C4 T – CELL BIOLOGY

UNIT 3: Cytoplasmic organelles I

Protein sorting & Mechanism of vesicular transport (Part – I)

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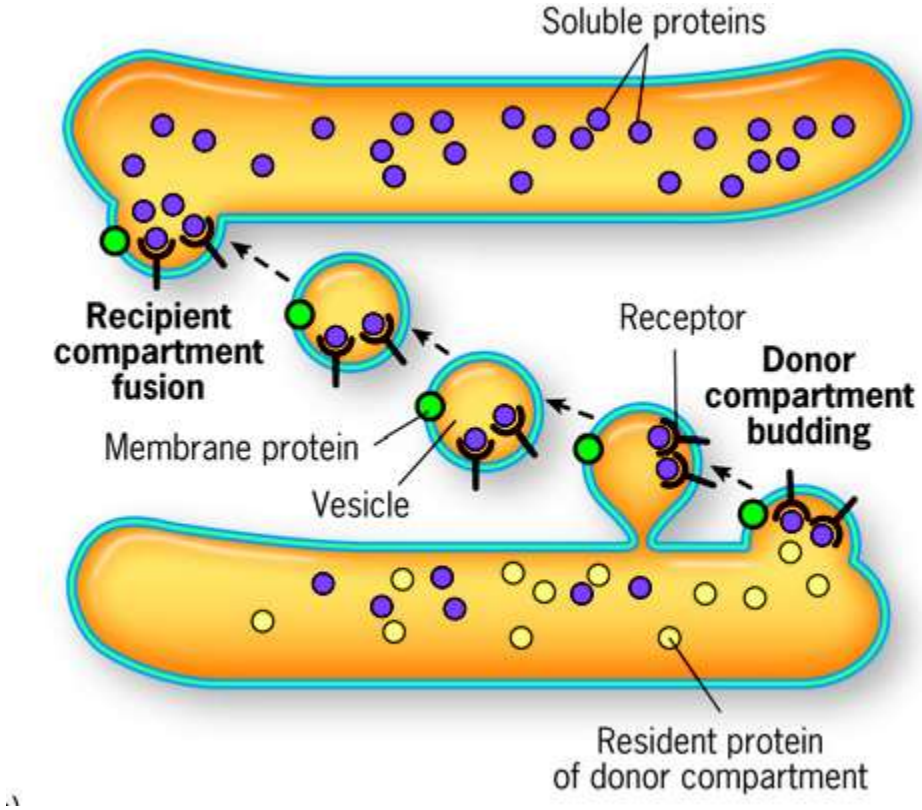
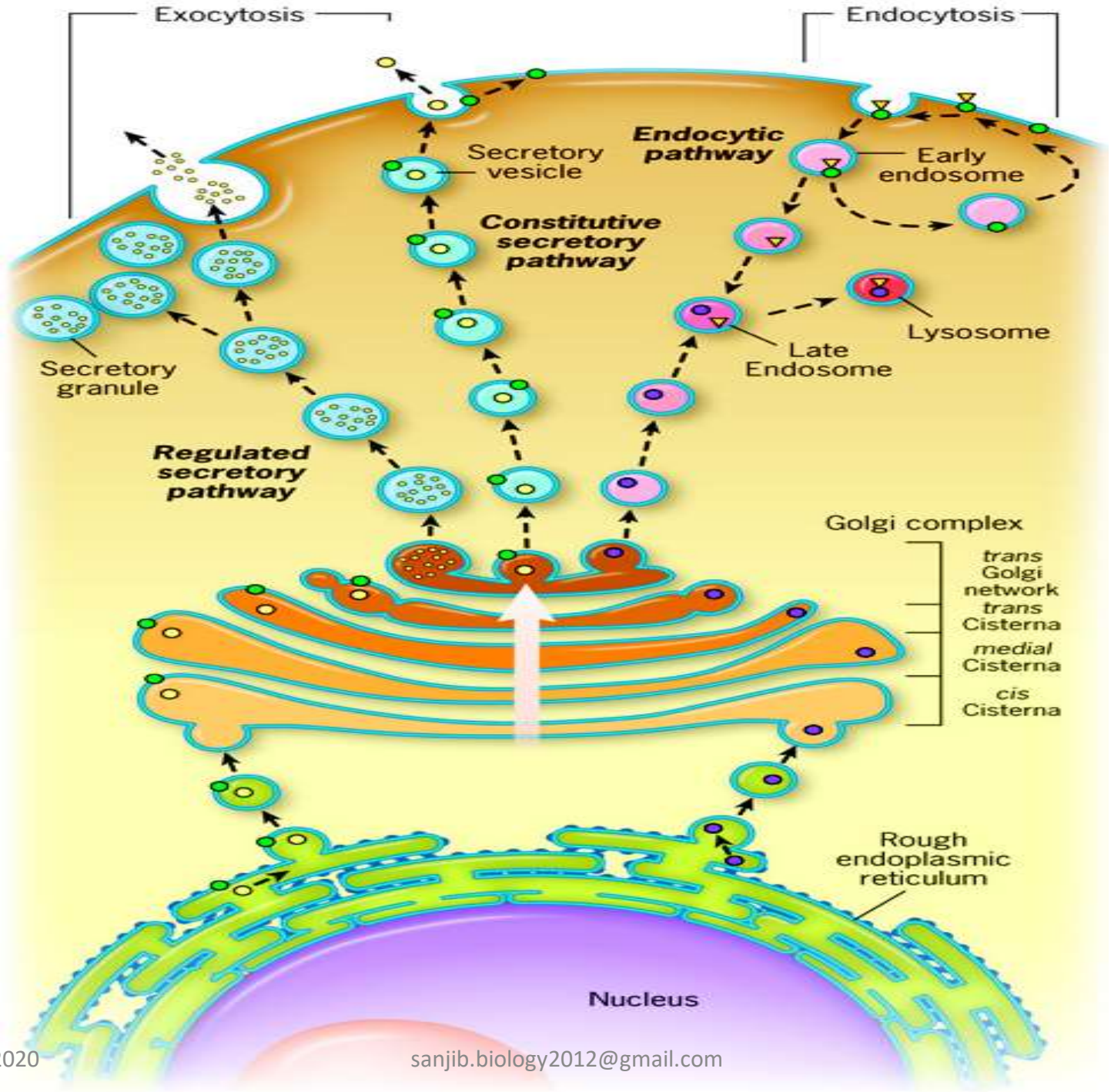
The Endomembrane System



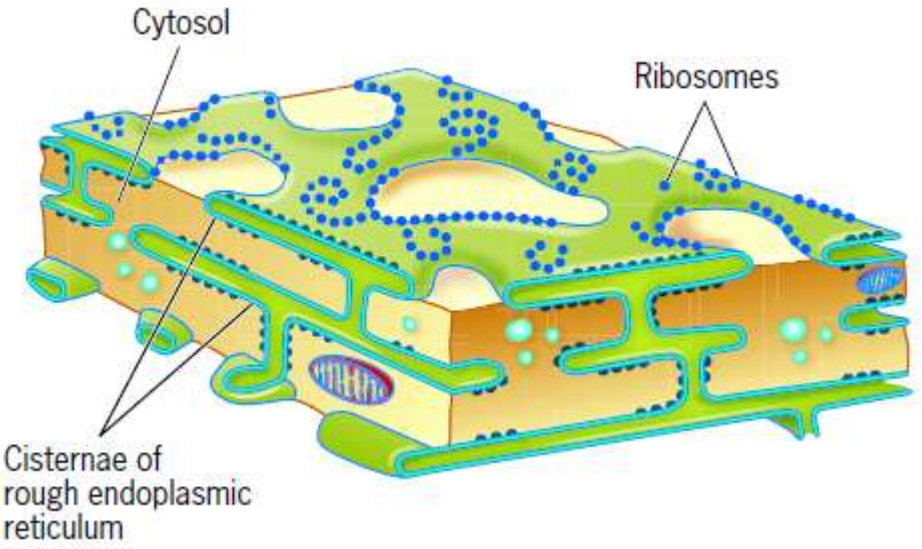
An overview of the biosynthetic/secretory and endocytic pathways that unite endomembranes into a dynamic, interconnected network.

Pathways:

- BIOSYNTHETIC / SECRETORY
  - CONSTITUTIVE SECRETION
  - REGULATED SECRETION


Exocytosis
Endocytosis
Secretory vesicle
Endocytic pathway
Early endosome
Constitutive secretory pathway
Lysosome
Late Endosome
Secretory granule
Regulated secretory pathway
Golgi complex
trans Golgi network
trans Cisterna
medial Cisterna
cis Cisterna
Rough endoplasmic reticulum
Nucleus

Endoplasmic Reticulum



Schematic
diagram showing the stacks of flattened cisternae that make up the rough ER. The cytosolic surface of the ER membrane contains bound ribosomes, which gives the cisternae their rough appearance.

General Features:

- a network of membrane enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm
- The entire ER is enclosed by a continuous membrane and is the largest organelle of most eukaryotic cells.
- Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume.

Types:

- **rough endoplasmic reticulum (RER)**
- **smooth endoplasmic reticulum (SER)**

FUNCTIONS: SER

- Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex.

- Detoxification in the liver of a wide variety of organic compounds, including barbiturates and ethanol, whose chronic use can lead to proliferation of the SER in liver cells

FUNCTIONS: SER

- Sequestering calcium ions within the cytoplasm of cells. The regulated release of Ca^{+2} from the SER of skeletal and cardiac muscle cells (known as the *sarcoplasmic reticulum* in muscle cells) triggers contraction

FUNCTIONS: RER

- it is the site of **synthesis of the proteins**, **carbohydrate chains**, and **phospholipids** that journey through the membranous compartments of the cell

- The RER is a major **protein processing plant**

FUNCTIONS: RER

- **Membrane Biosynthesis in the ER**
- **Glycosylation in the Rough Endoplasmic Reticulum**
- **Mechanisms that Ensure the Destruction of Misfolded Proteins**

FUNCTIONS: RER

The ER is also the site of -

- Protein folding
- Assembly of multisubunit proteins
- Disulfide bond formation
- The initial stages of glycosylation, and
- The addition of glycolipid anchors to some plasma membrane proteins.

Synthesis of Proteins on Membrane-Bound vs Free Ribosomes

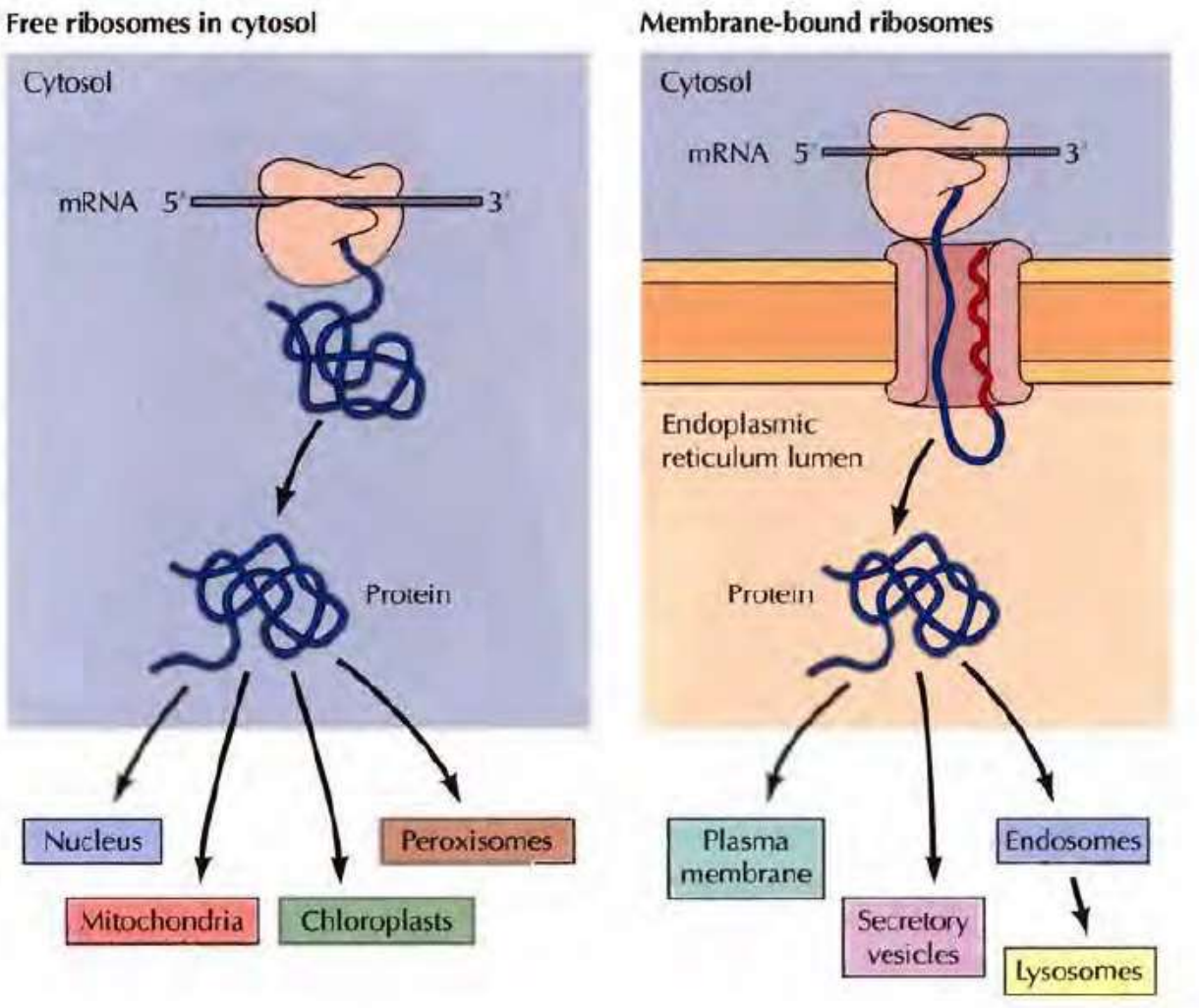
- **one-third of the proteins encoded by a mammalian genome are synthesized on ribosomes attached to the cytosolic surface of the RER membranes**

- These include (a) secreted proteins, (b) integral membrane proteins, and (c) soluble proteins that reside within compartments of the endomembrane system, including the ER, Golgi complex, lysosomes, endosomes, vesicles, and plant vacuoles.

