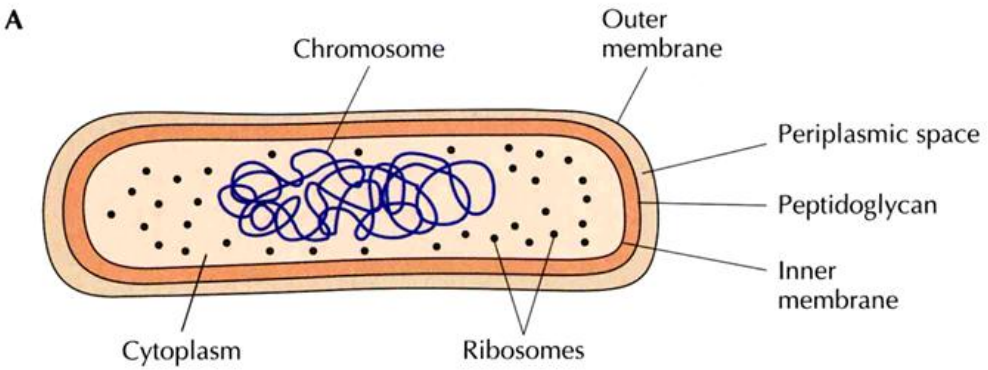
Chapter 2.
Molecular Biotechnology
Biological System

1. Prokaryotic and Eukaryotic Organisms

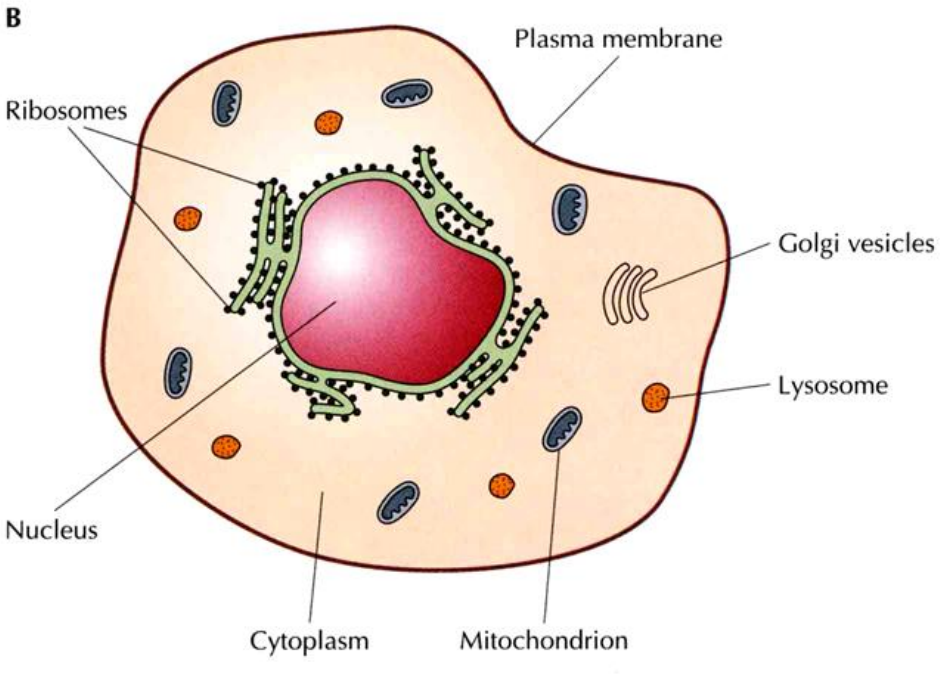
Prokaryotic and Eukaryotic Organisms

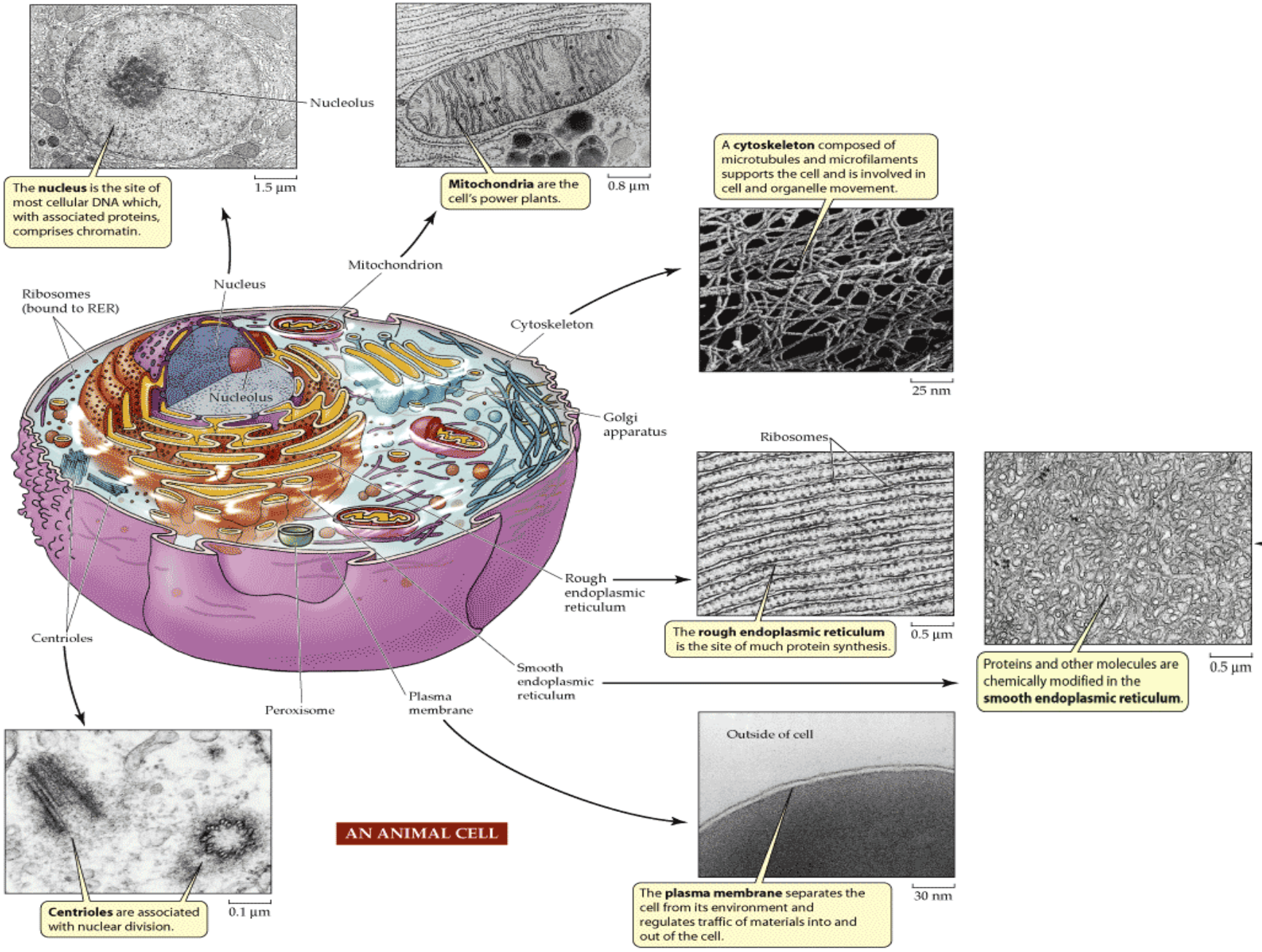
- Prokaryotic cells
  - Prokaryote (pro; before, karyon: kernel or nucleus)
  - No nuclear membrane
  - Small (0.2-2 μm), mostly single-celled organisms
    - Eubacteria : common bacteria, e.g. *E.coli*, blue-green algae
    - Archaea (Archaebacteria)
- Eukaryotic cells
  - Eukaryote (well-formed nucleus)
  - Nuclear and internal membranes → organelles
  - Larger than prokaryotes (10-100 μm)
    - Single-celled: yeast, green algae, amoebae
    - Multicellular: fungi, plant, animal

Prokaryotic cell

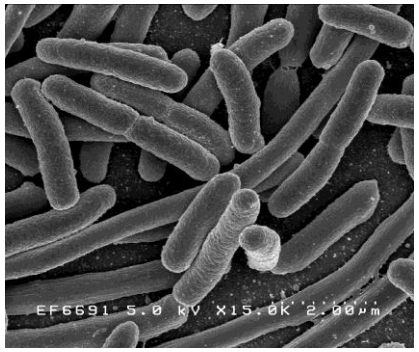


Eukaryotic cell



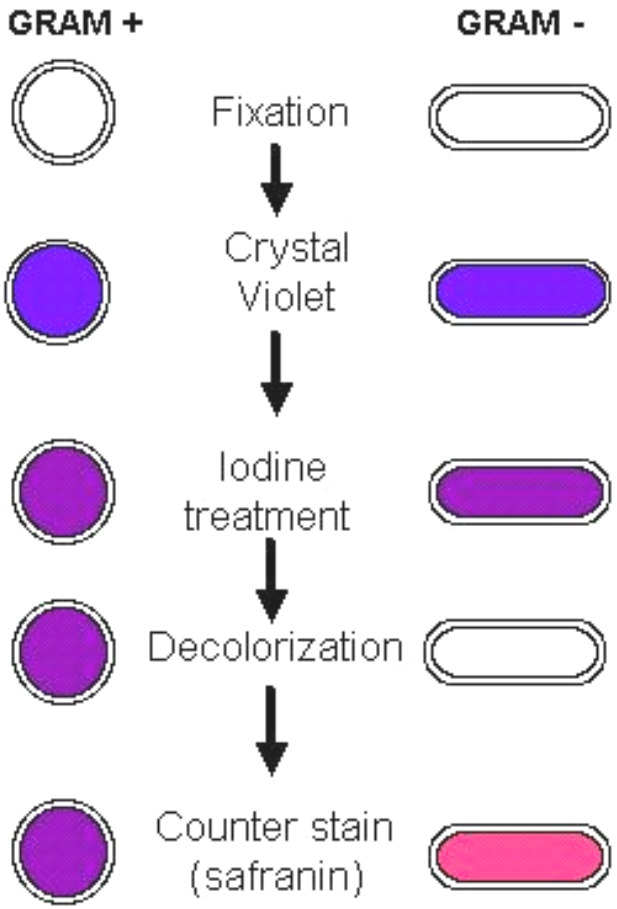

Nucleolus
1.5 µm
The **nucleus** is the site of most cellular DNA which, with associated proteins, comprises chromatin.
Mitochondria are the cell's power plants.
0.8 µm
A **cytoskeleton** composed of microtubules and microfilaments supports the cell and is involved in cell and organelle movement.
25 nm
Nucleus
Mitochondrion
Ribosomes (bound to RER)
Cytoskeleton
Nucleolus
Golgi apparatus
Ribosomes
Rough endoplasmic reticulum
The **rough endoplasmic reticulum** is the site of much protein synthesis.
0.5 µm
Centrioles
Smooth endoplasmic reticulum
Proteins and other molecules are chemically modified in the **smooth endoplasmic reticulum**.
0.5 µm
Peroxisome
Plasma membrane
Outside of cell
AN ANIMAL CELL
Centrioles are associated with nuclear division.
0.1 µm
The **plasma membrane** separates the cell from its environment and regulates traffic of materials into and out of the cell.
30 nm

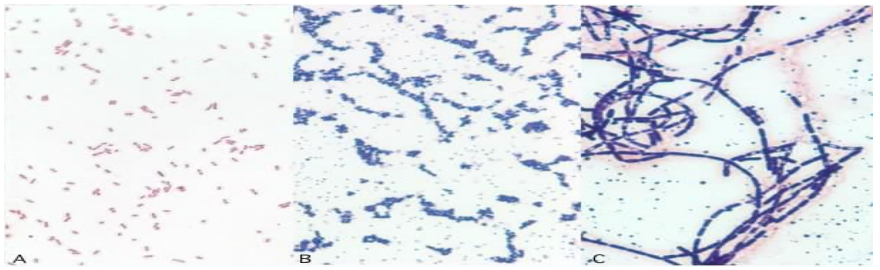
Escherichia coli



- One of the most studied organisms
- Gram-negative, nonpathogenic, motile, rod shaped
- Naturally found in the intestines of humans
- About 22 min doubling time in rich medium at 37^{o}C
- Facultatively anaerobic
  - Both aerobic and anaerobic growth
- Useful for recombinant protein production
  - Aeration is important for productivity
- Other microorganisms for molecular biotechnology applications
  - *Bacillus*, *Corynebacterium*, *Streptomyces*, *Pseudomonas*, *Zymomonas* etc.

