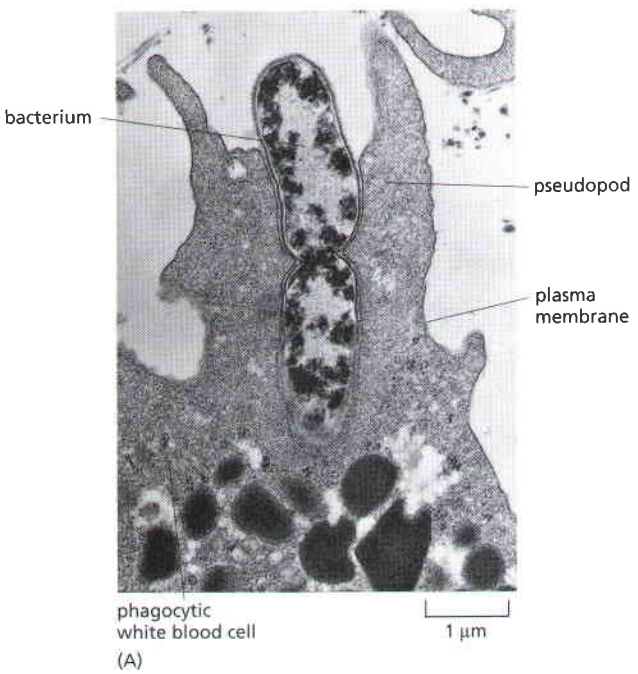


Figure 13–46 Phagocytosis by a macrophage. A scanning electron micrograph of a mouse macrophage phagocytosing two chemically altered red blood cells. The *red arrows* point to edges of thin processes (pseudopods) of the macrophage that are extending as collars to engulf the red cells. (Courtesy of Jean Paul Revel.)



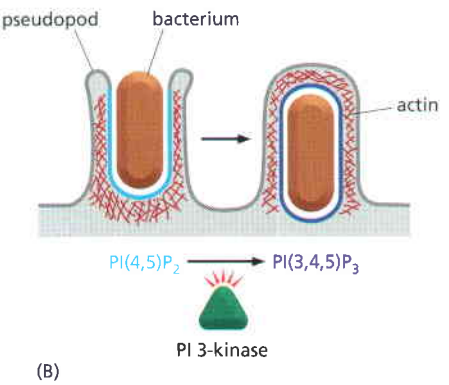


Figure 13–47 A neutrophil reshaping a plasma membrane during phagocytosis. (A) An electron micrograph of a neutrophil phagocytosing a bacterium, which is in the process of dividing. (B) Pseudopod extension and phagosome formation are driven by actin polymerization and reorganization, which respond to the accumulation of specific phosphoinositides in the membrane of the forming phagosome. (A, courtesy of Dorothy F. Bainton, Phagocytic Mechanisms in Health and Disease. New York: Intercontinental Medical Book Corporation, 1971.)

Remarkably, macrophages will also phagocytose a variety of inanimate particles—such as glass or latex beads and asbestos fibers—yet they do not phagocytose live animal cells. Living animal cells seem to display "don't-eat-me" signals in the form of cell-surface proteins that bind to inhibiting receptors on the surface of macrophages. The inhibitory receptors recruit tyrosine phosphatases that antagonize the intracellular signaling events required to initiate phagocytosis, thereby locally inhibiting the phagocytic process. Thus phagocytosis, like many other cell processes, depends on a balance between positive signals that activate the process and negative signals that inhibit it. Apopototic cells are thought both to gain "eat-me" signals (such as extracellularly exposed phosphatidylserine) and to lose their "don't-eat-me" signals, causing them to be very rapidly phagocytosed by macrophages.

