



UNIVERSITY OF JORDAN
FACULTY OF MEDICINE
BATCH 2013-2019



GENETICS & MOLECULAR BIOLOGY

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Lecture #2

Title: Protein Sorting
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DESIGNED BY NADEEN AL-FREIHAT

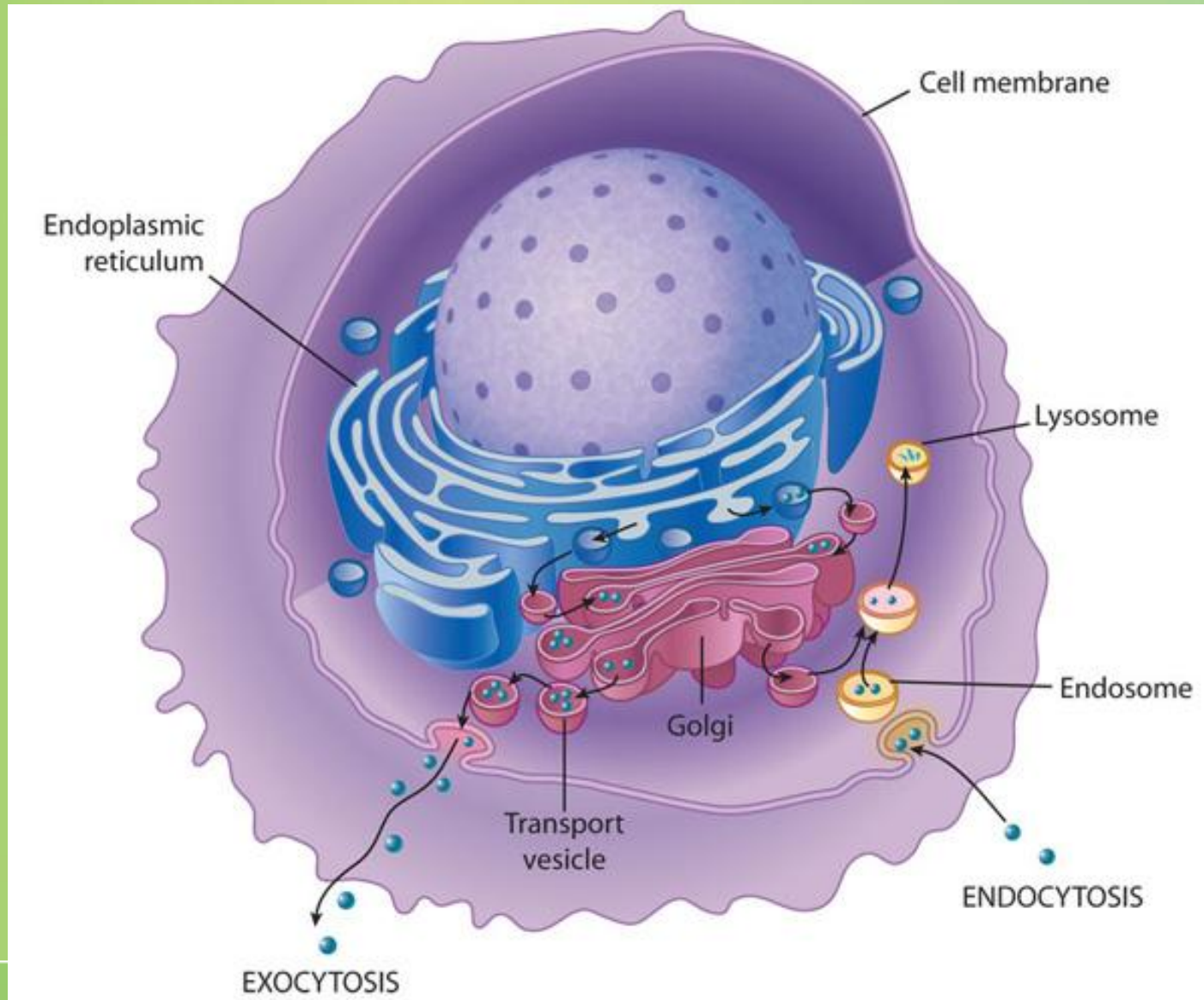


Lecture 2: Protein sorting (endoplasmic reticulum)

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Second year, Second semester, 2014-2014

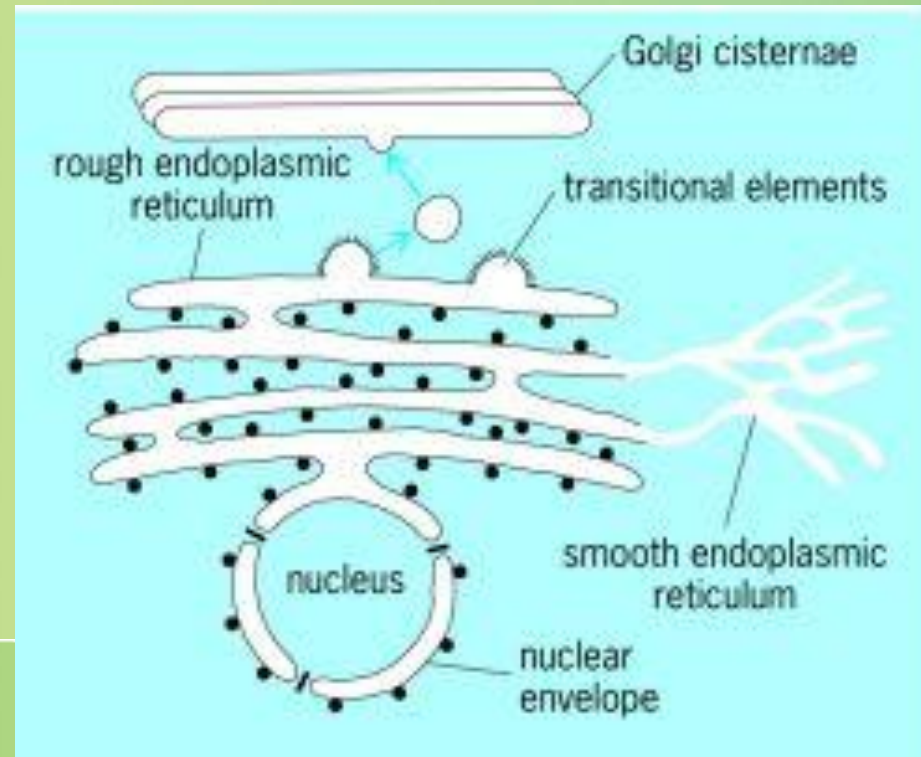
Principles of Genetics and Molecular Biology