Gram negative vs. Gram positive

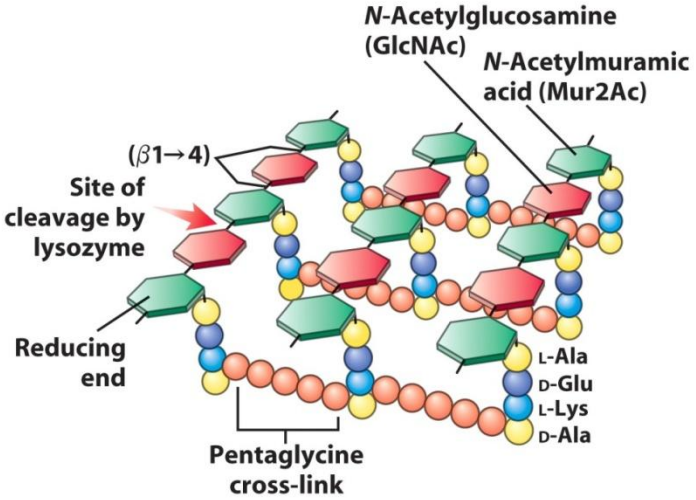


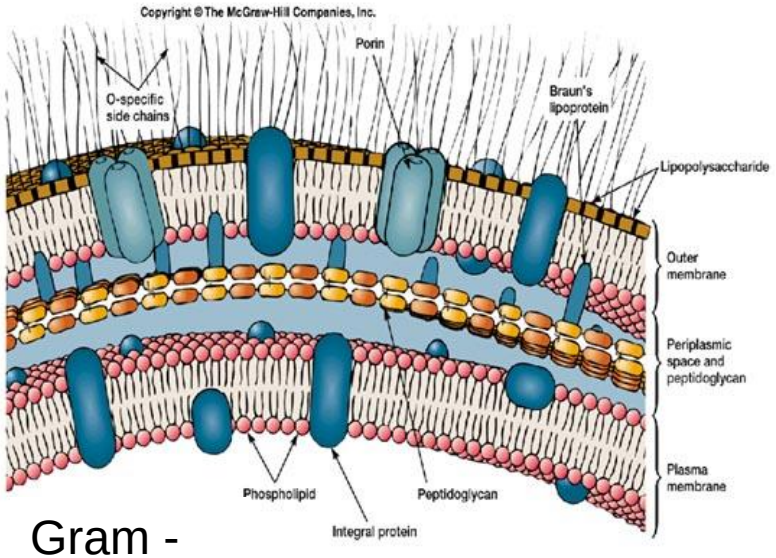


E. coli *Staphylococcus epidemidis* *Bacillus cereus*

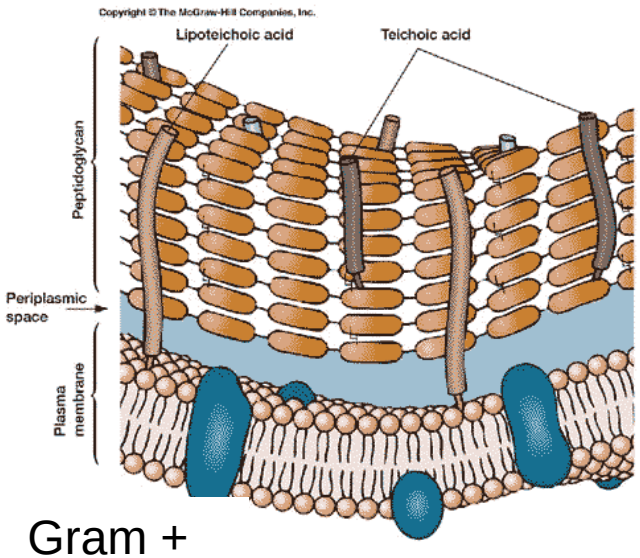
Bacterial Cell Walls (Peptidoglycan)

- Bacterial cell wall
  - Alternating N-acetylglucosamine (β1→4) N-acetymuramic acid
  - Polysaccharide chains are linked by bacterial specific peptide linkage





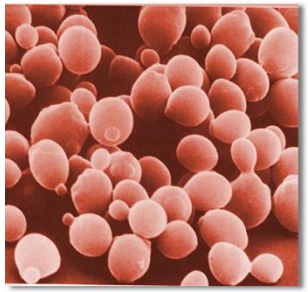
Gram -



Gram +

Saccharomyces cerevisiae

- Eukaryotic version of *E. coli*
- Nonpathogenic, single-celled (5 μm) yeast
- Basic research
  - Eukaryotic model system
    - e.g. cell cycle regulation, disease model
  - The first eukaryotic genome sequenced (1996)
  - Yeast artificial chromosome
- Biotechnology
  - Production of ethanol
    - Alcoholic beverages and bread, bioenergy
  - Production of recombinant proteins from eukaryotic organisms
    - Provide protein modification
  - Other yeast for protein production
    - *Pichia pastoris*, *Hansenula polymorpha*
    - *Schizosaccharomyces pombe*, *Yarrowia lipoltica*



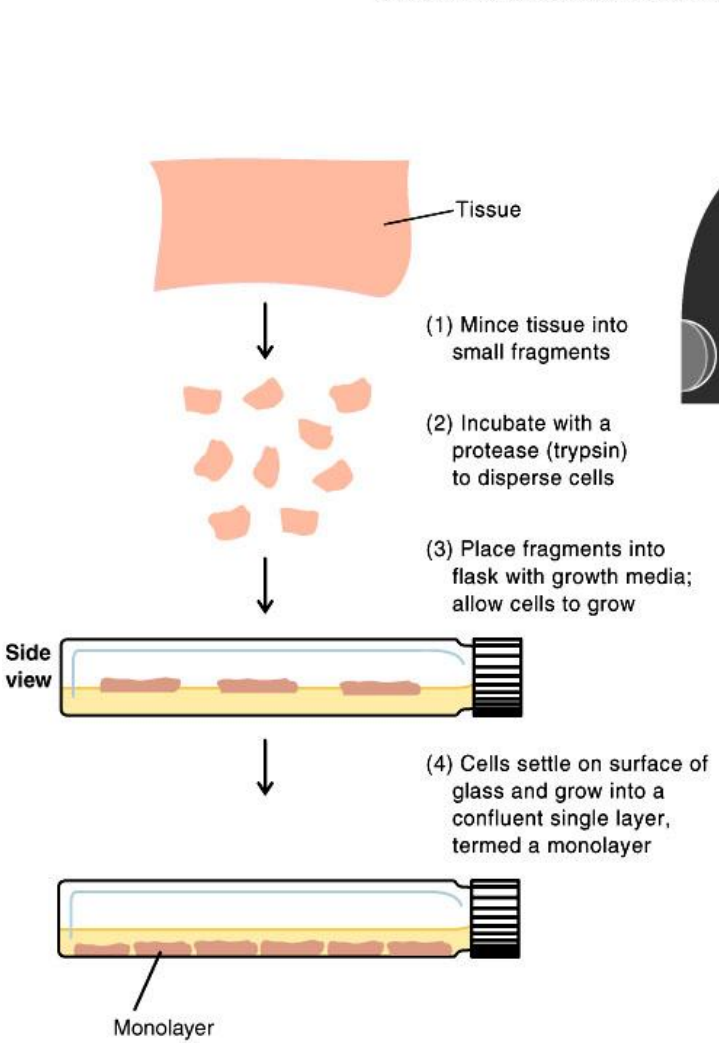
S. cerevisiae

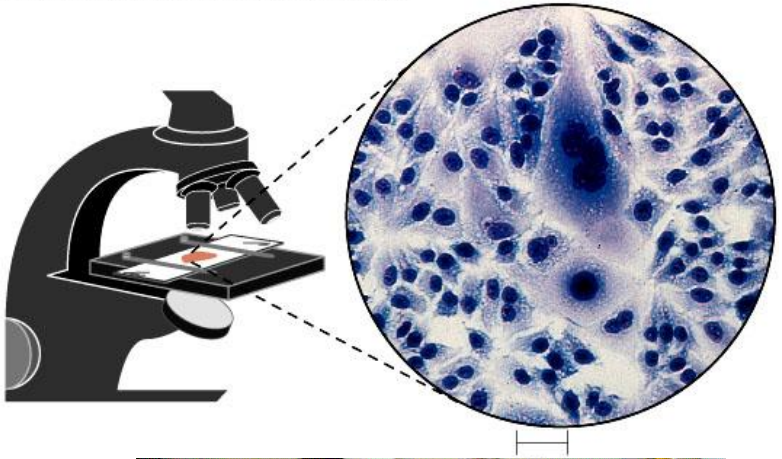
S. pombe

Eukaryotic Cell Culture

- Primary cell culture
  - Isolation of a small sample of tissue
  - Treatment with protease to release cells from extracellular matrix
  - Growth in medium containing nutrients and growth factors
  - Monolayer growth
  - Passage or subculture after confluent growth
  - Limited cell division: 50~ 100 cell generations
  - Useful to study biochemical features of various tissues
- Established cell lines
  - Indefinite growth by genetic changes (chromosomal change)
  - Useful for maintaining virus and protein expressions

Animal Cell Culture





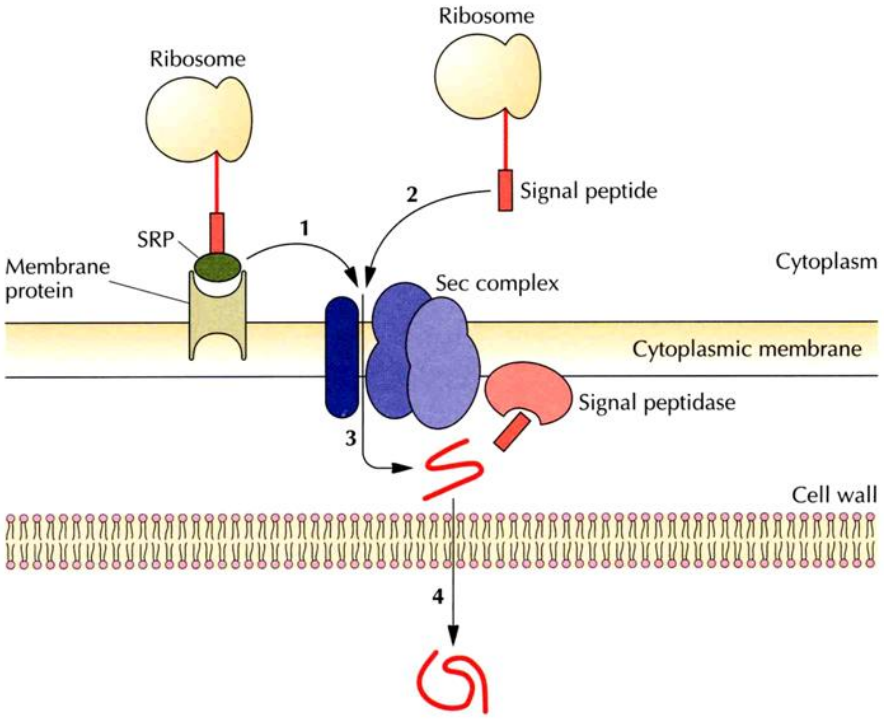
2. Secretion Pathway in Prokaryotes

Secretion Pathways

- Function of secretory proteins
  - Nutrient acquisition
  - Cell-to-cell communication
  - Protection
  - Structure of outer surface of cell membrane
- N-terminal amino acid sequence determines secretion
  - Signal peptide, signal sequence, leader sequence, leader peptide
- Membrane barrier of prokaryotes
  - Gram negative bacteria
    - Inner membrane, periplasmic space, outer membrane
  - Gram positive bacteria
    - Single membrane
- Eukaryotic secretory proteins
  - Modifications of secretory proteins during secretion
    - Glycosylation, acetylation, sulfation, posphorylation

