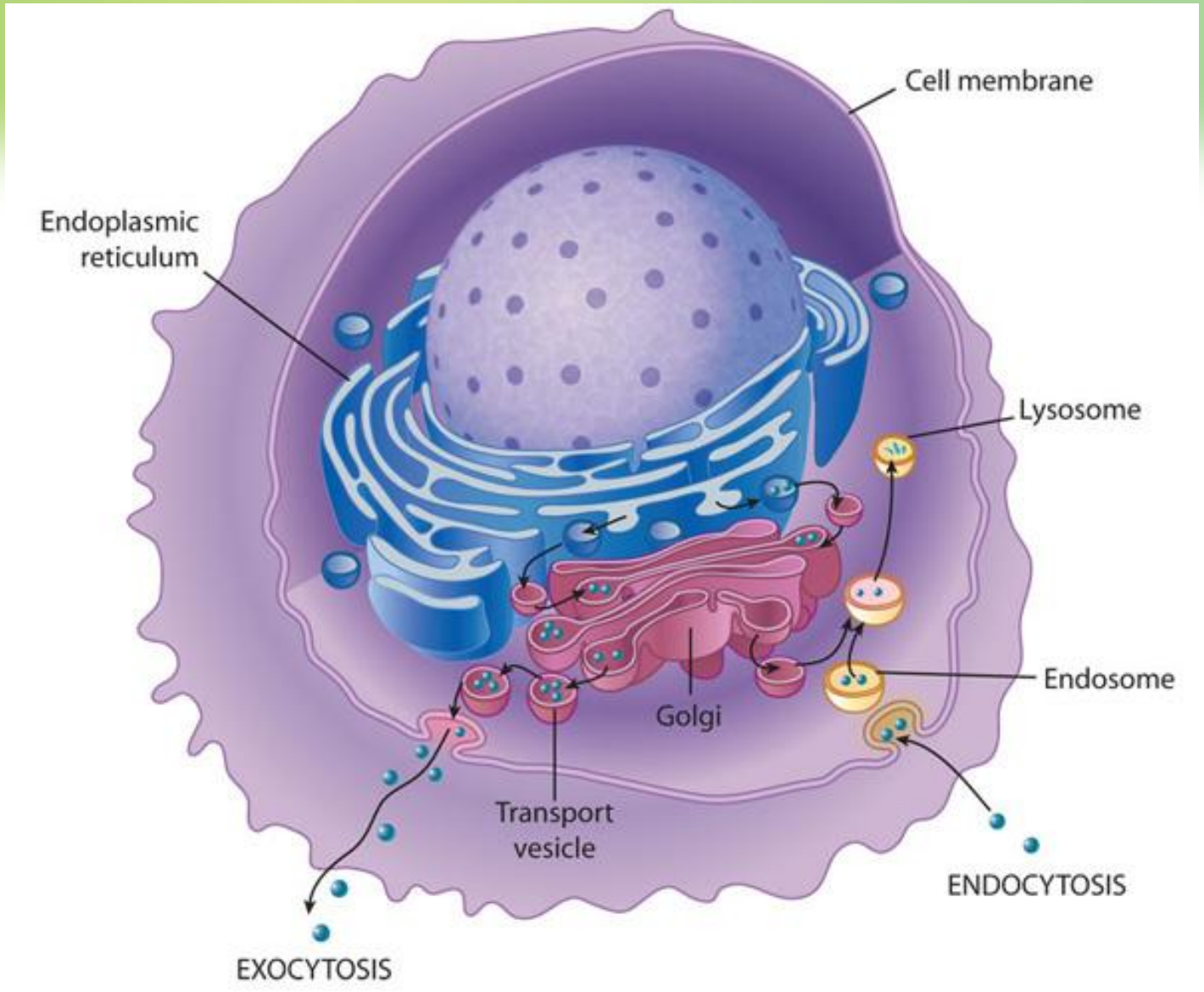
# Lecture 2: Protein sorting (endoplasmic reticulum)

**Dr. Diala Abu-Hsasan**
**School of Medicine**
**dr.abuhassand@gmail.com**

***Principles of Genetics and Molecular Biology***

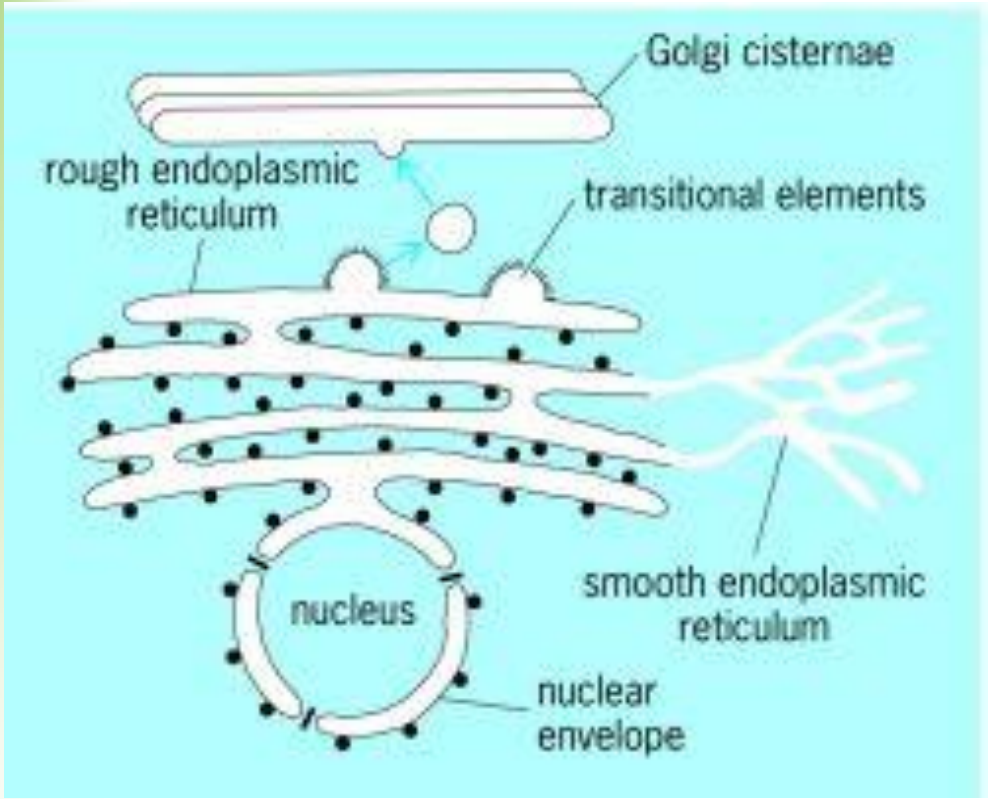
# An overview of cellular components



# Endoplasmic reticulum (ER)

**It is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm.**

**It is the largest organelle of most eukaryotic cells.**

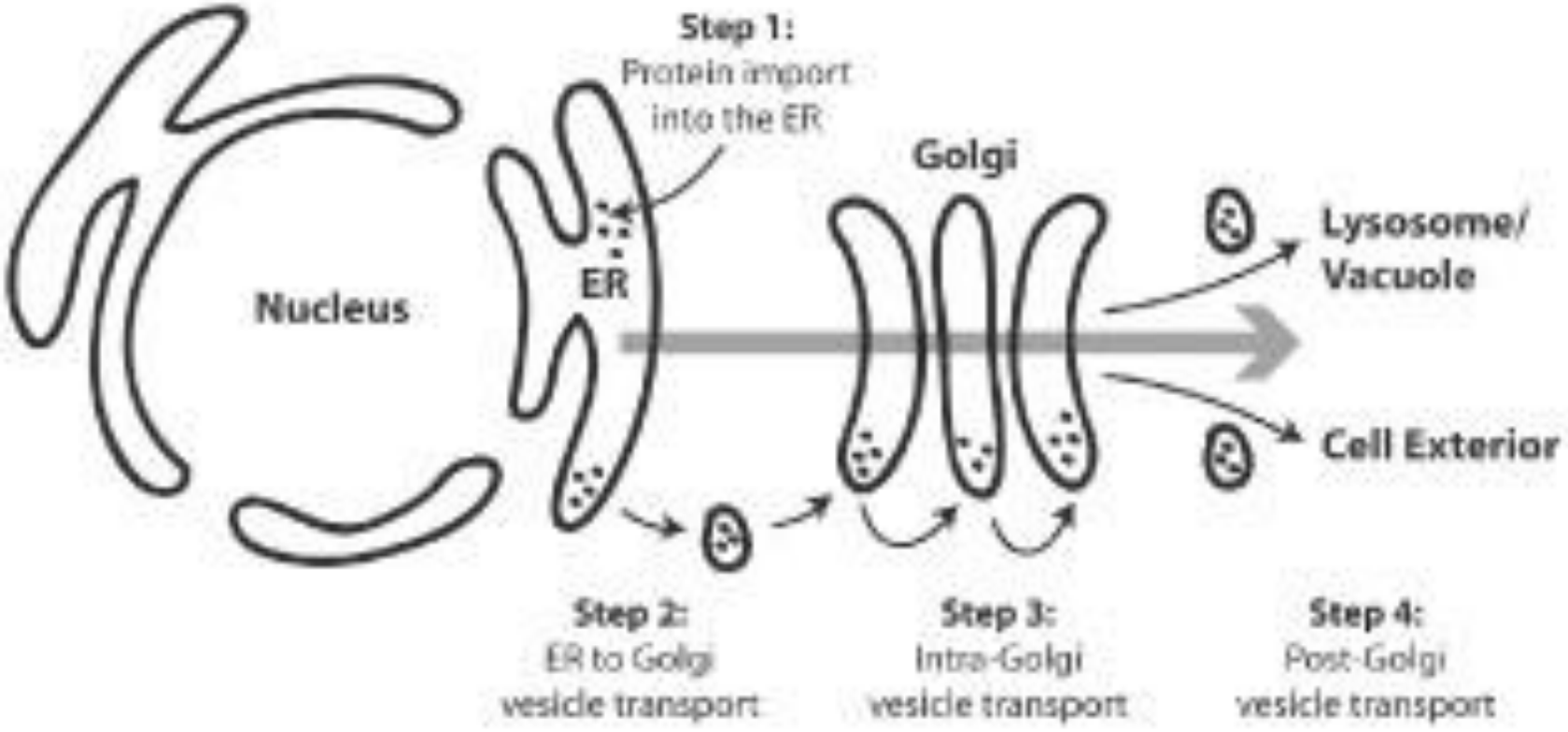



- **The rough ER: covered by ribosomes on its outer surface and functions in protein processing.**
- **The smooth ER: lipid metabolism**
- **Transitional ER: exit of vesicles to Golgi apparatus**

