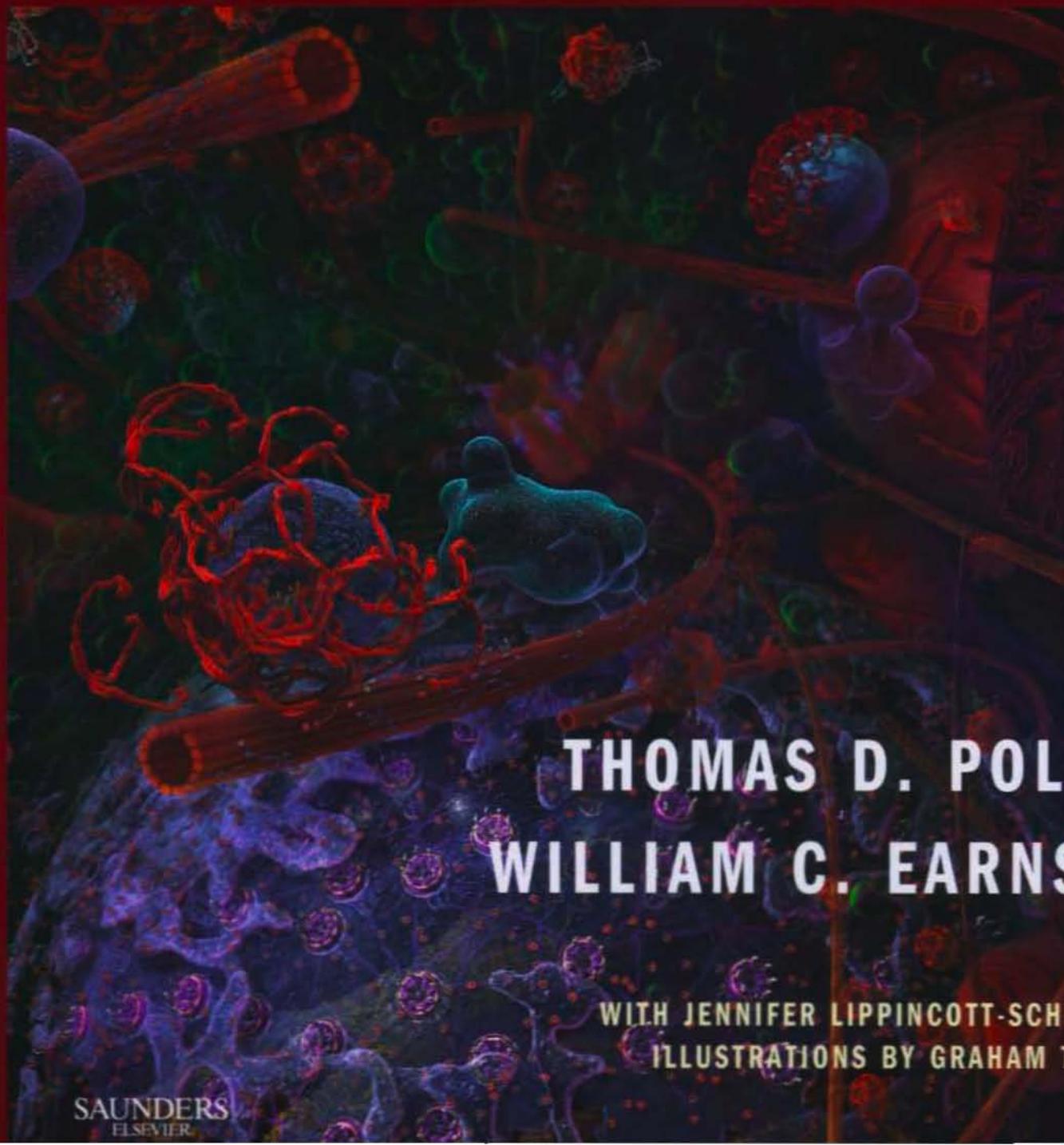


SECOND EDITION



CELL BIOLOGY



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Secretory Membrane System and Golgi Apparatus

Eukaryotic cells transport newly synthesized proteins destined for the extracellular space, the plasma membrane, or the endocytic/lysosomal system through a series of functionally distinct, membrane-bound compartments, including the **endoplasmic reticulum (ER)**, **Golgi apparatus**, and vesicular transport intermediates. This is the secretory membrane system (Fig. 21-1), which allows eukaryotic cells to perform three major functions: (1) distribute proteins and lipids synthesized in the ER to the cell surface and other cellular sites, (2) modify and/or store protein and lipid molecules after their export from the ER, and (3) generate and maintain the unique identity and function of the ER, Golgi apparatus, and plasma membrane. This chapter describes how the secretory membrane system is organized and operates to fulfill these functions. It also provides a detailed description of the Golgi apparatus whose conserved features are central for the operation of the secretory membrane system.

Overview of the Secretory Membrane System

The secretory membrane system uses membrane-enclosed transport carriers to move thousands of diverse macromolecules—including proteins, proteoglycans, and glycoproteins—efficiently and precisely among different membrane-bound compartments (i.e., the ER, Golgi apparatus, and plasma membrane). Within the large cytoplasmic volume of the eukaryotic cell (up to 10^3 times that of the volume of a prokaryotic cell), this is essential for coordinating cellular needs in response to the constantly changing environment and organismal physiology.

Newly synthesized transmembrane and luminal proteins transported through the secretory system are called **cargo**. These include luminal proteins destined to be stored within a compartment or secreted to the cell exterior, as well as transmembrane proteins that are retained in a particular compartment (e.g., Golgi processing enzymes), delivered to the plasma membrane, or recycled among compartments (e.g., transport machinery). Transfer of cargo molecules through the secretory system begins with their cotranslational insertion into or across the ER bilayer (see Fig. 20-7). The cargo molecules are next folded and assembled into forms that can be sorted and concen-

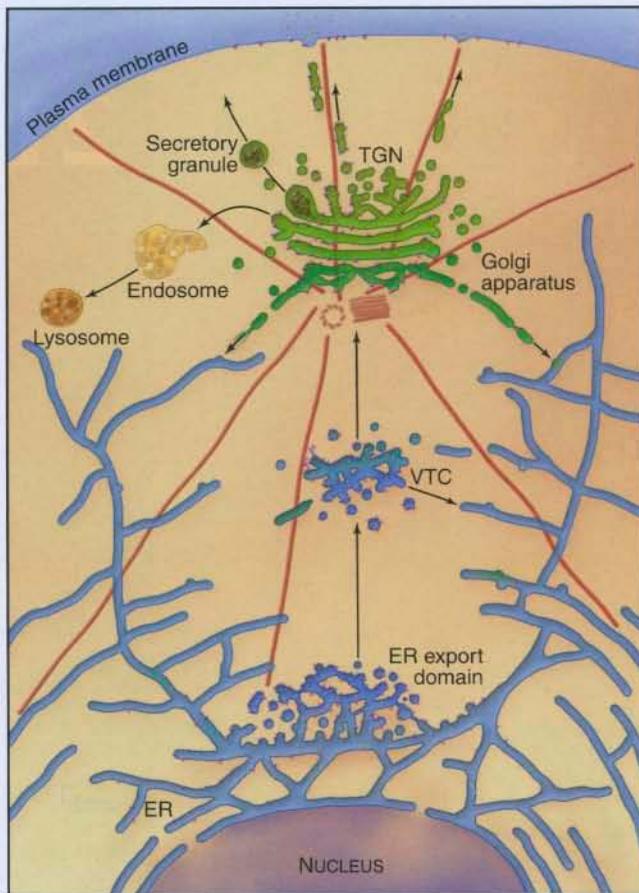


Figure 21-1 OVERVIEW OF THE SECRETORY MEMBRANE SYSTEM. The three principal organelles of the secretory pathway—the ER, Golgi apparatus, and plasma membrane—communicate with one another and the cell exterior by way of transport carriers. The carriers (either small vesicles or larger vesicle-tubule elements) move along cytoskeletal elements (red lines) to transfer newly synthesized proteins, called cargo, from the ER to the Golgi, and from the Golgi to the plasma membrane (or to the endosome/lysosomal system). Carriers form from the ER at specialized regions, called ER export domains, producing pre-Golgi structures called vesicular tubular carriers (VTCs) that move to the Golgi. Retrograde transport carriers bud off from the VTC or Golgi apparatus to retrieve proteins and lipids back to the ER for repeated use and to balance the anterograde flow of membrane to the plasma membrane. The luminal spaces enclosed by the carriers and organelles of the secretory membrane system are all topologically equivalent to the outside of the cell.

trated within membrane-bound transport intermediates (called **vesicular tubular carriers [VTCs]**) destined for the Golgi apparatus. Once packaged into and transported by such a carrier, cargo enters the Golgi apparatus, which serves as the central processing and sorting station in the secretory membrane system. Within the Golgi apparatus, numerous enzymes modify the cargo molecules by trimming or elongating the cargo's glycan side chains or cleaving its polypeptides. Processed cargo is then sorted into membrane-bound carriers that bud out from the Golgi apparatus and move to the plasma membrane, to the endosome/lysosomal system, or back

to the ER. In specialized cell types, the Golgi apparatus can sort certain classes of cargo into secretory granules (for storage and later release to the cell exterior in response to specific stimuli) or give rise to transport carriers that target to different polarized plasma membrane domains.

Membrane-enclosed **carriers** mediate transport within the secretory membrane system (Fig. 21-2). Carriers are shaped as tubules, vesicles, or larger structures. The carriers are too large to diffuse freely in the crowded cytoplasm but are transported over long distances along microtubules or actin filaments by molecular motor proteins. Each carrier selects certain types of cargo before budding from a donor compartment and fuses only with an appropriate target membrane. Molecular markers on the cytoplasmic surface of the carrier, as well as on the acceptor membrane, steer the carrier through the cytoplasm and ensure that it fuses only with the correct target compartment. The carriers continuously shuttle among ER, Golgi apparatus, and plasma membranes, enabling cargo to be distributed to its appropriate target organelle.

Sorting of cargo into transport carriers is facilitated by the presence of specialized lipids in the donor organelle membrane (such as sphingomyelin, glycosphingolipids, and phosphoinositides in the Golgi apparatus) and by the recruitment of protein-based sorting and transport machinery (e.g., coat proteins and tethering/fusion factors). Together, the specialized lipids and protein-sorting machinery generate membrane microdomains that concentrate or exclude cargo. The domains then pinch off the membrane bilayer as membrane-enclosed carriers and travel to target membranes.

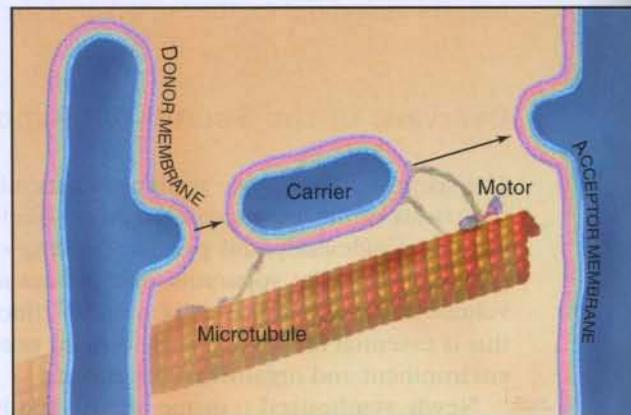


Figure 21-2 CARRIER TRANSPORT. Membrane-enclosed carriers (shaped as vesicles, tubules, or vesicle-tubule elements) bud off from a donor compartment after packaging both luminal and transmembrane cargo proteins. Carriers are moved through the cytoplasm along cytoskeletal elements (e.g., microtubules in mammalian cells) by motor proteins until they fuse with a target compartment. During this process, the relative topology of the lipids and transmembrane proteins is maintained.

During transport of a carrier, the relative orientation (called topology) of lipid and protein in the membrane bilayer, established during synthesis in the ER, is maintained (Fig. 21-2). Hence, one side of the membrane always faces the cytoplasm. The other side initially faces the lumen of the ER. This side remains inside each membrane compartment along the secretory pathway but is exposed on the cell surface if the carrier fuses with the plasma membrane. Selection of proteins and lipids by a carrier, budding of the carrier, and subsequent fusion of the carrier with an acceptor compartment all also occur without leakage of contents from the carrier or the donor and target compartments.

The flow of cargo and lipid forward through the secretory system toward the plasma membrane (**anterograde traffic**) is balanced by selective **retrograde traffic** of cargo and lipids back toward the ER (Fig. 21-1). Retrograde traffic allows proteins and lipids involved in membrane transport and fusion to be retrieved for repeated use. Retrograde traffic also returns proteins that have been inadvertently carried forward through the secretory system so they can be redirected to their proper destination. Both anterograde and retrograde flows of membrane within the secretory system are necessary for the ER, Golgi apparatus, and plasma membrane to generate and maintain their distinct functional and morphologic identities.

Advantages of the Secretory Membrane System

The secretory membrane system, found in all eukaryotic cells, offers numerous advantages over the simpler secretory process in prokaryotic cells, which involves insertion of newly synthesized proteins directly into or across the plasma membrane. First, synthesizing, folding, and processing membrane and secretory proteins within a series of distinct compartments provides a protective environment for cells to modify proteins before they are exposed on the cell surface. Newly synthesized proteins within the ER, for example, can fold into complex shapes and assemble into multisubunit complexes. Within the Golgi apparatus, the cargo molecules can be further modified by glycan processing and proteolytic cleavage. The resulting repertoire of protein structures that are expressed at the cell surface is significantly larger and capable of performing more diverse functions than that found in prokaryotes.

A second advantage is the capacity of the secretory membrane system to regulate protein secretion and expression at the cell surface. Eukaryotic cells can store proteins in membrane compartments before releasing them at the cell surface in response to internal or external signals. By exploiting these capabilities, eukaryotic cells have evolved elaborate ways to control the types of proteins located on or secreted from the cell surface.

A third advantage relates to the differentiation of the plasma membrane. Prokaryotic cells synthesize their proteins at the plasma membrane, so they must keep this surface enriched in loosely packed glycerophospholipids that are pliable enough that newly synthesized proteins can enter into and fold in a hydrophobic environment. Consequently, prokaryotic cells secrete a rigid cell wall as a protective barrier to the outside. In eukaryotes, concentrating protein synthesis in the ER frees the plasma membrane to become enriched in lipids such as cholesterol and sphingolipids that can arrange into highly ordered, flexible arrays. The ordered, flexible arrays of cholesterol and sphingolipids in the plasma membrane provide mechanical stability and an impermeable barrier to water-soluble molecules. As a consequence, eukaryotic cells do not require a cell wall to survive (although some eukaryotes, such as plant and fungal cells, make cell walls) and can employ their plasma membrane in a wide range of functions, such as membrane protrusion for engulfing large extracellular objects (see Chapter 22) and for crawling (see Chapter 38).

Building and Maintaining the Secretory Membrane System

Effective operation of the secretory membrane system depends on several features. The system must generate and maintain the specialized character of each secretory compartment (including the different lipid and protein environments of the ER, Golgi apparatus, and plasma membrane) in the face of continual exchange of protein and lipid components. Cargo must be concentrated selectively in or excluded from each transport carrier. Each carrier must be directed along a specific route and fuse only with an appropriate target membrane.

Two mechanisms, described in more detail in the following sections, play important roles in accomplishing these tasks. First, a lipid-based sorting mechanism uses the inherent capacity of lipids to self-organize into different domains to create a gradient of phospholipid composition across the secretory pathway. On the basis of the length of their transmembrane segments, transmembrane proteins partition into particular membranes that differ in the thickness of the lipid bilayer. Second, protein-based sorting machinery generates transport carriers capable of concentrating specific cargo proteins and targeting to appropriate acceptor membranes, where they fuse and deliver their cargo.

