CHAPTER 4 LECTURE SLIDES

Prepared by
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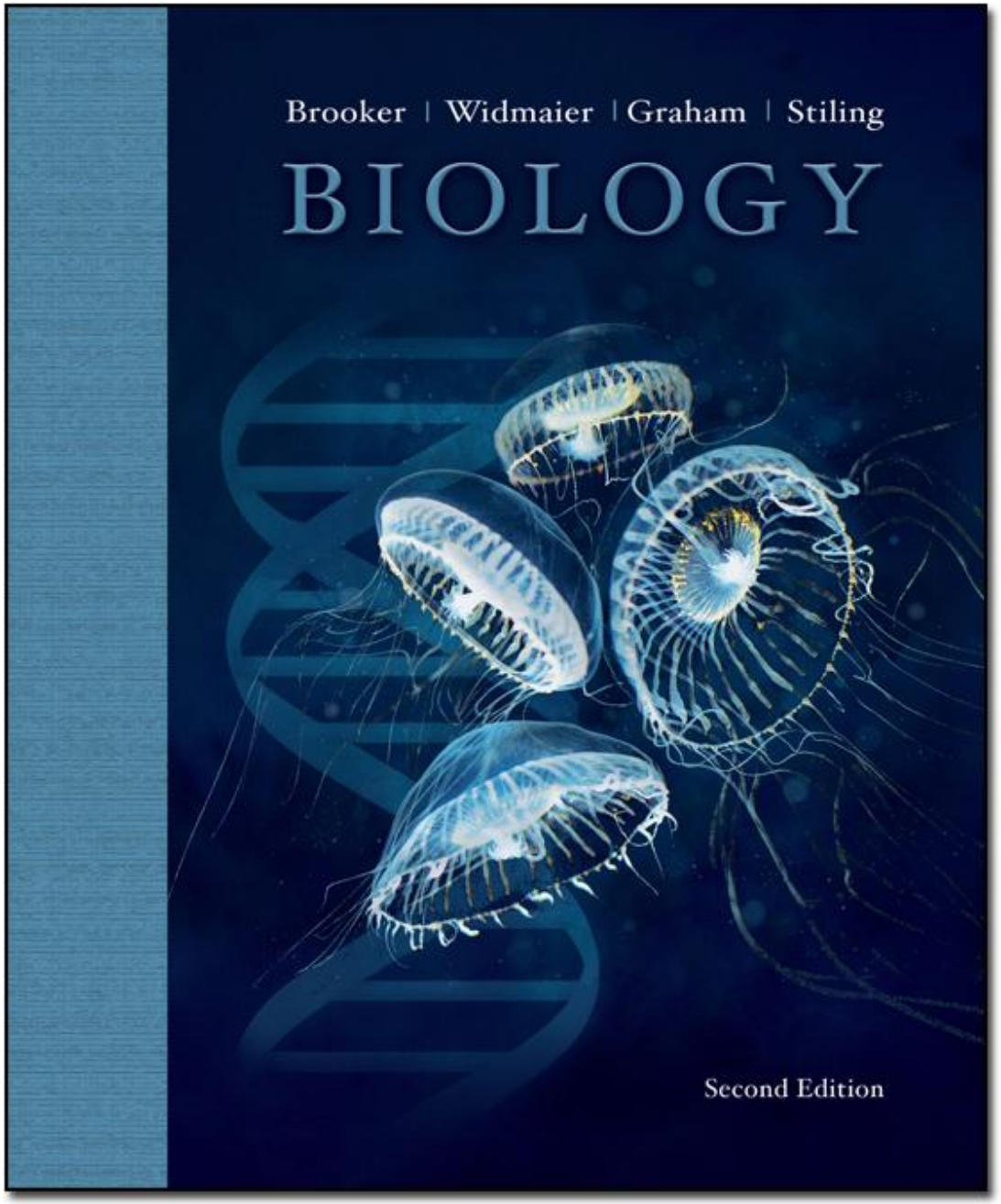
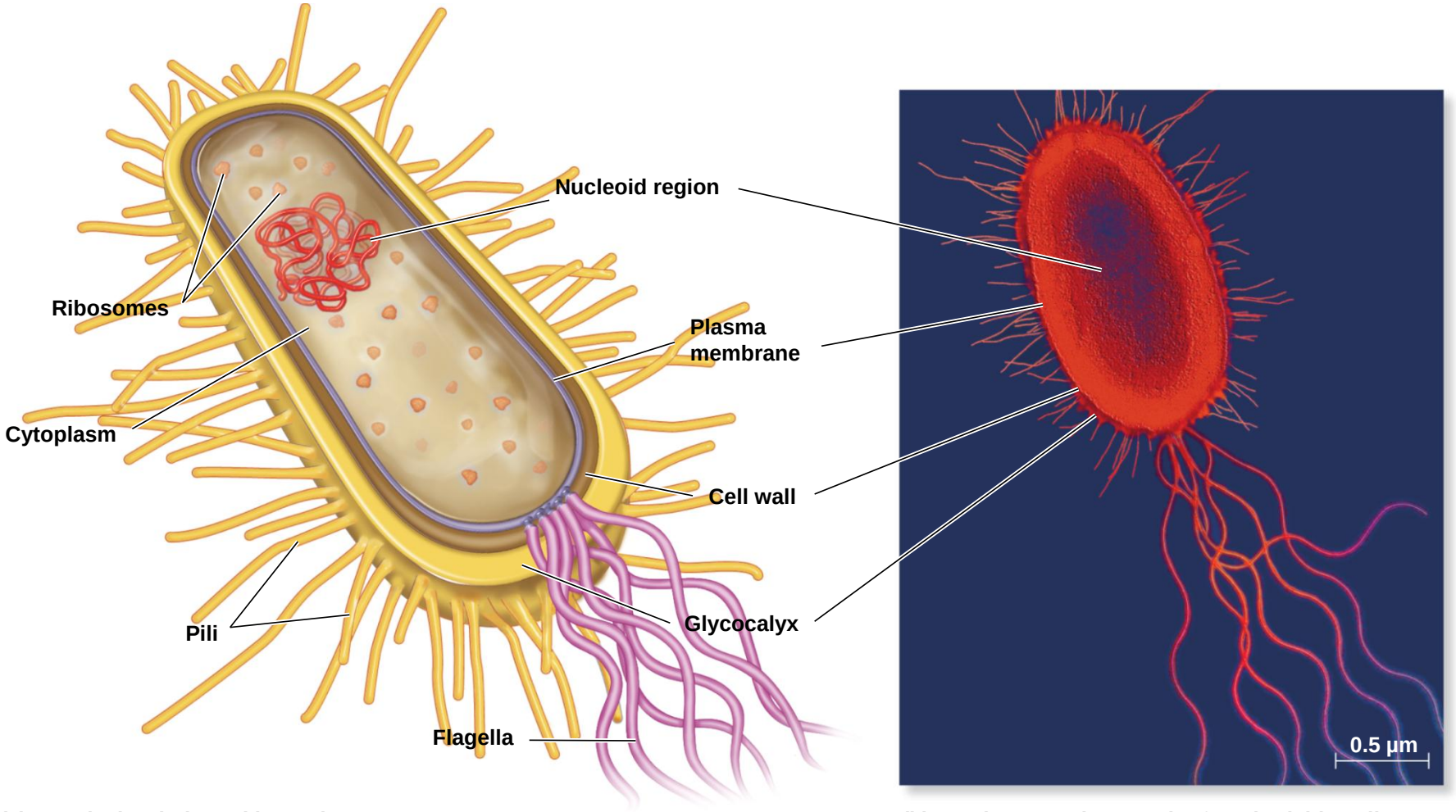


Prokaryotic cells

- Simple cell structure
- Lack a membrane-enclosed nucleus
- 2 categories- bacteria and archaea
  - Both small
  - Bacteria- abundant, most not harmful
  - Archaea- less common, often found in extreme environments

