CHAPTER 13

Intracellular Membrane 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 and internal compartments in rapid response to need. They use an elaborate internal membrane system to add and remove cell-surface proteins, such as receptors, ion channels, and transporters (**Figure 13–1**). Through the process of *exocytosis*, the secretory pathway delivers newly synthesized proteins, carbohydrates, and lipids either to the plasma membrane or the extracellular space. By the converse process of *endocytosis*, 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 be delivered to lysosomes for degradation. Cells also use endocytosis to capture important nutrients, such as vitamins, cholesterol, and iron; these are taken up together with the macromolecules to which they bind and are then moved on to endosomes and lysosomes, from where they can be transported into the cytosol for use in various biosynthetic processes.

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

Within a eukaryotic cell, transport vesicles continually bud off from one membrane and fuse with another, carrying membrane components and soluble lumenal molecules, which are referred to as **cargo** (**Figure 13–2**). This vesicular traffic flows along highly organized, directional routes, which allow the cell to secrete, eat, and remodel its plasma membrane and organelles. The *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, *retrieval pathways*

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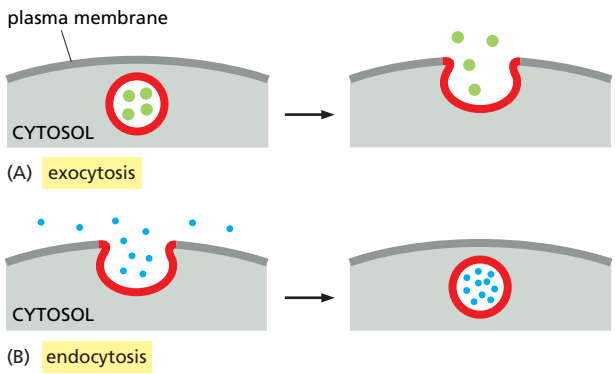


Figure 13–1 Exocytosis and endocytosis. (A) In exocytosis, a transport vesicle fuses with the plasma membrane. Its content is released into the extracellular space, while the vesicle membrane *(red)* becomes continuous with the plasma membrane. (B) In endocytosis, a plasma membrane patch *(red)* is internalized,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

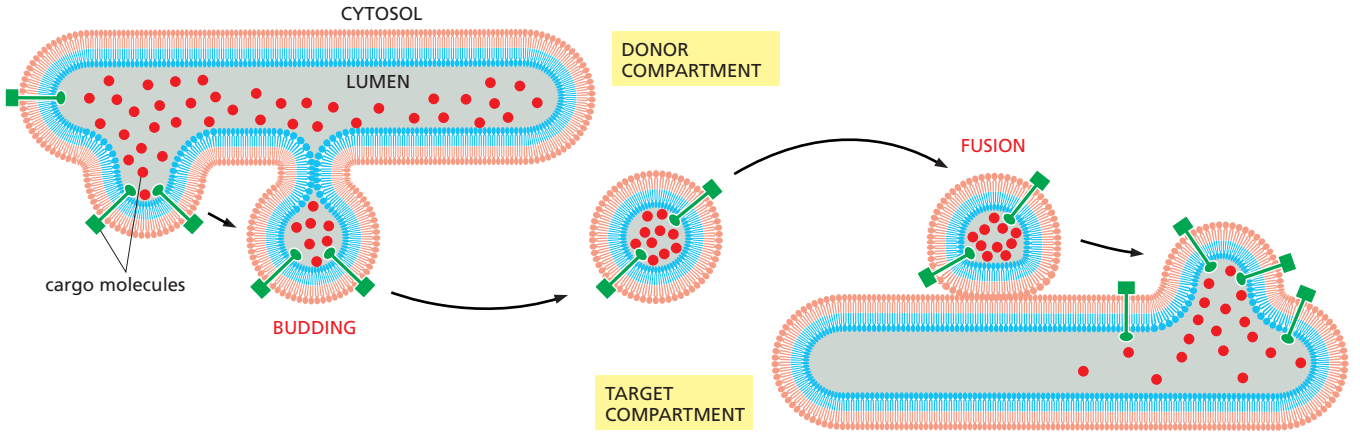


Figure 13–2 Vesicle transport. Transport vesicles bud off from one compartment and fuse with another. As they do so, they carry material as cargo from the *lumen* (the space within a membrane-enclosed compartment) and membrane of the donor compartment to the lumen and membrane of the target compartment, as shown.

balance the flow of membrane between compartments 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 ER to the Golgi apparatus, for example, must exclude most proteins that are to stay in the ER, and it must fuse only with the Golgi apparatus and not with any other organelle.

We begin this chapter by considering the molecular mechanisms of budding and fusion that underlie all vesicle transport. We then discuss the fundamental problem of how, in the face of this transport, the cell maintains the molecular and

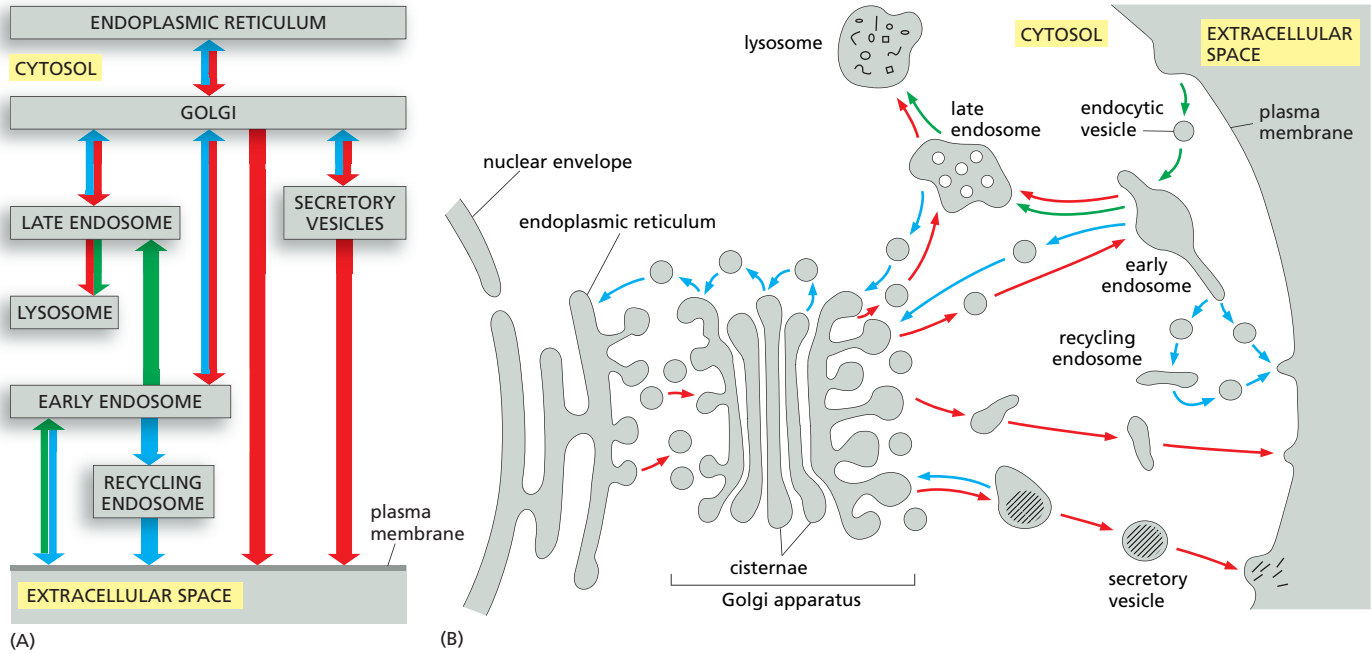


Figure 13–3 A "road-map" of the secretory and endocytic pathways. (A) In this schematic roadmap, which was introduced in Chapter 12, the endocytic and secretory pathways are illustrated with *green* and *red arrows*, respectively. In addition, *blue arrows* denote retrieval pathways for the backflow of selected components. (B) The compartments of the eukaryotic cell involved in vesicle 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 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 endocytic 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 (some via recycling endosomes) 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).

functional differences between its compartments. Finally, we consider the function of the Golgi apparatus, lysosomes, secretory vesicles, and endosomes, as we trace the pathways that connect these organelles.

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

Vesicle transport mediates a continuous exchange of components between the ten or more chemically distinct, membrane-enclosed compartments that collectively comprise the 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 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.

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 used to extract specific cargo components from a membrane and compartment lumen for delivery to another compartment. Finally, we discuss how transport vesicles dock at the appropriate target membrane and then 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 that are reflected in a common two-layered structure. First, an inner coat layer concentrates specific membrane proteins in a specialized patch, which then gives rise to the vesicle membrane. In this way, the inner layer selects the appropriate membrane molecules for transport. Second, an outer coat layer assembles into a curved, basketlike lattice that deforms the membrane patch and thereby shapes the vesicle.

There are three well-characterized types of coated vesicles, distinguished by their major 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, 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

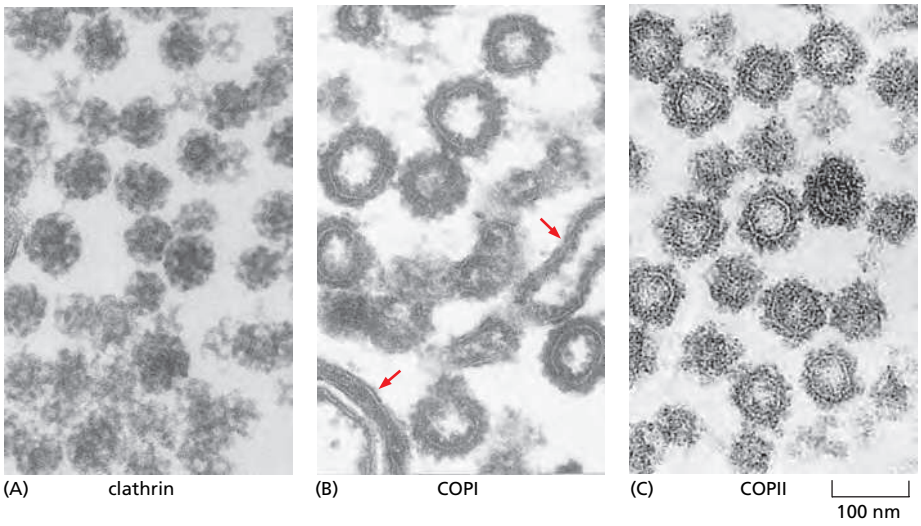


Figure 13–4 Electron micrographs of clathrin-coated, COPI-coated, and COPII-coated vesicles. All are shown in electron micrographs at the same scale. (A Clathrin-coated vesicles. (B) COPI-coated vesicles and Golgi cisternae *(red arrows)* from a cell-free system in which COPI-coated vesicles bud in the 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.)

in the secretory pathway: COPI-coated vesicles bud from Golgi compartments, and COPII-coated vesicles bud from the ER (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, which forms the outer layer of the coat. Each clathrin subunit consists of three large and three small polypeptide chains that together form a three-legged structure called a *triskelion* (**Figure 13–6**A,B). Clathrin triskelions assemble into a basketlike framework of hexagons and pentagons to form coated pits (buds) on the cytosolic surface of membranes (**Figure 13–7**). 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 13–6C,D). Thus, the clathrin triskelions determine the geometry of the clathrin cage (Figure 13–6E).

Adaptor Proteins Select Cargo into Clathrin-Coated Vesicles

Adaptor proteins, another major coat component in clathrin-coated vesicles, form a discrete inner layer of the coat, positioned between the clathrin cage and the membrane. They bind the clathrin coat to the membrane and trap various transmembrane proteins, including transmembrane receptors that capture soluble cargo molecules inside the vesicle—so-called *cargo receptors*. In this way, the adaptor proteins select a specific set of transmembrane proteins, together with the soluble proteins that interact with them, and package them into each newly formed clathrin-coated transport vesicle (**Figure 13–8**).

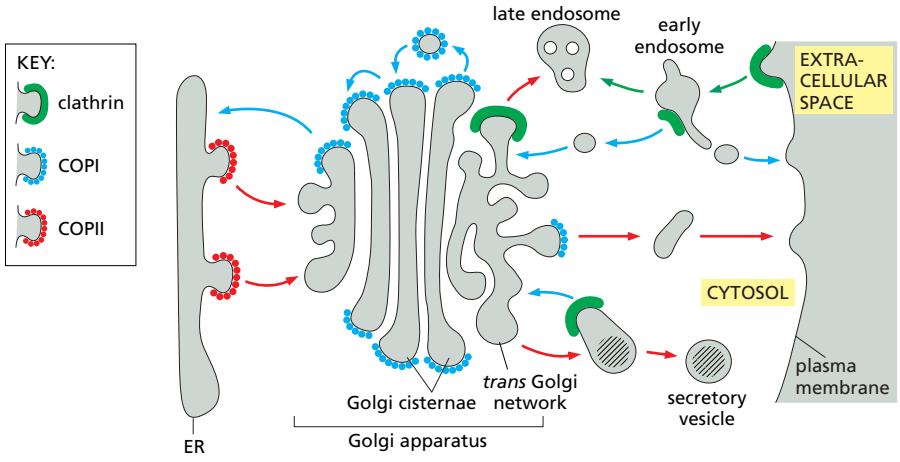


Figure 13–5 Use of different coats for different steps in vesicle traffic. Different coat proteins select different cargo and shape the transport vesicles that mediate the various steps in the secretory and endocytic pathways. When the same coats function in different places in the cell, they usually 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 nerve terminals of neurons (see Figure 11–36). The arrows are colored as in Figure 13–3.

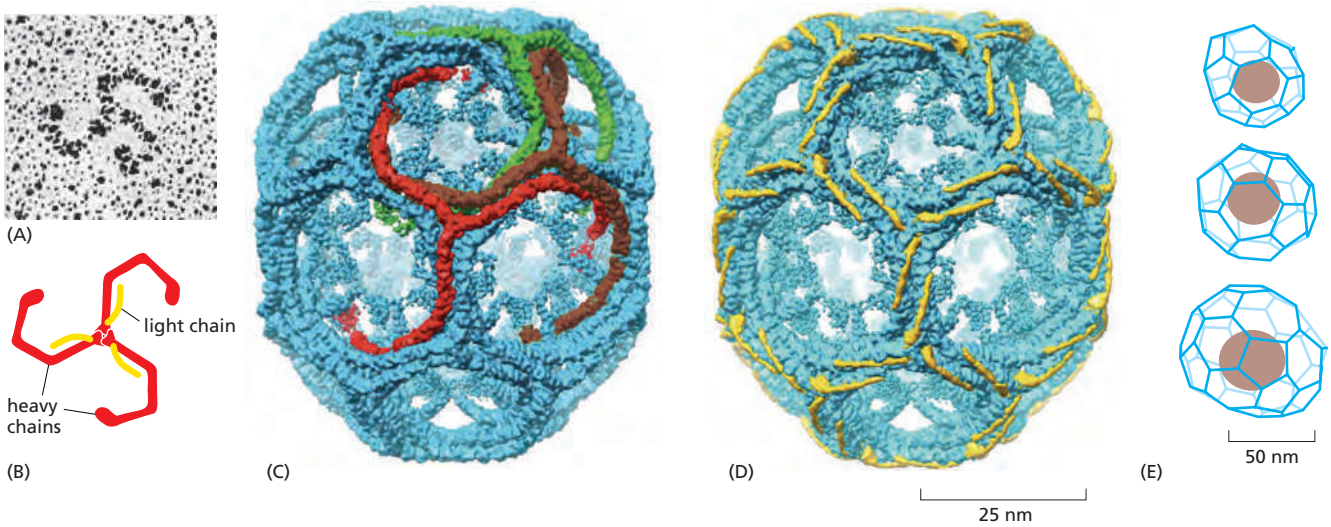


Figure 13–6 The structure of a clathrin coat. (A) Electron micrograph of a clathrin triskelion shadowed with platinum. (B) Each triskelion is composed of three clathrin heavy chains and three clathrin light chains, as shown in the diagram. (C and D) A cryoelectron micrograph taken of a clathrin coat composed of 36 triskelions organized in a network of 12 pentagons and 6 hexagons, with some heavy chains (C) and light chains (D) highlighted (**Movie 13.1**). The light chains link to the actin cytoskeleton, which helps generate force for membrane budding and vesicle movement, and their phosphorylation regulates clathrin coat assembly. The interwoven legs of the clathrin triskelions form an outer shell from which the N-terminal domains of the triskelions protrude inward. These domains bind to the adaptor proteins shown in Figure 13–8. The coat shown was assembled biochemically from pure clathrin triskelions and is too small to enclose a membrane vesicle. (E) Images of clathrin-coated vesicles isolated from bovine brain. The clathrin coats are constructed in a similar but less regular way, from pentagons, a larger number of hexagons, and sometime heptagons, resembling the architecture of deformed soccer balls. The structures were determined by cryoelectron microscopy and tomographic reconstruction. (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; E, from Y. Cheng et al., *J. Mol. Biol*. 365:892–899, 2007. With permission from Elsevier.)

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. Clathrin-coated vesicles budding from different membranes use different adaptor proteins and thus package different receptors and cargo molecules.

The assembly of adaptor proteins on the membrane is tightly controlled, in part by the cooperative interaction of the adaptor proteins with other components of the coat. The adaptor protein AP2 serves as a well-understood example. When it binds to a specific phosphorylated phosphatidylinositol lipid (a *phosphoinositide*), it alters its conformation, exposing binding sites for cargo receptors in the membrane. The simultaneous binding to the cargo receptors and lipid head groups greatly enhances the binding of AP2 to the membrane (**Figure 13–9**).

Because several requirements must be met simultaneously to stably bind AP2 proteins to a membrane, the proteins act as *coincidence detectors* that only assemble at the right time and place. Upon binding, they induce membrane curvature, which makes the binding of additional AP2 proteins in its proximity more likely. The cooperative assembly of the AP2 coat layer then is further amplified by clathrin binding, which leads to the formation and budding of a transport vesicle.

Adaptor proteins found in other coats also bind to phosphoinositides, which not only have a major role in directing when and where coats assemble in the cell, but also are used much more widely as molecular markers of compartment identity. This helps to control vesicular traffic, as we now discuss.

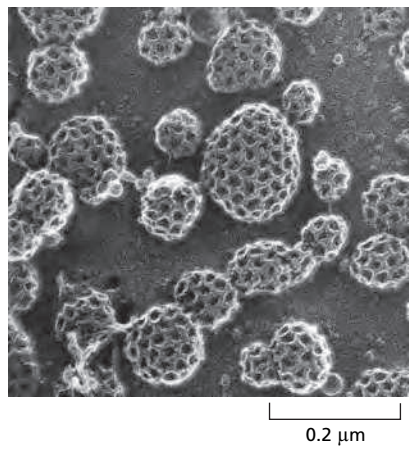


Figure 13–7 Clathrin-coated pits and vesicles. This rapid-freeze, deep-etch electron micrograph shows numerous clathrin-coated pits and vesicles on the inner surface of the plasma membrane of cultured fibroblasts. The cells were rapidly frozen in liquid helium, fractured, and deep-etched to expose the cytoplasmic surface of the plasma membrane. (Courtesy of John Heuser.)

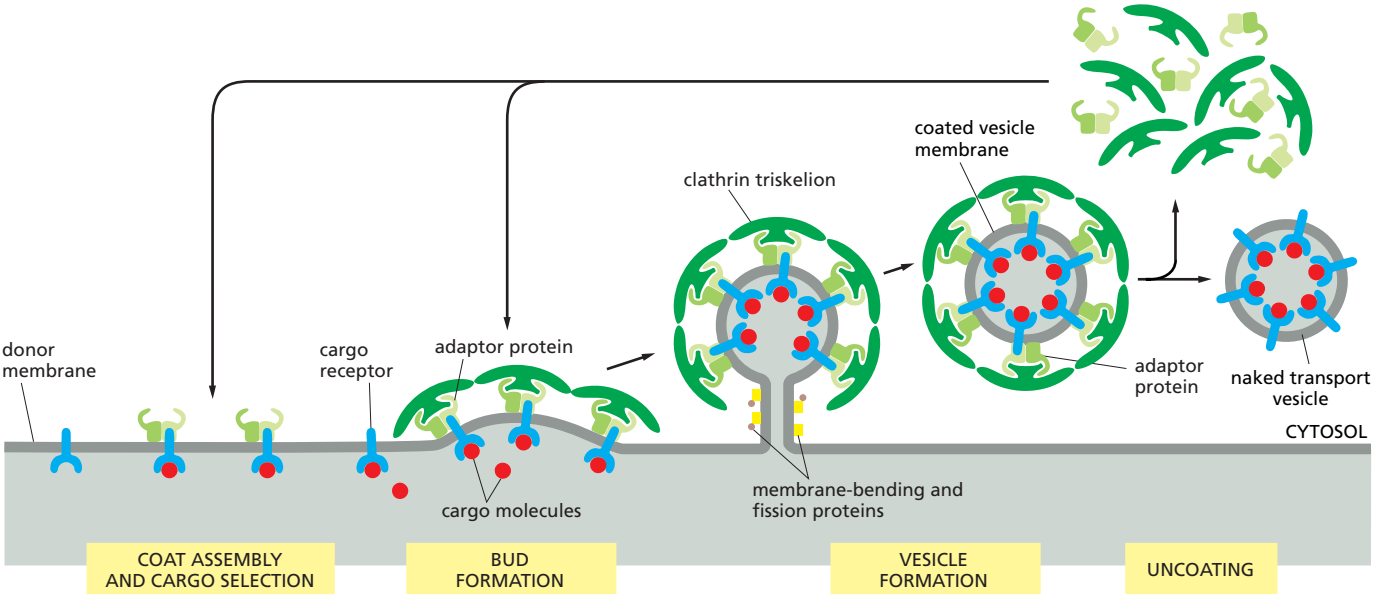


Figure 13–8 The assembly and disassembly of a clathrin coat. The assembly of the coat introduces curvature into the membrane, which leads in turn to the formation of a coated bud (called a coated pit if it is in the plasma membrane). The adaptor proteins bind both clathrin triskelions and membrane-bound cargo receptors, thereby mediating the selective recruitment of both membrane and soluble cargo molecules into the vesicle. Other membrane-bending and fission proteins are recruited to the neck of the budding vesicle, where sharp membrane curvature is introduced. The coat is rapidly lost shortly after the vesicle buds off.

Phosphoinositides Mark Organelles and Membrane Domains

Although inositol phospholipids typically comprise less than 10% of the total phospholipids in a membrane, they have important regulatory functions. They can undergo rapid cycles of phosphorylation and dephosphorylation at the 3′, 4′, and 5′ positions of their inositol sugar head groups to produce various types of **phosphoinositides** (**phosphatidylinositol phosphates**, or **PIPs**). The interconversion of phosphatidylinositol (PI) and PIPs is highly compartmentalized: different organelles in the endocytic and secretory pathways have distinct sets of PI and PIP kinases and PIP phosphatases (**Figure 13–10**). The distribution, regulation, and local balance of these enzymes determine the steady-state distribution of each PIP species. As a consequence, the distribution of PIPs varies from organelle to organelle, and often within a continuous membrane from one region to another, thereby defining specialized membrane domains.

Many proteins involved at different steps in vesicle transport contain domains that bind with high specificity to the head groups of particular PIPs, distinguishing one phosphorylated form from another (see Figure 13–10 E and F). Local control of the PI and PIP kinases and PIP phosphatases can therefore be used to rapidly control the binding of proteins to a membrane or membrane domain. The production of a particular type of PIP recruits proteins containing matching PIP-binding domains. The PIP-binding proteins then help regulate vesicle formation and other steps in the control of vesicle traffic (**Figure 13–11**). The same strategy is widely used to recruit specific intracellular signaling proteins to the plasma membrane in response to extracellular signals (discussed in Chapter 15).

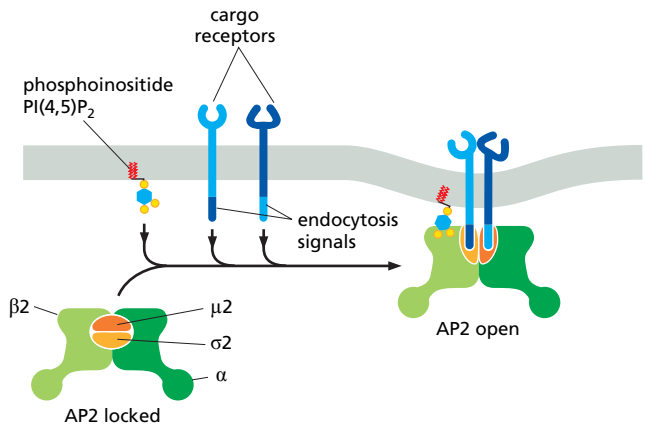


Figure 13–9 Lipid-induced conformation switching of AP2. The AP2 adaptor protein complex has four subunits (α, β2, μ2, and σ2). Upon interaction with the phosphoinositide PI(4,5)P_2 (see Figure 13–10) in the cytosolic leaflet of the plasma membrane, AP2 rearranges so that binding sites for cargo receptors become exposed. Each AP2 complex binds four PI(4,5)P_2 molecules (for clarity, only one is shown). In the open AP2 complex, the μ2 and σ2 subunits bind the cytosolic tails of cargo receptors that display the appropriate endocytosis signals. These signals consist of short amino acid sequence motifs. When AP2 binds tightly to the membrane, it induces curvature, which favors the binding of additional AP2 complexes in the vicinity.

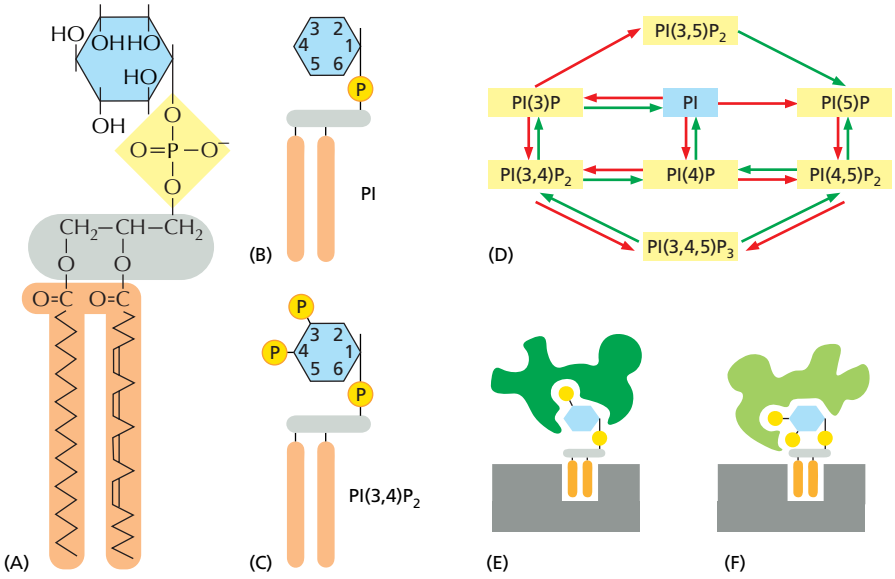


Figure 13–10 Phosphatidylinositol (PI) and phosphoinositides (PIPs). (A, B) The structure of PI shows the free hydroxyl groups in the inositol sugar that can in principle be modified. (C) Phosphorylation of one, two, or three of the hydroxyl groups on PI by PI and PIP kinases produces a variety of PIP species. They are named according to the ring position (in parentheses) and the number of phosphate groups (subscript) added to PI. $PI(3,4)P_2$ is shown. (D) Animal cells have several PI and PIP kinases and a similar number of PIP phosphatases, which are localized to different organelles, where they are regulated to catalyze the production of particular PIPs. The *red* and *green arrows* show the kinase and phosphatase reactions, respectively. (E, F) Phosphoinositide head groups are recognized by protein domains that discriminate between the different forms. In this way, select groups of proteins containing such domains are recruited to regions of membrane in which these phosphoinositides are present. PI(3)P and $PI(4,5)P_2$ are shown. (D, modified from M.A. de Matteis and A. Godi, *Nat. Cell Biol.* 6:487–492, 2004. With permission from Macmillan Publishers Ltd.)

Membrane-Bending Proteins Help Deform the Membrane During Vesicle Formation

The forces generated by clathrin coat assembly alone are not sufficient to shape and pinch off a vesicle from the membrane. Other membrane-bending and force-generating proteins participate at every stage of the process. Membrane-bending proteins that contain crescent-shaped domains, called *BAR domains,* bind to and impose their shape on the underlying membrane via electrostatic interactions with the lipid head groups (**Figure 13–12**; also see Figure 10–40). Such BAR-domain proteins are thought to help AP2 nucleate clathrin-mediated endocytosis by shaping the plasma membrane to allow a clathrin-coated bud to form. Some of these proteins also contain amphiphilic helices that induce membrane curvature after being inserted as wedges into the cytoplasmic leaflet of the membrane. Other BAR-domain proteins are important in shaping the neck of a budding vesicle, where stabilization of sharp membrane bends is essential. Finally, the clathrin machinery nucleates the local assembly of actin filaments that introduce tension to help pinch off and propel the forming vesicle away from the membrane.

Cytoplasmic Proteins Regulate the Pinching-Off and Uncoating of Coated Vesicles

As a clathrin-coated bud grows, soluble cytoplasmic proteins, including **dynamin**, assemble at the neck of each bud (**Figure 13–13**). Dynamin contains a PI(4,5)P_2-binding domain, which tethers the protein to the membrane, and a GTPase domain, which regulates the rate at which vesicles pinch off from the membrane.