Protein Sorting by the Lipid Gradient across the Secretory Membrane System

A conserved feature of the secretory membrane system is the differential distribution of various classes of lipids

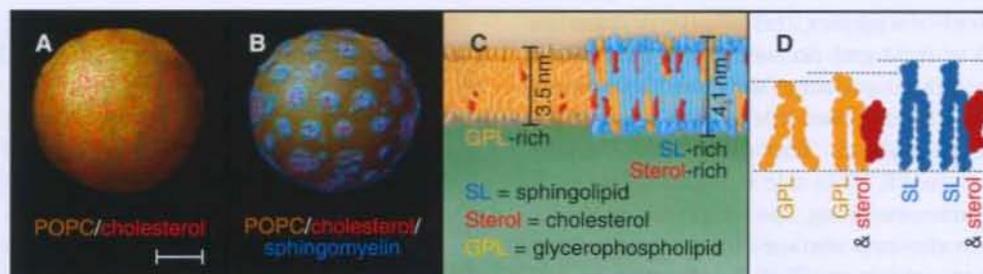


Figure 21-3 PROPERTIES OF LIPIDS WITHIN MEMBRANES. **A–B**, Cartoon depiction of an artificial bilayer containing a POPC/cholesterol mixture of 2:1 (**A**) and a POPC/cholesterol/sphingomyelin mixture of 2:1:1 (POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) (**B**). The blue spherical spots in part **B** are cholesterol- and sphingomyelin-enriched domains that have segregated from POPC because of the affinity between sphingomyelin and cholesterol. **C**, A bilayer enriched in cholesterol and sphingomyelin has a greater thickness than a bilayer composed mainly of glycerophospholipids, owing to the long saturated hydrocarbon chains of glycosphingolipids that attract proteins with longer transmembrane domains. **D**, Glycerophospholipid-containing membranes with high concentrations of cholesterol have a greater thickness than those with low concentrations, owing to a tighter alignment of the hydrocarbon chains. Scale bar is 5 μ m.

along the pathway. These classes of lipids include **glycerophospholipids** (phosphoglycerides), **sphingolipids** (e.g., sphingomyelin and glycosphingolipids), and **cholesterol** (see Figs. 7-4 and 20-13). These lipids play a major role in the sorting of proteins within the secretory membrane system because of their immiscibility (i.e., the property of not mixing) in membranes with different lipid compositions. By not mixing with some lipids while mixing with others, these lipid classes form lateral lipid assemblies, termed *microdomains*, that can concentrate or exclude specific membrane proteins.

Studies using artificial membranes have demonstrated how lipid immiscibility allows a continuous lipid bilayer to self-organize into distinct **lipid domains** with unique lipid compositions and biophysical properties. A prime example is an artificial bilayer containing glycerophospholipids and cholesterol to which sphingolipid is added; after sphingolipid is added, the cholesterol and glycerophospholipids partition into distinct domains (Fig. 21-3A–B). Because of van der Waals attraction between the sphingolipid's long, saturated hydrocarbon chain and cholesterol's rigid, flat-cylindrical steroid backbone, the cholesterol and sphingolipids associate in the plane of the membrane, whereas glycerophospholipids, which have unsaturated, kinked hydrocarbon chains with much less affinity for cholesterol, are largely excluded from the cholesterol/sphingolipid domains. The domains enriched in cholesterol/sphingolipid are thicker than the surrounding membrane composed of shorter, unsaturated, kinked glycerophospholipids (Fig. 21-3C). Tension on the bilayer (i.e., from binding of proteins that bend or curve the membrane) enhances the tendency of lipids that have different physical properties to separate into distinct phases.

In addition to prompting separation of sphingolipids from glycerophospholipids, cholesterol can affect a bilayer composed of glycerophospholipids alone (Fig. 21-3D). In this case, the cholesterol fills the space between the floppy hydrocarbon chains of glycerophos-

pholipids in the bilayer. This forces the glycerophospholipids into a tighter alignment and increases the distance between their head groups. As a result, the bilayer becomes thicker, resembling the thickness of bilayers enriched in sphingomyelin alone or sphingomyelin plus cholesterol.

Sphingolipids (e.g., glycosphingolipids and sphingomyelin) are synthesized in the Golgi apparatus, while the ER produces cholesterol and glycerophospholipids. Synthesis of these lipids at two different sites, combined with the self-organizing capacity of sphingolipids, cholesterol, and glycerophospholipids, gives rise to a pattern of lipid circulation within the secretory system that plays important roles in membrane sorting (Fig. 21-4A). Newly synthesized cholesterol is continually removed from the ER and redistributed to the Golgi apparatus, where high affinity interactions with sphingolipids prevent it from returning to the ER. The association of cholesterol with sphingolipids in the Golgi apparatus, in turn, triggers the lateral differentiation of domains enriched in these lipids. Through the additional activity of protein-based sorting and trafficking machinery, these domains bud off the Golgi apparatus and move to the plasma membrane, redistributing sphingolipids and cholesterol to the cell surface.

The forward flow of cholesterol, sphingolipids, and glycerophospholipids toward the plasma membrane is balanced by selective retrograde flow. Glycerophospholipids transferred from the ER to the Golgi apparatus are recycled back to the ER. Similarly, sphingolipids delivered to the plasma membrane from the Golgi apparatus are returned to the Golgi apparatus. Cholesterol, in contrast, is not returned through these retrograde pathways to either the ER or the Golgi apparatus but enters and circulates within the endocytic pathway leading to lysosomes. This pattern of lipid circulation creates a gradient of cholesterol, sphingolipids, and glycerophospholipids across the secretory membrane system. Within this gradient, the ER has a low concen-

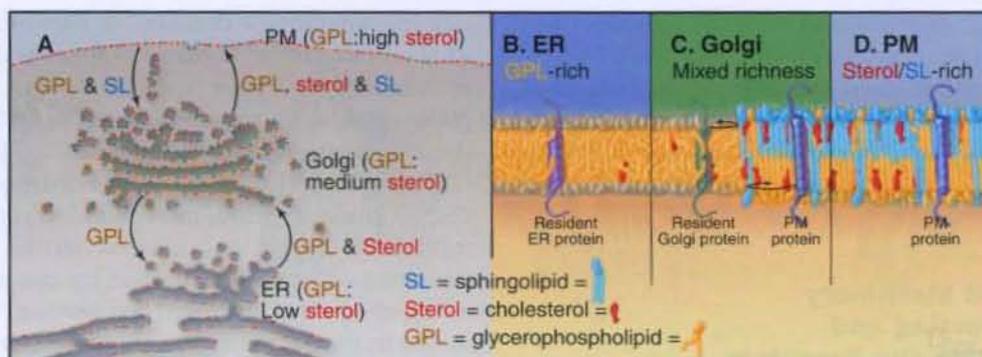


Figure 21-4 A LIPID GRADIENT ARISES ACROSS THE SECRETORY PATHWAY AS A RESULT OF THE SELF-ORGANIZING PROPERTIES OF GLYCEROPHOSPHOLIPIDS, SPHINGOLIPIDS, AND CHOLESTEROL AND THEIR DIFFERENTIAL SITES OF SYNTHESIS. The gradient helps to sort and transport proteins to different sites within the secretory system. **A**, Lipid circulation and sorting within the secretory membrane system. Glycerophospholipids (GPL) and cholesterol (sterol) are synthesized in the ER, whereas sphingolipids (SL), including sphingomyelin and glycosphingolipids, are synthesized in the Golgi apparatus. Cholesterol that moves to the Golgi from the ER associates with SL and is carried to the plasma membrane. This gives rise to different concentrations of these lipids in these organelles at steady state and results in lipid environments in the ER and plasma membrane that are compatible with their functions (e.g., protein translocation for the ER and low permeability for the plasma membrane). **B–D**, Sorting of transmembrane proteins based on the length of their transmembrane domains. The distinct lipid compositions of the ER, Golgi apparatus, and plasma membrane result in bilayers that differ in thickness (with the ER bilayer depleted of SL/sterols and thin, the plasma membrane bilayer enriched in SL/sterols and thick, and the Golgi bilayer intermediate in SL/sterol content and having mixed thickness). To avoid hydrophobic mismatch, transmembrane proteins move to the organelle whose bilayer thickness best matches that of the protein's transmembrane domain length.

tration of cholesterol (e.g., sterols) and sphingolipids, the Golgi apparatus has an intermediate concentration, and the plasma membrane has a high concentration (Fig. 21-4A).

The lipid gradient serves two important functions. First, it generates different lipid environments in the ER, Golgi apparatus, and plasma membrane compatible with their distinct functions. The low concentration of sterols and sphingolipids in the ER membrane means that it is composed primarily of glycerophospholipids (i.e., phosphatidylcholine, PC; phosphatidylserine, PS; and phosphatidylethanolamine, PE). The loosely packed acyl chains of PC, PS, and PE are readily deformable, permitting newly synthesized membrane proteins to insert into and fold in the ER bilayer. This feature explains why the ER is used as the sole site of cotranslational protein synthesis in the cell. By contrast, the high concentration of sterols and sphingolipids makes the plasma membrane bilayer thicker and less permeable to small molecules. This allows the plasma membrane to form a flexible but impermeable barrier between the cytoplasm and cell exterior. The intermediate concentration of sterols and sphingolipids in the Golgi apparatus allows it to serve as a membrane-sorting station.

A second function of the lipid gradient is to promote sorting of transmembrane proteins within the secretory system. Each integral membrane protein seeks a lipid bilayer with a thickness that matches the lengths of its transmembrane segments (Fig. 21-4B–D). Because most transmembrane segments are stiff hydrophobic α -helices, it is energetically unfavorable to expose hydro-

phobic residues of a transmembrane polypeptide to the aqueous environment of the cytoplasm or vesicle lumen or to bury hydrophilic amino acids with the lipid acyl chains in the interior of the membrane. To avoid such hydrophobic mismatches, integral membrane proteins of the secretory system have evolved with transmembrane segments that are matched to the thickness of their target membranes. Hence, resident membrane proteins in the ER and Golgi apparatus typically have shorter transmembrane segments (around 15 amino acids) than do resident plasma membrane proteins (approximately 20 to 25 amino acids). Retention and/or transport of these proteins occurs because the lipid bilayers of carriers budding out from either the ER (toward the Golgi apparatus) or the Golgi apparatus (toward the plasma membrane) are thicker than the bilayers of the donor organelles. Only transmembrane proteins with transmembrane segments long enough to span this thickness enter such carriers.

If the transmembrane segment of a plasma membrane protein is shortened experimentally by using recombinant DNA techniques, the new protein is retained in the thinner bilayers of the ER and/or Golgi apparatus rather than moving on to the thicker plasma membrane. Similarly, when the transmembrane segment of a Golgi protein is extended, the protein is no longer retained in the Golgi apparatus but is transported to the plasma membrane.

This lipid-based protein sorting mechanism takes advantage of the lipid gradient established by the self-organizing properties of glycerophospholipids,

cholesterol, and sphingolipids to sort and transport proteins within the secretory system. It is not, however, the only mechanism used by cells to organize and transport proteins along the secretory pathway. In addition, a complex protein-based machinery is relied on to bring far greater specificity and efficiency to these processes.

Protein-Based Machinery for Protein Sorting and Transport within the Secretory Membrane System

Sorting and transporting proteins within the secretory membrane system depend on several types of proteins (Fig. 21-5): Specialized “coats” help to generate both small and large transport carriers and sort proteins into them; motor proteins move carriers along the cytoskeleton; “tethering factors” attach carriers to the cytoskeleton and to their destination organelles prior to fusion; and fusion proteins mediate fusion of the carrier with an acceptor membrane. These components also associate with specific organelles, providing organelles with an identity that is both unique and dynamic. Many of the components are peripheral membrane proteins that

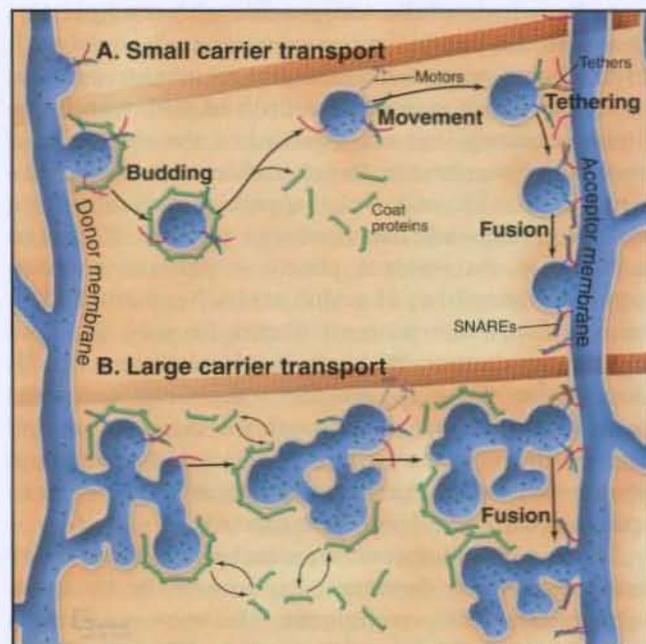


Figure 21-5 PROTEIN MACHINERY FOR SECRETORY TRANSPORT. Coat proteins that cluster into polymerized arrays help to sort soluble cargo and transmembrane proteins into a coated bud that pinches off a donor membrane as a coated vesicle (A) or larger vesicular tubular carriers (B). The carriers move by motor proteins along either microtubules or actin. Tethering factors, including long coiled-coil proteins or multimeric tethering complexes, tether the carriers to an acceptor membrane. SNARE proteins on the carrier and acceptor membrane then form a complex that drives membrane fusion, leading to delivery of the carrier's content to the acceptor membrane.

lack transmembrane domains, so they must be recruited to the cytoplasmic surface of appropriate membranes by binding to either specific lipids, such as phosphoinositides, or to activated GTPases. Cells regulate the distributions of these organelle-specific lipids and GTPases. When infectious agents or stressful conditions disrupt these targeting molecules, secretory membrane trafficking can be disorganized and/or inhibited. The following sections describe the six major protein-based mechanisms that are used for sorting, transport, and fusion in the secretory membrane system.

Arf GTPases

The Arf family of GTPases includes Sar1, Arf1-6 and several distantly related Arf-like GTPases. These small GTPases mediate the association of a wide variety of protein effectors with specific membranes, which, in turn, leads to the differentiation of membrane domains that give rise to transport carriers and create compartmental identity.

Like other GTPases (see Figs. 4-6 and 4-7), Arfs are molecular switches that alternate between a GTP-bound active form that interacts with effector targets and a GDP-bound inactive form that does not (Fig. 21-6). Active Arf GTPases associate with membranes, whereas inactive GTPases are cytoplasmic. Specific GTP exchange factors (GEFs) recruit Arf proteins to particular membrane surfaces and then catalyze the exchange of GDP for GTP. When associated with particular membranes active Arfs bind their effectors until a GTPase-activating protein (GAP) induces hydrolysis of GTP, reversing membrane association and effector binding. The distribution of GEFs on particular membranes determines the location of specific active Arfs. Similarly, the location of GAPs determines where each type of Arf is inactivated.

Activation of Arfs by exchange of GDP for GTP not only creates a binding site for target proteins (i.e., effectors) but also promotes interaction with the lipid bilayer. A myristoyl group covalently bound to the N-terminus of most Arfs allows them to interact transiently and nonspecifically with membranes. When a specific Arf-GEF on a membrane catalyzes the exchange of GDP for GTP, an amphipathic (hydrophobic on one side, hydrophilic on the other) N-terminal, α -helix is released from a hydrophobic pocket on the GTPase so that the hydrophobic side of the helix can interact with the bilayer (Fig. 21-6D). The membrane-associated GEFs that are responsible for activating Arfs all contain an evolutionarily conserved domain (referred to as the Sec7 domain). Association of this domain with Arf1-GDP is stabilized in the presence of the toxic fungal metabolite **brefeldin A (BFA)** [Fig. 21-6D]). This prevents Arf1 conversion to its active, GTP-bound state and thereby blocks Arf1 activity, similar to that of a GDP-locked Arf1 mutant.

