Intracellular vesicular traffic

B. Balen

Three types of transport in eukaryotic cells

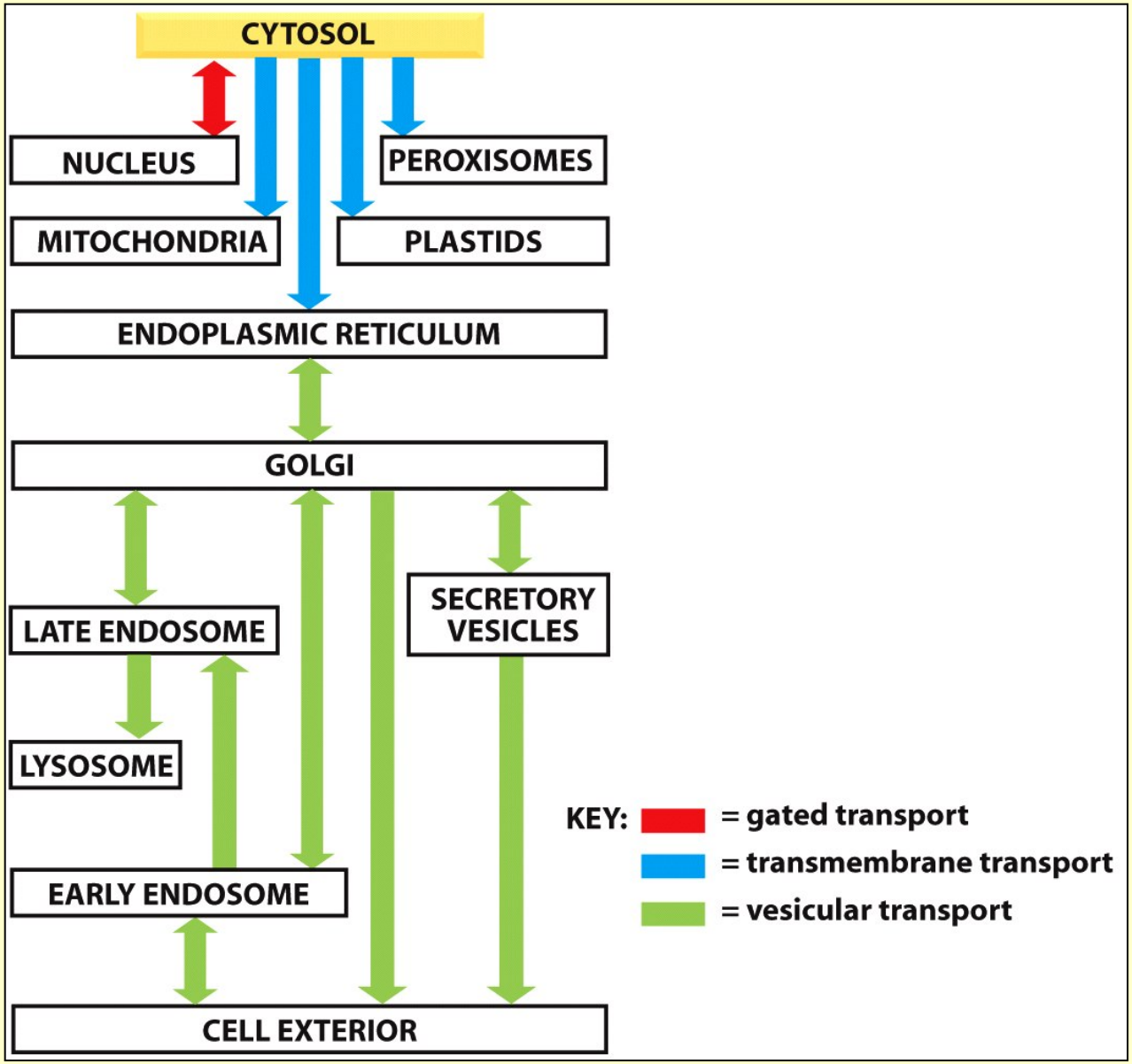
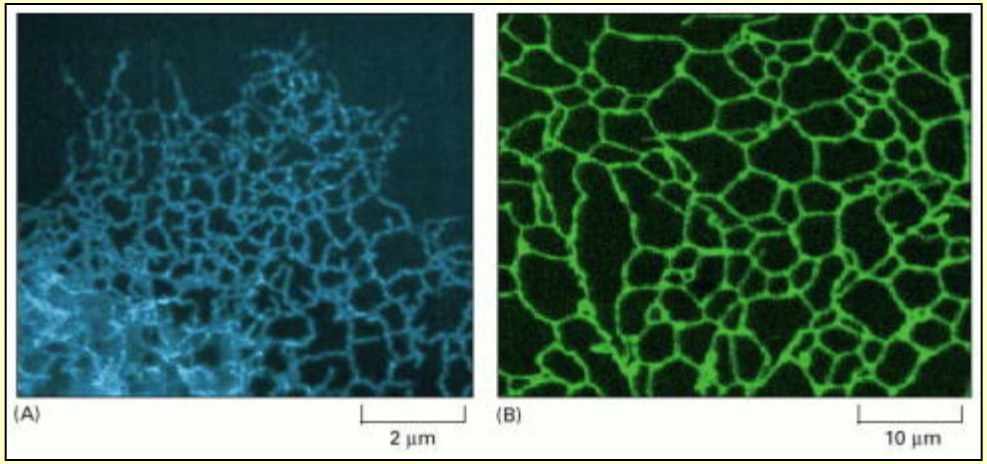


Figure 12-6 *Molecular Biology of the Cell* (© Garland Science 2008)

Endoplasmic reticulum

Endoplasmic reticulum (ER)

- ✓ in all eucaryotic cells
- ✓ its membrane typically constitutes ½ of the total membrane of an average animal cell
- ✓ organized into a netlike labyrinth of branching tubules and flattened sacs extending throughout the cytosol



(A) Part of the ER network in a cultured mammalian cell, stained with an antibody that binds to a protein retained in the ER

(B) Part of an ER network in a living plant cell that was genetically engineered to express a fluorescent protein in ER

Figure 12-34a *Molecular Biology of the Cell* (© Garland Science 2008)

ER has a central role in protein and lipid biosynthesis

✓ ER membrane:

→ production of all the transmembrane proteins for most of the cell organelles (ER, GA, lysosomes, endosomes, secretory vesicles and plasma membrane)

→ production of all lipids for most of organelles (ER, GA, lysosomes, endosomes, secretory vesicles and plasma membrane)

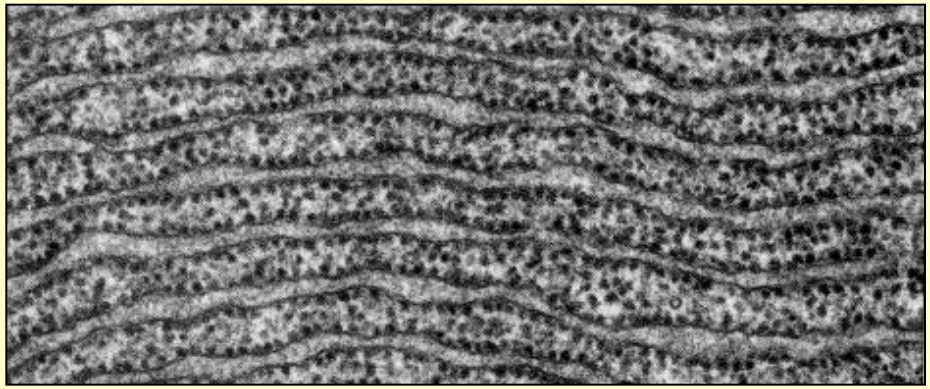
→ most of the lipids of mitochondrial and peroxisomal membranes

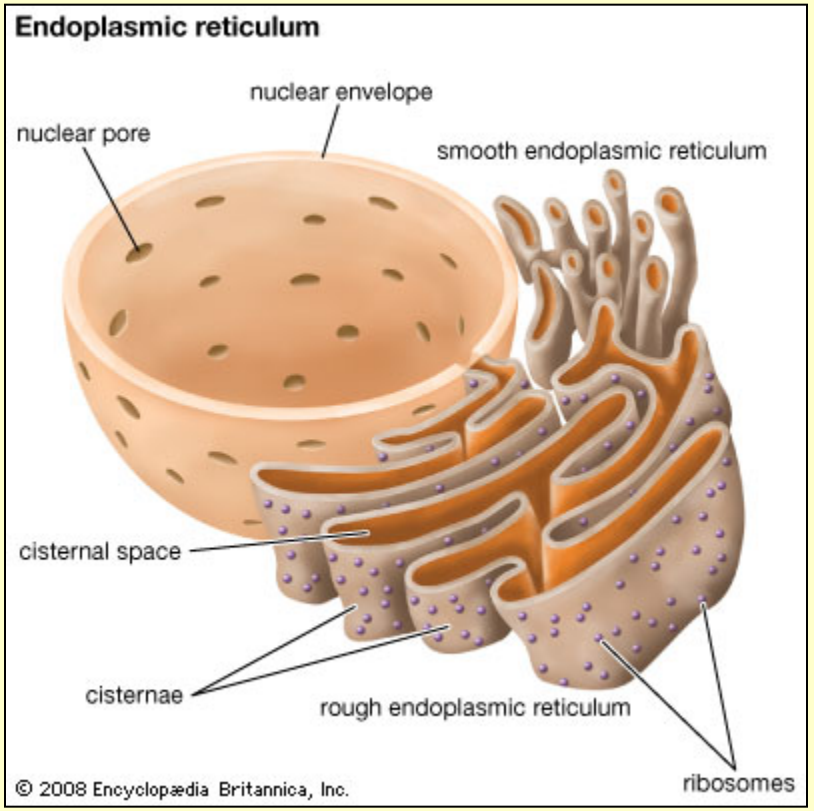
✓ ER lumen:

→ almost all of the proteins that will be secreted to the cell exterior and those destined for the lumen of the ER, GA or lysosomes → are initially delivered to the ER lumen

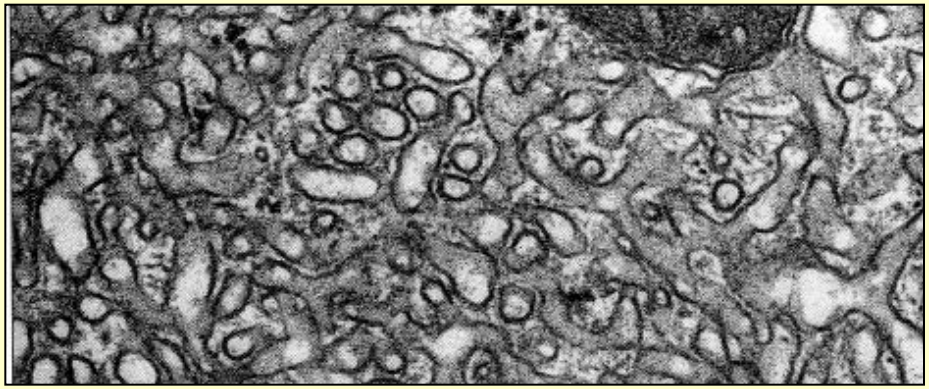
Two parts of ER

✓ **rough ER (rER) –** ribosomes



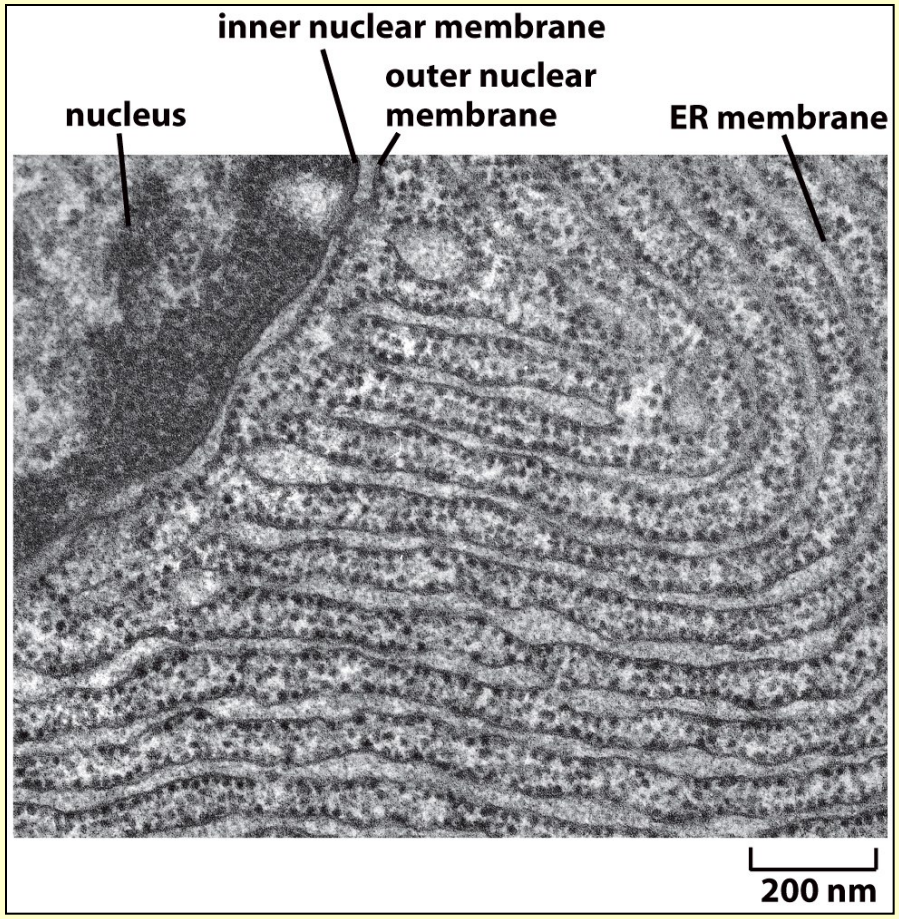


✓**smooth ER (sER)**



Rough ER

- ribosomes on cytoplasmic side of ER membrane
- protein synthesis



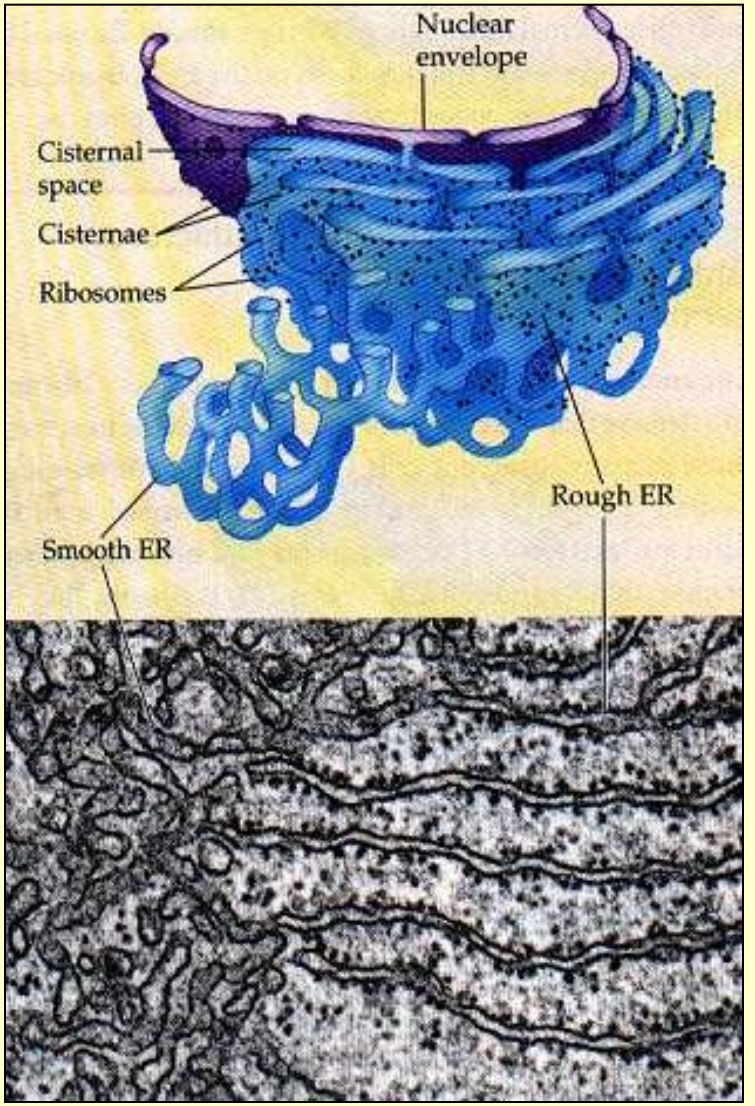


Figure 12-36a *Molecular Biology of the Cell* (© Garland Science 2008)

2002. Alberts et al.

Import of proteins into ER

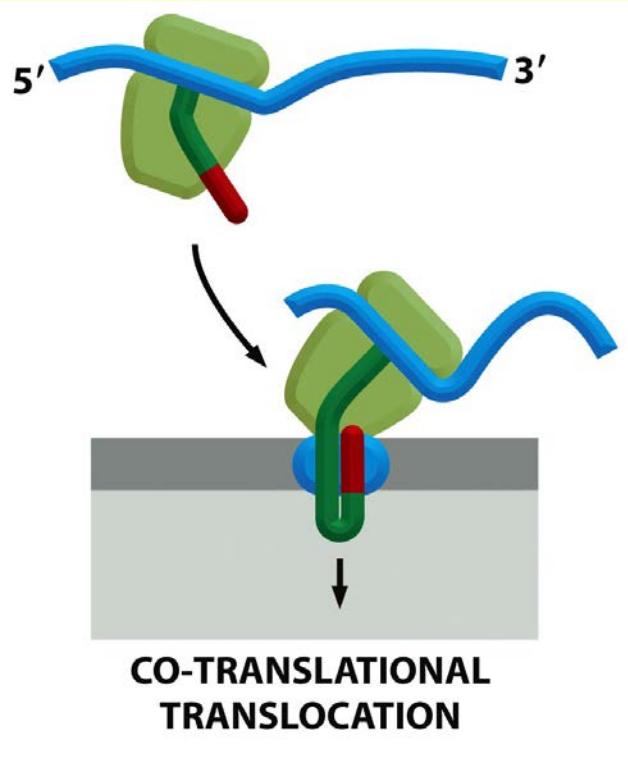
✓before the polypeptide chain is completely synthesized → **co-translational process**

✓one end of the protein is translocated into the ER as the rest of the polypeptide chain is being made

✓protein is never released into the cytosol and is never in danger of folding up before reaching the translocator in the ER membrane

✓chaperone proteins are not required to keep the protein unfolded

✓ ribosome that is synthesizing the protein is directly attached to the ER membrane



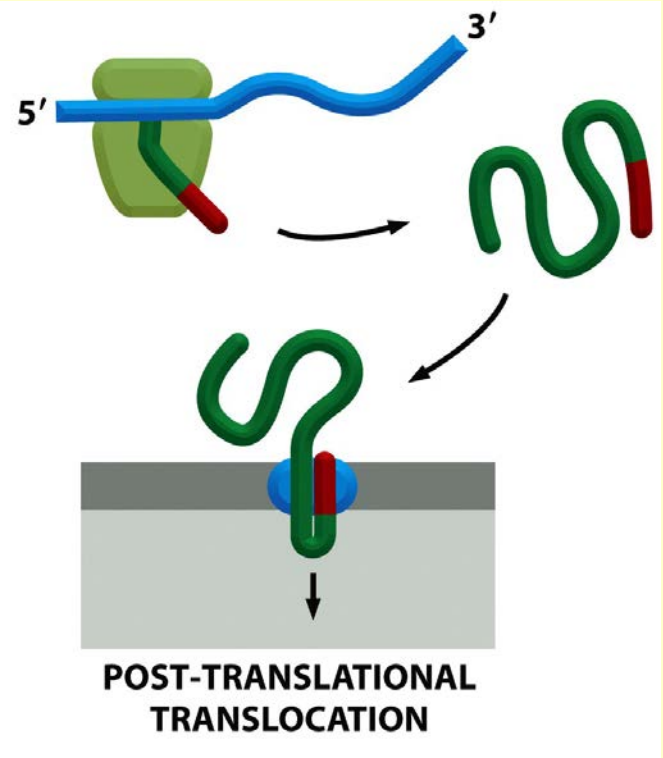


Figure 12-35a *Molecular Biology of the Cell* (© Garland Science 2008)

Two spatially separate populations of ribosomes in the cytosol

✓free ribosomes
→ unattached to any membrane
→ synthesize all proteins encoded by the nuclear genome that are not translocated to ER

✓membrane-bound ribosomes
→ attached to the cytosolic side of the ER
→ engaged in the synthesis of proteins concurrently translocated into ER

➢membrane-bound and free ribosomes are structurally and functionally identical

➢they differ only in the proteins they are making at any given time

➢signal directs the ribosome to the ER

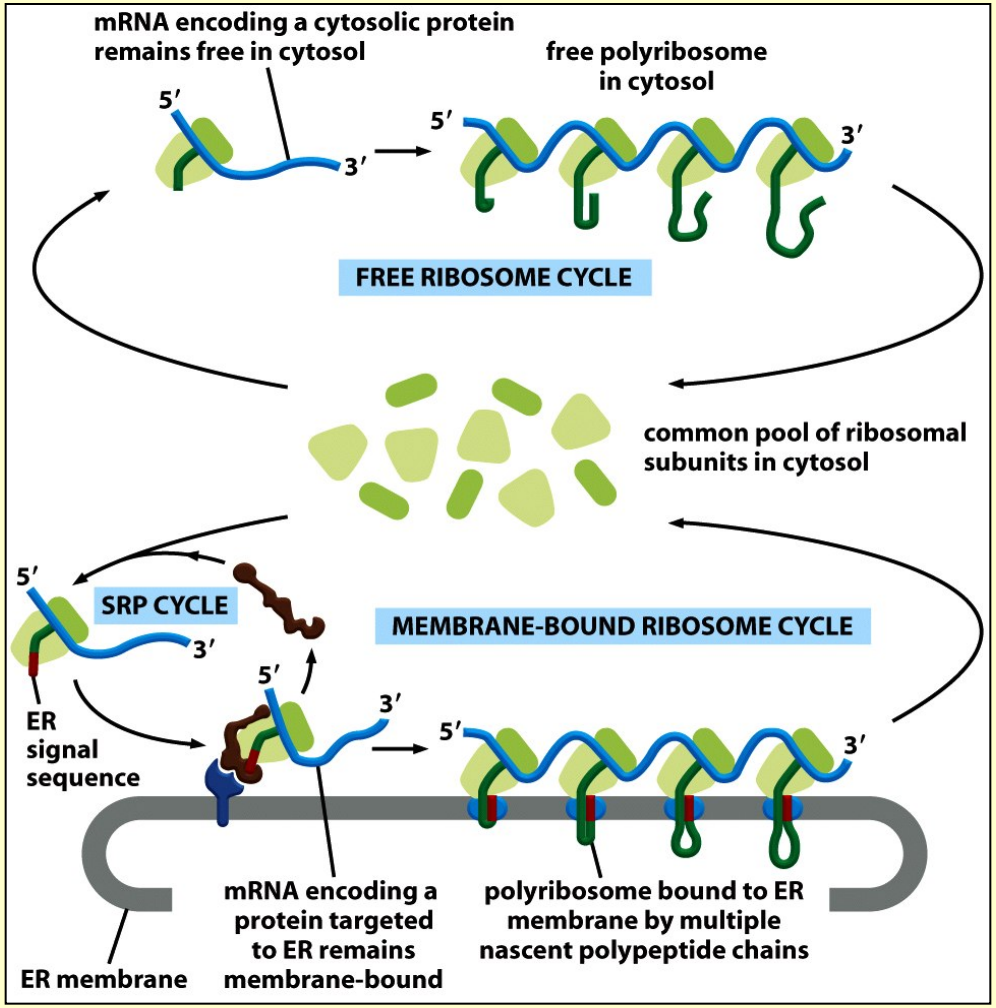


Figure 12-41a *Molecular Biology of the Cell* (© Garland Science 2008)