Pinocytic Vesicles Form from Coated Pits in the Plasma Membrane

Virtually all eucaryotic cells continually ingest bits of their plasma membrane in the form of small pinocytic (endocytic) vesicles, which are later returned to the cell surface. The rate at which plasma membrane is internalized in this process of **pinocytosis** varies between cell types, but it is usually surprisingly large. A macrophage, for example, ingests 25% of its own volume of fluid each hour. This means that it must ingest 3% of its plasma membrane each minute, or 100% in about half an hour. Fibroblasts endocytose at a somewhat lower rate (1% of their plasma membrane per minute), whereas some amoebae ingest their plasma membrane even more rapidly. Since a cell's surface area and volume remain unchanged during this process, it is clear that the same amount of membrane being removed by endocytosis is being added to the cell surface by the converse process of *exocytosis*. In this sense, endocytosis and exocytosis are linked processes that can be considered to constitute an *endocytic–exocytic cycle*. The coupling between exocytosis and endocytosis is particularly strict in specialized structures characterized by high membrane turnover, such as the neuronal synapse.

The endocytic part of the cycle often begins at **clathrin-coated pits**. These specialized regions typically occupy about 2% of the total plasma membrane area. The lifetime of a clathrin-coated pit is short: within a minute or so of being formed, it invaginates into the cell and pinches off to form a clathrin-coated vesicle (**Figure 13–48**). It has been estimated that about 2500 clathrin-coated vesicles leave the plasma membrane of a cultured fibroblast every minute. The coated vesicles are even more transient than the coated pits: within seconds of being formed, they shed their coat and are able to fuse with early endosomes. Since extracellular fluid is trapped in clathrin-coated pits as they invaginate to

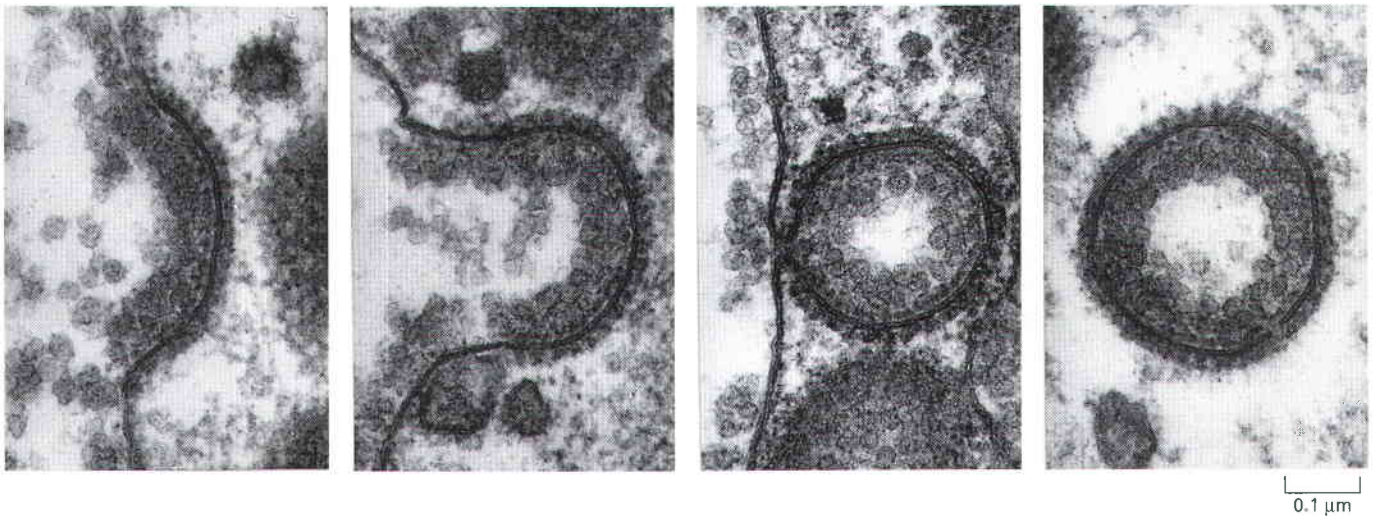


Figure 13–48 The formation of clathrin-coated vesicles from the plasma membrane. These electron micrographs illustrate the probable sequence of events in the formation of a clathrin-coated vesicle from a clathrin-coated pit. The clathrin-coated pits and vesicles shown are larger than those seen in normal-sized cells. They take up lipoprotein particles into a very large hen oocyte to form yolk. The lipoprotein particles bound to their membrane-bound receptors appear as a dense, fuzzy layer on the extracellular surface of the plasma membrane—which is the inside surface of the vesicle. (Courtesy of M.M. Perry and A.B. Gilbert, *J. Cell Sci.* 39:257–272, 1979. With permission from The Company of Biologists.)