- **Other polypeptides are synthesized on "free" ribosomes, that is, on ribosomes that are not attached to the RER, and are subsequently released into the cytosol.**

- This class includes (a) proteins destined to remain in the cytosol (such as the enzymes of glycolysis and the proteins of the cytoskeleton), (b) peripheral proteins of the cytosolic surface of membranes (such as spectrins and ankyrins that are only weakly associated with the plasma membrane's cytosolic surface), (c) proteins that are transported to the nucleus, and (d) proteins to be incorporated into peroxisomes, chloroplasts, and mitochondria. Proteins in the latter two groups are synthesized to completion in the cytosol and then imported *posttranslationally into the* appropriate organelle across its boundary membrane(s)

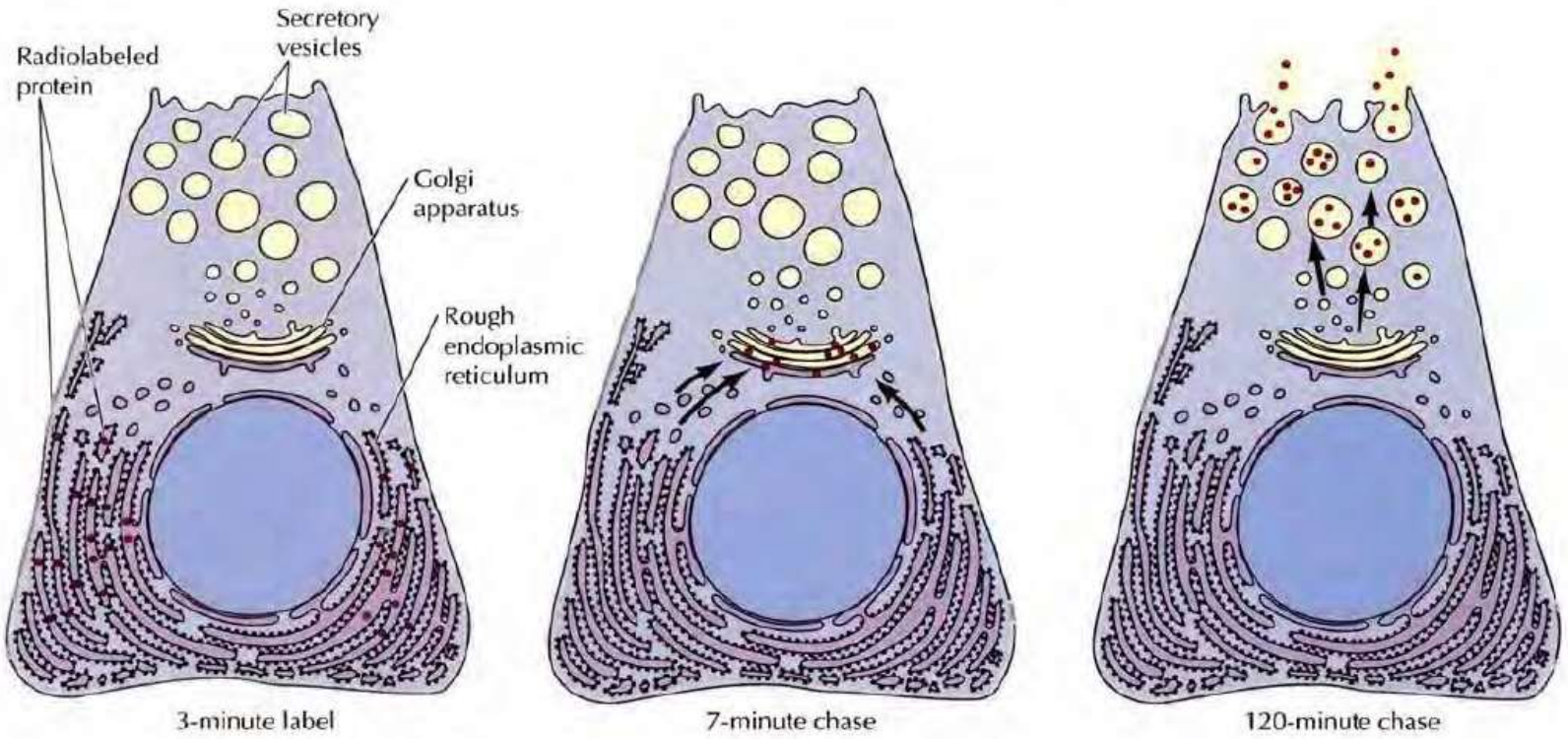
Overview of protein sorting



Secretory pathway:

rough ER ⟶ Golgi ⟶ secretory vesicles ⟶ cell exterior

- The role of the endoplasmic reticulum in protein processing and sorting was first demonstrated by George Palade and his colleagues in the 1960s.



Targeting Proteins to the Endoplasmic Reticulum

Co-translational translocation

Post-translational translocation

- **Free** and **membrane** bound **ribosomes** are functionally indistinguishable, and 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 chain being synthesized**, rather than by intrinsic properties of the ribosome itself

What determines the location in a cell where a protein is synthesized?

- **Signal hypothesis**

  David Sabatini and Gunter Blobel first proposed in 1971 that the signal for ribosome attachment to the ER might be an amino acid sequence near the amino terminus of the growing polypeptide chain

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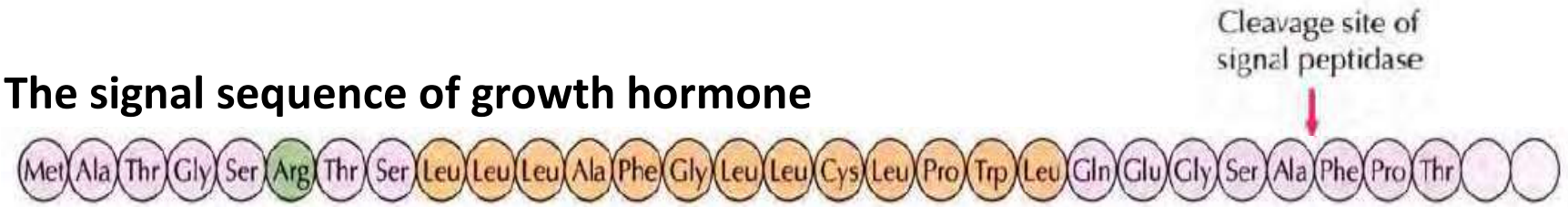
Signal sequence

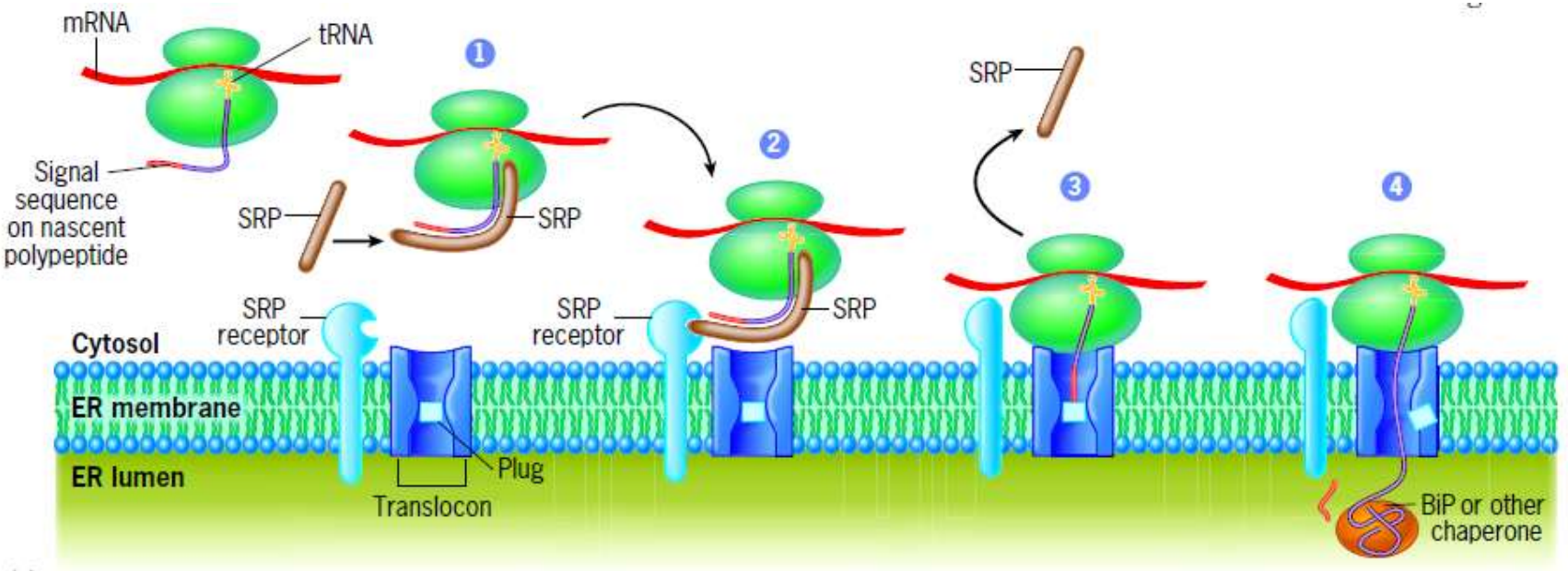
- signal sequence—typically includes a stretch of 6–15 **hydrophobic amino acid** residues—that targets the nascent polypeptide to the ER membrane and leads to the compartmentalization of the polypeptide within the ER lumen

- Although the signal sequence is usually located at or near the N-terminus, it occupies an internal position in some polypeptides

The signal sequence of growth hormone



A schematic model of the synthesis of a secretory protein (or a lysosomal enzyme) on a membrane-bound ribosome of the RER

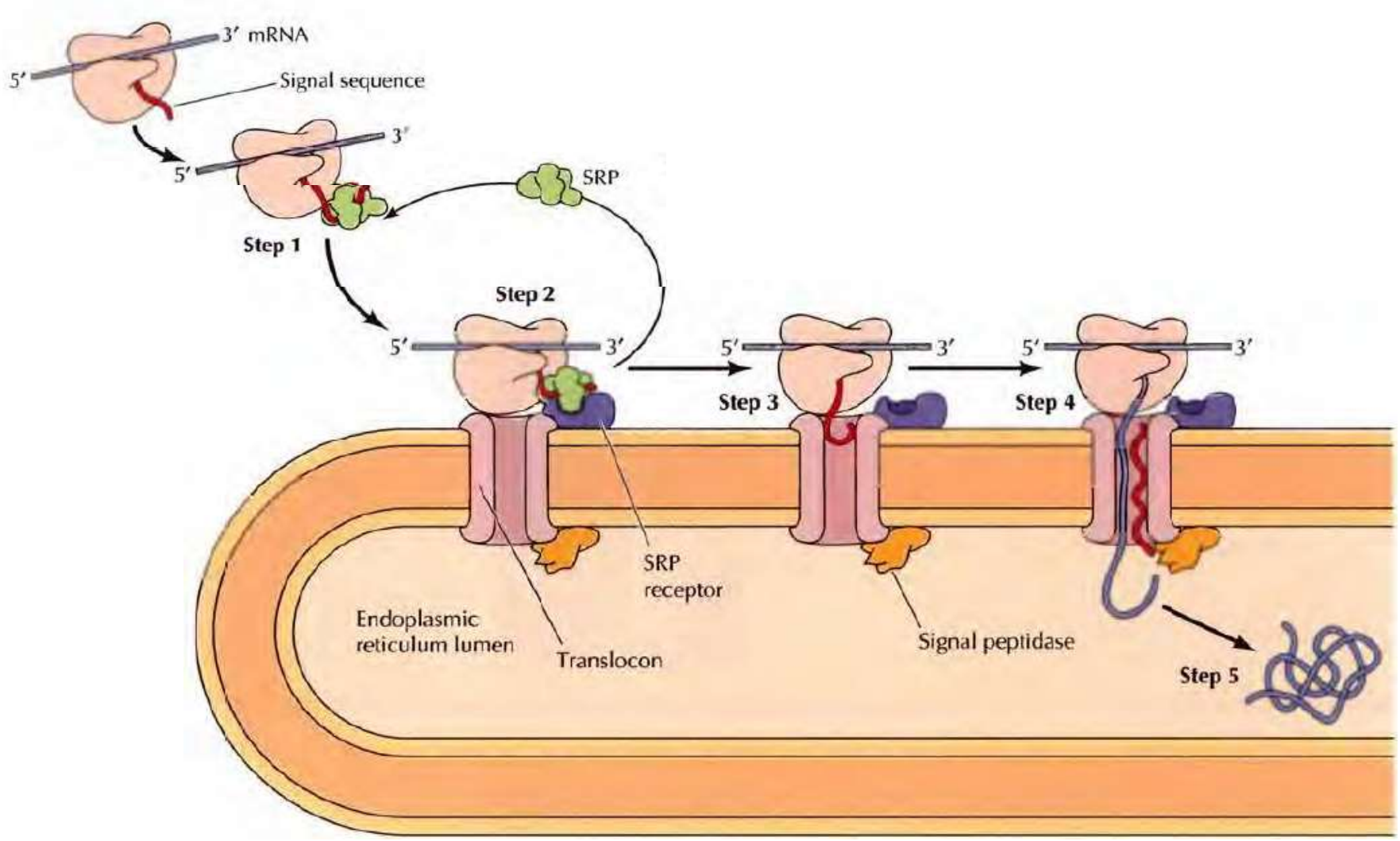


STEPS:

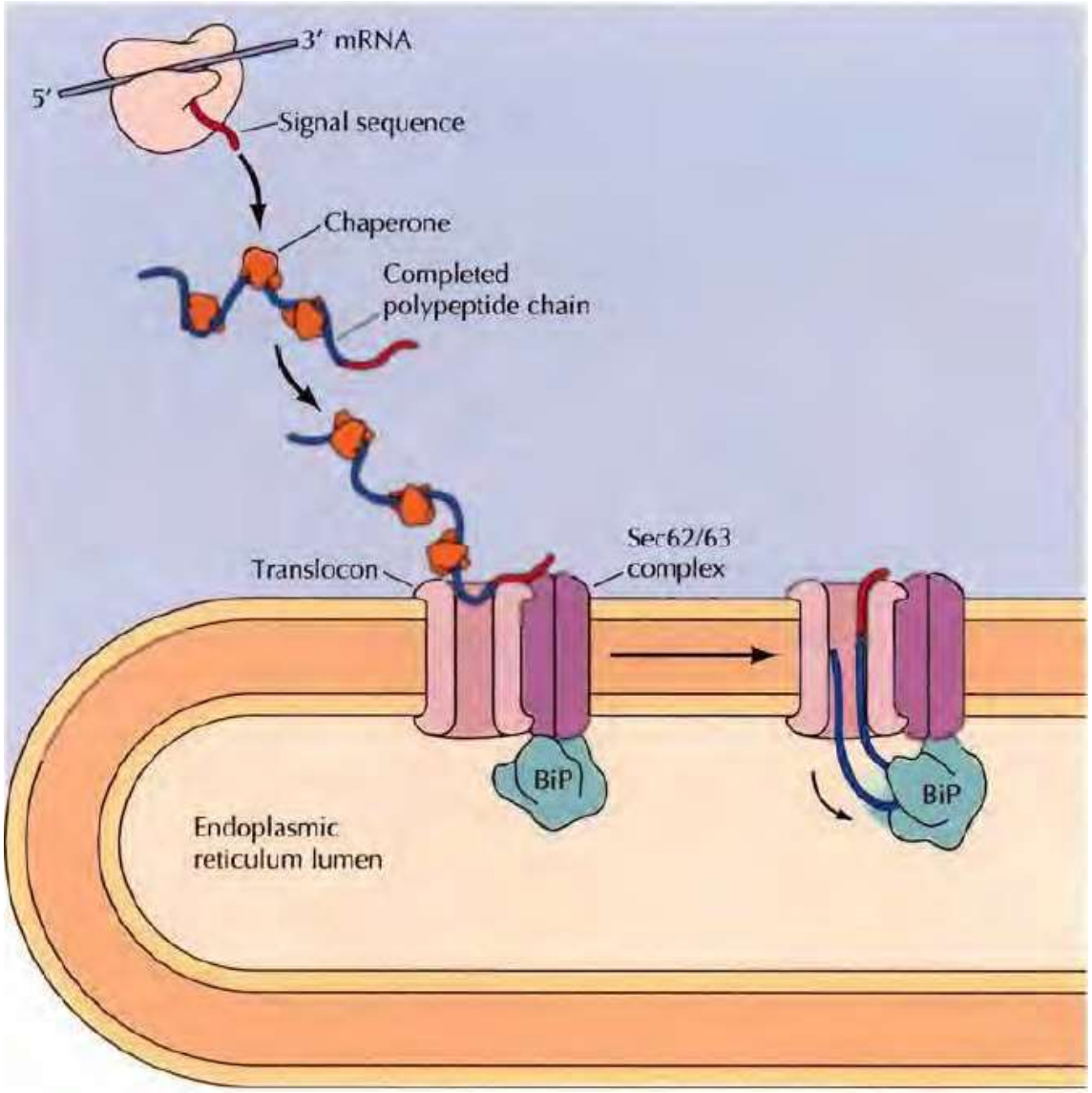
- **STEP 1**: Synthesis of the polypeptide begins on a free ribosome. As the signal sequence (shown in red) emerges from the ribosome, it binds to the SRP, which stops further translation until the SRP-ribosome-nascent chain complex can make contact with the ER membrane.
- **STEP 2**: The SRP-ribosome complex then collides with and binds to an SRP receptor (SR) situated within the ER membrane.
- **STEP 3**: Attachment of this complex to the SRP receptor is followed by release of the SRP and the association of the ribosome with a translocon of the ER membrane.
- **STEP 4**: These latter events are accompanied by the reciprocal hydrolyis of GTP molecules (not shown) bound to both the SRP and its receptor.