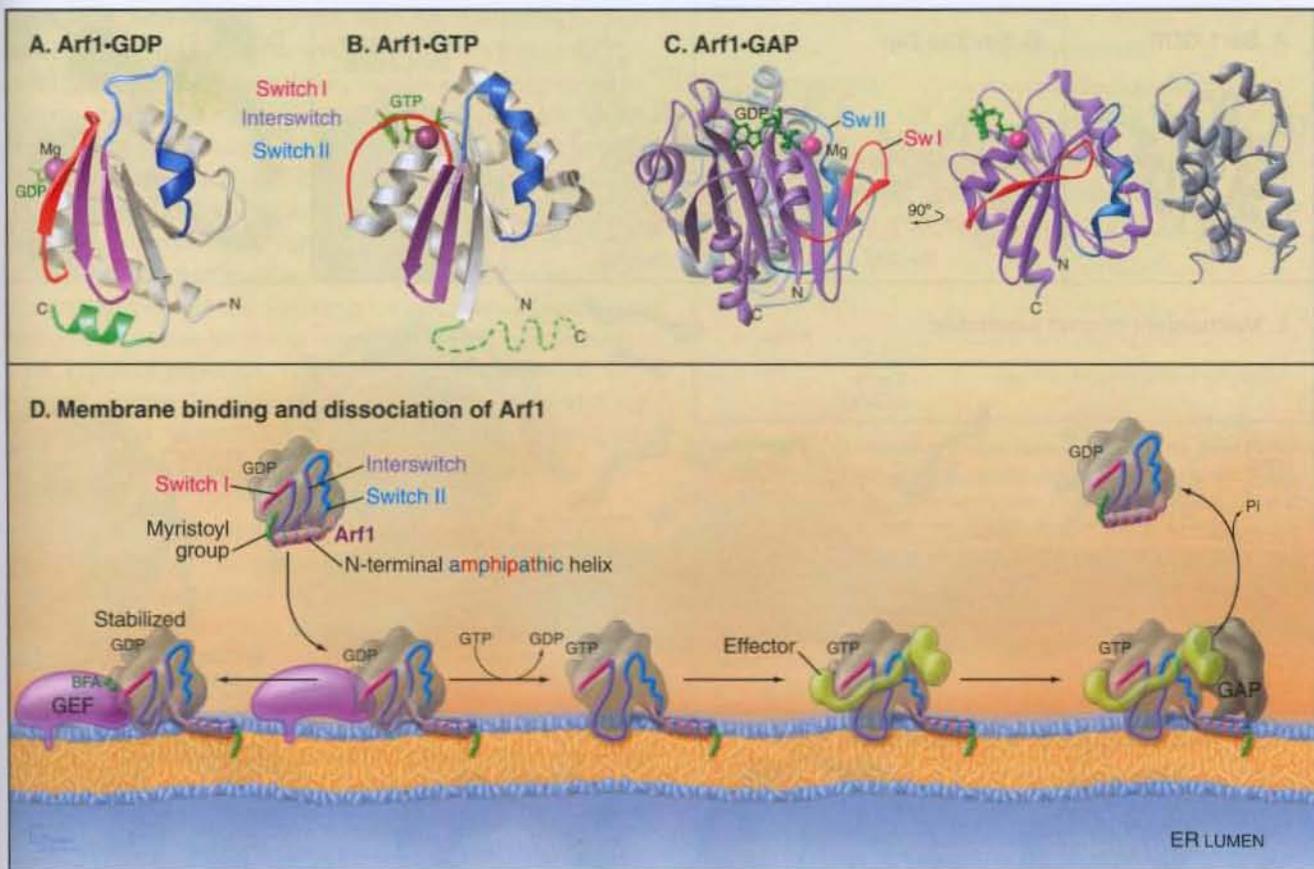


Figure 21-6 Arf-GTPase cycle. **A–C**, Ribbon diagrams of Arf1-GDP (**A**), Arf1-GTP (**B**), and free Arf1 and Arf1 bound to its GAP (**C**). **D**, Membrane binding and dissociation of Arf1. In the cytoplasm, Arf1 exists in its GDP-bound form with its N-terminal amphipathic helix tucked into a hydrophobic pocket. An N-terminal myristoyl group allows Arf1 to reversibly bind to membranes for activation by a GEF. The exchange of GDP for GTP induces a conformational change in switch 1 and 2, as well as in the interswitch loop, which displaces the N-terminal helix out of its pocket. This causes Arf1-GTP to bind tightly to membranes, since both the hydrophobic residues of the N-terminal helix and the myristoyl anchor associate with the bilayer. Arf1-GTP then recruits effectors. Association of a GAP with the Arf1-GTP-effector complex stimulates GTP hydrolysis. Arf1-GDP returns to the cytoplasm, and GAP and effector proteins dissociate from the membrane. Note that GDP-bound Arf1 has its N-terminal amphipathic helix (striped blue and pink) retracted into a hydrophobic pocket and its interswitch region (purple) retracted. The N-terminal myristoyl group (green) is still free to associate with membrane, but the binding is weak, resulting in reversible binding. On exchange of GDP for GTP, the switch 1 and 2 domains move, and the interswitch toggles out of the hydrophobic pocket, allowing tighter membrane binding. The drug BFA interferes with exchange of GDP for GTP on Arf1 by stabilizing the association between Arf1-GDP and its GEF. As a result, Arf1 cannot recruit effectors to the membrane, leading to disruption of membrane traffic between the ER and Golgi apparatus.

Arf GTPases of the secretory pathway, in particular Sar1 and Arf1, recruit to membranes many types of effector proteins. These include the coat protein complexes of **COPII**, **COPI**, and **clathrin/adapters** plus other effectors such as phospholipid modifiers (e.g., phospholipase D, a lipid metabolizing enzyme), phosphoinositides, and cytoskeletal components. The coat protein complexes assemble into large polymeric structures (called **protein coats**) at the cytoplasmic surface of ER, pre-Golgi, and Golgi membranes, from which they sort cargo and promote the budding of transport carriers. The other Arf effectors play roles in differentiating the membrane environment of these carriers and enabling them to move to different locations within the cell. The four other mammalian Arf proteins (Arfs 2 to 6) regulate vesicle formation at other locales in the exocytic and endocytic pathways.

Sar1 assembles the COPII coat complex that is involved in differentiating ER export domains, which are the sites from which transport carriers bud out from the ER. Arf1, by contrast, assembles the COPI coat complex that is involved in the creation of retrograde transport carriers that bud from pre-Golgi and Golgi structures. Arf1 also recruits the clathrin/adaptor coat complexes that are involved in budding of transport carriers from the Golgi en route to the endosome/lysosomal system. Disruption of the GTPase cycles of either Sar1 or Arf1 has dramatic consequences for secretory transport and the organization of the secretory pathway (Fig. 21-14). When the GTPase cycles of Sar1 or Arf1 are disrupted, the Golgi apparatus disassembles, and Golgi enzymes return to the ER or to ER exit sites with all secretory transport out of the ER inhibited.

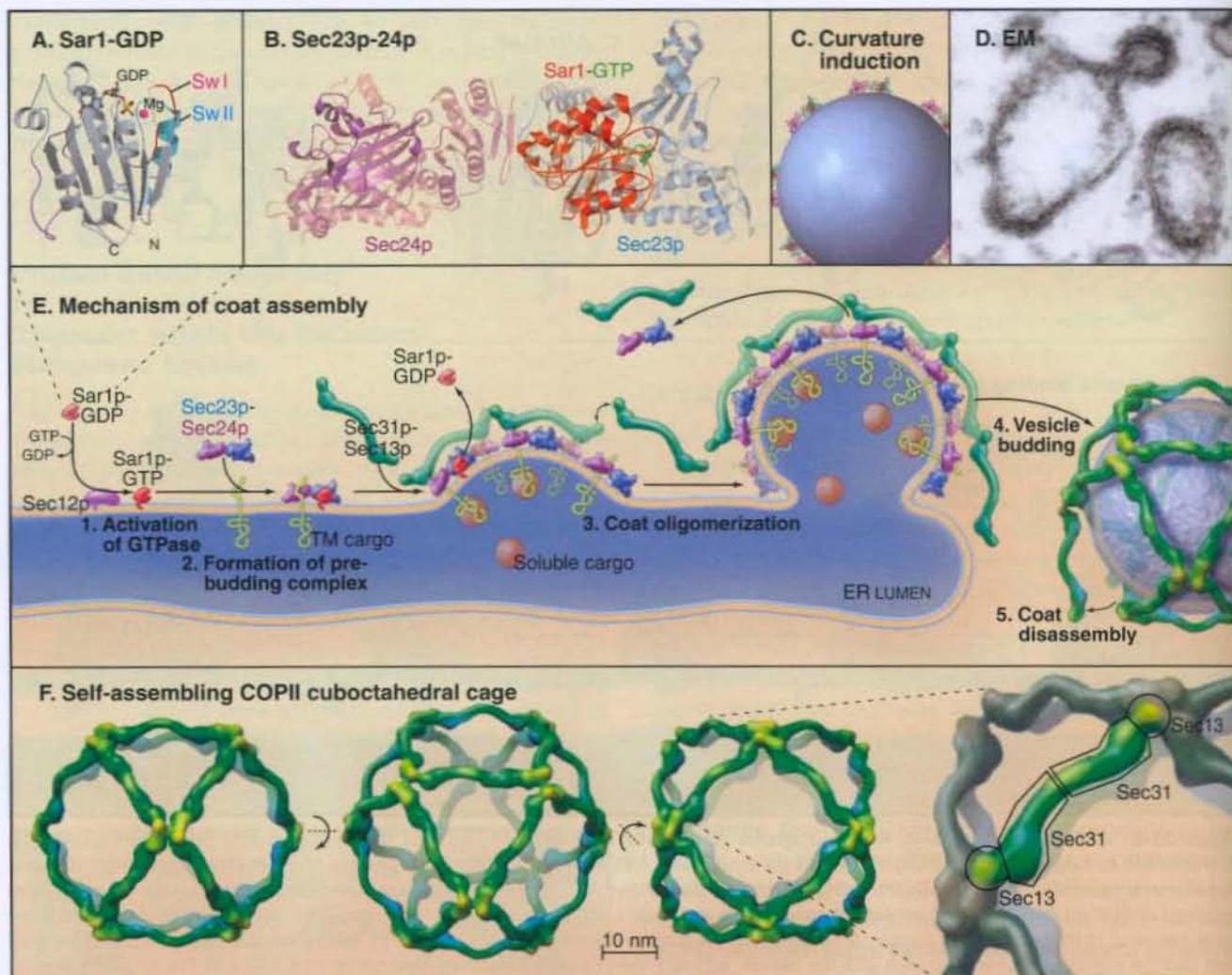


Figure 21-7 COPII COAT ASSEMBLY ON ENDOPLASMIC RETICULUM MEMBRANE. **A–B**, Ribbon diagrams of Sar1-GDP (PDB file: 1F6B) and the Sec23p-24p complex with Sec24p bound to Sar1-GTP. **C**, The bow-tie structure of Sec23p-Sec24p provides an extensive membrane-interaction surface that is concave, positively charged, and suitable for curving the bilayer when the subcomplex is bound to a membrane surface. **D**, Electron micrograph of a thin section illustrates the formation of a typical COPII vesicle when ER membranes are incubated in a test tube with cytosol and ATP. **E**, Sec12p activates Sar1 by promoting exchange of GDP for GTP, bringing Sar1 to the membrane. Sar1p-GTP then recruits the Sec23p-Sec24p subcomplex. Binding of Sec13p-Sec31p to Sec23p-Sec24p clusters these complexes into a coat. Transmembrane cargo is recruited into the coat by binding to Sec24p. Coat complexes dissociate from the lattice after Sar1-GTP converts to Sar1-GDP and releases into the cytosol. As long as coat oligomerization occurs faster than Sar1-dependent coat complex release, the lattice grows into a coated bud that can pinch off the membrane as a coated vesicle. Coat disassembly on the coated vesicle results from continued Sar1-dependent coat complex release in the absence of further coat complex addition due to Sec12 not being packaged into the coated vesicle membrane. **F**, Three-dimensional reconstruction of COPII cage at 30-Å resolution using cryoelectron microscopy and single-particle analysis. (C, Adapted from Bickford LC, Mossessova E, Goldberg J: A structural view of the COPII vesicle coat. *Curr Opin Struct Biol* 14:147–153, 2004, with permission from Elsevier. D, Courtesy of W. Balch, Scripps Research Institute, La Jolla, California. F, Adapted by permission from Macmillan Publishers Ltd. from Stagg SM, Gurkan C, Fowler DM, et al: Structure of the Sec13/31 COPII cage. *Nature* 439:234–238, 2006. Copyright 2006.)

The COPII Coat

The COPII coat complex (Fig. 21-7) is essential for sorting and trafficking secretory cargo out of the ER. It consists of Sar1p GTPase, the Sec23p-Sec24p subcomplex, and the Sec13p-Sec31p subcomplex. These components self-assemble into a polymeric, two-dimensional scaffold (called a coat) that then collects specific types

of cargo. The intrinsic curvature of the coat promotes the formation of membrane buds that are capable of pinching off the membrane as coated vesicles.

COPII coats assemble by a sequential process (Fig. 21-7E). A GEF called Sec12p recruits Sar1p-GDP from the cytoplasm to the ER membrane and activates it by exchanging GDP for GTP. Activated Sar1p-GTP then recruits the two COPII subcomplexes: Sec23p-Sec24p,

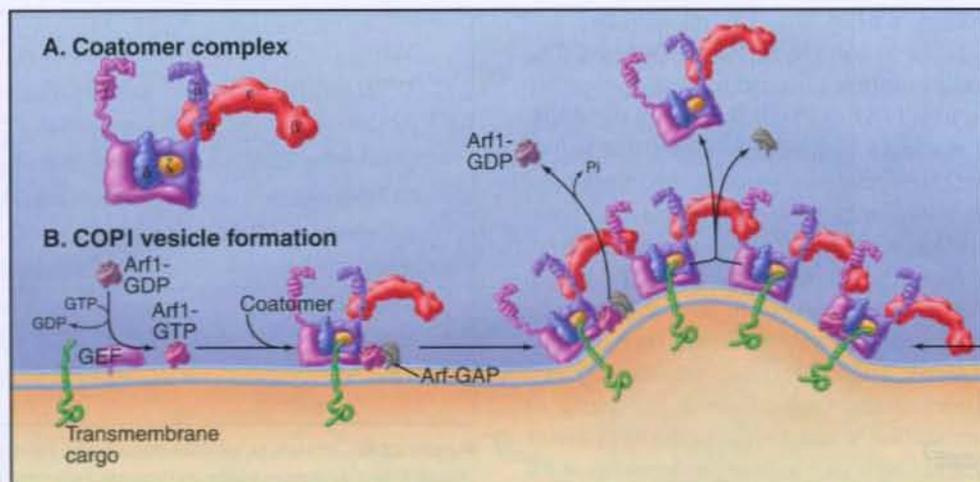


Figure 21-9 COPI COAT ASSEMBLY ON MEMBRANES. **A**, Depiction of COPI subunits within the coatomer complex. **B**, Activation of Arf1 to the GTP-bound form by the Sec7 domain of Arf1-specific GEFs results in the coupled recruitment of cargo, vesicle tethering factors, and fusion factors through binding of the cytoplasmic coatomer complex. Low-affinity interactions among Arf1, coatomer, and GAP cause them to polymerize into a coat that bends the patch of membrane to which they are associated. The increased curvature activates the GAP that stimulates Arf1 to hydrolyze GTP triggering its release from the membrane. After Arf1 is released, coatomer and GAP are destabilized and dissociate from the coat. The continuous cycle of coatomer binding, polymerization, and dissociation mediated by Arf1 GTPase activity leads to the formation of a coated bud that can pinch off the membrane as a coated vesicle or remain as a meta-stable coated bud that imparts curvature and tension to the membrane.

lattice of coatomer and Arf-GAP1. This leads to coat disassembly.