Overview of protein sorting

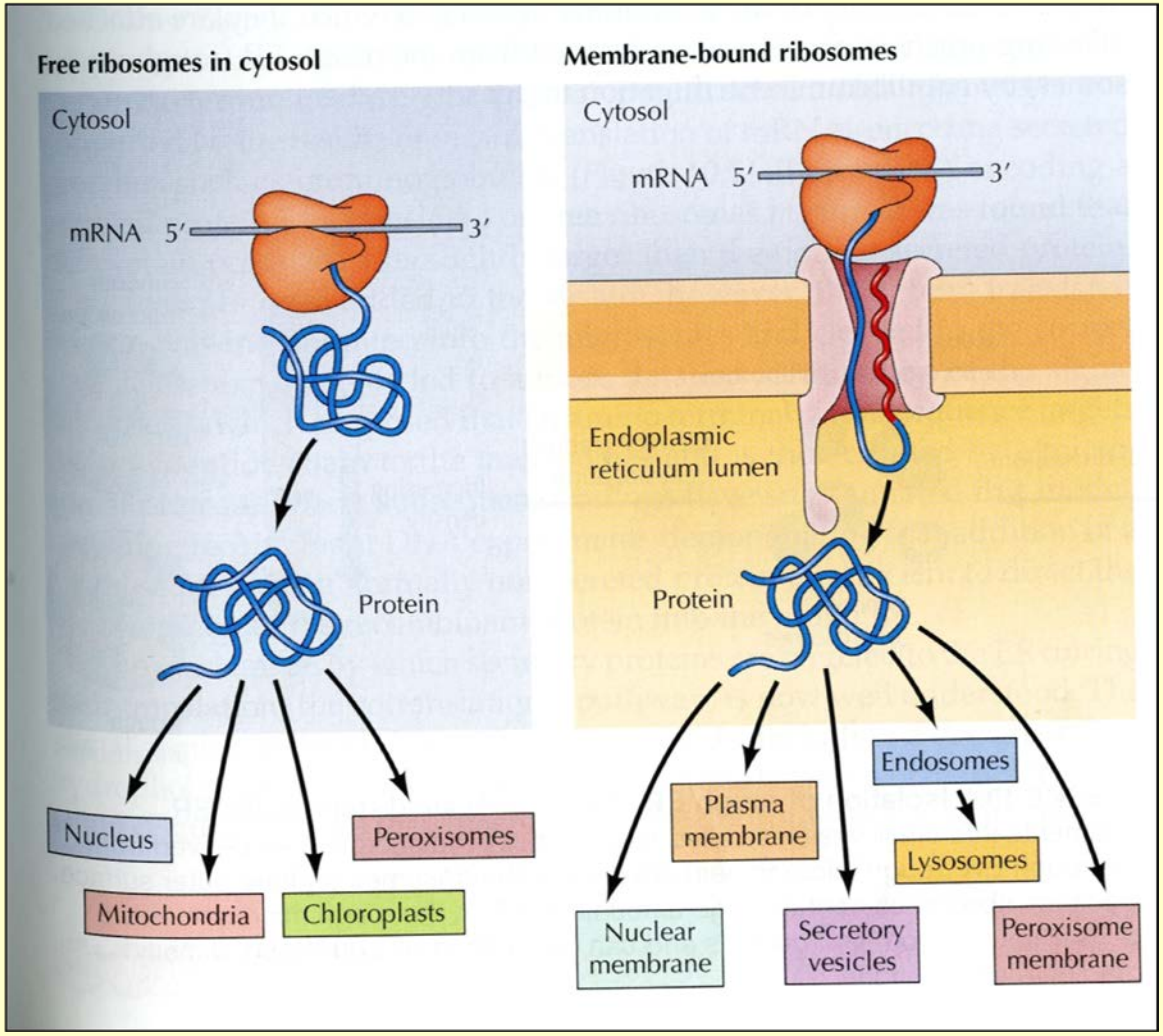


Figure 10-3. 2013. Cooper

Microsomes

✓ signal sequences were first discovered in the early 1970s in secreted proteins that are translocated across the ER membrane

✓ in the key experiment, mRNA encoding a secreted protein was translated by ribosomes *in vitro* on microsomes

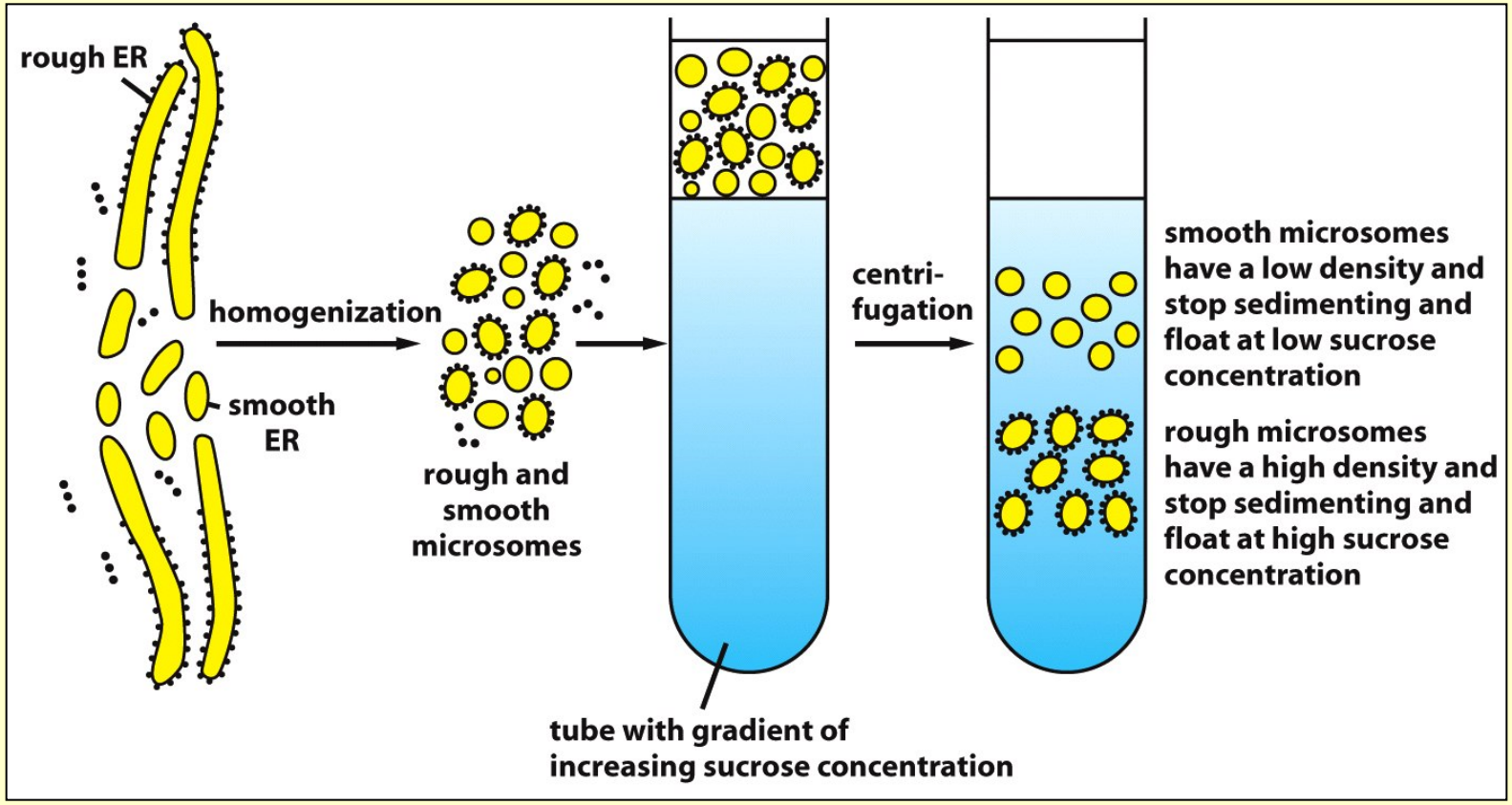
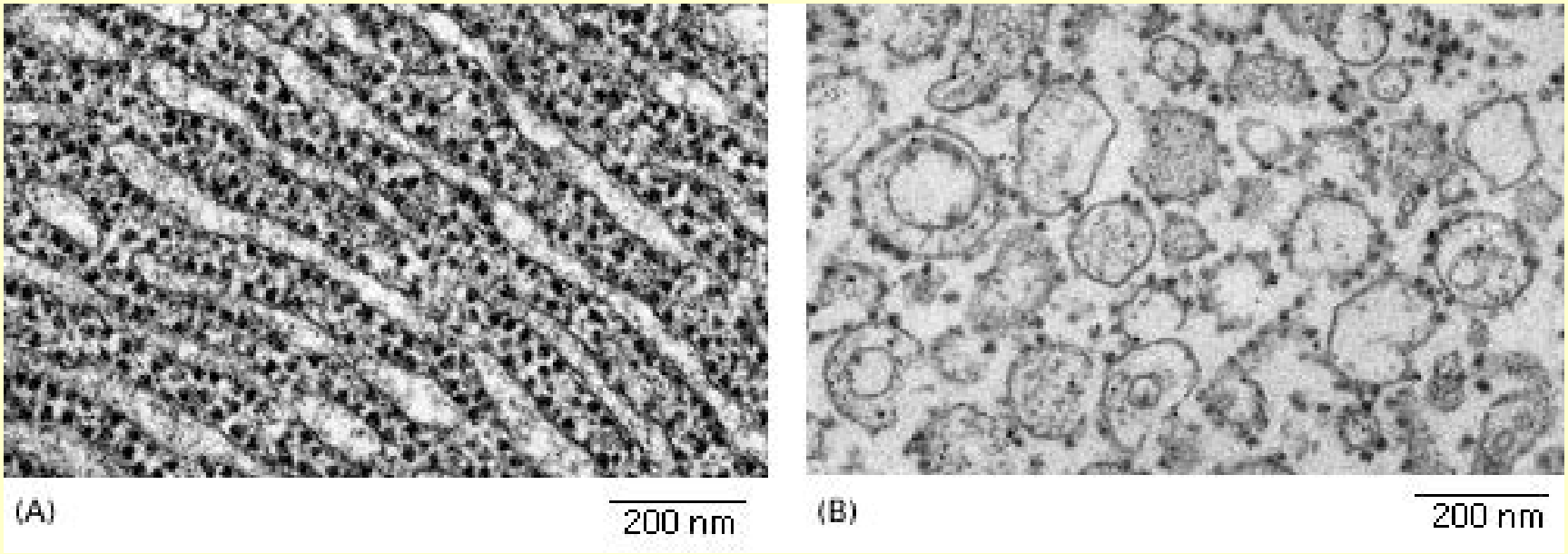


Figure 12-37b *Molecular Biology of the Cell* (© Garland Science 2008)



(A) rough ER

(B) rough microsomes

Figure. 12-39. 2002. Alberts et al.

Protein synthesis on microsomes

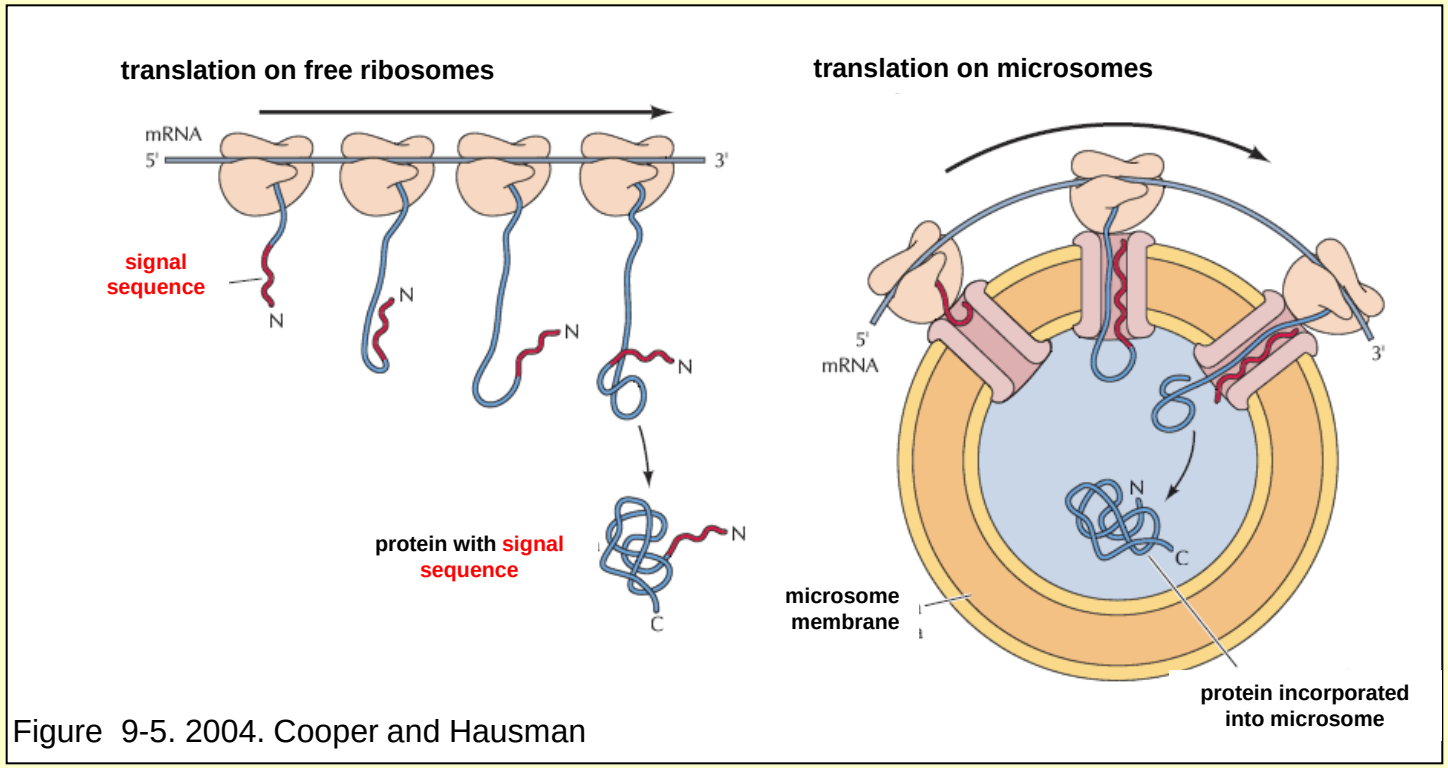


Figure 9-5. 2004. Cooper and Hausman

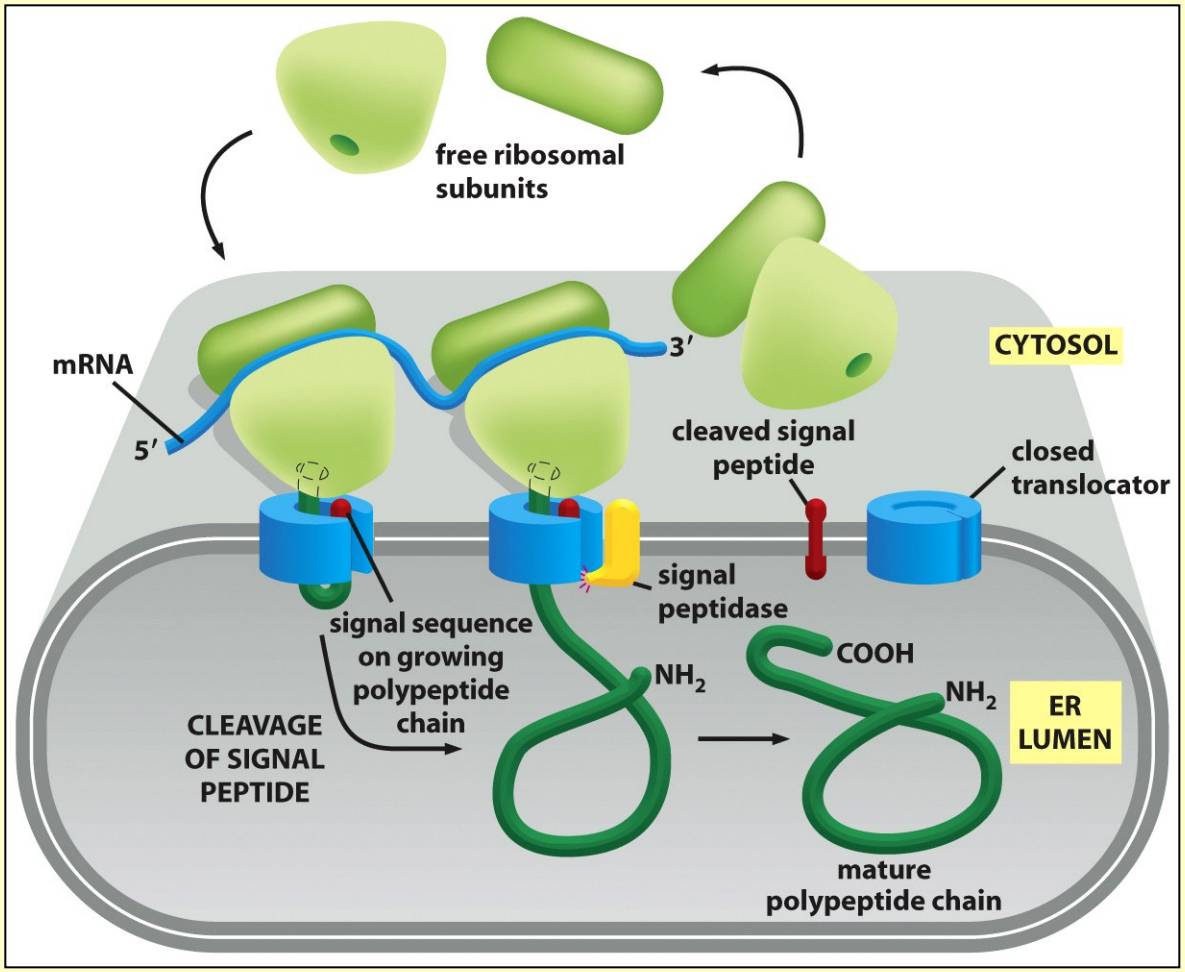
✓ when microsomes were omitted from cell-free system

→ synthesized protein slightly larger than the normal secreted protein

→ extra length being the N-terminal signal sequence

✓ in the presence of rough microsomes → protein of the correct size was produced

Signal hypothesis



→ N-terminal signal sequence serves as an ER signal sequence

→ directs the secreted protein to the ER membrane and is then cleaved off by a signal peptidase in the ER membrane before the polypeptide chain has been completed

Figure 12-38 *Molecular Biology of the Cell* (© Garland Science 2008)

How ER signal sequences direct ribosomes to the ER membrane?

