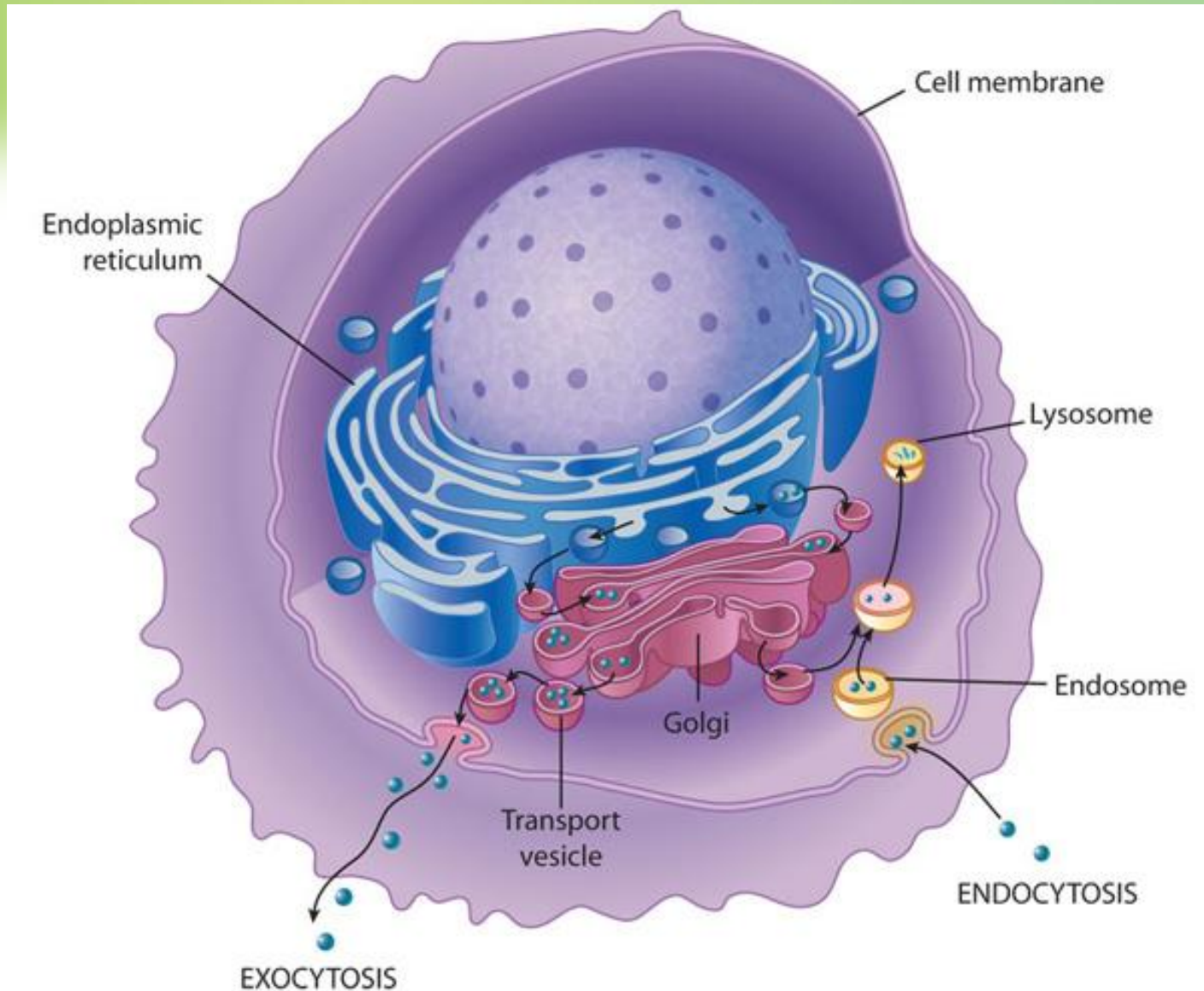


# Lecture 2: Protein sorting (endoplasmic reticulum)

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

*Principles of Genetics and Molecular Biology*

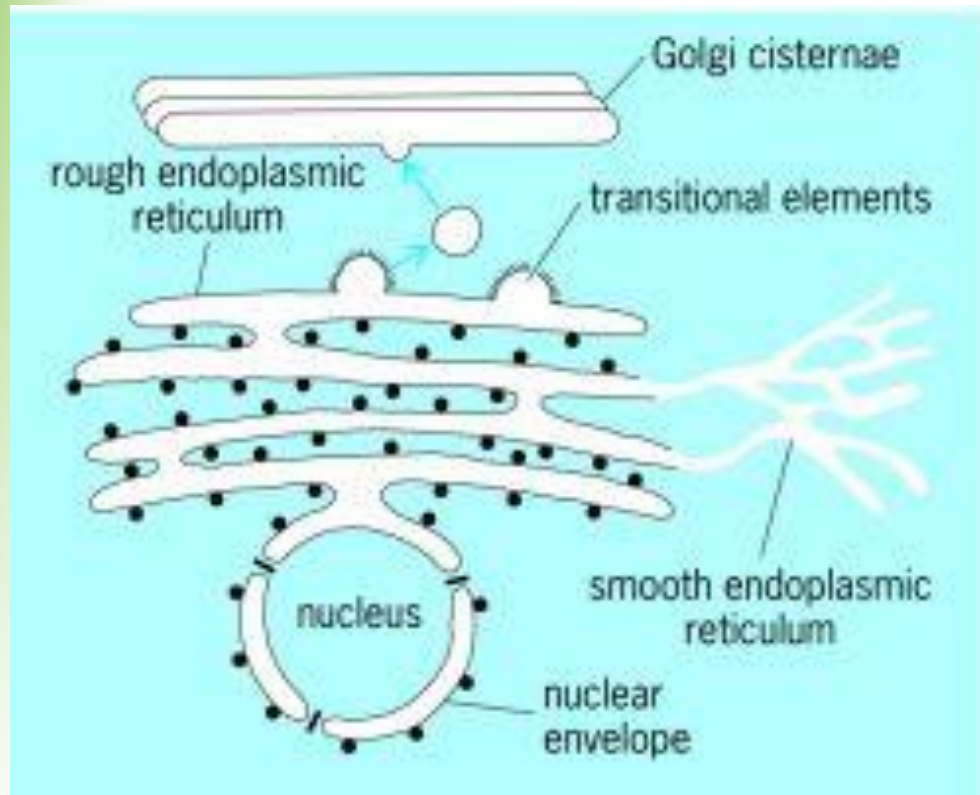
# An overview of cellular components



# 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.

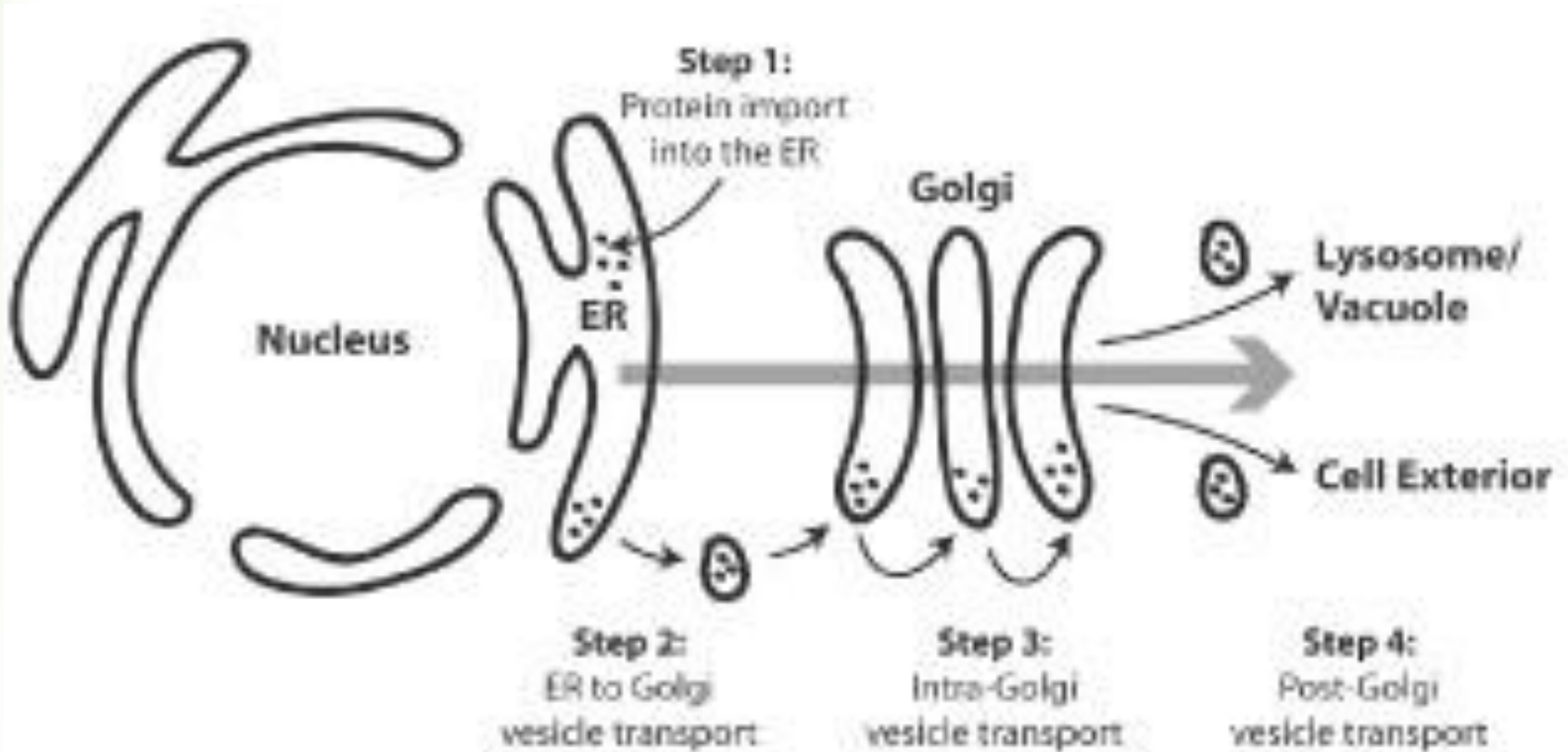


- The rough ER: covered by ribosomes on its outer surface and functions in protein processing.
- The smooth ER: lipid metabolism
- Transitional ER: exit of vesicles to Golgi apparatus

