MCDB/CHEM 103/203; BMSE 233

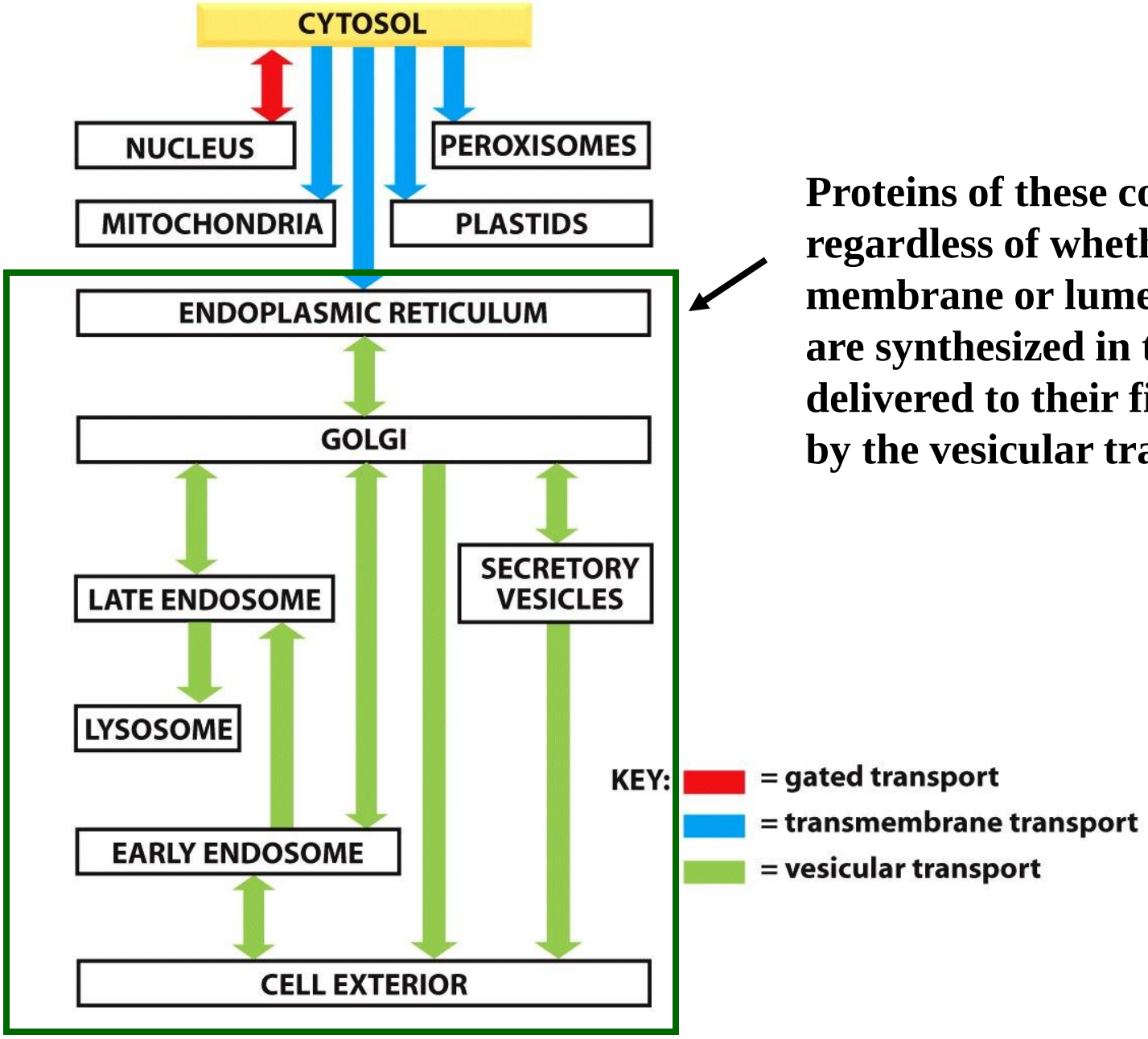
Molecular Trafficking and Signaling

Lecture 16

Associated Textbook Reading:

pp. 723-732

Cytosol to ER Transport

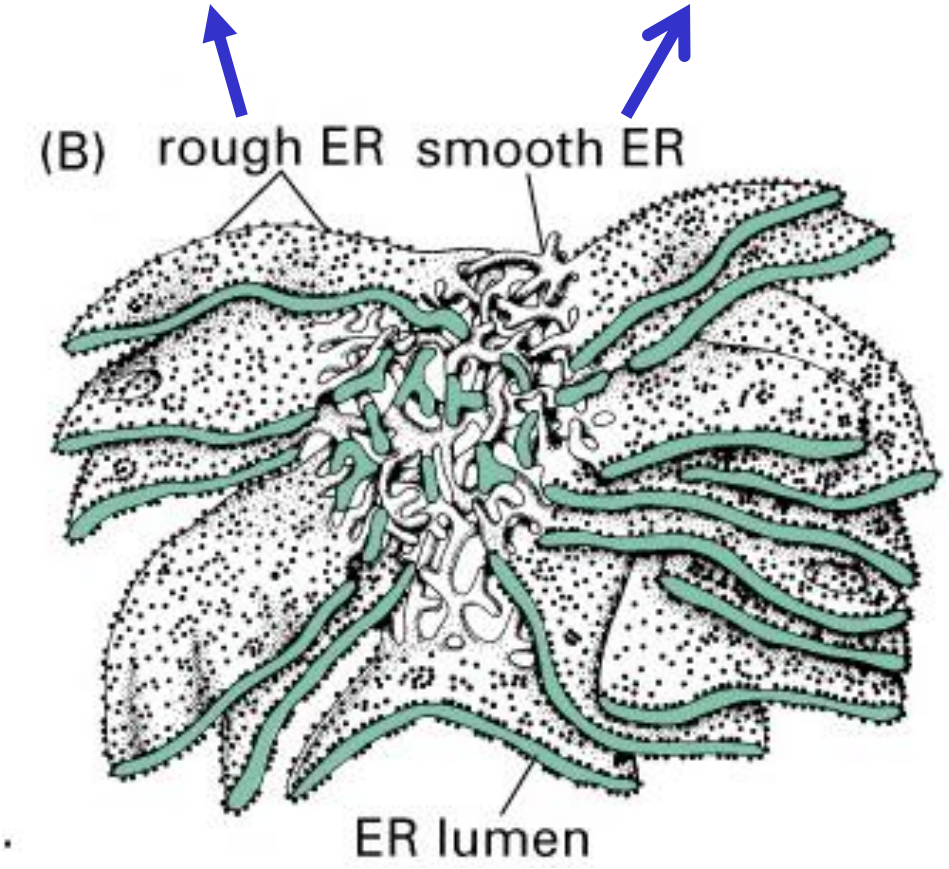


Proteins of these compartments, regardless of whether they are membrane or lumenal proteins, are synthesized in the ER and delivered to their final destinations by the vesicular transport pathway.

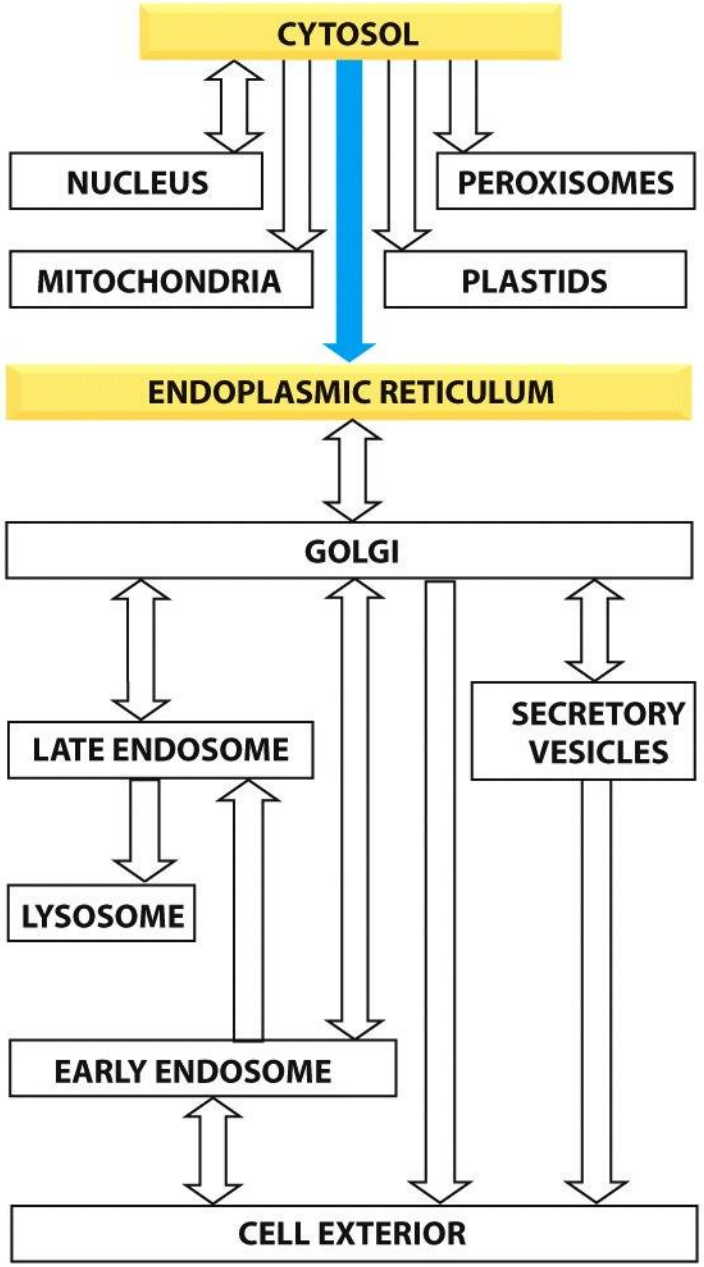
Two Types of ER Membrane Domains

polyribosomes attached
the biosynthesis of proteins

no ribosomes attached
the biosynthesis of lipids
& steroids
regulation of Ca2+



Cytosol to ER Transport



General Features:

Translocator-mediated

ER signal sequence required (often cleaved after translocation)

bi-directional transport (although it seems uni-directional from the diagram) (e.g. misfolded proteins, pathogens)

usually co-translational import

imported as unfolded polypeptide chains

ER Signal Sequence versus:

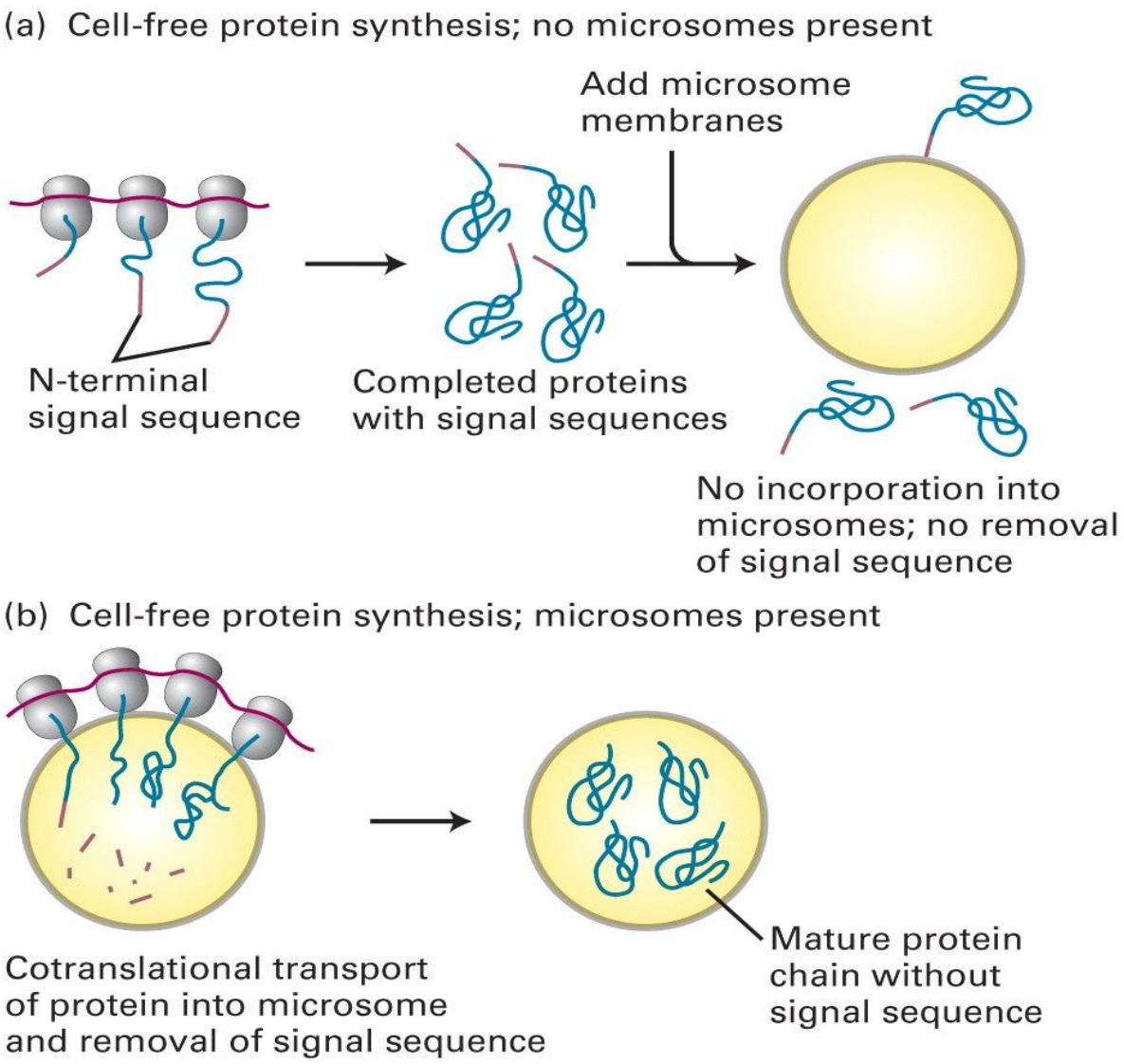
Different types of transport signals of intracellular transport

Uptake-Targeting Sequences That Direct Proteins from the Cytosol to Organelles

Target Organelle	Location of Sequence Within Protein	Removal of Sequence	Nature of Sequence
Endoplasmic reticulum (lumen)	N-terminus	Yes	Core of 6–12 hydrophobic amino acids, often preceded by one or more basic amino acids (Arg, Lys)
Mitochondrion (matrix)	N-terminus	Yes	Amphipathic helix, 20–50 residues in length, with Arg and Lys residues on one side and hydrophobic residues on the other
Chloroplast (stroma)	N-terminus	Yes	No common motifs; generally rich in Ser, Thr, and small hydrophobic residues and poor in Glu and Asp
Peroxisome (matrix)	C-terminus (most proteins); N-terminus (few proteins)	No	PTS1 signal (Ser-Lys-Leu) at extreme C-terminus; PTS2 signal at N-terminus

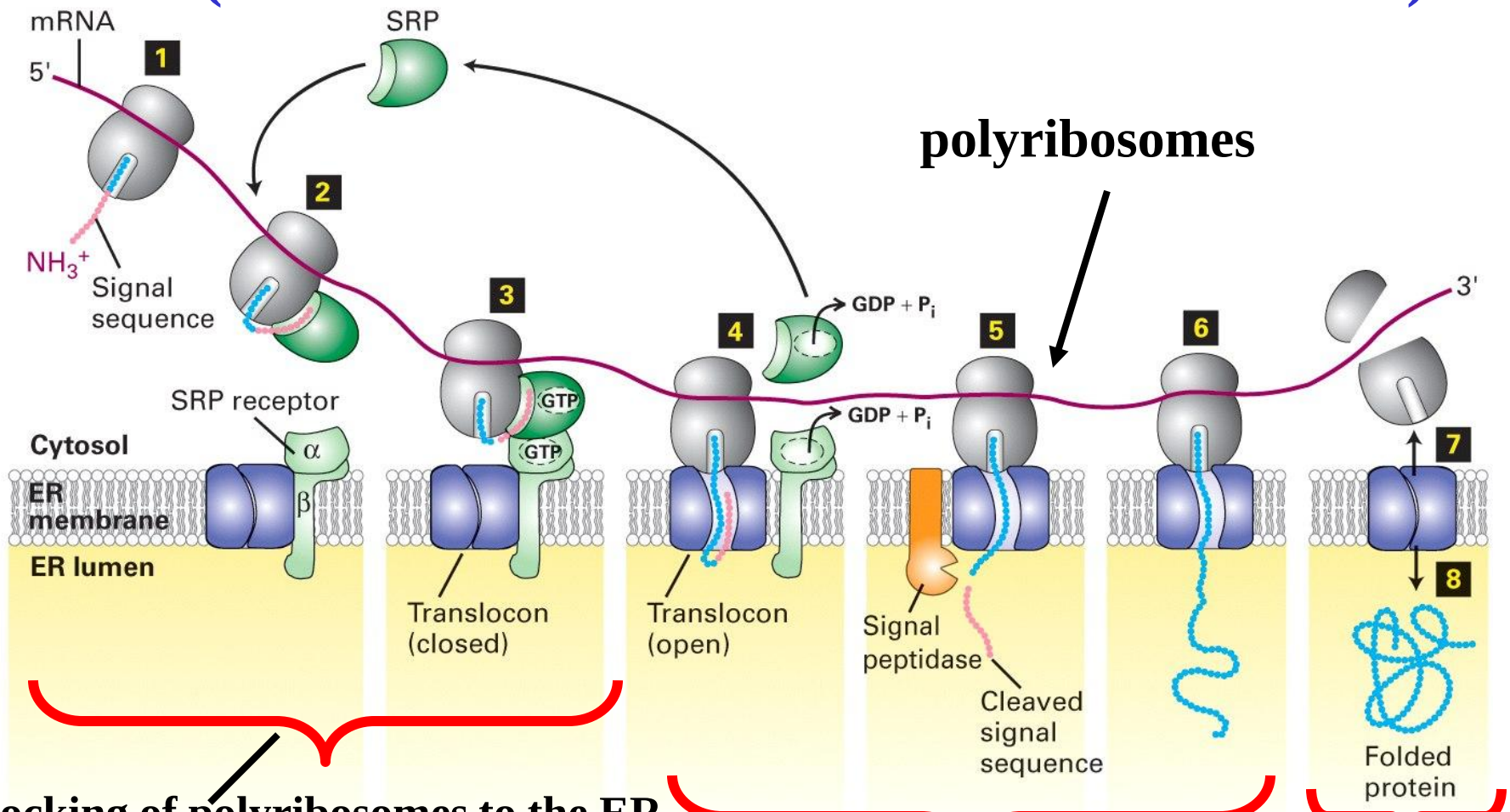
nucleus import	**anywhere**	**no**	**1 or 2 clusters of basic a.a.**
nucleus export	**anywhere**	**no**	**leucine rich sequence**

How Do We Know ER Translocation Occurs Co-Translationally?



In the following slides, we will utilize a souble secretory protein as a model cargo protein to discuss important events during ER translocation. The same principles can be applied to integral membrane proteins, except that the latter require the presence of additional signals within them to direct the insertion of their transmembrane domains into the membrane.

Overview of Cytosol-to-ER Transport
(a co-translational event in most animal cells)

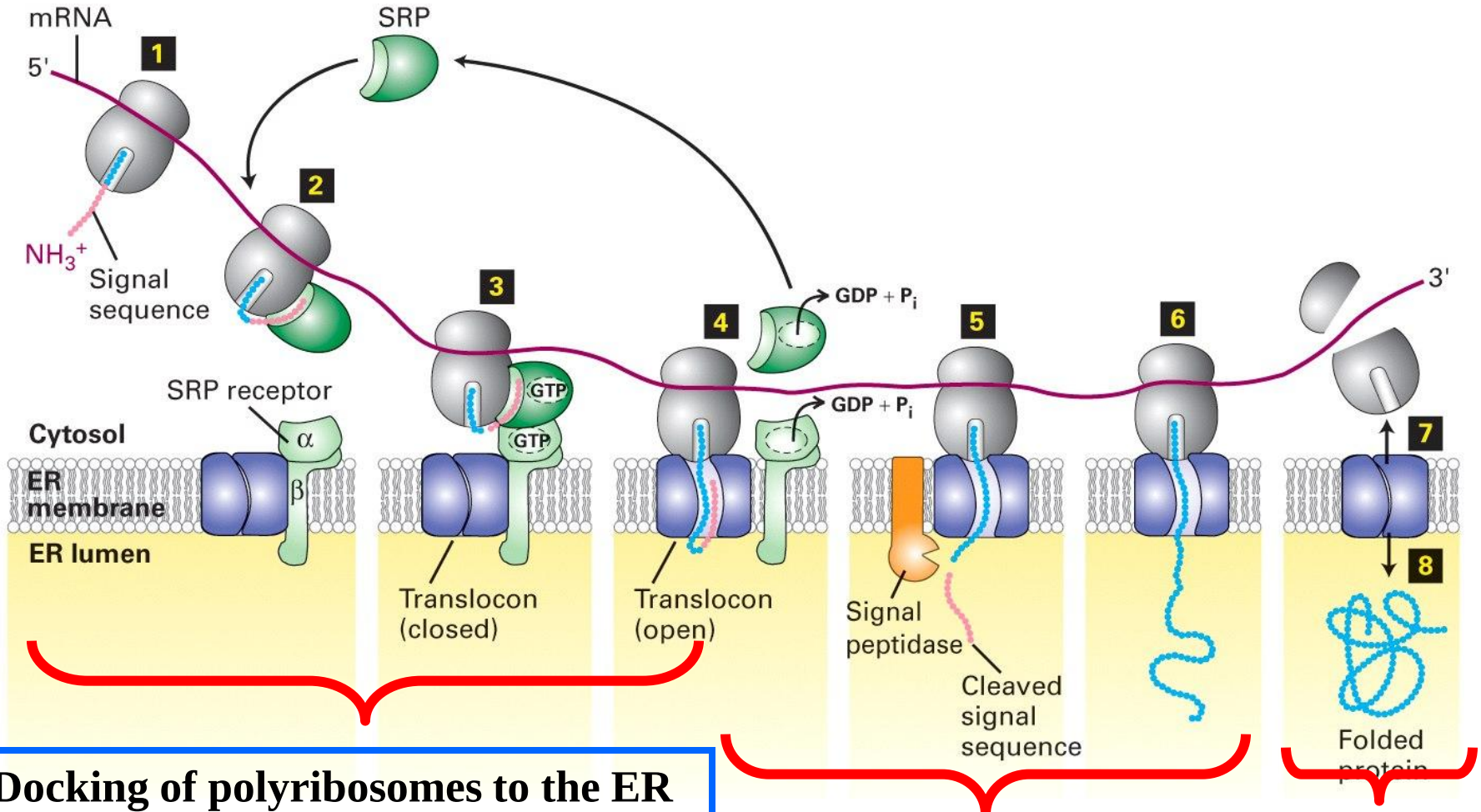


docking of polyribosomes to the ER
(docking occurs during translation)

translocation
(newly synthesized proteins move into ER)

folding
(translocated proteins are folded)

Co-translational docking of polyribosomes to the rough ER requires the ER signal sequence (on a cargo), SRP (in the cytosol), as well as SRP receptor (on the rough ER membrane)



Docking of polyribosomes to the ER

ER signal sequence

SRP

SRP receptor

ER signal sequences (also called ER signal peptides) SRP (signal recognition particle), and SRP receptor

ER signal sequence:

a short stretch of hydrophobic residues (usually 6-12 a.a. long) located at the N-terminus of a cargo protein targeted to the ER

SRP:

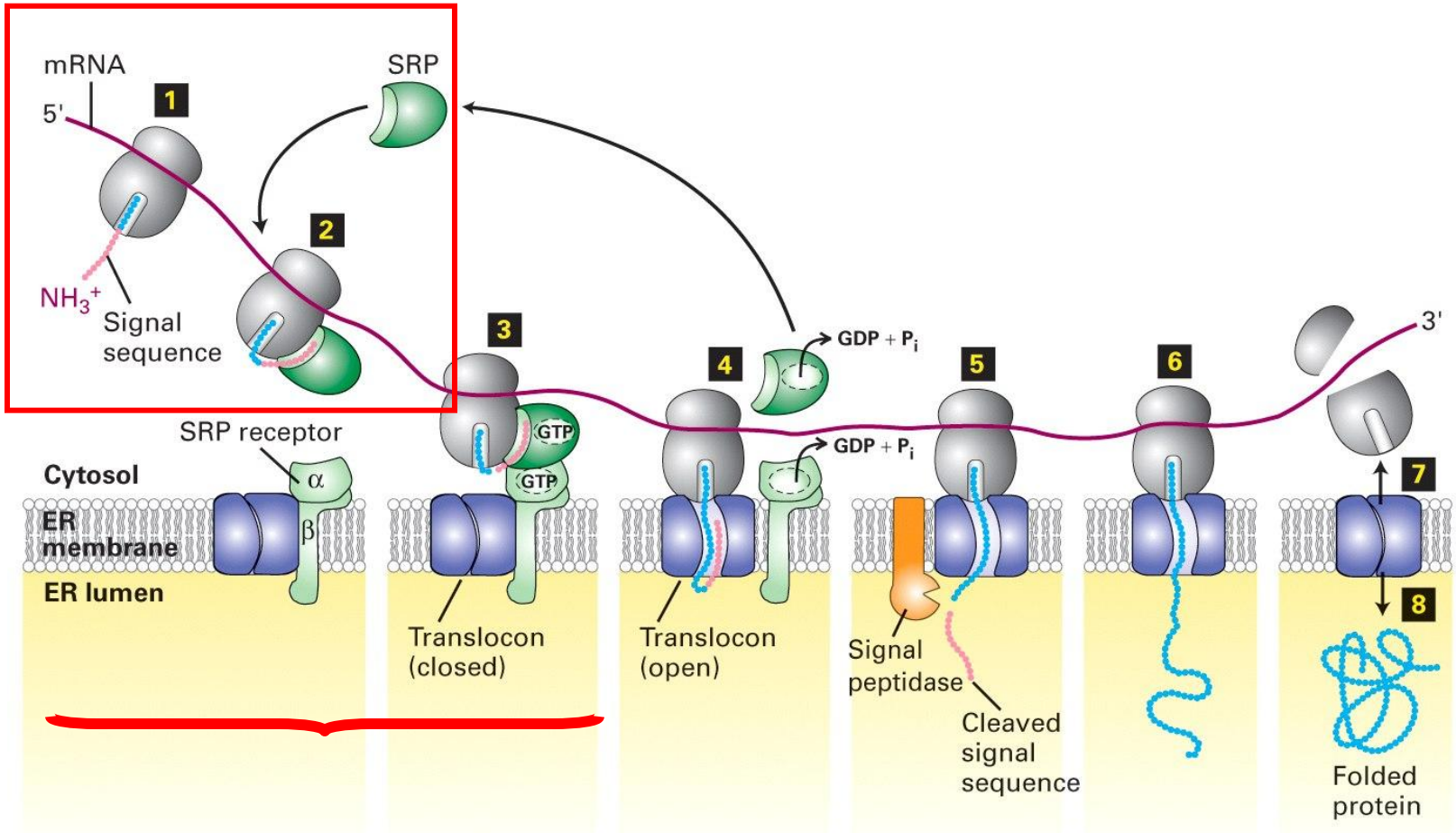
a complex (containing both RNAs and proteins) recognizing the ER signal sequences of a cargo protein

SRP receptor:

an ER integral membrane protein composed of two subunits, α & β. It recognizes SRP.

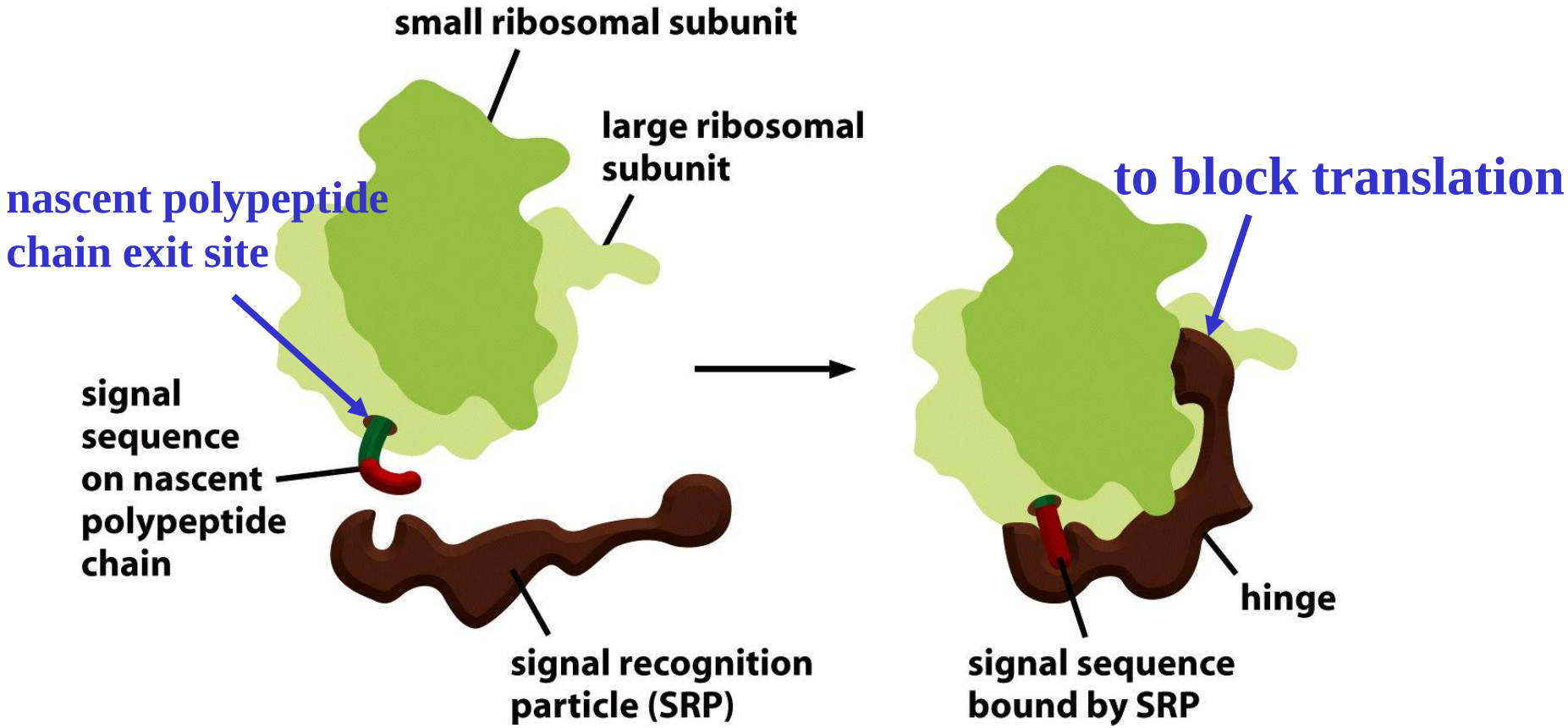
Both SRP and SRP receptor are GTPases.

SRP Binds to the ER Signal Sequence



Due to its N-terminal location, the signal peptide is recognized by SRP soon after its synthesis (before translation is completed).

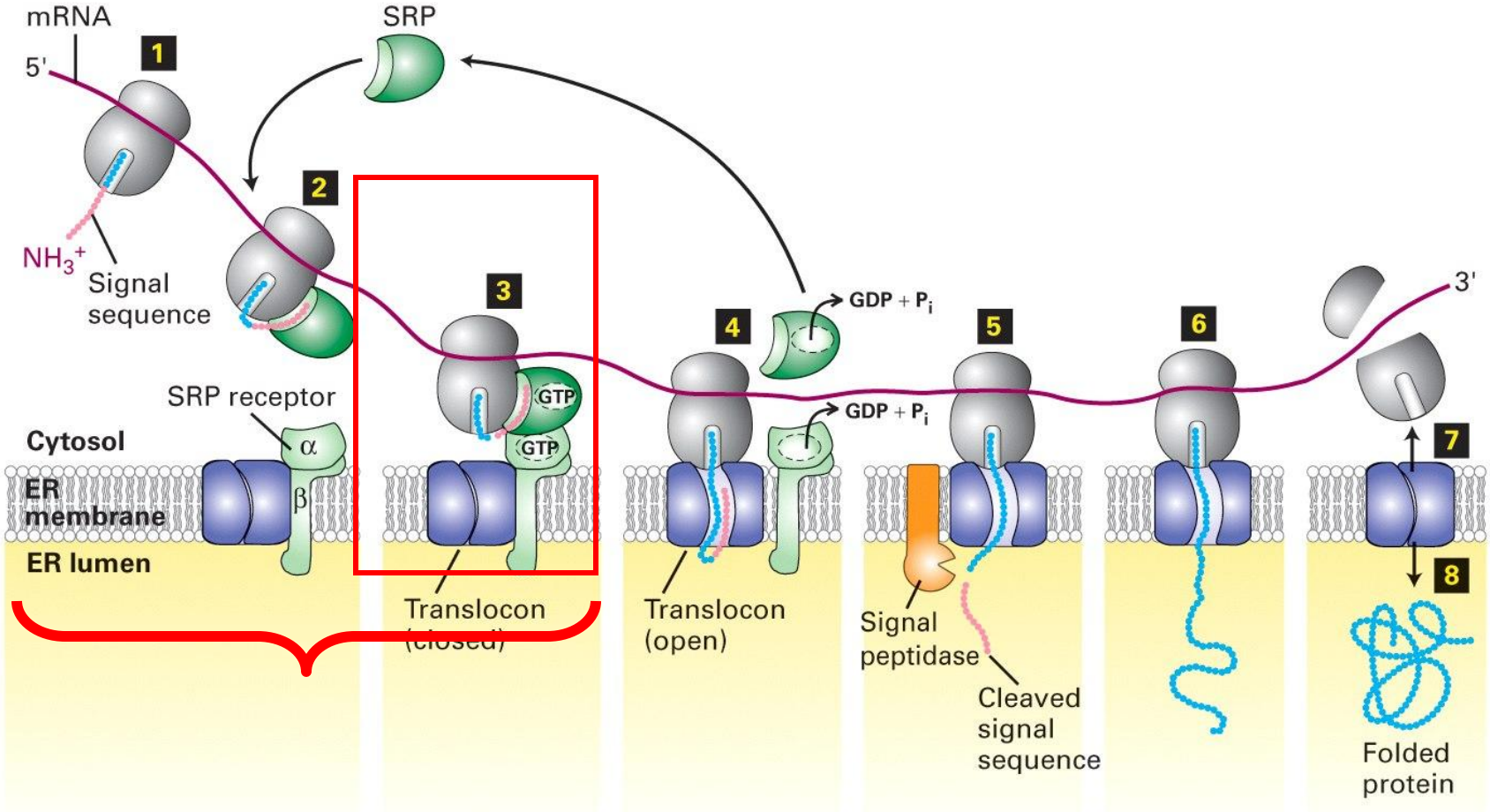
Once Bound to ER Signal Sequence, SRP Interacts with the Ribosome to Pause Translation



another example of protein conformational change

In addition to binding to the signal sequence, SRP also interacts with the ribosome to cause a pause in translation. This assures co-translational transport.