Secretion in Gram-positive Bacteria

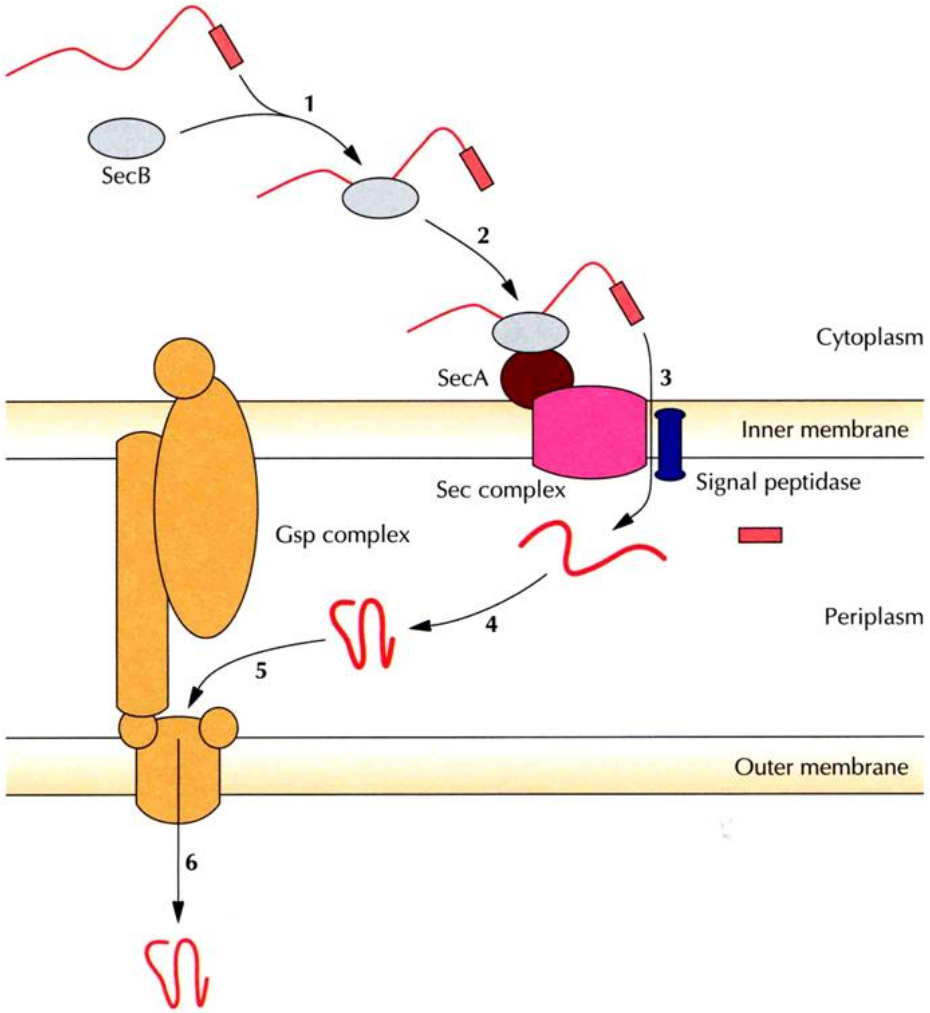
- Sec complex
  - Membrane-bound
  - Facilitate secretion of protein through a channel
    - Direct contact with the signal peptide
    - Signal recognition complex + signal peptide
    - → SRP receptor → Sec complex
- Signal peptidase
  - Removal of signal peptide
  - Releasing the protein
- Crossing the porous cell wall and proper folding



Secretion in Gram-negative bacteria

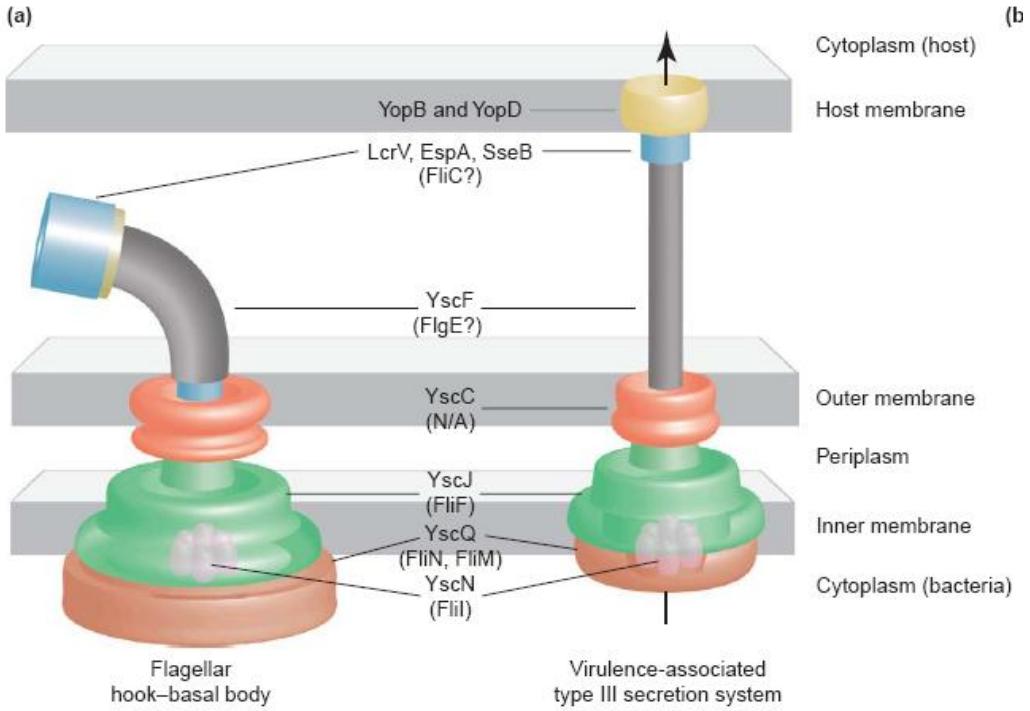
- Sec-dependent :General secretion pathway (GSP)
  - Passage through inner membrane
    - SecB + signal peptide-containing protein → SecA → Sec complex
    - Removal of signal peptide after transport and folding in periplasm
  - Passage through outer membrane
    - Autotransporter pathway
      - A region of the protein generates channel structure and removed after transport of the other part
      - Release of the functional portion of the protein by proteolytic cleavage
    - Single accessory pathway
      - Transport across a single outer membrane channel
    - Chaperone/usher pathway
      - Proteins that form P pili
    - Type II secretion pathway
      - Gsp complex spans the periplasmic space

Type II Secretion Pathway in Gram-negative Bacteria



Secretion in Gram-negative bacteria

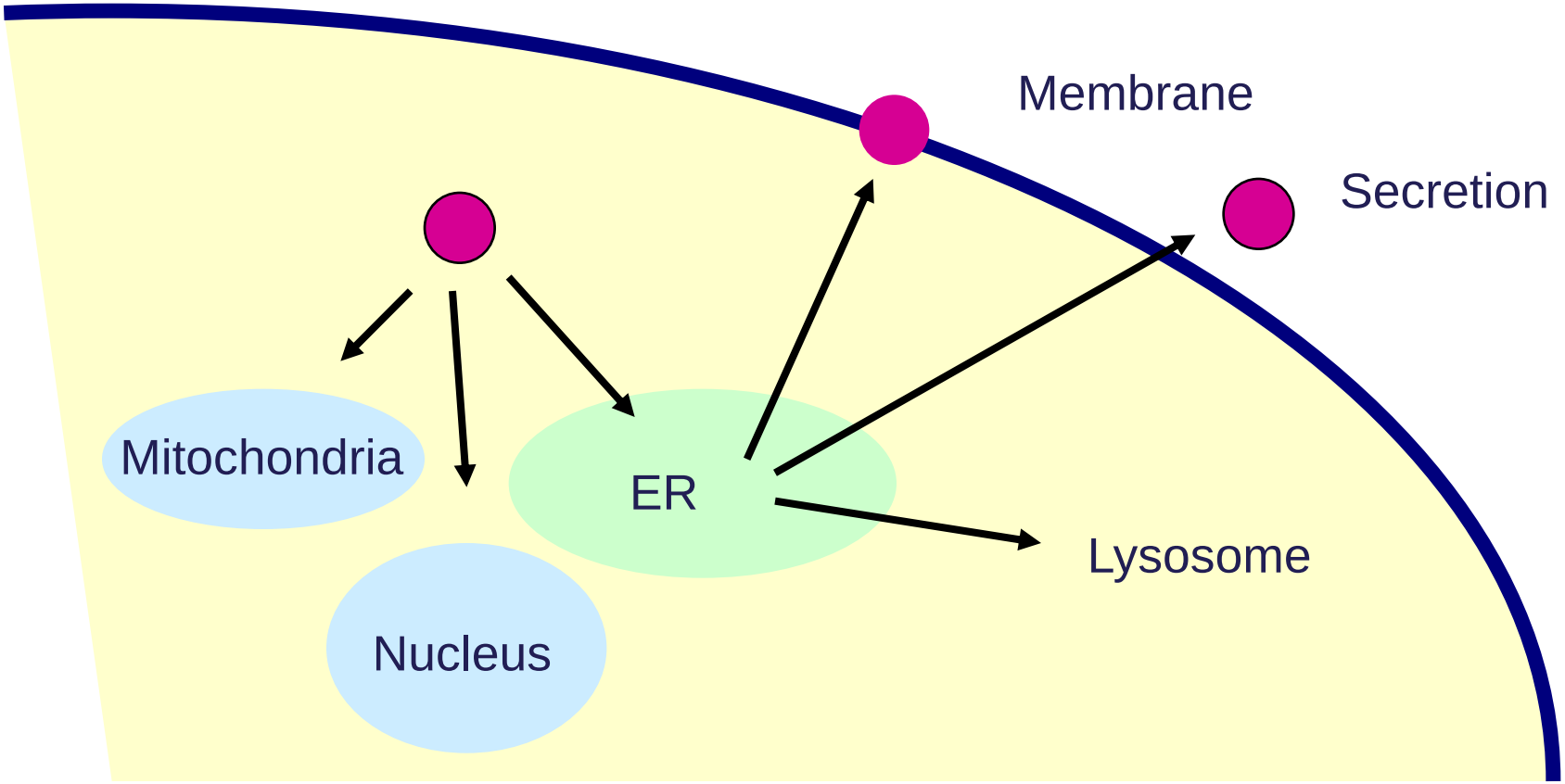
- Sec-independent
  - Own protein complex that extends from the inner to the outer membrane
  - Type I secretion system
  - Type III secretion system
    - Bacterial flagella protein



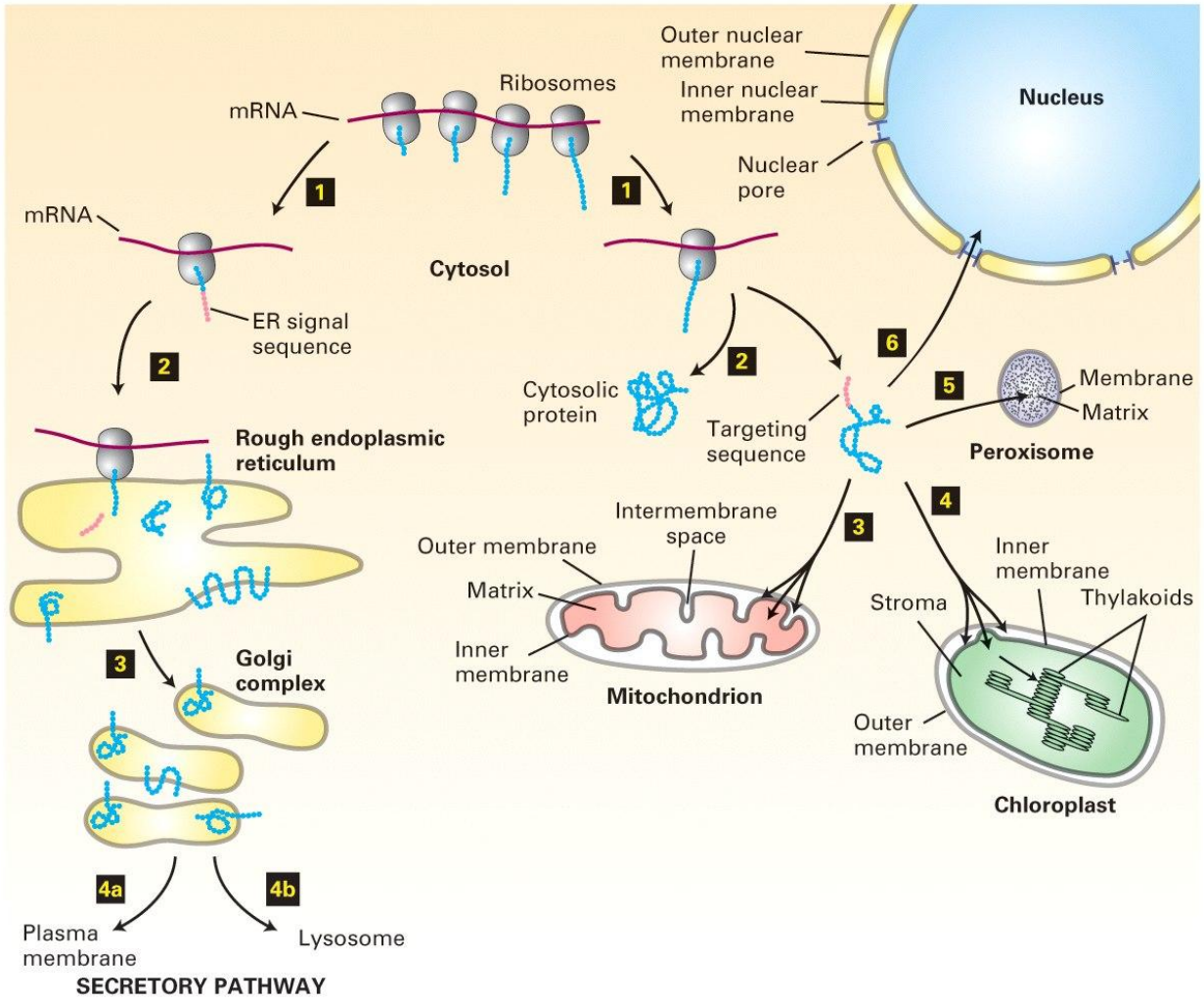
3. Secretion Pathway in Eukaryotes

Protein Targeting in Eukaryotes

- Protein Targeting to specific compartment (ER, Nucleus, Mitochondria) is guided by **signal peptide (tags)**