# The secretory pathway

ER-Golgi- secretory vesicles- cell exterior

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

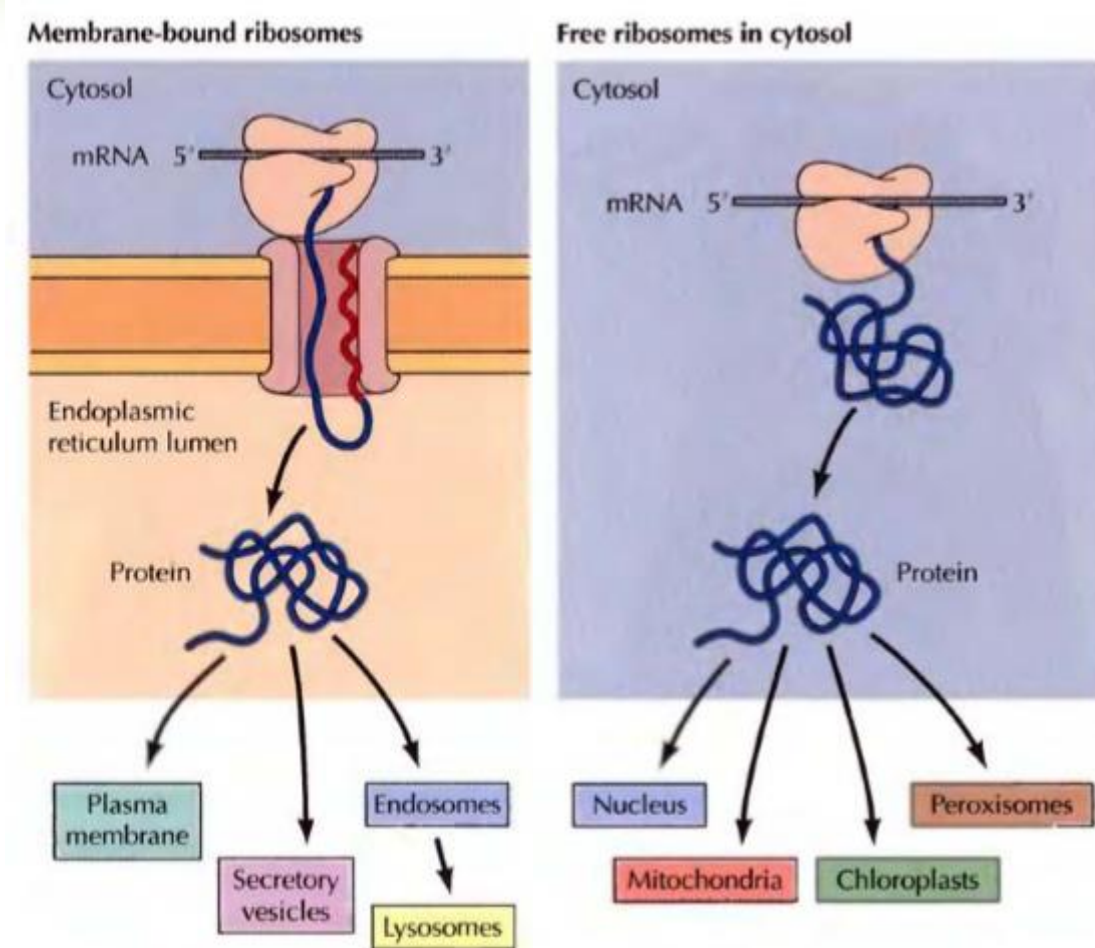


# Pulse chase

**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).



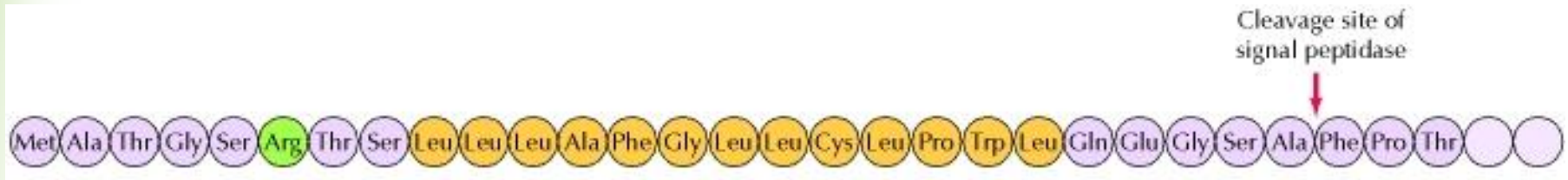
Cytosolic, interior nuclear, peroxisomal, and mitochondrial proteins are synthesized on free ribosomes and released into the cytosol after translation is complete

# 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

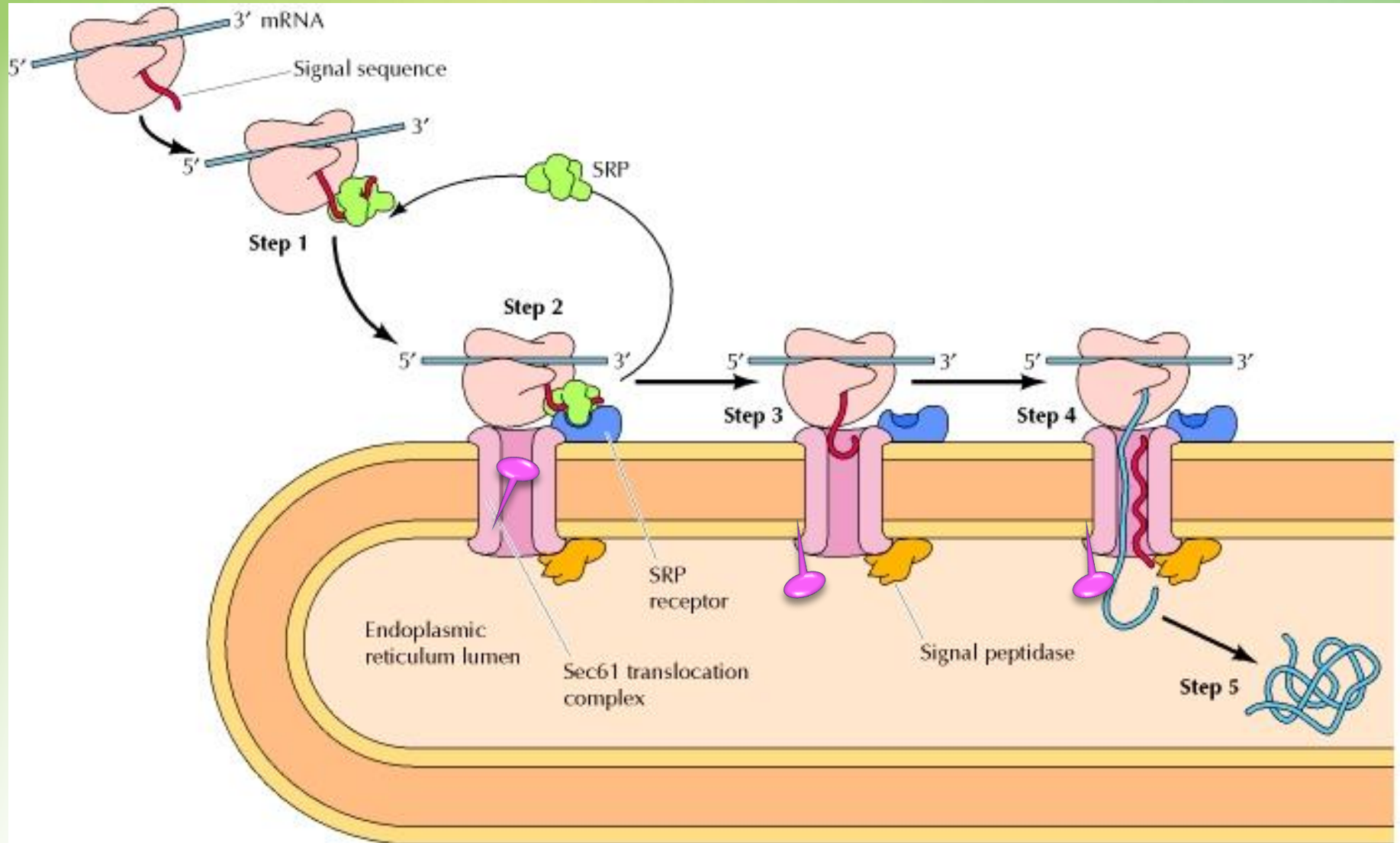
## Translocation Animations

<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)

[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).**

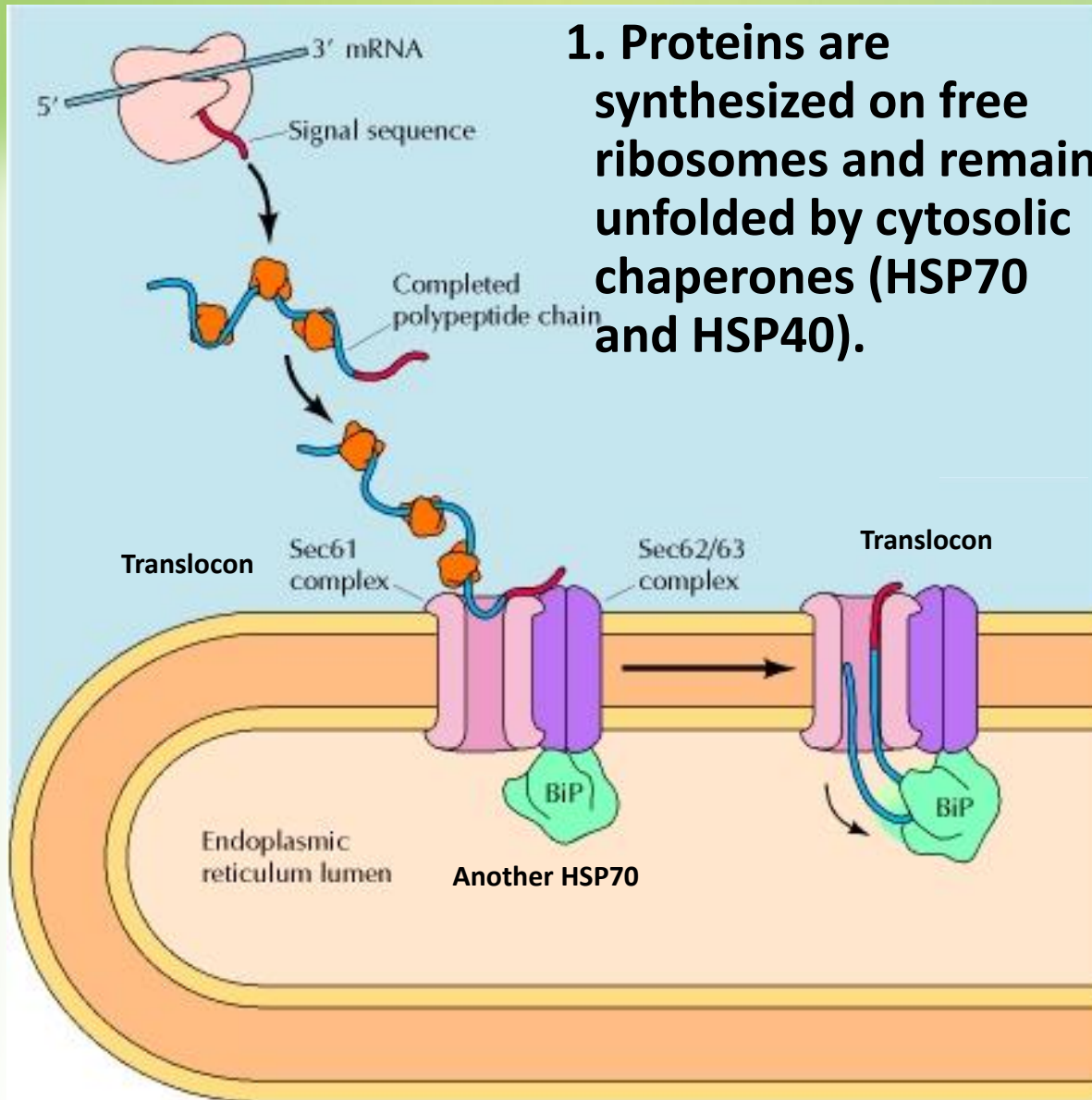
**Step 2: The SRP inhibits translation and escorts the complex to the ER membrane, where it binds to the SRP receptor.**