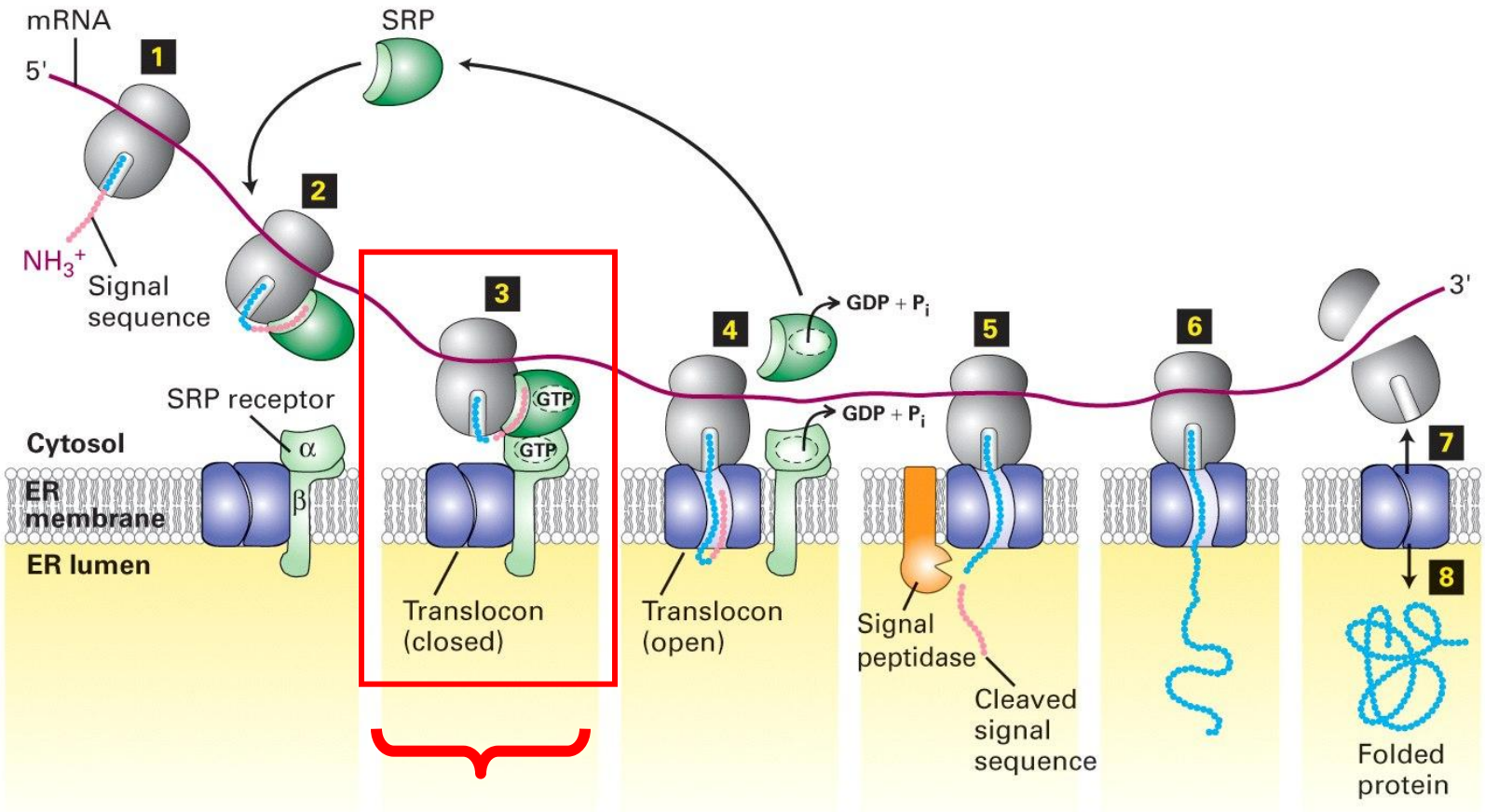
Interaction of SRP & SRP Receptor Leads to Recruitment of Polyribosomes to the Rough ER Membrane



The paused complex containing mRNA, the ribosomes & nascent polypeptides is recruited to the rough ER membrane by the SRP-SRP receptor interaction.

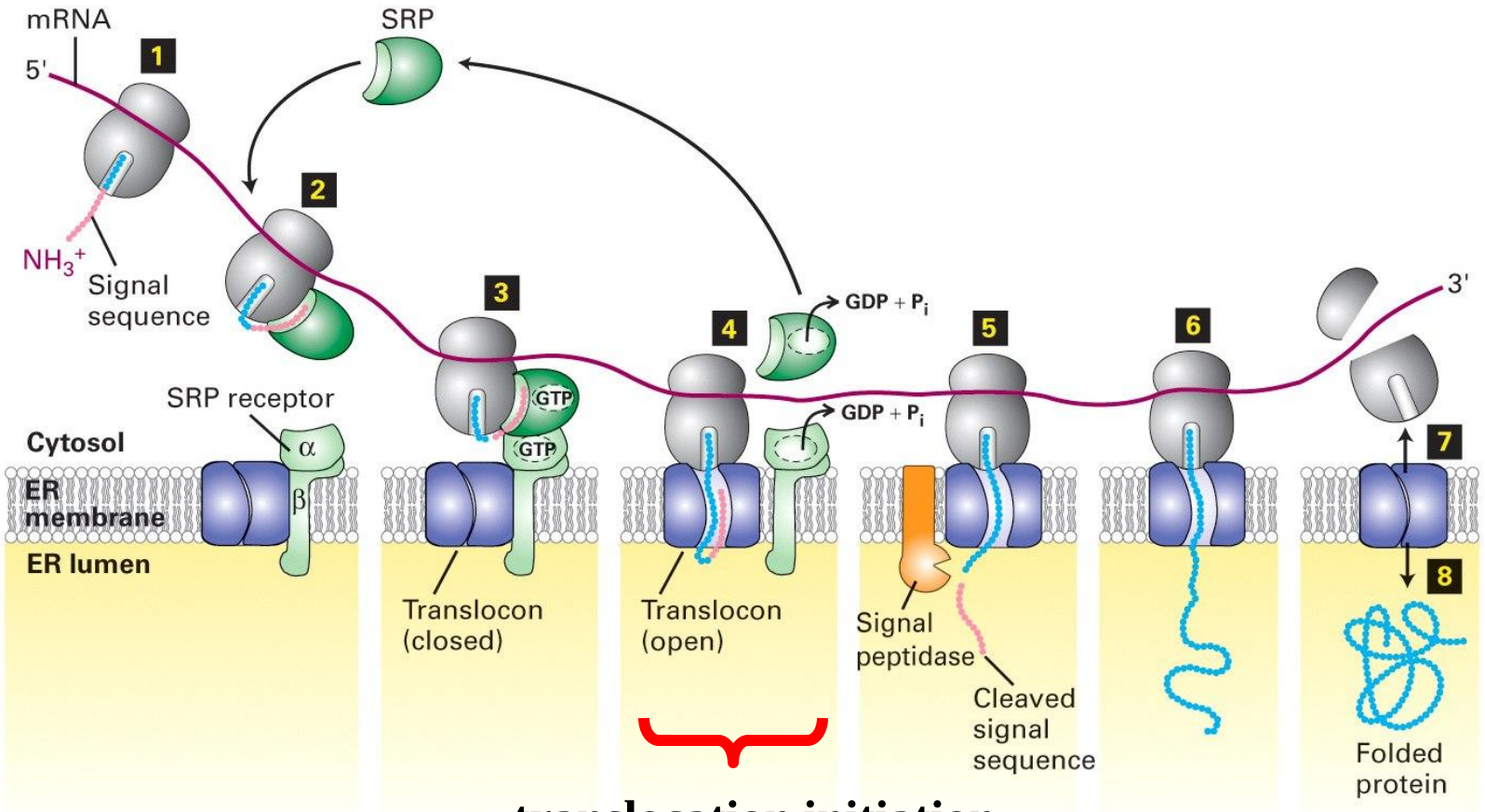
SRP & SRP Receptor Also Function to Proof-Read ER Signal Sequences

Translation will not resume until SRP leaves the polyribosome complex, which is controlled by GTP hydrolysis



A bad signal sequence will dissociate with the SRP before the hydrolysis of GTP. Thus, SRP/SRP receptor function as a molecular clock to allow sufficient time to check the quality of the signal sequences.

SRP/Signal Sequence Communicate with the Translocon to Initiate Translocation



translocation initiation

If the signal sequence is good, the mRNA-ribosome-nascent polypeptide chain complex is transferred from the SRP/SRP receptor to the translocon and the GTP hydrolysis of SRP/SRP receptor occurs. As a result, translation resumes. Moreover, the signal sequence opens up the translocon and functions as a signal to start the translocation

Multiple Functions of the SRP

interacts with the signal sequence
(to recruit the polyribosomes to the ER)

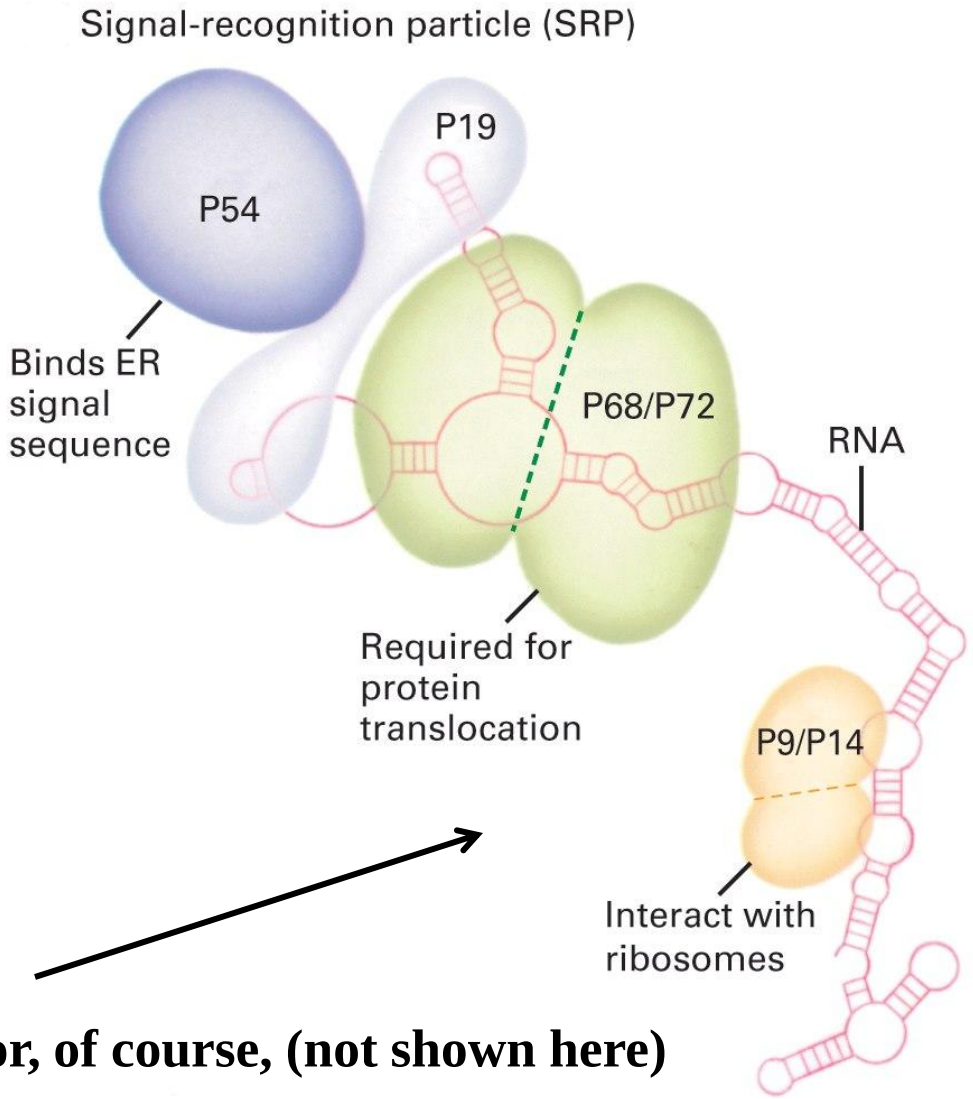
interacts with the ribosome
(to pause translation and ensure co-translational translocation)

interacts with the SRP receptor
(to dock polyribosomes to the ER)
(to proof-read the signal sequence)

communicates with the translocon
(to initiate the translocation process)

