14.4 Later Stages of the Secretory Pathway

As cargo proteins move from the *cis*- to the *trans*-Golgi by cisternal maturation, modifications to their oligosaccharide chains are carried out by Golgi-resident enzymes. The retrograde trafficking of COPI vesicles from later to earlier Golgi compartments maintains sufficient levels of these carbohydrate-modifying enzymes in the appropriate compartments. Eventually, properly processed cargo proteins reach the *trans*-Golgi network, the most distal Golgi compartment. Here they are sorted into a number of different kinds of vesicles for delivery to their final destination. Each of the target destinations, such as the plasma membrane, endosomes, and lysosomes, has a unique composition of lipids and membrane proteins, and it is primarily the sorting in the *trans*-Golgi network that gives each of these organelles its unique identity. In this section, we discuss the different kinds of vesicles that bud from the *trans*-Golgi network, the mechanisms that segregate cargo proteins among them, and key processing events that occur late in the secretory pathway. The various types of vesicles that bud from the *trans*-Golgi and will be described in this section are summarized in Figure 14-18.

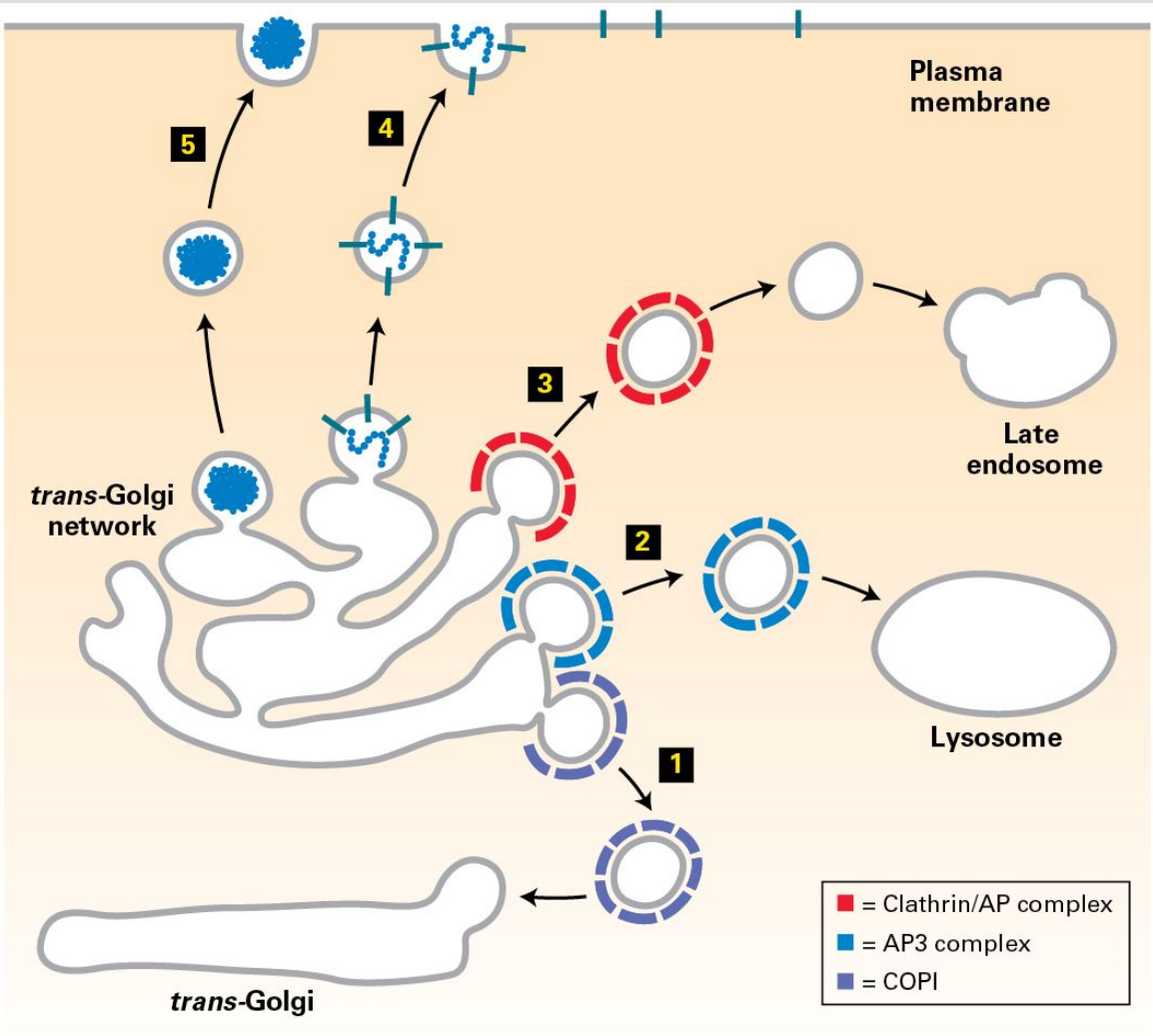


FIGURE 14-18 Vesicle-mediated protein trafficking from the *trans*-Golgi network. COPI (purple) vesicles mediate retrograde transport within the Golgi (1). Proteins that function in the lumen or in the membrane of the lysosome are first transported from the *trans*-Golgi network via AP complex and clathrin-coated (red) vesicles (2); after uncoating, these vesicles fuse with late endosomes, which deliver their contents to the lysosome. Some vesicles from the *trans*-Golgi carrying cargo destined for the lysosome fuse with the lysosome directly (3), bypassing the late endosome. These vesicles are coated with AP3 complex (blue); it is unknown whether these vesicles also contain clathrin. The coat proteins surrounding constitutive (4) and regulated (5) secretory vesicles have not yet been characterized; these vesicles carry secreted proteins and plasma-membrane proteins from the *trans*-Golgi network to the cell surface.

Description
The illustration shows a trans-Golgi network out of which C O P 1 coated vesicles, A P 3 complex coated vesicles, clathrin or A P complex coated vesicles, uncoated vesicles with secreted proteins and plasma membrane proteins budding out.

1. The C O P 1 coated vesicles budding from the trans-Golgi network returns to the trans-Golgi.

2. The A P 3-complex coated vesicle moves towards the lysosome to fuse with it.

3. The clathrin-coated vesicles lose their coat to fuse with the late endosome.

4. Secreted proteins are held by receptor proteins inside the uncoated vesicles. The secreted protein is represented by small blue dotted lines. They move towards the cell surface to fuse with the plasma membrane.

5. Some secreted proteins inside uncoated vesicles are represented by a large tangle of blue lines. These vesicles move towards the cell surface to eliminate the secreted proteins.

Vesicles Coated with Clathrin and Adapter Proteins Mediate Transport from the *trans*-Golgi

The best characterized vesicles that bud from the *trans*-Golgi network have a two-layered coat: an outer layer composed of the fibrous protein clathrin and an inner layer composed of **adapter protein (AP) complexes**. Purified clathrin molecules, which have a three-limbed shape, are called *triskelions*, from the Greek for "three-legged" (Figure 14-19a). Each limb contains one clathrin heavy chain (180 kDa) and one clathrin light chain (~35–40 kDa). Triskelions polymerize to form a polygonal lattice with an

intrinsic curvature (Figure 14-19b). When clathrin polymerizes on a donor membrane, it does so in association with AP complexes, which fill the space between the clathrin lattice and the membrane. Each AP complex (340 kDa) contains one copy each of four different adapter subunit proteins. A specific association between the globular domain at the end of each clathrin heavy chain in a triskelion and one subunit of the AP complex both promotes the co-assembly of clathrin triskelions with AP complexes and adds to the stability of the completed vesicle coat.