- In the model depicted here, the signal peptide then binds to the interior of the translocon, displacing the plug from the channel and allowing the remainder of the polypeptide to translocate through the membrane cotranslationally .After the nascent polypeptide passes into the lumen of the ER, the signal peptide is cleaved by a membrane protein (the signal peptidase, not shown), and the protein undergoes folding with the aid of ER chaperones, such as BiP

Co-translational targeting of secretory proteins to the ER



Post-translational translocation of proteins into the ER



Processing of Newly Synthesized Proteins in the ER

Signal peptidase

- Removes **signal peptide**

Oligosaccharyltransferase

- Add **carbohydrates**

Processing of Newly Synthesized Proteins in the ER

- Both the **signal peptidase** and **oligosaccharyltransferase** are integral membrane proteins associated with the **translocon** that act on nascent proteins as they enter the ER lumen.

- Carbohydrates are added to the nascent protein by the enzyme oligosaccharyltransferase

- The N terminal portion containing the signal peptide is removed from most nascent polypeptides by a proteolytic enzyme, the **signal peptidase**

- RER lumen is packed with molecular chaperones that recognize and bind to unfolded or misfolded proteins and give them the opportunity to attain their correct (native) three-dimensional structure

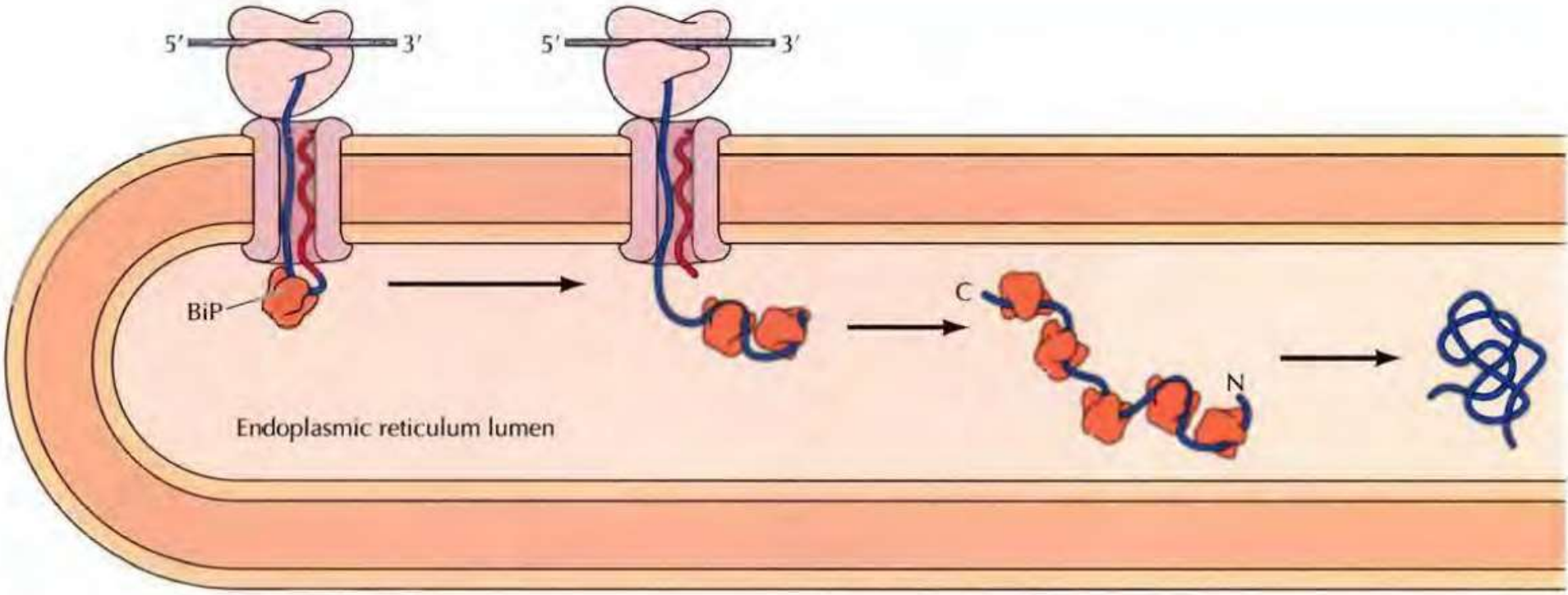
- The formation (and rearrangement) of disulfide bonds is catalyzed by PDI **protein disulfide isomerase** (PDI).

Protein Folding and Processing in the ER

- proteins are translocated across the ER membrane as unfolded polypeptide chains while their translation is still in progress.
- These polypeptides, therefore, fold into their three-dimensional conformations within the ER, assisted by molecular chaperones that facilitate the folding of polypeptide chains

Protein Folding

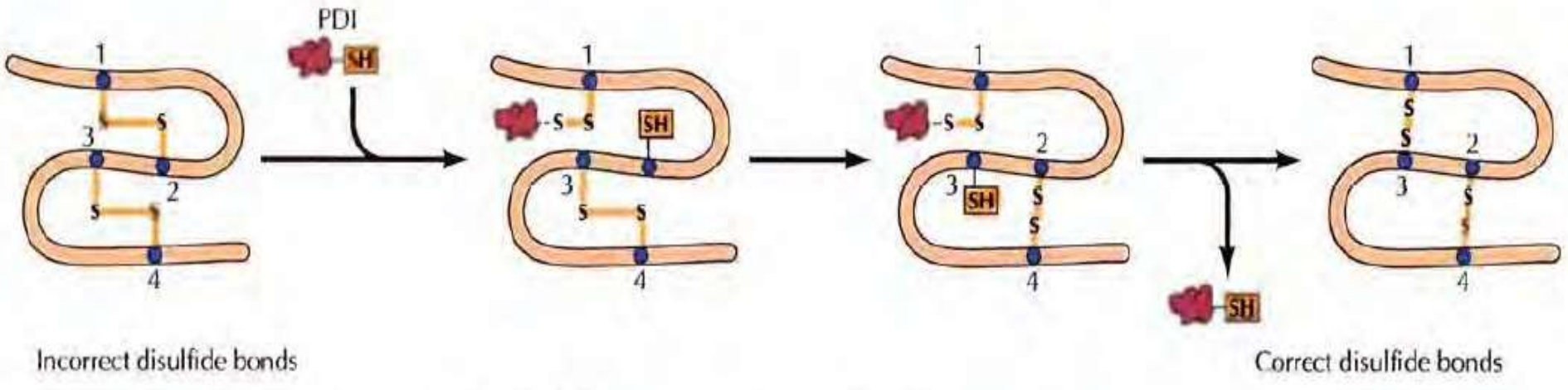
- The Hsp70 chaperone, BiP, is thought to bind to the unfolded polypeptide chain as it crosses the membrane and then mediate protein folding and the assembly of multisubunit proteins within the ER.
- Correctly assembled proteins are released from BiP (and other chaperones) and are available for transport to the Golgi apparatus.
- Abnormally folded or improperly assembled proteins are targets for degradation, as will be discussed later.



Protein folding in the ER

Protein Folding

- The formation of disulfide bonds between the side chains of **cysteine residues** is an important aspect of protein folding and assembly within the ER.
- These bonds do not form in the cytosol, which is characterized by a reducing environment that maintains **cysteine residues in their reduced (- SH) state**.
- In the ER, however, an oxidizing environment promotes disulfide (S- S) bond formation, and disulfide bonds formed in the ER play important roles in the structure of secreted and cell surface proteins.
- Disulfide bond formation is facilitated by the enzyme protein disulfide isomerase (see Figure 8.24), which is located in the ER lumen

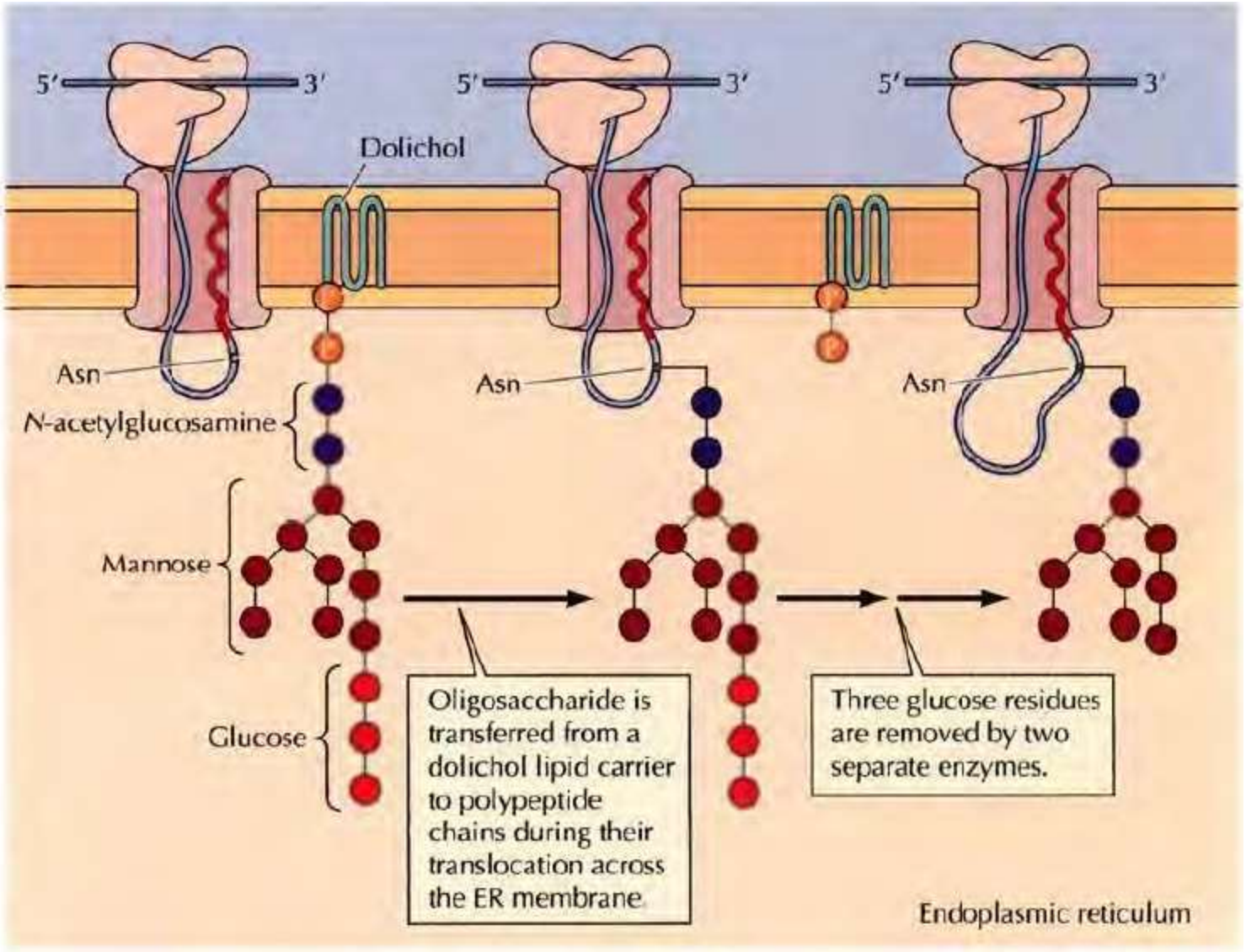


The action of protein disulfide isomerase

GLYCOSYLATION

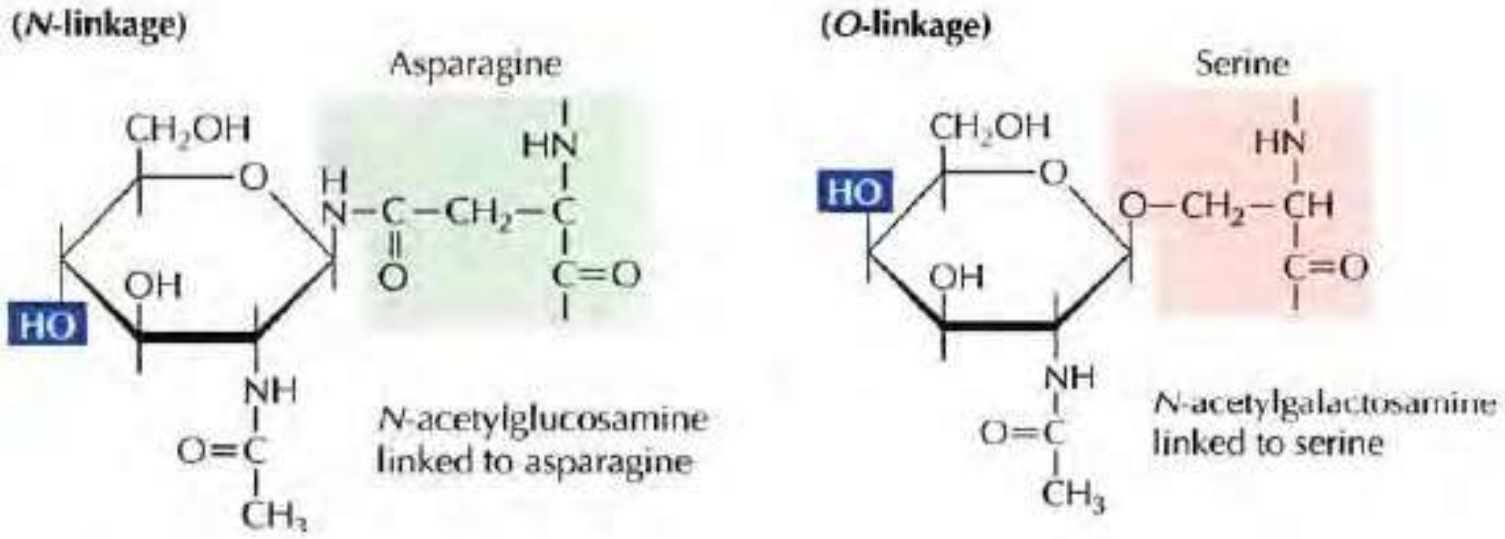
- Proteins are glycosylated on specific **asparagine** residues (N-linked glycosylation) within the ER while their translation is still in process.
- Oligosaccharide units consisting of 14 sugar residues are added to acceptor asparagine residues of growing polypeptide chains as they are translocated into the ER.