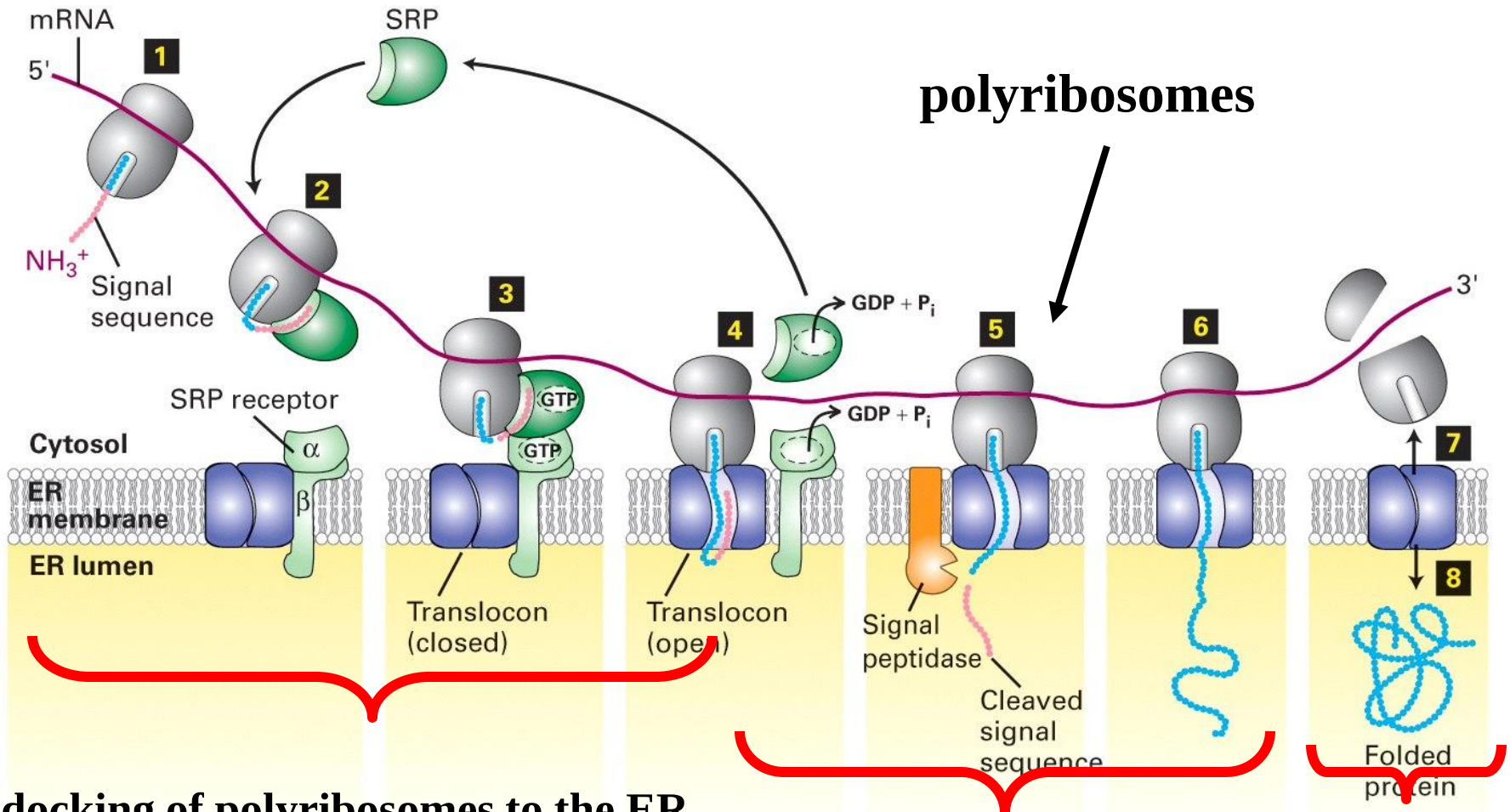
The Multiple Functions of SRP Explains Why Its Structure is Complex, Compared to Other Types of Signal Receptors Such as the Importins

SRP is a ribonucleoprotein particle (i.e. containing RNA and protein)



SRP also interacts with SRP receptor, of course, (not shown here)

Translocation and Cleavage of the Signal Sequence



polyribosomes

docking of polyribosomes to the ER

ER signal sequence

SRP

SRP receptor

translocation

translocon

signal peptidase

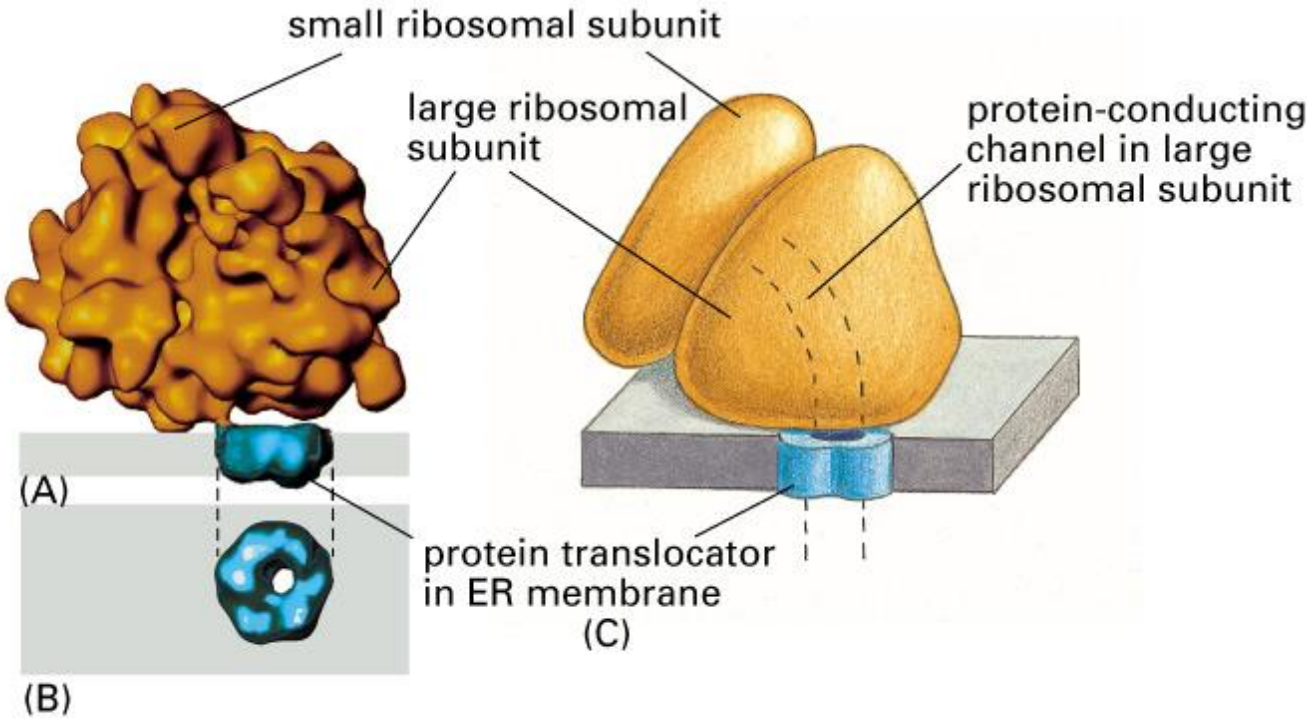
ribosomes

folding

chaperones

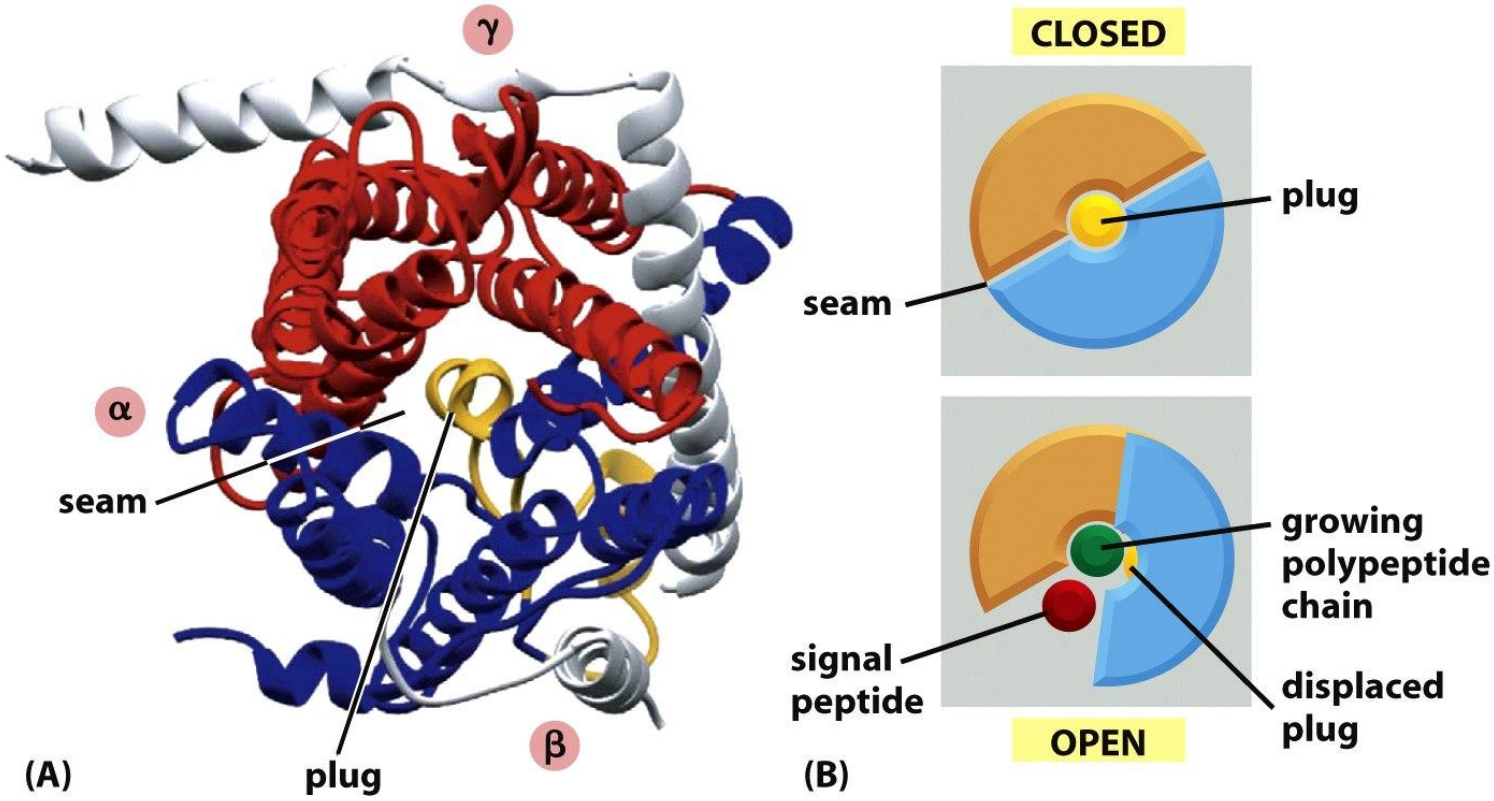
other proteins

Translocation of the Nascent Polypeptide Chain by an Open Translocon (Translocator)



After attaching to the ER membrane, the ribosomes and nascent chains are transferred to the translocon. As translation resumes, the elongating chain passes directly from the large ribosomal subunit to the central pore of the translocon