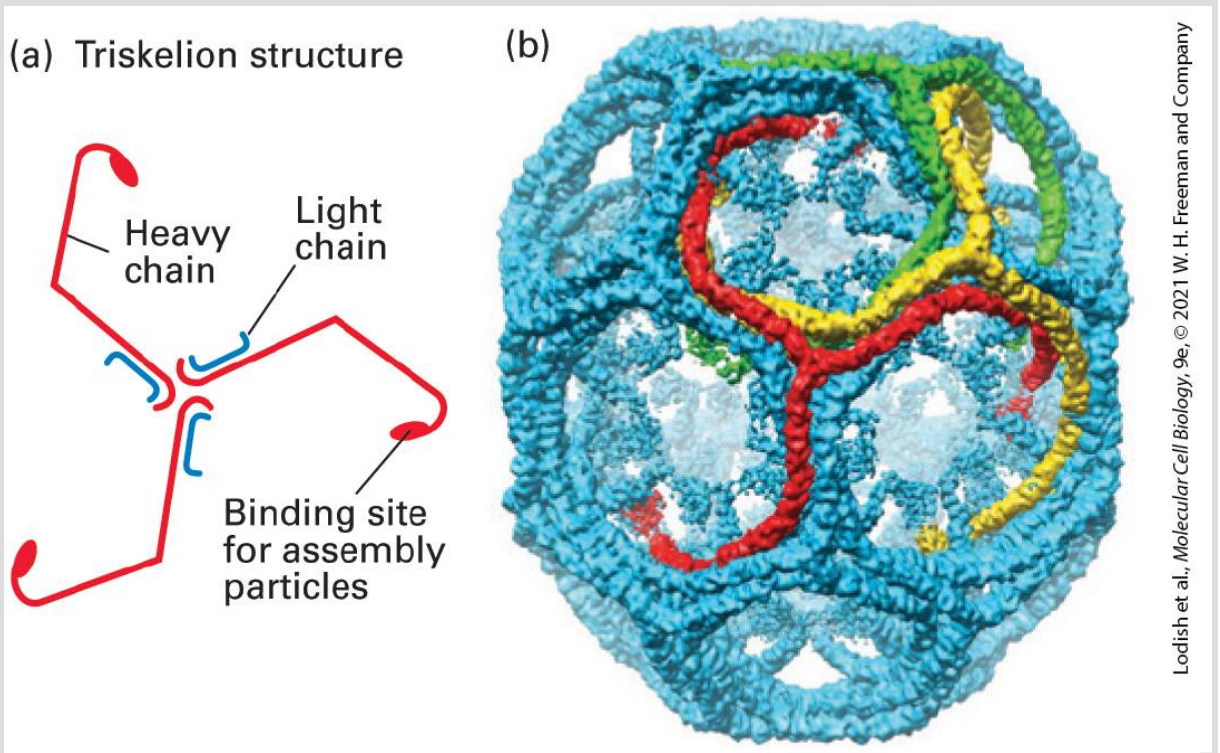


FIGURE 14-19 Structure of clathrin coats. (a) A clathrin molecule, called a *triskelion*, is composed of three heavy and three light chains. It has an intrinsic curvature due to the bend in the heavy chains. (b) Clathrin coats were formed in vitro by mixing purified clathrin heavy and light chains with AP2 complexes in the absence of membranes. Cryoelectron micrographs of more than 1000 assembled hexagonal clathrin barrel particles were analyzed by digital image processing to generate an average structural representation. The processed image shows only the clathrin heavy chains in a structure composed of 36

triskelions. Three representative triskelions are highlighted in red, yellow, and green. Some of the AP2 complexes packed into the interior of the clathrin cage are also visible in this representation. See B. Pishvaee and G. Payne, 1998, *Cell* **95**:443.

[Part (b) Republished with permission from *Nature*, from A. Fotin et al., 2004, "Molecular Model for a Complete Clathrin Lattice from Electron Cryomicroscopy," *Nature* **432**:573–579; permission conveyed through Copyright Clearance Center, Inc.]

Description

The illustration labeled (a) shows the Triskelion structure of clathrin with three heavy chains and three light chains, arranged in a three-armed, right-handed propeller arrangement. At the tips of the 'propeller' are binding sites for assembly particles.

The cryoelectron micrograph labeled (b) shows the sections of clathrin coat. The clathrin coat is composed of hexagonal sections and triskelion structures highlighted in red, green, and yellow.

Adapter proteins determine which cargo proteins are specifically included in a budding transport vesicle by binding to the cytosolic face of membrane proteins. Thus each of the multiple adapter protein complexes that operate in the late secretory pathway can be classified based on the types of cargo proteins that they select as well as the specific transport step that they mediate (see Table 14-1). Three different AP complexes are known (AP1, AP2, and AP3), each with four subunits of different, though related, proteins. A second general type of adapter protein, known as *GGA*, consists of a single 70 kDa polypeptide. This monomeric adapter protein has been shown to contain both clathrin- and cargo-binding elements similar to those found in the much larger hetero-tetrameric AP complexes. All vesicles whose coats contain one of these complexes use ARF to initiate coat assembly on the donor membrane. As discussed previously,

ARF also initiates assembly of COPI coats. The additional membrane features or protein factors that determine which type of coat will assemble after ARF attachment are not well understood at this time.

Vesicles that bud from the *trans*-Golgi network en route to the lysosome by way of the late endosome (see Figure 14-18, **3**) have clathrin coats associated with either AP1 or GGA. One of the AP1 subunits in the vesicle coat binds to membrane proteins containing a Tyr-X-X-Φ sequence. Proteins bearing this *YXXΦ sorting signal* are recruited into clathrin/AP1-coated vesicles budding from the *trans*-Golgi network. As we discuss in the next section, vesicles with clathrin/AP2 coats, which bud from the plasma membrane during endocytosis, can also recognize the YXXΦ sorting signal. Vesicles coated with GGA proteins and clathrin bind cargo molecules with different kinds of sorting sequences including Asp-X-Leu-Leu and Asp-Phe-Gly-X-Φ sequences.

Some vesicles that bud from the *trans*-Golgi network have coats composed of the AP3 complex. Although the AP3 complex does contain a binding site for clathrin similar to those in the AP1 and AP2 complexes, it is not clear whether clathrin is necessary for the functioning of AP3-containing vesicles because mutant versions of AP3 that lack the clathrin binding site appear to be fully functional. AP3-coated vesicles mediate trafficking to the lysosome, but they appear to bypass the late endosome and fuse directly with the lysosomal membrane (see Figure 14-18, **2**). In certain types of cells, such AP3 vesicles mediate protein transport to specialized storage compartments related to the lysosome. For example, AP3 is required for delivery of proteins to melanosomes, which contain the black

pigment melanin in skin cells, and to platelet storage vesicles in megakaryocytes, large cells that fragment into dozens of platelets. Mice with mutations in either of two different subunits of AP3 not only have abnormal skin pigmentation but also exhibit bleeding disorders. The latter occur because platelets require normal storage vesicles in order to repair tears in blood vessels.

Dynamin Is Required for Pinching Off of Clathrin-Coated Vesicles

A fundamental step in the formation of a transport vesicle that we have not yet considered is how a vesicle bud is pinched off from the donor membrane. In the case of clathrin-coated vesicles, a cytosolic protein called **dynamin** is essential for the release of complete vesicles. At the later stages of bud formation, dynamin polymerizes around the neck portion of the bud and then hydrolyzes GTP. The energy derived from GTP hydrolysis is thought to drive a conformational change in dynamin that stretches the neck until the vesicle pinches off (Figure 14-20).

