

Cytoplasm, organelles and nucleus

Intracellular Compartments:

Endoplasmic Reticulum and Protein Sorting

Golgi Apparatus and Intracellular Vesicular Traffic

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Compartmentalization of Cells

- A prokaryotic cell consists of a single compartment: the cytosol enclosed by the plasma membrane.
- A eukaryotic cell is subdivided by internal membranes - creating enclosed compartments where sets of enzymes can operate without interference

Compartmentalization of Cells

Major intracellular compartments of an animal cell

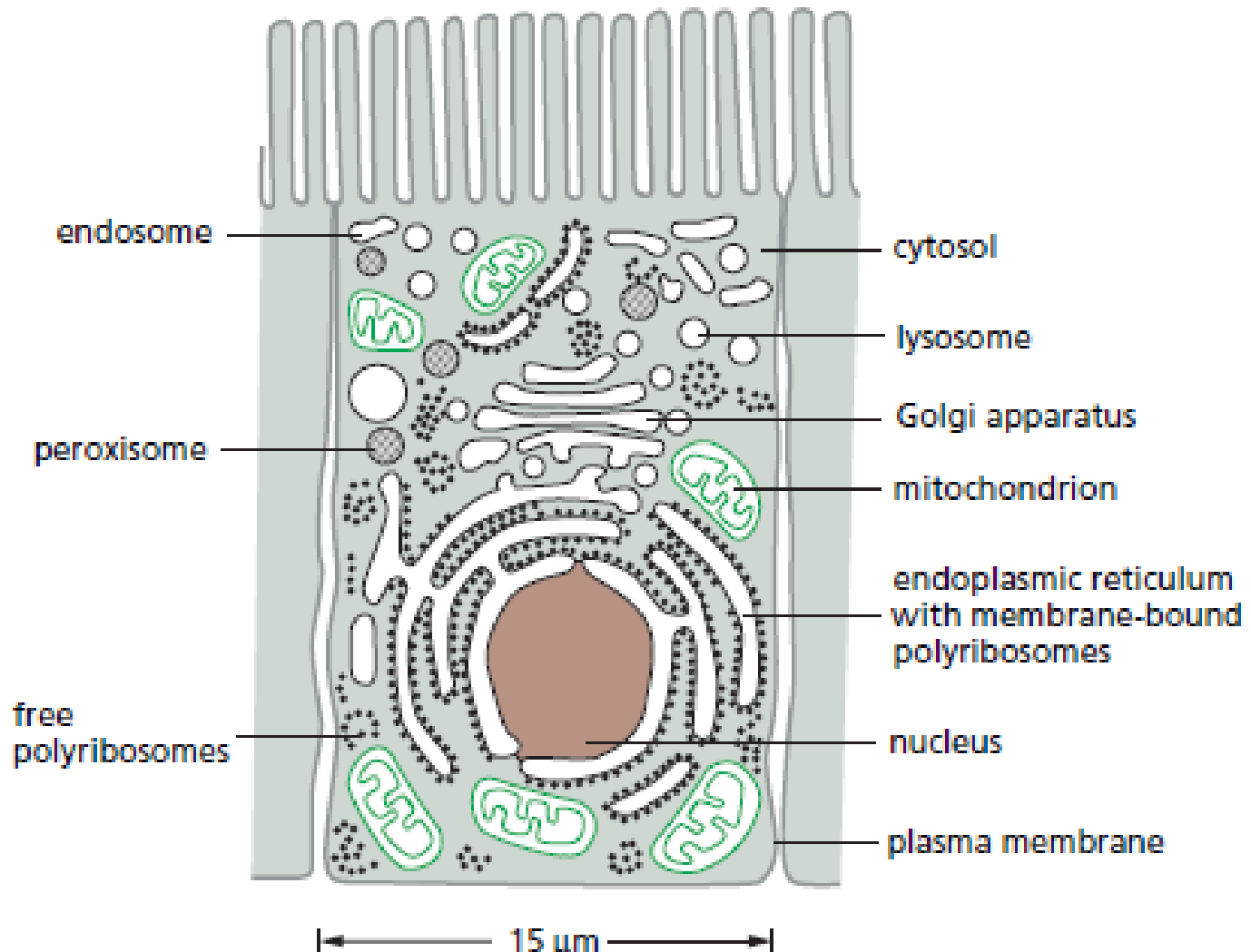
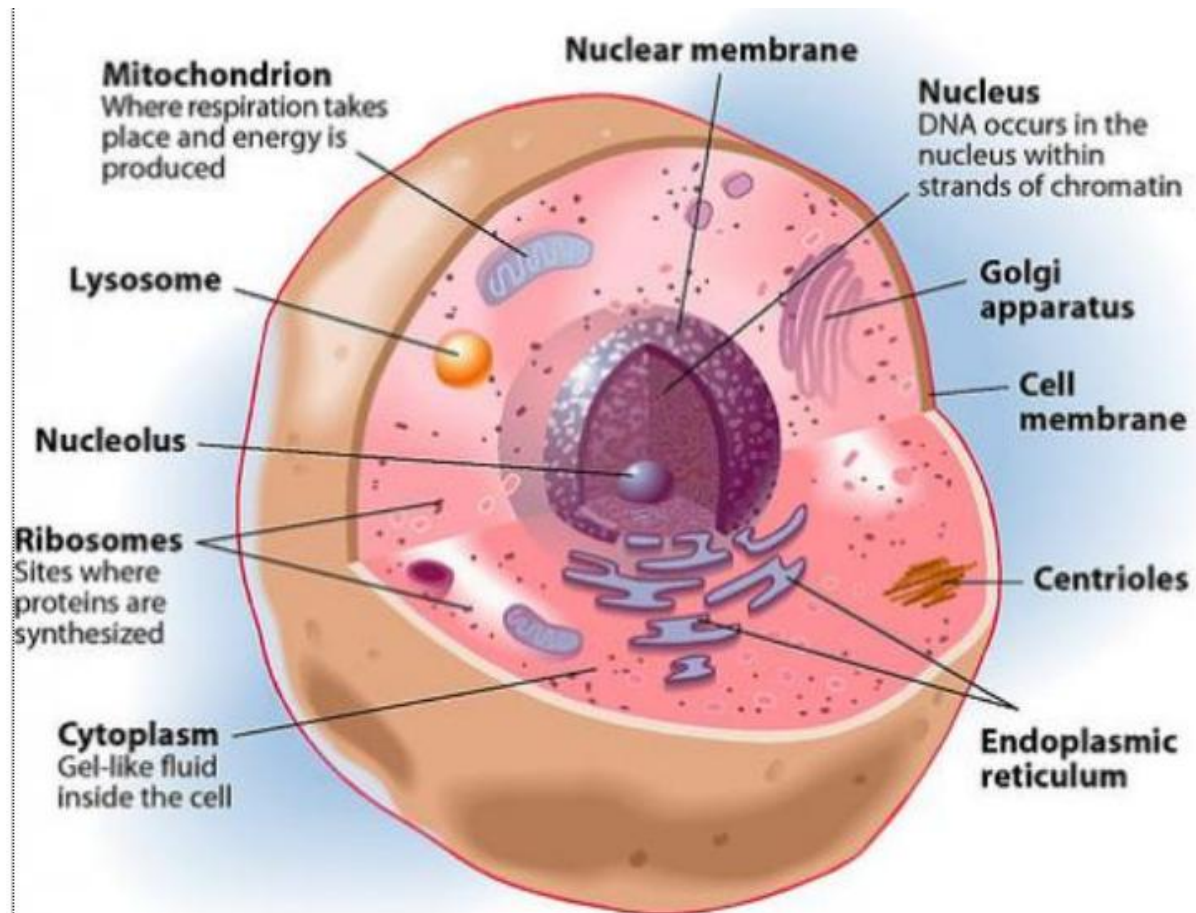


Figure 12–1 The major intracellular compartments of an animal cell. Molecular Biology of the Cell, 5th ed.

Cytoplasm

- The cytoplasm consists of the cytosol and the cytoplasmic organelles



Cytosol

- intracellular fluid
- part of the cytoplasm
- constitutes a little more than half the total volume of the cell
- Performs most of the cell's intermediary metabolism (degradation of some small molecules, proteins and synthesize others to provide the building blocks for macromolecules)

Organelles

Organelles Cannot be Constructed *de novo*

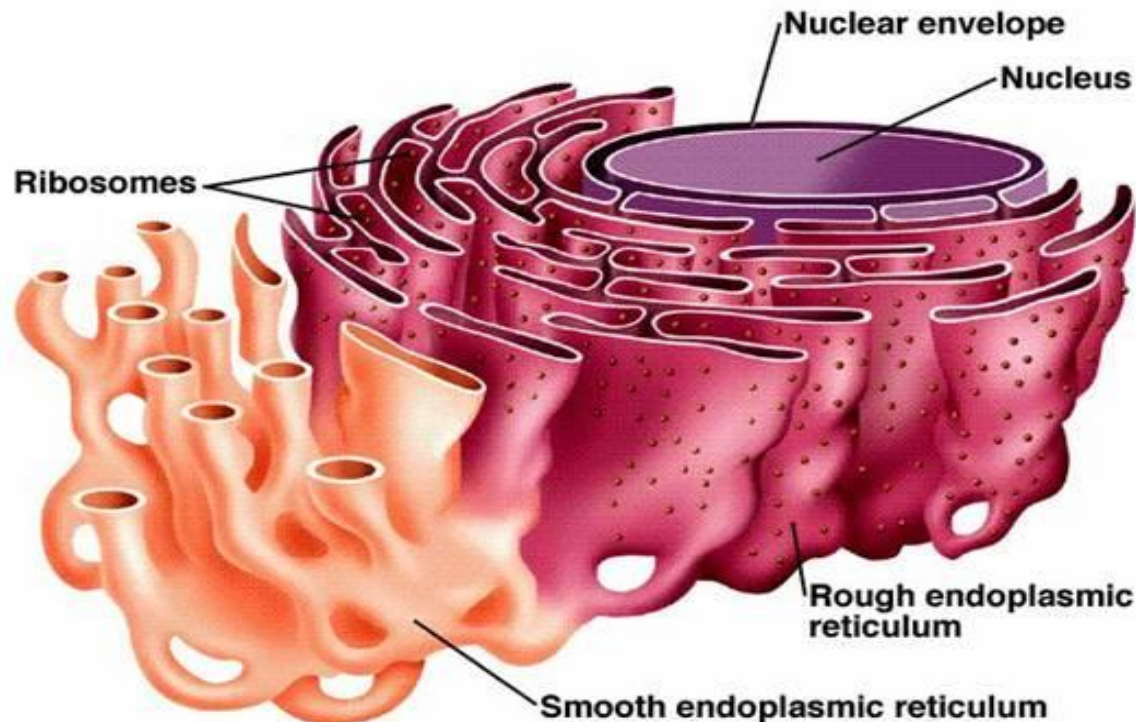
- Organelles reproduced via binary fission
- Organelles cannot be reconstructed from DNA alone
- Information in form of one protein that pre-exists in organelle membrane is required and passed on from parent to progeny

Functions of major intracellular compartments:

Organelle	Function
Nucleus	contains main genome, DNA and RNA synthesis
Endoplasmic reticulum (ER)	synthesis of proteins, lipid synthesis
Golgi apparatus	covalent modification of proteins from ER, sorting of proteins for transport to other parts of the cell
Mitochondria and chloroplasts (plants)	ATP synthesis
Lysosomes	degradation of defunct intracellular organelles and material taken in from the outside of the cell by endocytosis
Endosomes	sorts proteins received from both the endocytic pathway and from the Golgi apparatus
Peroxisomes	oxidize a variety of small molecules

Endoplasmic Reticulum (ER)

- All eukaryotic cells have an endoplasmic reticulum
- Constitutes more than half the total area of membrane in a eukaryotic cell



The structure of ER

- ER has branching tubules and flattened sacs that extends throughout the cytosol.
- The tubules and sacs is continuous with the outer nuclear membrane so material in the ER lumen can move freely into the perinuclear space (between the two layers of the nuclear envelope)

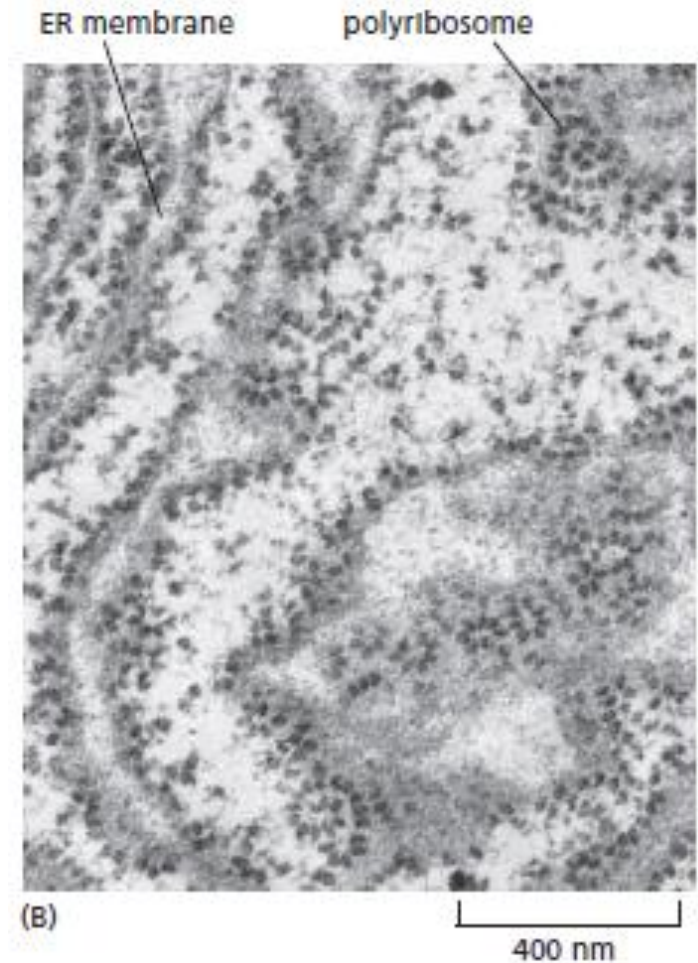
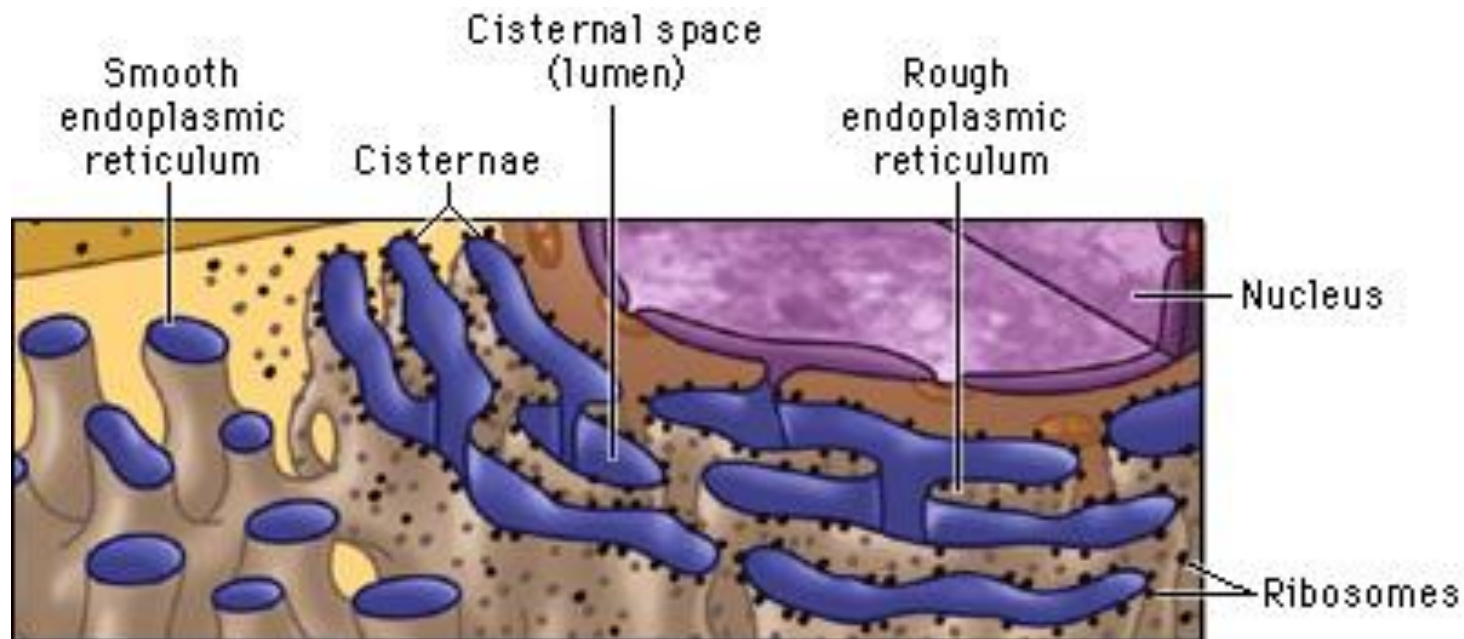


Figure 12–41 Free and membrane-bound ribosomes. Molecular Biology of the Cell, 5th ed.