A consequence of these dynamic events is that COPI units move into the lattice from the rims and are released from the interior after Arf1 hydrolyzes its GTP, paralleling events occurring in the COPII coat. This results in

a continuous flux of coat units through the lattice whether or not a coated vesicle detaches from the membrane. This dynamic behavior of coat units allows for several outcomes (Fig. 21-10A-C). The lattice can grow, disassemble (after budding off the membrane as a coated vesicle), or persist as a coated bud. In the latter case,

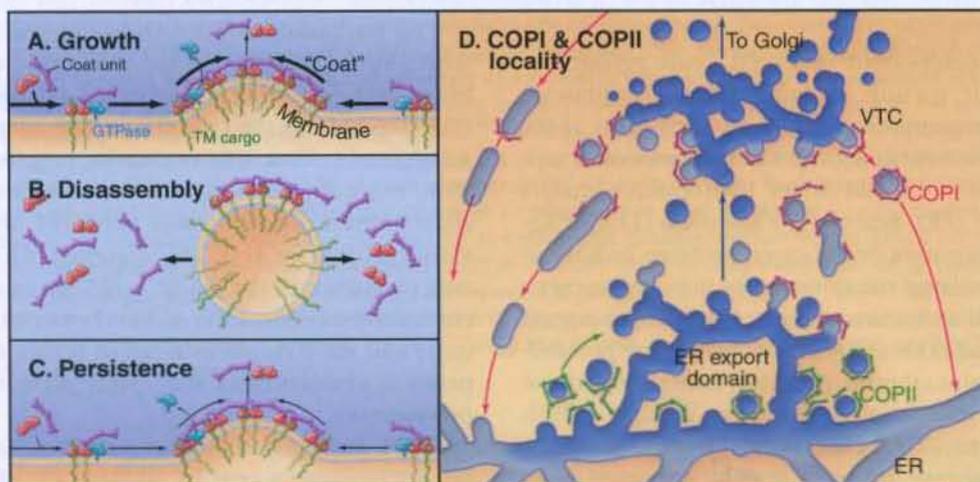


Figure 21-10 POTENTIAL FATES OF COAT COMPLEXES ON MEMBRANES. **A**, When binding of coat units is faster than release, the coat grows and forms a coated vesicle. **B**, After a coated vesicle forms, coat binding becomes slower than release (owing to GEF not being incorporated into the coated bud), and the coat disassembles. **C**, When coat units bind at the same rate as they release, then the coat is metastable (it neither shrinks nor grows but imparts curvature to the membrane). By increasing curvature in the membrane, metastable coats increase membrane tension, which can cause lipid partitioning. **D**, Cartoon diagram of the distribution of COPII and COPI coats on ER export domains and VTCs. COPII coats are restricted to ER membranes, where they recruit cargo into the ER export domain. COPI coats are present on the vesicular/tubular elements of the ER export domain, VTC and Golgi apparatus, where they orchestrate retrieval of proteins back to the ER.

the rate of addition of coat units to the bud is equal to the rate of unit loss. These behaviors of the coat lattice play key roles in orchestrating the protein sorting and morphologic events that occur at ER export domains to allow for VTC formation (Fig. 21-10D).

Incorporation of recycling proteins into COPI coated buds requires a specific sorting motif. Generally, this is a dilysine motif in a sequence of Lys-Lys-x-x-COOH (KKxx), where x is any amino acid. Two arginine residues substitute for the lysines in some proteins. Dilysine motifs are generally found at the cytoplasmic C-terminus of transmembrane proteins. They function in retrieval and possibly in retention of proteins within post-ER compartments by interacting with specific subunits of the COPI complex.

Rab GTPases

The Rab family of GTPases are the molecular switches that control the protein-protein interactions between transport carriers and docking complexes on target membranes (Fig. 21-11). These complexes recruit motor proteins that transport carriers on actin filaments or microtubules and then tether carrier vesicles to an organelle prior to fusion. Mammals express about 70 different Rab proteins to provide specificity at numer-

ous transport steps in the secretory membrane systems of various cell types.

Rab proteins are posttranslationally modified by two geranylgeranyl lipids on conserved cysteine residues at their C-termini. This modification is essential for function and facilitates Rab association with the membrane bilayer. The cysteines are included in a variable segment of 30 amino acids that targets each Rab to its correct subcellular location.

Rab proteins cycle between the cytoplasm, where they are found in the GDP-bound form, and membranes, where they contain bound GTP (Fig. 21-11). In the cytoplasm, Rabs are complexed with a carrier protein called a **guanin nucleotide dissociation inhibitor** (GDI), which prevents exchange of GDP to GTP. GDI also sequesters the hydrophobic geranylgeranyl groups. Proteins called GDI displacement factors facilitate Rab recruitment to membranes by displacing GDI.

Rab-specific GEFs activate and recruit Rabs to form carrier vesicles. Rab-GTP then recruits the targeting and docking components to be used subsequently to recognize the target membrane and initiate bilayer fusion. Following fusion, a Rab-specific GAP stimulates GTP hydrolysis, recycling Rab-GDP back to the cytoplasm through binding to GDI. Using this GTPase cycle, Rab proteins regulate the timing of the assembly and disassembly of diverse multiprotein complexes involved in the trafficking of transport containers.

Tethering Factors

Tethering factors are rod-shaped proteins that extend about 15 nm from membranes into the cytoplasm (Fig. 21-11). They tether membrane carriers to target organelles prior to fusion and play structural roles as components of a Golgi matrix or scaffold for the assembly of other factors important for fusion and/or cargo sorting. Heterogeneous in sequence and structure, tethering factors can be divided into two general classes:

- Coiled-coiled tethering factors interact exclusively with active Rabs and function as Rab effectors. For example, the tethering factor p115/Usolp functions in ER-Golgi transport. It is a homodimer with a long tail consisting of a coiled-coil of parallel α -helices and two globular heads at the C-terminus, reminiscent of myosin II (see Fig. 36-1). An internal hinge-like region in the tail collapses once the tether brings the membrane-enclosed carrier close to an acceptor membrane.
- Multisubunit tethering factors, such as TRAP1/II, the exocyst, and COG, bind to inactive Rabs and participate in their activation (functioning as GEFs). The TRAPP I (transport protein particle)

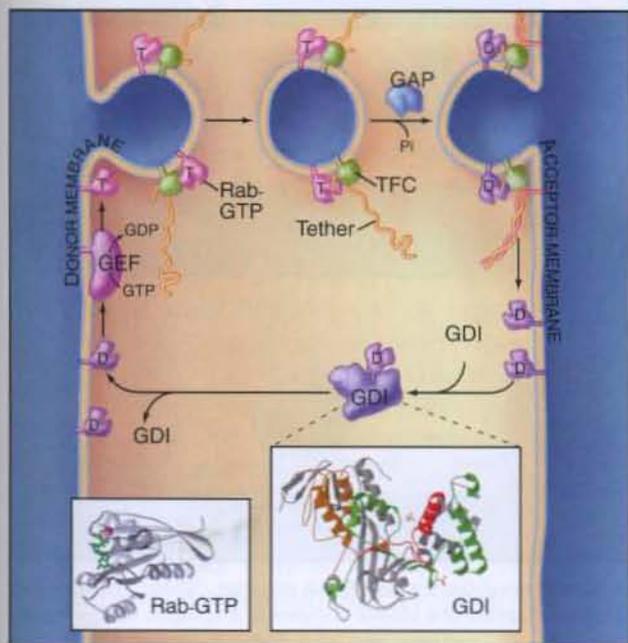


Figure 21-11 Rab GTPase cycle. Rab GTPases in their GDP-bound form are complexed with GDI in the cytoplasm. Following delivery to the membrane involving interactions with a GTPase dissociation factor, they are activated by a membrane-associated, Rab-specific GEF. In the GTP-bound form, they recruit effectors, such as tethering factor complexes (TFCs), which aid in targeting and docking the vesicle. Rab-GTP is returned to the GDP-bound form by a GAP, wherein it binds again to GDI. Insets show ribbon diagrams of Rab-GTP and GDI (PDB files: 3RAB and 1D5T). Pi, inorganic phosphate.

complex contains seven subunits, whereas the COG (conserved oligomeric Golgi) complex contains eight subunits. Recent structural studies suggest that the mechanism for TRAPP association with membranes involves a membrane-interacting surface that is flat, wide, and decorated with positively charged residues. Cells that are defective in COG function exhibit pleiotropic defects in virtually all *N*-linked, *O*-linked and lipid-linked conjugates, suggesting that the COG complex regulates glycosylation reactions in the Golgi in addition to interacting with Rabs.

SNAP Receptor Components

The SNAP receptor (SNARE) family of proteins participates in the fusion of carriers with their appropriate acceptor compartment (Fig. 21-12). Most SNAREs are

transmembrane proteins with their functional *N*-terminal domains in the cytoplasm and their *C*-termini anchored to the bilayer. Each contains a heptad repeat (i.e., "SNARE motif") of 60 to 70 amino acids that can form a coiled-coil. Multiple SNAREs assemble a SNARE complex consisting of a bundle of α -helices. Members of the SNARE protein family were originally grouped according to whether they were *v*-SNAREs or *t*-SNAREs, referring to whether they conferred function to the vesicle (*v*-SNARE) or target (*t*-SNARE) compartment. For example, synaptobrevin is a *v*-SNARE found on synaptic vesicles involved in neurotransmission (see Fig. 11-9), whereas syntaxin 1 is a *t*-SNARE found on presynaptic densities to which synaptic vesicles fuse to trigger neurotransmitter release.

The formation of a SNARE complex occurs by the pairing of cognate *v*- and *t*-SNAREs. This generates a four-helix bundle with one α -helix contributed by one

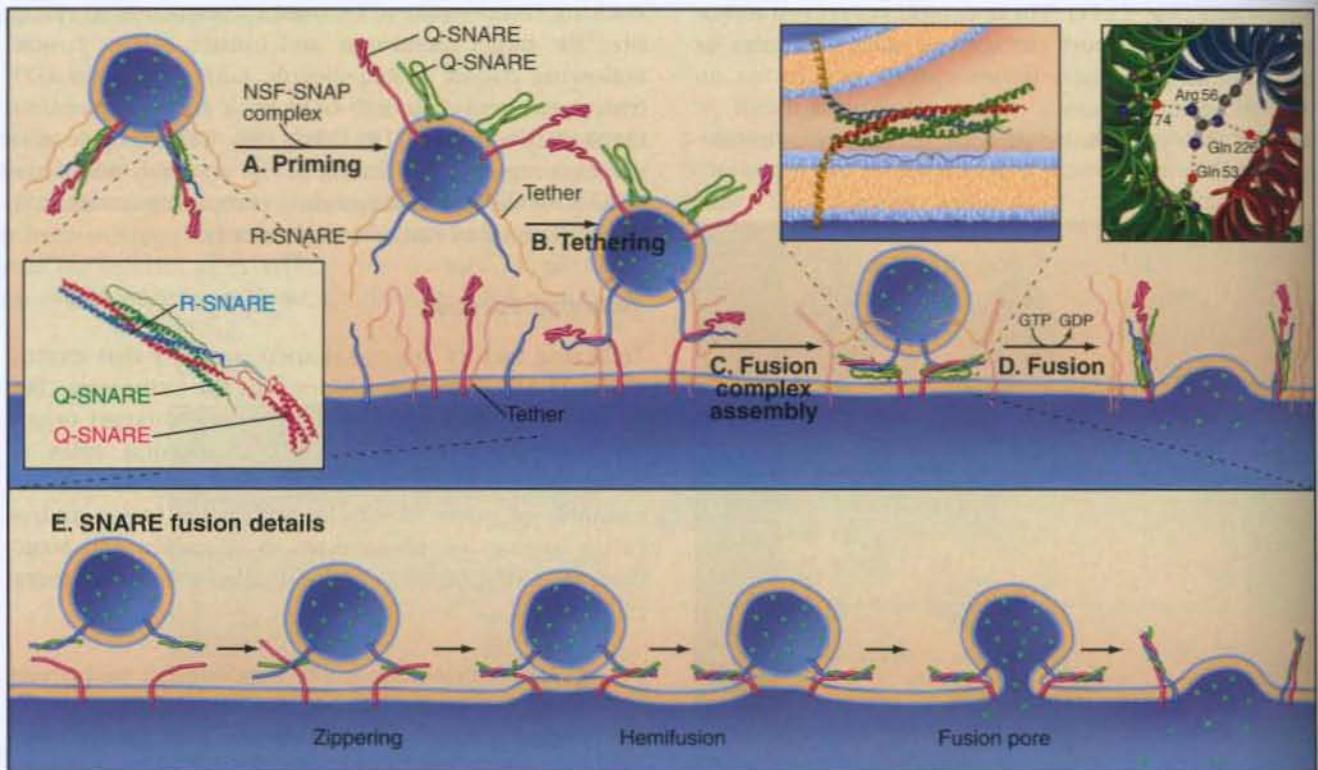


Figure 21-12 GENERIC SNAP RECEPTOR TARGETING AND DOCKING MACHINERY. **A**, Tethering factors and SNAP receptor (SNARE) proteins form *cis*-SNARE complexes during carrier formation. The *lower left inset* shows a ribbon diagram of a SNARE complex of a synaptic vesicle involved in neurotransmitter release involving R-SNARE (synaptobrevin, blue) and Q-SNARE (syntaxin, red) and SNAP25 (green) SNARE. (PDB file:1SFC.) **B–C**, Interaction of a vesicle with its target membrane through tethers results in the formation of *trans*-SNARE pairs involving extensive coiled-coiled regions of the interacting SNARE proteins. The *middle inset* shows the *trans*-SNARE pair. The *upper right panel* is a ribbon diagram looking down the coiled-coil, illustrating the critical Arg (arginine) residue of R-SNAREs that stabilizes interaction with glutamines (Glns) of Q-SNAREs forming the four-helix bundle in a SNARE complex. **D**, Following *trans*-SNARE pairing, hydrolysis of GTP bound to Rab (not shown) leads to vesicle fusion. This results in the incorporation of the *trans*-SNARE pair into the bilayer of the target membrane, where it and tethering complexes are disassembled for reuse. **E**, Overview of SNARE-mediated fusion. (*Top right inset*, Adapted from Ossig R, Schmitt HD, de Groot B, et al: Exocytosis requires asymmetry in the central layer of the SNARE complex. EMBO J 22:6000–6010, 2000, by permission of Oxford University Press.)

SNARE and the other three α -helices contributed by an oligomerized t-SNARE. The four-helix bundle of SNAREs depends on interactions of an arginine from one helix with glutamines from three other helices. This requirement has led to an alternative classification of these proteins as either R-SNAREs or Q-SNAREs, based on the presence of these critical arginine (R) or glutamine (Q) residues.

The v-SNAREs and t-SNAREs in separate membranes can pair to form a *trans*-SNARE complex, or v-SNAREs and t-SNAREs in the same membrane can pair to form a *cis*-SNARE complex. Assembly of a *trans*-SNARE complex, also called the "SNAREpin," is thought to supply the free energy needed to bring two membranes close enough to fuse. This is similar to the operation of viral fusion proteins.