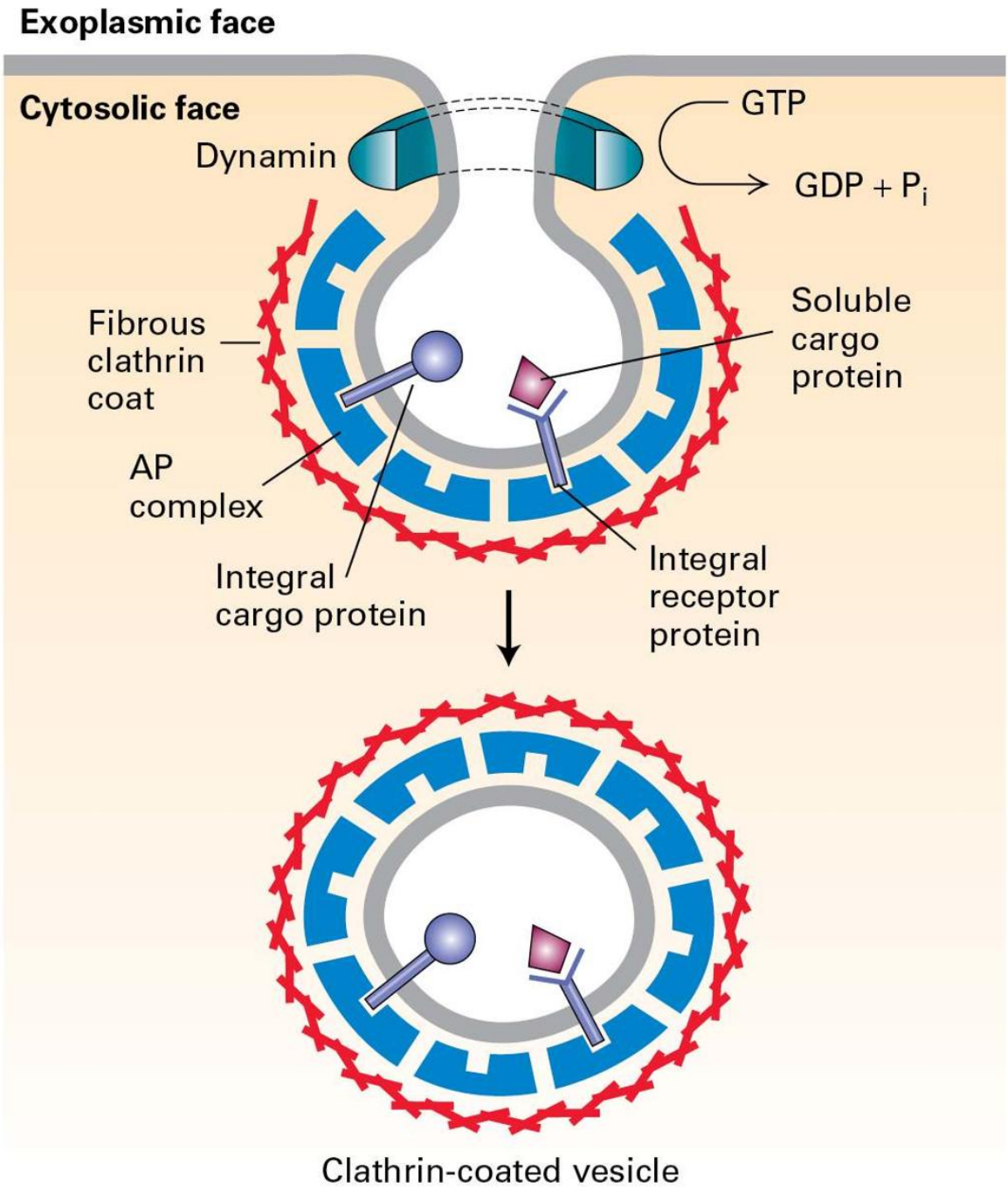
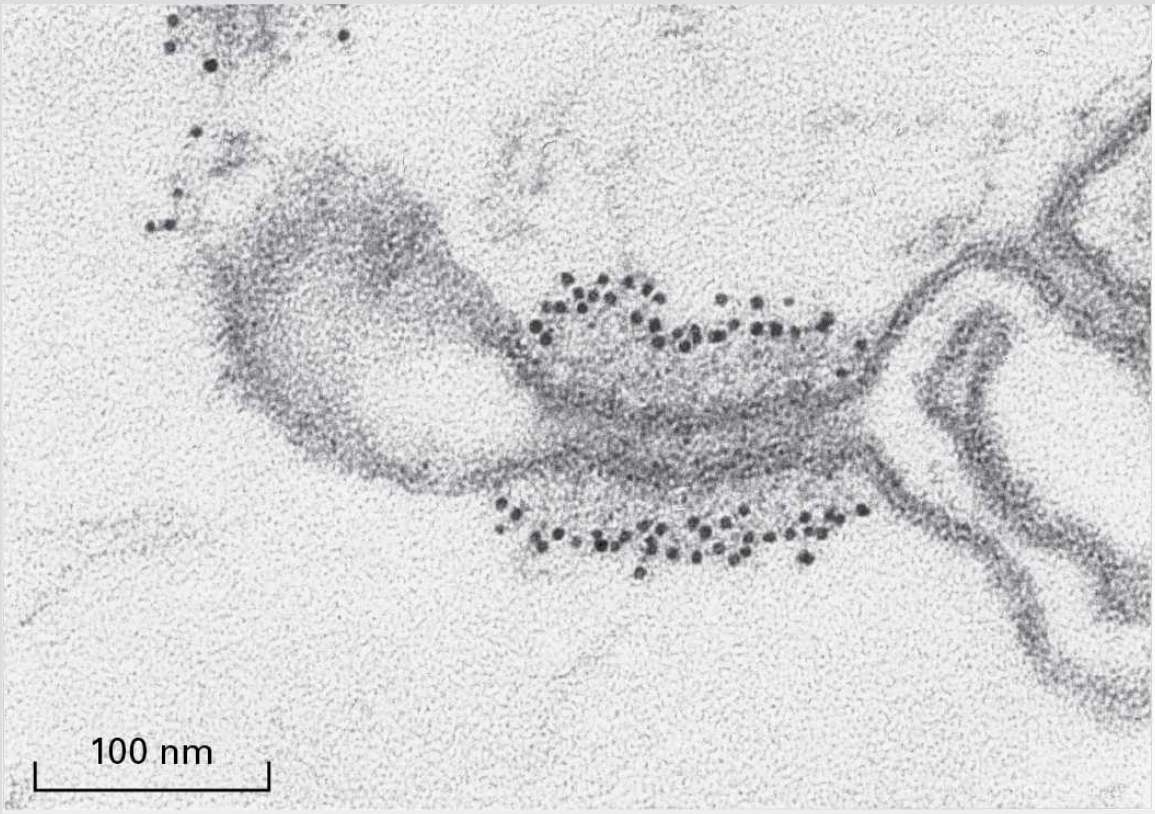


FIGURE 14-20 Model for dynamin-mediated pinching off of clathrin-coated vesicles. After a vesicle bud forms, dynamin polymerizes over the neck. By a mechanism that is not well understood, dynamin-catalyzed hydrolysis of GTP leads to release of the vesicle from the donor membrane. Note that membrane proteins in the donor membrane are incorporated into vesicles by interacting with AP complexes in the coat. See K. Takel et al., 1995, *Nature* **374**:186.

Description
A vesicle starts to buds into the cytosol. The vesicle contains an integral cargo protein and an integral receptor protein bound to a soluble cargo protein. The vesicle is coated with A P complex proteins and a fibrous clathrin coat. Dynamin forms a ring around the budding vesicle just below the membrane (cytosolic face). Hydrolysis of G T P to G D P and a molecule of inorganic phosphate results in the pinching and budding of the clathrin-coated vesicle.

Incubation of cell extracts with a nonhydrolyzable derivative of GTP provides dramatic evidence for the importance of dynamin in the pinching off of clathrin/AP2-coated vesicles during endocytosis. Such treatment leads to accumulation of clathrin-coated vesicle buds with excessively long necks that are surrounded by polymeric dynamin but do not pinch off (Figure 14-21). Likewise, cells expressing mutant forms of dynamin that cannot bind GTP do not form clathrin-coated vesicles and instead accumulate similar long-necked vesicle buds encased with polymerized dynamin.



