❖ **Single-particle tracking (SPT):**

A technique for studying movement of membrane proteins that consists of two steps:

(1) Linking the protein molecules to visible substances such as colloidal gold particles, and

(2) Monitoring the movements of the individual tagged particles under the microscope.

❖ **Scramblase:**

In eukaryotic cells, new phospholipids are manufactured by enzymes bound to the cytosolic surface of the endoplasmic reticulum. Using free fatty acids as substrates, the enzymes deposit the newly made phospholipids exclusively in the cytosolic half of the bilayer. The transfer of lipids from one monolayer to the other rarely occur spontaneously. Instead, they are catalyzed by enzymes called *scramblases*, which remove randomly selected phospholipids from one half of the lipid bilayer and insert them in the other. As a result of this scrambling, newly made phospholipids are redistributed equally between each monolayer of the ER membrane.

❖ **Flippase:**

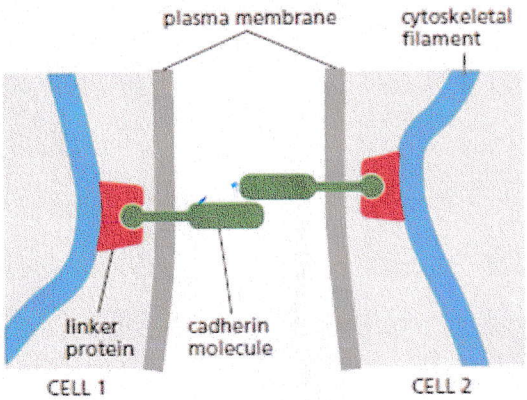
The Golgi membrane contains another family of phospholipid-handling enzyme, called *flippases*. These enzymes remove specific phospholipids from the side of the bilayer facing the exterior space and flip them into the monolayer that faces the cytosol. The action of these flippases—and similar enzymes in the plasma membrane—initiates and maintains the asymmetric arrangement of phospholipids that is characteristic of the membranes of animal cells.

❖ **Cadherins:**

The cell junctions that hold an epithelium together by forming mechanical attachments are of three main types. Adherens junctions and desmosomes bind one epithelial cell to another, while hemidesmosomes bind epithelial cells to the basal lamina. All of these junctions provide mechanical strength by the same strategy: the proteins that form the cell adhesion span the plasma membrane and are linked inside the cell to cytoskeletal filaments. In this way, the cytoskeletal filaments are tied into a network that extends from cell to cell across the whole expanse of the epithelial sheet. Adherens junctions and desmosomes are both built around transmembrane proteins that belong to the cadherin family: a cadherin molecule in the plasma membrane of one cell binds directly to an identical cadherin molecule in the plasma membrane of its neighbor Such binding of like-to-like is called homophilic binding. In the case of cadherins, binding also requires that Ca2+ be present in the extracellular medium—hence the name.

Identical cadherin molecules in the plasma membranes of adjacent cells bind to each other extracellularly; inside the cell, they are attached, via linker proteins, to cytoskeletal filaments—either actin

filaments or keratin intermediate filaments. As cells touch one another, their cadherins become concentrated at the point of attachment.