The structure of ER

- The sacs are called cisternae, the space enclosed is the ER Lumen.



Functions of Endoplasmic Reticulum in the cell

- Storage, release and reuptake of calcium from the cytosol
- Biosynthesis of protein for most of the cell's organelles:
 - for ER, Golgi apparatus, lysosomes, endosomes, secretory vesicles and the plasma membrane
 - proteins that will be secreted to the cell exterior

Functions of Endoplasmic Reticulum in the cell

- Biosynthesis of lipids for most of the cell's organelles:
 - transmembrane lipids for ER, Golgi apparatus, lysosomes, endosomes, secretory vesicles and the plasma membrane
 - lipids for mitochondrial and peroxisomal membranes
- Initiation site for N-linked glycosylation of proteins
 - for proper folding in the ER

Two types of ER

- Smooth ER
- Rough ER
- Smooth and Rough ER are continuous : material can travel from one to the other

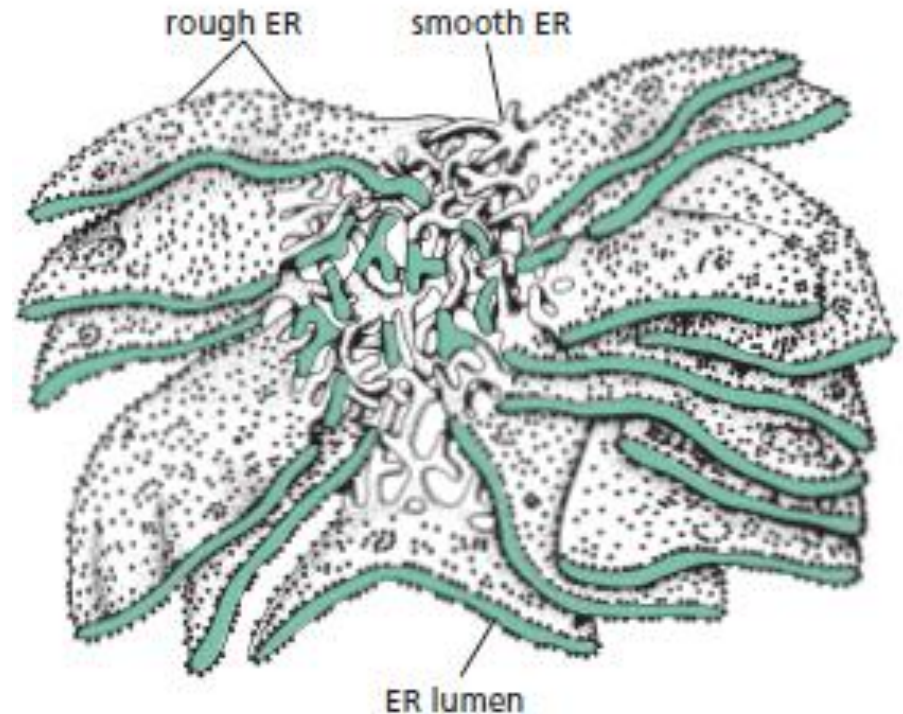


Figure 12–36 The rough and smooth ER. Molecular Biology of the Cell, 5th ed.

Smooth ER

- Lack ribosomes

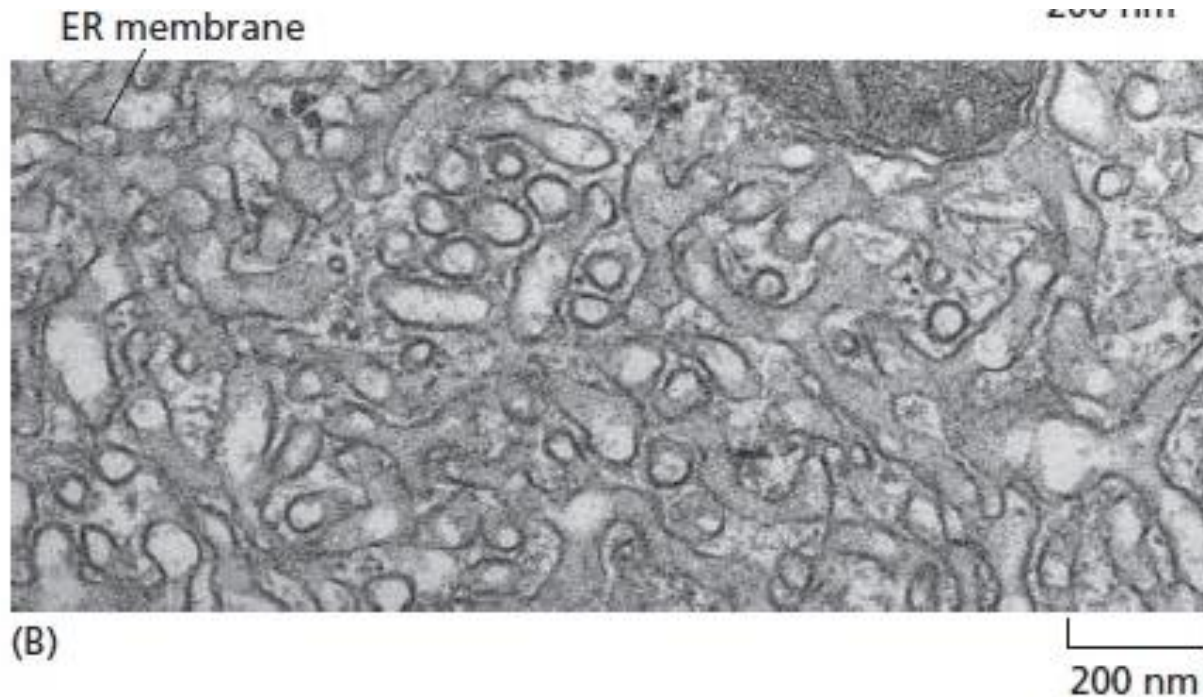


Figure 12–36 The rough and smooth ER. Molecular Biology of the Cell, 5th ed.

Function of the smooth ER in the cell

- Carbohydrate metabolism
- Calcium storage
- Steroid biosynthesis
- Membrane biosynthesis

Rough ER

- Many ribosomes bound to its cytosolic surface

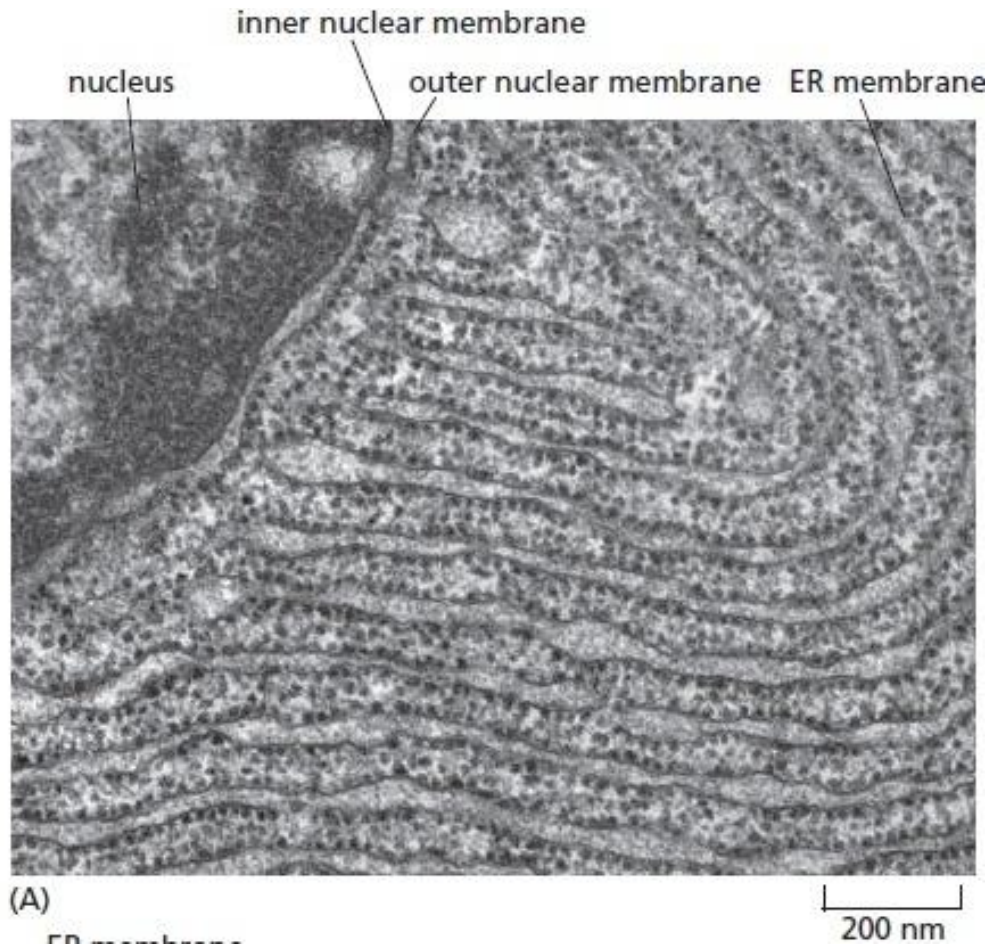


Figure 12–36 The rough and smooth ER. Molecular Biology of the Cell, 5th ed.

Rough ER

- Synthesis of both membrane-bound (organelle and plasma membrane proteins) and soluble proteins (organelle and secreted proteins)
- Most proteins that enter the endomembrane system, enter the ER co-translationally: as translation is occurring
- Most proteins of other membrane bound organelles (mitochondria, chloroplasts, peroxisomes) are transported there posttranslationally.

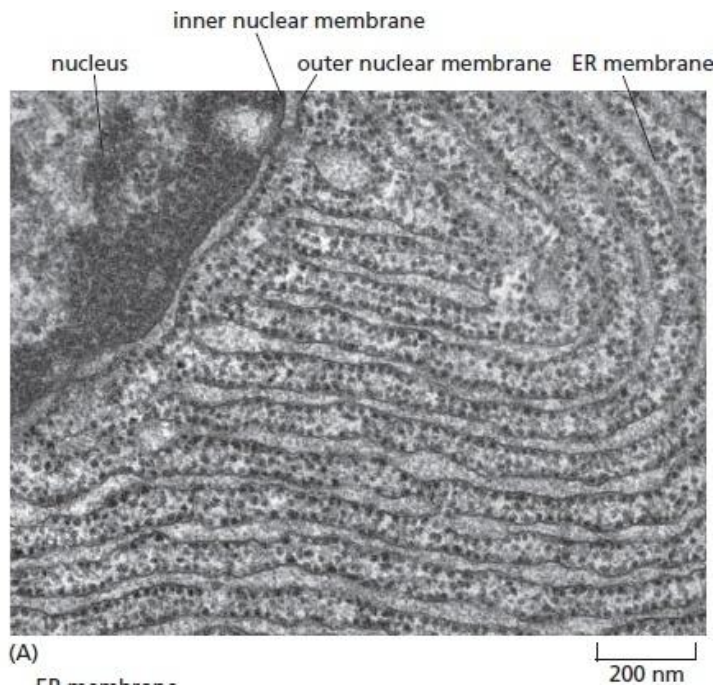


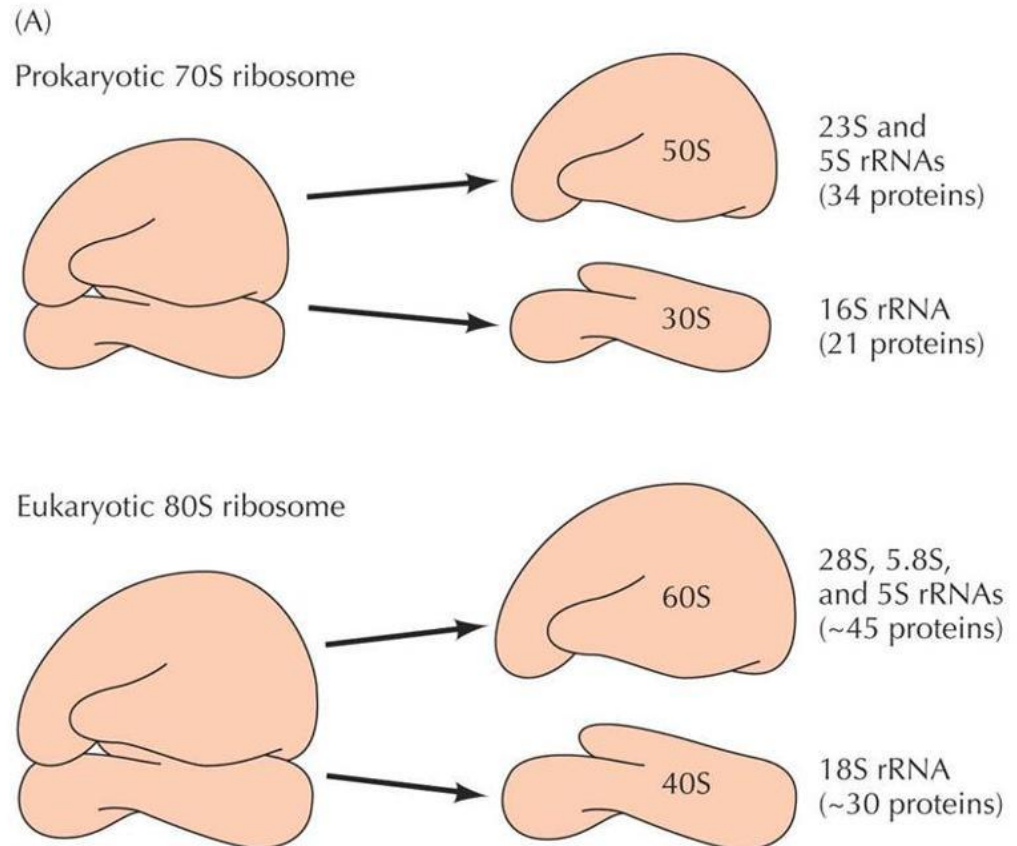
Figure 12–36 The rough and smooth ER. Molecular Biology of the Cell, 5th ed.

Rough ER

- Initial steps of carbohydrate addition (glycosylation)
- Folding of proteins
- Assembly of multimeric proteins
- “Quality control”: improperly folded or modified proteins are retained or degraded

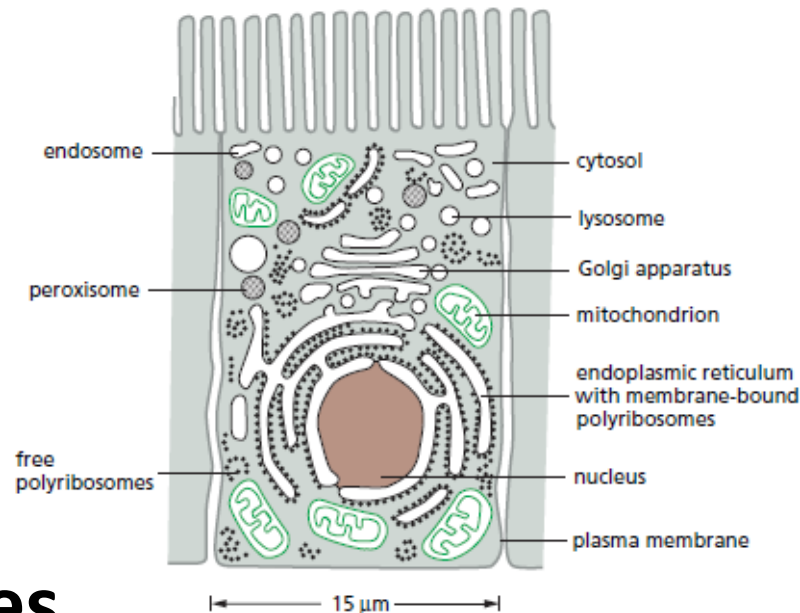
Ribosomes

- Serve as the primary site for protein synthesis
- Consist of two major components:
 - the small ribosomal subunit which reads the RNA
 - the large subunit which joins amino acids to form a polypeptide chain



Ribosomes

- There are two types of ribosomes:
 - Membrane-bound
 - Free ribosomes



- **Membrane-bound ribosomes**
 - attached to the cytosolic side of the ER membrane
 - engaged in the synthesis of proteins that are being concurrently translocated into the ER.