An overview



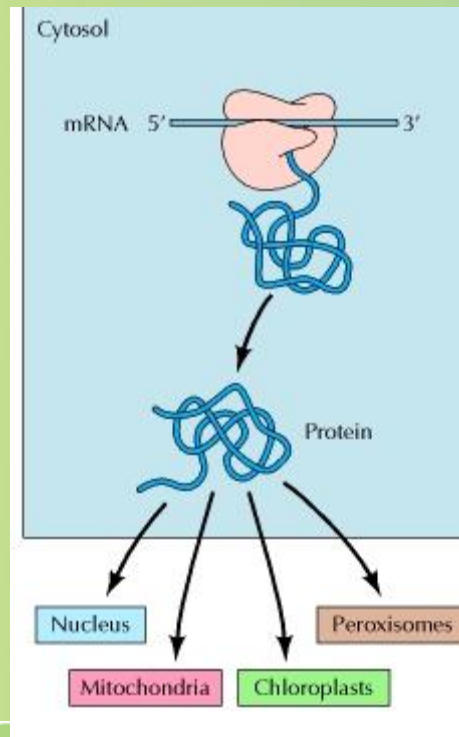
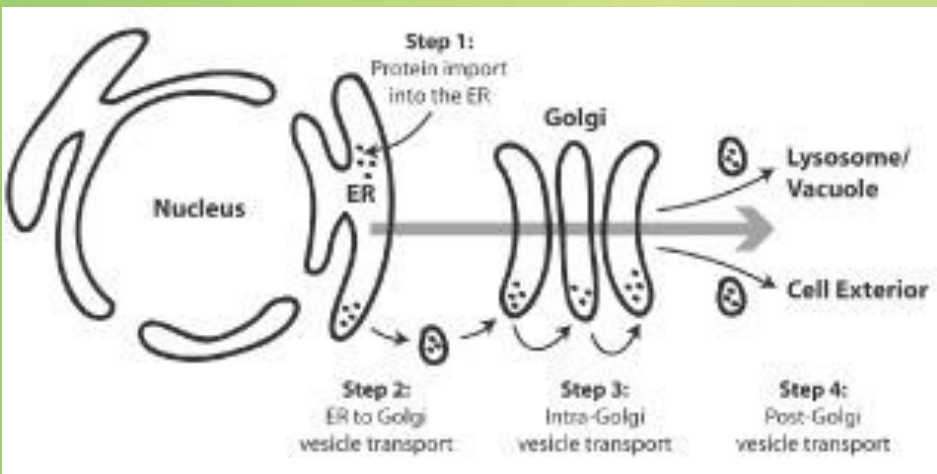
Endoplasmic reticulum (ER)

- 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

- Secretory, ER, Golgi apparatus, and lysosomal proteins are initially targeted to the ER.
 - Most proteins are transferred into the ER while they are being translated on membrane-bound ribosomes (cotranslational translocation).



Cytosolic, nuclear, peroxisomal, and mitochondrial proteins are synthesized on free ribosomes and released into the cytosol.

Ribosomal and protein targeting



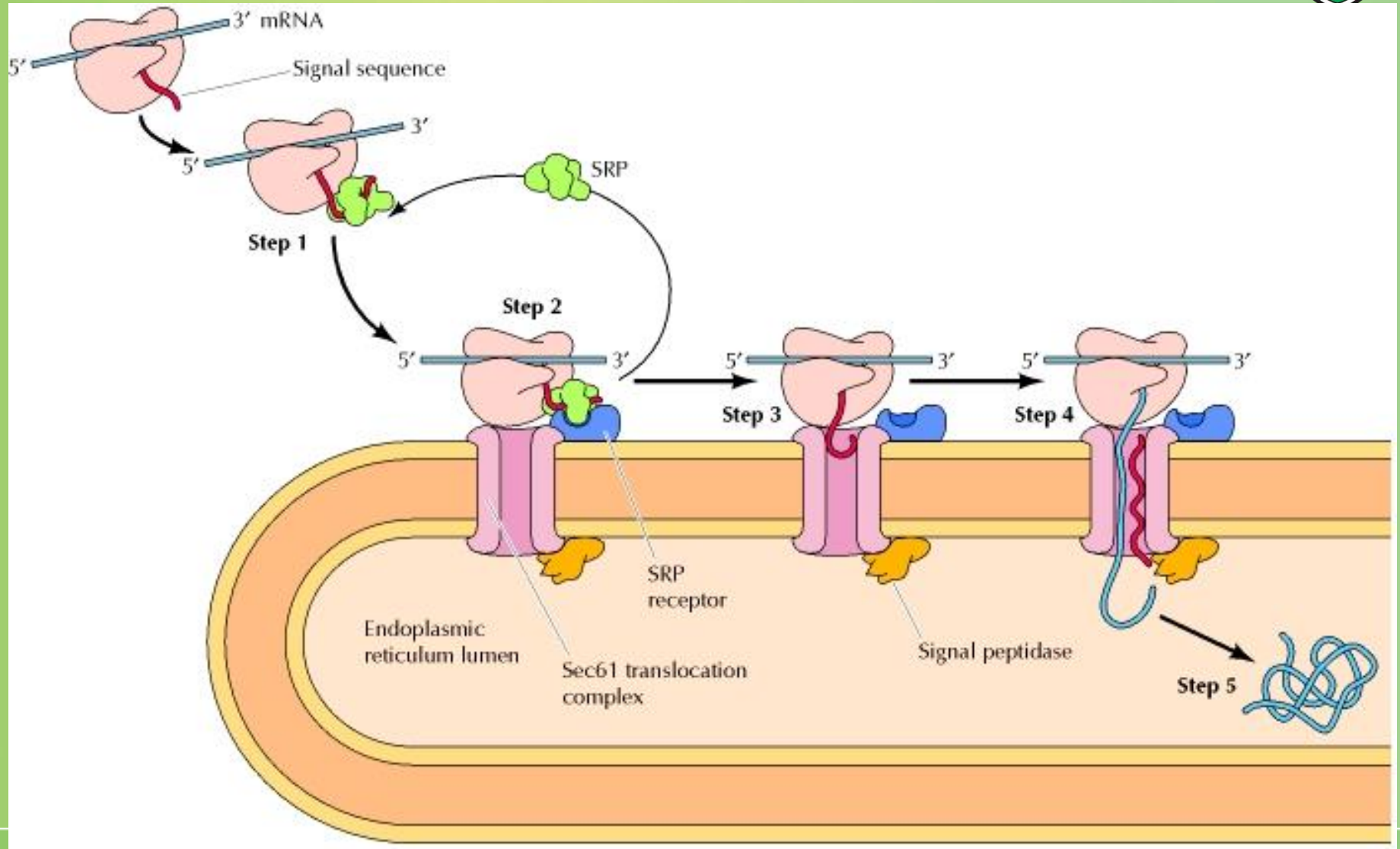
- All protein synthesis initiates on ribosomes that are free in the cytosol.
- Ribosomes are targeted for binding to the ER membrane by the amino acid sequence of the polypeptide at the amino terminus called a signal sequence.
- It is then cleaved from the polypeptide chain during its transfer into the ER lumen.



Mechanism of translocation

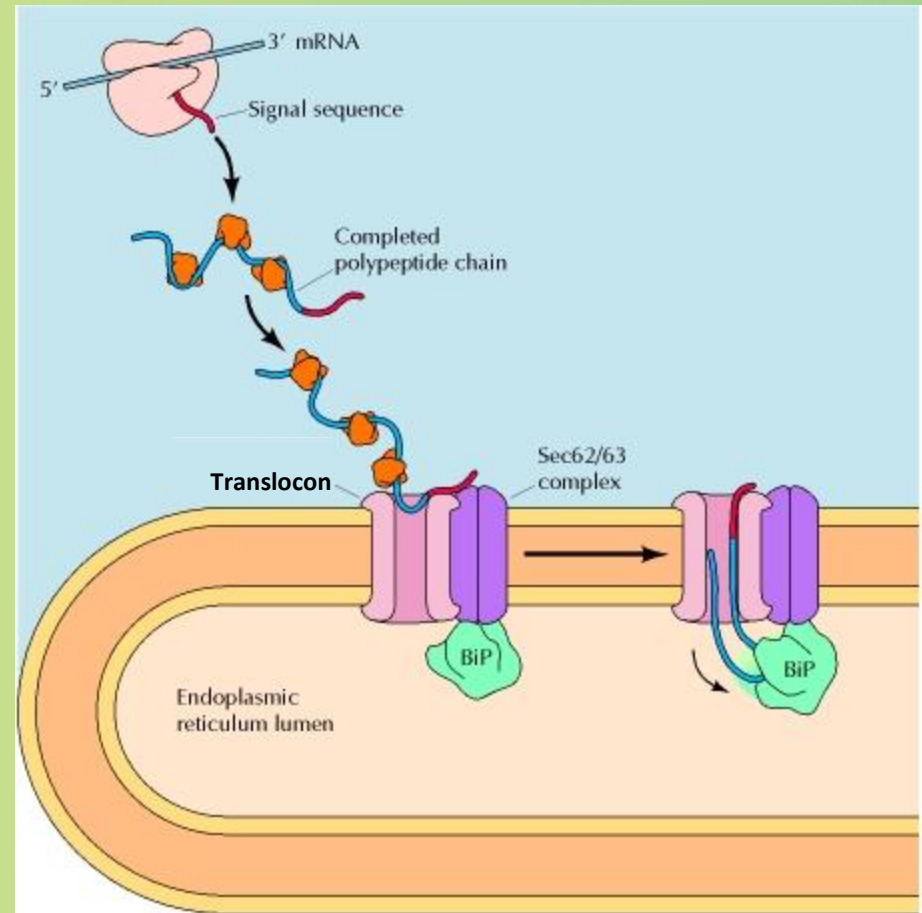


- **Step 1:** As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).
- **Step 2:** The SRP escorts the complex to the ER membrane, where it binds to the SRP receptor.
- **Step 3:** The SRP is released, the ribosome binds to a membrane translocation complex of 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

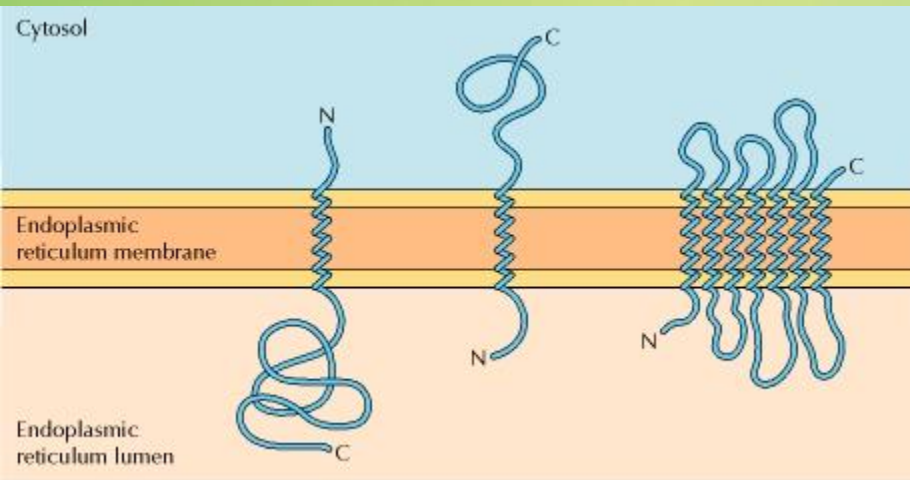
- Proteins are synthesized on free ribosomes and remain unfolded by cytosolic chaperones.
- Their signal sequences are recognized by a protein complex, which is associated with the translocon in the ER membrane.
- The protein complex is also associated with a chaperone protein (BiP), which drives protein translocation into the ER.



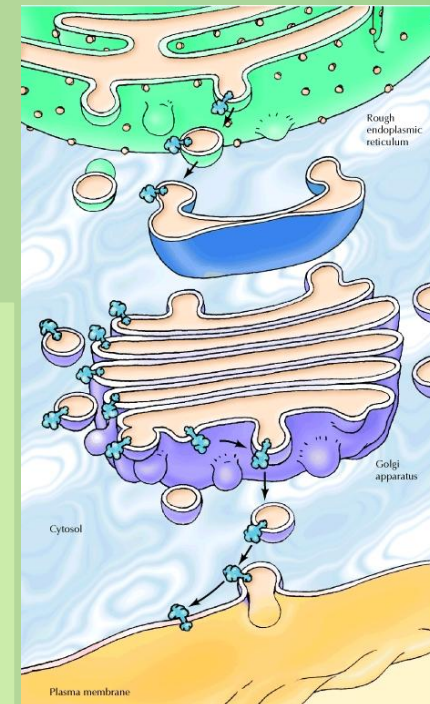
Pathways of protein sorting



- Secretory, ER, Golgi apparatus, and lysosomal proteins are released into the lumen of the ER.
- Membranous proteins are initially inserted into the ER membrane.
- Considerations
 - Single vs. multiple membrane spanning region
 - Orientation of N- and C-termini



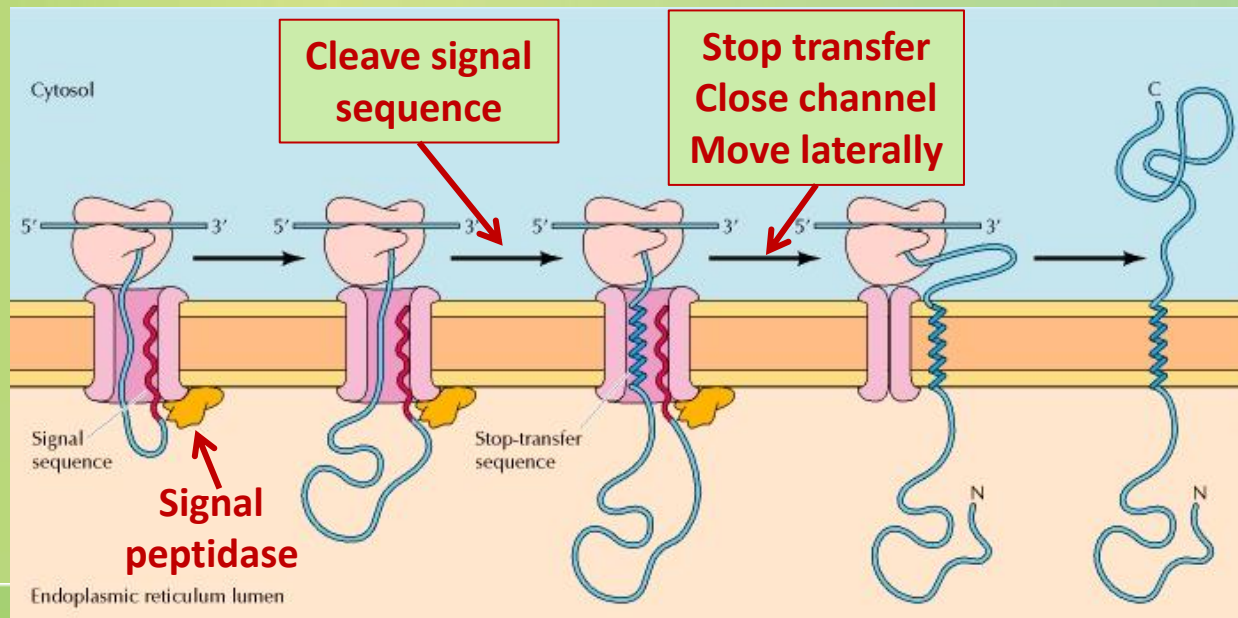
The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.



Insertion of a membrane protein (1)

N-terminus: in C-terminus: out

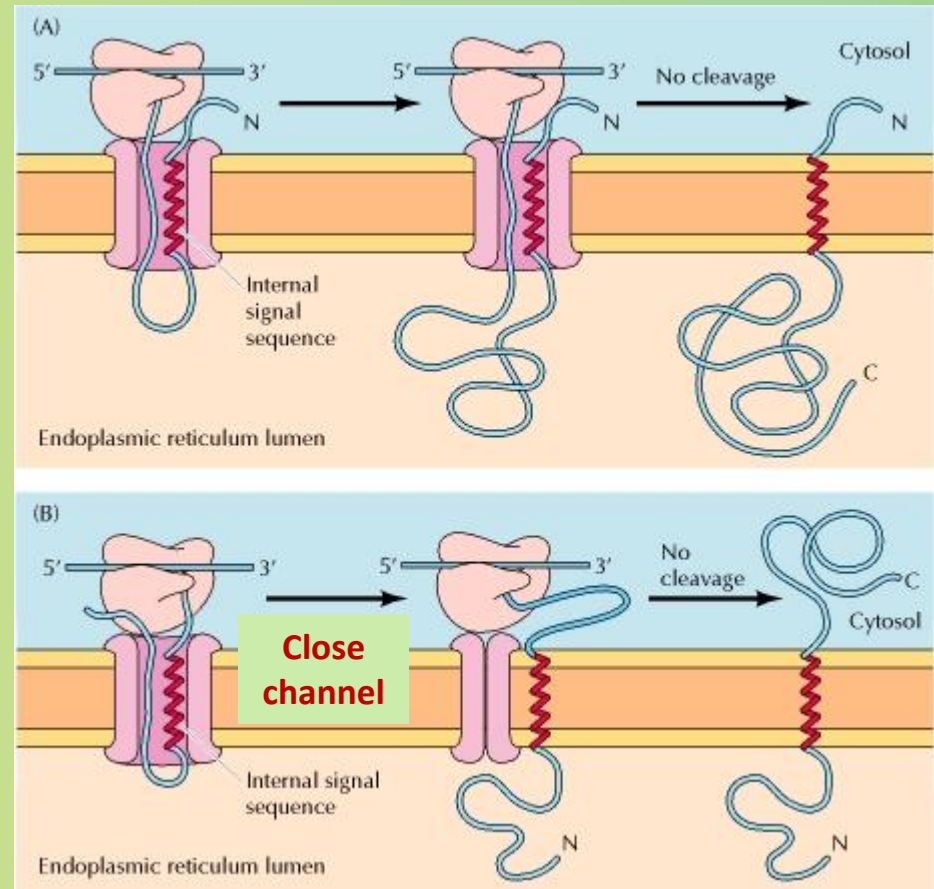
- The insertion of such proteins in the membrane involves two distinct elements:
 - A cleavable amino-terminal signal sequence that initiates translocation across the membrane
 - A transmembrane stop-transfer sequence that anchors the protein in the membrane.



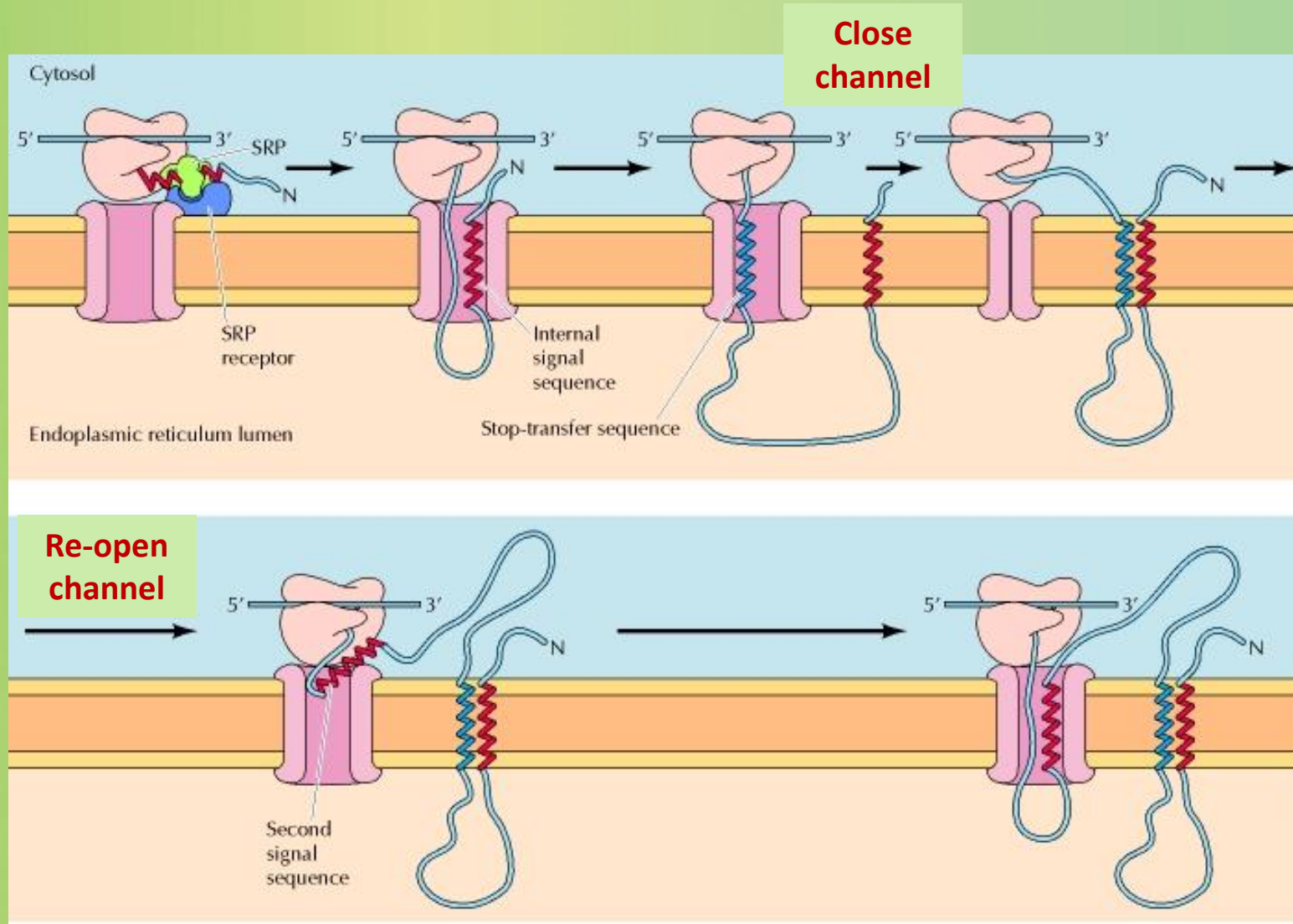
Insertion of a membrane protein (2)



- The signal sequence is not cleaved and acts as a membrane-spanning sequence.

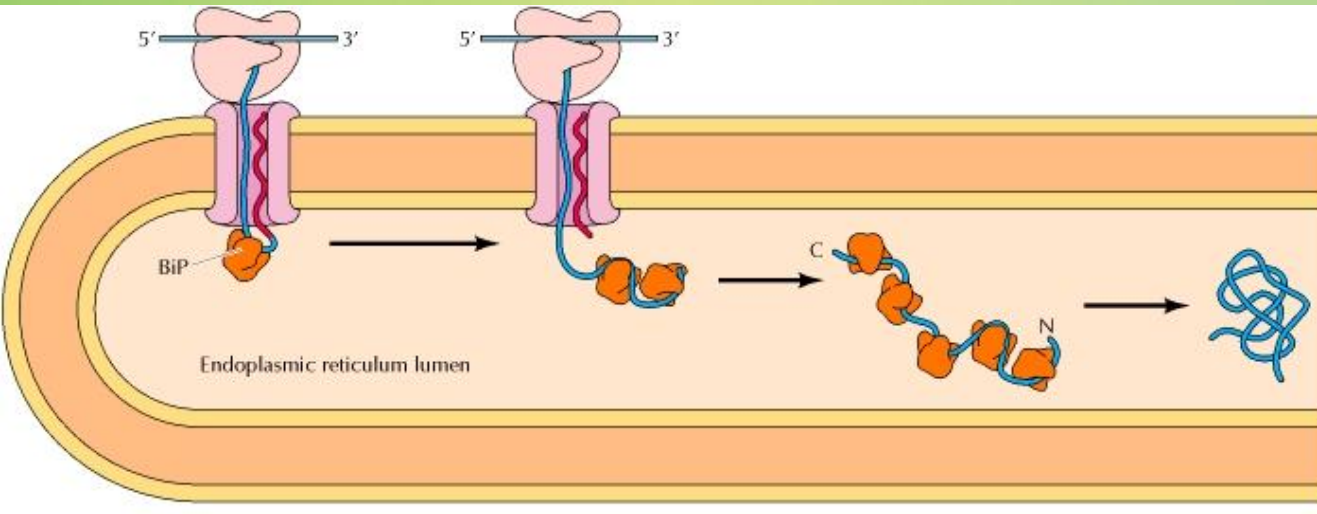


Insertion of a membrane protein (3)



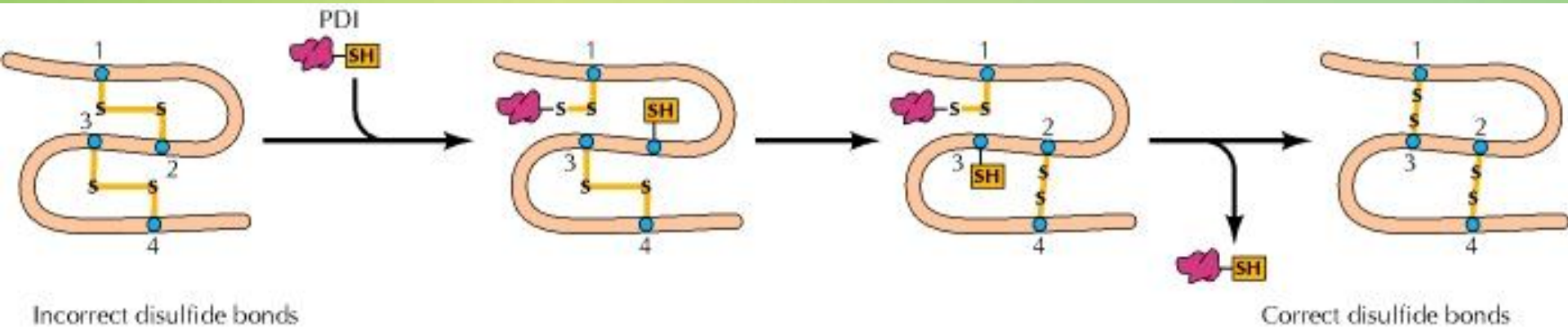
Once inside...

Assembly of multisubunit proteins

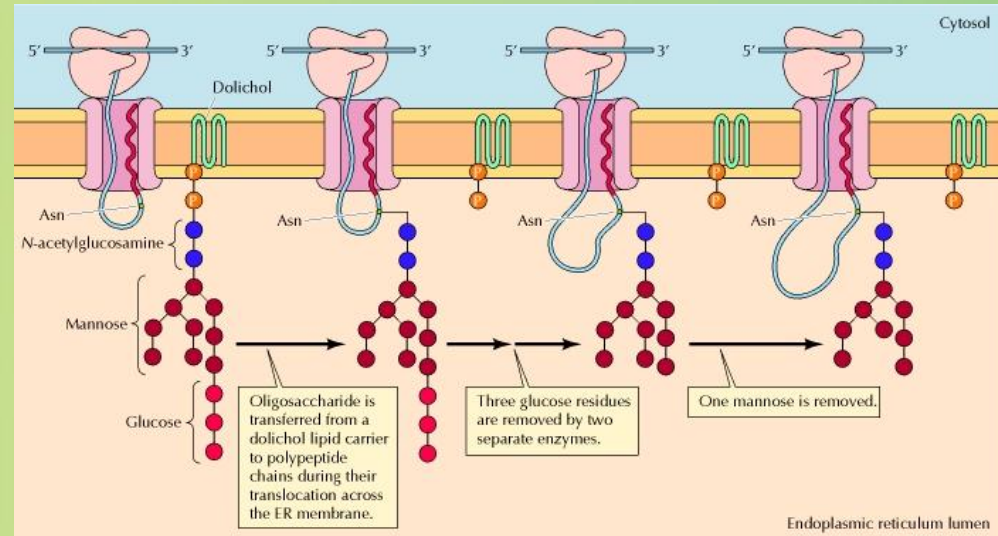
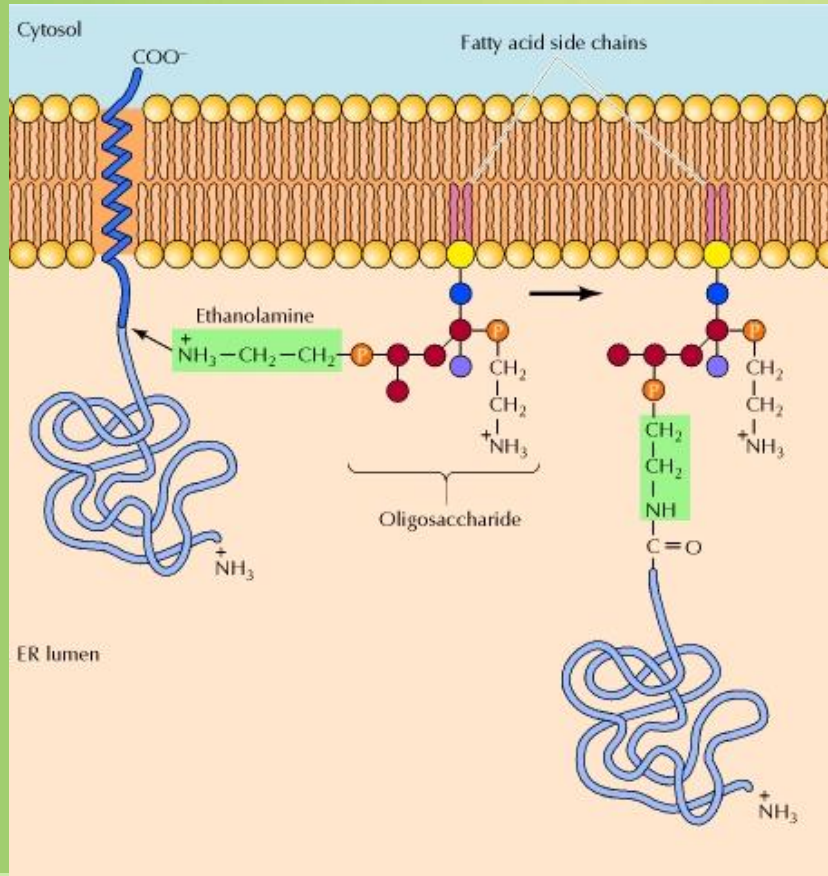


protein folding, assisted by the molecular chaperone, that keep protein folded until properly folded

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



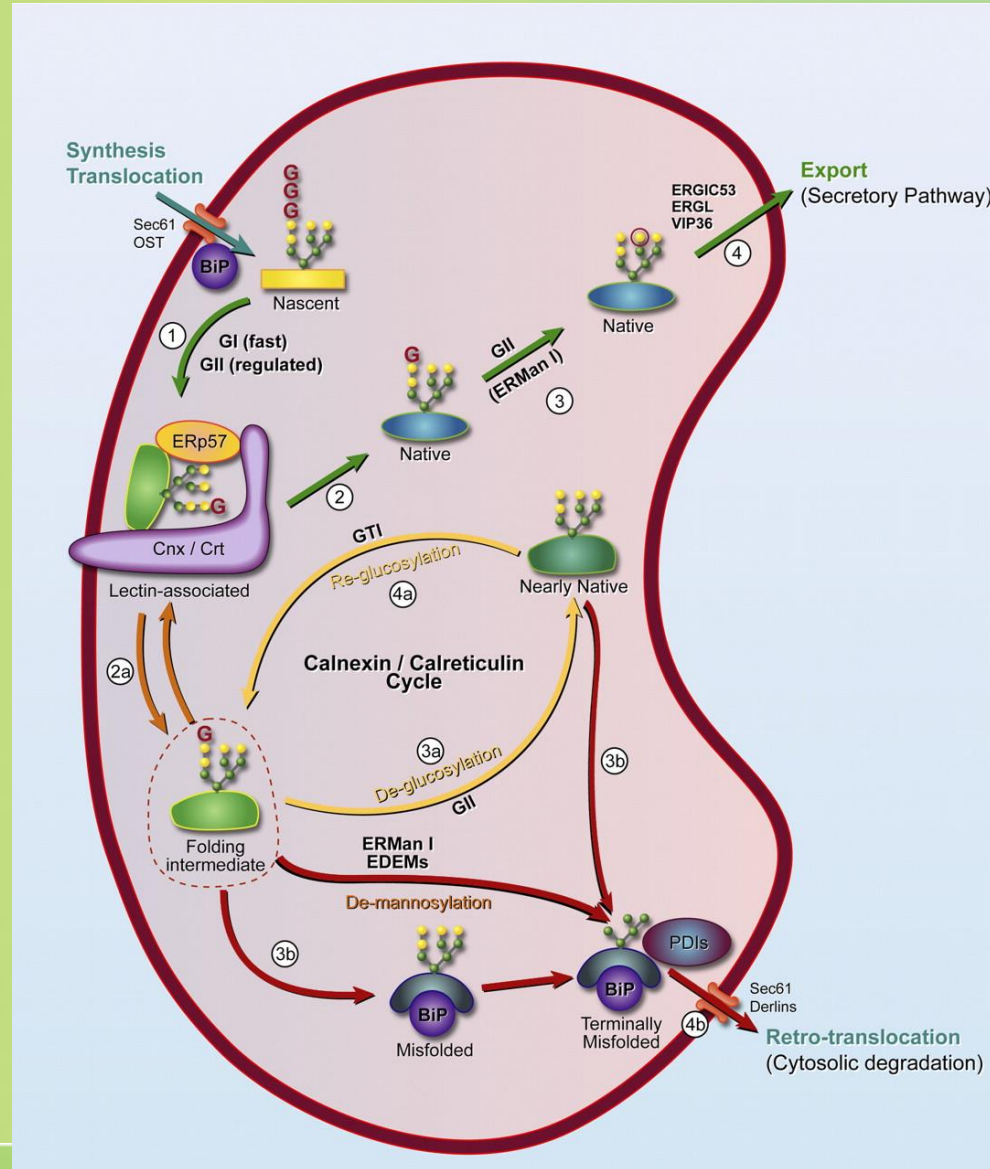
Once inside...



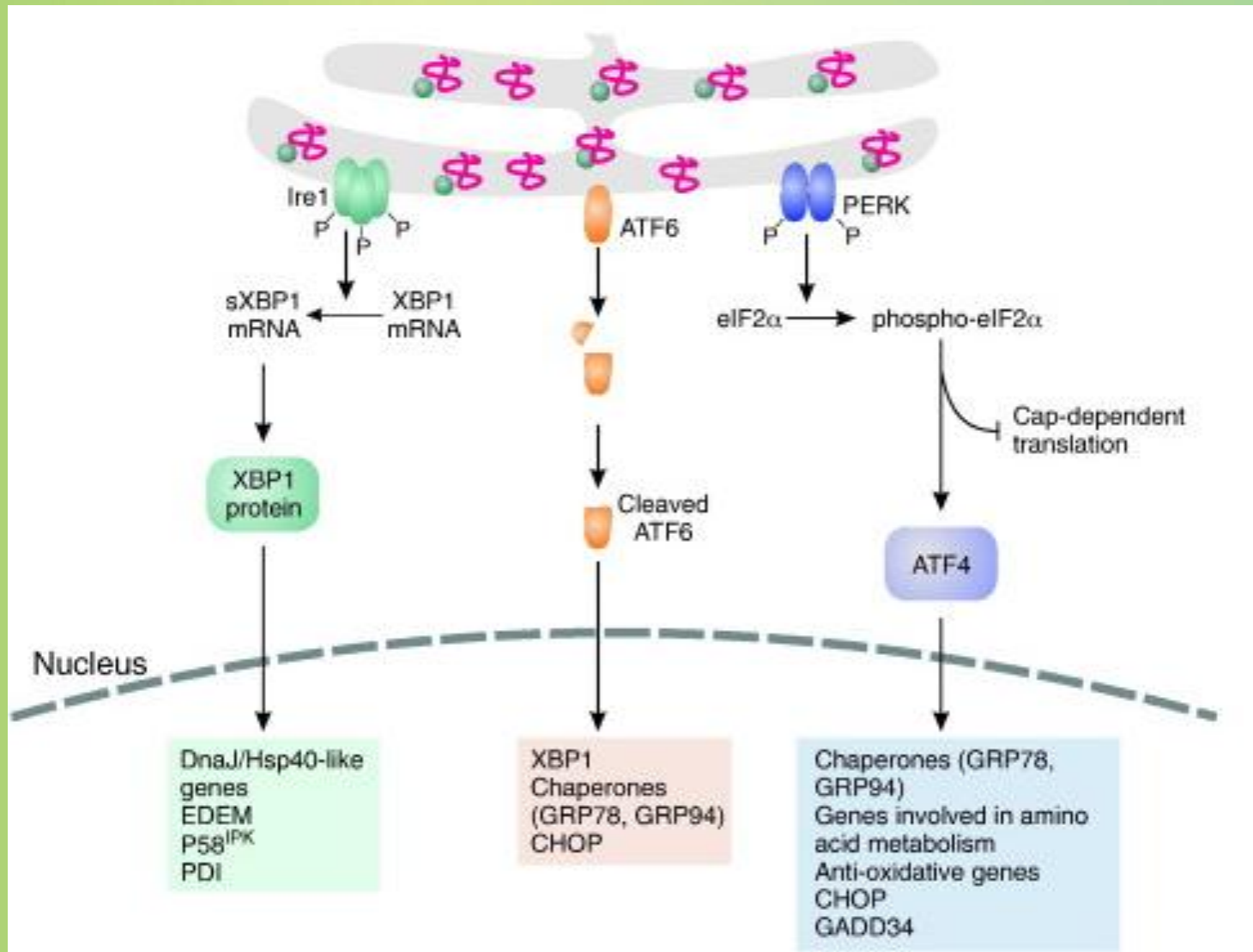
**the initial stages
of glycosylation
at Asn-X-Ser/Thr**

Addition of glycolipid anchors to some plasma membrane proteins.

Fate of a glycoprotein



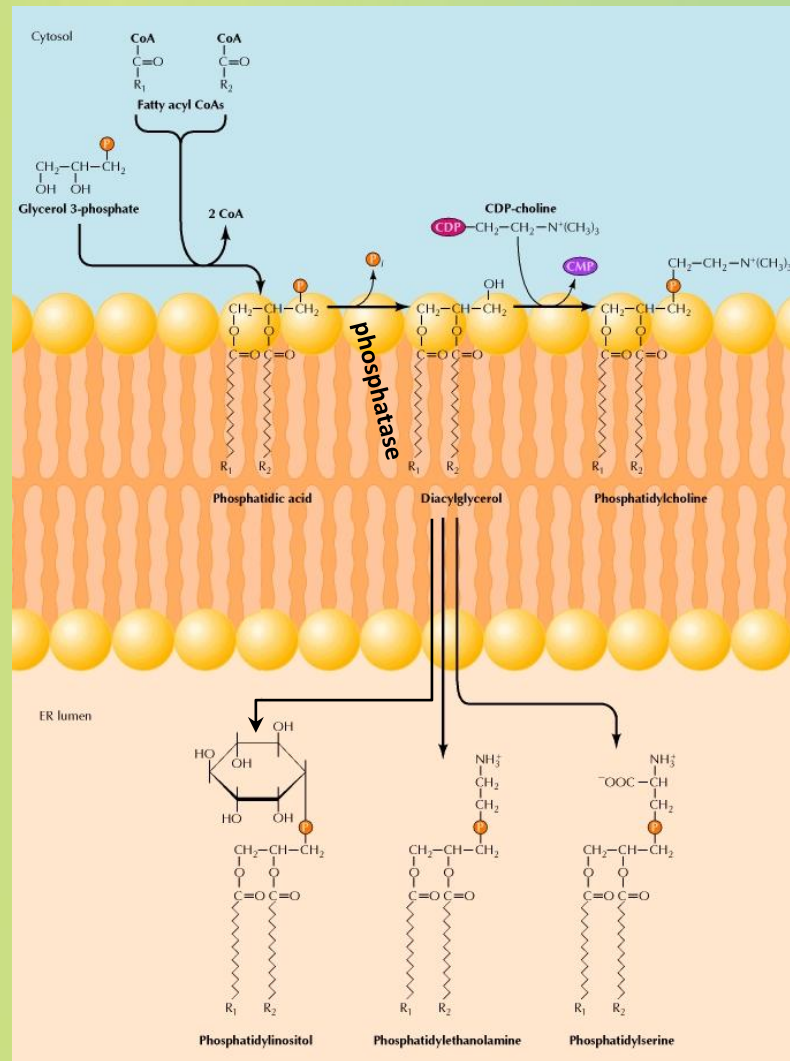
Unfolded protein response (UPR)