EXPERIMENTAL FIGURE 14-21 GTP hydrolysis by dynamin is required for the pinching off of clathrin-coated vesicles in cell-free extracts. A preparation of nerve terminals, which undergo extensive endocytosis, was lysed by treatment with distilled water and incubated with GTP-γ-S, a nonhydrolyzable derivative of GTP. After sectioning, the preparation was treated with gold-tagged anti-dynamin antibody and viewed in the electron microscope. This image, which shows a long-necked clathrin/AP-coated bud with polymerized dynamin lining the neck, reveals that buds can form in the absence of GTP hydrolysis, but vesicles cannot pinch off. The extensive polymerization of dynamin that occurs in the presence of GTP-γ-S probably does not occur during the normal budding process.

[Republished by permission from *Nature*, from K. Takei et al., 1995, "Tubular Membrane Invaginations Coated by Dynamin Rings Are Induced by GTP-Gamma S in Nerve Terminals," *Nature* **374**:(6518):186–190; permission conveyed through Copyright Clearance Center, Inc.]

Like COPI and COPII vesicles, clathrin-coated vesicles normally lose their coat soon after their formation. Cytosolic Hsp70, a constitutive chaperone protein found in all eukaryotic cells, is thought to use energy derived from the hydrolysis of ATP to drive depolymerization of the clathrin coat into triskelions. In the case of endocytic vesicles, uncoating not only releases triskelions for reuse in the formation of additional vesicles but also exposes v-SNAREs for use in fusion with target membranes. Vesicle uncoating by cytosolic Hsp70 appears to be activated by a co-chaperone, auxillin, that contains a domain that stimulates the ATP hydrolysis by Hsp70. Conformational changes that occur when ARF switches from the GTP-bound to the GDP-bound state are thought to regulate the timing of clathrin coat depolymerization, but how the action of Hsp70 and auxillin is coupled to ARF switching is not well understood.

Mannose 6-Phosphate Residues Target Resident Enzymes to Lysosomes

As we have seen, many of the sorting signals that direct cargo-protein trafficking in the secretory pathway are short amino acid sequences in the targeted protein. In contrast, the sorting signal that directs soluble lysosomal enzymes from the *trans*-Golgi network to the late endosome is a carbohydrate residue, **mannose 6-phosphate (M6P)**, which is formed in the *cis*-Golgi. The addition and initial processing of one or more preformed *N*-linked oligosaccharide precursors in the rough ER is the same for lysosomal enzymes as for membrane and secreted proteins,

yielding core $\mathrm{Man_8(GlcNAc)_2}$ chains (see Figure 13-18). In the *cis*-Golgi, the *N*-linked oligosaccharides present on most lysosomal enzymes undergo a two-step reaction sequence that generates M6P residues (Figure 14-22). The addition of M6P residues to the oligosaccharide chains of soluble lysosomal enzymes prevents these proteins from undergoing the further processing reactions characteristic of secreted and membrane proteins (see Figure 14-15).

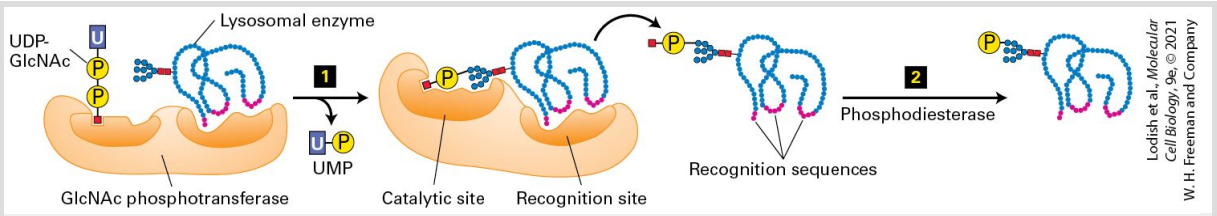


FIGURE 14-22 Formation of mannose 6-phosphate (M6P) residues that target soluble enzymes to lysosomes. The M6P residues that direct proteins to lysosomes are generated in the *cis*-Golgi by two Golgi-resident enzymes. Step 1: An *N*-acetylglucosamine (GlcNAc) phosphotransferase transfers a phosphorylated GlcNAc group to carbon atom 6 of one or more mannose residues. Because only lysosomal enzymes contain sequences (red) that are recognized and bound by this enzyme, phosphorylated GlcNAc groups are added specifically to lysosomal enzymes. Step 2: After release of the modified protein from the phosphotransferase, a phosphodiesterase removes the GlcNAc group, leaving a phosphorylated mannose residue on the lysosomal enzyme. See A. B. Cantor et al., 1992, *J. Biol. Chem.* **267**:23349; and S. Kornfeld, 1987, *FASEB J.* **1**:462.

Description

Step 1: U D P-N-acetyl glucosamine and a lysosomal enzyme with attached oligosaccharide bind to the N-acetyl glucosamine phosphotransferase recognition sites. U M P is eliminated in this process. The N-acetyl glucosamine phosphate unit in the catalytic site and the mannose unit of the lysosomal enzyme in the recognition site of N-acetyl glucosamine phosphotransferase bind together.

Step 2. The targeting protein is released and phosphodiesterase catalyzes the removal of N-acetyl glucosamine on the lysosomal enzyme.

As shown in Figure 14-23, the segregation of M6P-bearing lysosomal enzymes from secreted and membrane proteins occurs in the *trans*-Golgi network. Here transmembrane *mannose 6-phosphate receptors* bind the M6P residues on lysosome-destined proteins very tightly and specifically. Clathrin/AP1-coated vesicles containing the M6P receptor and bound lysosomal enzymes then bud from the *trans*-Golgi network, lose their coats, and subsequently fuse with a late endosome by mechanisms described previously (Figure 14-18). Because M6P receptors can bind M6P at the slightly acidic $\mathrm{pH}\ (\sim 6.5)$ of the *trans*-Golgi network, but not at a pH of less than 6, the bound lysosomal enzymes are released within late endosomes, which have an internal pH of 5.0–5.5. Furthermore, a phosphatase within late endosomes usually removes the phosphate from M6P residues on lysosomal enzymes, preventing any rebinding to the M6P receptor that might occur in spite of the low pH there. Vesicles budding from late endosomes, coated with a protein complex known as the **retromer**, recycle the M6P receptor back to the *trans*-Golgi network. Eventually, mature late endosomes fuse with lysosomes, delivering the lysosomal enzymes to their final destination.