# The secretory pathway

**ER-Golgi- secretory vesicles- cell exterior**

**ER, Golgi apparatus, and lysosomal proteins are initially targeted to the ER.**

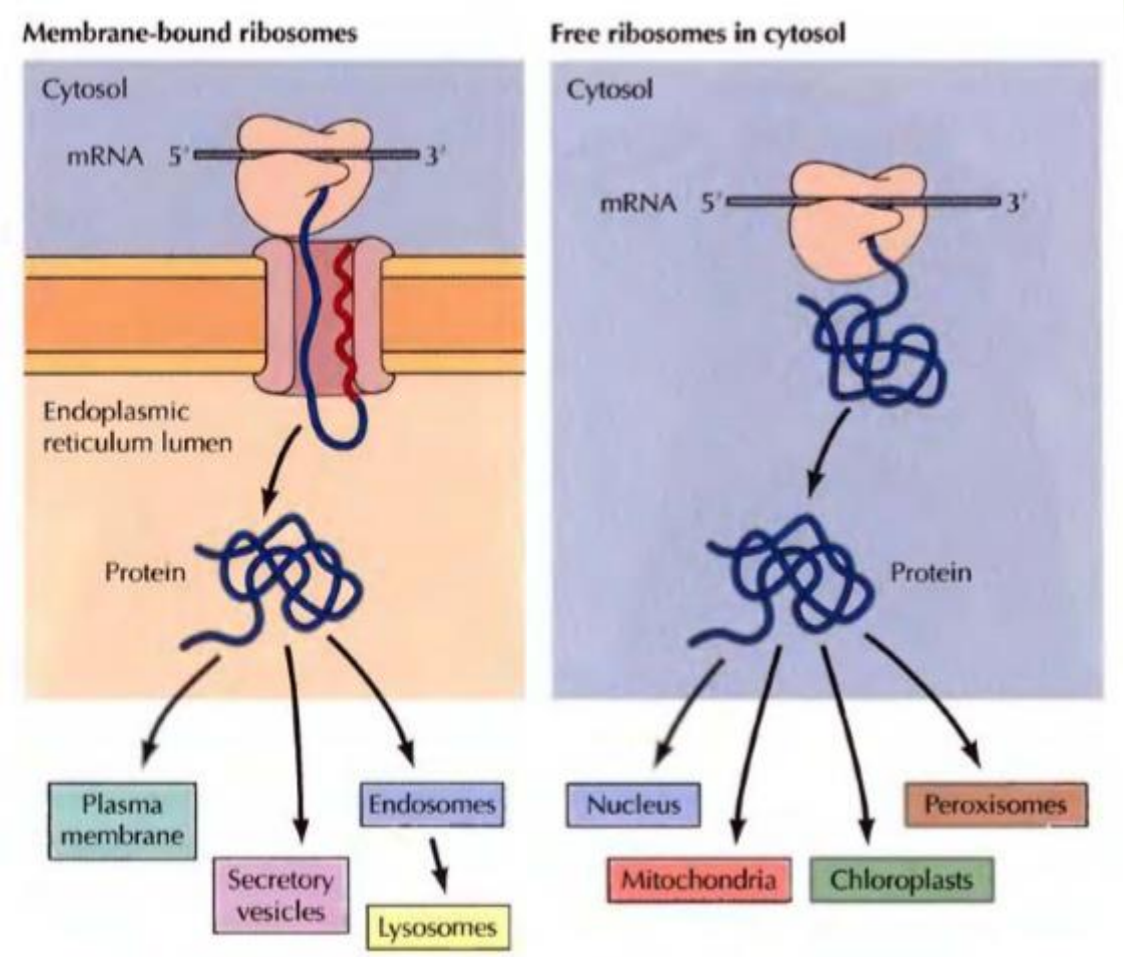


# Pulse chase

**Read 374-375**

# The secretory pathway

**Most proteins are transferred into the ER while they are being translated on membrane-bound ribosomes (co-translational translocation).**



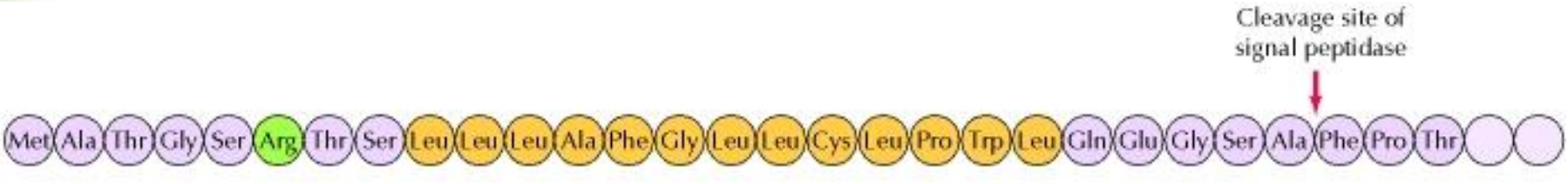

**Cytosolic, interior nuclear, peroxisomal, and mitochondrial proteins are synthesized on free ribosomes and released into the cytosol after translation is complete**

# Ribosomal and protein targeting

**All protein synthesis initiates on ribosomes that are free in the cytosol.**

**Ribosomes are targeted for binding to the ER membrane by the amino acid sequence of the polypeptide at the N-terminus called a signal sequence.**

**Signal sequence is a short stretch of hydrophobic amino acids that are then cleaved from the polypeptide chain during its transfer into the ER lumen.**



# Resources

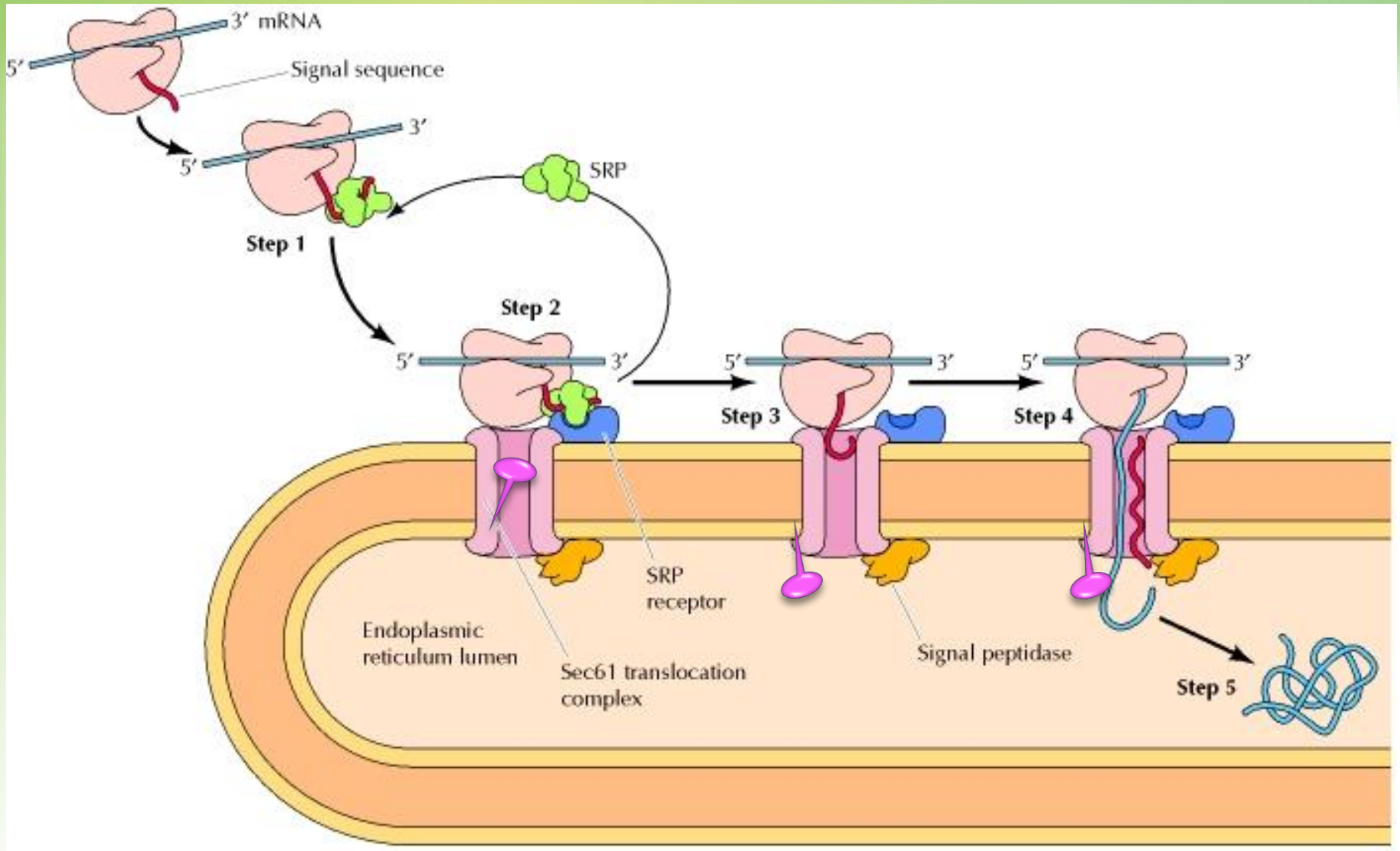
**Translocation Animations**

http://www.ohsu.edu/research/skachlab/animations.shtml

http://biochem.web.utah.edu/iwasa/projects/HMS/translocation/downloads/posttranslational_prok.mov

http://biochem.web.utah.edu/iwasa/projects/HMS/translocation/downloads/posttranslational_prok.mov

# Co-translational Translocation of polypeptides to ER



# Mechanism of translocation

**Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).**

**Step 2: The SRP inhibits translation and escorts the complex to the ER membrane, where it binds to the SRP receptor.**

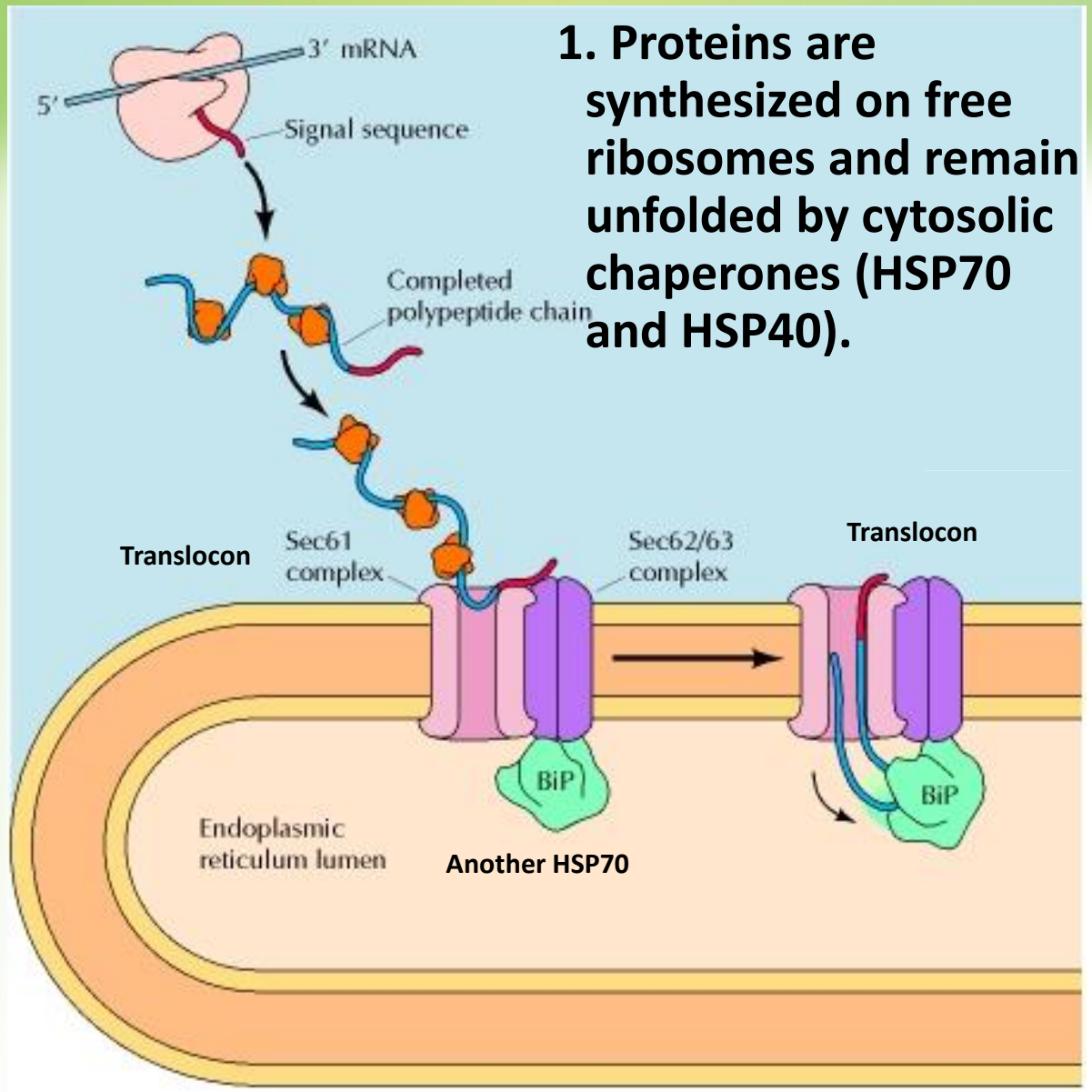
**Step 3: The SRP is released, the ribosome binds to a translocon protein (Sec61 proteins), and the signal sequence is inserted into a membrane channel.**