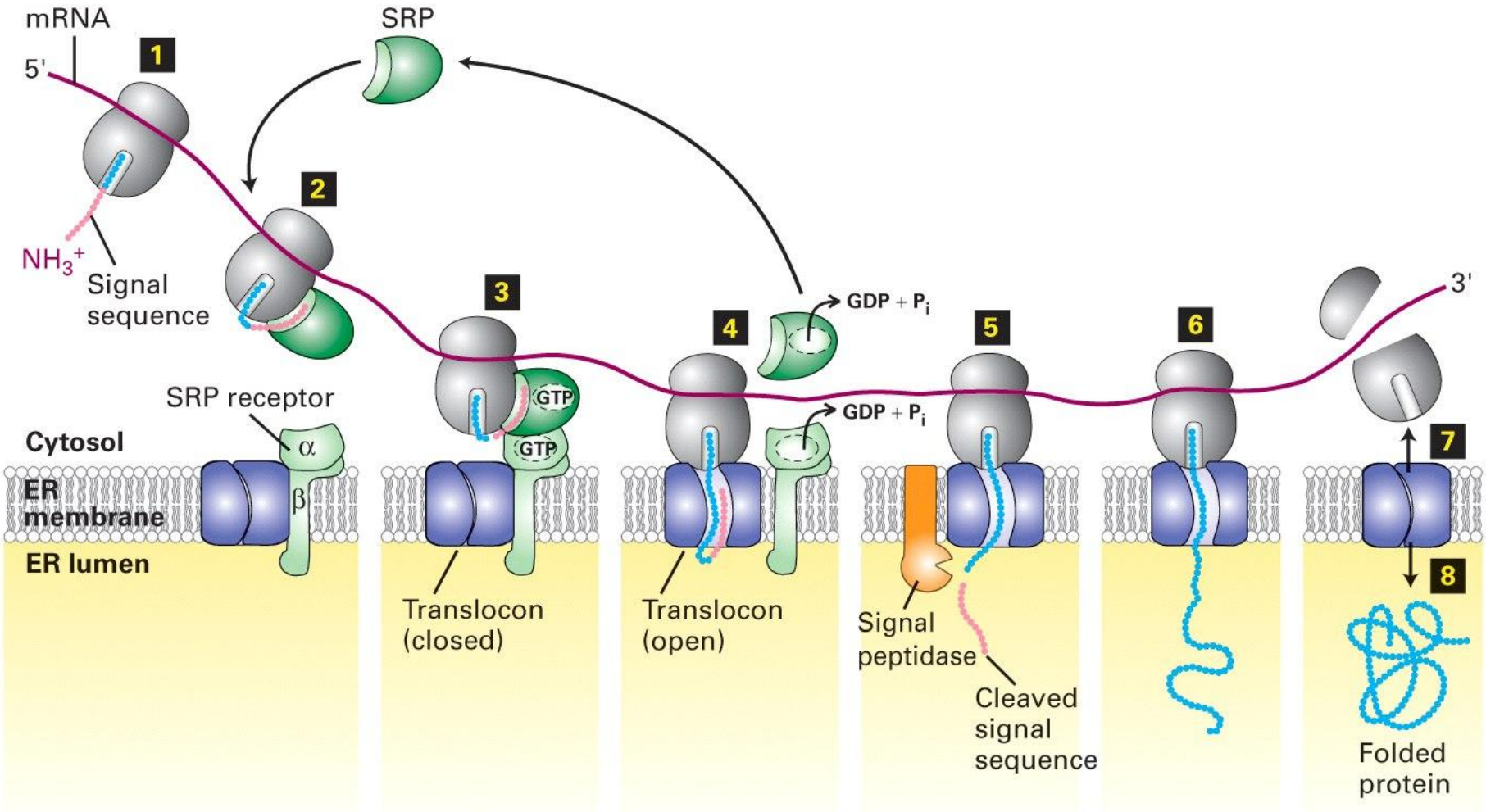
Conformational Changes Between the Closed and Open States of Sec61 Translocon



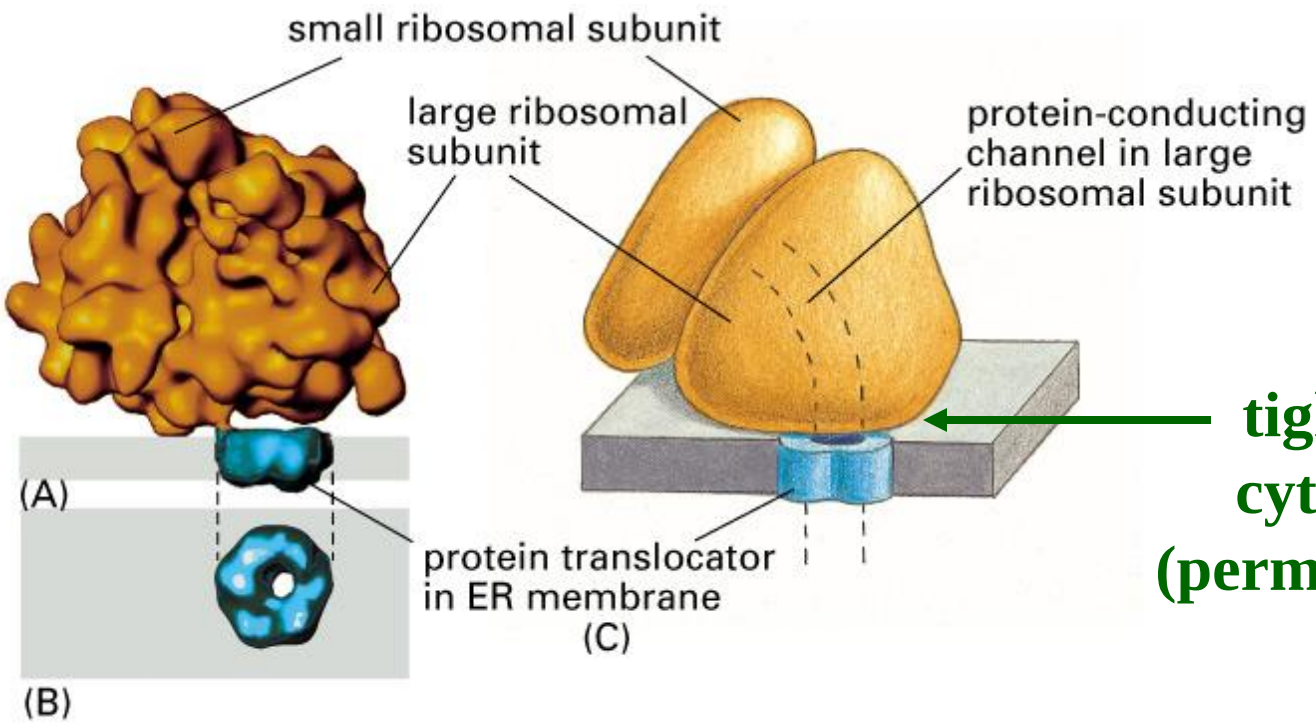
Signal sequence interacts with the translocon and induces a conformational change, which leads to the removal of the plug to allow the passage of nascent polypeptide chain into the lumen.

The pore can probably open to the lateral side as well to allow the signal sequence and the transmembrane domains to be released to the lipid bilayer where it can be subsequently cleaved or inserted

How is the Permeability Barrier Maintained While the Translocon is Open (since the plug is removed)?



The Ribosome Forms a Tight Seal with an Open Translocon

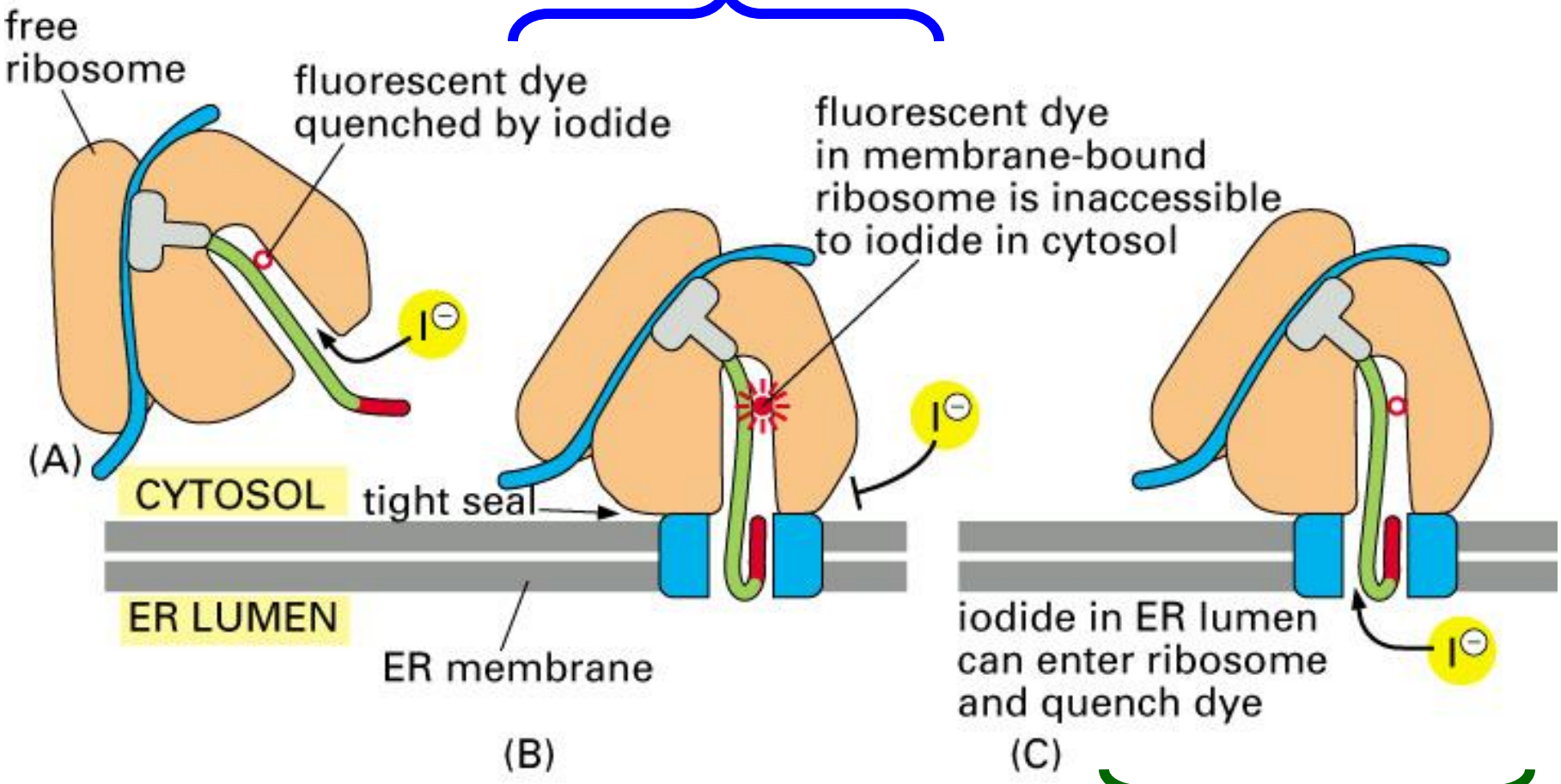


tight seal on the cytoplasmic side (permeability barrier)

After attaching to the ER membrane, the ribosomes and nascent chains are transferred to the translocon. As translation resumes, the elongating chain passes directly from the large ribosomal subunit to the central pore of the translocon (without being exposed to the cytoplasm)

