14.2 Molecular Mechanisms of Vesicle Budding and Fusion

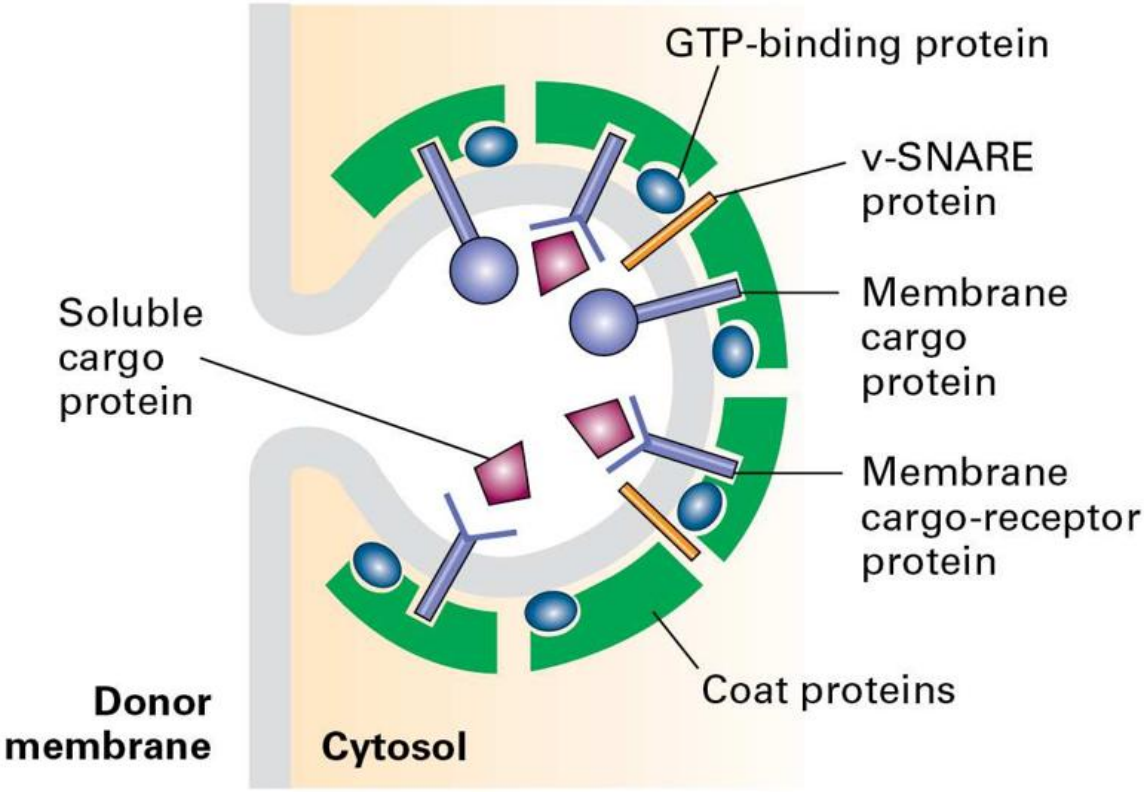
Small membrane-bounded vesicles that transport proteins from one organelle to another are the fundamental functional elements in the secretory and endocytic pathways (see Figure 14-1). These vesicles bud from the membrane of a particular *parent (donor) organelle* and fuse with the membrane of a particular *target (destination) organelle*. Although each step in the secretory and endocytic pathways employs a different type of vesicle, studies employing genetic and biochemical techniques have revealed that each of the different vesicular transport steps is simply a variation on a common theme. In this section, we explore the basic mechanisms underlying vesicle budding and fusion that all vesicle types have in common, before discussing the details unique to each step in the pathway.

Assembly of a Protein Coat Drives Vesicle Formation and Selection of Cargo Molecules

The budding of a vesicle from its parent membrane is triggered by the activation of a GTP-binding protein followed by the polymerization of soluble protein complexes on the membrane to form a proteinaceous

vesicle coat (Figure 14-7a). Interactions between the cytosolic portions of integral membrane proteins and the vesicle coat gather the appropriate cargo proteins into the forming vesicle. Thus the coat gives curvature to the membrane to form a vesicle and acts as the filter to determine which proteins are admitted into the vesicle.

(a) Coated vesicle budding



(b) Uncoated vesicle fusion

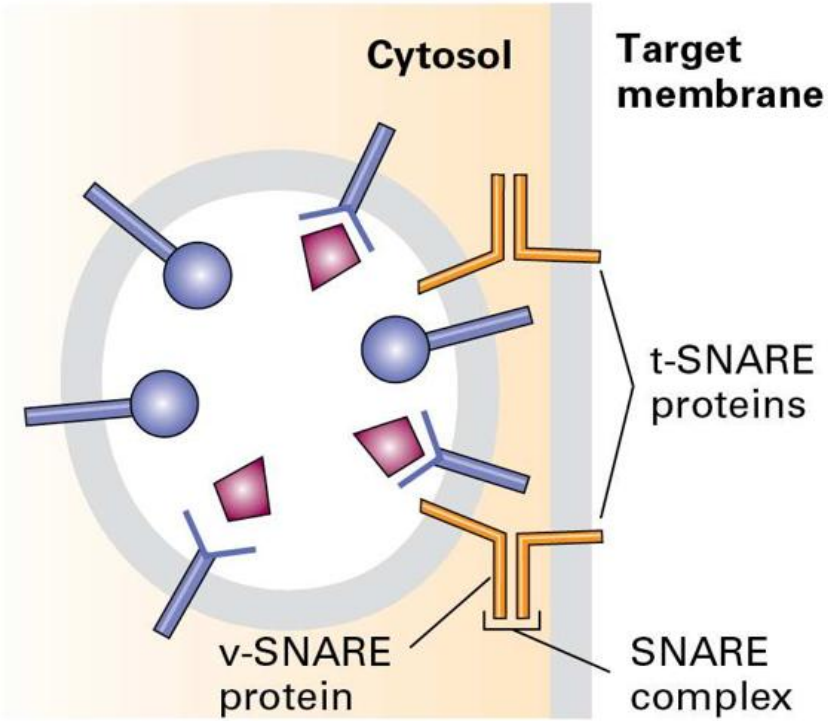


FIGURE 14-7 Overview of vesicle budding and fusion with a target membrane. (a) Budding is initiated by recruitment of a small GTP-binding protein to a patch of donor membrane. Complexes of coat proteins in the cytosol then bind to the cytosolic domain of membrane cargo proteins, some of which also act as receptors that bind soluble proteins in the lumen, thereby recruiting luminal cargo proteins into the budding vesicle. (b) After being released and shedding its coat, a vesicle fuses with its target membrane in a process that involves interaction of cognate SNARE proteins.