**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.**

# 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.**

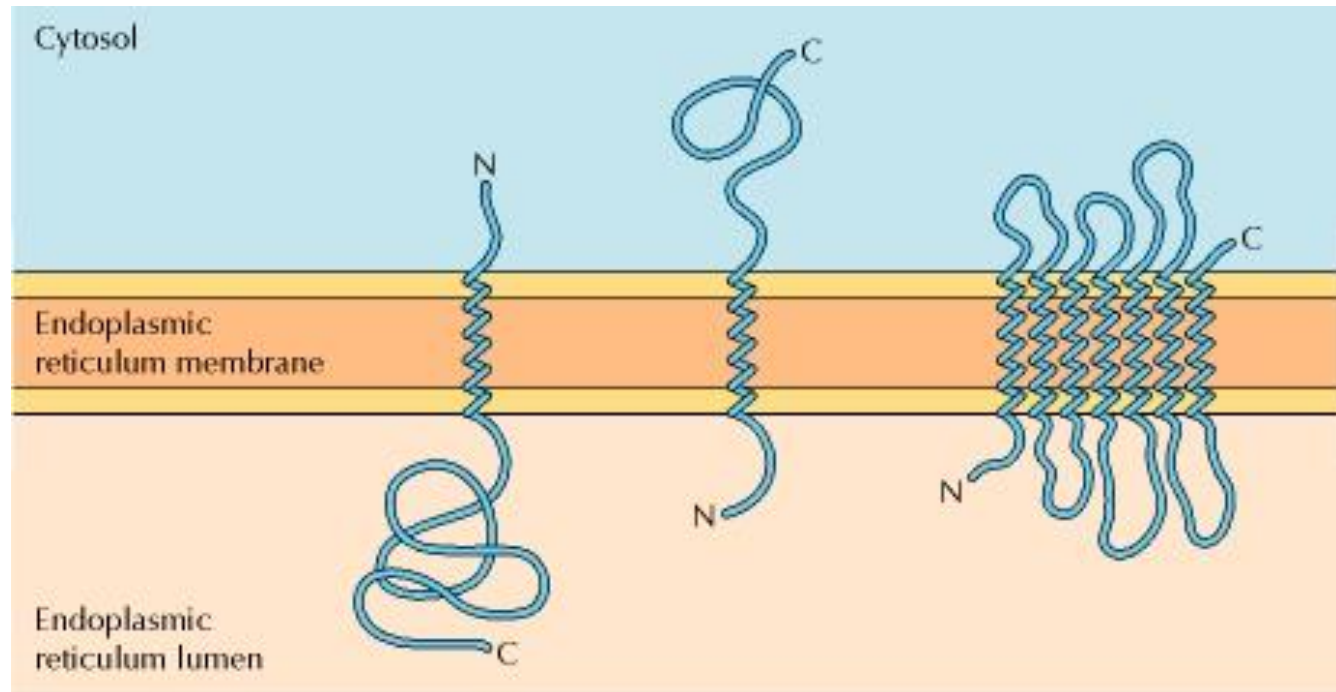
# Insertion of proteins into the ER membrane

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

Membrane proteins are initially inserted into the ER membrane.

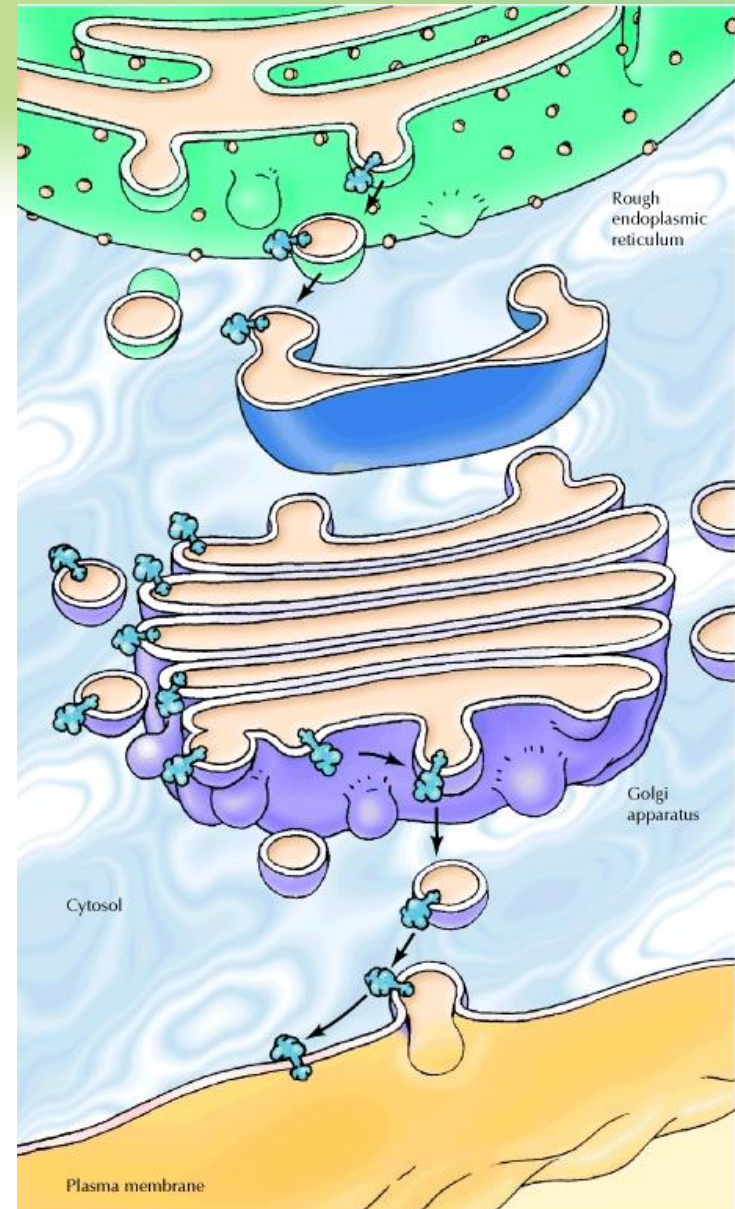
Factors that affect protein insertion into the ER membrane:

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

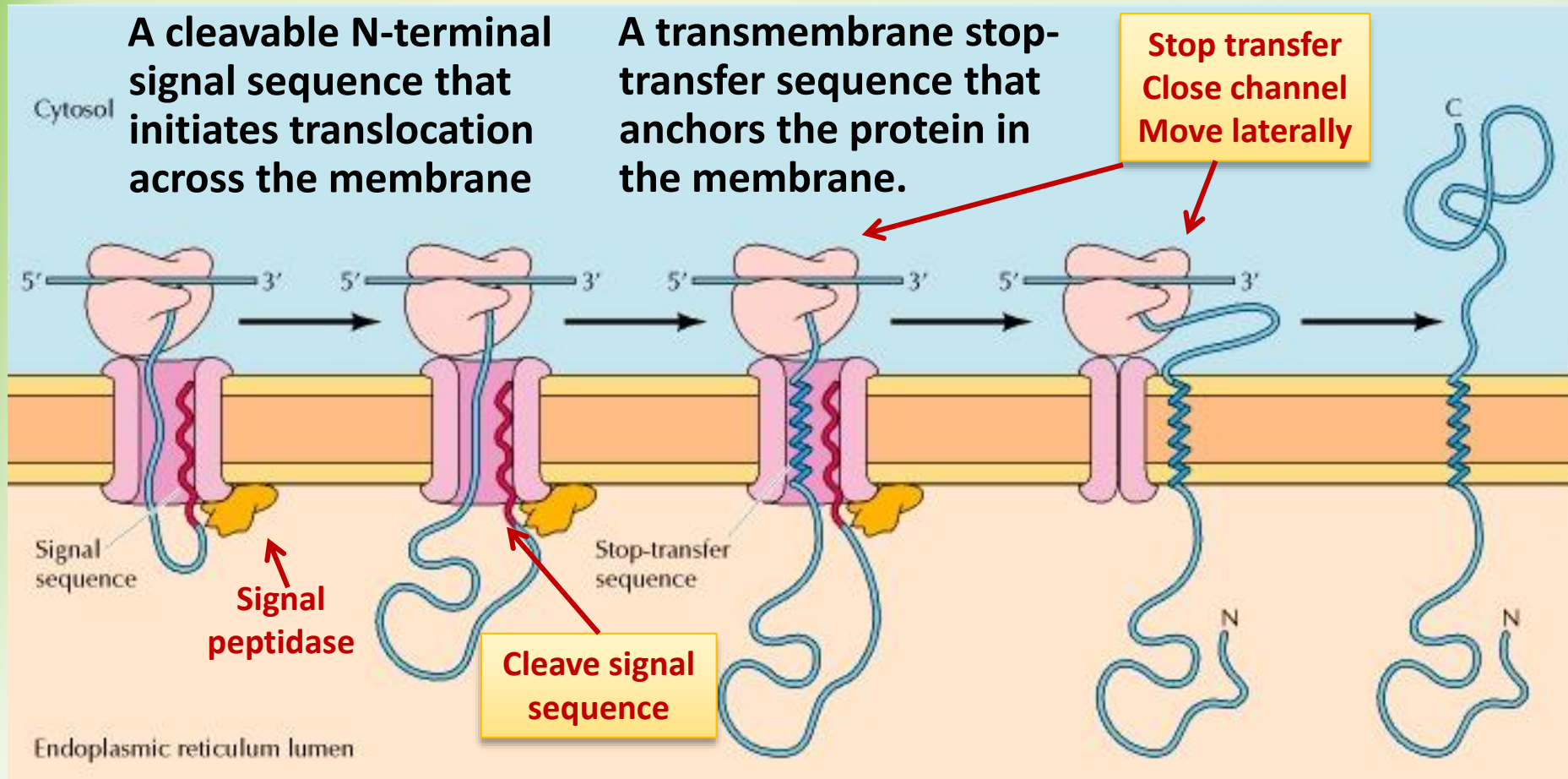


# Membrane protein orientation

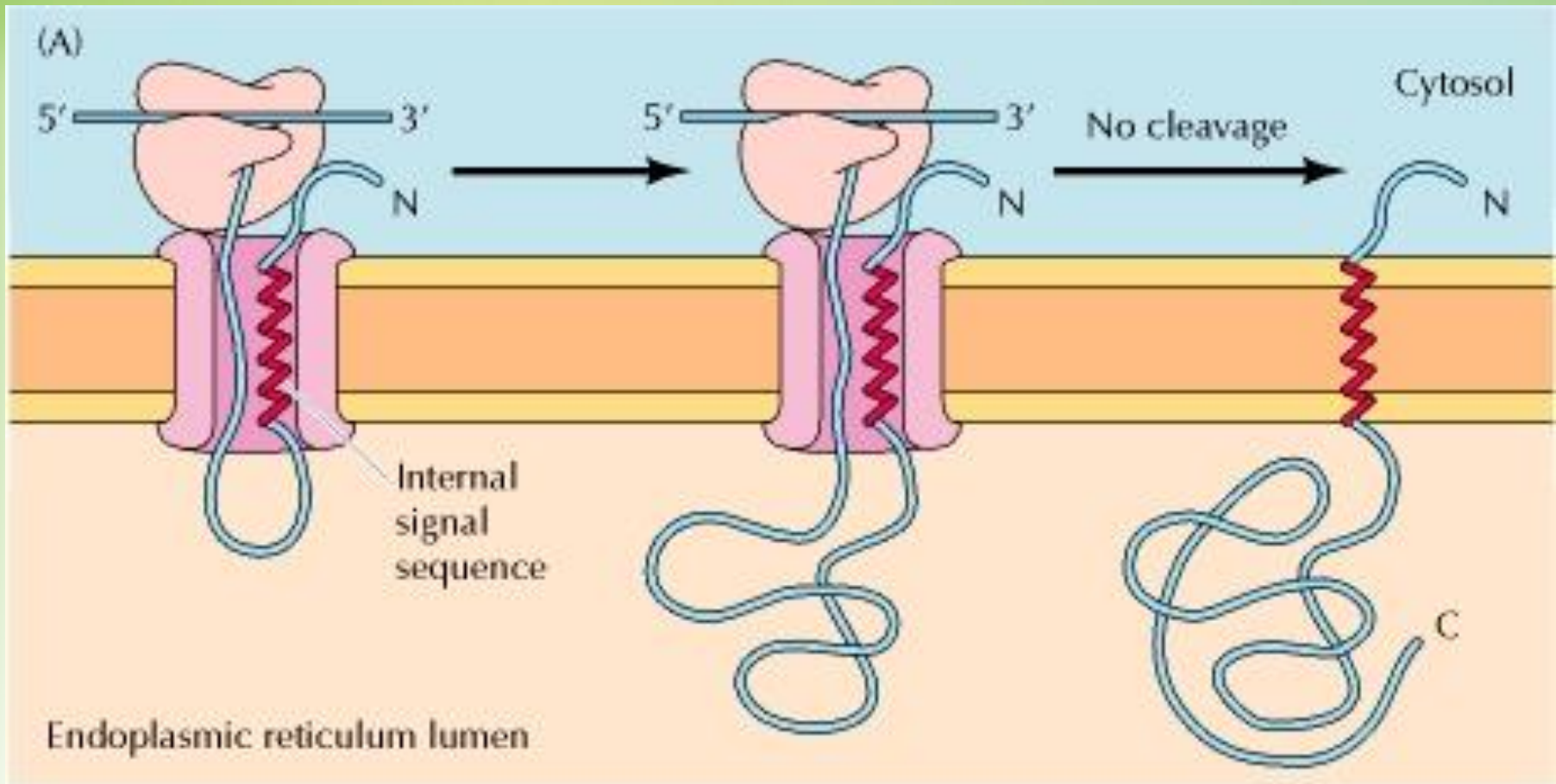
**The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.**



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

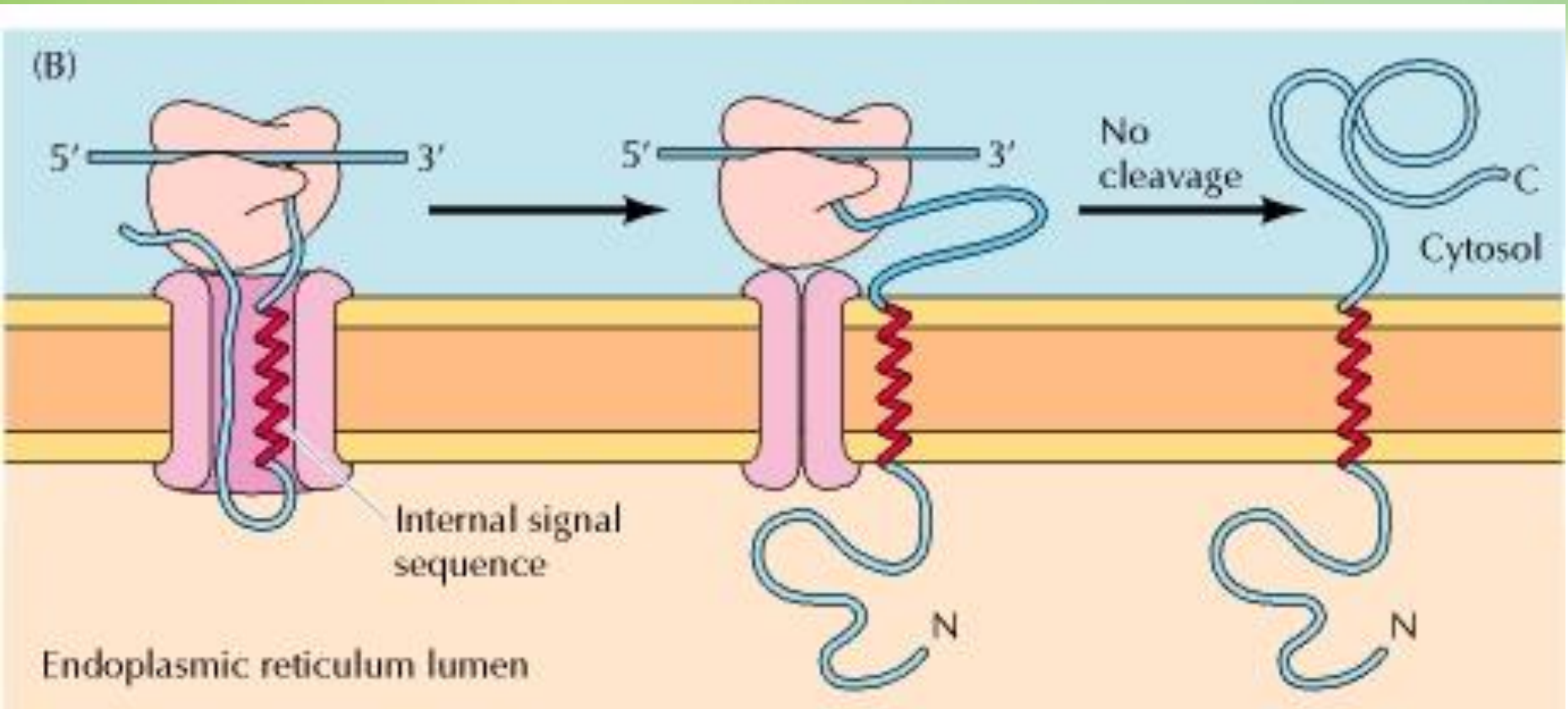


## Case 2a: Insertion of membrane proteins C-terminus in and N-terminus out



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

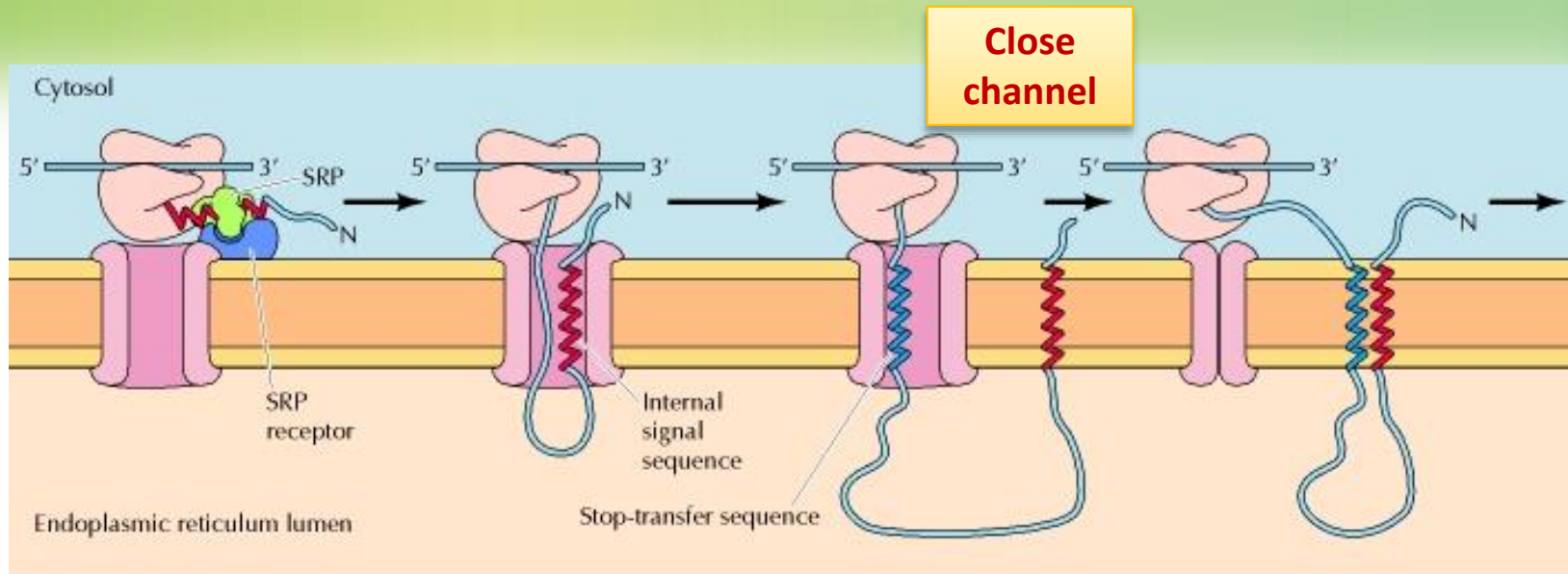
## Case 2b: Insertion of membrane proteins N-terminus in and C-terminus out