Evidence for a Tight Seal on the Cytoplasmic Side but not on the Lumenal Side

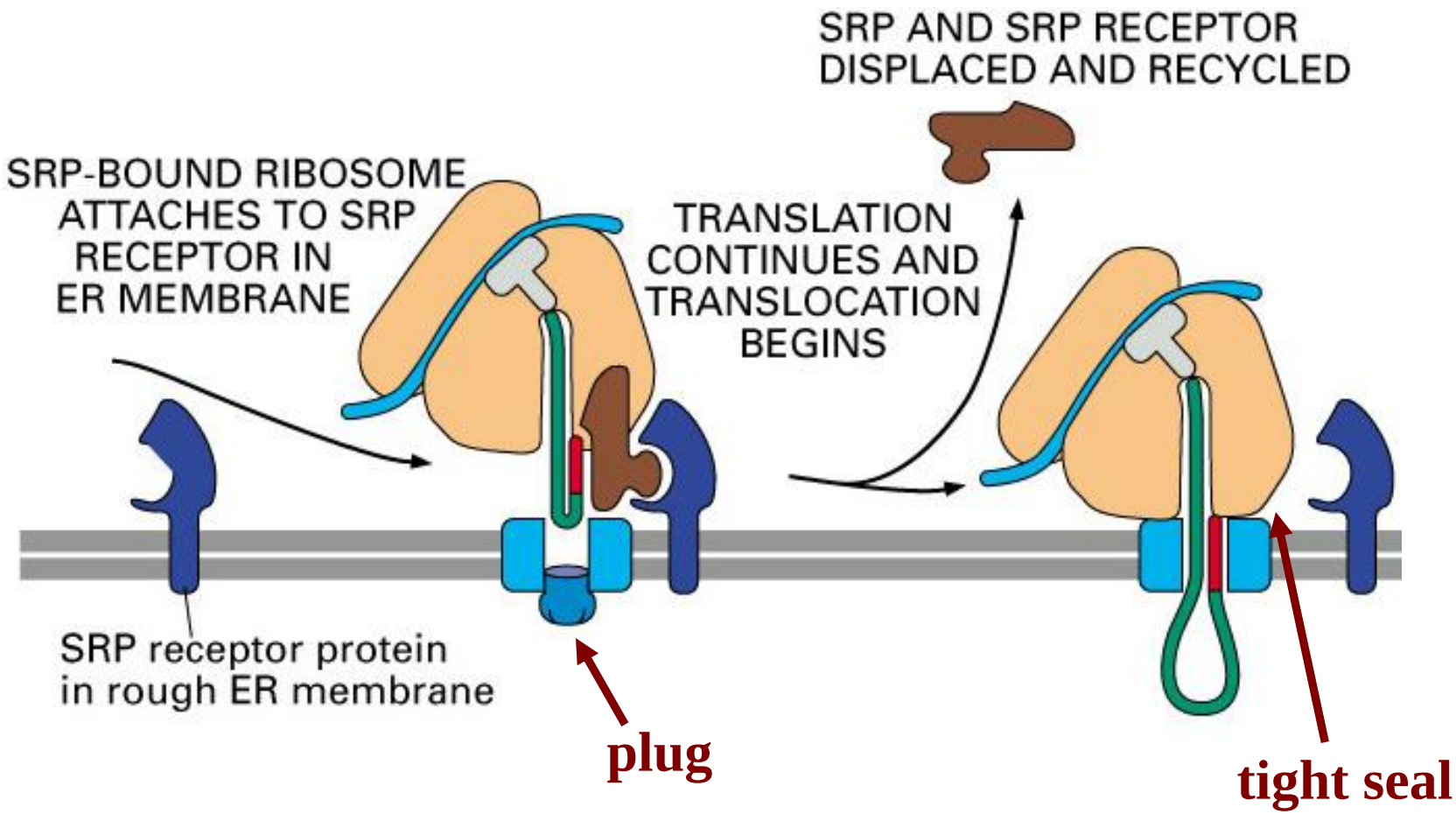
Pore closes to the cytoplasmic side



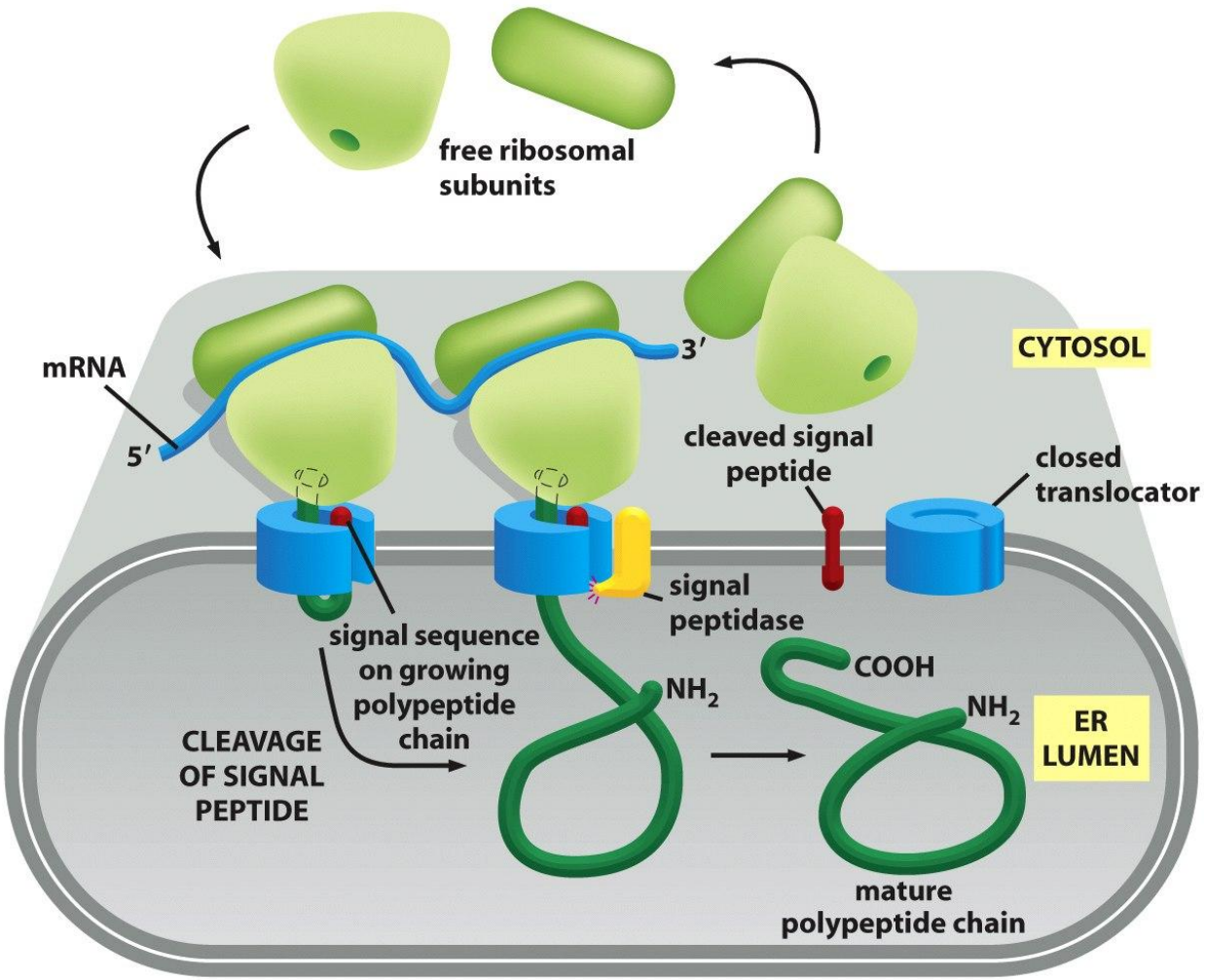
the iodide ion is membrane impermeable

pore opens to the lumenal side

Gating Model of the Sec61 Translocon

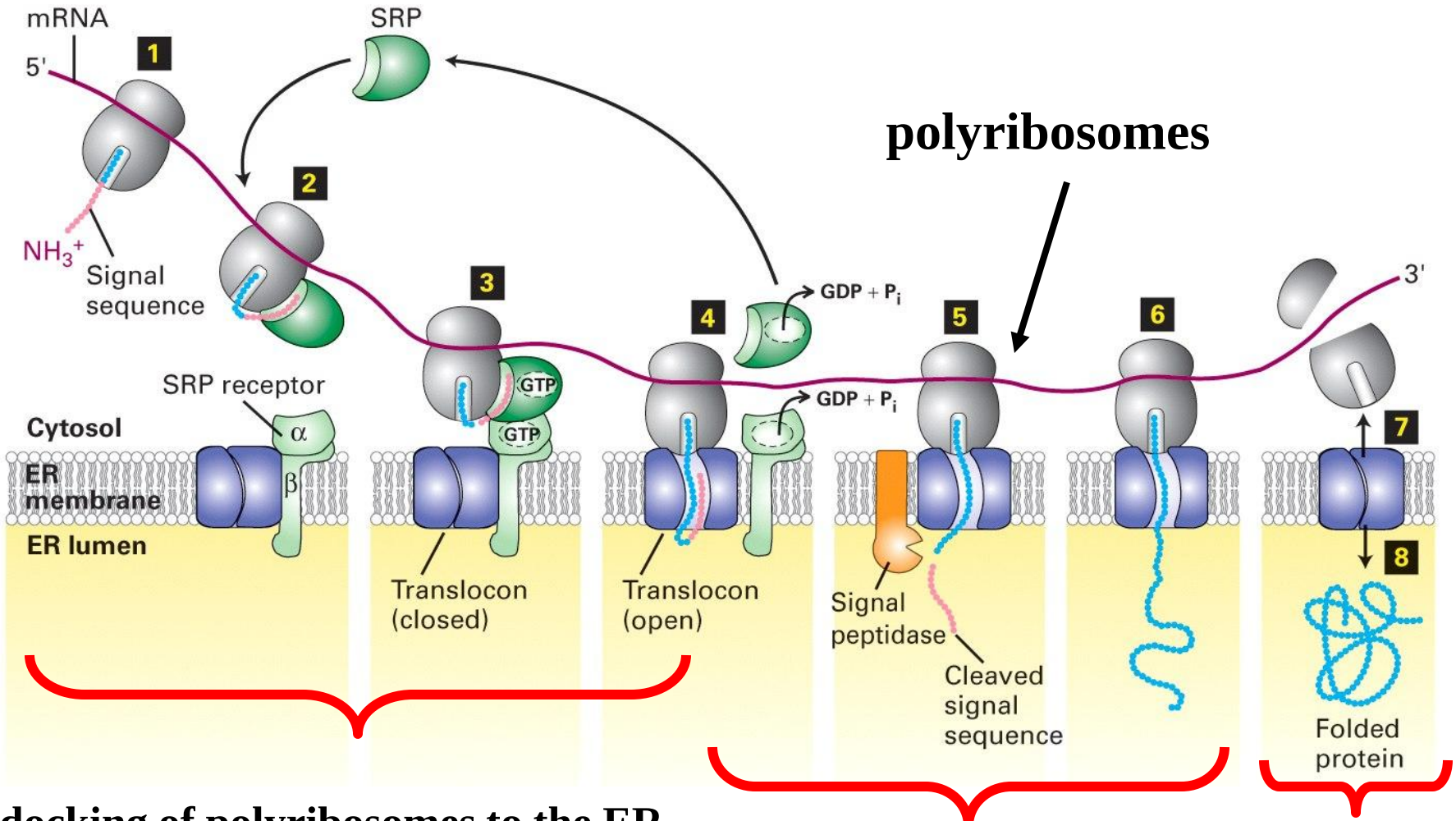


The ER Signal Sequence is Often Removed During Translocation



The signal peptidase closely associates with the translocon and clips off the signal sequence at the lumenal side of the membrane