**Step 4: Translation resumes, and the growing polypeptide chain is translocated across the membrane.**

**Step 5: Cleavage of the signal sequence by signal peptidase releases the polypeptide into the lumen of the ER.**


stylusvisuals

# Posttranslational translocation




**1. Proteins are synthesized on free ribosomes and remain unfolded by cytosolic chaperones (HSP70 and HSP40).**

**2.Their signal sequences are recognized by a protein complex (Sec62/63), which is associated with the translocon in the ER membrane.**

**3. The protein complex is also associated with a chaperone protein (BiP), which pulls protein through the channel.**

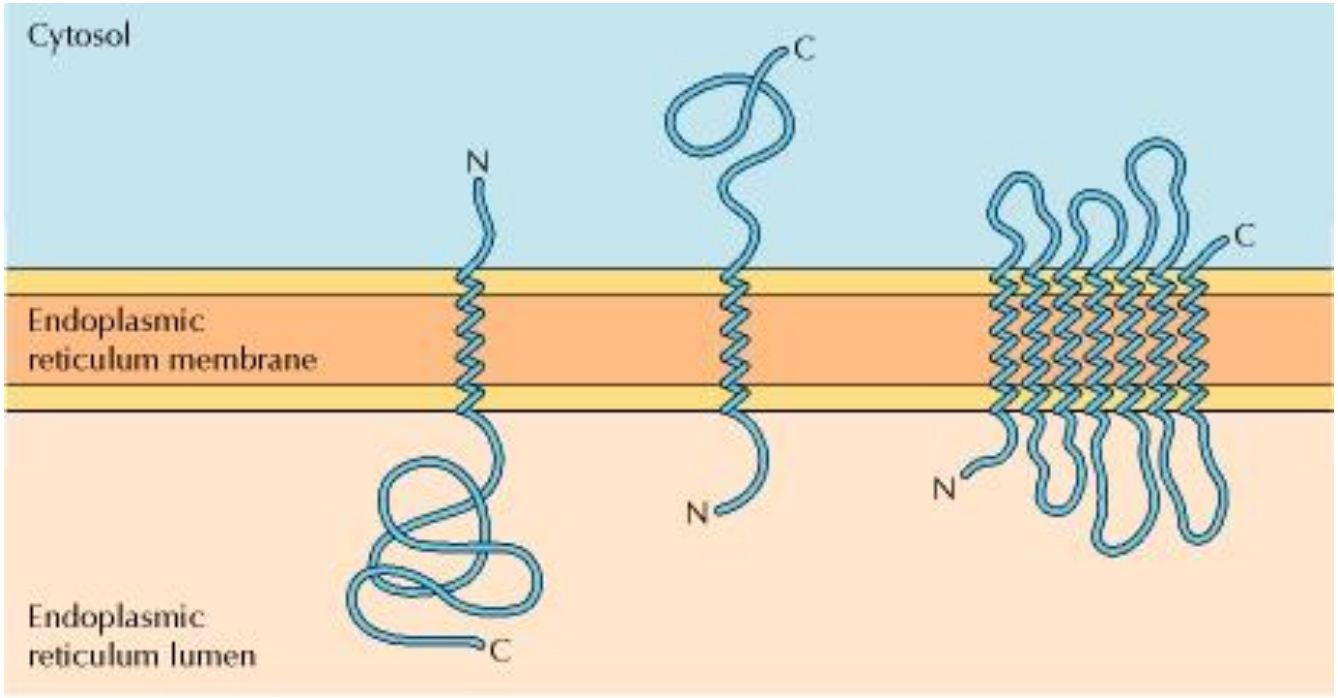
# Insertion of proteins into the ER membrane

**Secretory, ER, Golgi apparatus, and lysosomal proteins are released into the lumen of the ER.**

**Membrane proteins are initially inserted into the ER membrane.**

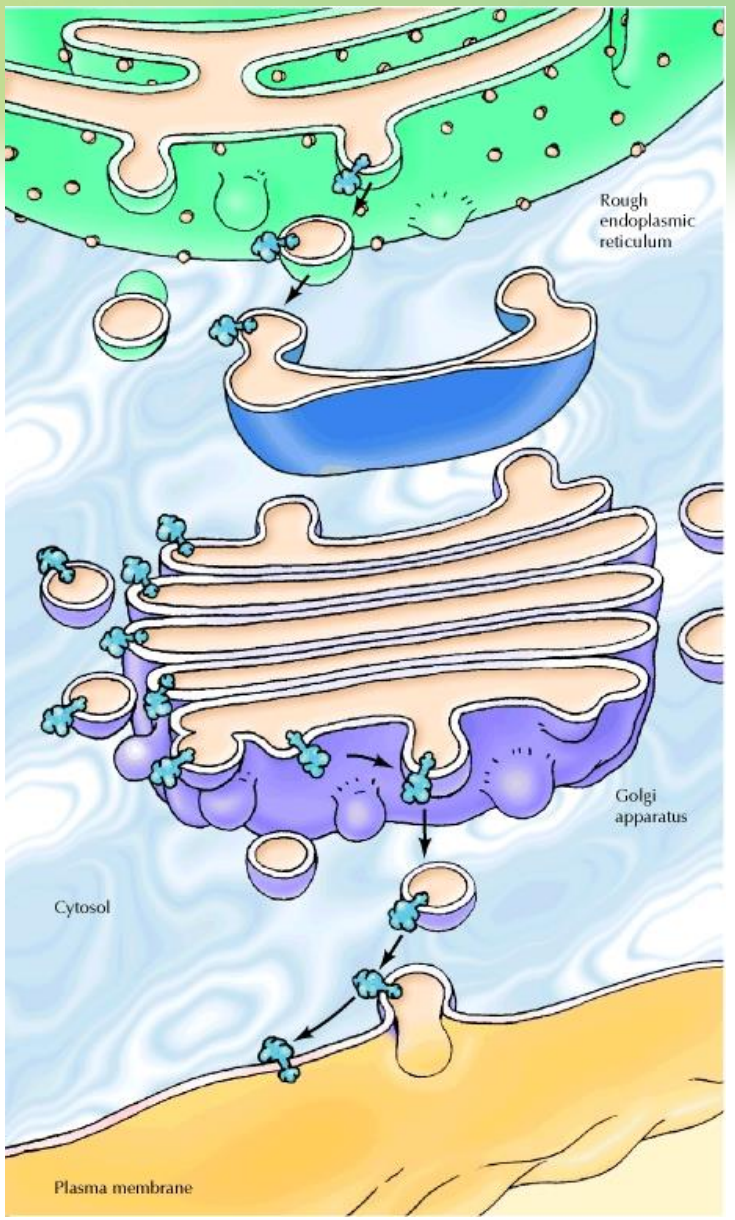
**Factors that affect protein insertion into the ER membrane:**

1. **Single vs. multiple membrane spanning region**
2. **Orientation of N- and C-termini**