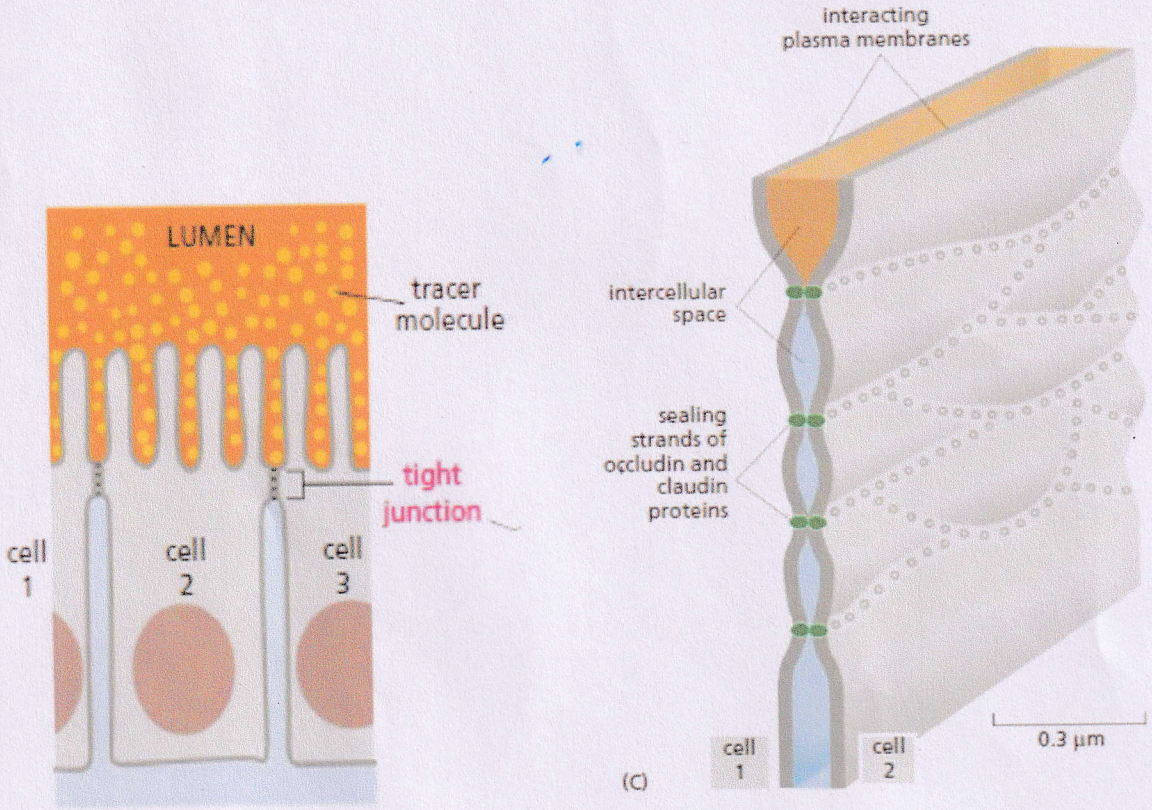


The results of experiments with L cells, a line of cultured mouse fibroblasts grown in the laboratory, demonstrated that E-cadherin preferentially mediate homophilic interactions. L cells express no cadherins and adhere poorly to themselves or to other types of cultured cells. When gene encoding either E-cadherin was introduced into L cells with the use of transfection technique, the resulting engineered L cells expressed the encoded cadherin. These cadherin-expressing L cells were found to adhere preferentially to cells expressing the same type of cadherin molecules; that is, they mediate homophilic interactions. The L cells expressing E-cadherin also exhibited the polarized distribution of a membrane protein similar to that in epithelial cells, and they formed epithelial-like aggregates with one another and with epithelial cells isolated from lungs.

Tight junctions:

Epithelial cell junctions can be classified according to their function. Some provide a tight seal to prevent the leakage of molecules across the epithelium through the gaps between its cells. The sealing function is served (in vertebrates) by tight junctions. These junctions seal neighboring cells together so that water-soluble molecules cannot easily leak between them. If a tracer molecule is added to one side of an epithelial cell sheet, it will usually not pass beyond the tight junction. The tight junction is formed from proteins called *claudins* and *occludins*, which are arranged in strands along the lines of the junction to create the seal. Without tight junctions to prevent leakage, the pumping activities of absorptive cells such as those in the gut would be futile, and the composition of the extracellular fluid would become the same on both sides of the epithelium. Tight junctions also play a key part in maintaining the polarity of the individual epithelial cells in two ways. First, the tight junctions around the apical region of each cell prevents diffusion of proteins within the plasma membrane and so keeps the apical domain of the plasma

membrane different from the basal (or basolateral) domain. Second, in many epithelia, the tight junctions are sites of assembly for the complexes of intracellular proteins that govern the apico-basal polarity of the cell interior.