- The oligosaccharide is synthesized on a lipid (dolichol) carrier anchored in the ER membrane.
- It is then transferred as a unit to acceptor asparagine residues in the consensus sequence Asn-X-Ser / Thr by a membrane-bound enzyme called oligosaccharyl transferase.
- Three glucose residues are removed while the protein is still within the ER, and the protein is modified further after being transported to the Golgi apparatus

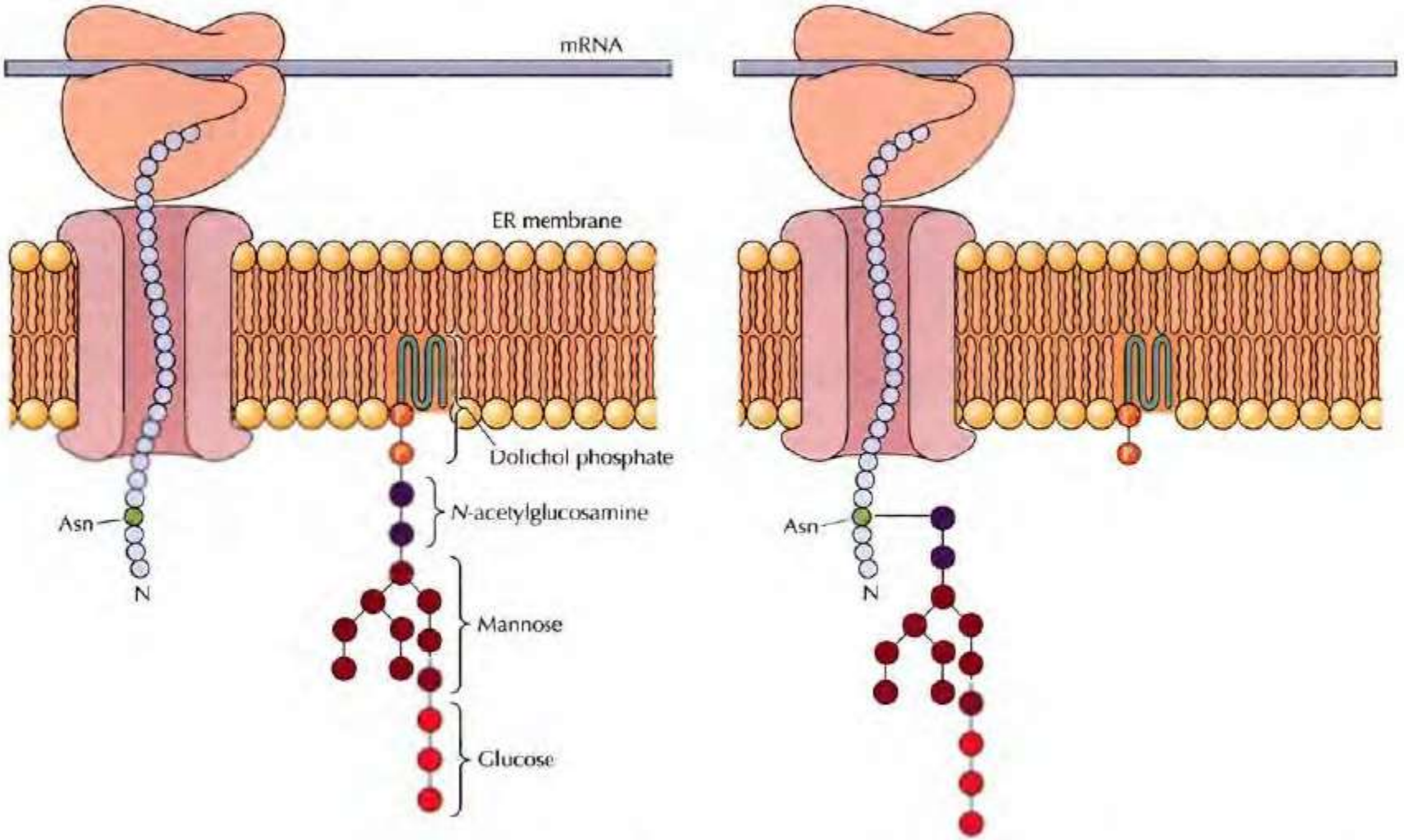


Protein glycosylation in the ER

Glycosylation in the Rough Endoplasmic Reticulum

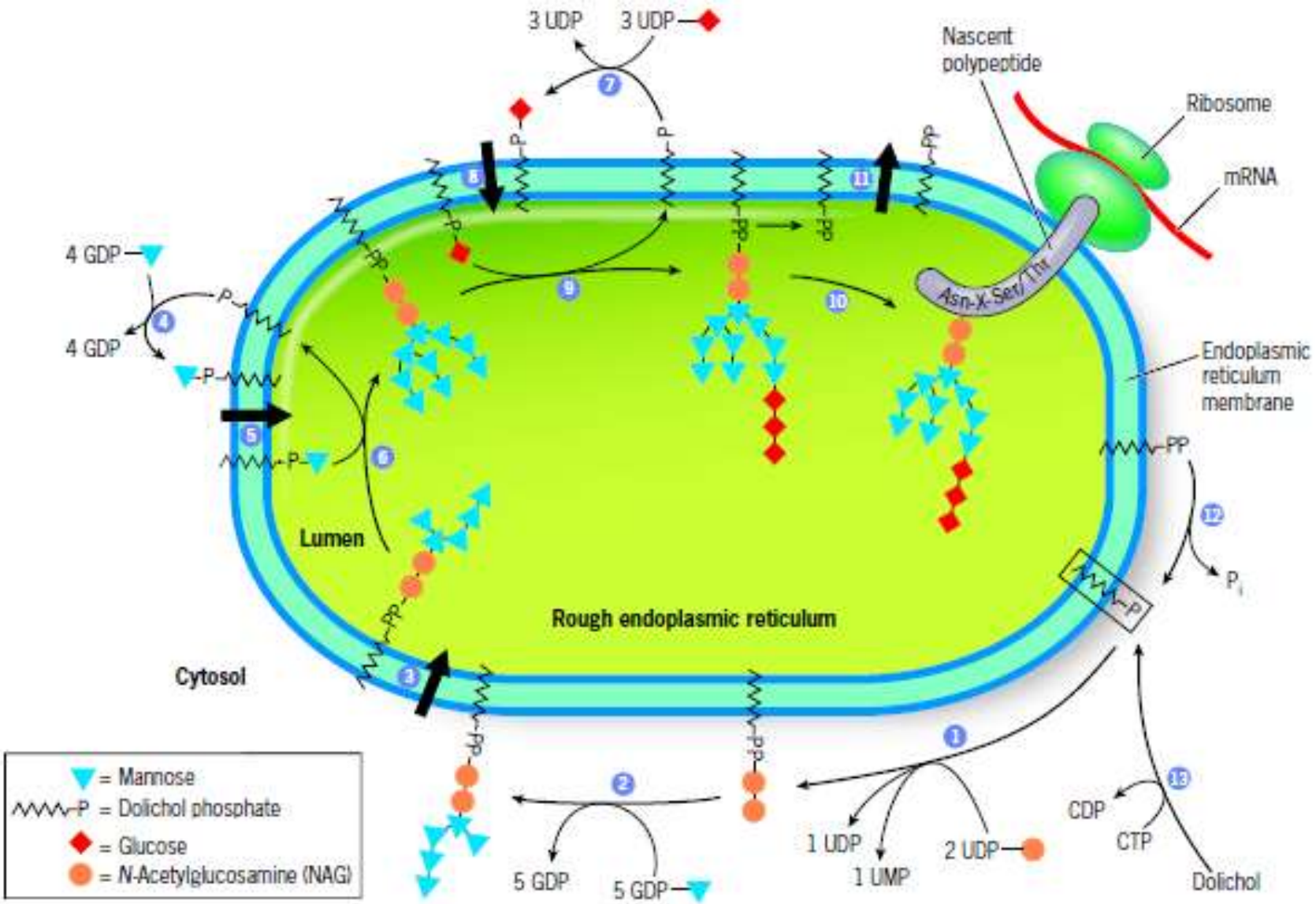


Linkage of carbohydrate side chains to glycoproteins

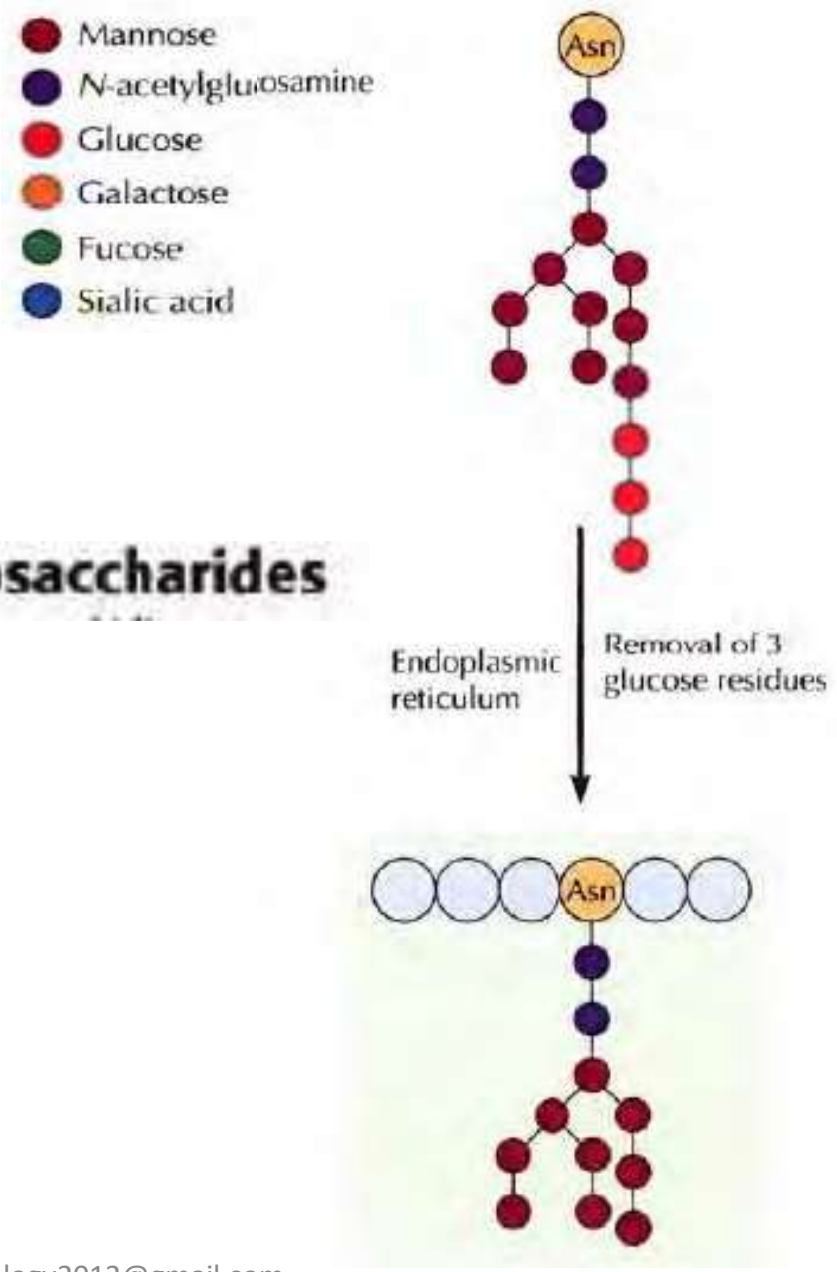


Synthesis of *N*-linked glycoproteins

Steps in the synthesis of the core portion of an N-linked oligosaccharide in the rough ER



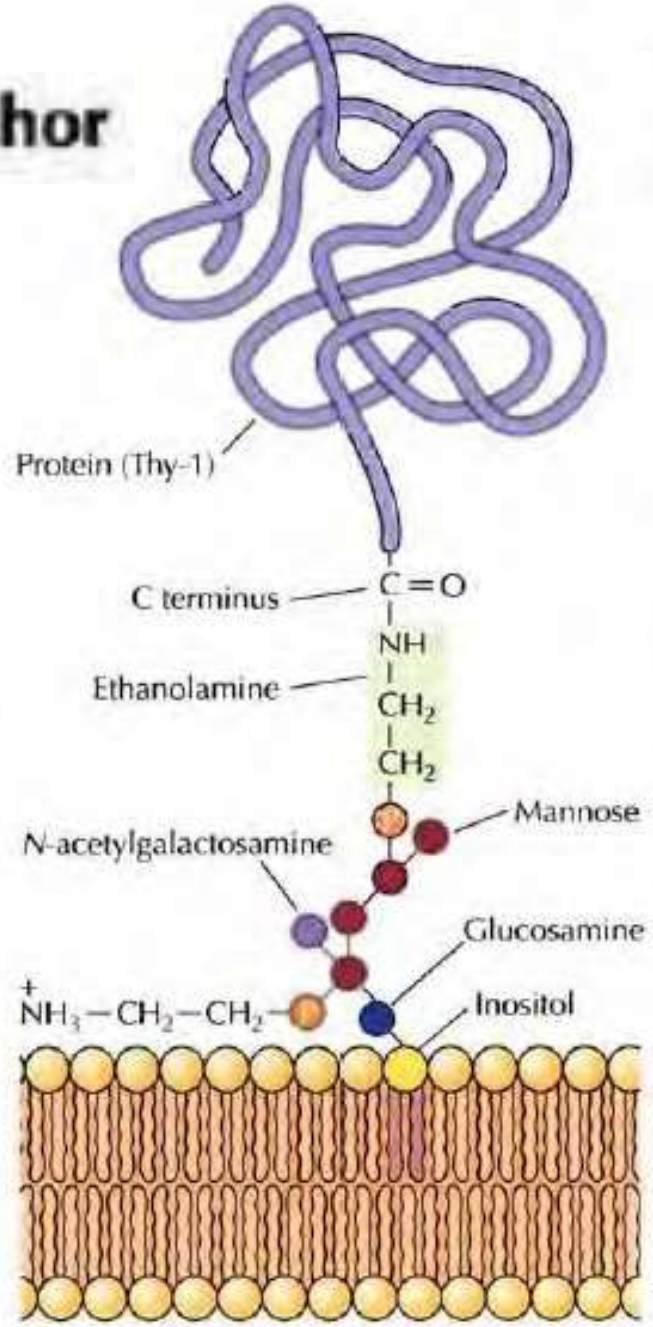
Processing of *N*-linked oligosaccharides