Typical bacterial cell



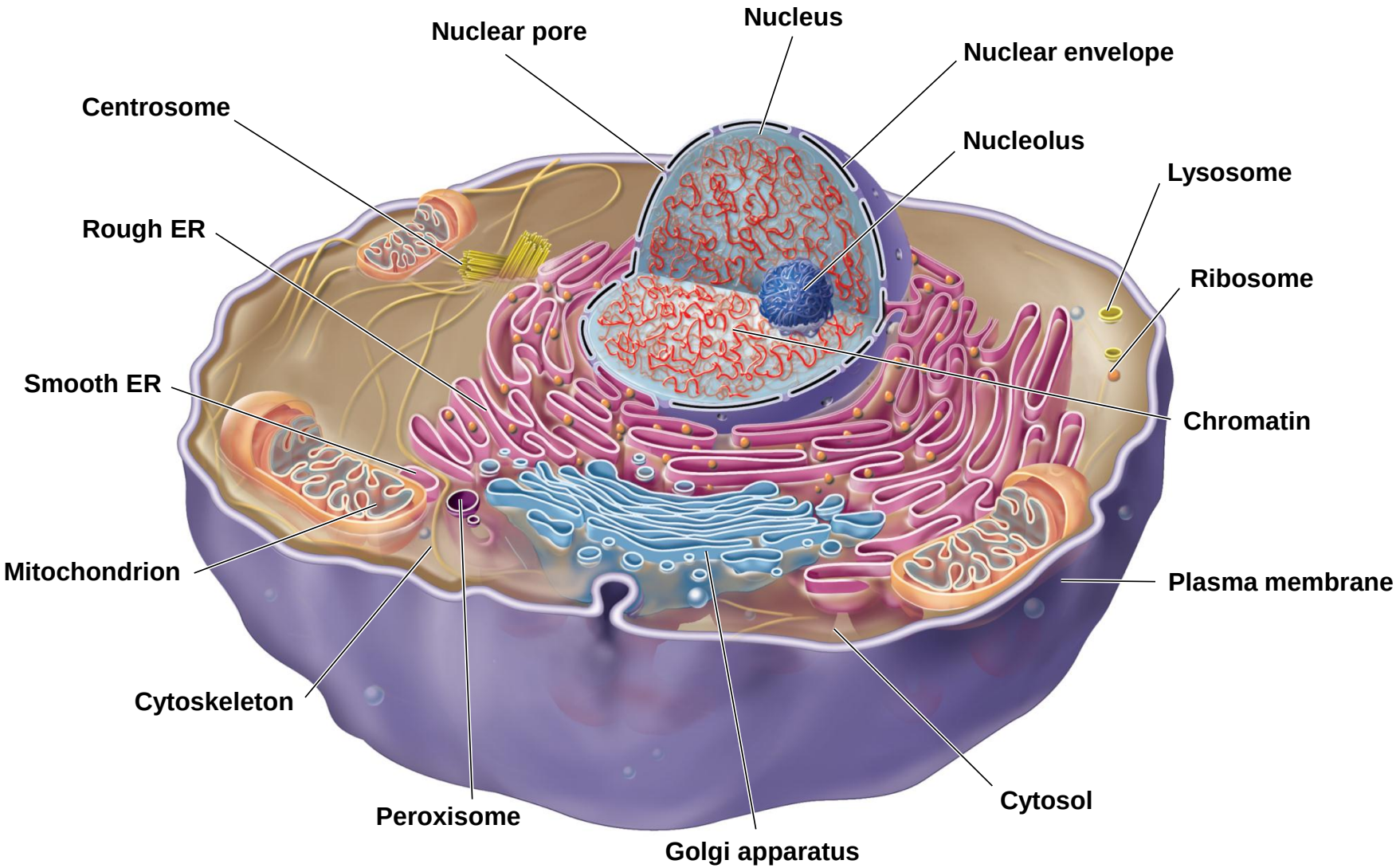
(a) A typical rod-shaped bacterium

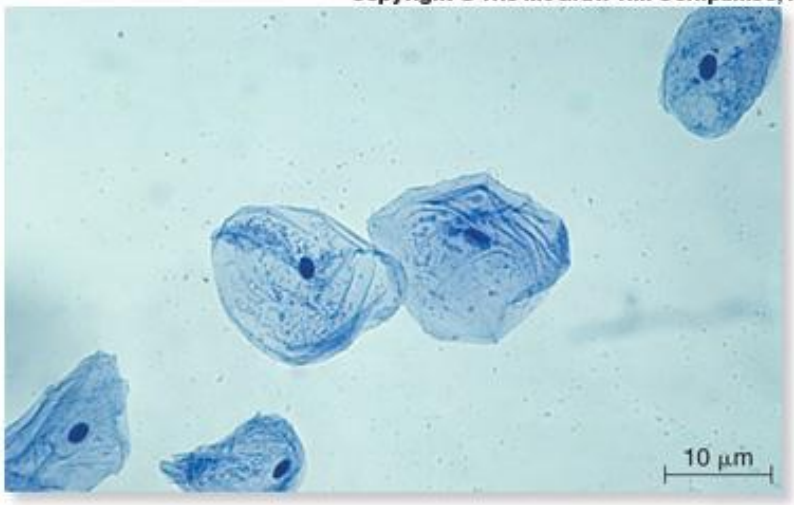
(b) An electron micrograph of Escherichia coli

b: © Dr. Dennis Kunkel Microscopy/Visuals Unlimited

Eukaryotic cells

- DNA housed inside nucleus
- Eukaryotic cells exhibit compartmentalization
- Organelle- membrane-bound compartment with its own unique structure and function
- Shape, size, and organization of cells vary considerably among different species and even among different cell types of the same species

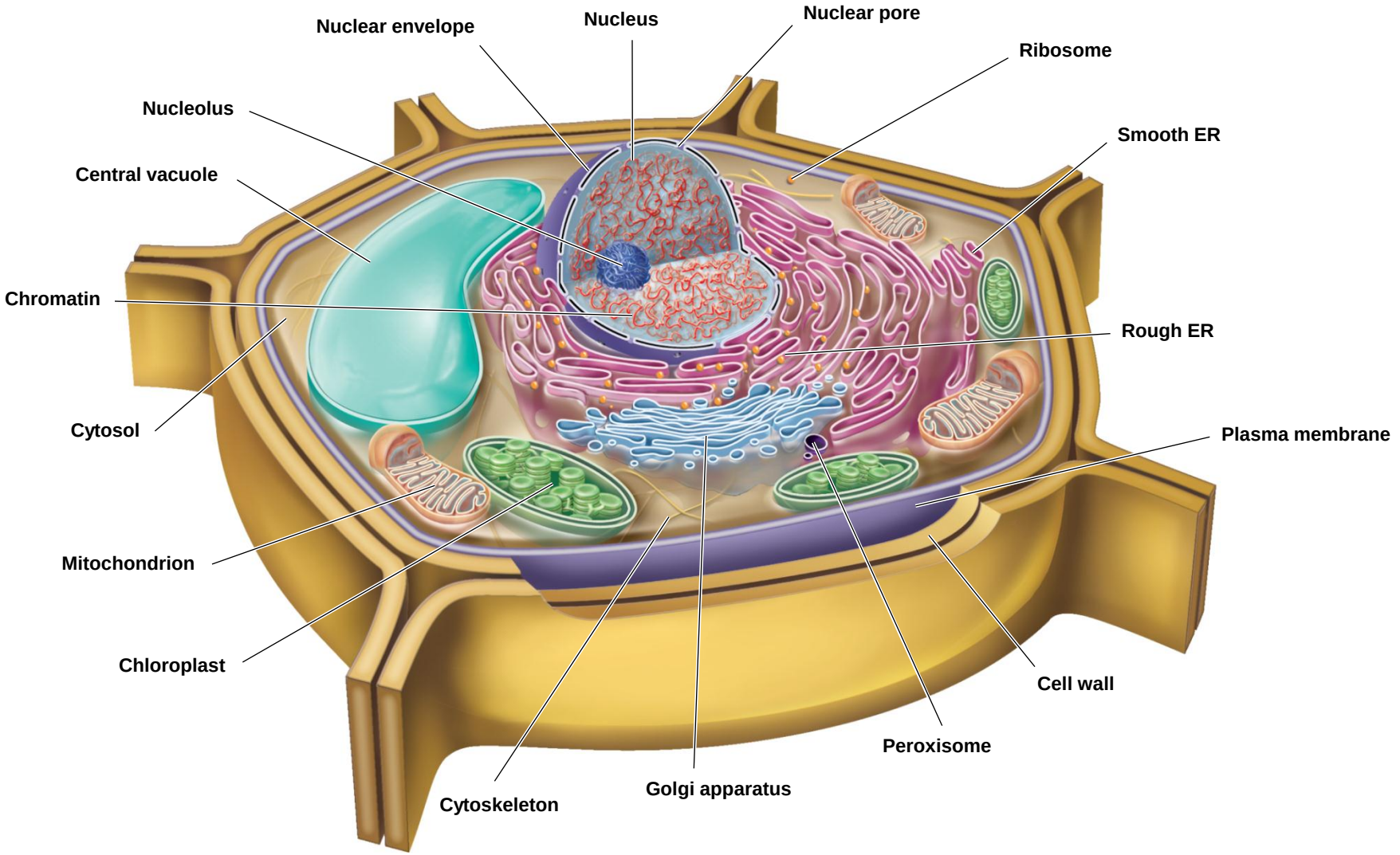




Human skin cell

Human nerve cell

(b)