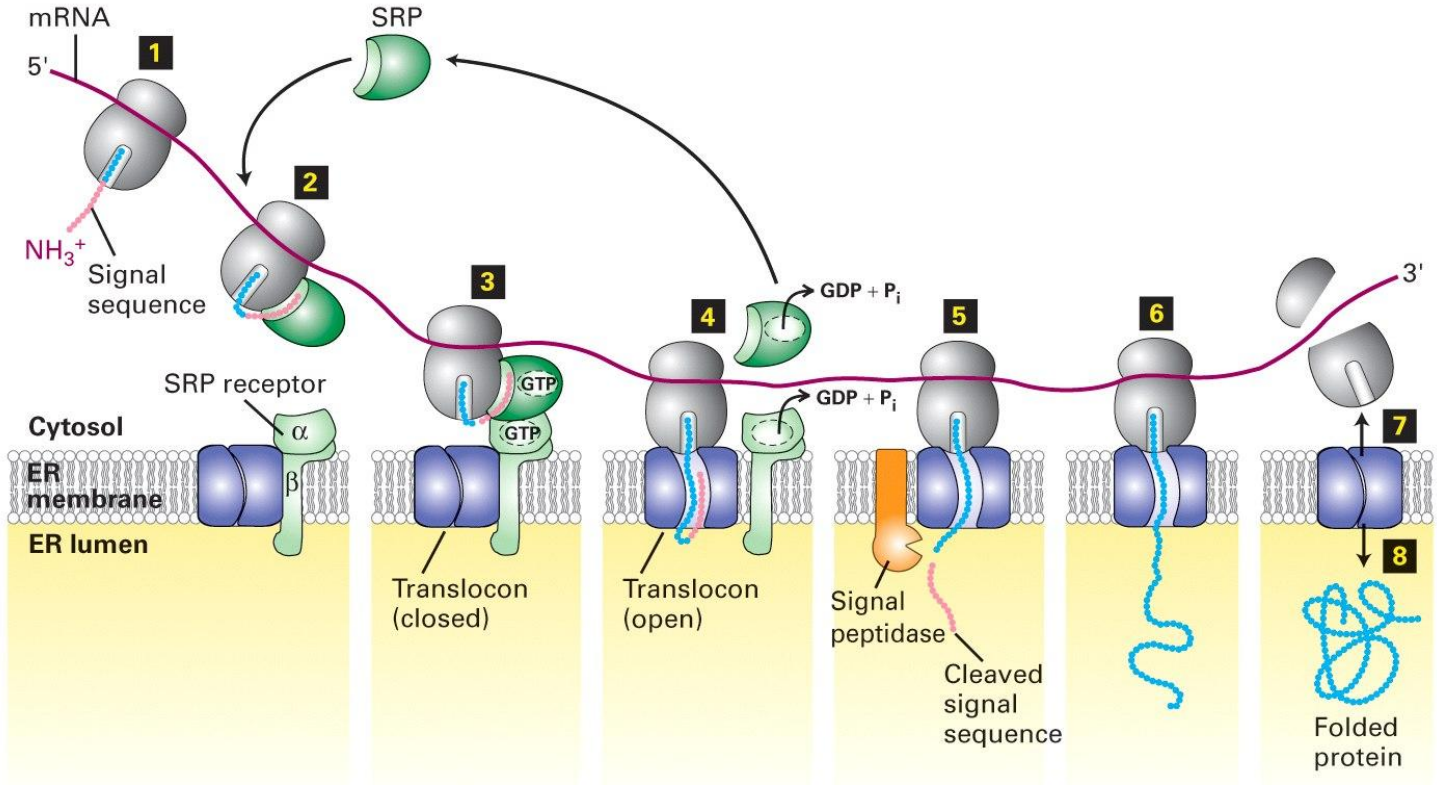


Overview of Protein Sorting Pathway

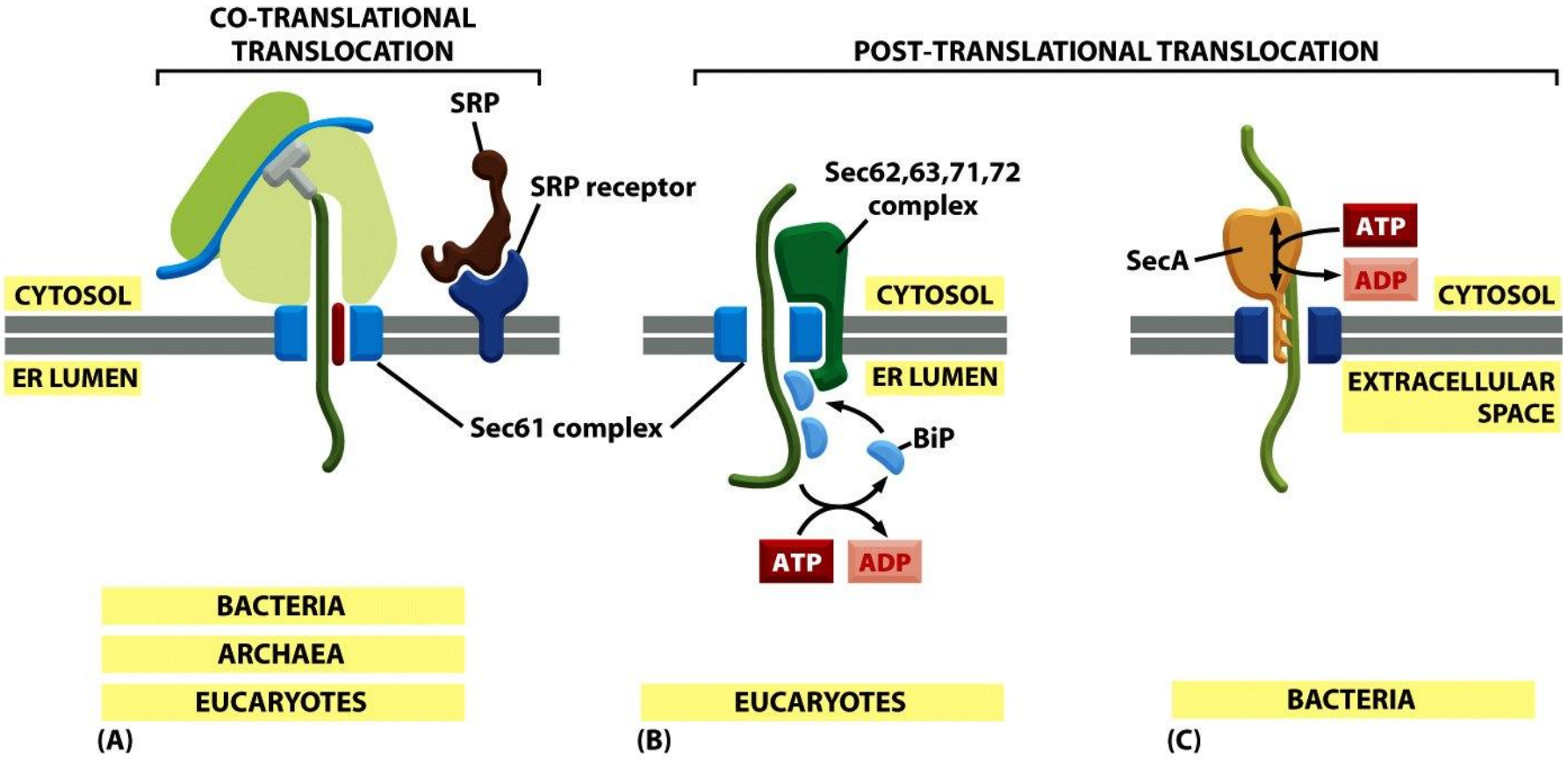


Translocation of Secretory Proteins into ER

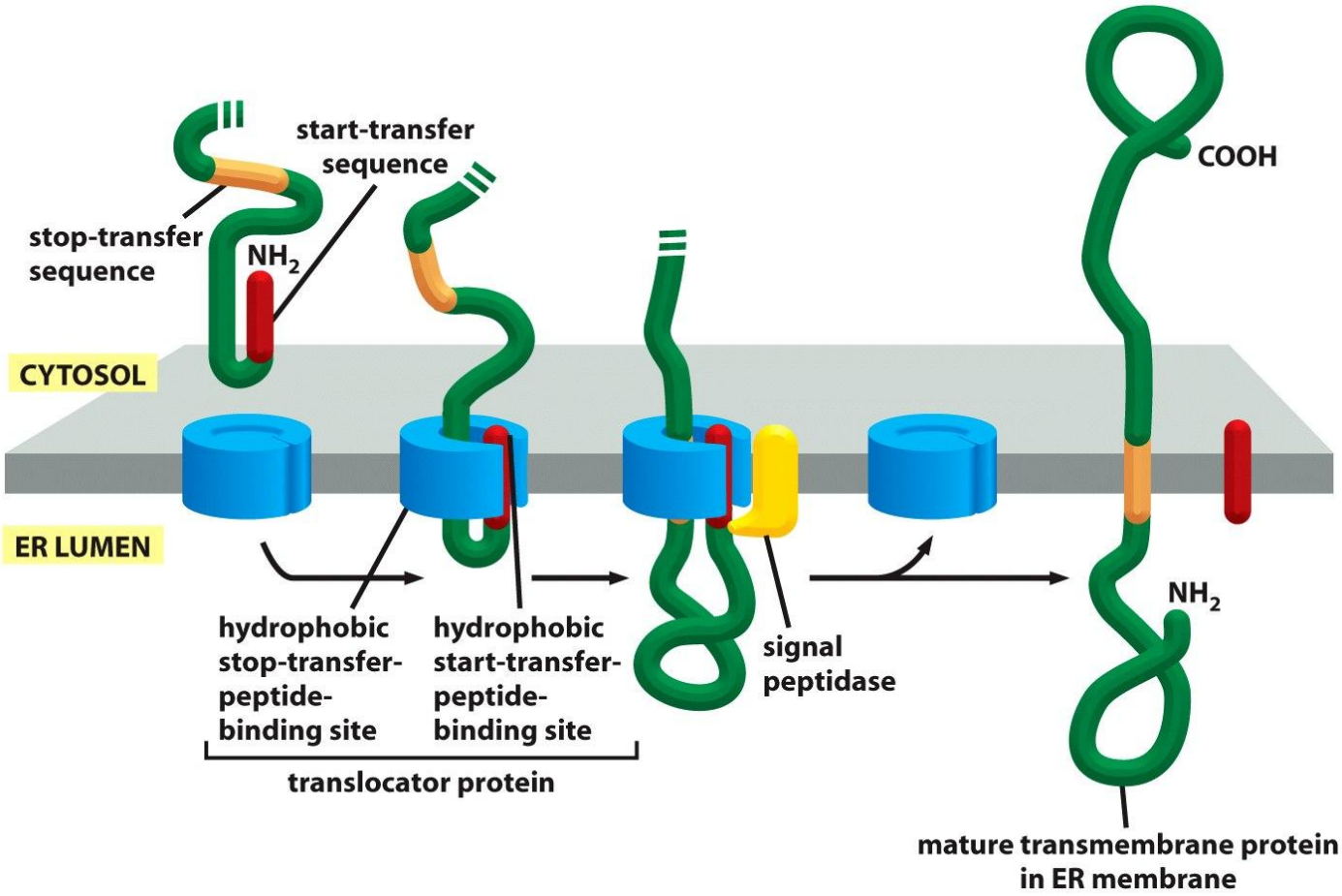
- **Signal sequence**
  - N-terminal hydrophobic 16 to 30 residues
  - Direct the nascent proteins to ER
  - Binds to SRP (signal recognition particle)