- Signal Recognition Particle (SRP)
- SRP receptor
- Protein translocator

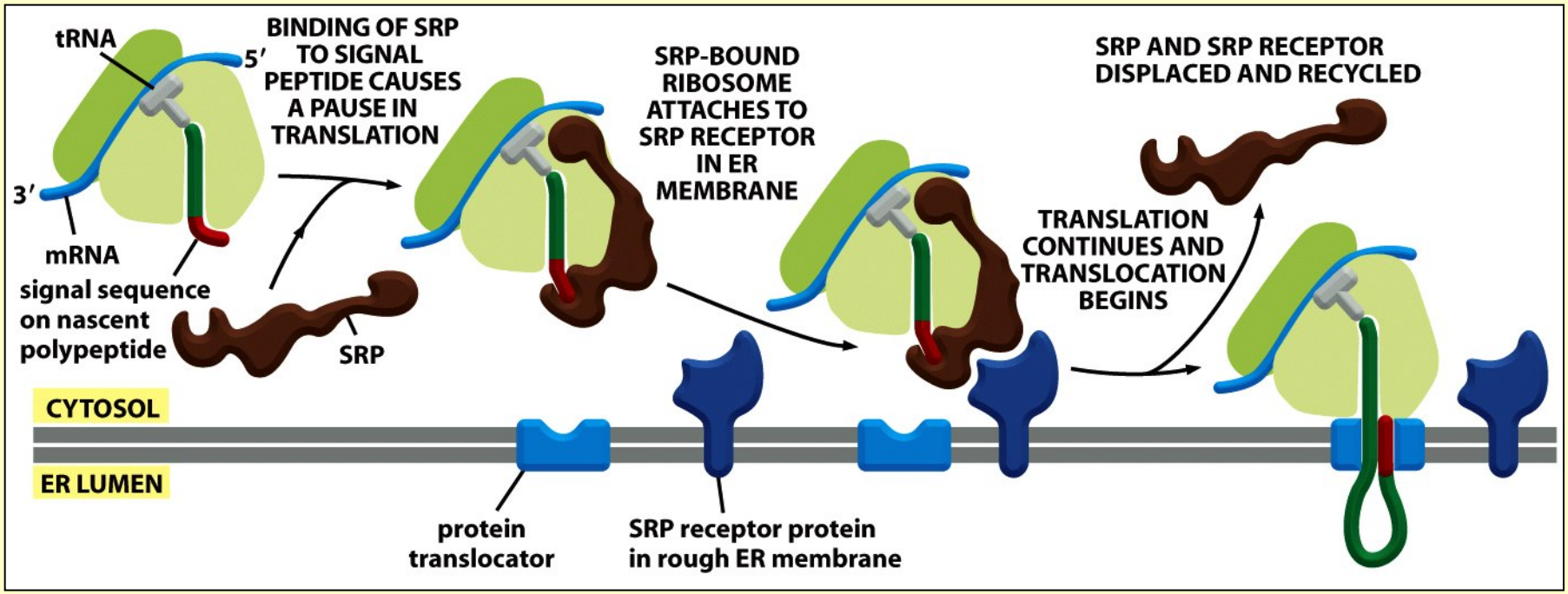


Figure 12-40 *Molecular Biology of the Cell* (© Garland Science 2008)

Co-translational targeting of secretory proteins to the ER

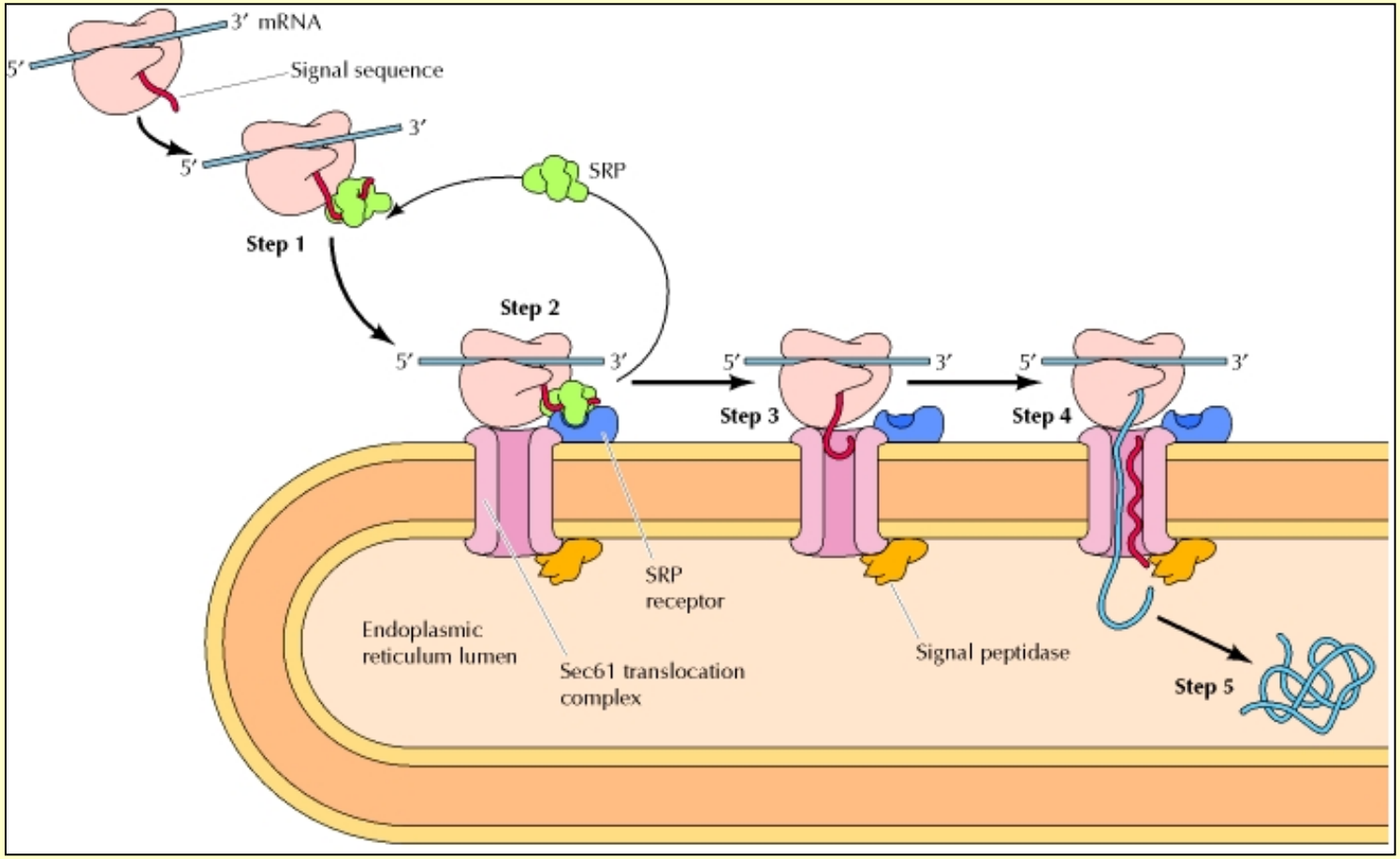


Figure 9-7. 2000. Cooper

Post-translational translocation of proteins into the ER

– signal sequence is recognized by complex **Sec62/63,** which is associated with the Sec61 translocation channel in the ER membrane

– requires additional energy from ATP

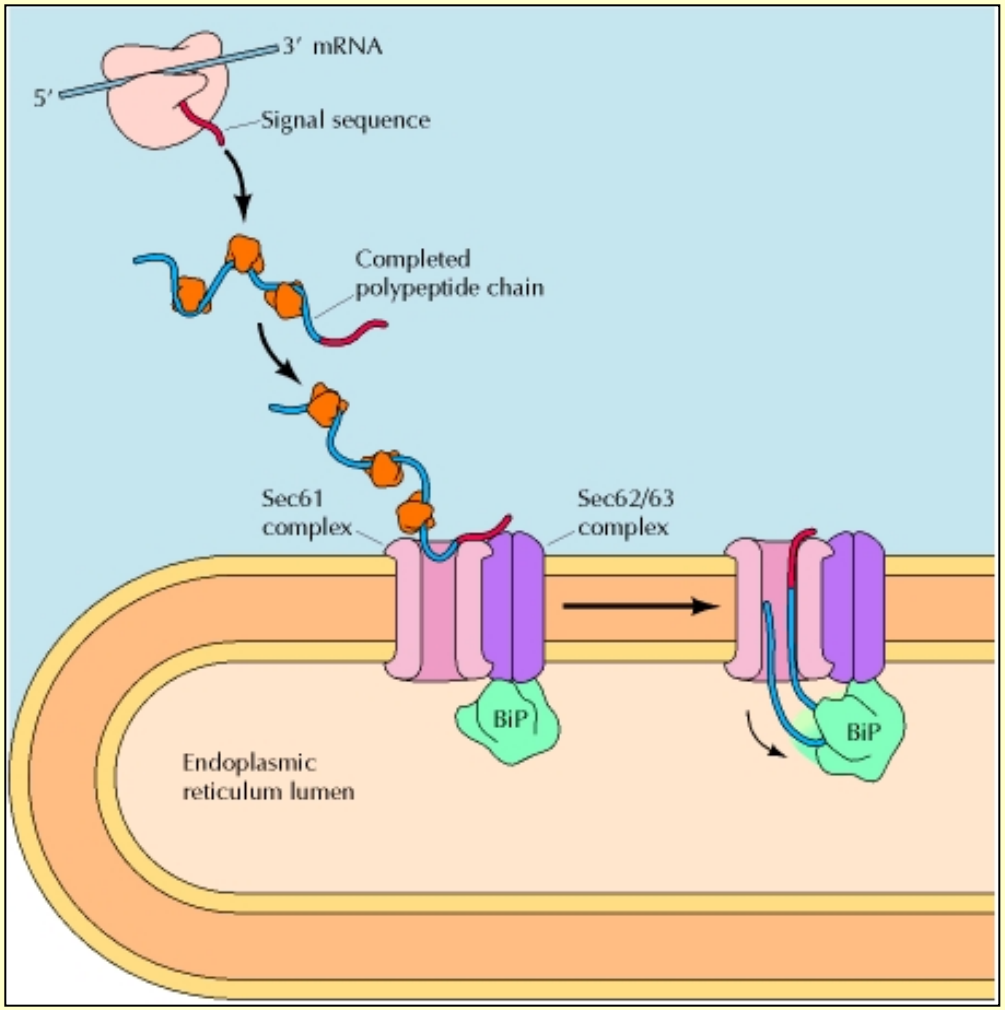


Figure 9-8. 2000. Cooper

How a soluble protein is translocated across the ER membrane

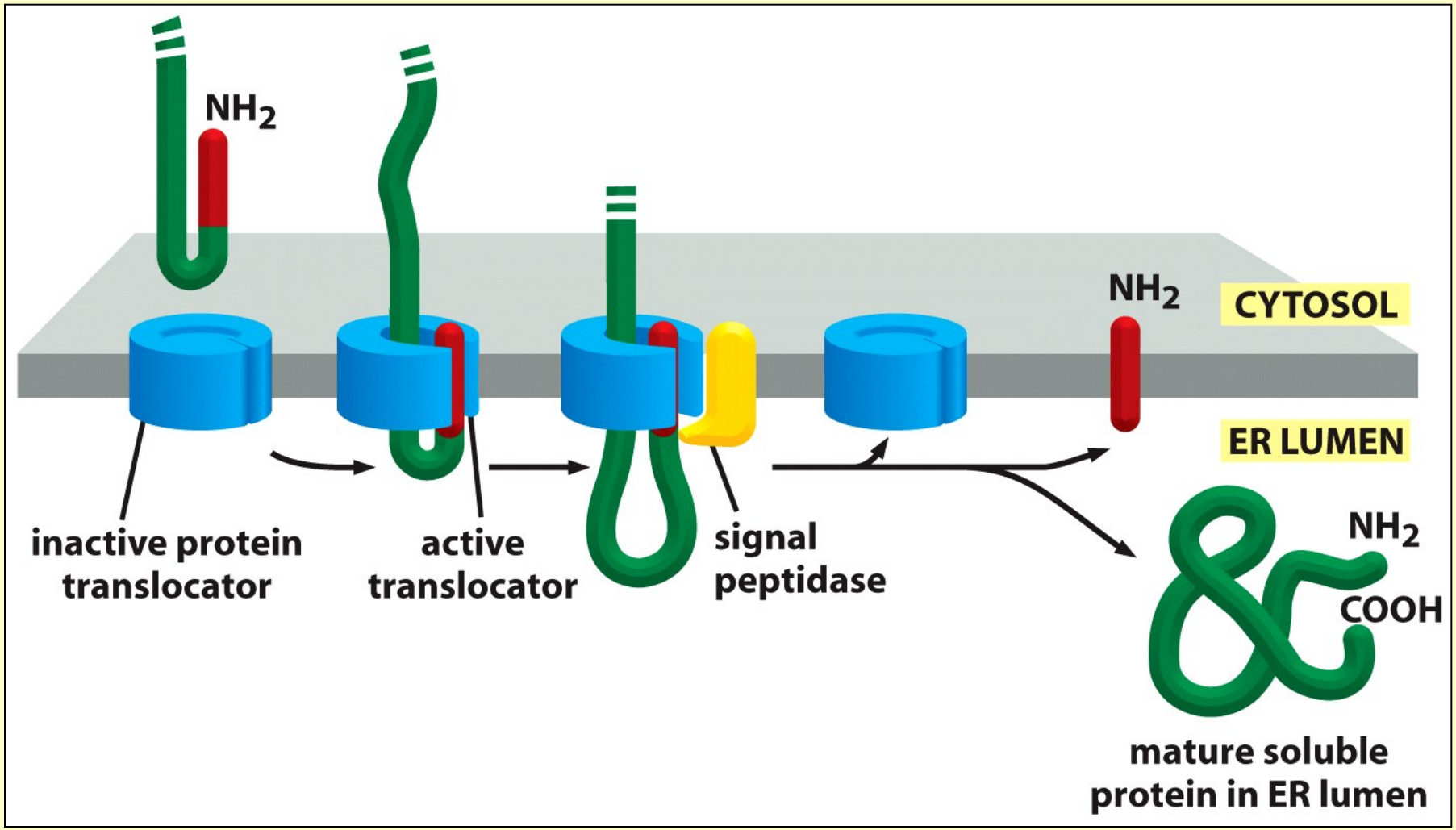


Figure 12-45 *Molecular Biology of the Cell* (© Garland Science 2008)

How a single-pass transmembrane protein is integrated into the ER membrane

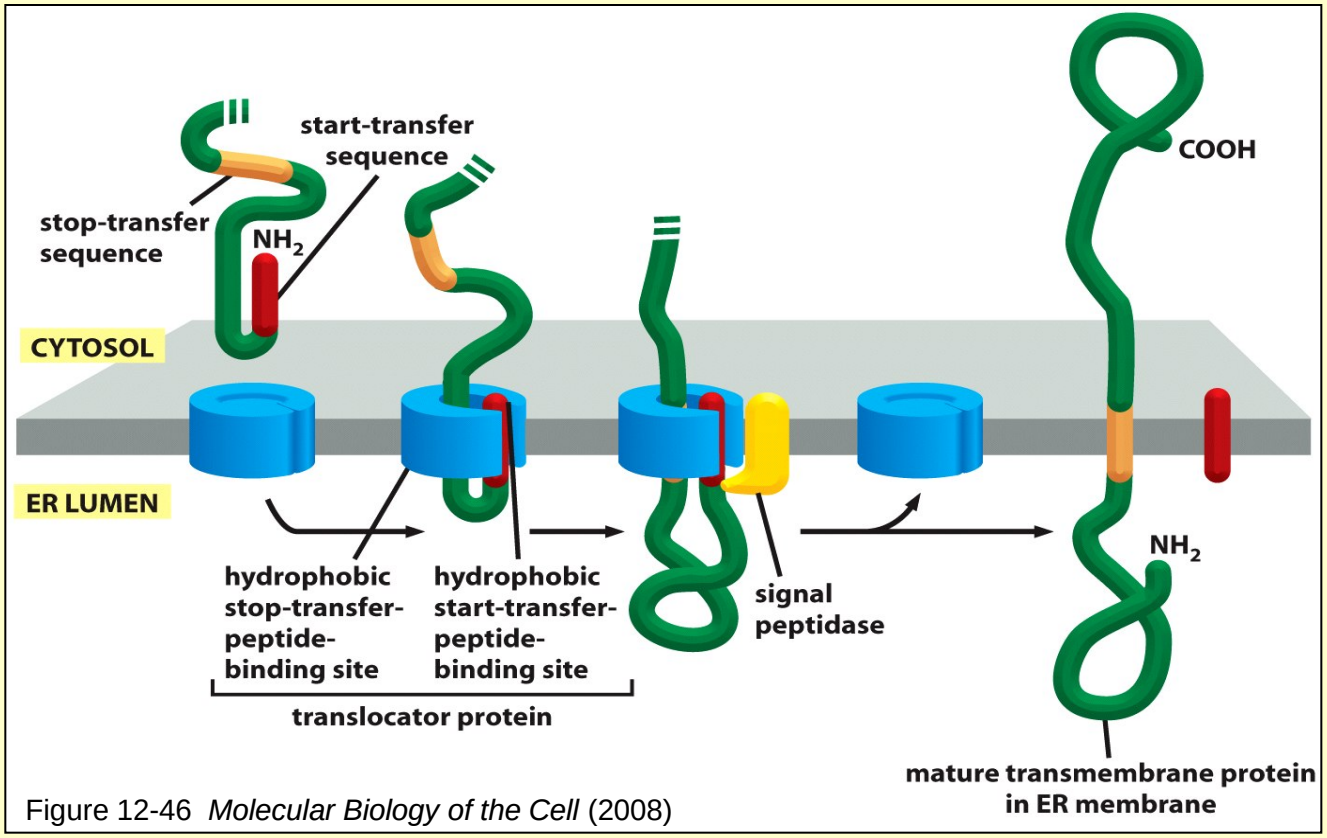


Figure 12-46 *Molecular Biology of the Cell* (2008)

✓co-translation is initiated by **N-terminal ER signal sequence** - start-transfer signal

✓ protein also contains a **stop-transfer sequence**

✓ when the stop-transfer sequence enters the translocator and interacts with a binding site, the translocator changes its conformation and discharges the protein laterally into the lipid bilayer

Smooth ER

✓ parts of ER-a without ribosomes

✓ great majority of cells
→ such regions are scanty and often partly smooth and partly rough
→ sometimes called **transitional ER** because they contain ER exit sites

✓ certain specialized cells → abundant smooth ER with additional functions

✓ usually prominent in cells that specialize in lipid metabolism
→ cells that synthesize steroid hormones from cholesterol
→ to accommodate the enzymes needed to make cholesterol and to modify it to form the hormones

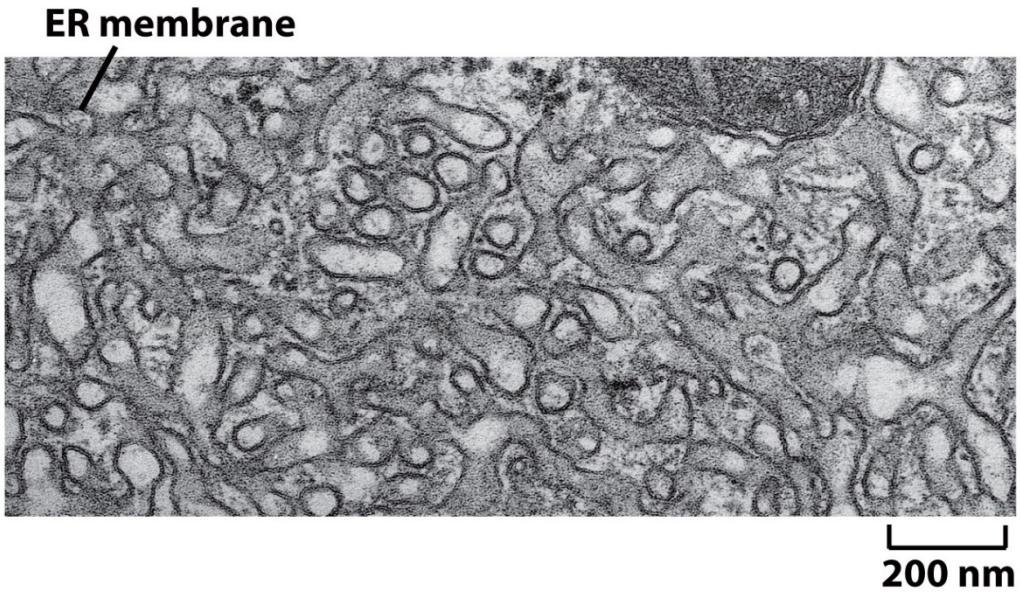
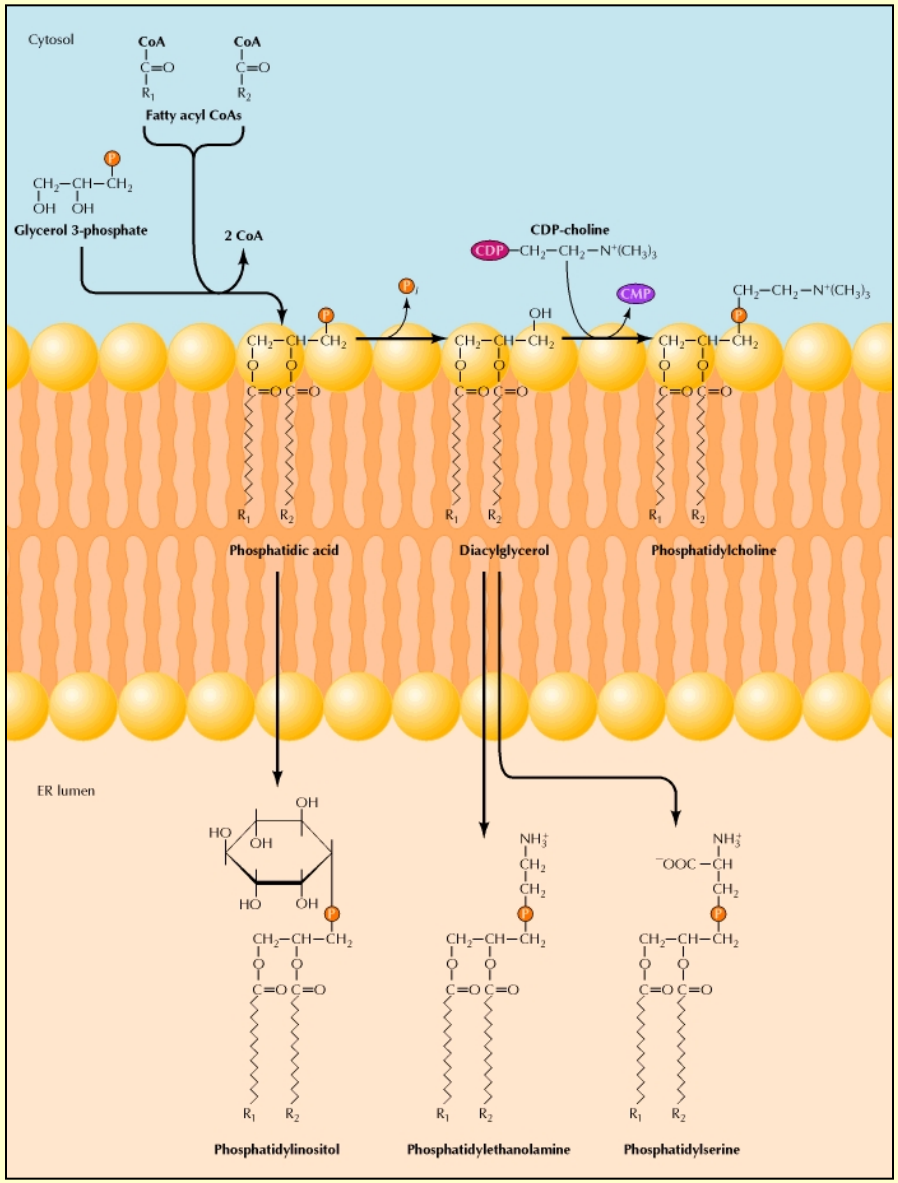


Figure 12-36b *Molecular Biology of the Cell* (© Garland Science 2008)