Description

The illustration labeled (a) titled coated vesicle budding shows a vesicle buds in the cytosol from the donor membrane. On the cytosolic side of the budding membrane, coat proteins are present. The coat proteins contain G T P-binding proteins and membrane cargo receptor proteins. Several v-S N A R E proteins are embedded in the vesical membrane. The membrane cargo-receptor proteins are y-shaped and are bound to soluble cargo proteins. In addition to the membrane cargo receptors, membrane cargo proteins from the coat protein are also embedded in the vesicle.

The illustration labeled (b) titled uncoated vesicle fusion shows a spherical vesicle inside the cytosol approaching the target membrane. It has no coat. The vesicle contains membrane cargo proteins and membrane cargo-receptor proteins embedded in the vesicular membrane and several v-S N A R E proteins. The target membrane contains two t-S N A R E proteins, which can interact with the v-S N A R E proteins in the membrane.

Proteins responsible for the eventual fusion of a vesicle with the target membrane, known as **v-SNAREs**, are incorporated into the vesicle membrane during assembly of the vesicle coat. Once the coat is at least partially shed from a completed vesicle, v-SNARE proteins embedded in the vesicle membrane become accessible to join with cognate **t-SNAREs** in the target membrane to which the vesicle is docked. This joining brings the membranes into close apposition, allowing the two bilayers to fuse

(Figure 14-7b). Regardless of target organelle, all transport vesicles use v-SNAREs and t-SNAREs to fuse.

Three major types of coated vesicles have been characterized, each with a different type of protein coat and each formed by reversible polymerization of a distinct set of protein subunits (Table 14-1). Each type of vesicle, named for its primary coat proteins, transports cargo proteins from particular parent organelles to particular target organelles:

TABLE 14-1 • Coated Vesicles Involved in Protein Trafficking

Vesicle Type	Transport Step Mediated	Coat Proteins	Associated GTPase
COPII	ER to *cis*-Golgi	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1
COPI	*cis*-Golgi to ER later to earlier Golgi cisternae	Coatomers containing seven different COP subunits	ARF
Clathrin and adapter proteins[i]	*trans*-Golgi to endosome	Clathrin + AP1 complexes	ARF
	trans-Golgi to endosome	Clathrin + GGA	ARF
	Plasma membrane to endosome	Clathrin + AP2 complexes	ARF
	Golgi to lysosome, melanosome, or platelet vesicles	AP3 complexes	ARF

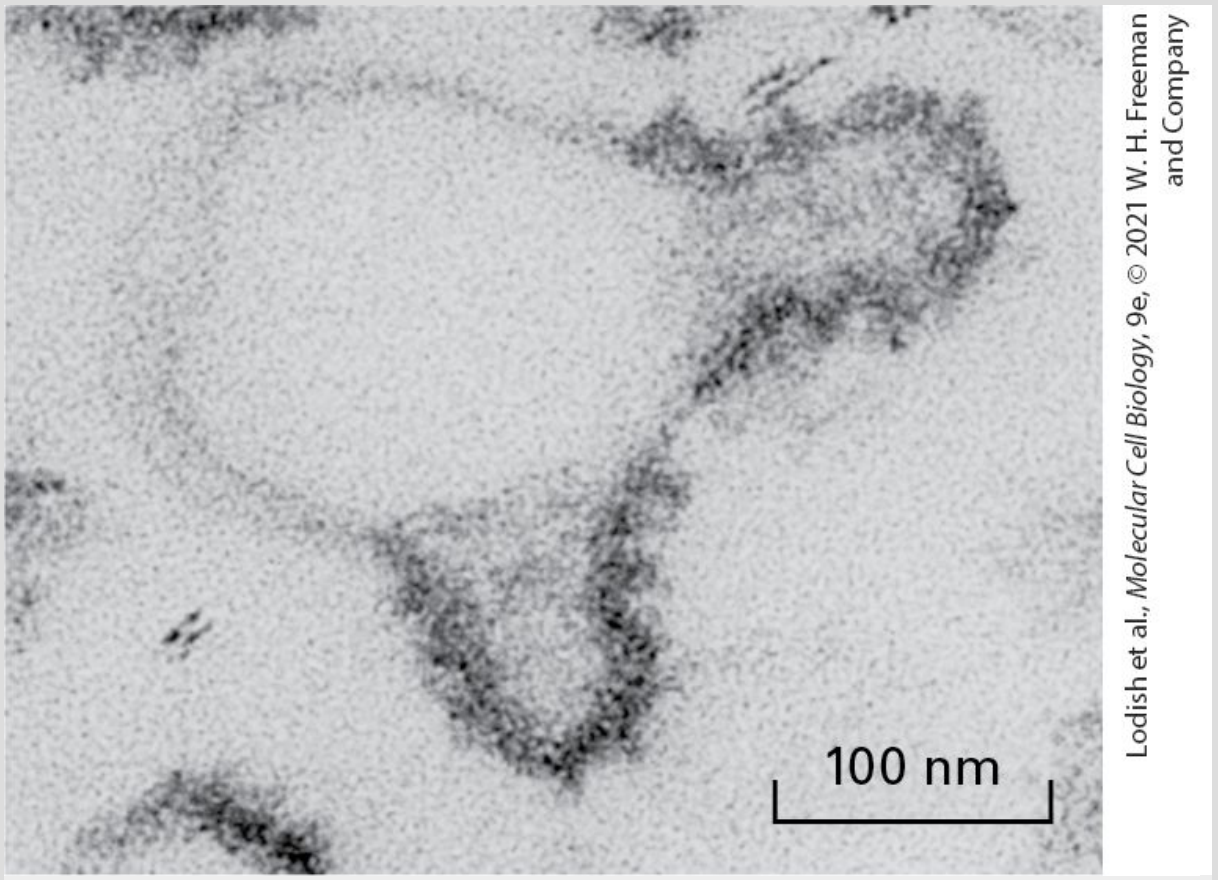
[i]Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

- **COPII** vesicles transport proteins from the ER to the Golgi.
- **COPI** vesicles mainly transport proteins in the retrograde direction between Golgi cisternae and from the *cis*-Golgi back to the ER.
- **Clathrin**-coated vesicles transport proteins from the plasma membrane (cell surface) and the *trans*-Golgi network to late endosomes.