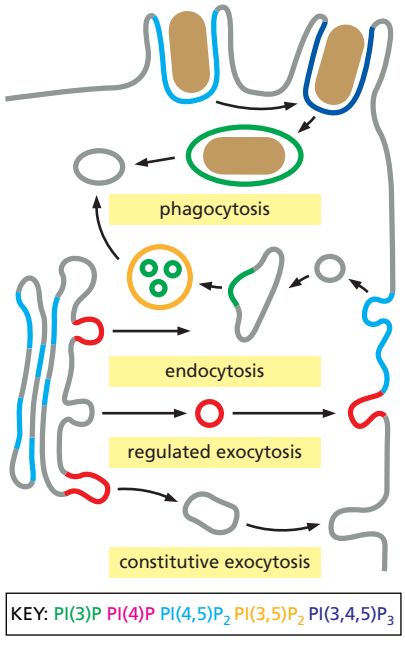


Figure 13–11 The intracellular location of phosphoinositides. Different types of PIPs are located in different membranes and membrane domains, where they are often associated with specific vesicle transport events. The membrane of secretory vesicles, for example, contains PI(4)P. When the vesicles fuse with the plasma membrane, a PI 5-kinase that is localized there converts the PI(4)P into $PI(4,5)P_2$. The $PI(4,5)P_2$, in turn, helps recruit adaptor proteins, which initiate the formation of a clathrin-coated pit, as the first step in clathrin-mediated endocytosis. Once the clathrin-coated vesicle buds off from the plasma membrane, a PI(5)P phosphatase hydrolyzes $PI(4,5)P_2$, which weakens the binding of the adaptor proteins, promoting vesicle uncoating. We discuss phagocytosis and the distinction between regulated and constitutive exocytosis later in the chapter. (Modified from M.A. de Matteis and A. Godi, *Nat. Cell Biol.* 6:487–492, 2004. With permission from Macmillan Publishers Ltd.)

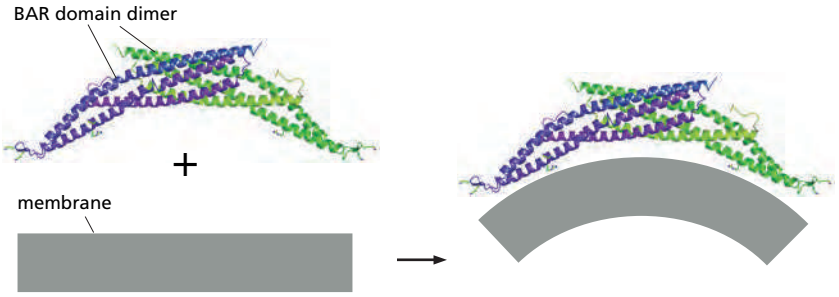


Figure 13–12 The structure of BAR domains. BAR-domain proteins are diverse and enable many membrane-bending processes in the cell. BAR domains are built from coiled coils that dimerize into modules with a positively charged inner surface, which preferentially interacts with negatively charged lipid head groups to bend membranes. Local membrane deformations caused by BAR-domain proteins facilitate the binding of additional BAR-domain proteins, thereby generating a positive feedback cycle for curvature propagation. Individual BAR-domain proteins contain a distinctive curvature and often have additional features that adapt them to their specific tasks: some have short amphiphilic helices that cause further membrane deformation by wedge insertion; others are flanked by PIP-binding domains that direct them to membranes enriched in cognate phosphoinositides.

The pinching-off process brings the two noncytosolic leaflets of the membrane into close proximity and fuses them, sealing off the forming vesicle (see Figure 13–2). To perform this task, dynamin recruits other proteins to the neck of the bud. Together with dynamin, they help bend the patch of membrane—by directly distorting the bilayer structure, or by changing its lipid composition through the recruitment of lipid-modifying enzymes, or by both mechanisms.

Once released from the membrane, the vesicle rapidly loses its clathrin coat. A PIP phosphatase that is co-packaged into clathrin-coated vesicles depletes PI(4,5)P_2 from the membrane, which weakens the binding of the adaptor proteins. In addition, an hsp70 chaperone protein (see Figure 6–80) functions as an uncoating ATPase, using the energy of ATP hydrolysis to peel off the clathrin coat. *Auxilin*, another vesicle protein, is thought to activate the ATPase. The release of the coat, however, must not happen prematurely, so additional control mechanisms must somehow prevent the clathrin from being removed before it has formed a complete vesicle (discussed below).

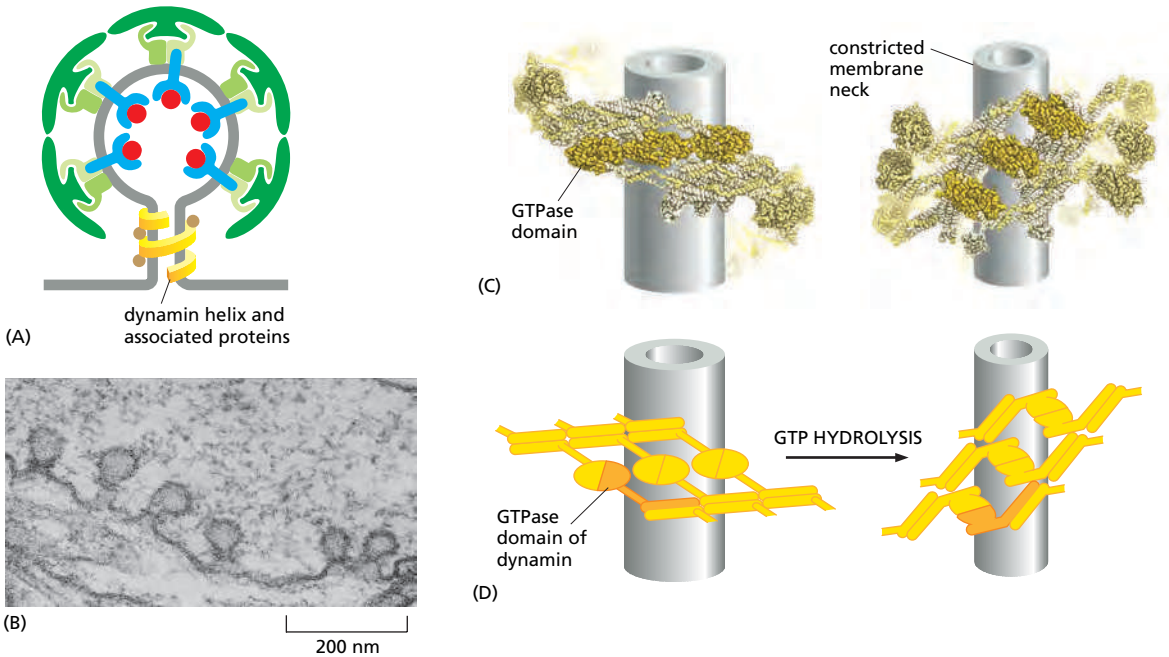


Figure 13–13 The role of dynamin in pinching off clathrin-coated vesicles. (A) Multiple dynamin molecules assemble into a spiral around the neck of the forming bud. The dynamin spiral is thought to recruit other proteins to the bud neck, which, together with dynamin, destabilize the interacting lipid bilayers so that the noncytoplasmic leaflets flow together. The newly formed vesicle then pinches off from the membrane. Specific mutations in dynamin can either enhance or block the pinching-off process. (B) Dynamin was discovered as the protein defective in the *shibire* mutant of *Drosophila.* These mutant flies become paralyzed because clathrin-mediated endocytosis stops, and the synaptic vesicle membrane fails to recycle, blocking neurotransmitter release. Deeply invaginated clathrin-coated pits form in the nerve endings of the fly's nerve cells, with a belt of mutant dynamin assembled around the neck, as shown in this thin-section electron micrograph. The pinching-off process fails because the required membrane fusion does not take place. (C, D) A model of how conformational changes in the GTPase domains of membrane-assembled dynamin may power a conformational change that constricts the neck of the bud. A single dynamin molecule is shown in *orange* in D. (B, from J.H. Koenig and K. Ikeda, *J. Neurosci.* 9:3844–3860, 1989. With permission from the Society of Neuroscience; C and D, adapted from M.G.J. Ford, S. Jenni and J. Nunnari, *Nature* 477:561–566, 2011. With permission from Macmillan Publishers.)

Monomeric GTPases Control Coat Assembly

To balance the vesicle traffic to and from a compartment, coat proteins must assemble only when and where they are needed. While local production of PIPs plays a major part in regulating the assembly of clathrin coats on the plasma membrane and Golgi apparatus, cells superimpose additional ways of regulating coat formation. *Coat-recruitment GTPases*, for example, control the assembly of clathrin coats on endosomes and the COPI and COPII coats on Golgi and ER membranes.

Many steps in vesicle transport depend on a variety of GTP-binding proteins that control both the spatial and temporal aspects of vesicle formation and fusion. As discussed in Chapter 3, GTP-binding proteins regulate most processes in eukaryotic cells. They act as molecular switches, which flip between an active state with GTP bound and an inactive state with GDP bound. Two classes of proteins regulate the flipping: *guanine nucleotide exchange factors* (*GEFs*) activate the proteins by catalyzing the exchange of GDP for GTP, and *GTPase-activating proteins* (*GAPs*) inactivate the proteins by triggering the hydrolysis of the bound GTP to GDP (see Figures 3–68 and 15–7). Although both monomeric GTP-binding proteins (monomeric GTPases) and trimeric GTP-binding proteins (G proteins) have important roles in vesicle transport, the roles of the monomeric GTPases are better understood, and we focus on them here.

Coat-recruitment GTPases are members of a family of monomeric GTPases. They include the **ARF proteins**, which are responsible for the assembly of both COPI and clathrin coats assembly at Golgi membranes, and the **Sar1 protein**, which is responsible for the assembly of COPII coats at the ER membrane. Coat-recruitment GTPases are usually found in high concentration in the cytosol in an inactive, GDP-bound state. When a COPII-coated vesicle is to bud from the ER membrane, for example, a specific Sar1-GEF embedded in the ER membrane binds to cytosolic Sar1, causing the Sar1 to release its GDP and bind GTP in its place. (Recall that GTP is present in much higher concentration in the cytosol than GDP and therefore will spontaneously bind after GDP is released.) In its GTP-bound state, the Sar1 protein exposes an amphiphilic helix, which inserts into the cytoplasmic leaflet of the lipid bilayer of the ER membrane. The tightly bound Sar1 now recruits adaptor coat protein subunits to the ER membrane to initiate budding (**Figure 13–14**). Other GEFs and coat-recruitment GTPases operate in a similar way on other membranes.

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, it has been proposed that the GTPases work like timers, which hydrolyze GTP at slow but predictable rates, to ensure that vesicle formation is synchronized with the requirements of the moment. COPII coats accelerate GTP hydrolysis by Sar1, and 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, perhaps at a more appropriate time and place. Once a vesicle pinches off, GTP hydrolysis releases Sar1, but the sealed coat is sufficiently stabilized through many cooperative interactions, including binding to the cargo receptors in the membrane, that it may stay on the vesicle until the vesicle docks at a target membrane. There, a kinase phosphorylates the coat proteins, which completes coat disassembly and readies the vesicle for fusion.

Clathrin- and COPI-coated vesicles, by contrast, shed their coat soon after they pinch off. For COPI vesicles, the curvature of the vesicle membrane serves as a trigger to begin uncoating. An ARF-GAP is recruited to the COPI coat as it assembles. It interacts with the membrane, and senses the lipid packing density. It becomes activated when the curvature of the membrane approaches that of a transport vesicle. It then inactivates ARF, causing the coat to disassemble.

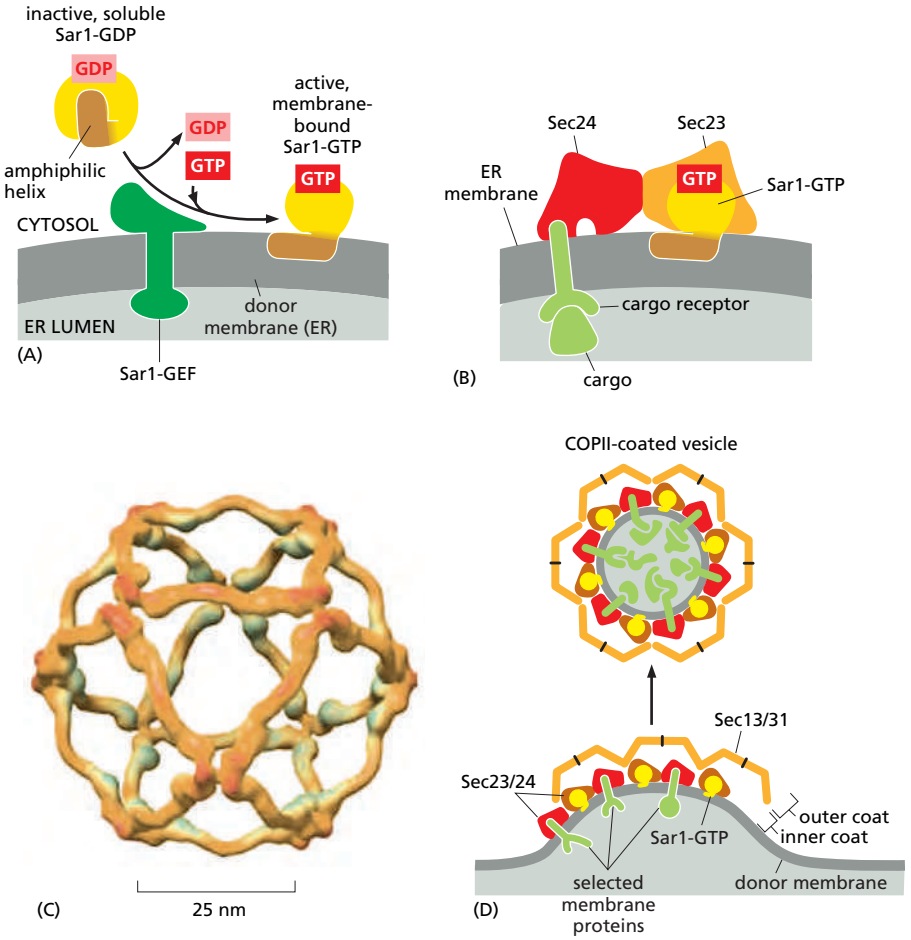


Figure 13–14 Formation of a COPII-coated vesicle. (A) Inactive, soluble Sar1-GDP binds to a Sar1-GEF in the ER membrane, causing the Sar1 to release its GDP and bind GTP. A GTP-triggered conformational change in Sar1 exposes an amphiphilic helix, which inserts into the cytoplasmic leaflet of the ER membrane, initiating membrane bending (which is not shown). (B) GTP-bound Sar1 binds to a complex of two COPII adaptor coat proteins, called Sec23 and Sec24, which form the inner coat. Sec24 has several different binding sites for the cytosolic tails of cargo receptors. The entire surface of the complex that attaches to the membrane is gently curved, matching the diameter of COPII-coated vesicles. (C) A complex of two additional COPII coat proteins, called Sec13 and Sec 31, forms the outer shell of the coat. Like clathrin, they can assemble on their own into symmetrical cages with appropriate dimensions to enclose a COPII-coated vesicle. (D) Membrane-bound, active Sar1-GTP recruits COPII adaptor proteins to the membrane. They select certain transmembrane proteins and cause the membrane to deform. The adaptor proteins then recruit the outer coat proteins which help form a bud. A subsequent membrane fusion event pinches off the coated vesicle. Other coated vesicles are thought to form in a similar way. (C, modified from S.M. Stagg et al., *Nature* 439:234–238, 2006. With permission from Macmillan Publishers Ltd.)

Not All Transport Vesicles Are Spherical

Although vesicle-budding is similar at various locations in the cell, 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 actin-rich cortex. Thus, the coordinated action of clathrin coats and membrane-bending proteins has to produce sufficient force to introduce curvature, especially at the neck of the bud where sharp bends are required for the pinching-off processes. 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 also occur in various sizes and shapes. Diverse COPII vesicles are required for the transport of large cargo molecules. Collagen, for example, is assembled in the ER as 300-nm-long, stiff procollagen rods that then are secreted from the cell where they are cleaved by proteases to collagen, which is embedded into the extracellular matrix (discussed in Chapter 19). Procollagen rods do not fit into the 60–80 nm COPII vesicles normally observed. To circumvent this problem, the procollagen cargo molecules bind to transmembrane *packaging proteins* in the ER, which control the assembly of the COPII coat components (**Figure 13–15**). These events drive the local assembly of much larger COPII vesicles that accommodate the oversized cargo. Human mutations in genes encoding such packaging proteins result in collagen defects with severe consequences, such as skeletal abnormalities and other developmental defects. Similar mechanisms must regulate the sizes of vesicles required to secrete other large macromolecular complexes, including the lipoprotein particles that transport lipids out of cells.

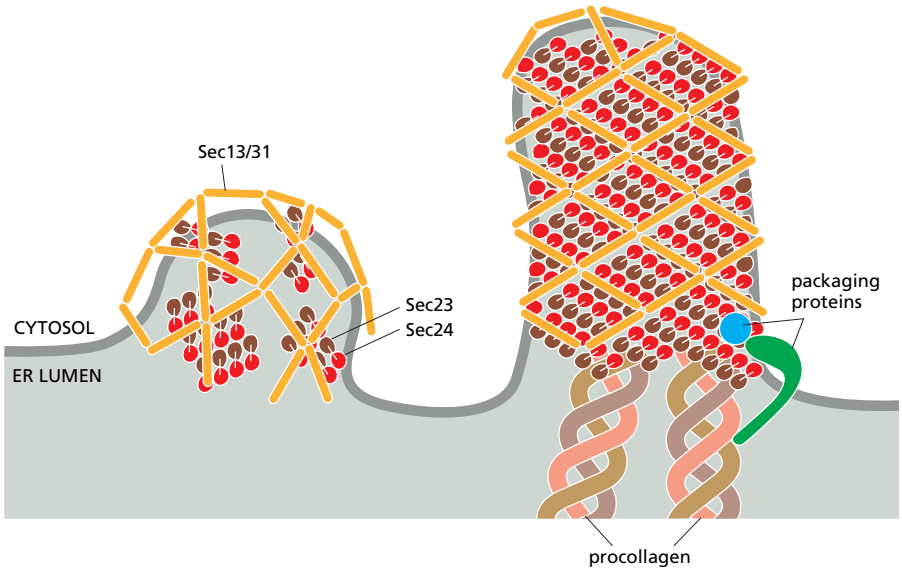


Figure 13–15 Packaging of procollagen into large tubular COPII-coated vesicles. The cartoons show models for two COPII coat assembly modes. The models are based on cryoelectron tomography images of reconstituted COPII vesicles. On a spherical membrane (left), the Sec23/24 inner coat proteins assemble in patches that anchor the Sec13/31 outer coat protein cage. The Sec13/31 rods assemble a cage of triangles, squares, and pentagons. When procollagen needs to be packaged (right), special packaging proteins sense the cargo and modify the coat assembly process. This interaction recruits the COPII inner coat protein Sec24 and locally enhances the rate with which Sar1 cycles on and off the membrane (not shown). In addition, a monoubiquitin is added to the Sec31 protein, changing the assembly properties of the outer cage. Sec23/24 proteins arrange in larger arrays and Sec13/31 arrange in a regular lattice of diamond shapes. As the result, a large tubular vesicle is formed that can accommodate the large cargo molecules. The packaging proteins are not part of the budding vesicle but remain in the ER. (Modified from G. Zanetti et al., *eLife* 2:e00951, 2013.)

Many other vesicle budding events likewise involve variations of common mechanisms. 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 membrane 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 of different sizes and shapes.

Tubules have a higher surface-to-volume ratio than the larger 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 an important feature for sorting proteins in endosomes.

Rab Proteins Guide Transport Vesicles to Their Target Membrane

To ensure an orderly flow of vesicle traffic, transport vesicles must be highly accurate 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 occurs in two steps. First, *Rab proteins* and *Rab effectors* direct the vesicle to specific spots on the correct target membrane. Second, *SNARE proteins* and *SNARE regulators* mediate the fusion of the lipid bilayers.

Rab proteins play a central part in the specificity of vesicle transport. Like the coat-recruitment GTPases discussed earlier (see Figure 13–14), Rab proteins are also monomeric GTPases. With over 60 known members, the Rab subfamily is the largest of the monomeric GTPase subfamilies. Each Rab protein is associated with one or more membrane-enclosed organelles of the secretory or endocytic pathways, and each of these organelles has at least one Rab protein on its cytosolic surface (**Table 13–1**). Their highly selective distribution on these membrane systems makes Rab proteins ideal molecular markers for identifying each membrane type and guiding vesicle traffic between them. Rab proteins can function on transport vesicles, on target membranes, or both.

Like the coat-recruitment GTPases, Rab proteins cycle between a membrane and the cytosol and regulate the reversible assembly of protein complexes on the membrane. In their GDP-bound state, they are inactive and bound to another protein (*Rab-GDP dissociation inhibitor*, or *GDI*) that keeps them soluble in the

TABLE 13–1 Subcellular Locations of Some Rab Proteins

Protein	Organelle
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	Synaptic vesicles, secretory vesicles
Rab4/Rab11	Recycling endosomes
Rab5	Early endosomes, plasma membrane, clathrin-coated vesicles
Rab6	Medial and *trans* Golgi
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, *trans* Golgi

cytosol; in their GTP-bound state, they are active and tightly associated with the membrane of an organelle or transport vesicle. Membrane-bound Rab-GEFs activate Rab proteins on both transport vesicle and target membranes; for some membrane fusion events, activated Rab molecules are required on both sides of the reaction. Once in the GTP-bound state and membrane-bound through a now-exposed lipid anchor, Rab proteins bind to other proteins, called **Rab effectors**, which are the downstream mediators of vesicle transport, membrane tethering, and membrane fusion (Figure 13–16). The rate of GTP hydrolysis sets the concentration of active Rab and, consequently, the concentration of its effectors on the membrane.

In contrast to the highly conserved structure of Rab proteins, the structures and functions of Rab effectors vary greatly, and the same Rab proteins can often bind to many different effectors. Some Rab effectors are *motor proteins* that propel vesicles along actin filaments or microtubules to their target membrane. Others are *tethering proteins*, some of which have long, threadlike domains that serve as "fishing lines" that can extend to link two membranes more than 200 nm apart; other tethering proteins are large protein complexes that link two membranes that are closer together and interact with a wide variety of other proteins that facilitate the membrane fusion step. The tethering complex that docks COPII-coated

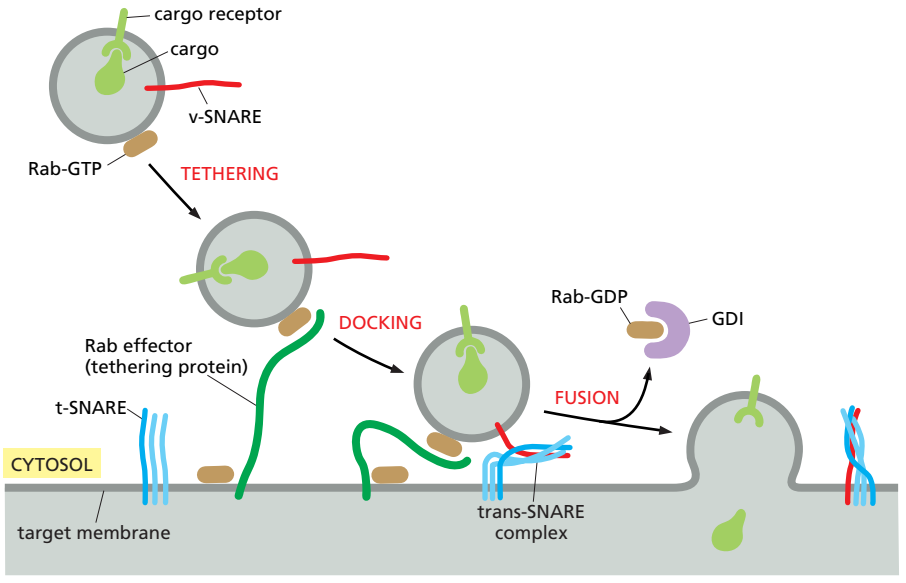
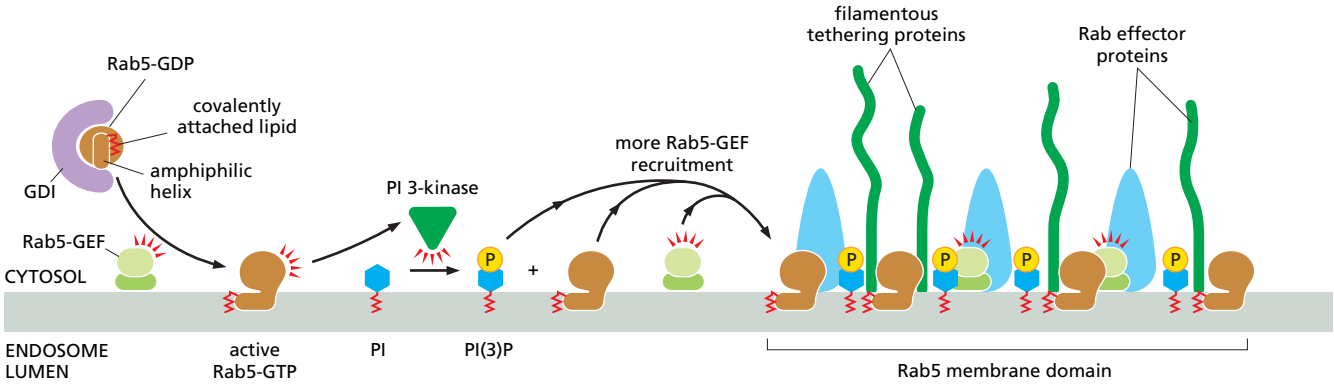


Figure 13–16 Tethering of a transport vesicle to a target membrane. Rab effector proteins interact with active Rab proteins (Rab-GTPs, *yellow*) located on the target membrane, vesicle membrane, or both, to establish the first connection between the two membranes that are going to fuse. In the example shown here, the Rab effector is a filamentous tethering protein *(dark green)*. Next, SNARE proteins on the two membranes (*red* and *blue*) pair, docking the vesicle to the target membrane and catalyzing the fusion of the two apposed lipid bilayers. During docking and fusion, a Rab-GAP (not shown) induces the Rab protein to hydrolyze its bound GTP to GDP, causing the Rab to dissociate from the membrane and return to the cytosol as Rab-GDP, where it is bound by a GDI protein that keeps the Rab soluble and inactive.



vesicles, for example, contains a protein kinase that phosphorylates the coat proteins to complete the uncoating process. Coupling uncoating to vesicle delivery helps to ensure directionality of the transport process and fusion with the proper membrane. Rab effectors can also interact with SNAREs to couple membrane tethering to fusion (see Figure 13–16).

The assembly of Rab proteins and their effectors on a membrane is cooperative and results in the formation of large, specialized membrane patches. Rab5, for example, assembles on endosomes and mediates the capture of endocytic vesicles arriving from the plasma membrane. The experimental depletion of Rab5 causes disappearance of the entire endosomal and lysosomal membrane system, highlighting the crucial role of Rab proteins in organelle biogenesis and maintenance.

A Rab5 domain concentrates tethering proteins that catch incoming vesicles. Its assembly on endosomal membranes begins when a Rab5-GDP/GDI complex encounters a Rab-GEF. GDI is released and Rab5-GDP is converted to Rab5-GTP. Active Rab5-GTP becomes anchored to the membrane and recruits more Rab5-GEF to the endosome, thereby stimulating the recruitment of more Rab5 to the same site. In addition, active Rab5 activates a PI 3-kinase, which locally converts PI to PI(3)P, which in turn binds some of the Rab effectors including tethering proteins and stabilizes their local membrane attachment (**Figure 13–17**). This type of positive feedback greatly amplifies the assembly process and helps to establish functionally distinct membrane domains within a continuous membrane.

The endosomal membrane provides a striking example of how different Rab proteins and their effectors help to create multiple specialized membrane domains, each fulfilling a particular set of functions. Thus, while the Rab5 membrane domain receives incoming endocytic vesicles from the plasma membrane, distinct Rab11 and Rab4 domains in the same membrane organize the budding of recycling vesicles that return proteins from the endosome to the plasma membrane.

Figure 13–17 The formation of a Rab5 domain on the endosome membrane. A Rab5-GEF on the endosome membrane binds a Rab5 protein and induces it to exchange GDP for GTP. GDI is lost and GTP binding alters the conformation of the Rab protein, exposing an amphiphilic helix and a covalently attached lipid group, which together anchor the Rab5-GTP to the membrane. Active Rab5 activates PI 3-kinase, which converts PI into PI(3)P. PI(3)P and active Rab5 together bind a variety of Rab effector proteins that contain PI(3)P-binding sites, including filamentous tethering proteins that catch incoming clathrin-coated endocytic vesicles from the plasma membrane. With the help of another effector, active Rab5 also recruits more Rab5-GEF, further enhancing the assembly of the Rab5 domain on the membrane.

Controlled cycles of GTP hydrolysis and GDP–GTP exchange dynamically regulate the size and activity of such Rab domains. Unlike SNAREs, which are integral membrane proteins, the GDP/GTP cycle, coupled to the membrane/cytosol translocation cycle, endows the Rab machinery with the ability to undergo assembly and disassembly on the membrane. (Adapted from M. Zerial and H. McBride, *Nat. Rev. Mol. Cell Biol.* 2:107–117, 2001. With permission from Macmillan Publishers Ltd.)

Rab Cascades Can Change the Identity of an Organelle

A Rab domain can be disassembled and replaced by a different Rab domain, changing the identity of an organelle. Such ordered recruitment of sequentially acting Rab proteins is called a **Rab cascade**. Over time, for example, Rab5 domains are replaced by Rab7 domains on endosomal membranes. This converts an early endosome, marked by Rab5, into a late endosome, marked by Rab7. Because the set of Rab effectors recruited by Rab7 is different from that recruited by Rab5, this change reprograms the compartment: as we discuss later, it alters the membrane dynamics, including the incoming and outgoing traffic, and repositions the organelle away from the plasma membrane toward the cell interior. All of the cargo contained in the early endosome that has not been recycled to the plasma membrane is now part of a late endosome. This process is also referred to as *endosome maturation*. The self-amplifying nature of the Rab domains renders the process of endosome maturation unidirectional and irreversible (**Figure 13–18**).