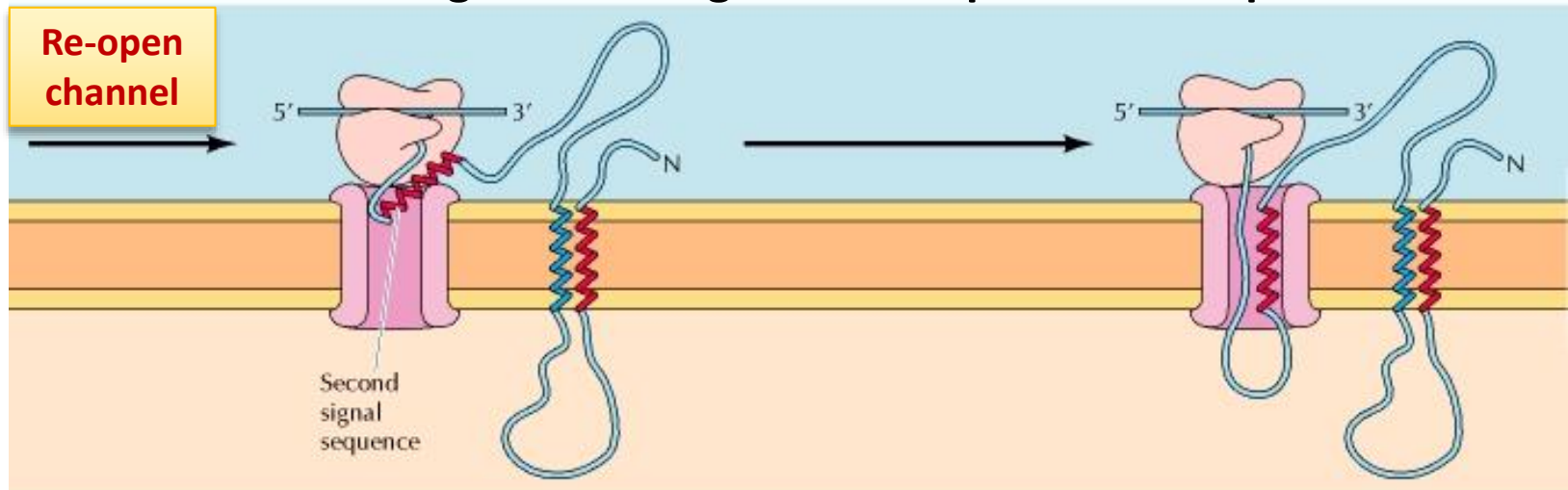
**The signal sequence is not cleaved by signal peptidase and acts as a transmembrane alpha helix.**

# Case 3: Insertion of membrane proteins

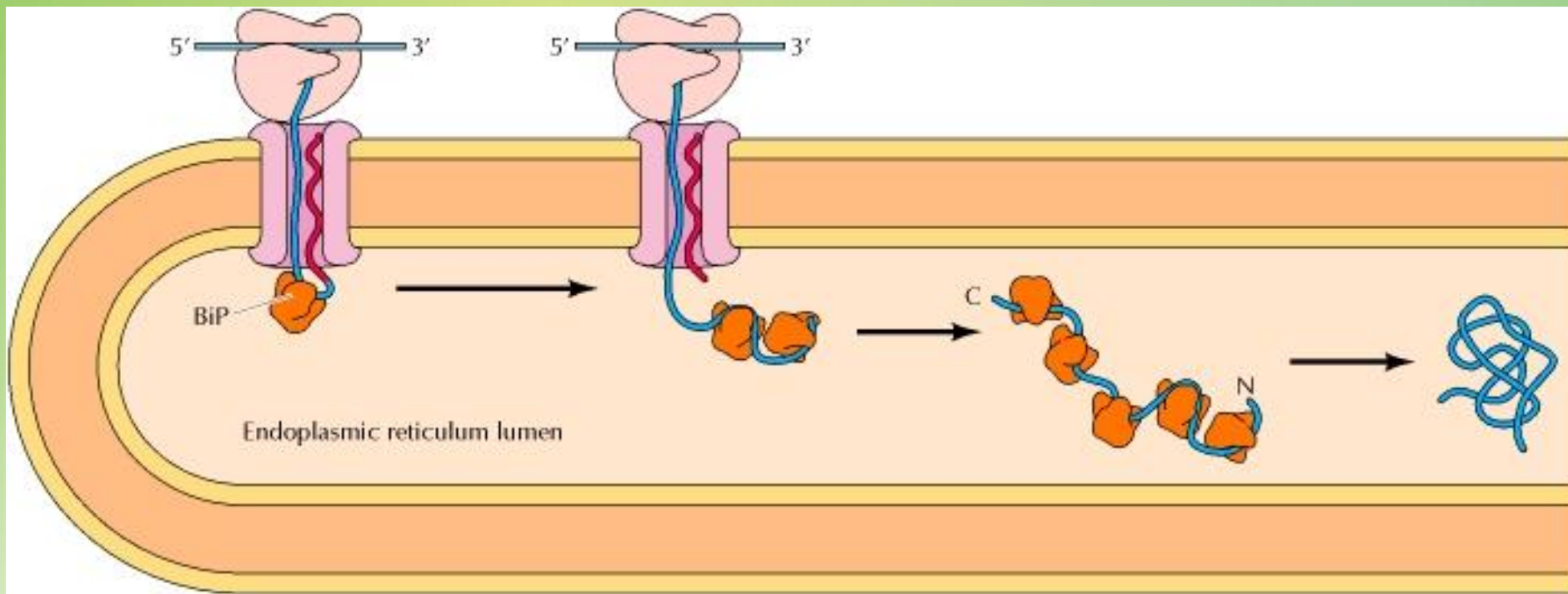
## Multiple membrane spanning regions



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



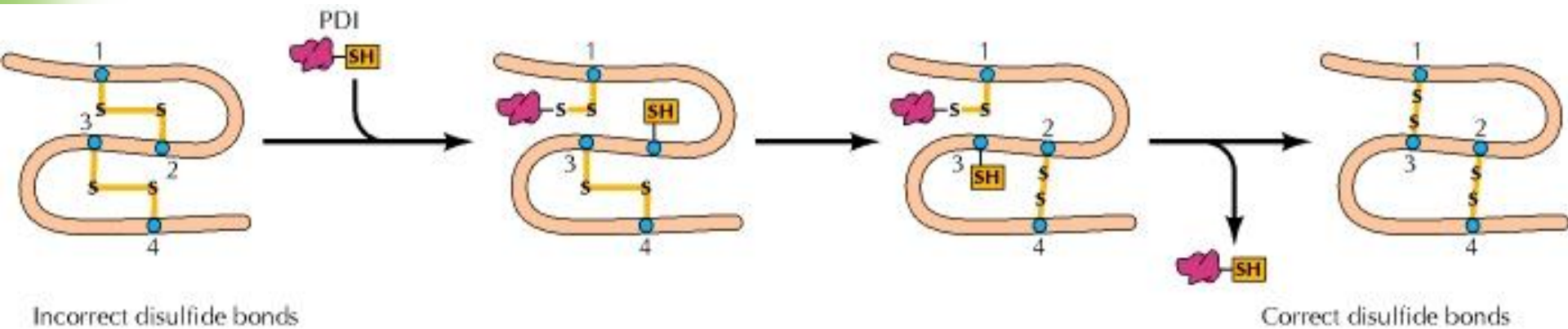
# Protein folding and processing in the ER



**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.**

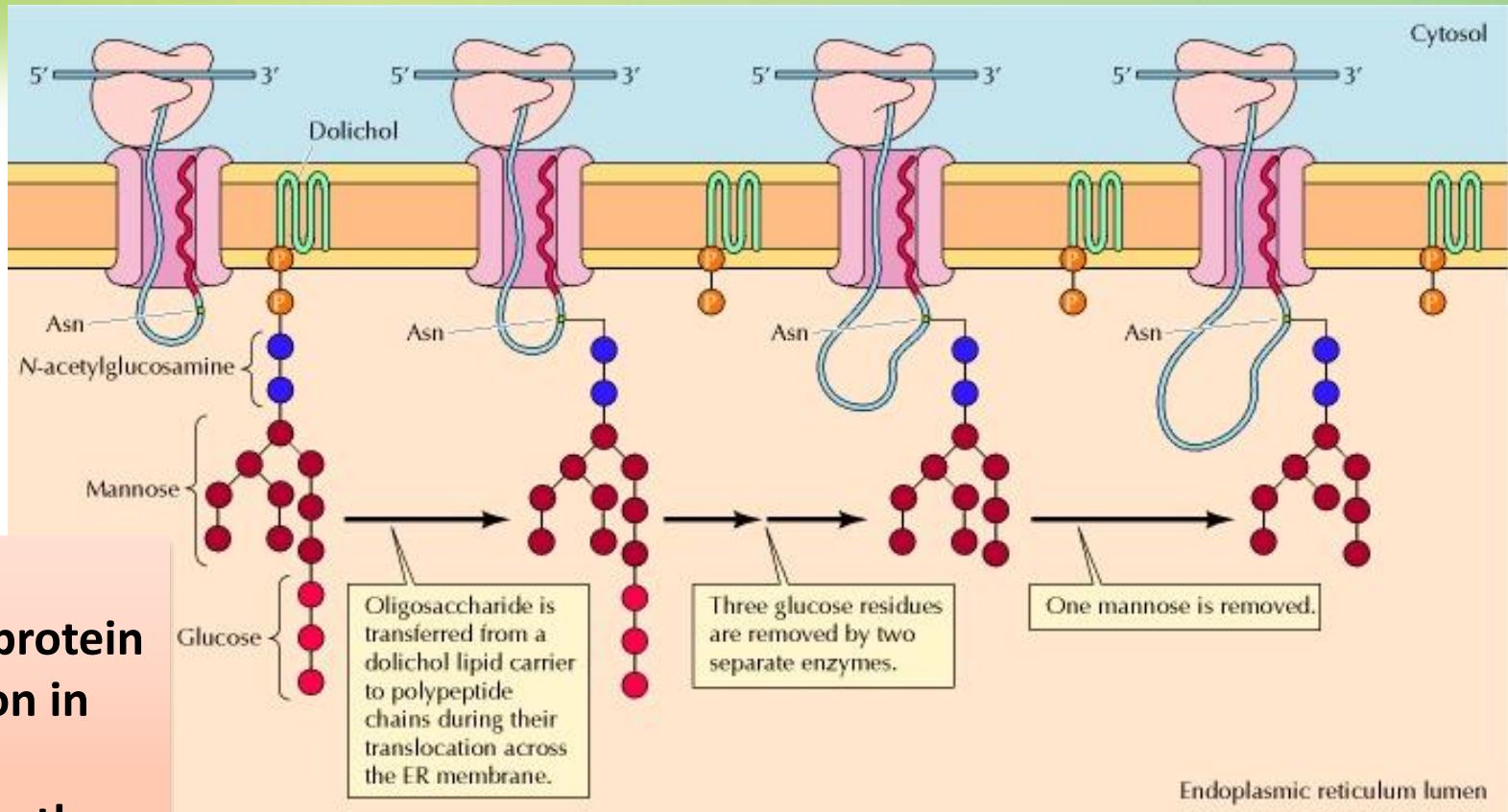
# Protein folding and processing in the ER-Disulfide bonds