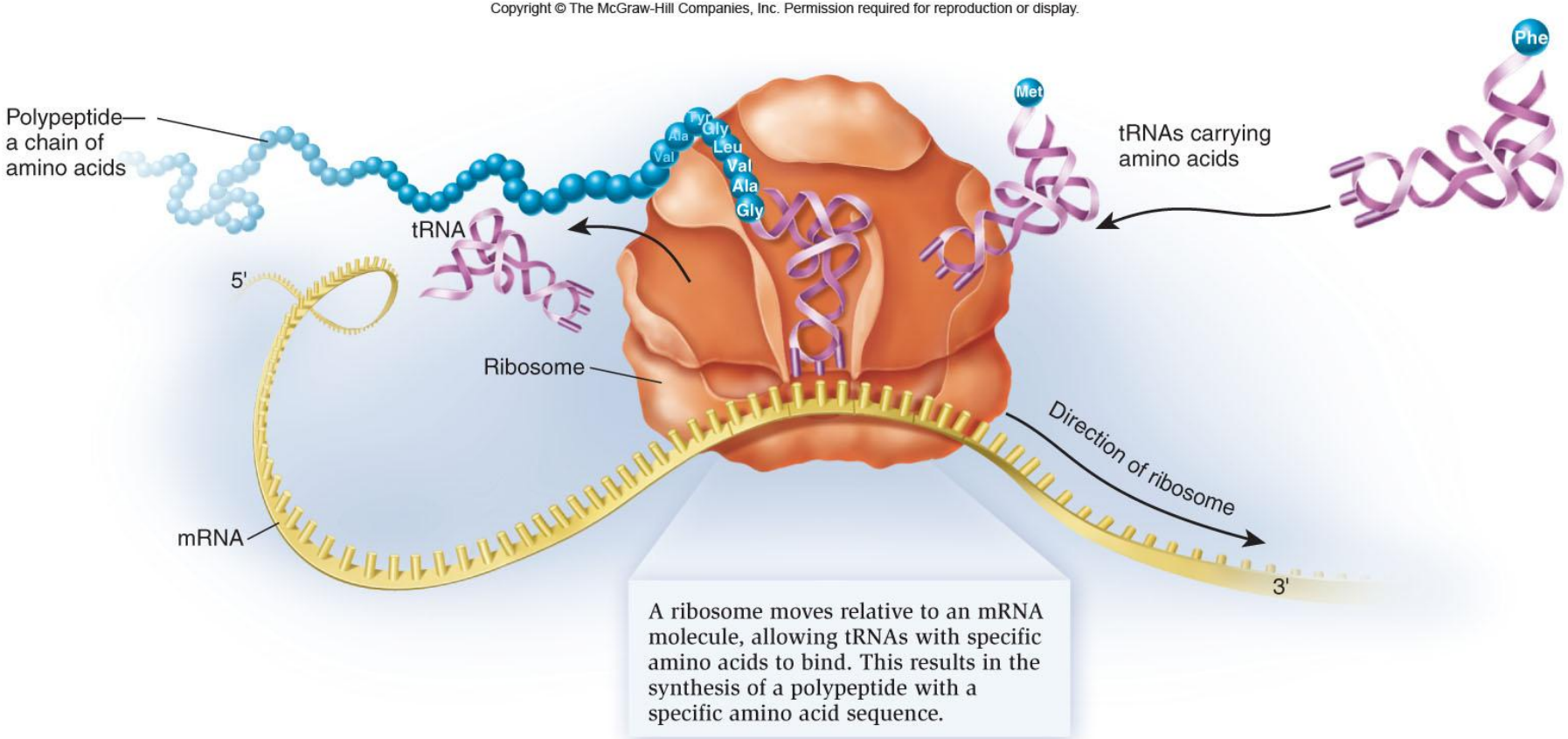


The Proteome Determines the Characteristics of a Cell

- How does a single organism produce different types of cells?
- Identical DNA in different cells but different proteomes
- The proteome of a cell determines its structure and function
- Gene regulation, amount of protein, amino acid sequence of a particular protein, and protein modification can influence a cell's proteome
- Proteomes in healthy cells are different from the proteomes of cancerous cells

Translation

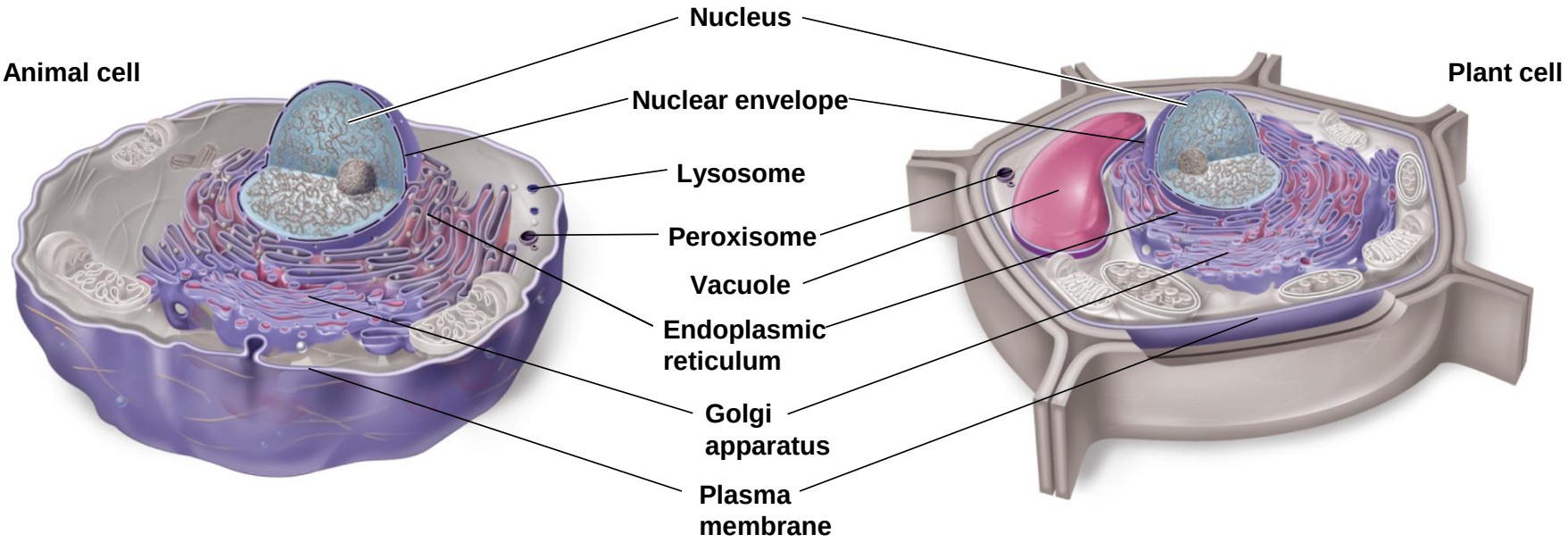
- Process of polypeptide synthesis
- Information within a gene is ultimately translated into the sequence of amino acids in a polypeptide
- Ribosome- site of synthesis
- Transfer RNA (tRNA)- brings amino acids
- Messenger RNA (mRNA)- information to make a polypeptide



A ribosome moves relative to an mRNA molecule, allowing tRNAs with specific amino acids to bind. This results in the synthesis of a polypeptide with a specific amino acid sequence.

