PROTEIN TRAFFICKING

6.27.1 Introduction

Key Terms

A **sorting signal (targeting signal)** is the part of a protein that allows it to be incorporated into a nascent transport vesicle. The vesicle moves the protein from one compartment to another. Sorting signals consist of either a short sequence of amino acids or a covalent modification.

A great variety of molecules move out of and into the cell. At one extreme of the size range, proteins may be secreted from the cell into the extracellular fluid or may be internalized from the cell surface. At the other extreme, ions such as K^+, Na^+, and Ca^{2+} may be pumped out of or into the cell. In this chapter, we are concerned with the processes by which proteins are physically transported through membranous systems to the plasma membrane or other organelles, or from the cell surface to organelles within the cell. In *Molecular Biology 6.28 Signal transduction* we discuss the pathways by which an interaction at the surface can trigger internal pathways.

Proteins enter the pathway that leads to secretion by co-translational transfer to the membranes of the endoplasmic reticulum (for introduction see *Molecular Biology Supplement 32.6 ER and Golgi*). They are then transferred to the Golgi apparatus, where they are *sorted* according to their final intended destination. **Figure 27.1** summarizes the routes by which proteins are carried forward or diverted to other organelles. Their destinations are determined by specific **sorting signals**, which take the form of short sequences of amino acids or covalent modifications that are made to the protein.

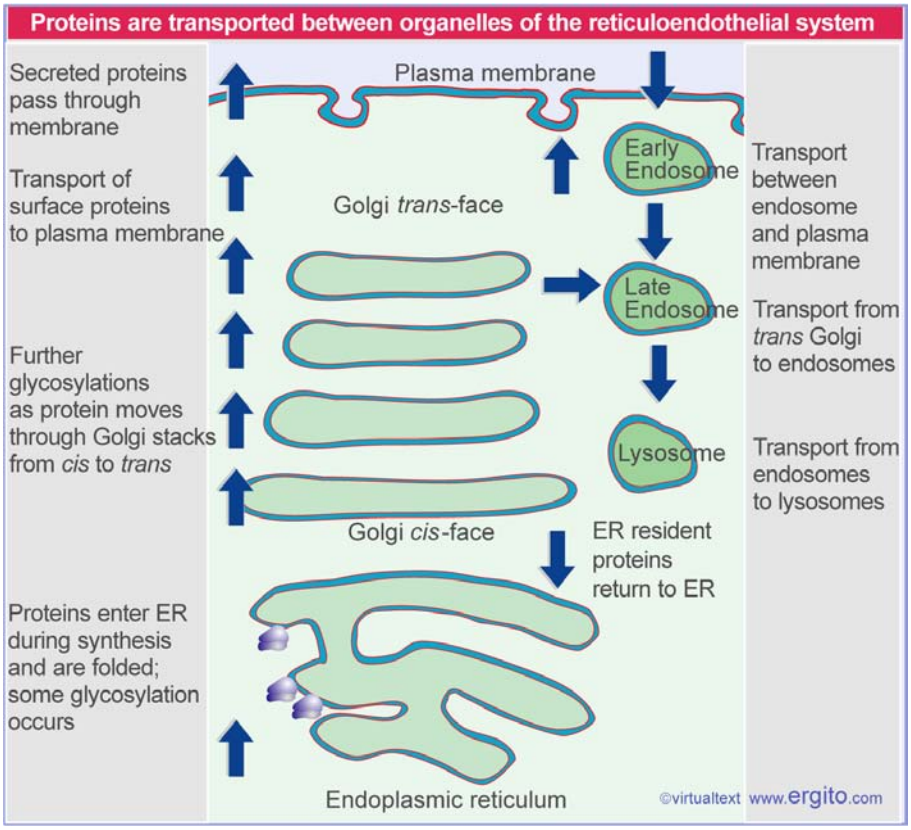

Figure 27.1 Proteins that enter the endoplasmic reticulum are transported to the Golgi and towards the plasma membrane. Specific signals cause proteins to be returned from the Golgi to the ER, to be retained in the Golgi, to be retained in the plasma membrane, or to be transported to endosomes and lysosomes. Proteins may be transported between the plasma membrane and endosomes.

The transport machinery consists of small membranous vesicles. A soluble protein is carried within the lumen of a vesicle, and an integral membrane protein is carried within its membrane. **Figure 27.2** illustrates the nature of the budding and fusion events by which the vesicles move between adjacent compartments. A vesicle buds off from a donor surface and then fuses with a target surface. Its proteins are released into the lumen or into the membrane of the target compartment, depending on their nature, and must be loaded into new vesicles for transport to the next compartment. The series of events is repeated at each transition between membrane surfaces, for example, during passage from the ER to the Golgi, or between cisternae of the Golgi stacks.

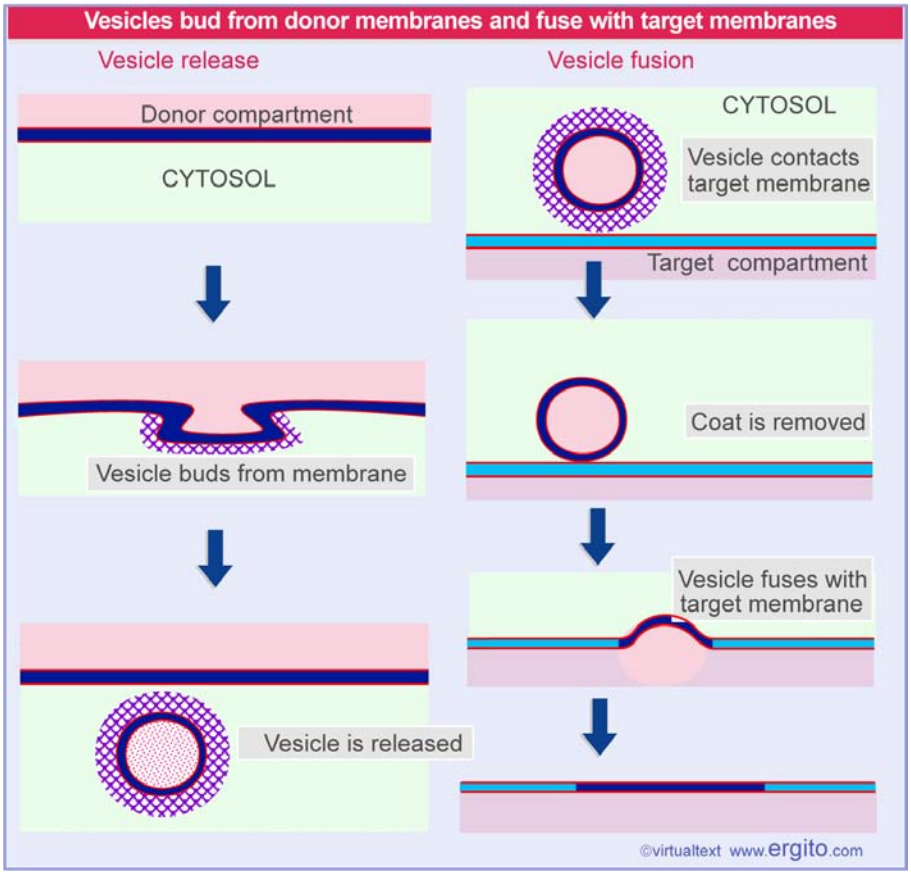

Figure 27.2 Vesicles are released when they bud from a donor compartment and are surrounded by coat proteins (left). During fusion, the coated vesicle binds to a target compartment, is uncoated, and fuses with the target membrane, releasing its contents (right).

Once a protein enters a membranous environment, it remains in the membrane until it reaches its final destination. A membrane protein that enters the endoplasmic reticulum is inserted into the membrane with the appropriate orientation (N-terminal lumenal, C-terminal cytosolic for group I proteins, the reverse for group II proteins). The orientation is retained as it moves through the system. The process starts in the same way irrespective of whether the protein is destined to reside in the Golgi, lysosome, or plasma membrane. In each case, it is transported in membrane vesicles along the secretory pathway to the appropriate destination, where some structural feature of the protein is recognized and it is permanently secured (or secreted from the cell).

Two important changes occur to a protein in the endoplasmic reticulum: it becomes folded into its proper conformation; and it is modified by glycosylation.

A protein is translocated into the ER in unfolded form. Folding occurs as the protein enters the lumen; probably a series of domains each folds independently as the protein passes through the membrane. Folding of a 50 kD protein is complete in <3-4 minutes, compared with the ~1 minute required to synthesize the chain (see *Molecular Biology Supplement 32.4 Protein folding*).

Folding in the ER is associated with modification and is assisted by accessory proteins. Addition of carbohydrate may be required for correct folding; in fact, this may be an important function of the modification. Reshuffling of disulfide bonds by the enzyme PDI (protein disulfide isomerase) may be involved. Association with chaperones in the ER may be necessary to recognize the partially folded forms of proteins as they emerge from transport through the membrane, and to assist them in acquiring their proper conformation. Some or all of these activities could be exercised by a complex of enzymes as a protein enters the ER; that is, the necessary functions all could associate with the protein as it translocates through the membrane. Calculations of the spontaneous rates of folding and oligomerization suggest that these accessory activities are needed to catalyze the process in order it to enable it to occur rapidly enough in the cell (for review see 286).

Multimeric glycoproteins usually oligomerize in the ER. In fact, oligomerization may be necessary for further transport. Oligomers are rapidly transported from the ER to the Golgi, but unassembled subunits or misassembled proteins are held back. Misfolded proteins are often associated with ER-specific chaperones. In due course, they are removed by degradation. So a protein is allowed to move forward into the Golgi only if it has been properly folded previously in the ER (for review see 283).

Reviews

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PROTEIN TRAFFICKING

6.27.2 Oligosaccharides are added to proteins in the ER and Golgi

Key Terms

Dolichol is a lipid that consists of a long chain of isoprenoid units and is present in the membrane of the rough endoplasmic reticulum. It is part of the precursor in the synthesis of N-linked oligosaccharides. An oligosaccharide is assembled onto dolichol via a pyrophosphoryl linkage, then transferred to particular asparagine residues of a nascent polypeptide.

A **high mannose oligosaccharide** is an N-linked oligosaccharide that contains N-acetylglucosamine linked only to mannose residues. It is covalently added to transmembrane proteins in the rough endoplasmic reticulum and is trimmed and modified in the Golgi apparatus.

A **complex oligosaccharide** is an N-linked oligosaccharide that is made during transit through the Golgi apparatus. Mannose residues are trimmed from the high mannose precursor in the rough endoplasmic reticulum and cis Golgi, and other sugars are added by enzymes in the medial and trans Golgi cisternae to form a complex oligosaccharide.

The **inner core** is an intermediate in the synthesis of N-linked oligosaccharides. It is produced upon the removal of mannose residues from a high mannose oligosaccharide in the cis Golgi and is resistant to degradation by endoglycosidase H.

The **terminal region** is the part of an N-linked oligosaccharide that consists of all the sugar residues added subsequent to formation of the inner core.

Key Concepts

- A major function of the ER and Golgi is to glycosylate proteins as they pass through the system.
- N-linked oligosaccharides are initiated by transferring the saccharide from the lipid dolichol to an asparagine of the target protein in the ER.
- Sugars are trimmed in the ER to give a high mannose oligosaccharide.
- A complex oligosaccharide is generated in those cases in which further residues are added in the Golgi.

Virtually all proteins that pass through the secretory apparatus are glycosylated. Glycoproteins are generated by the addition of oligosaccharide groups either to the NH_2 group of asparagine (N-linked glycosylation) or to the OH group of serine, threonine, or hydroxylysine (O-linked glycosylation). N-linked glycosylation is initiated in the endoplasmic reticulum and completed in the Golgi; O-linked glycosylation occurs in the Golgi alone. The stages of N-glycosylation are illustrated in the next three figures.

The addition of all N-linked oligosaccharides starts in the ER by a common route, as illustrated in **Figure 27.3**. An oligosaccharide containing 2 N-acetyl glucosamine, 9 mannose, and 3 glucose residues is formed on a special lipid, **dolichol**. Dolichol is a highly hydrophobic lipid that resides within the ER membrane, with its active group facing the lumen. The oligosaccharide is constructed by adding sugar residues individually; it is linked to dolichol by a pyrophosphate group, and is transferred as a unit to a target protein by a membrane-bound glycosyl transferase enzyme whose active site is exposed in the lumen of the endoplasmic reticulum.

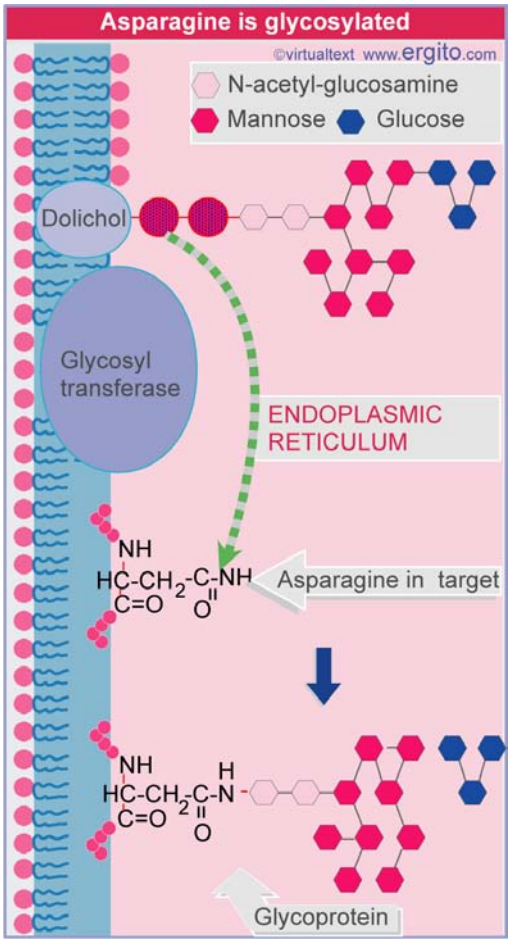

Figure 27.3 An oligosaccharide is formed on dolichol and transferred by glycosyl transferase to asparagine of a target protein.

The acceptor group is an asparagine residue, located within the sequence Asn-X-Ser or Asn-X-Thr (where X is any amino acid except proline). It is recognized as soon as the target sequence is exposed in the lumen, when the nascent protein crosses the ER membrane.

Some trimming of the oligosaccharide occurs in the ER, after which a nascent glycoprotein is handed over to the Golgi. The oligosaccharide structures generated during transport through the ER and Golgi fall into two classes, determined by the fate of the mannose residues. Mannose residues are added only in the ER, although they can be trimmed subsequently.

- **High mannose oligosaccharides** are generated by trimming the sugar residues in the ER. **Figure 27.4** shows that almost immediately following addition of the oligosaccharide, the 3 glucose residues are removed by the enzymes glucosidases I and II. For proteins that reside in the ER, a mannosidase removes some of the mannose residues to generate the final structure of the oligosaccharide. The ER mannosidase attacks the first mannose quickly, and the next 3 more slowly; the total number of mannose residues that is removed varies with the individual substrate protein.