**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

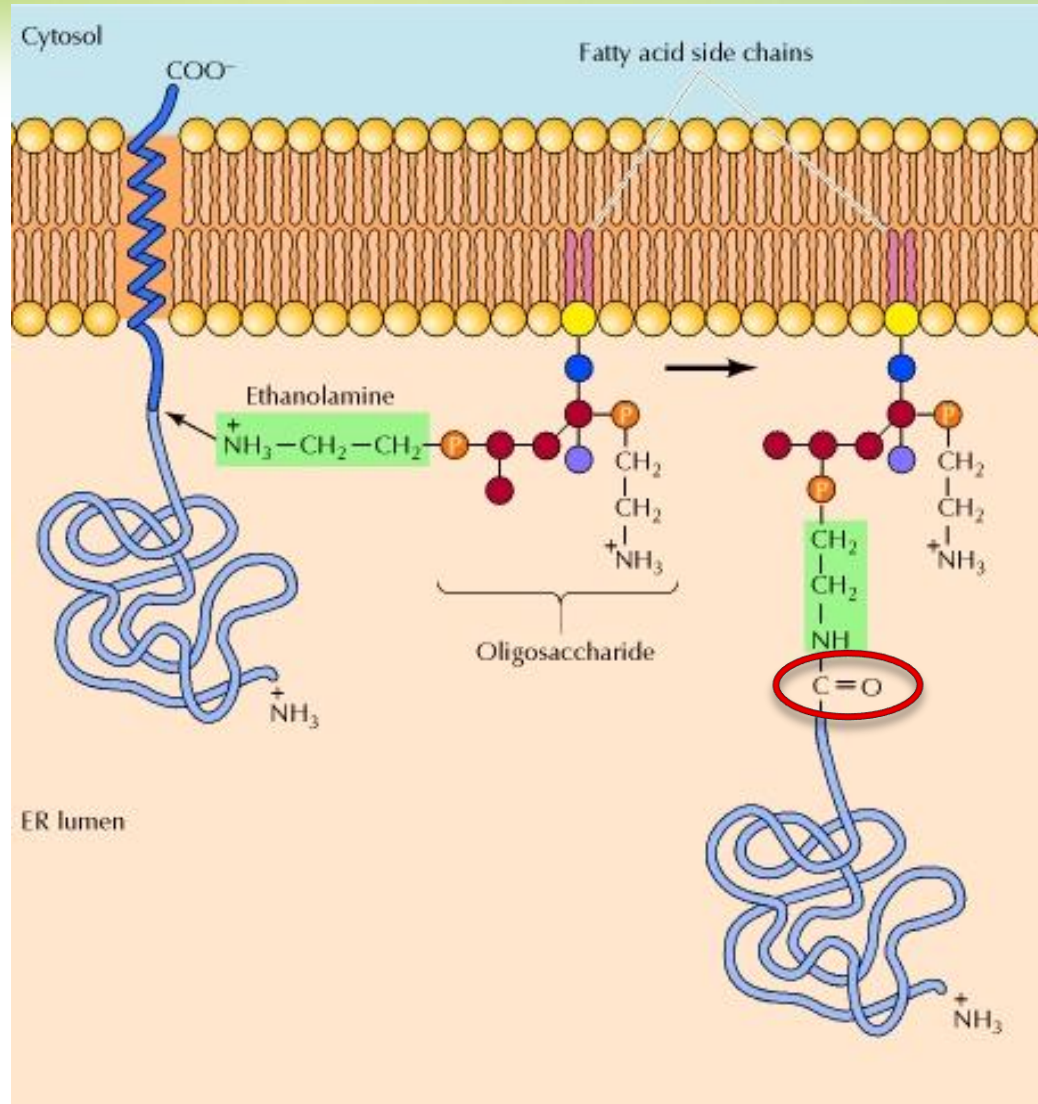


### Functions:

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

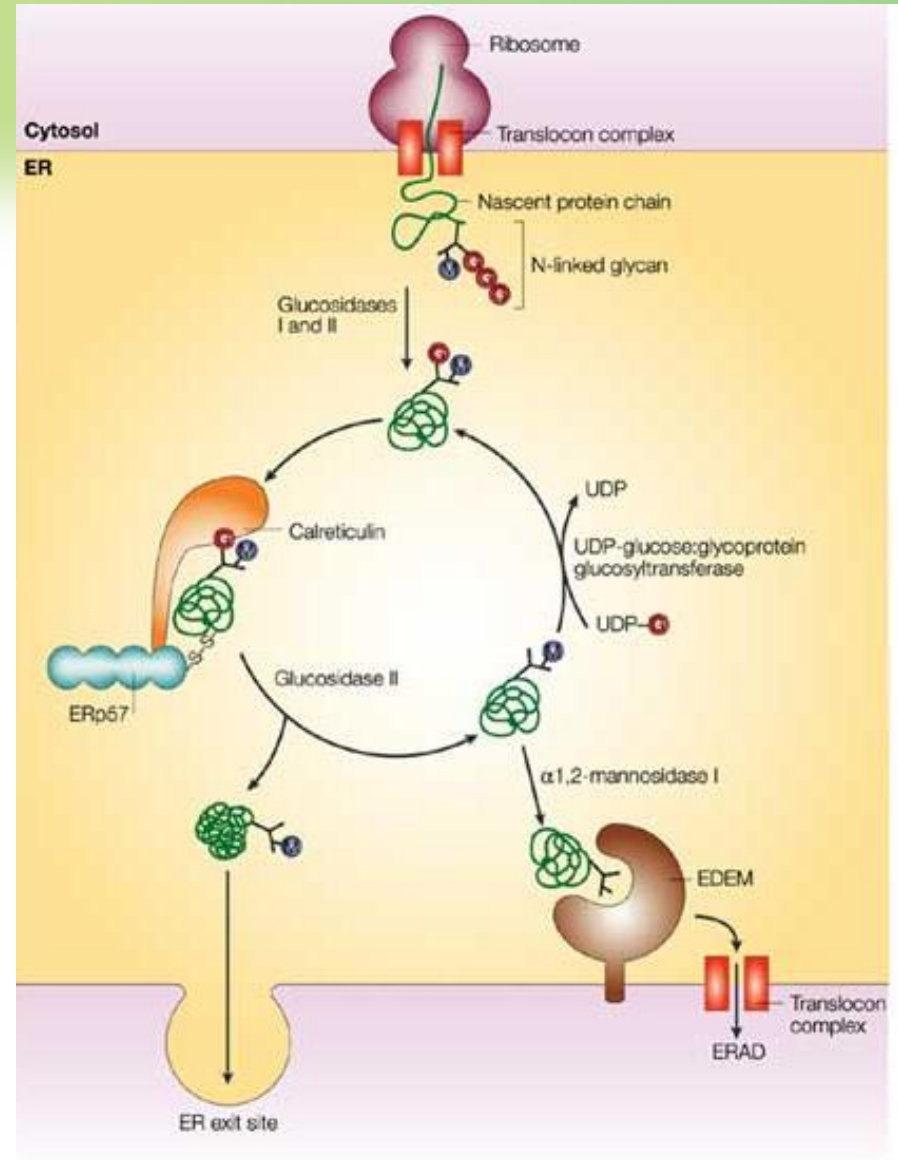


**Addition of glycolipid anchors to some plasma membrane proteins.**

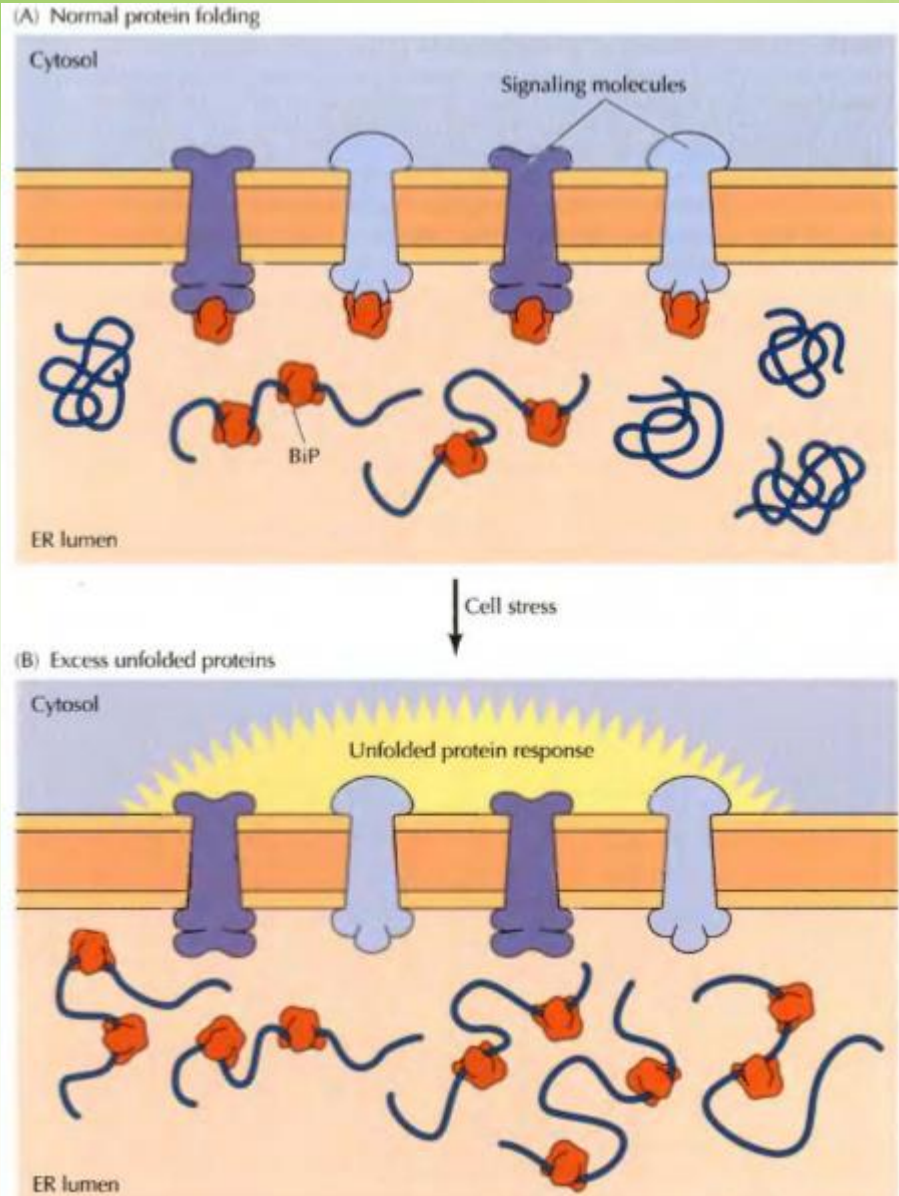
# Quality control in the ER

## 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 folded, the protein is degraded by ubiquitin proteosomal system



# Unfolded protein response (UPR)

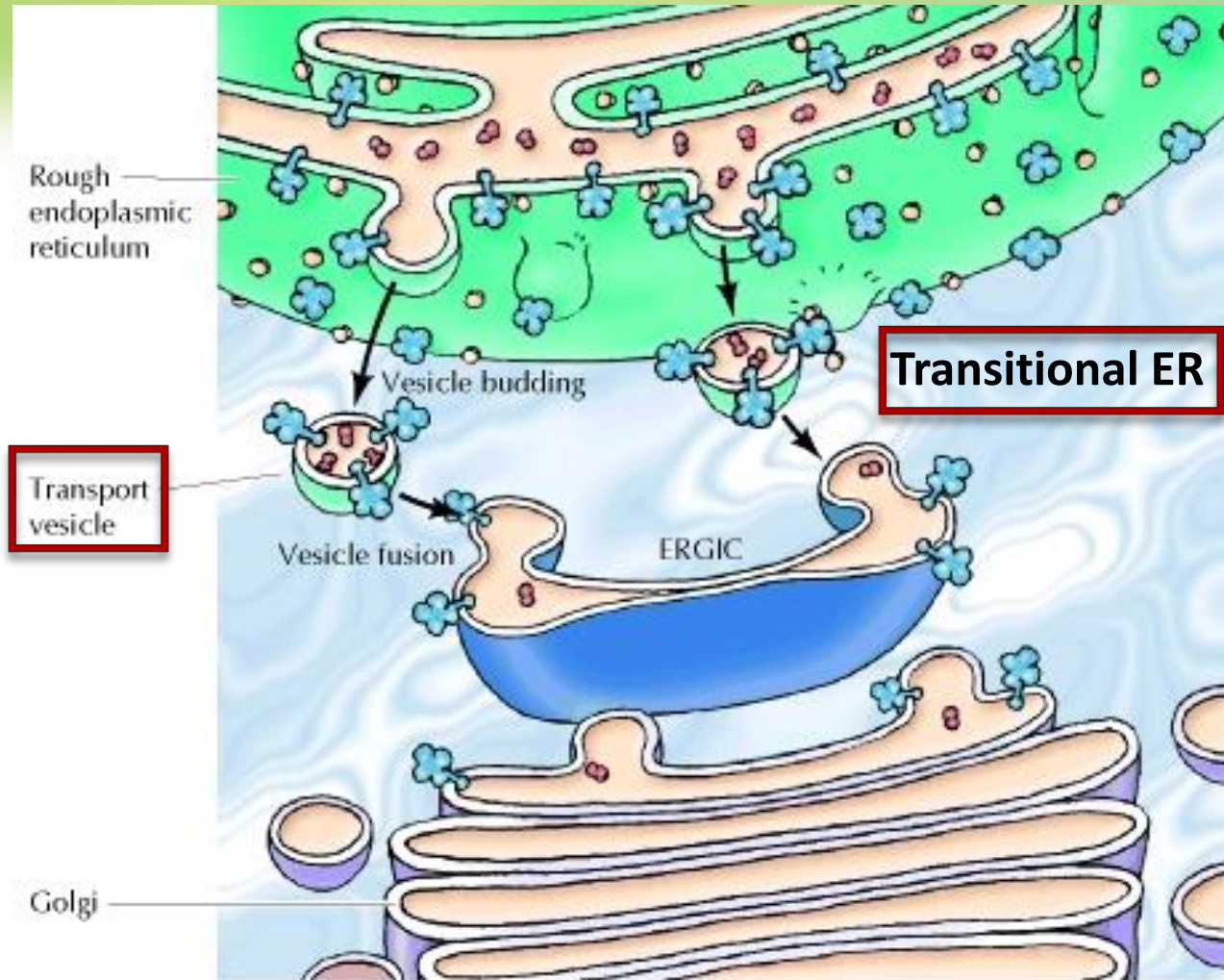


Read UPR in details Page 391-392

- ✓ 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**