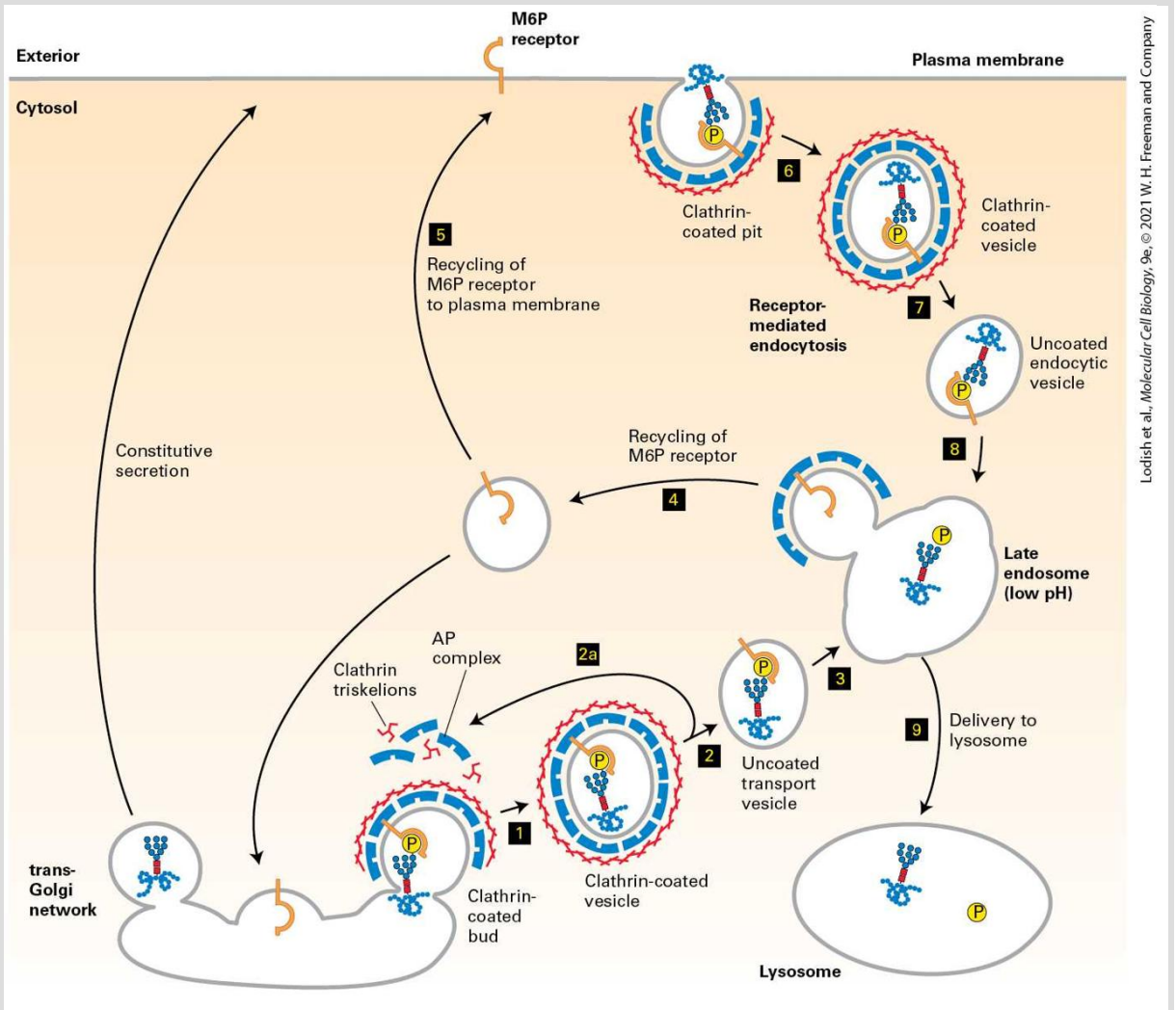


FIGURE 14-23 Trafficking of soluble lysosomal enzymes from the *trans*-Golgi network and cell surface to lysosomes. Newly synthesized lysosomal enzymes, produced in the ER, acquire mannose 6-phosphate (M6P) residues in the *cis*-Golgi (see Figure 14-22). For simplicity, only one phosphorylated oligosaccharide chain is depicted, although lysosomal enzymes typically have many such chains. In the *trans*-Golgi network, proteins that bear the M6P sorting signal interact with M6P receptors in the membrane and thereby are directed into clathrin/AP1-coated vesicles (step 1). The coat surrounding released vesicles is rapidly depolymerized (step 2), and the uncoated transport vesicles fuse with late endosomes (step 3). Note that disassembled coat proteins are recycled for additional rounds of vesicle assembly (step 2a). After the phosphorylated enzymes dissociate from the M6P receptors in late endosomes, free M6P receptors are recycled by incorporation into vesicles that bud from the late endosome (step 4). Vesicles carrying free M6P receptors can return to the *trans*-Golgi or fuse with the plasma membrane delivering M6P receptors to

the cell surface (step 5). Phosphorylated lysosomal enzymes are also occasionally sorted from the *trans*-Golgi to the cell surface and secreted. These secreted enzymes can be retrieved by receptor-mediated endocytosis (steps 6–8). Ultimately, fusion of the late endosome with the lysosome delivers phosphorylated lysosomal enzymes to the interior of the lysosomes (step 9). See G. Griffiths et al., 1988, *Cell* **52**:329; S. Kornfeld, 1992, *Annu. Rev. Biochem.* **61**:307; and G. Griffiths and J. Gruenberg, 1991, *Trends Cell Biol.* **1**:5.

Description

Step 1: The illustration shows a trans-Golgi network in cytosol with a clathrin-coated bud containing mannose 6 phosphate (M 6 P) receptor with associated targeted proteins. The trans-Golgi network also has another M 6 P receptor and an uncoated bud. The uncoated bud undergoes constitutive secretion. The clathrin-coated bud becomes a vesicle.

Step 2: The clathrin coating of the vesicle gets removed to become the uncoated transport vesicle.

Step 2 a: The disassembled clathrin triskelion and A P complex coats are reused by the trans-Golgi network.

Step 3: The vesicle merges with the late endosome at a low p H, depositing the glycoprotein.

Step 4 and 5: The late endosome releases the M 6 P receptor inside an empty vesicle, which either gets recycled back to the trans-Golgi complex or to the plasma membrane.

Step 6: From the surface of the cell, labeled proteins bind with the mannose 6 phosphate receptor on the surface of the vesicle to form clathrin-coated pits in the cell membrane. Via receptor-mediated endocytosis, a clathrin-coated vesicle is formed.

Step 7: The clathrin coating of the vesicle gets removed to become the uncoated endocytic vesicle.

Step 8: The uncoated endocytic vesicle moves to bind with the late endosome.

Step 9: The late endosome delivers proteins and phosphate to a lysosome.

The sorting of soluble lysosomal enzymes in the *trans*-Golgi network (Figure 14-23, steps **1**–**4**) shares many features with the trafficking of proteins between the ER and *cis*-Golgi compartments mediated by COPII and COPI vesicles. First, M6P acts as a sorting signal by interacting with the luminal domain of a receptor protein in the donor membrane. Second, the membrane-embedded receptors with their bound ligands are incorporated into the appropriate vesicles — in this case, either GGA- or AP1-containing clathrin-coated vesicles — by interacting with the vesicle coat. Third, these transport vesicles fuse with only one specific organelle, here the late endosome, as the result of interactions between specific v-SNAREs and t-SNAREs. And finally, intracellular transport receptors dissociated from their bound ligand are recycled by retrograde vesicle trafficking.

Study of Lysosomal Storage Diseases Revealed Key Components of the Lysosomal Sorting Pathway

A group of genetic disorders termed *lysosomal storage diseases* are caused by the absence of one or more lysosomal enzymes. As a result, undigested glycolipids and extracellular components that would normally be degraded by lysosomal enzymes accumulate in lysosomes as large inclusions. Patients with lysosomal storage diseases can have a variety of developmental, physiological, and neurological abnormalities depending on the type and severity of the storage defect. *I-cell disease* is a

particularly severe type of lysosomal storage disease in which multiple enzymes are missing from the lysosomes. Cells from affected individuals lack the *N*-acetylglucosamine phosphotransferase that is required for formation of M6P residues on lysosomal enzymes in the *cis*-Golgi (see Figure 14-22). Biochemical comparison of lysosomal enzymes from normal individuals with those from patients with I-cell disease led to the initial discovery of M6P as the lysosomal sorting signal. Lacking this signal, the lysosomal enzymes of affected individuals are secreted rather than being sorted to and sequestered in lysosomes.