- **Complex oligosaccharides** result from additional trimming and further additions carried out in the Golgi. Golgi modifications occur in the fixed order illustrated in **Figure 27.5**. The first step is further trimming of mannose residues by Golgi mannosidase I. Then a single sugar residue is added by the enzyme N-acetyl-glucosamine transferase. Then Golgi mannosidase II removes further mannose residues. This generates a structure called the **inner core**, consisting of the sequence NAc-Glc·NAc-Glc·Man_3. At this point, the oligosaccharide becomes resistant to degradation by the enzyme endoglycosidase H (Endo H). *Susceptibility to Endo H is therefore used as an operational test to determine when a glycoprotein has left the ER.*

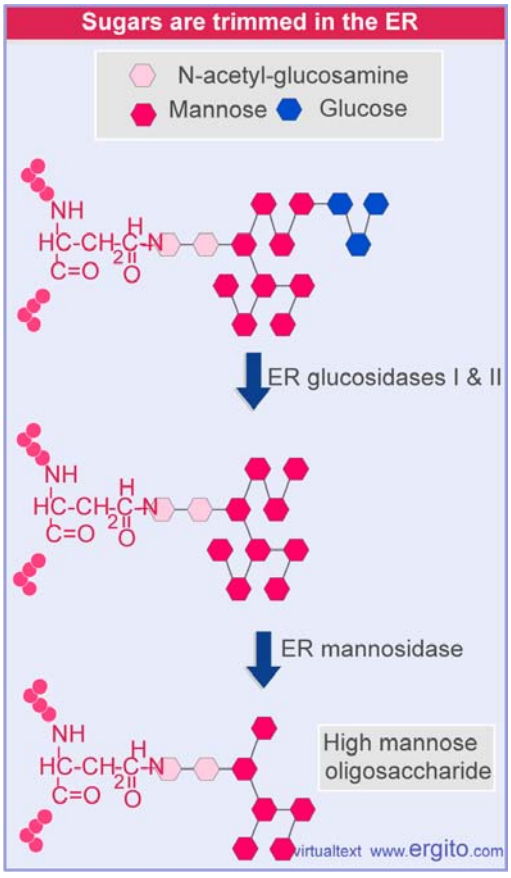

Figure 27.4 Sugars are removed in the ER in a fixed order, initially comprising 3 glucose and 1-4 mannose residues. This trimming generates a high mannose oligosaccharide.

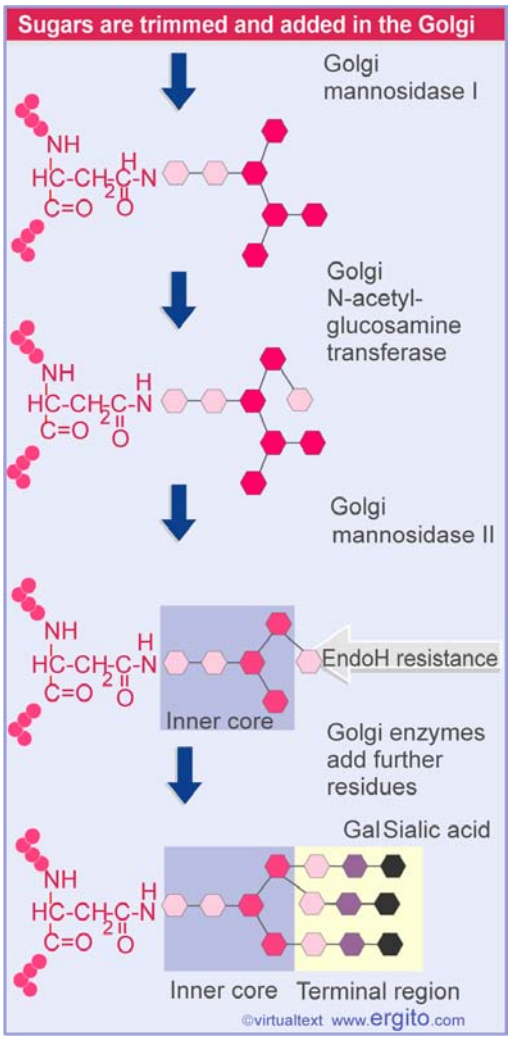

Figure 27.5 Processing for a complex oligosaccharide occurs in the Golgi and trims the original preformed unit to the inner core consisting of 2 N-acetyl-glucosamine and 3 mannose residues. Then further sugars can be added, in the order in which the transfer enzymes are encountered, to generate a terminal region containing N-acetyl-glucosamine, galactose, and sialic acid.

Additions to the inner core generate the **terminal region**. The residues that can be added to a complex oligosaccharide include N-acetyl-glucosamine, galactose, and sialic acid (N-acetyl-neuraminic acid). The pathway for processing and glycosylation is highly ordered, and the two types of reaction are interspersed in it. Addition of one sugar residue may be needed for removal of another, as in the example of the addition of N-acetyl-glucosamine before the final mannose residues are removed.

We do not know what determines how each protein undergoes its specific pattern of processing and glycosylation. We assume that the necessary information resides in the structure of the polypeptide chain; it cannot lie in the oligosaccharide, since all proteins subject to N-linked glycosylation start the pathway by addition of the same (preformed) oligosaccharide.

PROTEIN TRAFFICKING

6.27.3 The Golgi stacks are polarized

Key Concepts

- The Golgi stacks change in lipid and protein constitution proceeding from the *cis*-face near the ER to the *trans*-face near the plasma membrane.

The individual cisternae of the Golgi are organized into a series of *stacks*, somewhat resembling a pile of plates. A typical stack consists of 4-8 flattened cisternae. **Figure 27.6** shows an example. A major feature of the Golgi apparatus is its *polarity*. The *cis* side faces the endoplasmic reticulum; the *trans* side in a secretory cell faces the plasma membrane. The Golgi consists of compartments, which are named *cis*, *medial*, *trans*, and *TGN (trans-Golgi network)*, proceeding from the *cis* to the *trans* face. Proteins enter a Golgi stack at the *cis* face and are modified during their transport through the successive cisternae of the stack. When they reach the *trans* face, they are directed to their destination.

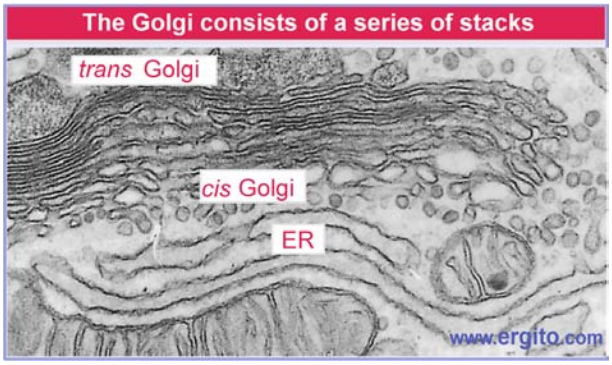

Figure 27.6 The Golgi apparatus consists of a series of individual membrane stacks. Photograph kindly provided by Alain Rambourg.

Membrane structure changes across the Golgi stack. The main difference is an increase in the content of cholesterol proceeding from *cis* to *trans.* As a result, fractionation of Golgi preparations generates a gradient in which the densest fractions represent the *cis* cisternae, and the lightest fractions represent the *trans* cisternae. The positions of enzymes on the gradient, and *in situ* immunochemistry with antibodies against individual enzymes, suggest that certain enzymes are differentially distributed proceeding from *cis* to *trans.* The difference between the *cis* and *trans* faces of the Golgi is clear, but it is not clear how the concept of compartments relates to individual cisternae; there may rather be a continuous series of changes proceeding through the cisternae.

Nascent proteins encounter the modifying enzymes as they are transported through the Golgi stack. **Figure 27.7** illustrates the order in which the enzymes function. This may be partly determined by the fact that the modification introduced by one enzyme is needed to provide the substrate for the next, and partly by the availability of the

enzymes proceeding through the cisternae.

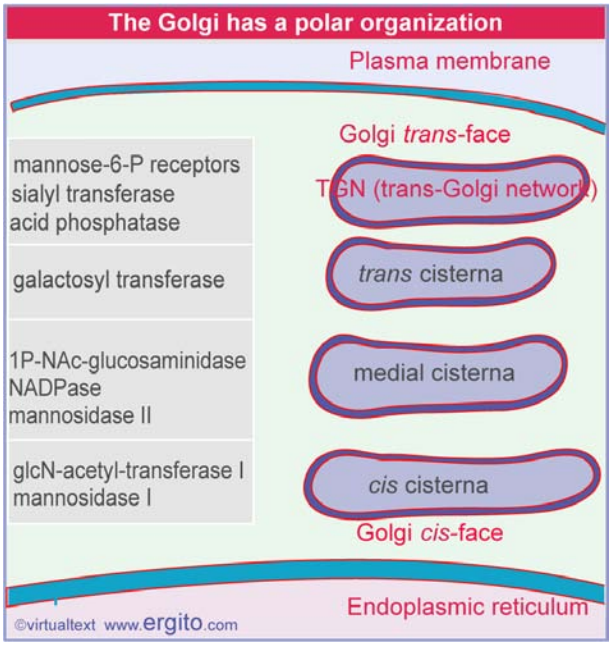

Figure 27.7 A Golgi stack consists of a series of cisternae, organized with *cis* to *trans* polarity. Protein modifications occur in order as a protein moves from the *cis* face to the *trans* face.

The addition of a complex oligosaccharide can change the properties of a protein significantly. Glycoproteins often have a mass with a significant proportion of oligosaccharide. What is the significance of these extensive glycosylations? In some cases, the saccharide moieties play a structural role, for example, in the behavior of surface proteins that are involved in cell adhesion. Another possible role could be in promoting folding into a particular conformation. One modification – the addition of mannose-6-phosphate – confers a targeting signal.

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.6.27.3

PROTEIN TRAFFICKING

6.27.4 Coated vesicles transport both exported and imported proteins

Key Terms

Exocytosis is the process whereby cells secrete cargo molecules that are originally synthesized at the endoplasmic reticulum. Exocytosis occurs by the fusion of secretory vesicles with the plasma membrane.

Endocytosis is the process whereby cells internalize small molecules and particles from their surroundings. There are several forms of endocytosis, all of which involve the formation of a membranous vesicle from the plasma membrane.

Coated vesicles are formed by the pinching off of a coated pit from a membrane. The membrane of a coated vesicle has on its cytoplasmic surface proteins such as clathrin, COPI or COPII.

Cargo describes any macromolecule (such as RNA, soluble or membrane protein, lipid) that is transported from one compartment to another. Cargo may contain sequences or modifications that specify their destination. Some cargo molecules are carried in transport vesicles, but others (such as those that move between the nucleus and cytosol) are not.

Anterograde transport is the direction of membrane transport specified by the movement of macromolecules through the secretory pathway (from the rough endoplasmic reticulum, through the Golgi complex, and to their final destinations). It is also called forward transport.

Retrograde transport is the movement of material from the Golgi apparatus (or from the plasma membrane) to the endoplasmic reticulum.

Key Concepts

- Protein transport through the ER-Golgi system occurs in coated vesicles.
- Secreted proteins move in the forward, anterograde direction toward the plasma membrane.
- There is also movement within the system in the reverse, retrograde direction.
- Proteins that are imported through the plasma membrane also are incorporated in coated vesicles.
- Coated vesicles bud from a donor membrane surface and fuse with a target membrane surface.

Secreted and transmembrane proteins start on the route to localization when they are translocated into the endoplasmic reticulum during synthesis. Transport from the ER, through the Golgi, to the plasma membrane occurs in vesicles. A protein is incorporated into a vesicle at one membrane surface, and is released from the vesicle at the next membrane surface. Progress through the system requires a series of such

transport events. A protein changes its state of glycosylation as it passes through the Golgi from *cis* to *trans* compartments (for review see 277; 281).

Vesicles are used to transport proteins both out of the cell and into the cell. The secretion of proteins is called **exocytosis**; internalization of proteins is called **endocytosis**. The pathways for vesicle movement are pictured in **Figure 27.8**. The cycle for each type of vesicle is similar, whether they are involved in export or import of proteins: *budding from the donor membrane is succeeded by fusion with the target membrane.*

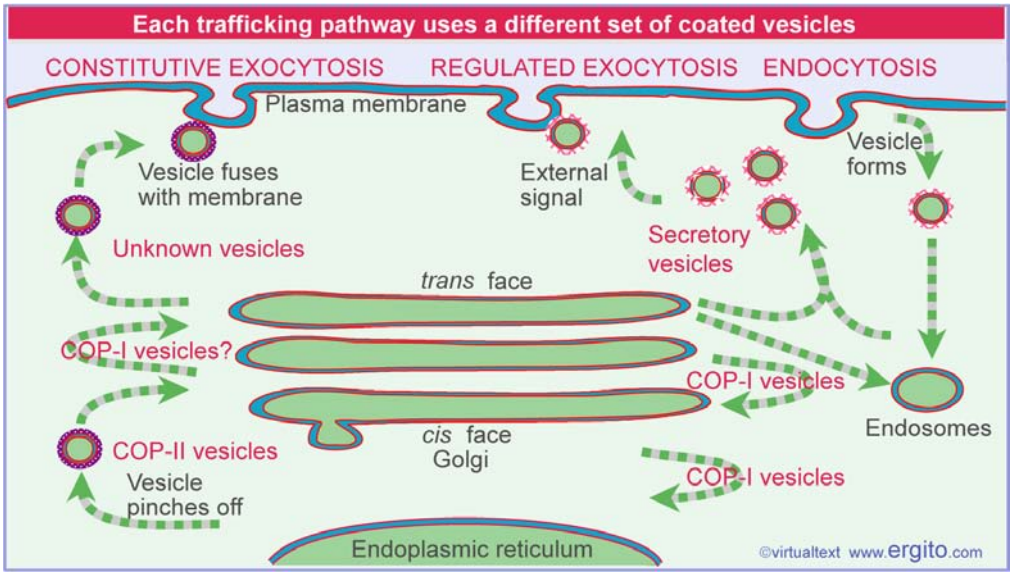

Figure 27.8 Proteins are transported in coated vesicles. Constitutive (bulk flow) transport from ER through the Golgi takes place by COP-coated vesicles. Clathrin-coated vesicles are used for both regulated exocytosis and endocytosis.

Vesicles involved in transporting proteins have a protein layer surrounding their membranes, and for this reason are called **coated vesicles**. Examples are shown in the electron micrograph of **Figure 27.9**. Different types of vesicles are distinguished by the protein coats. The coat serves two purposes: it is involved with the processes of budding and fusion; and it enables the type of vesicle to be identified, so that it is directed to the appropriate target membrane. The coat may also play a role in the selection of proteins to be transported.

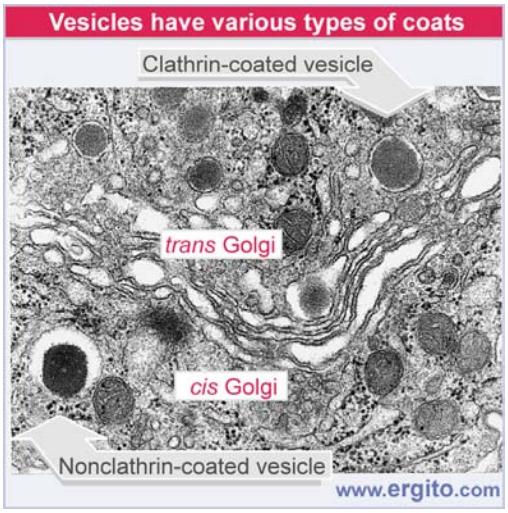

Figure 27.9 Coated vesicles are released from the *trans* face of the Golgi. The diameter of a vesicle is ~70 nm. Photograph kindly provided by Lelio Orci.

One of the most remarkable features of protein trafficking is the conservation of the vesicular apparatus, including structural components of the vesicles, and proteins required for budding or fusion. Many of these functions have been identified through mutations of the *sec* genotype in *S. cerevisiae*, which are unable to export proteins through the ER-Golgi pathway (774). Many of the genes identified by *sec* mutants in yeast have direct counterparts in animal cells. In particular, the proteins involved in budding, fusion, and targeting in mammalian brain (where release of proteins from the cell provides the means of propagating nerve impulses) have homologues in the yeast secretory pathway (785).

The process of generating a vesicle requires a membrane bilayer to protrude a vesicle that eventually pinches off as a bud (see **Figure 27.2**). Such events require deformation of the membrane, as illustrated in **Figure 27.10**. Proteins concerned with this process are required specifically for budding, and become part of the coat.