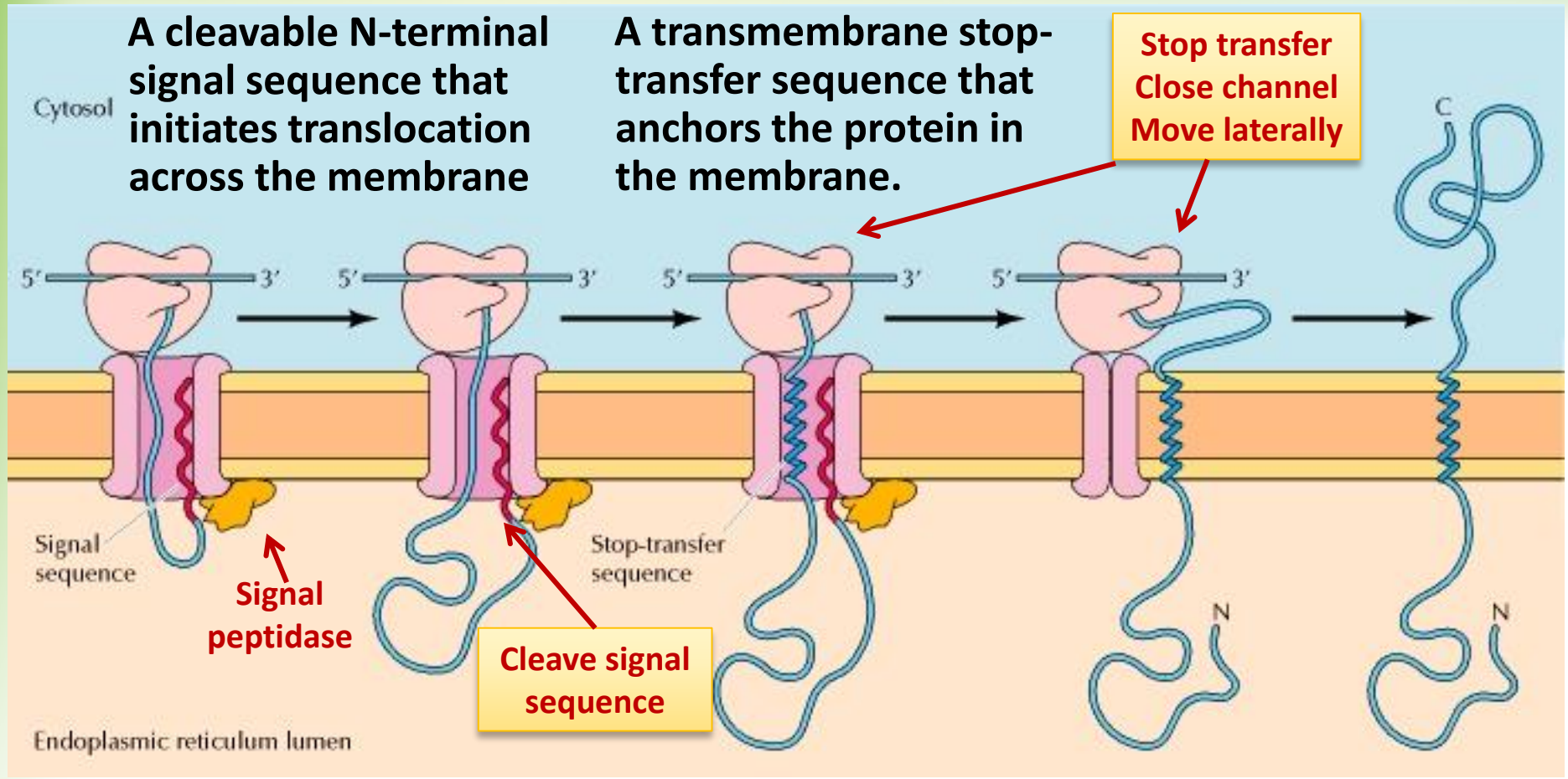


# Membrane protein orientation

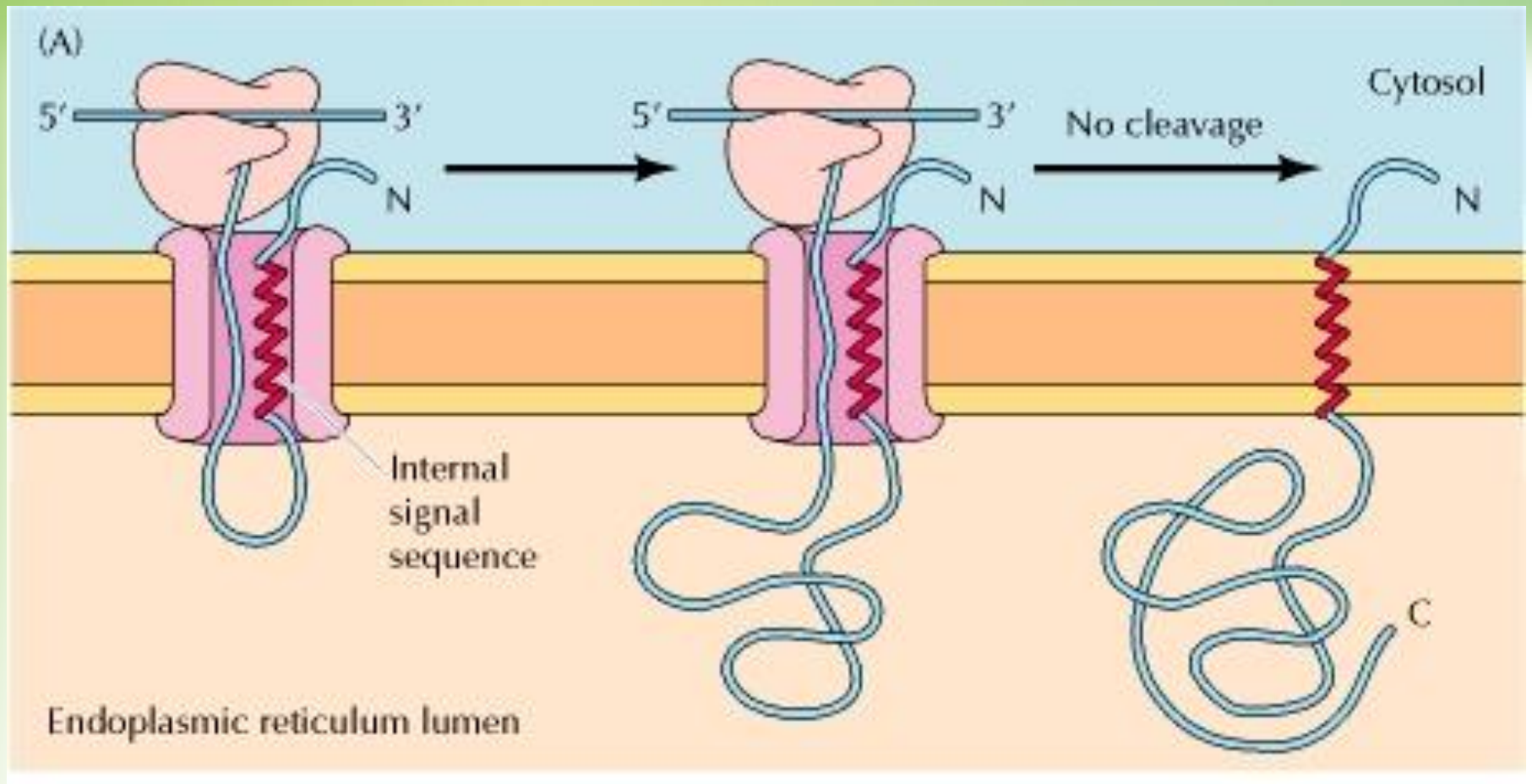
**The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.**