When fibroblasts from patients with I-cell disease are grown in a medium containing lysosomal enzymes bearing M6P residues, the diseased cells acquire a nearly normal intracellular content of lysosomal enzymes. This finding indicates that the plasma membrane of these cells contains M6P receptors, which can internalize extracellular phosphorylated lysosomal enzymes by receptor-mediated endocytosis. This process, used by many cell-surface receptors to bring bound proteins or particles into the cell, is discussed in detail in the next section. It is now known that even in normal cells, some M6P receptors are transported to the plasma membrane and some phosphorylated lysosomal enzymes are secreted (see Figure 14-23). The secreted enzymes can be retrieved by receptor-mediated endocytosis and directed to lysosomes. This pathway thus scavenges any lysosomal enzymes that escape the usual M6P sorting pathway.

Hepatocytes from patients with I-cell disease contain a normal complement of lysosomal enzymes and no inclusions, even in the absence of an exogeneous source of lysosomal enzymes bearing M6P residues. This finding implies that hepatocytes (the most abundant type of liver cell) employ a pathway for sorting lysosomal enzymes that does not rely on a targeting signal based on M6P. This pathway probably relies on some yet unidentified protein-based sorting signal present in lysosomal enzymes. ■

Protein Aggregation in the *trans*-Golgi May Function in Sorting Proteins to Regulated Secretory Vesicles

As noted in the chapter introduction, all eukaryotic cells continuously secrete certain proteins (constitutive secretion). Specialized secretory cells store other proteins in vesicles and secrete them only when triggered by a specific stimulus. One example of such regulated secretion occurs in pancreatic β cells, which store newly made insulin in specialized secretory vesicles and secrete it in response to an elevation in blood glucose (see Figure 21-1b). These and other secretory cells simultaneously use two different types of secretory vesicles to move proteins from the *trans*-Golgi network to the cell surface: unregulated transport vesicles (also called *constitutive secretory vesicles*) and regulated transport vesicles.

A common mechanism appears to sort regulated proteins as diverse as ACTH (adrenocorticotropic hormone), insulin, and trypsinogen into

regulated secretory vesicles. Evidence for such a common mechanism comes from experiments in which recombinant DNA techniques were used to induce the synthesis of insulin and trypsinogen in pituitary tumor cells already synthesizing ACTH. In these cells, which do not normally express insulin or trypsinogen, all three proteins segregate into the same regulated secretory vesicles and are secreted together when a hormone binds to a receptor on the pituitary cells and causes a rise in cytosolic Ca^{2+}.

Morphologic evidence suggests that the ability to form protein aggregates is a common feature of all three proteins that governs their packaging into regulated secretory vesicles. For instance, immature vesicles in this pathway — those that have just budded from the *trans*-Golgi network — contain diffuse aggregates of secretory protein that are visible in the electron microscope. These aggregates are also found in vesicles that are in the process of budding, indicating that proteins destined for regulated secretory vesicles selectively aggregate together before their incorporation into the vesicles.

Some Proteins Undergo Proteolytic Processing After Leaving the *trans*-Golgi

For some secretory proteins (e.g., growth hormone) and certain viral membrane proteins (e.g., VSV G protein), removal of the N-terminal ER signal sequence from the nascent chain is the only known proteolytic cleavage required to convert the polypeptide to the mature, active protein

(see Figure 13-10). However, some membrane proteins and many soluble secretory proteins are initially synthesized as relatively long-lived, inactive precursors, termed *proproteins*, that require further proteolytic processing to generate the mature, active proteins. Examples of proteins that undergo such processing are soluble lysosomal enzymes; many membrane proteins, such as influenza hemagglutinin (HA); and secreted proteins such as serum albumin, insulin, glucagon, and the yeast α mating factor. In general, the proteolytic conversion of a proprotein to the corresponding mature protein occurs after the proprotein has been sorted in the *trans*-Golgi network to appropriate vesicles.

In the case of soluble lysosomal enzymes, the proproteins, called *proenzymes*, are sorted by the M6P receptor as catalytically inactive enzymes. Once in the late endosome or lysosome, a proenzyme undergoes a proteolytic cleavage that generates a smaller but enzymatically active polypeptide. Delaying the activation of lysosomal proenzymes until they reach the lysosome prevents them from digesting macromolecules in earlier compartments of the secretory pathway.

The proproteins of most constitutively secreted proteins (e.g., albumin) are cleaved only once at a site C-terminal to a dibasic recognition sequence such as Arg-Arg or Lys-Arg (Figure 14-24a). Proteolytic processing of proteins whose secretion is regulated generally entails additional cleavages. In the case of proinsulin, multiple cleavages of the single polypeptide chain yield the N-terminal B chain and the C-terminal A chain of mature insulin, which are linked by disulfide bonds, and the

central C peptide, which is lost and subsequently degraded (Figure 14-24b).