Ribosomes

- **Free ribosomes:**
 - unattached to any membrane
 - synthesize all other proteins encoded by the nuclear genome.
- Membrane-bound and free ribosomes are structurally and functionally identical.
- They differ only in the proteins they are making at any given time.

Why do cells need proteins?

- Proteins have diverse functions in the cell
 - Regulation of cell and body functions
 - Cell signaling
 - Cell cycle
 - Cell adhesion
 - Catalysis
 - Motion
 - Actin and myosin in the muscle

Protein Sorting

- Each compartment contains a unique set of proteins to regulate the cell functions
- Proteins have to be transferred selectively to the compartment in which they will be used:

Protein Sorting

Protein Sorting

- Proteins contain specific sequences that signal to the cell's machinery what the fate of the protein is to be.
- Protein sorting depends on signals built into the amino acid sequence of the proteins.
 - Ex: KDEL (lysine, aspartate, glutamate, leucine) at the carboxy terminus of a protein signals that it should be retained in the ER

Protein Sorting

Transport guided by:

1. Sorting signals in transported proteins

- Signal Sequence (amino acid sequence)
- Signal Patches (three-dimensional arrangement of atoms on the protein's surface)

2. Complementary receptor proteins

Protein Sorting: Roadmap of protein traffic

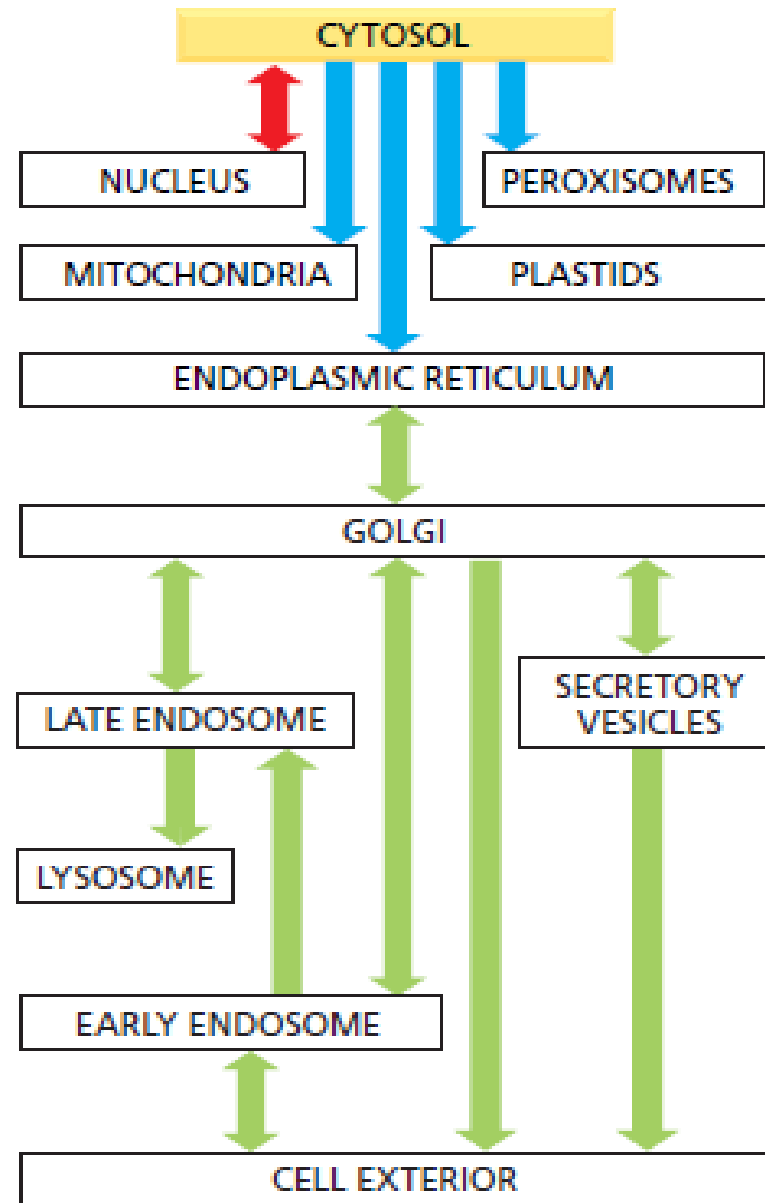
3 Types of Transport Mechanisms

1. Gated Transport:

The nuclear pore complexes function as selective gates actively transport specific macromolecules

KEY: █ = gated transport
█ = transmembrane transport
█ = vesicular transport

Figure 12–6 A simplified “roadmap” of protein traffic. Molecular Biology of the Cell, 5th ed.



Protein Sorting: Roadmap of protein traffic

2. Transmembrane Transport:

Directly transport specific proteins across a membrane from the cytosol into a space

3. Vesicular transport:

Transport of proteins spherical transport from one compartment to another

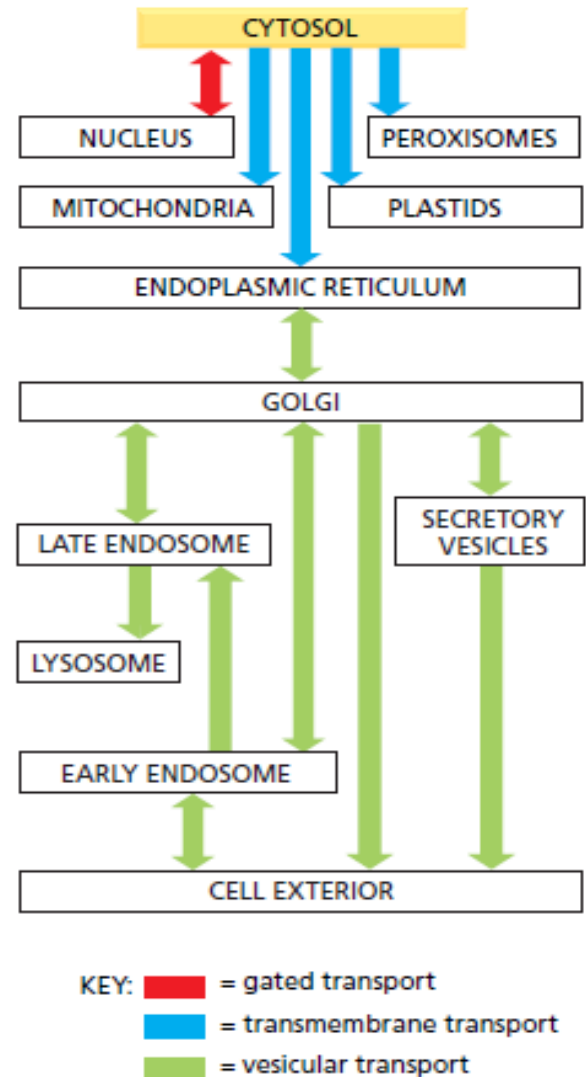


Figure 12–6 A simplified “roadmap” of protein traffic. Molecular Biology of the Cell, 5th ed.

Protein Sorting: Nucleus

- Proteins enter or leave the nucleus through nuclear pores.

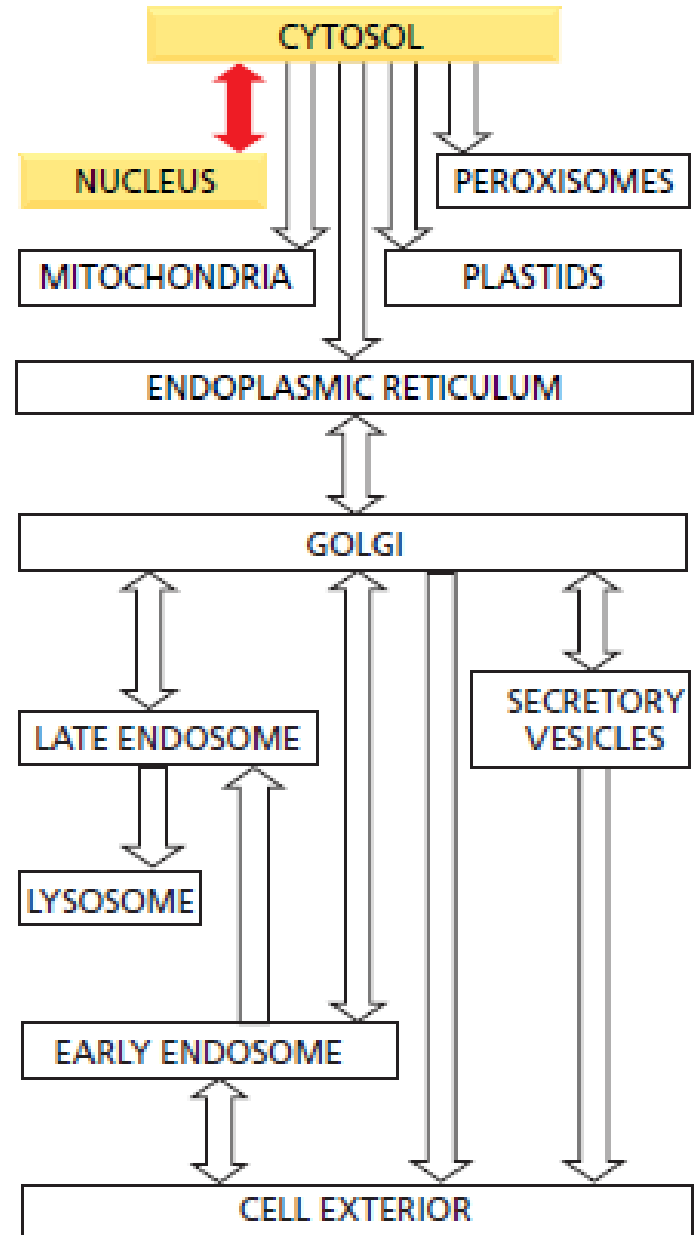


Figure 12–6 A simplified “roadmap” of protein traffic. Molecular Biology of the Cell, 5th ed.

Protein Sorting: Nucleus

- The composition of the outer nuclear membrane closely resembles the ER.
- The inner membrane contains proteins that act as binding sites for chromosomes and for the nuclear lamina

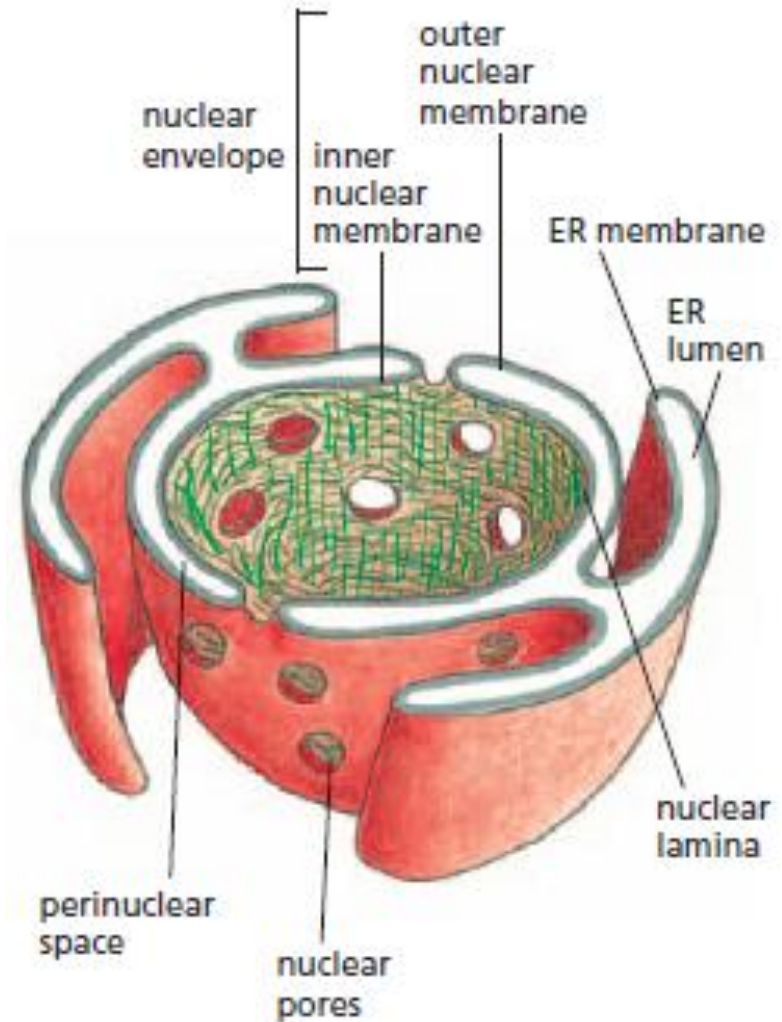


Figure 12–8 The nuclear envelope.
Molecular Biology of the Cell, 5th ed.

Protein Sorting: Mitochondria and Chloroplast

- Most mitochondrial and chloroplast proteins are encoded by nuclear genes and imported from the cytosol.
- Proteins unfold to enter mitochondria and chloroplasts
- The protein is translocated simultaneously across both the inner and outer membranes at specific sites where the two membranes are in contact with each other

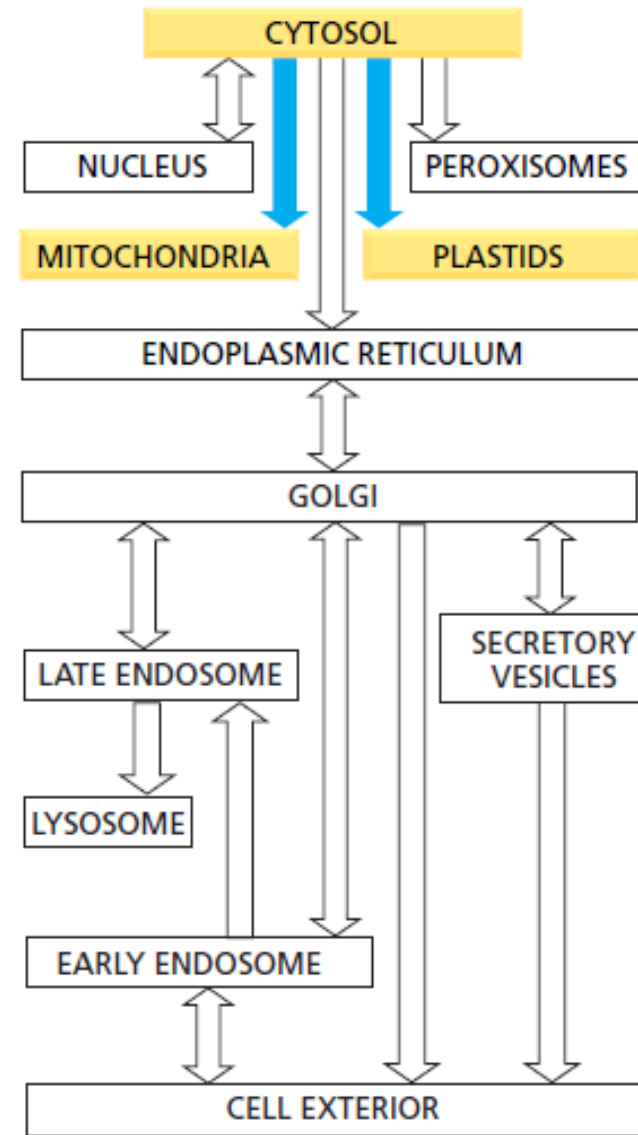


Figure 12–6 A simplified “roadmap” of protein traffic.
Molecular Biology of the Cell, 5th ed.