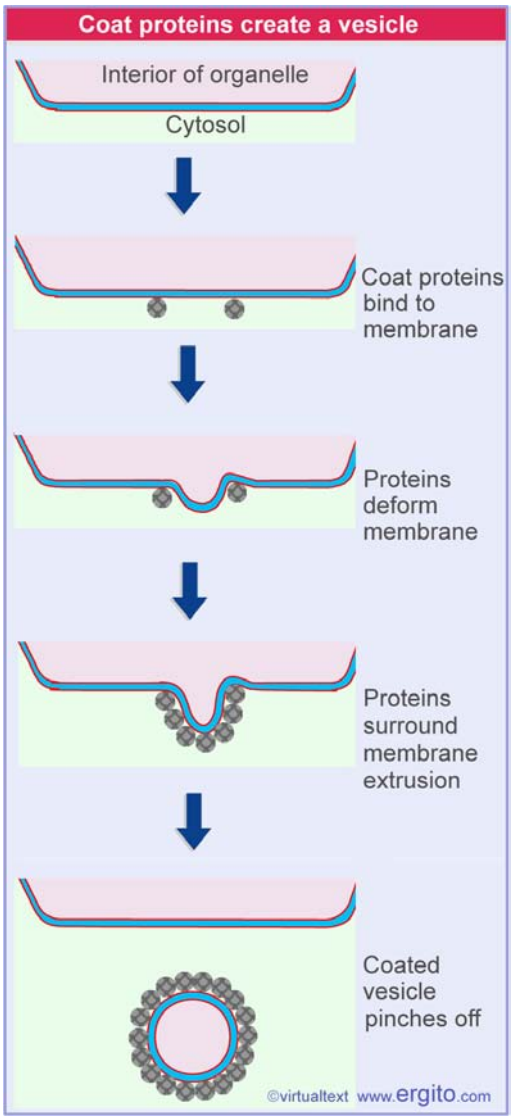

Figure 27.10 Vesicle formation results when coat proteins bind to a membrane, deform it, and ultimately surround a membrane vesicle that is pinched off.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.27.4 to view properly.

In the reverse reaction, fusion is a property of membrane surfaces. In order to fuse with a target membrane, a vesicle must be "uncoated" by removal of the protein layer. A coated vesicle recognizes its destination by a reaction between a protein in the vesicle membrane and a receptor in the target membrane.

A vesicle therefore follows a cycle in which it gains its coat, is released from a donor membrane, moves to the next membrane, becomes uncoated, and fuses with the target membrane. When a vesicle is generated, it carries proteins that were resident in (or associated with) the stretch of membrane that was pinched off. The interior of the

vesicle has the constitution of the lumen of the organelle from which it was generated. When the vesicle fuses with its target membrane, its components become part of that membrane or the lumen of the compartment. Proteins that are transported by vesicles (that is, which are not part of the structure of the vesicle itself) are called the **cargo**. Proteins must be sorted at each stage, when either they remain in the compartment or are incorporated into new vesicles and transported farther along the system.

The dynamic state of transport through the ER-Golgi system poses a dilemma. There is continuous movement of vesicles carrying proteins from the ER and through the Golgi. Movement in this direction is called *forward* or **anterograde transport**. It takes a typical protein ~20 minutes to pass through the system. A significant proportion of the membrane surface of the ER and Golgi is incorporated into vesicles that move to the plasma membrane. Such a flow of membrane should rapidly denude the Golgi apparatus and enormously enlarge the plasma membrane, yet both are stable in size. The net amount (and types) of lipid in each membrane must remain unperturbed in spite of vesicle movement.

The need to maintain the structure of the reticuloendothelial system suggests that there is a pathway for returning membrane segments from the Golgi to ER, so that there is no net flow of membrane. Movement in this direction is called **retrograde transport**. We do not yet understand the balance of forward and retrograde flow. One possibility is that some vesicles engaged in retrograde movement do not carry cargo, except for returning components to earlier parts of the system. Alternatively, reverse flow might occur by structures that have a high surface to volume ratio, such as tubules, which could thus return large amounts of material.

Reviews

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PROTEIN TRAFFICKING

6.27.5 Different types of coated vesicles exist in each pathway

Key Terms

A **transition vesicle** is a small membrane-bounded compartment that mediates transport between organelles, especially the rough endoplasmic reticulum and Golgi complex. It is also known as a transport vesicle. COPI- and COPII-coated vesicles are transition vesicles.

A **COPI** coat consists of coatomer and ADP-ribosylation factor. COPI-coated vesicles are transport vesicles that bud from the cytoplasmic face of the Golgi complex and mediate retrograde transport from the Golgi complex to the endoplasmic reticulum. COPI-coated vesicles may also mediate transport between Golgi cisternae.

A **COPII** coat is made up of protein complexes comprising a GTPase (Sar1p) and two heterodimers (Sec 23/24 and Sec 13/31). COPII-coated vesicles are transport vesicles that bud from the cytoplasmic face of the rough endoplasmic reticulum and mediate anterograde transport to the Golgi apparatus.

Constitutively secreted macromolecules are transported to the plasma membrane or secreted at a relatively constant rate. They include lipids and soluble and membrane proteins that exit to the plasma membrane from the *trans*-Golgi network but are not secreted by regulated exocytosis.

A **secretory vesicle** is transport vesicle that carries newly synthesized proteins and lipids from the Golgi apparatus to the plasma membrane.

A **secretory granule** is a membrane-bounded compartment that contains molecules to be released from cells by regulated exocytosis (that is, the molecules are concentrated and stored in secretory granules, and are released only in response to a signal).

Endocytic vesicles are membranous particles that transport proteins through endocytosis; also known as clathrin-coated vesicles.

Clathrin proteins interact with adaptor proteins to form the coat on some of the vesicles that bud from the cytoplasmic face of the plasma membrane and the *trans*-Golgi network. Clathrin is composed of heavy and light chains that form triskelions, which then assemble into polyhedral curved lattices during the formation of clathrin-coated pits and vesicles.

A **clathrin-coated vesicle** is a membrane-bounded compartment that mediates endocytosis, formation of secretory granules at the trans-Golgi network, and transport from the trans-Golgi network to the endocytic pathway. In addition to clathrin, its major constituents include cargo and adaptor proteins.

The subunit of a clathrin coat is a **triskelion.** It is a three-legged structure; each leg is composed of a clathrin heavy chain and a clathrin light chain.

A **coated pit** is an infolding of membrane formed during clathrin-mediated endocytosis. It is pinched off to form a clathrin-coated vesicle.

Adaptor complexes bind to signals in the cytoplasmic tails of transmembrane cargo proteins and recruit clathrin molecules during the assembly of clathrin-coated pits. Different types of adaptor complexes function at different compartments. Each adaptor complex contains four different subunits.

Adaptins are the large subunits of the cytosolic adaptor protein complexes that mediate formation of clathrin-coated vesicles. There are several types of adaptin subunits.

Coatomer is the complex of coat proteins on COPI-coated vesicles. It consists of seven proteins.

Key Concepts

- Clathrin-coated vesicles are used for endocytosis.
- The clathrin coat forms when triskelions form a lattice at a coated pit on the plasma membrane.
- The clathrin is joined to the membrane by an AP adaptor, which is a heterotetramer of adaptin subunits.
- Different types of AP adaptors are found in vesicles used in different locations.
- COPI coated vesicles have a coat consisting of a 7-component coatomer and are required for retrograde transport in the ER-Golgi system.
- COPII coated vesicles have a coat consisting of a different protein complex.
- Cargo proteins may be bound by components of the vesicle coat.

Different groups of coated vesicles can be identified by the types of transport they undertake. In some cases, the vesicles can be distinguished by the biochemical components of their coats.

Newly synthesized proteins enter the ER and may be transported along the ER-Golgi system. This transport is undertaken by **transition vesicles**, and is common to all eukaryotic cells. Two different types of transition vesicles have been identified on the basis of their coats.

- The vesicles that were originally identified in transport between Golgi cisternae are now called **COPI**-coated vesicles. (COP is an acronym for coat protein.) They are involved in retrograde transport. It is an open question whether they also undertake anterograde transport (776).
- Transition vesicles that proceed from the ER to the Golgi have a different coat, called **COPII**. Their major role appears to be forward transport (777).
- Some proteins are **constitutively secreted**, moving from the *trans*-Golgi to the plasma membrane. The vesicles that undertake this process have not been characterized biochemically.

The export of some proteins is regulated. These proteins are packaged into **secretory vesicles**. These vesicles provide a storage medium, and release their contents at the plasma membrane only following receipt of a particular signal (triggered, for example, by a hormone or Ca^{2+}). This occurs in cells that are specialized to produce the appropriate proteins.Vesicles that form at the *trans*-face of the Golgi for use in the regulated pathway may fuse to form **secretory granules**. They may also transport their cargoes to endosomes. Secretory vesicles may form at endosomes to transport proteins to the plasma membrane. The most common route for regulated transport is probably via the endosome.

Proteins enter the cell by packaging into **endocytic vesicles**, which are released from the plasma membrane, and transport their contents toward the interior of the cell. The cargo is released when the endocytic vesicle fuses with the membrane of a target compartment such as an endosome.

What controls the specificity of the cargo carried by a vesicle? It is necessary to distinguish those proteins that should be transported out of the compartment from resident proteins that should remain there. This may be a function of the coat. The COPII coat can cause vesicles to bud when liposomes are mixed with the coat proteins. When the liposomes contain membrane proteins that are resident in the ER and other membrane proteins that are involved in targeting vesicles to the Golgi, only the latter class enter the vesicles. This suggests that specificity may be determined by a direct interaction with the coat proteins.

Endocytic and secretory vesicles have **clathrin** as the most prominent protein in their coats, and are therefore known as **clathrin-coated vesicles**. Their structure is known in some detail. The 180 kD chain of clathrin, together with a smaller chain of 35 kD, forms a polyhedral coat on the surface of the coated vesicle. The subunit of the coat consists of a **triskelion**, a three-pronged protein complex consisting of 3 light and 3 heavy chains. The triskelions form a lattice-like network on the surface of the coated vesicle, as revealed in the electron micrograph of **Figure 27.11** (for review see 2273). Endocytic vesicles form and are coated at invaginations of the plasma membrane that are called **coated pits**. Similar structures can be observed at the *trans* face of the Golgi, where vesicles destined for endosomes and secretory vesicles originate (for review see 287).

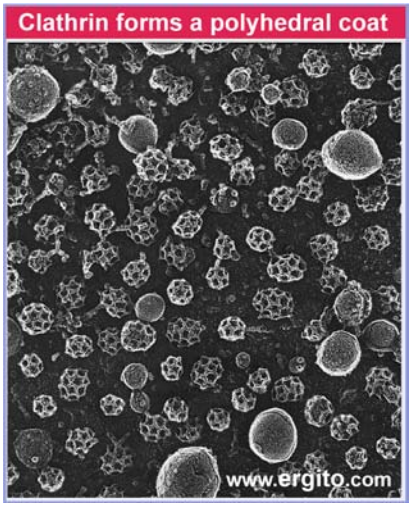

Figure 27.11 Coated vesicles have a polyhedral lattice on the surface, created by triskelions of clathrin. Photograph kindly provided by Tom Kirchhausen.

The structure of clathrin-coated vesicles is shown schematically in **Figure 27.12**. The inner shell of the coat is made by proteins called **adaptors**, which bind both to clathrin and to integral membrane proteins of the vesicle. Different types of adaptors identify coated vesicles with different origins. There are several types of adaptors. Each is identified as AP (for adaptor complex) and a number. The most abundant adaptor is AP2, which is found at plasma membrane coated pits and identifies endocytic vesicles. These vesicles also contain an additional adaptor protein, AP180, which controls the size of the vesicle (1002). AP4 is associated with the *trans*-Golgi network. AP1 (founding member of the family) is found on vesicles that transport 6-mannose-phosphate receptors from the *trans*-Golgi network to the endosomes (for review see 998).

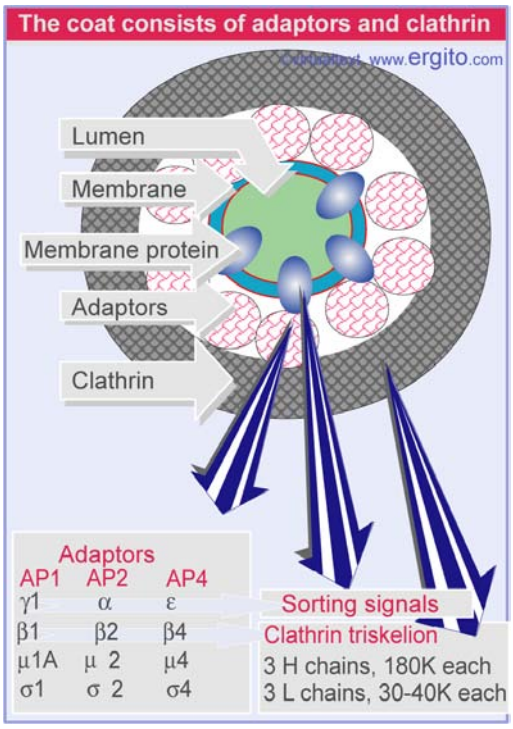

Figure 27.12 Clathrin-coated vesicles have a coat consisting of two layers: the outer layer is formed by clathrin, and the inner layer is formed by adaptors, which lie between clathrin and the integral membrane proteins.

Each AP is a heterotetramer. The individual subunits are called **adaptins**. The β adaptin usually binds to the clathrin skeleton (999). It may also interact with dileucine (KK) sorting signals in the membrane proteins (1001). The μ adaptins recognize tyrosine-based sorting signals for internalization (1000). The best characterized case is μ2, where phosphorylation of the subunit triggers a conformational change that enables it to recognize the target motif in a protein that is to be endocytosed (3333). The α or γ adaptins (and presumably also the δ and ε adaptins) are involved in interactions with the membrane where the vesicles are formed, that is, they are responsible for assembly of the full AP complex at the appropriate membrane, after which a clathrin coat assembles. The adaptor is therefore responsible not only for connecting the membrane of the vesicle to the clathrin skeleton, but also for incorporating the cargo proteins into the vesicle.

A variant of AP1, which contains the adaptin μ1B instead of the more common μ1A, is found in polarized epithelial cells, where it is involved in transporting proteins from the apical to the basolateral surface (for review see 292). We may expect that more variants like this will be discovered.

The variety of adaptor complexes, their localization to specific transport pathways, and their roles in recognizing cargo proteins, all argue that they are a key component of the targeting system that ensures that proteins are taken to the right location.

How many types of cargo protein can be carried by a single endocytic vesicle? It is

not yet clear how many types of vesicle exist and what variety they display on the coats and in their cargo. We do know that some endocytic vesicles carry more than one type of cargo protein. Generally they are viewed as fairly specific carriers.

The coats of the COPI-coated transition vesicles have 7 major protein components, called COPs. They exist as a high molecular weight complex (~700 kD), called **coatomer**, which is the precursor to the COP coat. The β-COP component has some homology to β-and β ′ –adaptins. This suggests a similar organization in which β-COP plays a similar role to the β- and β ′ –adaptins in connecting an outer coat protein (an analog of clathrin) to the membrane proteins in the vesicle.

COPI-coated vesicles appear to be capable of performing both anterograde and retrograde transport. Such vesicles can be found carrying types of cargo that are transported in either direction; but any individual vesicles carries only anterograde or retrograde cargo, not both. Certain mutations in COP proteins block retrograde transport, which suggests that COPI vesicles provide the sole (or at least major) capacity for retrograde transport. We do not know how COPI vesicles moving in one direction are distinguished from those moving in the opposite direction: presumably there is some further component that has yet to be identified. Both directions of transport are probably supported through every level of the Golgi stack (779).

The coats of COPII-coated vesicles consist of the protein complexes Sec23p/Sec24p (found as a 400 kD tetramer), Sec13p/Sec31p (which form a 700 kD complex), and Sar1p (777). There is no homology between the Sec protein components and the components of COPI-coated vesicles. Sar1p is a small GTP-binding protein that regulates coat formation, and Sec23p is the GAP (GTPase-activating protein) that acts on Sar1p (see *Molecular Biology 6.27.7 Vesicles can bud and fuse with membranes*). Sec24p is responsible for recruiting the cargo (3332).