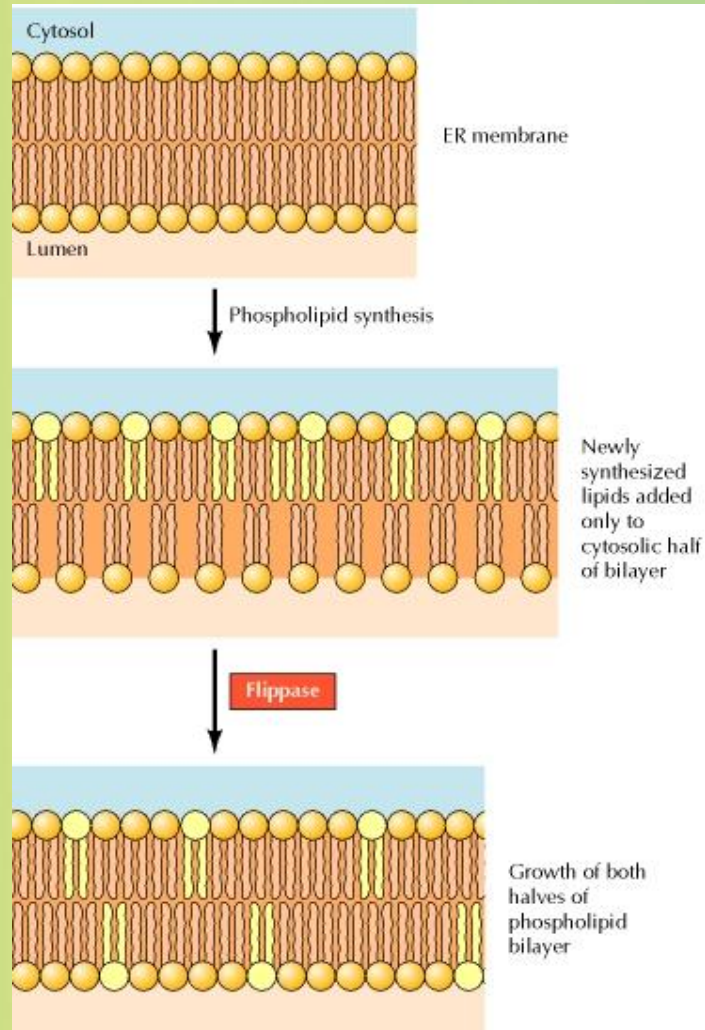
Synthesis of phospholipids in ER



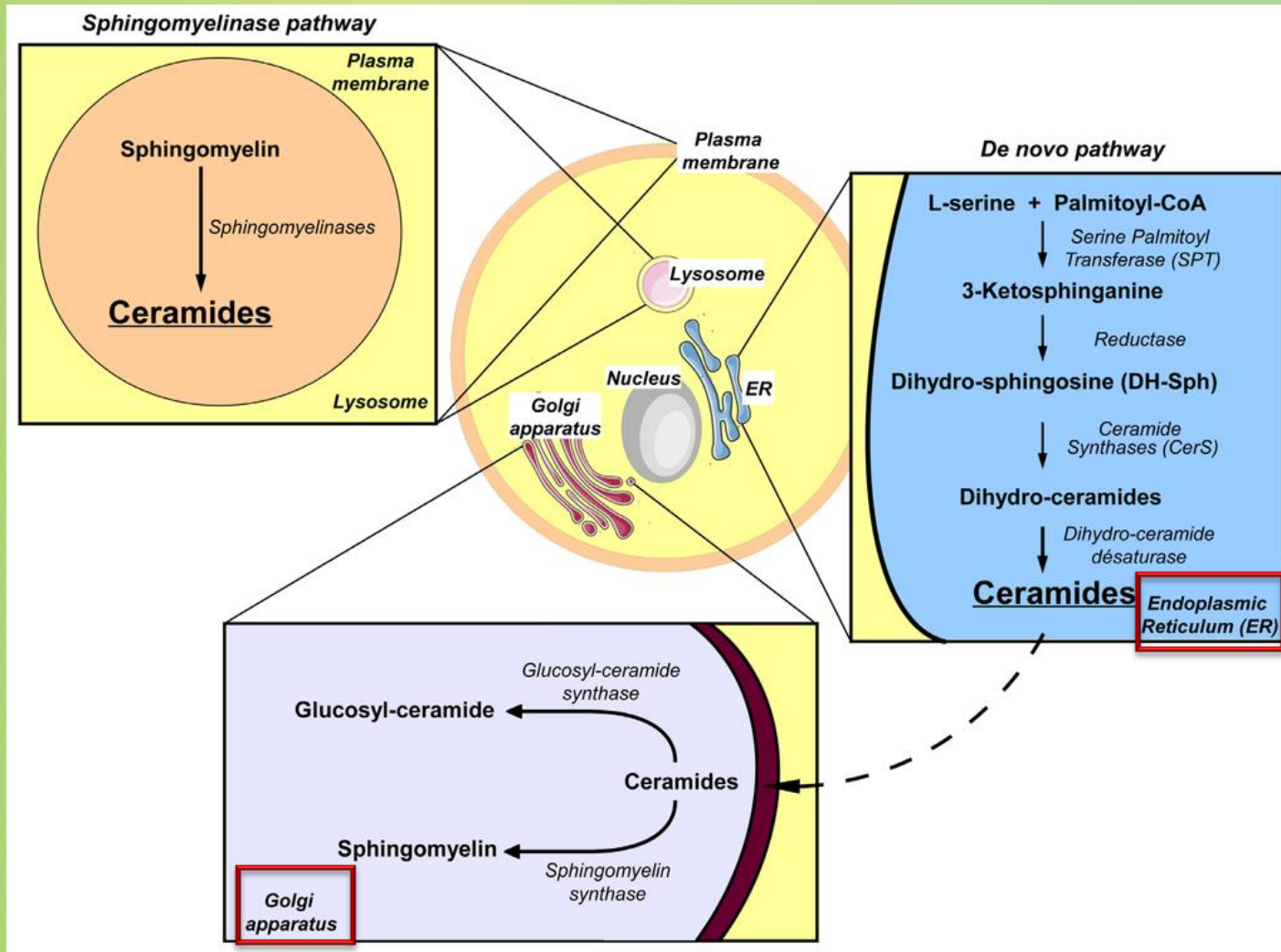
Enzymes are buried inside the membrane



Translocation of phospholipids across the ER membrane



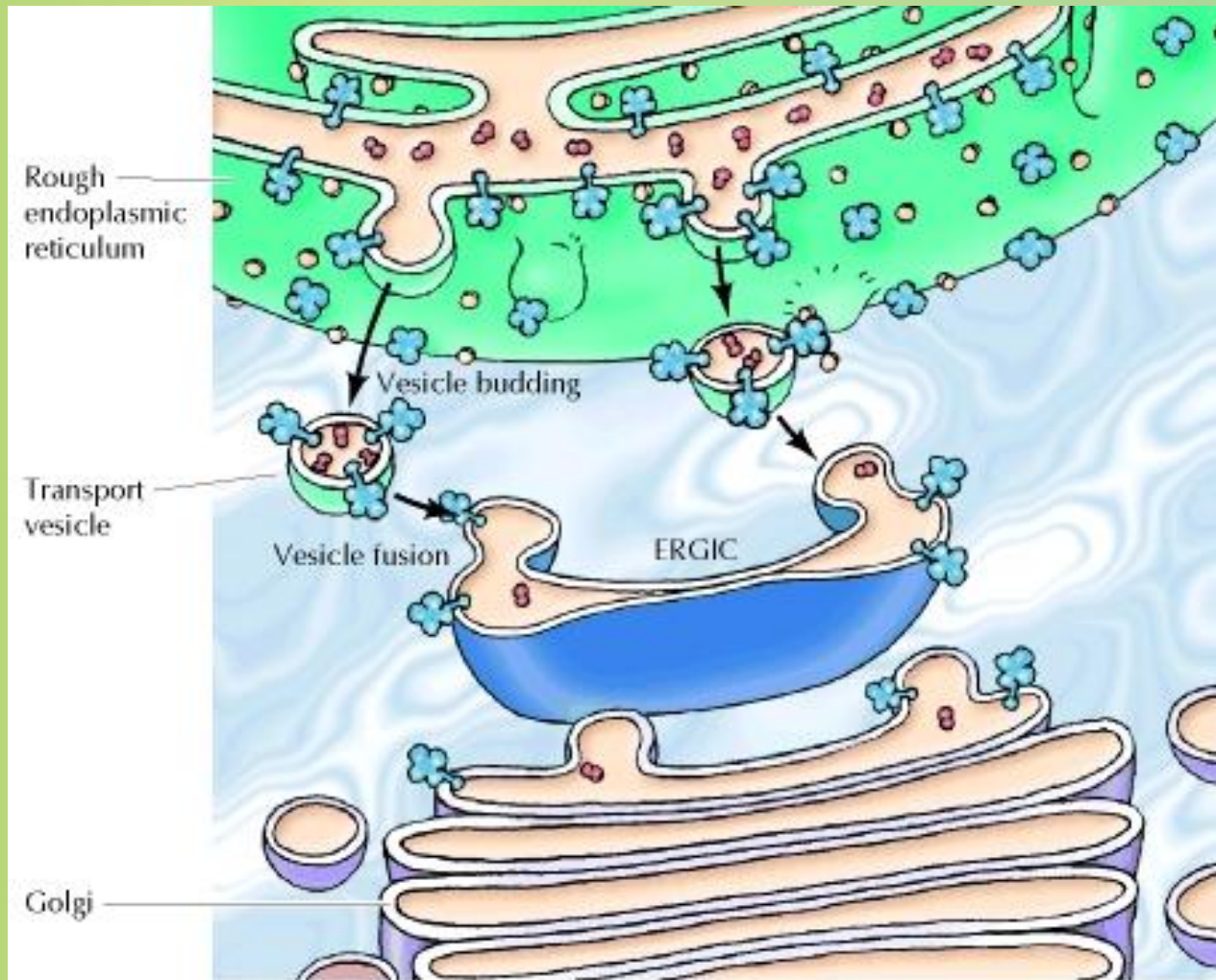
Synthesis of ceramide



ER-Golgi intermediate compartment (ERGIC)



Note that topological orientation is maintained

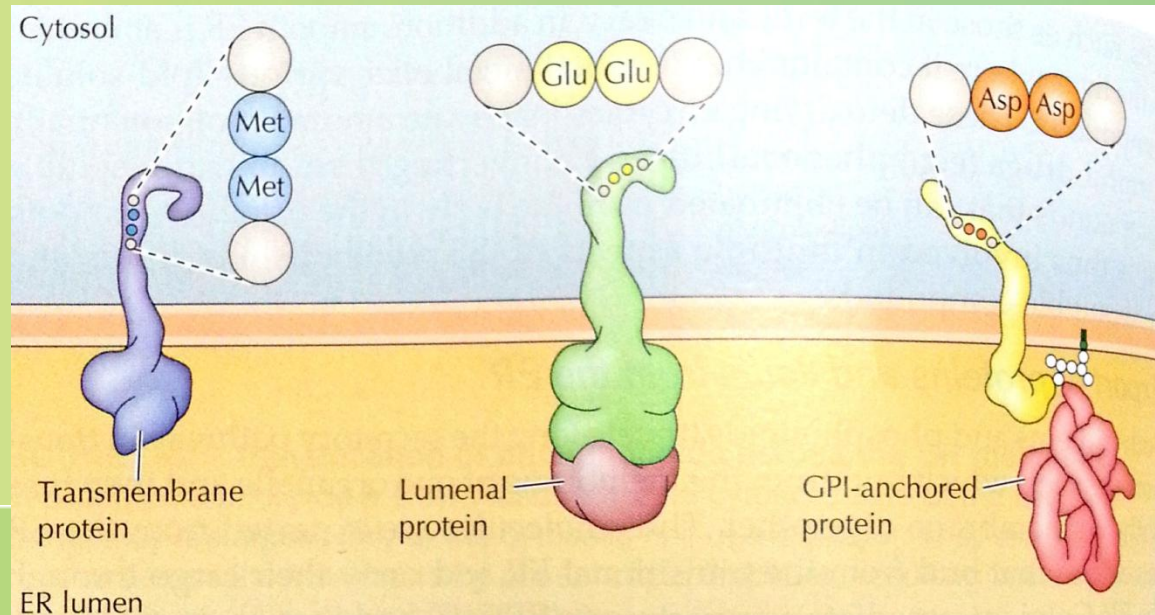


Protein sorting and retention

- 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 appear to be recycled back to the ER.

Membrane proteins contain di-acidic or di-Met signal sequences.

They can also function as carriers of GPI-anchored and luminal proteins.



Synthesis of other lipids



- **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
- **Smooth ER is abundant in the liver, where it 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