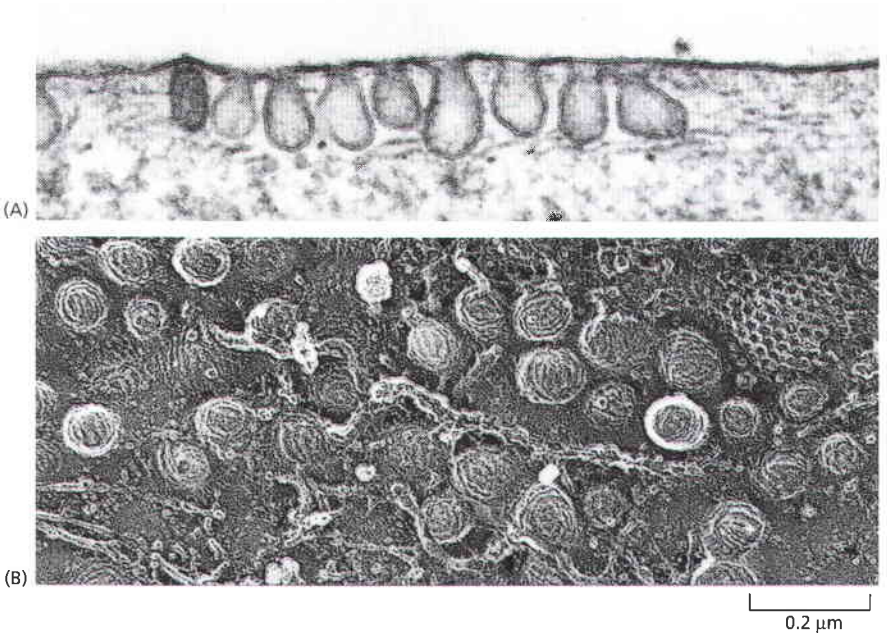


Figure 13–49 Caveolae in the plasma membrane of a fibroblast. (A) This electron micrograph shows a plasma membrane with a very high density of caveolae. Note that no cytosolic coat is visible. (B) This rapid-freeze deep-etch image demonstrates the characteristic "cauliflower" texture of the cytosolic face of the caveolae membrane. The regular texture is thought to result from aggregates of caveolin in the membrane. A clathrin-coated pit is also seen at the upper right. (Courtesy of R.G.W. Anderson, from K.G. Rothberg et al., *Cell* 68:673–682, 1992. With permission from Elsevier.)

form coated vesicles, any substance dissolved in the extracellular fluid is internalized—a process called *fluid-phase endocytosis.*

Not All Pinocytic Vesicles Are Clathrin-Coated

In addition to clathrin-coated pits and vesicles, there are other, less well-understood mechanisms by which cells can form pinocytic vesicles. One of these pathways initiates at **caveolae** (from the Latin for "little cavities"), originally recognized by their ability to transport molecules across endothelial cells, which form the inner lining of blood vessels. Caveolae are present in the plasma membrane of most cell types, and in some of these they are seen in the electron microscope as deeply invaginated flasks (**Figure 13–49**). They are thought to form from membrane microdomains, or *lipid rafts,* which are patches of the plasma membrane that are especially rich in cholesterol, glycosphingolipids, and GPI-anchored membrane proteins (see Figure 10–14). The major structural proteins in caveolae are **caveolins**, which are a family of unusual integral membrane proteins that each insert a hydrophobic loop into the membrane from the cytosolic side but do not extend across the membrane.

In contrast to clathrin-coated and COPI- or COPII-coated vesicles, caveolae are thought to invaginate and collect cargo proteins by virtue of the lipid composition of the calveolar membrane, rather than by the assembly of a cytosolic protein coat. Caveolins may stabilize these raft domains, into which certain plasma membrane proteins partition. Caveolae pinch off from the plasma membrane using dynamin, and they deliver their contents either to an endosome-like compartment (called a *caveosome*) or to the plasma membrane on the opposite side of a polarized cell (in a process called *transcytosis,* which we discuss later). Because caveolins are integral membrane proteins, they do not dissociate from the vesicles after endocytosis; instead they are delivered to the target compartments, where they are maintained as discrete membrane domains. Some animal viruses such as SV40 and papilloma virus (which causes warts) enter cells in vesicles derived from caveolae. The viruses are first delivered to caveosomes, and they move from there in specialized transport vesicles to the ER. The viral genome exits from the ER across the ER membrane into the cytosol, from where it is imported into the nucleus to start the infection cycle.