Every vesicle-mediated trafficking step is thought to use some kind of vesicle coat; however, a specific coat protein complex has not been identified for every type of vesicle. For example, vesicles that move proteins from the *trans*-Golgi to the plasma membrane during either constitutive or regulated secretion exhibit a uniform size and morphology, which suggests that their formation is driven by assembly of a regular coat structure, yet researchers have not identified specific coat proteins surrounding these vesicles.

The general scheme of vesicle budding shown in Figure 14-7a applies to all three known types of coated vesicles. Experiments with isolated or artificial membranes and purified coat proteins have shown that polymerization of the coat proteins on the cytosolic face of the parent membrane is necessary to produce the high curvature of the membrane that is typical of a transport vesicle that is about 50 nm in diameter. Electron micrographs of in vitro budding reactions often reveal structures that exhibit discrete regions of the parent membrane bearing a dense coat accompanied by the curvature characteristic of a completed vesicle (Figure 14-8). Such structures, usually called *vesicle buds*, appear to be

intermediates that are visible after the coat has begun to polymerize but before the completed vesicle pinches off from the parent membrane. The polymerized coat proteins form a curved lattice that drives the formation of a vesicle bud by adhering to the cytosolic face of the membrane.



EXPERIMENTAL FIGURE 14-8 Vesicle buds can be visualized during in vitro budding reactions. When purified COPII coat components are incubated with isolated ER vesicles or artificial phospholipid vesicles (liposomes), polymerization of the coat proteins on the vesicle surface induces emergence of highly curved buds. In this electron micrograph of an in vitro budding reaction, note the distinct membrane coat, visible as a dark protein layer, present on the vesicle buds.

[Republished with permission from Elsevier, from K. Matsuoka et al., 1998, "COPII-Coated Vesicle Formation Reconstituted with Purified Coat Proteins and Chemically Defined Liposomes," *Cell* **93**(2):263–275; permission conveyed through Copyright Clearance Center, Inc.]

A Conserved Set of GTPase Switch Proteins Controls the Assembly of Different Vesicle Coats

Using in vitro vesicle-budding reactions among isolated membranes and purified coat proteins, scientists have determined the minimum set of coat components required to form each of the three major types of vesicles. Although most of the coat proteins differ considerably from one type of vesicle to another, the coats of all three vesicles contain a small GTP-binding protein that acts as a regulatory subunit to control coat assembly (see Figure 14-7a). A GTP-binding protein known as **ARF protein** plays this role in COPI and clathrin-coated vesicles. A different but related GTP-binding protein known as *Sar1 protein* is present in the coat of COPII vesicles. Both ARF and Sar1 are monomeric proteins with a structure generally similar to that of Ras, a key intracellular signal-transducing protein (see Figure 16-12). ARF and Sar1 proteins, like Ras, belong to the GTPase superfamily of switch proteins that cycle between GDP-bound and GTP-bound forms (see Figure 3-9 to review the mechanism of GTPase switch proteins).

As depicted in Figure 14-9, activation of Sar1 by binding to GTP is the initial event that triggers assembly of the COPII vesicle coat. Note that Sar1 is the only GTPase switch protein activated at the ER membrane and thus only COPII-coated vesicles bud from the ER membrane. This

initiating event begins with an ER membrane protein known as *Sec12*, which acts as a guanine nucleotide exchange factor (GEF) for Sar1 by catalyzing the release of GDP from cytosolic Sar1·GDP and the binding of GTP. Sec12 apparently receives and integrates multiple as yet unknown signals, probably including the presence of cargo proteins that are ready to be transported at the ER membrane. Binding of GTP causes a conformational change in Sar1; this exposes its amphipathic N-terminus, which then becomes embedded in the phospholipid bilayer and tethers Sar1·GTP to the ER membrane (Figure 14-9, step **1**). The membrane-attached Sar1·GTP drives the polymerization of cytosolic complexes of COPII subunits on the membrane, eventually leading to formation of vesicle buds (step **2**). Once COPII vesicles are released from the donor membrane, the Sar1 GTPase activity hydrolyzes Sar1·GTP in the vesicle membrane to Sar1·GDP with the assistance of one of the coat subunits (step **3**). This hydrolysis triggers disassembly of the COPII coat (step **4**). Thus Sar1 couples a cycle of GTP binding and hydrolysis to the formation and then disassembly of the COPII coat.