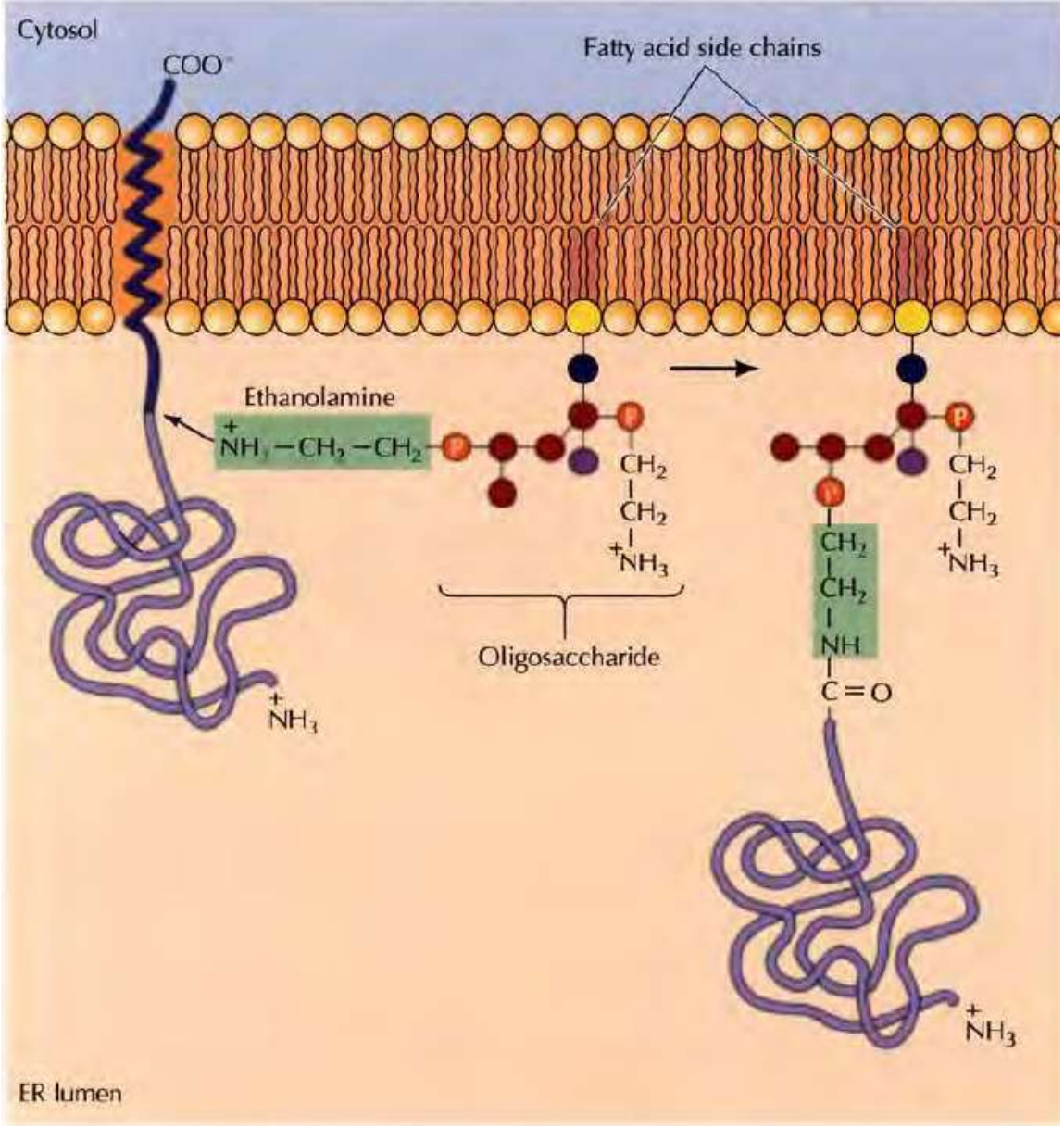


ADDITION TO **GPI** ANCHOR:

- Some proteins are attached to the plasma membrane by glycolipids rather than by membrane-spanning regions of the polypeptide chain.
- Because these membrane-anchoring glycolipids contain phosphatidylinositol, they are called glycosylphosphatidylinositol (GPI) anchors

Structure of a GPI anchor





Addition of GPI anchors

- The GPI anchors are assembled in the ER membrane. They are then added immediately after completion of protein synthesis to the carboxy terminus of some proteins that are retained in the membrane by a C-terminal hydrophobic sequence
- The C-terminal sequence of the protein is cleaved and exchanged for the GPI anchor, so these proteins remain attached to the membrane only by their associated glycolipid.

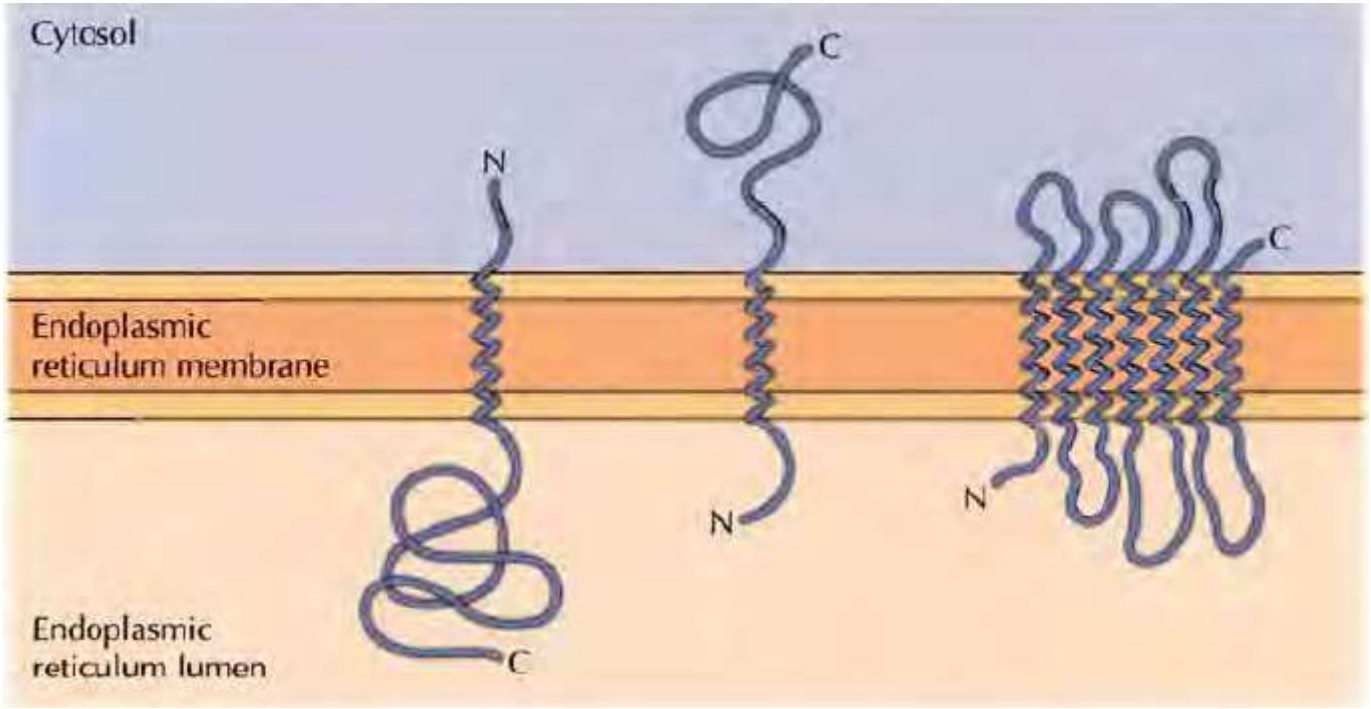
Insertion of Proteins into the ER Membrane

proteins destined for incorporation into the plasma membrane or the membranes of these compartments are initially inserted into the ER membrane instead of being released into the lumen

ER ⟶ Golgi⟶ plasma membrane or endosomes ⟶ lysosomes.

However, these proteins are transported along this pathway as membrane components rather than as soluble proteins

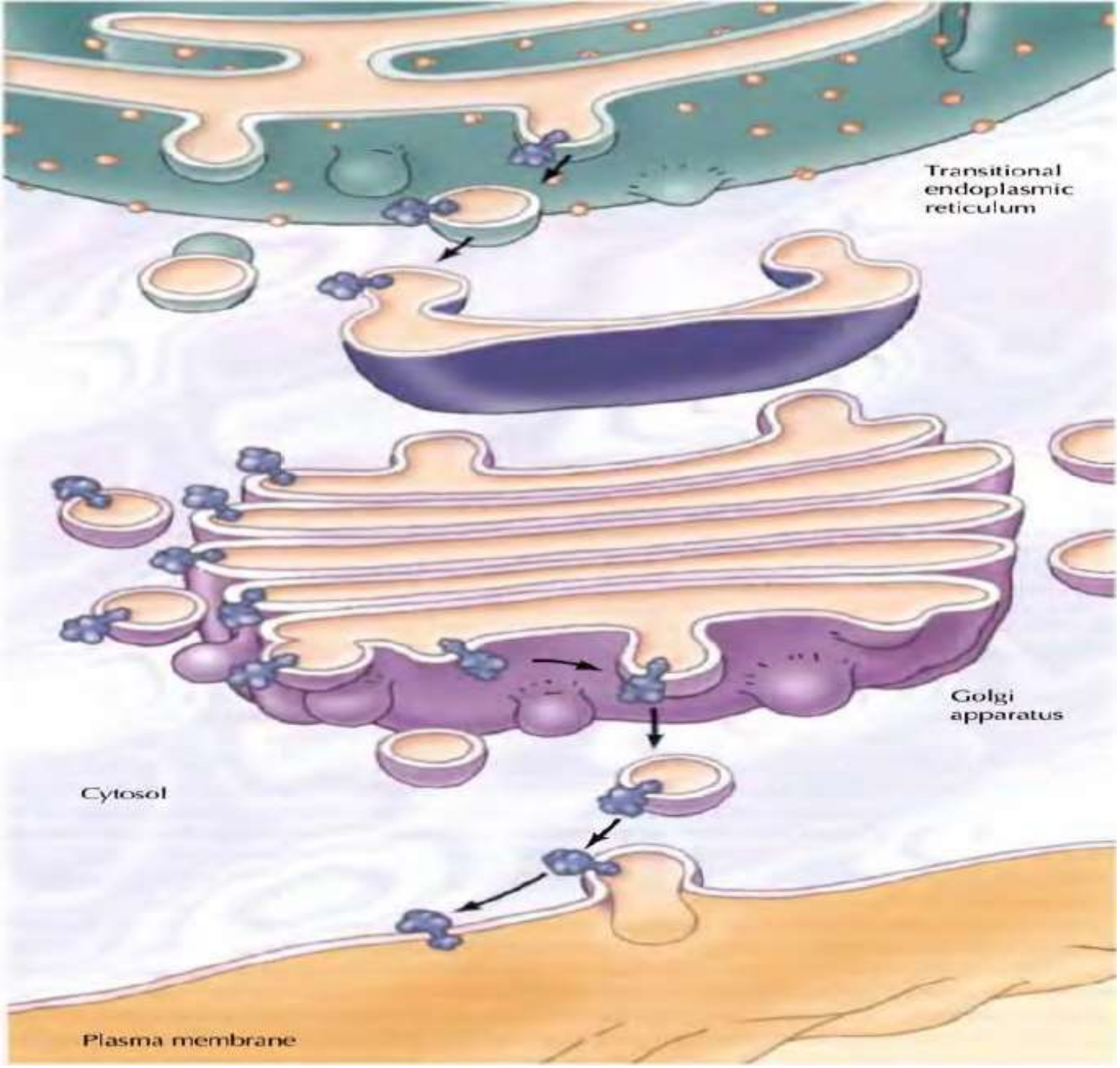
Orientations of membrane proteins



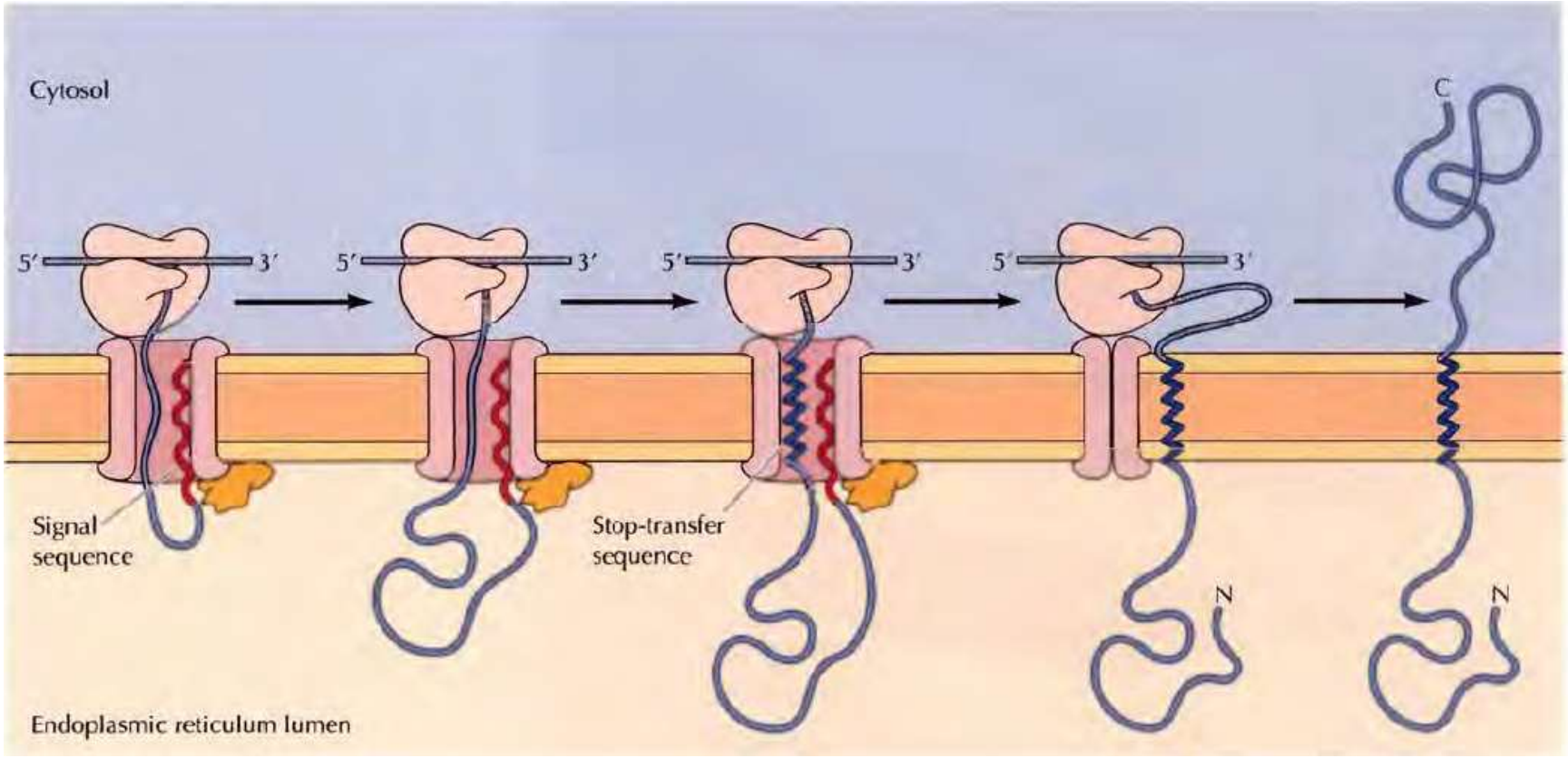
- The membrane spanning portions of these integral membrane proteins are usually a **helical regions** consisting of **20 to 25 hydrophobic amino acids**. The formation of an **α helix** maximizes hydrogen bonding between the peptide bonds, and the **hydrophobic amino acid side chains interact with the fatty acid tails of the phospholipids in the bilayer**.

