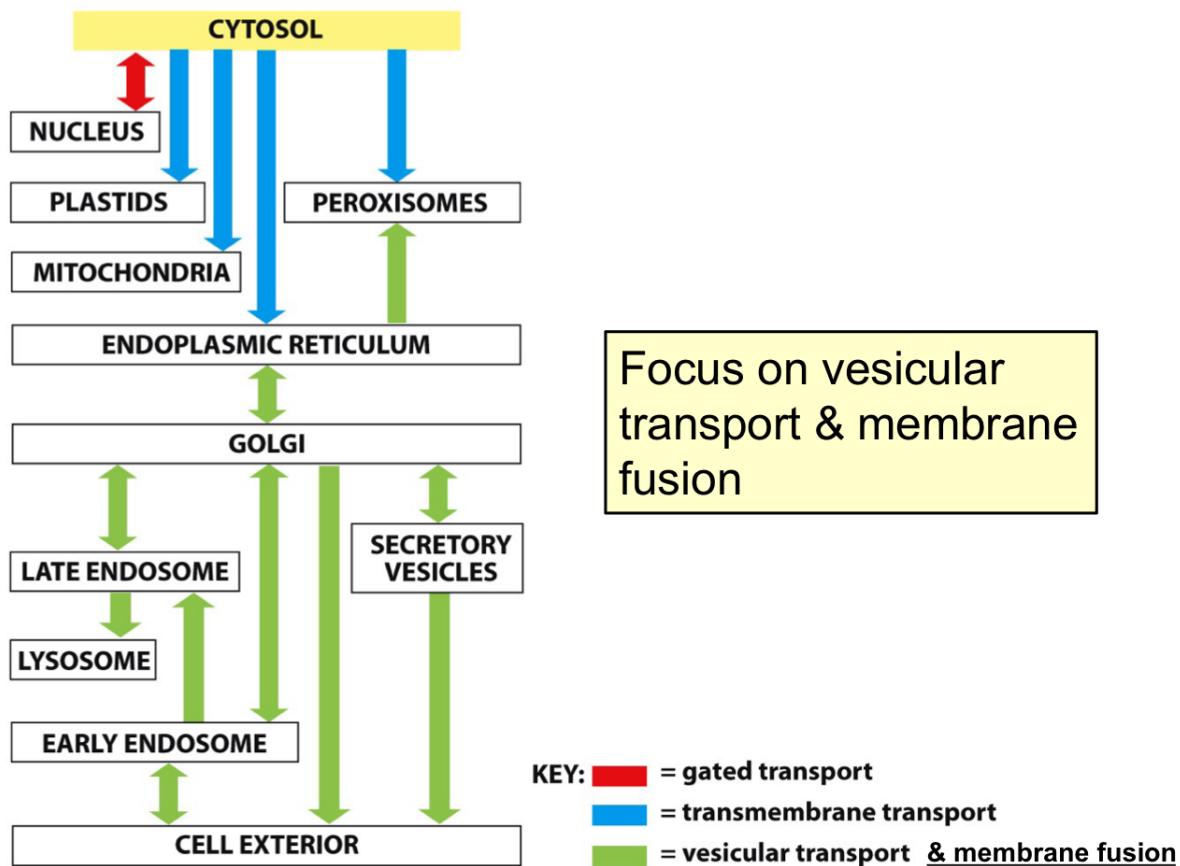


Membrane Trafficking

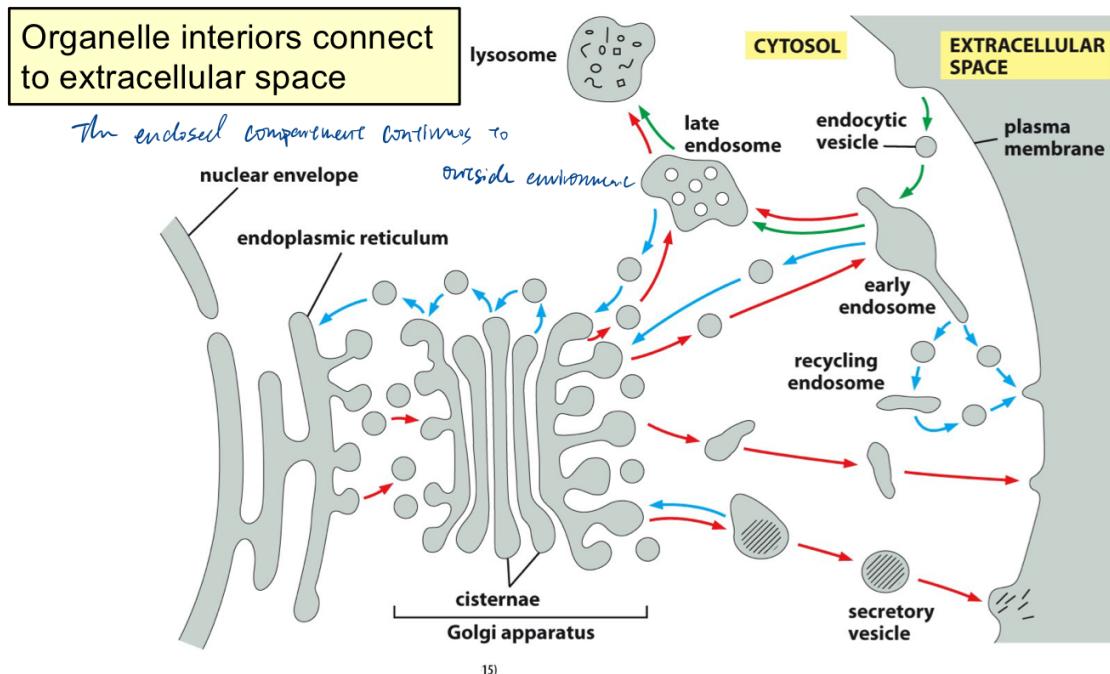
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SECRETION/EXOCYTOSIS/ORGANELLE BIOGENESIS