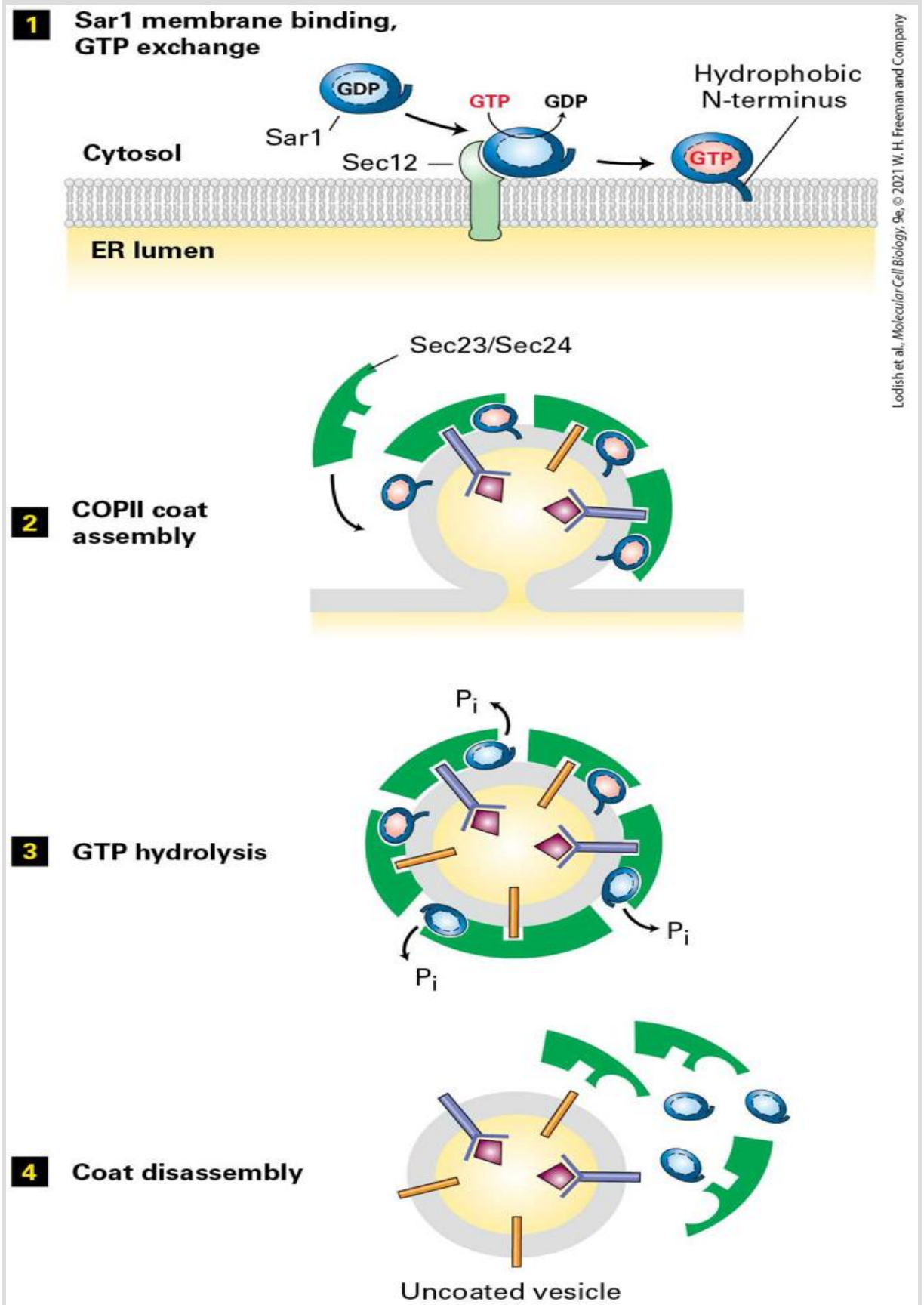

1
Sar1 membrane binding, GTP exchange
GDP
GTP
GDP
Hydrophobic N-terminus
Sar1
Cytosol
Sec12
GTP
ER lumen
Sec23/Sec24
2
COPII coat assembly
Pi
3
GTP hydrolysis
Pi
Pi
4
Coat disassembly
Uncoated vesicle
Lodish et al., Molecular Cell Biology, 9e, © 2021 W.H. Freeman and Company

FIGURE 14-9 Model for the role of Sar1 in the assembly and disassembly of the COPII coat. Step 1: Interaction of soluble GDP-bound Sar1 with the GEF Sec12, an ER integral membrane protein, catalyzes exchange of GTP for GDP on Sar1. The hydrophobic N-terminus of the GTP-bound form of Sar1 extends outward from the protein's surface and anchors Sar1 to the ER membrane. Step 2: Once attached to the membrane, Sar1 serves as a binding site for the Sec23/Sec24 coat protein complex. Membrane cargo proteins are recruited to the forming vesicle bud by binding of specific short sequences (sorting signals) in their cytosolic regions to sites on the Sec23/Sec24 complex. Some membrane cargo proteins also act as receptors that bind soluble proteins in the lumen. The coat is completed by assembly of a second type of coat complex composed of Sec13 and Sec31 (not shown). Step 3: After the vesicle coat is complete, the Sec23 coat subunit promotes GTP hydrolysis by Sar1. Step 4: Release of Sar1·GDP from the vesicle membrane causes disassembly of the coat. See S. Springer et al., 1999, *Cell* **97**:145.

Description

Step 1: A s e c 12 protein is embedded in the plasma membrane separating the cytosol from the E R lumen. In the cytosol, S A R 1, a G D P-containing protein binds with S E C 12. In this step G T P is converted to G D P. The hydrophobic N-terminus of S A R 1 gets embedded in the plasma membrane, binding the protein to the cytosolic face of the membrane.

Step 2: S e c 23 and S e c 24 coat proteins bind with the S A R 1 proteins in the budding vesicle. During this process, membrane target receptor proteins bind with the coating proteins. Thus, C O P 2 coat assembly is complete.

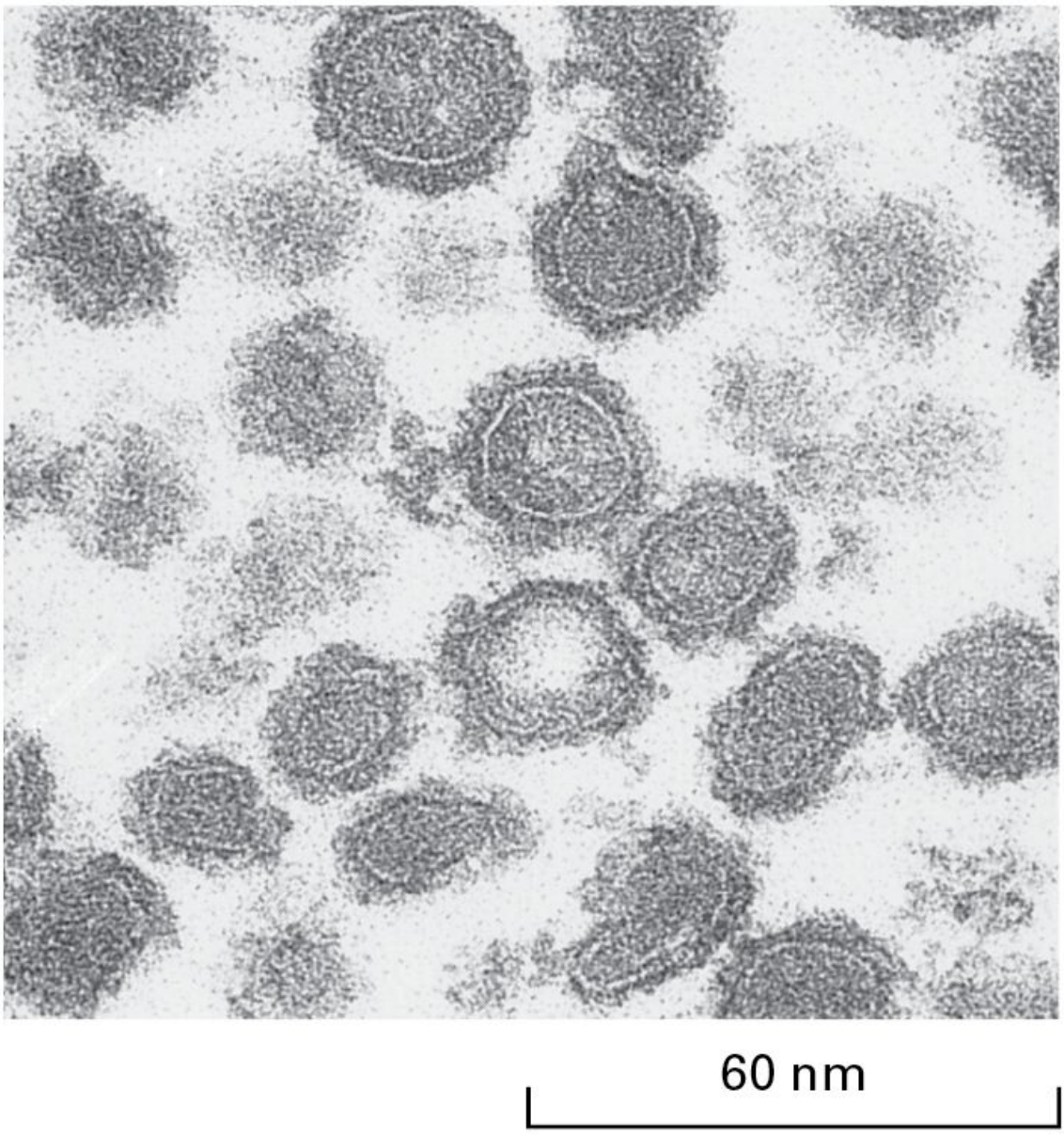
Step 3: The fully coated vesicle undergoes G T P hydrolysis. Three molecules of inorganic phosphates are removed from the S A R 1 proteins.