Mechanisms of Protein Translocation

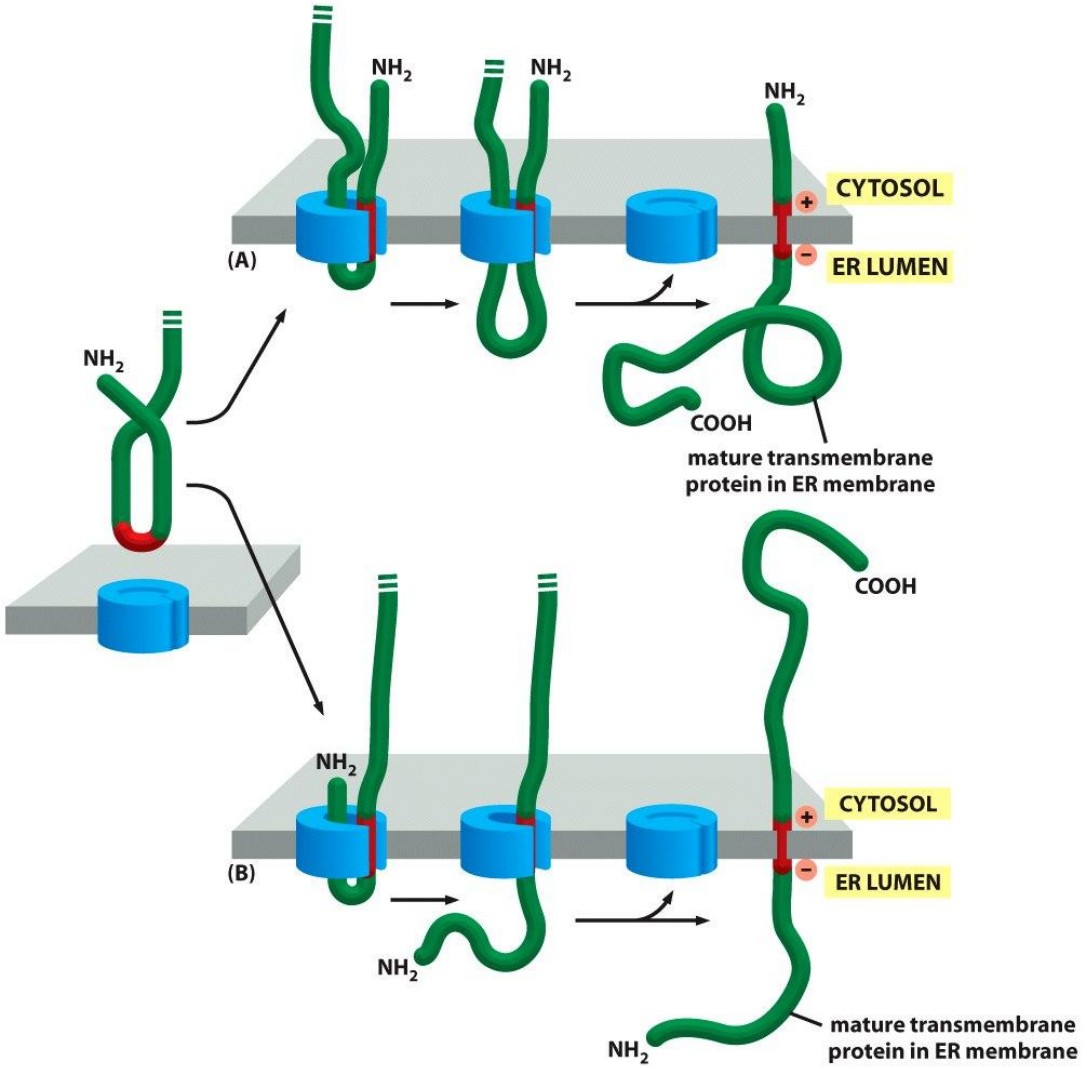


Single-Pass Transmembrane Protein with Start and Stop Transfer Sequence

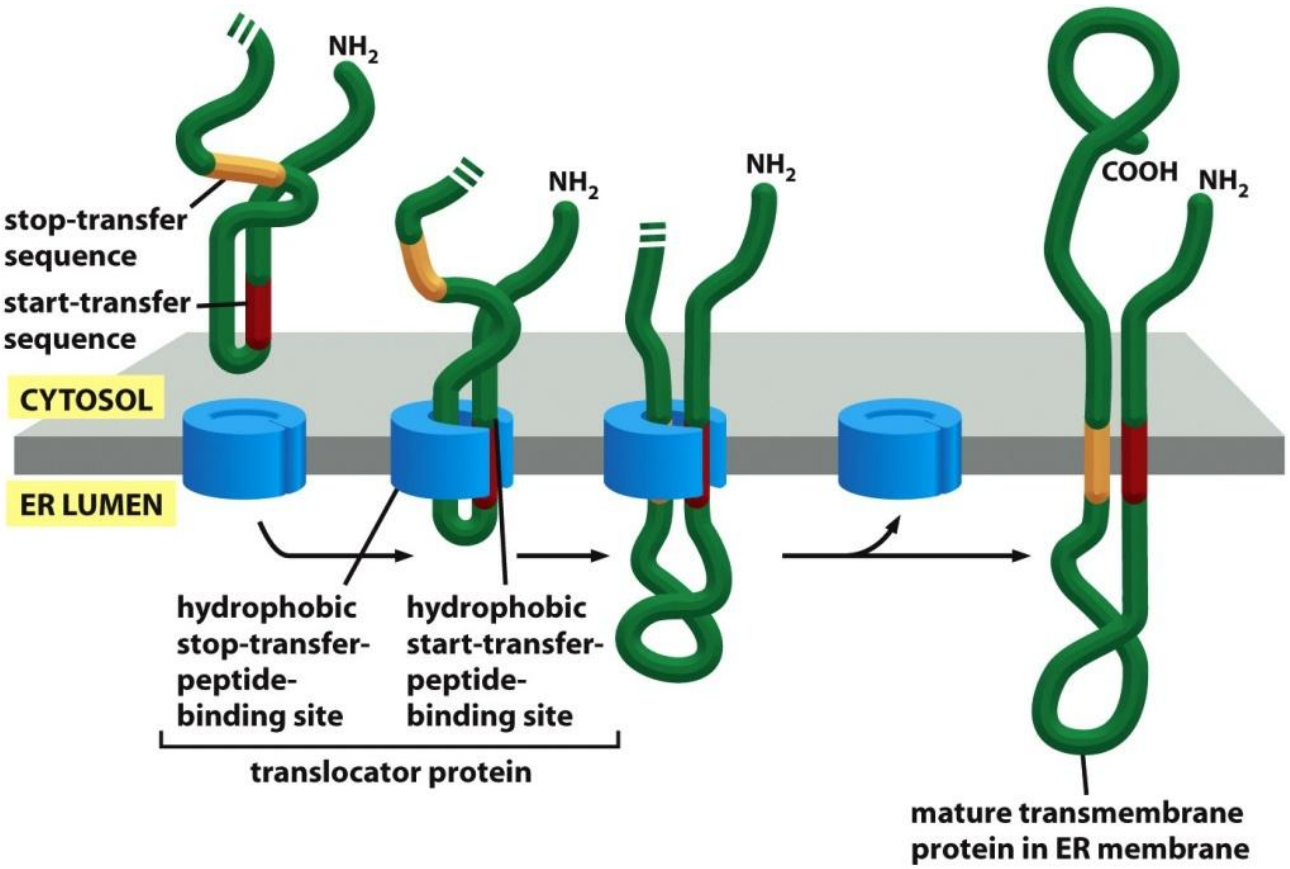


Single-Pass Transmembrane Protein with Internal Signal Sequence

- Location of + charged region relative to the signal sequence determines the orientation of the protein

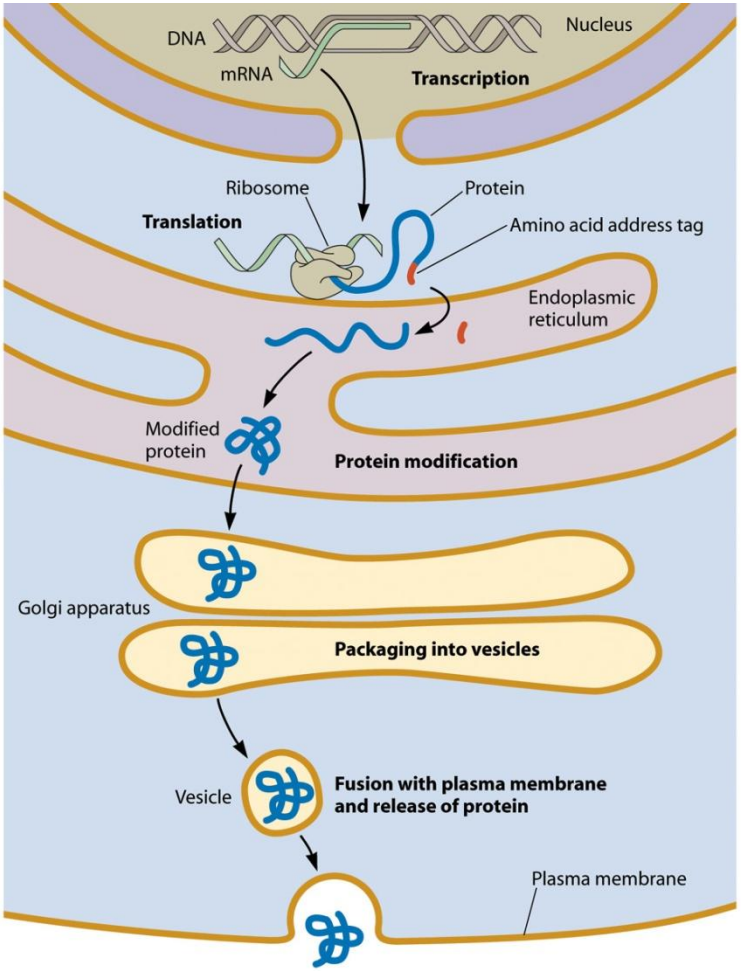


Double Transmembrane Protein



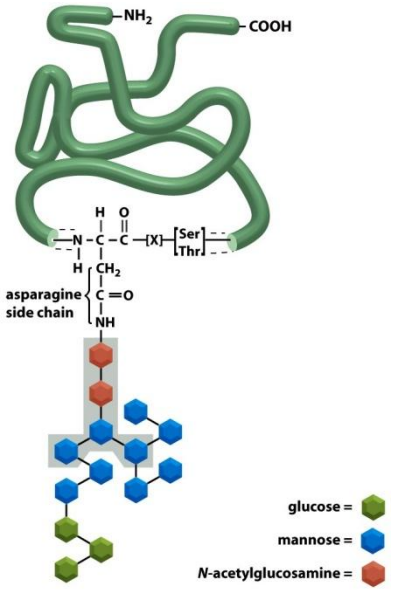
Protein Secretion

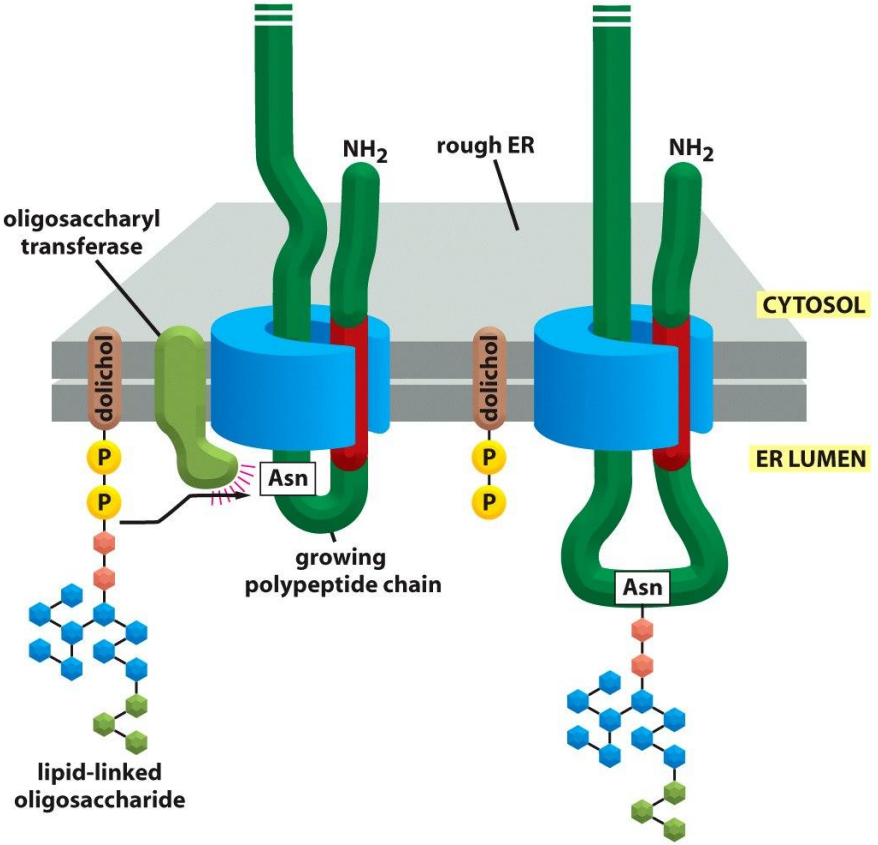
- ER lumen
  - Protein folding
  - Glycosylation
- Golgi apparatus
  - Budding off from ER and fusion with cis Golgi
  - Trans Golgi
  - Posttranslational modification
- Secretory vesicle
- Fusion with the membrane