ENDOCYTOSIS and the ENDOCYTIC PATHWAY

RETROGRADE TRANSPORT or RECYCLING



▼ Coated vesicles

- Clathrin: mediate transport originating **from Golgi to late endosome; early endosome to plasma membrane & from plasma membrane to early endosome — mostly endocytosis**
- COPI-coated: from Golgi to cytosol; between Golgi cisternae; from Golgi to ER
- COPII: ER to Golgi
- Retromer: early endosome to late endosome
- **The coats are usually double layered**

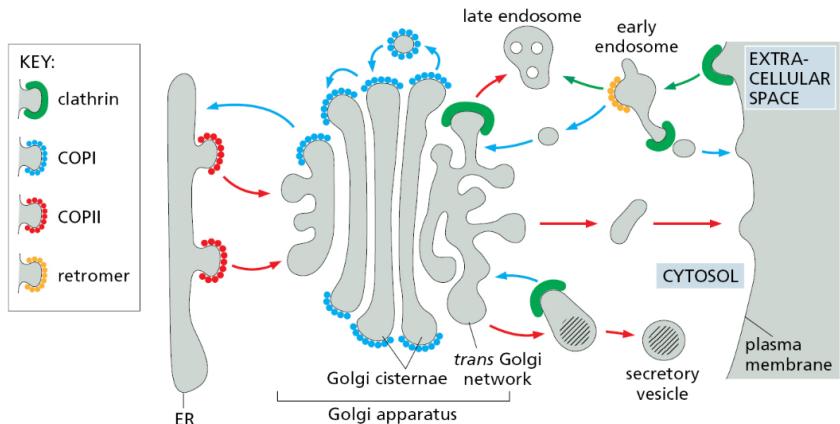


Figure 13–5 Use of different coats for different steps in vesicle traffic. Different coat proteins select different cargo and shape the transport vesicles that mediate the various steps in the secretory and endocytic pathways. When the same coats function in different places in the cell, they usually incorporate different coat protein subunits that modify their properties (not shown). Many differentiated cells have additional pathways besides those shown here, including a sorting pathway from the *trans* Golgi network to the apical surface of epithelial cells and a specialized recycling pathway for proteins of synaptic vesicles in the nerve terminals of neurons (see Figure 11–38). The arrows are colored as in Figure 13–3.

▼ Clathrin structure — outer layer of the coat

- heavy chain (large subunit) and light chain (small subunit) form a triskelion
- triskelions then assemble into a basketlike framework of hexagons or pentagons on the cytosolic surfaces of membranes
 - can spontaneously self-assemble into the cage-like structure — help bend the membranes to form vesicles — clathrin triskelions (the coat proteins) determine the geometry
 - clathrin assembly induces formation of coated buds, which then pinch off to become clathrin-coated vesicles

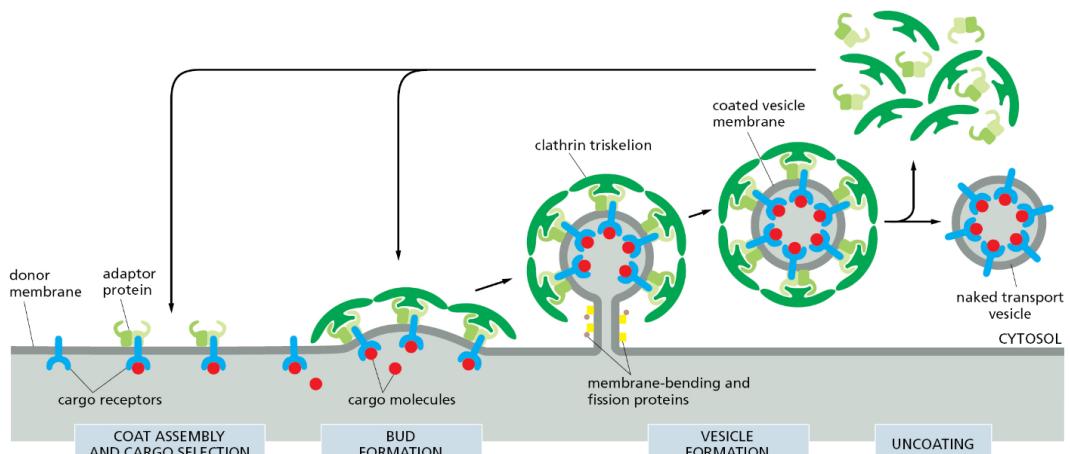


Figure 13–8 The assembly and disassembly of a clathrin coat. The assembly of the coat introduces curvature into the membrane, which leads in turn to the formation of a coated bud (called a coated pit if it is in the plasma membrane). The adaptor proteins bind both clathrin triskelions and membrane-bound cargo receptors, thereby mediating the selective recruitment of both membrane and soluble cargo molecules into the vesicle. Other membrane-bending and fission proteins are recruited to the neck of the budding vesicle, where sharp membrane curvature is introduced. The coat is rapidly lost shortly after the vesicle buds off.

- formation of lattice is energetically favourable! — uncoating is E unfavourable
- ▼ Adaptor protein — inner layer of the coat: between clathrin cage and cytosolic surface of membrane
 - cargo receptors: transmembrane receptors that capture soluble cargo
 - Different membranes have different types of **recognition molecules** for recruiting adaptor proteins, but many of them are **different types of phosphoinositides** → different types of adaptor proteins can recognise **different head groups for phosphoinositides**
 - each type of adaptor protein is specific for transmembrane cargoes and cargo receptors sharing amino acid sequence motif on the cytosolic tail
 - protein modifications such as ubiquitination & phosphorylation offer extra recognition motif for adaptor proteins
 - each type of adaptor protein directs assembly of clathrin to only particular membranes
 - **AP2 as an example, is a tetramer**
- ▼ The mechanism of association of adaptor proteins and initiate endocytosis process
 - AP2 in locked conformation binds to the head groups of PI(4,5)P₂ located at the membrane — conformational change and expose σ₂ and μ₂ — able to bind to the cytosolic tail of cargo receptors — induces curvature
 - For AP1 — binds PI(4)P on Golgi & endosomal membrane