Synthesis of phospholipids



→ glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors

→ formation of
phosphatidylcholine
phosphatidylethanolamine
phosphatidylserine

→phosphatidylinositol is formed from phosphatidic acid

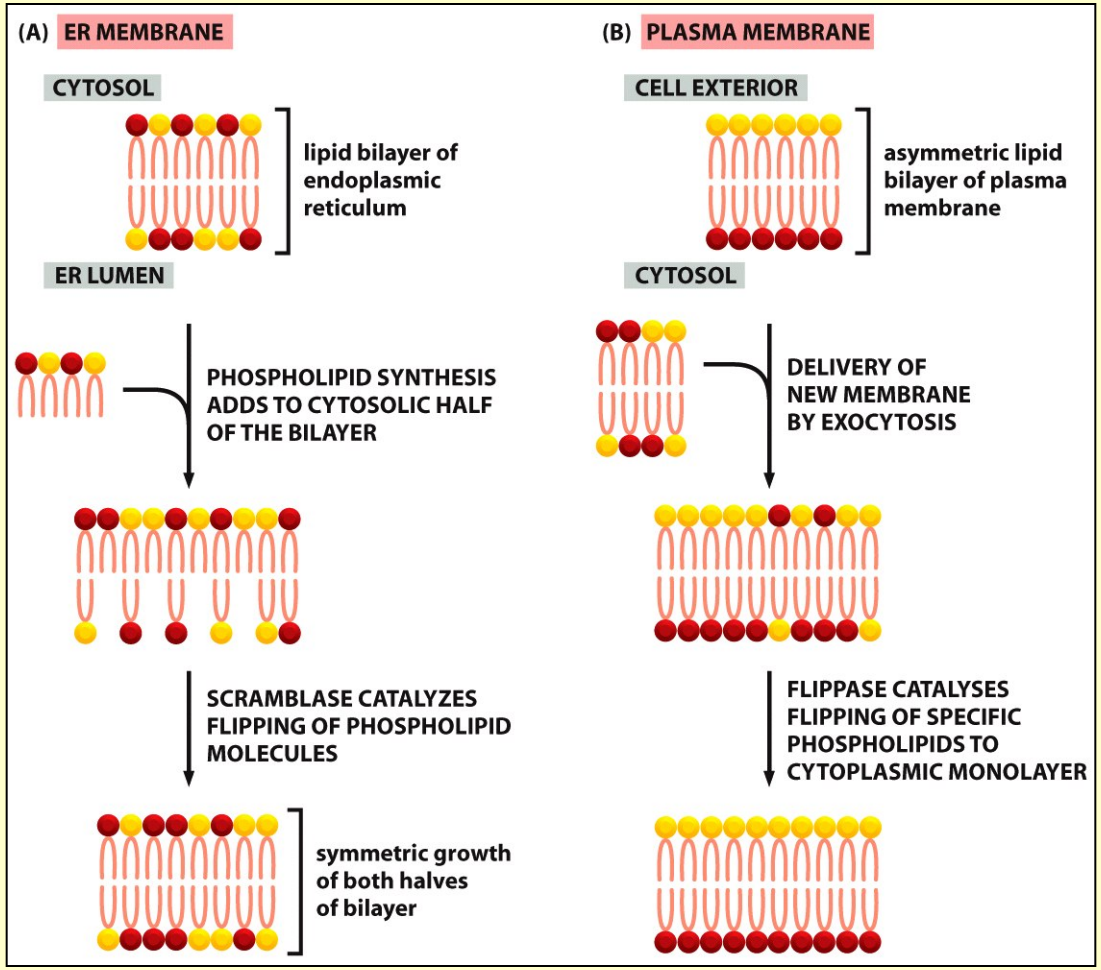
Figure 9-17. 2000. Cooper

Role of phospholipid translocators in lipid bilayer synthesis

✓ new lipids are added only to the cytosolic half of the bilayer

→ phospholipid translocator **scramblase** transfers lipid molecules from the cytosolic to the luminal half so that membrane grows as a bilayer

→ scramblase is <u>not specific</u> for particular phospholipid head groups and therefore equilibrates the different phospholipids between the two monolayers



✓<u>head-group-specific</u> **flippase** in the plasma membrane

→ flips phosphatidylserine and phosphatidylethanolamine from the extracellular to the cytosolic leaflet, creating the characteristically asymmetric lipid bilayer of the plasma membrane

Figure 12-58 *Molecular Biology of the Cell* (© Garland Science 2008)

Cholesterol and ceramide

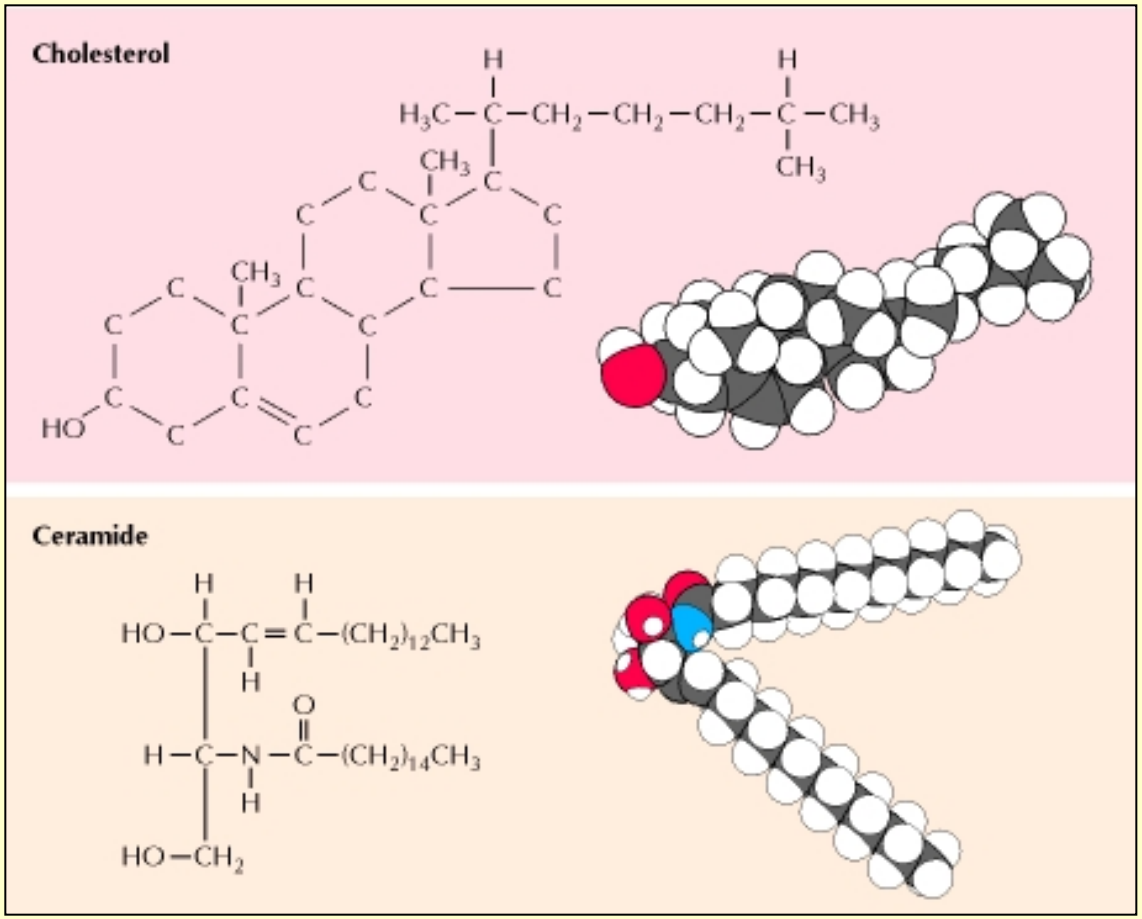
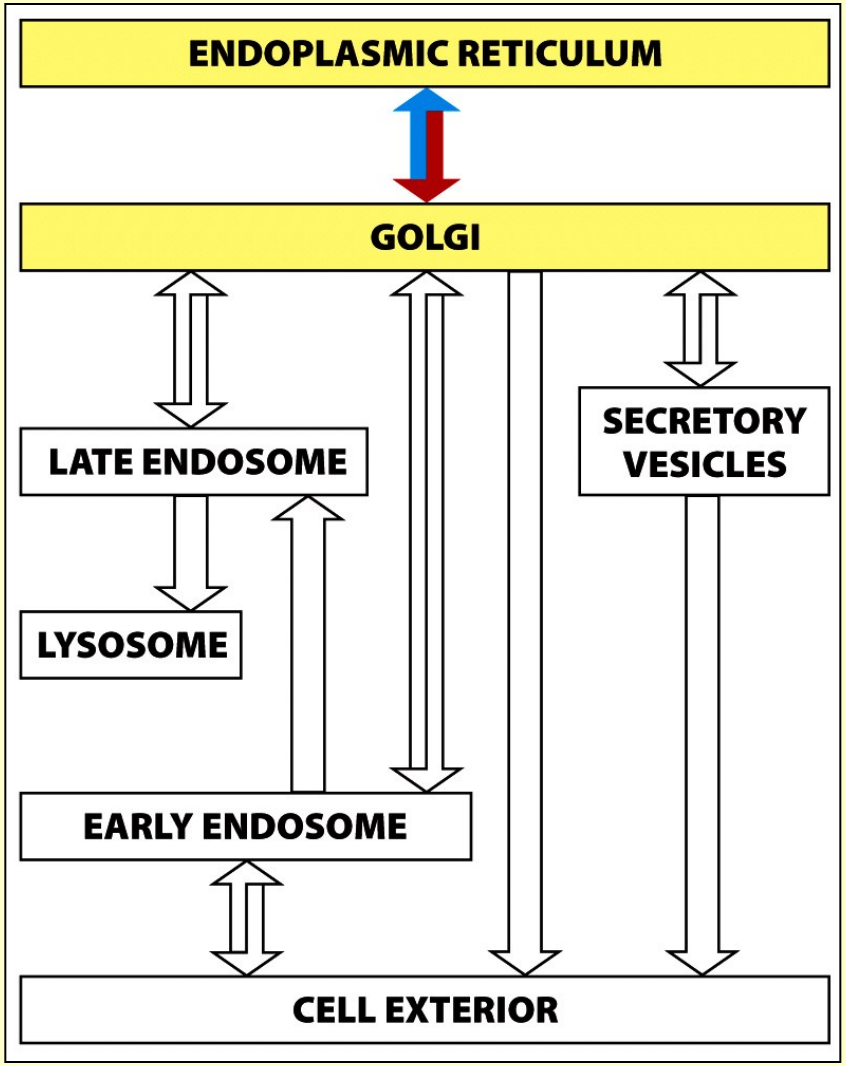


Figure 9.19. 2000. Cooper

Transport from ER to GK



Vesicular transport

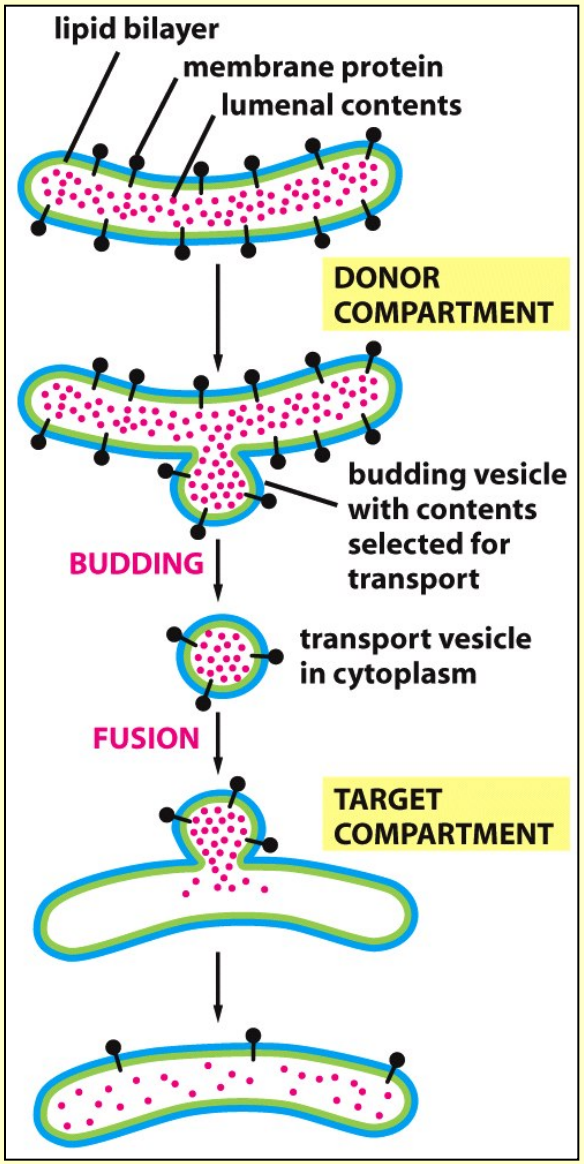
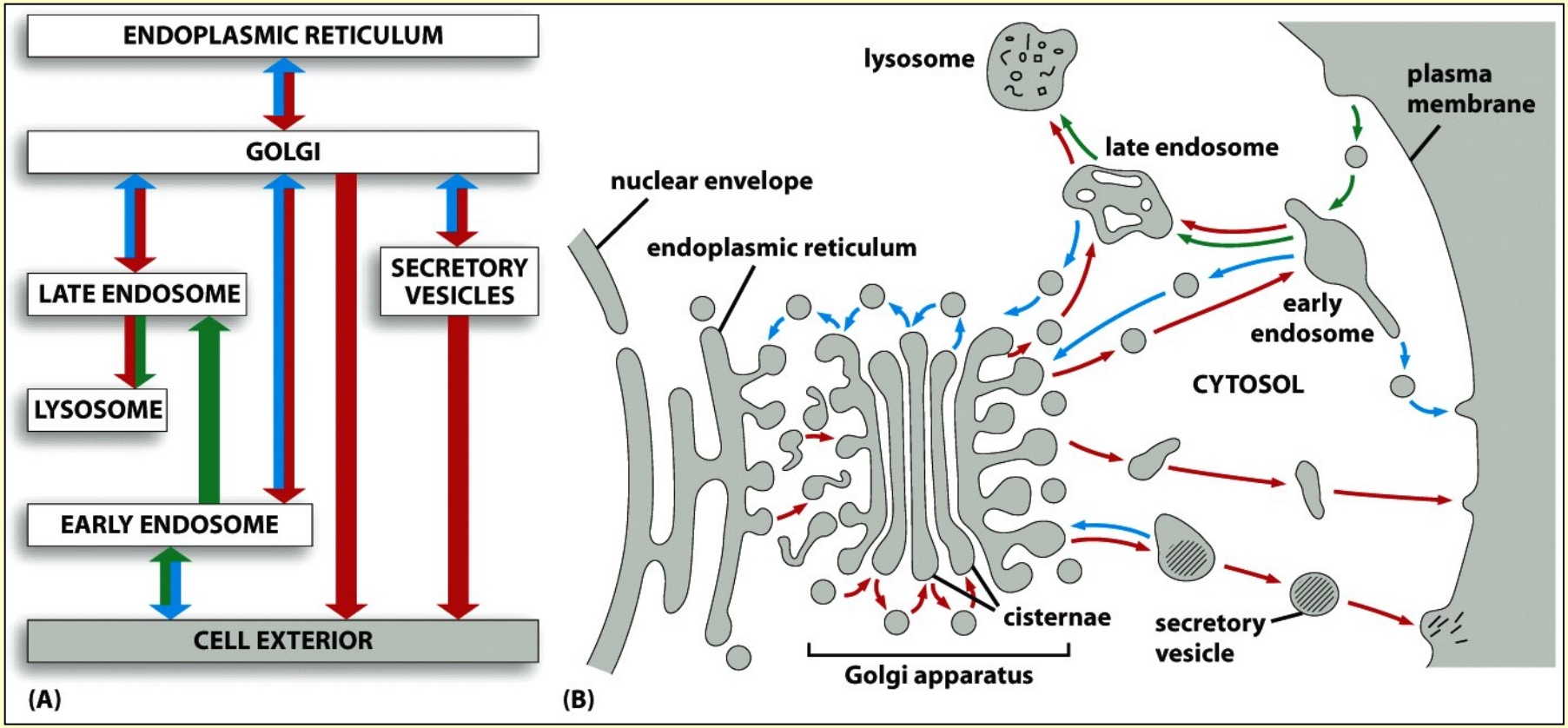


Figure 12-7 *Molecular Biology of the Cell* (© Garland Science 2008)

Intracellular compartments of eukaryotic cell involved in vesicular transport



✓ each compartment encloses a space – *lumen* – that is topologically equivalent to the outside of the cell

✓ all compartments shown communicate with one another and the outside of the cell by means of transport vesicles

Figure 13-3 *Molecular Biology of the Cell* (© Garland Science 2008)

Three types of coated vesicles are involved in transportation of molecules

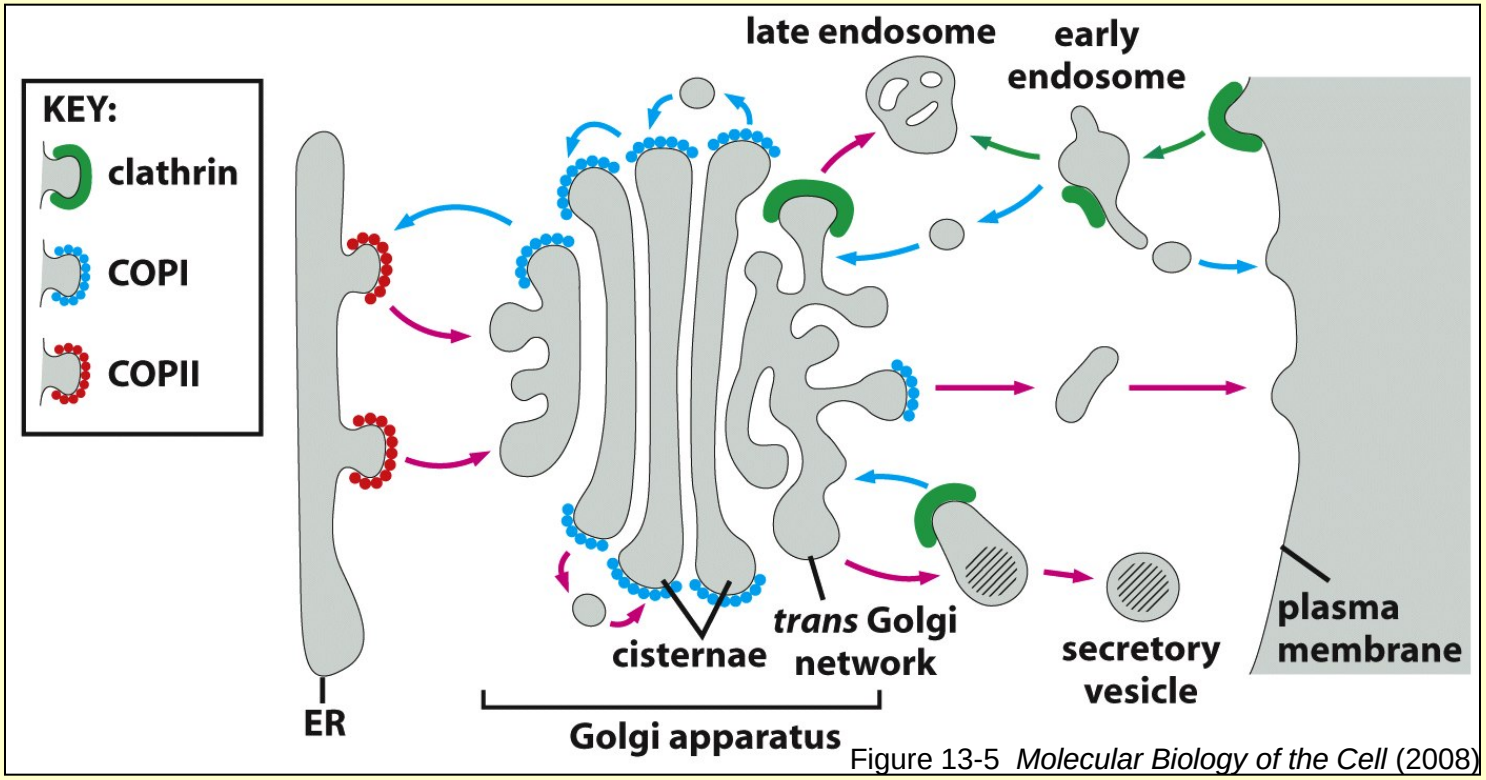


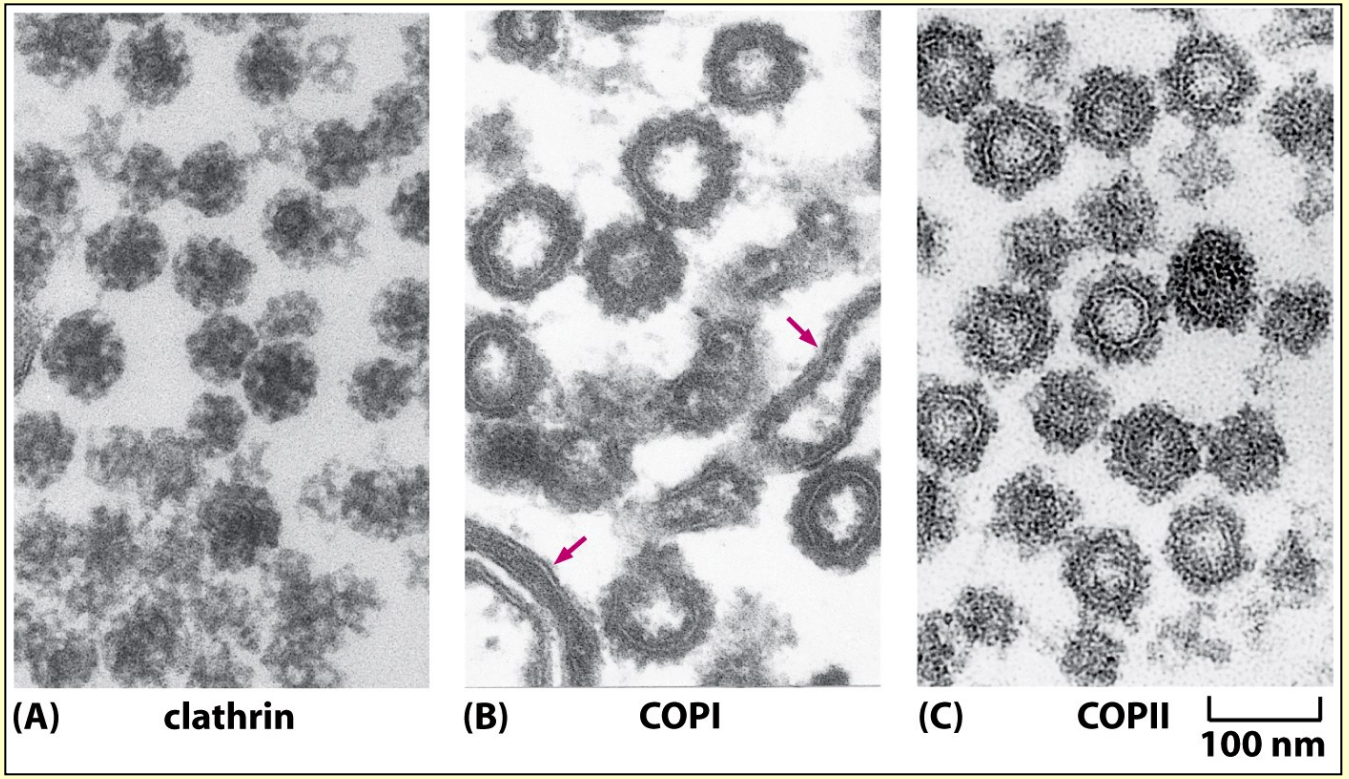
Figure 13-5 *Molecular Biology of the Cell* (2008)

✓**COPI**-coated transport vesicles (***Co****at* ***P****rotein*) → involved in the retrograde transport from GA to ER

✓**COPII**-coated transport vesicles → involved in the transport from ER to GA

✓**Clathrin**-coated transport vesicles → involved in the transport from GA to lysosomes and plasma membrane as well as from the plasma membrane

Three types of coated vesicles are involved in transportation of molecules



Different coat proteins select different cargo and shape the transport vesicles that mediate various steps in the biosynthetic-secretory and endocytic pathways

Figure 13-4 *Molecular Biology of the Cell* (© Garland Science 2008)

Exit from ER

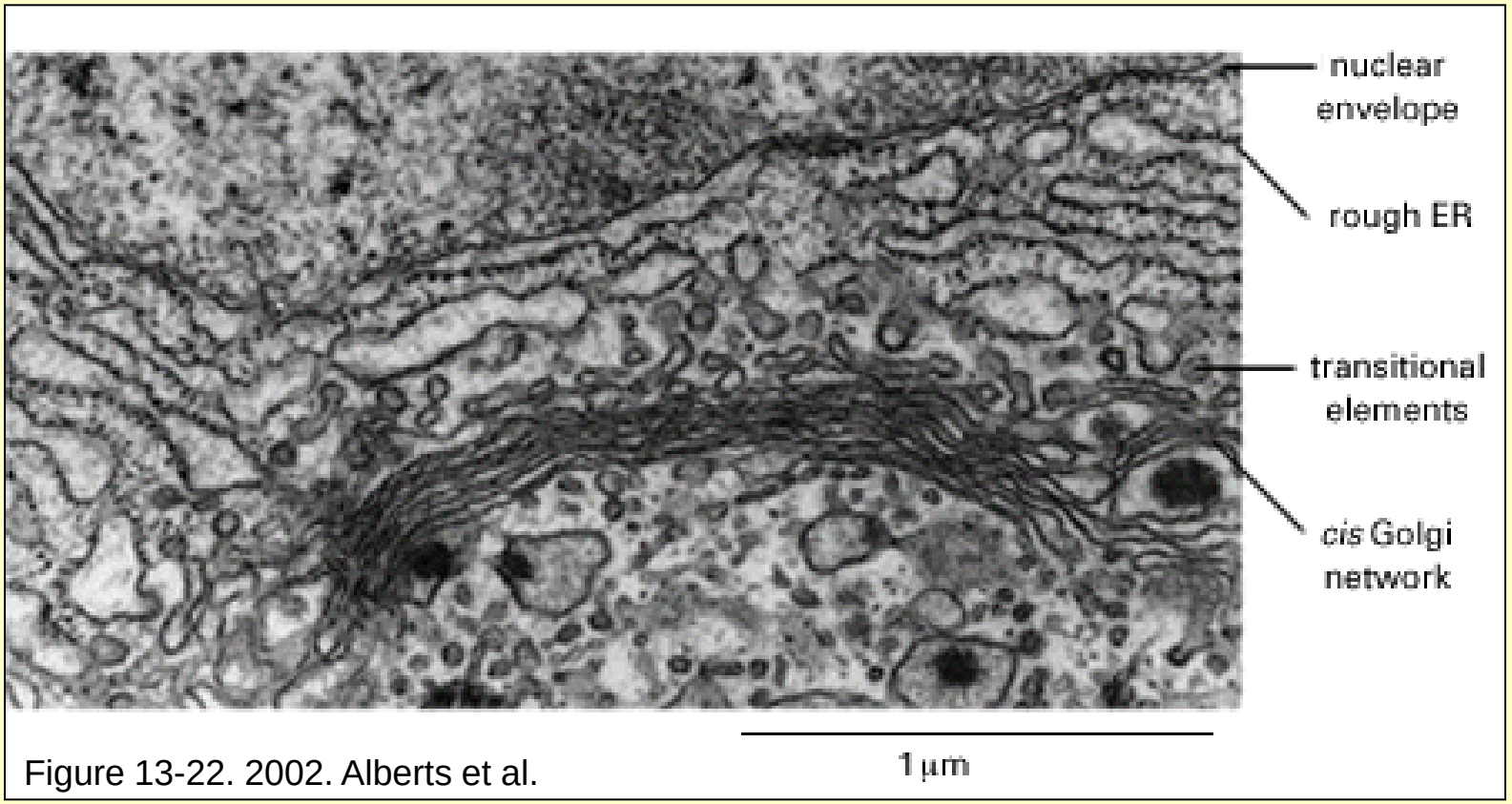


Figure 13-22. 2002. Alberts et al.

✓ electron micrograph emphasizing the transitional zone between the ER and the Golgi apparatus in an animal cell

- ✓ vesicles budding from ER are not highly selective
- ✓ transportation of all properly folded proteins
- ✓ misfolded proteins are kept in ER and degraded
- ✓ ER proteins have a retention signal

→ **KDEL** (Lys-Asp-Glu-Leu) - luminal ER proteins

→ **KKXX** (Lys-Lys-X-X) - transmembrane ER proteins

Proteins that are properly folded and assembled can leave the ER

- ✓ quality control
- ✓ molecular chaperons – calnexin and calreticulin

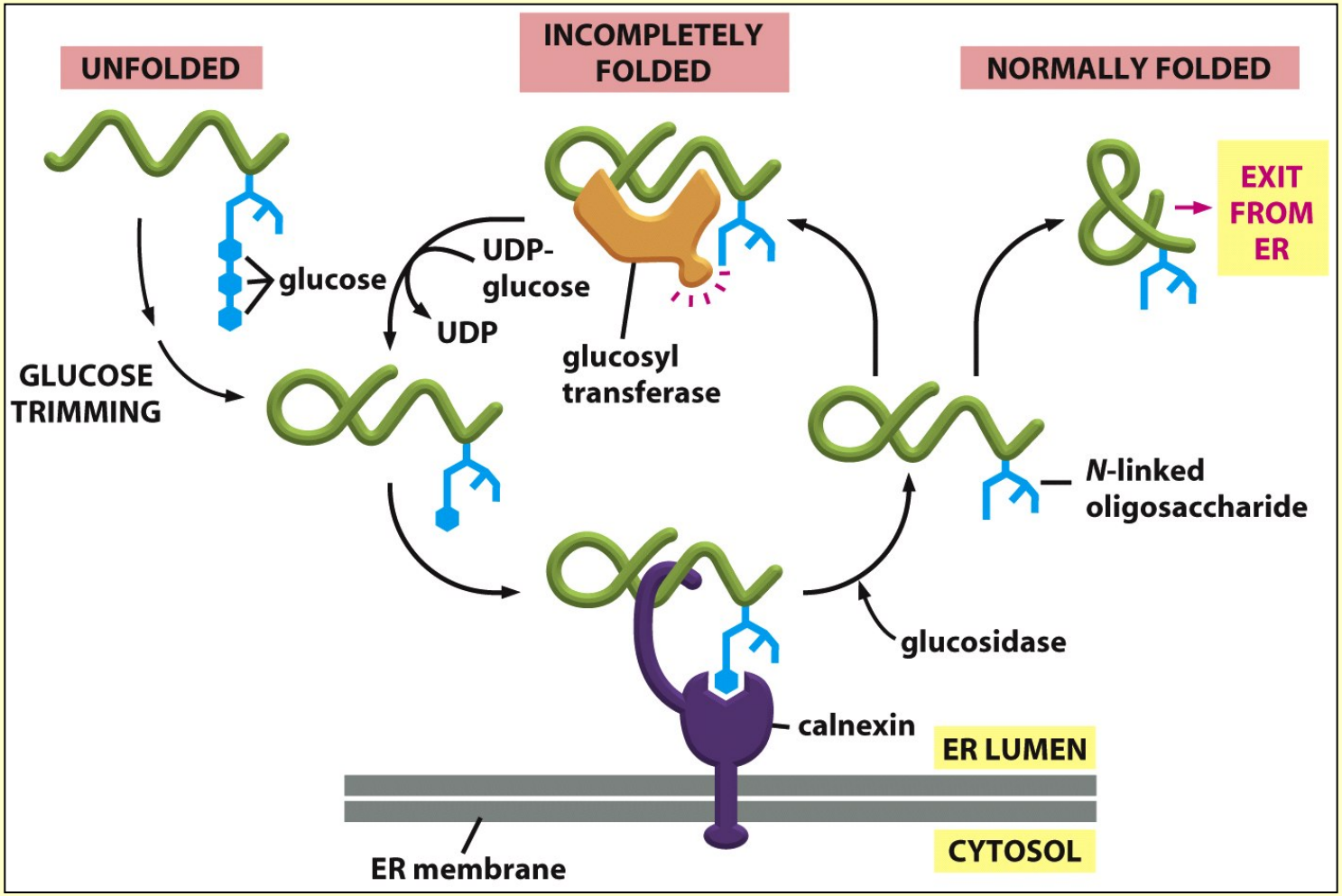


Figure 12-53 *Molecular Biology of the Cell* (© Garland Science 2008)

Degradation of misfolded proteins

✓ misfolded protein is expelled from the ER, ubiquitinated and degraded in proteasome

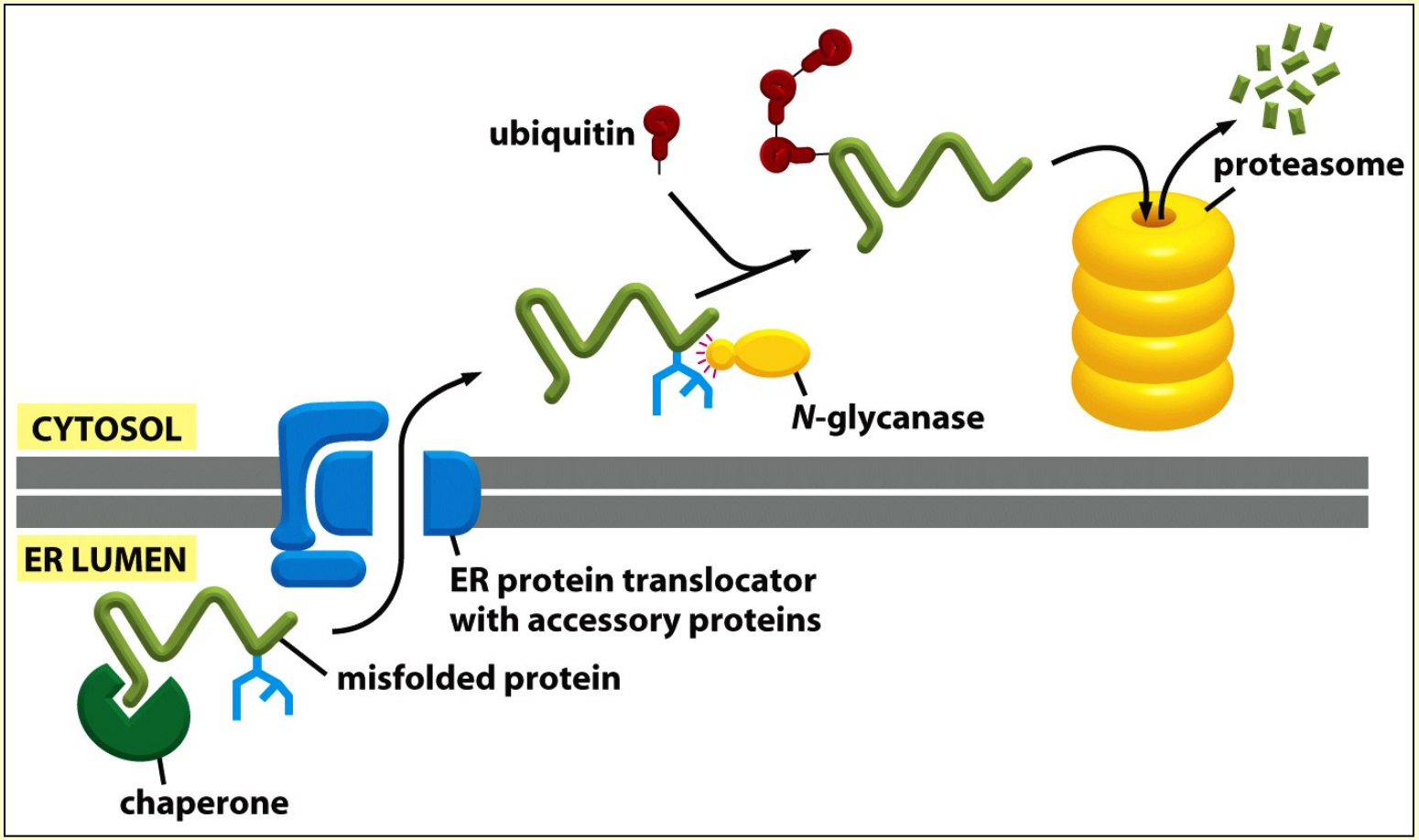
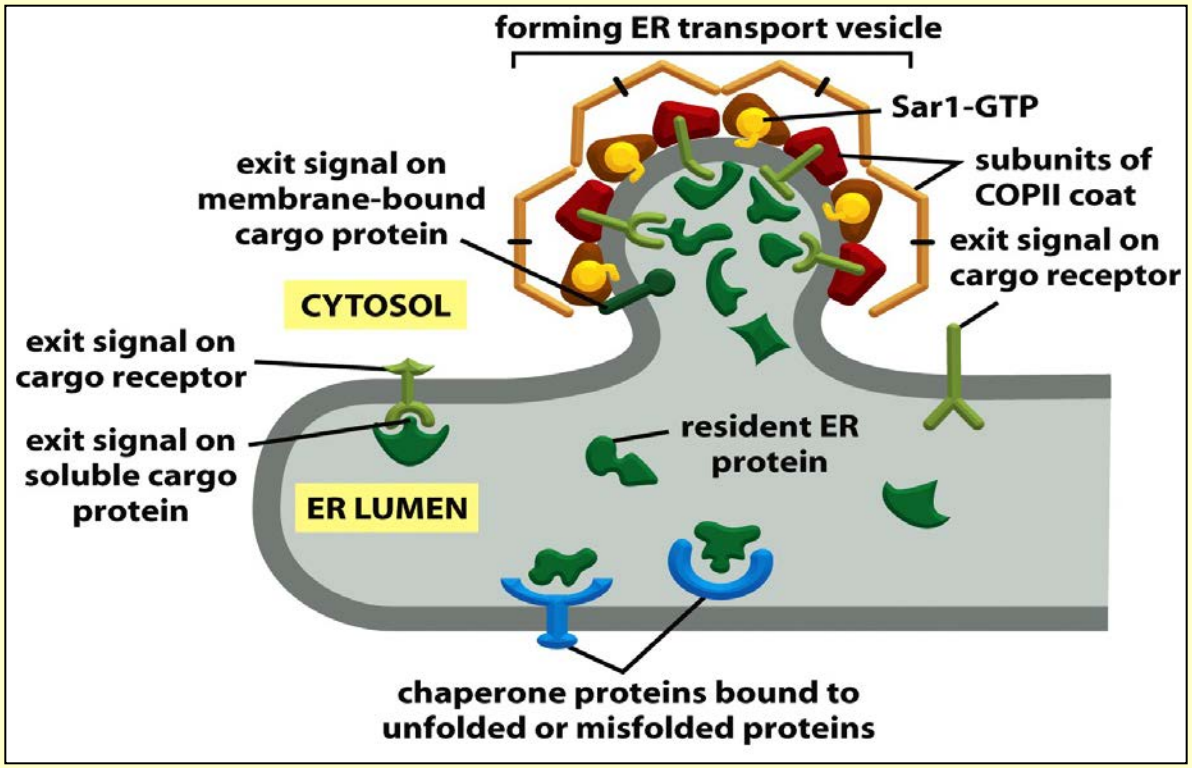


Figure 12-54 *Molecular Biology of the Cell* (© Garland Science 2008)

The recruitment of cargo molecules into ER transport vesicles



✓ by binding to the COPII coat, membrane and cargo proteins become concentrated in the transport vesicles as they leave the ER

✓ membrane proteins are packaged into budding vesicles through the interactions of exit signals on their cytosolic tails with the COPII coat

✓some of the membrane proteins trapped by the coat function as cargo receptors, binding soluble proteins in the lumen and helping to package them into vesicles

Figure 13-20 *Molecular Biology of the Cell* (© Garland Science 2008)

Transport from the ER to the Golgi apparatus is mediated by vesicular tubular clusters

✓ correctly folded and assembled proteins in the ER are packaged into **COPII**-coated transport vesicles that pinch off from the ER membrane

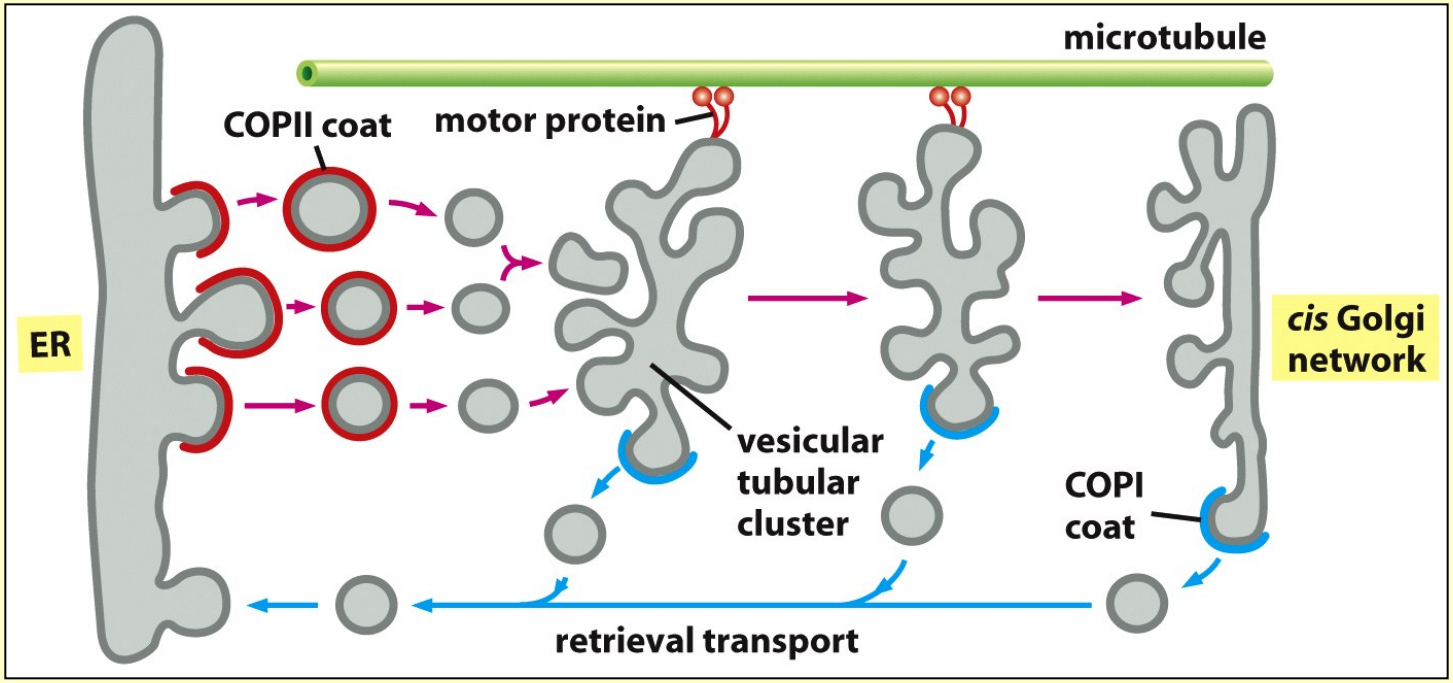
✓ **COPII** transport vesicles bud from specialized regions of the ER called **ER exit sites (transitional ER)**

✓ shortly thereafter the coat is shed and the vesicles fuse with one another to form **vesicular tubular clusters**, which move on microtubule tracks to GA

✓ many resident ER proteins slowly escape, but they are returned to the ER from the vesicular tubular clusters and the GA by retrograde transport in **COPI**-coated vesicles

Vesicular tubular clusters

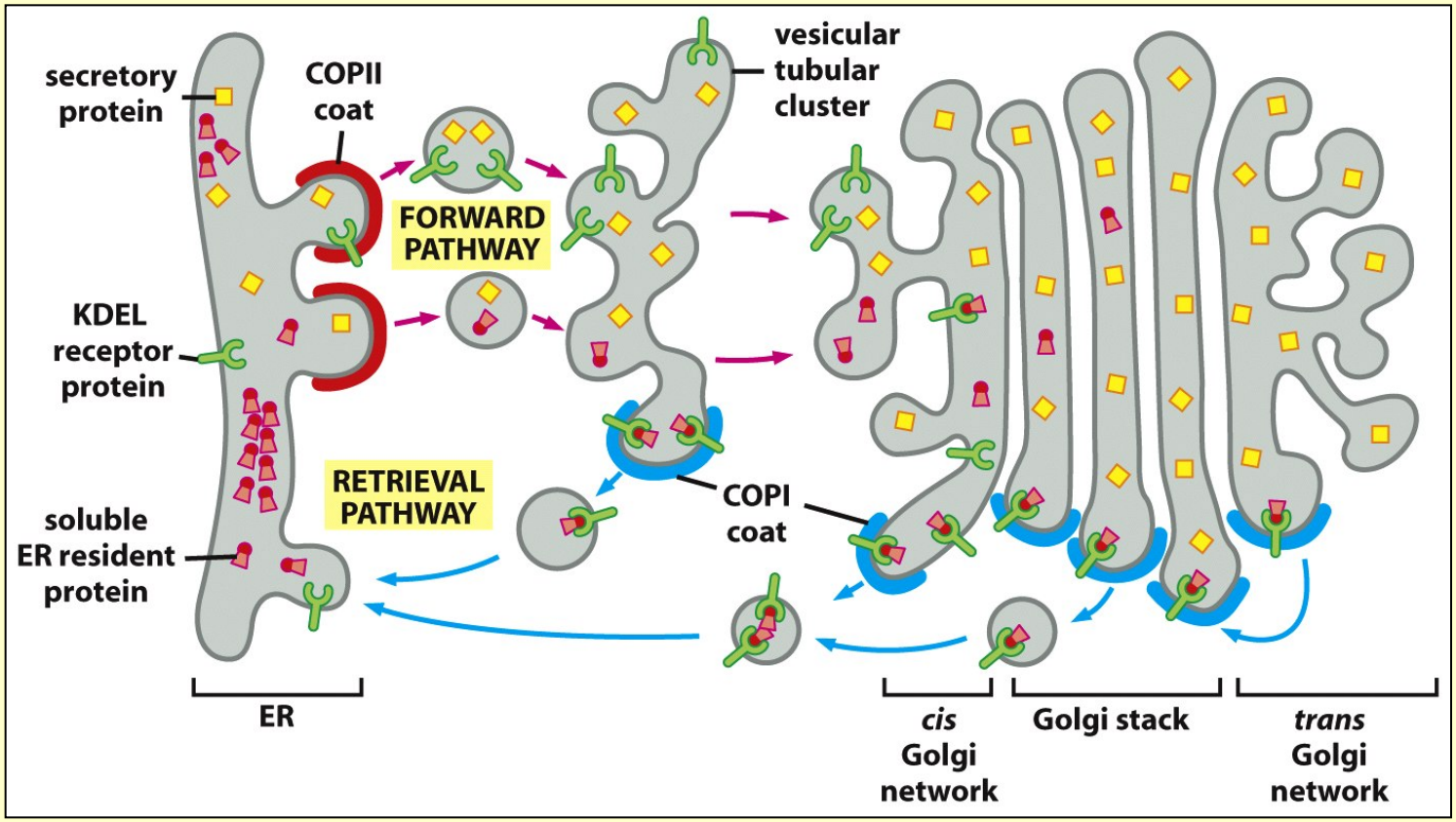
- ✓ move along the microtubules to carry proteins from ER to GA
- ✓ **COPI** coats mediate the budding of vesicles that return to the ER
- ✓ coats quickly disassemble after the vesicles have formed



✓**COPI** vesicles carry back to the ER resident proteins that have escaped, as well as proteins that participated in the ER budding reaction and are being returned

Figure 13-23b *Molecular Biology of the Cell* (© Garland Science 2008)

A model for retrieval of ER resident proteins

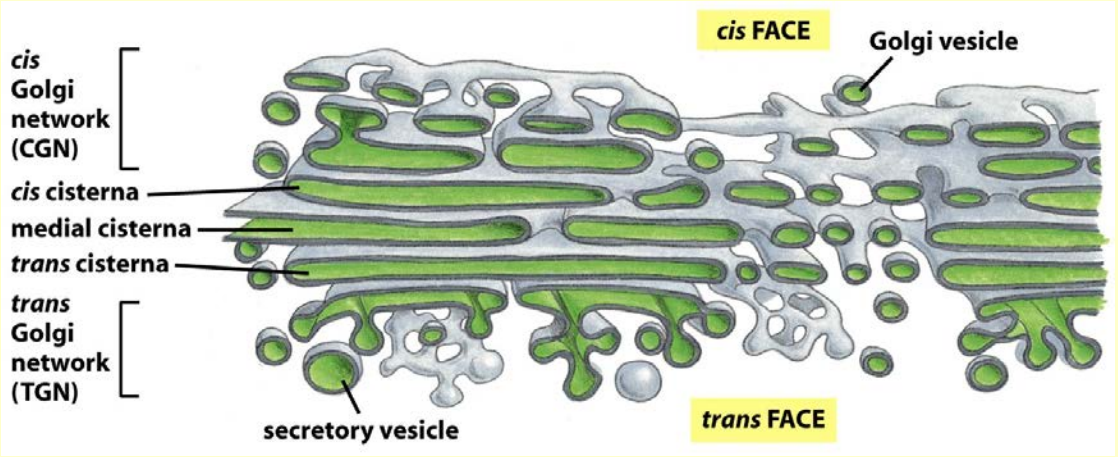


- ✓ retrieval of ER proteins begins in VTC and continues from all parts of GA
- ✓ in neutral pH environment of ER, ER-proteins dissociate from KDEL receptor
- ✓ receptors are returned to GA for reuse

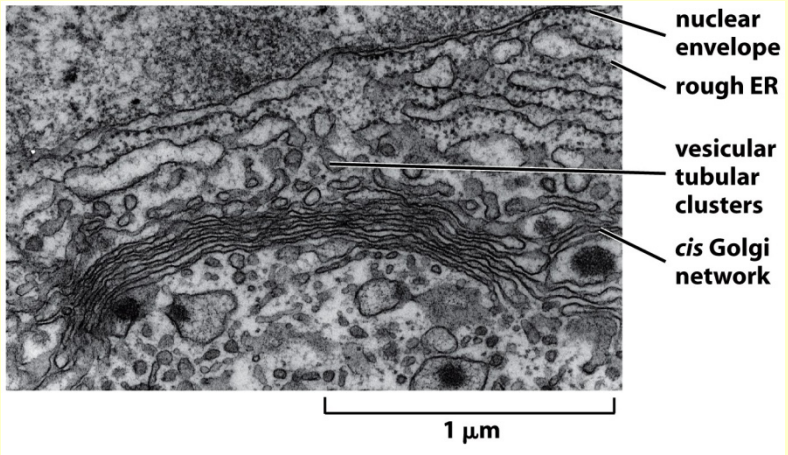
Figure 13-24b *Molecular Biology of the Cell* (© Garland Science 2008)

Golgi apparatus

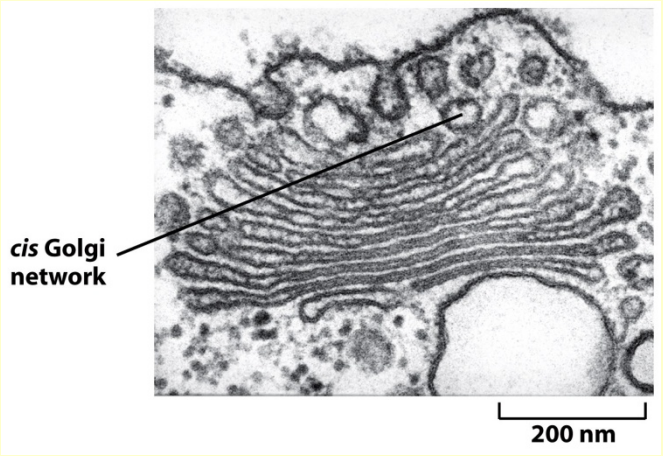
Golgi apparatus (GA)



Three-dimensional reconstruction from electron micrographs of the GA in a secretory animal cell



Electron micrograph of transitional zone between ER and GA in animal cell



Electron micrograph of a GA in a plant
→ GA is generally more distinct and more clearly separated from other intracellular membranes than in animal cells

Figure 13-25c *Molecular Biology of the Cell* (© Garland Science 2008)

Regions of GA

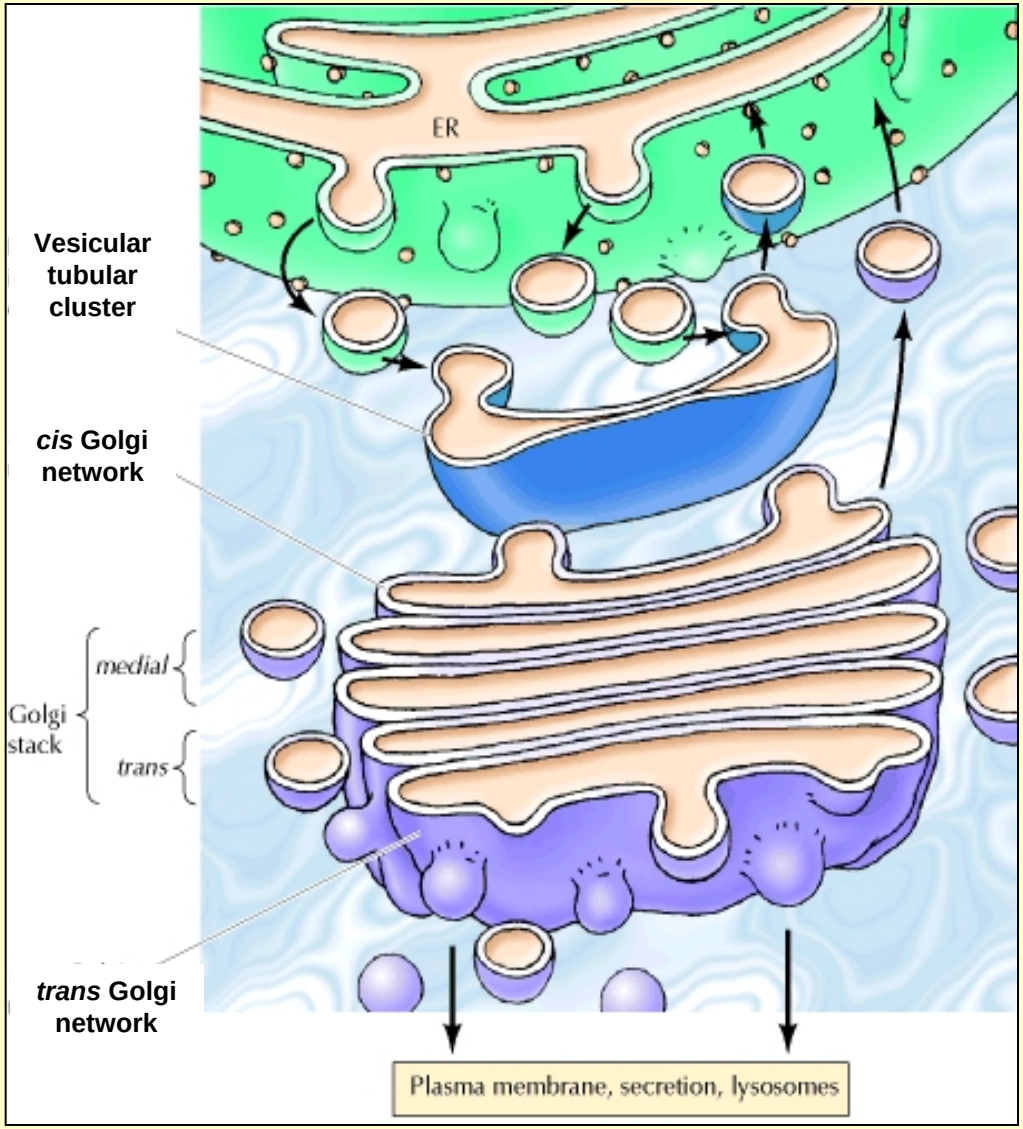


Figure 9-23. 2000. Cooper

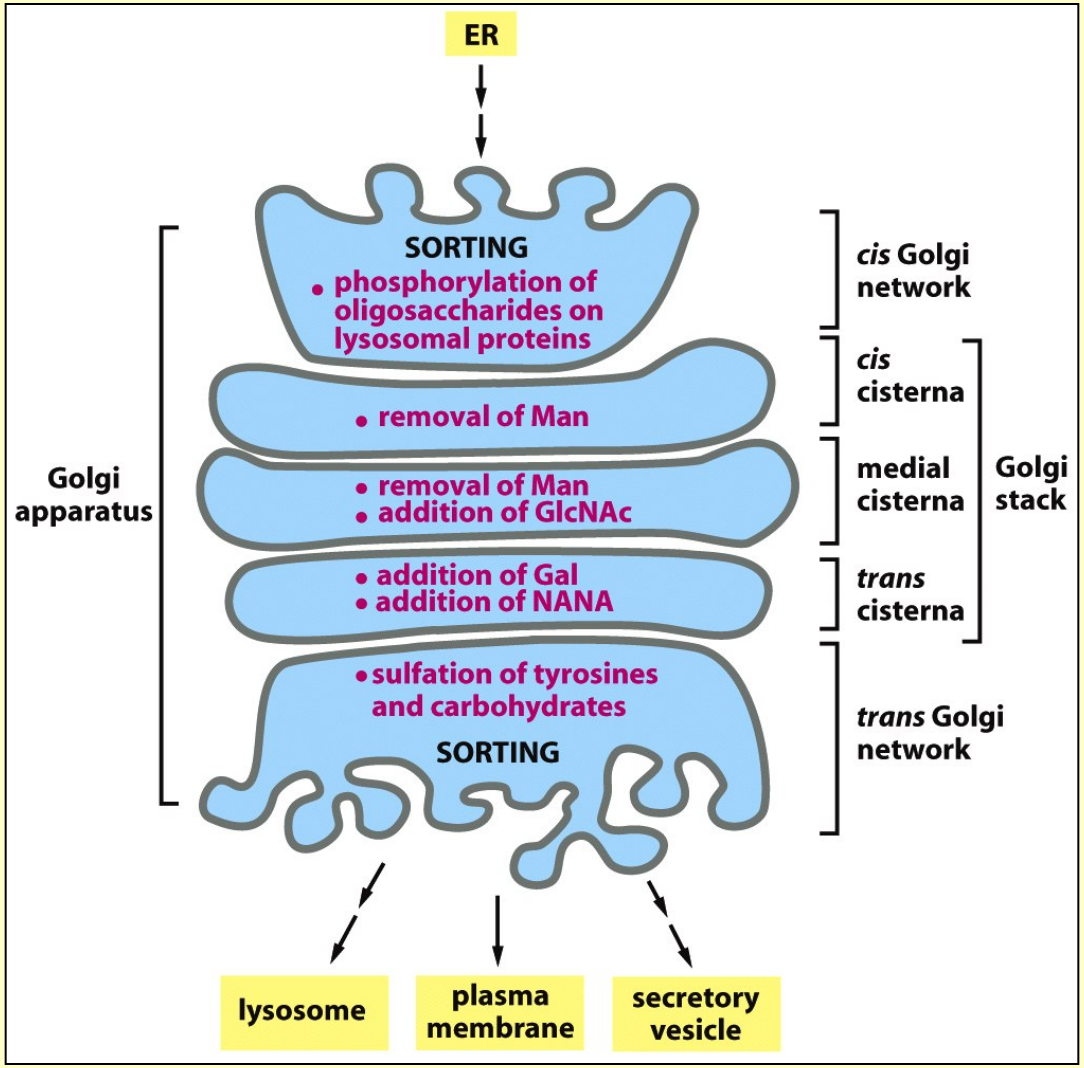
✓ vesicles from ER (COPII) fuse to form the VTC → proteins from the ER are transported to the ***cis* Golgi network**

✓ **GA *medial* and *trans* compartments** of Golgi stack correspond to the cisternae in the middle of GA → sites of most protein modifications

✓ proteins are then carried to the ***trans* Golgi network** (TGN), where they are sorted for transport to:

plasma membrane, secretion, lysosomes

GA biochemical compartments



✓ each compartment has its own specific set of enzymes for protein modification

Figure 13-28 *Molecular Biology of the Cell* (© Garland Science 2008)

Synthesis of sphingomyelin and glycolipids

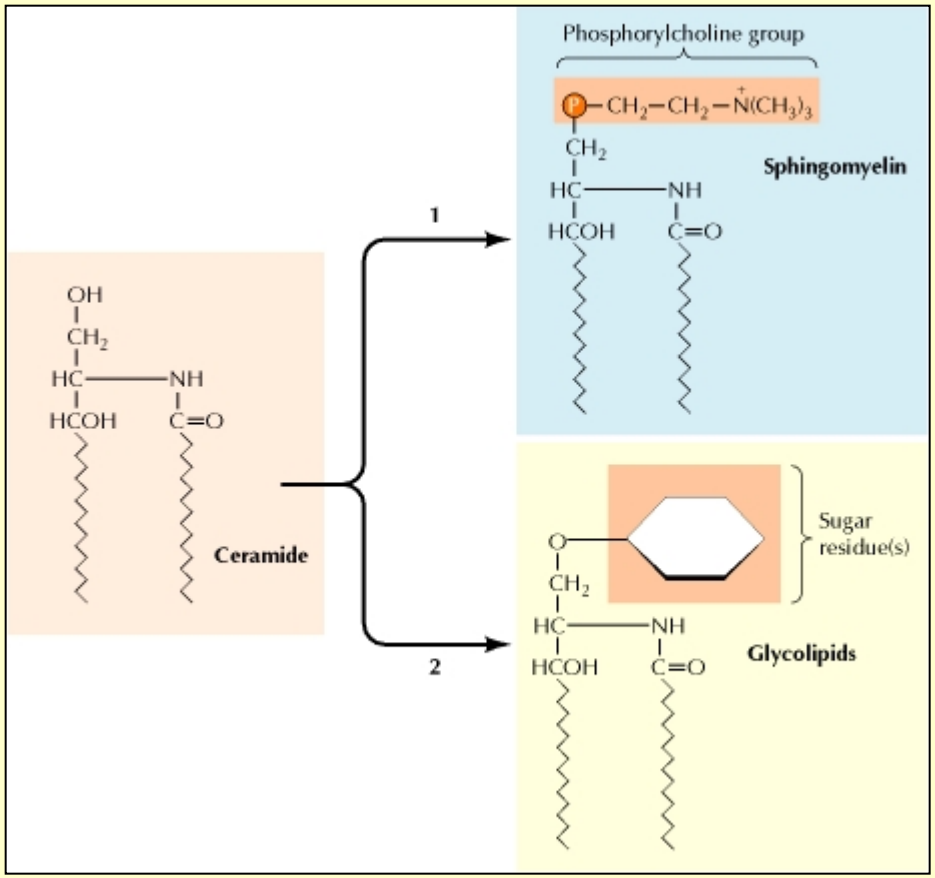


Figure 9-26. 2000. Cooper

✓ceramide, which is synthesized in the ER, is converted either to sphingomyelin or to glycolipids

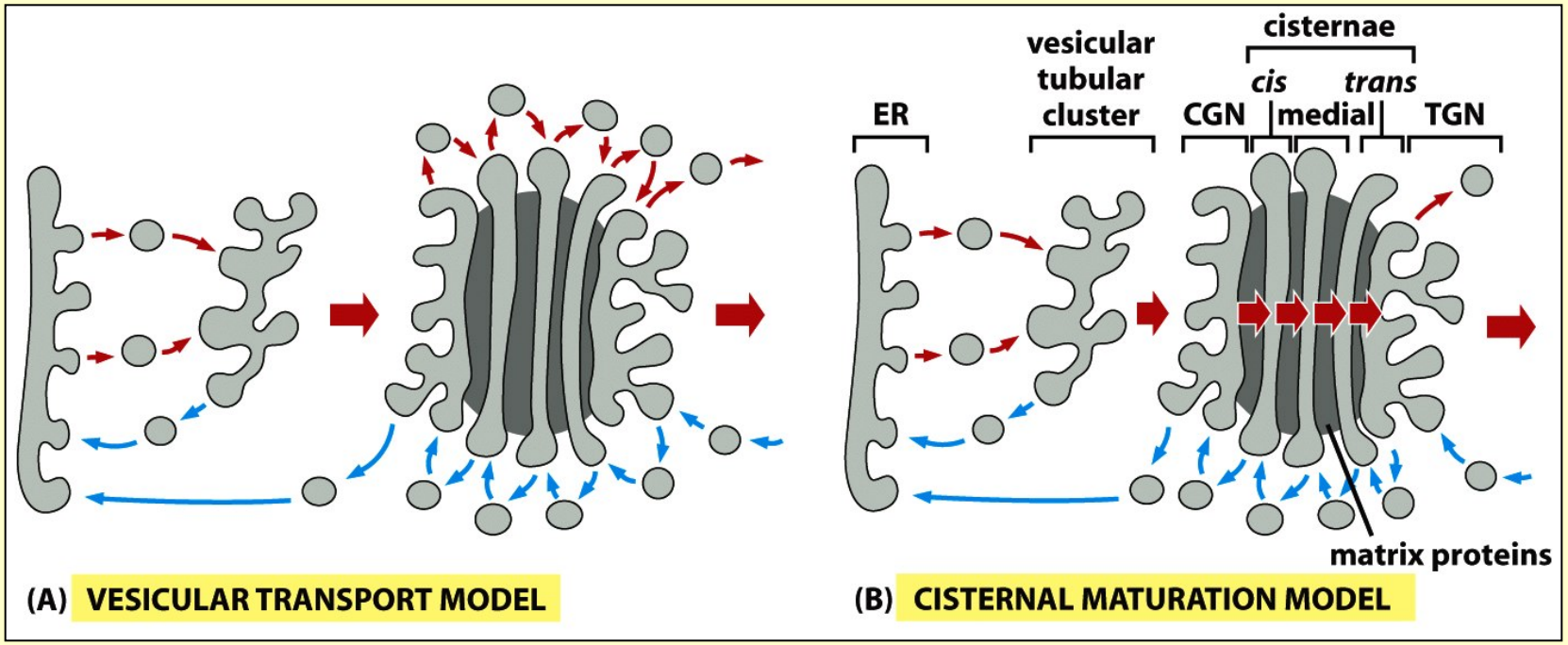
✓1 - phosphorylcholine group is transferred from phosphatidylcholine to ceramide

✓2 - a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (e.g., glucose)

Two possible models explaining the organization of GA and transport of proteins from one cisterna to the next

✓ **vesicular transport model**
→ cisternaes are static and contain a characteristic complement of resident enzymes
→ passing of molecules through GA is accomplished by forward-moving transport vesicles, which bud from one cisterna and fuse with the next in a cis-to-trans direction

✓ **cisternal maturation model**
→ each cisterna matures as it migrates outwards through a stack.



Animation
http://sites.sinauer.com/cooper7e/animation1102.html

Figure 13-35 *Molecular Biology of the Cell* (© Garland Science 2008)

Chlatrin-coated vesicles

- ✓ **chlatrin** – triskelion is composed of 3 clathrin heavy chains and 3 clathrin light chains
- ✓ **adaptin** – connects chlatrin with transmembrane receptors
- ✓ **transmembrane receptors** → selection of **cargo molecules** within the vesicles
- ✓ **dynamin** – helps the pinching-off of the bud to form a vesicle

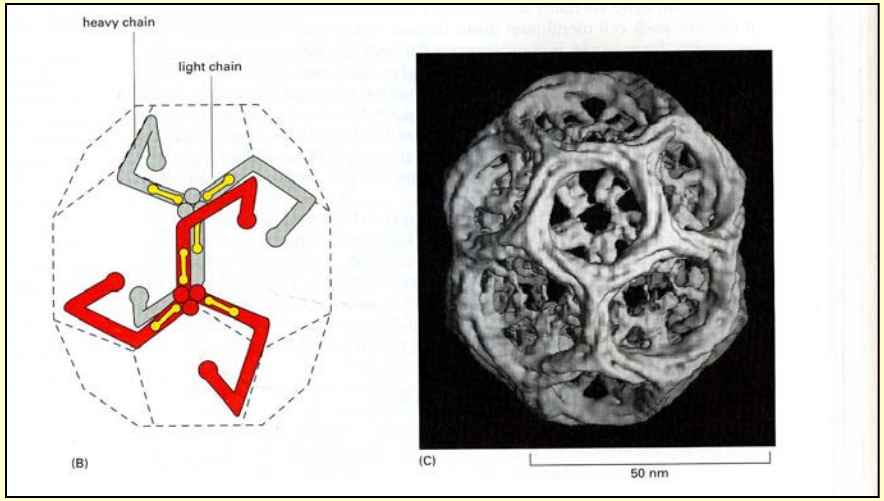
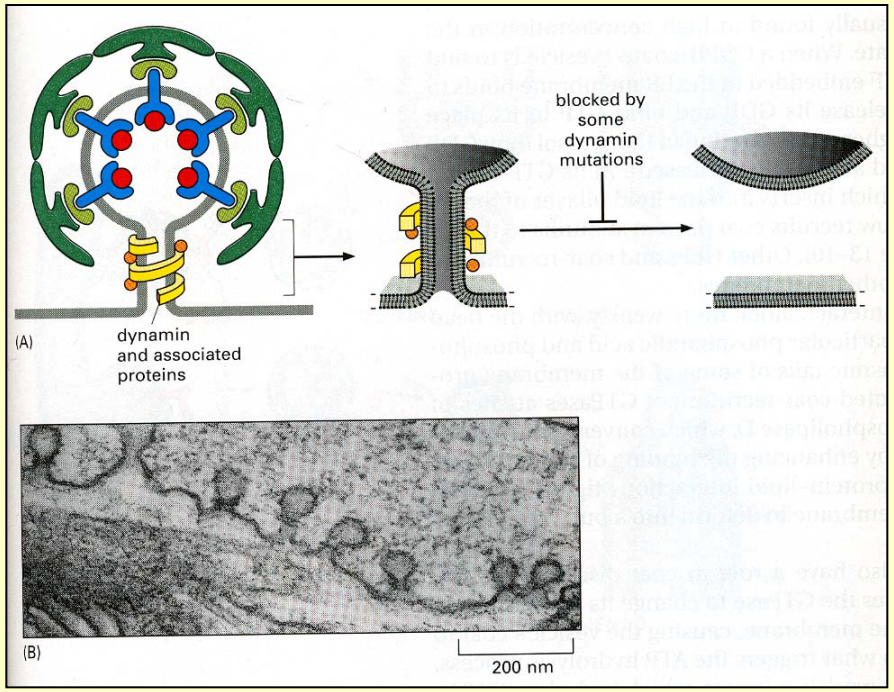


Figure 13-7. The structure of a clathrin coat



2002. Alberts et al.

Formation of chlatrin vesicles

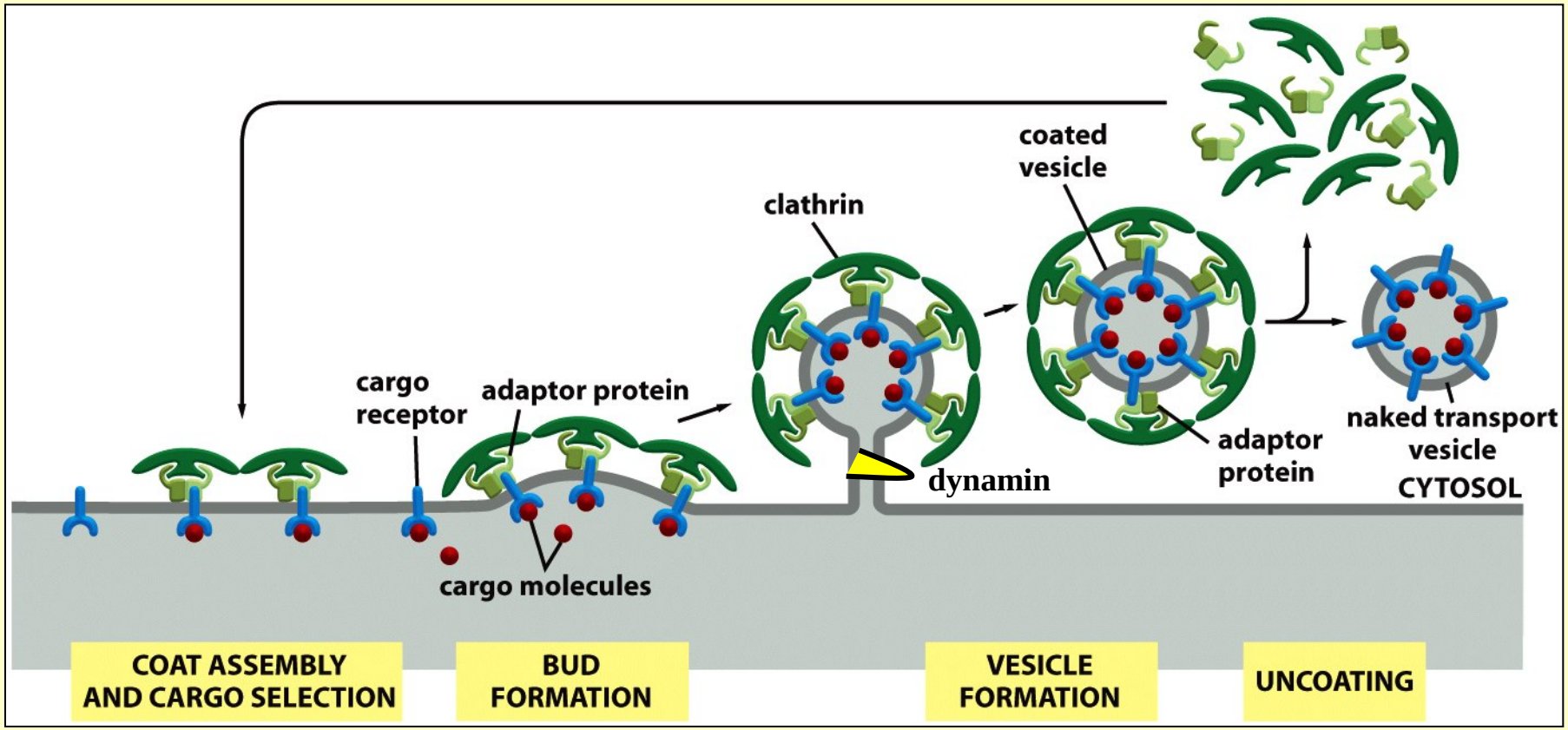


Figure 13-8. 2008. Alberts et al.

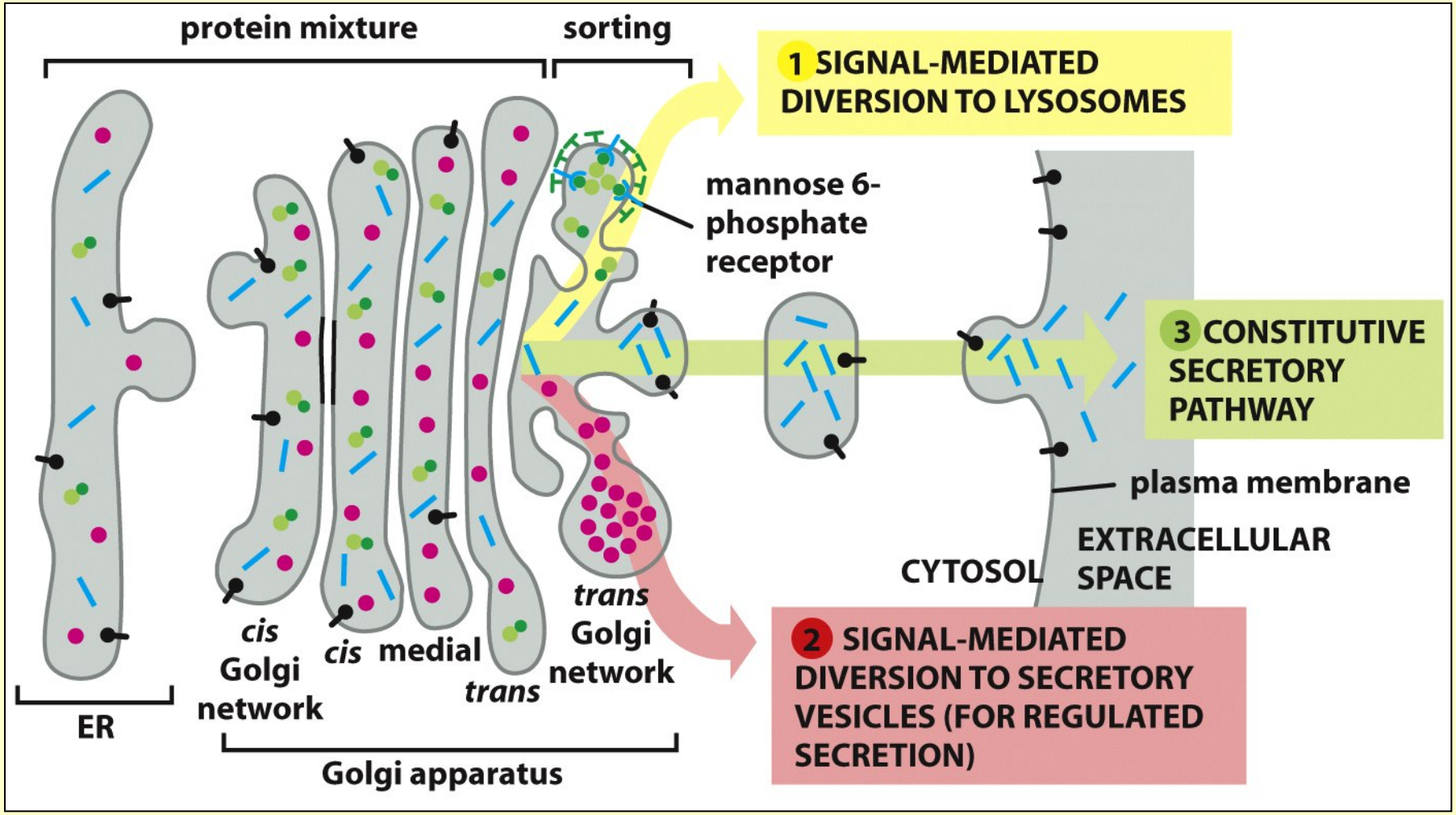
✓ budding of vesicle in TGN → **chlatrin coat** formation

✓ **adaptins** bind both clathrin triskelions and membrane-bound **cargo receptors**
→ mediating the selective recruitment of **cargo molecules** into the vesicle

✓ pinching-off of the bud to form a vesicle involves membrane fusion
→ helped by protein **dynamin**, which assembles around the neck of the bud

✓coat of clathrin-coated vesicles is rapidly removed shortly after the vesicle forms

The three best-understood pathways of protein sorting in the TGN

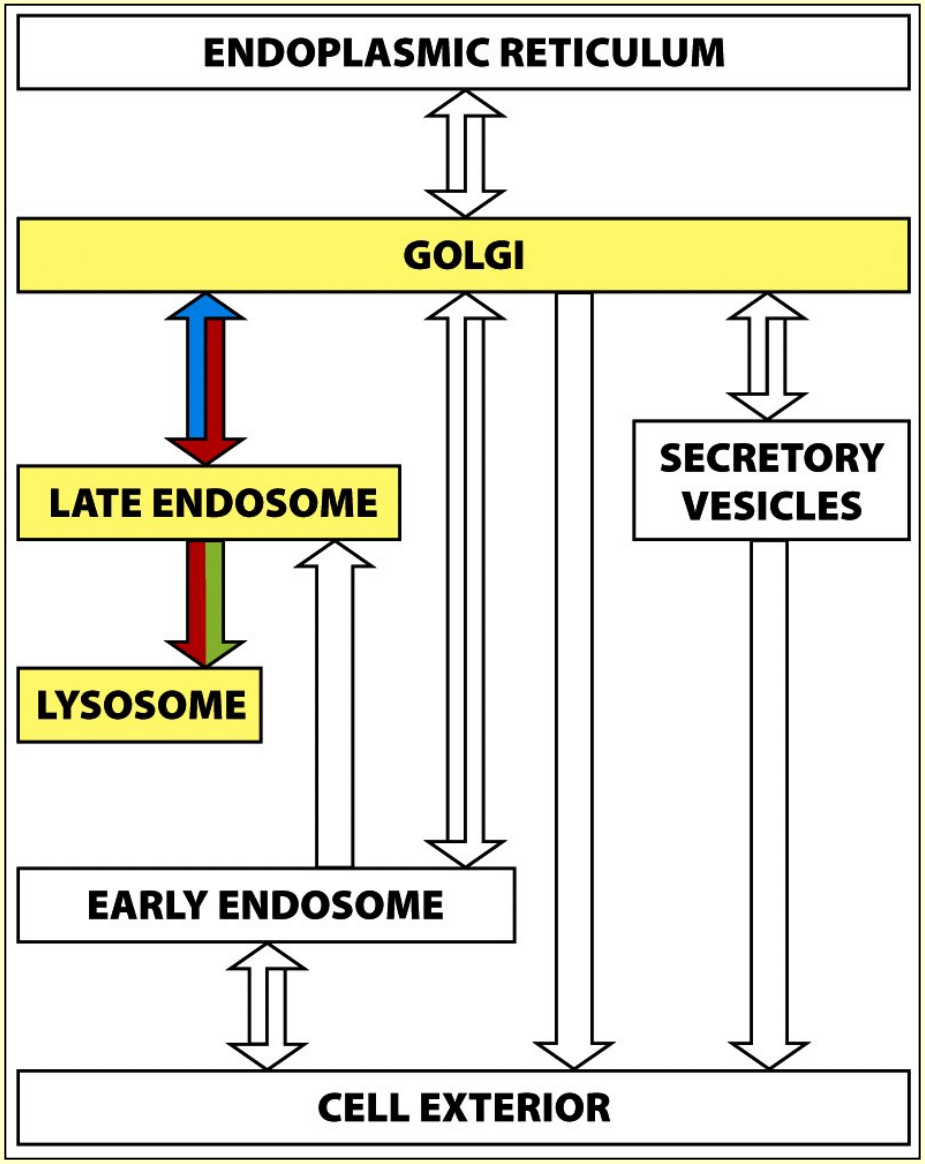


Animation

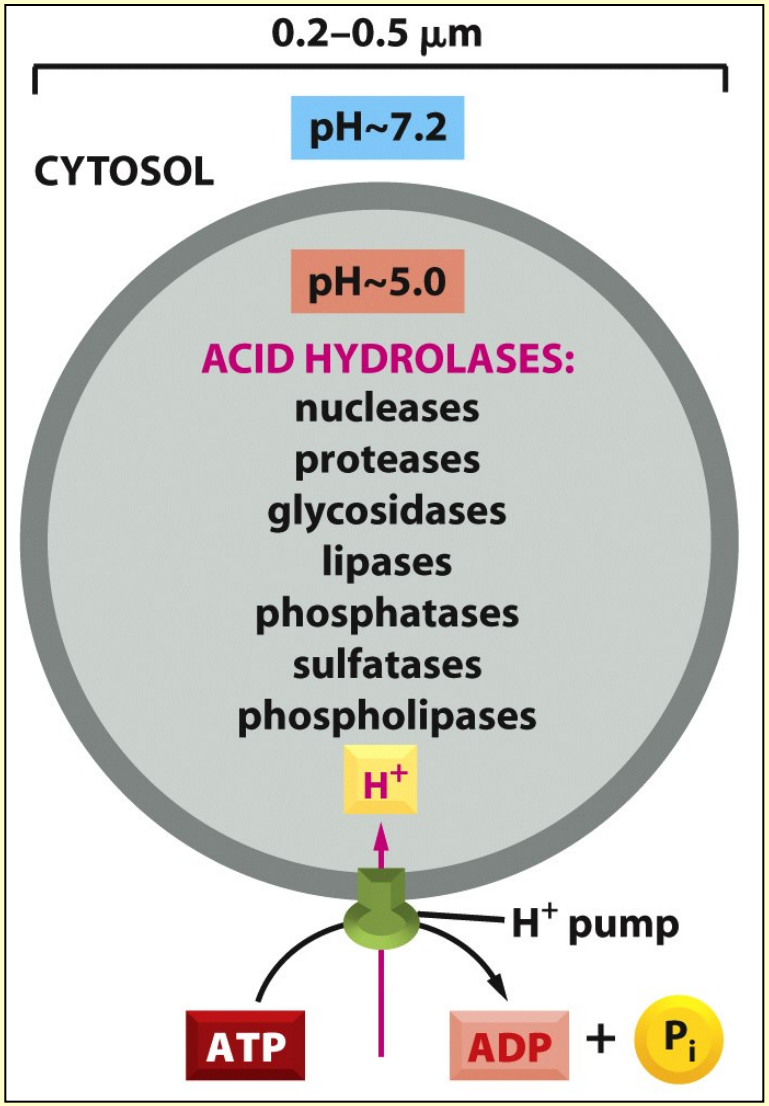
https://www.youtube.com/watch?v=rvfvRgk0MfA

Figure 13-64 *Molecular Biology of the Cell* (© Garland Science 2008)

Transport from *trans* Golgi network to lysosomes



Lysosomes



✓ acid hydrolases are hydrolytic enzymes that are active under acidic conditions

✓ lumen is maintained at acidic pH by H^+-ATPase in the membrane – pumps H^+ into lysosome

Figure 13-36 *Molecular Biology of the Cell* (© Garland Science 2008)

Histochemical visualization of lysosomes

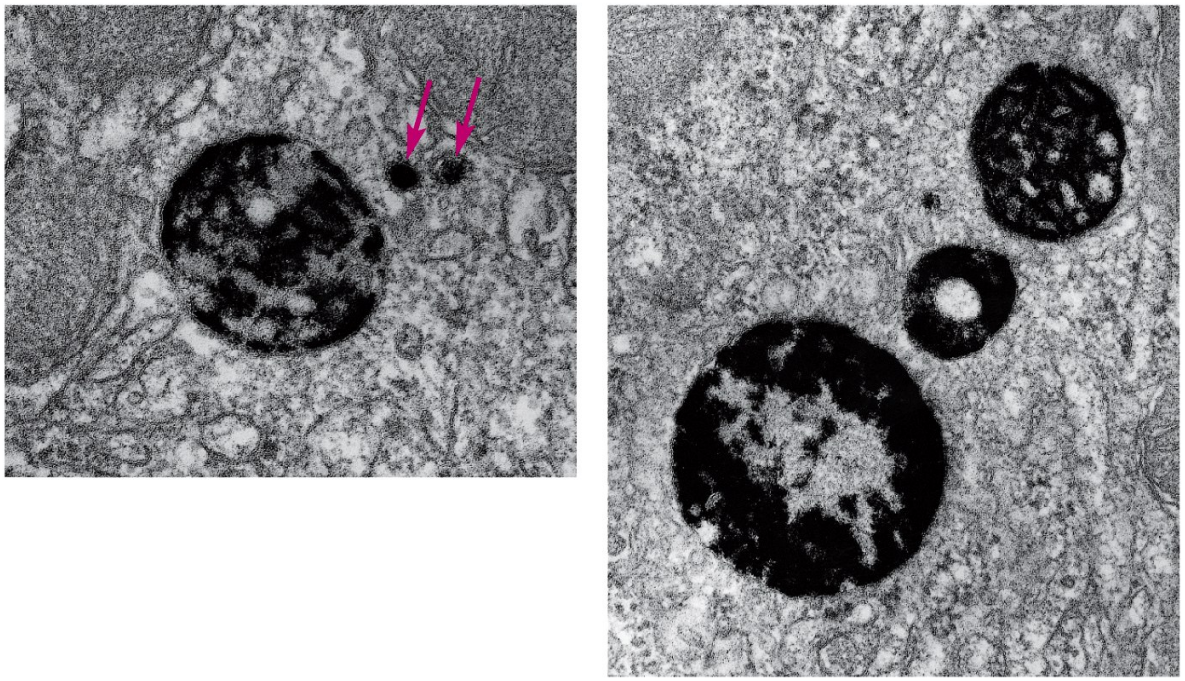


Figure 13-37 *Molecular Biology of the Cell* (© Garland Science 2008)

✓ acid phosphatase → marker enzyme for lysosomes

✓ their diverse morphology reflects variations in the amount and nature of the material they are digesting

✓precipitates are produced when tissue fixed with glutaraldehyde (to fix the enzyme in place) is incubated with a phosphatase substrate in the presence of lead ions

✓ **arrows** → two small vesicles carrying acid hydrolases from the GA

The transport of newly synthesized lysosomal hydrolases to lysosomes

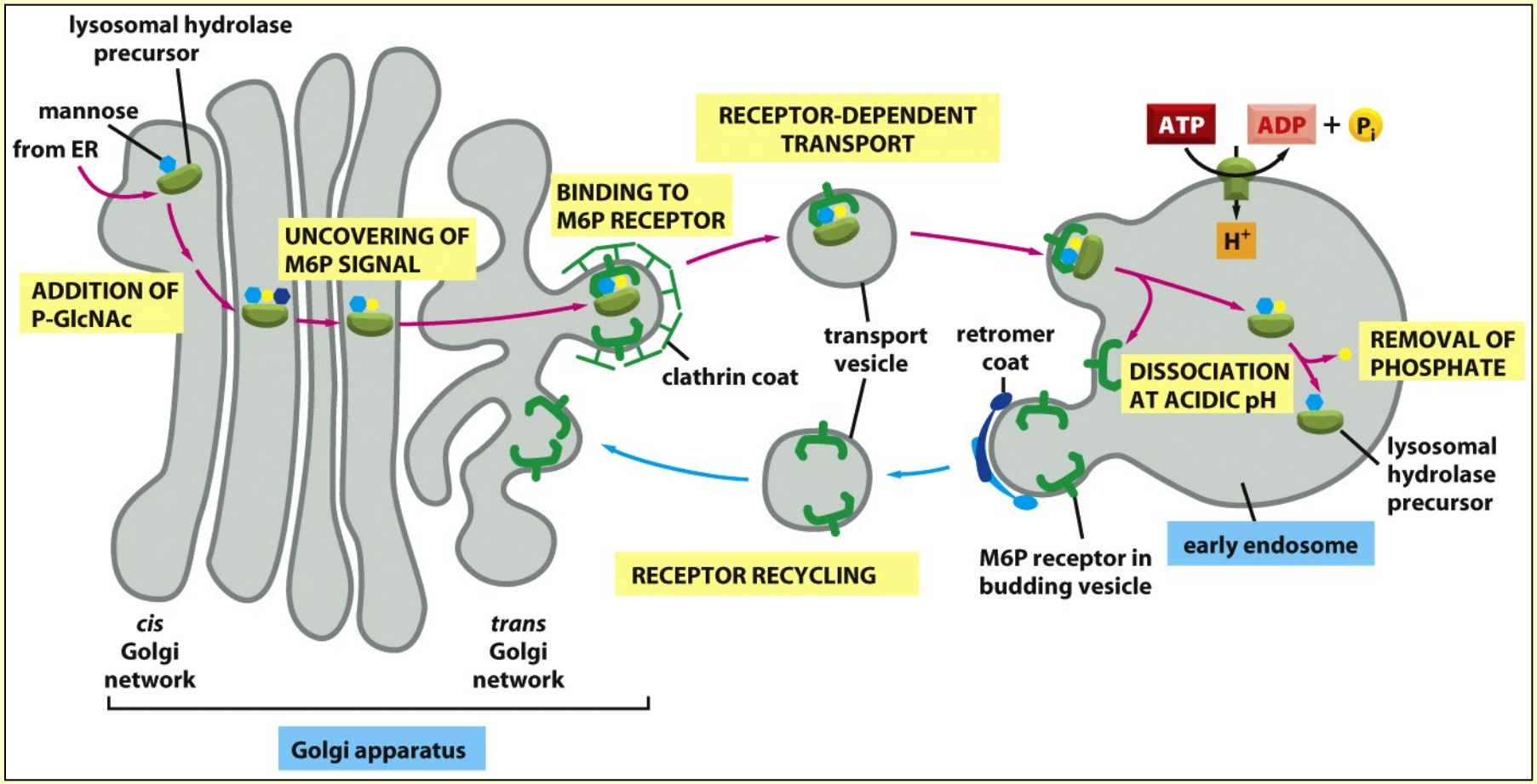


Figure 13-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Three pathways to degradation in lysosomes