- The **lumen of the ER** is **topologically equivalent** to the **exterior of the cell**, so the domains of plasma membrane proteins that are exposed on the cell surface correspond to the regions of polypeptide chains that are translocated into the ER lumen

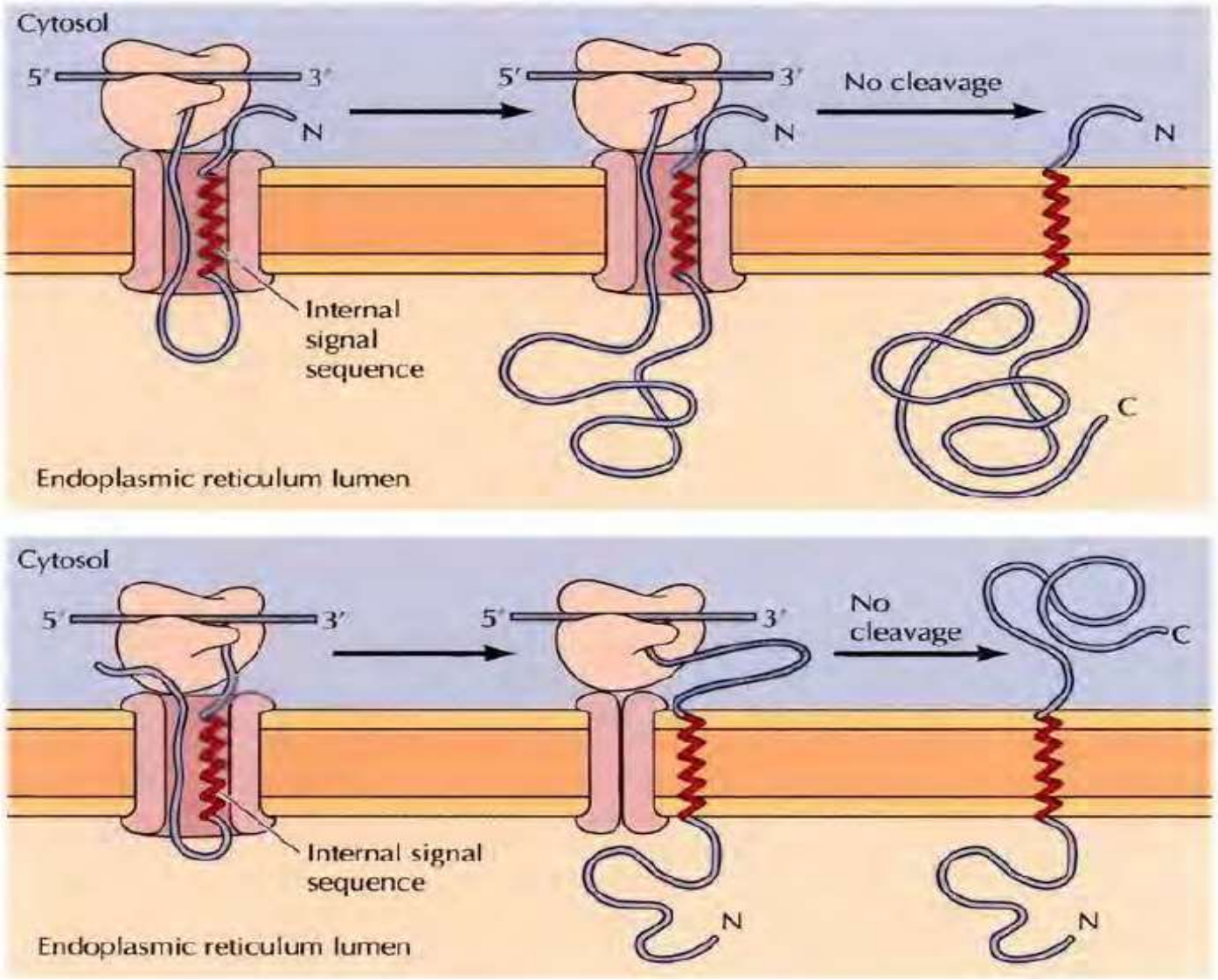
Topology of the secretory pathway



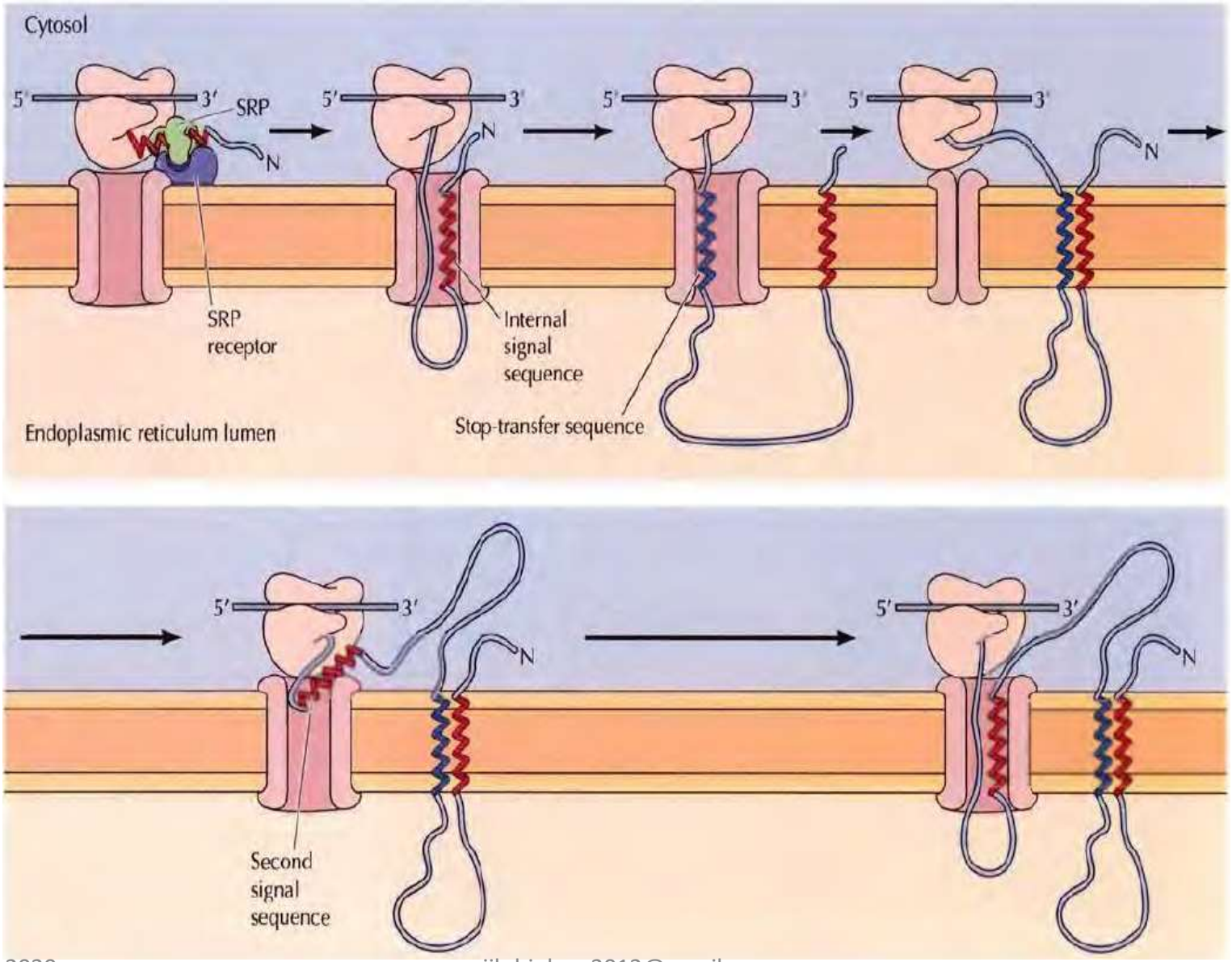
Insertion of a membrane protein with a cleavable signal sequence and a single stop-transfer sequence



Insertion of membrane proteins with internal non-cleavable signal sequences



Insertion of a protein that spans the membrane multiple times



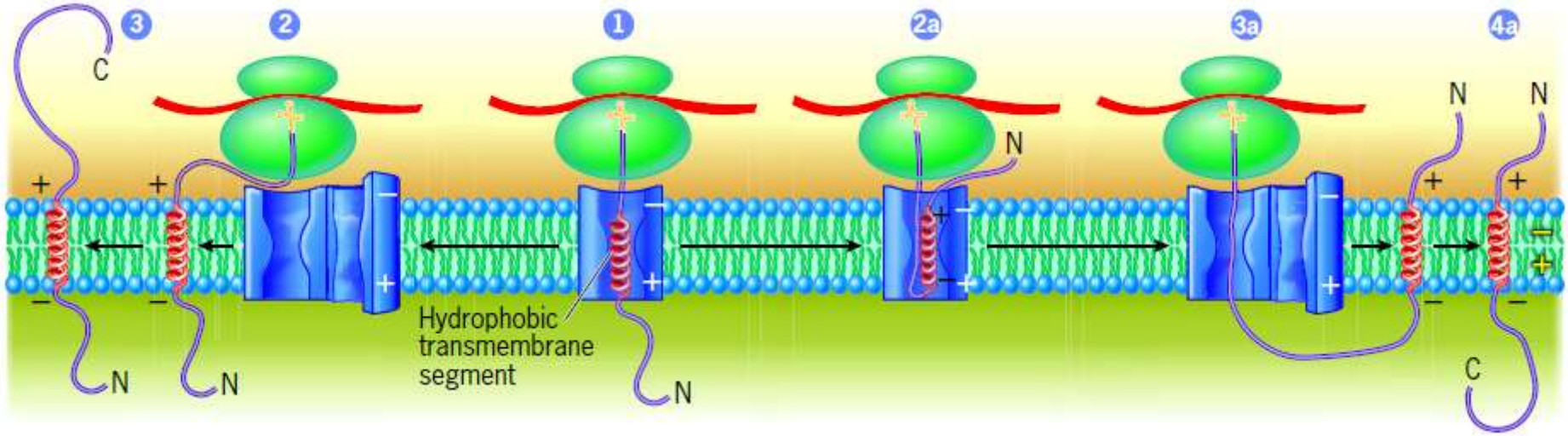
Mechanism

- Translocon to have a clam-shaped conformation with a groove or seam along one side of the wall where the channel might open and close.

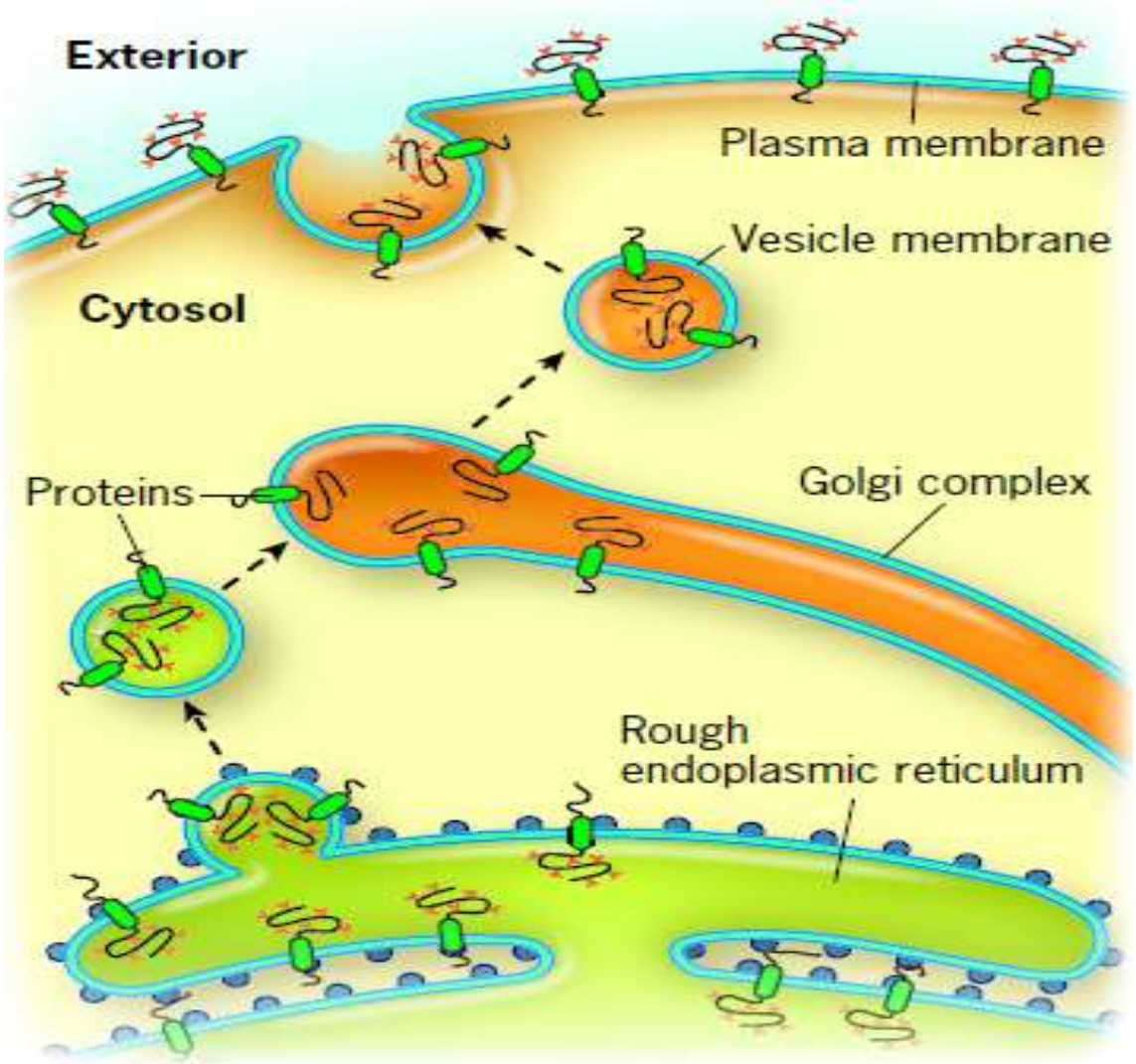
- As a polypeptide passes through the translocon, it is proposed that this lateral “gate” in the channel continually opens and closes, which gives each segment of the nascent polypeptide an opportunity to partition itself according to its solubility properties into either the aqueous compartment within the translocon channel or the surrounding hydrophobic core of the lipid bilayer.