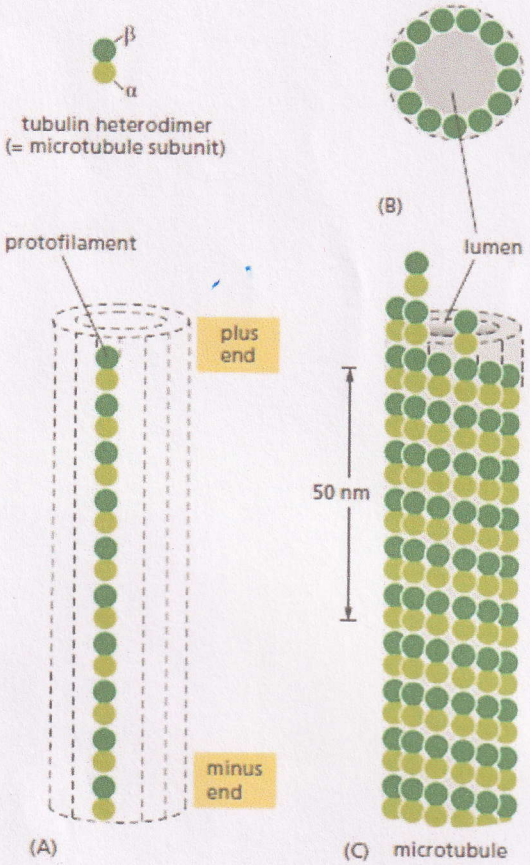


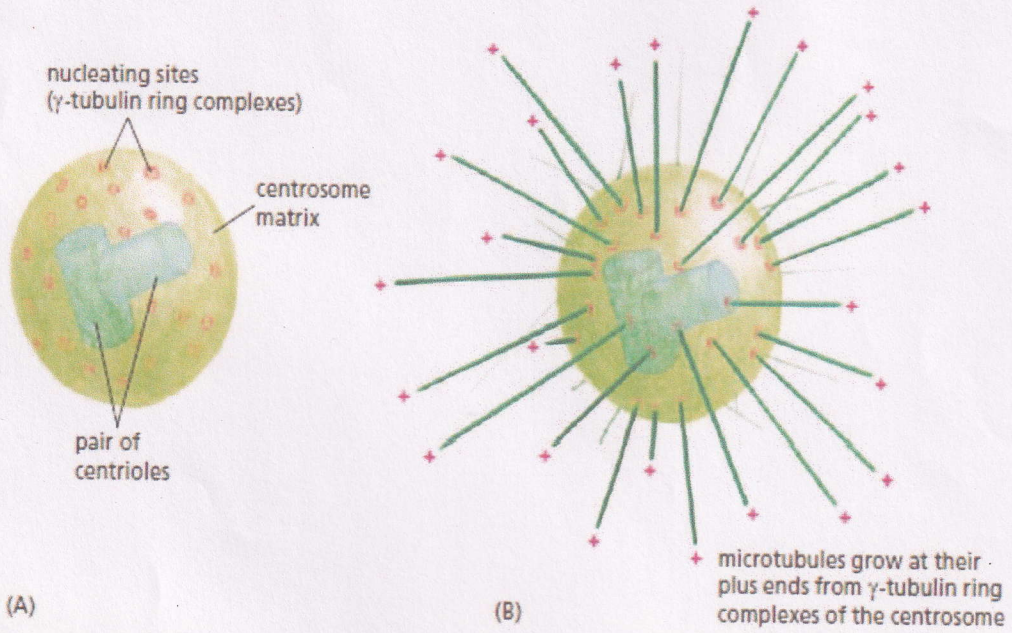
❖ **Tubulin:**

Microtubules are built from subunits—molecules of tubulin—each of which is itself a dimer composed of two very similar globular proteins called α-tubulin and β-tubulin, bound tightly together by noncovalent interactions. The tubulin dimers stack together, again by noncovalent bonding, to form the wall of the hollow cylindrical microtubule. This tubelike structure is made of 13 parallel protofilaments, each a linear chain of tubulin dimers with α- and β-tubulin alternating along its length. Each protofilament has a structural polarity, with α-tubulin exposed at one end and β-tubulin at the other, and this polarity—the directional arrow embodied in the structure—is the same for all the protofilaments, giving a structural polarity to the microtubule as a whole. One end of the microtubule, thought to be the β-tubulin end, is called its *plus end*, and the other, the α-tubulin end, its *minus end*.

Inside cells, microtubules grow from specialized organizing centers **(Microtubule organizing centers, MTOC)** that control the location, number, and orientation of the microtubules. In animal cells, for example, the centrosome—which is typically close to the cell nucleus when the cell is not in mitosis—organizes an array of microtubules that radiates outward through the cytoplasm. The centrosome consists of a pair of centrioles, surrounded by a matrix of proteins. The centrosome matrix includes hundreds of ring shaped structures formed from a special type of tubulin, called *γ-tubulin*, and each *γ-tubulin ring complex* serves as the starting point, or *nucleation site*, for the growth of one microtubule. The αβ-tubulin dimers add to each γ-tubulin ring complex in a specific orientation, with the result that the minus end of each microtubule is embedded in the centrosome, and growth occurs only at the plus end that extends into the cytoplasm.

Once a microtubule has been nucleated, it typically grows outward from the organizing center for many minutes by the addition of αβ-tubulin dimers to its plus end. Then, without warning, the microtubule can suddenly undergo a transition that causes it to shrink rapidly inward by losing tubulin dimers from its free plus end. It may shrink partially and then, no less suddenly, start growing again, or it may disappear completely, to be replaced by a new microtubule that grows from the same γ-tubulin ring complex. This remarkable behavior—switching back and forth between polymerization and depolymerization—is known as **dynamic instability**. It is driven by GTP hydrolysis. It allows microtubules to undergo rapid remodeling, and is crucial for their function. In a normal cell, the centrosome (or other organizing center) is continually shooting out new microtubules in different directions in an exploratory fashion, many of which then retract. A microtubule growing out from the centrosome can, however, be prevented from disassembling if its plus end is stabilized by attachment to another molecule or cell structure so as to prevent its depolymerization. If stabilized by attachment to a structure in a more distant region of the cell, the microtubule will establish a relatively stable link between that structure and the centrosome.


β
α
tubulin heterodimer
(= microtubule subunit)
protofilament
plus end
minus end
(A)
(B)
lumen
50 nm
(C) microtubule


nucleating sites
(γ-tubulin ring complexes)
centrosome matrix
pair of centrioles
(A)
(B)
microtubules grow at their plus ends from γ-tubulin ring complexes of the centrosome

❖ **Drugs modifying Microtubule Dynamics:**

Drugs that prevent the polymerization or depolymerization of tubulin dimers can have a rapid and profound effect on the organization of microtubules— and thereby on the behavior of the cell. Consider the mitotic spindle, the microtubule-based apparatus that guides the chromosomes during mitosis. If a cell in mitosis is exposed to the drug ***colchicine***, which binds tightly to free tubulin dimers and prevents their polymerization into microtubules, the mitotic spindle rapidly disappears, and the cell stalls in the middle of mitosis, unable to partition the chromosomes into two groups. This finding, and others like it, demonstrates that the mitotic spindle is normally maintained by a continuous balanced addition and loss of tubulin subunits: when tubulin addition is blocked by colchicine, tubulin loss continues until the spindle disappears. The drug ***Taxol*** has the opposite effect. It binds tightly to microtubules and prevents them from losing subunits. Because new subunits can still be added, the microtubules can grow but cannot shrink. However, despite this difference in their mechanism of action, Taxol has the same overall effect as colchicine—arresting dividing cells in mitosis. These experiments show that for the mitotic spindle to function, microtubules must be able to assemble and disassemble. The inactivation or destruction of the mitotic spindle eventually kills dividing cells. Because cancer cells divide in a less controlled way than do normal cells of the body, they can sometimes be killed preferentially by microtubule-stabilizing or microtubule-destabilizing ***antimitotic drugs.*** These drugs include colchicine, Taxol, vincristine, and vinblastine—all of which are used in the treatment of human cancer.

❖ **Kinesin & Dynein:**

If a living cell is observed in a light microscope, its cytoplasm is seen to be in continual motion. Mitochondria and the smaller membrane enclosed organelles and vesicles travel in small, jerky steps—moving for a short period, stopping, and then moving again. This *saltatory* movement can occur along either microtubules or actin filaments. In both cases, the movements are driven by motor proteins, which use the energy derived from repeated cycles of ATP hydrolysis to travel steadily along the microtubule or actin filament in a single direction. Because the motor proteins also attach to other cell components, they can transport this cargo along the filaments. There are dozens of different motor proteins; they differ in the type of filament they bind to, the direction in which they move along the filament, and the cargo they carry.

The motor proteins that move along cytoplasmic microtubules, such as those in the axon of a nerve cell, belong to two families: the kinesins generally move toward the plus end of a microtubule (outward from the cell body; the dyneins move toward the minus end (toward the cell body). Both kinesins and dyneins are generally dimers that have two globular ATP-binding heads and a single tail. The heads

interact with microtubules in a stereospecific manner, so that the motor protein will attach to a microtubule in only one direction. The tail of a motor protein generally binds stably to some cell component, such as a vesicle or an organelle, and thereby determines the type of cargo that the motor protein can transport. The globular heads of kinesin and dynein are enzymes with ATP-hydrolyzing (ATPase) activity. This reaction provides the energy for driving a directed series of conformational changes in the head that enable it to move along the microtubule by a cycle of binding, release, and rebinding to the microtubule. For a discussion of the discovery and study of motor proteins.

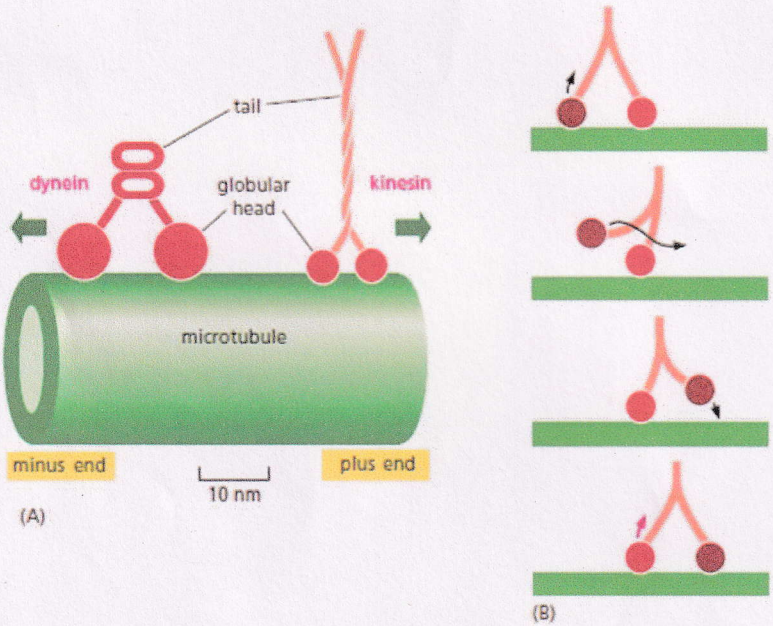


Figure 17–18 Both kinesins and dyneins move along microtubules using their globular heads. (A) Kinesins and cytoplasmic dyneins are microtubule motor proteins that generally move in opposite directions along a microtubule. Each of these proteins (drawn here roughly to scale) is a dimer composed of two identical subunits. Each dimer has two globular heads at one end, which bind and hydrolyze ATP and interact with microtubules, and a single tail at the other end, which interacts with cargo (not shown). (B) Schematic diagram of a generic motor protein "walking" along a filament; these proteins use the energy of ATP hydrolysis to move in one direction along the filament, as illustrated in Figure 4–46. (See also Figure 17–22B.)

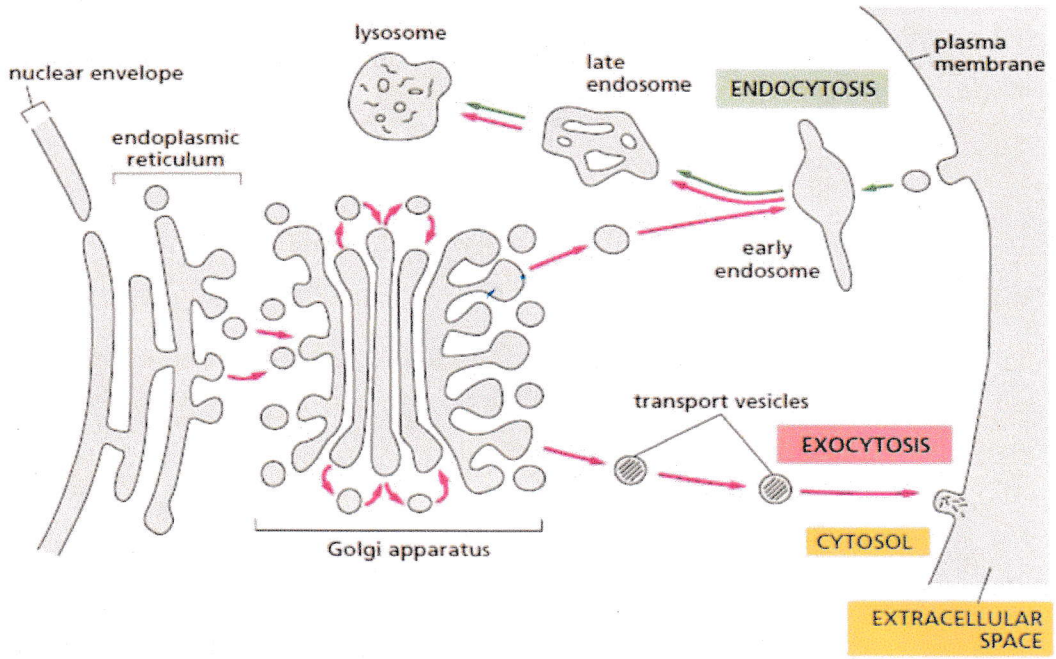


Figure 15–18 Transport vesicles bud from one membrane and fuse with another, carrying membrane components and soluble proteins between compartments of the endomembrane system and the plasma membrane. The membrane of each compartment or vesicle maintains its orientation, so the cytosolic side always faces the cytosol and the noncytosolic side faces the lumen of the compartment or the outside of the cell (see Figure 11–18). The extracellular space and each of the membrane-enclosed compartments (shaded *gray*) communicate with one another by means of transport vesicles, as shown. In the outward secretory pathway (*red* arrows), protein molecules are transported from the ER, through the Golgi apparatus, to the plasma membrane or (via early and late endosomes) to lysosomes. In the inward endocytic pathway (*green* arrows), extracellular molecules are ingested (endocytosed) in vesicles derived from the plasma membrane and are delivered to early endosomes and, usually, on to lysosomes via late endosomes.

❖ **Clathrin Coated vesicles:**

Vesicles that bud from membranes usually have a distinctive protein coat on their cytosolic surface and are therefore called coated vesicles. After budding from its parent organelle, the vesicle sheds its coat, allowing its membrane to interact directly with the membrane to which it will fuse. Cells produce several kinds of coated vesicles, each with a distinctive protein coat. The coat serves at least two functions: it helps shape the membrane into a bud and captures molecules for onward transport.

The best-studied vesicles are those that have an outer coat made of the protein **clathrin.** These *clathrin-coated vesicles* bud from both the Golgi apparatus on the outward secretory pathway and from the plasma membrane on the inward endocytic pathway. At the plasma membrane, for example, each vesicle starts off as a *clathrin-coated pit*. Clathrin molecules assemble into a basketlike network on the cytosolic surface of the membrane, and it is this assembly process that starts shaping the membrane into a vesicle. A small GTP-binding protein called ***dynamin*** assembles as a ring around the neck of each deeply invaginated coated pit. Together with other proteins recruited to the neck of the vesicle, the dynamin causes the ring to constrict, thereby pinching off the vesicle from its parent membrane. Other kinds of transport vesicles, with different coat proteins, are also involved in vesicular transport. They form in a similar way and carry their own characteristic sets of molecules between the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane.

The mechanism is best understood for clathrin-coated vesicles. Clathrin itself plays no part in choosing specific molecules for transport. This is the function of a second class of coat proteins called ***adaptins,*** which both secure the clathrin coat to the vesicle membrane and help select cargo molecules for transport. Molecules for onward transport carry specific *transport signals* that are recognized by *cargo receptors* in the Golgi or plasma membrane. Adaptins help capture specific cargo molecules by trapping the cargo receptors that bind them. In this way, a selected set of cargo molecules, bound to their specific receptors, is incorporated into the lumen of each newly formed clathrin-coated vesicle. There are different types of adaptins: the adaptins that bind cargo receptors in the plasma membrane, for example, are not the same as those that bind cargo receptors in the Golgi apparatus, reflecting the differences in the cargo molecules to be transported from each of these sources.

Another class of coated vesicles, called ***COP-coated vesicles*** (COP being shorthand for "coat protein"), is involved in transporting molecules between the ER and the Golgi apparatus and from one part of the Golgi apparatus to another.

Once a transport vesicle has reached its target, it must recognize and dock with its specific organelle. Only then can the vesicle membrane fuse with the target membrane and unload the vesicle's

cargo. The identification process depends on a diverse family of monomeric GTPases called **Rab** proteins. Specific Rab proteins on the surface of each type of vesicle are recognized by corresponding *tethering proteins* on the cytosolic surface of the target membrane. Each organelle and each type of transport vesicle carries a unique combination of Rab proteins, which serve as molecular markers for each membrane type. The coding system of matching Rab and tethering proteins helps to ensure that transport vesicles fuse only with the correct membrane.

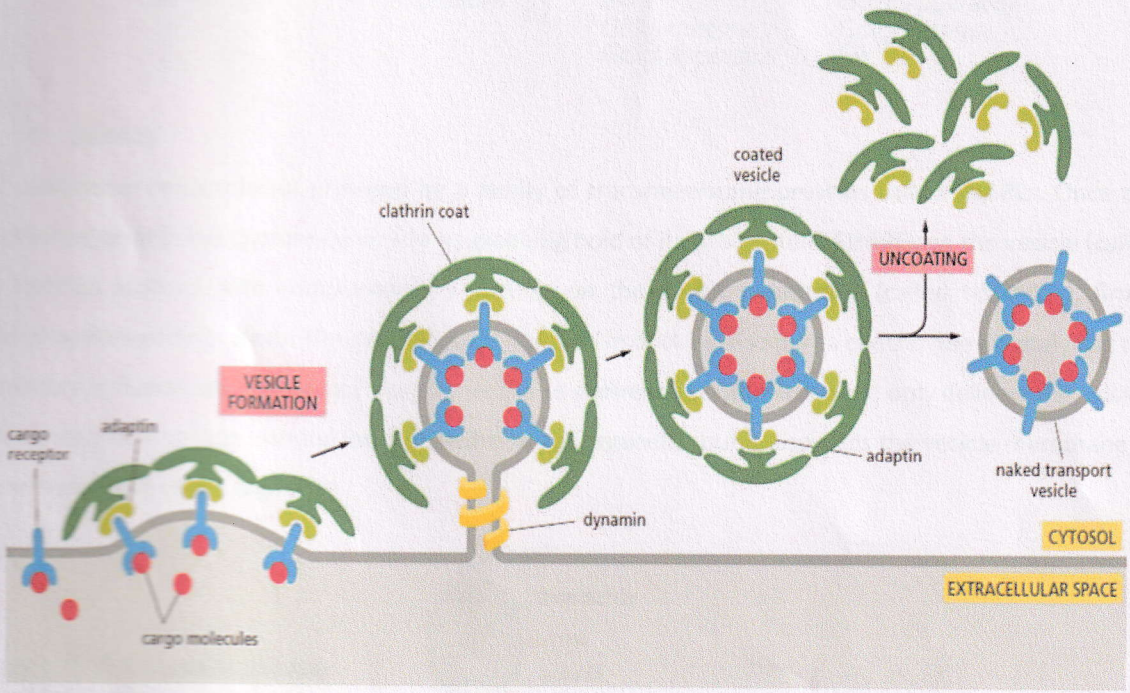


Figure 15–20 Clathrin-coated vesicles transport selected cargo molecules. Here, as in Figure 15–19, the vesicles are shown budding from the plasma membrane. Cargo receptors, with their bound cargo molecules, are captured by adaptins, which also bind clathrin molecules to the cytosolic surface of the budding vesicle (**Movie 15.5**). Dynamin proteins assemble around the neck of budding vesicles; once assembled, the dynamin molecules—which are monomeric GTPases (discussed in Chapter 16)—hydrolyze their bound GTP and, with the help of other proteins recruited to the neck (not shown), pinch off the vesicle. After budding is complete, the coat proteins are removed, and the naked vesicle can fuse with its target membrane. Functionally similar coat proteins are found in other types of coated vesicles.

TABLE 15–4 SOME TYPES OF COATED VESICLES			
Type of Coated Vesicle	**Coat Proteins**	**Origin**	**Destination**
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COP-coated	COP proteins	ER Golgi cisterna Golgi apparatus	Golgi apparatus Golgi cisterna ER

❖ **SNAREs:**

Additional recognition is provided by a family of transmembrane proteins called SNAREs. Once the tethering protein has captured a vesicle by grabbing hold of its Rab protein, SNAREs on the vesicle (called v-SNAREs) interact with complementary SNAREs on the target membrane (called t-SNAREs), firmly docking the vesicle in place. The same SNAREs involved in docking also play a central role in catalyzing the membrane fusion required for a transport vesicle to deliver its cargo. Fusion not only delivers the soluble contents of the vesicle into the interior of the target organelle, but it also adds the vesicle membrane to the membrane of the organelle.

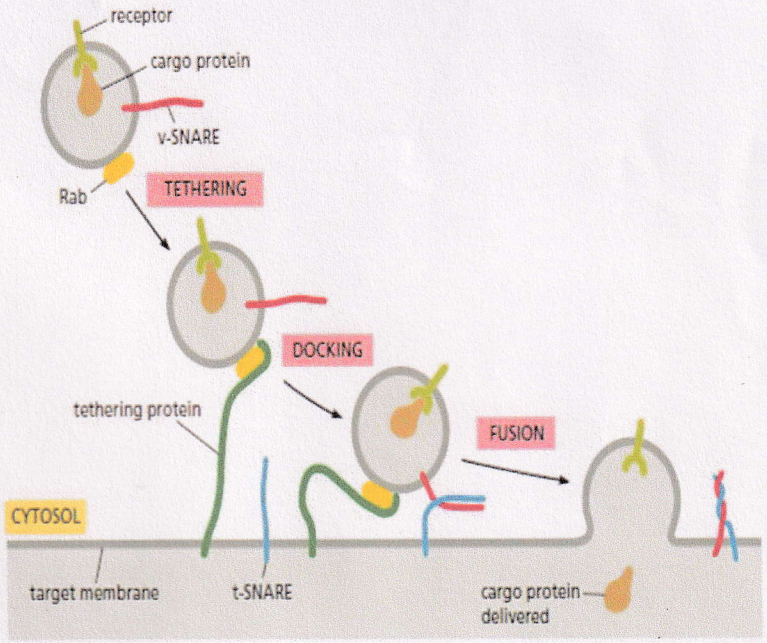


Figure 15–21 Rab proteins, tethering proteins, and SNAREs help direct transport vesicles to their target membranes. A filamentous tethering protein on a membrane binds to a Rab protein on the surface of a vesicle. This interaction allows the vesicle to dock on its particular target membrane. A v-SNARE on the vesicle then binds to a complementary t-SNARE on the target membrane. Whereas Rab and tethering proteins provide the initial recognition between a vesicle and its target membrane, complementary SNARE proteins ensure that transport vesicles dock at their appropriate target membranes. These SNARE proteins also catalyze the final fusion of the two membranes (see Figure 15–22).