Another class of vesicle has the AP3 coat, whose subunits (δ, β3, μ3, σ3) are related to those of the AP1 and AP2 adaptor complexes. This coat complex is found on some synaptic vesicles, which form at endosomes. This type of coat complex is also found on vesicles that transport cargo from the Golgi to lysosomes, and on storage vesicles (780).

Last updated on 1-25-2003

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PROTEIN TRAFFICKING

6.27.6 Cisternal progression occurs more slowly than vesicle movement

Key Terms

Cisternal maturation is one of two popular models for the mechanism of cargo transport through the Golgi stack. It is also called cisternal migration or cisternal progression. In this model, a new Golgi cisterna forms at the *cis* face, then moves forward in the stack as the enzyme content of the cisterna changes from *cis* to *medial* to *trans*. Enzymes that belong in earlier cisternae are retrieved by retrograde transport vesicles.

Key Concepts

- An alternative model for ER-Golgi protein transport is that the stacks are mobile structures, and that *cis*-stacks mature into *trans*-stacks by a series of changes in lipid and protein composition.

Are coated vesicles responsible for all transport between membranous systems? There are conflicting models for the nature of forward transport from the ER, through Golgi cisternae, and then from the TGN to the plasma membrane.

The vesicular model for anterograde transport proposes that the Golgi cisternae are fixed structures that gain and lose proteins by the processes of vesicle fusion and budding. The process starts when COPII coated vesicles bud at the ER and transport cargo to the Golgi. It is still not clear whether the vesicles that transport proteins along the Golgi are COPII or COPI coated. The natures of the coat(s) of vesicles that proceed from the Golgi to the plasma membrane remain unknown (for review see 288; 290; 291).

An alternative model for anterograde transport suggests that there is **cisternal maturation**. Instead of being fixed structures, cisternae move from the *cis* side of the Golgi to the *trans* side, maturing into more *trans*-like types of cisternae by changes in their protein constitution. Evidence for cisternal maturation has been provided by following the fate of a substrate protein that is too large to be incorporated into vesicles. Procollagen type I assembles into rod-like triple helices that are ~300 nm long in the lumen of the ER. These rods can be followed as they move into the *cis*-Golgi and through the Golgi to the TGN. Because they remain intact, and are too large to be incorporated into vesicles (COPcoated vesicles are 60-90 nm in diameter), this means that the membrane-bound compartment containing the rods must itself have moved from the *cis* to the *trans* side of the Golgi. This shows at least the plausibility of cisternal maturation, although it does not demonstrate whether normal cargo proteins are carried by vesicles or also move by cisternal maturation (781).

To take the model for cisternal maturation to its extremes, the *cis*-Golgi could be formed by fusion between COPII coated vesicles that bud from the ER; this process might also involve larger tubules. The *cis*-Golgi cisternae would move steadily

forward until they mature into the *trans*-Golgi cisternae. At the TGN, secretory vesicles might form by fragmenting into tubular structures, without requiring any special type of coat. Of course, as cisternae mature, proteins that belong to more *cis*-like cisternae must be retrieved; this would occur by COPI-mediated retrograde vesicular transport.

The outstanding question is the relative quantitative importance of cisternal maturation and vesicular transport for the anterograde direction. It is likely that cisternal progression is much slower than vesicle movement. This may mean that there is a two-track system, in which proteins can be transported rapidly in vesicles, but this will be accompanied by the much slower maturation of *cis*-stacks into *trans*-stacks (for review see 1221).

Whichever model applies, the TGN provides the sorting center for directing proteins on the anterograde route to the plasma membrane, endosomes, or other membrane surfaces (for review see 279).

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PROTEIN TRAFFICKING

6.27.7 Vesicles can bud and fuse with membranes

Key Terms

Dynamin is a cytosolic protein that is a GTPase and is required for clathrin-mediated vesicle formation. Although the exact role of dynamin is debated, dynamin polymers are involved in the scission of clathrin-coated pits from membranes. A variant of dynamin functions in mitochondrial septation.

Key Concepts

- Budding and fusion are controlled by a monomeric G protein, ARF/Sar1p.
- Budding requires ARF/Sar1p to be bound to GTP so that its N-terminus is available to insert into the membrane.
- Fusion occurs when the coat is destabilized by the hydrolysis of GTP.

Budding and fusion are essentially reversible reactions. Budding occurs when coat proteins assemble on a patch of membrane, ultimately causing its release as an independent vesicle. Fusion occurs when the coat proteins are removed, exposing the membrane surface, which can then fuse with a target membrane. Whether the coat proteins assemble or disassemble is controlled by the state of a monomeric G protein (for introduction see *Molecular Biology Supplement 32.10 G proteins*).

Budding of COPI, clathrin-coated, and AP3 vesicles is initiated by ARF (ADP-ribosylation factor). Sar1p is a closely related protein that serves the same role for COPII-coated vesicles. ARF is myristoylated at the N-terminus and can insert spontaneously into lipid bilayers. ARF-GTP is the active form; ARF-GDP is inactive. Sar1p behaves similarly, except that its N-terminus functions without requiring modification. ARF/Sar1p's activity (and ability to recycle) is controlled by GTP hydrolysis. The type of guanine nucleotide controls the conformation, so that the N-terminus is exposed only when GTP is bound. ARF/Sar1p is the key component that triggers both the budding and fusion processes in response to the condition of its guanine nucleotide.

Figure 27.13 shows how the conformation of ARF/Sar1p is controlled by its guanine nucleotide in such a way that the N-terminus is available to sponsor membrane insertion only when GTP is bound. The N-terminus has an amphipathic helix that can insert its hydrophobic side chains into a lipid bilayer. Contacts with the γ phosphate of GTP change the localization of a pair of β-sheets. This causes them to move within the protein, releasing the N-terminal region, which then protrudes and can insert into the membrane (3334).

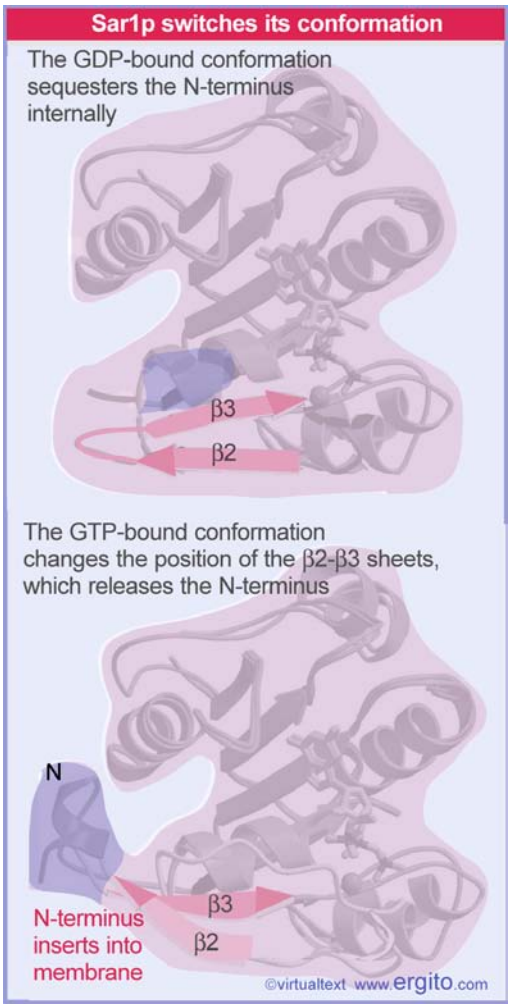

Figure 27.13 The conformation of Sar1p is controlled by the guanine nucleotide. Its N-terminal sequence is a hydrophobic stretch that can insert into membranes. This sequence is bound to the β 2- β 3 sheets and localized internally in the GDP-bound conformation. When Sar1p is bound to GTP, the β 2- β 3 sheets change their position and release the N-terminus, which projects out of the protein so that it can bind to membranes.

Figure 27.14 illustrates the initiation of budding when ARF/Sar1p is converted to the GTP-bound form. ARF/Sar1p is recruited to the appropriate membranes by interacting with a receptor (called ARF-GAP) that activates the factor. The receptor does not become a component of the vesicle, but acts catalytically to activate its target. The function of ARF/Sar1p is to provide the binding sites at which the other coat proteins can assemble; after ARF/Sar1p inserts into the membrane, the other coat proteins bind to it stoichiometrically. Coat proteins surround the membrane as a prerequisite for budding, but another function may be needed to complete the process of "pinching off."

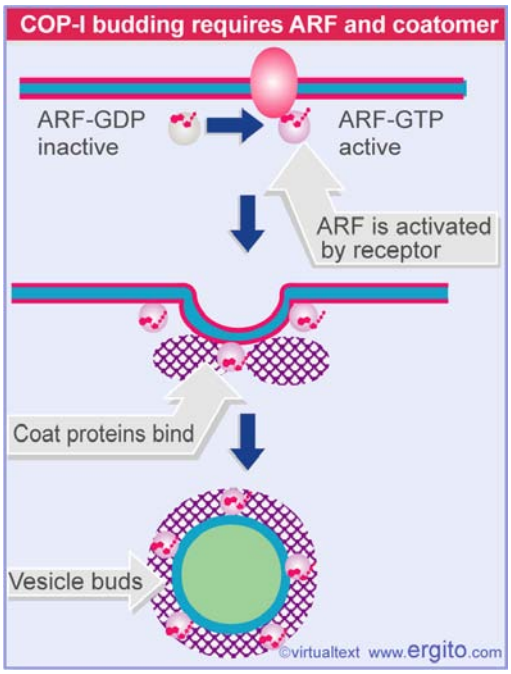

Figure 27.14 ARF and coatomer are required for the budding of COP-I-coated vesicles.

Formation of a vesicle is energetically unfavorable – the membrane must be deformed and finally a small sphere is pinched off. Most is known about the process during endocytosis. When clathrin polymerizes into a basket-like structure at a coated pit, it pulls the membrane inward toward the cytoplasm. Then it is necessary to release the vesicle by separating it at the "neck" where it is joined to the plasma membrane. This requires input of energy in order to deform the membrane

The first protein to be discovered that may act at the scission step was a GDP/GTP-binding protein called **dynamin**. The GDP-bound form of dynamin binds to the clathrin lattice. Replacement of the GDP by GTP (which is catalyzed by an exchange factor; see *Molecular Biology Supplement 32.10 G proteins*) causes the dynamin to form a ring around the neck of the forming vesicle. One model proposes that the dynamin uses hydrolysis of GTP to provide the energy for the deformation and scission. (Dynamin is also involved in septation of mitochondria; see *Molecular Biology 4.13.24 How do mitochondria replicate and segregate?*) Other proteins have also since been discovered that can accomplish vesiculation, including amphiphysin and endophilin. The common feature in all these proteins is their ability to bind to the membrane, typically by interacting with a phosphoinositide head of a lipid.

The original evidence for the involvement of dynamin was the discovery that the *Drosophila* mutation *shibire*, which causes a temperature-sensitive paralysis as the result of a block in endocytosis, lies in a gene for dynamin (2315). It is not clear whether dynamin is required for other vesicle trafficking, but one possibility is that different variants of dynamin (produced by alternative splicing) are required for different trafficking pathways (for review see 2301).

Fusion is a reversal of budding. The coat of the vesicle is an impediment to fusion, and must be removed. If we suppose that uncoated vesicles would have an ability to

fuse spontaneously with membranes in the cell, we can view the coat as a protective layer that preserves the vesicle until it reaches its destination. **Figure 27.15** shows that dissociation of the coat is triggered by hydrolysis of the GTP bound to ARF. This causes ARF to withdraw from the membrane of the vesicle. The coat of COPI-coated vesicles is unstable in the absence of ARF, so the coatomers then dissociate from the vesicle. In the case of clathrin-coated vesicles, the coat is stable, and further components, including a chaperone-like protein and an ATPase, are necessary to remove it. However, a significant proportion of the clathrin and the adaptors in the cell are found in a pool of free molecules, which suggests that both components are removed when vesicles become uncoated prior to fusion with their membrane targets.

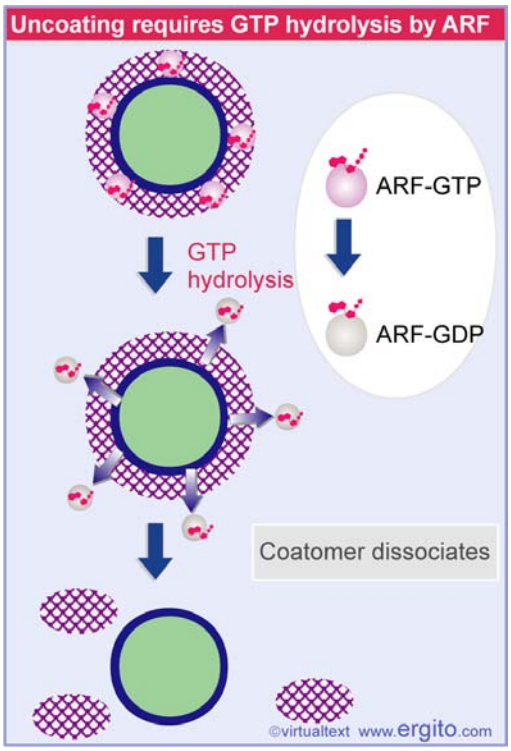

Figure 27.15 Vesicle uncoating is triggered by hydrolysis of GTP bound to ARF.

Components involved in the fusion between the donor membrane of the vesicle and the target membrane were identified via a mammalian protein called NSF, identified by its sensitivity to the sulfhydryl agent NEM (N-ethyl-maleimide). NSF is the homologue for the product of yeast gene *sec18*, which is required for vesicle fusion during movement of transition vesicles. Fusion requires a 20S complex that consists of NSF (a soluble ATPase), a SNAP (*S*oluble NSF-*A*ttachment *P*rotein), and SNAREs (*SNA*P-*re*ceptors) located in the membrane. The fusion particle is a basic part of the vesicular apparatus; it functions at all surfaces where vesicles fuse in the secretory and endocytic pathways. It functions in conjunction with the components necessary for vesicle-membrane recognition and uses ATP hydrolysis to allow them to recycle (782; 783; 784).

Last updated on 1-28-2003

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PROTEIN TRAFFICKING

6.27.8 The exocyst tethers vesicles by interacting with a Rab

Key Terms

The **exocyst** is a complex of 8 proteins that is found at sites on the plasma membrane where secretion occurs. It tethers secretory vesicles to the membrane as the first step in the process of membrane fusion.

Rab proteins are small Ras-like GTPases. Different Rabs are required for protein trafficking between different membrane compartments. Although their exact role is not clear, Rabs appear to regulate membrane targeting and fusion.

Key Concepts

- A tethering complex consists of a group of proteins that are localized to a target membrane and recognize a protein on a suitable vesicle.
- Rabs are prenylated monomeric G proteins that are specifically distributed to different membrane surfaces.
- One mode of action for a tethering complex is to recognize a specific Rab on the vesicle.

What controls the specificity of vesicle targeting? When a vesicle buds from a particular membrane, it has a specific target: vesicles leaving the ER have the *cis*-Golgi as their destination, vesicles leaving the *trans*-Golgi fuse with the plasma membrane, etc. The apparatus for budding and fusion is ubiquitous, so some additional component(s) must allow a vesicle to recognize the appropriate target membrane.

Specific interactions occur between the vesicle and its target membrane at more than one stage, as summarized in **Figure 27.16**. A vesicle approaches a membrane either by diffusion or by some transport mechanism. Tethering complexes are found in the vicinity of the membrane and make the initial recognition of a suitable vesicle. Monomeric GTP-binding proteins called Rabs are important in the tethering reaction. They are either recognized by the tethering apparatus or involved in its activation. Tethering basically holds the vesicle in the vicinity of the membrane, but does not itself cause fusion (for review see 3452). Tethering is followed by interactions between SNARE proteins that trigger fusion (see *Molecular Biology 6.27.9 SNARES are responsible for membrane fusion*).