Protein Sorting: Peroxisome

- Proteins for peroxisome are encoded in the nucleus
- Peroxisomes acquire most of these proteins by selective import from the cytosol
- Some proteins enter the peroxisome membrane via the ER

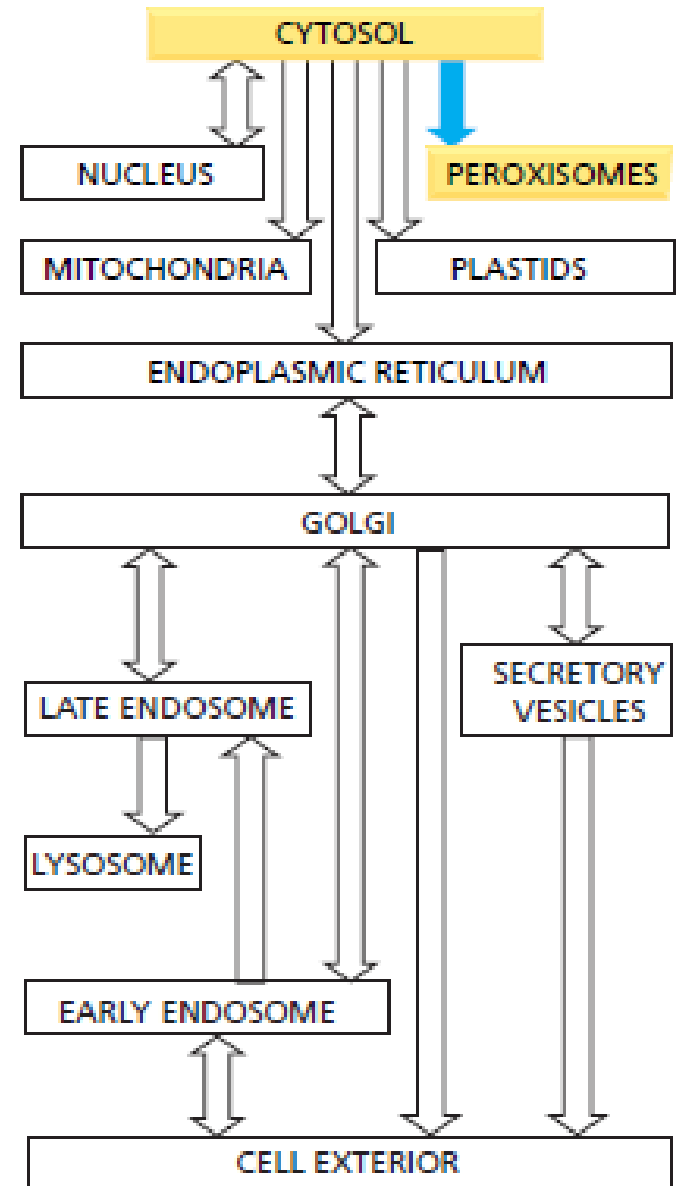


Figure 12–6 A simplified “roadmap” of protein traffic.
Molecular Biology of the Cell, 5th ed.

Protein Sorting: ER

- ER is the entry point for proteins destined for other organelles (as well as ER).
- Once inside the ER, proteins will not reenter the cytosol. They are transferred by transport vesicles to various organelles.

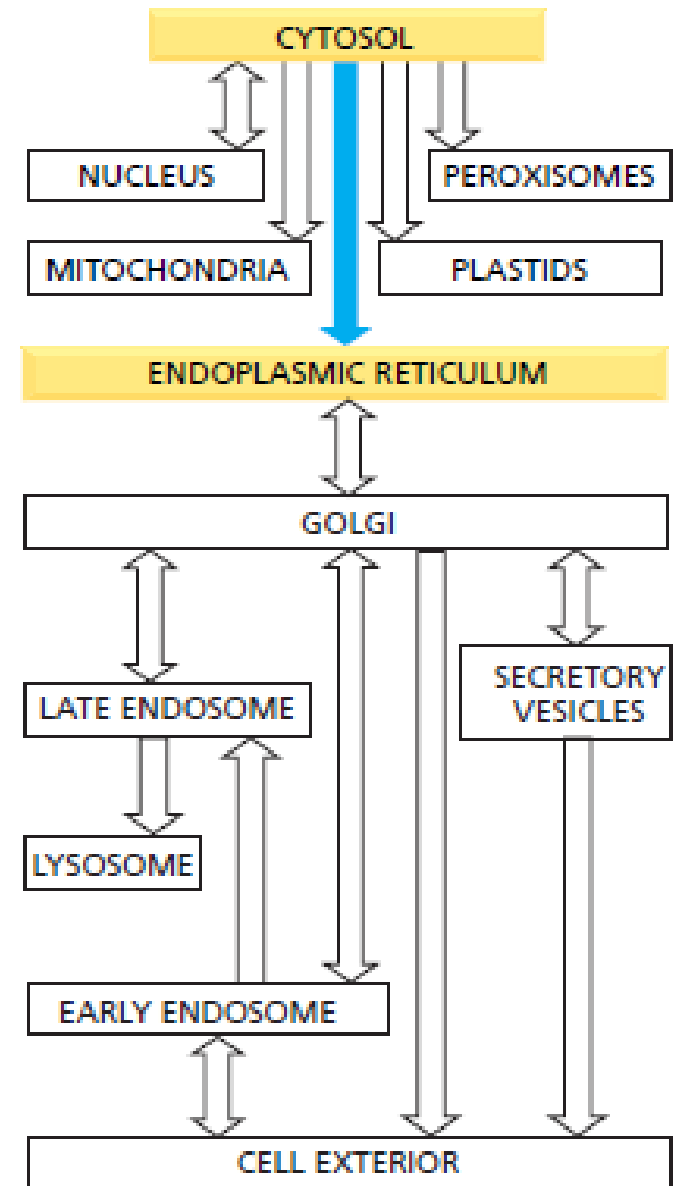


Figure 12–6 A simplified “roadmap” of protein traffic.
Molecular Biology of the Cell, 5th ed.

Protein Sorting: ER

ER Trafficking removes 2 types of proteins from cytosol:

1. Transmembrane proteins partly translocated across ER
 - transported into the membrane of another organelle or the plasma membrane.
2. Water soluble proteins translocated into lumen
 - secreted or will be transported into the lumen of an organelle.

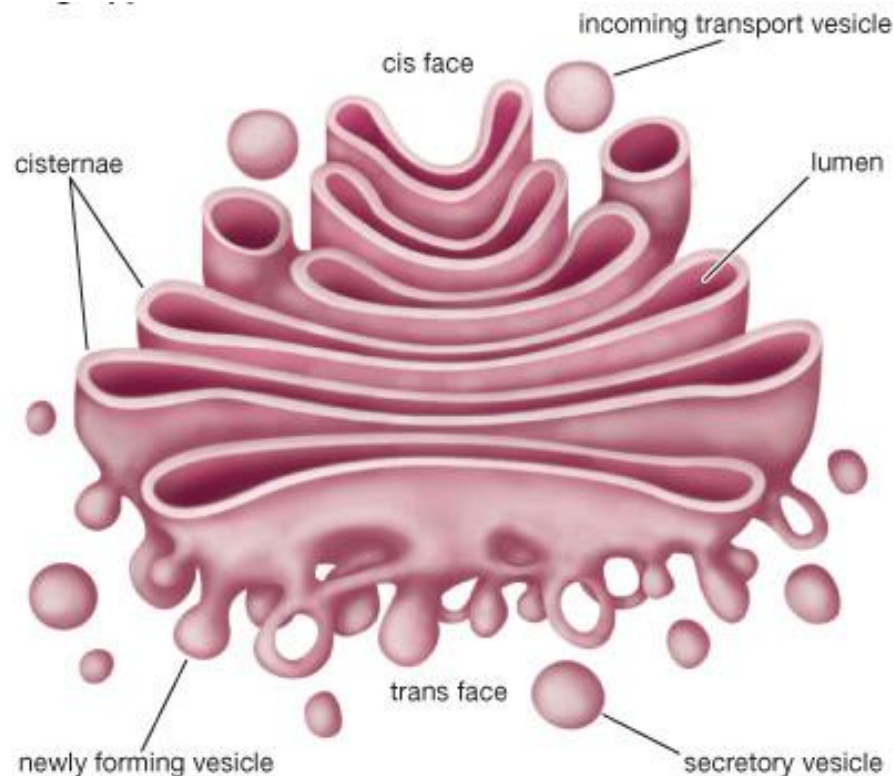
Protein Sorting: ER

Import of Proteins into ER

- Occurs co-translationally: Translation and translocation proceed in unison
- Since the ribosome masks about 30 amino acids, the signal sequence isn't fully exposed until the newly forming protein is about 50 amino acids long.

Golgi Apparatus

- Golgi apparatus consists of organized stacks of disc-like compartments called Golgi cisternae.
- Located near the nucleus and close to centrosome



Golgi Apparatus

- One of the first organelles described by light microscope.
- Each Golgi stack usually consists of 4-6 cisternae, but some unicellular flagellates can have up to 60.

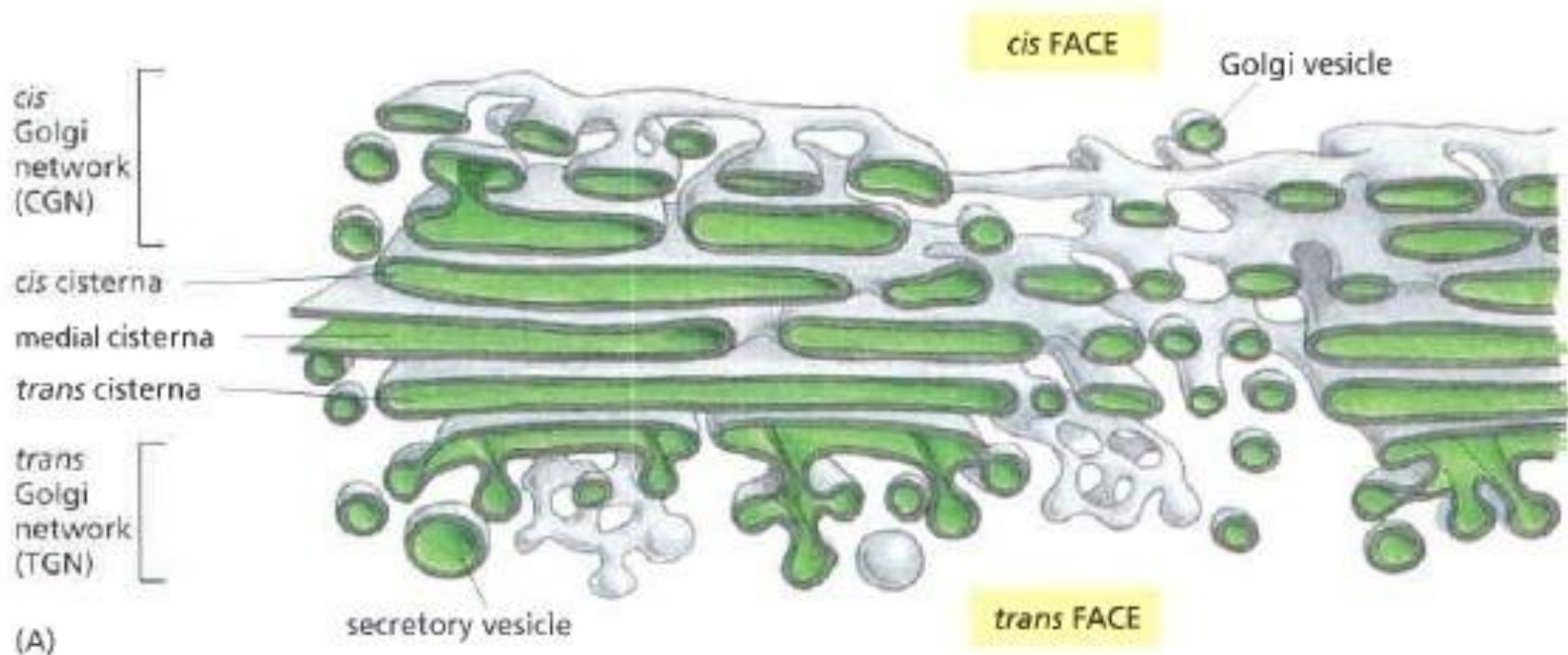


Figure 13-25 The Golgi apparatus. Molecular Biology of the Cell, 5th ed.

Golgi Apparatus

- Cis face: Entry face
 - Cis Golgi network: Proteins and lipids enter through cis Golgi network.
- Trans face: Exit face
 - Trans Golgi network: Proteins and lipids exit through cis Golgi network.
 - The Golgi apparatus is especially prominent in cells that are specialized for secretion of glycoproteins. They have unusually large vesicles on the trans side of the Golgi apparatus.

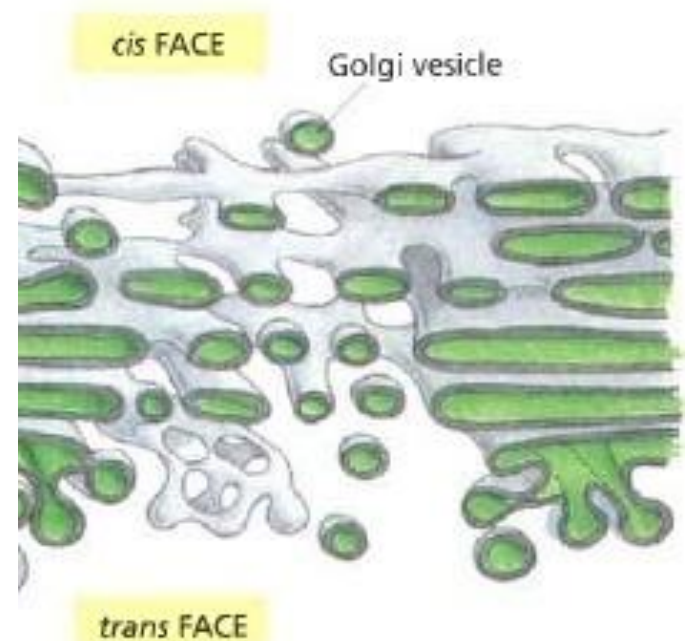


Figure 13-25 The Golgi apparatus. Molecular Biology of the Cell, 5th ed.

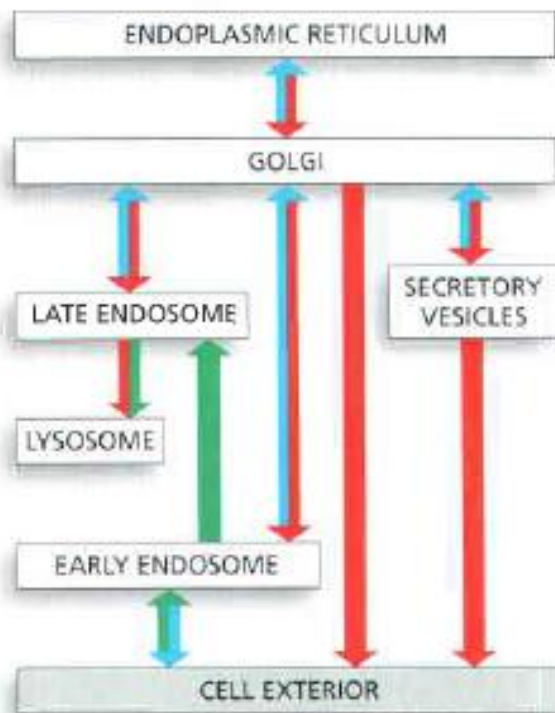
Why do cells need the Golgi Apparatus?