Glycosylation

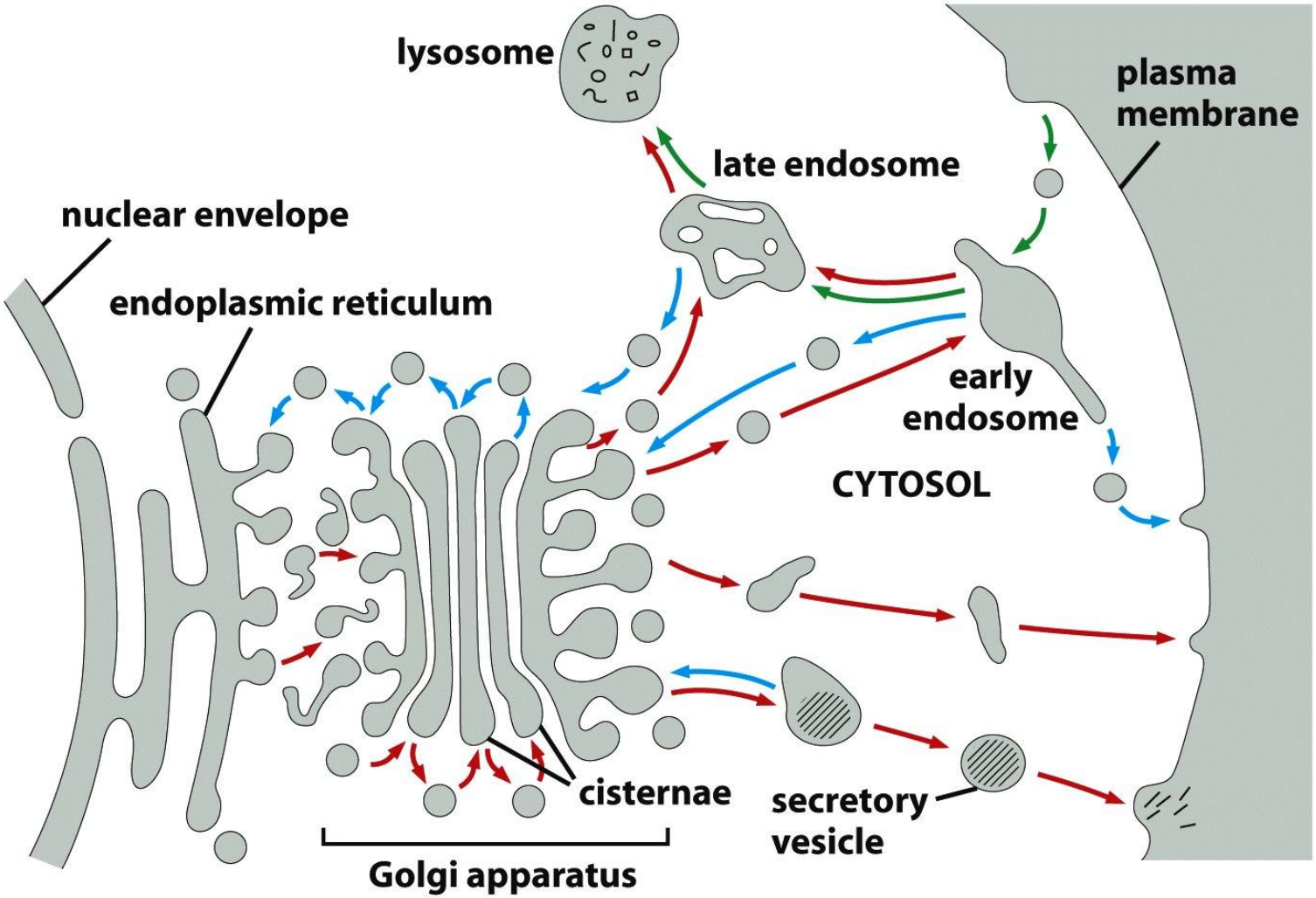
- N-linked glycosylation
  - In ER lumen
  - 90% of glycoproteins



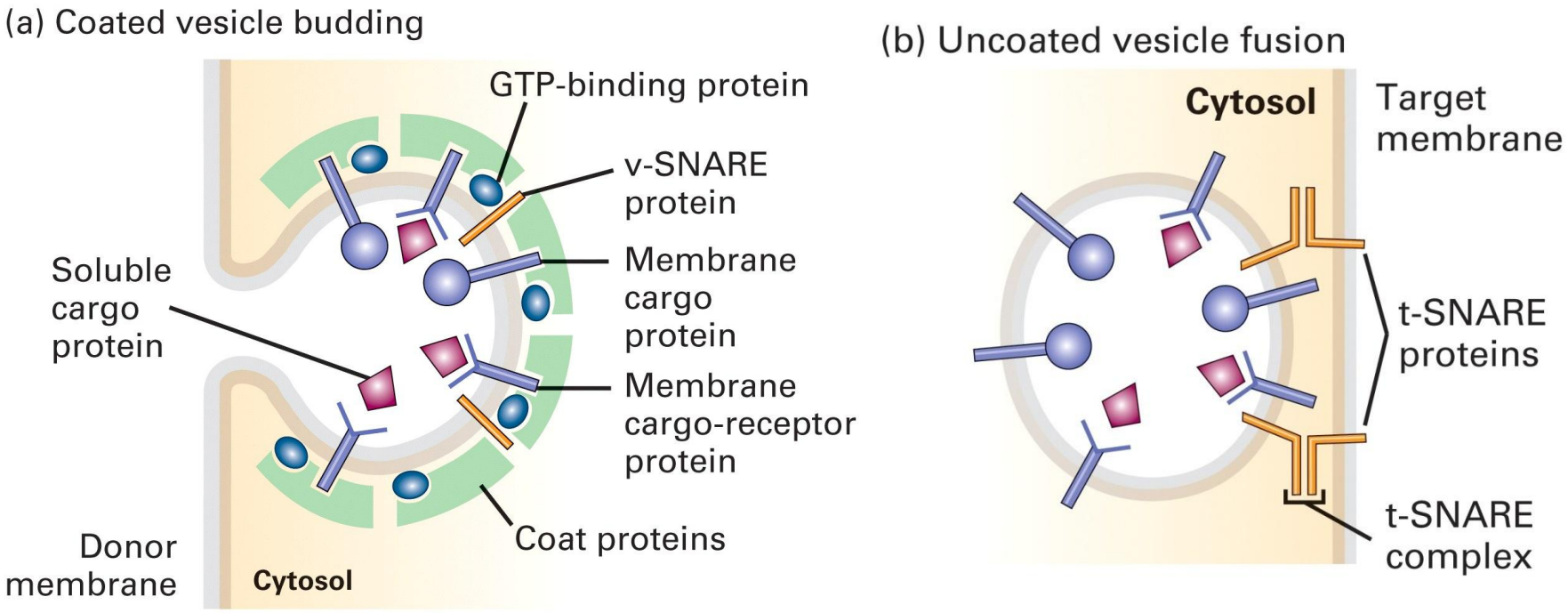


- O-linked glycosylation
  - In Golgi
  - Ser, Thr, hydroxylysine

Secretory and Enodocytic Pathway



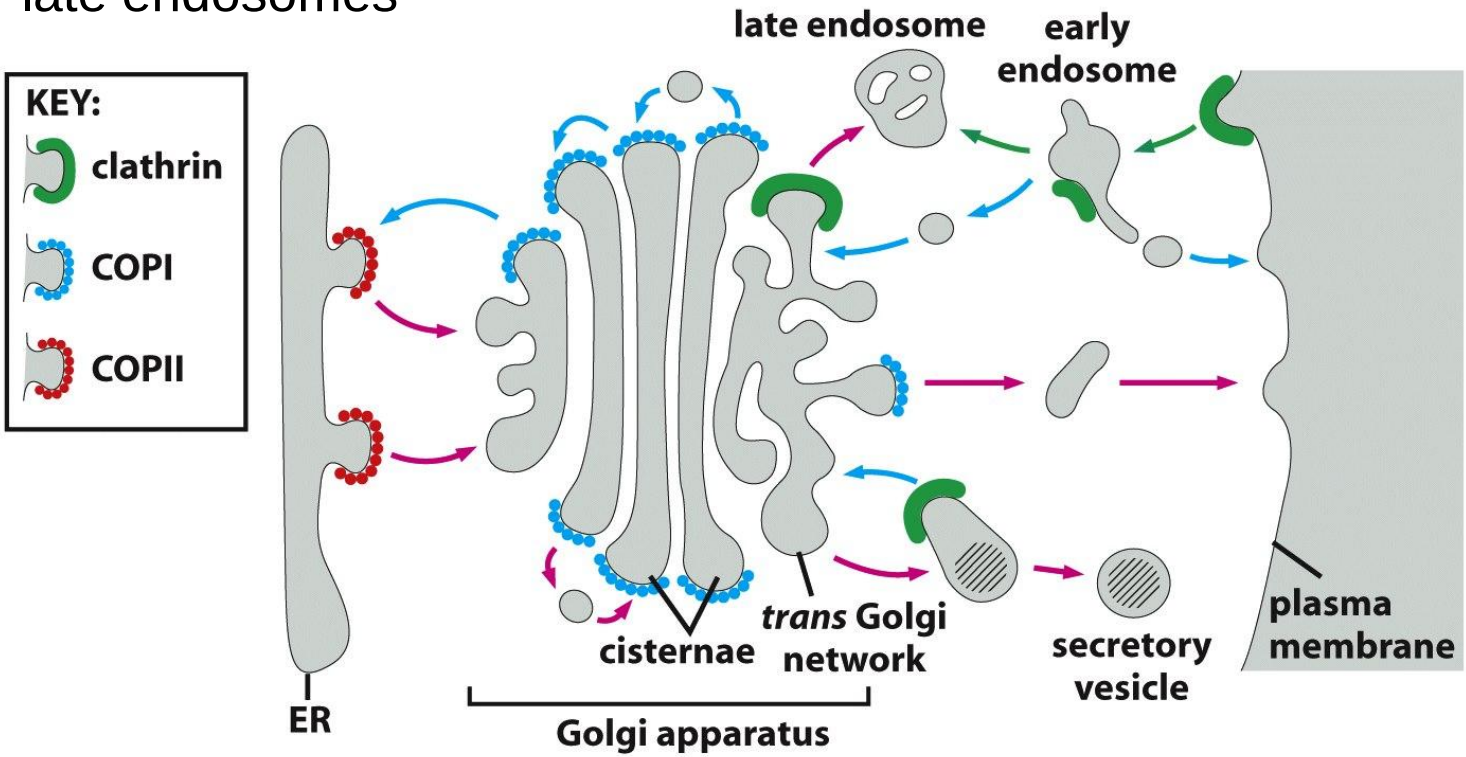
Vesicle Budding and Fusion



- Coat proteins: bind to cytosolic domain of cargo proteins
  → Removed after budding
- Fusion of membranes by joining of v-SNARE with t-SNARE

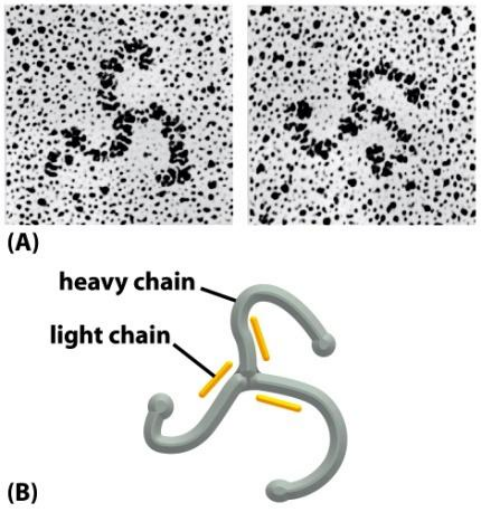
Coated Vesicles in Vesicular Traffic

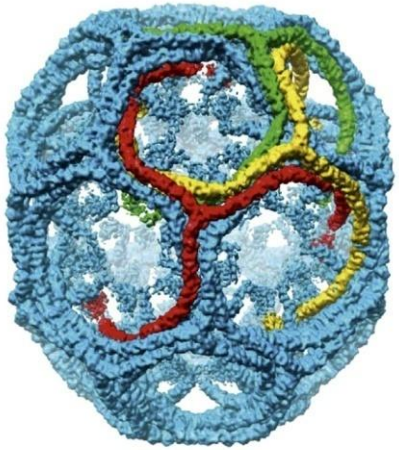
- COPII : from ER to Golgi
- COP I: Retrograde between Golgi cisternae, and from the cis Golgi to the rough ER
- Clathrin: From the plasma membrane and trans-Golgi network to late endosomes