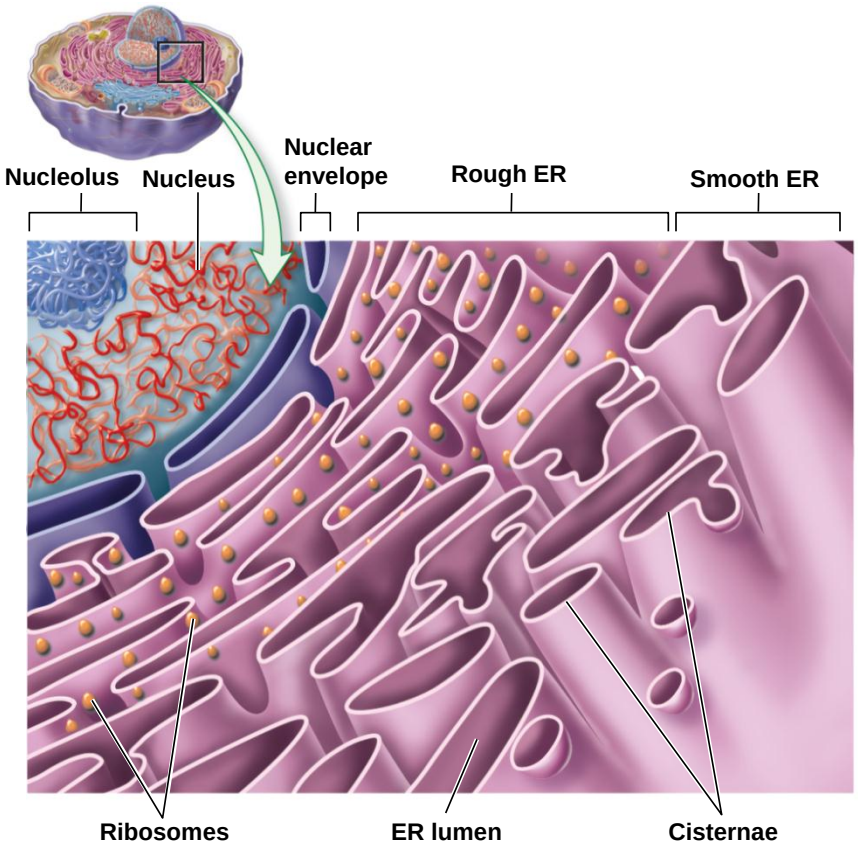
Endomembrane system

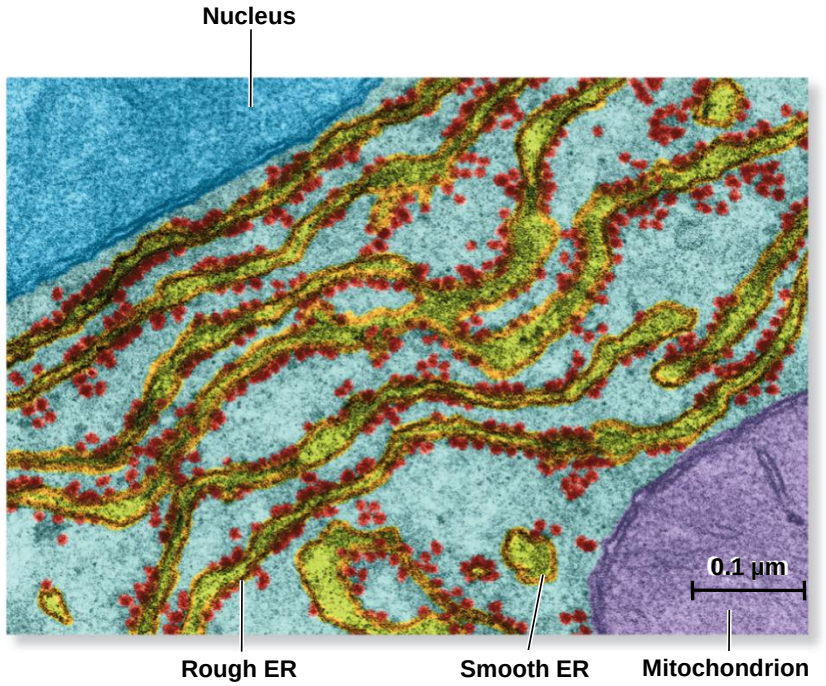
- Network of membranes enclosing the nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, and vacuoles
- Also includes plasma membrane
- May be directly connected to each other or pass materials via vesicles



Endoplasmic reticulum

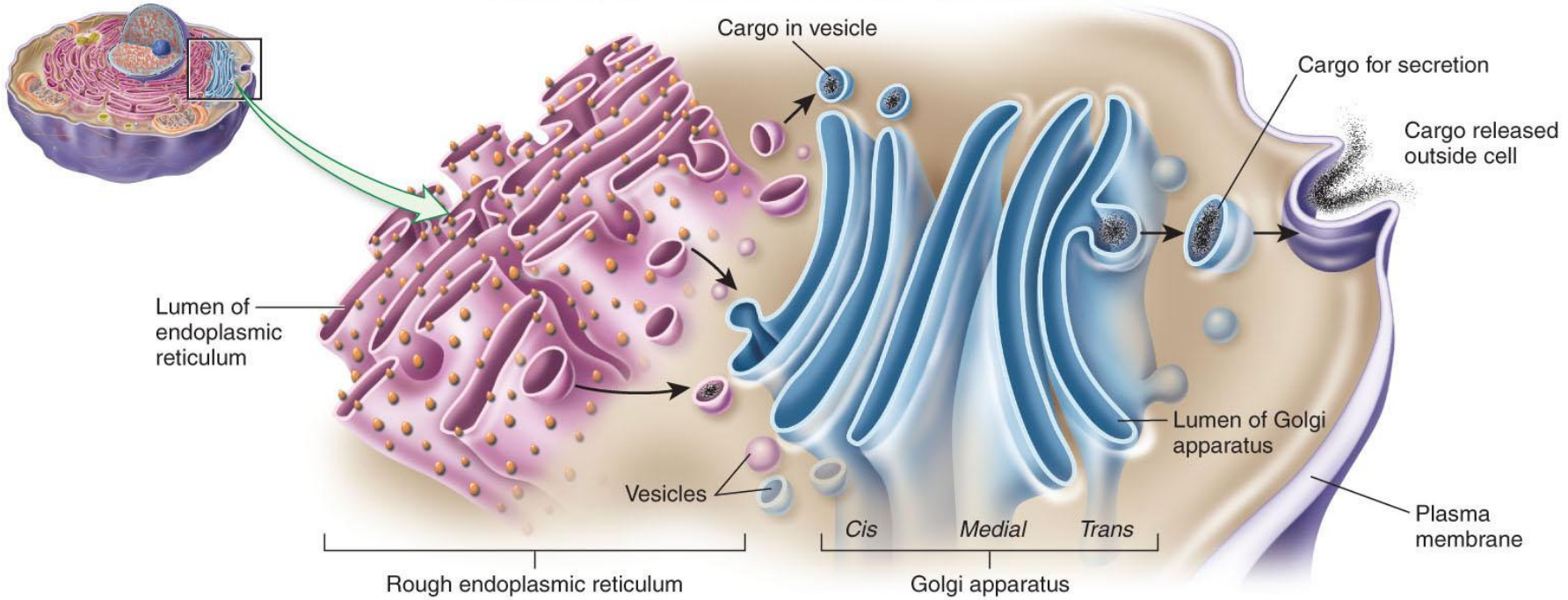
- Network of membranes that form flattened, fluid-filled tubules or cisternae
- ER membrane encloses a single compartment called the ER lumen
- Rough endoplasmic reticulum (rough ER)
  - Studded with ribosomes
  - Involved in protein synthesis and sorting
- Smooth endoplasmic reticulum (smooth ER)
  - Lacks ribosomes
  - Detoxification, carbohydrate metabolism, calcium balance, synthesis, and modification of lipids





Golgi apparatus

- Also called the Golgi body, Golgi complex, or simply Golgi
- Stack of flattened, membrane-bounded compartments
- Vesicles transport materials between stacks
- Three overlapping functions
  - Secretion, processing, and protein sorting