Step 4: The coat disassembles along with S A R 1 proteins, leaving an uncoated vesicle behind.

ARF protein undergoes a similar cycle of nucleotide exchange and hydrolysis coupled to the assembly of vesicle coats composed either of

COPI or of clathrin and other coat proteins (AP complexes), discussed later. A covalent protein modification known as a *myristate anchor* on the N-terminus of the ARF protein weakly tethers ARF·GDP to the Golgi membrane. When GTP is exchanged for the bound GDP by a GEF attached to the Golgi membrane, the resulting conformational change in ARF allows hydrophobic residues in its N-terminal segment to insert into the membrane bilayer. The resulting tight association of ARF·GTP with the membrane serves as the foundation for further coat assembly.

Drawing on the structural similarities of Sar1 and ARF to other small GTPase switch proteins, researchers have constructed genes encoding mutant versions of the two proteins that have predictable effects on vesicular traffic when transfected into cultured cells. For example, in cells expressing mutant versions of Sar1 or ARF that cannot hydrolyze GTP, vesicle coats form and vesicle buds pinch off. However, because the mutant proteins cannot trigger disassembly of the coat, all available coat subunits eventually become permanently assembled into coated vesicles that are unable to fuse with target membranes. Addition of a nonhydrolyzable GTP analog to in vitro vesicle-budding reactions causes a similar blocking of coat disassembly. The vesicles that form in such reactions have coats that never dissociate, allowing their composition and structure to be more readily analyzed. The purified COPI vesicles shown in Figure 14-10 were produced in such a budding reaction.



Courtesy of L. Orci (University of Geneva, Switzerland).

EXPERIMENTAL FIGURE 14-10 Coated vesicles accumulate during in vitro budding reactions in the presence of a nonhydrolyzable analog of GTP. When isolated Golgi membranes are incubated with a cytosolic extract containing COPI coat proteins, vesicles form and bud off from the membranes. Inclusion of a nonhydrolyzable analog of GTP in the budding reaction prevents disassembly of the coat after vesicle release. This micrograph shows COPI vesicles generated in such a reaction and separated from membranes by centrifugation. Coated vesicles prepared in this way can be analyzed to determine their components and properties.

A second general function of small GTPases in vesicle formation is in the pinching off of a completed vesicle from the parent membrane. In vitro budding experiments show that the Sar1 GTPase that accumulates at the neck of the budding vesicle is required for the pinching off of COPII vesicles and that the ARF GTPase similarly drives the pinching off of COPI vesicles. The mechanism by which these small GTPases convert the energy from GTP hydrolysis to a mechanical force to complete the pinching off of the membrane is not understood. As we will see in Section 14.4, a large polymeric GTPase known as *dynamin* plays this role in clathrin-coated vesicles.

Targeting Sequences on Cargo Proteins Make Specific Molecular Contacts with Coat Proteins

In order for transport vesicles to move specific proteins from one compartment to the next, vesicle buds must be able to discriminate among potential membrane and soluble cargo proteins, accepting only those cargo proteins that should advance to the next compartment and excluding those that should remain as residents in the donor compartment. In addition to sculpting the curvature of a donor membrane, the vesicle coat functions in selecting specific proteins as cargo. Membrane cargo proteins and soluble cargo proteins carry two different kinds of **sorting signals.** The sorting signals for membrane cargo proteins usually lie in the cytosolic portion of the protein and bind to one or another of the vesicle coat proteins (see Figure 14-7a). The polymerized coat thus acts as an affinity matrix to

cluster selected membrane cargo proteins into forming vesicle buds. Because soluble proteins within the lumen of the parent organelle cannot contact the coat directly, they require a different kind of sorting signal. Soluble luminal proteins often contain what can be thought of as *luminal sorting signals*, which bind to the luminal domains of certain membrane cargo proteins. The properties of some of the known sorting signals in membrane and soluble proteins are summarized in Table 14-2. We describe the role of these signals in more detail in later sections.

TABLE 14-2 • Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

Signal Sequence[ii]	Signal-Bearing Protein	Proteins with Signal	Signal Receptor
Cytoplasmic Sorting Signals			
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins	COPI α and β subunits	COPI
Di-arginine (X-Arg-Arg-X)	ER-resident membrane proteins	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER	COPII Sec24 subunit	COPII
Asn-Pro-X-Tyr (NPXY)	LDL receptor in plasma membrane	AP2 complex	Clathrin/AP2
Tyr-X-X-Φ (YXX Φ)	Membrane proteins in *trans*-Golgi	AP1 ($\mu 1$ subunit)	Clathrin/AP1
	Plasma membrane proteins	AP2 ($\mu 2$ subunit)	Clathrin/AP2
Leu-Leu (LL)	Plasma membrane proteins	AP2 complexes	Clathrin/AP2

Luminal Sorting Signals

Lys-Asp-Glu-Leu (KDEL)	ER-resident soluble proteins	KDEL receptor in *cis*-Golgi membrane	COPI
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2

[ii] $\mathrm{X} = \text{any amino acid};\ \Phi = \text{hydrophobic amino acid}$. Single-letter amino acid abbreviations are in parentheses.