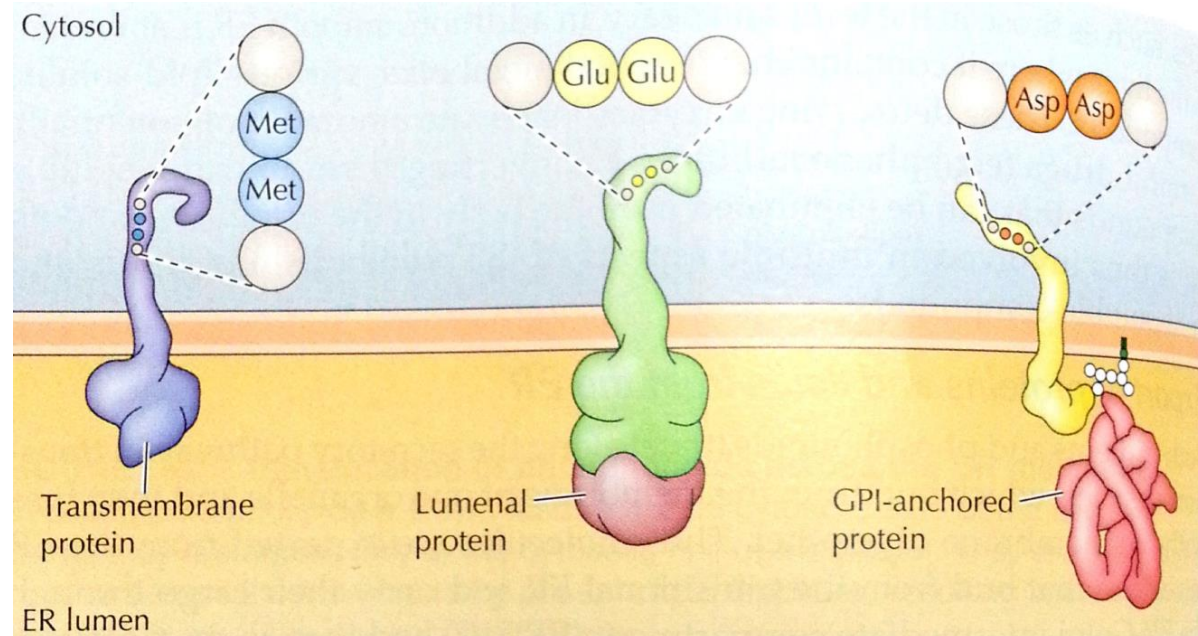


**Proteins and lipids are transported**

# Protein sorting and retention

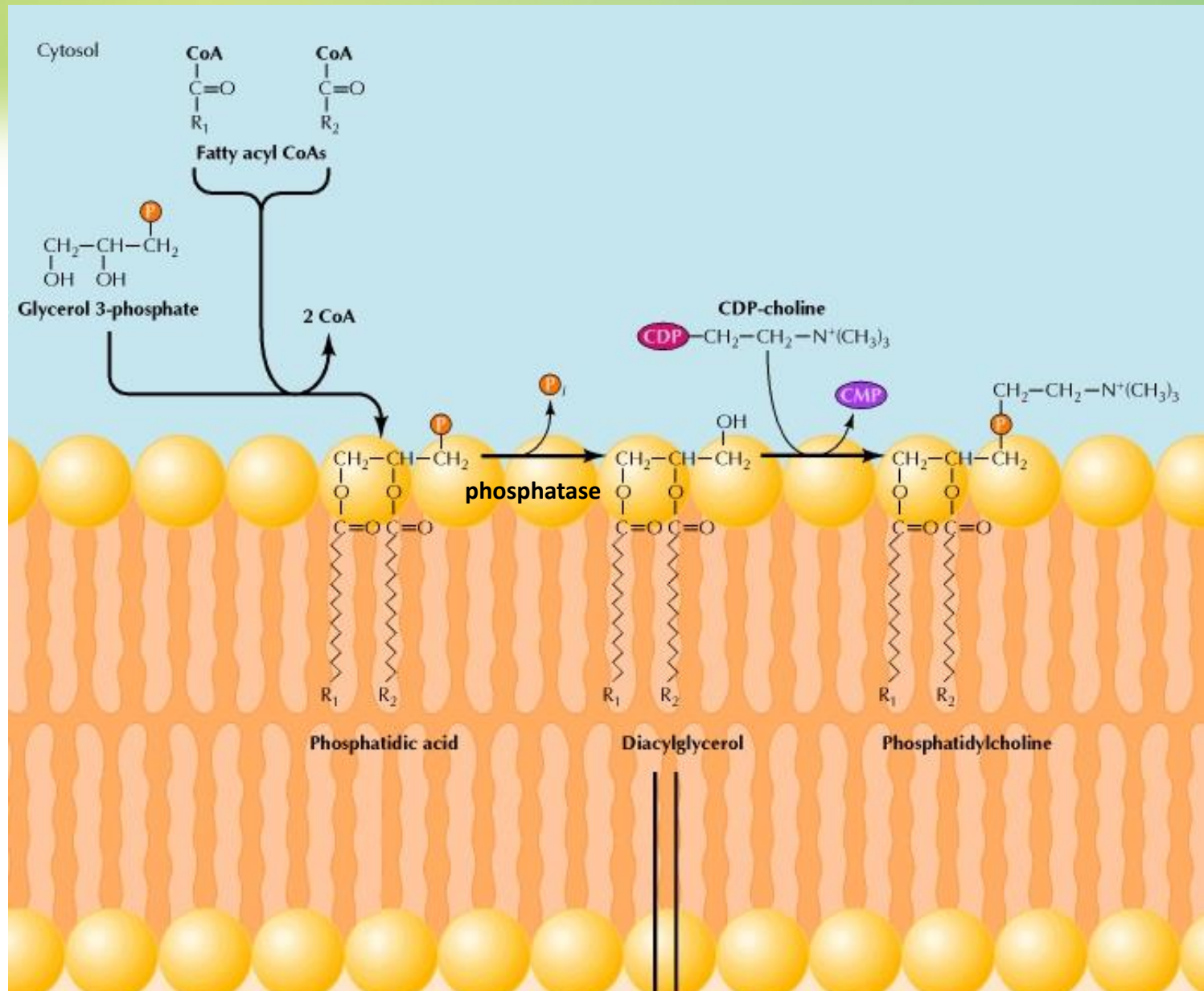
- Many proteins with KDEL sequence (Lys-Asp-Glu-Leu) at C-terminus are retained in the ER lumen.
  - ✓ If sequence is deleted, the protein is transported to the Golgi and secreted from the cell.
  - ✓ Addition of the sequence causes a protein to be retained in the ER.
- The retention of some transmembrane proteins in the ER is dictated by short C-terminal KKXX sequences.
- Proteins bearing the KDEL and KKXX sequences are 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 luminal proteins.**