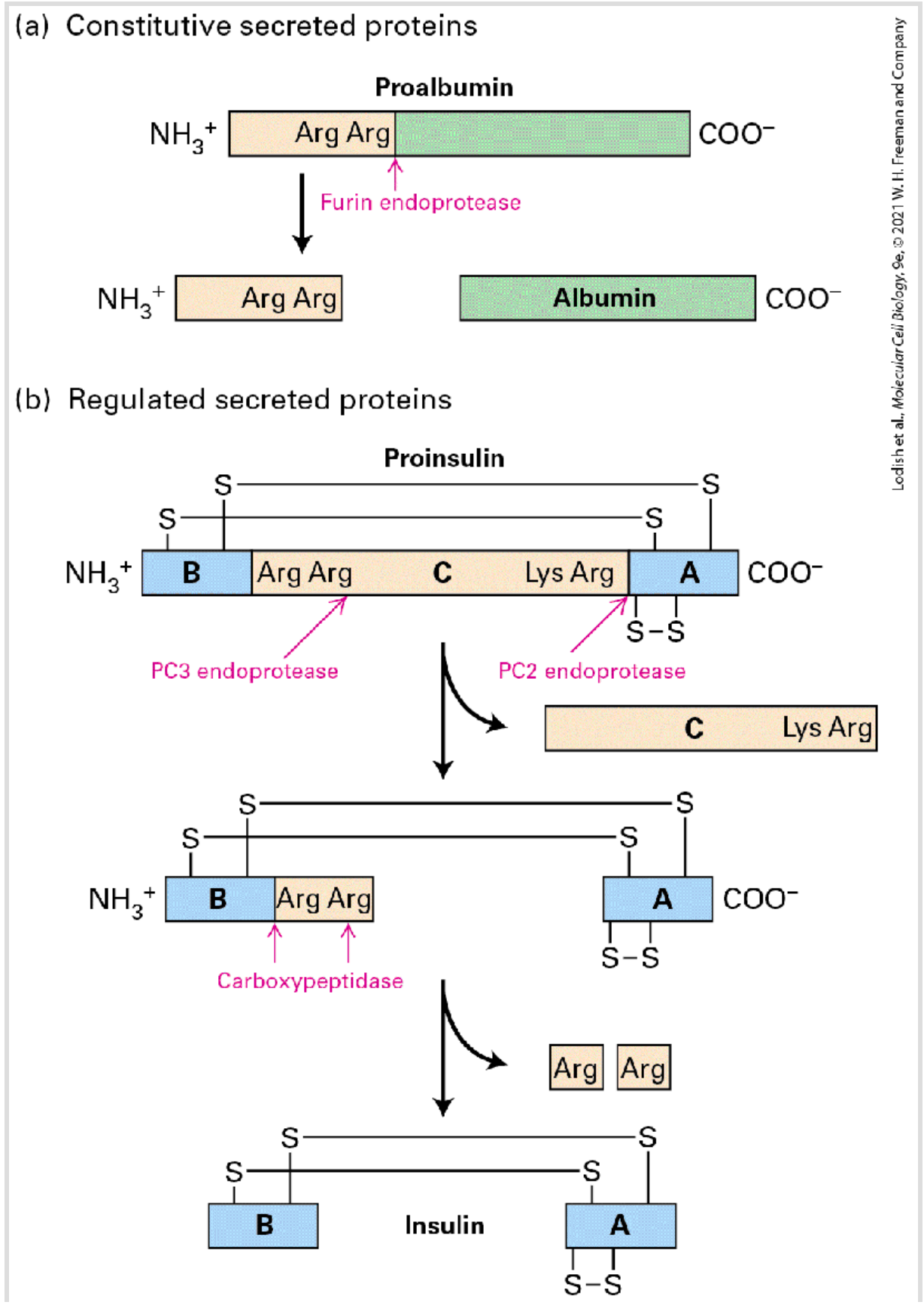


FIGURE 14-24 Proteolytic processing of proproteins in the constitutive and regulated secretory pathways. The processing of proalbumin and proinsulin is typical of the constitutive and regulated pathways, respectively. The endoproteases that function in such processing cleave at the C-terminal end of a sequence of two consecutive amino acids. (a) The endoprotease furin acts on the precursors of constitutive secreted proteins. (b) Two endoproteases, PC2 and PC3, act on the precursors of regulated secreted proteins. The final processing of many such proteins is catalyzed by a carboxypeptidase that sequentially removes two basic amino acid residues at the C-terminus of a polypeptide. See D. Steiner et al., 1992, *J. Biol. Chem.* **267**:23435.

Description

The illustration labeled (a) is titled constitutive secreted proteins. Proalbumin consists of the albumin protein which has a C-terminal (C O O superscript minus) end and a double arginine sequence at the N-terminal (N H subscript 3 superscript plus) end. A furin endoprotease cleaves the terminal arginines.

The illustration labeled (b) is titled regulated secreted proteins. Proinsulin contains a B chain at the N-terminal, an A chain at the c-terminal, and a C chain in the center. An arginine-arginine sequence is located after the B chain and a lysine arginine sequence is located before the A chain. The B and A chains are linked by disulfide bonds, and there is a disulfide bond present in the A chain. P C 3 and P C 2 endoproteases act to cut out the C chain at the end of the arginine- arginine sequence. Carboxypeptidase removes the arginine-arginine sequence leaving mature insulin.

The processing cleavages are carried out by the members of a family of endoproteases located in the late secretory pathway, all of which cleave a protein chain on the C-terminal side of an Arg-Arg or Lys-Arg sequence. One, called *furin*, is found in all mammalian cells; it processes proteins such as albumin that are secreted constitutively. In contrast, the *PC2* and *PC3 endoproteases* are found only in cells that exhibit regulated secretion;

these enzymes are localized to regulated secretory vesicles and proteolytically cleave the precursors of many hormones at specific sites.

Distinct Pathways Sort Membrane Proteins to the Apical or Basolateral Region of Polarized Cells

The plasma membrane of a polarized epithelial cell is divided into two domains: *apical* and *basolateral*. Tight junctions located between the two domains prevent the movement of plasma-membrane proteins between them (see Figure 20-19). Several sorting mechanisms direct newly synthesized membrane proteins to either the apical or the basolateral domain of epithelial cells, and any one protein may be sorted by more than one mechanism. As a result of this sorting and the restriction on protein movement within the plasma membrane by tight junctions, distinct sets of proteins are found in the apical and basolateral domains. This preferential localization of certain transport proteins is critical to a variety of important physiological functions, such as absorption of nutrients from the intestinal lumen and acidification of the stomach lumen (see Figures 11-30 and 11-31).

In one mechanism, the sorting takes place in the *trans*-Golgi network. Microscopic and cell-fractionation studies indicate that proteins destined for either the apical or the basolateral membrane are initially transported together to the membranes of the *trans*-Golgi network. In some cases, proteins destined for the apical membrane are sorted into their own

transport vesicles that bud from the *trans*-Golgi network and then move to the apical region, whereas proteins destined for the basolateral membrane are sorted into other vesicles that move to the basolateral region. The different vesicle types can be distinguished by their protein constituents, including distinct Rab and v-SNARE proteins, which apparently target them to the appropriate plasma-membrane domain. In this mechanism, segregation of proteins destined for the two domains occurs as cargo proteins are incorporated into particular types of vesicles budding from the *trans*-Golgi network.

Such direct basolateral-apical sorting has been investigated in cultured Madin-Darby canine kidney (MDCK) cells, a line of cultured polarized epithelial cells (see Figure 4-4). In MDCK cells infected with the influenza virus, progeny viruses bud only from the apical membrane, whereas in cells infected with vesicular stomatitis virus, progeny viruses bud only from the basolateral membrane. This difference occurs because the HA glycoprotein of influenza virus is transported from the Golgi complex exclusively to the apical membrane and the VSV G protein is transported only to the basolateral membrane (Figure 14-25).