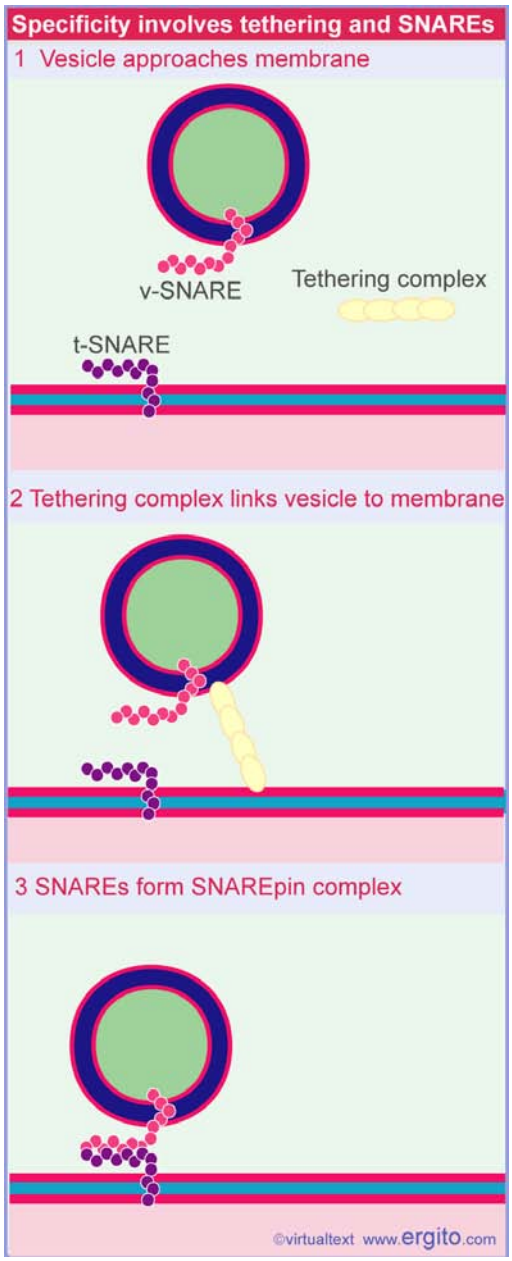

Figure 27.16 A vesicle makes its first contact with a target membrane via a tethering complex. Rab proteins are involved in the tethering reaction. When the v-SNARE and t-SNARE come into close proximity, they interact to bring the vesicle into contact with the membrane. Then the membranes fuse.

The best characterized tethering complex is the **exocyst**, an assembly of eight protein subunits that were originally identified as the products of yeast genes in which mutation causes the accumulation of vesicles that should have released proteins through the plasma membrane (3454; 3455). The absence of any of the subunits makes the vesicles unable to fuse with the membrane, even though the fusion apparatus is not itself affected. The complex is localized at sites where exocytosis

occurs. The homologues of this complex in mammalian cells are called the Sec6/8 complex (3456).

The tethering complex recognizes an appropriate vesicle by interacting with a **Rab** protein on the vesicle. Rabs are attached to membranes via the addition of prenyl or palmityl groups at the C-terminus. There are ~30 Rabs, distributed to different membrane systems in the cell. **Figure 27.17** summarizes their distribution. Different Rabs are involved in ER to Golgi transport, in the constitutive and regulated pathways from the Golgi to the plasma membrane, and in stages of transport between endosomes (for review see 3450). For example, mutations in the yeast genes *YPT1* or *SEC4* that code for two such (related) proteins block transport and cause the accumulation of vesicles in the Golgi stacks or between Golgi and plasma membrane, respectively (for review see 289).

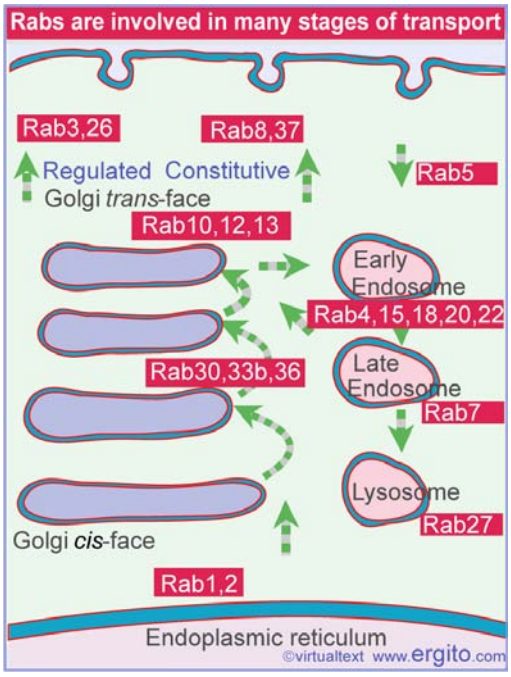

Figure 27.17 Rab proteins affect particular stages of vesicular transport.

The Rabs are GTP-binding proteins that are active in the form bound to GTP; but hydrolysis of the GTP converts the protein to an inactive form. As with other monomeric G proteins, their activities are affected by other proteins that influence the hydrolysis of GTP (for introduction see *Molecular Biology Supplement 32.10 G proteins*). There may be GAP (GTP-hydrolyzing) activities specific for certain Rabs, GEF proteins that stimulate dissociation of the guanine nucleotide, and GDI proteins that prevent dissociation of guanine nucleotide.

The exocyst binds to Sec4p, which is a Rab found on secretory vesicles. The reaction involves several of the exocyst subunits. Two of the exocyst subunits (Sec10p and Sec15p) bind to activated (GTP-bound) Sec4p. This subcomplex binds to Sec3p, which is a component of the exocyst responsible for localization at polarized secretion sites. The result is to tether the vesicle at the secretion site.

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PROTEIN TRAFFICKING

6.27.9 SNARES are responsible for membrane fusion

Key Terms

The **SNARE hypothesis** proposes that the specificity of a transport vesicle for its target membrane is mediated by the interaction of SNARE proteins. In this hypothesis, a SNARE on the vesicle (v-SNARE) binds specifically to its cognate SNARE on the target membrane (t-SNARE).

Key Concepts

- Vesicle-membrane fusion is specified by an interaction between a membrane-bound v-SNARE on the vesicle and t-SNARE on the target membrane.
- The SNAREs have a rod-like structure that lies parallel to the membrane surface.
- The v-SNARE and t-SNARE interact by a zipper-like reaction that creates a bundle of 4 helices parallel to the membrane, and which pulls the membranes into close contact.

The **SNARE hypothesis** proposes that the fusion reaction results from the interaction of a v-SNARE membrane protein carried by the vesicle with a t-SNARE membrane protein that is present on the target membrane (see *Great Experiments 6.6 The SNARE complex and its role in the specificity of membrane fusion*) (for review see 2299). Every membrane surface is marked by a particular constellation of SNAREs, as summarized in **Figure 27.18**, so that vesicle-membrane interactions are determined by pairwise combinations of v-SNAREs and t-SNAREs. This imposes further specificity at the step following the tethering reaction (for review see 3437).

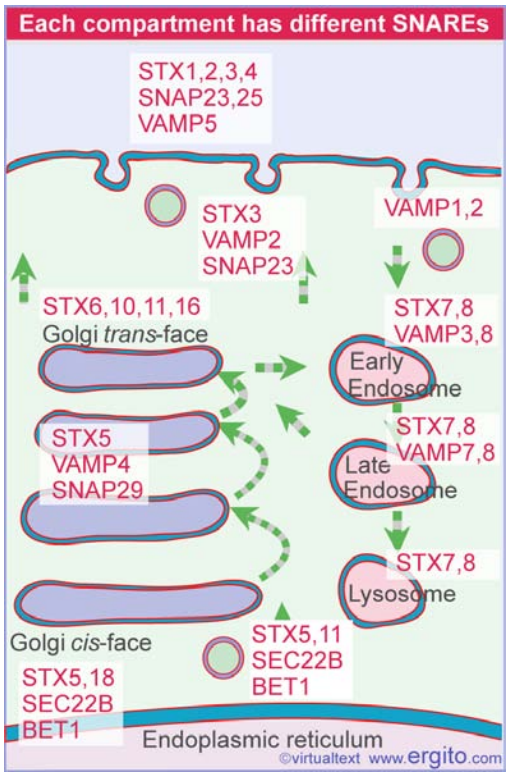

Figure 27.18 Each compartment or vesicle has a characteristic set of SNAREs. The constituent proteins fall into several families, including syntaxins (STX), VAMPs, and SNAP-25s.

Figure 27.19 illustrates the interaction between SNAREs. The v-SNARE is a transmembrane protein carried by the vesicle. The t-SNARE includes two proteins; syntaxin is a transmembrane protein, and SNAP-25 is connected to the membrane by a fatty acyl linkage. (The name of SNAP-25 has an independent origin, and it has no connection with the SNAPs of the fusion particle.) Homologues to these SNAREs are found in other systems, including other animal cell types and yeast cells (785).

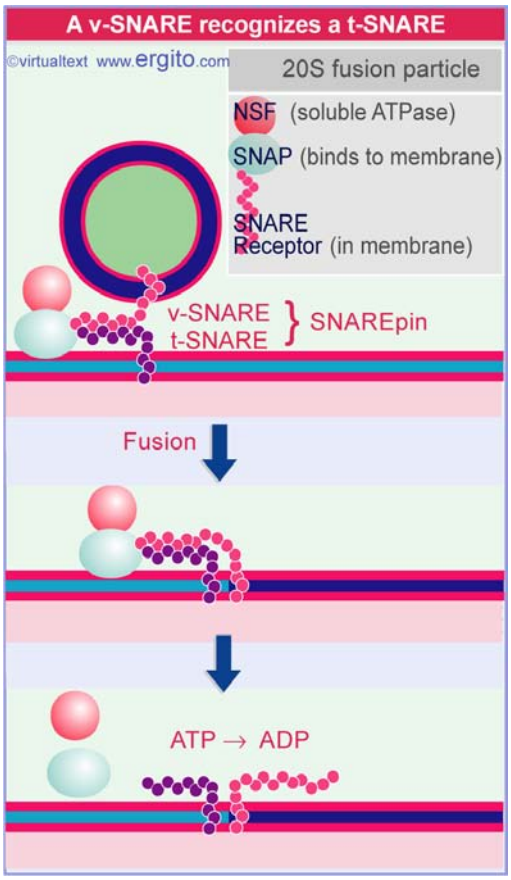

Figure 27.19 Specificity for docking is provided by SNAREs. The v-SNARE carried by the vesicle binds to the t-SNARE on the plasma membrane to form a SNAREpin. NSF and SNAP remain bound to the far end of the SNAREpin during fusion. After fusion, ATP is hydrolyzed and NSF and SNAP dissociate to release the SNAREs.

The major part of each SNARE is exposed in the cytoplasm, and includes an extensive coiled-coil structure. Such structures commonly participate in protein-protein interactions. In fact, v-SNAREs can bind directly to the t-SNAREs *in vitro*, even without the other components of the fusion particle. The interaction between v-SNARE and t-SNARE is sufficient to sponsor membrane fusion. Liposomes containing v-SNAREs can fuse with liposomes containing t-SNAREs in the absence of any additional components. An energy source is not required, suggesting that activation energy is provided by changes in the conformation of the proteins. This *in vitro* reaction is slow, occurring over a time course of minutes. Comparison with the millisecond time course of fusion *in vivo* implies that other components will be needed to facilitate the reaction. But the basic apparatus involved in bringing the membranes together consists of the SNAREs. The idea that individual SNAREs influence the specificity of the reaction is supported by the observation that only certain pairs of SNAREs allow one liposome to interact with another (1183).

A SNARE complex has a rod-like structure (~4 × 14 nm) in which the v-SNARE and t-SNARE are bound in parallel. Their membrane anchors are at the same end,

implying that the rod must lie in a plane between the two membrane surfaces. This structure is called a SNAREpin. **Figure 27.20** is based on the crystal structure, which shows that the complex consists of a 4-helix bundle. **Figure 27.21** shows a model for the SNAREpin superimposed at the appropriate scale on an electron micrograph of the complex (786).

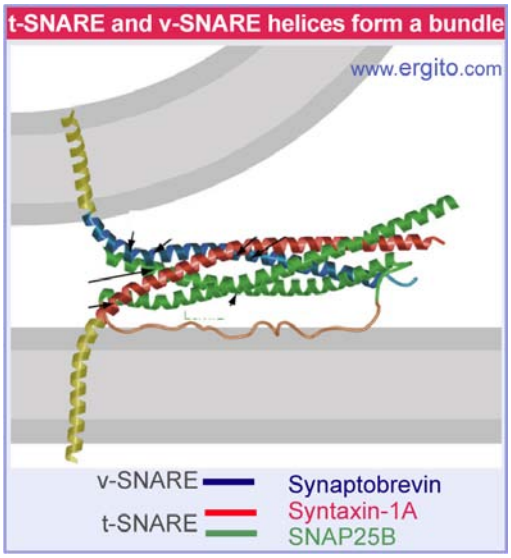

Figure 27.20 A SNAREpin forms by a 4-helix bundle. Photograph kindly provided by Axel Brunger.

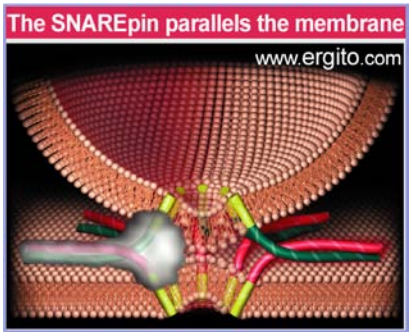

Figure 27.21 A SNAREpin complex protrudes parallel to the plane of the membrane. An electron micrograph of the complex is superimposed on the model. Photograph kindly provided by James Rothman.

The other components of the fusion particle bind to the far end of the complex. Hydrolysis of ATP and dissociation of the fusion complex is probably necessary not for fusion as such, but in order to release the SNAREs from the SNAREpin in order to allow them to be used again. (The 20S fusion complex was originally envisaged to play a role prior to fusion, possibly in providing the energy for fusion, but now we believe it is more likely to function post-fusion.)

It requires a lot of energy to fuse two membranes. When the v-SNARE and t-SNARE interact, they form the SNAREpin by a zipping reaction that moves along the rod. As this generates the 4-helical bundle, the facing leaflets of the two membranes are brought closer and closer, and ultimately they fuse together spontaneously to form the structure drawn in **Figure 27.22**. This stage is called hemifusion. The structure is energetically unstable because of the void space between the membranes. This leads to contacts between the two distal membrane layers. When they break down and reform, there is a small area of contact between the aqueous environment on either side. This is called the fusion pore. It rapidly expands and the membrane relaxes. The fusion pore is probably entirely lipid, with proteins acting to deform the exterior surface (for review see 3459). The energy for driving the transformation of the membrane may come from the interactions between the SNARE proteins (for review see 3437).

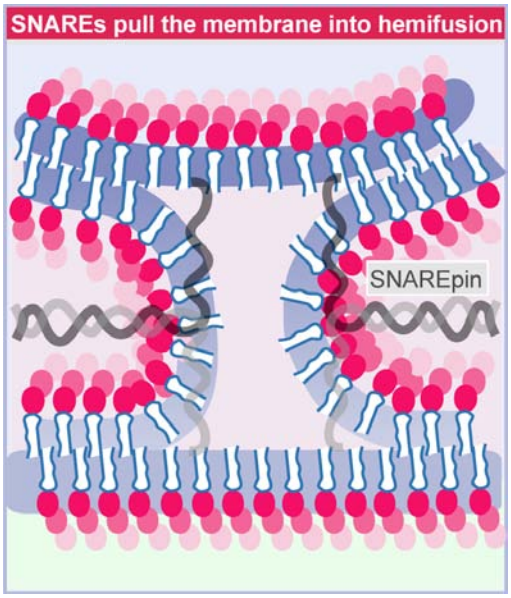

Figure 27.22 When a SNAREpin forms, it pulls the apposing membrane leaflets into juxtaposition in a state of hemifusion.