Folding of the Translocated Nascent Polypeptide Chain



docking of polyribosomes to the ER

ER signal sequence

SRP

SRP receptor

translocation

translocon

signal peptidase

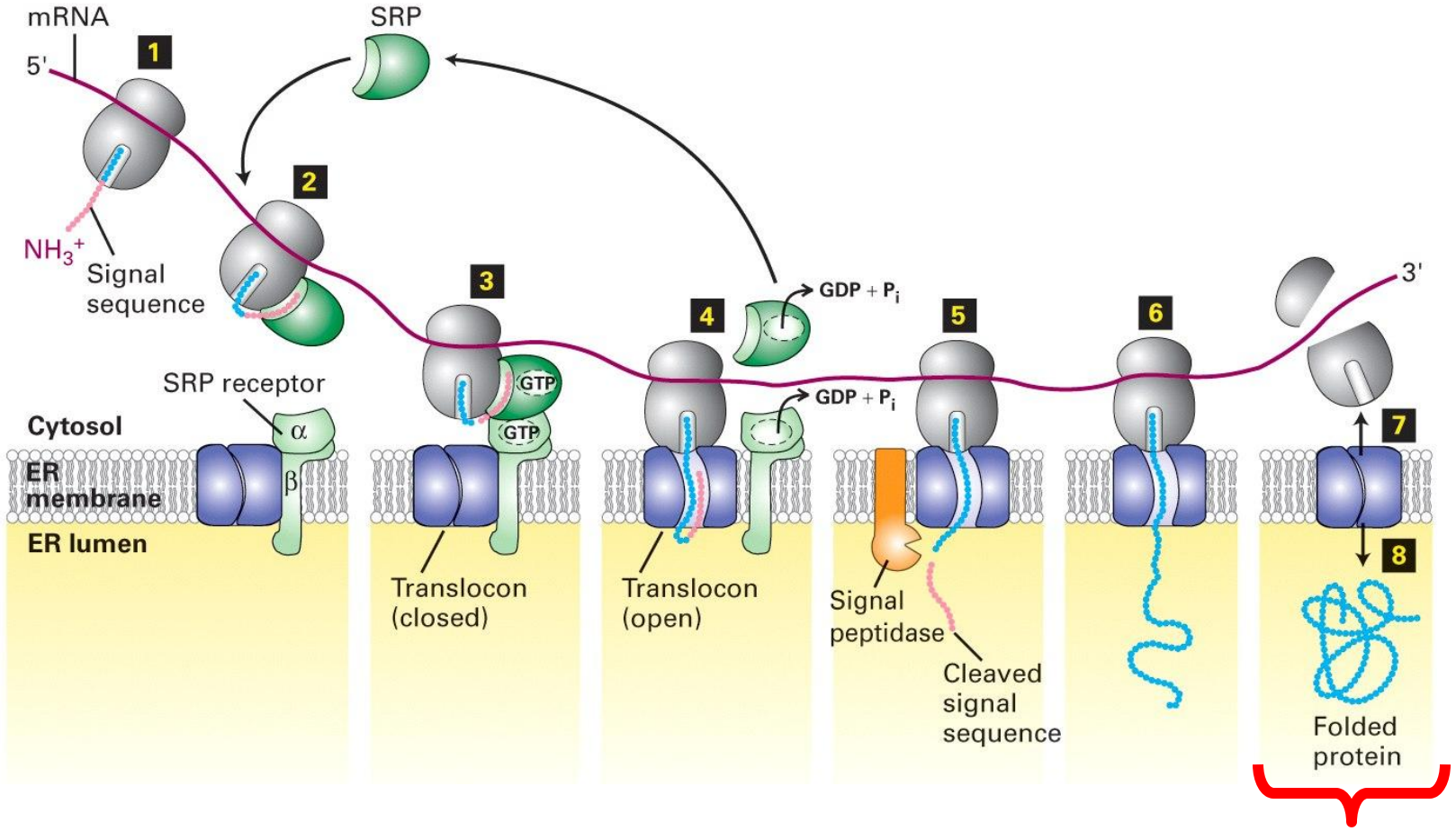
ribosomes

folding

chaperones

other proteins

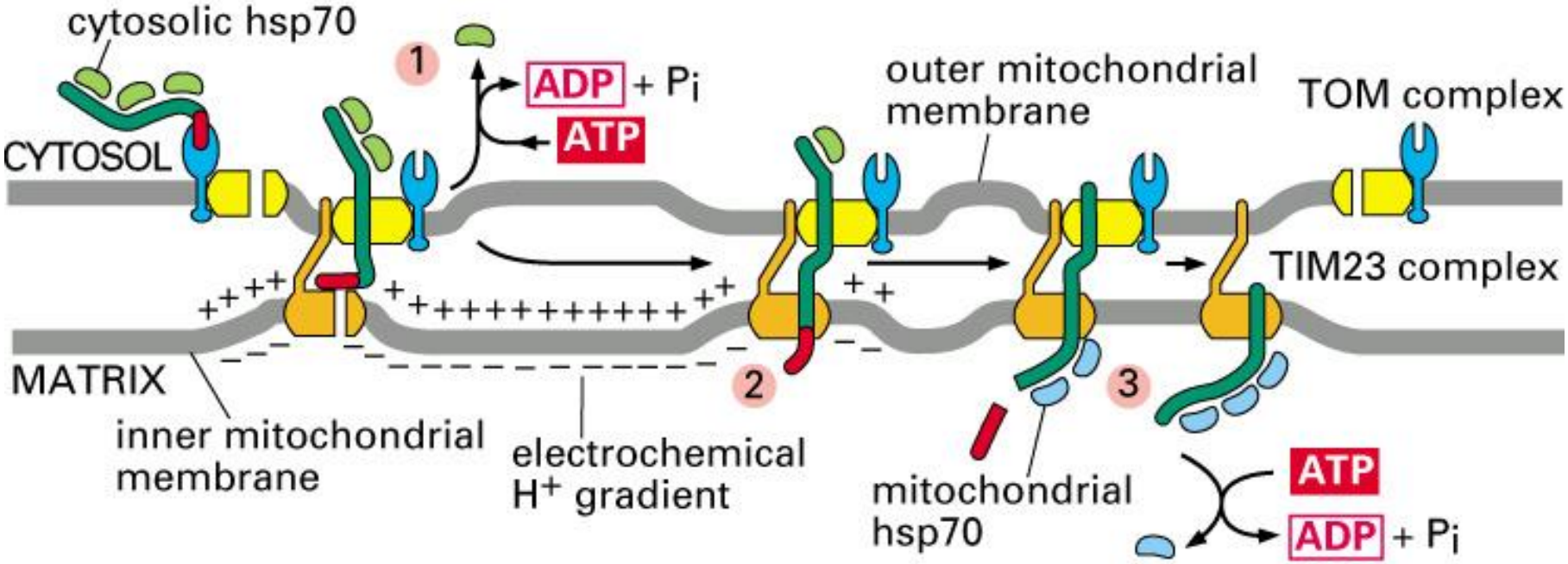
Folding of the Translocated Polypeptide Requires ER Chaperones



Chaperones are needed in ER lumen to facilitate the folding of a nascent protein. However, no chaperones are needed in the cytoplasmic side during translocation.

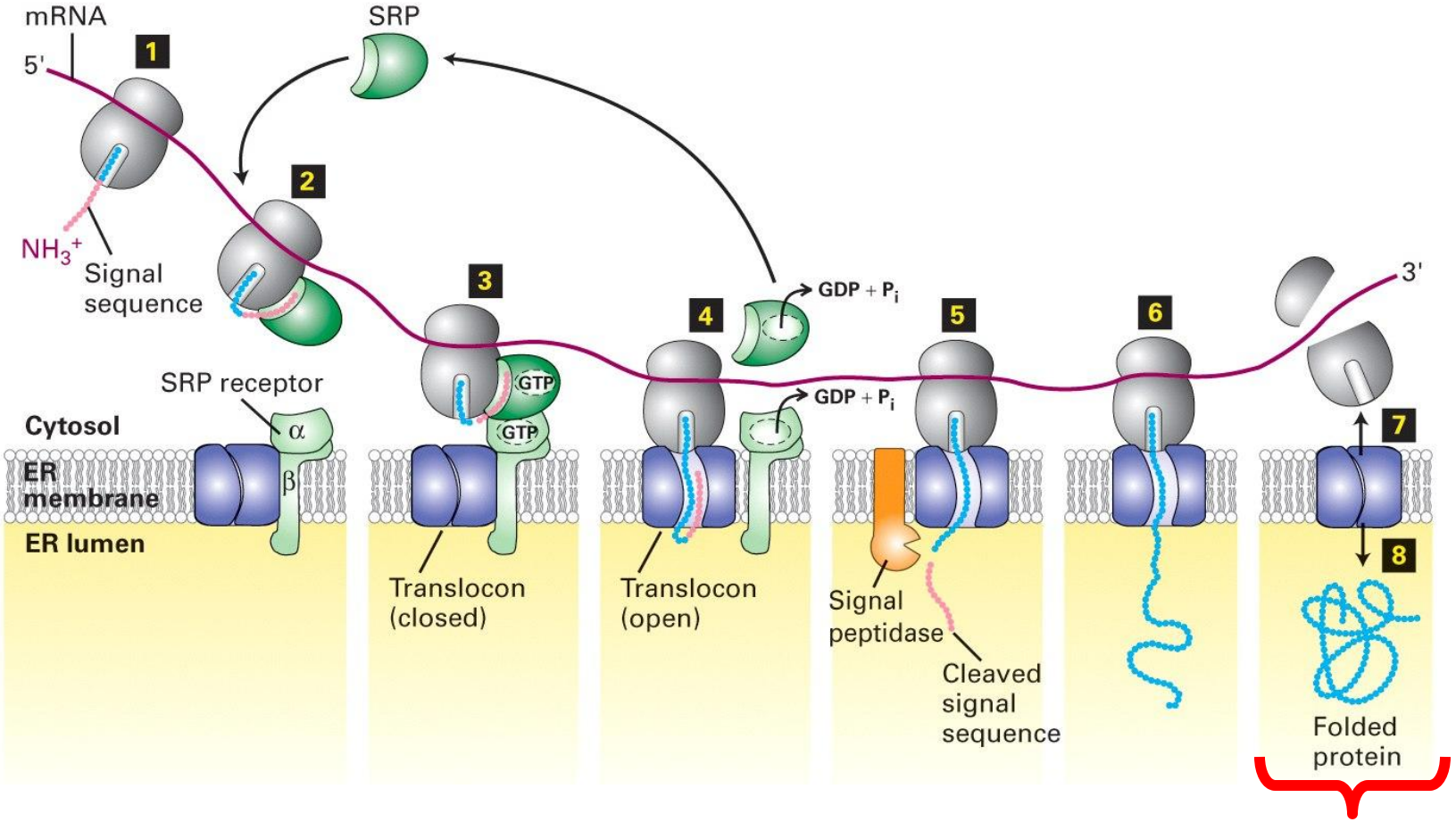
Recall: Chaperones are Required on Both Cytosolic and Lumenal (Matrix) Sides During Mitochondrial Cytosol-to-Matrix Transport

For import into mitochondria, cytosolic chaperone hsp70 is required to maintain the unfolded status of the transported protein (due to its post-translational folding)

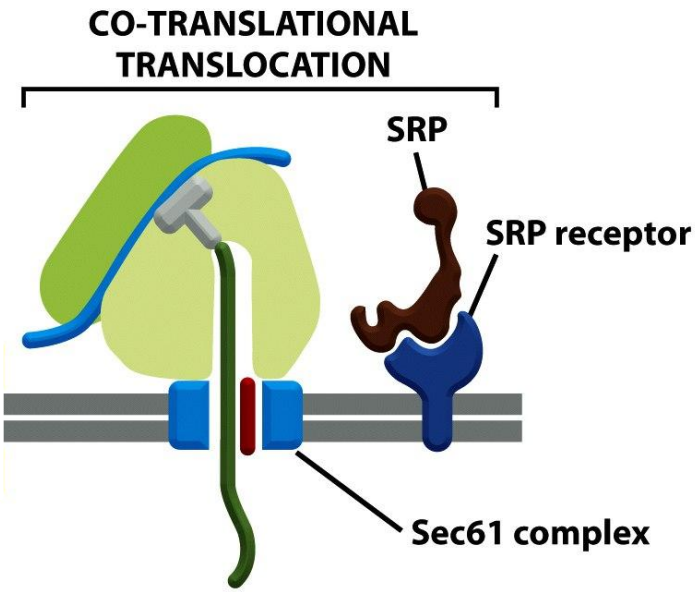


Why are Chaperones Needed Only in the Lumenal Side During Co-Translational ER Translocation?

During co-translational translocation, the length of nascent protein chain is short on the cytoplasmic side during translocation. As a result, the protein cannot fold before being pushed into the lumen and therefore there is no need of chaperones to unfold the protein.



A Common Theme Between Co-Translational ER Transport in Eukaryotes and Co-Translational Transport Across the Cell Membrane of Bacteria and Archaea



BACTERIA

ARCHAEA

EUCARYOTES

However, some proteins enter ER post-translationally in eukaryotes

Post-Translational ER Transport Requires Additional Accessory Proteins