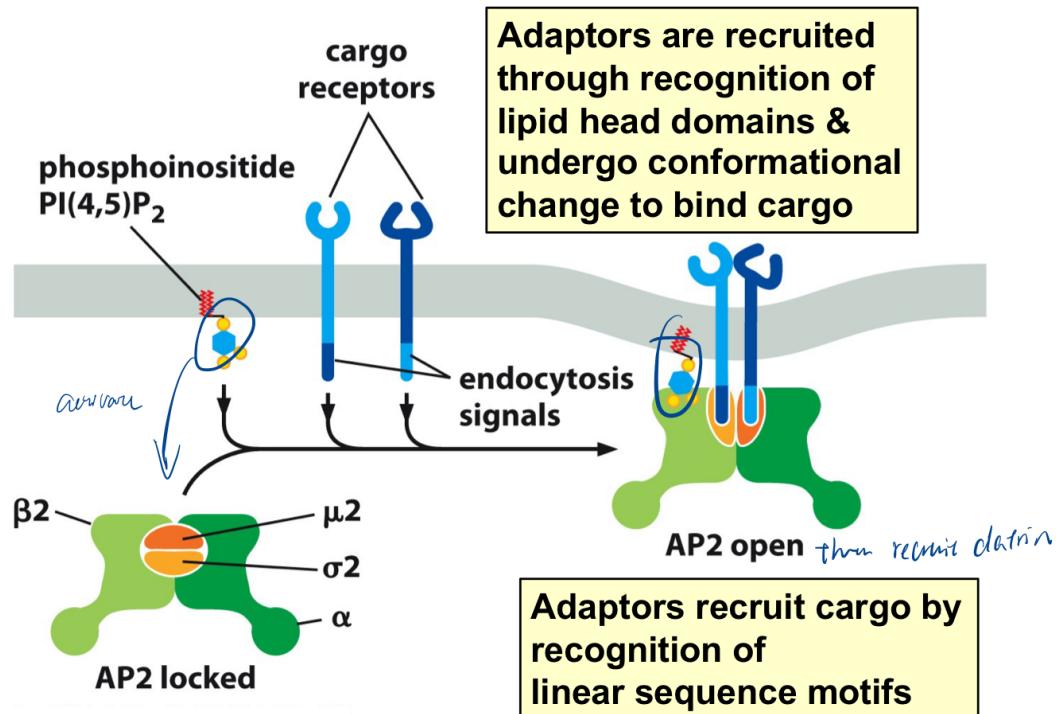
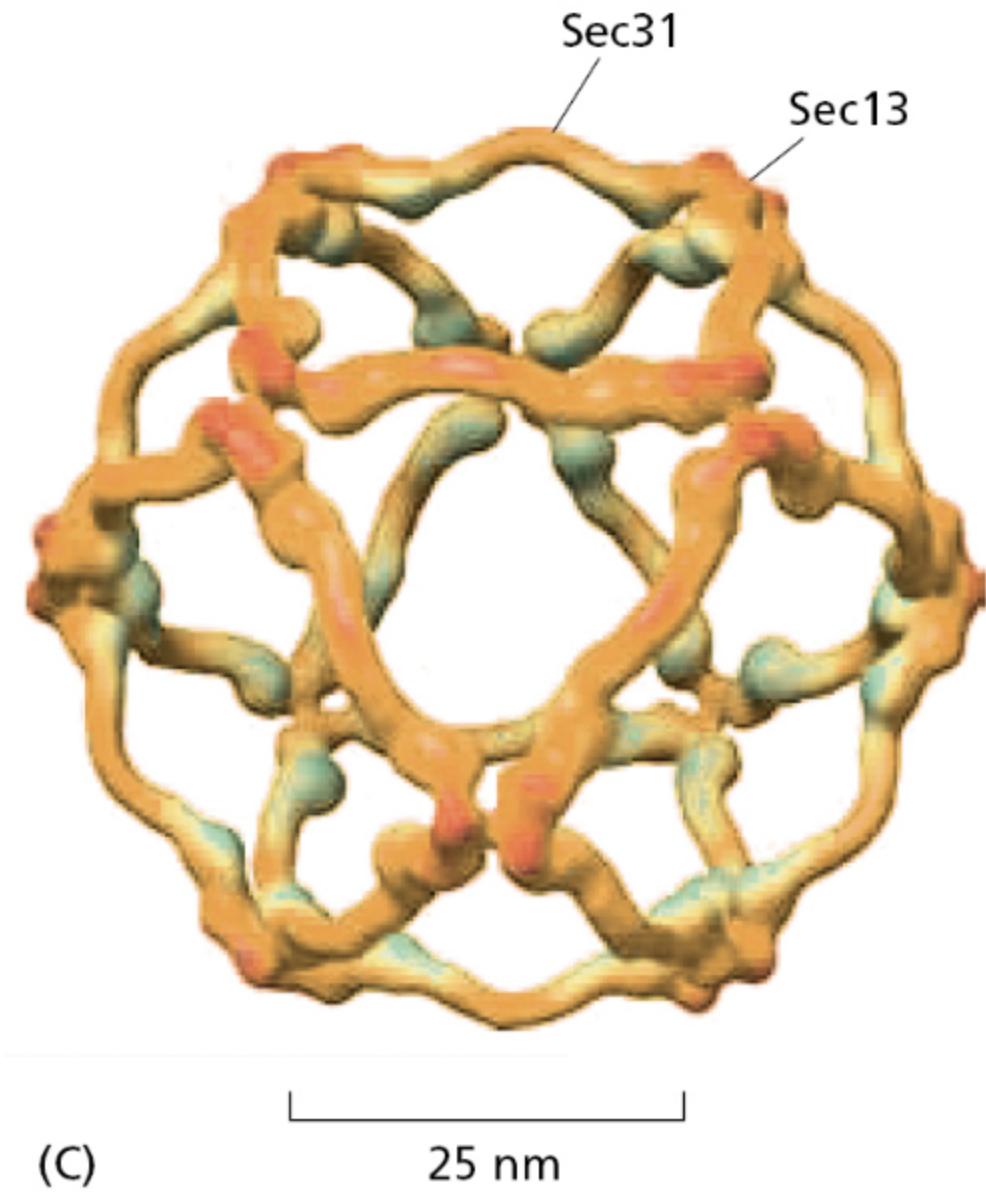