- Involved in modifying, sorting and packaging the macromolecules that the cells synthesize.
- Transports lipids around the cell.
- The cell makes many polysaccharides in the Golgi apparatus
 - the pectin and hemicellulose of the cell wall in plants
 - the glycosaminoglycans of the extracellular matrix in animals
- Carbohydrate synthesis

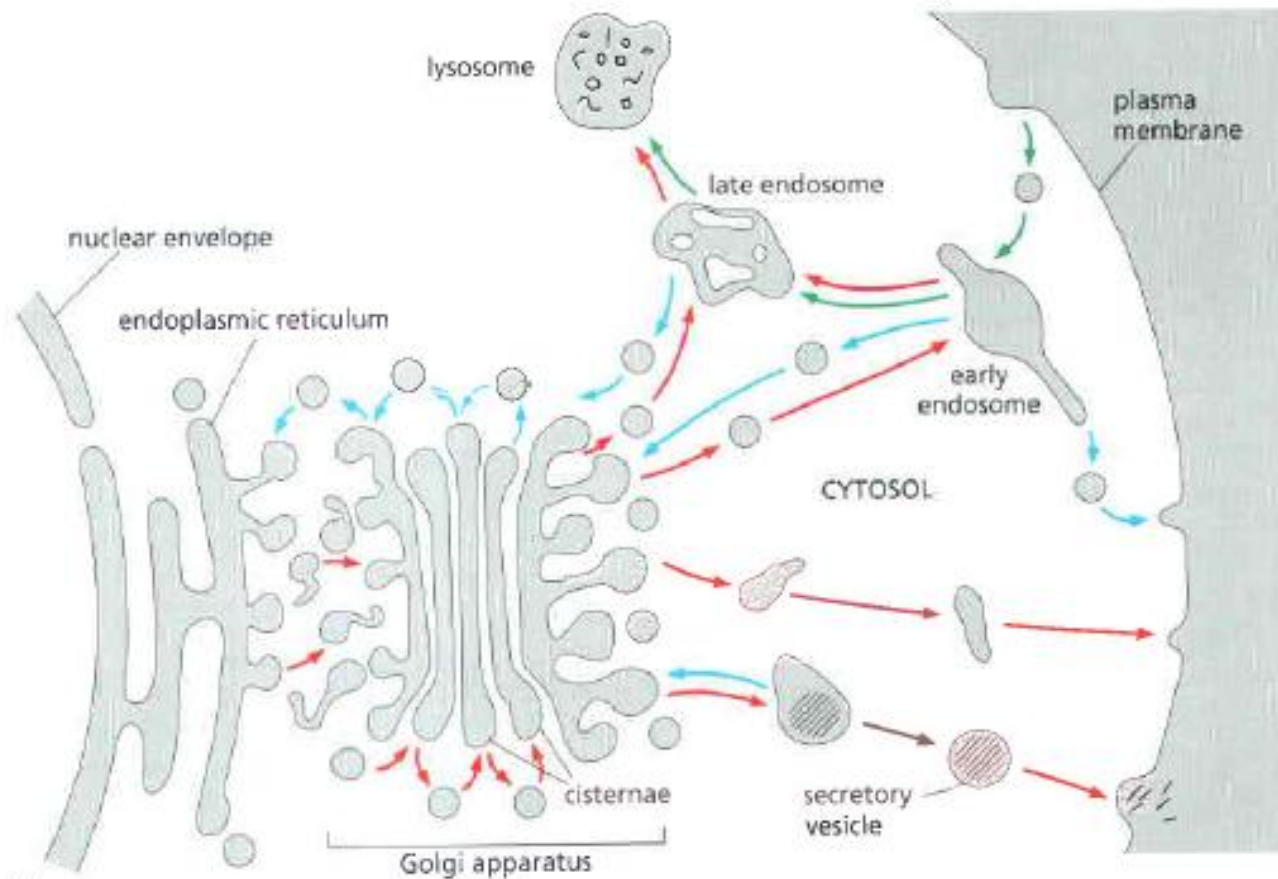
Functions of Golgi Apparatus

- Mainly modifies proteins delivered from the rough ER
- Protects protein from degradation
- Retains proteins in the ER until the proper folding is completed
- Protein sorting: proteins are selectively transported.
 - Guide the proteins to appropriate organelle
 - Signals in the protein and receptors in the membrane are involved in this process
- Glycosylation and phosphorylation
 - Forms the signal sequences required for protein transport

Intracellular Vesicular Traffic



(A)



(B)

Figure 13-3 A "road-map " of the biosynthetic-secretory and endocytic pathways. Molecular Biology of the Cell, 5th ed.

Intracellular Vesicular Traffic

- Cells communicate with their surroundings, adjust the composition of the plasma membrane by adding/removing cell-surface proteins, ion channels and transporters
- How do cells achieve this cell communication and transport of proteins, carbohydrates and lipids?
 - Vesicular transport
 - Exocytosis
 - Endocytosis

Intracellular Vesicular Traffic:

Vesicular Transport

- **Vesicular transport:** exchange of components between the membrane-enclosed compartments
- Carry soluble proteins (in the lumen), membrane proteins (in the bilayer) and lipids between compartments
 - Collectively comprise the biosynthetic-secretory and endocytic pathways
 - Molecular markers displayed on the cytosolic surface of the membrane ensures that transport vesicles fuse only with the correct compartment.

Intracellular Vesicular Traffic:

Vesicular Transport

Three main steps of vesicular transport:

1. Cargo Selection

- Adaptor proteins couple sorting signals on specific molecules to the transport machinery

2. Container formation

- Proteins form coat complexes

3. Targeting and fusion of the container with the next compartment.

- Membrane associated joining and fusion of proteins of the transport container to the right target compartment

Intracellular Vesicular Traffic: Vesicular Transport

- Transport vesicles bud off from donor compartment and fuse with target compartment

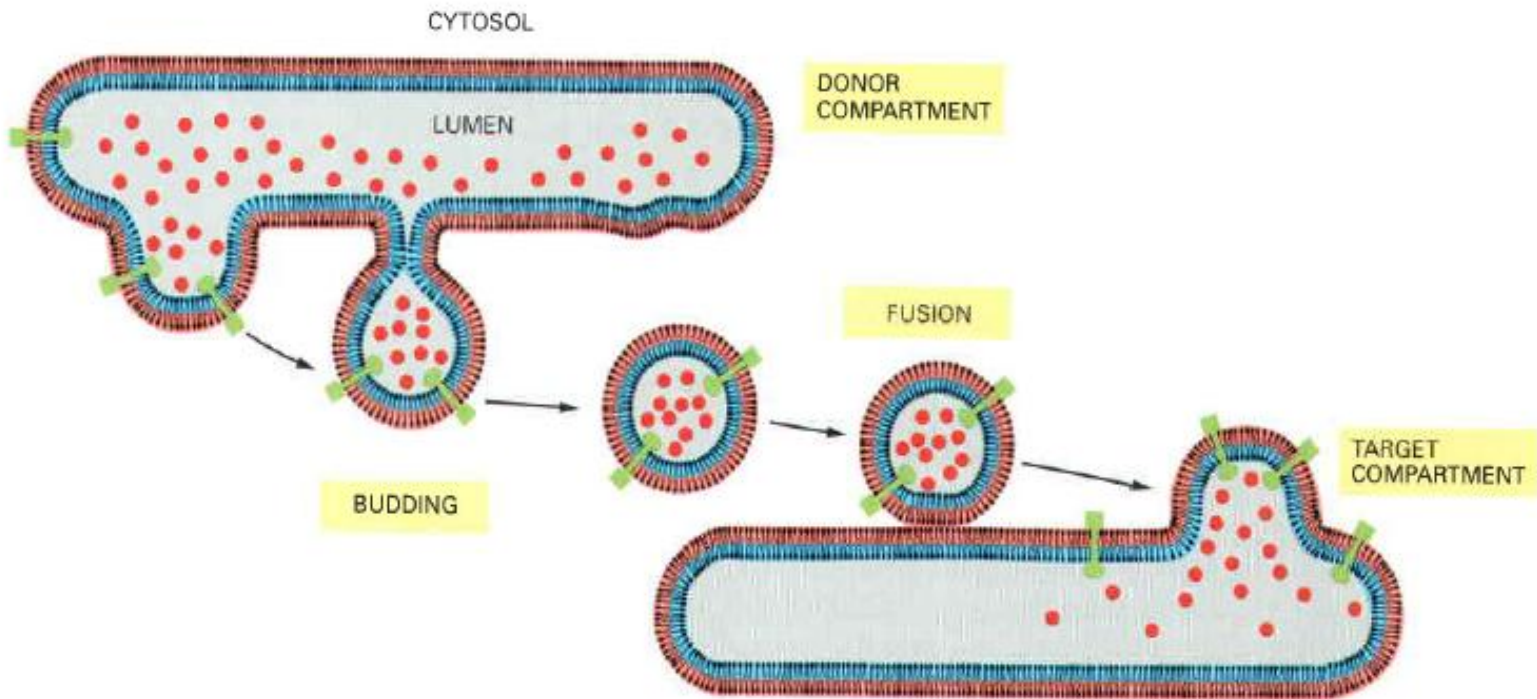


Figure 13-2 Vesicular transport. Molecular Biology of the Cell, 5th ed.

Vesicular Transport: Vesicle budding

- Vesicle budding requires
 - Membrane fusion initiated from the lumenal of the membrane and transported along microtubules by motor proteins
 - Vesicle fusion requires a membrane fusion initiated from the cytoplasmic side of both donor and target membranes.

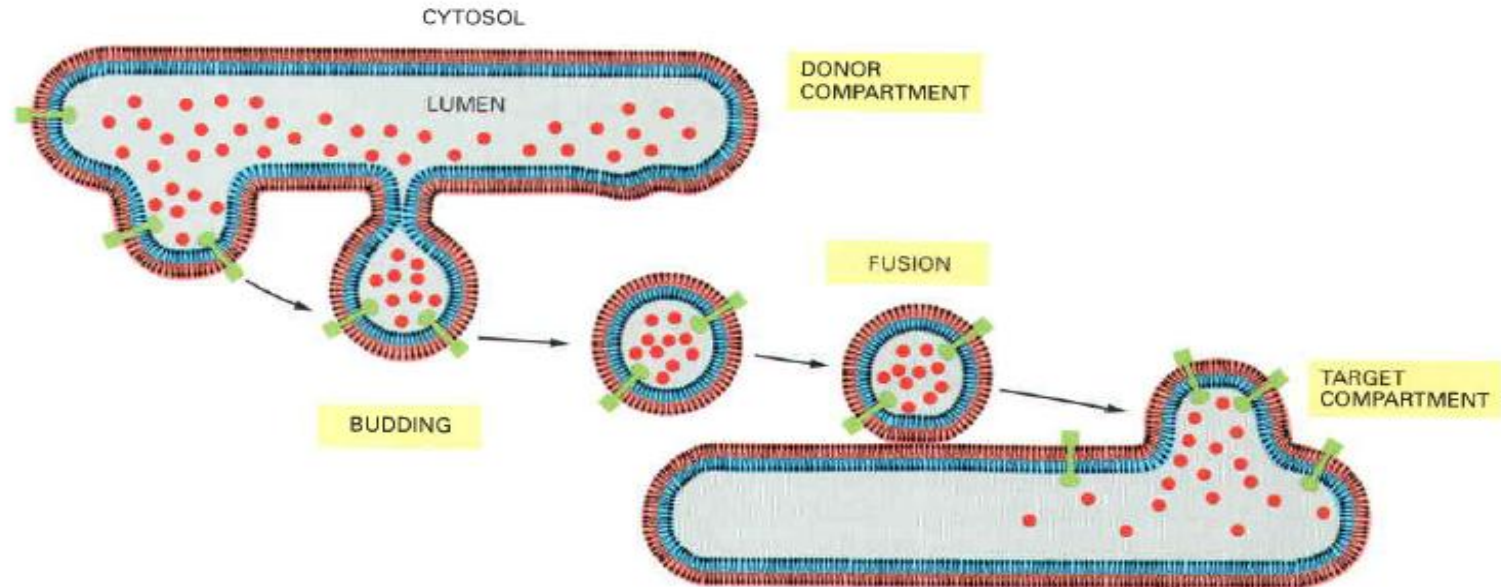


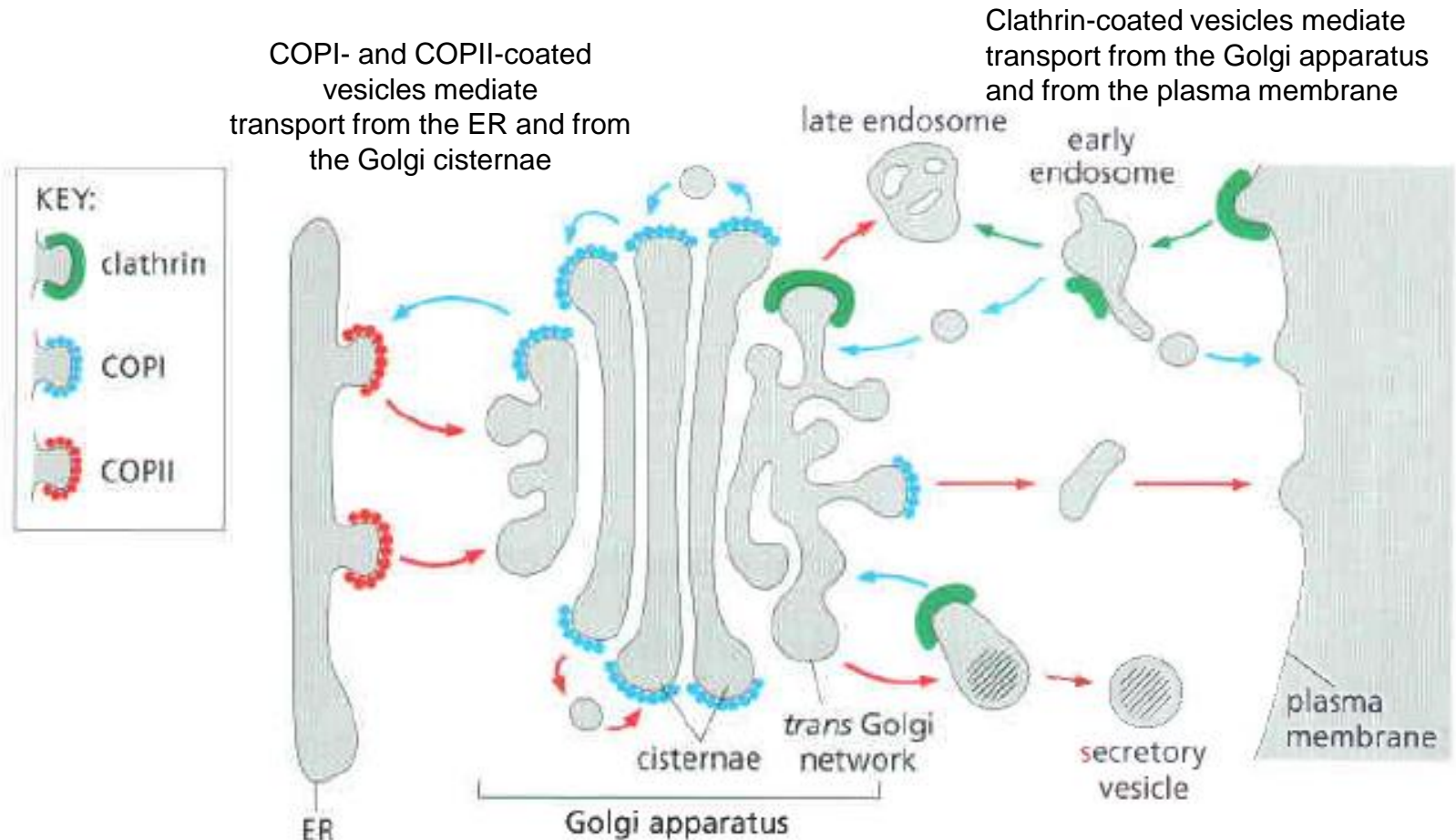
Figure13 -2 Vesicular transport. Molecular Biology of the Cell, 5th ed.