Fusion of a carrier with a target membrane transforms a *trans*-SNARE complex into a *cis*-SNARE complex on the cytoplasmic face of the fused membrane. Following completion of fusion, the *cis*-SNARE complex is disassembled by a ubiquitous AAA ATPase (see Box 36-1) called NSF (for *N*-ethyl maleimide [NEM]-sensitive factor). The sulfhydryl alkylating reagent NEM inactivates NSF and prevents all carrier transport in the cell. SNAP proteins recruit NSF to the membrane. NSF uses the energy from ATP hydrolysis to dissociate the *cis*-SNARE complex and recycles the SNAREs for another round of membrane fusion.

Most SNARE proteins are anchored to membranes by a transmembrane segment that inserts into ER membranes after translation (i.e., they are tail-anchored proteins; see Chapter 20). Thus, SNAREs must traverse the secretory pathway to reach specific organelles, but little is known about the mechanism they use. The length of their transmembrane domains and their capacity to interact with protein coats are likely to be important. For example, SNAREs involved in ER to Golgi transport are packaged into COPII coats at ER export sites for delivery to the Golgi apparatus, where they mediate homotypic fusion (i.e., fusion of two like transport containers that have identical *cis*-SNARE pairs) among incoming carriers as well as heterotypic fusion (i.e., fusion of two distinct membrane structures that have different *cis*-SNARE pairs) of these carriers with the Golgi membrane. The SNAREs are then packaged into COPI coats for retrieval to the ER. This allows them to function repeatedly in ER-to-Golgi apparatus transport.

Particular v-SNARE and t-SNARE complexes help to ensure the specificity of fusion at different steps along the secretory membrane pathway. SNAREs do not work alone in membrane fusion. Tethering factors assembled with the aid of Rab GTPases link specific apposing membranes prior to SNARE complex formation. Thus, SNAREs, tethers, and Rabs work together to ensure that

membranes fuse at the correct time and place within the secretory system.

Secretory Transport from the Endoplasmic Reticulum to the Golgi Apparatus

Transport of newly synthesized proteins out of the ER takes place in specialized areas called **ER export domains**. These structures are approximately 1 to 2 μm in diameter and appear in fluorescent images as dispersed, punctate structures that are scattered over the surface of the ER (Fig. 21-13A). An individual ER export domain is organized into two zones (Fig. 21-13B-C). One is a region of smooth ER membrane studded with COPII-coated buds and uncoated tubules. The other is a central cluster of vesicles and tubules with the capacity to detach and traffic to the Golgi apparatus. The ER membrane is continuous between these two zones until the vesicle-tubule cluster and its associated cargo detach from the ER and move to the Golgi apparatus as a transport intermediate, called vesicular tubular carrier (VTC) (Fig. 21-10D). Cargo proteins are actively sorted into ER export domains through binding of signal motifs within their cytoplasmic tails to the COPII coat, and/or by lateral partitioning into the specialized lipid environment of this region. Partitioning is thought to occur once the transmembrane segments of the cargo proteins match the thickness of the ER exit site lipid bilayer.

The morphologic and biochemical differentiation of the ER export domain into a motile VTC is a multistep process orchestrated by the sequential action of the Sar1, Rab1, and Arf1 GTPases and their effectors (Fig. 21-14). Sar1 GTPase initiates ER export domain formation through COPII-mediated sorting of specific integral membrane proteins (including the p24 family proteins and SNAREs) and the formation of coated buds. The presence of coated buds and specialized cargo in this region, together with the membrane tension produced by the coated buds, leads to changes in bilayer lipid composition. This, in turn, promotes partitioning of other transmembrane proteins into the ER export domain, including proteins with longer-than-average transmembrane domains that lack COPII recognition motifs in their cytoplasmic domains. Additional cytoplasmic proteins are then also recruited to the ER exit site, including Rab1 and p115, which interact with tethering factors (such as GM130 and giantin), SNARE proteins, and GBF1 (the GEF for Arf1). Together, these molecules stimulate the membrane budding and fusion events that differentiate the ER export domain and VTC. The SNARE proteins, for example, allow the COPII-coated vesicles and membrane tubules that bud out

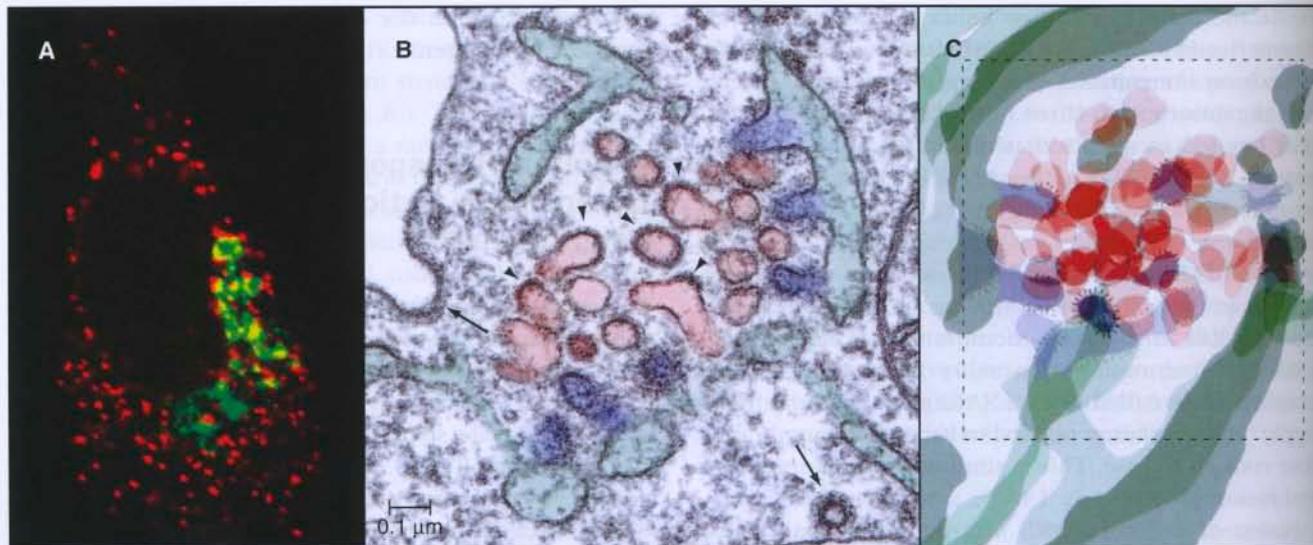


Figure 21-13 MORPHOLOGY AND OVERALL DISTRIBUTION OF ENDOPLASMIC RETICULUM EXPORT DOMAIN AND VESICLE-TUBULE CARRIER. **A**, Light micrograph showing the distribution of ER export domains and Golgi apparatus within a fibroblast cell. The cell was transfected with cDNAs encoding an ER export domain marker, Sec31-YFP (red), and Golgi marker, galactosyltransferase-CFP (green), which were labeled with different color variants of green fluorescent protein. Note that the ER exit sites are distributed throughout the cytoplasm as punctate structures, whereas the Golgi apparatus is localized in a juxtanuclear site. **B**, Electron micrograph of a thin cross section of a typical ER export domain containing a central vesicle tubule carrier that can detach and traffic to the Golgi apparatus. ER is green, ER-associated-coated buds are blue, the VTC is red, arrowheads mark COPI coats, and arrows mark clathrin-coated vesicles from the plasma membrane. **C**, Reconstruction from four consecutive serial-thin sections illustrating the three dimensional organization of ER export domain demarcated by the box. (A, Adapted from Altan-Bonnet N, Sougrat R, Liu W, et al: Golgi inheritance in mammalian cells is mediated through endoplasmic reticulum export activities. *Mol Biol Cell* 17:990–1005, 2006. B–C, From Bannykh SI, Nishimura N, Balch WE: Getting into the Golgi. *Trends Cell Biol* 8:21–25, 1998.)

from the smooth ER to fuse with themselves to form a tubule cluster; GM130 and giantin tether these membranes to the cytoskeleton; and Arf1 effectors differentiate the membrane further by initiating retrieval of specific proteins back to the ER. Disruption of the GTPase cycle of Sar1 through expression of a GDP-locked form prevents ER export domain formation, whereas disruption of the GTPase cycle of Arf1—through expression of a GDP-locked form of Arf1 or by BFA treatment—blocks VTC formation (Fig. 21-14). By blocking membrane delivery into the secretory pathway, both treatments also cause the disappearance of the Golgi apparatus, which depends on continuous membrane input to maintain its structure.

Detachment of the VTC from the ER export domain and its maturation and delivery to the Golgi apparatus are the next steps in protein trafficking from ER to Golgi apparatus. Mammalian cells use motors to detach VTCs from ER export domains and to carry them along microtubules toward the Golgi apparatus located near the microtubule-organizing center (Fig. 21-1). During this process, the VTC matures by a process that is orchestrated by Arf1 and its effectors. Activated Arf1 recruits dozens of cytoplasmic proteins to VTCs (and to Golgi membranes). Among these, the COPI coat binds to and clusters specific proteins, enabling them to be retrieved back to the ER. Lipid-modifying enzymes such as

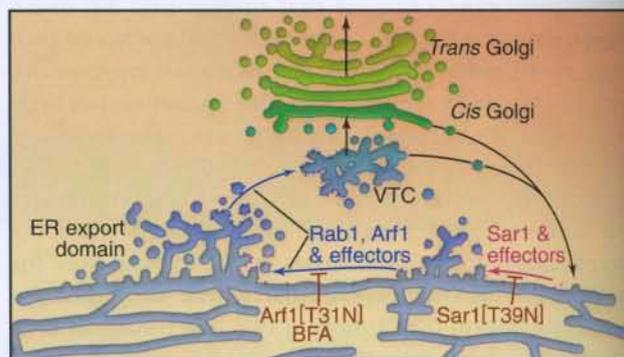


Figure 21-14 TRANSPORT FROM THE ENDOPLASMIC RETICULUM TO THE GOLGI APPARATUS. ER to Golgi transport is orchestrated by the combined activities of many molecules. Sar1 and its effectors initiate COPII-coated bud formation and clustering of cargo at regions called ER export domains. This induces p115 and Rab1 to bind to these regions, which in turn recruits GBF1, the GEF for Arf1. Subsequent recruitment of Arf1 and its effectors further differentiates the ER export domain into a VTC. The VTC detaches from the ER and targets the Golgi apparatus, where it fuses with the cis face of the Golgi. The cargo in the VTC is then released into the Golgi and moves to the trans Golgi (where it will exit from the TGN). Expression of a constitutively inactive Sar1 mutant, Sar1[T39N], blocks COPII recruitment, and no ER exit sites form. Expression of an inactive Arf1 mutant, Arf1[T31N], or BFA treatment blocks recruitment of Arf1 effectors, which prevents ER exit sites from differentiating into VTCs. This causes the shrinkage and disappearance of the Golgi apparatus because new membrane from the ER cannot be delivered to the Golgi.

phosphatidylinositol kinases and phosphatases create a lipid environment that is distinct from that in the ER membrane, permitting tethering factors and matrix proteins to bind to the motile VTC membrane. Ankyrin and spectrin proteins (see Fig. 7-10) form a scaffold for other cytoskeletal proteins, including actin, tubulin, dynactin, and dynein. Among these, the dynactin complex (see Fig. 37-2) mediates dynein-dependent clustering of VTCs by movement on microtubules toward centrioles at the center of the cell.

After VTCs have clustered by movement inward along microtubules, they undergo fusion with the Golgi apparatus. This occurs at the *cis* or entry face of the Golgi apparatus, also called the *cis*-Golgi network (CGN) because of its elaborate tubular appearance. The membrane fusion releases cargo proteins and lipids of the VTC into the Golgi system for processing by enzymes that modify the cargo's oligosaccharide side chains.

Exactly how biosynthetic cargo is then transferred through the Golgi apparatus system has not been clarified experimentally, but three mechanisms are likely to contribute. The first mechanism uses vesicular transport to transfer cargo between the distinct cisternal elements that make up the Golgi apparatus. Vesicles derived from one cisternum transfer cargo to a neighboring cisternum. In a second mechanism, cargo is conveyed across the Golgi system by directed maturation of cisternal elements. A third mechanism involves diffusion and/or lateral partitioning of cargo within the membrane or luminal spaces between interconnected cisternal Golgi elements. The contributions of each

mechanism are still unclear and may vary depending on the cargo being transported through the Golgi system.

Sorting from the Trans-Golgi Network

After transport through the Golgi system, cargo leaves the *trans* or exit face of the Golgi apparatus (Fig. 21-15). The exit region is called the *trans*-Golgi network (TGN) because of its tubular network organization. This organization is characteristic of other sorting compartments, such as that of the VTC, the *cis* Golgi, and sorting endosomes (see Chapter 22). Depending on the cell type, the cargo that arrives in the TGN can be distributed, via distinct transport carriers, to several different intracellular locations, including the plasma membrane or cell exterior, the endosome/lysosomal system, or specialized secretory organelles or granules. The intracellular route taken by each protein depends on sorting properties that are encoded in the polypeptide chain.

Constitutive Transport of Cargo to the Plasma Membrane or Cell Exterior

A steady stream of both proteins and lipids from the TGN to the cell surface occurs constitutively through tubular transport carriers that bud out from the TGN (Fig. 21-16). No known coat proteins function in the formation of these structures. Instead, cargo proteins conveyed to the plasma membrane by these structures

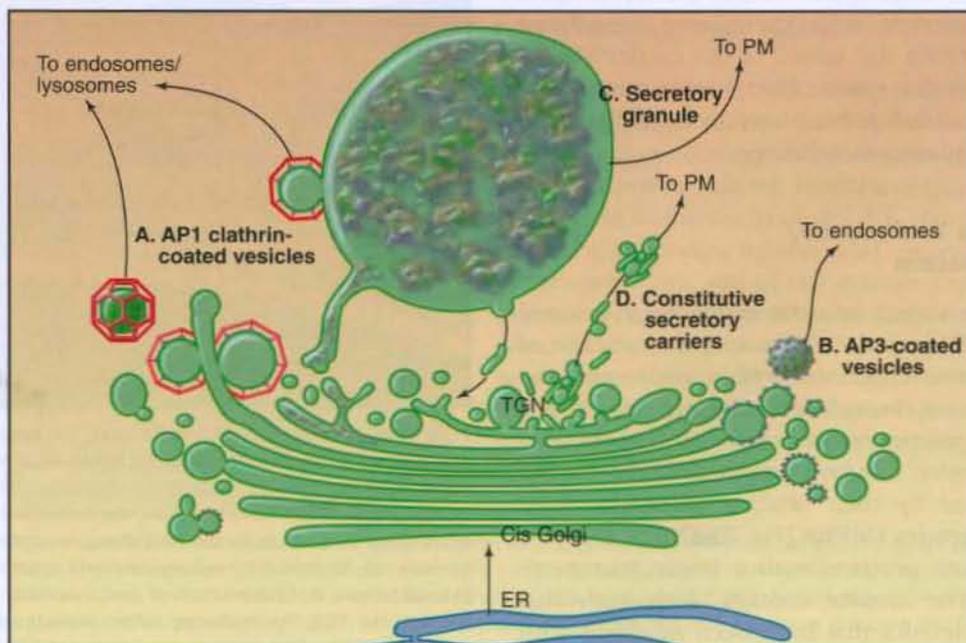


Figure 21-15 DIVERGENCE OF BIOSYNTHETIC/EXOCYTTIC CARGOES AT THE TRANS-GOLGI NETWORK. A–D, Cargo destined for secretion or distinct intracellular locations is sorted and packaged into distinct transport carriers. The tubular/vesicular geometry of the TGN plays an important role in protein sorting. PM, plasma membrane.