- Those segments of the nascent polypeptide that are sufficiently hydrophobic will spontaneously “dissolve” into the lipid bilayer and ultimately become transmembrane segments of an integral membrane protein

A schematic model for the synthesis of an integral membrane protein



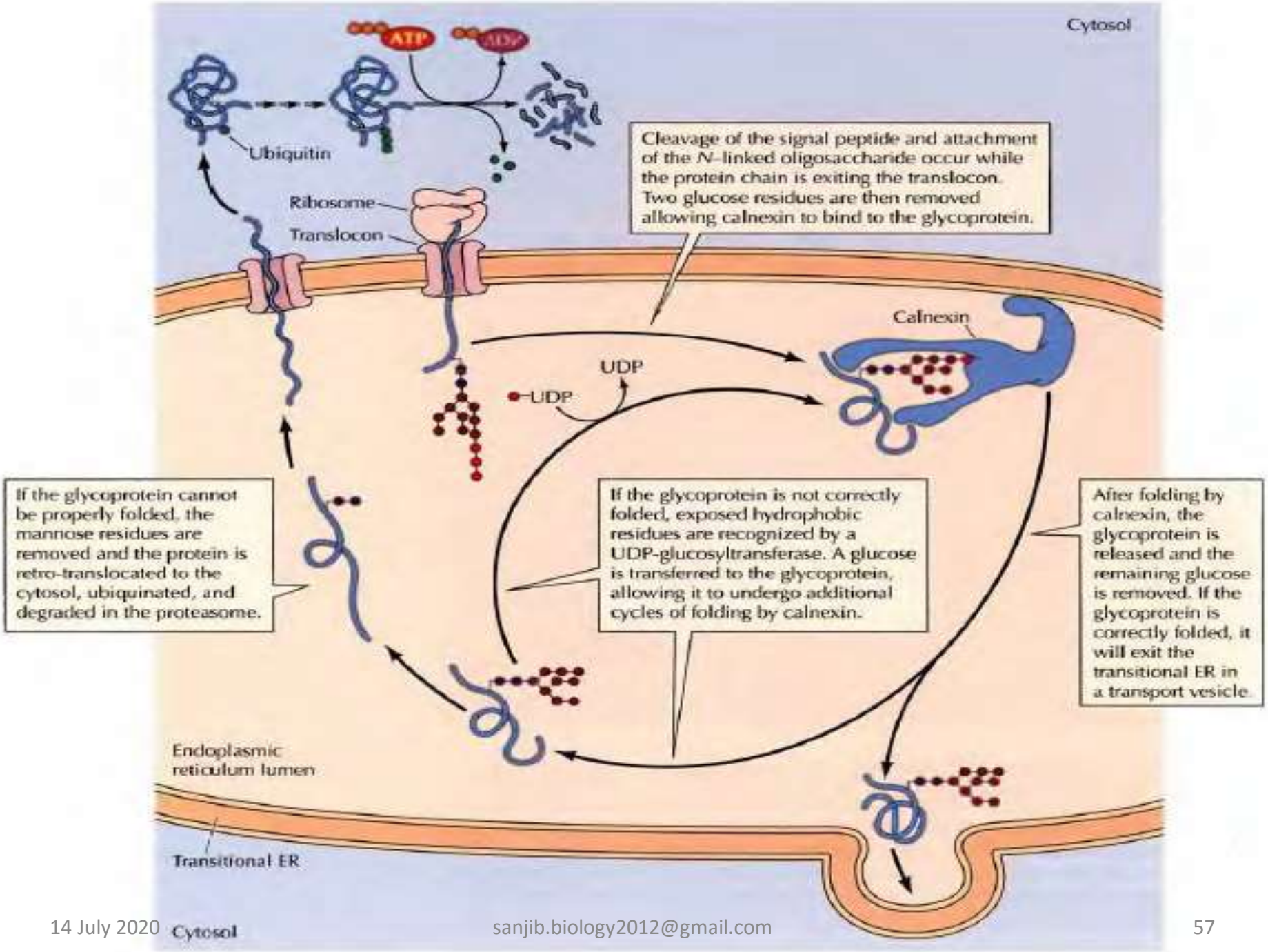
Membrane Biosynthesis in the ER



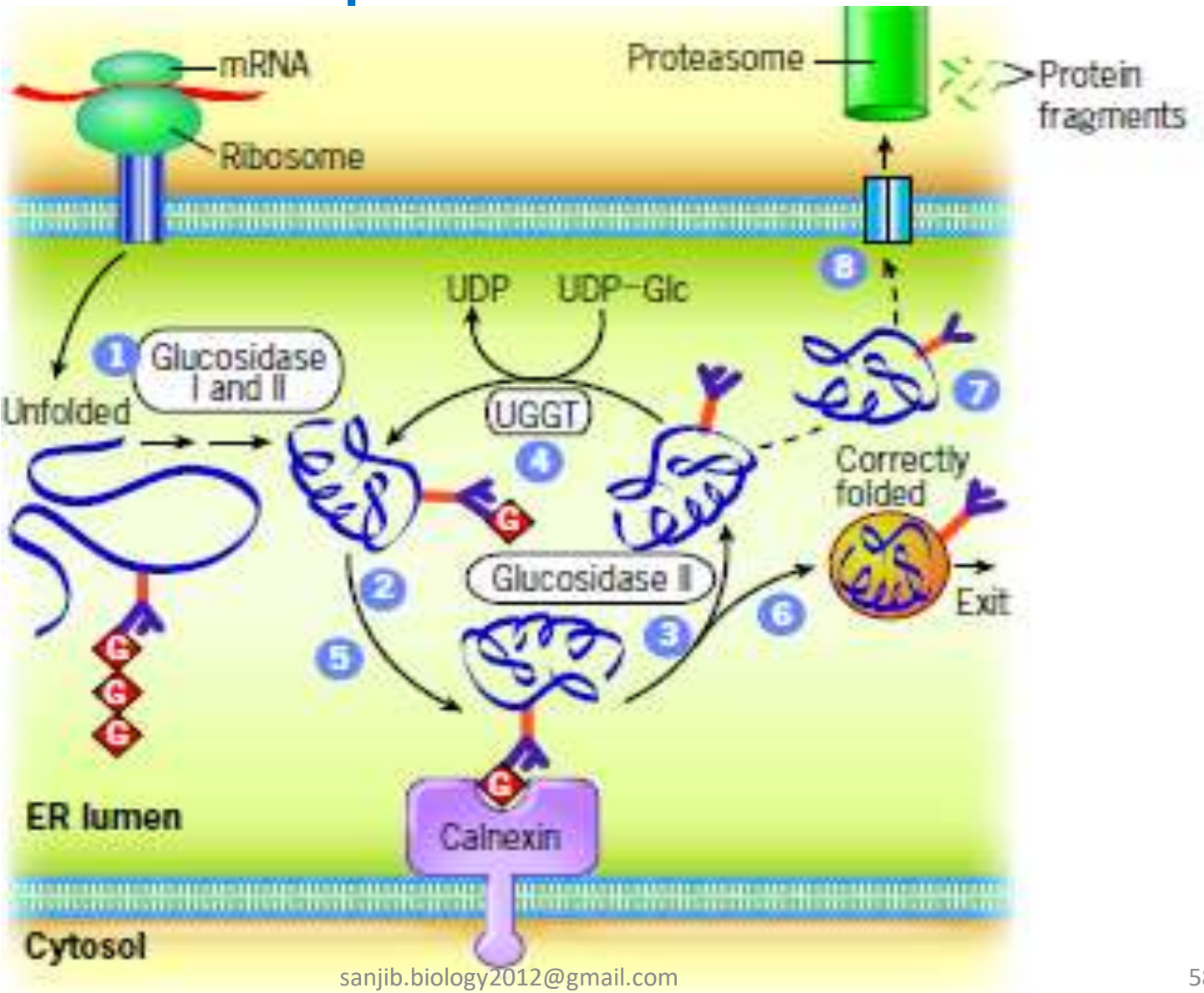
Maintenance of membrane asymmetry

Quality control

- the oligosaccharide chain undergoes a process of gradual modification shortly after it is transferred to the nascent polypeptide. This modification begins in the ER with the enzymatic removal of two of the three terminal glucose residues.
- This sets the stage for an important event in the life of a newly synthesized glycoprotein in which it is screened by a system of **quality control** **that determines** whether or not it is fit to move on to the next compartment of the biosynthetic pathway or not.

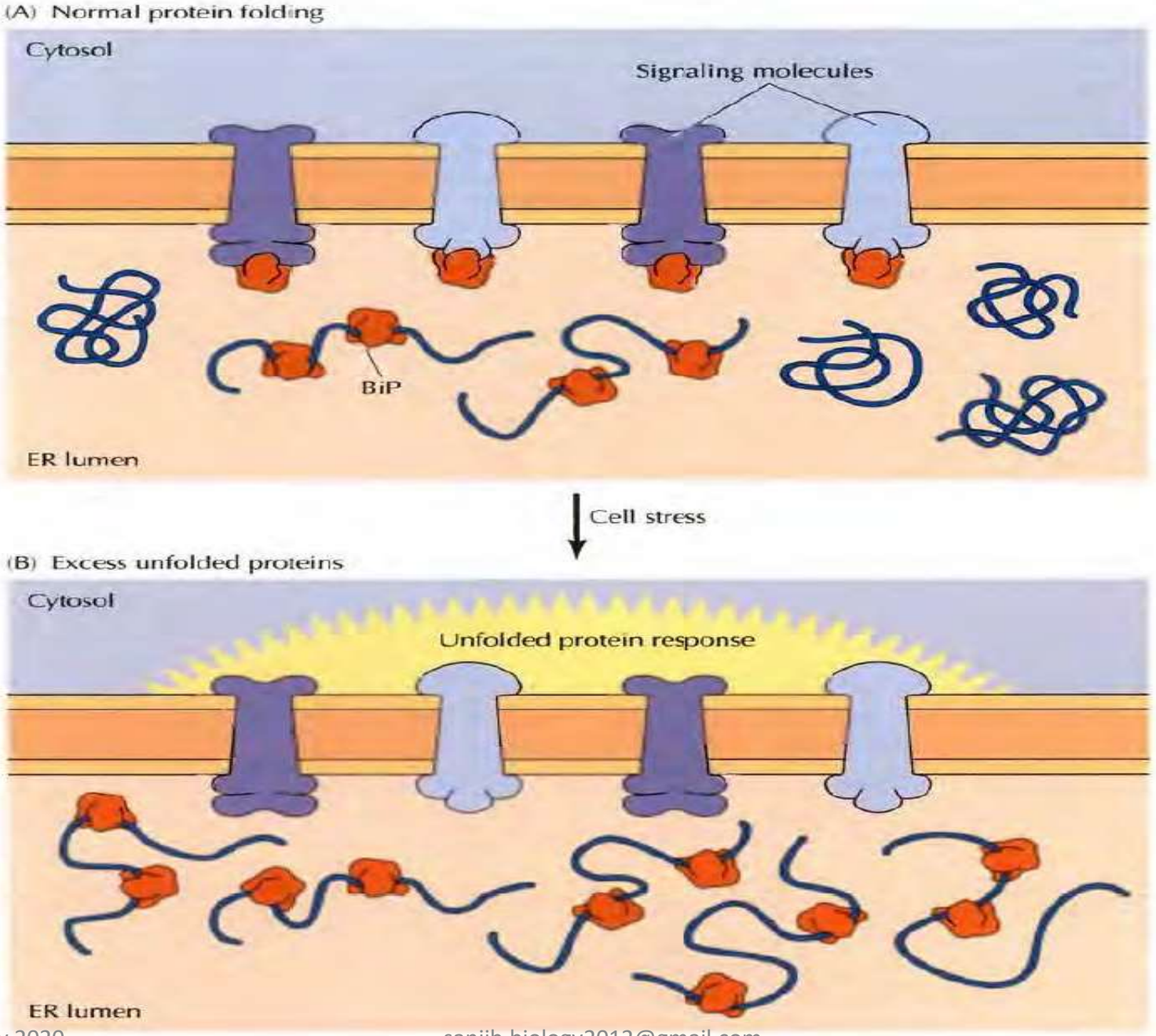

Cytosol
ATP
ADP
Ubiquitin
Ribosome
Translocon
Cleavage of the signal peptide and attachment of the N-linked oligosaccharide occur while the protein chain is exiting the translocon. Two glucose residues are then removed allowing calnexin to bind to the glycoprotein.
Calnexin
UDP
UDP
If the glycoprotein cannot be properly folded, the mannose residues are removed and the protein is retro-translocated to the cytosol, ubiquinated, and degraded in the proteasome.
If the glycoprotein is not correctly folded, exposed hydrophobic residues are recognized by a UDP-glucosyltransferase. A glucose is transferred to the glycoprotein, allowing it to undergo additional cycles of folding by calnexin.
After folding by calnexin, the glycoprotein is released and the remaining glucose is removed. If the glycoprotein is correctly folded, it will exit the transitional ER in a transport vesicle.
Endoplasmic reticulum lumen
Transitional ER
Cytosol

Quality control: ensuring that misfolded proteins do not proceed forward

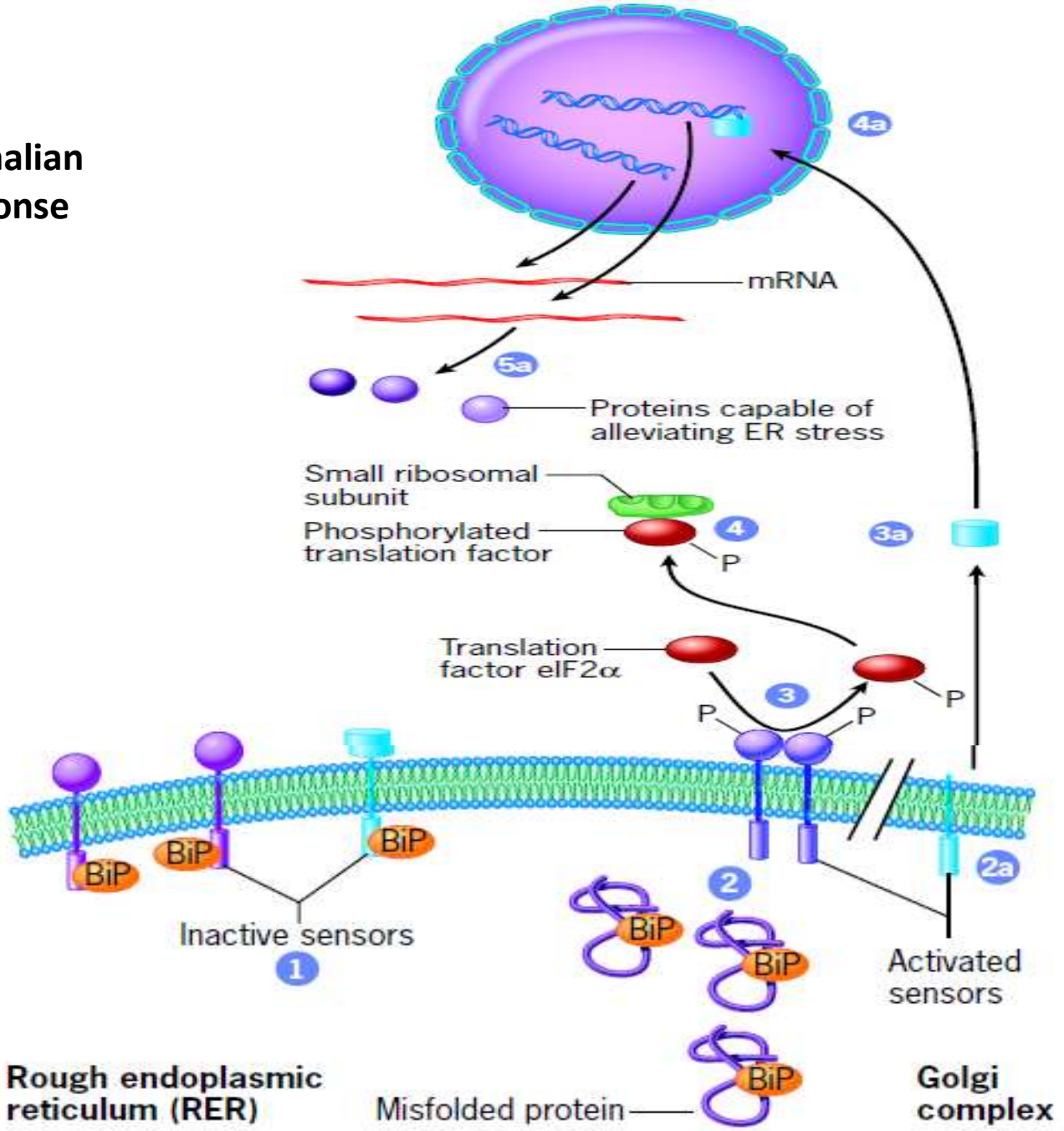


Mechanisms that Ensure the Destruction of Misfolded Proteins

- Misfolded proteins are not destroyed in the ER, but instead are transported into the cytosol by a process of *dislocation*.
- In the cytosol, the oligosaccharide chains are removed, and the misfolded proteins are destroyed in proteasomes, which are protein-degrading machines
- This process, known as *ER-associated degradation (ERAD),* ensures that aberrant proteins are not transported to other parts of the cell


(A) Normal protein folding
Cytosol
Signaling molecules
BiP
ER lumen
Cell stress
(B) Excess unfolded proteins
Cytosol
Unfolded protein response
ER lumen

A model of the mammalian unfolded protein response (UPR).