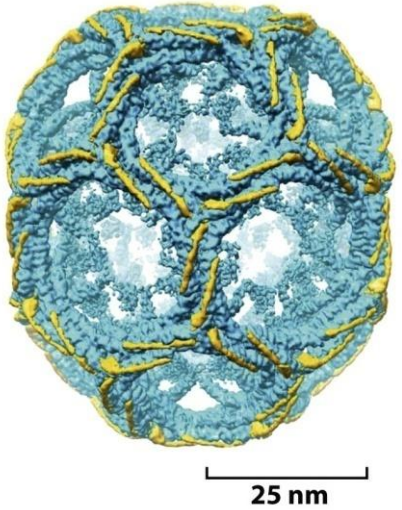


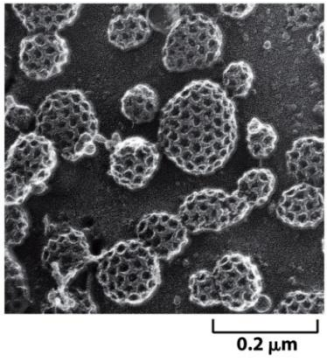
Clathrin-Coated Vesicles

- Outer layer: clathrin
  - Triskelions
  - 3 Heavy chain (180 kDa) and 3 light chain (35-40 kDa)
  - Clathin coat contain 36 triskelions
- Inner layer: adapter protein (AP) complex
  - Determine specificity of cargo proteins