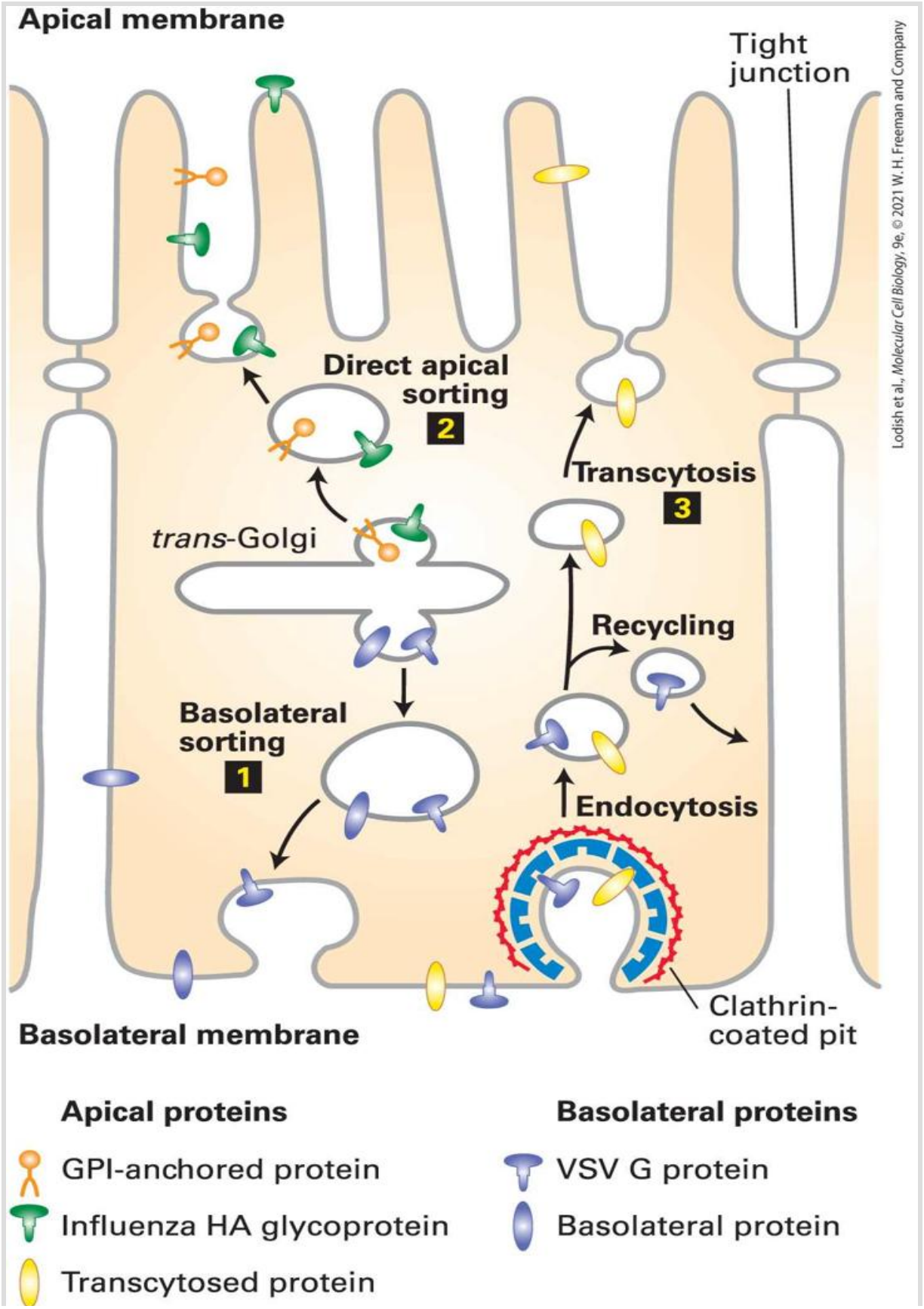

Apical membrane
Tight junction
Direct apical sorting
2
Transcytosis
3
trans-Golgi
Recycling
Basolateral sorting
1
Endocytosis
Clathrin-coated pit
Basolateral membrane
Apical proteins
GPI-anchored protein
Influenza HA glycoprotein
Transcytosed protein
Basolateral proteins
VSV G protein
Basolateral protein
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FIGURE 14-25 Sorting of proteins destined for the apical and basolateral plasma membranes of polarized cells. Cells that form an epithelial cell layer contain distinct apical and basolateral domains of the plasma membrane separated by tight junctions. Distinct sorting pathways in the late secretory pathway govern sorting to either the basolateral or apical membrane. **1**: In the *trans*-Golgi, one type of vesicle transports proteins such as VSV G protein and endogenous proteins that carry a basolateral targeting sequence directly to the basolateral membrane. **2**: A second type of vesicle budding from the *trans*-Golgi carries influenza HA protein and GPI-linked proteins directly to the apical membrane. **3**: Through a process known as *transcytosis*, proteins can move from the basolateral to the apical membrane. Transcytosis involves endocytosis in clathrin/AP coated vesicles from the basolateral membrane, recycling from an endocytic compartment of basolateral proteins back to the basolateral membrane, and delivery of transcytosed apical proteins to the apical membrane. See K. Simons and A. Wandinger-Ness, 1990, *Cell* **62**:207; and K. Mostov et al., 1992, *J. Cell Biol.* **116**:577.

Description

The illustration has a legend at the bottom with figures that represent 5 different proteins namely G P I-anchored protein, Influenza H A glycoprotein, transcytosed protein, V S V G protein, and basolateral protein. Each protein is represented by differently shaped structures.

1. Basolateral sorting: A vesicle with V S V G and basolateral proteins buds out of the trans-Golgi to move towards the basolateral membrane to fuse with the same.

2. Direct apical sorting: From the same trans-Golgi another vesicle with G P I-anchored protein and Influenza H A glycoprotein moves towards the apical membrane at the top. The vesicle fuses with the apical membrane.

3. Endocytosis: A coated vesicle covered in clathrin-coated pits with transcytosed protein and V S V G protein moves up from the basolateral membrane through endocytosis, where V S V G protein gets eliminated for recycling. The uncoated vesicle undergoes transcytosis into the apical membrane. A section of the membrane at the right is labeled tight junction.

Mutational studies on proteins, such as the VSV G protein, that are specifically targeted to the basolateral domain have defined Tyr-X-X-Φ and Asp-X-Leu-Leu sorting signals. As we have seen, proteins bearing these motifs are recruited into clathrin/AP-coated vesicles, strongly implicating clathrin-coated vesicles in the sorting of proteins to the basolateral membrane.

Among the cellular proteins that undergo similar apical-basolateral sorting in the Golgi are those with a *glycosylphosphatidylinositol (GPI) membrane anchor*. In MDCK cells and most other types of epithelial cells, GPI-anchored proteins are targeted to the apical membrane. In membranes, GPI-anchored proteins are clustered into lipid rafts, which are rich in sphingolipids (see Chapter 10). This finding suggests that lipid rafts are localized to the apical membrane along with proteins that preferentially partition them in many cells. However, the GPI anchor is not an apical sorting signal in all polarized cells; in thyroid cells, for example, GPI-anchored proteins are targeted to the basolateral membrane. Other than GPI anchors, no unique sequences have been identified that are both necessary and sufficient to target proteins to either the apical or basolateral domain. Instead, each membrane protein may contain multiple sorting signals, any one of which can target it to the appropriate plasma-membrane domain. The identities of these complex signals and of the vesicle coat proteins that recognize them are currently being pursued for a number of different proteins that are sorted to specific plasma-membrane domains of polarized epithelial cells.

Another mechanism for sorting apical and basolateral proteins, also illustrated in Figure 14-25, operates in hepatocytes. The basolateral membranes of hepatocytes face the blood (like those of intestinal epithelial cells), and the apical membranes line the small intercellular channels into which bile is secreted. In hepatocytes, newly made apical and basolateral proteins are first transported in vesicles from the *trans*-Golgi network to the basolateral region and incorporated into the plasma membrane by exocytosis (i.e., fusion of the vesicle membrane with the plasma membrane). From there both basolateral and apical proteins are endocytosed in the same vesicles, but then their paths diverge. The endocytosed basolateral proteins are sorted into transport vesicles that recycle them to the basolateral membrane. In contrast, the apically destined endocytosed proteins are sorted into transport vesicles that move across the cell and fuse with the apical membrane, a process called **transcytosis**. This process is also used to move extracellular materials from one side of an epithelium to another. Even in epithelial cells, such as MDCK cells, in which apical-basolateral protein sorting occurs in the Golgi, transcytosis may provide an editing function by which an apical protein sorted incorrectly to the basolateral membrane is subjected to endocytosis and then correctly delivered to the apical membrane.

KEY CONCEPTS OF SECTION 14.4

Later Stages of the Secretory Pathway

- The *trans*-Golgi network is a major branch point in the secretory pathway where soluble secreted proteins, lysosomal proteins, and in some cells, membrane proteins destined for the basolateral or apical plasma membrane are segregated into different transport vesicles.

- Many vesicles that bud from the *trans*-Golgi network as well as endocytic vesicles bear a coat composed of AP (adapter protein) complexes and clathrin (see Figure 14-19).
- The pinching off of clathrin-coated vesicles requires dynamin, which forms a collar around the neck of the vesicle bud and hydrolyzes GTP (see Figure 14-20).
- Soluble enzymes destined for lysosomes are modified in the *cis*-Golgi by the addition of multiple mannose 6-phosphate (M6P) residues to their oligosaccharide chains.
- M6P receptors in the membrane of the *trans*-Golgi network bind proteins bearing M6P residues and direct them to late endosomes, where receptors and their ligand proteins dissociate. The receptors are then recycled to the Golgi or plasma membrane, and the lysosomal enzymes are delivered to lysosomes (see Figure 14-23).
- Regulated secreted proteins are concentrated and stored in secretory vesicles to await a neuronal or hormonal signal for exocytosis. Protein aggregation within the *trans*-Golgi network may play a role in sorting secreted proteins to the regulated secretory pathway.
- Many proproteins transported through the secretory pathway undergo post-Golgi proteolytic cleavages that yield the mature, active proteins. This proteolytic maturation can occur in vesicles carrying proteins from the *trans*-Golgi network to the cell surface, in the late endosome, or in the lysosome.
- In polarized epithelial cells, membrane proteins destined for the apical and basolateral domains of the plasma membrane are sorted in the *trans*-Golgi network into different transport vesicles (see Figure 14-25). Tyr-X-X-Φ and Asp-X-Leu-Leu sorting signals target proteins to the basolateral membrane, whereas a GPI anchor targets proteins to the apical membrane.
- In hepatocytes and some other polarized cells, all plasma-membrane proteins are directed first to the basolateral membrane. Apically destined proteins are then endocytosed and moved across the cell to the apical membrane (transcytosis).