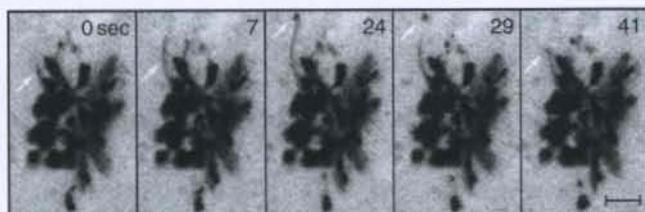


Figure 21-16 FLUORESCENCE MICROGRAPHS OF A TISSUE CULTURE CELL EXPRESSING A FLUORESCENTLY TAGGED TRANSMEMBRANE PROTEIN, VSVG-GFP, EN ROUTE TO THE PLASMA MEMBRANE. The images were collected over time and show long tubules enriched in the labeled protein (arrows) emanating from the Golgi apparatus. The tubules later detach from the Golgi and traffic to the plasma membrane. Scale bar is 5 μm . (Reproduced from Hirschberg K, Miller CM, Ellenberg J, et al: Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. *J Cell Biol* 143:1485–1503, 1998. Copyright 1998 The Rockefeller University Press.)

have transmembrane segments that partition into lipid domains containing sphingolipids and cholesterol. Activation of specific lipid-modifying enzymes such as phosphatidylinositol 4-kinase in the sphingolipid/cholesterol-enriched sorting domain of the TGN then results in the domains forming tubules that pinch off the TGN. Because the tubules have a higher volume-to-surface ratio than small vesicles, bulk soluble markers are also carried to the plasma membrane by these structures. Tubule extension is facilitated by motors moving on microtubules and/or by actin filaments, while tubule severing is mediated by dynamin-2, a GTPase localized in TGN. In mammalian cells, motor proteins such as kinesins move the constitutive membrane carriers outward from the Golgi apparatus along microtubules. Fusion of the carriers with the plasma membrane releases cargo within the lumen of the carrier vesicle into the extracellular space. After fusion, membrane lipids and proteins redistribute laterally by diffusion in the plane of the plasma membrane.

Sorting to the Endosome/Lysosomal System

Proteins that are sorted into the endosome/lysosomal system (Fig. 21-17) include a large and diverse class of hydrolytic enzymes contained within lysosomes, the digestive centers of the cell (see Chapter 23). Newly synthesized hydrolytic enzymes are prevented from entering constitutive tubular carriers destined for the plasma membrane by their binding to **mannose-6-phosphate receptors (MPRs)** [Fig. 21-17A)]. MPRs are integral membrane proteins with a single transmembrane domain. The luminal domain binds individual prohydrolase molecules that have been modified with mannose-6-phosphate (M6P), whereas the cytoplasmic domain encodes sorting motifs that interact with the clathrin/adaptor sorting machinery (see Chapter 22)

that directs packaging into carriers as they leave the TGN destined for endosomes. After MPRs discharge their cargo, other carriers transfer the unoccupied MPRs back from the endosome to the TGN (Fig. 21-17).

AP1 complexes direct clathrin coat assembly at the TGN (Fig. 21-17B). They interact directly with either tyrosine-based or dileucine sorting motifs on the MPR receptor tail. Recruitment and assembly of the AP1-containing clathrin coat on the TGN occur through direct interaction with the same small guanosine tri-

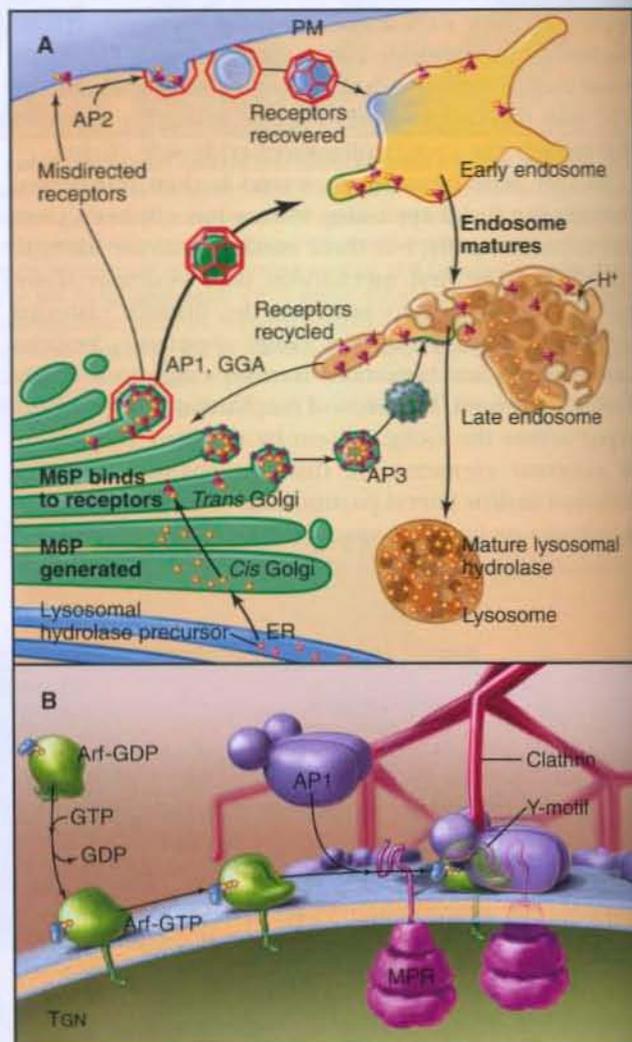


Figure 21-17 SORTING PATHWAYS USED BY MANNOSE 6-PHOSPHATE RECEPTORS AND COAT ASSEMBLY AT THE TRANS-GOLGI NETWORK. **A**, MPRs carry newly synthesized lysosomal hydrolases containing mannose-6-phosphate (M6P) from the TGN, via endosomes, to lysosomes, after which they return to the TGN. Receptors missorted to the cell surface are recovered by endocytosis and returned to the pathway in endosomes. **B**, Coordination of coat assembly and cargo recruitment at the TGN. An exchange factor activates the small GTPase Arf to bind GTP, which triggers recruitment of AP1 coat constituents to the TGN membrane. The MPR is concentrated in the emerging coated vesicle through interactions between a tyrosine-based sorting motif in its cytoplasmic domain and the μ -subunit of AP1.

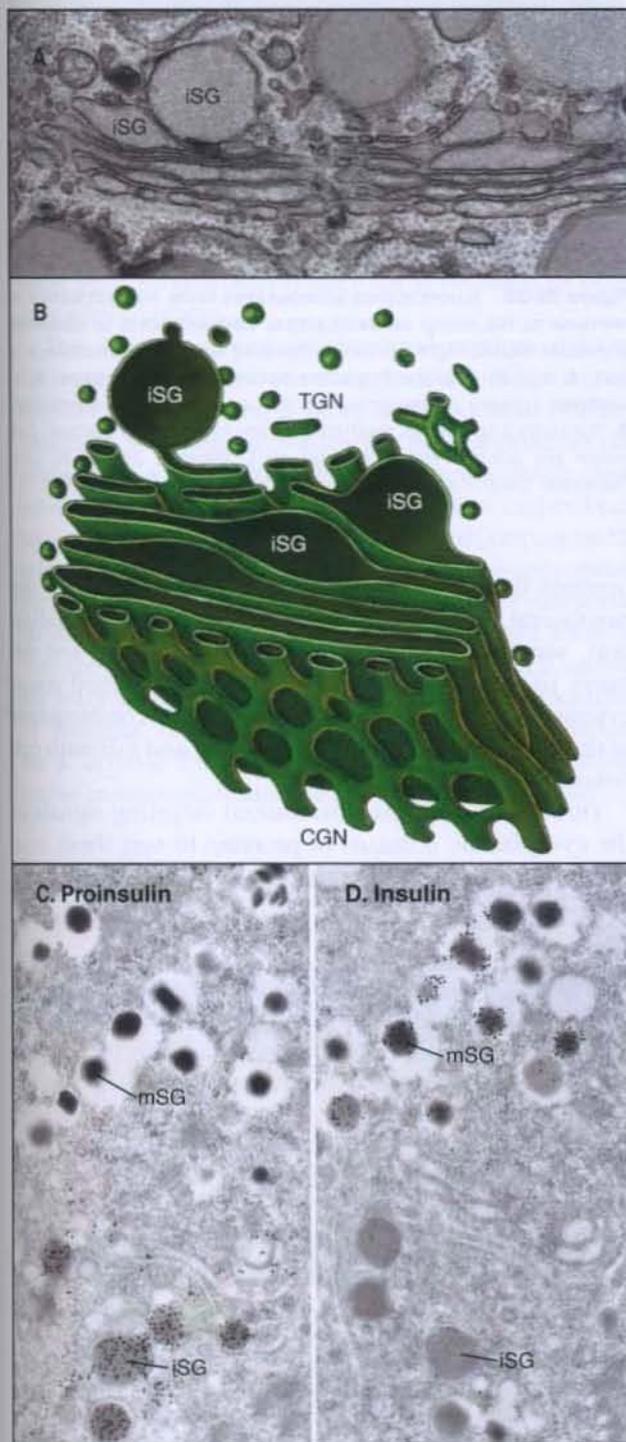


Figure 21-18 FORMATION OF SECRETORY GRANULES. Transmission electron micrograph of a thin section (**A**) and a diagram (**B**) show immature secretory granules (ISG) as they emerge from the TGN. Much of the TGN surface is consumed by forming immature secretory granules. **C–D**, Cryoelectron micrographs of frozen sections reacted with gold-labeled antibodies to proinsulin (**C**) or insulin (**D**). Proinsulin is concentrated in immature secretory granules. After processing, insulin is concentrated in mature, dense-core secretory granules (mSG). (A, Courtesy of J. Clermont, McGill University but by permission of Wiley-Liss, Inc. B, Redrawn from Clermont Y, Rambourg A, Hermo L: *Trans-Golgi network (TGN) of different cell types: Three-dimensional structural characteristics and variability*. *Anat Rec* 242:289–301, 1995. Copyright © 1995. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. C–D, Courtesy of L. Orci, University of Geneva, Switzerland.)

phosphate (GTPase) Arf1 that also triggers COPI assembly (Fig. 21-6). The assembly of the clathrin-API coat drives receptor clustering and budding off the TGN membrane of clathrin-coated transport vesicles. Several lysosomal membrane proteins are also sorted into these clathrin-coated vesicles by virtue of tyrosine-based sorting motifs on the cytoplasmic domains of the membrane protein. After budding off the TGN in clathrin-API-coated vesicles, both lysosomal hydrolases and lysosomal membrane proteins are delivered to lysosomes by way of endosomal intermediates.

Secretory Granule Formation and Transport

An additional sorting pathway from the TGN occurs in specialized endocrine, exocrine, or neuronal cells that concentrate and package selected proteins in storage granules for eventual mobilization and discharge from the cell in response to hormonal or neural stimulation. This is the so-called **regulated secretory pathway** (Fig. 21-18), which is used for discharging most of the body's polypeptide hormones, enzymes used in the digestive tract, and many other products that are needed intermittently rather than continuously.

Our mechanistic understanding of secretory granule formation and sorting processes is hindered by the apparent lack of a universal sorting signal on proteins that are destined for inclusion into regulated secretory granules. Instead, secretory granule formation appears to involve physical sorting, selective retention, and condensation (Fig. 21-19). Condensation of luminal content during secretory granule biogenesis involves charge neutralization, protein aggregation and active extrusion of ions.

In some cells that produce and store peptide hormones, aggregation involves only selected products of proteolytic processing of hormone precursors. For example, production of **insulin** requires proteolytic enzymes in immature granules that cleave proinsulin at two sites, generating insulin and C-peptide. Insulin condenses with zinc ion in the granule core, whereas C-peptide is excluded and so accumulates around this core. As a consequence, more C-peptide is shed into unstimulated secretory pathways that originate from the immature granule. Very tight regulation of insulin secretion is important for controlling the glucose

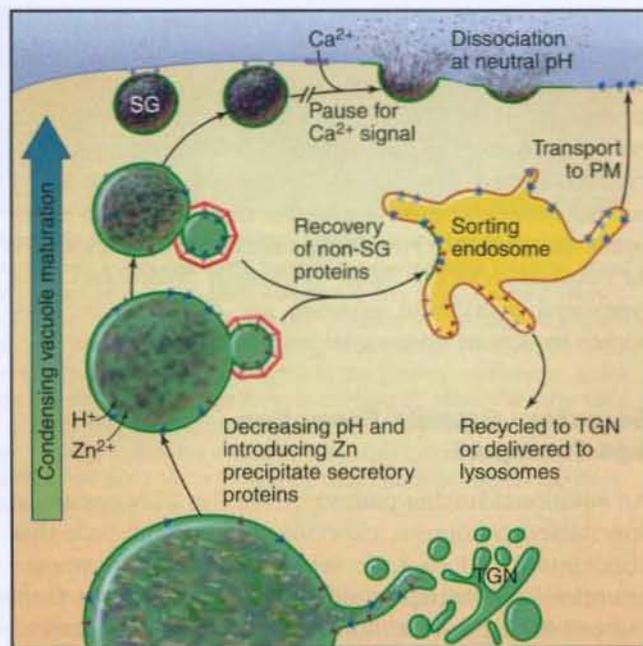


Figure 21-19 MATURATION OF NASCENT SECRETORY GRANULES/CONDENSING VACUOLES. The vacuolar H^+ -ATPase in the secretory granule (SG) membrane lowers the internal pH. This drives condensation and concentration of the contents. Dense-core, mature secretory granules are stored in the cytoplasm until a Ca^{2+} -mediated signaling event triggers fusion and release of their contents. Proteins inadvertently included in large, immature secretory granules emerging from the TGN are captured by clathrin-coated vesicles and recycled to endosomes and the TGN. PM, plasma membrane.

concentration in the blood plasma. This regulation is compromised in certain forms of **diabetes**.

Trafficking to the Plasma Membrane in Polarized Cells

In contrast to nonpolarized cells, polarized cells have functionally (and thus compositionally) distinct apical and basolateral domains separated by tight junctions that cement neighboring cells together and prevent diffusion between the domains (Fig. 21-20). Most of our knowledge of membrane sorting in polarized cells has come from studying epithelial cells. As expected, the trafficking complexity increases as destination options increase, and three distinct mechanisms for the polarized sorting of plasma membrane proteins have been revealed (Fig. 21-21). One mechanism involves selective packaging of apically or basolaterally destined proteins into distinct carrier vesicles at the TGN for delivery to the appropriate surface. A second mechanism involves the random delivery of newly synthesized proteins to both surfaces, followed by selective retention or depletion so that, at steady state, they become differentially abundant because they are more stable at one surface than at the other. A third mechanism

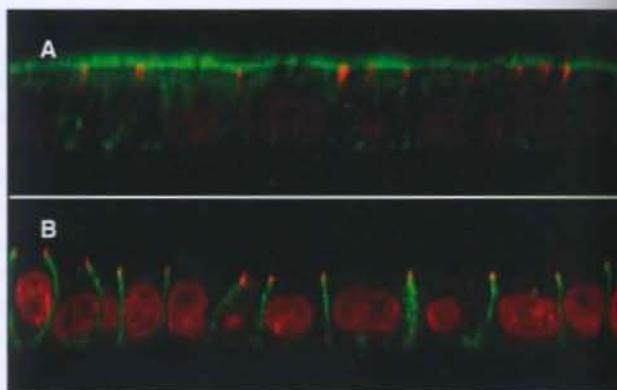


Figure 21-20 FLUORESCENCE MICROGRAPHS SHOW THE RESTRICTION OF PROTEINS TO THE APICAL OR BASOLATERAL COMPARTMENTS OF COLUMNAR EPITHELIAL CELLS. Tight junctions (marked with *red* fluorescence in both **A** and **B**) seal the boundary between these domains. **A**, E-cadherin (*green*) is restricted to the apical plasma membrane. **B**, Syntaxin-3 (*green*) is restricted to the basolateral surface. Cell nuclei are stained *red*. (Courtesy of T. Weimbs and S. H. Low, Cleveland Clinic Foundation, Ohio.)

involves delivery of newly synthesized proteins to the basolateral surface, followed by selective internalization, sorting in the endosomal compartment, and delivery to the apical surface in a process termed transcytosis. Most epithelial cells use different combinations of these three mechanisms to generate and maintain cell polarity.