Fig 13-9 MBoC-6th Edition-Garland Science 2015

- Other than local production of PIPs to assemble clathrin coats on **plasma membrane & golgi** — **coat-recruitment GTPases** regulate the assembly of COPI coats on Golgi membrane; COPII coats on ER and retromer & clathrin on endosome
- ▼ COPII — less dense than Clathrin → ↑flexible
 - composed of Sec31 & 13



▼ coat-recruitment GTPase — monomeric GTPase — **they are not adaptor proteins!**

- ARF → **COPⅠ** & clathrin coats at Golgi membranes
- Sar1 → **COPⅡ** at ER
- Rab7 → retromer

- disassembly of Clathrin and COPII requires GTP hydrolysis of their active adaptor proteins (ARF and Sar1)

Vesicle uncoating requires energy

*x energy
favour*

- In addition to lipid recognition, the GTPases ARF1 and Sar1 control coat recruitment for some coats.

COPI coats are recruited by ARF1

COPII coats are recruited by SAR1

Clathrin adaptor AP1 is recruited by ARF1

ATPase

- GTP hydrolysis of ARF1 and SAR1 triggers disassembly of COPI and COPII coated vesicles.

hsc70 ATPase

- Clathrin-coated vesicles at the plasma membrane *only* (AP2 adaptor) require Hsp70 family ATPase activity for uncoating

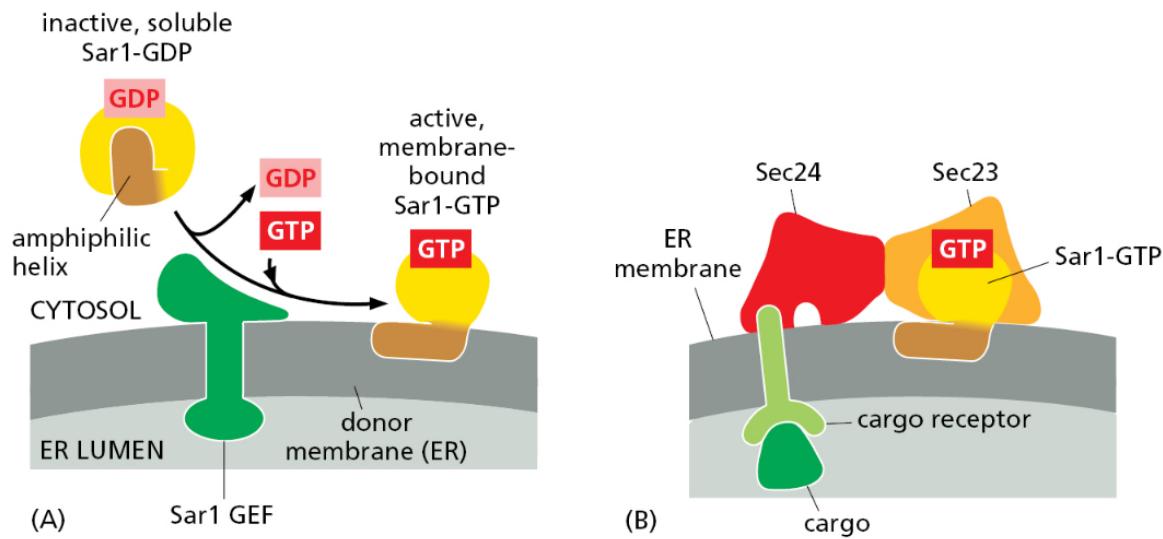
binds to clathrin - hydrolyse ATP → twist structure.

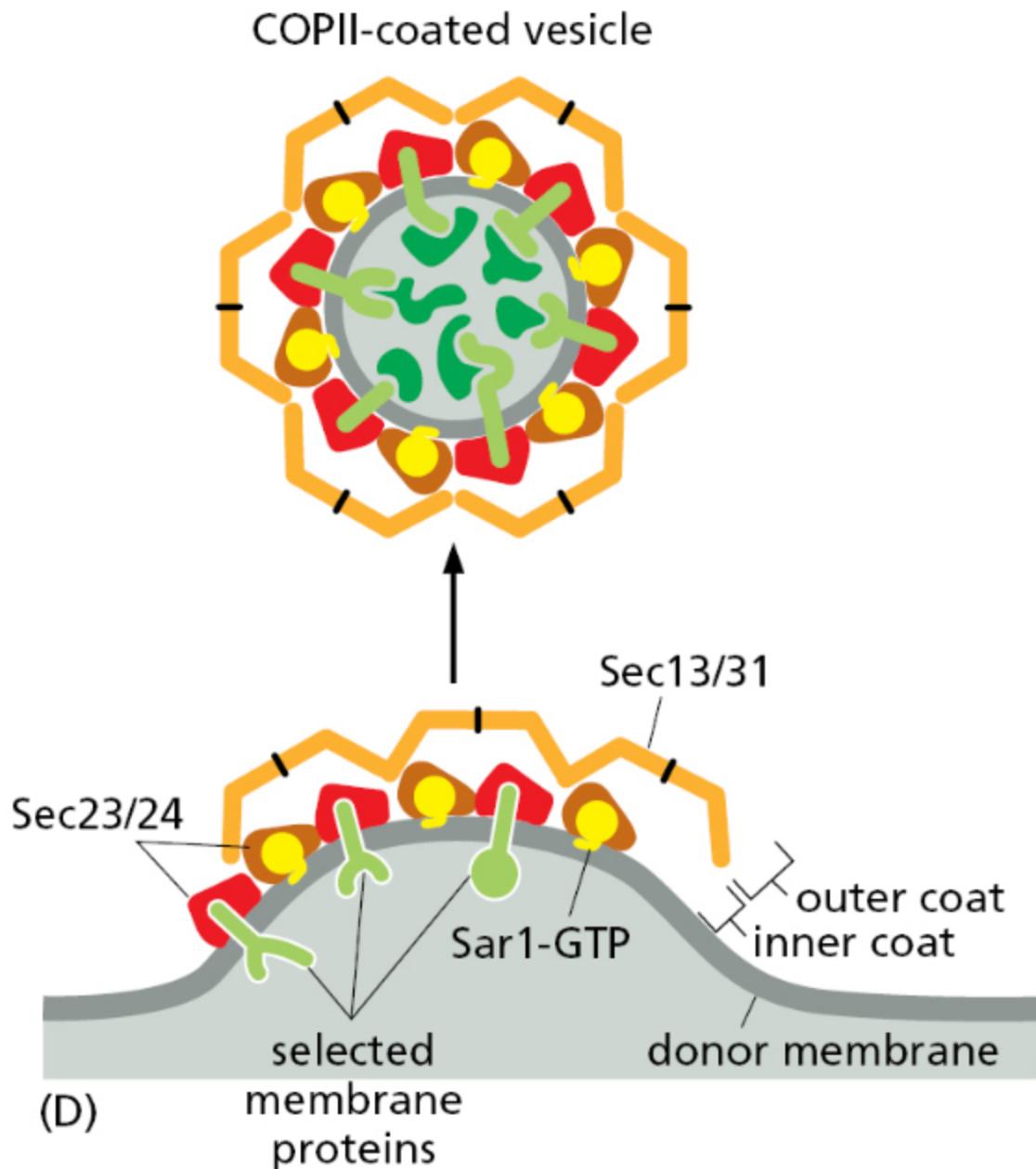
- Clathrin-coated vesicles formed internally (AP1 adaptor) require Hsp70 activity plus GTP hydrolysis of ARF1