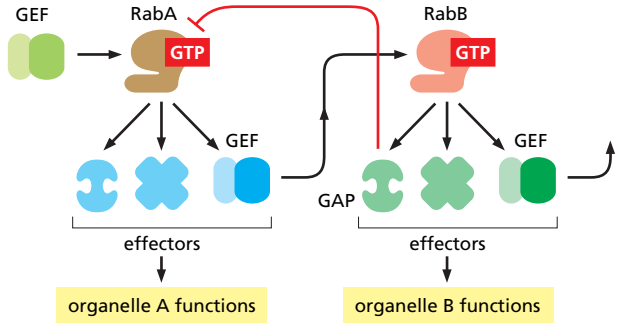


Figure 13–18 A model for a generic Rab cascade. The local activation of a RabA-GEF leads to assembly of a RabA domain on the membrane. Active RabA recruits its effector proteins, one of which is a GEF for RabB. The RabB-GEF then recruits RabB to the membrane, which in turn begins to recruit its effectors, among them a GAP for RabA. The RabA-GAP activates RabA GTP hydrolysis leading to the inactivation of the RabA and the disassembly of the RabA domain as the RabB domain grows. In this way, the RabA domain is irreversibly replaced by the RabB domain. In principle, this sequence can continued by the recruitment of a next GEF by RabB. (Adapted from A.H. Hutagalung and P.J. Novick, *Physiol. Rev*. 91:119–149, 2011. With permission from The American Physiological Society.)

SNAREs Mediate Membrane Fusion

Once a transport vesicle has been tethered to its target membrane, it unloads its cargo by membrane fusion. Membrane fusion requires bringing the lipid bilayers of two membranes to within 1.5 nm of each other so that they can merge. When the membranes are in such close apposition, lipids can flow from one bilayer to the other. For this close approach, water must be displaced from the hydrophilic surface of the membrane—a process that is highly energetically unfavorable and requires specialized *fusion proteins* that overcome this energy barrier. We have already discussed the role of dynamin in a related task during the pinching-off of clathrin-coated vesicles (see Figure 13–13).

The **SNARE proteins** (also called **SNAREs**, for short) catalyze the membrane fusion reactions in vesicle transport. There are at least 35 different SNAREs in an animal cell, each associated with a particular organelle in the secretory or endocytic pathways. These transmembrane proteins exist as complementary sets, with **v-SNAREs** usually found on vesicle membranes and **t-SNAREs** usually found on target membranes (see Figure 13–16). A v-SNARE is a single polypeptide chain, whereas a t-SNARE is usually composed of three proteins. The v-SNAREs and t-SNAREs have characteristic helical domains, and when a v-SNARE interacts with a t-SNARE, the helical domains of one wrap around the helical domains of the other to form a very stable four-helix bundle. The resulting *trans-SNARE complex* locks the two membranes together. Biochemical membrane fusion assays with all different SNARE combinations show that v- and t-SNARE pairing is highly specific. The SNAREs thus provide an additional layer of specificity in the transport process by helping to ensure that vesicles fuse only with the correct target membrane.

The trans-SNARE complexes catalyze membrane fusion by using the energy that is freed when the interacting helices wrap around each other to pull the membrane faces together, simultaneously squeezing out water molecules from the interface (**Figure 13–19**). When liposomes containing purified v-SNAREs are mixed with liposomes containing complementary t-SNAREs, their membranes fuse, albeit slowly. In the cell, other proteins recruited to the fusion site, presumably Rab effectors, cooperate with SNAREs to accelerate fusion. Fusion does not always follow immediately after v-SNAREs and t-SNAREs pair. As we discuss later, in the process of regulated exocytosis, fusion is delayed until secretion is triggered by a specific extracellular signal.

Rab proteins, which can regulate the availability of SNARE proteins, exert an additional layer of control. t-SNAREs in target membranes are often associated with inhibitory proteins that must be released before the t-SNARE can function. Rab proteins and their effectors trigger the release of such SNARE inhibitory

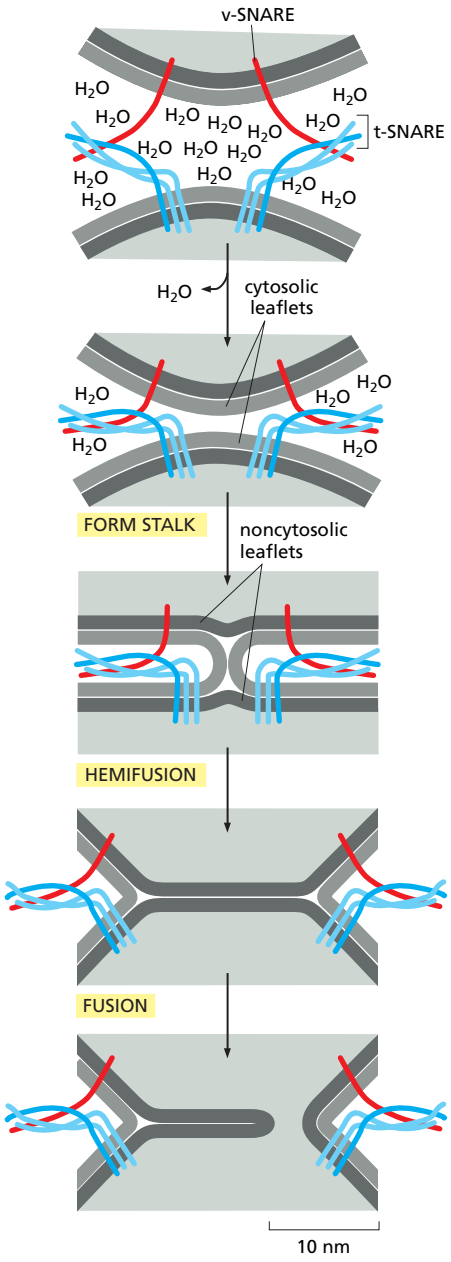


Figure 13–19 A model for how SNARE proteins may catalyze membrane fusion. Bilayer fusion occurs in multiple steps. A tight pairing between v- and t-SNAREs forces lipid bilayers into close apposition and expels water molecules from the interface. Lipid molecules in the two interacting (cytosolic) leaflets of the bilayers then flow between the membranes to form a connecting stalk. Lipids of the two noncytosolic leaflets then contact each other, forming a new bilayer, which widens the fusion zone (*hemifusion*, or half-fusion). Rupture of the new bilayer completes the fusion reaction.

proteins. In this way, SNARE proteins are concentrated and activated in the correct location on the membrane, where tethering proteins capture incoming vesicles. Rab proteins thus speed up the process by which appropriate SNARE proteins in two membranes find each other.

For vesicle transport to operate normally, transport vesicles must incorporate the appropriate SNARE and Rab proteins. Not surprisingly, therefore, many transport vesicles will form only if they incorporate the appropriate complement of SNARE and Rab proteins in their membrane. How this crucial control process operates during vesicle budding remains a mystery.

Interacting SNAREs Need to Be Pried Apart Before They Can Function Again

Most SNARE proteins in cells have already participated in multiple rounds of vesicle transport and are sometimes present in a membrane as stable complexes with partner SNAREs. The complexes have to disassemble before the SNAREs can mediate new rounds of transport. A crucial protein called **NSF** cycles between membranes and the cytosol and catalyzes the disassembly process. NSF is a hexameric ATPase of the family of AAA-ATPases (see Figure 6–85) that uses the energy of ATP hydrolysis to unravel the intimate interactions between the helical domains of paired SNARE proteins (**Figure 13–20**). The requirement for NSF-mediated reactivation of SNAREs by SNARE complex disassembly helps prevent membranes from fusing indiscriminately: if the t-SNAREs in a target membrane were always active, any membrane containing an appropriate v-SNARE might fuse whenever the two membranes made contact. It is not known how the activity of NSF is controlled so that the SNARE machinery is activated at the right time and place. It is also not known how v-SNAREs are selectively retrieved and returned to their compartment of origin so that they can be reused in newly formed transport vesicles.

Membrane fusion is important in other processes beside vesicle transport. The plasma membranes of a sperm and an egg fuse during fertilization, and myoblasts fuse with one another during the development of multinucleate muscle fibers (discussed in Chapter 22). Likewise, the ER network and mitochondria fuse and fragment in a dynamic way (discussed in Chapters 12 and 14). All cell membrane fusions require special proteins and are tightly regulated to ensure that only appropriate membranes fuse. The controls are crucial for maintaining both the identity of cells and the individuality of each type of intracellular compartment.

The membrane fusions catalyzed by viral fusion proteins are well understood. These proteins have a crucial role in permitting the entry of enveloped viruses (which have a lipid-bilayer-based membrane coat) into the cells that they infect (discussed in Chapters 5 and 23). For example, viruses such as the human immunodeficiency virus (HIV), which causes AIDS, bind to cell-surface receptors and then fuse with the plasma membrane of the target cell (**Figure 13–21**). This fusion event allows the viral nucleic acid inside the nucleocapsid to enter the cytosol, where it replicates. Other viruses, such as the influenza virus, first enter the cell by receptor-mediated endocytosis (discussed later) and are delivered to endosomes; the low pH in endosomes activates a fusion protein in the viral envelope that catalyzes the fusion of the viral and endosomal membranes, releasing the viral nucleic acid into the cytosol. Viral fusion proteins and SNAREs promote lipid bilayer fusion in similar ways.

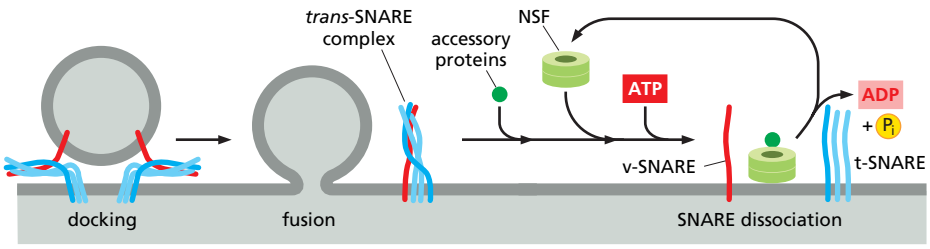


Figure 13–20 Dissociation of SNARE pairs by NSF after a membrane fusion cycle. After a v-SNARE and t-SNARE have mediated the fusion of a transport vesicle with a target membrane, NSF binds to the SNARE complex and, with the help of accessory proteins, hydrolyzes ATP to pry the SNAREs apart.

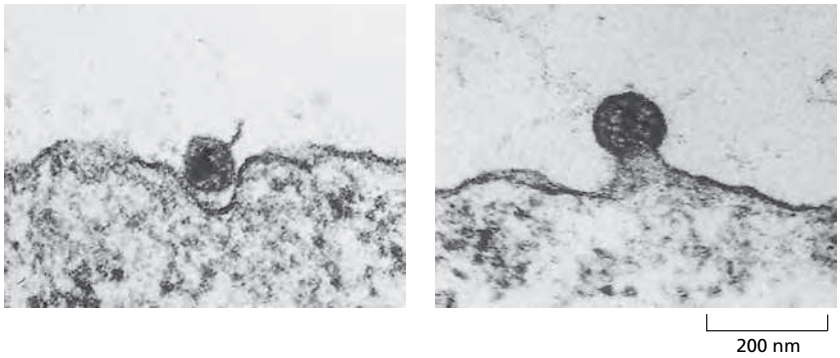


Figure 13–21 The entry of enveloped viruses into cells. Electron micrographs showing how HIV enters a cell by fusing its membrane with the plasma membrane of the cell. (From B.S. Stein et al., *Cell* 49:659–668, 1987. With permission from Elsevier.)

Summary

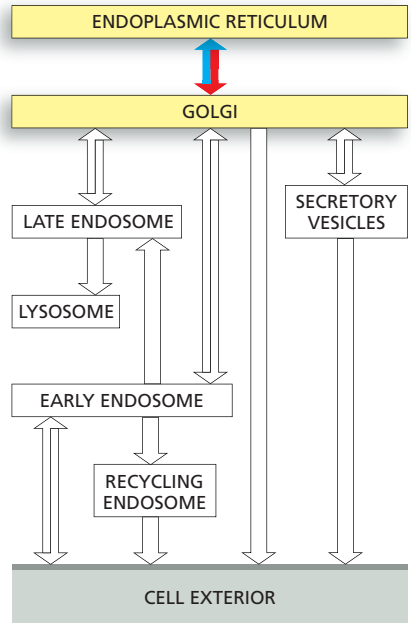
Directed and selective transport of particular membrane components from one membrane-enclosed compartment to another in a eukaryotic cell maintains the differences between those compartments. Transport vesicles, which can be spherical, tubular, or irregularly shaped, bud from specialized coated regions 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. Coats have a common two-layered structure: an inner layer formed of adaptor proteins links the outer layer (or cage) to the vesicle membrane and also traps specific cargo molecules for packaging into the vesicle. The coat is shed before the vesicle fuses with its appropriate target membrane.*

Local synthesis of specific phosphoinositides creates binding sites that trigger clathrin coat assembly and vesicle budding. In addition, monomeric GTPases help regulate various steps in vesicle 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 both, forming 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 along actin filaments or microtubules, and filamentous tethering proteins, which help ensure that the vesicles deliver their contents only to the appropriate target membrane. 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 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 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 secretory pathway, proteins that have entered the ER and are destined for the Golgi apparatus or beyond are first packaged into 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.

Entry into vesicles that leave the ER can be a selective process or can happen by default. Many membrane proteins are actively recruited into such vesicles, where they become concentrated. These cargo membrane proteins display exit (transport) signals on their cytosolic surface that adaptor proteins of the inner COPII coat recognize (**Figure 13–22**); some of these 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. Proteins without exit signals can also enter transport vesicles, including protein molecules that normally function in the ER (so-called *ER resident proteins*), some of which slowly leak out of the ER and are delivered to the Golgi apparatus. Different cargo proteins enter the transport vesicles with substantially different rates and efficiencies, which may result from differences in their folding and oligomerization efficiencies and kinetics, as well as the factors already discussed. The exit step from the ER is a major checkpoint at which quality control is exerted on the proteins that a cell secretes or displays on its surface, as we discussed in Chapter 12.

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 on the secreted

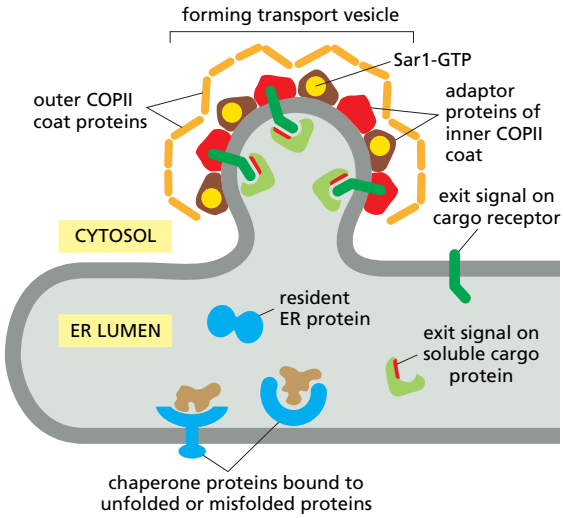


Figure 13–22 The recruitment of membrane and soluble cargo molecules into ER transport vesicles. Membrane proteins are packaged into budding transport vesicles through interactions of exit signals on their cytosolic tails with adaptor proteins of the inner COPII coat. Some of these membrane proteins function as cargo receptors, binding soluble proteins in the ER lumen and helping to package them into vesicles. Other proteins may enter the vesicle by bulk flow. 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 transiently retained in the ER compartment.

proteins. One such lectin, for example, binds to mannose on two secreted blood-clotting factors (Factor V and Factor VIII), thereby packaging the proteins into transport vesicles in the ER; its 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 multiprotein complexes, they need to be completely assembled. Those that are misfolded or incompletely assembled transiently 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 the ER. 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. Such failures are surprisingly common. More than 90% of the newly synthesized subunits of the T cell receptor (discussed in Chapter 24) 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 some protein molecules to produce a select few that fold, assemble, and function properly.

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 normally if it reached the plasma membrane, it is retained in the ER and then is degraded by cytosolic proteasomes. 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. The 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 13–23**).

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

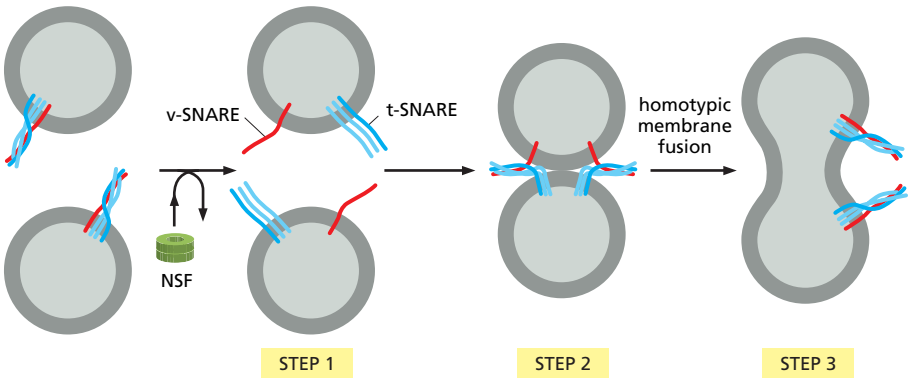


Figure 13–23 Homotypic membrane fusion. In step 1, NSF pries apart identical pairs of v-SNAREs and t-SNAREs in both membranes (see Figure 13–20). In steps 2 and 3, the separated matching SNAREs on adjacent identical membranes interact, which leads to membrane fusion and the formation of one continuous compartment. Subsequently, the compartment grows by further homotypic fusion with vesicles from the same kind of membrane, displaying matching SNAREs. Homotypic fusion occurs when ER-derived transport vesicles fuse with one another, but also when endosomes fuse to generate larger endosomes. Rab proteins help regulate the extent of homotypic fusion and hence the size of a cell's compartments (not shown).

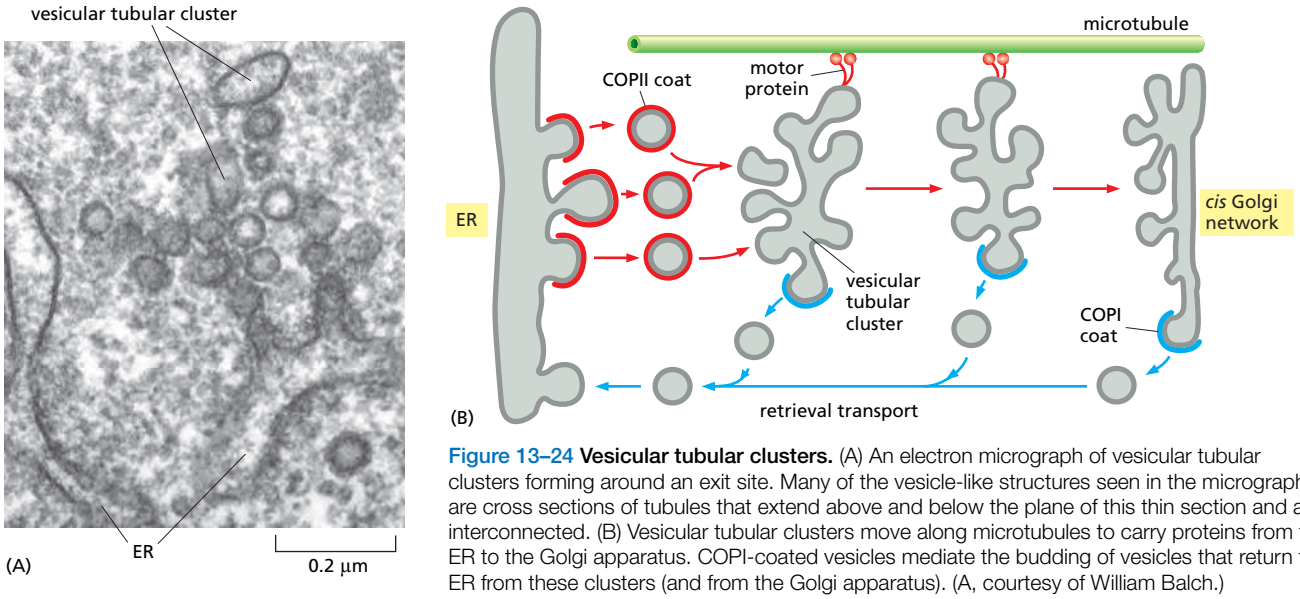


Figure 13–24 Vesicular tubular clusters. (A) An electron micrograph of vesicular tubular clusters forming around an exit site. Many of the vesicle-like structures seen in the micrograph are cross sections of tubules that extend above and below the plane of this thin section and are interconnected. (B) Vesicular tubular clusters move along microtubules to carry proteins from the ER to the Golgi apparatus. COPI-coated vesicles mediate the budding of vesicles that return to the ER from these clusters (and from the Golgi apparatus). (A, courtesy of William Balch.)

the electron microscope (**Figure 13–24**A). These clusters constitute a 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 move quickly along microtubules to the Golgi apparatus with which they fuse (Figure 13–24B and **Movie 13.2**).

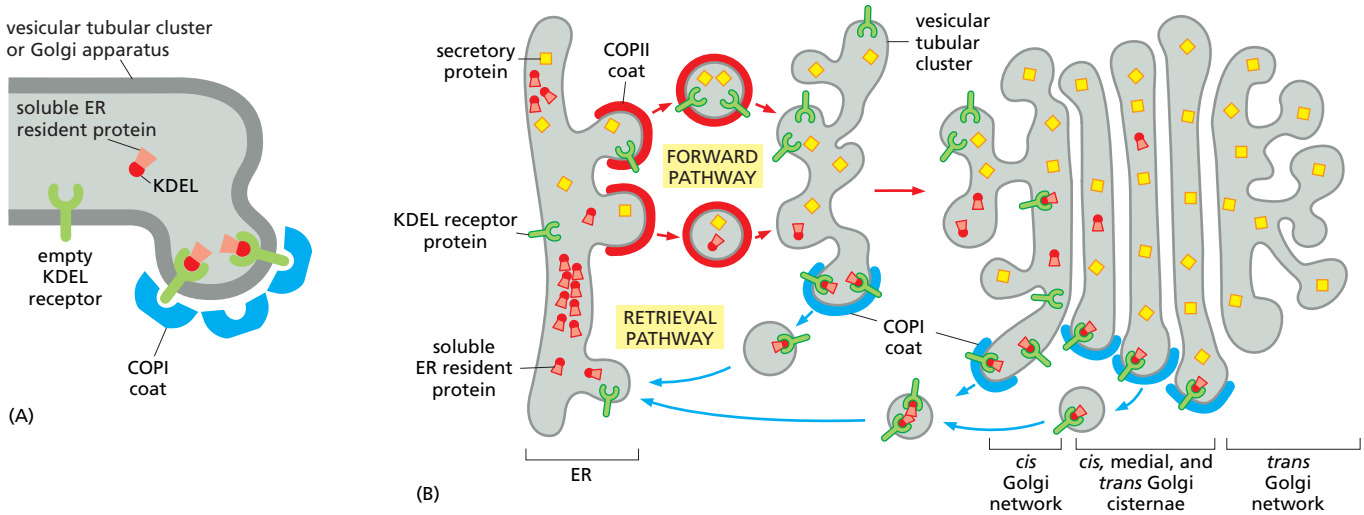
As soon as vesicular tubular clusters form, they begin to bud off transport vesicles of their own. Unlike the COPII-coated vesicles that bud from the ER, these vesicles are COPI-coated (see Figure 13–24A). COPI-coated vesicles are unique in that the components that make up the inner and outer coat layers are recruited as a preassembled complex, called *coatomer*. They function as a *retrieval pathway*, carrying back ER resident proteins that have escaped, as well as proteins such as cargo receptors and SNAREs that participated in the ER budding and vesicle fusion reactions. 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, and remains a mystery how this switch in coat assembly is controlled.

The retrieval (or retrograde) transport continues as the vesicular tubular clusters move toward the Golgi apparatus. Thus, the clusters continuously mature, gradually changing their composition as selected proteins are returned to the ER. The retrieval 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 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 ER 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 13–25**). 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 soluble 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 likely related to the lower pH in the Golgi compartments, which is regulated by H^+ pumps. As we discuss later, pH-sensitive protein–protein interactions form the basis for many of the protein 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, also enter the retrieval pathway back to the ER.

Figure 13–25 Retrieval of soluble ER resident proteins. ER resident proteins that escape from the ER are returned by vesicle transport. (A) The KDEL receptor present in both 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. (Recall that the COPI-coated vesicles shed their coats as soon as they are formed.) Upon binding its ligands in the tubular cluster or Golgi, 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 later 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. We discuss the different compartments of the Golgi apparatus shortly.

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 mentioned, 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 normally retains 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 retain most of the proteins in such complexes.

Aggregation of proteins that function in the same compartment 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.

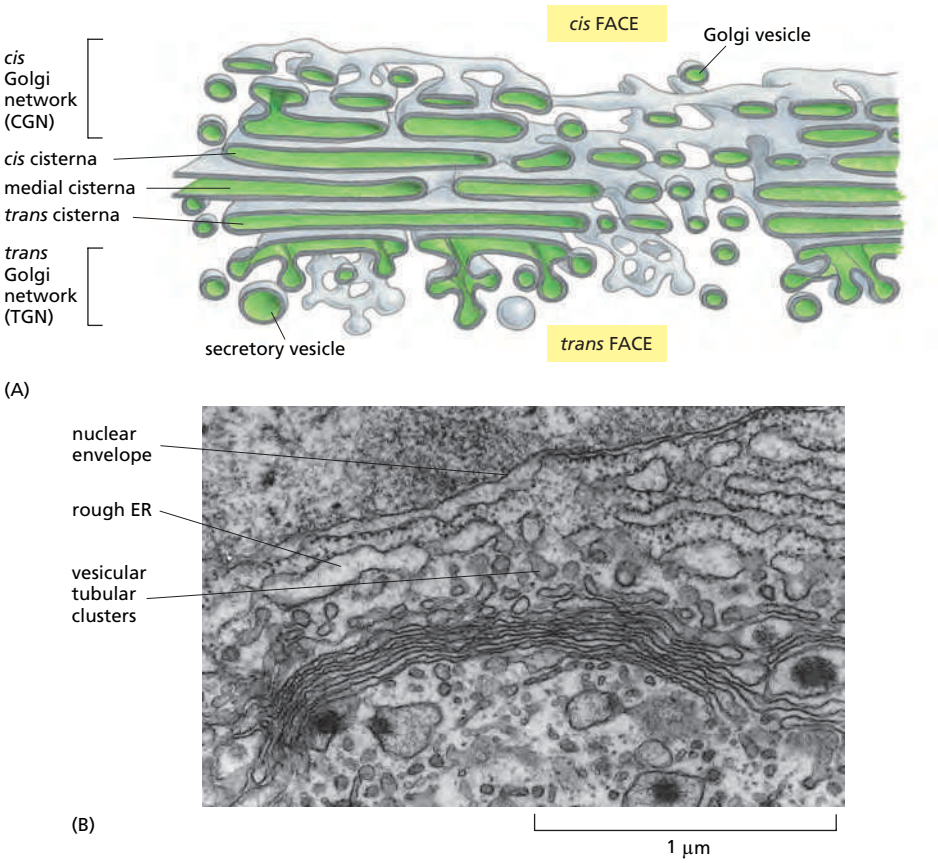


Figure 13–26 The Golgi apparatus. (A) Three-dimensional reconstruction from electron micrographs of the Golgi apparatus in a secretory animal cell. The *cis* face of the Golgi stack is that closest to the ER. (B) A thin-section electron micrograph of an animal cell. In plant cells, the Golgi apparatus 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.)

The Golgi Apparatus Consists of an Ordered Series of Compartments

Because it could be selectively visualized by silver stains, 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 typically consists of four to six cisternae (**Figure 13–26**), although some unicellular flagellates can have more than 20. 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 13–27**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 where they are typically found adjacent to ER exit sites (Figure 13–27B).

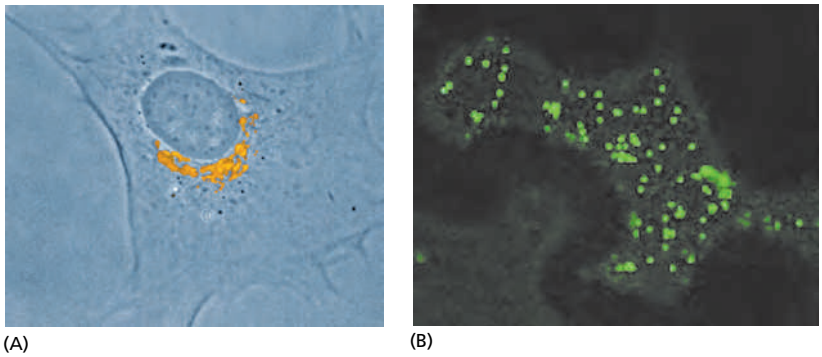


Figure 13–27 Localization of the Golgi apparatus in animal and plant cells. (A) The Golgi apparatus in a cultured fibroblast stained with a fluorescent antibody that recognizes a Golgi resident protein *(bright orange)*. The Golgi apparatus is polarized, facing the direction in which the cell was crawling 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. (A, courtesy of John Henley and Mark McNiven; B, courtesy of Chris Hawes.)

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: endosomes, secretory vesicles, or the cell surface. They also can be returned to an earlier compartment. Some membrane proteins are retained in the part of the Golgi apparatus where they function.

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 in proteasomes. 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 reutilizes the oligosaccharides for new functions. This begins in the Golgi apparatus, which generates the heterogeneous oligosaccharide structures seen in mature proteins. After arrival in the CGN, proteins enter 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 mixture 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.