# Case 1: Insertion of membrane proteins N-terminus in and C-terminus out

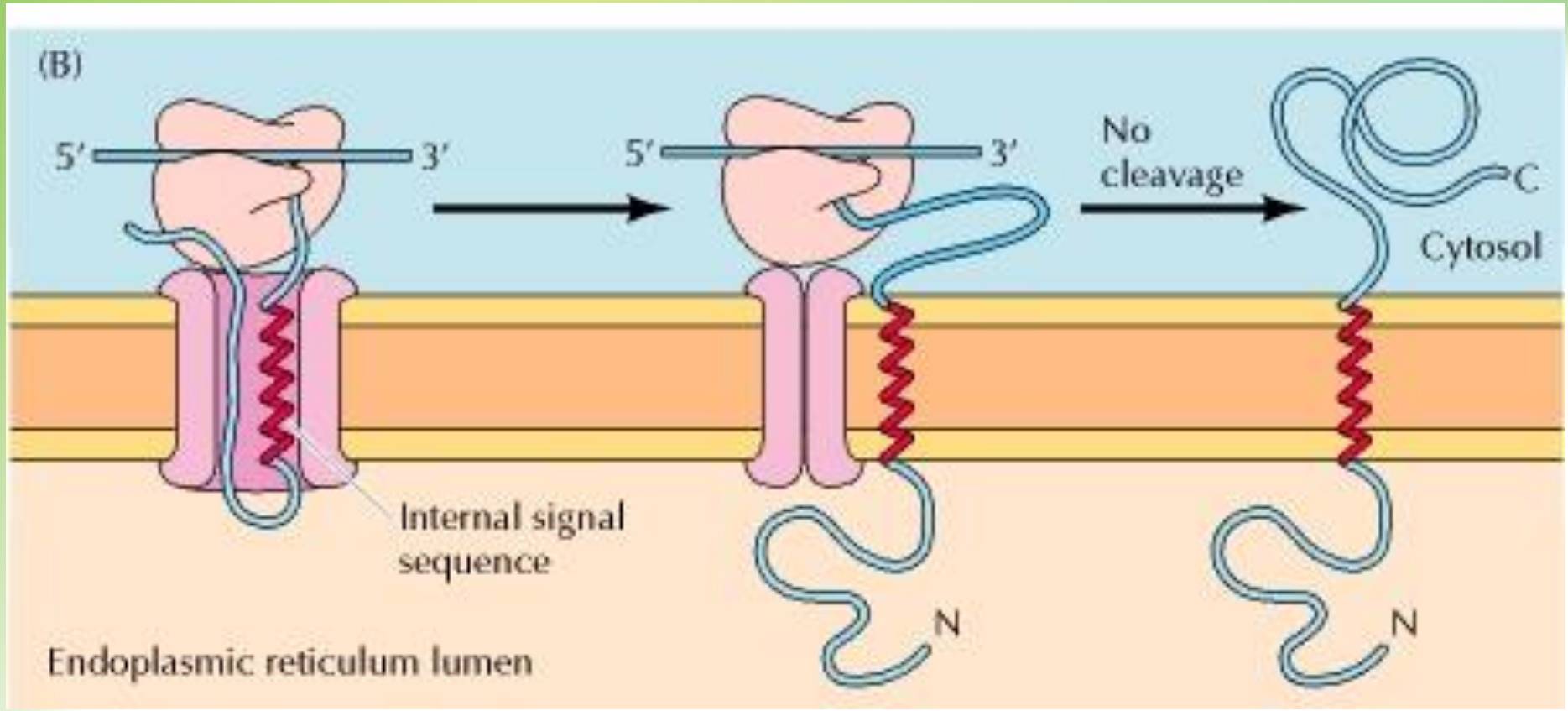


# Case 2a: Insertion of membrane proteins C-terminus in and N-terminus out

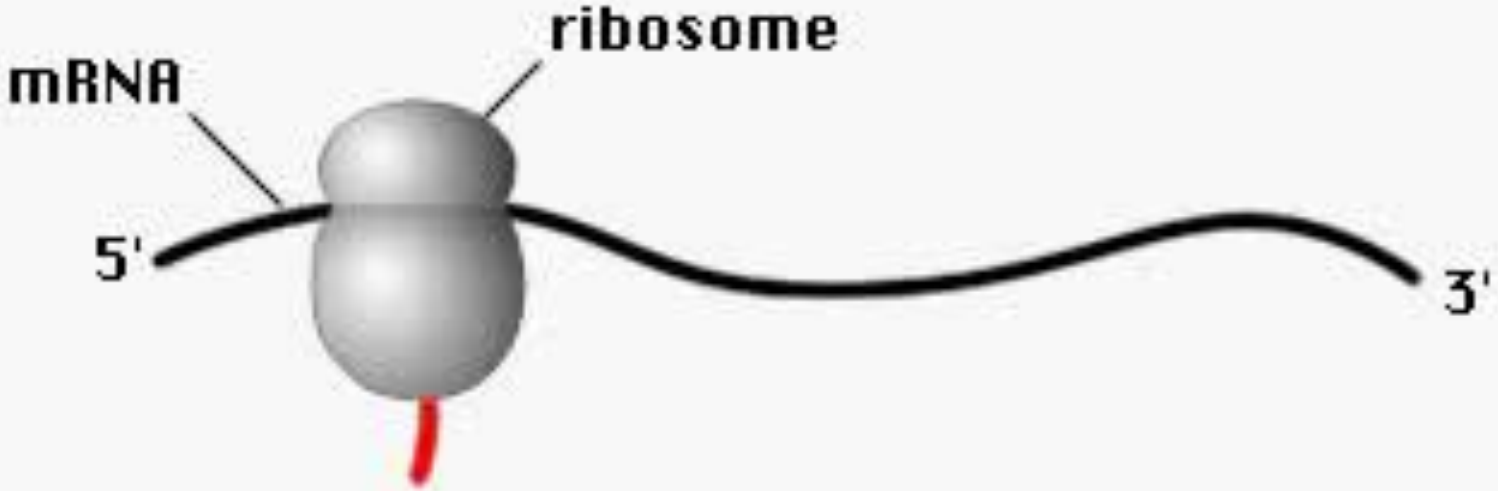



**The signal sequence is not cleaved by signal peptidase and acts as a transmembrane alpha helix.**

# Case 2b: Insertion of membrane proteins N-terminus in and C-terminus out




**The signal sequence is not cleaved by signal peptidase and acts as a transmembrane alpha helix.**

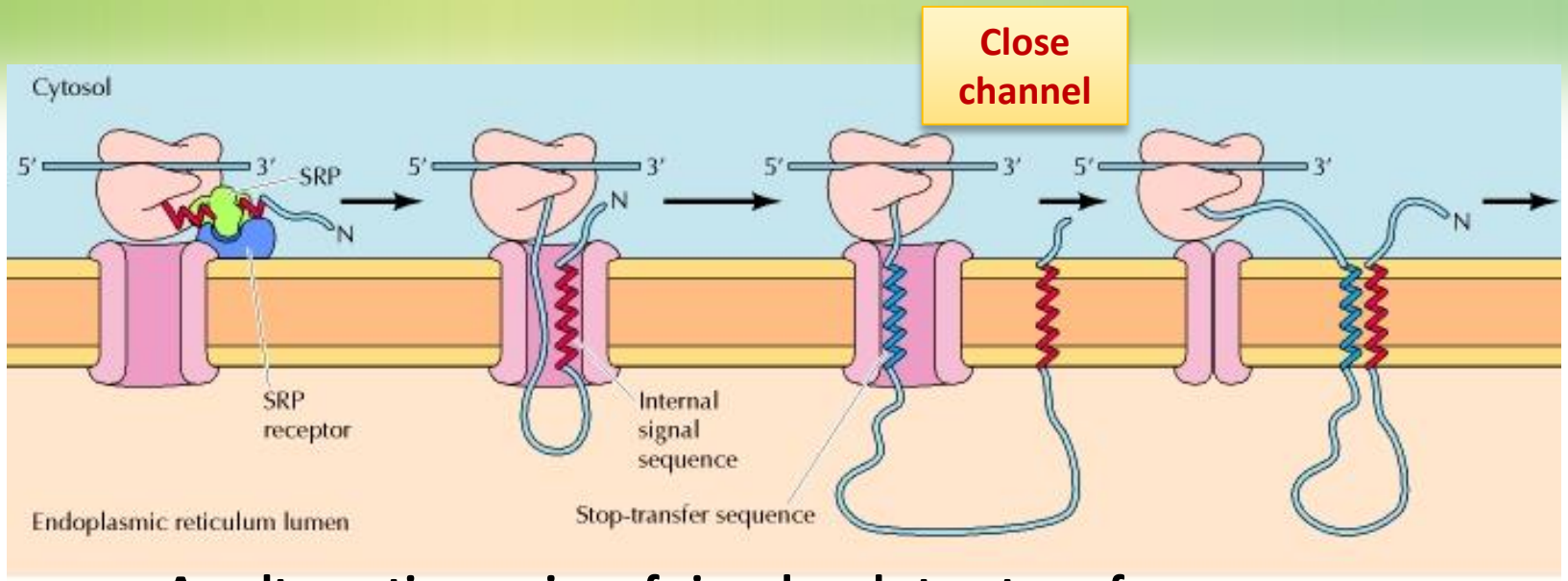



cytosol

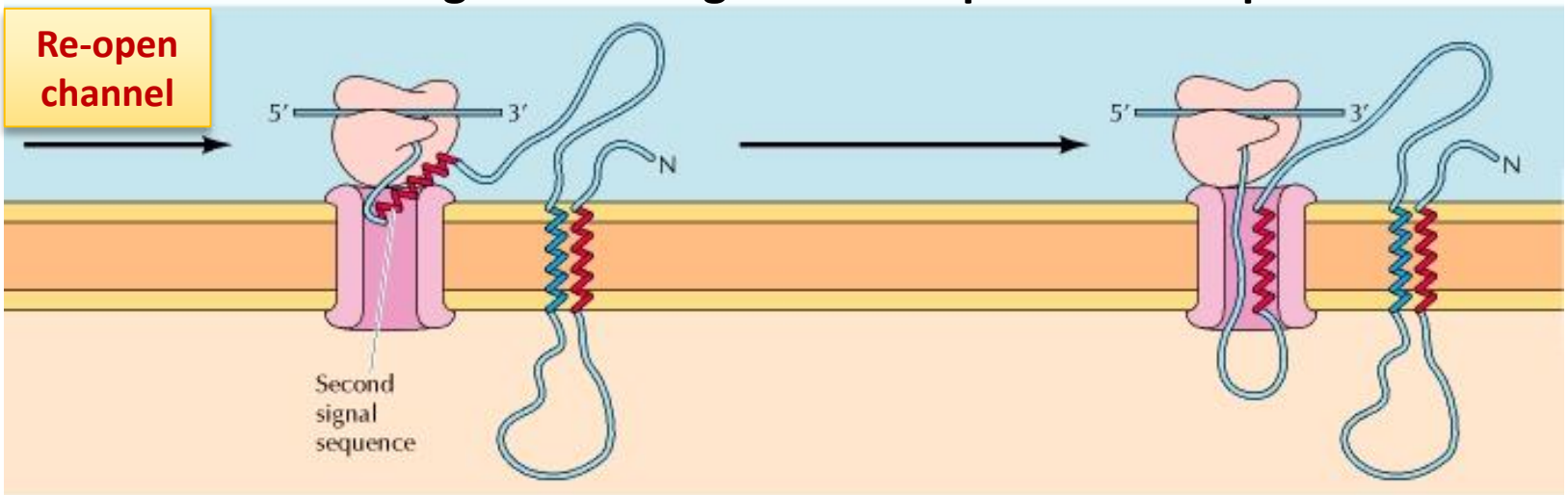
ER
membrane

ER
lumen

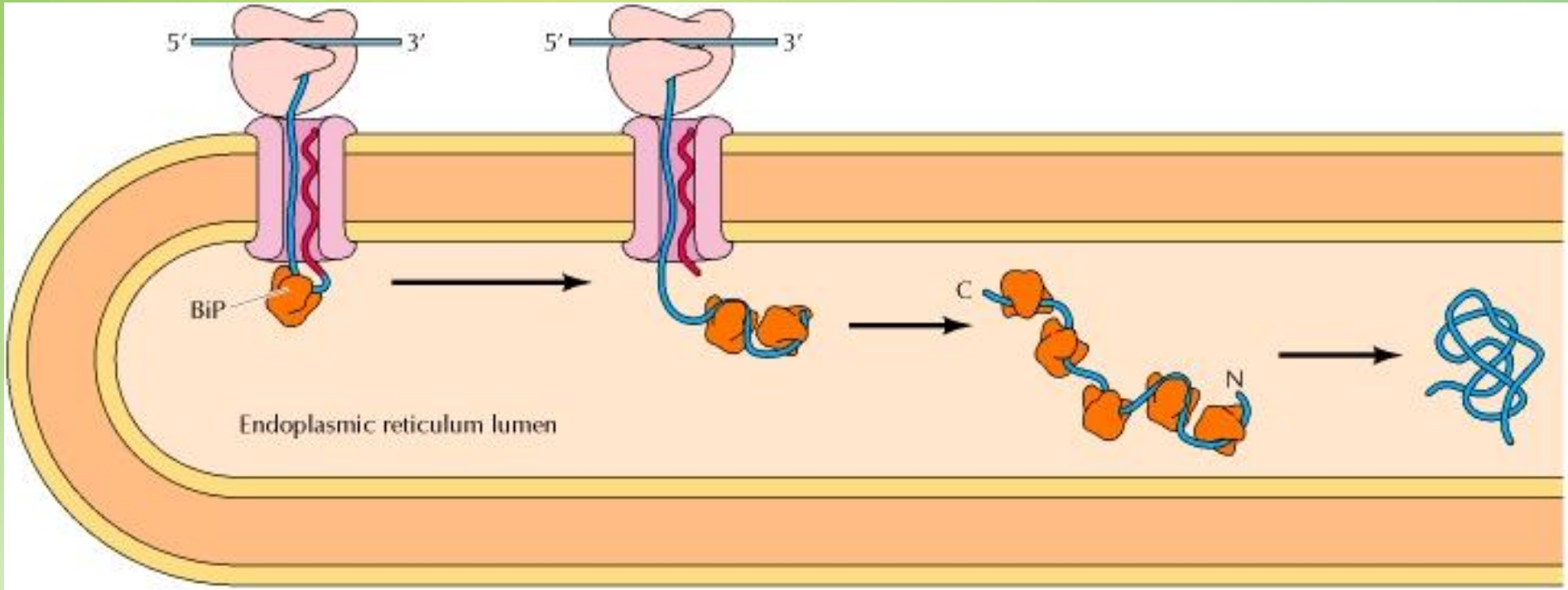
# Case 3: Insertion of membrane proteins
# Multiple membrane spanning regions




**An alternating series of signal and stop transfer sequences**



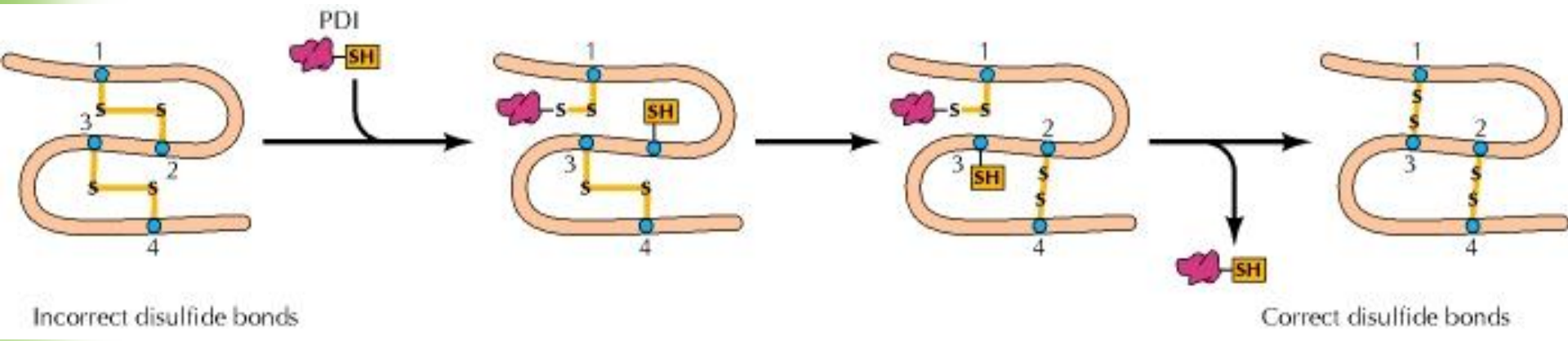
# Protein folding and processing in the ER




**Protein folding, assembly of multisubunit proteins and covalent modifications occur either during translocation to the ER or in the ER lumen**

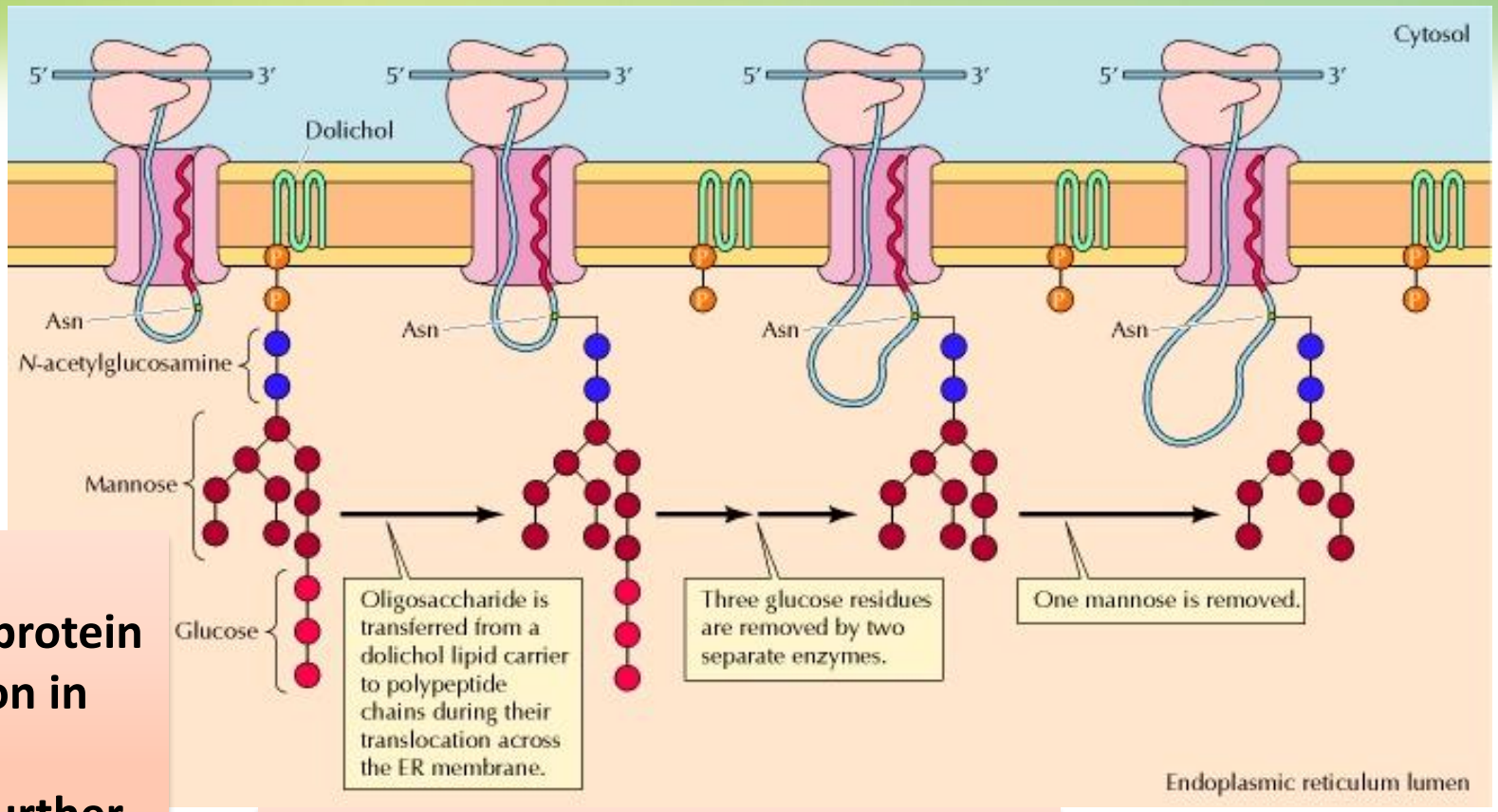
**Protein folding, assisted by the molecular chaperone, that keep protein unfolded until translocated.**

# Protein folding and processing in the ER-Disulfide bonds




**Disulfide bond formation by providing an oxidizing environment (the cytosol has a reducing environment) assisted by protein disulfide isomerase (PDI)**