A model of the mammalian unfolded protein response (UPR).

- The ER contains transmembrane proteins that function as sensors of stressful events that occur within the ER lumen.
- **Step 1** :Under normal conditions, these sensors are present in an inactive state as the result of their association with chaperones, particularly BiP .
- If the number of unfolded or misfolded proteins should increase to a high level, the chaperones are recruited to aid in protein folding, which leaves the sensors in their unbound, activated state and capable of initiating a UPR.
- At least three distinct UPR pathways have been identified in mammalian cells, each activated by a different protein sensor. Two of these pathways are depicted in this illustration.
- **Step 2** : In one of these pathways, the release of the inhibitory BiP protein leads to the dimerization of a sensor (called **PERK**).

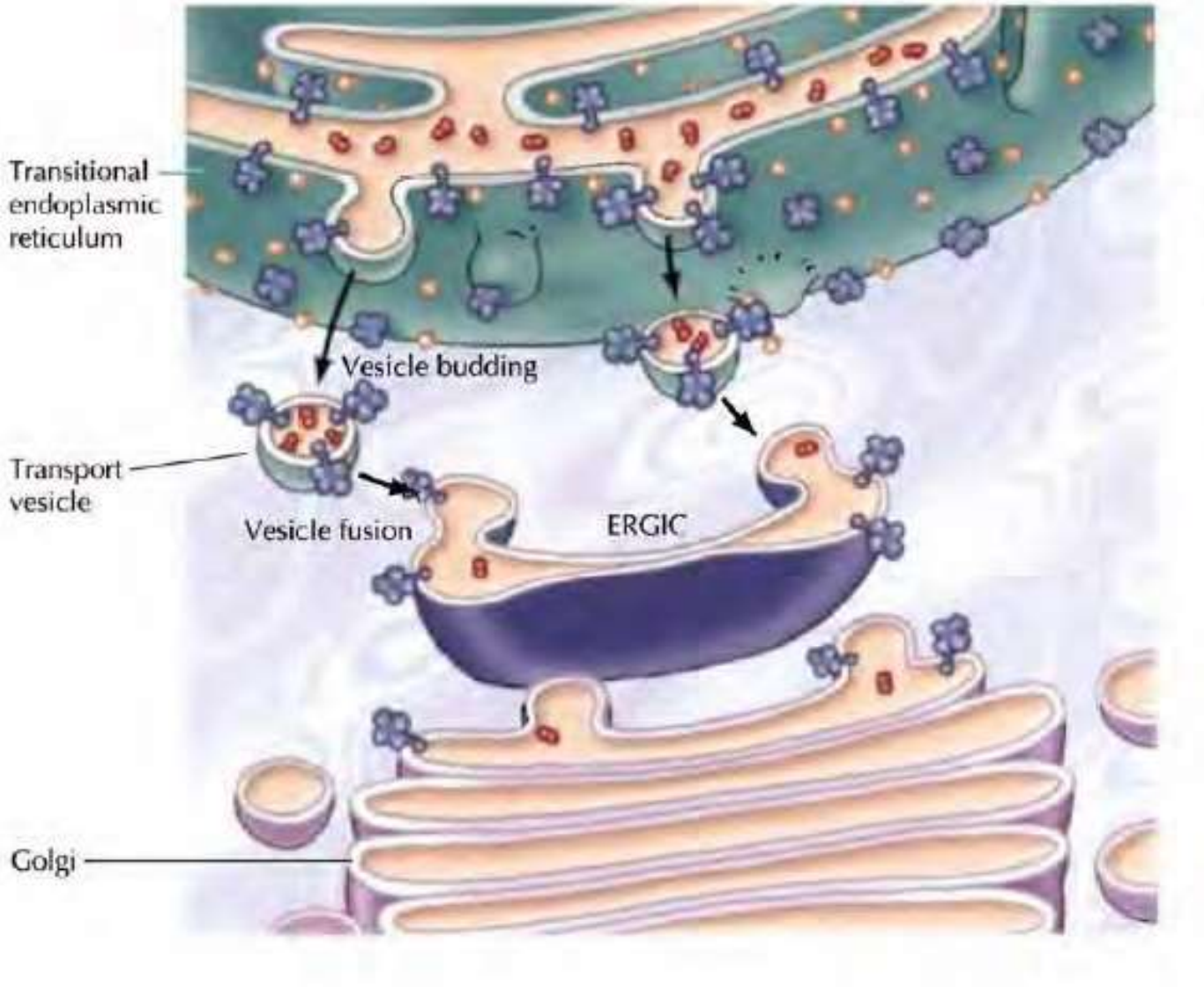
- **Step 3** : In its dimeric state, PERK becomes an activated protein kinase that phosphorylates a protein (eIF2) that is required for the initiation of protein synthesis .
- **Step 4** : This translation factor is inactive in the phosphorylated state, which stops the cell from synthesizing additional proteins in the ER, giving the cell more time to process those proteins already present in the ER lumen.
- **Step 2a** : In the second pathway depicted here, release of the inhibitory BiP protein allows the sensor (called **ATF6**) to move on to the Golgi complex where the cytosolic domain of the protein is cleaved away from its transmembrane domain.
- **Step 3a** : The cytosolic portion of the sensor diffuses through the cytosol and into the nucleus (**step 4a**), where it stimulates the expression of genes whose encoded proteins can alleviate the stress in the ER (**step 5a**). These include chaperones, coat proteins that form on transport vesicles, and proteins of the quality-control machinery.

Export of Proteins from the ER

From the ER to the Golgi Complex: The First Step in Vesicular Transport

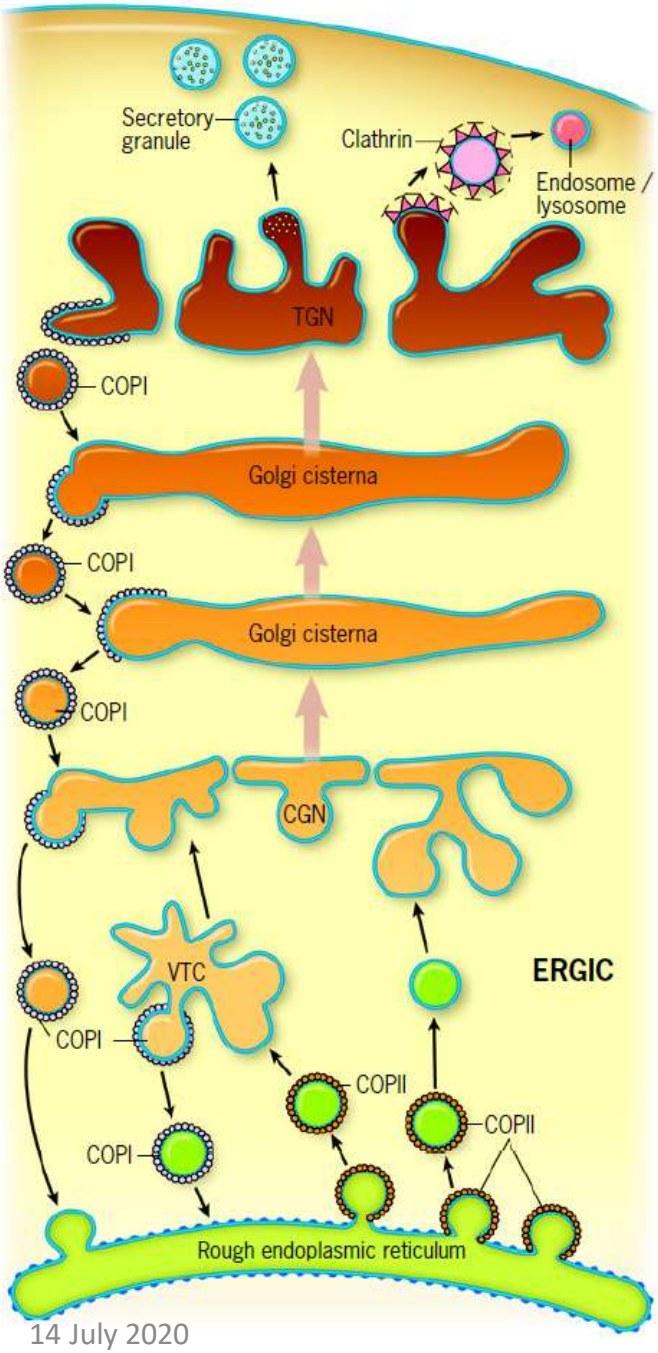
- The RER cisternae contain specialized exit sites that are **devoid of ribosomes** and serve as places where the first transport vesicles in the biosynthetic pathway are formed.
- soon after they bud from the ER membrane, transport vesicles fuse with one another to form larger vesicles and interconnected tubules in the region between the ER and Golgi complex.

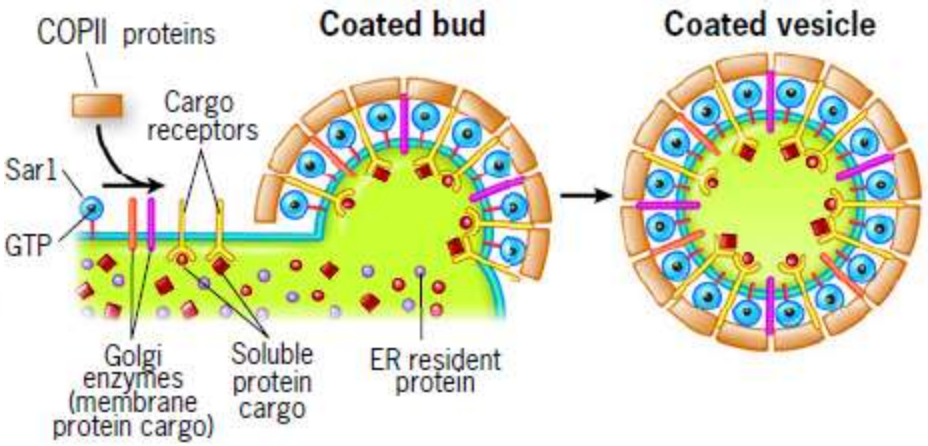
- This region has been named the ***ERGIC*** *(endoplasmic reticulum Golgi intermediate compartment),* and the ***vesicular-tubular carriers*** that form there are called ***VTCs***
- Once formed, the VTCs move farther away from the ER toward the Golgi complex
- Movement of VTCs occurs along tracks composed of microtubules.



Vesicular transport from the ER to the Golgi

Proposed movement of materials by vesicular transport between membranous compartments of the biosynthetic/secretory pathway





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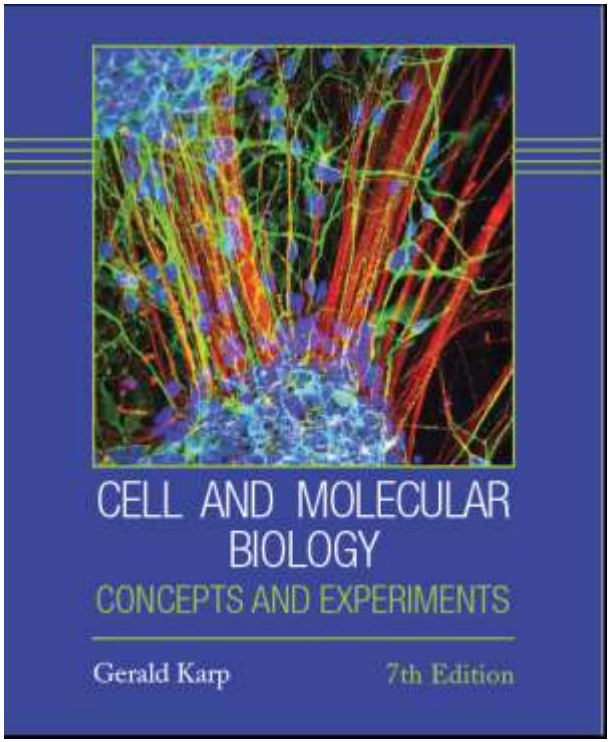
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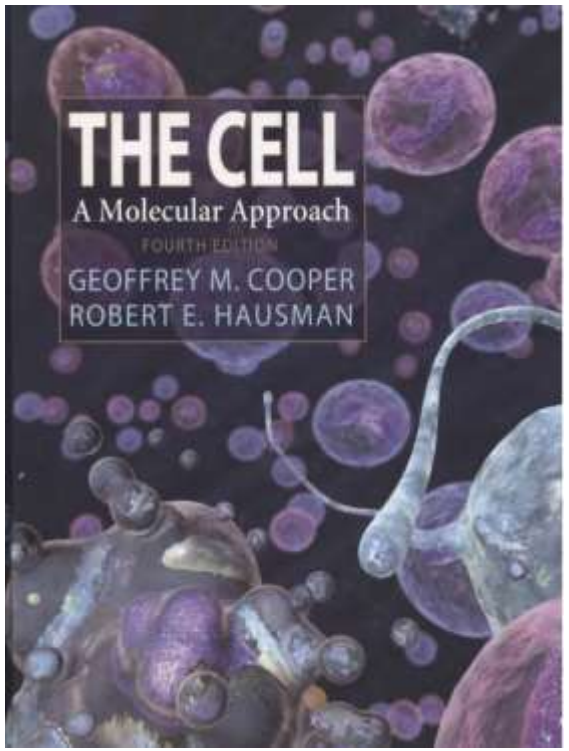
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