Direct targeting uses basolateral targeting signals in the cytoplasmic domains of proteins to sort these molecules during secretory transport or during endocytosis

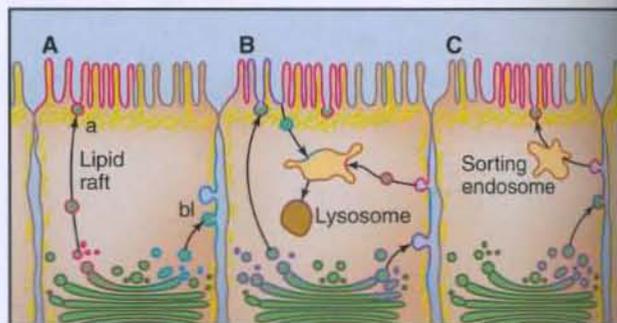


Figure 21-21 Three pathways for the distribution of integral membrane proteins destined for either the apical (*a* [*red*]) or basolateral (*bl* [*blue*]) membranes of polarized epithelial cells. **A**, Direct sorting from the TGN to either the apical or basolateral surface. Apical transport involves inclusion into lipid rafts, whereas proteins destined for direct transport to the basolateral surface carry a cytoplasmic sorting motif for inclusion into specific transport vesicles. **B**, Indirect pathway. Newly synthesized proteins are randomly targeted to both surfaces followed by selective retention and/or selective degradation from one surface or the other, resulting in a polarized distribution. **C**, Indirect pathway. Newly synthesized proteins are transported to the basolateral surface, followed by retention of basolateral proteins and selective transcytosis of apical proteins to the apical surface.

by recycling the proteins from endosomes back to the appropriate membrane domain. Examples include receptors for low-density lipoprotein, transferrin, MPRs, and polymeric immunoglobulin receptor. Alternatively, direct targeting occurs by lateral partitioning of proteins into sphingomyelin- and cholesterol-rich subdomains (called **lipid rafts**) (see Fig. 7-7) formed in the TGN or at the plasma membrane. GPI-anchored proteins or other integral membrane proteins that directly associate with these lipid rafts based on physical properties of their transmembrane domains are selectively targeted to the apical surface. The unique physical properties of these lipid subdomains render them resistant to detergent solubilization.

The second sorting mechanism—random delivery followed by selective rearrangements—is particularly relevant to establishing polarity during cellular differentiation. In this case, uniformly distributed proteins that preexist on a nonpolarized cell will redistribute themselves in a polarized fashion in response to cell-cell contacts that initiate polarization. Often, this occurs by the selective retention of a specific protein at the appropriate surface through intracellular (cytoskeletal) or extracellular (cell-cell or cell-matrix) interactions, or both. Proteins that are not actively retained on the other cell surface are internalized and degraded in lysosomes. Examples of proteins that are polarized in this way include $\text{Na}^+\text{K}^+\text{-ATPase}$ and the cell adhesion molecule *uvomorulin*, an immunoglobulin-like cell adhesion molecule.

Regulated Fusion with the Plasma Membrane

All transport carriers leaving the TGN contain components of the vesicle targeting and fusion machinery (i.e., v-SNAREs, members of the syntaxin/VAMP family, Rab proteins) required to direct their fusion with the appropriate target organelle containing cog-

nate t-SNAREs (members of the syntaxin, SNAP, and Sec1p families). Secretory granules carry additional regulatory factors that are superimposed on this constitutive machinery for docking and fusion. This ensures that fusion takes place only on demand. Regulated fusion has been studied extensively in the context of synaptic vesicle release, in endocrine cells, and in mast cells. In all cases, regulated secretion can be divided into three steps: docking, priming, and fusion (Fig. 21-22). Docking is the slowest step and is believed to involve interactions of v-SNARE and t-SNAREs regulated by Rab GTPases. In vitro reconstitution studies have suggested a role for a phosphatidylinositol transfer protein, a phosphatidylinositol 5-kinase, and phosphatidylinositol 4,5-bisphosphate (PIP_2 , the product of PI-5 kinase), in priming steps required for regulated secretion in neuroendocrine cells. A cytoplasmic protein, CAPS (calcium activator protein for secretion), is recruited to the secretory vesicle via interactions with PIP_2 and is required for calcium-triggered fusion of dense core secretory granules. In most cases, fusion is triggered by an influx of Ca^{2+} , a process called *calcium-secretion coupling*. Synaptotagmins, part of a family of transmembrane vesicle proteins that also bind calcium and interact with the fusion machinery, are believed to act as clamps, inhibiting fusion until calcium triggers their release.

Diverse signals lead to the calcium influx that triggers fusion. These include ligand activation of G-protein-coupled receptors on neuroendocrine cells, activation of immunoglobulin E receptors and kinase cascades in mast cells (see Fig. 28-5), and membrane depolarization in neurons (see Figs. 11-8 and 11-9).

The Golgi Apparatus: Function, Structure, and Dynamics

The Golgi apparatus (Fig. 21-23) performs three primary functions within the secretory membrane system. First,

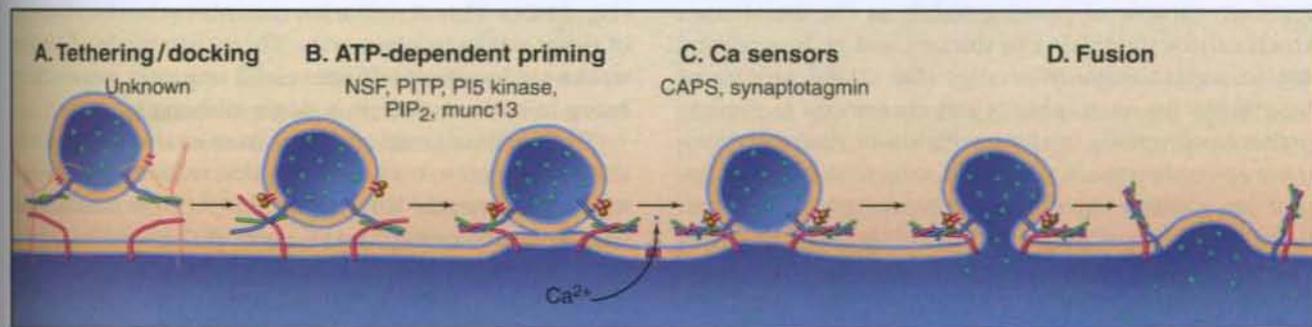


Figure 21-22 Four terminal steps (A–D) in Ca^{2+} -triggered membrane fusion during regulated secretion. Docking/tethering and fusion are mechanistically similar to other vesicle fusion events (Fig. 21-15). Additional steps prepare proteins on both the secretory vesicles and plasma membrane to respond rapidly to Ca^{2+} influx, which triggers fusion. CAPS, calcium activator protein for secretion; munc 13, mammalian homolog for *Caenorhabditis elegans* UNC13 (unknown function, critical for Ca^{2+} -triggered fusion of primary vesicles); NSF, NEM-sensitive factor; PTP, phosphatidylinositol transfer protein.

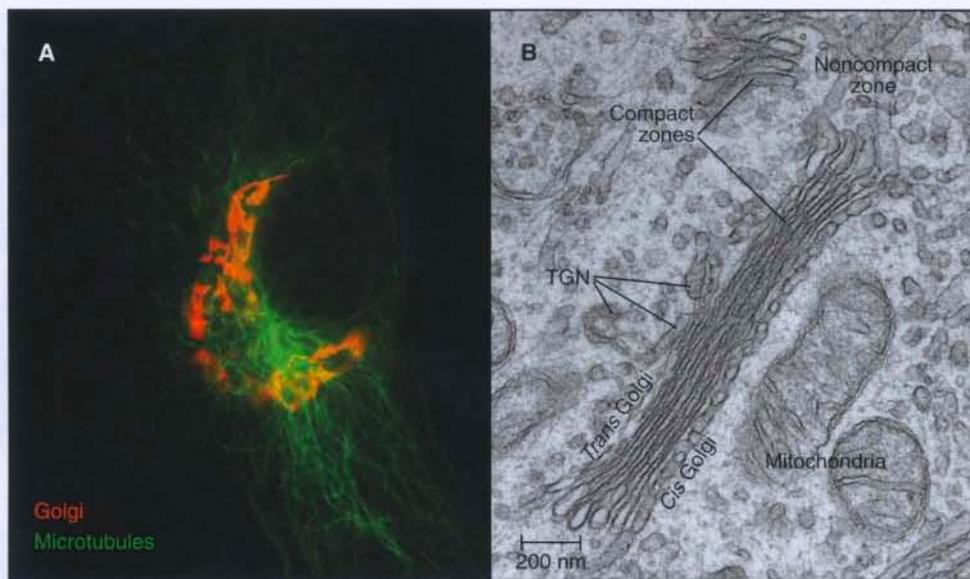


Figure 21-23 LOCALIZATION AND MORPHOLOGY OF THE GOLGI APPARATUS IN ANIMAL CELLS. **A**, Immunofluorescent micrograph of a rat fibroblast stained with antibodies to galactosyltransferase (a Golgi enzyme) (red) and antibodies to tubulin (green). The Golgi typically extends as a ribbon-like structure around the microtubule organizing center, which is localized to one side of the nucleus. **B**, Electron micrograph of a rat epithelial cell showing a single Golgi stack of cisternae cut transversely. The *cis* and *trans* faces of the Golgi are at opposite ends of the stack, with the TGN extending off from the *trans* face. The compact zones are the stacked regions of the Golgi, whereas the noncompact zones are tubular-vesicular regions of the Golgi that interconnect stacks and participate in membrane trafficking through the Golgi apparatus. (Courtesy of J. Lippincott-Schwartz and Rachid Sougrat, National Institutes of Health, Bethesda, Maryland. Reprinted from Zaal K, Smith CL, Polishchuk RS, et al: Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* 99:589–601, 1999. Copyright 1999, with permission from Elsevier.)

it acts as a carbohydrate factory in which glycoproteins, polysaccharides (in plants) and proteoglycans received from the ER are further processed. Such processing permits these molecules to participate in numerous specialized biological functions at the cell surface. Second, the Golgi apparatus functions as a protein-sorting station for the delivery of proteins to many different destinations within the cell. This includes transport to the plasma membrane, secretion to the cell exterior, sorting to the endosome/lysosomal system, or retrieval back to the ER. Third, the Golgi apparatus serves as the site where sphingomyelin and glycosphingolipids are synthesized within the cell. These lipids are capable of packing tightly in the membrane, which causes the bilayer to thicken and be less permeable to water-soluble molecules (Fig. 21-3). Affinity of these lipids for each other when cholesterol is present furthermore results in the formation of discrete membrane microdomains called lipid rafts that can concentrate or exclude specific membrane proteins. Such domains can serve as platforms for the association of diverse signaling molecules and can initiate the formation of transport carriers that bud out from the Golgi apparatus.

Golgi Morphology and Dynamics

The Golgi apparatus in many animal cells appears as a ribbon-like structure adjacent to the nucleus and close

to centrosomes, which are the microtubule organizing centers of the cell (Fig. 21-23A). In electron microscope images, the Golgi apparatus exhibits a distinctive morphology consisting of a series of stacked, flattened, membrane-enclosed cisternae that resemble a stack of pancakes (Fig. 21-23B). Cross-linking of cisternae by Golgi-associated tethering factors results in their tight, parallel alignment within the stack. Tubules and vesicles at the rims of the stacks interconnect the stacks into a single ribbon-like structure by a process dependent on microtubules. If microtubules are experimentally depolymerized, the ribbon-like Golgi structure reorganizes into single stacks (i.e., fragments) found at ER exit sites (Fig. 21-24). This distribution resembles the distribution of Golgi stacks in plant cells. There, hundreds of single stacks are localized adjacent to ER exit sites rather than being joined together as a single ribbon.

The stacks of Golgi cisternae in animal and plant cells all exhibit a *cis* to *trans* polarity that reflects the passage of cargo through this organelle. As was mentioned before, proteins from the ER enter at the stack's *cis* face (entry face). After passing through the cisternae in the middle of the stack, cargo then leaves the Golgi at the *trans* face, which is at the opposite end of the stack. Membrane sorting and transport activities of the Golgi are thought to be especially high at the *cis* and *trans* faces and within the tubular-vesicular elements (noncompact zone) that interconnect the stacks (Fig. 21-23B).

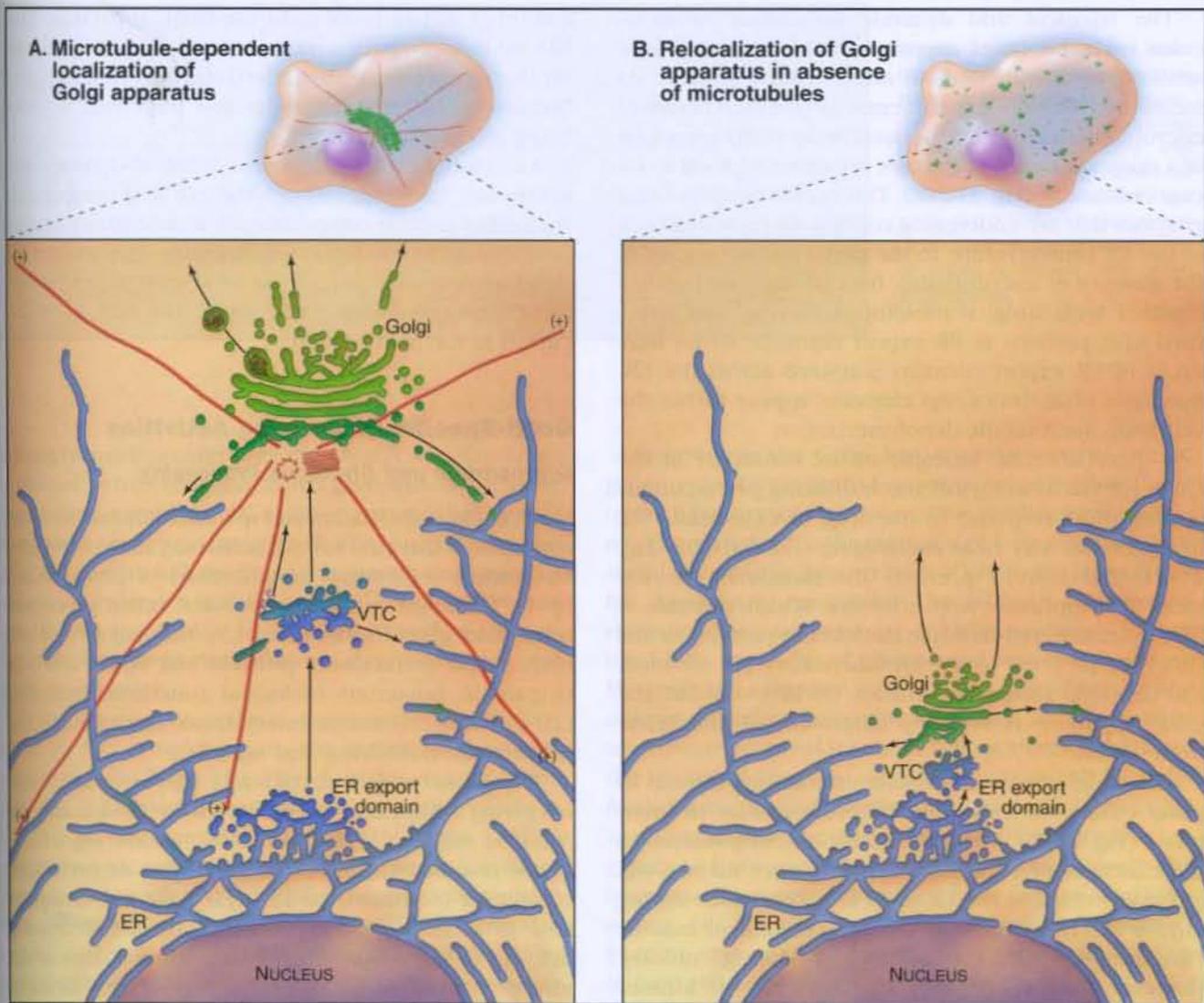


Figure 21-24 EFFECT OF MICROTUBULE DISRUPTION ON THE DISTRIBUTION OF THE GOLGI APPARATUS. **A**, Microtubules radiating out from the centrosome (red barrels) with their plus ends at the cell periphery help localize the Golgi apparatus in many animal cells by serving as tracks for the inward movement of membrane-bound carriers (VTC) derived from the ER. The carriers deliver secretory cargo, as well as Golgi enzymes, to the Golgi apparatus. Retrograde transport of Golgi enzymes back to the ER is not dependent on microtubules (since the ER is widely distributed throughout the cytoplasm). Because of this, when microtubules are disassembled (**B**), the Golgi apparatus reforms at sites adjacent to ER export domains, owing to the accumulation of cycling Golgi enzymes at these sites.