# Protein processing in the ER
# N-linked glycosylation

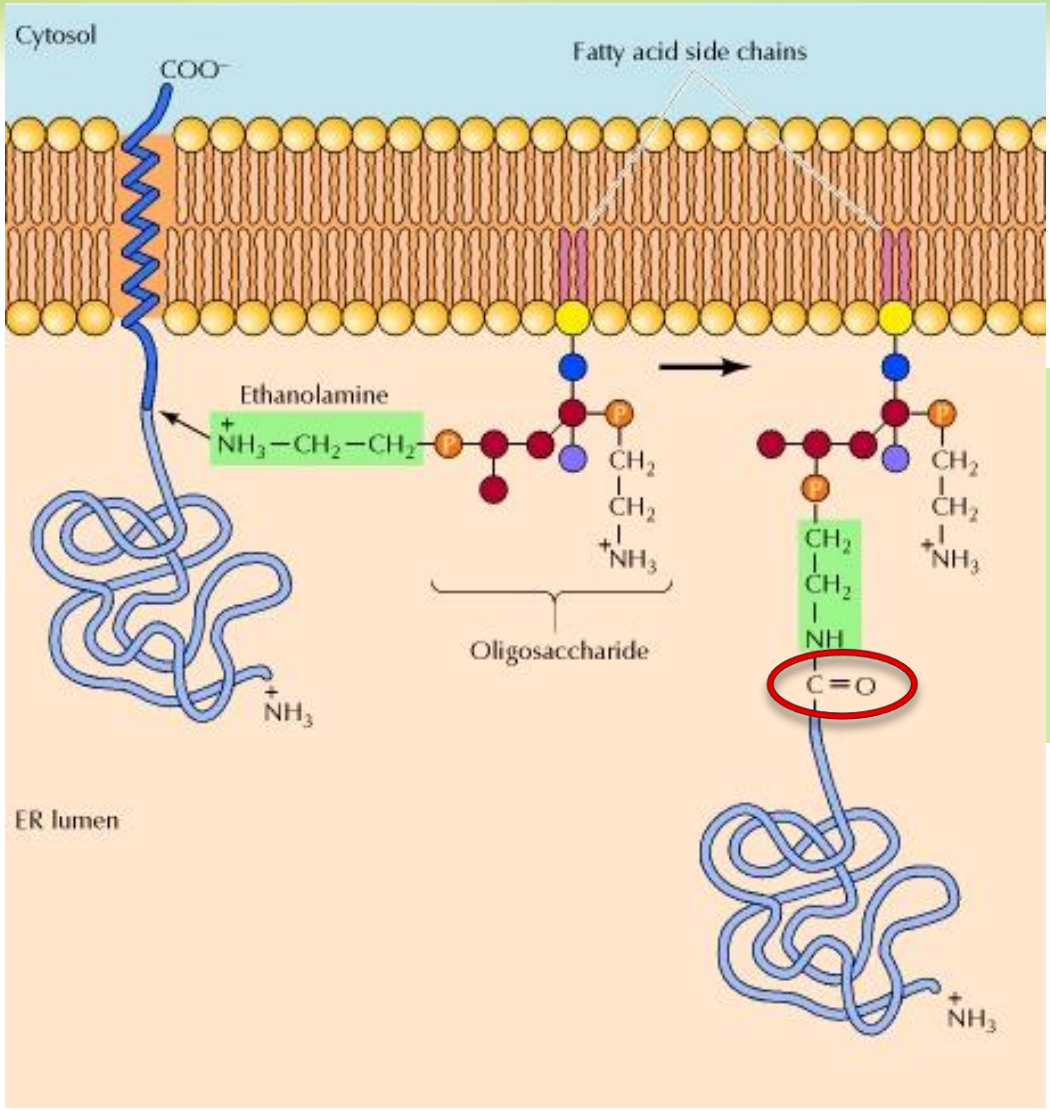



**Functions:**

1. **Prevents protein aggregation in the ER**
2. **Helps in further protein sorting**

**Glycosylation at**
**Asn-X-Ser/Thr**
**By oligosaccharyl transferase**

# Protein processing in the ER-GPI anchors

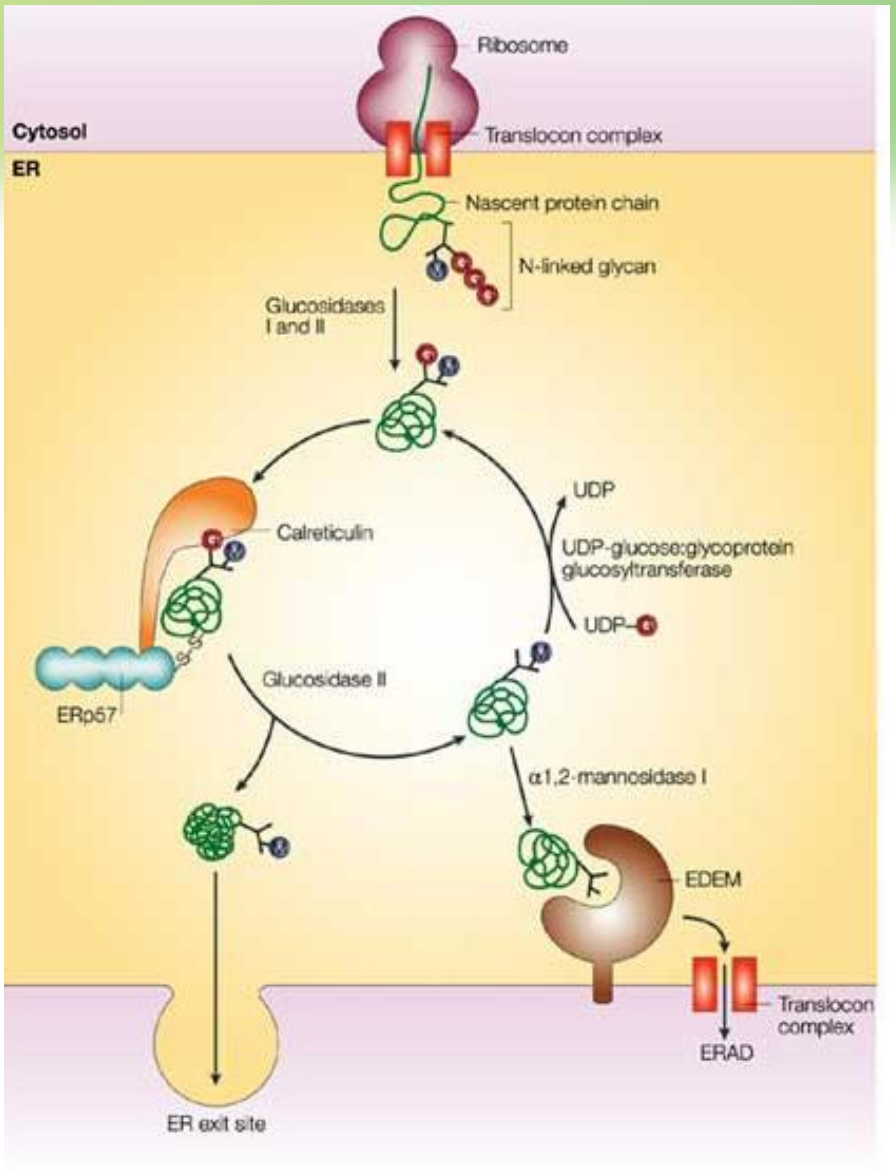



**Addition of glycolipid anchors to some plasma membrane proteins.**

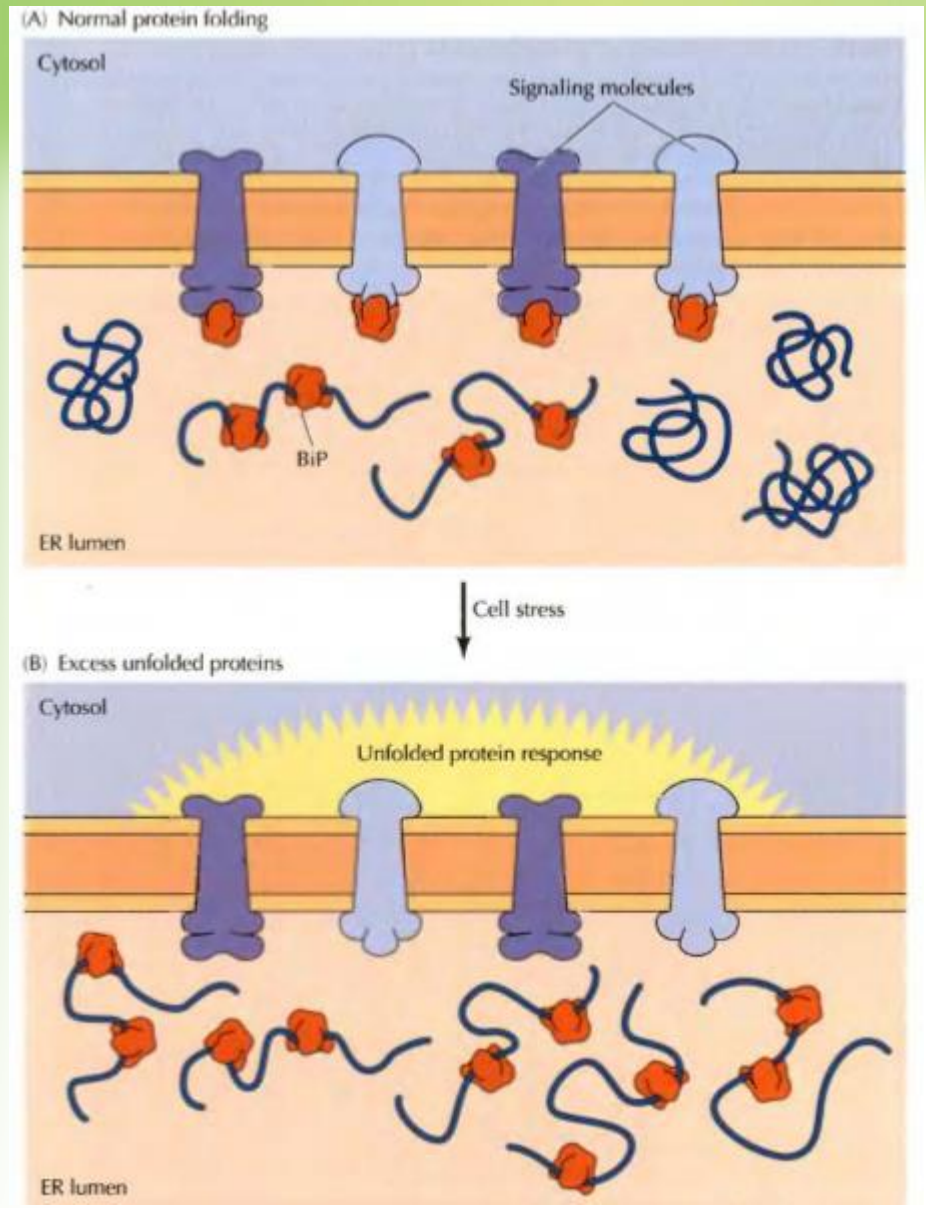
# Quality control in the ER

## ER-associated degradation (ERAD)

- **Misfolded proteins are identified, returned to the cytosol and degraded by ubiquitin proteosomal system.**
- **Chaperone and protein-processing enzymes are misfolded protein sensors**
- **Calreticulin , a chaperone, helps in folding of glycoprotein, and releases it when glucose is removed.**
- **A folding sensor binds to the protein.**
- **If correctly folded, the protein moves to transitional ER.**
- **If misfolded, glucose is added, calreticulin re-folds the proteins.**
- **If severely misfolded, the protein is degraded by ubiquitin proteosomal system**