Vesicular Transport: Vesicle budding

- Vesicle budding is driven by the assembly of a protein coat on their cytosolic surface: **coated vesicles**.
- After budding is complete, the coat is lost.
- There are several kinds of coated vesicles, each with distinctive protein coats.
- Functions of the coats:
 - Shapes the membrane into a vesicle
 - Helps to capture the appropriate molecules to be transported.
 - Each coat is used for different transport steps.
Example: transport from ER to Golgi Apparatus or from Golgi Apparatus to lysosome

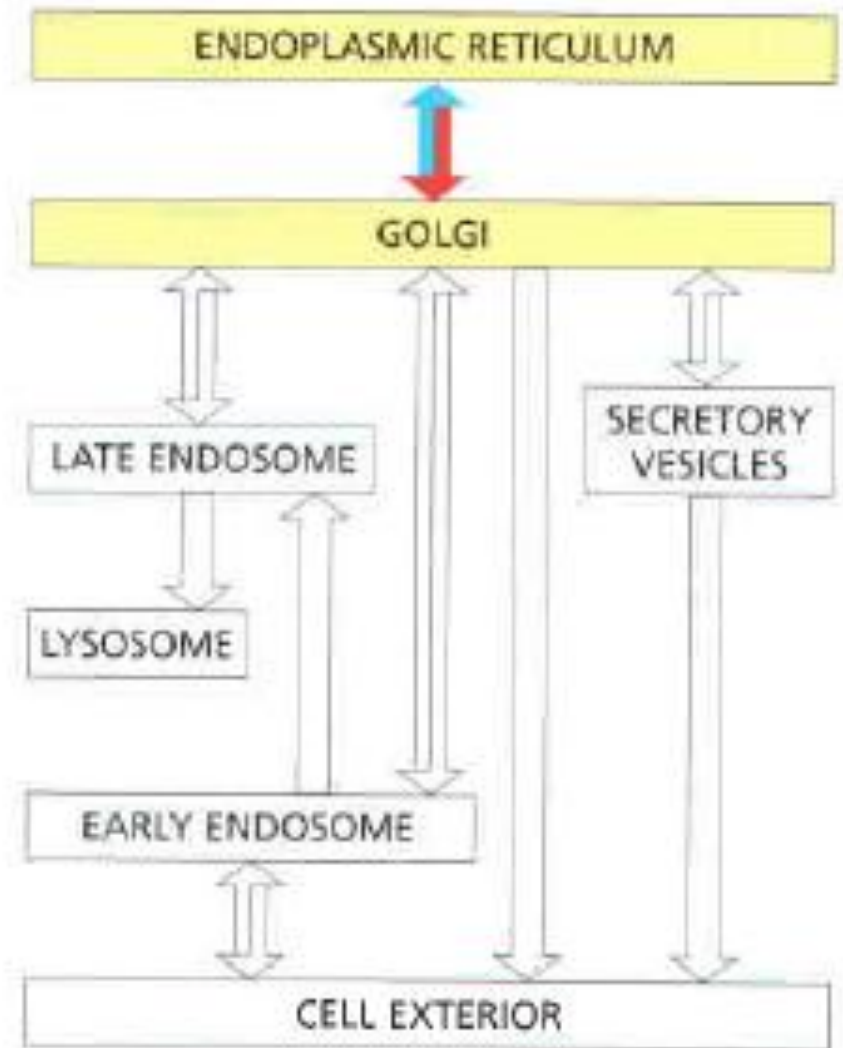
Vesicular Transport: Vesicle budding

- Examples of coats:



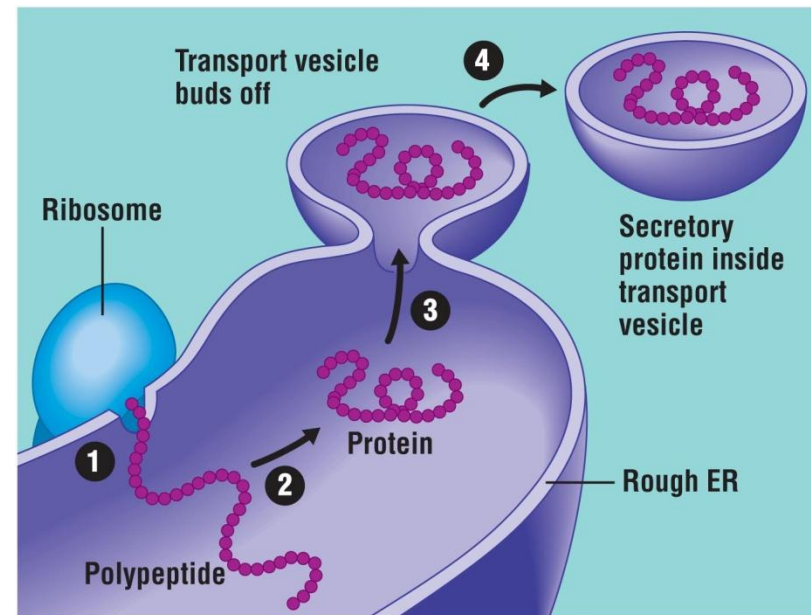
Intracellular Vesicular Traffic: ER to Golgi Apparatus

- Proteins that don't normally reside in the ER are transported to the Golgi apparatus by vesicular transport.



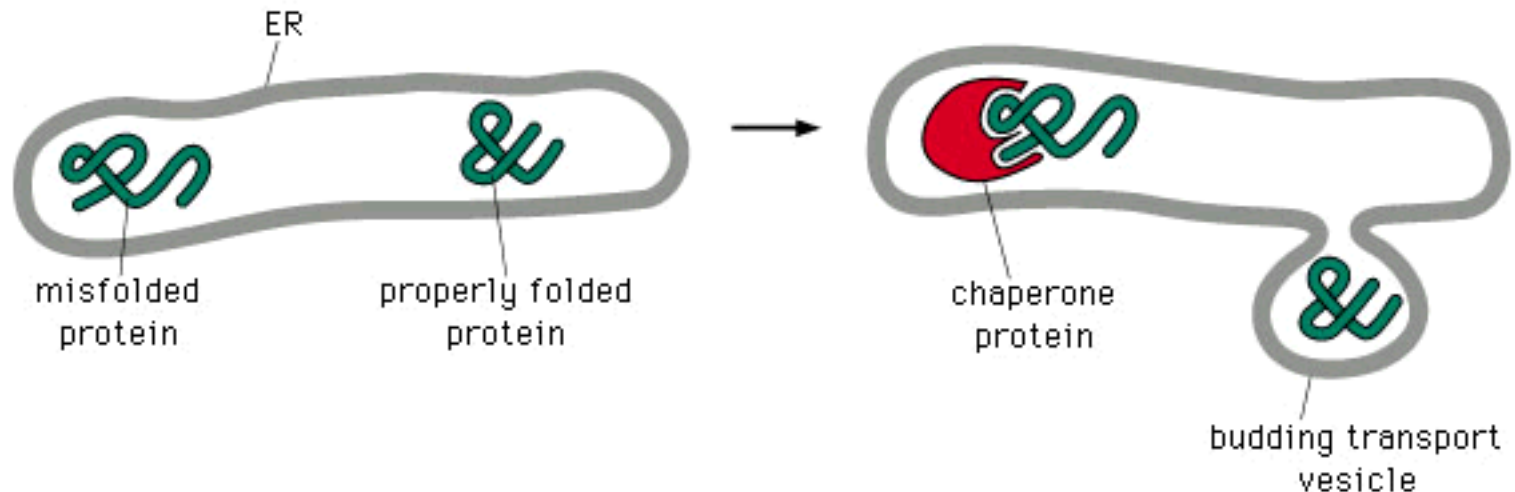
Intracellular Vesicular Traffic: ER to Golgi Apparatus

- Each transport vesicle takes only the proteins and lipids appropriate to its destination and fuse only with the appropriate target membrane
- Some transport vesicles retrieve escaped proteins and return them to the previous compartment where they normally function
- Transport involves selecting membrane and soluble luminal proteins for packaging and transport-in vesicles or organelle fragments
- Only properly folded proteins are transported



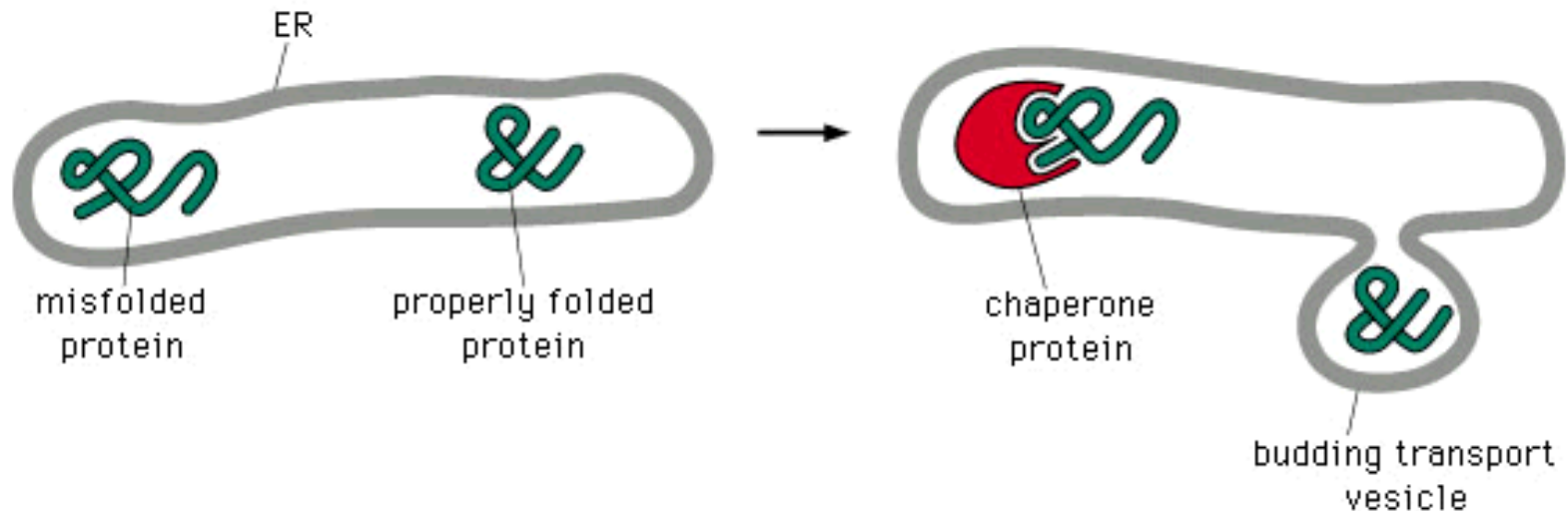
Intracellular Vesicular Traffic: ER to Golgi Apparatus

- To exit from the ER, proteins must be properly folded.
- Chaperone proteins in the ER hold proteins until they fold and assemble properly.



Intracellular Vesicular Traffic: ER to Golgi Apparatus

- Misfolded proteins are degraded since they could potentially interfere with the functions of normal proteins
 - Ex. cystic fibrosis – mutant plasma membrane transport protein



ER to Golgi Apparatus: Vesicular tubular clusters

- Transport vesicles are budded from ER exit sites and they begin to fuse with one another called **homotypic fusion**
- Vesicular tubular clusters are formed when ER-derived vesicles fuse with one another

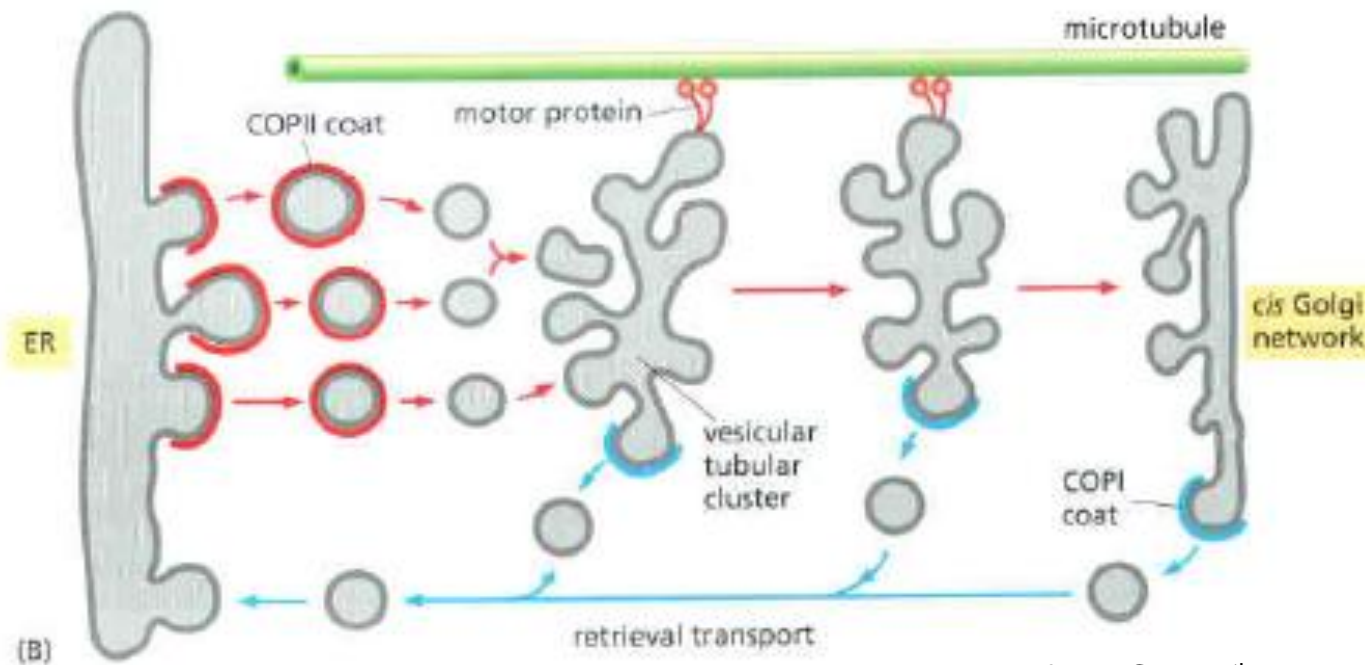


Figure 13-23 Vesicular tubular clusters. Molecular Biology of the Cell, 5th ed.

ER to Golgi Apparatus: Vesicular tubular clusters

- Vesicular tubular clusters function as transport containers from the ER to the Golgi apparatus.