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 (**Figure 13–28**). The removal of mannose and the addition of *N*-acetylglucosamine, for example, occur in the *cis* and medial cisternae, while the addition of galactose and sialic acid occurs in the *trans* cisterna and *trans* Golgi network. **Figure 13–29** summarizes the functional compartmentalization of the Golgi apparatus.

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, as the enzymatic reactions apparently occur entirely on membrane surfaces. All of the Golgi glycosidases and glycosyl transferases, for example, are single-pass transmembrane proteins, many of which are organized in multienzyme complexes.

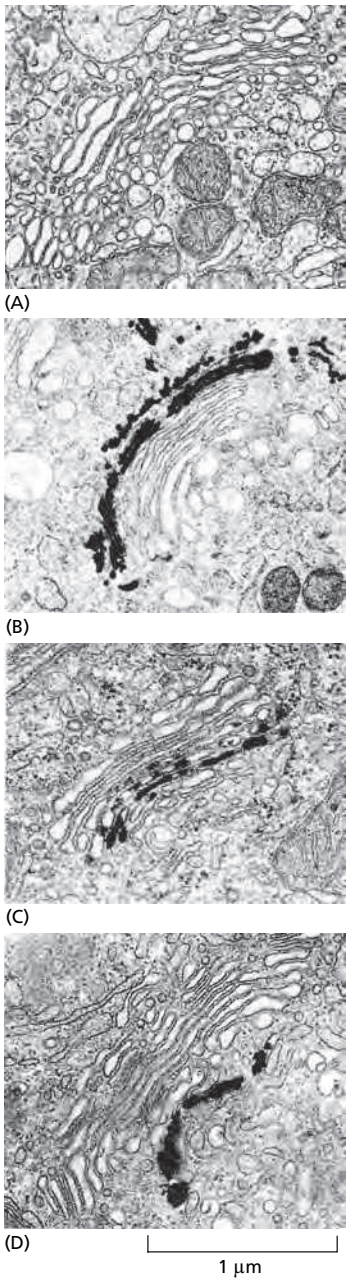


Figure 13–28 Molecular compartmentalization of the Golgi apparatus. A series of electron micrographs shows the Golgi apparatus (A) unstained, (B) stained with osmium, which preferentially labels the cisternae of the *cis* compartment, and (C and D) stained to reveal the location of specific enzymes. 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.)

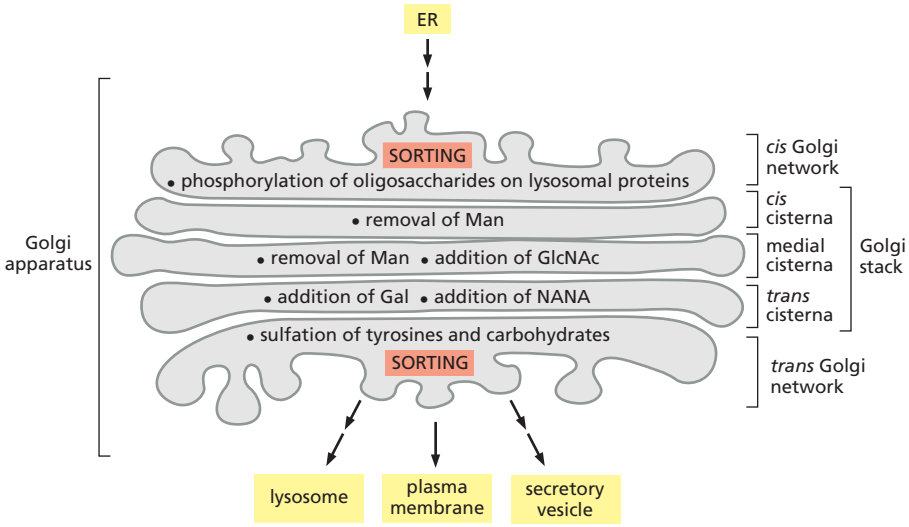


Figure 13–29 Oligosaccharide processing in Golgi compartments. The localization of each processing step shown was determined by a combination of techniques, including biochemical subfractionation of the Golgi apparatus membranes and electron microscopy after staining with antibodies specific for some 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. Man, mannose; GlcNAc, *N*-acetylglucosamine; Gal, galactose; NANA, *N*-acetylneuraminic acid (sialic acid).

Two broad classes of *N*-linked oligosaccharides, the **complex oligosaccharides** and the **high-mannose oligosaccharides**, are attached to mammalian glycoproteins. 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 (Figure 13–30). The sialic acids in the

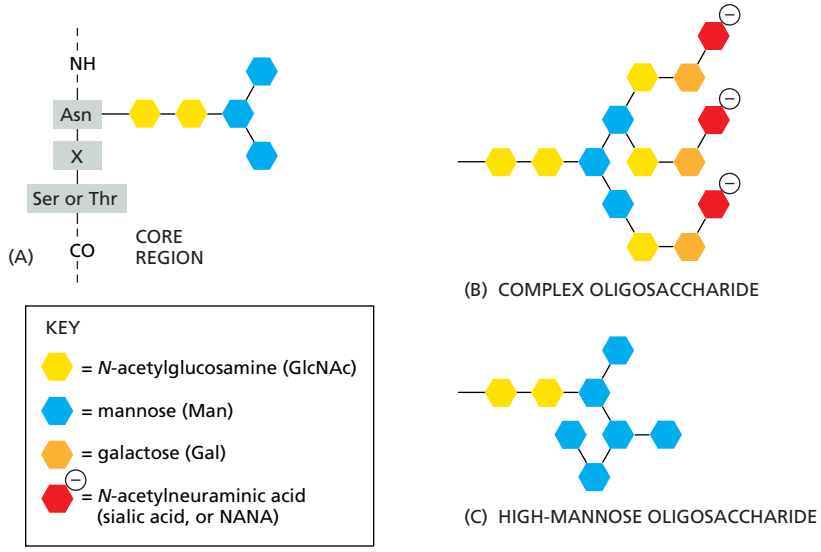


Figure 13–30 The two main classes of asparagine-linked (*N*-linked) oligosaccharides found in mature mammalian glycoproteins. (A) Both complex oligosaccharides and high-mannose oligosaccharides share a common *core region* derived from the original *N*-linked oligosaccharide added in the ER (see Figure 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 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 mannoses. 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.

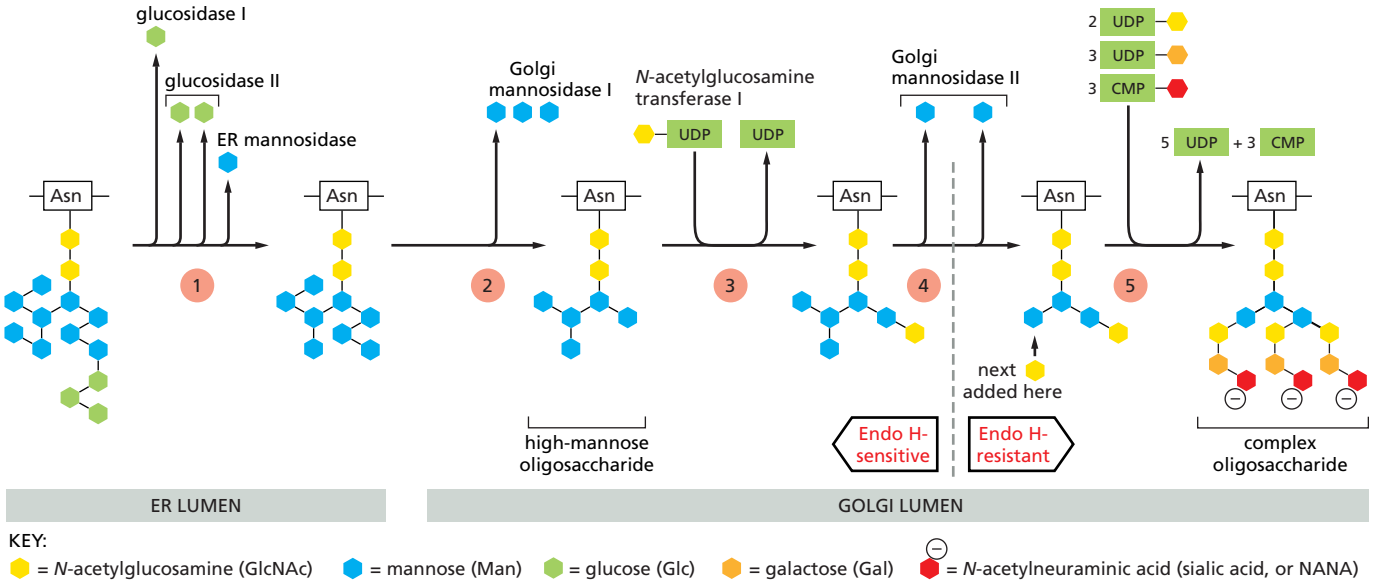


Figure 13–31 Oligosaccharide processing in the ER and the Golgi apparatus. The processing pathway is highly ordered, so that each step shown depends on the previous one. Step 1: Processing begins in the ER with the removal of the glucoses from the oligosaccharide initially transferred to the protein. Then a mannosidase in the ER membrane removes a specific mannose. The remaining steps occur in the Golgi stack. Step 2: Golgi mannosidase I 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 from the ER: all sugars in the Golgi are assembled inside the lumen from sugar nucleotide, whereas in the ER, the *N*-linked precursor oligosaccharide is assembled partly in the cytosol and partly in the lumen, and most lumenal reactions use dolichol-linked sugars as their substrates (see Figure 12–51).

complex oligosaccharides are of special importance because they bear 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 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 of study called glycobiology. The human genome, for example, encodes hundreds of different Golgi glycosyl transferases and many glycosidases, 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 the Golgi apparatus in other ways. Some proteins have sugars added to the hydroxyl groups of selected serines or threonines,

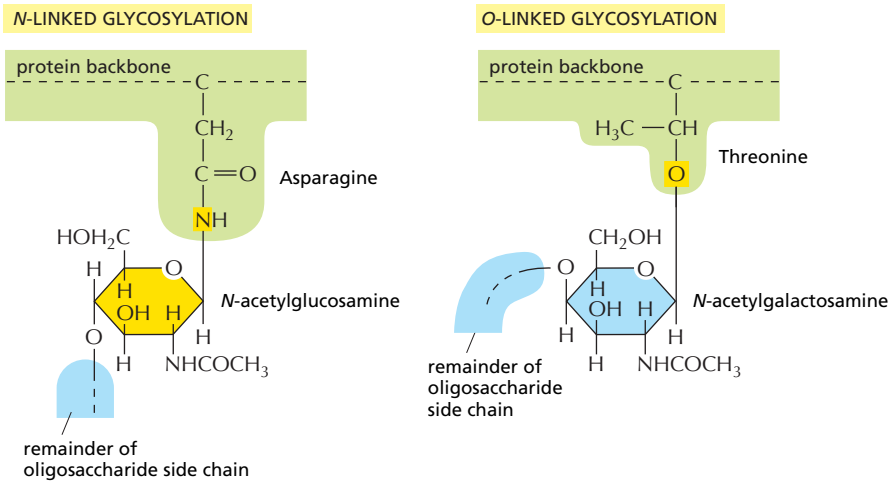


Figure 13–32 *N*- and *O*-linked glycosylation. In each case, only the single sugar group that is directly attached to the protein chain is shown.

or, in some cases—such as collagens—to hydroxylated proline and lysine 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 add sugars to a protein one at a time. Usually, *N*-acetylgalactosamine is added first, followed by a variable number of additional sugars, ranging from just a few to 10 or more.

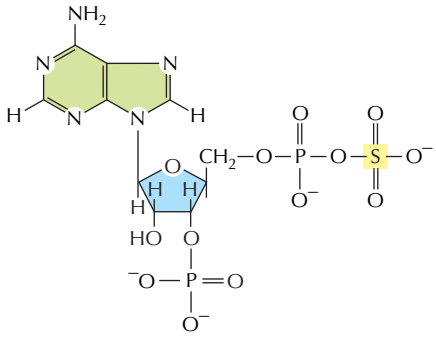
The Golgi apparatus confers the heaviest *O*-linked glycosylation of all on *mucins*, the glycoproteins in mucus secretions, and on *proteoglycan core proteins*, which it modifies to produce **proteoglycans**. As discussed in Chapter 19, this process involves the polymerization of one or more *glycosaminoglycan chains* (long, unbranched polymers composed of repeating disaccharide units; see Figure 19–35) 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 emphasize that the oligosaccharides on glycoproteins and glycosphingolipids have very important functions.

N-linked glycosylation, for example, is prevalent in all eukaryotes, 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



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

Figure 13–33 The structure of PAPS.

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.

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, such a sugar 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 transmembrane lectins that function in cell–cell adhesion during blood cell migration, as discussed in Chapter 19. The presence of oligosaccharides may modify a protein's antigenic and functional 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, is an important factor in determining the cell's fate in development (discussed in Chapter 21). 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 Notch for the cell-surface signal proteins that activate it.

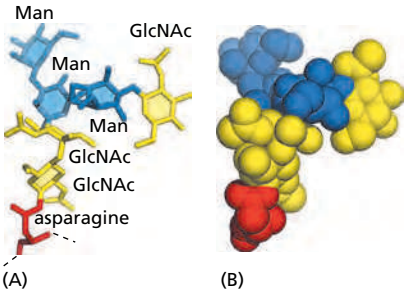


Figure 13–34 The three-dimensional structure of a small *N*-linked oligosaccharide. The structure was determined by x-ray crystallographic analysis of a glycoprotein. This oligosaccharide contains only 6 sugars, whereas there are 14 sugars in the *N*-linked oligosaccharide that is initially transferred to proteins in the ER (see Figure 12–47). (A) A backbone model showing all atoms except hydrogens; only the asparagine of the protein is shown. (B) A space-filling model, with the asparagine and sugars indicated using the same color scheme as in (A). (B, courtesy of Richard Feldmann.)

Transport Through the Golgi Apparatus May Occur 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, and it is likely that more than one mechanism is involved in each case. One hypothesis, called the **cisternal maturation model**, views the Golgi cisternae as dynamic structures that mature from early to late by acquiring and then losing specific Golgi-resident proteins. According to this view, new *cis* cisternae continually form as vesicular tubular clusters arrive from the ER and progressively mature to become a medial cisterna and then a *trans* cisterna (**Figure 13–35**A). A cisterna therefore moves through the Golgi stack with cargo in its lumen. Retrograde transport of the Golgi enzymes by budding COPI-coated vesicles explains their characteristic distribution. 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 it to the *trans* Golgi network.

The cisternal maturation model is supported by studies using Golgi enzymes from different cisternae that were fluorescently labeled with different colors. Such studies performed in yeast cells where Golgi cisternae are not stacked reveal that individual Golgi cisternae change their color, thereby demonstrating that they change their complement of resident enzymes as they mature, even though they are not stacked. In further support of the model, electron microscopic observations found that large structures such as procollagen rods in fibroblasts and scales in certain algae move progressively through the Golgi stack.

An alternative view holds that Golgi cisternae are long-lived structures that retain their characteristic set of Golgi-resident proteins firmly in place, and cargo proteins are transported from one cisterna to the next by transport vesicles (Figure 13–35B). According to this **vesicle transport model**, retrograde flow of vesicles

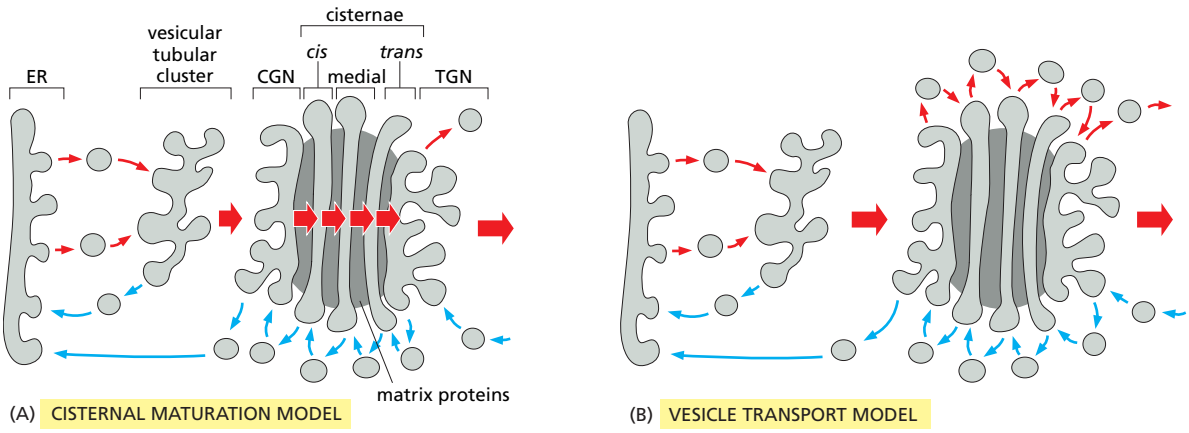


Figure 13–35 Two possible models explaining the organization of the Golgi apparatus and how proteins move through it. It is likely that the transport through the Golgi apparatus in the forward direction *(red arrows)* involves elements of both models. (A) According to the cisternal maturation model, each Golgi cisterna matures as it migrates outward through the stack. At each stage, the Golgi resident proteins that are carried forward in a maturing cisterna are moved backward to an earlier compartment in COPI-coated vesicles. When a newly formed cisterna moves to a medial position, for example, "leftover" *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 and then *trans* cisterna as it moves outward. (B) In the vesicle transport model, Golgi cisternae are static compartments, 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.

retrieves escaped ER and Golgi proteins and returns them to upstream compartments. Directional flow could be achieved because forward-moving cargo molecules are selectively packaged into forward-moving vesicles. Although both forward- and backward-moving vesicles would likely be COPI-coated, the coats may contain different adaptor proteins that confer selectivity on the packaging of cargo molecules. Alternatively, transport vesicles shuttling between Golgi cisternae might not be directional at all, transporting cargo randomly back and forth; directional flow would then occur because of the continual input to the *cis* cisterna and output from the *trans* cisterna.

The vesicle transport model is supported by experiments that show that cargo molecules are present in small COPI-coated vesicles and that these vesicles can deliver them to Golgi cisternae over large distances. In addition, when experimentally aggregated membrane proteins are introduced into Golgi cisternae, they can be observed staying in place, while soluble cargo, even if present as large aggregates, traverses the Golgi at normal rates.

It is likely that aspects of both models are true. A stable core of long-lasting cisternae might exist in the center of each Golgi cisterna, while regions at the rim may undergo continuous maturation, perhaps utilizing Rab cascades that change their identity. As matured pieces of the cisternae are formed, they might break off and fuse with downstream cisternae by homotypic fusion mechanisms, taking large cargo molecules with them. In addition, small COPI-coated vesicles might transport small cargo in the forward direction and retrieve escaped Golgi enzymes and return them to their appropriate upstream cisternae.

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, called *golgins*, form long tethers composed of stiff coiled-coil domains with interspersed hinge regions. Golgins form a forest of tentacles that can extend 100–400 nm from the surface of the Golgi stack. They are thought to help retain Golgi transport vesicles close to the organelle through interactions with Rab proteins (**Figure 13–36**). When the cell prepares to divide, mitotic protein kinases phosphorylate the Golgi matrix proteins, causing the Golgi

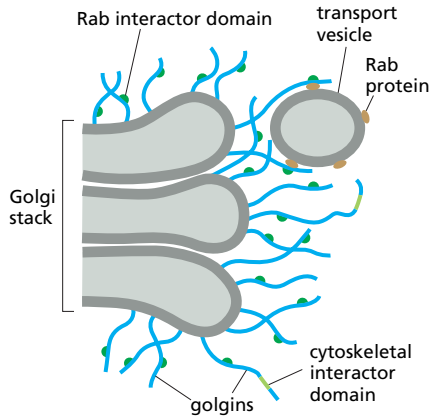


Figure 13–36 A model of golgin function. Filamentous golgins anchored to Golgi membranes capture transport vesicles by binding to Rab proteins on the vesicle surface.

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 stack. Similarly, during apoptosis, proteolytic cleavage of golgins by caspases ensues (discussed in Chapter 18), fragments the Golgi apparatus as the cell self-destructs.

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. In animal cells, 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 glycosylate 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.*

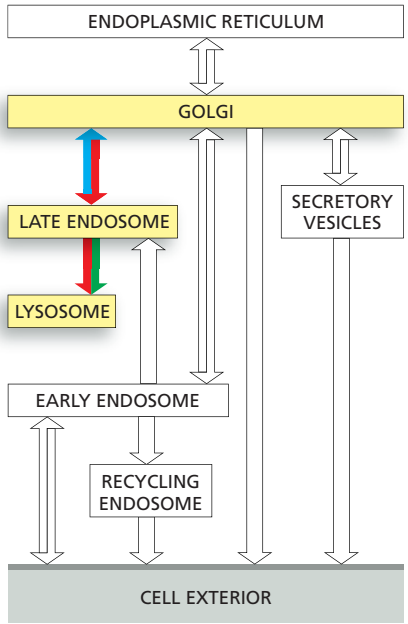
The Golgi apparatus modifies the many proteins and lipids that it receives from the ER and then distributes them to the plasma membrane, lysosomes, and secretory vesicles. The Golgi apparatus is a polarized organelle, consisting of one or more stacks of disc-shaped cisternae. Each stack is organized as a series of at least three functionally distinct compartments, termed cis*, medial, and* trans *cisternae. The* cis *and* trans *cisternae are each 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 vesicle 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 vesicle transport from upstream to more downstream 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 organelles filled with soluble hydrolytic enzymes that digest macromolecules. Lysosomes contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. All are **acid hydrolases**; that is, hydrolases that work best at acidic pH. For optimal activity, they need to be activated by proteolytic cleavage, which also requires an acid environment. The lysosome provides this acidity, maintaining an interior pH of about 4.5–5.0. 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 membrane-enclosed organelles, the lysosome not only contains a unique collection of enzymes, but also has a unique surrounding membrane. Most of the lysosome membrane proteins, for example, are highly glycosylated, which helps to protect them from the lysosome proteases in the lumen. Transport proteins in the lysosome 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 lysosome membrane uses the energy of ATP hydrolysis to pump H^+ into the lysosome, thereby maintaining the lumen at its acidic pH (**Figure 13–37**). The lysosome 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, some 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.

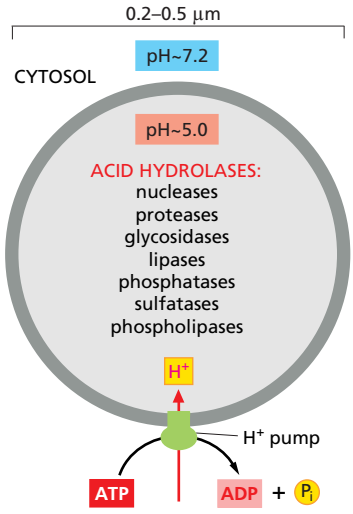


Figure 13–37 Lysosomes. The acid hydrolases are hydrolytic enzymes that are active under acidic conditions. An H^+ ATPase in the membrane pumps H^+ into the lysosome, maintaining its lumen at an acidic pH.

Lysosomes Are Heterogeneous

Lysosomes are found in all eukaryotic cells. They 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 histochemical techniques that reveal which organelles contain acid hydrolase (**Figure 13–38**).

The heterogeneous morphology of lysosomes contrasts with the relatively uniform structures of many 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. Their morphological diversity, however, also reflects the way lysosomes form. Late endosomes containing material received from both the plasma membrane by endocytosis and newly synthesized lysosomal hydrolases fuse with preexisting lysosomes to form structures that are sometimes referred to as *endolysosomes*, which then fuse with one another (**Figure 13–39**). When the majority of the endocytosed material within an endolysosome has been digested so that only resistant or slowly digestible residues remain, these organelles become "classical" lysosomes. These are relatively dense, round, and small, but they can enter the cycle again by fusing with late endosomes or endolysosomes. Thus, there is no real distinction between endolysosomes 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

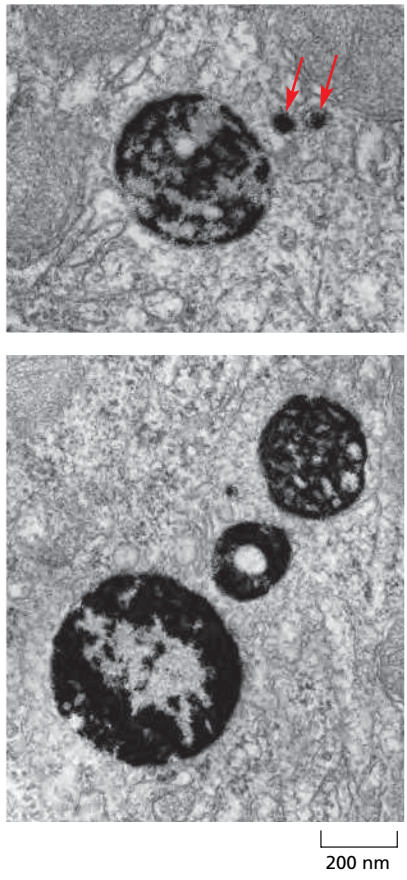


Figure 13–38 Histochemical visualization of lysosomes. These electron micrographs show two sections of a cell stained to reveal the location of acid phosphatase, a marker enzyme for lysosomes. The larger membrane-enclosed organelles, containing dense precipitates of lead phosphate, are lysosomes. Their 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.)

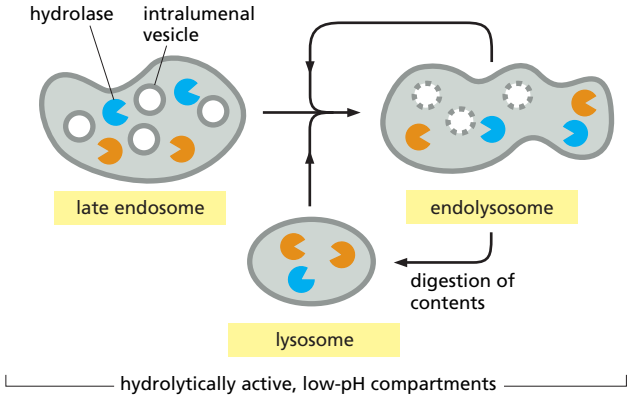


Figure 13–39 A model for lysosome maturation. Late endosomes fuse with preexisting lysosomes *(bottom arrow)* or preexisting endolysosomes *(top arrow)*. Endolysosomes eventually mature into lysosomes as hydrolases complete the digestion of their contents, which can include intralumenal vesicles. Lysosomes also fuse with phagosomes, as we discuss later.

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 13–40**). 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, and as a controller of *turgor pressure* (the osmotic pressure that pushes outward on the cell wall and keeps the plant from wilting) (**Figure 13–41**). 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 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,

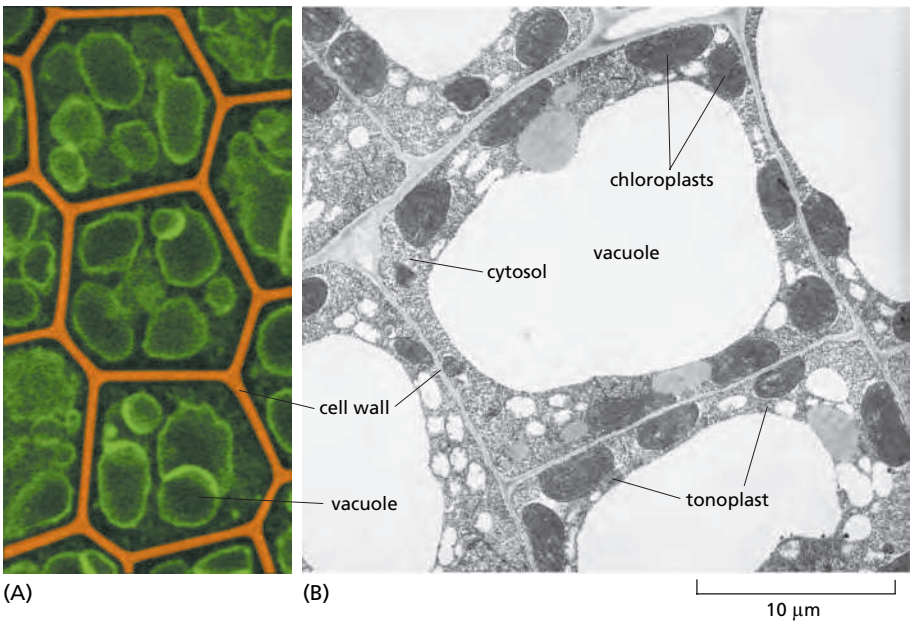


Figure 13–40 The plant cell vacuole. (A) A confocal image of cells from an *Arabidopsis* embryo that is expressing an aquaporin—YFP (yellow fluorescent protein) fusion protein in its tonoplast, or vacuole membrane *(green)*; the cell walls have been false-colored *orange*. Each cell contains several large vacuoles. (B) 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. (A, courtesy of C. Carroll and L. Frigerio, based on S. Gattolin et al., *Mol. Plant* 4:180–189, 2011. With permission from Oxford University Press; B, courtesy of J. Burgess.)

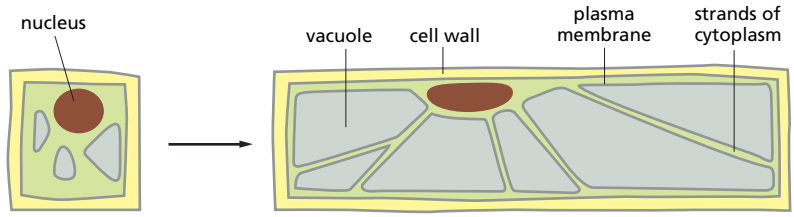


Figure 13–41 The role of the vacuole in controlling the size of plant cells. A plant cell can achieve a large increase in volume without increasing the volume of the cytosol. Localized weakening of the cell wall orients a turgor-driven cell enlargement that accompanies the uptake of water into an expanding vacuole. The cytosol is eventually confined to a thin peripheral layer, which is connected to the nuclear region by strands of cytosol stabilized by bundles of actin filaments (not shown).