The size, the appearance, and even the existence of the Golgi apparatus depend on the amount and speed of cargo movement through the secretory pathway. The yeast *Saccharomyces cerevisiae*, for example, has a poorly developed Golgi apparatus because secretory transport is normally too fast for elaborate Golgi structures to accumulate. However, conditions that slow cargo transport out of the Golgi apparatus in these cells lead to the Golgi apparatus enlarging and rearranging into compact stacks similar to those seen in most animal and plant cells.

The Golgi apparatus is a continuously renewed organelle rather than a permanent cellular structure because both its proteins and lipids move continuously along

various pathways. No class of Golgi protein is stably associated within this organelle. Integral membrane proteins associated with the Golgi apparatus, including processing enzymes and SNAREs, continuously exit and reenter the Golgi apparatus by membrane-trafficking pathways leading to and from the ER. Peripheral membrane proteins associated with the Golgi apparatus (including Arf1, coatamer, Rab proteins, matrix proteins, tethering factors, and GEFs) exchange constantly between Golgi membranes and cytoplasmic pools. Newly synthesized secretory cargo coming from the ER enters the Golgi apparatus on the *cis* face of the stack, traverses across the stack, and then leaves from the *trans* face.

The transient and dynamic association of molecules with the Golgi apparatus makes this organelle sensitive to malfunctions of many cellular systems. As mentioned before, experimental depolymerization of microtubules causes the pericentriolar Golgi apparatus of a mammalian cell to become relocated adjacent to ER export domains (Fig. 21-24B). This occurs because Golgi enzymes that are undergoing continuous recycling back to the ER cannot return to the pericentriolar region in the absence of microtubules. Instead, they accumulate together with Golgi scaffolding, tethering, and structural coat proteins at ER export domains. Given hundreds of ER export domains scattered across the ER, hundreds of distinct Golgi elements appear within the cell upon microtubule depolymerization.

A more dramatic example of the sensitivity of the Golgi apparatus to membrane trafficking perturbations is the Golgi's response to the drug BFA (brefeldin A). BFA prevents Arf1 from exchanging GTP for GDP (Fig. 21-6D) and thereby prevents the membrane recruitment of cytoplasmic Arf1 effectors. Within minutes of BFA treatment, resident transmembrane proteins of the Golgi are recycled to the ER where they are retained, and the Golgi apparatus vanishes. On BFA washout, the Golgi apparatus reforms by outgrowth of membrane from the ER.

The Golgi apparatus disassembles during mitosis in many eukaryotic cells and then reassembles in interphase (Fig. 21-25). This process superficially resembles the effects of BFA and BFA washout, since many Golgi enzymes return to the ER or to ER export sites during mitosis and reemerge from the ER at the end of mitosis. Furthermore, Arf1 is inactivated during mitosis. However, mitotic cells also inactivate mitotic kinases (see Chapter 40) that phosphorylate tethering factors

and other matrix proteins of the Golgi apparatus. This has led to a competing explanation for Golgi disassembly during mitosis in which the Golgi undergoes a direct breakdown into small vesicles and fragments without being absorbed into the ER.

Although the Golgi apparatus is highly dynamic and continually exchanges its protein and lipid components with other cellular compartments, it maintains a unique biochemical and morphologic identity. This allows the Golgi apparatus to participate in several major biosynthetic and processing pathways in the cell, as is discussed in the next section.

Golgi-Specific Processing Activities

Glycoprotein and Glycolipid Processing

Much of the organization and specialization of the Golgi apparatus is directed toward achieving the correct **glycosylation** (i.e., sugar modification) of proteins and lipids. The sugar-modified molecules, called **glycoproteins and glycolipids**, constitute the majority of cell surface and extracellular proteins and lipids, and participate in numerous biological functions, including cell-cell and cell-matrix interactions, intracellular and intercellular trafficking, and signaling.

The most widely recognized glycosylation event occurring within the Golgi involves the modification of *N*-linked oligosaccharides on glycoproteins (Fig. 21-26). These *N*-linked sugar chains are added as preformed complexes (consisting of 14 sugar residues) to asparagine side chains of the protein in the ER. Following delivery to the Golgi, the *N*-linked sugar chains of the glycoprotein undergo extensive further modifications in an ordered sequence. The first modification is the removal of mannose residues. This is followed by the sequential addition of *N*-acetylglucosamine, the further removal of mannoses, the addition of fucose and more *N*-acetylglucosamine, and the final addition of galactose and sialic acid residues. Cell biologists have used the *N*-linked glycan-processing steps that take place in the mammalian Golgi apparatus as experimental signposts for the passage of glycoproteins through the secretory pathway.

Many oligosaccharides are further chemically modified after growing by simple addition of monosaccharide units. Enzymes add substituents such as phosphate, sulfate, acetate or methyl groups or isomerize specific carbons. These modifications as well as differential processing of *N*-linked oligosaccharide structures (producing high-mannose type, complex type, and hybrid structures) contribute to the diversity of sugar residues exposed at the cell surface and can impart specific functions to the sugar chains.

More than 200 Golgi enzymes participate in the biosynthesis of glycoproteins and glycolipids. Enzymes

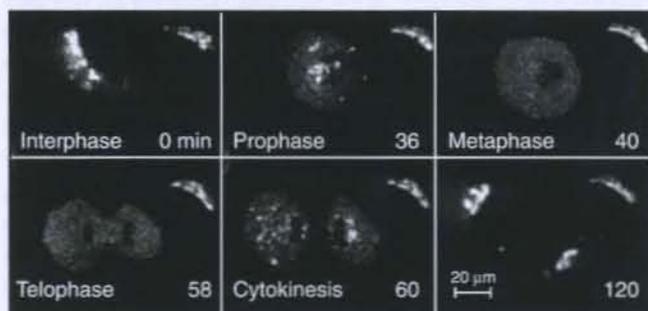


Figure 21-25 TIME-LAPSE IMAGING OF A CELL EXPRESSING A FLUORESCENTLY TAGGED GOLGI ENZYME, GALACTOSYLTRANSFERASE-GFP, THAT IS PROGRESSING THROUGH MITOSIS. As the cell in the left of the image passes through prophase and metaphase, its Golgi membranes fragment and then disperse. During cytokinesis, the Golgi membranes reappear as fragments. These fragments then coalesce into a juxtannuclear Golgi ribbon at the end of mitosis. (From Zaal K, Smith CL, Polishchuk RS, et al: Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* 99:589–601, 1999.)

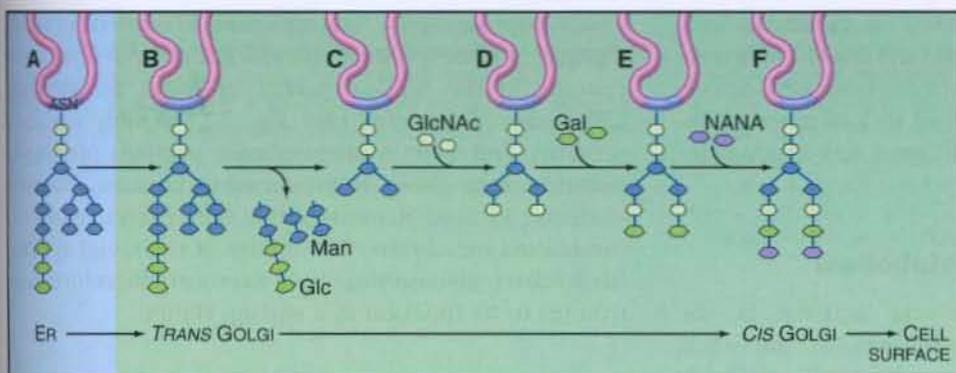


Figure 21-26 PROCESSING OF *N*-LINKED CORE OLIGOSACCHARIDES IN THE GOLGI APPARATUS. **A–F**, Sequential steps trim the mannose (Man)/glucose (Glc) core and then add *N*-acetylglucosamine (GlcNAc), galactose (Gal), and sialic acid (NANA) to form a variety of complex oligosaccharides, one of which is shown here, ASN, asparagines.

called **glycosyltransferases** add specific sugar residues to glycans, while enzymes called **glycosidases** remove specific sugar residues. All of these enzymes are type II transmembrane proteins with a short cytoplasmic amino terminal domain followed by a transmembrane segment and catalytic domain within the Golgi lumen. Additional constituents involved in Golgi oligosaccharide processing include **transporters**, donor sugar-nucleotides, and pyrophosphatases.

Transporters first transfer sugar-nucleotide donors made in the cytoplasm into the lumen of the Golgi apparatus (see Chapter 9). They function as antiporters (see Fig. 9-4), exchanging nucleotide sugars (such as UDP-*N*-acetylglucosamine, UDP-galactose, and CMP-*N*-acetylneuramic acid) for nucleoside monophosphates formed during glycosyl transfer. Glycosyltransferases then use the high-energy sugar-nucleotides as substrates to add new sugars to an oligosaccharide chain. Most glycosyltransferases are specific for sugar-nucleotide donors and particular oligosaccharide acceptors, but the oligosaccharides are synthesized without a template, so they vary more than polypeptides and polynucleotides, which are synthesized on templates. Finally, glycosidases trim sugars from the branched core oligosaccharides prior to addition of other sugars. They include mannosidase I and II, which clip outer-branch mannose residues on *N*-linked oligosaccharides prior to the addition of *N*-acetylglucosamine.

The Golgi enzymes also add oligosaccharides to the hydroxyl groups of serine and threonine residues of selected proteins, such as **proteoglycans**, heavily glycosylated proteins in secretory granules, and the extracellular matrix (see Figs. 29-13 and 29-14). This process, called **O-linked glycosylation**, begins with the addition of one of three short oligosaccharides to selected serine and threonine residues of a proteoglycan core protein. Glycosyltransferases in the Golgi then add many copies of the same disaccharide unit to the growing polysaccharide. Other enzymes then add sulfates to a few of the sugar residues before the molecule exits the Golgi system.

Enzymes in the Golgi apparatus also mark specific proteins for transport to lysosomes by phosphorylation of the 6-hydroxyl of mannose. This modification, as was mentioned before, is the sorting signal that enables lysosomal enzymes to interact with MPRs in the *trans* Golgi for targeting to lysosomes. The *N*-linked oligosaccharides on these enzymes are initially processed within the ER by trimming of glucose and mannose residues. However, on transport to the Golgi, they become the unique substrates for two enzymes that act sequentially to generate terminal mannose 6-phosphates, which are the lysosomal targeting signal. Human patients with the fatal disease mucopolipidosis II (called I-cell disease) fail to phosphorylate the mannose residues required for targeting to lysosomes (see Chapter 23). As a result, the lysosomal enzymes are secreted from the cell, and lysosomes fail to degrade waste materials. Lysosomes become engorged with undigested substrates, leading to fatal cell and tissue abnormalities.

Enzymes in the Golgi stacks further load noncovalently associated cholesterol and phospholipids onto high-density and **low-density lipoproteins** for secretion by liver cells into the blood. Golgi enzymes are also involved in the synthesis of complex polysaccharides in plant cells, which are an important constituent of the plant cell wall.

Proteolytic Processing of Protein Precursors

A number of proteins, particularly peptide hormones, are cleaved into active fragments in the Golgi apparatus and its secretory vesicles. Such proteins are synthesized as large precursors with one or more small hormones embedded in long polypeptides. One example is a yeast mating pheromone. Another is pro-opiomelanocortin, the precursor to no less than six small peptide hormones. Proteolytic enzymes called **prohormone convertases** cleave the precursor proteins into active hormones in the TGN and post-TGN transport intermediates. The mixture of products depends on the

prohormone convertases expressed in particular cells. Proteolysis in the Golgi also affects the final folding state and activity of many other proteins. Inherited defects in these processing pathways lead to a number of diseases, including hormone insufficiency and a hereditary amyloid disease.

Lipid Biosynthesis and Metabolism

Another Golgi-specific processing activity is the synthesis of sphingolipids. Sphingolipids, including **sphingomyelin** (SM) and the **glycosphingolipids** glucosylceramide and galactosylceramide, play central roles in membrane sorting within the Golgi apparatus as well as sorting within post-Golgi compartments. As was described earlier, these lipids have affinity for cholesterol, act as donors of intermolecular hydrogen bonds, and have saturated lipid chains, resulting in the denser packing of these lipids compared to glycerophospholipids. When densely packed, the sphingolipids and cholesterol form long cylinders that cause an increase in the thickness of the bilayer relative to a bilayer containing glycerophospholipids alone (e.g., ER [Fig. 21-3]). An enrichment of sphingolipids and cholesterol along the secretory pathway from Golgi to plasma membrane results in an increased thickness of the membranes along this route relative to the ER. This plays a vital role within the secretory system because of a protein's tendency to match the length of its transmembrane domain with that of the lipid bilayer.

The backbone of all sphingolipids, ceramide (see Chapter 20), is synthesized in the ER and then transported to the Golgi complex, where it is modified to form glucosylceramide and sphingomyelin. Glucosylceramide synthesis is catalyzed on the cytoplasmic surface of Golgi membranes by the enzyme UDP-glucose:ceramide glucosyltransferase. Glucosylceramide can then be transported to the plasma membrane or translocated to the luminal leaflet of Golgi membranes, where galactosylation of the head group results in the formation of lactosylceramide. Sequential glycosylation of lactosylceramide by glycosyltransferases of the Golgi lumen generates complex glycolipids and gangliosides for the plasma membrane.

Sphingomyelin synthesis is catalyzed by sphingomyelin-synthase, an enzyme on the luminal leaflet of Golgi membranes. The enzyme transfers phosphorylcholine from phosphatidylcholine to ceramide, releasing the signaling lipid diacylglycerol (DAG) in the process. This mechanism therefore couples consumption of the signaling lipid ceramide (see Fig. 26-11) with the production of the signaling lipid DAG (see Fig. 26-8). If DAG accumulates in the Golgi apparatus, phosphorylcholine can be transferred from sphingomyelin back to DAG, forming phosphatidylcholine and ceramide. Alternatively, DAG can be digested by lipases.

Glycosphingolipid microdomains, or rafts, are thought to form spontaneously by lateral lipid-lipid associations in the luminal leaflet of Golgi membranes. GPI-anchored proteins (see Fig. 7-7), doubly acylated proteins, and many transmembrane proteins physically partition into these microdomains and are thereby enriched in lipid structures that sort preferentially to the plasma membrane. The ability of the Golgi apparatus to drive glycosphingolipid synthesis therefore contributes to its function as a sorting station.

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