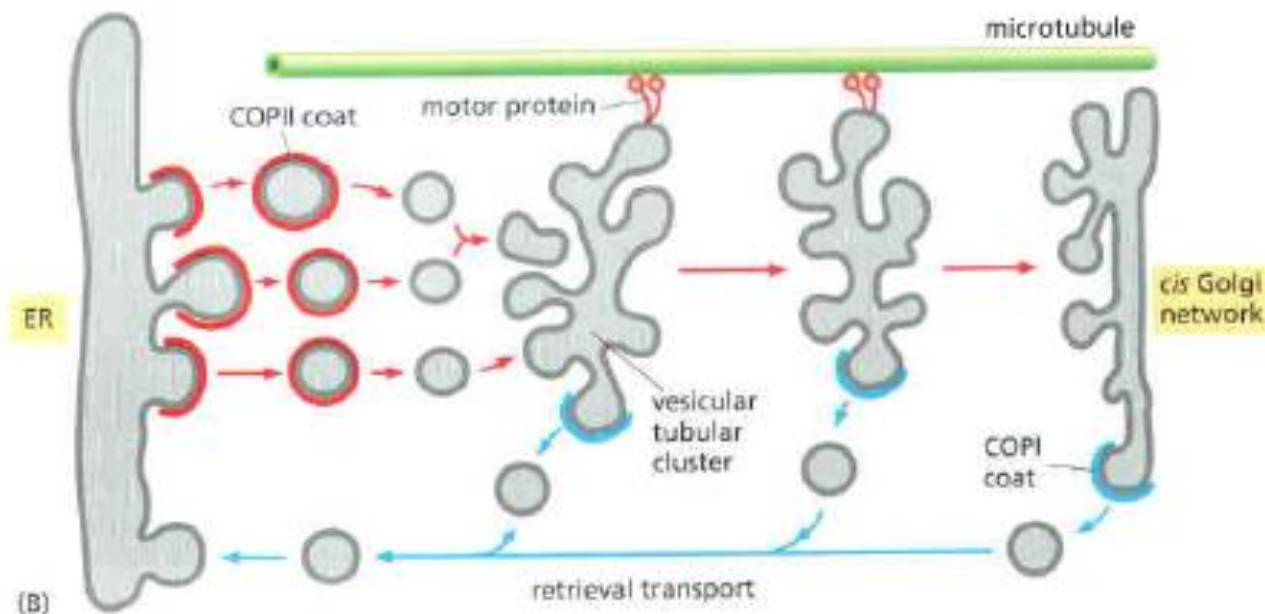
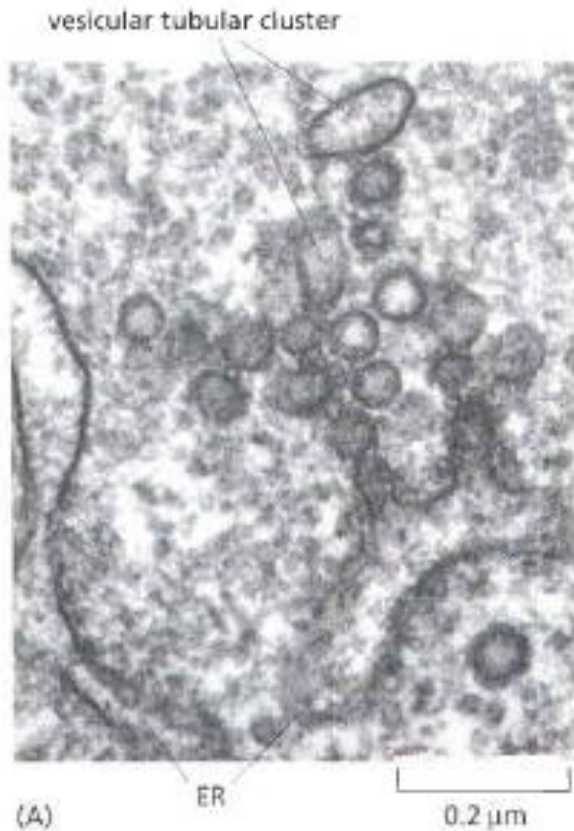
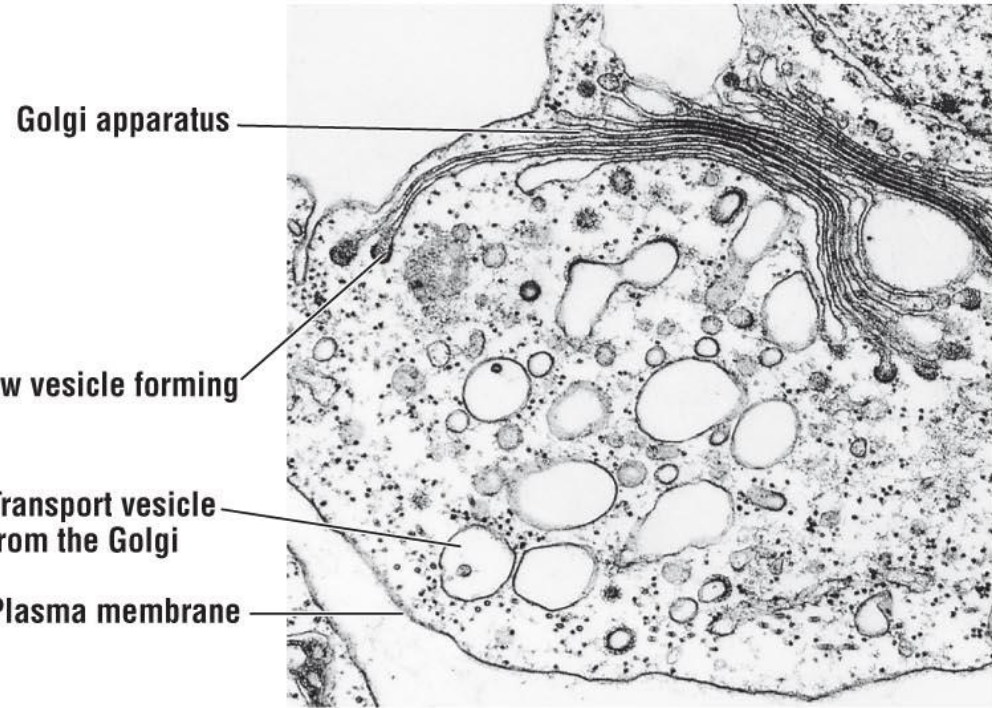
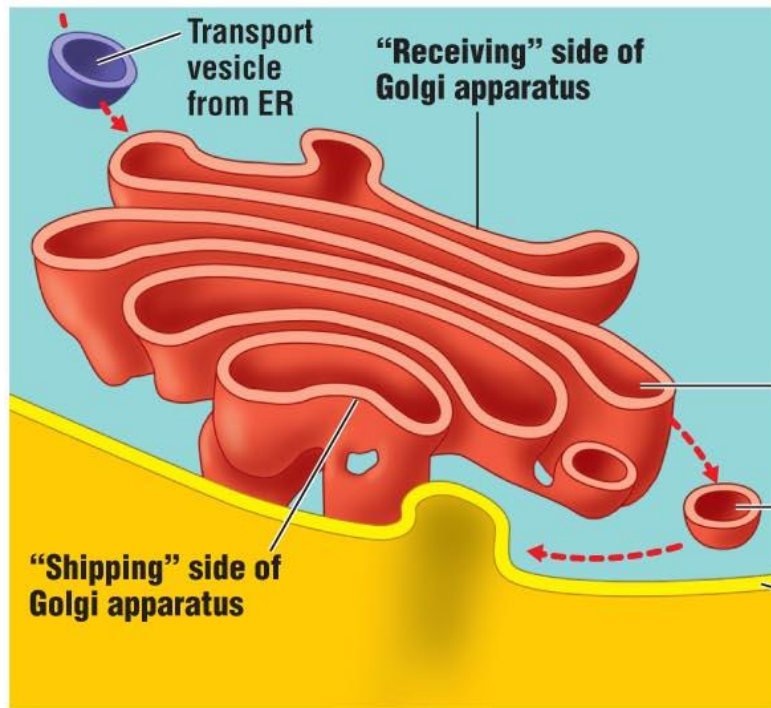


Figure 13-23 Vesicular tubular clusters. Molecular Biology of the Cell, 5th ed.

Intracellular Vesicular Traffic: ER to Golgi Apparatus



Protein transport from one cisterna to the next in Golgi Apparatus

Vesicular transport:

Molecules pass from cis to trans by forward-moving transport vesicles by budding from one cisterna and fuse with next from cis to trans direction

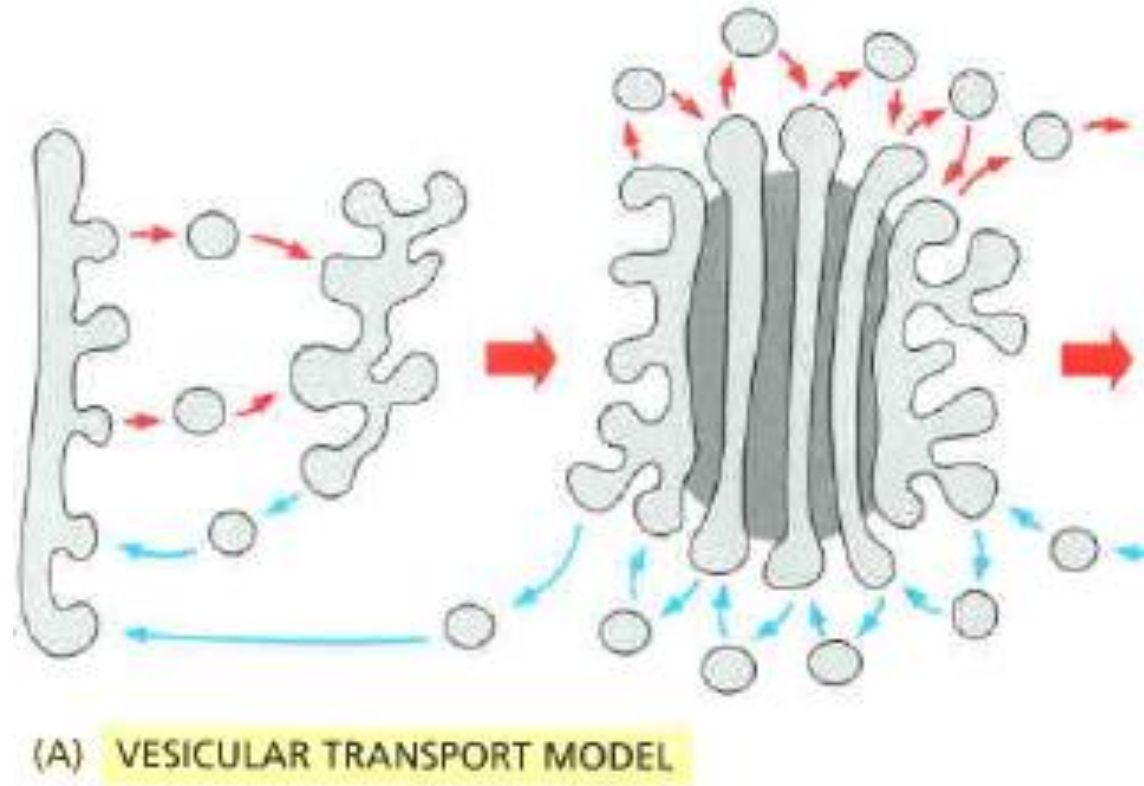


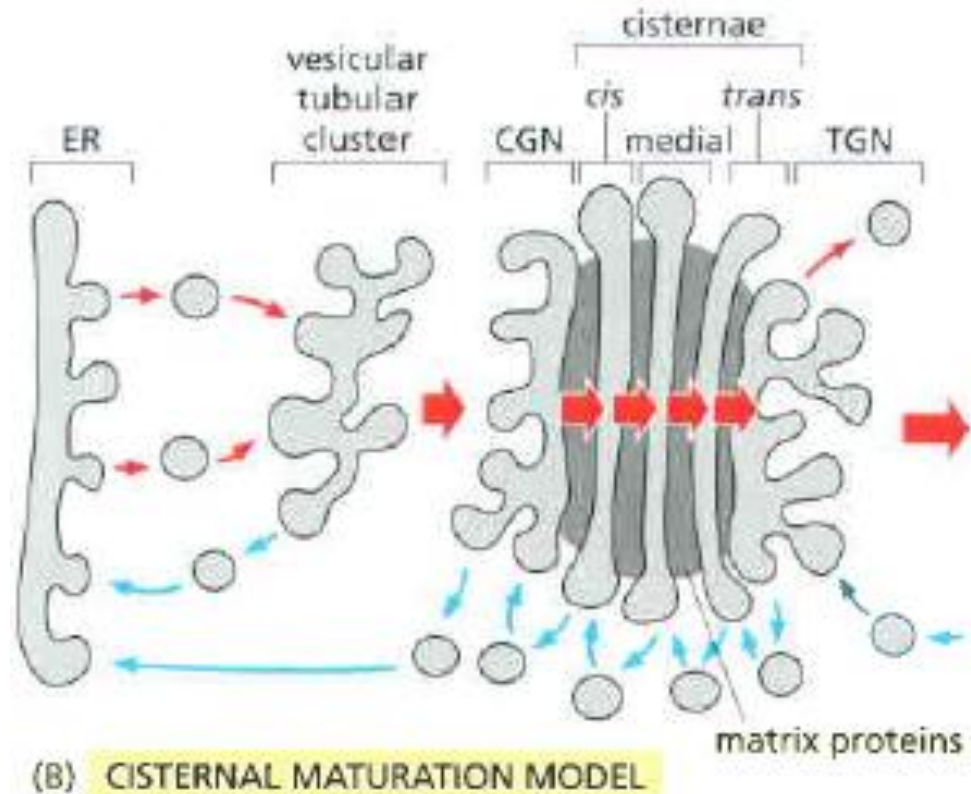
Figure 13-35 Two possible models explaining the organization of the Golgi apparatus and the transport of proteins from one cisterna to the next.
Molecular Biology of the Cell, 5th ed.

Protein transport from one cisterna to the next in Golgi Apparatus

Cisternal maturation:

Each cisterna matures as it migrates forward.

At each stage, the Golgi-resident proteins carried forward in a cisterna are moved backward to an earlier compartment by retrograde transport.

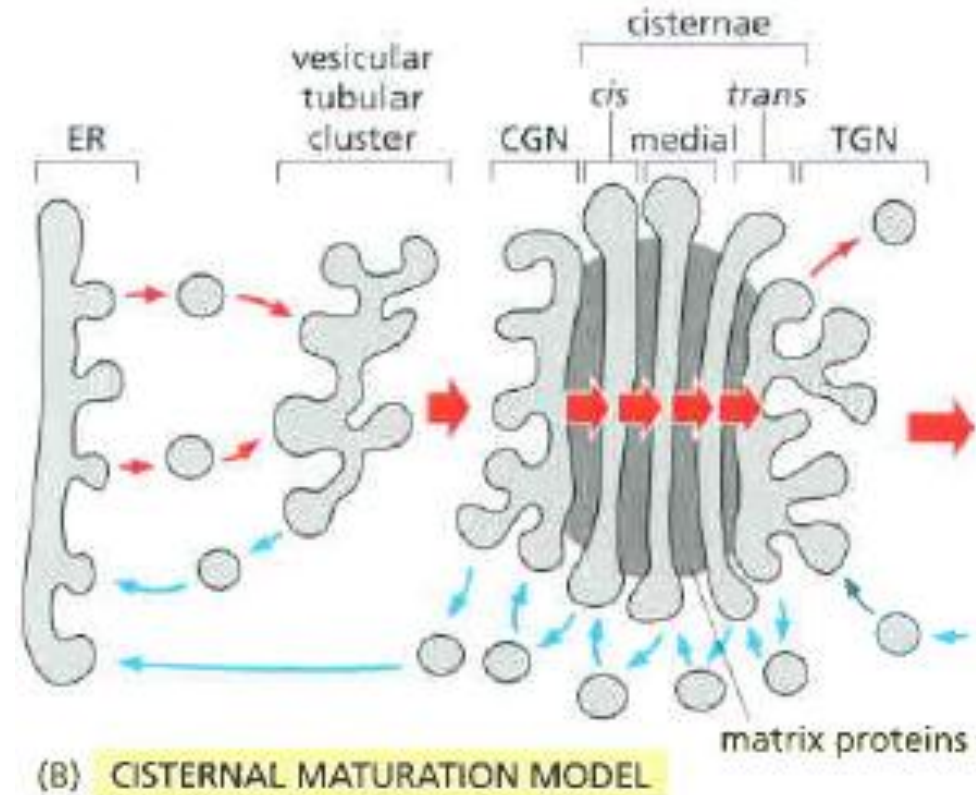


Protein transport from one cisterna to the next in Golgi Apparatus

Cisternal maturation:

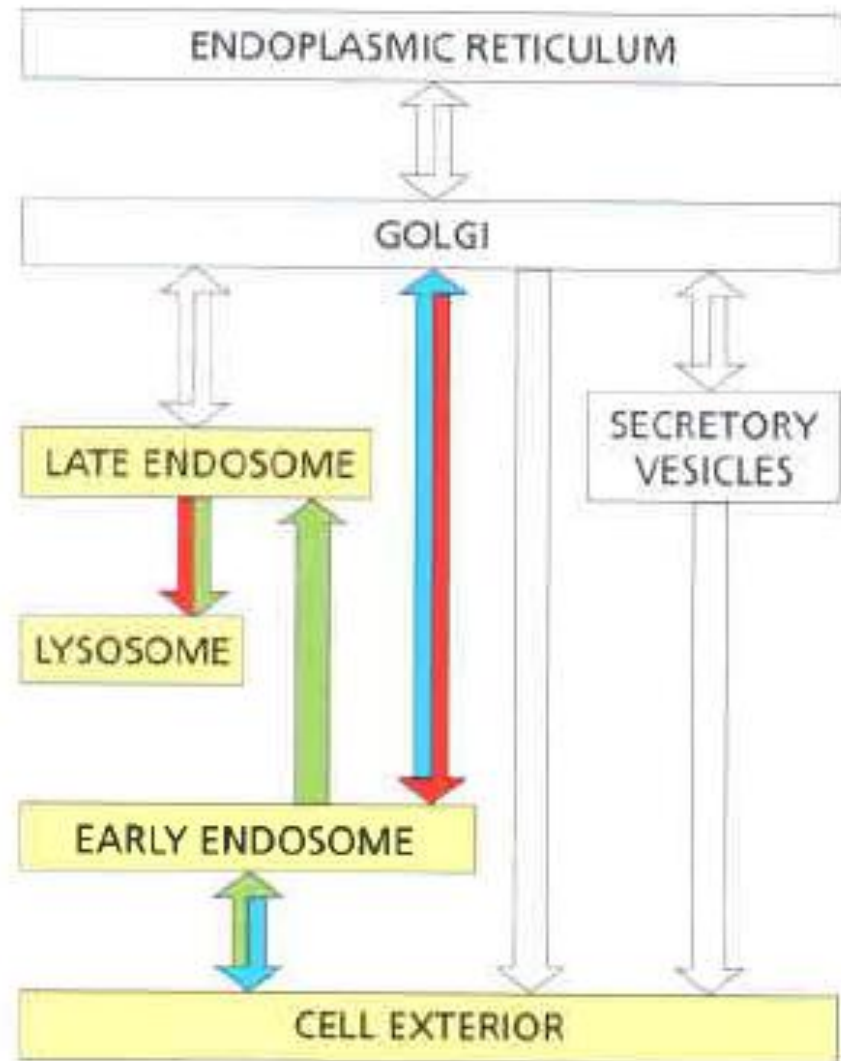
Vesicular tubular clusters arriving from ER fuse with each other forming cis Golgi network (CGN)

CGN matures as it migrates through the stack



Transport from Cell to Plasma Membrane: Endocytosis

- Endocytosis:
 - Occurs extensively in many cells and a large fraction of the plasma membrane is internalized every hour
- Two main types
 - Differ according to size of the endocytic vesicle formed
- Phagocytosis
- Pinocytosis



Transport from Cell to Plasma Membrane: Endocytosis

- **Endocytosis:** cells remove plasma membrane components and deliver them to internal compartments
 - Cells take up proteins by invaginating the plasma membrane

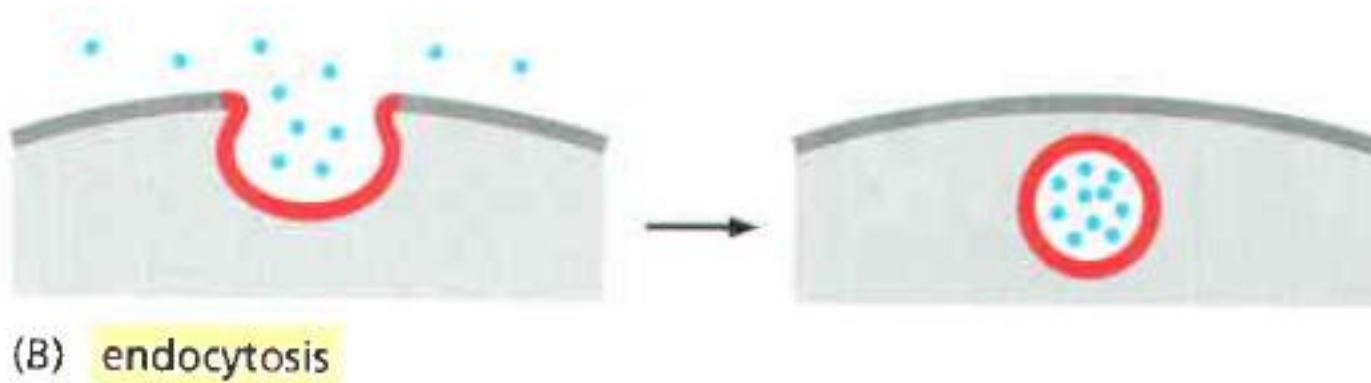


Figure 13-1 Exocytosis and endocytosis. Molecular Biology of the Cell, 5th ed.