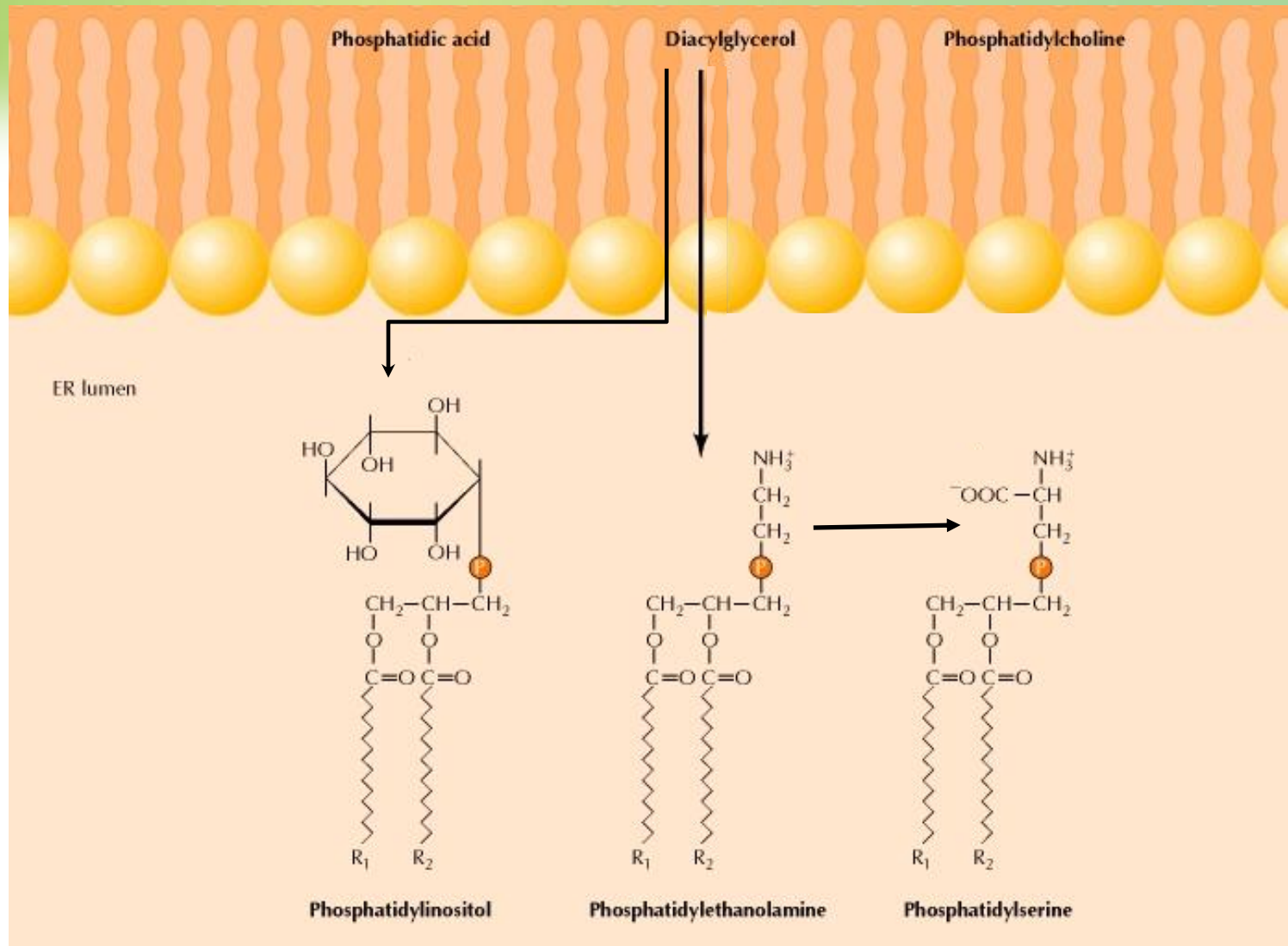


# Synthesis of phospholipids in SER

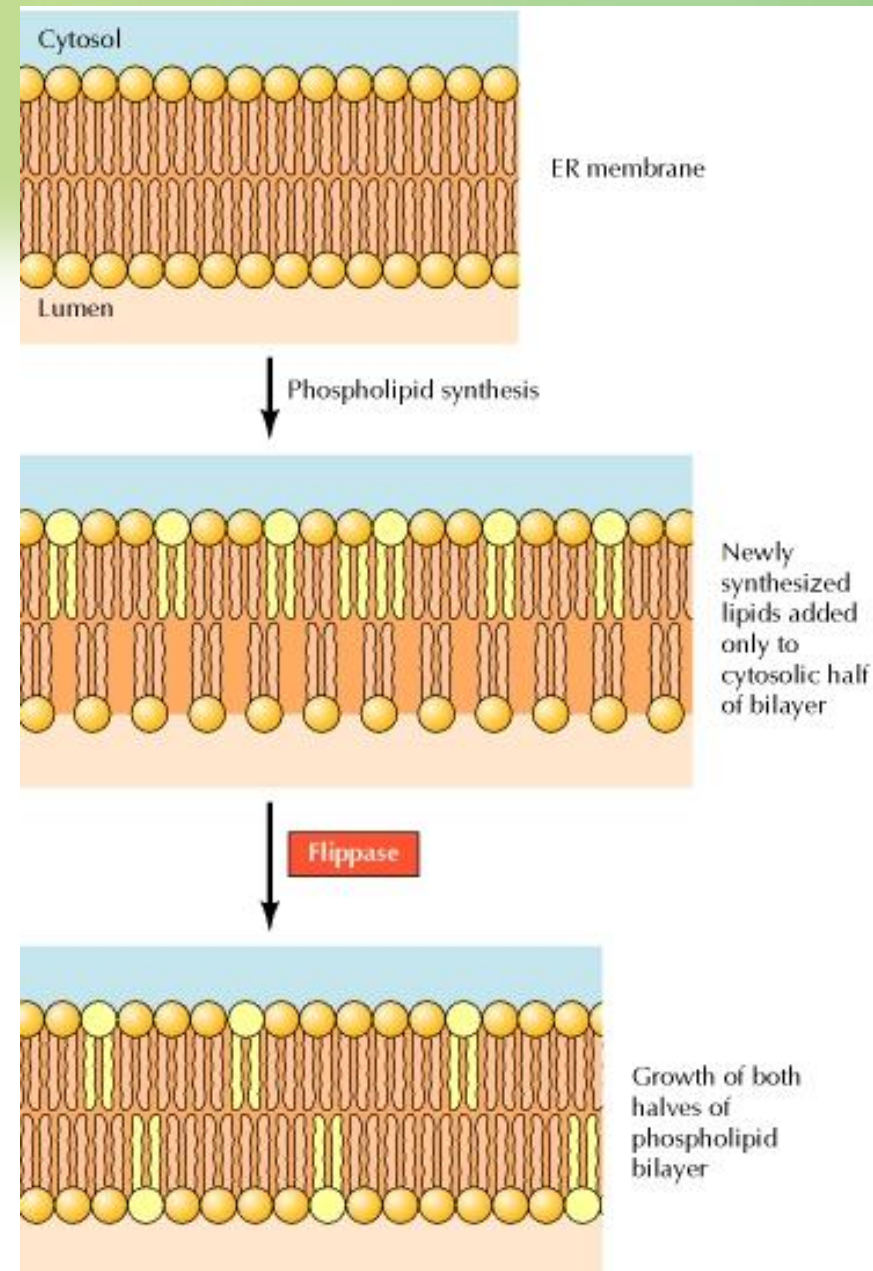
Enzymes are buried inside the membrane because the hydrophobic structure of lipids has to be maintained in close proximity to membranes



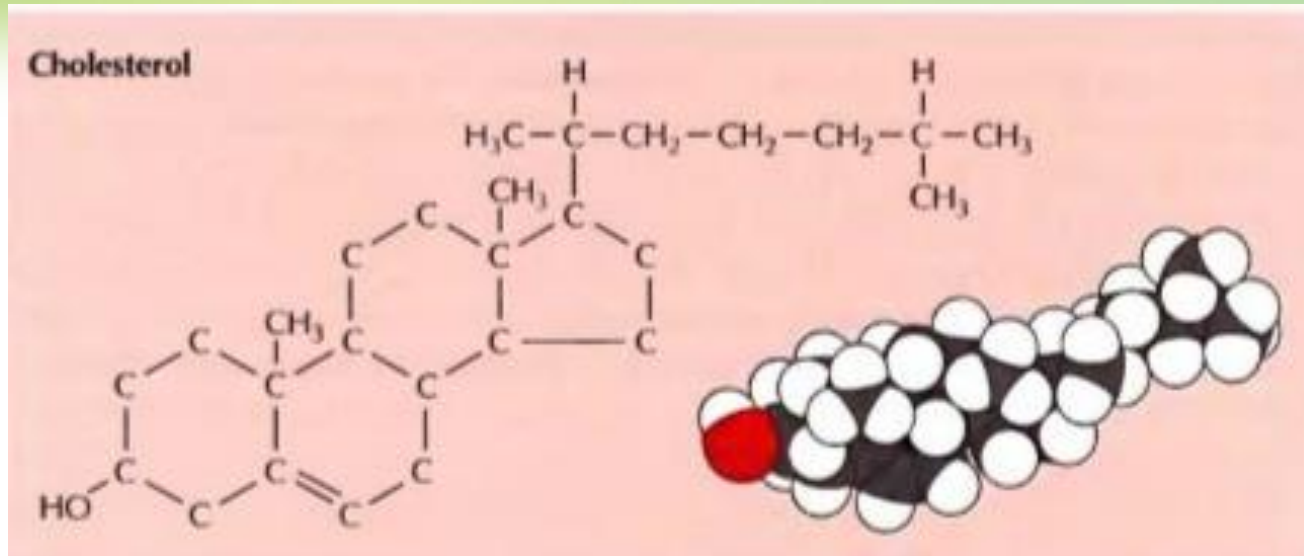
# Synthesis of phospholipids in SER



# Translocation of phospholipids across the ER membrane



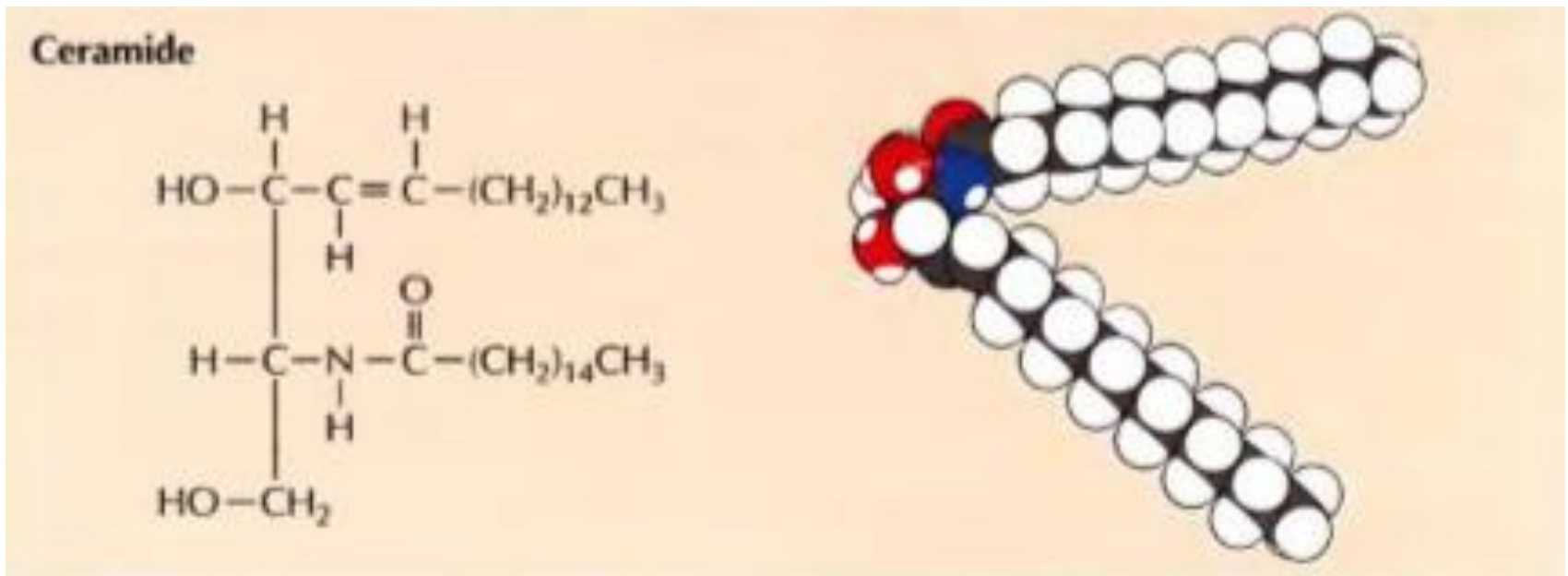
# Synthesis of cholesterol and its derivatives



**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**