more tense structure
↓
EFT to
disassembly

- ▼ Sar1 mechanism as a represented example

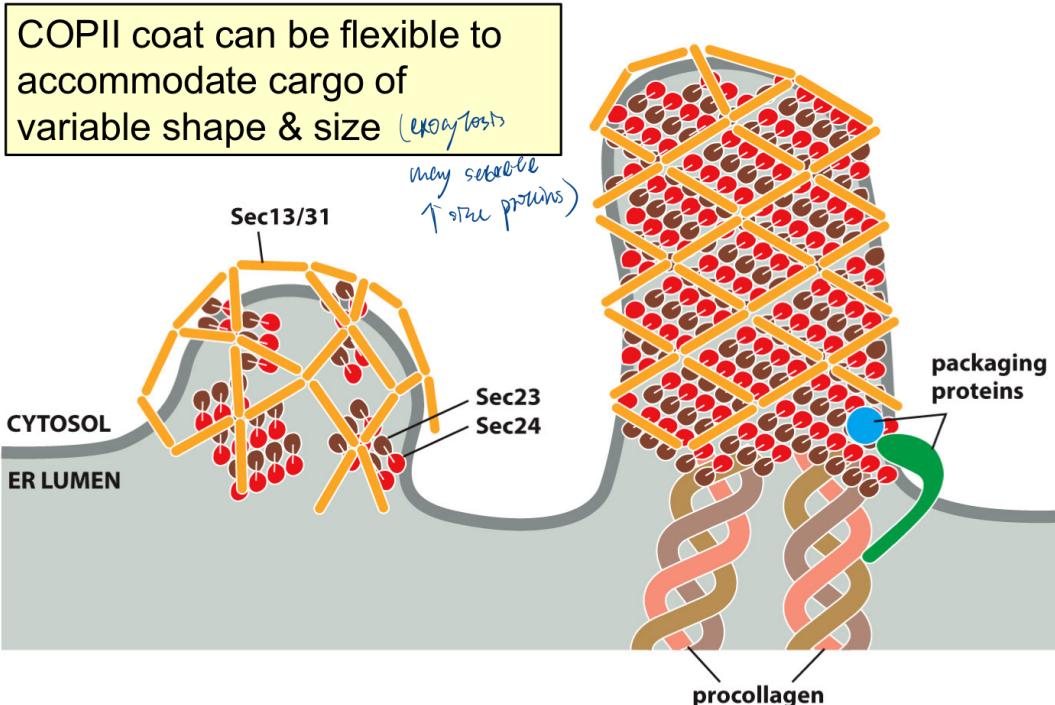
Sec24 is the adaptor protein for COPII coats!!





▼ For secretion (COPII particularly,) requires adaptation for larger molecules (collagen & lipoprotein)

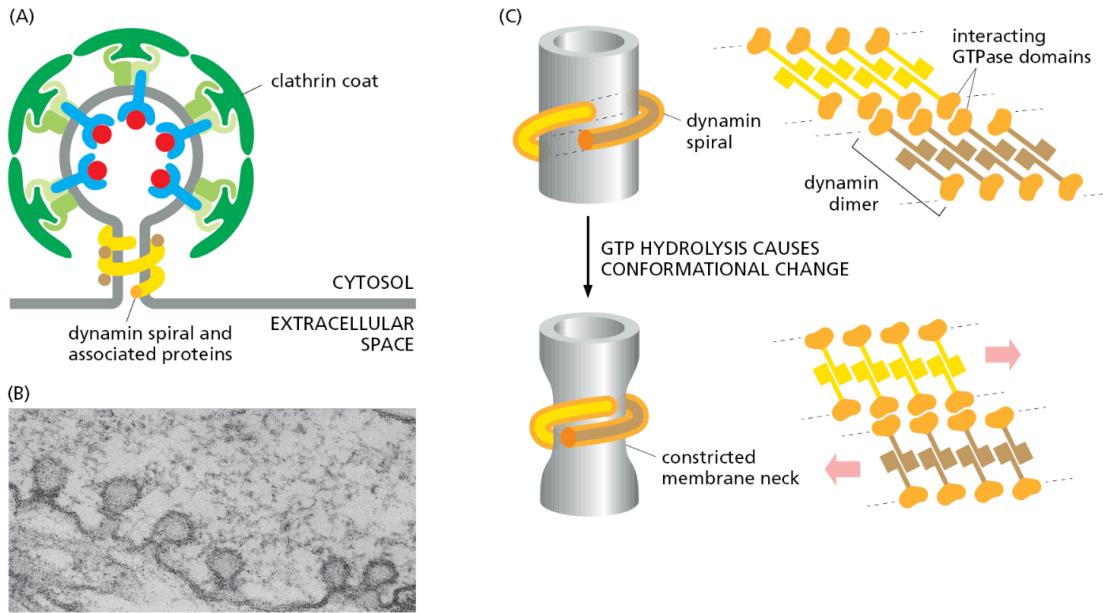
- collagen is synthesised in ER to be secreted to extracellular matrix; at ER, they are assembled as **procollagen rods**
- Those large cargoes bind to the transmembrane *protein packaging proteins* in ER → control the assembly of COPII coats:



special packaging proteins sense the cargo and modify the coat assembly process. This interaction recruits the COPII inner coat protein Sec24 and locally enhances the rate with which Sar1 cycles on and off the membrane (not shown). In addition, a monoubiquitin (not shown) is added to the Sec31 protein, changing the assembly properties of the outer cage. Sec23/24 proteins arrange in larger arrays, and Sec13/31 proteins arrange in a regular lattice of diamond shapes. As a result, a large tubular vesicle is formed that can accommodate the large cargo molecules. The packaging proteins are not part of the budding vesicle but remain in the ER.

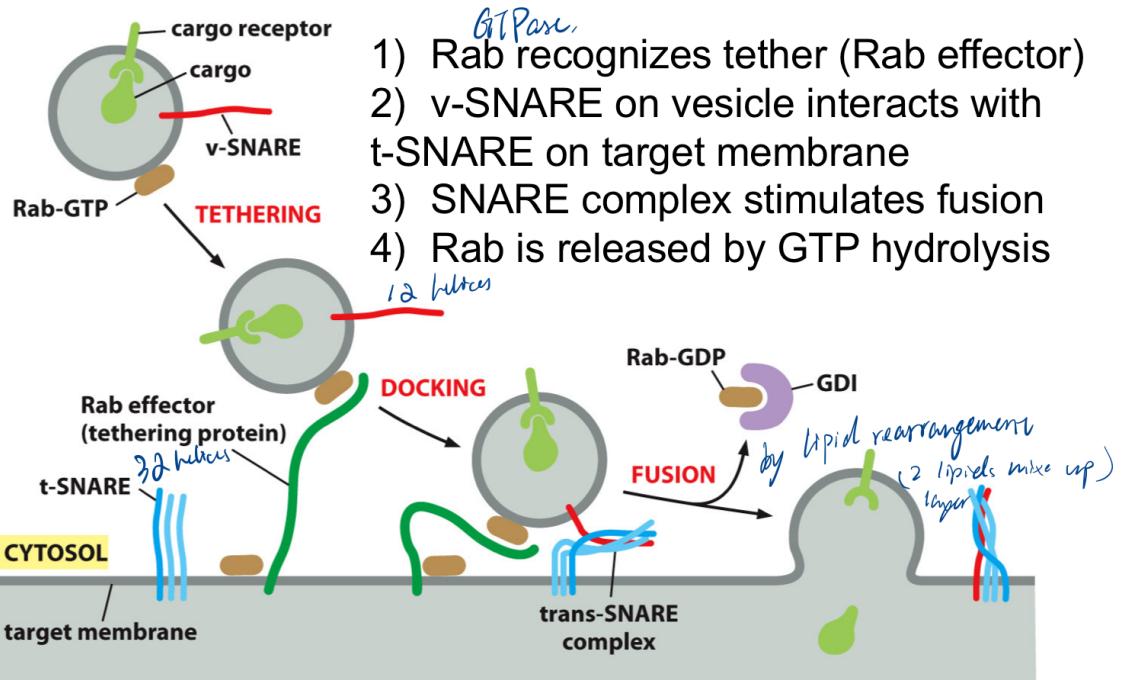
▼ Scission and formation of the coated vesicles — Dynamin: GTPase

- phosphoinositide-binding domain: recruit dynamin to membrane
- GTPase domain: regulate the pinching-off rate



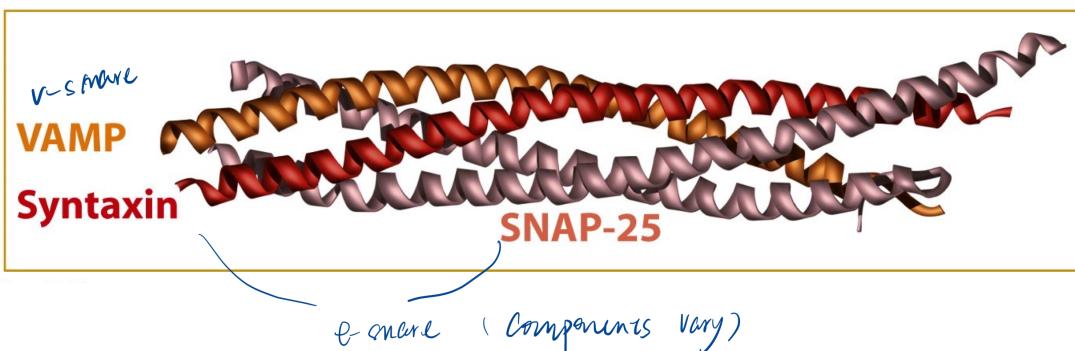
The pinching-off process brings the two noncytosolic leaflets of the membrane at the bud neck into close proximity and seals off the forming vesicle (see Figure 13–2). To perform this task, dynamin assembles in a ring around the neck, then undergoes a conformational change when it hydrolyzes its bound GTP. This constricts the dynamin ring together with the underlying membrane at the bud neck. In addition, dynamin may recruit lipid-modifying enzymes that change the lipid composition locally at the neck of the bud to facilitate membrane fusion.