# Unfolded protein response (UPR)

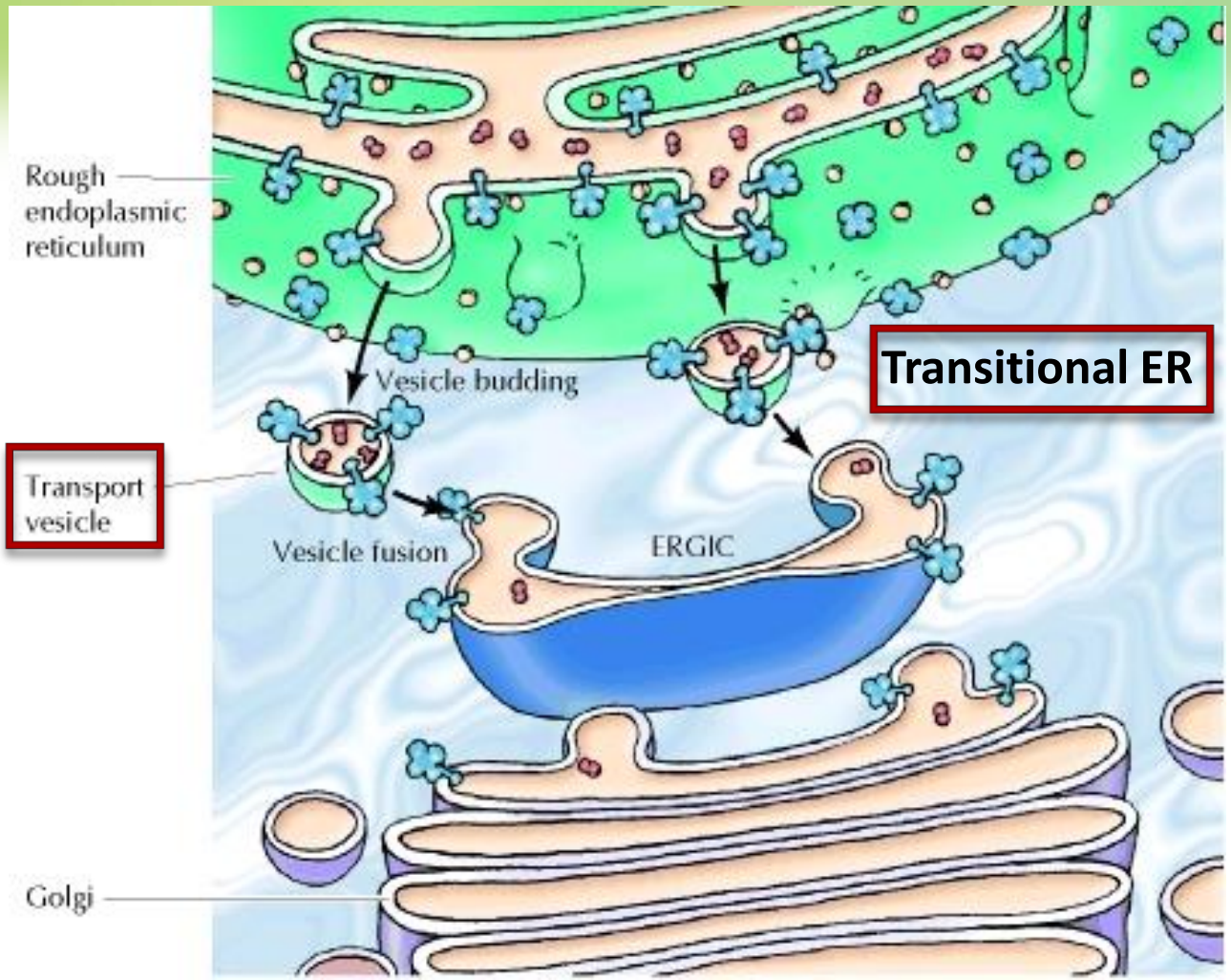



Read UPR in details Page 391-392

- ✓ **Coordinates protein folding capacity of the ER with the physiological needs of the cell.**
- ✓ **Is activated when excess unfolded proteins accumulate in the ER.**
- ✓ **ER expansion, activation of UPR target genes such as chaperones and transient reduction in new protein entry to ER**

# ER-Golgi intermediate compartment (ERGIC)

**Note that topological orientation is maintained**

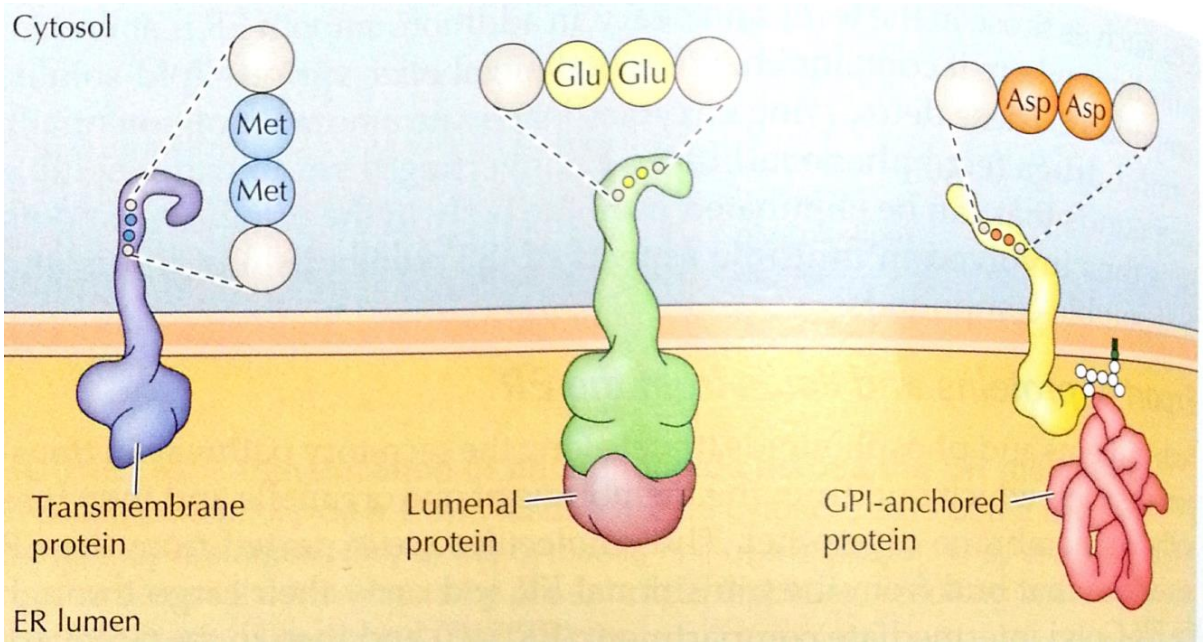



**Proteins and lipids are transported**

# Protein sorting and retention

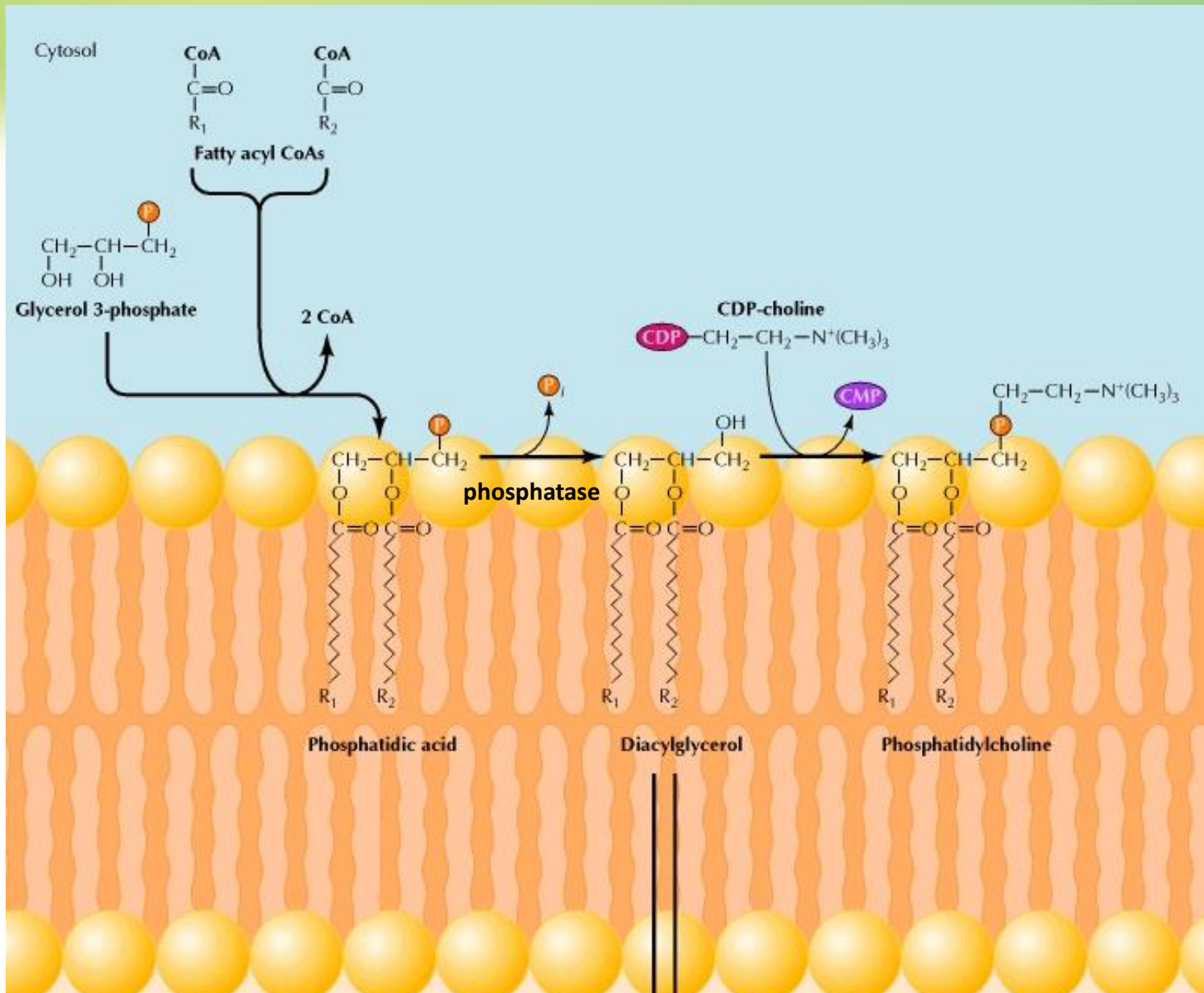
- **Many proteins with KDEL sequence (Lys-Asp-Glu-Leu) at C-terminus are retained in the ER lumen.**
  - ✓ If sequence is deleted, the protein is transported to the Golgi and secreted from the cell.
  - ✓ Addition of the sequence causes a protein to be retained in the ER.
- **The retention of some transmembrane proteins in the ER is dictated by short C-terminal KKXX sequences.**
- **Proteins bearing the KDEL and KKXX sequences are to recycled back to the ER but are not prevented from being carried to Golgi..**

**Membrane proteins contain di-acidic or di-hydrophobic amino acid signal sequences.**
**They also function as carriers of GPI-anchored and lumenal proteins.**