Rab GTPases Control Docking of Vesicles on Target Membranes

A second set of small GTP-binding proteins, known as **Rab proteins**, associate with transport vesicles and act as key regulators of vesicle trafficking to and fusion with the appropriate target membrane. Like Sar1 and ARF, Rab proteins belong to the GTPase superfamily of switch proteins. Rab proteins also contain an isoprenoid anchor that allows them to become tethered to the vesicle membrane. Association of an activated Rab protein with a specific vesicle type is generally a two-step process. In the first step, cytosolic Rab·GDP is targeted to the appropriate vesicle, becoming attached there by insertion of its isoprenoid anchor into the vesicle membrane. Often this attachment step is facilitated by a protein that can associate with Rab·GDP along with its isoprenoid anchor, usually known as a *guanine nucleotide dissociation inhibitor (GDI)*. In the second

step, a specific GEF located in the vesicle membrane converts membrane-bound Rab·GDP to Rab·GTP. Once localized and activated in this way, Rab·GTP is enabled to bind to a variety of different proteins, known as *Rab effectors*. Binding of Rab·GTP to a Rab effector can ultimately lead to docking of the vesicle on an appropriate target membrane (Figure 14-11a, step **1**). After vesicle fusion occurs, the GTP bound to the Rab protein is hydrolyzed to GDP, triggering the release of Rab·GDP, which can then undergo another cycle of GDP-GTP exchange, binding, and hydrolysis.

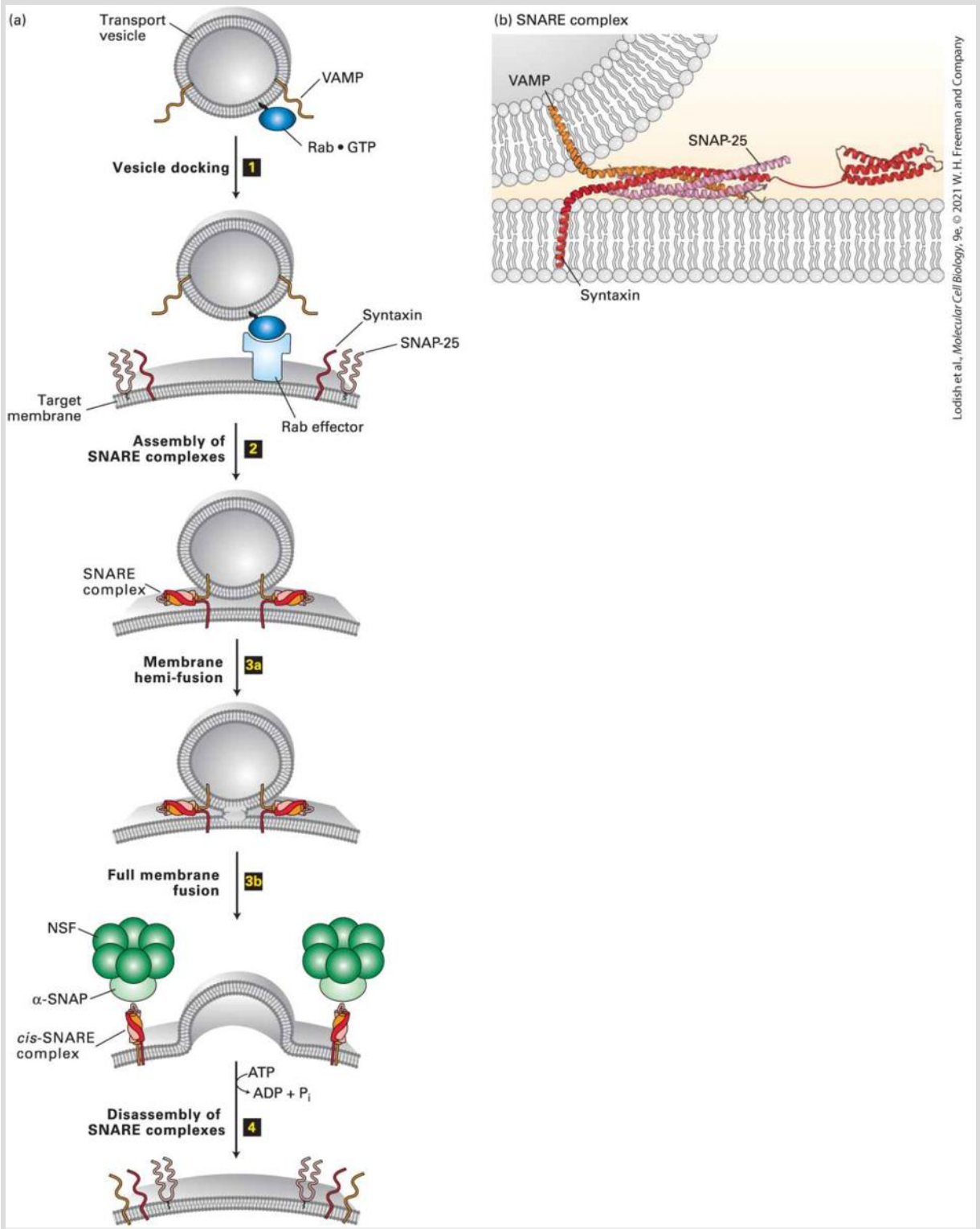


FIGURE 14-11 Model for docking and fusion of transport vesicles with their target membranes. (a) The proteins shown in this example participate in fusion of secretory vesicles with the plasma membrane, but similar proteins mediate all vesicle-fusion events.