Last updated on 1-28-2003

Reviews

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PROTEIN TRAFFICKING

6.27.10 The synapse is a model system for exocytosis

Key Terms

A **synapse** is a connection between a neuron and a target cell at which chemical information or electrical impulses may be transmitted.

Key Concepts

- The best characterized example of regulated exocytosis occurs at a synapse to release neurotransmitters from the donor neuron.

The **synapse** has been especially useful for investigating fusion because it offers the advantage of large numbers of vesicles of the same type which fuse with the plasma membrane when a specific trigger is applied.

Impulses in the nervous system are propagated by the passage of material from a donor (or presynaptic) cell to a recipient (or postsynaptic cell). **Figure 27.23** illustrates a nerve terminal. An impulse in the donor cell triggers the exocytic pathway. Stored coated vesicles (called synaptic vesicles) move to the plasma membrane and release their contents of neurotransmitters into the extracellular fluid. The neurotransmitters in turn act upon receptors at the plasma membrane of the recipient cell.

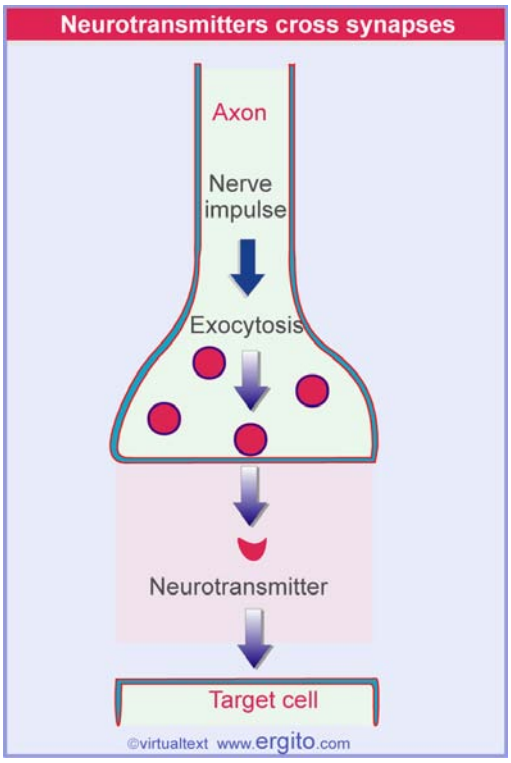

Figure 27.23 Neurotransmitters are released from a donor (presynaptic) cell when an impulse causes exocytosis. Synaptic (coated) vesicles fuse with the plasma membrane, and release their contents into the extracellular fluid.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.27.10 to view properly.

Exocytosis would lead to the accumulation of vesicle components in the plasma membrane if there were no means to retrieve them. There are two possibilities for the recycling of these vesicles, both of which may occur.

In the "kiss and run" model illustrated in **Figure 27.24**, a vesicle does not completely fuse with the plasma membrane, but contacts it transiently. The neurotransmitter is released through some sort of pore; then the vesicle reforms. Major questions about this pathway are how the vesicle maintains its integrity and what sort of structure forms the pore.

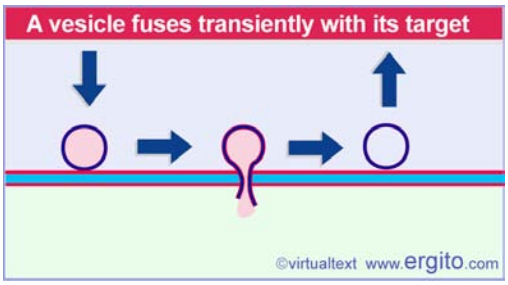

Figure 27.24 The kiss and run model proposes that a synaptic vesicle touches the plasma membrane transiently, releases its contents through a pore, and then reforms.

In the fusion model illustrated in **Figure 27.25**, the vesicle fuses with the plasma membrane in the conventional manner, releasing its contents into the extracellular space. Recycling occurs by the formation of clathrin-coated vesicles at coated pits, that is, by the endocytic pathway. This may occur at large invaginations of the plasma membrane. The importance of endocytosis in this pathway is emphasized by the fact that inhibition of the formation of the clathrin-coated vesicles affects neurotransmitter release from synaptic vesicles. A major question about the pathway is the relationship between the endocytic and exocytic vesicles. The synaptic vesicles are not clathrin-coated. It is probable that the clathrin-coated endocytic vesicles give rise to synaptic vesicles by losing their clathrin coats, but synaptic vesicles may also form by other pathways (as in the case of AP3-coated vesicles). It is probably true that removal of the clathrin coat takes place quite soon after budding for all classes of clathrin-coated vesicles; the process of removal is not well defined.

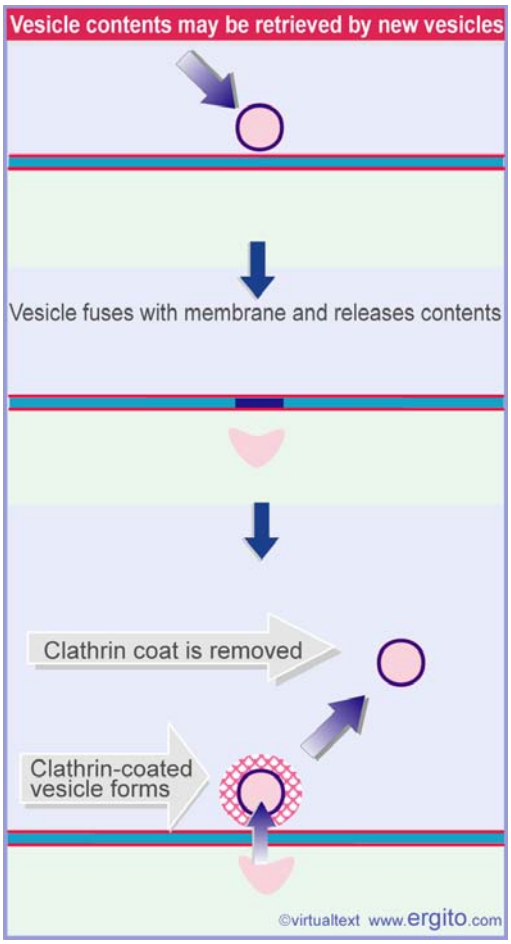

Figure 27.25 When synaptic vesicles fuse with the plasma membrane, their components are retrieved by endocytosis of clathrin-coated vesicles.

Last updated on 10-4-2000

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.6.27.10

PROTEIN TRAFFICKING

6.27.11 Protein localization depends on specific signals

Key Terms

A **transport signal** is the part of a cargo molecule that is recognized by coat proteins or cargo receptors for incorporation into budding transport vesicles. Examples of transport signals are short amino acid sequences, secondary structure, and a protein modification such as phosphorylation.

A **retention signal** is the part (or parts) of a protein that locates it in the correct membrane and prevents it from leaving the compartment. An example is the transmembrane domain of Golgi resident proteins.

A **lysosome** is an organelle that contains hydrolytic enzymes and has an acidic lumen (pH as low as 4.5). Its primary function is the degradation of endocytosed material.

Key Concepts

- A membrane protein is incorporated into a vesicle when a transport signal consisting of a short sequence of amino acids in its cytoplasmic tail binds an adaptor protein of the vesicle.

Various types of signals influence transport through the ER-Golgi system. A protein that has no special signals will presumably enter vesicles at a rate determined by its concentration in the compartment, and may move in the anterograde direction by bulk flow. However, most proteins appear to have specific signals that facilitate or retard transport.

A typical cargo protein has a **transport signal** that is responsible for its entry into budding vesicles. **Figure 27.26** shows that the transport signal in a transmembrane protein is usually a region in its cytoplasmic domain that binds to an adaptor protein of the vesicle coat. **Figure 27.27** shows that the transport signal in a soluble cargo protein (for example, a secreted protein that passes through the lumen) is a region that binds to the lumenal domain of a transmembrane cargo receptor, which in turn has an cytoplasmic domain that binds an adaptor protein. Interaction between the cargo and the coat thus directly or indirectly determines specificity of transport. Such mechanisms control anterograde transport from the ER to the cell surface and other destinations.

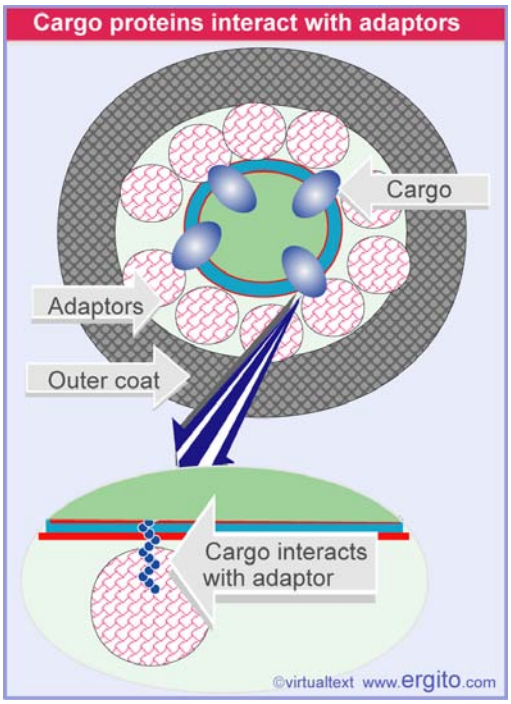

Figure 27.26 A transport signal in a *trans*-membrane cargo protein interacts with an adaptor protein.

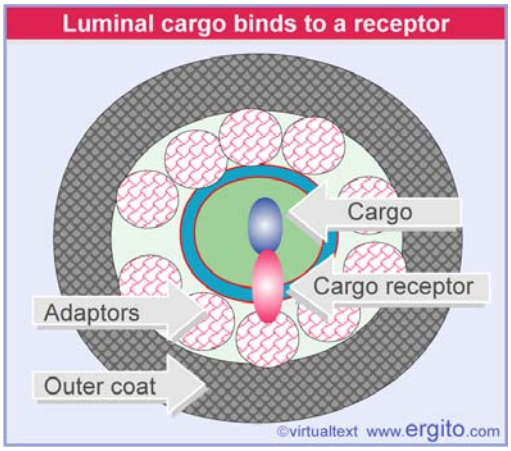

Figure 27.27 A transport signal in a luminal cargo protein interacts with a transmembrane receptor that interacts with an adaptor protein.

A protein may be prevented from leaving a compartment by a **retention signal**. Such signals are often found in transmembrane regions, perhaps because aggregation between them creates a structure that is too large to be incorporated into a budding vesicle.

We have detailed information about several types of signal: a conformation that is required for proteins to be internalized by endocytosis; an amino acid sequence that targets proteins to the ER; and a modification that targets proteins to **lysosomes**

(small membranous bodies, where proteins are degraded).

Internalization of receptors via coated pits requires information in their cytoplasmic tails. The sequence for internalization is usually a short amino acid motif located near the C-terminus. Typically it makes a tight turn in the structure and exposes a tyrosine. Two signals of this sort are NPXY and YXRF. Although these are the basic signals for internalization, other sequences in the cytoplasmic tail influence the efficiency (789).

Enzymes that will be transported to lysosomes are recognized as targets for high mannose glycosylation, and are trimmed in the ER as described in **Figure 27.5**. Then *mannose-6-phosphate* residues are generated by a two-stage process in the Golgi. First the moiety is added to the 6 position of mannose by GlcNAc-phosphotransferase; then a glucosaminidase removes the N-acetyl-glucosamine (GlcNAc).

The action of the phosphotransferase provides the critical step in marking a protein for lysosomal transport. It occurs early in ER-Golgi transfer, possibly between the ER and the *cis* Golgi. The basis for the enzyme's specificity is its ability to recognize a structure that is common to lysosomal proteins. The structure consists of two short sequences, which are separated in the primary sequence, but form a common surface in the tertiary structure. Each of these sequences has a crucial lysine residue. The nature of this signal explains how proteins with little identity of sequence may share a common pathway for localization (788).

Lysosomal proteins continue to be transported along the Golgi stacks until they encounter receptors for mannose-6-phosphate. Recognition of mannose-6-phosphate targets a protein for transport in a coated vesicle to the lysosome. This final stage of sorting for the lysosome occurs in the *trans* Golgi, where the proteins are collected by specific transport vesicles that are coated with clathrin. The vesicles transport the lysosomal proteins to the late endosome, where they join the pathway for movement to the lysosome. A single pool of mannose-6-phosphate receptors is probably used for directing proteins to the lysosome whether they are newly synthesized or endocytosed. Most of the receptors in fact are located on endosomes, where they could recognize endocytosed proteins (for review see 280; 284).

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PROTEIN TRAFFICKING

6.27.12 ER proteins are retrieved from the Golgi

Key Concepts

- The KDEL or KKXX C-terminal sequences are required for a soluble protein to be retained in the ER.
- The KDEL signal binds to a receptor in the Golgi, which causes the receptor-KDEL protein to be transported back to the ER.
- The KKXX signal binds to components of the coatomer of COP-I vesicles, which return the protein from the Golgi to the ER.

Proteins that reside in the lumen of the endoplasmic reticulum possess a short sequence at the C-terminus, Lys-Asp-Glu-Leu (KDEL in single letter code). The alternative signals HDEL or DDEL are used in yeast. If this sequence is deleted, or if it is extended by the addition of other amino acids, the protein is secreted from the cell instead of remaining in the lumen. Conversely, if this tetrapeptide sequence is added to the C-terminus of lysozyme, the enzyme is held in the ER lumen instead of being secreted from the cell. This suggests that there is a mechanism to recognize the C-terminal tetrapeptide and cause it to be localized in the lumen (for review see 285; 282).

An interesting question emerges from the behavior of proteins that have an ER-localization signal. Does this signal cause a protein to be held so that it cannot pass beyond the ER or is it the target for a more active localization process? The model shown in **Figure 27.28** suggests that *the KDEL sequence causes a protein to be returned to* the ER from an early Golgi stack (for an account of the discovery see *Great Experiments 6.5 The discovery of the KDEL retrieval signal*).

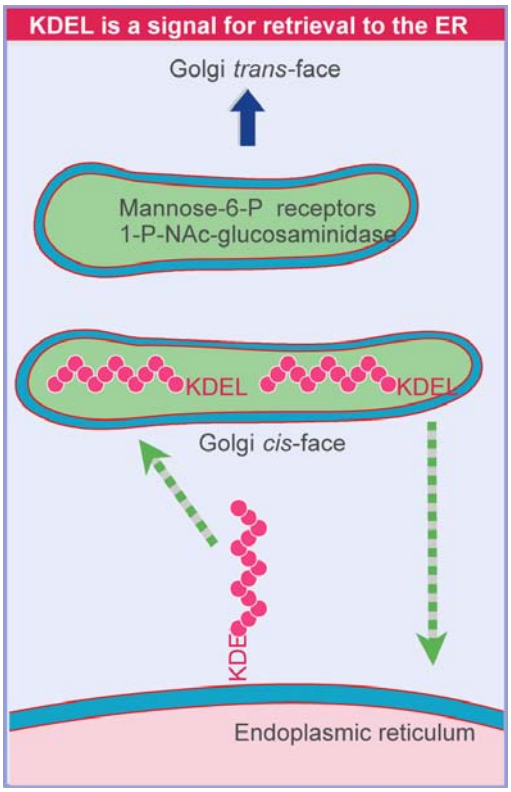

Figure 27.28 An (artificial) protein containing both lysosome and ER-targeting signals reveals a pathway for ER-localization. The protein becomes exposed to the first but not to the second of the enzymes that generates mannose-6-phosphate in the Golgi, after which the KDEL sequence causes it to be returned to the ER.