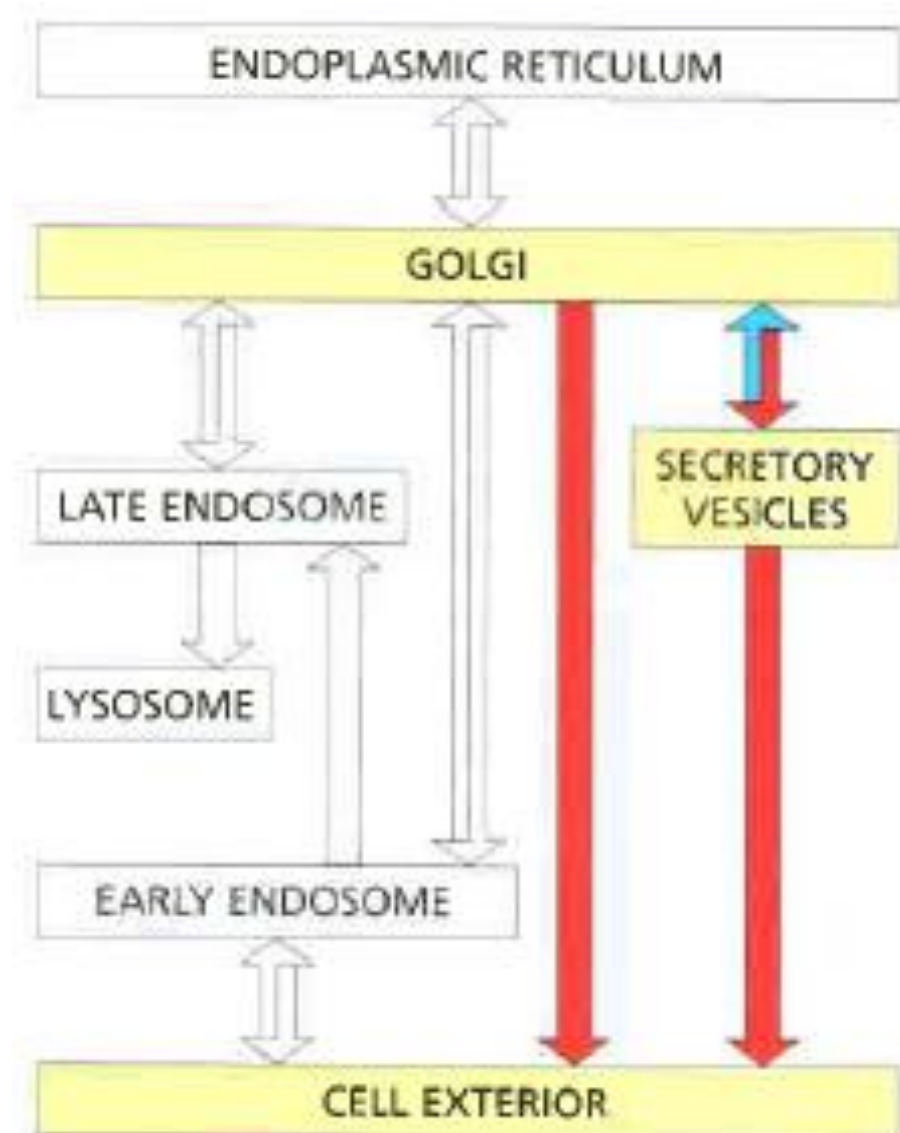
Transport from Cell to Plasma Membrane:

Endocytosis

- Phagocytosis: cell eating
 - Large particles ingested via large vesicles- called phagosomes
 - In mammals: two classes of white blood cells: macrophages and neutrophils. They ingest invading microorganisms to defend against infection.
- Pinocytes: cell drinking
 - uptake of extracellular fluid through endocytosis

Transport from trans Golgi to Cell Exterior: Exocytosis

- Transport vesicles destined for plasma membrane leave as irregularly shaped tubules:
- Soluble proteins inside vesicles are secreted to extracellular space
- Fusion of the vesicle and plasma membrane: exocytosis



Transport from trans Golgi to Cell Exterior: Exocytosis

- **Exocytosis:** newly synthesized proteins, carbohydrates and lipids enter to the plasma membrane or extracellular space
 - Material expelled from the cell

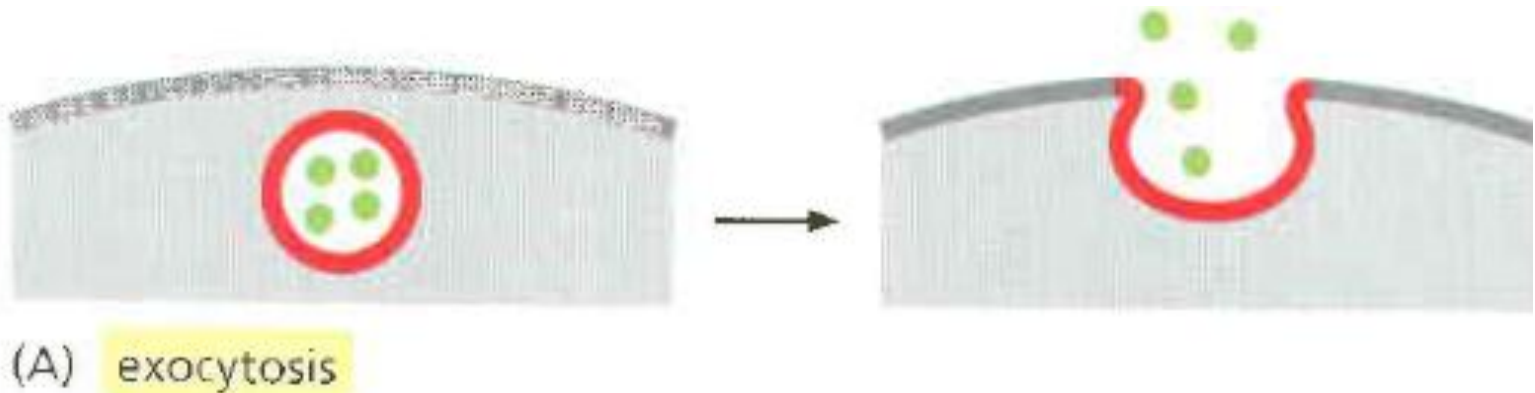


Figure 13-1 Exocytosis and endocytosis. Molecular Biology of the Cell, 5th ed.

Transport from trans Golgi to Cell Exterior: Exocytosis

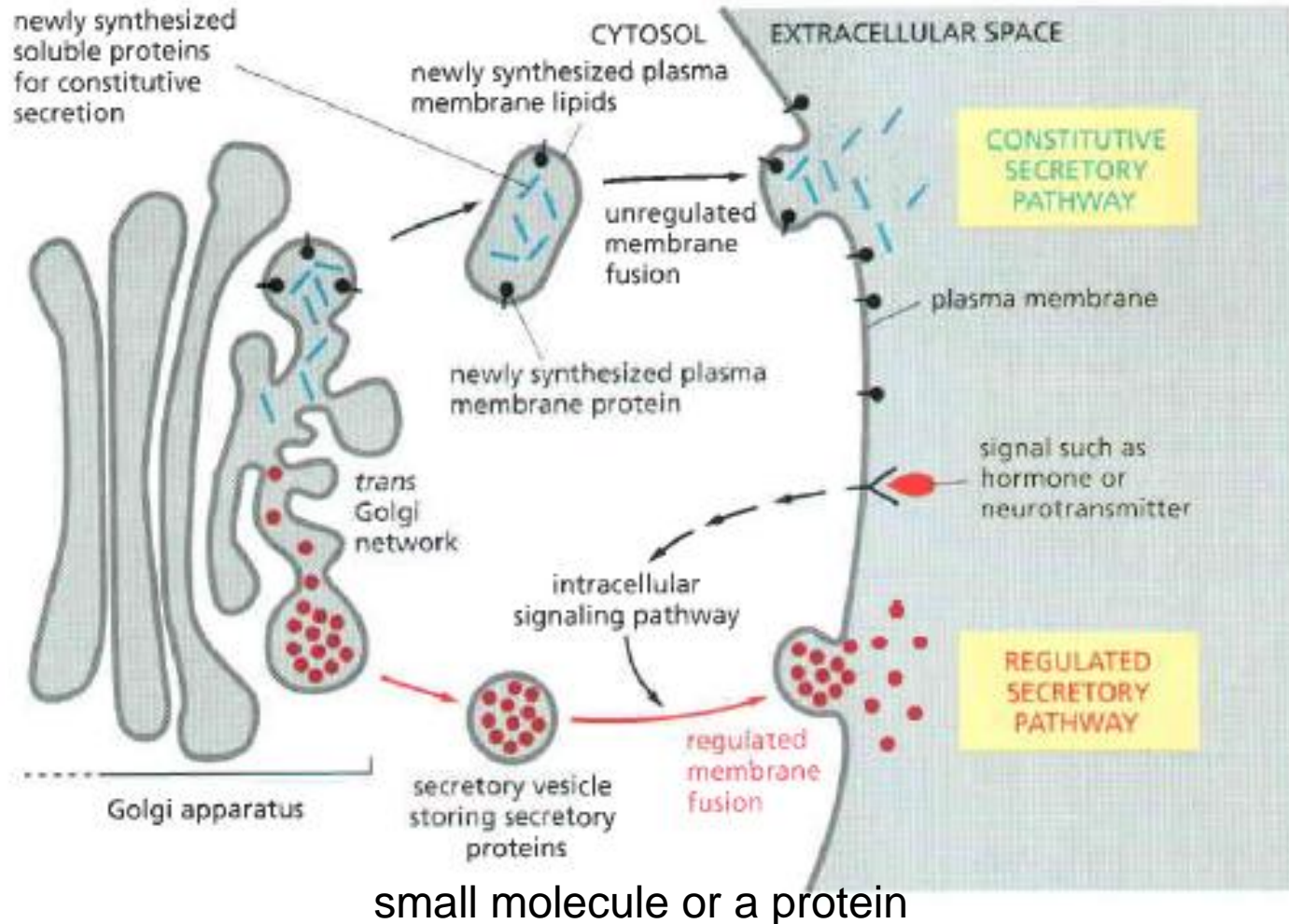


Figure 13.63 The constitutive and regulated secretory pathways. Molecular Biology of the Cell, 5th ed.

Transport from trans Golgi to Cell Exterior: Exocytosis

- Secretory vesicles are so densely filled with contents, the secretory cell can expel large amounts of material promptly by exocytosis when triggered

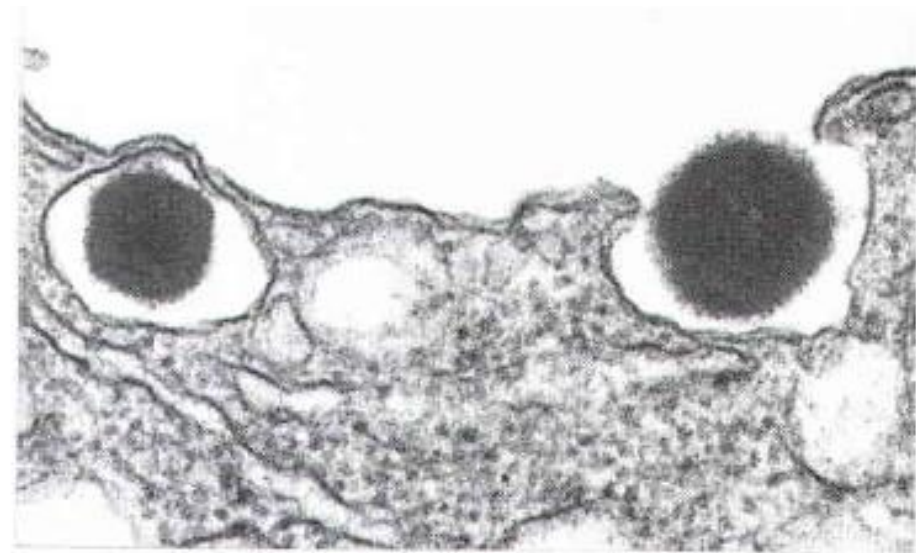
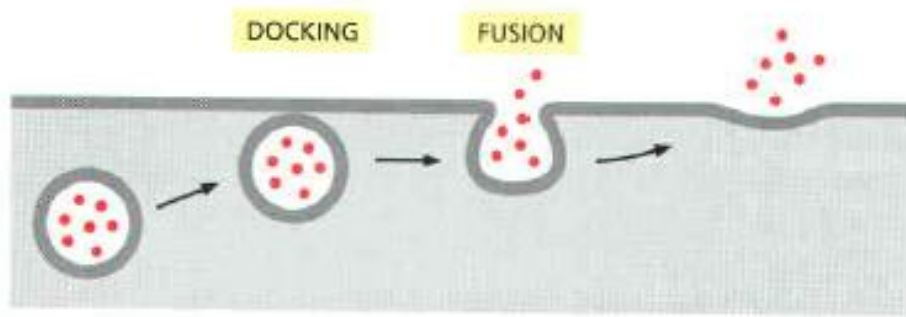


Figure 13-66 Exocytosis of secretory vesicles. Molecular Biology of the Cell, 5th ed.

Summary

- Compartmentalization of the cell: Eukaryotic cell is subdivided by internal membranes
- Organelles: Endoplasmic reticulum, Golgi Apparatus, Mitochondria, Chloroplast, Lysosome, Endosome, Peroxisome, Nucleus

Summary

- ER: Two types- smooth ER and rough ER
- Ribosomes: Main site for protein synthesis
- Events that occur in the ER: Proper protein folding, translocation of the protein, glycosylation, carbohydrate metabolism
- Protein sorting: 3 transport systems; gated, transmembrane and vesicular transport

Summary

- Golgi apparatus: stacks of disc-like compartments called Golgi cisternae
- Intracellular vesicular traffic
 - Exocytosis: Material expelled from the cell
 - Endocytosis: Cells take up substances by invaginating the plasma membrane
 - Vesicular transport: bud from membrane of one organelle & fuse with membrane of next organelle
 - ER to Golgi Apparatus
 - Cell to plasma membrane
 - Golgi Apparatus to Cell exterior

Summary

- Intracellular Vesicular Traffic: Complex process
 - thousands of different proteins are transported efficiently and accurately between different compartments without mixing-up at the same time.
- Transport is coordinated with the constantly changing needs of cells and tissues in response to the cell signaling and the organism's physiology.

Reading:

Chapter 11: 695-705, 723-725

Chapter 12: 749-755, 766-773, 787-790, 799-803