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—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 meeting places where several streams of intracellular traffic converge. A route that leads outward from the ER via the Golgi apparatus delivers most of the lysosome's digestive enzymes, while at least four paths from different sources feed substances into lysosomes for digestion.

The best studied of these degradation paths is the one followed by macromolecules taken up from extracellular fluid by *endocytosis*. A similar pathway found in phagocytic cells, such as macrophages and neutrophils in vertebrates, is dedicated to the engulfment, or *phagocytosis*, of large particles and microorganisms to form *phagosomes*. A third pathway called *macropinocytosis* specializes in the nonspecific uptake of fluids, membrane, and particles attached to the plasma membrane. We will return to discuss these pathways later in the chapter. A fourth pathway called *autophagy* originates in the cytoplasm of the cell itself and is used to digest cytosol and worn-out organelles, as we discuss next. The four paths to degradation in lysosomes are illustrated in **Figure 13–42**.

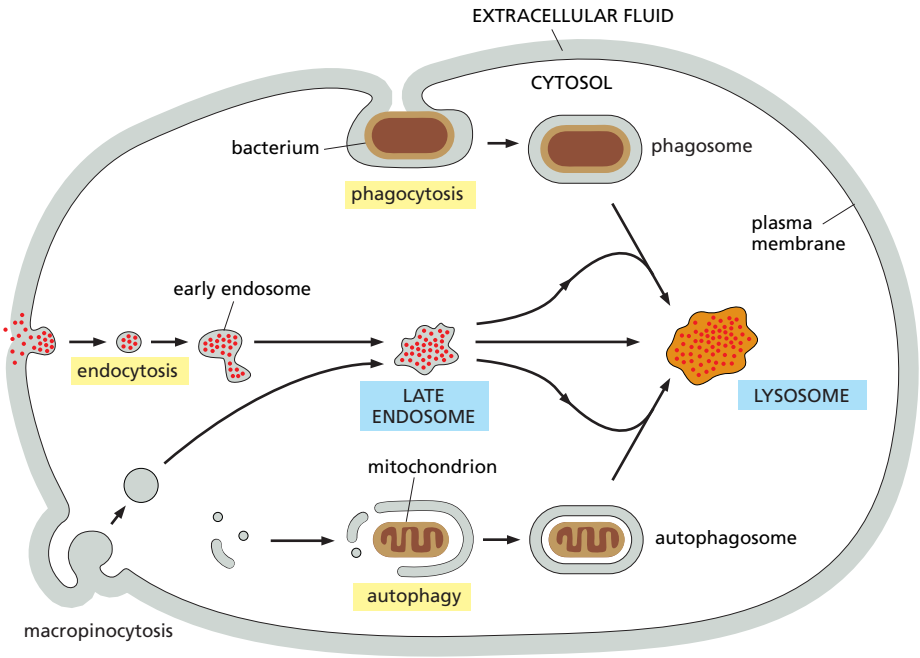
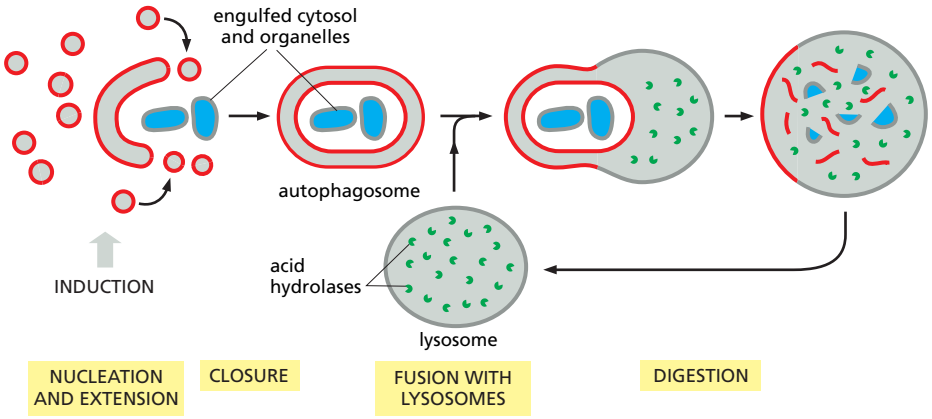


Figure 13–42 Four pathways to degradation in lysosomes. Materials in each pathway are derived from a different source. Note that the autophagosome has a double membrane. In all cases, the final step is the fusion with lysosomes.



(A)

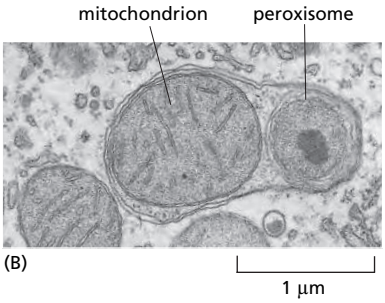


Figure 13–43 A model of autophagy. (A) Activation of a signaling pathway initiates a nucleation event in the cytoplasm. A crescent of autophagosomal membrane grows by fusion of vesicles of unknown origin and eventually fuses to form a double-membrane-enclosed autophagosome, which sequesters a portion of the cytoplasm. The autophagosome then fuses with lysosomes containing acid hydrolases that digest its content. During the formation of the autophagosome membrane, a ubiquitin-like protein becomes activated by covalent attachment of a phosphatidylethanolamine lipid anchor. These proteins then mediate vesicle tethering and fusion, leading to the formation of a crescent-shaped membrane structure that assembles around its target (not shown). (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.)

Autophagy Degrades Unwanted Proteins and Organelles

All cell types dispose of obsolete parts by a lysosome-dependent process called **autophagy**, or "self-eating." The degradation process is important during normal cell growth and in development, where it helps restructure differentiating cells, but also in adaptive responses to stresses such as starvation and infection. Autophagy can remove large objects—macromolecules, large protein aggregates, and even whole organelles—that other disposal mechanisms such as proteasomal degradation cannot handle. Defects in autophagy may prevent cells from clearing away invading microbes, unwanted protein aggregates and abnormal proteins, and thereby contribute to diseases ranging from infectious disorders to neurodegeneration and cancer.

In the initial stages of autophagy, cytoplasmic cargo becomes surrounded by a double membrane that assembles by the fusion of small vesicles of unknown origin, forming an **autophagosome** (Figure 13–43). A few tens of different proteins have been identified in yeast and animal cells that participate in the process, which must be tightly regulated: either too little or too much can be deleterious. The whole process occurs in the following sequence of steps:

1. Induction by activation of signaling molecules: Protein kinases (including the mTOR complex 1, discussed in Chapter 15) that relay information about the metabolic status of the cell, become activated and signal to the autophagic machinery.
2. Nucleation and extension of a delimiting membrane into a crescent-shaped cup: Membrane vesicles, characterized by the presence of ATG9, the only transmembrane protein involved in the process, are recruited to an assembly site, where they nucleate autophagosome formation. ATG9 is not incorporated into the autophagosome: a retrieval pathway must remove it from the assembling structure.
3. Closure of the membrane cup around the target to form a sealed double-membrane-enclosed autophagosome.
4. Fusion of the autophagosome with lysosomes, catalyzed by SNAREs.
5. Digestion of the inner membrane and the lumenal contents of the autophagosome.

Autophagy can be either nonselective or selective. In *nonselective autophagy*, a bulk portion of cytoplasm is sequestered in autophagosomes. It might occur, for example, in starvation conditions: when external nutrients are limiting, metabolites derived from the digestion of the captured cytosol might help the cell survive. In *selective autophagy* specific cargo is packaged into autophagosomes that tend to contain little cytosol, and their shape reflects the shape of the cargo. Selective autophagy mediates the degradation of worn out, or otherwise unwanted, mitochondria, peroxisomes, ribosomes, and ER; it can also be used to destroy invading microbes.

The selective autophagy of worn out or damaged mitochondria is called *mitophagy*. As discussed in Chapters 12 and 14, when mitochondria function normally, the inner mitochondrial membrane is energized by an electrochemical H^+ gradient that drives ATP synthesis and the import of mitochondrial precursor proteins and metabolites. Damaged mitochondria cannot maintain the gradient, so protein import is blocked. As a consequence, a protein kinase called Pink1, which is normally imported into mitochondria, is instead retained on the mitochondrial surface where it recruits the ubiquitin ligase Parkin from the cytosol. Parkin ubiquitylates mitochondrial outer membrane proteins, which mark the organelle for selective destruction in autophagosomes. Mutations in Pink1 or Parkin cause a form of early-onset Parkinson's disease, a degenerative disorder of the central nervous system. It is not known why the neurons that die prematurely in this disease are particularly reliant on mitophagy.

A Mannose 6-Phosphate Receptor Sorts Lysosomal Hydrolases in the *Trans* Golgi Network

We now consider the pathway that delivers lysosomal hydrolases from the TGN to lysosomes. The enzymes are first delivered to endosomes in transport vesicles that bud from the TGN, before they move on to endolysosomes and lysosomes (see Figure 13–39). The vesicles that leave the TGN incorporate the lysosomal proteins and exclude the many other proteins being packaged into different transport vesicles for delivery elsewhere.

How are lysosomal hydrolases recognized and selected in the TGN with the required accuracy? In animal cells 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 13–44**). Transmembrane **M6P receptor proteins**, which are present in the TGN, recognize the M6P groups and bind to the lysosomal hydrolases on the lumenal side of the membrane and to adaptor proteins in assembling clathrin coats on the cytosolic side. In this way, the receptors help package the hydrolases into clathrin-coated vesicles that bud from the TGN and deliver their contents to early endosomes.

The M6P receptor protein binds to M6P at pH 6.5–6.7 in the TGN lumen and releases it at pH 6, which is the pH in the lumen of endosomes. Thus, after the receptor is delivered, the lysosomal hydrolases dissociate from the M6P receptors, which are retrieved into transport vesicles that bud from endosomes. These vesicles are coated with *retromer*, a coat protein complex specialized for endosome-to-TGN transport, which returns the receptors to the TGN for reuse (**Figure 13–45**).

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 TGN. These signals are recognized by the retromer complex that recruits M6P receptors into transport vesicles that bud from 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* (discussed later) 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.

For the sorting system that segregates lysosomal hydrolases and dispatches them to endosomes to work, the M6P groups must be 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

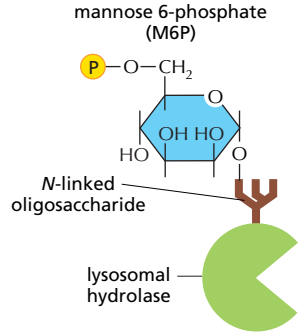
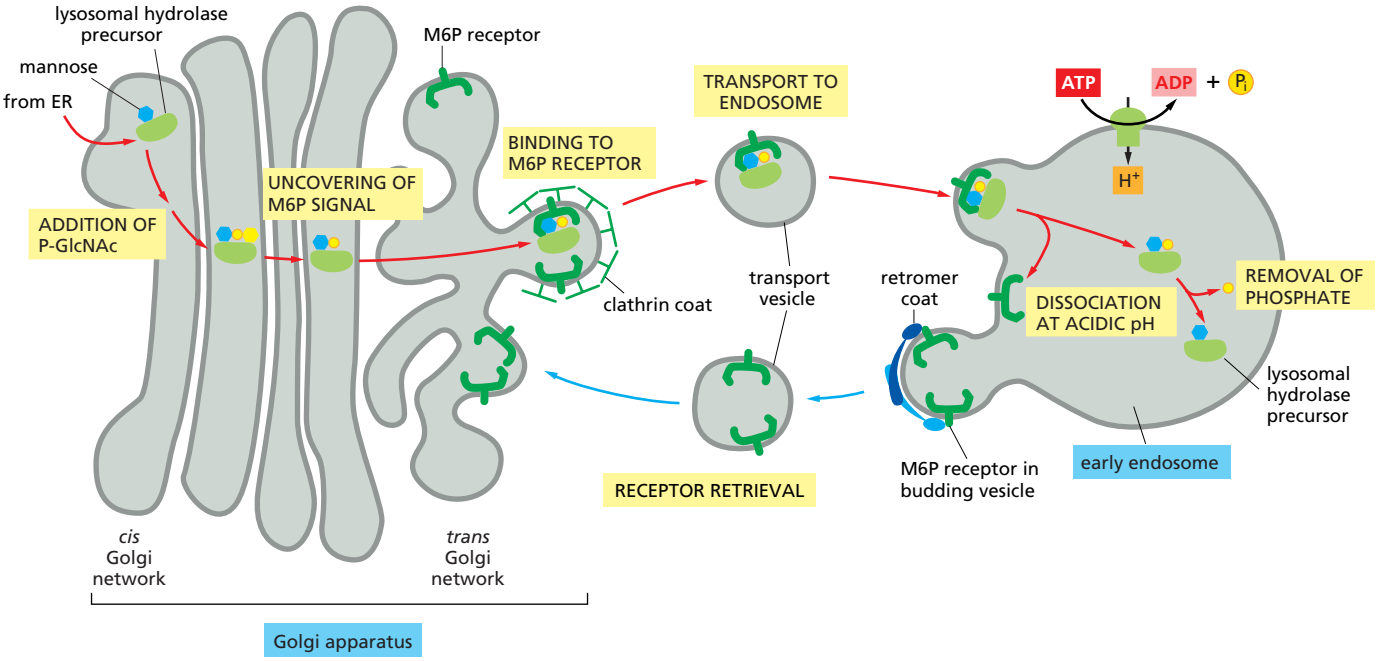


Figure 13–44 The structure of mannose 6-phosphate on a lysosomal hydrolase.



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* (**Figure 13–46**). Since most lysosomal hydrolases contain multiple oligosaccharides, they acquire many M6P groups, 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 inherited metabolic disorder called *inclusion-cell disease* (*I-cell disease*). In this condition, almost all of the hydrolytic enzymes are missing from the lysosomes of many cell types, and their undigested substrates accumulate in these lysosomes, which consequently form large *inclusions* in the cells. The consequent pathology is complex, affecting all organ systems, skeletal integrity, and mental development; individuals rarely live beyond six or seven years.

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, they are secreted rather than transported to lysosomes. The mis-sorting 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.

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

Figure 13–45 The transport of newly synthesized lysosomal hydrolases to endosomes. 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 13–46). The M6P-tagged hydrolases then segregate from all other types of proteins in the TGN because adaptor proteins (not shown) in the clathrin coat bind the M6P receptors, which, in turn, bind the M6P-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 retrieved in retromer-coated vesicles to the TGN for further rounds of transport. In the endosomes, the phosphate is removed from the M6P attached to the hydrolases, which may further ensure that the hydrolases do not return to the TGN with the receptor.

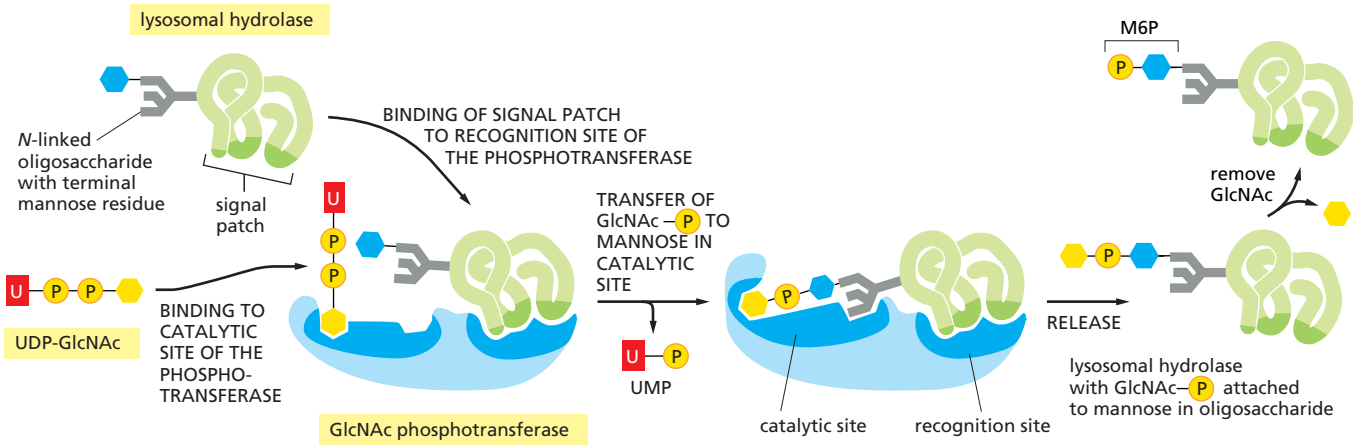


Figure 13–46 The recognition of a lysosomal hydrolase. A GlcNAc phosphotransferase recognizes lysosomal hydrolases in the Golgi apparatus. The enzyme has separate catalytic and recognition sites. The catalytic site binds both high-mannose *N*-linked oligosaccharides and UDP-GlcNAc. The recognition site binds to a signal patch that is present only on the surface of lysosomal hydrolases. A second enzyme cleaves off the GlcNAc, leaving the mannose 6-phosphate exposed.

pathway for directing hydrolases to lysosomes that is used by some cell types but not others. Alternative sorting receptors function in these M6P-independent pathways. 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.

Some Lysosomes and Multivesicular Bodies 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 indigestible 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). Under certain conditions, multivesicular bodies can also fuse with the plasma membrane. If that occurs, their intralumenal vesicles are released from cells. Circulating small vesicles, also called *exosomes*, have been observed in the blood and may be used to transport components between cells, although the importance of such a mechanism of potential communication between distant cells is unknown. Some exosomes may derive from direct vesicle budding events at the plasma membrane, which is a topologically equivalent process (see Figure 13–57).

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 transported from the lumen of the ER, through the Golgi apparatus; they are then carried from the trans Golgi network to endosomes by means of clathrin-coated transport vesicles, before moving on to lysosomes.

The lysosomal hydrolases contain N*-linked oligosaccharides that are covalently modified in a unique way in the* cis *Golgi so that their mannoses 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 the endosomes. The low pH in endosomes and the 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 to endosomes.*

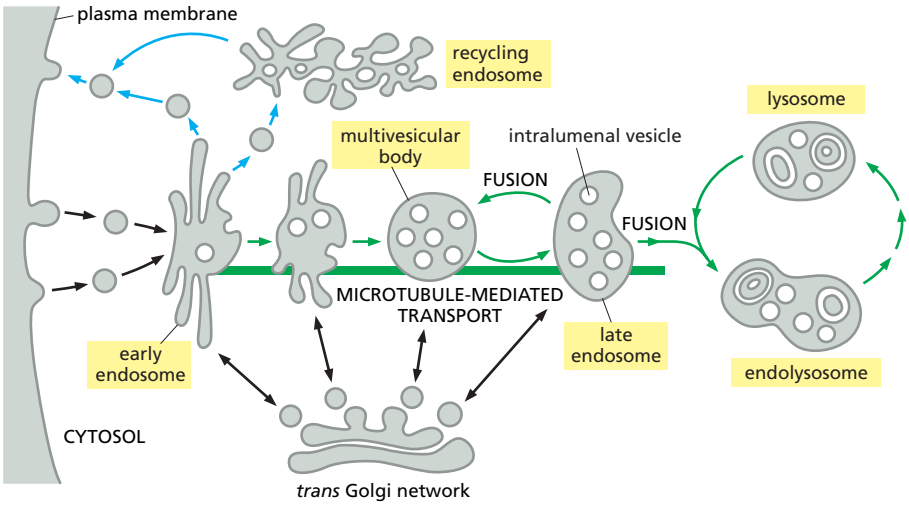
TRANSPORT INTO THE CELL FROM THE PLASMA MEMBRANE: ENDOCYTOSIS

The routes that lead inward from the cell surface start with the process of **endocytosis**, by which cells take up plasma membrane components, fluid, solutes, macromolecules, and particulate substances. Endocytosed cargo includes receptor–ligand complexes, a spectrum of nutrients and their carriers, extracellular matrix components, cell debris, bacteria, viruses, and, in specialized cases, even other cells. Through endocytosis, the cell regulates the composition of its plasma membrane in response to changing extracellular conditions.

In endocytosis, 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. Most eukaryotic cells constantly form endocytic vesicles, a process called *pinocytosis* ("cell drinking"); in addition, some specialized cells contain dedicated pathways that take up large particles on demand, a process called *phagocytosis* ("cell eating"). Endocytic vesicles form at the plasma membrane by multiple mechanisms that differ in both the molecular machinery used and how that machinery is regulated.

Once generated at the plasma membrane, most endocytic vesicles fuse with a common receiving compartment, the *early endosome*, where internalized cargo is sorted: some cargo molecules are returned to the plasma membrane, either directly or via a *recycling endosome*, and others are designated for degradation by inclusion in a *late endosome*. Late endosomes form from a bulbous, vacuolar portion of early endosomes by a process called *endosome maturation*. This conversion process changes the protein composition of the endosome membrane, patches of which invaginate and become incorporated within the organelles as *intralumenal vesicles*, while the endosome itself moves from the cell periphery to a location close to the nucleus. As an endosome matures, it ceases to recycle material to the plasma membrane and irreversibly commits its remaining contents to degradation: late endosomes fuse with one another and with lysosomes to form endolysosomes, which degrade their contents, as discussed earlier (**Figure 13–47**).

Each of the stages of endosome maturation—from the early endosome to the endolysosome—is connected through bidirectional vesicle transport pathways to



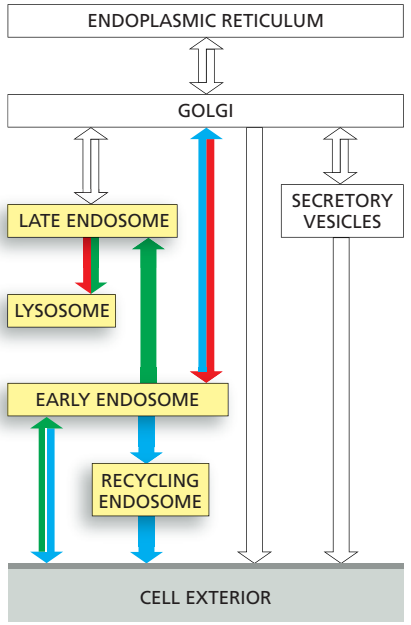


Figure 13–47 Endosome maturation: the endocytic pathway from the plasma membrane to lysosomes. Endocytic vesicles fuse near the cell periphery with an early endosome, which is the primary sorting station. Tubular portions of the early endosome bud off vesicles that recycle endocytosed cargo back to the plasma membrane—either directly, or indirectly via recycling endosomes. Recycling endosomes can store proteins until they are needed. Conversion of early endosomes to late endosomes is accompanied by loss of the tubular projections. Membrane proteins destined for degradation are internalized in intralumenal vesicles. The developing late endosome, or multivesicular body, moves on microtubules to the cell interior. Fully matured late endosomes no longer send vesicles to the plasma membrane, and they fuse with one another and with endolysosomes and lysosomes to degrade their contents. Each stage of endosome maturation is connected via transport vesicles with the TGN, providing a continuous supply of newly synthesized lysosomal proteins.

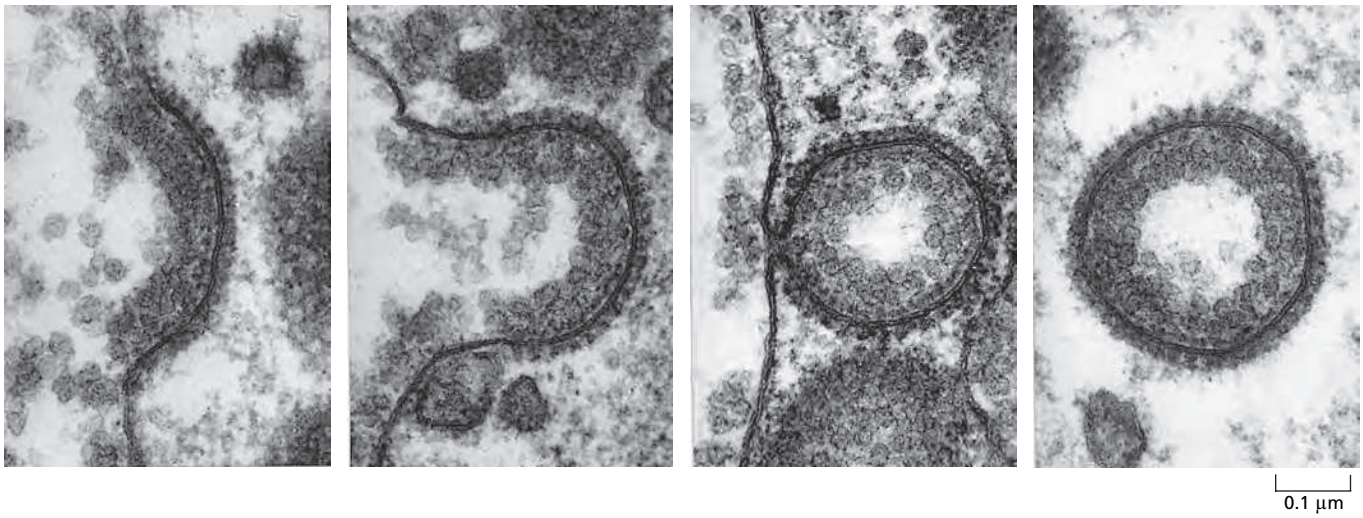


Figure 13–48 **The formation of clathrin-coated vesicles from the plasma membrane.** These electron micrographs illustrate the probable sequence of events in the formation of a clathrin-coated vesicle from a clathrin-coated pit. The clathrin-coated pits and vesicles shown are larger than those seen in normal-sized cells; they are from a very large hen oocyte and they take up lipoprotein particles 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 coated pit and vesicle. (Courtesy of M.M. Perry and A.B. Gilbert, *J. Cell Sci.* 39:257–272, 1979. With permission from The Company of Biologists.)

the TGN. These pathways allow insertion of newly synthesized materials, such as lysosomal enzymes arriving from the ER, and the retrieval of components, such as the M6P receptor, back into the early parts of the secretory pathway. We next discuss how the cell uses and controls the various features of endocytic trafficking.

Pinocytic Vesicles Form from Coated Pits in the Plasma Membrane

Virtually all eukaryotic cells continually ingest portions of their plasma membrane in the form of small pinocytic (endocytic) vesicles. The rate at which plasma membrane is internalized in this process of **pinocytosis** varies between cell types, but it is usually surprisingly high. 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 a nerve terminal.

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**). About 2500 clathrin-coated vesicles pinch off from 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 fuse with early endosomes.

Not All Pinocytic Vesicles Are Clathrin-Coated

In addition to clathrin-coated pits and vesicles, cells can form other types of pinocytic vesicles, such as **caveolae** (from the Latin for "little cavities"), originally recognized by their ability to transport molecules across endothelial cells that form the inner lining of blood vessels. Caveolae, sometimes seen in the electron microscope as deeply invaginated flasks, are present in the plasma membrane of most vertebrate cell types (**Figure 13–49**). They are thought to form from *lipid rafts* in the plasma membrane (discussed in Chapter 10), which are especially rich

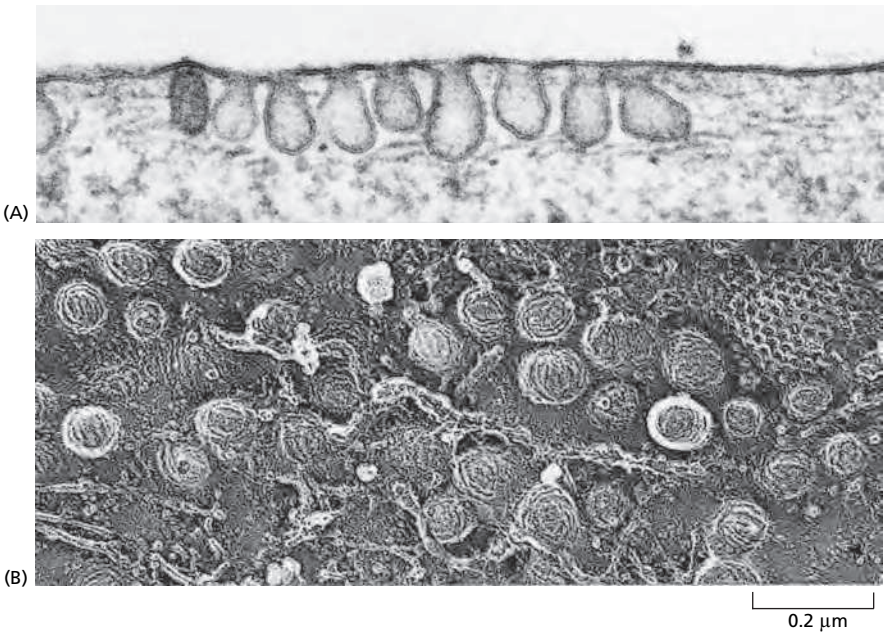


Figure 13–49 Caveolae in the plasma membrane of a fibroblast. (A) This electron micrograph shows a plasma membrane with a very high density of caveolae. (B) This rapid-freeze deep-etch image demonstrates the characteristic "cauliflower" texture of the cytosolic face of the caveolae membrane. The characteristic texture is thought to result from aggregates of caveolins and cavins. 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.)

in cholesterol, glycosphingolipids, and glycosylphosphatidylinositol (GPI)-anchored membrane proteins (see Figure 10–13). The major structural proteins in caveolae are **caveolins**, 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. On their cytosolic side, caveolins are bound to large protein complexes of caving proteins, which are thought to stabilize the membrane curvature.

In contrast to clathrin-coated and COPI- or COPII-coated vesicles, caveolae are usually static structures. Nonetheless, they can be induced to pinch off and serve as endocytic vesicles to transport cargo to early endosomes or to the plasma membrane on the opposite side of a polarized cell (in a process called *transcytosis*, which we discuss later). Some animal viruses such as SV40 and papillomavirus (which causes warts) enter cells in vesicles derived from caveolae. The viruses are first delivered to early endosomes and move from there in transport vesicles to the lumen of the ER. The viral genome exits across the ER membrane into the cytosol, from where it is imported into the nucleus to start the infection cycle. Cholera toxin (discussed in Chapters 15 and 19) also enters the cell through caveolae and is transported to the ER before entering the cytosol.

Macropinocytosis is another clathrin-independent endocytic mechanism that can be activated in practically all animal cells. In most cell types, it does not operate continually but rather is induced for a limited time in response to cell-surface receptor activation by specific cargoes, including growth factors, integrin ligands, apoptotic cell remnants, and some viruses. These ligands activate a complex signaling pathway, resulting in a change in actin dynamics and the formation of cell-surface protrusions, called *ruffles* (discussed in Chapter 16). When ruffles collapse back onto the cell, large fluid-filled endocytic vesicles form, called *macropinosomes* (**Figure 13–50**), which transiently increase the bulk fluid uptake of a cell by up to tenfold. Macropinocytosis is a dedicated degradative pathway: macropinosomes acidify and then fuse with late endosomes or endolysosomes, without recycling their cargo back to the plasma membrane.

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

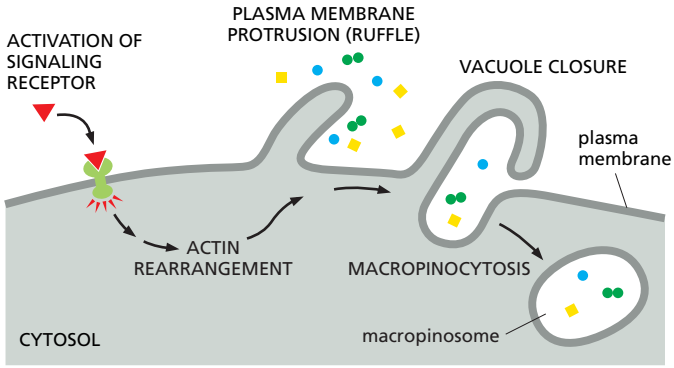


Figure 13–50 Schematic representation of macropinocytosis. Cell signaling events lead to a reprogramming of actin dynamics, which in turn triggers the formation of cell-surface ruffles. As the ruffles collapse back onto the cell surface, they nonspecifically trap extracellular fluid and macromolecules and particles contained in it, forming large vacuoles, or macropinosomes, as shown.

complementary transmembrane receptor proteins, which 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 efficiently taken up in large amounts. A particularly well-understood and physiologically important example is the process that mammalian cells use to import cholesterol.

Many animal 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 13–51). 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. There, an endocytosis signal in the cytoplasmic tail of the LDL receptors binds the membrane-bound adaptor protein AP2 after its conformation has been locally unlocked by binding to $PI(4,5)P_2$ on the plasma membrane. Coincidence detection, as discussed earlier, thus imparts both efficiency and selectivity to the process (see Figure 13–9). AP2 then recruits clathrin to initiate endocytosis.

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. Once the LDL and LDL receptors encounter the low pH in early 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 (Movie 13.3). 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 both 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 prematurely, and many would die at an early age of heart attacks resulting from coronary artery disease if they were not treated with drugs such as statins 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

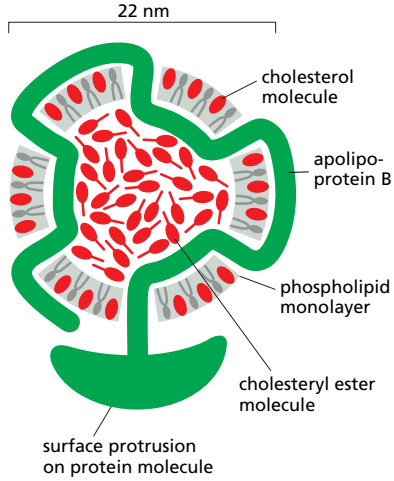


Figure 13–51 A low-density lipoprotein (LDL) particle. Each spherical particle has a mass of 3×10^6 daltons. It contains a core of about 1500 cholesterol molecules esterified to long-chain fatty acids. A lipid monolayer composed of about 800 phospholipid and 500 unesterified cholesterol molecules surrounds the core of cholesteryl esters. A single molecule of apolipoprotein B, a 500,000-dalton beltlike protein, organizes the particle and mediates the specific binding of LDL to cell-surface LDL receptors.

intracellular binding site that attaches the receptor AP2 adaptor protein in clathrin-coated pits. 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 accommodate more than 100 receptors of assorted varieties.

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

Early endosomes are the main sorting station in the endocytic pathway, just as the *cis* and *trans* Golgi networks serve this function in the 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 (although cholesterol is an exception, as just discussed), 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.

In the early endosome, the LDL receptor dissociates from its ligand, LDL, and is recycled back to the plasma membrane for reuse, leaving the discharged LDL to be carried to lysosomes (**Figure 13–52**). The recycling transport vesicles bud from long, narrow tubules that extend from the 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. The transport vesicles return the LDL receptor directly to 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

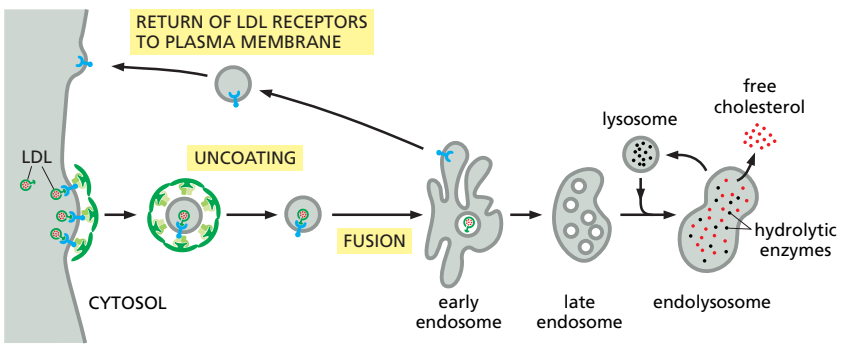


Figure 13–52 The receptor-mediated endocytosis of LDL. Note that the LDL dissociates from its receptors in the acidic environment of the early endosome. After a number of steps, the LDL ends up in endolysosomes and lysosomes, where it is degraded to release free cholesterol. In contrast, the LDL receptors are returned to the plasma membrane via 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 life-span.

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. 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 early endosomes, avoiding lysosomes and delivering iron to the cell interior, as needed for cells to grow and proliferate.

Plasma Membrane Signaling Receptors are Down-Regulated by Degradation in Lysosomes

A second pathway that endocytosed receptors can follow from endosomes is taken by many signaling receptors, including opioid receptors 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 their ligand, and most 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 downregulation*, that reduces the cell's subsequent sensitivity to EGF (see Figure 15–20).

Receptor downregulation is highly regulated. The activated receptors are first covalently modified on the cytosolic face with the small protein ubiquitin. Unlike *polyubiquitylation*, which adds a chain of ubiquitins that typically targets a protein for degradation in proteasomes (discussed in Chapter 6), ubiquitin tagging for sorting into the clathrin-dependent endocytic pathway adds just one or a few single ubiquitin molecules to the protein—a process called *monoubiquitylation* or *multiubiquitylation*, respectively. Ubiquitin-binding proteins recognize the attached ubiquitin and help direct the modified receptors into clathrin-coated pits. After delivery to the early endosome, other ubiquitin-binding proteins that are part of *ESCRT complexes* (ESCRT = Endosome Sorting Complex Required for Transport) recognize and sort the ubiquitylated receptors into intralumenal vesicles, which are retained in the maturing late endosome (see Figure 13–47). In this way, addition of ubiquitin blocks receptor recycling to the plasma membrane and directs the receptors into the degradation pathway, as we discuss next.

Early Endosomes Mature into Late Endosomes

The endosomal compartments 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 allowing varying lengths of time for the cell to endocytose the tracer. The distribution of the molecule after its uptake reveals the sequence of events. Within a minute or so after adding the tracer, it starts to appear in **early endosomes**, just beneath the plasma membrane (**Figure 13–53**). By 5–15 minutes later, it has moved to **late endosomes**, close to the Golgi apparatus and near the nucleus.

How early endosomes arise is not entirely clear, but their membrane and volume are mainly derived from incoming endocytic vesicles that fuse with one another (**Movie 13.4**). Early endosomes are relatively small and patrol the cytoplasm underlying the plasma membrane in jerky back-and-forth movements along microtubules, capturing incoming vesicles. Typically, an early endosome receives incoming vesicles for about 10 minutes, during which time membrane and fluid is rapidly recycled to the plasma membrane. Some of the incoming cargo, however, accumulates over the lifetime of the early endosome, eventually to be included in the late endosome.

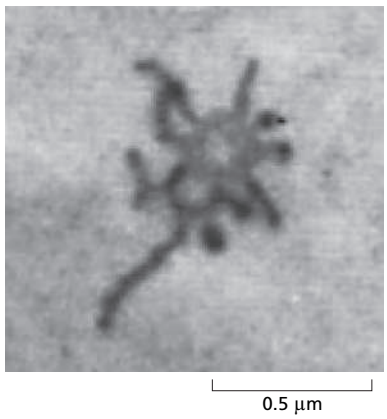


Figure 13–53 Electron micrograph of an early endosome. The endosome is labeled with endocytosed horseradish peroxidase, a widely used enzyme marker, detected in this case by an electron-dense reaction product. Many tubular extensions protrude from the central vacuolar space of the early endosome, which will later mature to give rise to a late endosome. (From J. Tooze and M. Hollinshead, *J. Cell Biol.* 118:813–830, 1992.)

Early endosomes have tubular and vacuolar domains (see Figure 13–53). Most of the membrane surface is in the tubules and most of the volume is in the vacuolar domain. During **endosome maturation**, the two domains have different fates: the vacuolar portions of the early endosome are retained and transformed into late endosomes; the tubular portions shrink. Maturing endosomes, also called *multivesicular bodies*, migrate along microtubules toward the cell interior, shedding membrane tubules and vesicles that recycle material to the plasma membrane and TGN, and receiving newly synthesized lysosomal proteins. As they concentrate in a perinuclear region of the cell, the multivesicular bodies fuse with each other, and eventually with endolysosomes and lysosomes (see Figure 13–47).

Many changes occur during the maturation process. (1) The endosome changes shape and location, as the tubular domains are lost and the vacuolar domains are thoroughly modified. (2) Rab proteins, phosphoinositide lipids, fusion machinery (SNAREs and tethers), and microtubule motor proteins all participate in a molecular makeover of the cytosolic face of the endosome membrane, changing the functional characteristics of the organelle. (3) A V-type ATPase in the endosome membrane pumps H^+ from the cytosol into the endosome lumen and acidifies the organelle. Crucially, the increasing acidity that accompanies maturation renders lysosomal hydrolases increasingly more active, influencing many receptor–ligand interactions, thereby controlling receptor loading and unloading. (4) Intralumenal vesicles sequester endocytosed signaling receptors inside the endosome, thus halting the receptor signaling activity. (5) Lysosome proteins are delivered from the TGN to the maturing endosome. Most of these events occur gradually but eventually lead to a complete transformation of the endosome into an early endolysosome.

In addition to committing selected cargo for degradation, the maturation process is important for lysosome maintenance. The continual delivery of lysosome components from the TGN to maturing endosomes, ensures a steady supply of new lysosome proteins. The endocytosed materials mix in early endosomes with newly arrived acid hydrolases. Although mild digestion may start here, many hydrolases are synthesized and delivered as proenzymes, called *zymogens*, which contain extra inhibitory domains that keep the hydrolases inactive until these domains are proteolytically removed at later stages of endosome maturation. Moreover, the pH in early endosomes is not low enough to activate lysosomal hydrolases optimally. By these means, cells can retrieve membrane proteins intact from early endosomes and recycle them back to the plasma membrane.

ESCRT Protein Complexes Mediate the Formation of Intralumenal Vesicles in Multivesicular Bodies

As endosomes mature, patches of their membrane invaginate into the endosome lumen and pinch off to form intralumenal vesicles. Because of their appearance in the electron microscope such maturing endosomes are also called **multivesicular bodies** (**Figure 13–54**).

The multivesicular bodies carry endocytosed membrane proteins that are to be degraded. As part of the protein-sorting process, receptors destined for degradation, such as the occupied EGF receptors described previously, selectively partition into the invaginating membrane of the multivesicular bodies. In this way, both the receptors and any signaling proteins strongly bound to them are sequestered away from the cytosol where they might otherwise continue signaling. They also are made fully accessible to the digestive enzymes that eventually will degrade them (**Figure 13–55**). In addition to endocytosed membrane proteins, multivesicular bodies include the soluble content of early endosomes destined for late endosomes and digestion in lysosomes.

As discussed earlier, sorting into intralumenal vesicles requires one or multiple ubiquitin tags, which are added to the cytosolic domains of membrane proteins. These tags initially help guide the proteins into clathrin-coated vesicles in the plasma membrane. Once delivered to the endosomal membrane, the ubiquitin tags are recognized again, this time by a series of cytosolic **ESCRT protein**

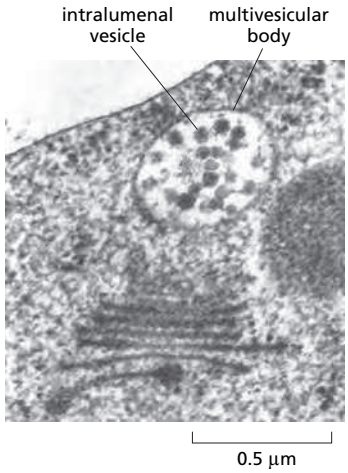


Figure 13–54 Electron micrograph of a multivesicular body. The large amount of internal membrane will be delivered to the lysosome, for digestion. (Courtesy of Andrew Staehelin, from A. Driouich, A. Jauneau and L.A. Staehelin; *Plant Physiol*. 113:487–492, 1997. With permission from the American Society of Plant Biologists.)

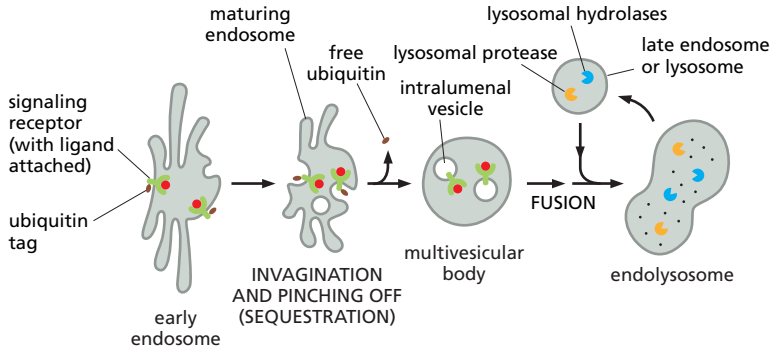


Figure 13–55 The sequestration of endocytosed proteins into intralumenal vesicles of multivesicular bodies. Ubiquitylated membrane proteins are sorted into domains on the endosome membrane, which invaginate and pinch off to form intralumenal vesicles. The ubiquitin marker is removed and returned to the cytosol for reuse before the intralumenal vesicle closes. Eventually, proteases and lipases in lysosomes digest all of the internal membranes. The invagination processes are essential for complete digestion of endocytosed membrane proteins: because the outer membrane of the multivesicular body becomes continuous with the lysosomal membrane, which is resistant to lysosomal hydrolases; the hydrolases, for example, could not digest the cytosolic domains of endocytosed transmembrane proteins, such as the EGF receptor shown here, if the protein were not localized in intralumenal vesicles.

complexes, (*ESCRT-0, -I, -II,* and *-III*), which bind sequentially and ultimately mediate the sorting process into the intralumenal vesicles. Membrane invagination into multivesicular bodies also depends on a lipid kinase that phosphorylates phosphatidylinositol to produce PI(3)P, which serves as an additional docking site for the ESCRT complexes; these complexes require both PI(3)P and the presence of ubiquitylated cargo proteins to bind to the endosomal membrane. ESCRT-III forms large multimeric assemblies on the membrane that bend the membrane (**Figure 13–56**).

Mutant cells compromised in ESCRT function display signaling defects. In such cells, activated receptors cannot be down-regulated by endocytosis and packaging into multivesicular bodies. The still-active receptors therefore mediate prolonged signaling, which can lead to uncontrolled cell proliferation and cancer.

Processes that shape membranes often use similar machinery. Because of strong similarities in their protein sequences, researchers think that ESCRT complexes are evolutionarily related to components that mediate cell-membrane deformation in cytokinesis in archaea. Similarly, the ESCRT machinery that drives the internal budding from the endosome membrane to form intralumenal vesicles is also used in animal cell cytokinesis and virus budding, which are topologically equivalent, as both processes involve budding away from the cytosolic surface of the membrane (**Figure 13–57**).

Recycling Endosomes Regulate Plasma Membrane Composition

The fates of endocytosed receptors—and of any ligands remaining bound to them—vary according to the specific type of receptor. As we discussed, most receptors are recycled and returned to the same plasma membrane domain from which they came; some proceed to a different domain of the plasma membrane, thereby mediating **transcytosis**; and some progress to lysosomes, where they are degraded.

Receptors on the surface of polarized epithelial cells can transfer specific macromolecules from one extracellular space to another by transcytosis. A newborn, for example, obtains antibodies from its mother's milk (which help protect it against infection) by transporting them across the epithelium of its gut. The lumen of the gut is acidic, and, at this low pH, the antibodies in the milk bind to specific receptors on the apical (absorptive) surface of the gut epithelial cells. The receptor–antibody complexes are internalized via clathrin-coated pits and

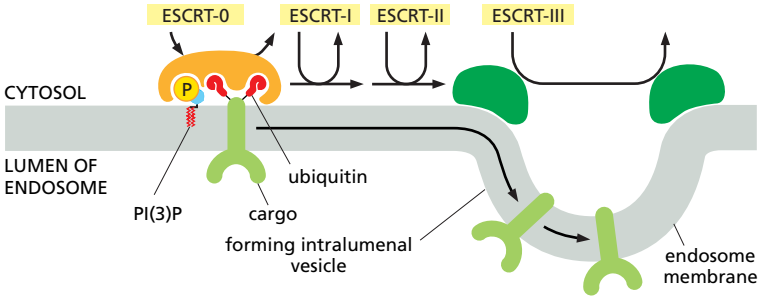


Figure 13–56 Sorting of endocytosed membrane proteins into the intralumenal vesicles of a multivesicular body. A series of complex binding events passes the ubiquitylated cargo proteins sequentially from one ESCRT complex (ESCRT-0) to the next, eventually concentrating them in membrane areas that bud into the lumen of the endosome to form intralumenal vesicles. ESCRT-III assembles into expansive multimeric structures and mediates invagination. The mechanisms of how cargo molecules are shepherded into the vesicles and how the vesicles are formed without including the ESCRT complexes themselves remain unknown. ESCRT complexes are soluble in the cytosol, are recruited to the membrane sequentially as needed, and are then released back into the cytosol as the vesicle pinches off.

vesicles and are delivered to early endosomes. The complexes remain intact and are retrieved in transport vesicles that bud from the early endosome and subsequently fuse with the basolateral domain of the plasma membrane. On exposure to the neutral pH of the extracellular fluid that bathes the basolateral surface of the cells, the antibodies dissociate from their receptors and eventually enter the baby's bloodstream.

The transcytotic pathway from the early endosome back to the plasma membrane is not direct. The receptors first move from the early endosome to the **recycling endosome**. The variety of pathways that different receptors follow from early endosomes implies that, in addition to binding sites for their ligands and binding sites for coated pits, many receptors also possess sorting signals that guide them into the appropriate transport pathway (**Figure 13–58**).

Cells can regulate the release of membrane proteins from recycling endosomes, thus adjusting the flux of proteins through the transcytotic pathway according to need. This regulation, the mechanism of which is uncertain, allows recycling endosomes to play an important part in adjusting the concentration of specific plasma membrane proteins. Fat cells and muscle cells, for example, contain large intracellular pools of the glucose transporters that are responsible for the uptake of glucose across the plasma membrane. These membrane transport proteins are stored in specialized recycling endosomes until the hormone *insulin* stimulates the cell to increase its rate of glucose uptake. In response to the insulin signal, transport vesicles rapidly bud from the recycling endosome and deliver large numbers of glucose transporters to the plasma membrane, thereby greatly increasing the rate of glucose uptake into the cell (**Figure 13–59**). Similarly, kidney cells regulate the insertion of aquaporins and V-ATPase into the plasma membrane to increase water and acid excretion, respectively, both in response to hormones.

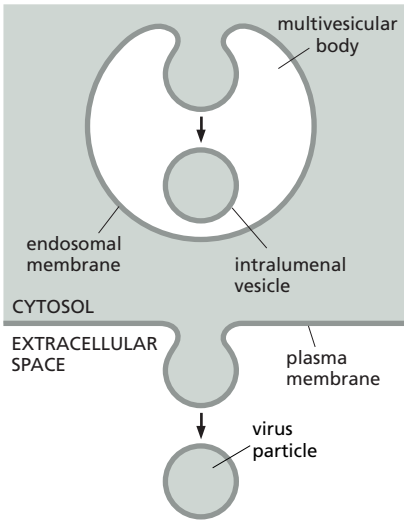


Figure 13–57 Conserved mechanism in multivesicular body formation and virus budding. In the two topologically equivalent processes indicated by the arrows, ESCRT complexes (not shown) shape membranes into buds that bulge away from the cytosol.

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. Phagocytosis is distinct, both in purpose and mechanism, from macropinocytosis, which we discussed earlier. 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 products of hydrolysis.

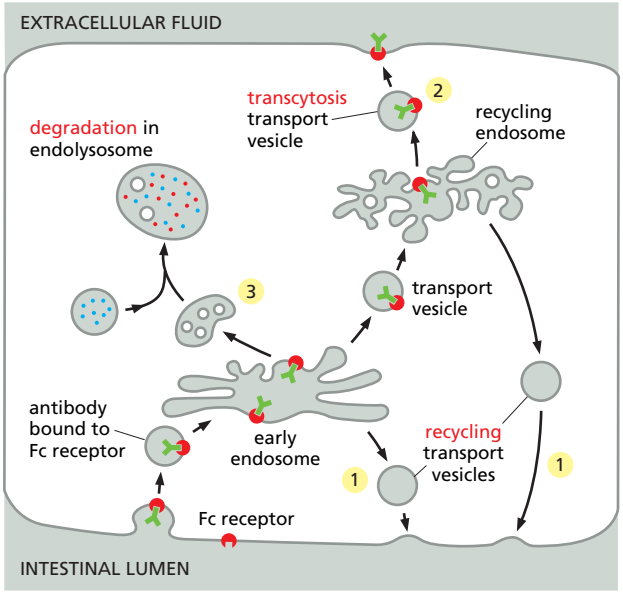


Figure 13–58 Possible fates for transmembrane receptor proteins that have been endocytosed. Three pathways from the early endosomal compartment in an epithelial cell are shown. Retrieved receptors are returned (1) to the same plasma membrane domain from which they came (*recycling*) or (2) via a recycling endosome to a different domain of the plasma membrane (*transcytosis*). (3) Receptors that are not specifically retrieved from early or recycling endosomes follow the pathway from the endosomal compartment to lysosomes, where they are degraded (*degradation*). If the ligand that is endocytosed with its receptor stays bound to the receptor in the acidic environment of the endosome, it shares the same fate as the receptor; otherwise, it is delivered to lysosomes. Recycling endosomes are a way-station on the transcytotic pathway. In the transcytosis example shown here, an antibody Fc receptor on a gut epithelial cell binds antibody and is endocytosed, eventually carrying the antibody to the basolateral plasma membrane. The receptor is called an Fc receptor because it binds the Fc part of the antibody (discussed in Chapter 24).

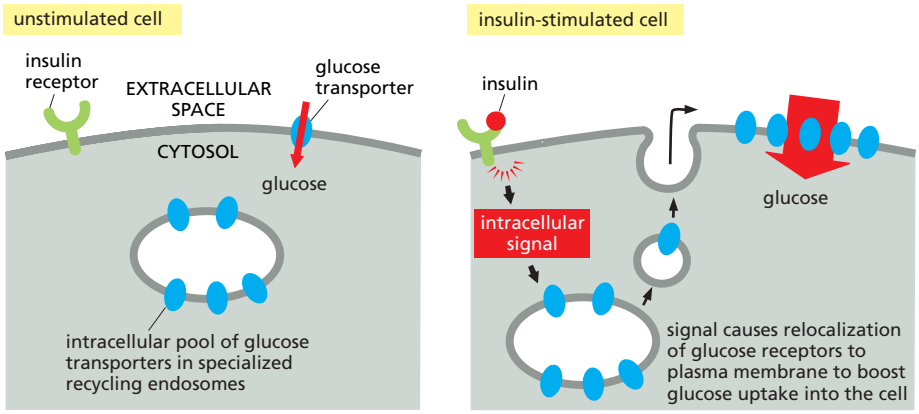


Figure 13–59 Storage of plasma membrane proteins in recycling endosomes. Recycling endosomes can serve as an intracellular storage site for specialized plasma membrane proteins that can be mobilized when needed. In the example shown, insulin binding to the insulin receptor triggers an intracellular signaling pathway that causes the rapid insertion of glucose transporters into the plasma membrane of a fat or muscle cell, greatly increasing its glucose intake.

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 important classes of white blood cells that act as professional phagocytes are **macrophages** and **neutrophils** (Movie 13.5). These cells develop from hemopoietic stem cells (discussed in Chapter 22), 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, for example, phagocytose more than 10^{11} senescent red blood cells in each of us every day.

The diameter of a phagosome is determined by the size of its ingested particles, and those particles can be almost as large as the phagocytic cell itself (Figure 13–60). Phagosomes fuse with lysosomes, and the ingested material is then degraded. Indigestible substances remain in the lysosomes, forming *residual bodies* that can be excreted from cells by exocytosis, as mentioned earlier. 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.

Some pathogenic bacteria have evolved elaborate mechanisms to prevent phagosome–lysosome fusion. The bacterium *Legionella pneumophila*, for example, which causes Legionnaires' disease (discussed in Chapter 23), injects into its unfortunate host a Rab-modifying enzyme that causes certain Rab proteins to misdirect membrane traffic, thereby preventing phagosome–lysosome fusion. The bacterium, thus spared from lysosomal degradation, remains in the modified phagosome, growing and dividing as an intracellular pathogen, protected from the host's adaptive immune system.

Phagocytosis is a cargo-triggered process. That is, it requires the activation of cell-surface receptors that transmit signals to the cell interior. Thus, to be phagocytosed, particles must first bind to the surface of the phagocyte (although not all particles that bind are ingested). Phagocytes have a variety of cell surface receptors that are functionally linked to the phagocytic machinery of the cell. The best-characterized triggers of phagocytosis are antibodies, which protect us by binding to the surface of infectious microorganisms (pathogens) and initiating a series of events that culminate in the invader being phagocytosed. When antibodies initially attack a pathogen, they coat it with antibody molecules that bind to *Fc receptors* on the surface of macrophages and neutrophils, activating the receptors to induce the phagocytic cell to extend pseudopods, which engulf the particle and fuse at their tips to form a phagosome (Figure 13–61A). Localized actin polymerization, initiated by Rho family GTPases and their activating RhoGEFs (discussed in Chapters 15 and 16), shapes the pseudopods. The activated Rho GTPases switch on the kinase activity of local PI kinases to produce $PI(4,5)P_2$ in the membrane (see Figure 13–11), which stimulates actin polymerization. To seal off the phagosome and complete the engulfment, actin is depolymerized by a PI 3-kinase that converts the $PI(4,5)P_2$ to $PI(3,4,5)P_3$, which is required for closure

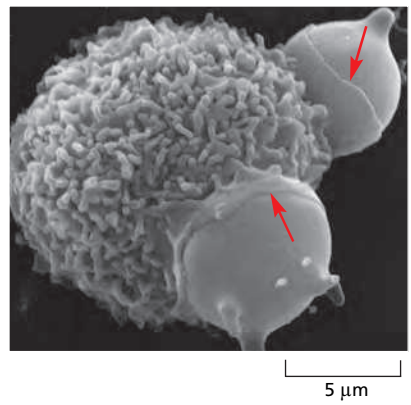


Figure 13–60 Phagocytosis by a macrophage. A scanning electron micrograph of a mouse macrophage phagocytosing two chemically altered red blood cells. The *red arrows* point to edges of thin processes (pseudopods) of the macrophage that are extending as collars to engulf the red cells. (Courtesy of Jean Paul Revel.)