Because the modification of proteins as they pass through the Golgi is ordered, we can use the types of sugar groups that are present on any particular species as a marker for its progress on the exocytic pathway. When a KDEL sequence is added to a protein that usually is targeted to the lysosome (because its oligosaccharide gains mannose-6-P residues), it causes the protein to be held in the ER. But the protein is modified by the addition of GlcNAc-P, which happens only in the Golgi. The GlcNAc is not removed, so the protein cannot have proceeded far enough through the Golgi stacks to encounter the second of the enzymes in the mannose-6-P pathway. This suggests that KDEL is recognized by a receptor which is located in Golgi before the stack containing the second enzyme (787).

Mutations in the *S. cerevisiae* genes *ERD1* and *ERD2* prevent retention of proteins with the HDEL signal in the ER; instead the proteins are secreted from the cell. The products of both these genes are integral membrane proteins. The *ERD1* mutation causes a general defect in the Golgi; this supports the idea that sorting of the ER proteins occurs by salvage from the Golgi. The *ERD2* mutation identifies the receptor for the HDEL sequence. One model for its role is that it cycles between the Golgi salvage compartment and the ER. This idea is supported by the localization of the corresponding receptor in mammalian cells: it is found largely in the Golgi, but overexpression of a protein with KDEL sequence causes it to concentrate in the ER.

So binding of a KDEL-protein causes the receptor to move from Golgi to ER. It may have a high affinity for the HDEL sequence under the conditions prevailing in the Golgi, but a low affinity in the ER. This could enable ERD2 to seize proteins by their HDEL tails in the Golgi, and take them back to the ER, where they are then released (790; 791).

Another signal is responsible for the localization of transmembrane proteins in the ER. This is KKXX, and thus consists of two Lys residues, located in the cytoplasmic tail just prior to the C-terminus. The dilysine motif of KKXX proteins binds to the β′ – and α-COP components of coatomer. Yeast mutants that affect β′–, α-, or γ-COP are defective in retrieval of KKXX proteins from the Golgi. This suggests that vesicles with the COP-I coat are involved in retrieving proteins from the Golgi and returning them to the ER, that is, COP-I vesicles are responsible for retrograde transport (778).

Overall protein transport is a unidirectional process: proteins enter the ER and are transported through the Golgi, unless stopped *en route*. COP-II-coated vesicles are thought to provide the major capacity for anterograde transport from the ER to the Golgi. COP-I-coated vesicles provide transport capacity along the Golgi stacks. However, both COP-I- and COP-II coated vesicles can be observed to bud from the ER, so there is the possibility that they are involved at multiple stages (perhaps in transporting different types of cargoes).

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PROTEIN TRAFFICKING

6.27.13 Brefeldin A reveals retrograde transport

Key Concepts

- Brefeldin A blocks the action of COP-II vesicles and thereby prevents anterograde (forward) transport.

Retrograde transport usually is obscured by anterograde transport, but is revealed when cells are treated with the drug brefeldin A (BFA), which specifically blocks the forward direction of transport. BFA blocks conversion of ARF from the GDP-bound to the GTP-bound form, and therefore prevents budding of coated vesicles. As a result of the block, a network of tubules forms between the cisternae of the Golgi (abolishing their usual independence) and joins them to the endoplasmic reticulum. There is resorption of most of the membranes of the *cis*-medial Golgi into the endoplasmic reticulum, which is accompanied by the redistribution of Golgi proteins into the ER, effecting a retrograde transport (775).

This happens because the COP-II vesicles involved in forward movement are more sensitive to the drug than the COP-I vesicles involved in retrograde movement. Retrograde transport may serve to retrieve membrane components to compensate for anterograde movement, and of course also provides for the retrieval of ER-proteins from the Golgi. It is possible there may also be other retrograde transport systems: certain toxins that are endocytosed at the plasma membrane can be found in the ER, but this retrograde transport does not appear to depend on the known systems.

The effects of brefeldin on ER-Golgi transport are universal, but in addition it inhibits other transport processes differently in different cells. In some cell types it inhibits transcytosis (transport from the basolateral surface to the apical surface in polarized cells); in other cells it inhibits transport from the *trans* Golgi network to endosomes. A related phenomenon is revealed by isolating cells that can grow in the presence of brefeldin. This identifies mutants in which transport is resistant in particular locations (such as endosomes or Golgi) but remains sensitive in others.

Brefeldin acts by binding to a common domain (the Sec7 domain) in the exchange factors (GEFs) that are responsible for regenerating ARF-GTP from ARF-GDP. BFA stabilizes the association of the GEF with the GDP-bound form of the ARF. This causes the ARF to remain in its inactive state. The differing effects of BFA on individual transport processes probably means that there is a variety of GEFs that act on ARFs on different membrane surfaces, so that the apparatus involved in assembling coated vesicles is specific for individual types of surface. The characteristic susceptibility of each GEF explains the effect of brefeldin on budding from its particular membrane.

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PROTEIN TRAFFICKING

6.27.14 Vesicles and cargos are sorted for different destinations

Key Concepts

- Proteins are loaded into vesicles in the ER and are sorted only subsequently according to their destinations.
- GPI-linked proteins are an exception and are sorted into separate vesicles in the ER.

A key question is how the loading of cargo into vesicles is coordinated with the targeting of the vesicles to their destinations. The first issue is whether and when different types of cargos are sorted into different vesicles. Most sorting occurs in the Golgi, but some proteins are sorted in the ER.

Secretory proteins and plasma membrane proteins can be colocalized in vesicles released from the ER (2182). This suggests the general principle that proteins are packaged into vesicles in the ER irrespective of their final destination, and they are sorted only subsequently in the Golgi. However, there is at least one exception to this rule. Some proteins are linked to the extracellular side of the plasma membrane by glycosyl-phosphatidyl-inositol (GPI) (see *Molecular Biology Supplement 32.5 Membranes and membrane proteins*). GPI-linked proteins leave the ER in different vesicles from other secretory proteins, and so must be sorted from them at a very early stage (2181).

Other secreted and membrane-bound proteins appear to be transported in the same sets of vesicles from the ER through the Golgi to the *trans* face. **Figure 27.29** shows that at this point they are sorted into (at least) four groups of vesicles as defined by their destinations:

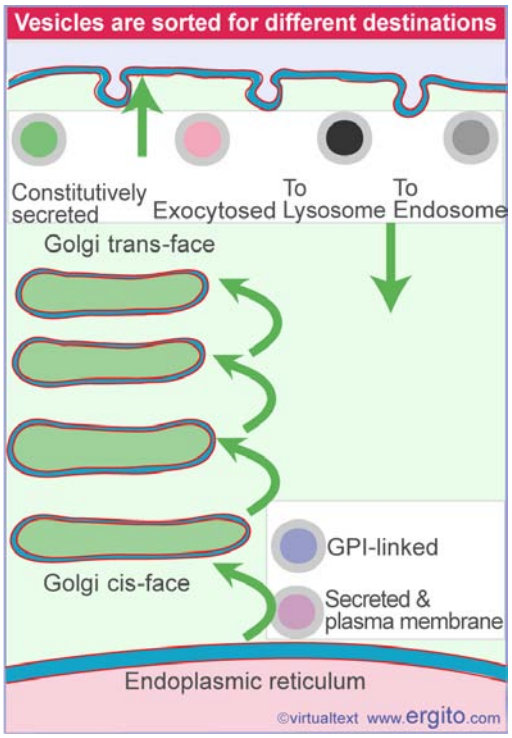

Figure 27.29 Sorting in the ER distinguishes GPI-linked proteins from other proteins, which are sorted into different vesicles at the Golgi *trans* face according to their ultimate destinations.

- Constitutively secreted proteins go in vesicles directly to the plasma membrane.
- Proteins whose exocytosis is regulated enter vesicles that will fuse with the plasma membrane when an appropriate signal is given.
- Vesicles containing proteins that have been modified by addition of mannose-6-phosphate are directed to endosomes (from where they continue to lysosomes).
- Other vesicles may also be directed to endosomes.

Last updated on 12-3-2001

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PROTEIN TRAFFICKING

6.27.15 Receptors recycle via endocytosis

Key Terms

Endocytosis is the process whereby cells internalize small molecules and particles from their surroundings. There are several forms of endocytosis, all of which involve the formation of a membranous vesicle from the plasma membrane.

An **endosome** is an organelle that functions to sort endocytosed molecules and molecules delivered from the trans-Golgi network and deliver them to other compartments, such as lysosomes. It consists of membrane-bounded tubules and vesicles.

Early endosomes are the first compartments of the endocytic pathway in which internalized molecules appear. They are slightly acidic (pH 6.5 to 6.8). In early endosomes, internalized molecules are sorted for recycling to the plasma membrane or for delivery to late endosomes and lysosomes.

Late endosomes are part of the endocytic pathway. They are formed upon the maturation of early endosomes, as proteins are recycled to the plasma membrane, and are more acidic than early endosomes.

Transcytosis is the transport of molecules from one type of plasma membrane domain to another in a polarized cell, such as a neuron and or epithelial cell.

Key Concepts

- Surface receptors are internalized by incorporation into clathrin-coated vesicles at a coated pit.
- Some receptors are internalized continuously, others are internalized upon binding ligand.
- Different receptors have different fates in the cell; the receptor and/or ligand may recycle to the surface or may be degraded.

The systems involved in importing proteins into the cell are closely related to those used for exporting secreted proteins. Ingestion of receptors starts by a common route, which leads to several pathways in which receptors have different fates. Some receptors are internalized continuously, but others remain exposed on the surface until a ligand is bound, which makes them susceptible to endocytosis. The signals that trigger internalization are different for ligand-independent and ligand-induced endocytosis.

In either case, the receptors slide laterally into coated pits, which are indented regions of the plasma membrane surrounded by clathrin. It is not clear whether simple lateral diffusion can adequately explain the movement into coated pits or whether some additional force is required. Coated pits invaginate into the cytoplasm and pinch off to form clathrin-coated vesicles. These vesicles move to early endosomes, are uncoated, fuse with the target membrane, and release their contents.

The process is called receptor-mediated **endocytosis** (for review see 278).

The immediate destination for endocytic (clathrin-coated) vesicles is the **endosome**, a rather heterogeneous structure consisting of membrane-bounded tubules and vesicles. There are at least two types of endosome, as indicated in **Figure 27.30**. **Early endosomes** lie just beneath the plasma membrane and are reached by endocytosed proteins within ~1 minute. **Late endosomes** are closer to the nucleus, and are reached within 5-10 minutes.

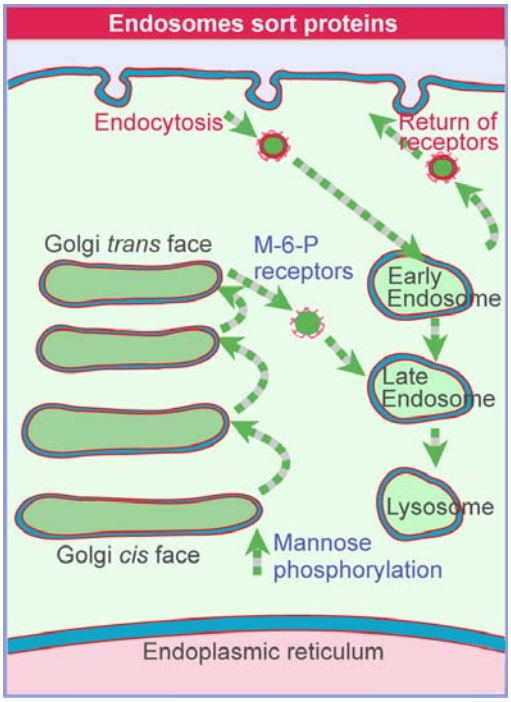

Figure 27.30 Endosomes sort proteins that have been endocytosed and provide one route to the lysosome. Proteins are transported via clathrin-coated vesicles from the plasma membrane to the early endosome, and may then either return to the plasma membrane or proceed further to late endosomes and lysosomes. Newly synthesized proteins may be directed to late endosomes (and then to lysosomes) from the Golgi stacks. The common signal in lysosomal targeting is the recognition of mannose-6-phosphate by a specific receptor.

The early endosome provides the main location for sorting proteins on the endocytic pathway. Its role is a counterpart to that played by the Golgi for newly synthesized proteins. The interior of the endosome is acidic, with a pH <6. Proteins that are transported to the endosome change their structure in response to the lowering of pH; this change is important in determining their fate.

Receptors that have been endocytosed to the early endosome behave in one of two ways. They may return to the plasma membrane (by vesicular transport). Or they may be transported further to the lysosome, where they may be degraded. Transport to the lysosome is the default pathway, and applies to any material that does not

possess a signal specifically directing it elsewhere.

The lysosome contains the cellular supply of hydrolytic enzymes, which are responsible for degradation of macromolecules. Like endosomes, the lysosome is an acidic compartment (pH = 5).

The relationship between the various types of endosomes and lysosomes is not yet clear. Vesicles may be used to transport proteins along the pathway from one pre-existing structure to the next; or early endosomes may "mature" into late endosomes, which in turn "mature" into lysosomes. At all events, the pathway is unidirectional, and a protein that has left the early endosome for the late endosome will end up in the lysosome.

There are two routes to the lysosome. Proteins endocytosed from the plasma membrane may be directed via the early endosome to the late endosome. Newly synthesized proteins may be directed from the *trans* Golgi via the late endosome, as described above.

The fate of a receptor-ligand complex depends upon its response to the acidic environment of the endosome. Exposure to low pH changes the conformation of the external domain of the receptor, causing its ligand to be released, and/or changes the structure of the ligand. But the receptor must avoid becoming irreversibly denatured by the acid environment; the presence of multiple disulfide bridges in the external domain may play an important role in maintaining this unusual stability.

Four possible fates for a receptor-ligand complex are described in the alternative pathways of the next four figures:

- *Receptor recycles to the surface in coated vesicles, while the ligand is degraded.***Figure 27.31** shows that this pathway is used by receptors that transport ligands into cells at high rates. A receptor recycles every 1-20 minutes, and can undertake >100 cycles during its lifetime of ~20 hours. The classic example of this pathway is the LDL receptor, whose ligands are the plasma low density lipoproteins apolipoprotein E and apolipoprotein B (collectively known as the LDLs). Apo-B is a very large (500 kD) protein that carries cholesterol and cholesterol esters. The LDL is released from its receptor in the endosome. The receptor recycles to the surface to be used again. The LDL and its cholesterol separate in the endosome; the LDL is sent on to the lysosome, where it is degraded, and the cholesterol is released for use by the cell. This constitutes the major route for removing cholesterol from the circulation. People with mutations in the LDL receptor accumulate large amounts of plasma cholesterol that cause the disease of familial hypercholesterolemia.

- *Receptor and ligand both recycle.* The transferrin receptor provides the classic example of this pathway, illustrated in **Figure 27.32**. The ligand for the receptor is the iron-carrying form of transferrin. When this reaches the endosome, the acid environment causes transferrin to release the iron. The iron-free ligand, called apo-transferrin, remains bound to the transferrin receptor, and recycles to the plasma membrane. In the neutral pH of the plasma membrane, apo-transferrin dissociates from the receptor. This leaves apo-transferrin free to bind another iron, while the transferrin receptor is available to internalize another

iron-carrying transferrin. Again this cycle is quite intensively used; a transferrin receptor recycles every 15-20 minutes, and has a half-life of >30 hours. It provides the cell with the means of taking up iron.

- *Receptor and ligand both are degraded.* The EGF receptor binds its ligand as a requirement for internalization. Although EGF and its receptor appear to dissociate at low pH, they are both carried on to the lysosome, where they may be degraded, as indicated in **Figure 27.33**. We do not know how and whether these events are related to the ability of EGF to change the phenotype of a target cell via binding to the receptor.