Endocytic vesicles can also bud from caveolin-free raft domains on the plasma membrane and deliver their cargo to caveosomes. Molecules that enter the cell through caveosomes avoid endosomes and lysosomes and are therefore shielded from exposure to low pH and lysosomal hydrolases; it is unknown how they move from caveosomes to other destinations in the cell.

Cells Use Receptor-Mediated Endocytosis to Import Selected Extracellular Macromolecules

In most animal cells, clathrin-coated pits and vesicles provide an efficient pathway for taking up specific macromolecules from the extracellular fluid. In this process, called **receptor-mediated endocytosis**, the macromolecules bind to complementary transmembrane receptor proteins, accumulate in coated pits, and then enter the cell as receptor–macromolecule complexes in clathrin-coated vesicles (see Figure 13–48). Because ligands are selectively captured by receptors, receptor-mediated endocytosis provides a selective concentrating mechanism that increases the efficiency of internalization of particular ligands more than a hundredfold. In this way, even minor components of the extracellular fluid can be specifically taken up in large amounts without taking in a large volume of extracellular fluid. A particularly well-understood and physiologically important example is the process that mammalian cells use to take up cholesterol.

Many animals cells take up cholesterol through receptor-mediated endocytosis and, in this way, acquire most of the cholesterol they require to make new membrane. If the uptake is blocked, cholesterol accumulates in the blood and can contribute to the formation in blood vessel (artery) walls of *atherosclerotic plaques,* deposits of lipid and fibrous tissue that can cause strokes and heart attacks by blocking arterial blood flow. In fact, it was a study of humans with a strong genetic predisposition for *atherosclerosis* that first revealed the mechanism of receptor-mediated endocytosis.

Most cholesterol is transported in the blood as cholesteryl esters in the form of lipid–protein particles known as **low-density lipoproteins** (**LDLs**) (**Figure 13–50**). When a cell needs cholesterol for membrane synthesis, it makes transmembrane receptor proteins for LDL and inserts them into its plasma membrane. Once in the plasma membrane, the *LDL receptors* diffuse until they associate with clathrin-coated pits that are in the process of forming (**Figure 13–51**A). Since coated pits constantly pinch off to form coated vesicles, any LDL particles bound to LDL receptors in the coated pits are rapidly internalized in coated vesicles. After shedding their clathrin coats, the vesicles deliver their contents to early endosomes, which are located near the cell periphery. Once the LDL and LDL receptors encounter the low pH in the endosomes, LDL is released from its receptor and is delivered via late endosomes to lysosomes. There, the cholesteryl esters in the LDL particles are hydrolyzed to free cholesterol, which is now available to the cell for new membrane synthesis. If too much free cholesterol accumulates in a cell, the cell shuts off both its own cholesterol synthesis and the synthesis of LDL receptors, so that it ceases either to make or to take up cholesterol.

This regulated pathway for cholesterol uptake is disrupted in individuals who inherit defective genes encoding LDL receptors. The resulting high levels of blood cholesterol predispose these individuals to develop atherosclerosis

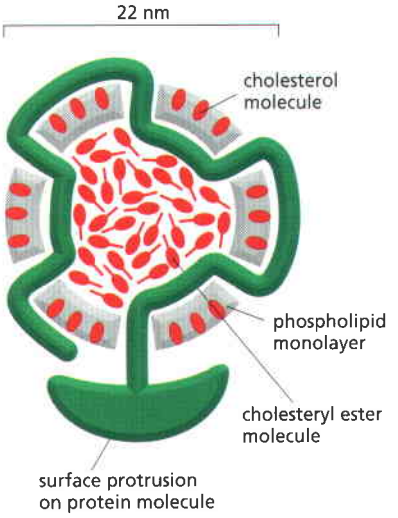


Figure 13–50 A low-density lipoprotein (LDL) particle. Each spherical particle has a mass of 3×10^6 daltons. It contains a core of about 1500 cholesterol molecules esterified to long-chain fatty acids. A lipid monolayer composed of about 800 phospholipid and 500 unesterified cholesterol molecules surrounds the core of cholesterol esters. A single molecule of a 500,000-dalton protein organizes the particle and mediates the specific binding of LDL to cell-surface LDL receptors.

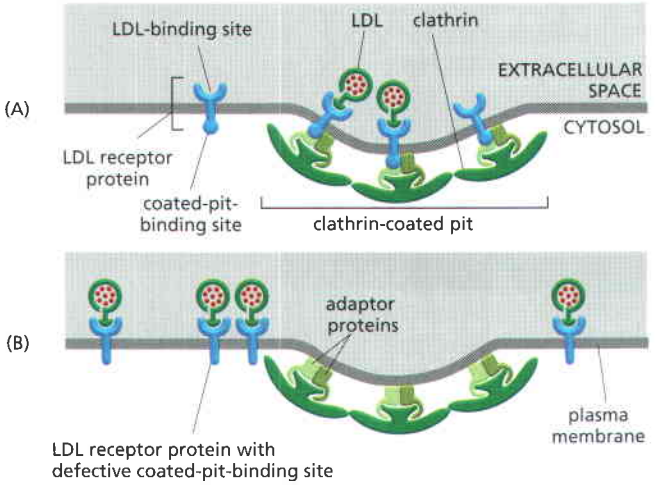


Figure 13–51 Normal and mutant LDL receptors. (A) LDL receptors binding to a coated pit in the plasma membrane of a normal cell. The human LDL receptor is a single-pass transmembrane glycoprotein composed of about 840 amino acids, only 50 of which are on the cytoplasmic side of the membrane. (B) A mutant cell in which the LDL receptors are abnormal and lack the site in the cytoplasmic domain that enables them to bind to adaptor proteins in the clathrin-coated pits. Such cells bind LDL but cannot ingest it. In most human populations, 1 in 500 individuals inherits one defective LDL receptor gene and, as a result, has an increased risk of a heart attack caused by atherosclerosis.

prematurely, and many would die at an early age of heart attacks resulting from coronary artery disease if they were not treated with drugs that lower the level of blood cholesterol. In some cases, the receptor is lacking altogether. In others, the receptors are defective—in either the extracellular binding site for LDL or the intracellular binding site that attaches the receptor to the coat of a clathrin-coated pit (see Figure 13–51B). In the latter case, normal numbers of LDL receptors are present, but they fail to become localized in clathrin-coated pits. Although LDL binds to the surface of these mutant cells, it is not internalized, directly demonstrating the importance of clathrin-coated pits for the receptor-mediated endocytosis of cholesterol.

More than 25 distinct receptors are known to participate in receptor-mediated endocytosis of different types of molecules. They all apparently use clathrin-dependent internalization routes and are guided into clathrin-coated pits by signals in their cytoplasmic tails that bind to adaptor proteins in the clathrin coat. Many of these receptors, like the LDL receptor, enter coated pits irrespective of whether they have bound their specific ligands. Others enter preferentially when bound to a specific ligand, suggesting that a ligand-induced conformational change is required for them to activate the signal sequence that guides them into the pits. Since most plasma membrane proteins fail to become concentrated in clathrin-coated pits, the pits serve as molecular filters, preferentially collecting certain plasma membrane proteins (receptors) over others.

Electron-microscope studies of cultured cells exposed simultaneously to different labeled ligands demonstrate that many kinds of receptors can cluster in the same coated pit, whereas some other receptors cluster in different clathrin-coated pits. The plasma membrane of one clathrin-coated pit can probably accommodate up to 1000 receptors of assorted varieties. Although all of the receptor–ligand complexes that use this endocytic pathway are apparently delivered to the same endosomal compartment, the subsequent fates of the endocytosed molecules vary, as we discuss next.

Endocytosed Materials That Are Not Retrieved from Endosomes End Up in Lysosomes

The endosomal compartments of a cell can be complex. They can be made visible in the electron microscope by adding a readily detectable tracer molecule, such as the enzyme peroxidase, to the extracellular medium and leaving the cells for various lengths of time to take it up by endocytosis. The distribution of the molecule after its uptake reveals the endosomal compartments as a set of heterogeneous, membrane-enclosed tubes extending from the periphery of the cell to the perinuclear region, where it is often close to the Golgi apparatus. Two sequential sets of endosomes can be readily distinguished in such labeling experiments. The tracer molecule appears within a minute or so in **early endosomes**, just beneath the plasma membrane. After 5–15 minutes, it has moved to **late endosomes**, close to the Golgi apparatus and near the nucleus. Early and late endosomes differ in their protein compositions. The transition from early to late endosomes is accompanied by the release of Rab5 and the binding of Rab7, for example.

As mentioned earlier, a vacuolar H^+ ATPase in the endosomal membrane, which pumps H^+ into endosomes from the cytosol, keeps the lumen of the endosomal compartments acidic (pH ~6). In general, later endosomes are more acidic than early endosomes. This gradient of acidic environments has a crucial role in the function of these organelles.

We have already seen how endocytosed materials mix in early endosomes with newly synthesized acid hydrolases and eventually end up being degraded in lysosomes. Many molecules, however, are specifically diverted from this journey to destruction. They are instead recycled from the early endosomes back to the plasma membrane via transport vesicles. Only molecules that are not retrieved from endosomes in this way are delivered to lysosomes for degradation.

Although mild digestion may start in early endosomes, many hydrolases are synthesized and delivered there as proenzymes, called *zymogens*, which contain extra inhibitory domains at their N-terminus that keep the hydrolase inactive until these domains are proteolytically removed. The hydrolases are activated when late endosomes become endolysosomes as the result of fusion with pre-existing lysosomes, which contain a full complement of active hydrolases that digest off the inhibitory domains from the newly synthesized enzymes. Moreover, the pH in early endosomes is not low enough to activate lysosomal hydrolases optimally. By these means, cells can retrieve most membrane proteins from early endosomes and recycle them back to the plasma membrane.

Specific Proteins Are Retrieved from Early Endosomes and Returned to the Plasma Membrane

Early endosomes form a compartment that acts as the main sorting station in the endocytic pathway, just as the *cis* and *trans* Golgi networks serve this function in the biosynthetic–secretory pathway. In the mildly acidic environment of the early endosome, many internalized receptor proteins change their conformation and release their ligand, as already discussed for the M6P receptors. Those endocytosed ligands that dissociate from their receptors in the early endosome are usually doomed to destruction in lysosomes, along with the other soluble contents of the endosome. Some other endocytosed ligands, however, remain bound to their receptors, and thereby share the fate of the receptors.

The fates of receptors—and of any ligands remaining bound to them—vary according to the specific type of receptor. (1) Most receptors are recycled and return to the same plasma membrane domain from which they came; (2) some proceed to a different domain of the plasma membrane, thereby mediating *transcytosis*; and (3) some progress to lysosomes, where they are degraded (**Figure 13–52**).

The LDL receptor follows the first pathway. It dissociates from its ligand, LDL, in the early endosome and is recycled back to the plasma membrane for reuse, leaving the discharged LDL to be carried to lysosomes (**Figure 13–53**). The recycling transport vesicles bud from long, narrow tubules that extend from the

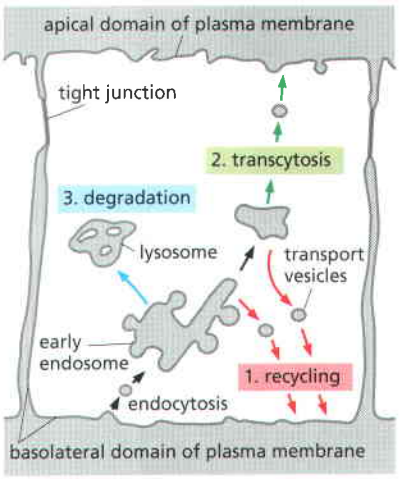


Figure 13–52 Possible fates for transmembrane receptor proteins that have been endocytosed. Three pathways from the endosomal compartment in an epithelial cell are shown. Retrieved receptors are returned (1) to the same plasma membrane domain from which they came *(recycling)* or (2) to a different domain of the plasma membrane *(transcytosis)*. (3) Receptors that are not specifically retrieved from endosomes follow the pathway from the endosomal compartment to lysosomes, where they are degraded *(degradation)*. The formation of oligomeric aggregates in the endosomal membrane may be one of the signals that guide receptors into the degradative pathway. If the ligand that is endocytosed with its receptor stays bound to the receptor in the acidic environment of the endosome, it follows the same pathway as the receptor; otherwise it is delivered to lysosomes.

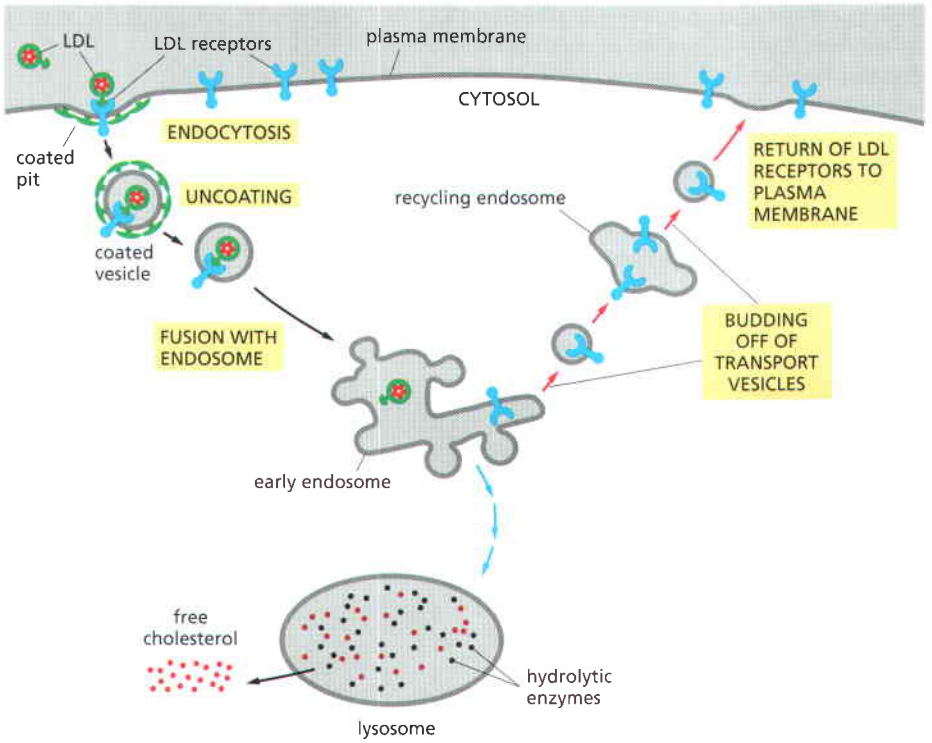


Figure 13–53 The receptor-mediated endocytosis of LDL. <GCTA> Note that the LDL dissociates from its receptors in the acidic environment of the early endosome. After a number of steps (shown in Figure 13–55), the LDL ends up in lysosomes, where it is degraded to release free cholesterol. In contrast, the LDL receptors are returned to the plasma membrane via clathrin-coated transport vesicles that bud off from the tubular region of the early endosome, as shown. For simplicity, only one LDL receptor is shown entering the cell and returning to the plasma membrane. Whether it is occupied or not, an LDL receptor typically makes one round trip into the cell and back to the plasma membrane every 10 minutes, making a total of several hundred trips in its 20-hour lifespan.

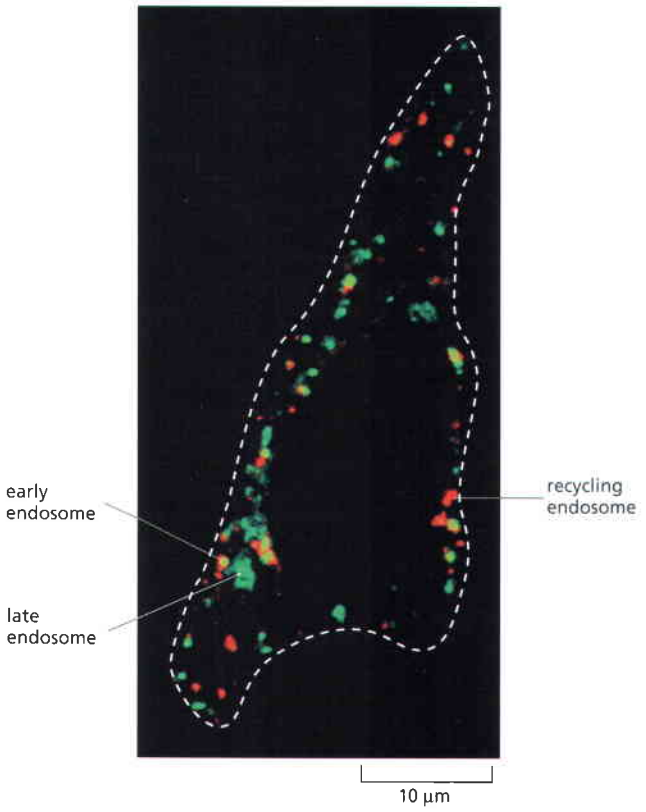


Figure 13–54 Sorting of membrane proteins in the endocytic pathway. Transferrin receptors mediate iron uptake and constitutively cycle between endosomes and the plasma membrane. By contrast, activated opioid receptors are down-regulated by endocytosis followed by degradation in lysosomes; they are activated by opiates such as morphine and heroin, as well as by endogenous peptides called enkephalins and endorphins. Endocytosis of both types of receptors starts in clathrin-coated pits. The receptors are then delivered to early endosomes, where their pathways part: transferrin receptors are sorted to the recycling endosomes, whereas opioid receptors are sorted to late endosomes. The micrograph shows both receptors—labeled with different fluorescent dyes—30 min after endocytosis (transferrin receptors are labeled in *red* and opioid receptors in *green*). At this time, some early endosomes still contain both receptors and are seen as *yellow*, due to the overlap of red and green light emitted from the fluorescent dyes. By contrast, recycling endosomes and late endosomes are selectively enriched in either transferrin or opioid receptors, respectively, and therefore appear as distinct red and green structures. (Courtesy of Mark von Zastrow.)

early endosomes. It is likely that the geometry of these tubules helps the sorting process: because tubules have a large membrane area enclosing a small volume, membrane proteins become enriched over soluble proteins. Transport vesicles that return material to the plasma membrane begin budding from the tubules, but tubular portions of the early endosome also pinch off and fuse with one another to form *recycling endosomes*, which serve as way-stations for the traffic between early endosomes and the plasma membrane. This recycling pathway operates continuously, compensating for the continuous endocytosis occurring at the plasma membrane.

The **transferrin receptor** follows a similar recycling pathway as the LDL receptor, but unlike the LDL receptor it also recycles its ligand. Transferrin is a soluble protein that carries iron in the blood. Cell-surface transferrin receptors deliver transferrin with its bound iron to early endosomes by receptor-mediated endocytosis. The low pH in the endosome induces transferrin to release its bound iron, but the iron-free transferrin itself (called apotransferrin) remains bound to its receptor. The receptor–apotransferrin complex enters the tubular extensions of the early endosome and from there is recycled back to the plasma membrane (**Figure 13–54**). When the apotransferrin returns to the neutral pH of the extracellular fluid, it dissociates from the receptor and is thereby freed to pick up more iron and begin the cycle again. Thus, transferrin shuttles back and forth between the extracellular fluid and the endosomal compartment, avoiding lysosomes and delivering iron to the cell interior, as needed for cells to grow and proliferate.

The second pathway that endocytosed receptors can follow from endosomes is taken by many signaling receptors, including opioid receptors (see Figure 13–54) and the receptor that binds *epidermal growth factor (EGF)*. EGF is a small, extracellular signal protein that stimulates epidermal and various other cells to divide. Unlike LDL receptors, EGF receptors accumulate in clathrin-coated pits only after binding EGF, and most of them do not recycle but are degraded in lysosomes, along with the ingested EGF. EGF binding therefore first activates intracellular signaling pathways and then leads to a decrease in the concentration of EGF receptors on the cell surface, a process called *receptor down-regulation* that reduces the cell's subsequent sensitivity to EGF (see Figure 15–29).