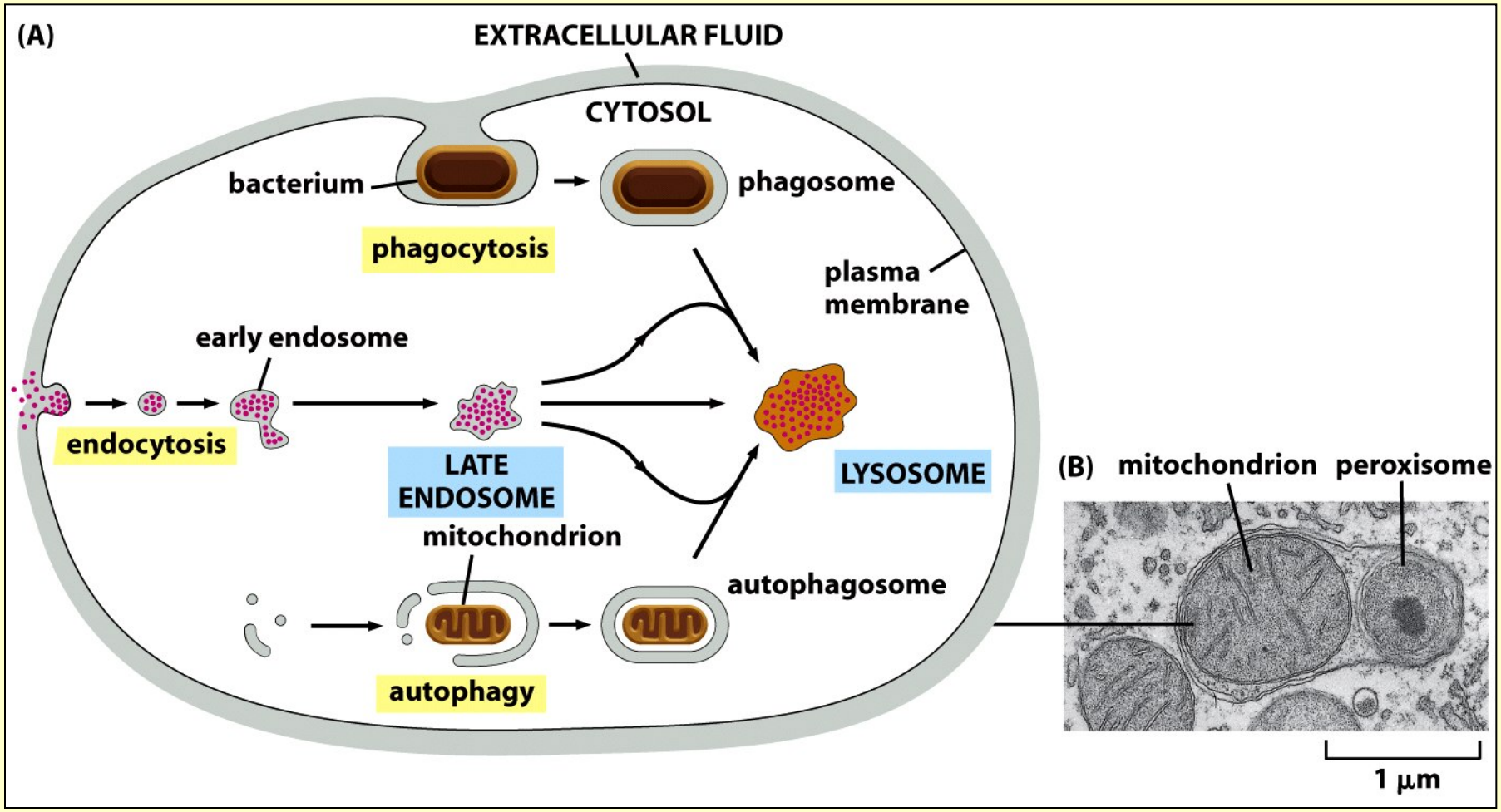


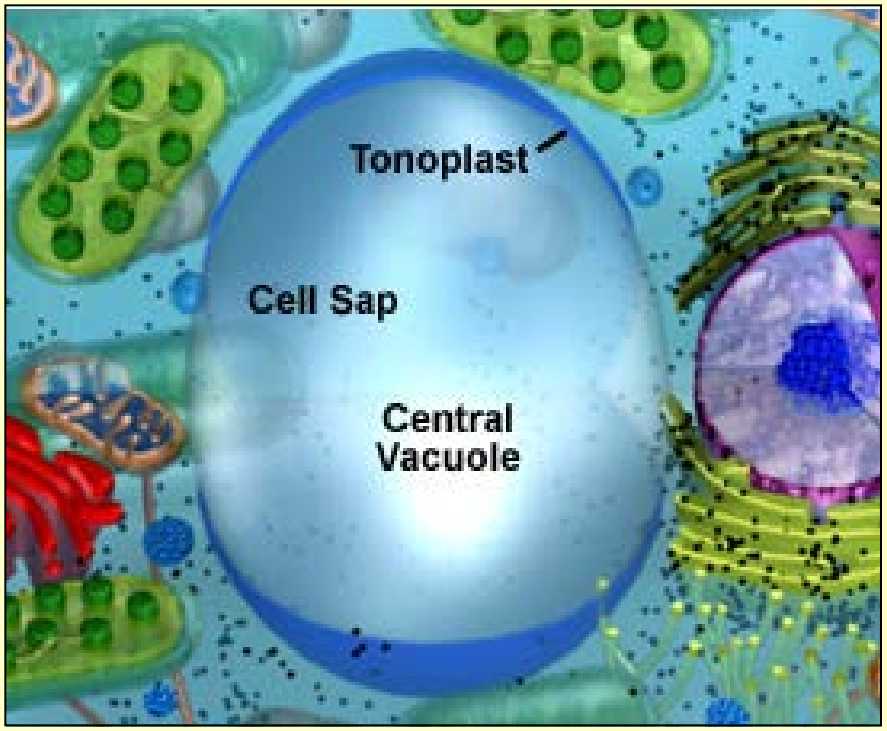
Figure 13-42a *Molecular Biology of the Cell* (© Garland Science 2008)

Plant and fungal vacuoles are remarkably versatile lysosomes

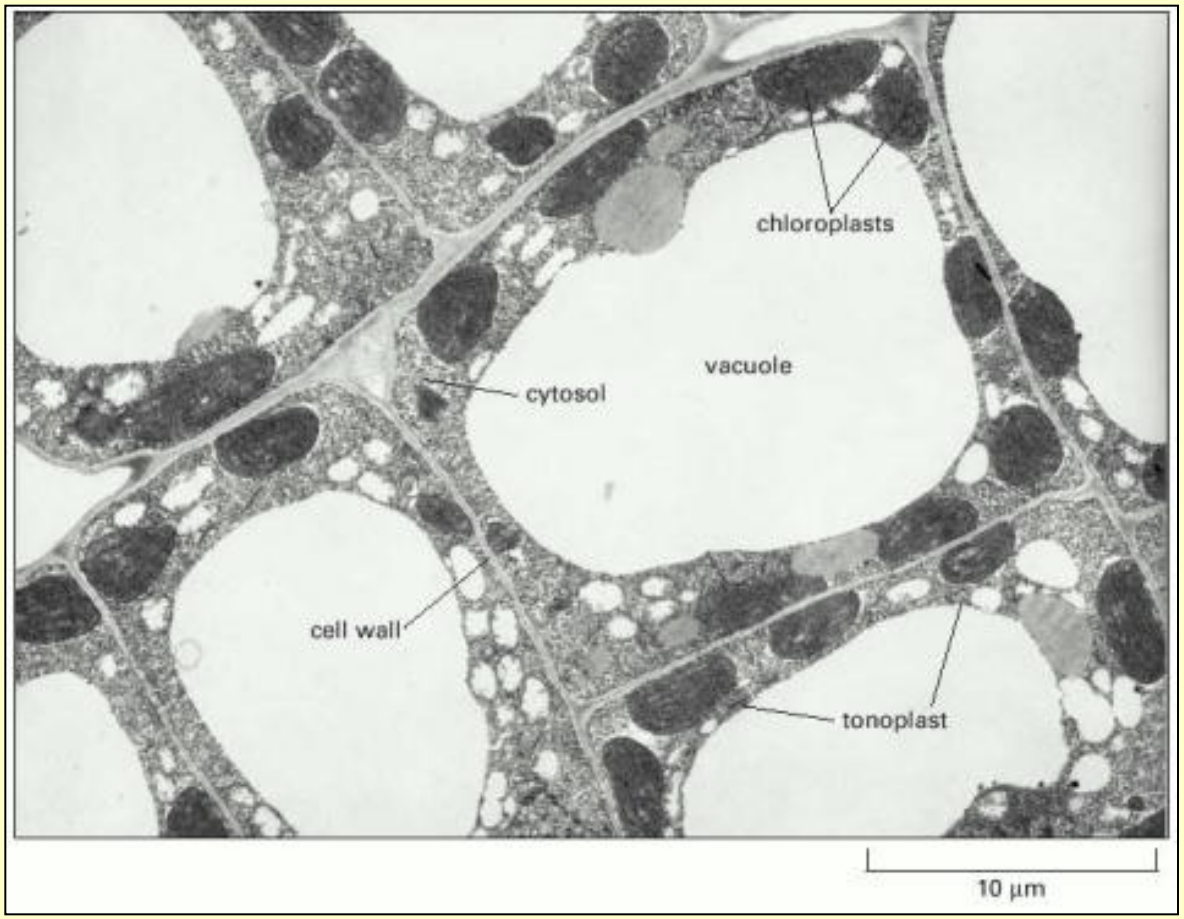
✓ most plant and fungal cells (including yeasts) contain one or several very large, fluid-filled vesicles → **vacuoles**

✓ occupy more than 30% of the cell volume, and as much as 90% in some cell types

✓ related to the lysosomes of animal cells, containing a variety of hydrolytic enzymes



Cell Biology and Microscopy 2004



❖ electron micrograph of cells in a young tobacco leaf

→ cytosol as a thin layer, containing chloroplasts, pressed against the cell wall by the enormous vacuole

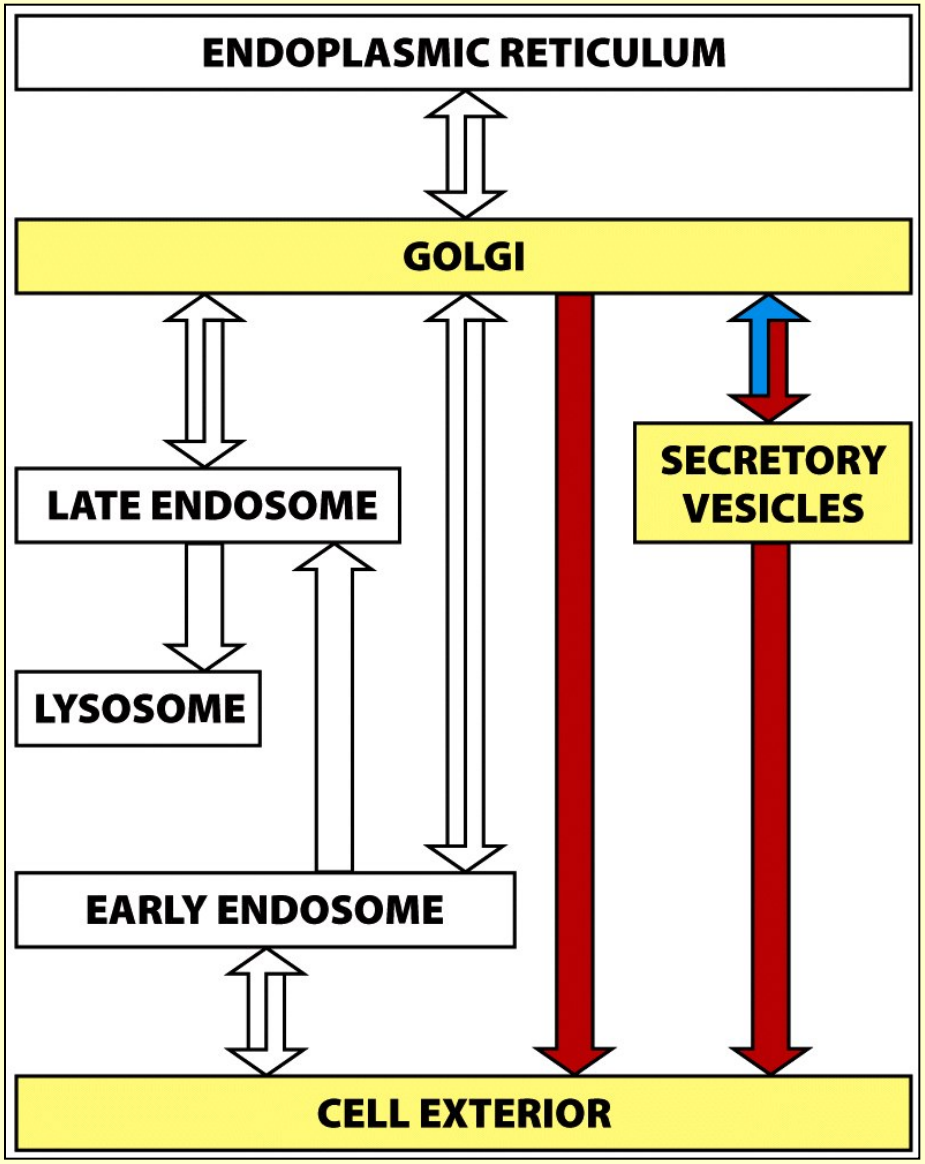
→ membrane of the vacuole is called the **tonoplast**

Figure 13-33. 2002. Alberts et al.

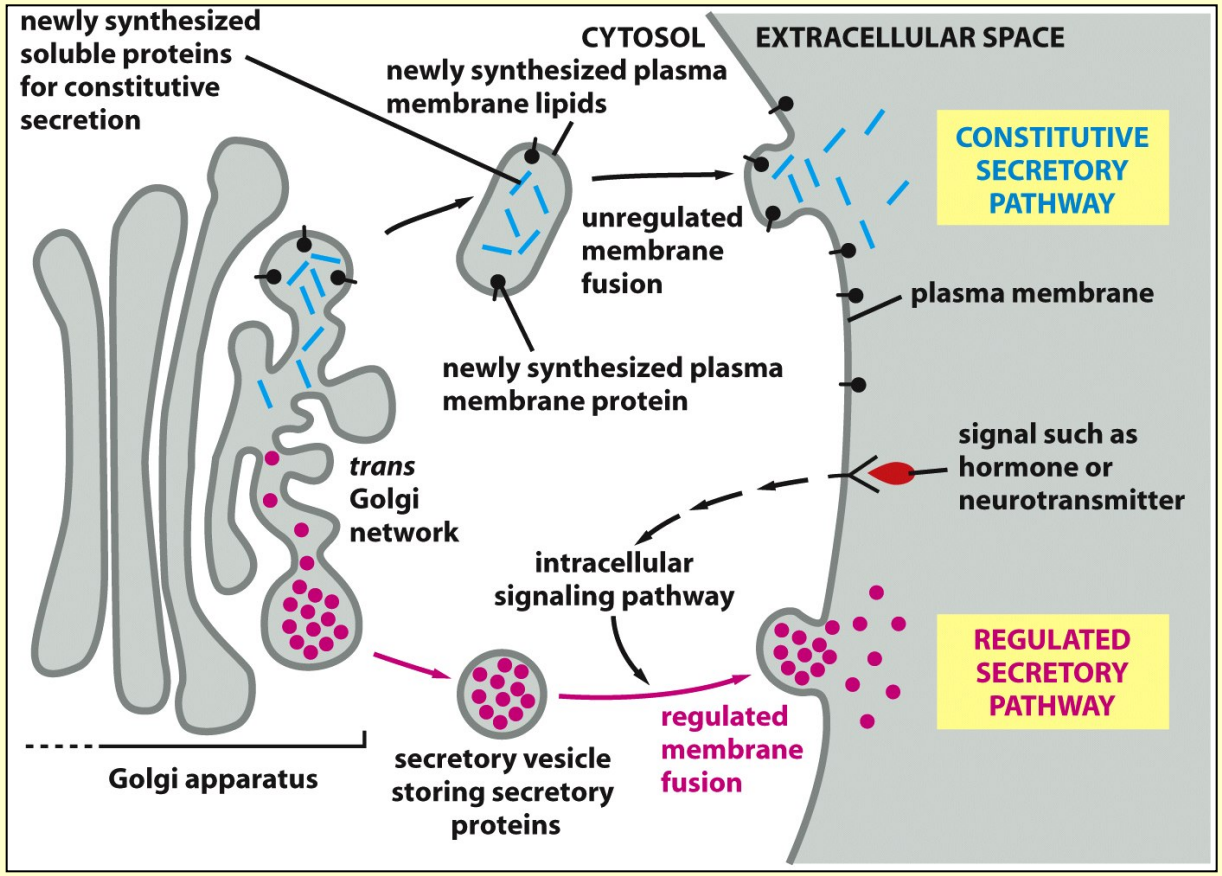
✓ their functions are remarkably diverse - can act as:
→ storage organelle for both nutrients and waste products as a degradative compartment
→ economical way of increasing cell size
→ controller of turgor pressure (the osmotic pressure that pushes outward on the cell wall and keeps the plant from wilting)

✓ different vacuoles with distinct functions (e.g., digestion and storage) are often present in the same cell

Secretion pathways



The constitutive and regulated secretory pathways



✓**constitutive secretory pathway** → operates in all cells and leads to continual unregulated protein secretion

✓ some cells also possess a distinct **regulated secretory pathway** → specific proteins are secreted in response to environmental signals

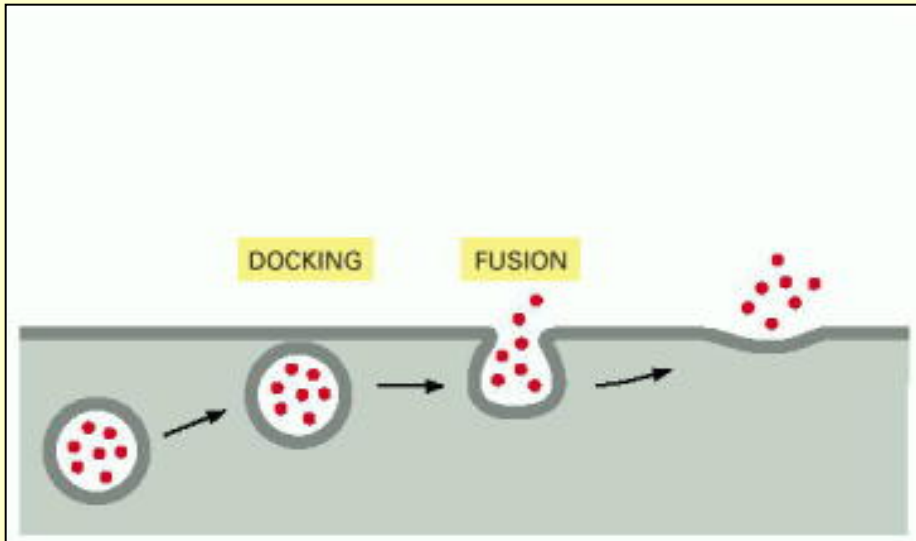
✓examples of regulated secretion:
- release of hormones from endocrine cells
- release of neurotransmitters from neurons
- release of digestive enzymes from the pancreatic acinar cells

Constitutive secretion - http://www.youtube.com/watch?v=MrHULUxAsGg&NR=1

Figure 13-63 *Molecular Biology of the Cell* (© Garland Science 2008)

An example of regulated secretory pathway

✓release of insulin from a secretory vesicle of a pancreatic β-cell



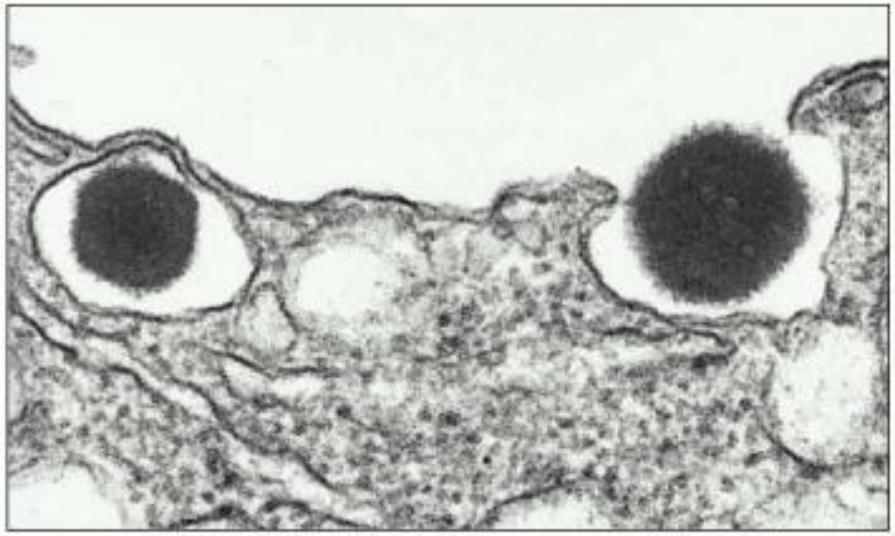


Figure 13-57. 2002. Alberts et al.

Regulated secretion animation -
http://www.youtube.com/watch?v=guqCEa7Y4RA&feature=related