- *Receptor and ligand are transported elsewhere.* The route illustrated in **Figure 27.34** is available in certain polarized cells. A receptor-ligand combination is taken up at one cell surface, transported to the endosome, and then released for transport to the far surface of the cell. This is called **transcytosis**. By this means, receptors can transport immunoglobulins across epithelial cells.

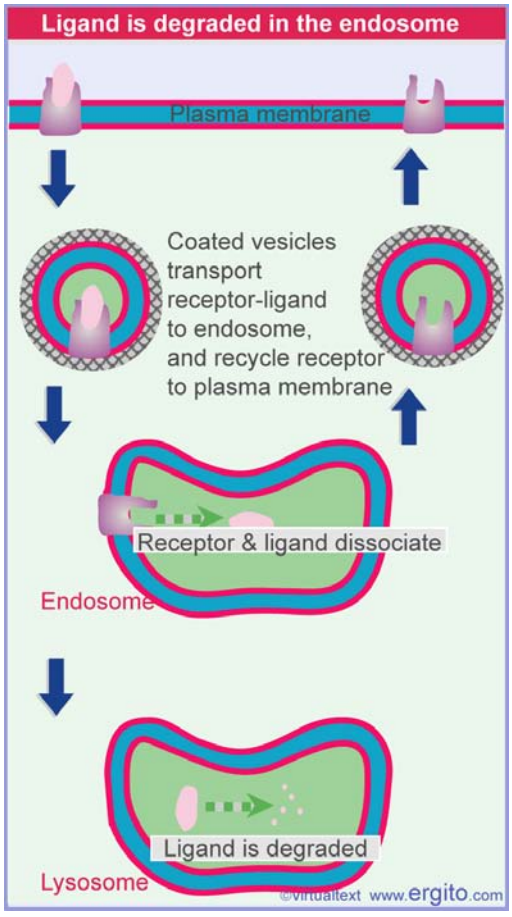

Figure 27.31 LDL receptor transports apo-B (and apo-E) into endosomes, where receptor and ligand separate. The receptor recycles to the surface, apo-B (or apo-E) continues to the lysosome and is degraded, and cholesterol is released.

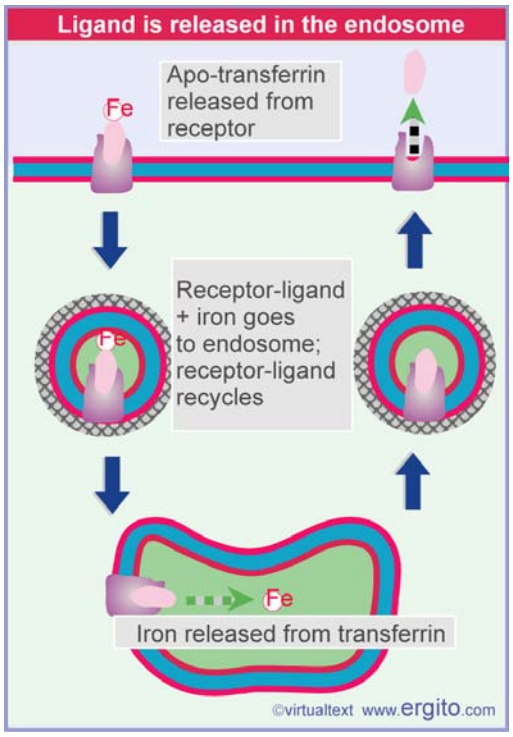

Figure 27.32 Transferrin receptor bound to transferrin carrying iron releases the iron in the endosome; the receptor now bound to apo-transferrin (lacking iron) recycles to the surface, where receptor and ligand dissociate.

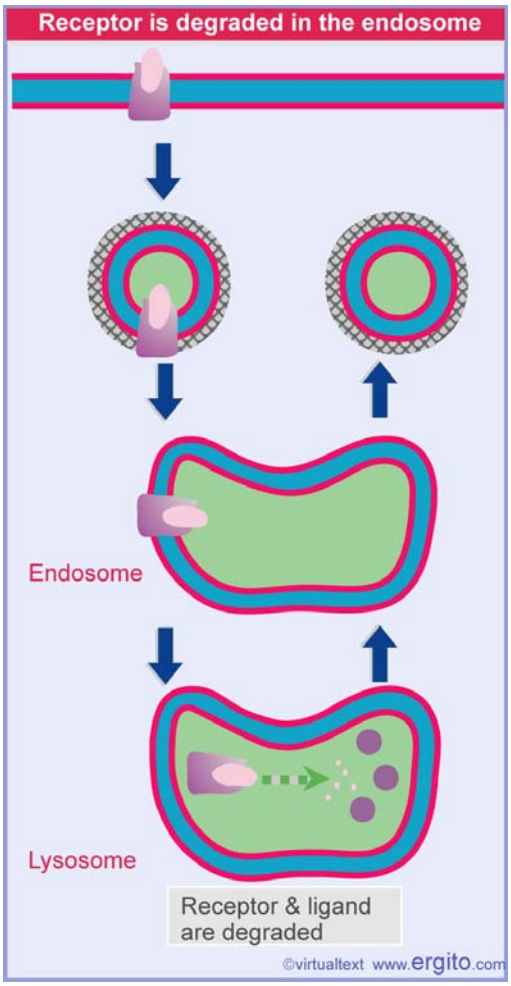

Figure 27.33 EGF receptor carries EGF to the lysosome where both the receptor and ligand are degraded.

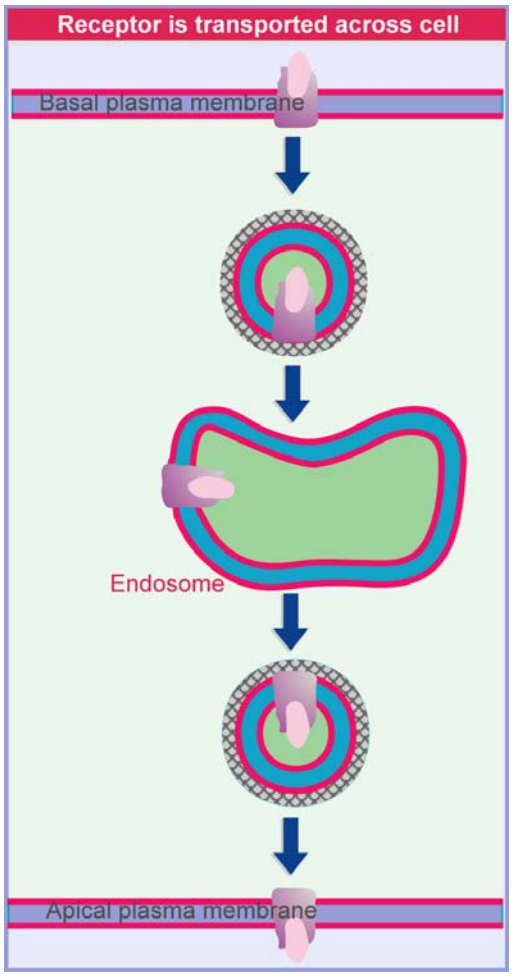

Figure 27.34 Ig receptor transports immunoglobulin across the cell from one surface to the other.

Rapid recycling in general occurs for receptors that bring ligands into the cell, not for those that trigger pathways of signal transduction. Receptors involved in signaling changes from the surface are usually degraded if they are endocytosed.

Reviews

278. Goldstein, J. L., Brown, M. S., Anderson, R. G., Russell, D. W., and Schneider, W. J. (1985). *Receptor-mediated endocytosis: concepts emerging from the LDL receptor system*. Annu. Rev. Cell Biol. 1, 1-39.

PROTEIN TRAFFICKING

6.27.16 Internalization signals are short and contain tyrosine

Key Concepts

- Signals for internalization are short (~4 amino acids), are located in the cytoplasmic tail near the plasma membrane, and usually contain a tyrosine residue that is exposed by the protein conformation.

What features of protein structure are required for endocytosis? Mutations that prevent internalization can be used to identify the relevant sequences in the receptors. In fact, the characterization of an internalization defect provided evidence that entry into coated pits is needed for receptor-mediated endocytosis of LDL. In cells from human patients with such defects in the LDL receptor, the receptor gathers in small clusters over the plasma membrane, and cannot enter coated pits in the manner observed for wild-type cells.

The mutations responsible for this type of defect all affect the *cytoplasmic domain* of the receptor, which functions independently in sponsoring endocytosis. A recombinant protein whose extracellular domain is derived from influenza virus hemagglutinin, which is not usually endocytosed, can be internalized if it is provided with a cytoplasmic domain from an endocytosed receptor.

We do not yet have a clear view of all of the sorting signals that mediate endocytosis, but two features are common. The relevant region of the protein comprises a relatively short part of the cytoplasmic tail close to the plasma membrane. And the presence of a tyrosine residue in this region often is necessary. The most common motif is YXX ϕ (where ϕ is a hydrophobic amino acid). Removal of the tyrosine prevents internalization; conversely, substituting tyrosine in the relevant region of a protein that is not endocytosed (such as influenza virus hemagglutinin) allows it to be internalized.

Tyrosine is also found in another signal for internalization: NPXY (Asn-Pro-X-Tyr). The essential tyrosine can be replaced by other aromatic amino acids, but not by other types of amino acids. The NPXY motif is found in several group I proteins that are internalized, and is usually located close to the plasma membrane. (It was first identified in the LDL receptor.) It could be a general signal for endocytosis. In proteins that are internalized in response to ligand binding, the internalization signal may be generated by a change in conformation as a result of the binding.

Do internalized receptors interact directly with proteins on the vesicles that transport them? Clathrin forms an outer polyhedral layer on clathrin-coated vesicles, but other proteins form an inner layer. The adaptins recognize the appropriate sequences in the cytoplasmic domains of receptors that are to be internalized (see **Figure 27.12**). **Figure 27.35** illustrates a model in which, as a coated pit forms, the adaptins bind to the receptor cytoplasmic domain, immobilizing the receptor in the pit. As a result,

the receptor is retained by the coated vesicle when it pinches off from the plasma membrane.

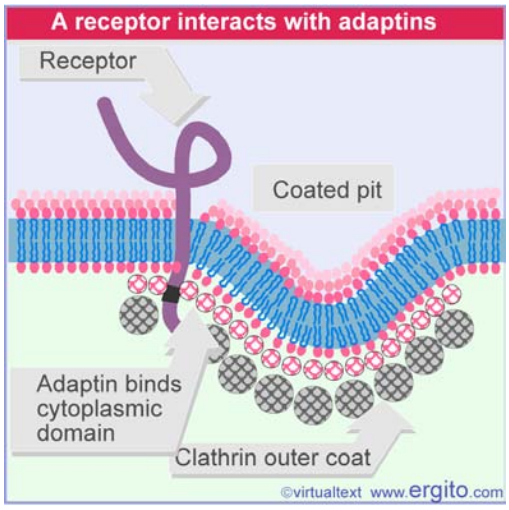

Figure 27.35 The cytoplasmic domain of an internalized receptor interacts with proteins of the inner layer of a coated pit.

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.6.27.16

PROTEIN TRAFFICKING

6.27.17 Summary

Proteins that reside within the reticuloendothelial system or that are secreted from the plasma membrane enter the ER by cotranslational transfer directly from the ribosome. They are transported through the Golgi in the anterograde (forward) direction. Specific signals may cause them to be retained in the ER or a Golgi stack, or directed to other organelles such as endosomes. The default pathway is to be transported to the plasma membrane. Retrograde transport is less well characterized, but proteins that reside in the ER are retrieved from the Golgi by virtue of specific signals; an example is the C-terminal KDEL.

Proteins are transported between membranous surfaces as cargoes in membrane-bound coated vesicles. The vesicles form by budding from donor membranes; they unload their cargos by fusing with target membranes. The protein coats are added when the vesicles are formed and must be removed before they can fuse with target membranes. Anterograde transport does not result in any net flow of membrane from the ER to the Golgi and/or plasma membrane, so membrane moving with anterograde transport must be returned to the ER by a retrograde mechanism.

Modification of proteins by addition of a preformed oligosaccharide starts in the endoplasmic reticulum. High mannose oligosaccharides are trimmed. Complex oligosaccharides are generated by further modifications that are made during transport through the Golgi, determined by the order in which the protein encounters the enzymes localized in the various Golgi stacks. Proteins are sorted for different destinations in the *trans* Golgi. The signal for sorting to lysosomes is the presence of mannose-6-phosphate.

Different types of vesicles are responsible for transport to and from different membrane systems. The vesicles are distinguished by the nature of their protein coats.

COP-I-coated vesicles are responsible for retrograde transport from the Golgi to the ER. COP-I vesicles are coated with coatomer. One of the proteins of coatomer, β-COP, is related to the β-adaptin of clathrin-coated vesicles, suggesting the possibility of a common type of structure between COP-I-coated and clathrin-coated vesicles.

COP-II vesicles undertake forward movement from the ER to Golgi. Vesicles that transport proteins along the Golgi stacks have not yet been identified. Vesicles responsible for constitutive (bulk) movement from the Golgi to the plasma membrane also have not been identified. An alternative model for anterograde transport proposes that *cis*-Golgi cisternae actually become *trans*-Golgi cisternae, so that there is a continuous process of cisternal maturation from the *cis* to the *trans* face.

In the pathway for regulated secretion of proteins, proteins are sorted into clathrin-coated vesicles at the Golgi *trans* face. Some vesicles may fuse into (larger)

secretory granules. Vesicles also move to endosomes, which control trafficking to the cell surface. Secretory vesicles are stimulated to unload their cargos at the plasma membrane by extracellular signals. Similar vesicles are used for endocytosis, the pathway by which proteins are internalized from the cell surface. The predominant protein in the outer coat of these vesicles is clathrin. The inner coat contains an adaptor complex, consisting of adaptin subunits, which bind to clathrin and to cargo proteins. There are (at least) three types of adaptor complex, with different specificities.

Budding and fusion of all types of vesicles is controlled by a small GTP-binding protein. This is ARF for clathrin and COP-I-coated vesicles, and Sar1P for COP-II-coated vesicles. When activated by GTP, ARF/Sar1p inserts into the membrane and causes coat proteins to assemble. This leads ultimately to budding. Further proteins, such as dynamin, may be required to "pinch off" the budding vesicle from the donor membrane. When ARF/Sar1p is inactivated because GTP is hydrolyzed to GDP, it withdraws from the membrane and the coat proteins either disassemble spontaneously (COP-coated vesicles) or are caused to do so by other proteins (clathrin-coated vesicles).

Vesicles initially recognize appropriate target membranes by a tethering reaction in which a tethering complex recognizes a Rab protein on the vesicle and brings the vesicle close to the membrane. Rabs are prenylated monomeric GTP-binding proteins. The fusion reaction is triggered when a v-SNARE on the vesicle pairs specifically with a t-SNARE on the target membrane. Pairing occurs by a coiled-coil interaction in which the SNARE complex lies parallel to the membrane surface. This causes the inner leaflets of the membranes to fuse to form a hemifusion complex; this is followed by fusion of the outer leaflets . The 20S fusion complex includes the soluble ATPase NSF and SNAP, and uses hydrolysis of ATP to release the SNAREs after pairing, which allows them to recycle.

Receptors may be internalized either continuously or as the result of binding to an extracellular ligand. Receptor-mediated endocytosis initiates when the receptor moves laterally into a coated pit. The cytoplasmic domain of the receptor has a signal that is recognized by proteins that are presumed to be associated with the coated pit. An exposed tyrosine located near the transmembrane domain is a common signal; it may be part of the sequence NPXY. When a receptor has entered a pit, the clathrin coat pinches off a vesicle, which then migrates to the early endosome.

The acid environment of the endosome causes some receptors to release their ligands; the ligand are carried to lysosomes, where they are degraded, and the receptors are recycled back to the plasma membrane by means of coated vesicles. A ligand that does not dissociate may recycle with its receptor. In some cases, the receptor-ligand complex is carried to the lysosome and degraded.