Step 1: A Rab protein tethered via a lipid anchor to a secretory vesicle binds to an effector protein complex on the plasma membrane, thereby docking the transport vesicle on the appropriate target membrane. Step 2: A v-SNARE protein (in this case, VAMP) interacts with the cytosolic domains of the cognate t-SNAREs (in this case, syntaxin and SNAP-25). The very stable coiled-coil SNARE complexes that are formed hold the vesicle close to the target membrane. Step 3: Fusion of the two membranes immediately follows formation of SNARE complexes, first by fusion of one leaflet of the bilayer to form a hemi-fused state (3a), followed by fusion of the second leaflet (3b). Step 4: Following membrane fusion, NSF, in conjunction with α-SNAP, binds to the SNARE complexes. The NSF-catalyzed hydrolysis of ATP then drives dissociation of the SNARE complexes, freeing the SNARE proteins for another round of vesicle fusion. Also at this time, Rab·GTP is hydrolyzed to Rab·GDP and dissociates from the Rab effector (not shown). (b) The SNARE complex. Numerous noncovalent interactions between four long α helices, two from SNAP-25 and one each from syntaxin and VAMP, stabilize the coiled-coil structure. See J. E. Rothman and T. Söllner, 1997, *Science* **276**:1212; Y. A. Chen and R. H. Scheller, 2001, *Nat. Rev. Mol. Cell Biol.* **2**:98; and W. Weis and R. Scheller, 1998, *Nature* **395**:328.

[Part (b) Data from I. Fernandez et al., 1998, *Cell* **94**:841–849, PDB ID 1br0; and R. B. Sutton et al., 1998, *Nature* **395**:347–353, PDB ID 1sfc.]

Description

The illustration labeled (a) depicts the docking and fusion of vesicles in four steps. A circular transport vesicle has a Rab-G T P protein embedded in the cytosolic face of the vesicle. Two V A M P proteins extend from the vesicle into the cytosol.

Step 1: The target membrane contains a Rab effector, two syntaxins, and two S N A P-25 proteins. Once the vesicle is close to the target membrane, the Rab- G T P protein docks with the Rab effector.

Step 2: The V A M P proteins, S N A P – 25 proteins, and the syntaxin proteins bind together to form the S N A R E complex between the vesicle and target membrane.

Step 3 a: The vesicle membrane and the target membrane undergo membrane hemi-fusion between the S N A R E complexes.

Step 3 b: The vesicle undergoes full membrane fusion with the target membrane. The now released cis-S N A R E complexes bind with the alpha S N A P domain of the N-ethylmaleimide sensitive fusion protein (NSF).

Step 4: The S N A R E complexes disassemble, using a molecule of A T P in the process. A T P is converted to A D P and inorganic phosphate.

The illustration labeled (b) depicts the formation of the S N A R E complex is. A vesicle is in close proximity to the target membrane. V A M P protein is embedded in the vesicle; syntaxin is embedded in the target membrane, as is S N A P-25. The alpha-helices of these three proteins wrap around each other, forming a coiled-coil.

A well-understood example of a Rab protein that enables vesicle fusion with the correct target membrane is the Sec4 protein of yeast, which specifically tags secretory vesicles, enabling them to fuse with the plasma membrane. In experiments, yeast cells expressing mutant Sec4 proteins accumulate secretory vesicles that are unable to fuse with the plasma membrane (class E mutants in Figure 14-5). Sec4·GDP binds to secretory vesicles, where it is activated to Sec4·GTP by its cognate GEF, which is itself located on secretory vesicles. Sec4·GTP, in turn, binds to its effector, a large tethering complex composed of eight subunits, known as the *exocyst*. Tethering of secretory vesicles to the exocyst by binding of Sec4·GTP ultimately leads to vesicle fusion with the plasma membrane.

In mammalian cells, Rab5 protein is localized to endocytic vesicles, also known as *early endosomes*. These uncoated vesicles form from clathrin-coated vesicles just after they bud from the plasma membrane during endocytosis (see Figure 14-2, step **9**). The fusion of early endosomes with one another in cell-free systems requires the presence of Rab5, and

addition of Rab5 and GTP to cell-free extracts accelerates the rate at which these vesicles fuse with one another. A long coiled protein known as *EEA1 (e*arly *e*ndosome *a*ntigen *1*), which resides on the membrane of the early endosome, functions as the effector for Rab5. In this case, Rab5·GTP on one endocytic vesicle is thought to bind specifically to EEA1 on the membrane of another endocytic vesicle, setting the stage for fusion of the two vesicles.

Every type of transport vesicle appears to be labeled with one or more specific Rab proteins. These Rab proteins, through their specific association with effectors that are membrane tethers and molecular motors, ensure that the vesicles are directed to the correct target membrane address (Table 14-3). The tracks on which the molecular motors run to deliver the vesicles to their destinations — microfilaments — will be discussed in Chapter 17.

TABLE 14-3 • Rab Proteins Involved in Vesicle Tethering and Vesicle Movement

Rab Protein	Transport Step	Effector Proteins
Rab1/Ypt1	ER to *cis*-Golgi	TRAPP complex and GM130 complex in *cis*-Golgi
Sec4	*trans*-Golgi to plasma membrane	Exocyst on plasma membrane and Type V Myosin
Rab5	Plasma membrane to endosome	EEA1 tether on endosome
Rab6	Golgi movement along microtubules	Kinesin

Rab27	Melanosome movement to cell periphery	Type V Myosin

Paired Sets of SNARE Proteins Mediate Fusion of Vesicles with Target Membranes

As noted previously, shortly after a vesicle buds off from the donor membrane, the vesicle coat disassembles to uncover a vesicle-specific membrane protein, a v-SNARE (see Figure 14-7b). Likewise, each type of target membrane in a cell contains t-SNARE membrane proteins, which interact specifically with v-SNAREs. After Rab-mediated docking of a vesicle on its target membrane, the interaction of cognate SNAREs brings the two membranes close enough together that they can fuse.

One of the best understood examples of SNARE-mediated fusion occurs during exocytosis of secreted proteins (see Figure 14-11a, steps **1** and **3**). In this case, the v-SNARE, known as *VAMP* (*v*esicle-*a*ssociated *m*embrane *p*rotein), is incorporated into secretory vesicles as they bud from the *trans*-Golgi network. The t-SNAREs are *syntaxin*, an integral membrane protein in the plasma membrane, and *SNAP-25*, which is attached to the plasma membrane by a hydrophobic lipid anchor in the middle of the protein. The cytosolic region in each of these three SNARE proteins contains a repeating heptad sequence that allows four α helices — one from VAMP, one from syntaxin, and two from SNAP-25 — to coil around one another to form a four-helix bundle (Figure 14-11b). The

unusual stability of this bundled SNARE complex is conferred by the arrangement of hydrophobic and charged amino acid residues in the heptad repeats. The hydrophobic amino acids are buried in the central core of the bundle, and amino acids of opposite charge are aligned to form favorable electrostatic interactions between helices. As multiple four-helix bundles form, the embedded transmembrane domains of VAMP and syntaxin pull the vesicle and target membranes together into very close apposition. The energetically favorable formation of four-helix bundles can overcome the electrostatic repulsion of the generally negatively charged phospholipid head groups in the vesicle and target membranes, allowing the hydrophobic interiors of the two membranes to come into contact, creating an opening between the two membranes, and ultimately causing the vesicle membrane to fuse with the target membrane.

In vitro experiments have shown that when liposomes containing purified VAMP are incubated with other liposomes containing syntaxin and SNAP-25, the two classes of membranes fuse, albeit slowly. This finding is strong evidence that the close apposition of membranes resulting from formation of SNARE complexes is sufficient to bring about membrane fusion. Fusion of a vesicle and target membrane occurs more rapidly and efficiently in the cell than it does in liposome experiments in which fusion is catalyzed only by SNARE proteins. The likely explanation for this difference is that in the cell, other proteins, such as Rab proteins and their effectors, are involved in targeting vesicles to the correct membrane.

Yeast cells, like all eukaryotic cells, express more than 20 different related v-SNARE and t-SNARE proteins. Analyses of yeast mutants defective in

each of the SNARE genes have identified specific membrane-fusion events in which each SNARE protein participates. For all fusion events that have been examined, the SNAREs form four-helix bundled complexes similar to the VAMP/syntaxin/SNAP-25 complexes that mediate fusion of secretory vesicles with the plasma membrane. However, in other fusion events (e.g., fusion of COPII vesicles with the *cis*-Golgi network), each participating SNARE protein contributes only one α helix to the bundle (unlike SNAP-25, which contributes two helices); in these cases, the SNARE complexes comprise one v-SNARE and three t-SNARE molecules.

Using the in vitro liposome fusion assay, researchers have tested the ability of various combinations of individual v-SNARE and t-SNARE proteins to mediate fusion of donor and target membranes. Of the very large number of different combinations tested, only a small number could efficiently mediate membrane fusion. To a remarkable degree, the functional combinations of v-SNAREs and t-SNAREs revealed in these in vitro experiments correspond to the actual SNARE protein interactions that mediate known membrane-fusion events in the yeast cell. Thus together with the specificity of interaction between Rab and Rab effector proteins, the specificity of the interaction between SNARE proteins can account for most, if not all, of the specificity of fusion between a particular vesicle type and its target membrane.

Dissociation of SNARE Complexes After Membrane Fusion Is Driven by

ATP Hydrolysis

After a vesicle and its target membrane have fused, the SNARE complexes must dissociate to make the individual SNARE proteins available for additional fusion events. Because of the stability of SNARE complexes, which are held together by numerous noncovalent intermolecular interactions, their dissociation depends on additional proteins and the input of energy.

The first clue that dissociation of SNARE complexes required the assistance of other proteins came from in vitro transport reactions depleted of certain cytosolic proteins. The observed accumulation of vesicles in these reactions indicated that vesicles could form under these conditions, but were unable to fuse with a target membrane. Eventually two proteins, designated *NSF* and *α-SNAP*, were found to be required for ongoing vesicle fusion in the in vitro transport reaction.

Yeast mutants have also contributed to our understanding of SNARE function. Among the class C yeast *sec* mutants are strains that lack functional Sec18 or Sec17, the yeast counterparts of mammalian NSF and α-SNAP, respectively. When these class C mutants are kept at the nonpermissive temperature, they accumulate ER-to-Golgi transport vesicles; when the cells are shifted to the lower, permissive temperature, the accumulated vesicles are able to fuse with the *cis*-Golgi.

Subsequent to the initial biochemical and genetic studies that identified NSF and α-SNAP, more sophisticated in vitro transport assays were developed. Using these newer assays, researchers have shown that NSF and α-SNAP proteins are not necessary for actual membrane fusion, but rather are required for regeneration of free SNARE proteins. NSF, a hexamer of identical subunits, associates with a SNARE complex with the aid of α-SNAP (*s*oluble *N*SF *a*ttachment *p*rotein). The bound NSF then hydrolyzes ATP, releasing sufficient energy to dissociate the SNARE complex (see Figure 14-11a, step **4**). Evidently, the defects in vesicle fusion observed in the earlier in vitro fusion assays and in the yeast mutants after a loss of Sec17 or Sec18 were a consequence of free SNARE proteins rapidly becoming sequestered in undissociated SNARE complexes and thus being unavailable to mediate membrane fusion.

KEY CONCEPTS OF SECTION 14.2

Molecular Mechanisms of Vesicle Budding and Fusion

- The three well-characterized types of transport vesicles — COPI, COPII, and clathrin-coated vesicles — are distinguished by the proteins that form their coats and the transport routes they mediate (see Table 14-1).
- All types of coated vesicles are formed by polymerization of cytosolic coat proteins on a parent (donor) membrane to form vesicle buds that eventually pinch off from the membrane to release a complete vesicle. Shortly after vesicle release, the coat is shed, exposing proteins required for fusion with the target membrane (see Figure 14-7).
- Small GTP-binding proteins (ARF or Sar1) belonging to the GTPase superfamily control polymerization of coat proteins, the initial step in vesicle budding (see Figure 14-9). After vesicles are released from the donor membrane, hydrolysis of GTP bound to ARF or Sar1 triggers disassembly of the vesicle coats.
- Specific sorting signals in membrane and luminal proteins in donor organelles interact with coat proteins during vesicle budding, thereby recruiting cargo proteins to vesicles (see Table 14-2).

- A second set of GTP-binding proteins, the Rab proteins, label specific vesicle types and mediate their targeting to the appropriate membrane (see Table 14-3). Activated Rab·GTP in a vesicle can bind to a specific type of effector protein. One type of effector is a peripheral membrane protein complex that tethers the vesicle to the correct target membrane.
- Each v-SNARE in a vesicular membrane specifically binds to a complex of cognate t-SNARE proteins in the target membrane, inducing fusion of the two membranes. After fusion is completed, the SNARE complex is disassembled in an ATP-dependent reaction mediated by other cytosolic proteins (see Figure 14-11).