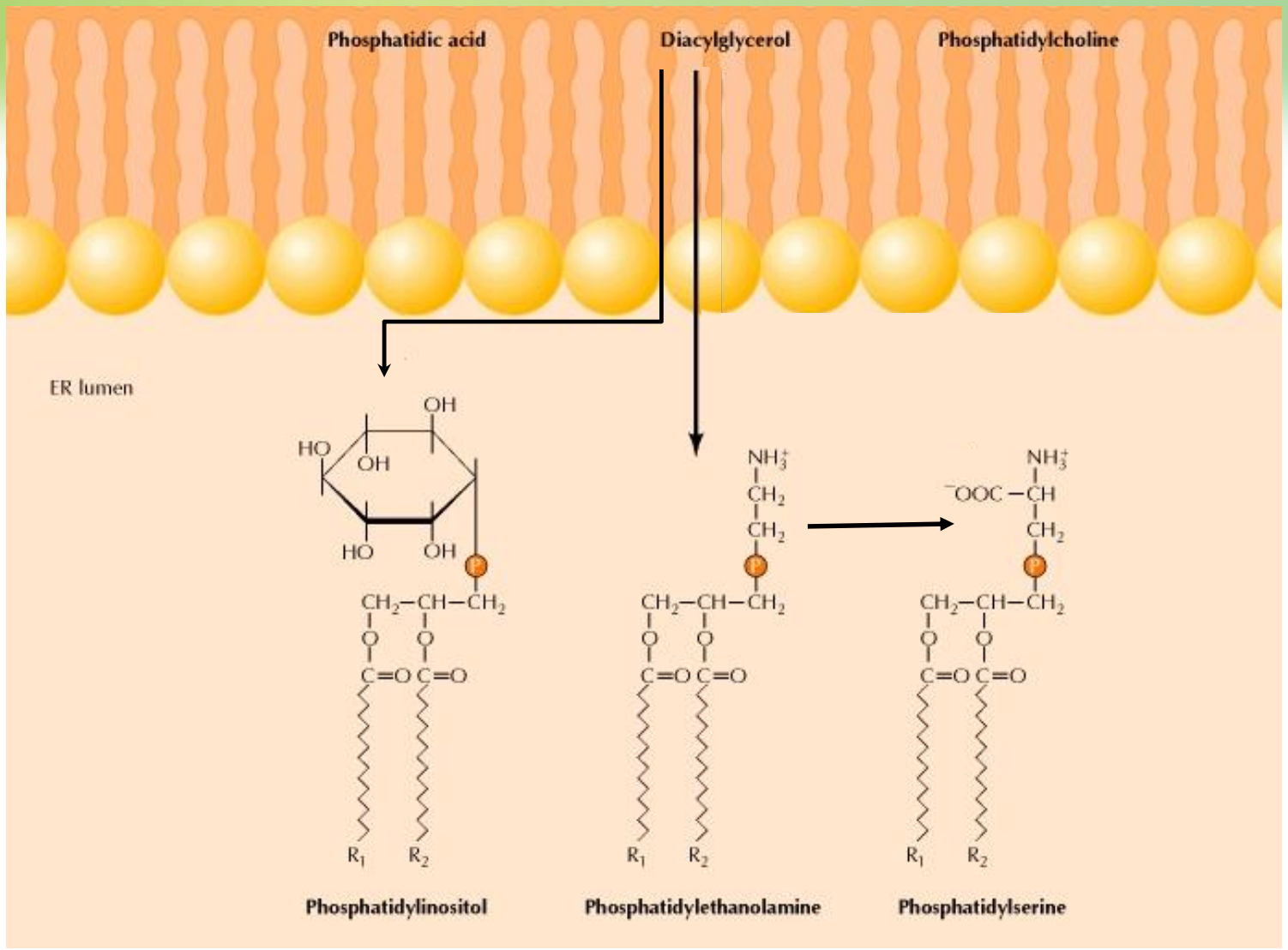


# Synthesis of phospholipids in SER

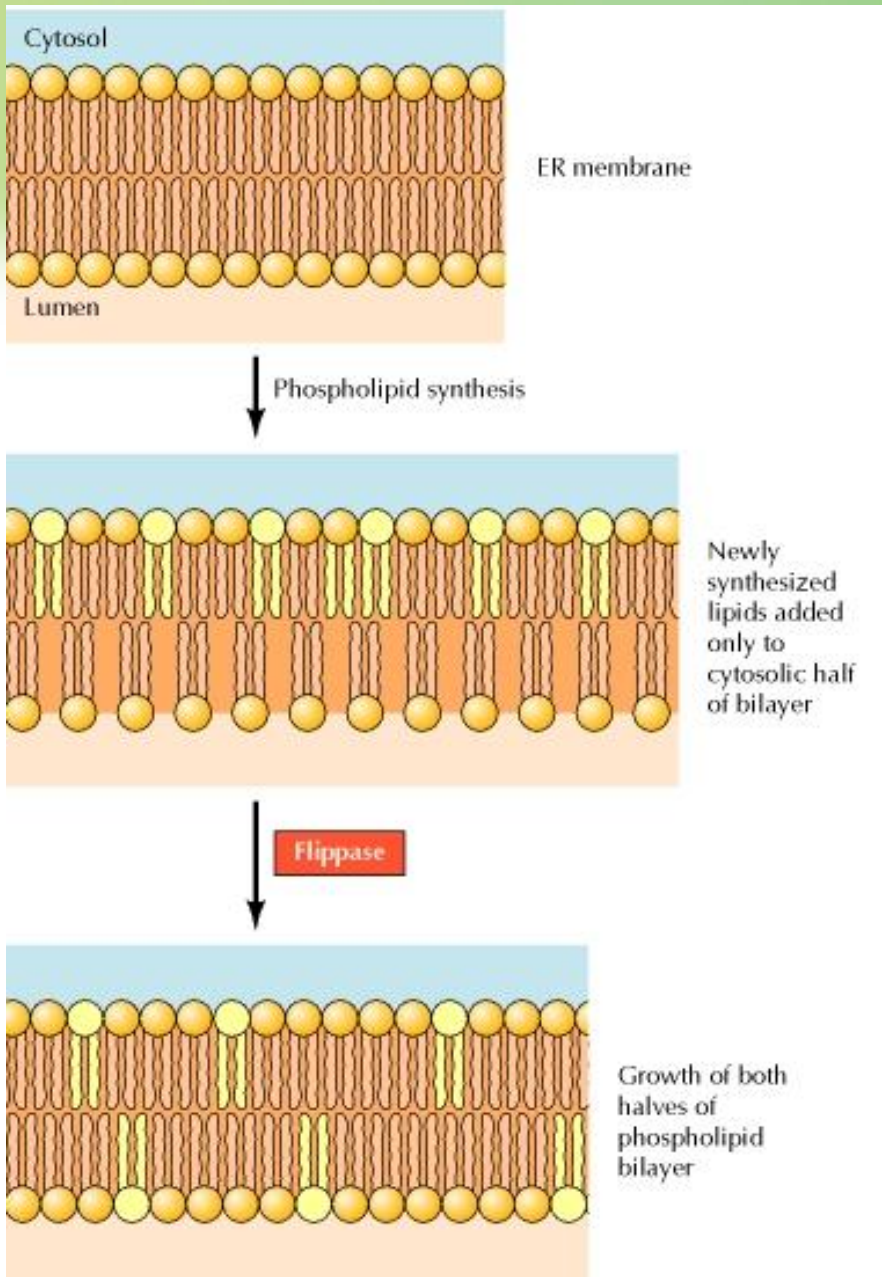
**Enzymes are buried inside the membrane because the hydrophobic structure of lipids has to be maintained in close proximity to membranes**



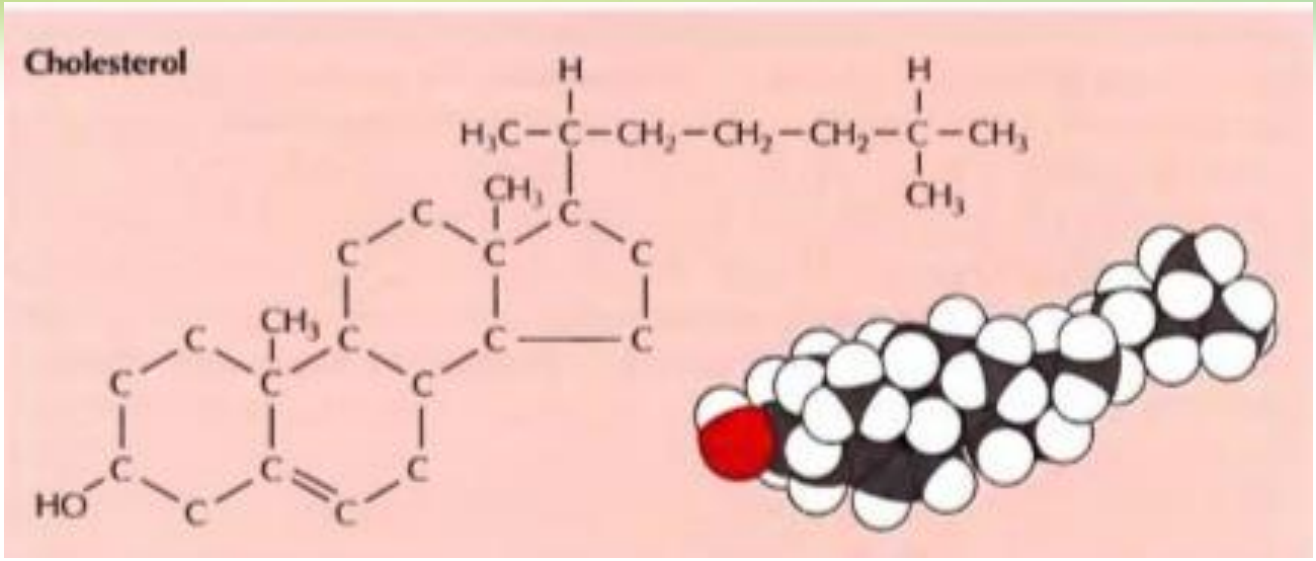
# Synthesis of phospholipids in SER



# Translocation of phospholipids across the ER membrane



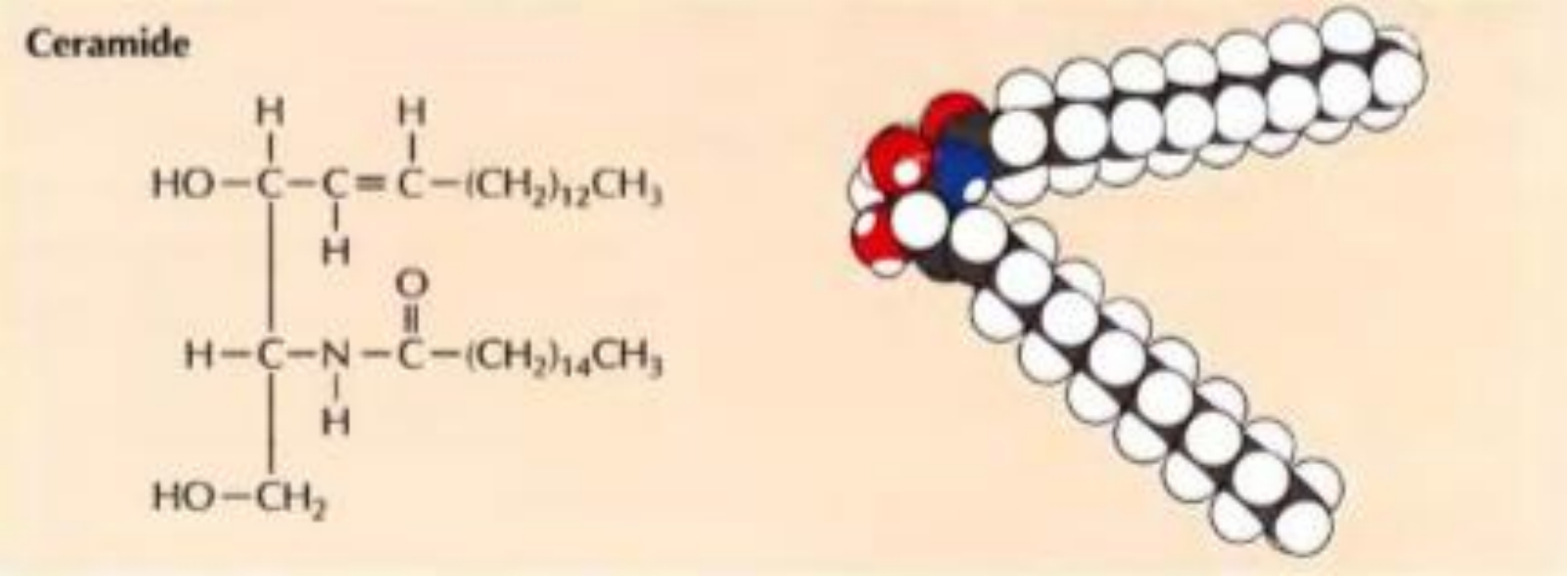
# Synthesis of cholesterol and its derivatives




**Steroid hormones are synthesized from cholesterol in the ER**

- **Large amounts of smooth ER are found in steroid-producing cells, such as those in the testis and ovary**

# Synthesis of ceramide




**Synthesis of glycolipids and sphingomyelin**

# Synthesis of other lipids

- **Smooth ER is abundant in the liver**
- **SER contains enzymes that metabolize various lipid-soluble compounds.**
  - ✓ **The detoxifying enzymes inactivate a number of potentially harmful drugs (e.g., phenobarbital) by converting them to water-soluble compounds that can be eliminated from the body in the urine**