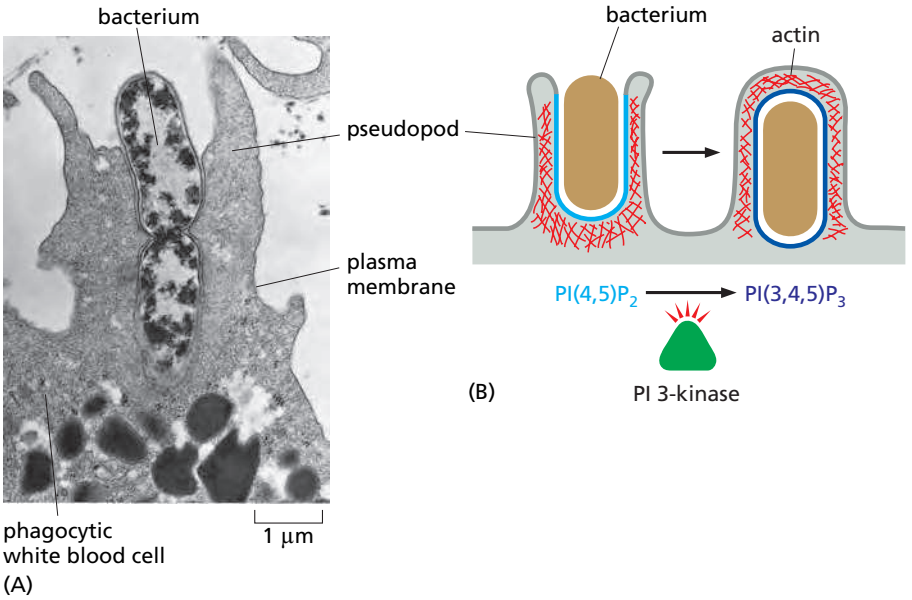


Figure 13–61 A neutrophil reshaping its plasma membrane during phagocytosis. (A) An electron micrograph of a neutrophil phagocytosing a bacterium, which is in the process of dividing. (B) Pseudopod extension and phagosome formation are driven by actin polymerization and reorganization, which respond to the accumulation of specific phosphoinositides in the membrane of the forming phagosome: $PI(4,5)P_2$ stimulates actin polymerization, which promotes pseudopod formation, and then $PI(3,4,5)P_3$ depolymerizes actin filaments at the base. (A, courtesy of Dorothy F. Bainton, Phagocytic Mechanisms in Health and Disease. New York: Intercontinental Medical Book Corporation, 1971.)

of the phagosome and may also contribute to reshaping the actin network to help drive the invagination of the forming phagosome (Figure 13–61B). 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 pathogens. 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.

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 cells in their own body. The living cells 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. Apoptotic 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.

Summary

Cells ingest fluid, molecules, and particles by endocytosis, in which localized regions of the plasma membrane invaginate and pinch off to form endocytic vesicles. In most cells, endocytosis internalizes a large fraction of the plasma membrane every hour. The cells remain the same size because most of the plasma membrane components (proteins and lipids) that are endocytosed are continually returned to the cell surface by exocytosis. This large-scale endocytic–exocytic cycle is mediated largely by clathrin-coated pits and vesicles but clathrin-independent endocytic pathways also contribute.

While many of the endocytosed molecules are quickly recycled to the plasma membrane, others eventually end up in lysosomes, where they are degraded. Most of the ligands that are endocytosed with their receptors dissociate from their receptors

in the acidic environment of the endosome and eventually end up in lysosomes, while most of the receptors are recycled via transport vesicles back to the cell surface for reuse. Many cell-surface signaling receptors become tagged with ubiquitin when activated by binding their extracellular ligands. Ubiquitylation guides activated receptors into clathrin-coated pits, they and their ligands are efficiently internalized and delivered to early endosomes.

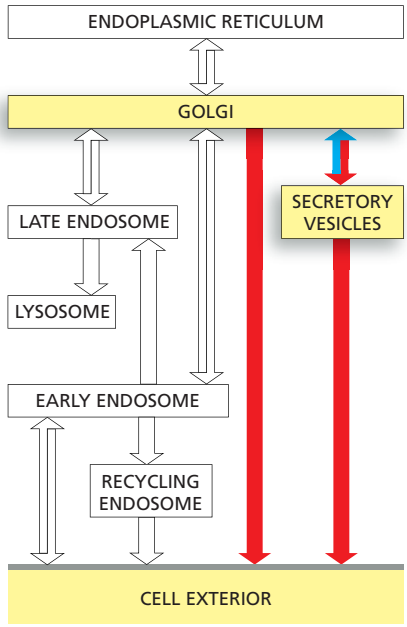
Early endosomes, rapidly mature into late endosomes. During maturation, patches of the endosomal membrane containing ubiquitylated receptors invaginate and pinch off to form intralumenal vesicles. This process is mediated by ESCRT complexes and sequesters the receptors away from the cytosol, which terminates their signaling activity. Late endosomes migrate along microtubules toward the interior of the cell where they fuse with one another and with lysosomes to form endolysosomes, where degradation occurs.

In some cases, both receptor and ligand are transferred to a different plasma membrane domain, causing the ligand to be released at a different surface from where it originated, a process called transcytosis. In some cells, endocytosed plasma membrane proteins and lipids can be stored in recycling endosomes, for as long as necessary until they are needed.

TRANSPORT FROM THE *TRANS* GOLGI NETWORK TO THE CELL EXTERIOR: EXOCYTOSIS

Having considered the cell's endocytic and internal digestive systems and the various types of incoming membrane traffic that converge on lysosomes, we now return to the Golgi apparatus and examine the secretory pathways that lead outward to the cell exterior. Transport vesicles destined for the plasma membrane normally leave the TGN in a steady stream as irregularly shaped tubules. The membrane proteins and the lipids in these vesicles provide new components for the cell's plasma membrane, while the soluble proteins inside the vesicles are secreted to the extracellular space. The fusion of the vesicles with the plasma membrane is called **exocytosis**. This is the route, for example, by which cells secrete most of the proteoglycans and glycoproteins of the *extracellular matrix,* as discussed in Chapter 19.

All cells require this **constitutive secretory pathway**, which operates continuously (**Movie 13.6**). Specialized secretory cells, however, have a second secretory pathway in which soluble proteins and other substances are initially stored in *secretory vesicles* for later release by exocytosis. This is the **regulated secretory pathway**, found mainly in cells specialized for secreting products rapidly on demand—such as hormones, neurotransmitters, or digestive enzymes (**Figure 13–62**). In this section, we consider the role of the Golgi apparatus in both of these pathways and compare the two mechanisms of secretion.



Many Proteins and Lipids Are Carried Automatically from the *Trans* Golgi Network (TGN) to the Cell Surface

A cell capable of regulated secretion must separate at least three classes of proteins before they leave the TGN—those destined for lysosomes (via endosomes), those destined for secretory vesicles, and those destined for immediate delivery to the cell surface (**Figure 13–63**). We have already discussed how proteins destined for lysosomes are tagged with M6P for packaging into specific departing vesicles, and analogous signals are thought to direct *secretory proteins* into secretory vesicles. The nonselective constitutive secretory pathway transports most other proteins directly to the cell surface. Because entry into this pathway does not require a particular signal, it is also called the **default pathway**. Thus, in an unpolarized cell such as a white blood cell or a fibroblast, it seems that any protein in the lumen of the Golgi apparatus is automatically carried by the constitutive pathway to the cell surface unless it is specifically returned to the ER, retained as a resident protein in the Golgi apparatus itself, or selected for the pathways that lead to regulated secretion or to lysosomes. In polarized cells, where different products have to be

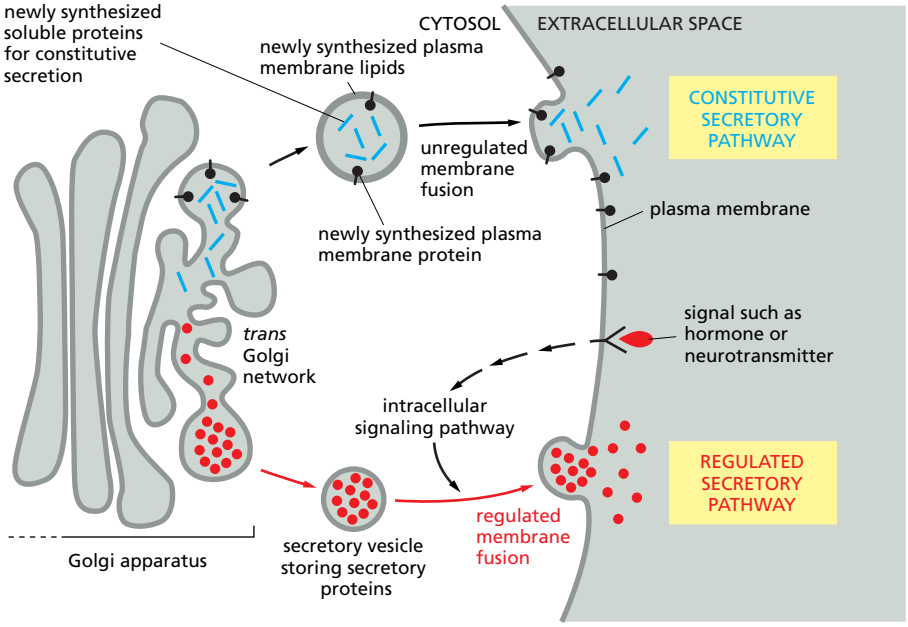


Figure 13–62 The constitutive and regulated secretory pathways. The two pathways diverge in the TGN. The constitutive secretory pathway operates in all cells. Many soluble proteins are continually secreted from the cell by this pathway, which also supplies the plasma membrane with newly synthesized membrane lipids and proteins. Specialized secretory cells also have a regulated secretory pathway, by which selected proteins in the TGN are diverted into secretory vesicles, where the proteins are concentrated and stored until an extracellular signal stimulates their secretion. The regulated secretion of small molecules, such as histamine and neurotransmitters, occurs by a similar pathway; these molecules are actively transported from the cytosol into preformed secretory vesicles. There they are often bound to specific macromolecules (proteoglycans, for histamine) so that they can be stored at high concentration without generating an excessively high osmotic pressure.

delivered to different domains of the cell surface, we shall see that the options are more complex.

Secretory Vesicles Bud from the *Trans* Golgi Network

Cells that are specialized for secreting some of their products rapidly on demand concentrate and store these products in **secretory vesicles** (often called *dense-core secretory granules* because they have dense cores when viewed in the electron microscope). Secretory vesicles form from the TGN, and they release their contents to the cell exterior by exocytosis in response to specific signals. The secreted product can be either a small molecule (such as histamine or a neuropeptide) or a protein (such as a hormone or digestive enzyme).

Proteins destined for secretory vesicles (called *secretory proteins*) are packaged into appropriate vesicles in the TGN by a mechanism that involves the selective aggregation of the secretory proteins. Clumps of aggregated, electron-dense material can be detected by electron microscopy in the lumen of the TGN. The signals that direct secretory proteins into such aggregates are not well defined and may be quite diverse. When a gene encoding a secretory protein is artificially expressed in a secretory cell that normally does not make the protein, the foreign protein is appropriately packaged into secretory vesicles. This observation

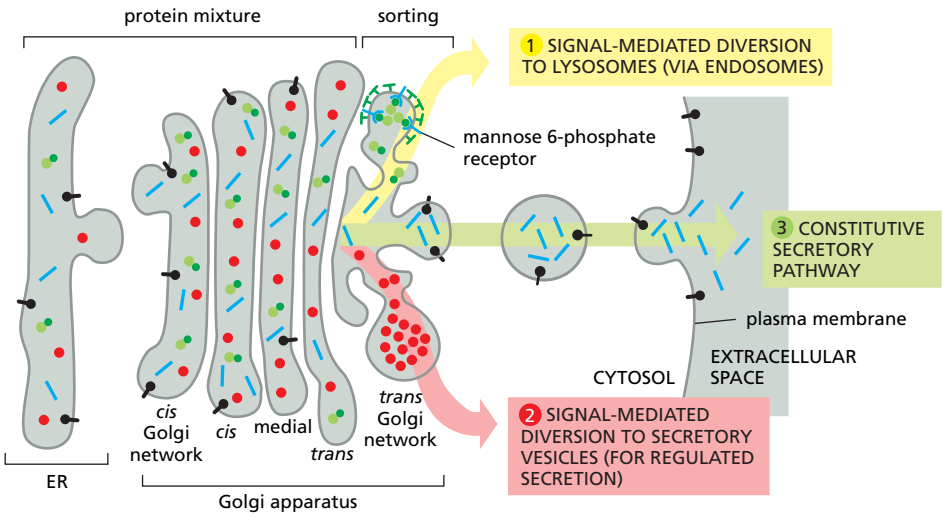


Figure 13–63 The three best-understood pathways of protein sorting in the *trans* Golgi network. (1) Proteins with the mannose 6-phosphate (M6P) marker (see Figure 13–45) are diverted to lysosomes (via endosomes) in clathrin-coated transport vesicles. (2) Proteins with signals directing them to secretory vesicles are concentrated in such vesicles as part of a regulated secretory pathway that is present only in specialized secretory cells. (3) In unpolarized cells, a constitutive secretory pathway delivers proteins with no special features to the cell surface. In polarized cells, such as epithelial cells, however, secreted and plasma membrane proteins are selectively directed to either the apical or the basolateral plasma membrane domain, so a specific signal must mediate at least one of these two pathways, as we discuss later.

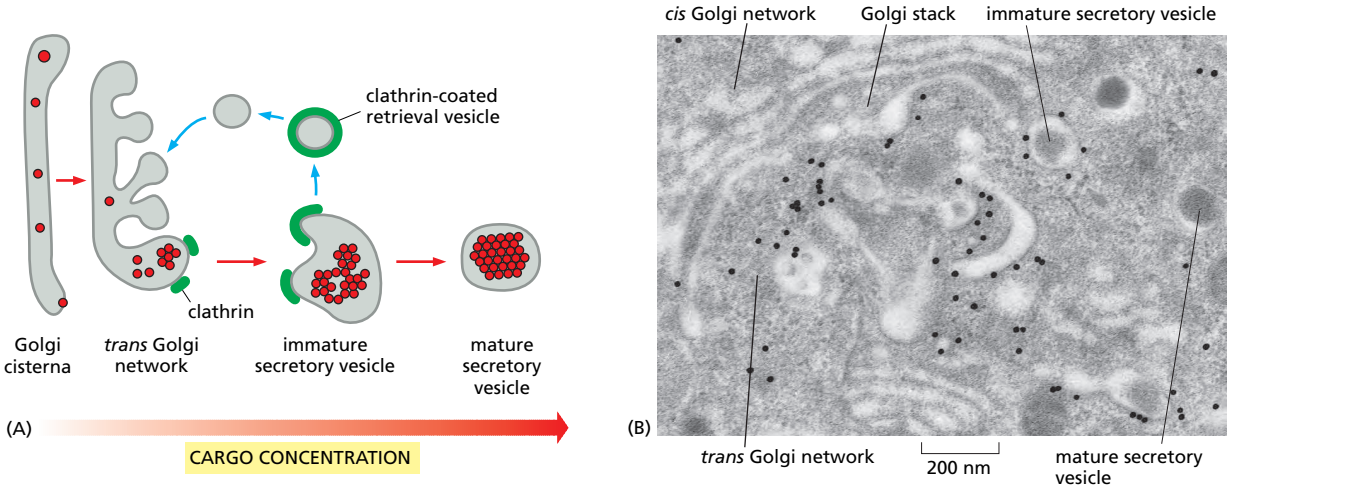


Figure 13–64 The formation of secretory vesicles. (A) Secretory proteins become segregated and highly concentrated in secretory vesicles by two mechanisms. First, they aggregate in the ionic environment of the TGN; often the aggregates become more condensed as secretory vesicles mature and their lumen becomes more acidic. Second, clathrin-coated vesicles retrieve excess membrane and lumenal content present in immature secretory vesicles as the secretory vesicles mature. (B) This electron micrograph shows secretory vesicles forming from the TGN in an insulin-secreting β cell of the pancreas. Anti-clathrin antibodies conjugated to gold spheres *(black dots)* have been used to locate clathrin molecules. The immature secretory vesicles, which contain insulin precursor protein (proinsulin), contain clathrin patches, which are no longer seen on the mature secretory vesicle. (B, courtesy of Lelio Orci.)

shows that, although the proteins that an individual cell expresses and packages in secretory vesicles differ, they contain common sorting signals, which function properly even when the proteins are expressed in cells that do not normally make them.

It is unclear how the aggregates of secretory proteins are segregated into secretory vesicles. Secretory vesicles have unique proteins in their membrane, some of which might serve as receptors for aggregated protein in the TGN. The aggregates are much too big, however, for each molecule of the secreted protein to be bound by its own cargo receptor, as occurs for transport of the lysosomal enzymes. The uptake of the aggregates into secretory vesicles may therefore more closely resemble the uptake of particles by phagocytosis at the cell surface, where the plasma membrane zippers up around large structures.

Initially, most of the membrane of the secretory vesicles that leave the TGN is only loosely wrapped around the clusters of aggregated secretory proteins. Morphologically, these *immature secretory vesicles* resemble dilated *trans* Golgi cisternae that have pinched off from the Golgi stack. As the vesicles mature, they fuse with one another and their contents become concentrated (**Figure 13–64**A), probably as the result of both the continuous retrieval of membrane that is recycled to the TGN, and the progressive acidification of the vesicle lumen that results from the increasing concentration of V-type ATPases in the vesicle membrane that acidify all endocytic and exocytic organelles (see Figure 13–37). The degree of concentration of proteins during the formation and maturation of secretory vesicles is only a small part of the total 200–400-fold concentration of these proteins that occurs after they leave the ER. Secretory and membrane proteins become concentrated as they move from the ER through the Golgi apparatus because of an extensive retrograde retrieval process mediated by COPI-coated transport vesicles that carry soluble ER resident proteins back to the ER, while excluding the secretory and membrane proteins (see Figure 13–25).

Membrane recycling is important for returning Golgi components to the Golgi apparatus, as well as for concentrating the contents of secretory vesicles. The vesicles that mediate this retrieval originate as clathrin-coated buds on the surface of immature secretory vesicles, often being seen even on budding secretory vesicles that have not yet pinched off from the Golgi stack (Figure 13–64B).

Because the final mature secretory vesicles are so densely filled with contents, the secretory cell can disgorge large amounts of material promptly by exocytosis when triggered to do so (**Figure 13–65**).

Precursors of Secretory Proteins Are Proteolytically Processed During the Formation of Secretory Vesicles

Concentration is not the only process to which secretory proteins are subjected as the secretory vesicles mature. Many protein hormones and small neuropeptides,

as well as many secreted hydrolytic enzymes, are synthesized as inactive precursors. Proteolysis is necessary to liberate the active molecules from these precursor proteins. The cleavages occur in the secretory vesicles and sometimes in the extracellular fluid after secretion. Additionally, many of the precursor proteins have an N-terminal *pro-peptide* that is cleaved off to yield the mature protein. These proteins are synthesized as *pre-pro-proteins*, the *pre-peptide* consisting of the ER signal peptide that is cleaved off earlier in the rough ER (see Figure 12–36). In other cases, peptide signaling molecules are made as *polyproteins* that contain multiple copies of the same amino acid sequence. In still more complex cases, a variety of peptide signaling molecules are synthesized as parts of a single polyprotein that acts as a precursor for multiple end products, which are individually cleaved from the initial polypeptide chain. The same polyprotein may be processed in various ways to produce different peptides in different cell types (**Figure 13–66**).

Why is proteolytic processing so common in the secretory pathway? Some of the peptides produced in this way, such as the *enkephalins* (five-amino-acid neuropeptides with morphine-like activity), are undoubtedly too short in their mature forms to be co-translationally transported into the ER lumen or to include the necessary signal for packaging into secretory vesicles. In addition, for secreted hydrolytic enzymes—or any other protein whose activity could be harmful inside the cell that makes it—delaying activation of the protein until it reaches a secretory vesicle, or until after it has been secreted, has a clear advantage: the delay prevents the protein from acting prematurely inside the cell in which it is synthesized.

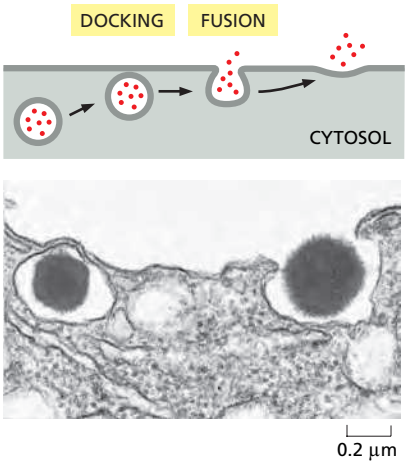


Figure 13–65 Exocytosis of secretory vesicles. The process is illustrated schematically (top) and in an electron micrograph that shows the release of insulin from a secretory vesicle of a pancreatic β cell. (Courtesy of Lelio Orci, from L. Orci, J.-D. Vassalli and A. Perrelet, *Sci. Am.* 259:85–94, 1988.)

Secretory Vesicles Wait Near the Plasma Membrane Until Signaled to Release Their Contents

Once loaded, a secretory vesicle has to reach the site of secretion, which in some cells is far away from the TGN. Nerve cells are the most extreme example. Secretory proteins, such as peptide neurotransmitters (neuropeptides), which will be released from nerve terminals at the end of the axon, are made and packaged into secretory vesicles in the cell body. They then travel along the axon to the nerve terminals, which can be a meter or more away. As discussed in Chapter 16, motor proteins propel the vesicles along axonal microtubules, whose uniform orientation guides the vesicles in the proper direction. Microtubules also guide transport vesicles to the cell surface for constitutive exocytosis.

Whereas transport vesicles containing materials for constitutive release fuse with the plasma membrane once they arrive there, secretory vesicles in the regulated pathway wait at the membrane until the cell receives a signal to secrete, and they then fuse. The signal can be an electrical nerve impluse (an action potential) or an extracellular signal molecule, such as a hormone: in either case, it leads to a transient increase in the concentration of free Ca^{2+} in the cytosol.

For Rapid Exocytosis, Synaptic Vesicles Are Primed at the Presynaptic Plasma Membrane

Nerve cells (and some endocrine cells) contain two types of secretory vesicles. As for all secretory cells, these cells package proteins and neuropeptides in dense-cored secretory vesicles in the standard way for release by the regulated secretory pathway. In addition, however, they use another specialized class of tiny (≈50 nm

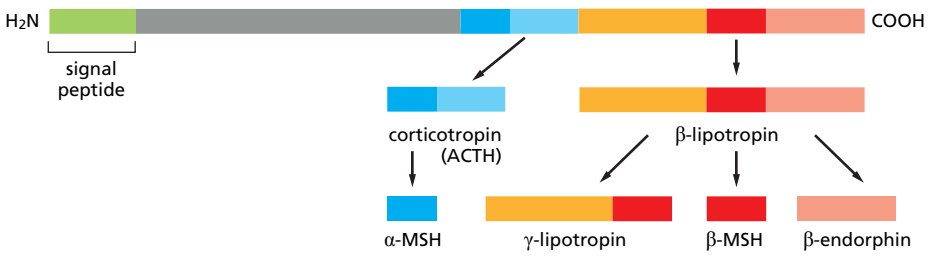


Figure 13–66 Alternative processing pathways for the prohormone polyprotein proopiomelanocortin. The initial cleavages are made by proteases that cut next to pairs of positively charged amino acids (Lys-Arg, Lys-Lys, Arg-Lys, or Arg-Arg pairs). Trimming reactions then produce the final secreted products. Different cell types produce different concentrations of individual processing enzymes, so that the same prohormone precursor is cleaved to produce different peptide hormones. In the anterior lobe of the pituitary gland, for example, only corticotropin (ACTH) and β-lipotropin are produced from proopiomelanocortin, whereas in the intermediate lobe of the pituitary gland mainly α-melanocyte stimulating hormone (α-MSH), γ-lipotropin, β-MSH, and β-endorphin are produced—α-MSH from ACTH and the other three from β-lipotropin, as shown.

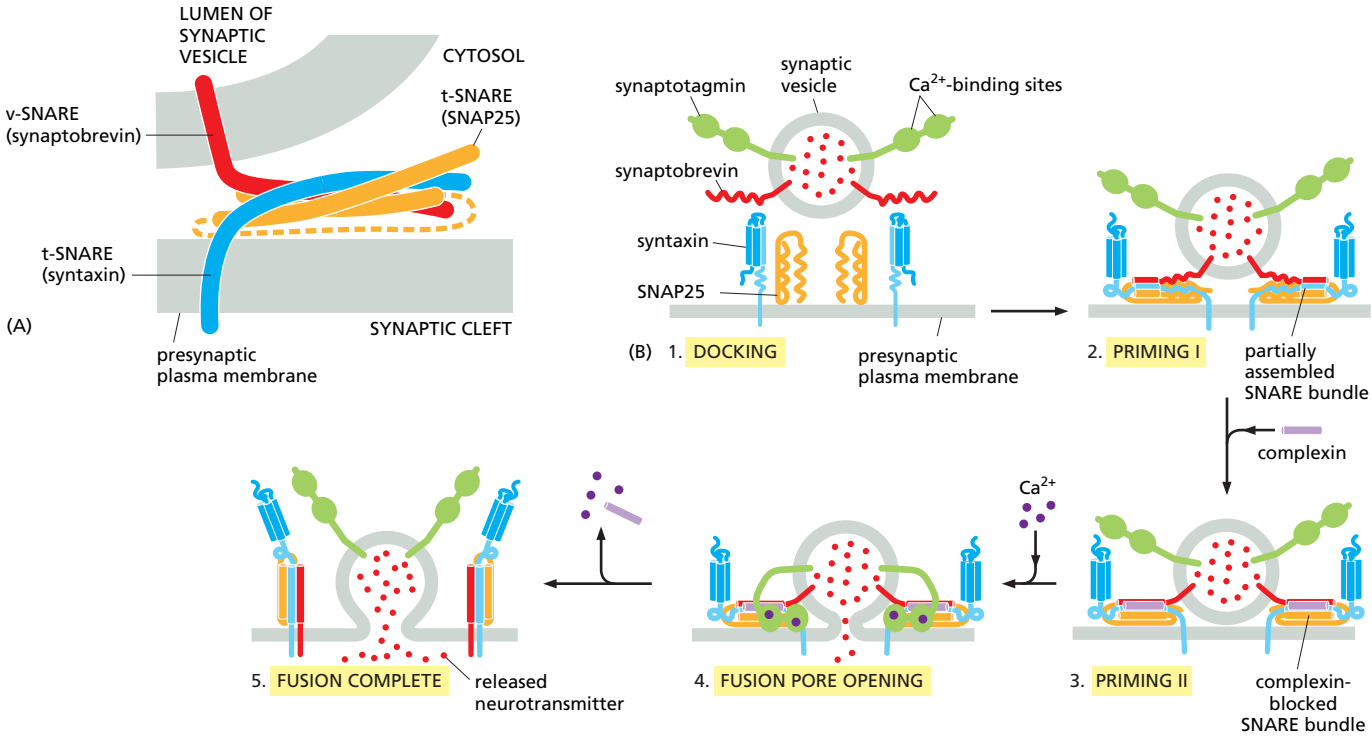


Figure 13–67 Exocytosis of synaptic vesicles. For orientation at a synapse, see Figure 11–36. (A) The trans-SNARE complex responsible for docking synaptic vesicles at the plasma membrane of nerve terminals consists of three proteins. The v-SNARE *synaptobrevin* and the t-SNARE *syntaxin* are both transmembrane proteins, and each contributes one α helix to the complex. By contrast to other SNAREs discussed earlier, the t-SNARE *SNAP25* is a peripheral membrane protein that contributes two α helices to the four-helix bundle; the two helices are connected by a loop *(dashed line)* that lies parallel to the membrane and has fatty acyl chains (not shown) attached to anchor it there. The four α helices are shown as rods for simplicity. (B) At the synapse, the basic SNARE machinery is modulated by the Ca^{2+} sensor *synaptotagmin* and an additional protein called *complexin*. Synaptic vesicles first dock at the membrane (Step 1), and the SNARE bundle partially assembles (Step 2), resulting in a "primed vesicle" that is already drawn close to the membrane. The SNARE bundle assembles further but the additional binding of complexin prevents fusion (Step 3). Upon arrival of an action potential, Ca^{2+} enters the cell and binds to synaptotagmin, which releases the block and opens a fusion pore (Step 4). Further rearrangements complete the fusion reaction (Step 5) and release the fusion machinery, which now can be reused. This elaborate arrangement allows the fusion machinery to respond on the millisecond time scale essential for rapid and repetitive synaptic signaling. (A, adapted from R.B. Sutton et al., *Nature* 395:347–353, 1998. With permission from Macmillan Publishers Ltd.; B, adapted from Z.P. Pang and T.C. Südhof, *Curr. Opin. Cell Biol*. 22:496–505, 2010. With permission from Elsevier.)

diameter) secretory vesicles called **synaptic vesicles**. These vesicles store small *neurotransmitter molecules*, such as acetylcholine, glutamate, glycine, and γ-aminobutyric acid (GABA), which mediate rapid signaling from nerve cell to its target cell at chemical synapses. When an action potential arrives at a nerve terminal, it causes an influx of Ca^{2+} through voltage-gated Ca^{2+} channels, which triggers the synaptic vesicles to fuse with the plasma membrane and release their contents to the extracellular space (see Figure 11–36). Some neurons fire more than 1000 times per second, releasing neurotransmitters each time.

The speed of transmitter release (taking only milliseconds) indicates that the proteins mediating the fusion reaction do not undergo complex, multistep rearrangements. Rather, after vesicles have been docked at the presynaptic plasma membrane, they undergo a priming step, which prepares them for rapid fusion. In the primed state, the SNAREs are partly paired, their helices are not fully wound into the final four-helix bundle required for fusion (**Figure 13–67**). Proteins called *complexins* freeze the SNARE complexes in this metastable state. The brake imposed by the complexins is released by another synaptic vesicle protein, synaptotagmin, which contains Ca^{2+}-binding domains. A rise in cytosolic Ca^{2+} triggers binding of synaptotagmin to phospholipids and to the SNAREs, displacing the complexins. As the SNARE bundle zippers up completely, a fusion pore

opens and the neurotransmitters are released. At a typical synapse, only a small number of the docked vesicles are primed and ready for exocytosis. The use of only a small number of vesicles at a time allows each synapse to fire over and over again in quick succession. With each firing, new synaptic vesicles dock and become primed to replace those that have fused and released their contents.

Synaptic Vesicles Can Form Directly from Endocytic Vesicles

For the nerve terminal to respond rapidly and repeatedly, synaptic vesicles need to be replenished very quickly after they discharge. Thus, most synaptic vesicles are generated not from the Golgi membrane in the nerve cell body but by local recycling from the presynaptic plasma membrane in the nerve terminals (**Figure 13–68**). Similarly, newly made membrane components of the synaptic vesicles are initially delivered to the plasma membrane by the constitutive secretory pathway and then retrieved by endocytosis. But instead of fusing with endosomes, most of the endocytic vesicles immediately fill with neurotransmitter to become synaptic vesicles.

The membrane components of a synaptic vesicle include transporters specialized for the uptake of neurotransmitter from the cytosol, where the small-molecule neurotransmitters that mediate fast synaptic signaling are synthesized. Once filled with neurotransmitter, the synaptic vesicles can be used again (see Figure 13–68). Because synaptic vesicles are abundant and relatively uniform in size, they can be purified in large numbers and, consequently, are the best-characterized organelle of the cell, in that all of their membrane components have been identified by quantitative proteomic analyses (**Figure 13–69**). Extending this analysis to a complete presynaptic terminal, allows us to model the crowded environment in which these reactions occur.

Secretory Vesicle Membrane Components Are Quickly Removed from the Plasma Membrane

When a secretory vesicle fuses with the plasma membrane, its contents are discharged from the cell by exocytosis, and its membrane becomes part of the plasma membrane. Although this should greatly increase the surface area of the plasma membrane, it does so only transiently, because membrane components are removed from the surface by endocytosis almost as fast as they are added by exocytosis, a process reminiscent of the endocytic–exocytic cycle discussed earlier. After their removal from the plasma membrane, the proteins of the secretory

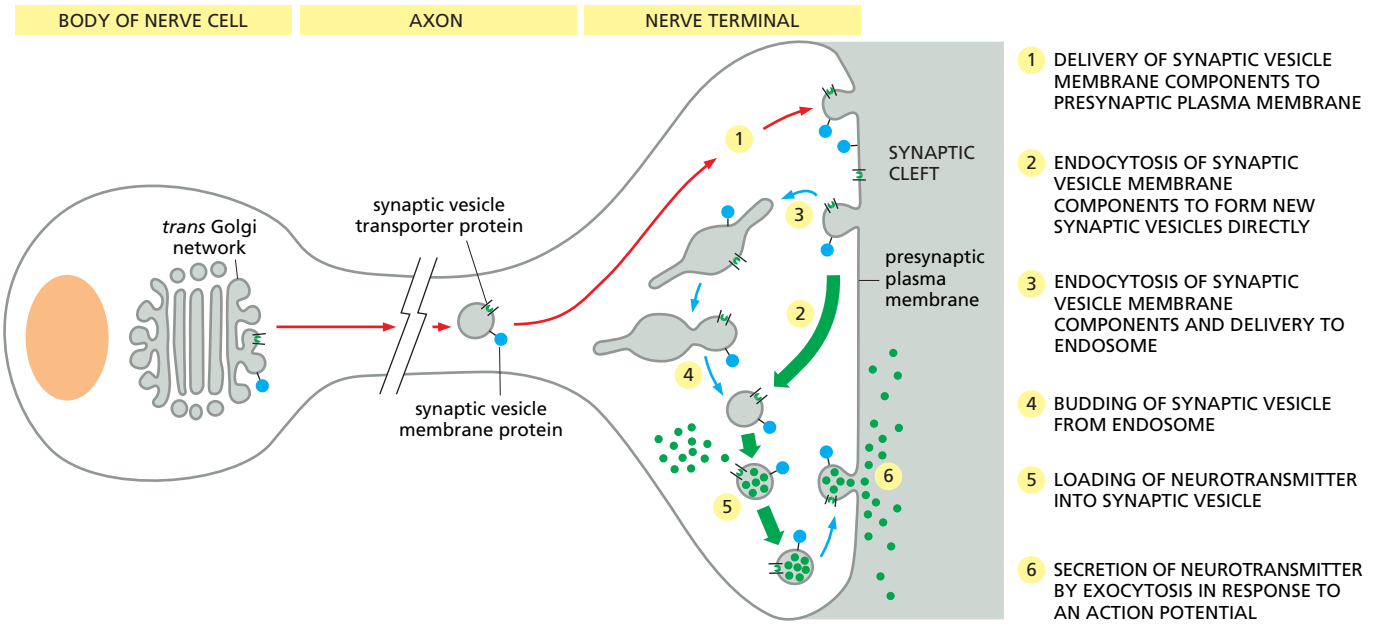
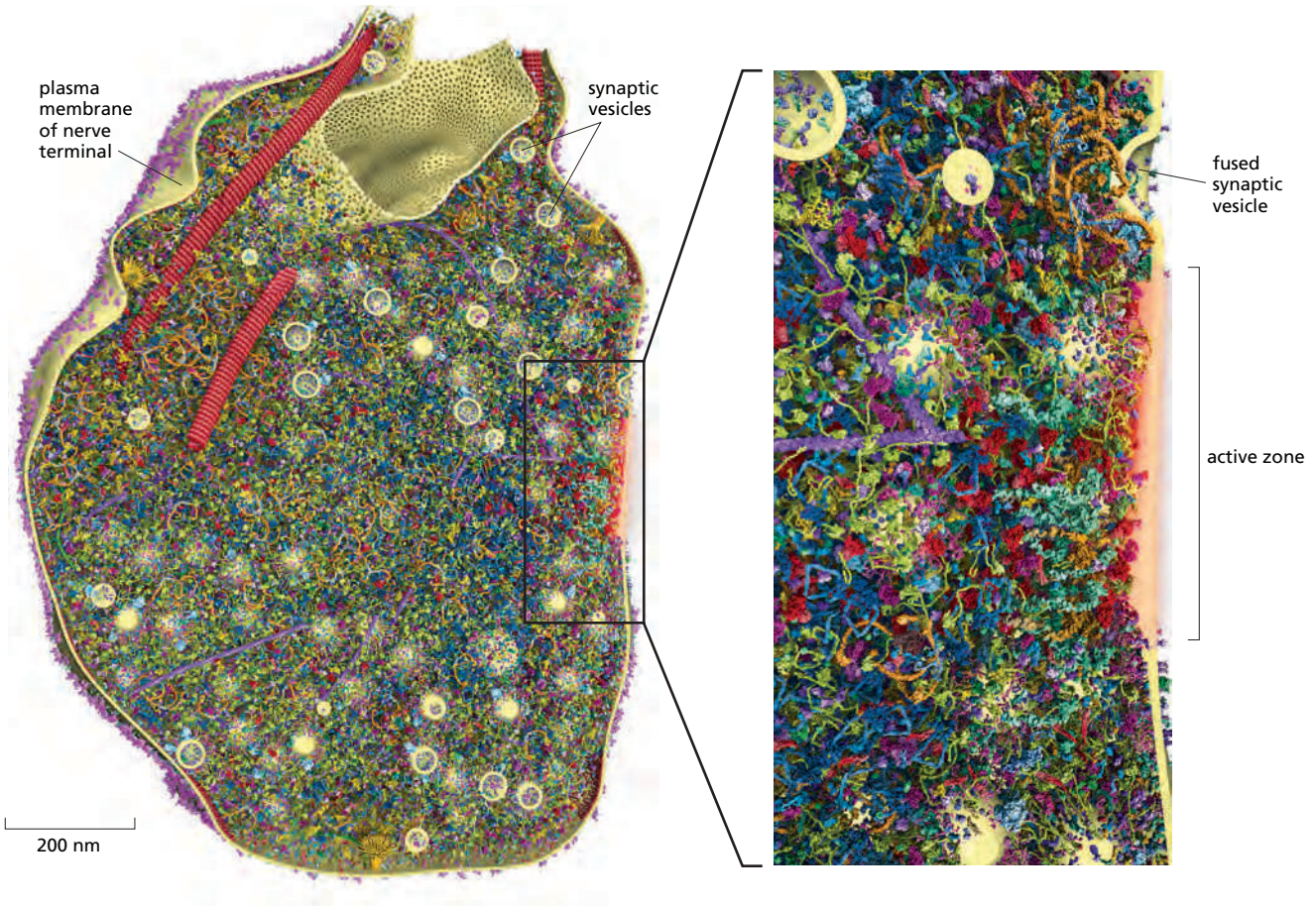


Figure 13–68 The formation of synaptic vesicles in a nerve cell. These tiny uniform vesicles are found only in nerve cells and in some endocrine cells, where they store and secrete small-molecule neurotransmitters. The import of neurotransmitter directly into the small endocytic vesicles that form from the plasma membrane is mediated by membrane transporters that function as antiports and are driven by an H^+ gradient maintained by V-ATPase H^+ pumps in the vesicle membrane (discussed in Chapter 11).



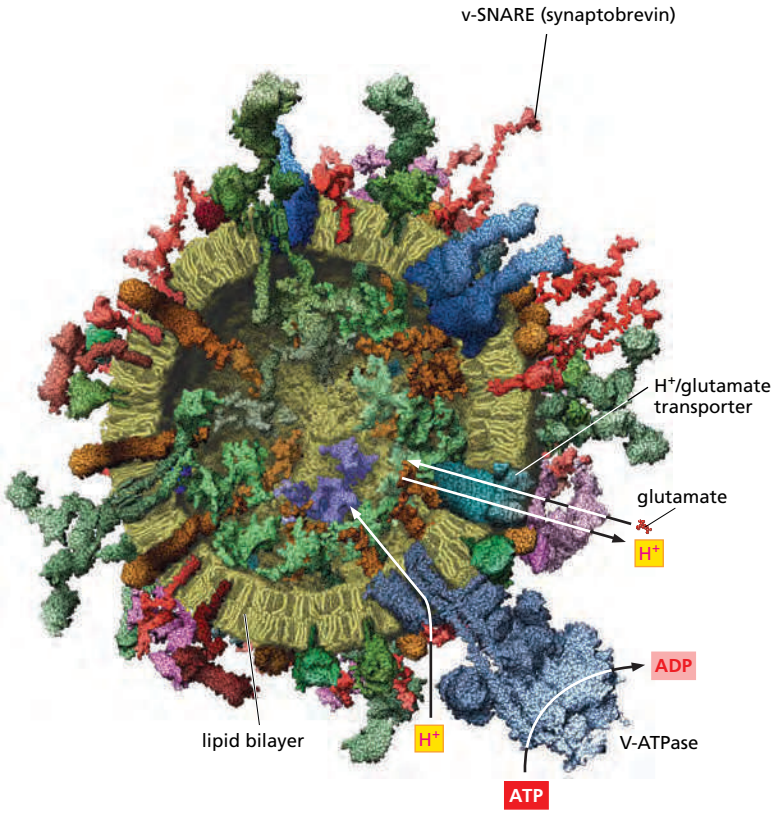


Figure 13–69 Scale models of a brain presynaptic terminal and a synaptic vesicle. The illustrations show sections through a pre-synaptic terminal (A; enlarged in B) and a synaptic vesicle (C) in which proteins and lipids are drawn to scale based on their known stoichiometry and either known or approximated structures. The relative localization of protein molecules in different regions of the presynaptic terminal was inferred from super-resolution imaging and electron microscopy. The model in (A) contains 300,000 proteins of 60 different kinds that vary in abundance from 150 copies to 20,000 copies. In the model in (C), only 70% of the membrane proteins present in the membrane are shown; a complete model would therefore show a membrane that is even more crowded than this picture suggests (Movie 13.7). Each synaptic vesicle membrane contains 7000 phospholipid molecules and 5700 cholesterol molecules. Each also contains close to 50 different integral membrane protein molecules, which vary widely in their relative abundance and together contribute about 600 transmembrane α helices. The transmembrane v-SNARE synaptobrevin is the most abundant protein in the vesicle (~70 copies per vesicle). By contrast, the V-ATPase, which uses ATP hydrolysis to pump H^+ into the vesicle lumen, is present in 1–2 copies per vesicle. The H^+ gradient provides the energy for neurotransmitter import by an H^+/neurotransmitter antiport, which loads each vesicle with 1800 neurotransmitter molecules, such as glutamate, one of which is shown to scale. (A and B, from B.G. Wilhelm et al., *Science* 344:1023–1028, 2014. With permission from AAAS; C, adapted from S. Takamori et al., *Cell* 127:831–846, 2006. With permission from Elsevier.)

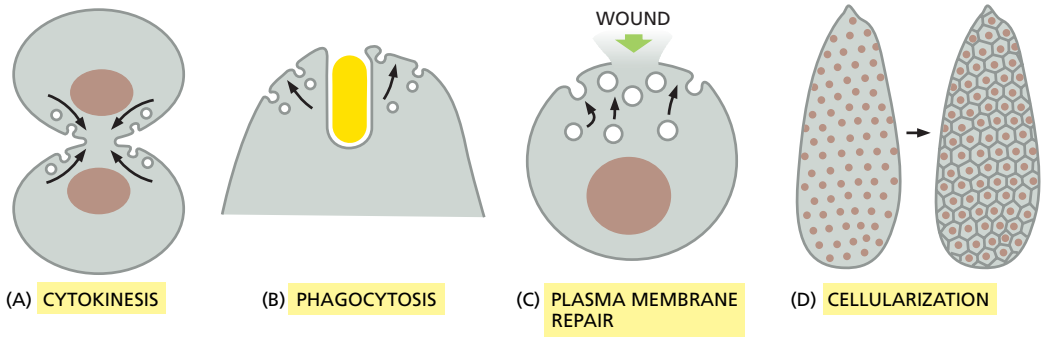


Figure 13–70 Four examples of regulated exocytosis leading to plasma membrane enlargement. The vesicles fusing with the plasma membrane during cytokinesis (A) and phagocytosis (B) are thought to be derived from endosomes, whereas those involved in wound repair (C) may be derived from plasma membranes. The vast amount of new plasma membrane inserted during cellularization in a fly embryo occurs by the fusion of cytoplasmic vesicles (D).

vesicle membrane are either recycled or shuttled to lysosomes for degradation. The amount of secretory vesicle membrane that is temporarily added to the plasma membrane can be enormous: in a pancreatic acinar cell discharging digestive enzymes for delivery to the gut lumen, about 900 μm^2 of vesicle membrane is inserted into the apical plasma membrane (whose area is only 30 μm^2) when the cell is stimulated to secrete.

Control of membrane traffic thus has a major role in maintaining the composition of the various membranes of the cell. To maintain each membrane-enclosed compartment in the secretory and endocytic pathways at a constant size, the balance between the outward and inward flows of membrane needs to be precisely regulated. For cells to grow, however, the forward flow needs to be greater than the retrograde flow, so that the membrane can increase in area. For cells to maintain a constant size, the forward and retrograde flows must be equal. We still know very little about the mechanisms that coordinate these flows.

Some Regulated Exocytosis Events Serve to Enlarge the Plasma Membrane

An important task of regulated exocytosis is to deliver more membrane to enlarge the surface area of a cell's plasma membrane when such a need arises. A spectacular example is the plasma membrane expansion that occurs during the cellularization process in a fly embryo, which initially is a syncytium—a single cell containing about 6000 nuclei surrounded by a single plasma membrane (see Figure 21–15). Within tens of minutes, the embryo is converted into the same number of cells. This process of *cellularization* requires a vast amount of new plasma membrane, which is added by a carefully orchestrated fusion of cytoplasmic vesicles, eventually forming the plasma membranes that enclose the separate cells. Similar vesicle fusion events are required to enlarge the plasma membrane when other animal cells or plant cells divide during *cytokinesis* (discussed in Chapter 17).

Many animal cells, especially those subjected to mechanical stresses, frequently experience small ruptures in their plasma membrane. In a remarkable process thought to involve both homotypic vesicle–vesicle fusion and exocytosis, a temporary cell-surface patch is quickly fashioned from locally available internal-membrane sources, such as lysosomes. In addition to providing an emergency barrier against leaks, the patch reduces membrane tension over the wounded area, allowing the bilayer to flow back together to restore continuity and seal the puncture. This membrane repair process, the fusion and exocytosis of vesicles is triggered by the sudden increase of Ca^{2+}, which is abundant in the extracellular space and rushes into the cell as soon as the plasma membrane is punctured. **Figure 13–70** shows four examples in which regulated exocytosis leads to plasma membrane expansion.

Polarized Cells Direct Proteins from the *Trans* Golgi Network to the Appropriate Domain of the Plasma Membrane

Most cells in tissues are *polarized*, with two or more molecularly and functionally distinct plasma membrane domains. This raises the general problem of how the

delivery of membrane from the Golgi apparatus is organized so as to maintain the differences between one cell-surface domain and another. A typical epithelial cell, for example, has an *apical domain*, which faces either an internal cavity or the outside world and often has specialized features such as cilia or a brush border of microvilli. It also has a *basolateral domain*, which covers the rest of the cell. The two domains are separated by a ring of *tight junctions* (see Figure 19–21), which prevent proteins and lipids (in the outer leaflet of the lipid bilayer) from diffusing between the two domains, so that the differences between the two domains are maintained.

In principle, differences between plasma membrane domains need not depend on the targeted delivery of the appropriate membrane components. Instead, membrane components could be delivered to all regions of the cell surface indiscriminately but then be selectively stabilized in some locations and selectively eliminated in others. Although this strategy of random delivery followed by selective retention or removal seems to be used in certain cases, deliveries are often specifically directed to the appropriate membrane domain. Epithelial cells lining the gut, for example, secrete digestive enzymes and mucus at their apical surface and components of the basal lamina at their basolateral surface. Such cells must have ways of directing vesicles carrying different cargoes to different plasma membrane domains. Proteins from the ER destined for different domains travel together until they reach the TGN, where they are separated and dispatched in secretory or transport vesicles to the appropriate plasma membrane domain (**Figure 13–71**).

The apical plasma membrane of most epithelial cells is greatly enriched in glycosphingolipids, which help protect this exposed surface from damage—for example, from the digestive enzymes and low pH in sites such as the gut or stomach, respectively. Similarly, plasma membrane proteins that are linked to the lipid bilayer by a GPI anchor (see Figure 12–52) are found predominantly in the apical plasma membrane. If recombinant DNA techniques are used to attach a GPI anchor to a protein that would normally be delivered to the basolateral surface, the protein is usually delivered to the apical surface instead. GPI-anchored proteins are thought to be directed to the apical membrane because they associate with glycosphingolipids in lipid rafts that form in the membrane of the TGN. As discussed in Chapter 10, lipid rafts form in the TGN and plasma membrane when glycosphingolipids and cholesterol molecules self-associate (see Figure 10–13).

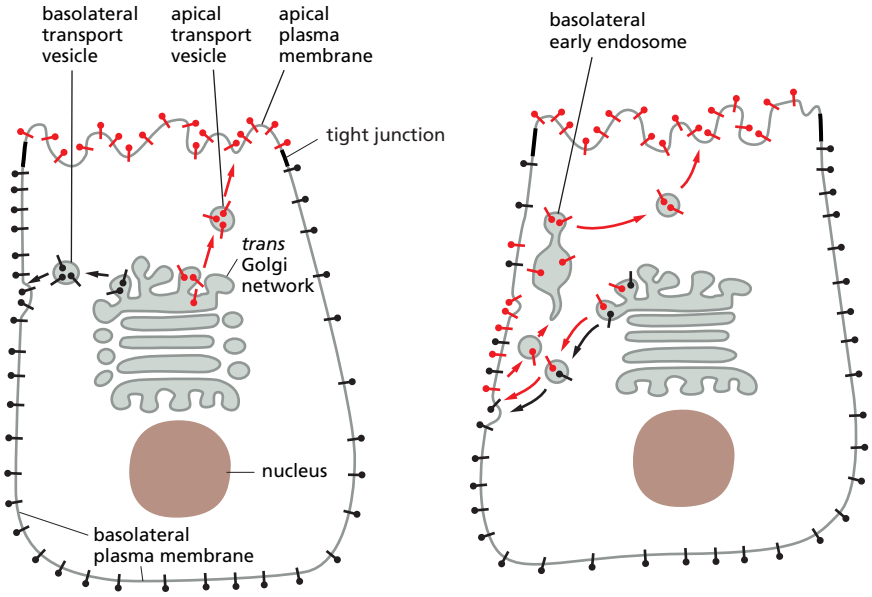


Figure 13–71 Two ways of sorting plasma membrane proteins in a polarized epithelial cell. (A) In the direct pathway, proteins destined for different plasma membrane domains are sorted and packaged into different transport vesicles. The lipid-raft-dependent delivery system to the apical domain described in the text is an example of the direct pathway. (B) In the indirect pathway, a protein is retrieved from the inappropriate plasma membrane domain by endocytosis and then transported to the correct domain via early endosomes—that is, by transcytosis. The indirect pathway, for example, is used in liver hepatocytes to deliver proteins to the apical domain that lines bile ducts.

Having selected a unique set of cargo molecules, the rafts then bud from the TGN into transport vesicles destined for the apical plasma membrane. This process is similar to the selective partitioning of some membrane proteins into the specialized lipid domains in caveolae at the plasma membrane discussed earlier.

Membrane proteins destined for delivery to the basolateral membrane contain sorting signals in their cytosolic tail. When present in an appropriate structural context, these signals are recognized by coat proteins that package them into appropriate transport vesicles in the TGN. The same basolateral signals that are recognized in the TGN also function in early endosomes to redirect the proteins back to the basolateral plasma membrane after they have been endocytosed.

Summary

Cells can secrete molecules by exocytosis in either a constitutive or a regulated fashion. Whereas the regulated pathways operate only in specialized secretory cells, a constitutive secretory pathway operates in all eukaryotic cells, characterized by continual vesicle transport from the TGN to the plasma membrane. In the regulated pathways, the molecules are stored either in secretory vesicles or in synaptic vesicles, which do not fuse with the plasma membrane to release their contents until they receive an appropriate signal. Secretory vesicles containing proteins for secretion bud from the TGN. The secretory proteins become concentrated during the formation and maturation of the secretory vesicles. Synaptic vesicles, which are confined to nerve cells and some endocrine cells, form from both endocytic vesicles and from endosomes, and they mediate the regulated secretion of small-molecule neurotransmitters at the axon terminals of nerve cells.

Proteins are delivered from the TGN to the plasma membrane by the constitutive pathway unless they are diverted into other pathways or retained in the Golgi apparatus. In polarized cells, the transport pathways from the TGN to the plasma membrane operate selectively to ensure that different sets of membrane proteins, secreted proteins, and lipids are delivered to the different domains of the plasma membrane.

WHAT WE DON'T KNOW

- How are targeting and fusion proteins such as SNAREs regulated, so that they can be returned to their respective donor compartments in an inactive state?
- How does a cell balance exocytic and endocytic events to keep its plasma membrane a constant size?
- Can newly formed daughter cells generate a Golgi apparatus *de novo*, or do they have to inherit it?
- How do lysosomes avoid digesting their own membranes?
- How does a cell maintain the right amount of every component (organelles, molecules), and how does it change these amounts as needed (for example, to greatly expand the endoplasmic reticulum when the cell needs to produce large amounts of secreted proteins)?

PROBLEMS

Which statements are true? Explain why or why not.

13–1 In all events involving fusion of a vesicle to a target membrane, the cytosolic leaflets of the vesicle and target bilayers always fuse together, as do the leaflets that are not in contact with the cytosol.

13–2 There is one strict requirement for the exit of a protein from the ER: it must be correctly folded.

13–3 All the glycoproteins and glycolipids in intracellular membranes have oligosaccharide chains facing the lumenal side, and all those in the plasma membrane have oligosaccharide chains facing the outside of the cell.

Discuss the following problems.

13–4 In a nondividing cell such as a liver cell, why must the flow of membrane between compartments be balanced, so that the retrieval pathways match the outward flow? Would you expect the same balanced flow in a gut epithelial cell, which is actively dividing?

13–5 Enveloped viruses, which have a membrane coat, gain access to the cytosol by fusing with a cell membrane. Why do you suppose that these viruses encode their own special fusion protein, rather than making use of a cell's SNAREs?

13–6 For fusion of a vesicle with its target membrane to occur, the membranes have to be brought to within 1.5 nm so that the two bilayers can join (**Figure Q13–1**). Assuming that the relevant portions of the two membranes at the fusion site are circular regions 1.5 nm in diameter, calculate the number of water molecules that would remain between the membranes. (Water is 55.5 M and the volume of a cylinder is $\pi r^2 h$.) Given that an average phospholipid

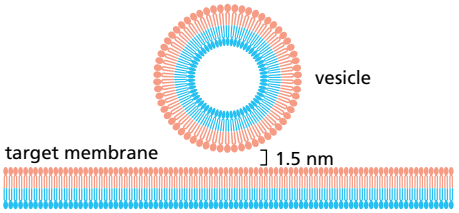


Figure Q13–1 Close approach of a vesicle and its target membrane in preparation for fusion (Problem 13–6).

occupies a membrane surface area of 0.2 nm^2, how many phospholipids would be present in each of the opposing monolayers at the fusion site? Are there sufficient water molecules to bind to the hydrophilic head groups of this number of phospholipids? (It is estimated that 10–12 water molecules are normally associated with each phospholipid head group at the exposed surface of a membrane.)

13–7 SNAREs exist as complementary partners that carry out membrane fusions between appropriate vesicles and their target membranes. In this way, a vesicle with a particular variety of v-SNARE will fuse only with a membrane that carries the complementary t-SNARE. In some instances, however, fusions of identical membranes (homotypic fusions) are known to occur. For example, when a yeast cell forms a bud, vesicles derived from the mother cell's vacuole move into the bud where they fuse with one another to form a new vacuole. These vesicles carry both v-SNAREs and t-SNAREs. Are both types of SNAREs essential for this homotypic fusion event?

To test this point, you have developed an ingenious assay for fusion of vacuolar vesicles. You prepare vesicles from two different mutant strains of yeast: strain B has a defective gene for vacuolar alkaline phosphatase (Pase); strain A is defective for the protease that converts the precursor of alkaline phosphatase (pro-Pase) into its active form (Pase) (**Figure Q13–2**A). Neither strain has active alkaline phosphatase, but when extracts of the strains are mixed, vesicle fusion generates active alkaline phosphatase, which can be easily measured (Figure Q13–2).

Now you delete the genes for the vacuolar v-SNARE, t-SNARE, or both in each of the two yeast strains. You prepare vacuolar vesicles from each and test them for their ability to fuse, as measured by the alkaline phosphatase assay (Figure Q13–2B).

What do these data say about the requirements for v-SNAREs and t-SNAREs in the fusion of vacuolar vesicles? Does it matter which kind of SNARE is on which vesicle?

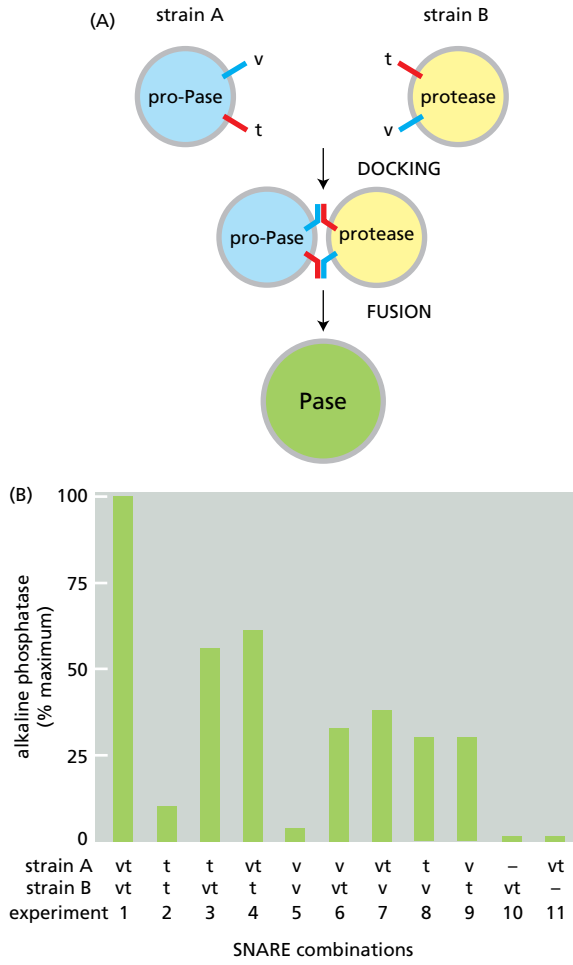


Figure Q13–2 SNARE requirements for vesicle fusion (Problem 13–7). (A) Scheme for measuring the fusion of vacuolar vesicles. (B) Results of fusions of vesicles with different combinations of v-SNAREs and t-SNAREs. The SNAREs present on the vesicles of the two strains are indicated as v (v-SNARE) and t (t-SNARE).

13–8 If you were to remove the ER retrieval signal from protein disulfide isomerase (PDI), which is normally a soluble resident of the ER lumen, where would you expect the modified PDI to be located?

13–9 The KDEL receptor must shuttle back and forth between the ER and the Golgi apparatus to accomplish its task of ensuring that soluble ER proteins are retained in the ER lumen. In which compartment does the KDEL receptor bind its ligands more tightly? In which compartment does it bind its ligands more weakly? What is thought to be the basis for its different binding affinities in the two compartments? If you were designing the system, in which compartment would you have the highest concentration of KDEL receptor? Would you predict that the KDEL receptor, which is a transmembrane protein, would itself possess an ER retrieval signal?

13–10 How does the low pH of lysosomes protect the rest of the cell from lysosomal enzymes in case the lysosome breaks?

13–11 Melanosomes are specialized lysosomes that store pigments for eventual release by exocytosis. Various cells such as skin and hair cells then take up the pigment, which accounts for their characteristic pigmentation. Mouse mutants that have defective melanosomes often have pale or unusual coat colors. One such light-colored mouse, the *Mocha* mouse (**Figure Q13–3**), has a defect in the gene for one of the subunits of the adaptor protein complex AP3, which is associated with coated vesicles budding from the *trans* Golgi network. How might the loss of AP3 cause a defect in melanosomes?

Figure Q13–3 A normal mouse and the *Mocha* mouse (Problem 13–11). In addition to its light coat color, the *Mocha* mouse has a poor sense of balance. (Courtesy of Margit Burmeister.)

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