Palade Demonstrated Secreted Proteins Move Sequentially

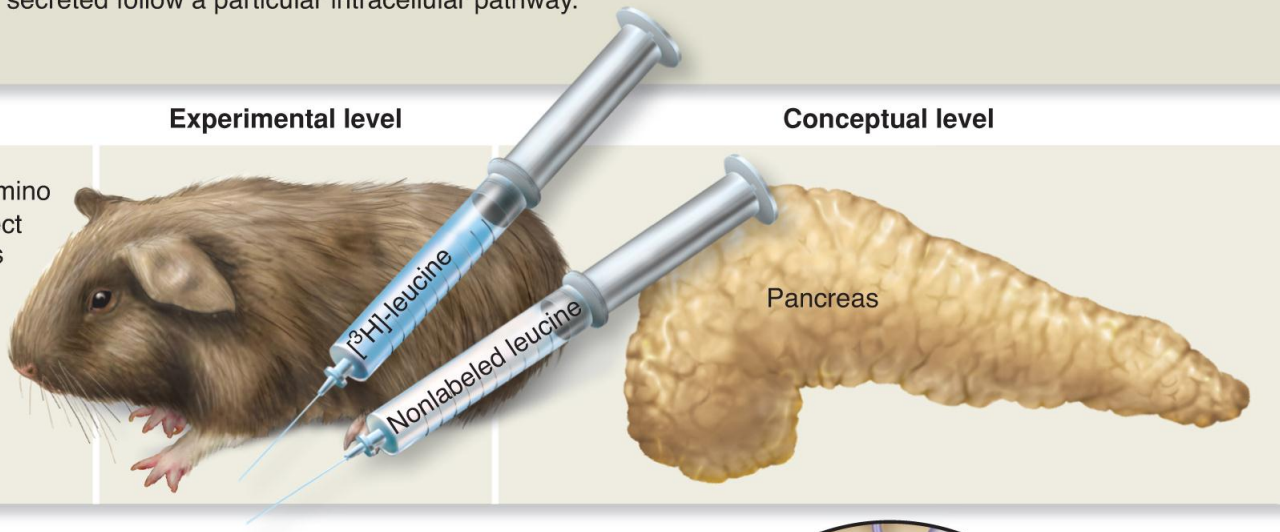
- Used pulse-chase experiments to trace path of radioactive proteins
- Studied pancreatic cells – primary function is protein secretion
- Dark spots in TEM images revealed radioactive proteins
- Experiments provided first evidence that secreted proteins are synthesized into rough ER and then move through a series of compartments before they are secreted

HYPOTHESIS Proteins that are to be secreted follow a particular intracellular pathway.

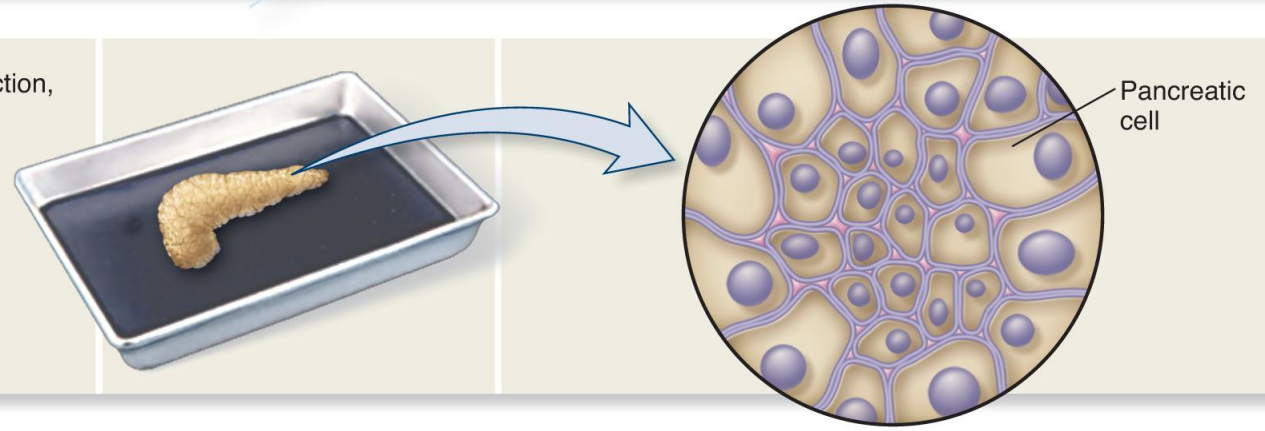
KEY MATERIALS Male guinea pigs.

Experimental level | **Conceptual level**

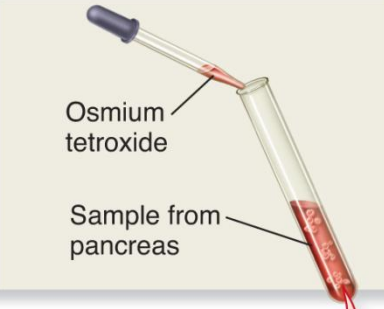
1 Inject guinea pigs with a radioactive amino acid, [^{3}H]-leucine. After 3 minutes, inject them with nonlabeled leucine, which is called a chase.



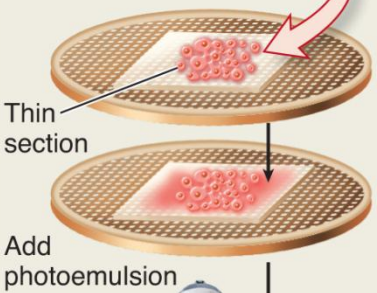
2 At various times after the second injection, remove samples of pancreatic cells.

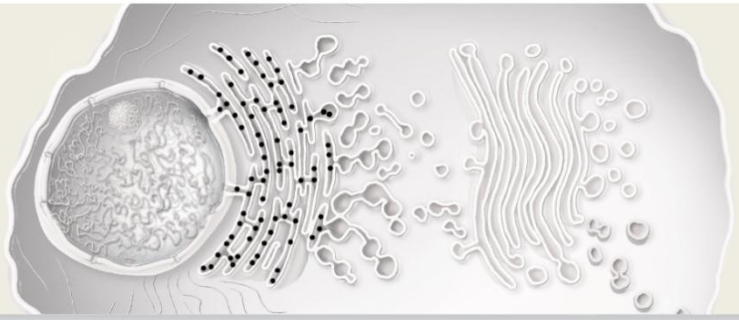


3 Stain the sample with osmium tetroxide, which is a heavy metal that binds to membranes.

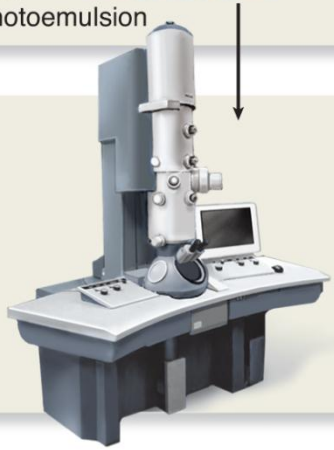


4 Cut thin sections of the samples, and place a thin layer of radiation-sensitive emulsion over the sample. Allow time for radioactive emission from radiolabeled proteins to precipitate silver atoms in the emulsion.





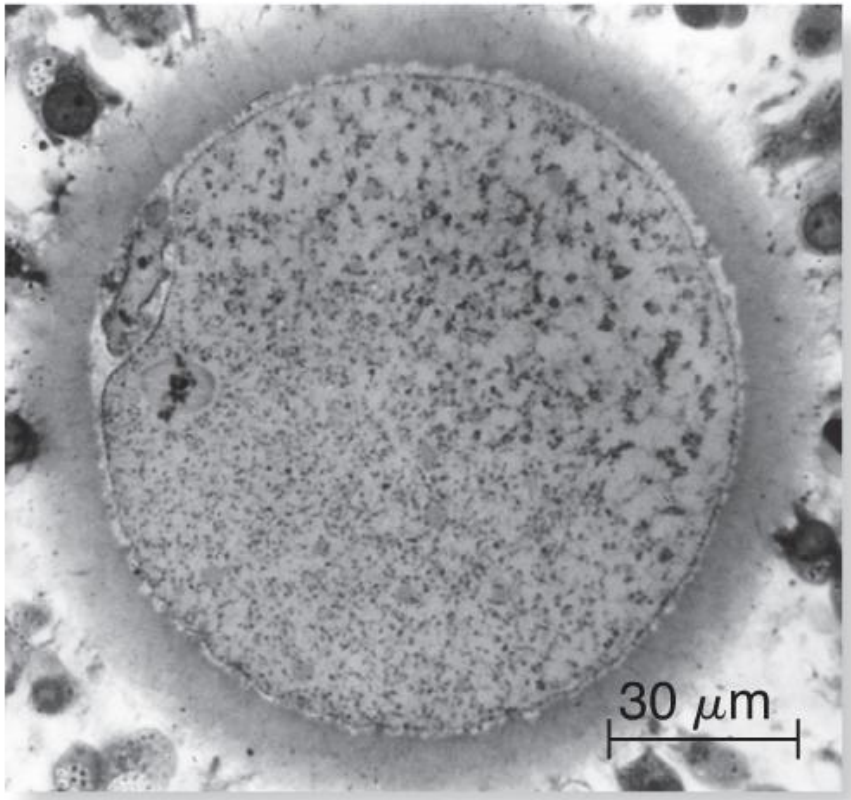
5 Observe the sample under a transmisson electron microscope.

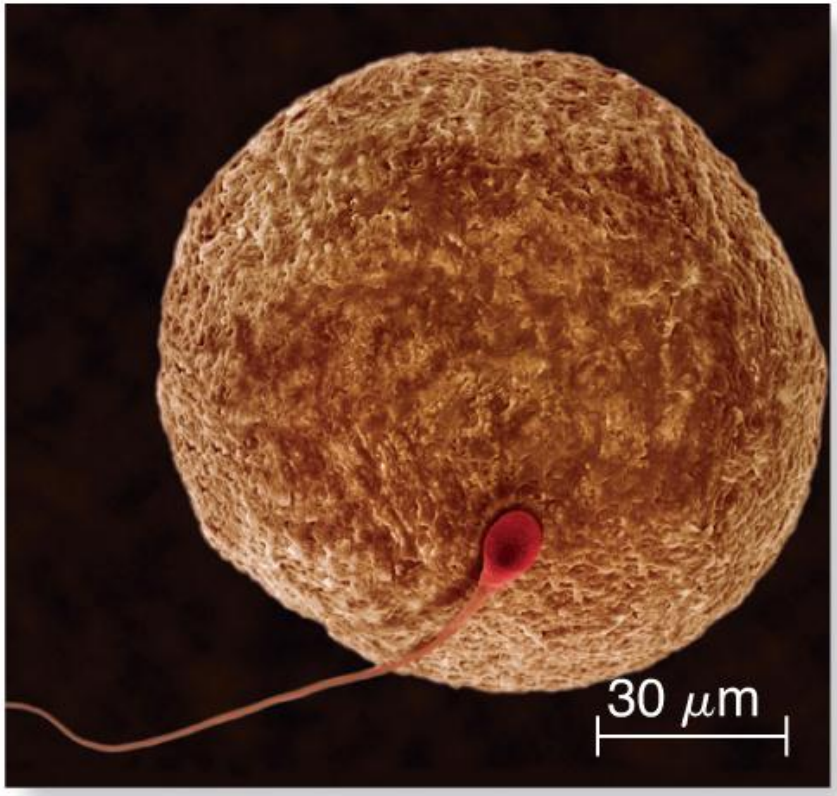


See Chapter 4 for a description of TEM.

Electron microscope types

- Transmission electron microscopy (TEM)
  - Beam of electrons transmitted through sample
  - Thin slices stained with heavy metals
  - Some electrons are scattered while others pass through to form an image
- Scanning electron microscopy (SEM)
  - Sample coated with heavy metal
  - Beam scans surface to make 3D image



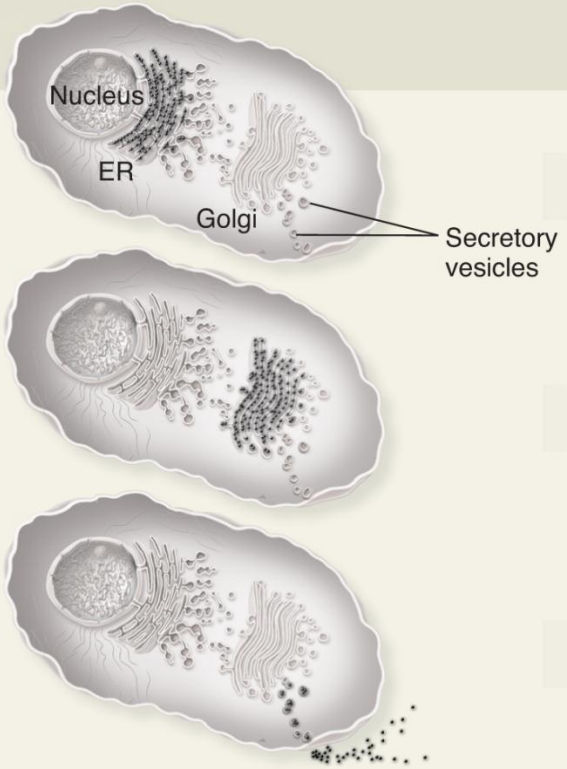


(a) Transmission electron micrograph

(b) Scanning electron micrograph

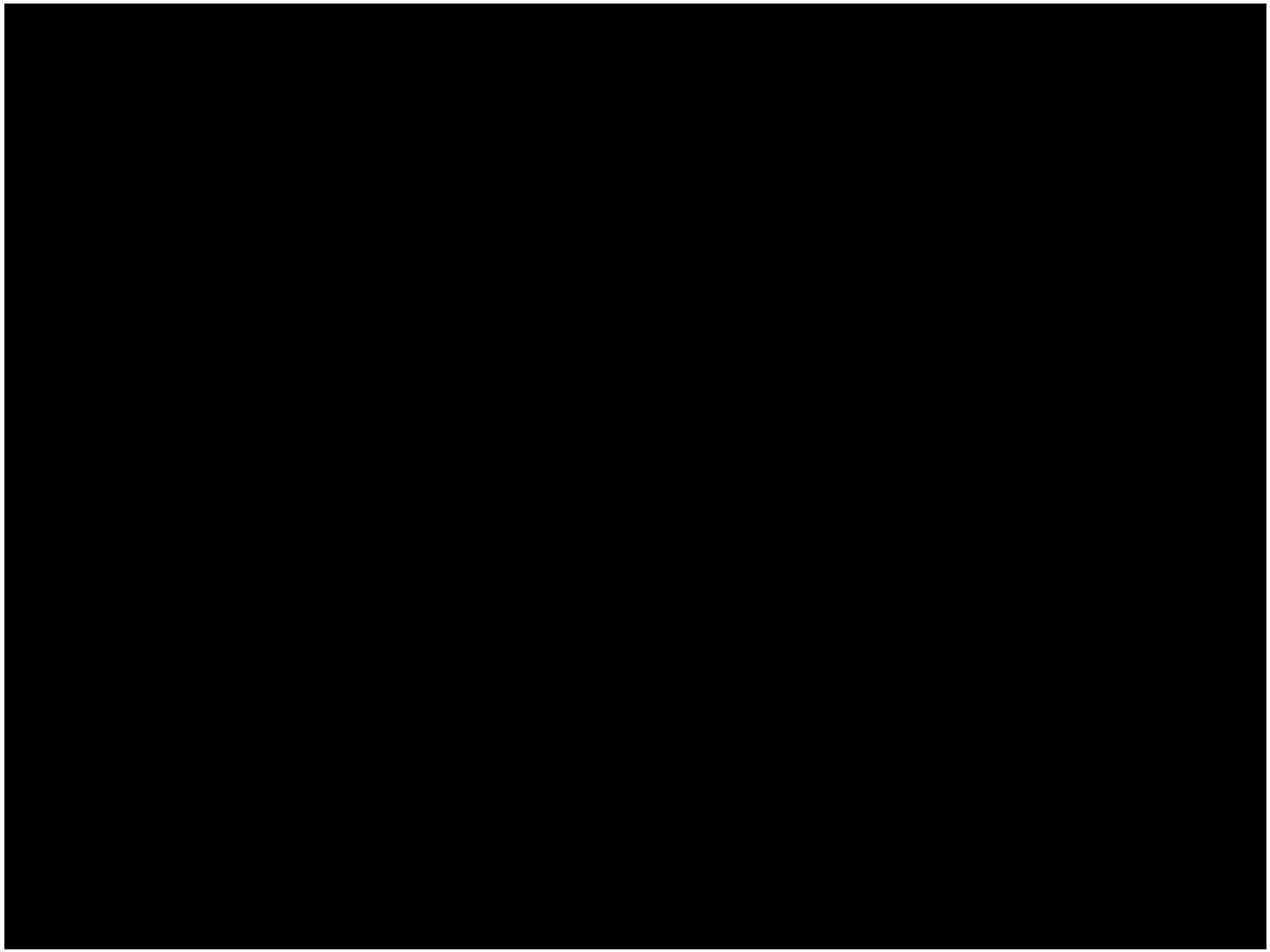
a(inset): © Dr. Donald Fawcett & L. Zamboni/Visuals Unlimited; b: © Dr. Dennis Kunkel Microscopy/Visuals Unlimited

6 THE DATA



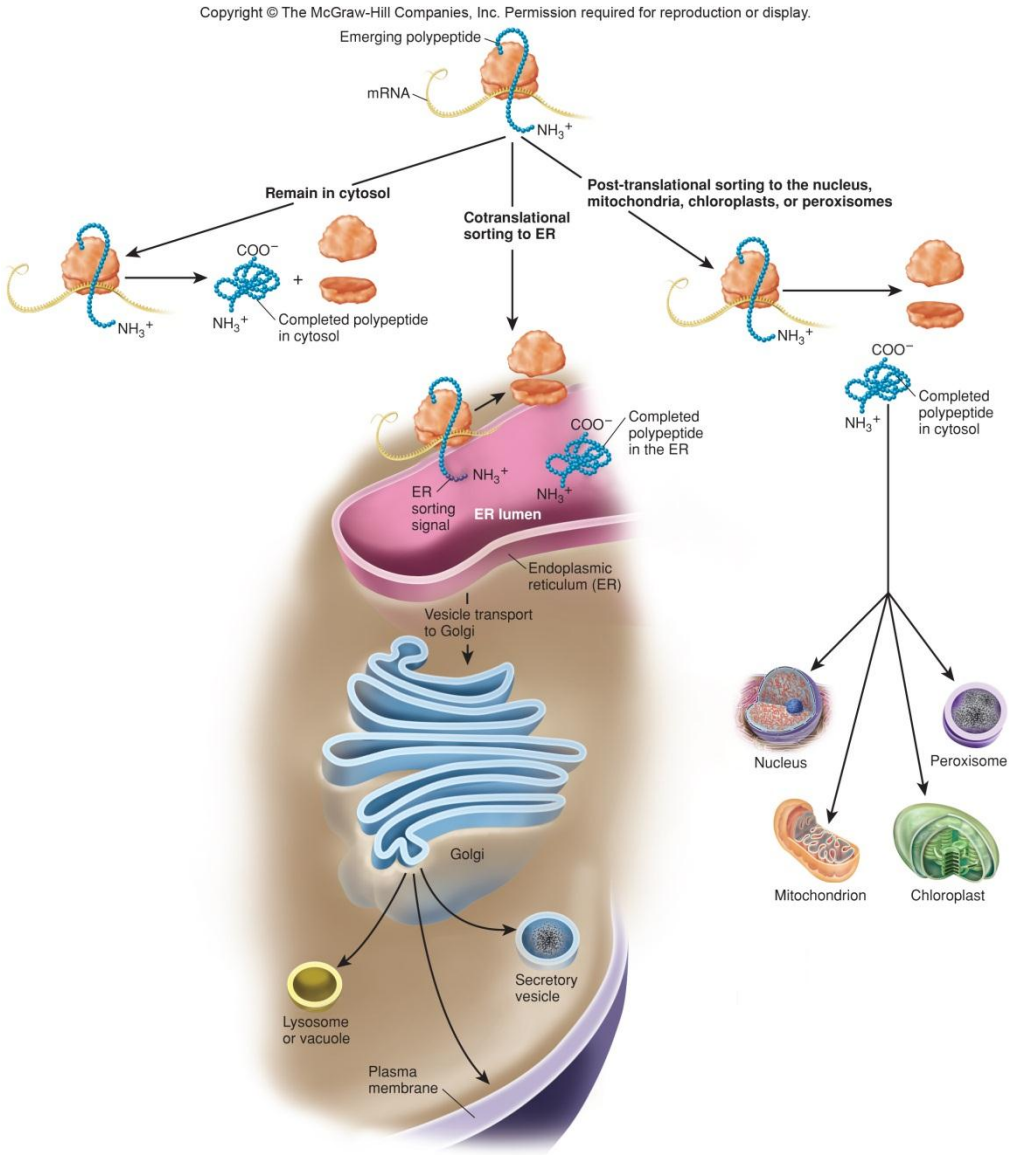
7 CONCLUSION To be secreted, proteins move from the ER to the Golgi to secretory vesicles and then to the plasma membrane, where they are released to the outside of the cell.

8 SOURCE Caro, L.G., and Palade, G.E. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. An autoradiographic study. *Journal of Cell Biology* 20:473–495.



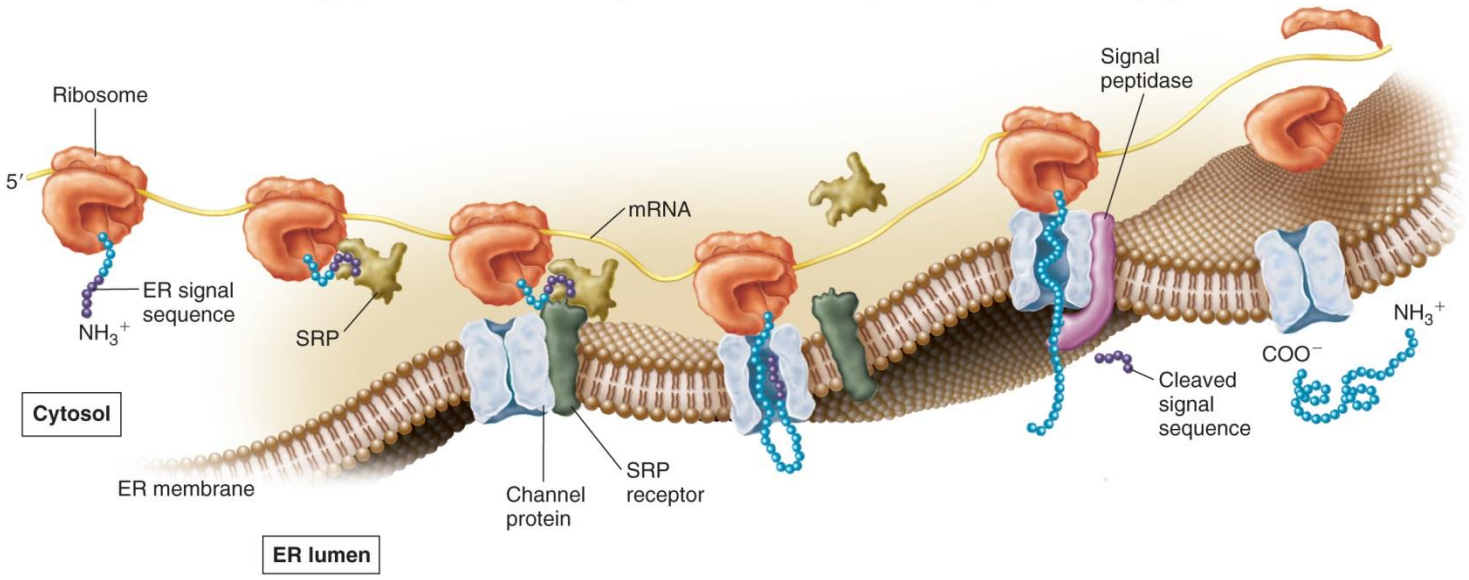
The Fate of Newly Synthesized Proteins

1. Cytosol
    a. **Example:** The enzymes of glycolysis.
2. Co-translational Sorting to the ER
    a. **Example:** Digestive enzymes of the small intestine.
3. Post-translational Sorting to other organelles
    a. **Example:** The enzymes of the peroxisome



Co-translational Modification

- Proteins destined for the rough ER are synthesized by free ribosomes in the cytoplasm.
- Through the actions of a **ER signal peptide sequence** and the **protein SRP**, the entire complex is shuttled to the rough ER.



ER signal sequence

- This is represented by a series of amino acids at the N-terminus targeted by SRP.

What types of amino acids are most prevalent in the N-terminus of the ER signal sequence?

H_2N-Met-Met-Ser-Phe-Val-Ser-Leu- Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln

Signal Recognition Particle (SRP)

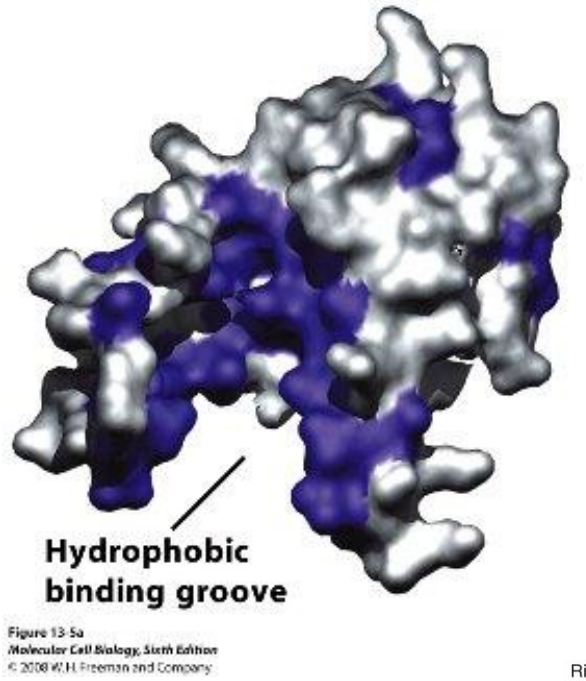
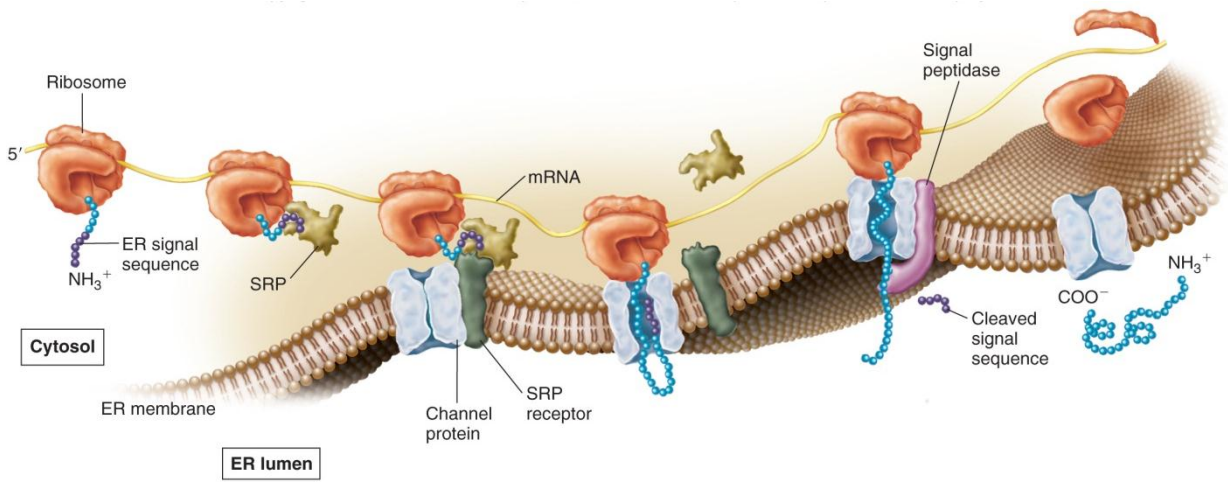


Figure 13-5a
Molecular Cell Biology, Sixth Edition

- This cytosolic protein binds to the ER signal sequence, halting translation and assisting the ribosome to the ER.



Errors in Trafficking Leading to Disease

Beyond the Signal Sequence: Protein Routing in Health and Disease

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Receptors, hormones, enzymes, ion channels, and structural components of the cell are created by the act of protein synthesis. Synthesis alone is insufficient for proper function, of course; for a cell to operate effectively, its components must be correctly compartmentalized. The mechanism by which proteins maintain the fidelity of localization warrants attention in light of the large number of different molecules that must be routed to distinct subcellular loci, the potential for error, and resultant disease. This review summarizes diseases known to have etiologies based on defective protein folding or failure of the cell's quality control apparatus and presents approaches for therapeutic intervention.

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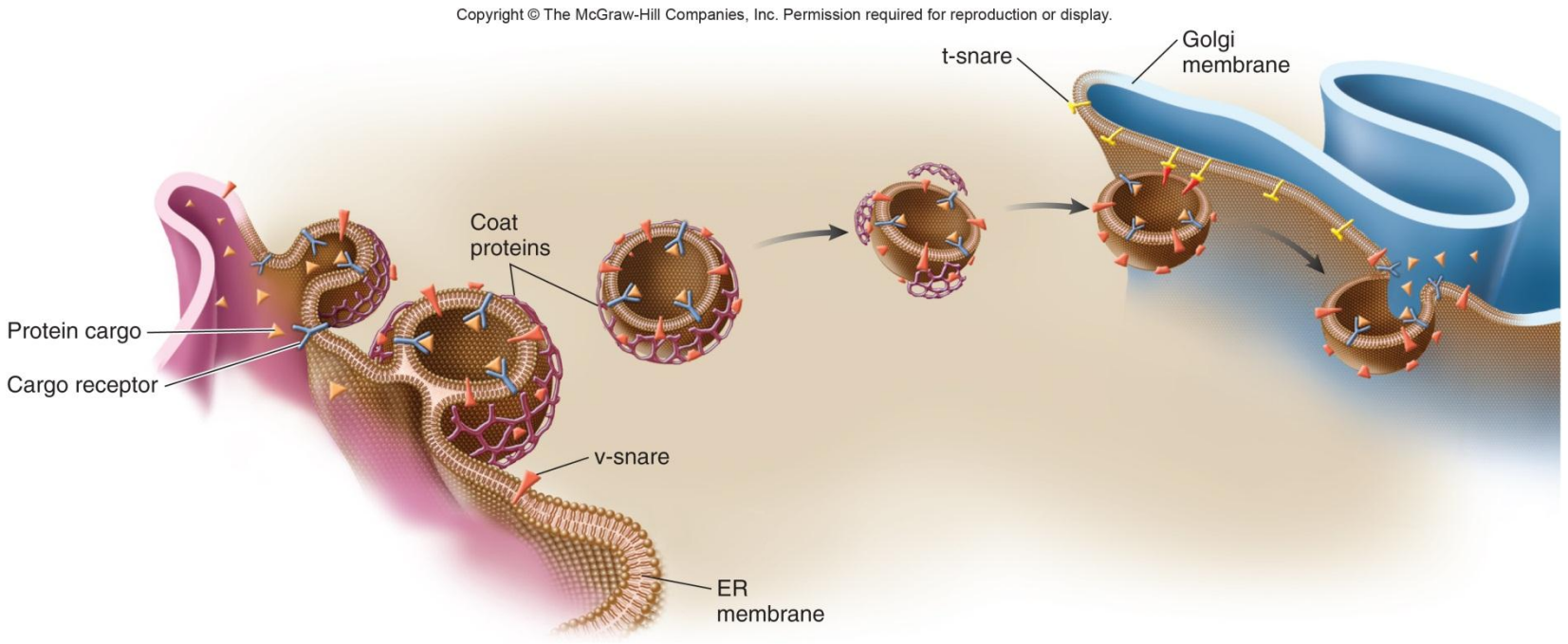
Connecting the Compartments

- **Vesicles** are parts of the ER membrane that are used to shuttle cargo to the Golgi.



Other Sequences

- There are other peptide signal sequences:
  - Nuclear localization sequences
  - ER retention sequences
  - Mitochondrial targeting sequences