▼ Membrane fusion

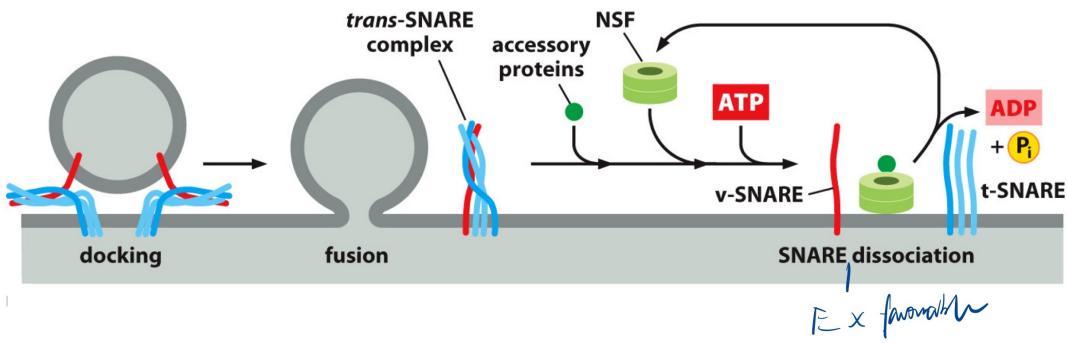


in 13-16 MRBC 6th Edition - Garland Science 2015

SNARE complex



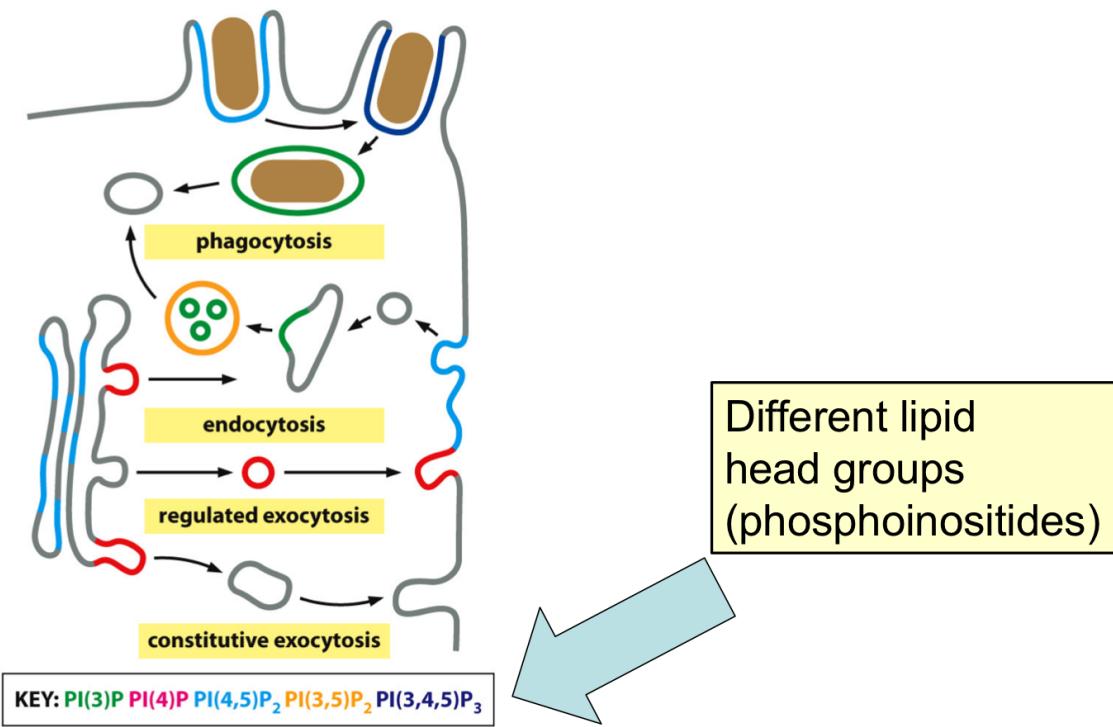
- disassembly of snare complex is not E favourable



- NSF is a ATPase — use the energy from ATP hydrolysis to disassemble paired SNARE complex.
- After the disassembly, the SNARE proteins are retrieved to original membrane, and the energy from disassembly can then drive a new round of vesicular transport