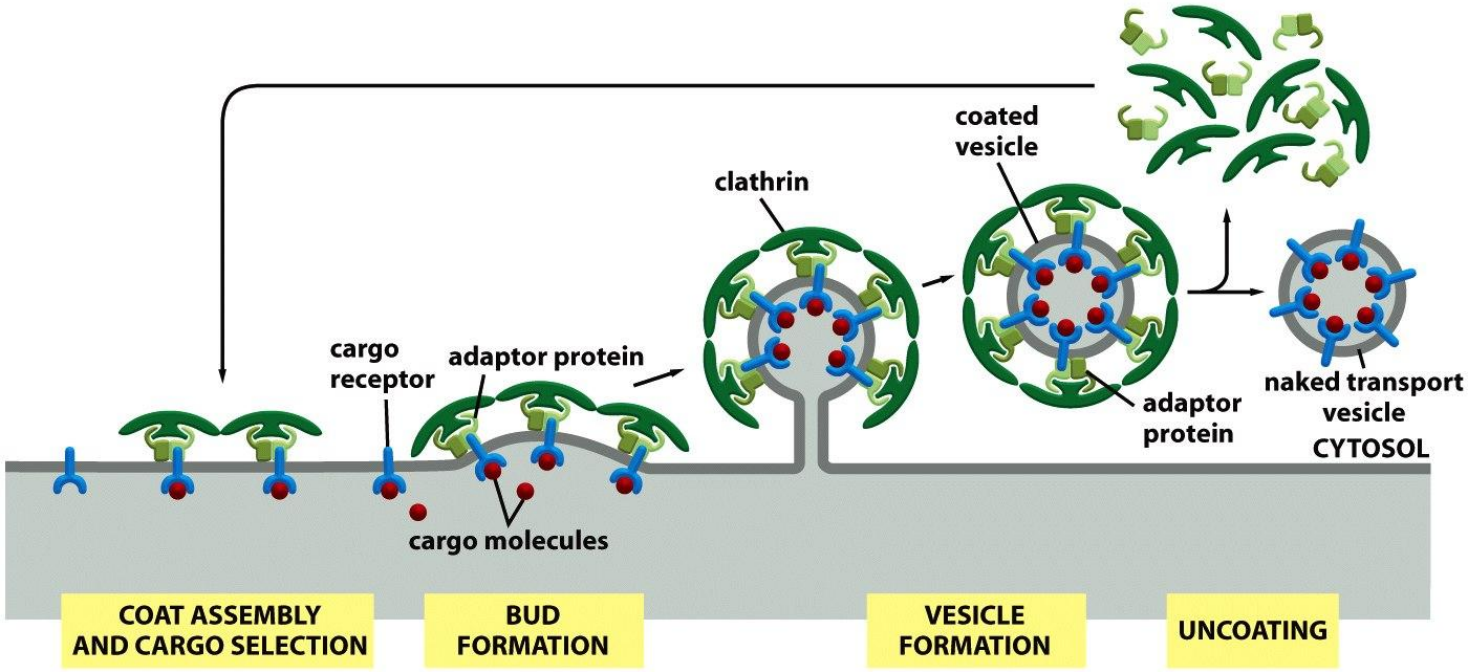


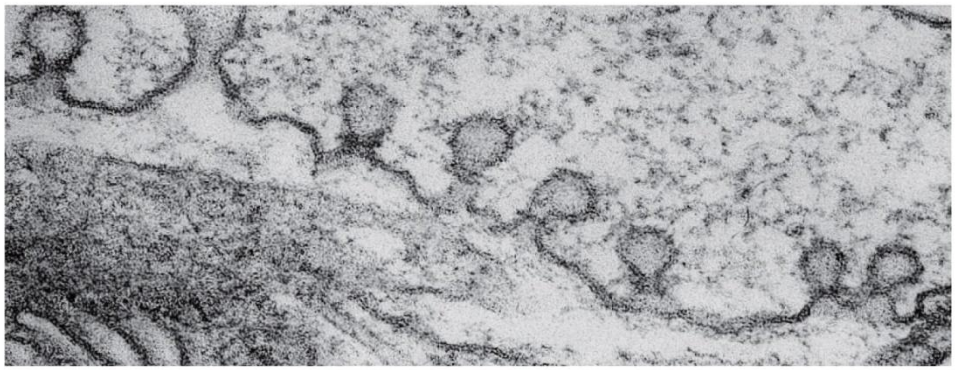




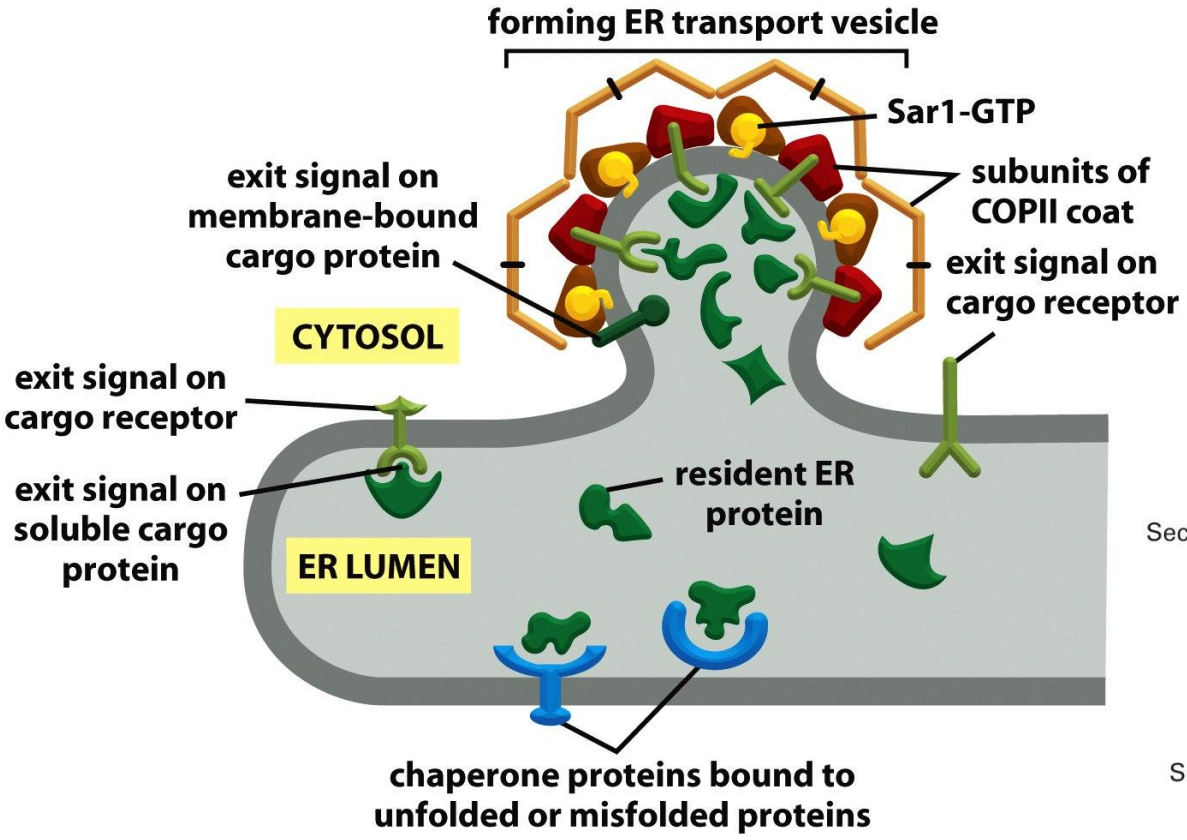


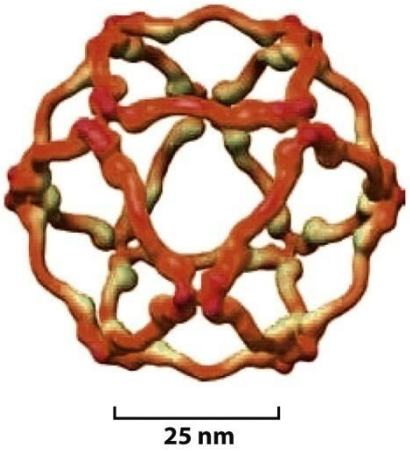
Formation of Clathrin Coated Vesicles

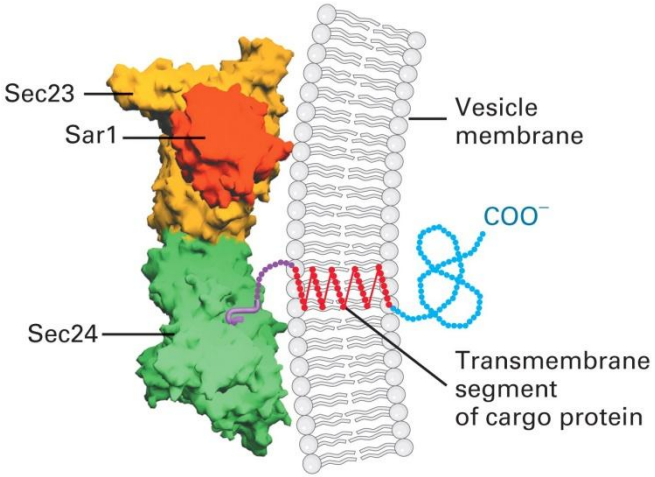




Formation of COPII Coated Vesicles

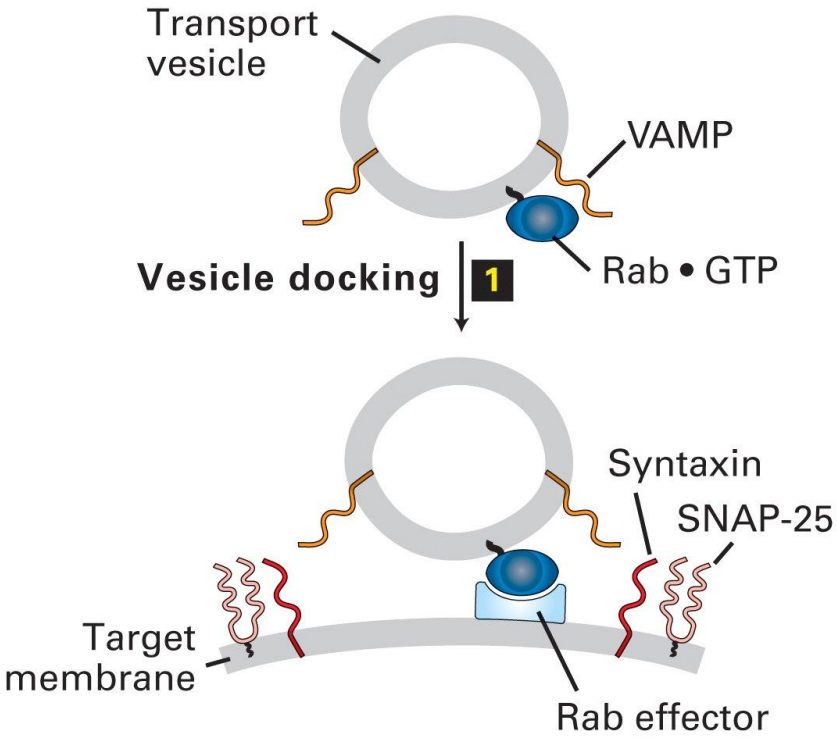






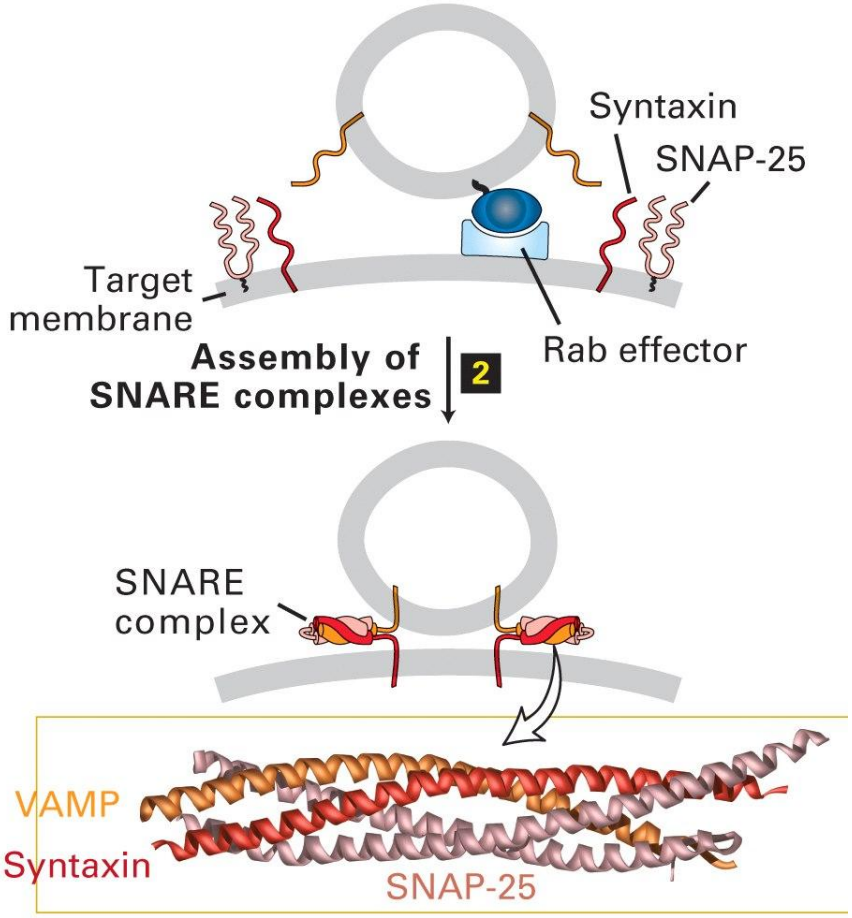
Docking of Transport Vesicles with Target Membranes

- GTP bound Rab
- Different Rab proteins depending on the vesicles
  - Interact with transport vesicle via a lipid anchor
  - Binds to an effector protein on target membrane

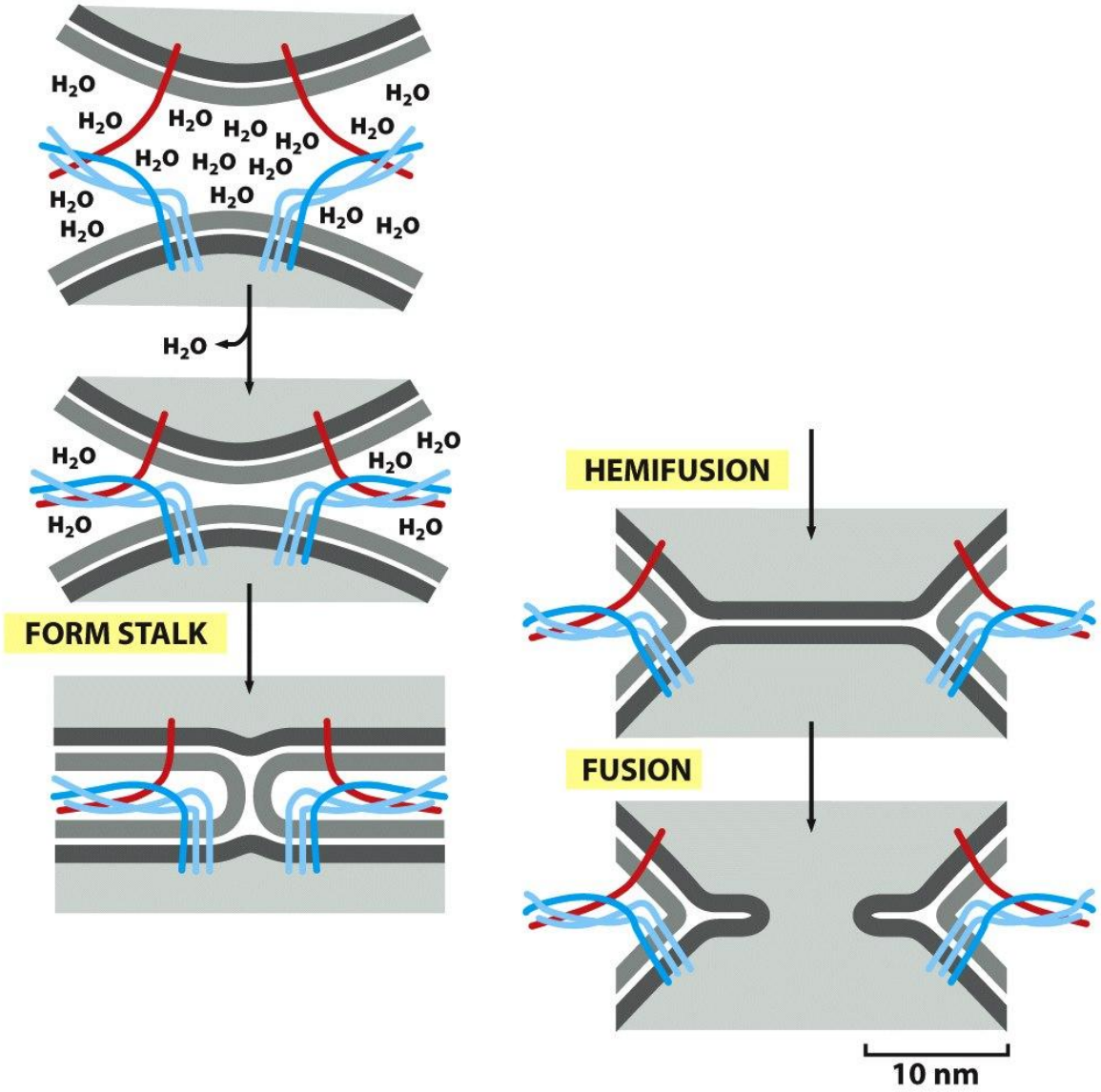


Fusion of Transport Vesicles with Target Membranes

- Assembly of SNARE complex
  - v-SNARE (VAMP)
  - t-SNARE (synthaxin)
  - SNAP-25
- Forms 4 a helices
- Membrane fusion after SNARE complex formation

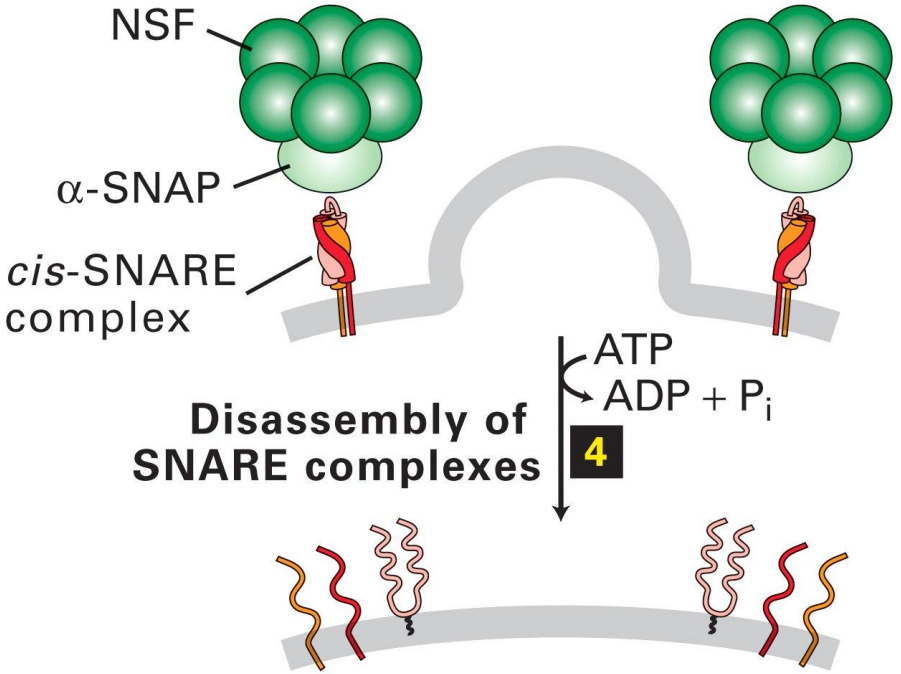


Model for SNARE-Mediated Membrane Fusion



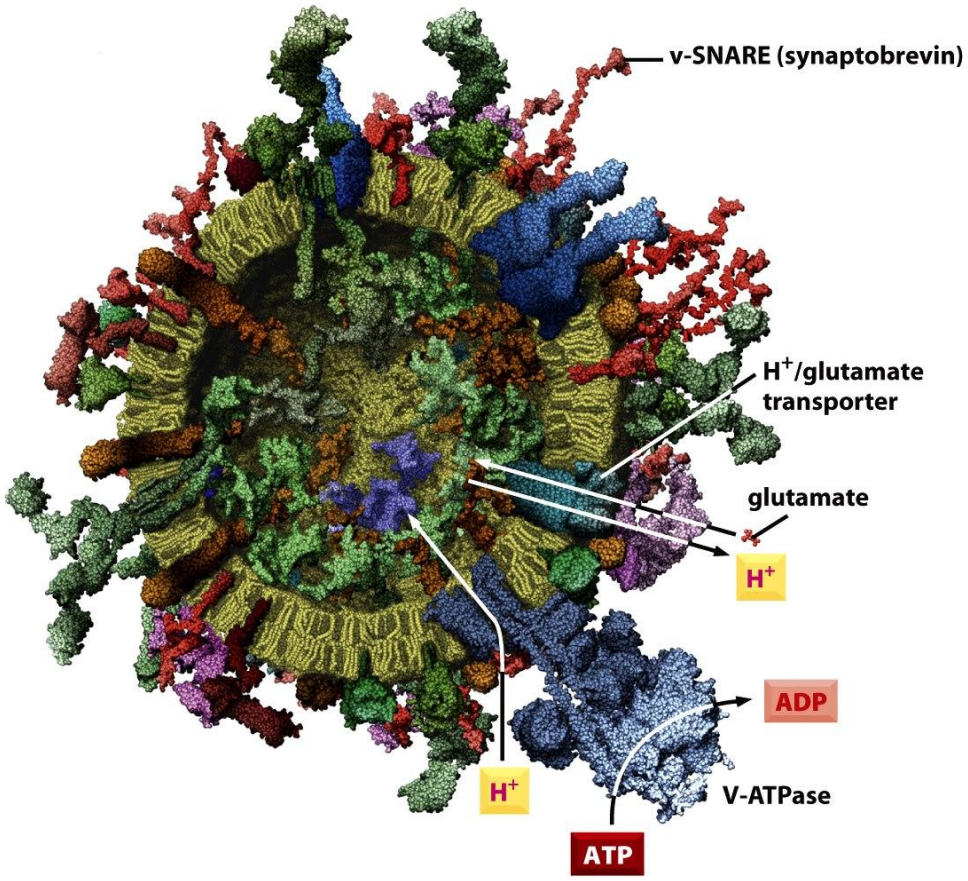
Dissociation of SNARE Complex after Fusion

- NSF with a-SNAP (soluble NSF-associated protein)
- Dissociate SNARE complex by ATP hydrolysis



Types of Secretion

- Constitutive secretion
  - Collagen from fibroblast
  - Serum protein from hepatocytes
  - Antibody from B cells
- Regulated secretion
  - Peptide hormones from endocrine cells
  - Precursors of digestive enzymes
  - Neurotransmitters



Synaptic vesicle