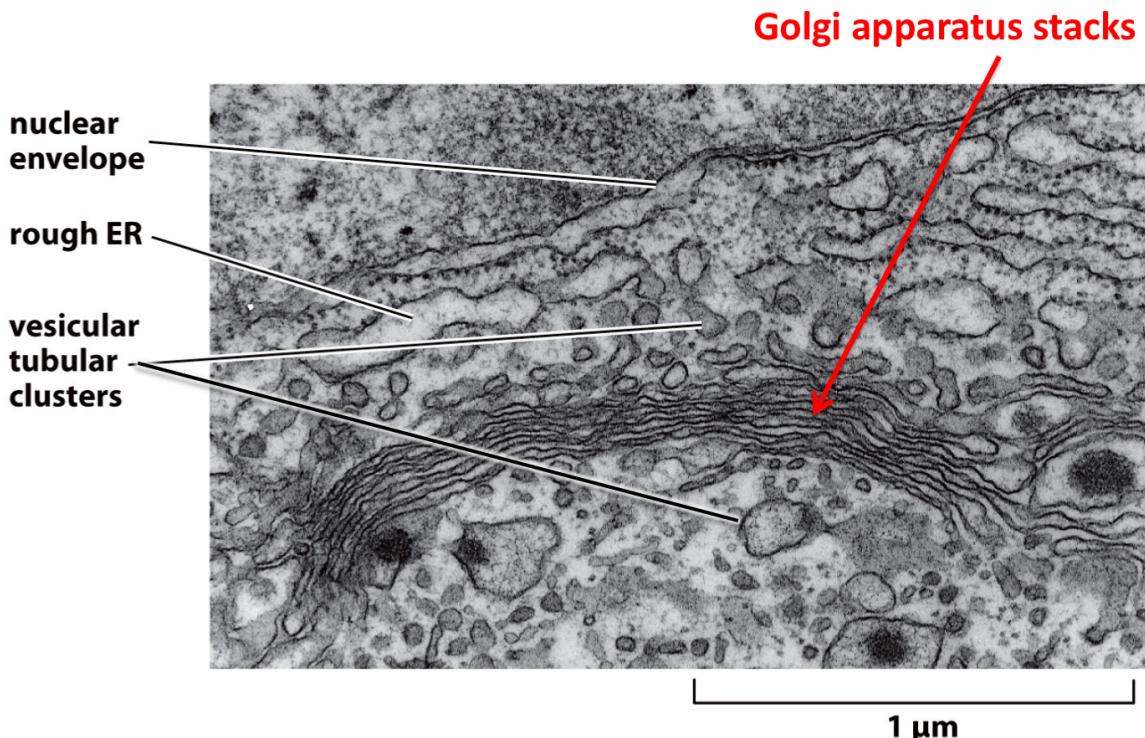
▼ dictating the trafficking specificity

- By the types of coating & types of Rab proteins
- therefore, the very essential determinant is the head groups of phosphoinositides on that organelle membrane



Transport through Golgi

- Homotypic fusion: fusion of membranes from the same compartment
- Heterotypic fusion: fusion of a membrane from one compartment with the membrane of a different compartment
- both fusion requires the matching and pairing of SNAREs complex



- Proteins in ER have to be correctly folded or assembled to be further transported to Golgi — quality control
 - The misfolded then are captured at ER by chaperone proteins
 - The exit step from ER is a major checkpoint where quality control is exerted on the proteins
 - The quality-control step prevents the onward transport of misfolded or misassembled proteins
 - Drawback of stringent quality control: Cl⁻ channels are retained in ER in cystic fibrosis — not solely because of mutation inactivate the protein (actually the mutant protein can function almost normally) but also the active degradation

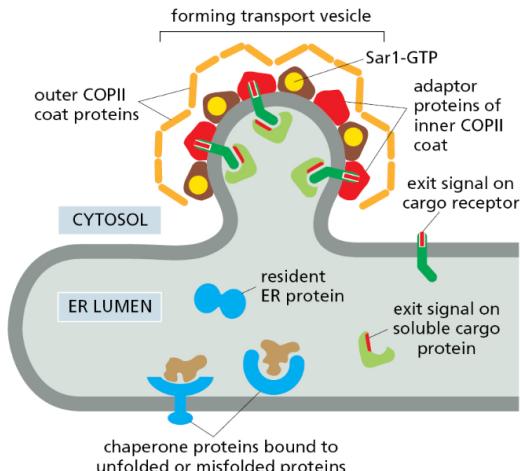


Figure 12-23 The recruitment of membrane and soluble cargo molecules into ER transport vesicles. Transmembrane proteins are packaged into budding transport vesicles through interactions of exit signals on their cytosolic tails with adaptor proteins of the inner COPII coat. Some of these transmembrane proteins function as cargo receptors, binding soluble proteins in the ER lumen and helping to package them into vesicles. Other proteins may enter the vesicle by bulk flow. A typical 50-nm transport vesicle contains about 200 transmembrane proteins, which can be of many different types. As indicated, unfolded or incompletely assembled proteins are bound to chaperones and transiently retained in the ER compartment.

The structures formed when ER-derived vesicles fuse with one another are called **vesicular tubular clusters**, because they have a convoluted appearance in the electron microscope (Figure 13-25A). These clusters constitute a compartment that is separate from the ER and lacks many of the proteins that function in the ER. They are generated continually and function as transport containers that bring material from the ER to the Golgi apparatus. The clusters move quickly along microtubules to the Golgi apparatus with which they fuse (Figure 13-25B).

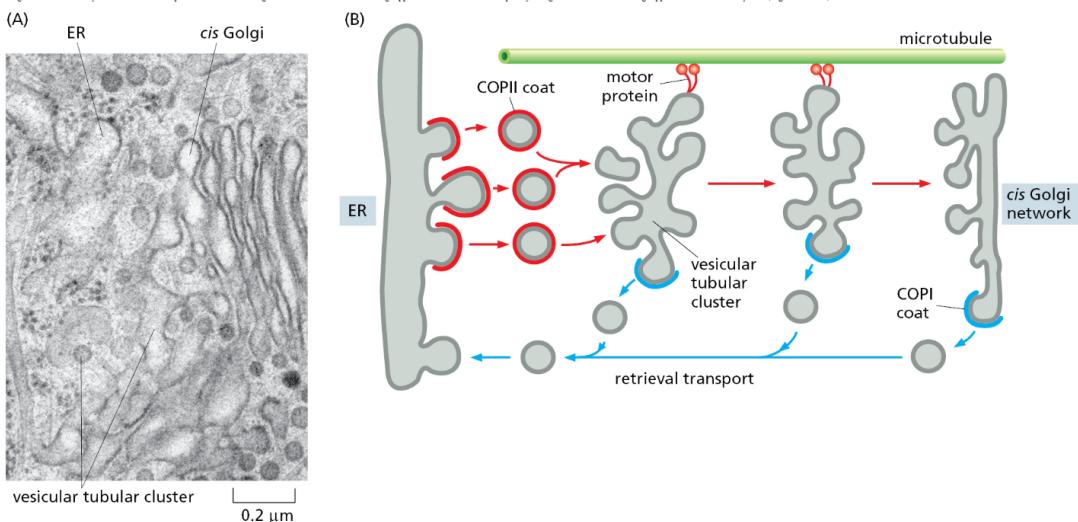


Figure 13-25 Vesicular tubular clusters. (A) An electron micrograph of vesicular tubular clusters forming around an exit site. Many of the vesicle-like structures seen in the micrograph are cross sections of tubules that extend above and below the plane of this thin section and are interconnected. (B) Vesicular tubular clusters move along microtubules to carry proteins from the ER to the Golgi apparatus. COPII coats mediate the budding of vesicles that return to the ER from these clusters (and from the Golgi apparatus). (A, courtesy of Judith Klimperman, from J.A. Martinez-Moreno et al., *Cell* 98:81–90, 1999.)

▼ Structure of Golgi network

- $cis \rightarrow trans$
- each layer contains specific modifying enzymes: