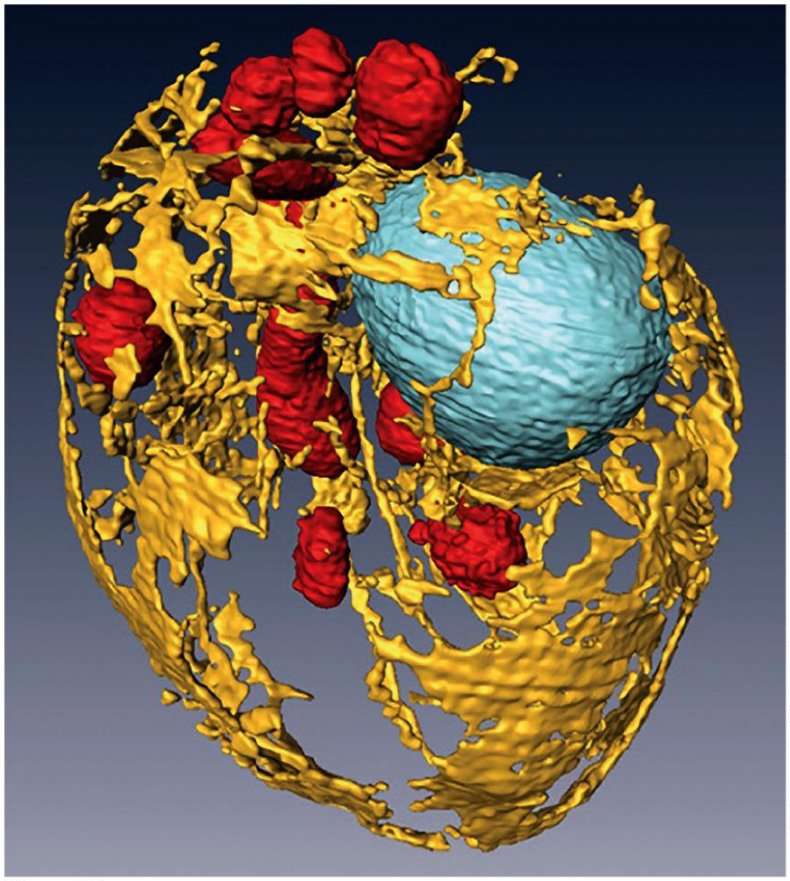
Chapter 13 – Moving Proteins into Membranes and Organelles



From Wei, D. et al., "High-resolution three-dimensional reconstruction of a whole yeast cell using focused-ion beam scanning electron microscopy," *Biotechniques*, 2012, 53(1):41–48.

Chapter 13 Opener
Molecular Cell Biology, Eighth Edition

Chapter 13 – Moving Proteins into Membranes and Organelles

13.1 Targeting Proteins To and Across the ER Membrane

13.2 Insertion of Membrane Proteins into the ER

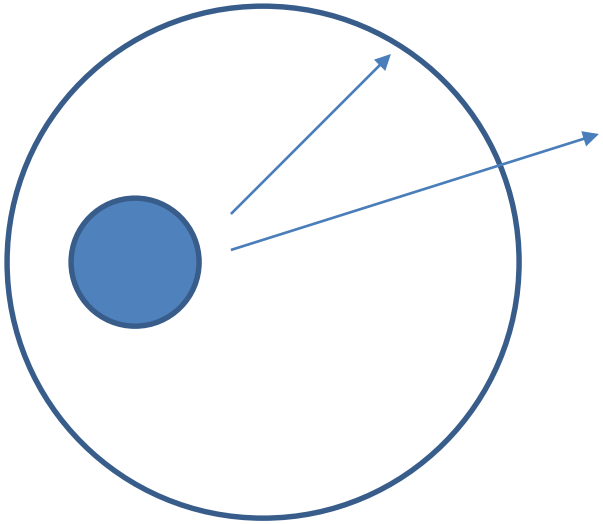
13.3 Protein Modifications, Folding, and Quality Control in the ER

13.4 Targeting of Proteins to Mitochondria and Chloroplasts

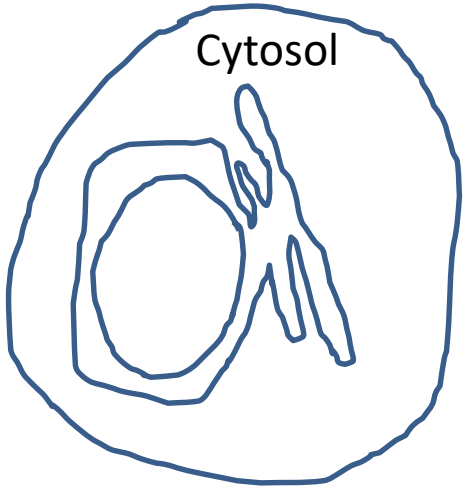
13.5 Targeting of Peroxisomal Proteins

13.6 Transport Into and Out of the Nucleus

What is the most reasonable way to translocate the protein into the membrane or outside of the cell?



Think about the inside and outside of cell!

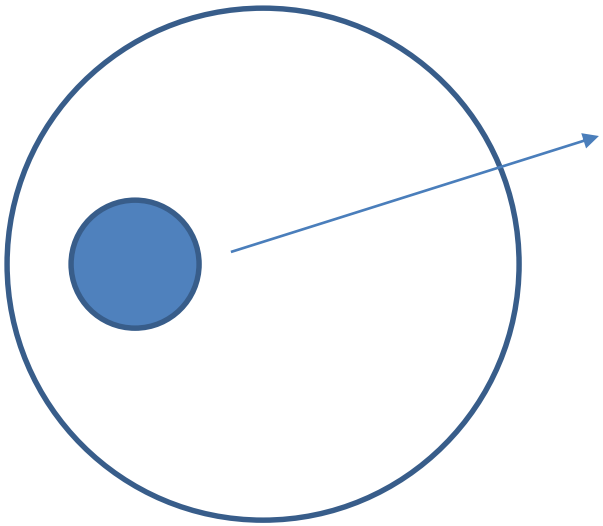


Outside of the cell

?

Evolution theory?

Secreted proteins



Moving Proteins into Membranes and Organelles

13.1 Targeting Proteins To and Across the ER Membrane

- Synthesis of secreted proteins, integral plasma-membrane proteins, and proteins destined for the ER, Golgi complex, plasma membrane, or lysosome begins on cytosolic ribosomes.
- A signal sequence, SRP, and SRP receptor system docks the ribosome on an ER translocon and cotranslationally inserts the nascent protein into or through the ER membrane.

Overview of major protein-sorting pathways in eukaryotes

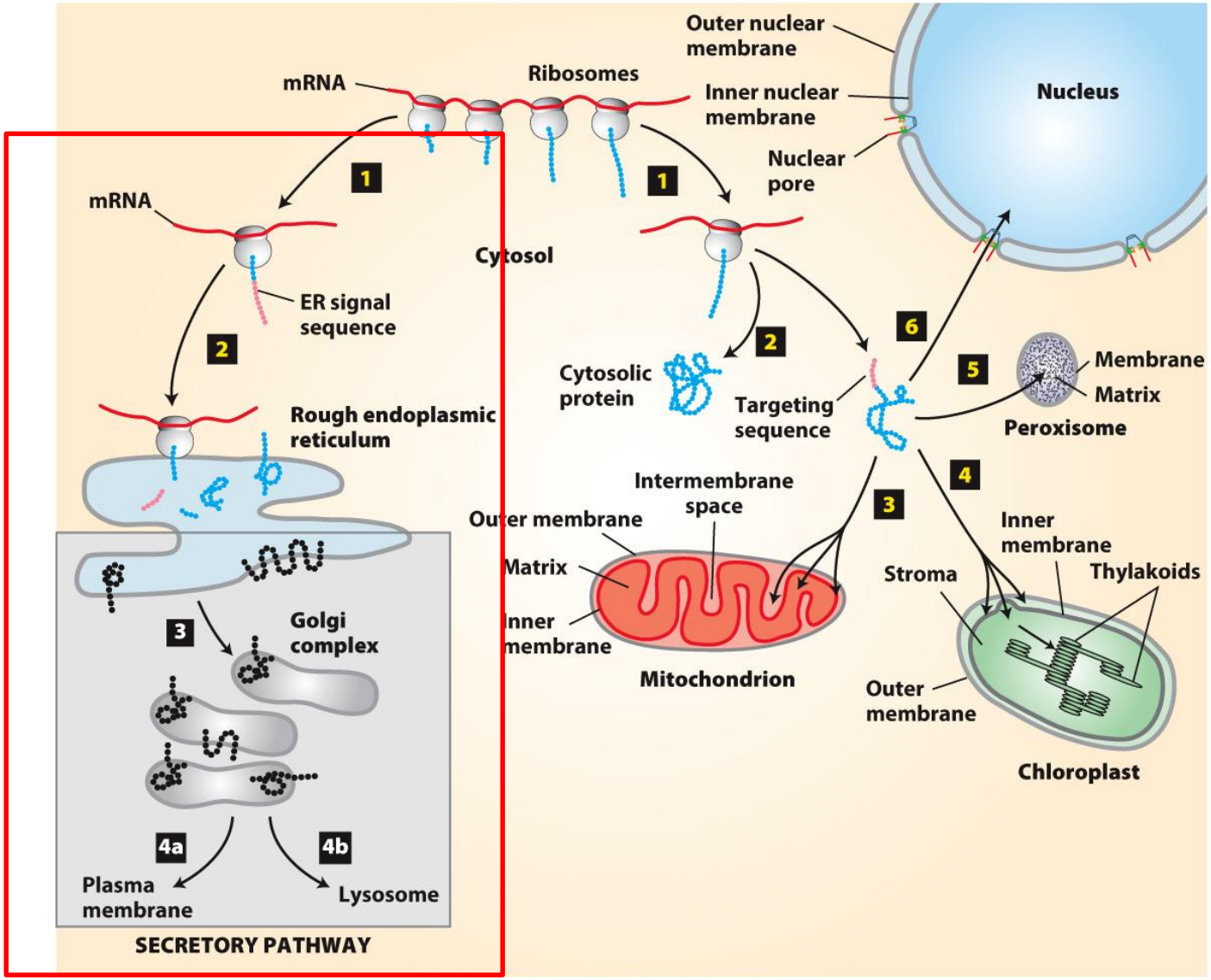


Figure 13-1
Molecular Cell Biology, Eighth Edition

Structure of the rough ER.

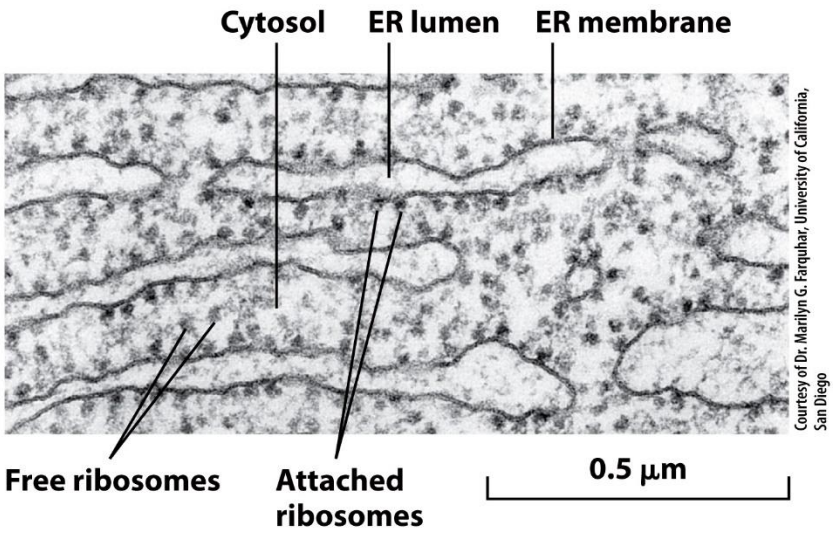


Figure 13-2a
Molecular Cell Biology, Eighth Edition
© 2016 W. H. Freeman and Company

- Protein synthesis on the ER:
  - ER membrane-bound and free cytosolic ribosomes are identical.
  - Membrane-bound ribosomes – recruited to the ER during synthesis of a polypeptide containing an ER signal sequence

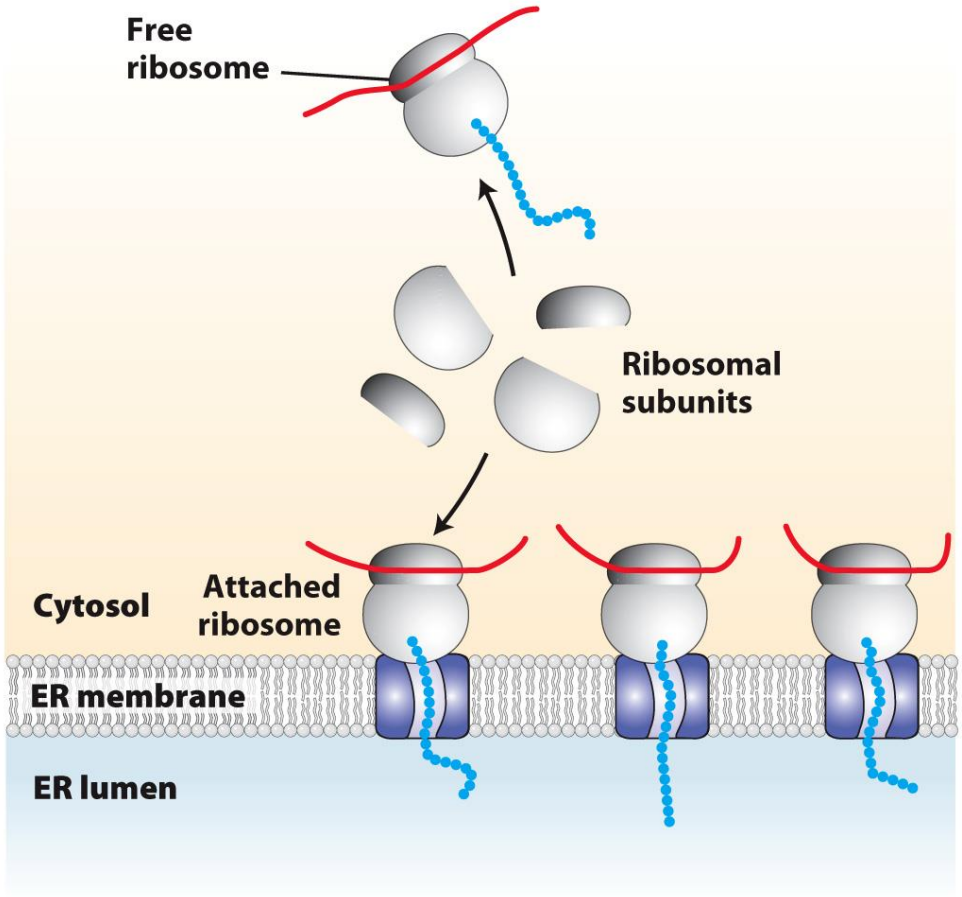


Figure 13-2b
Molecular Cell Biology, Eighth Edition
© 2016 W. H. Freeman and Company

How to test it?

Secretory proteins enter the ER lumen.

- Pulse-chase experiments with purified ER membranes: demonstrate that secretory proteins are localized in the ER lumen shortly after synthesis
- Experiment:
  - Cells are incubated for a brief time with radiolabeled amino acids – only newly synthesized proteins become labeled
  - Cells homogenized – fractures the plasma membrane and shears the rough ER into small vesicles called microsomes
  - Microsomes with bound ribosomes – isolated by differential and sucrose density-gradient centrifugation
  - Purified microsomes treated with a protease – results
  - (–) Detergent – proteins in ER are protected from digestion
  - (+) Detergent (control) – dissolves ER membrane – proteins no longer protected from digestion
- Conclusion: **newly made proteins are inside the microsomes (lumen of the rough ER) after synthesis.**

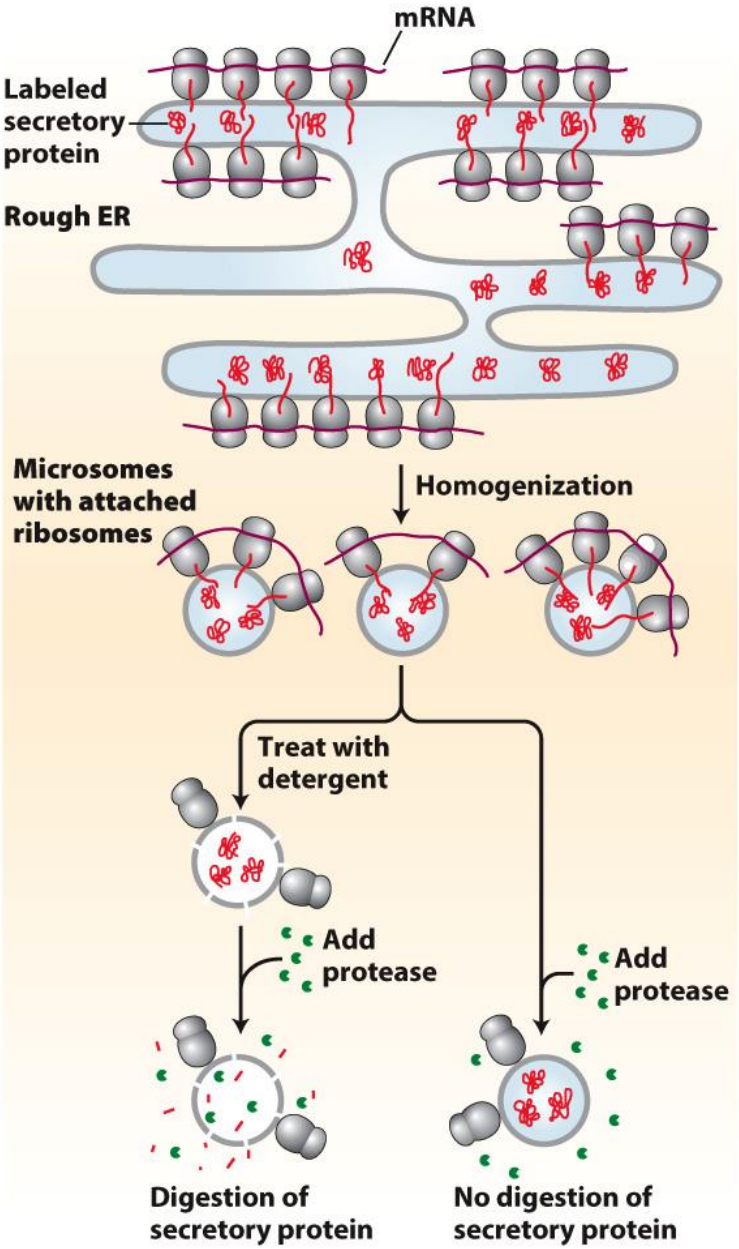


Figure 13-3
Molecular Cell Biology, Eighth Edition

Translation and translocation occur simultaneously.

When does the translocation occur?

- A hydrophobic N-terminal signal sequence targets nascent secretory proteins to the ER:

- N-terminal 16–30-residue ER targeting "signal" sequence –
  - First part of nascent protein to emerge from ribosome
  - Directs the ribosome to the ER membrane
  - Initiates translocation of the growing polypeptide across the ER membrane
  - Signal sequences of different secretory proteins –
    - All contain one or more positively charged amino acids adjacent to a continuous stretch of 6–12 hydrophobic core residues.

- (a) Add microsomes after protein synthesis (addition of mRNA) –
  - Protein not internalized in microsomes – digested by added protease
- (b) Add microsomes before protein synthesis (addition of mRNA) –
  - Protein is internalized in microsomes – protected from complete digestion by added protease
  - Signal sequence – removed by ER protease (decreases protein molecular weight)

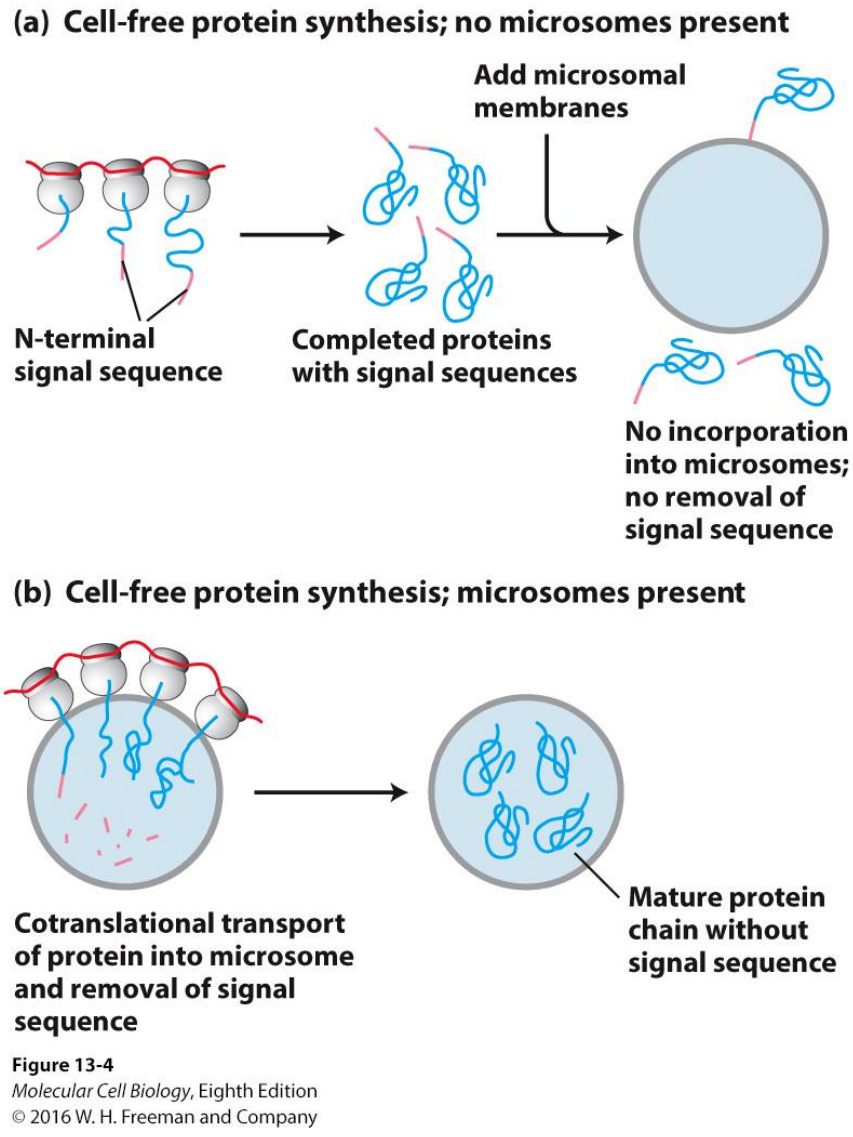


Figure 13-4
Molecular Cell Biology, Eighth Edition

Structure of the signal recognition particle (SRP).

N-terminal signal sequence? How to work?

- (a) Signal-sequence-binding domain: (bacterial Ffh protein – homologous to the P54 portion that binds ER signal sequences in eukaryotes.)
    - Large cleft lined with hydrophobic amino acids – binds to signal sequence hydrophobic core
- (b) GTP and receptor-binding domain: structure of GTP bound to FtsY (the archaeal homolog of the α subunit of the SRP receptor) and Ffh subunits from *Thermus aquaticus*
    - Interaction between SRP and receptor – controlled by GTP binding (SRP-receptor association) and hydrolysis (SRP-receptor dissociation) –
    - GTP bound to each subunit – fit in the interface between the Ffh and FtsY subunits and stabilize SRP-receptor association
    - Assembly of the pseudosymmetric dimer – forms two active sites for hydrolysis of both bound GTP molecules
    - Hydrolysis to GDP destabilizes the interface – causes SRP-receptor dissociation

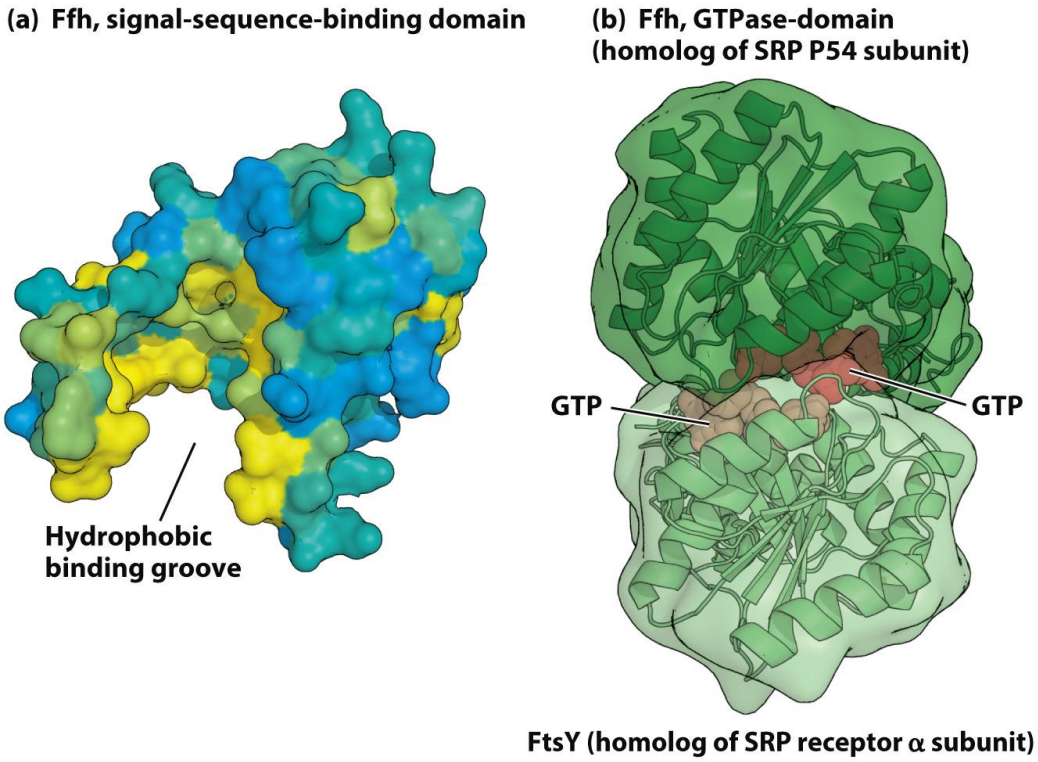
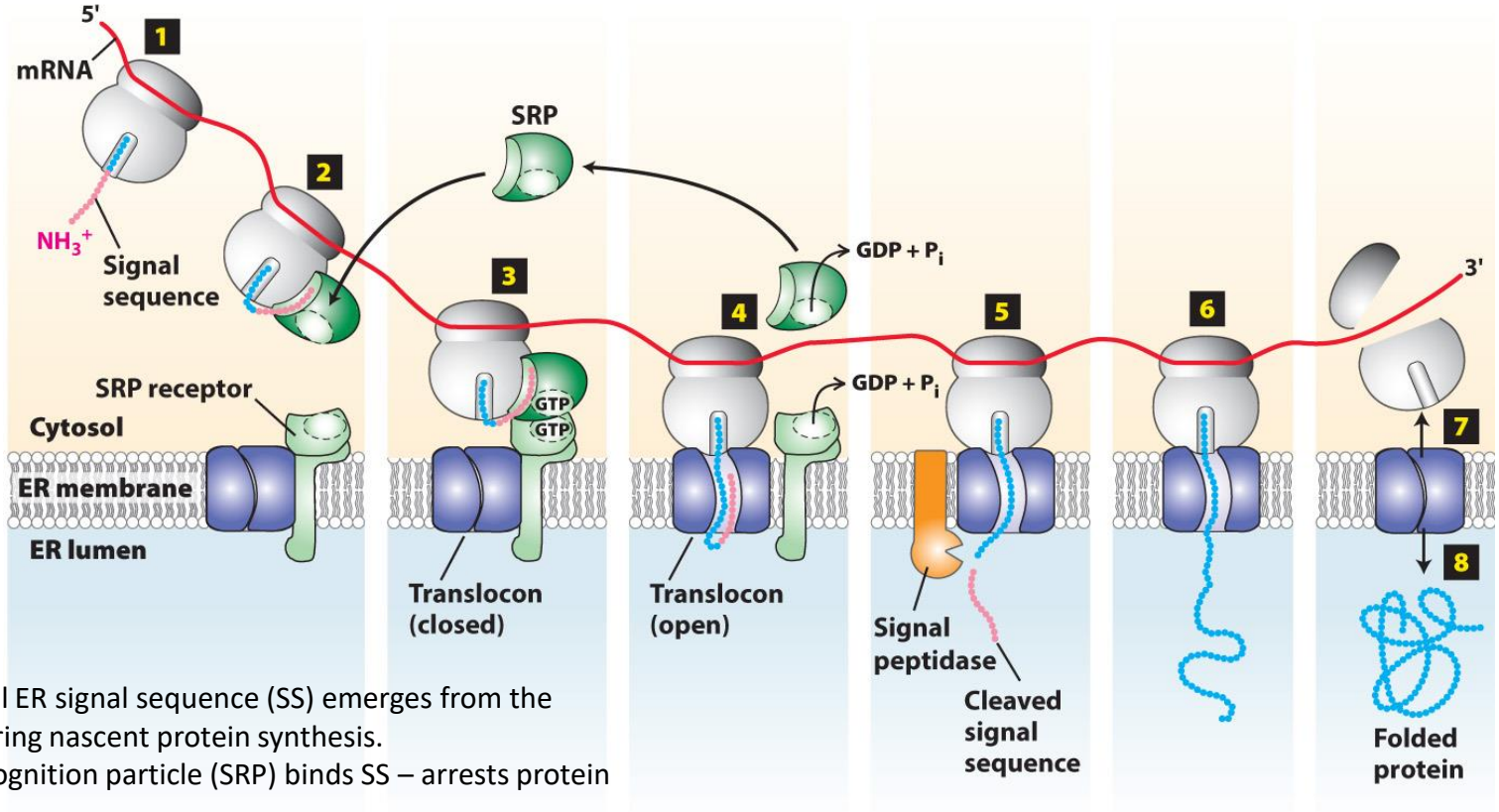


Figure 13-5
Molecular Cell Biology, Eighth Edition

Cotranslational translocation.



- Step 1: N-terminal ER signal sequence (SS) emerges from the ribosome first during nascent protein synthesis.
- Step 2: Signal recognition particle (SRP) binds SS – arrests protein synthesis
- Step 3: SRP-nascent polypeptide chain–ribosome complex –
  - Binds to the SRP receptor in the ER membrane
  - Interaction is strengthened by the binding of GTP to both the SRP and its receptor.
- Step 4: Transfer of the nascent polypeptide–ribosome to the translocon –
  - Opens translocation channel to admit the growing polypeptide
  - Signal sequence – transferred to a hydrophobic binding site next to the central pore
  - SRP and SRP receptor – hydrolyze bound GTP; dissociates SRP from ribosome and receptor; restarts protein synthesis (can initiate the insertion of another polypeptide chain)
- Step 5: Elongating polypeptide chain
  - Passes through the translocon channel into the ER lumen
  - **Signal sequence – cleaved by signal peptidase and rapidly degraded**
- Step 6: Growing peptide chain – continues extrusion through the translocon into the ER as the mRNA is translated toward the 3' end
- Step 7: Translation completes at mRNA stop codon – ribosome is released
- Step 8:
  - Nascent protein – remainder drawn into the ER lumen and folds into native conformation
  - Translocon closes.

Sec61α is a translocon component.

Is the protein really involved in the protein translocation?

- **Cross-linking experiment** – Sec61α translocon component; contacts nascent secretory proteins as they pass through the translocon into the ER lumen

- Experiment:
  - Cell-free system with liposomes containing only Sec61 complex
  - Addition of mRNA –
    - Encodes only the N-terminal 70 amino acids of the secreted protein prolactin
    - Lacks a stop codon – ribosome never dissociates
    - Contains one lysine codon – near the middle of the sequence
  - Add lysyl-tRNA – modified with a light-activated cross-linking reagent attached to the lysine side chain
  - Entire mRNA translated – "stuck" in translocon because ribosome not released without a mRNA stop codon
  - Exposure to intense light – covalently crosslinks nascent polypeptide chain to nearby proteins in the translocon

- Result: nascent chain covalently linked to Sec61α.
- Conclusion: Sec61 complex is translocon.

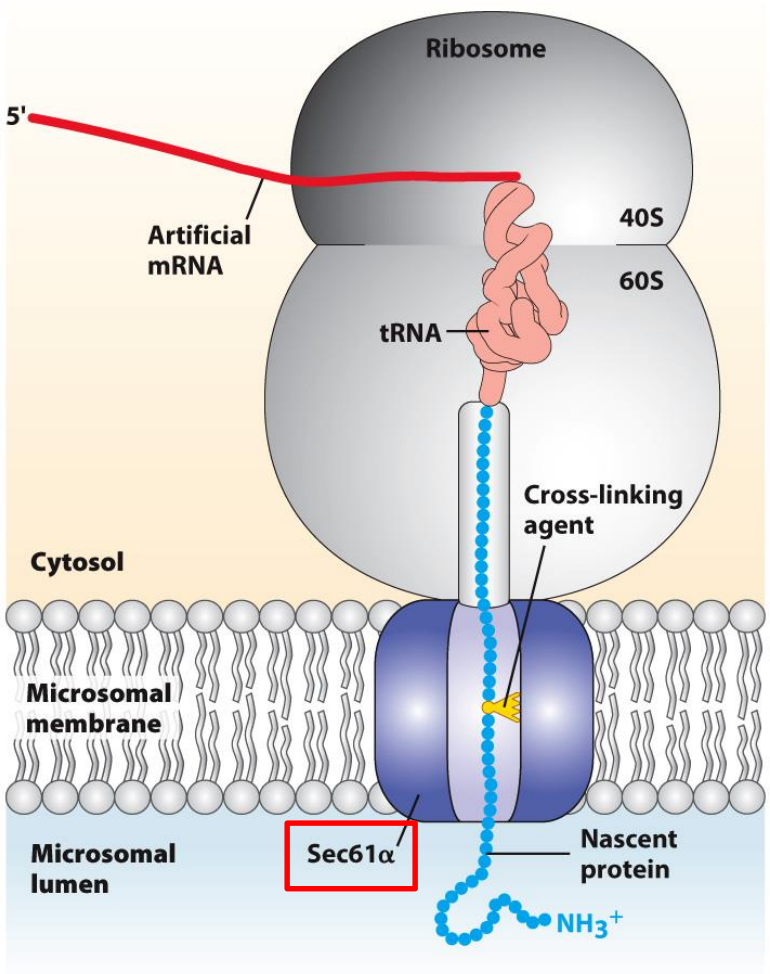


Figure 13-7
Molecular Cell Biology, Eighth Edition

Structure of an archaeal Sec61 complex.

Translocon – preserves integrity of the ER membrane by two gating mechanisms

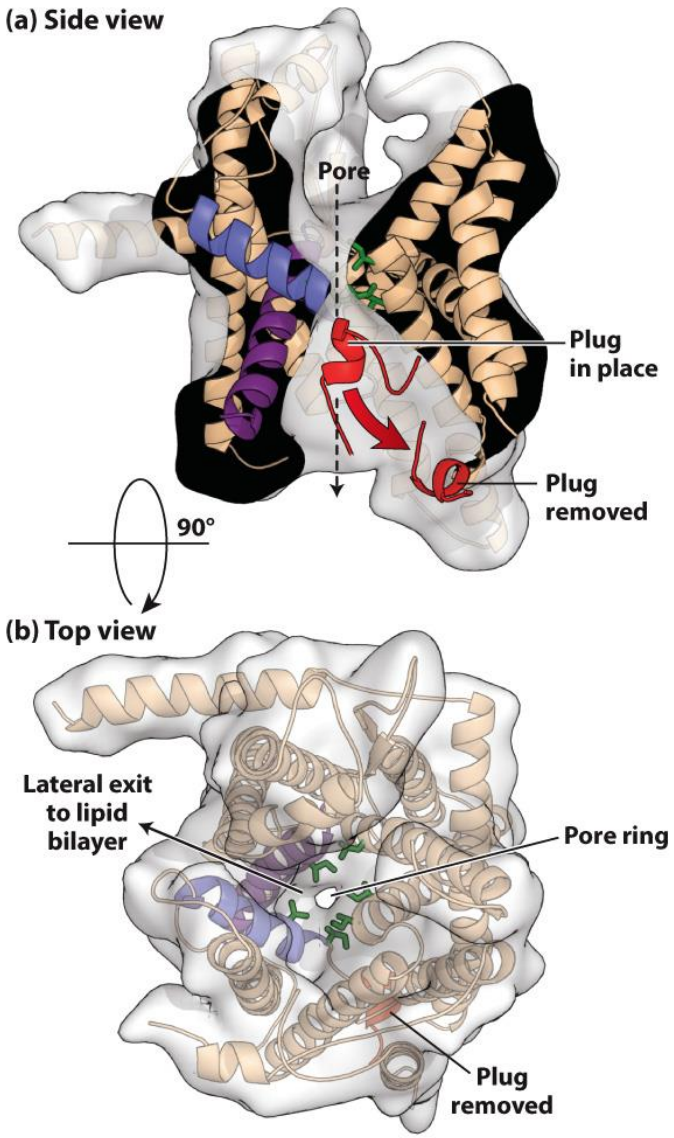


Figure 13-8
Molecular Cell Biology, Eighth Edition

Post-translational translocation

- Post-translational translocation – some yeast secretory proteins enter the ER lumen through the Sec61 translocon after translation has been completed (no SRP/receptor involvement)

Post-translational translocation.

- Step 1:
  - Direct interaction between the SS and the translocon – sufficient for targeting to the ER membrane
  - N-terminal segment of the protein enters the ER lumen – signal peptidase cleaves the signal sequence (just as in cotranslational translocation)
- Step 2: BiP (Hsp70 family protein of ATP-dependent molecular chaperones) and Sec63 complex – provide driving force for unidirectional translocation across the ER membrane
  - BiP interaction with Sec63 complex – stimulates BiP ATP hydrolysis
  - BiP-ADP conformational change – promotes binding to exposed polypeptide chain translocating through adjacent translocon
  - BiP binding prevents backsliding of peptide through translocon.
- Step 3: Random inward sliding – exposes more nascent protein
- Step 4: Successive BiP-ADP binding – ratchets nascent protein into ER
- Step 5: ATP for ADP exchange releases BiP from nascent protein.
- Step 6: Protein folds into native conformation.
- BiP ATP hydrolysis powers protein translocation across membrane.

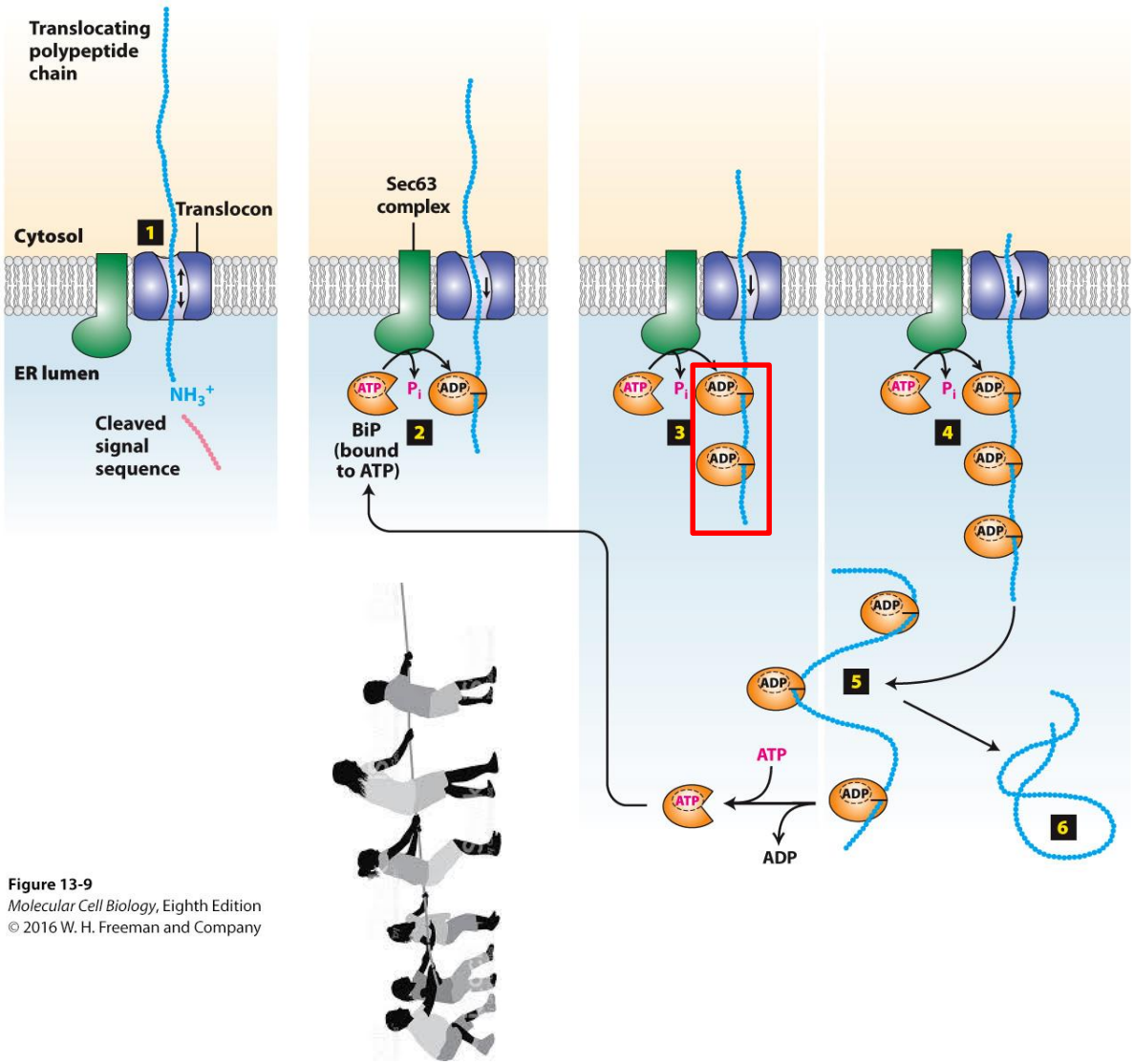
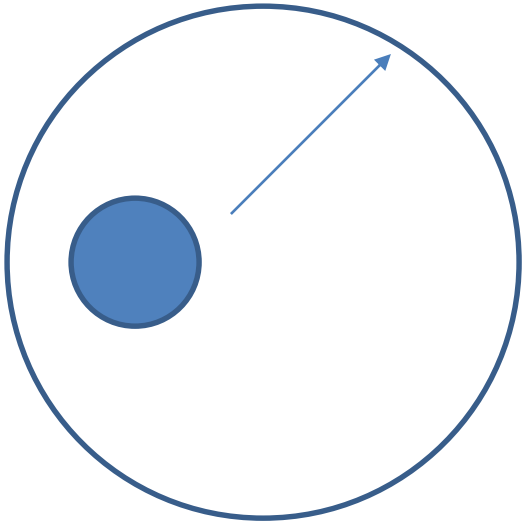


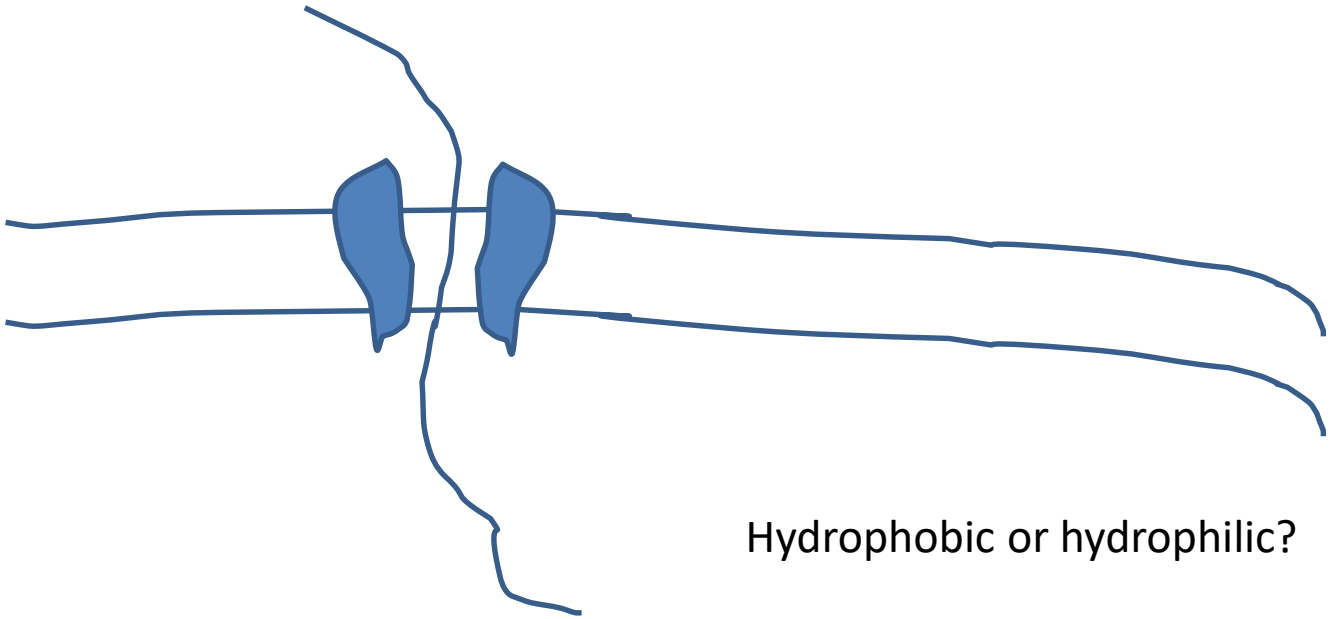
Figure 13-9
Molecular Cell Biology, Eighth Edition

Membrane proteins



Think! How?

Now you can think the process of how the membrane proteins are translocated!



Moving Proteins into Membranes and Organelles

13.2 Insertion of Membrane Proteins into the ER

- Topogenic sequences—N-terminal signal sequences, internal stop-transfer anchor sequences, and internal signal-anchor sequences—direct the insertion of nascent proteins into the ER membrane.
- Membrane protein topology can be predicted by computer programs that identify hydrophobic topogenic segments within the primary amino acid sequence.
- Some cell-surface proteins are initially synthesized as transmembrane proteins and transferred to a GPI anchor.

Classes of ER membrane proteins.

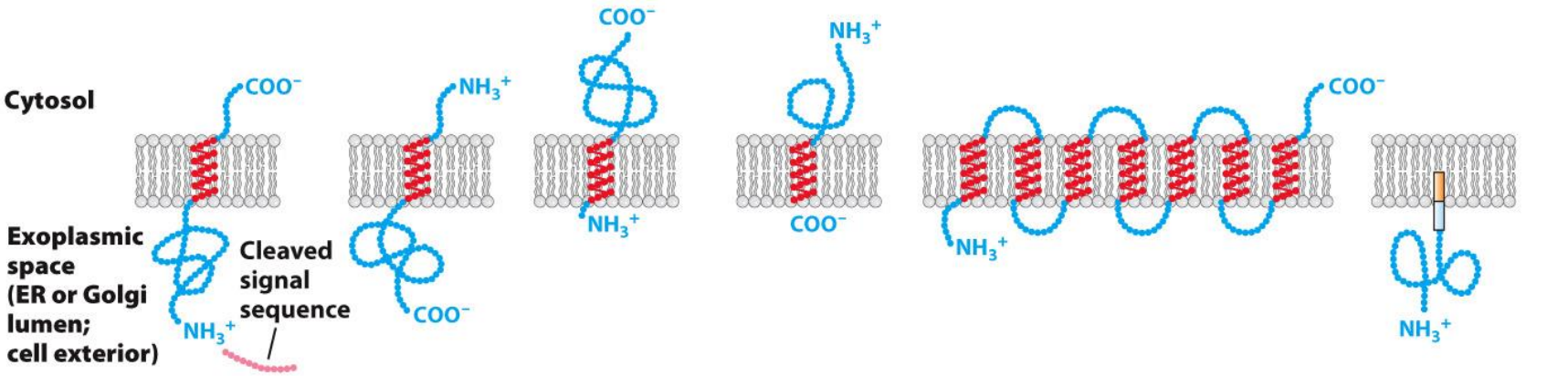


Figure 13-10
Molecular Cell Biology, Eighth Edition

You can get some idea for the integral mechanisms
from the topological classes of these integral membrane proteins

How about TYPE I first?

Membrane insertion and orientation of type I single-pass transmembrane proteins.

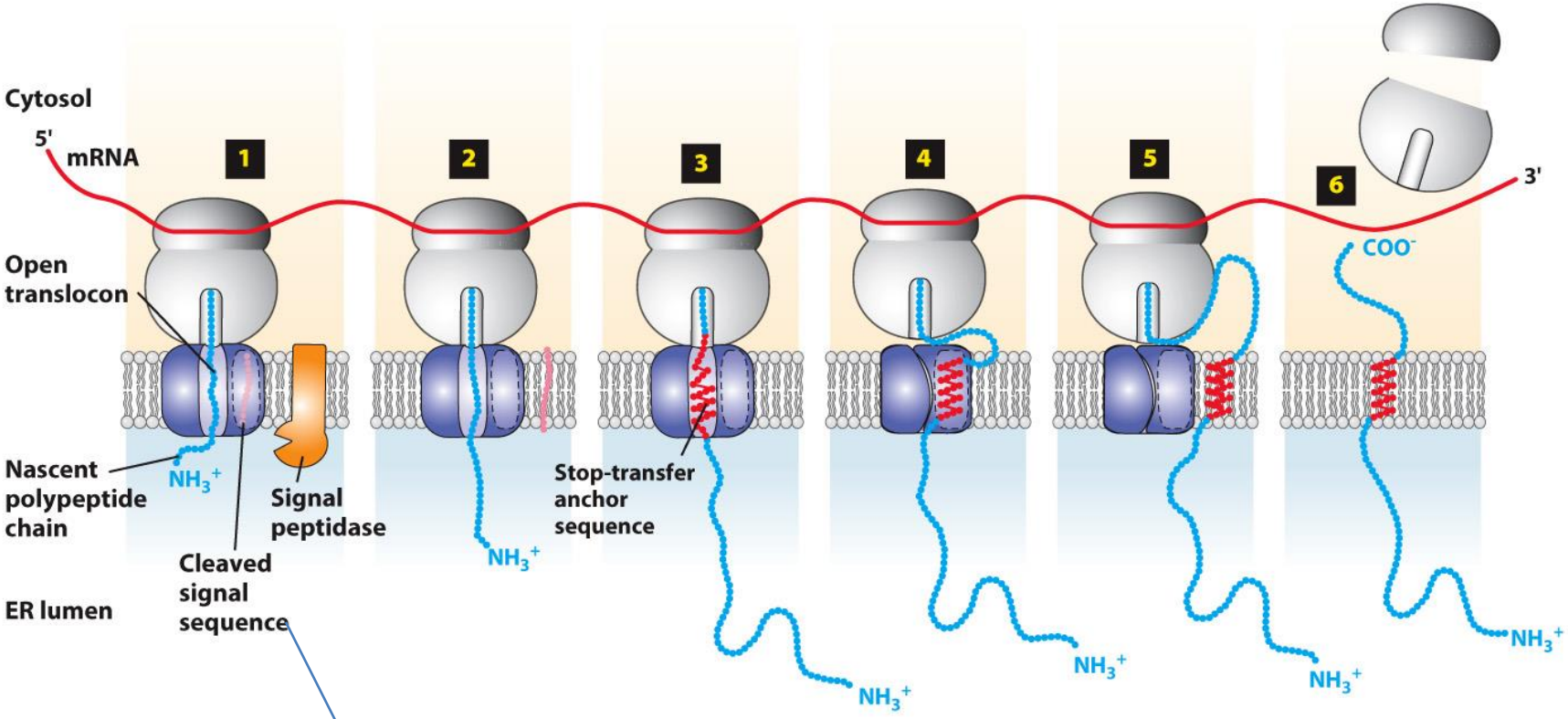


Figure 13-11
Molecular Cell Biology, Eighth Edition

- Step 1: Translocation initiation and SS cleavage by the same mechanism as for soluble secretory proteins

Classes of ER membrane proteins.

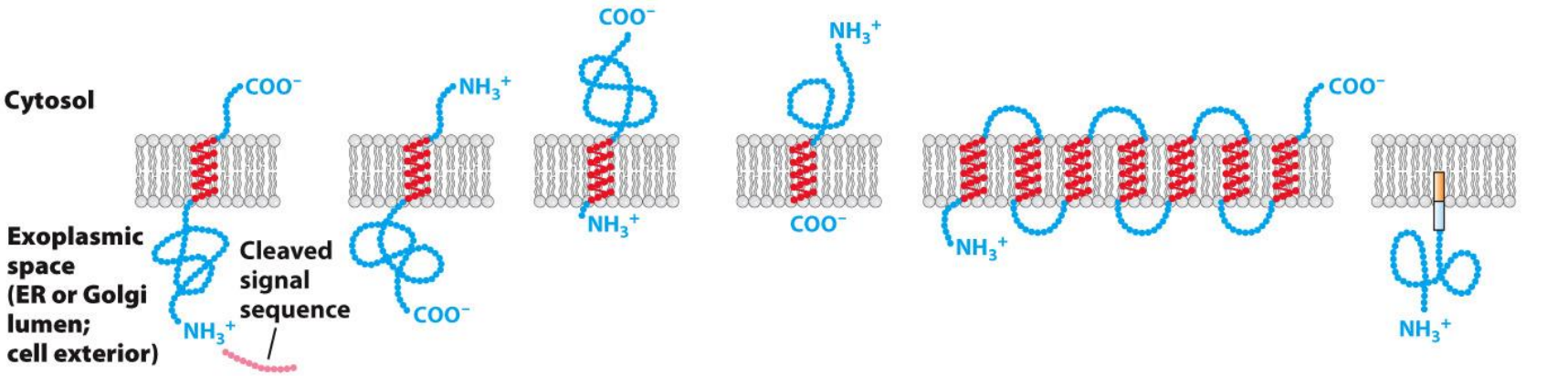


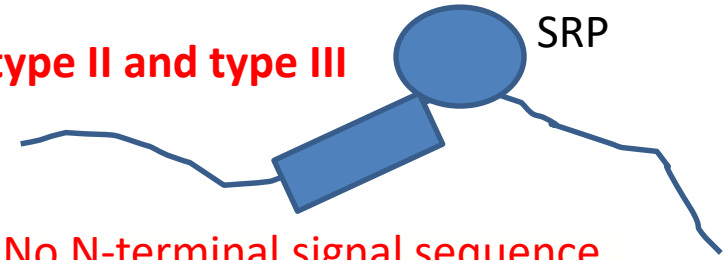
Figure 13-10
Molecular Cell Biology, Eighth Edition

You can get some idea for the integral mechanisms
from the topological classes of these integral membrane proteins

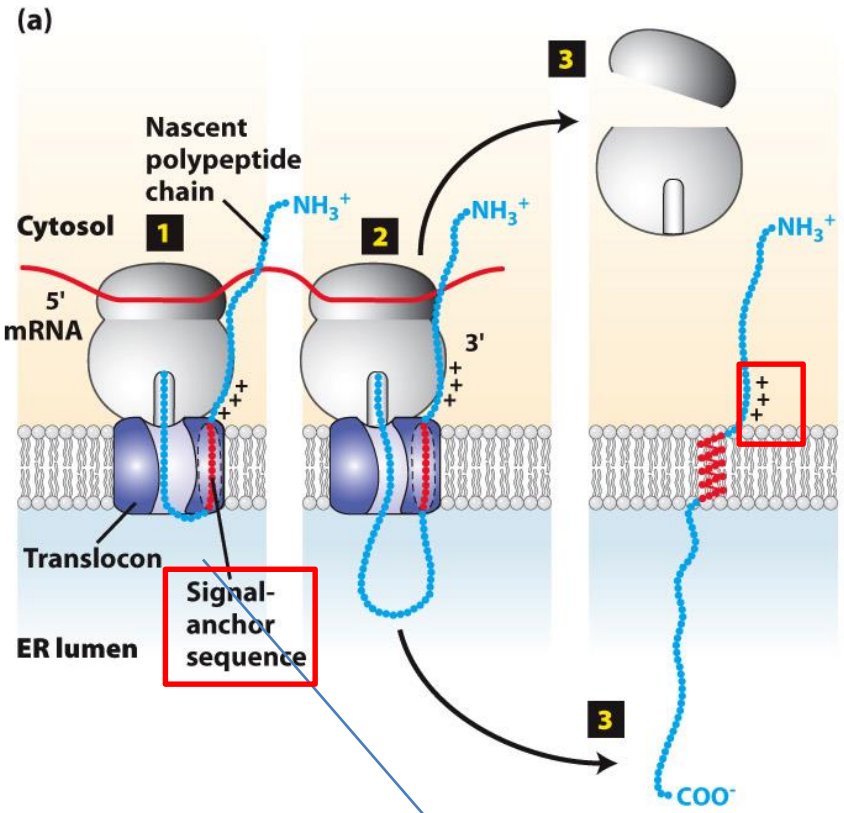
Then, TYPE II ?

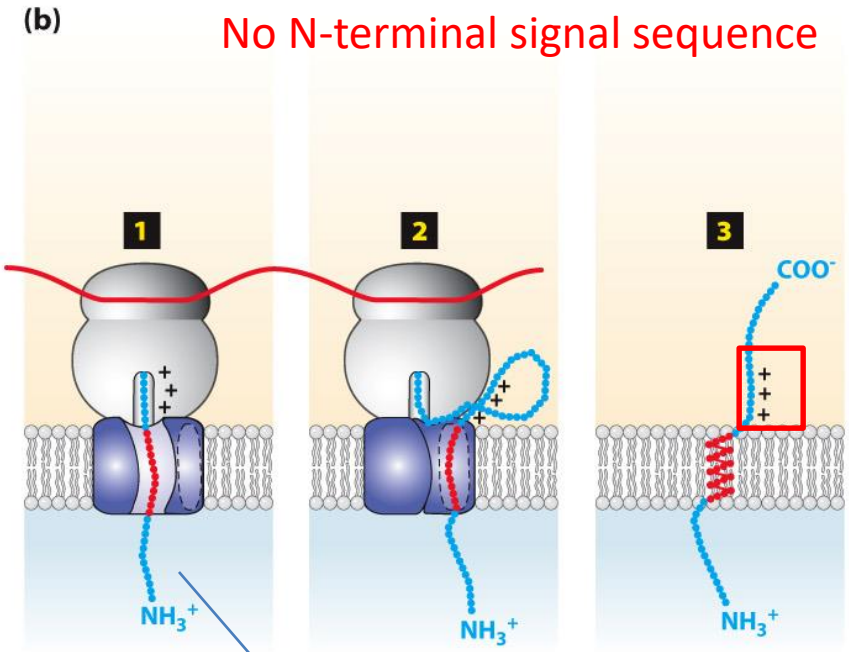
Membrane insertion and orientation of type II and type III single-pass transmembrane proteins.

Difference??



No N-terminal signal sequence

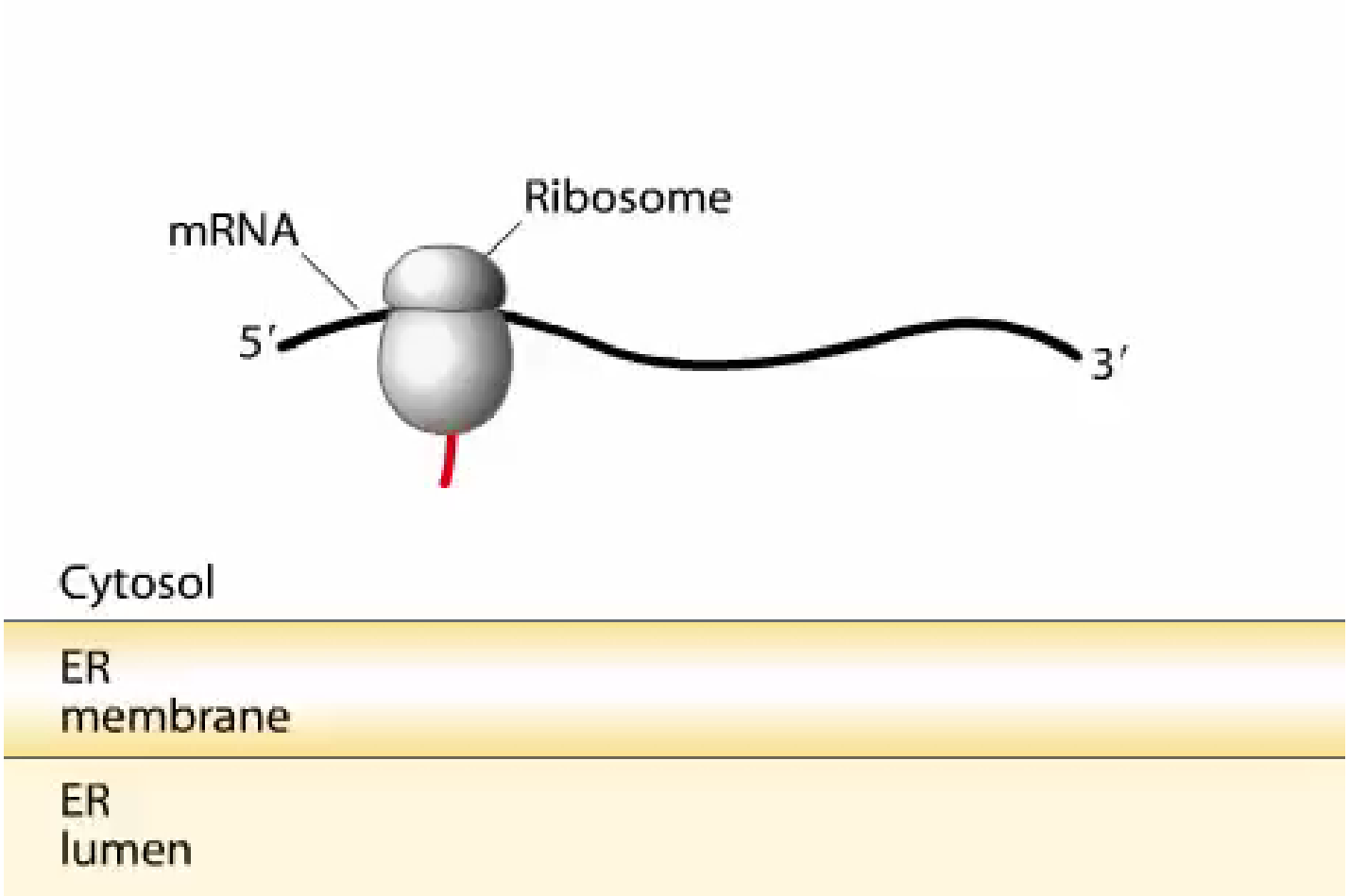




- Step 1:
  - SRP binds the internal signal-anchor sequence synthesized by a cytosolic ribosome (not shown) and interacts with the SRP receptor on the ER membrane.
  - Positively charged amino acids on N-terminal side of signal-anchor sequence orient nascent polypeptide chain in the translocon with the N-terminal portion in the cytosol.

- Step 1: Insertion similar to that of type II proteins – except positively charged residues on the signal-anchor sequence C-terminal side cause transmembrane segment orientation within the translocon with C-terminal end in the cytosol and the N-terminal end in the ER lumen

Secreted protein and membrane protein synthesis!

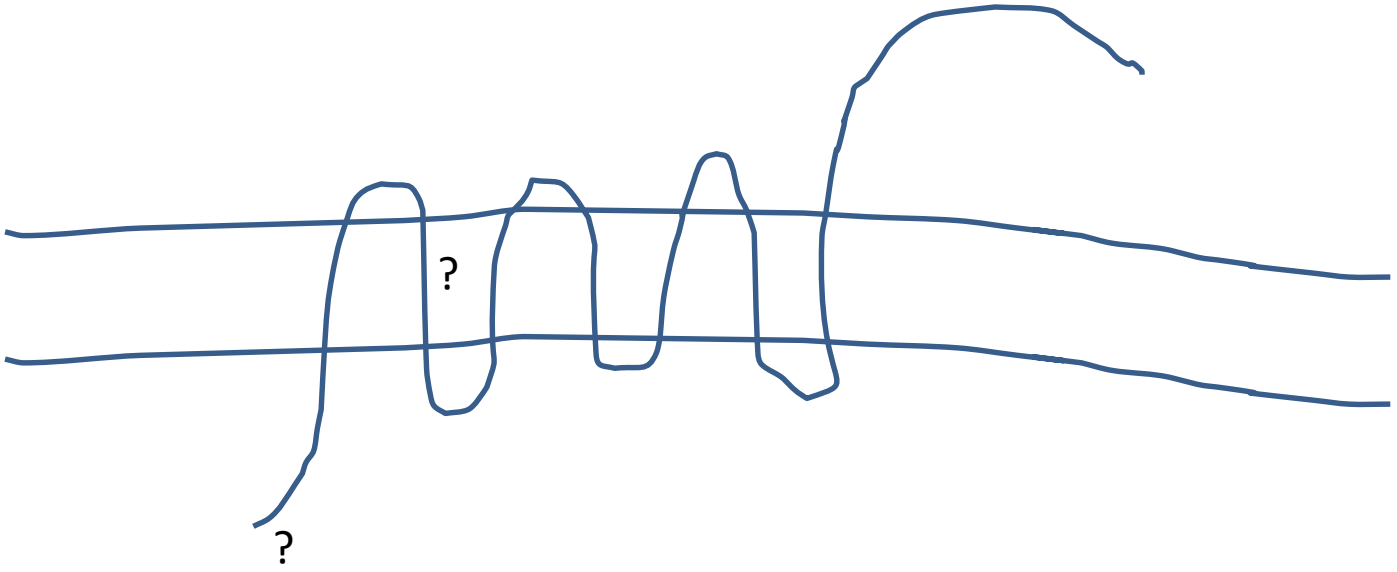


https://www.youtube.com/watch?v=SKMIzmAe0do

Question

What is the internal signal-anchor sequence used for the multi-transmembrane proteins?

Hint. Type I, II, III and IV?



Insertion of tail-anchored proteins.

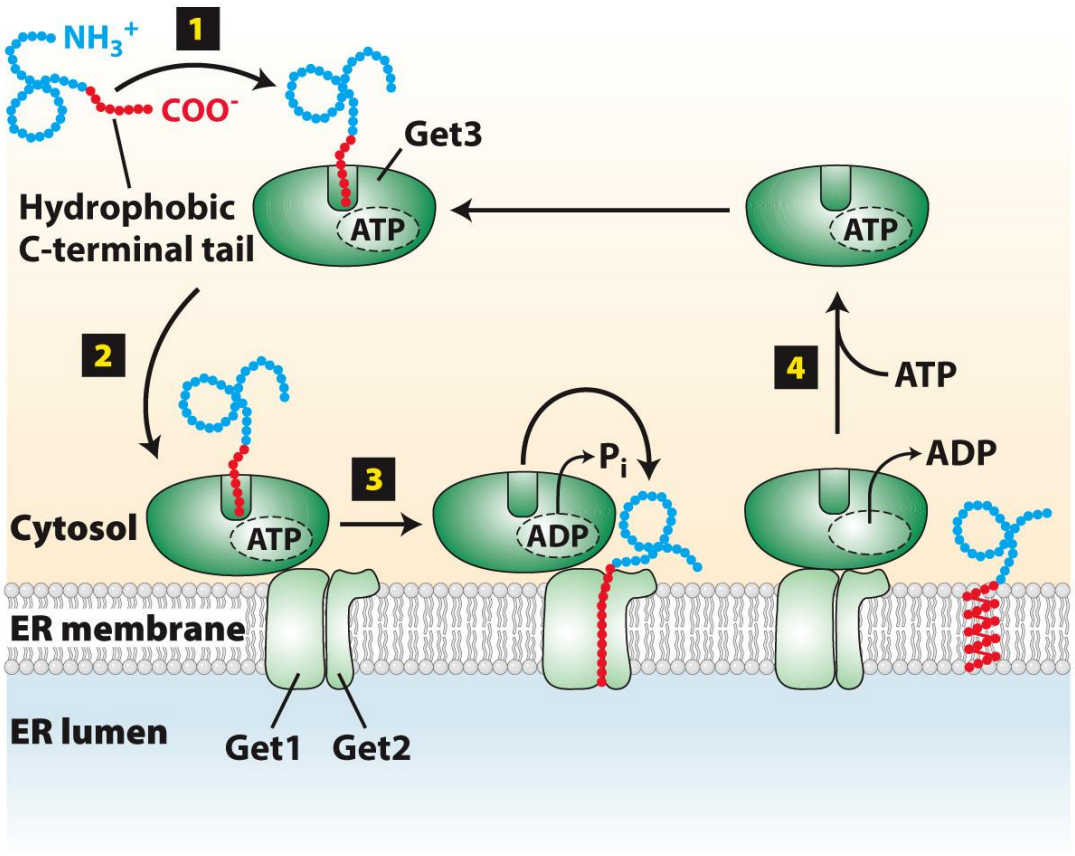
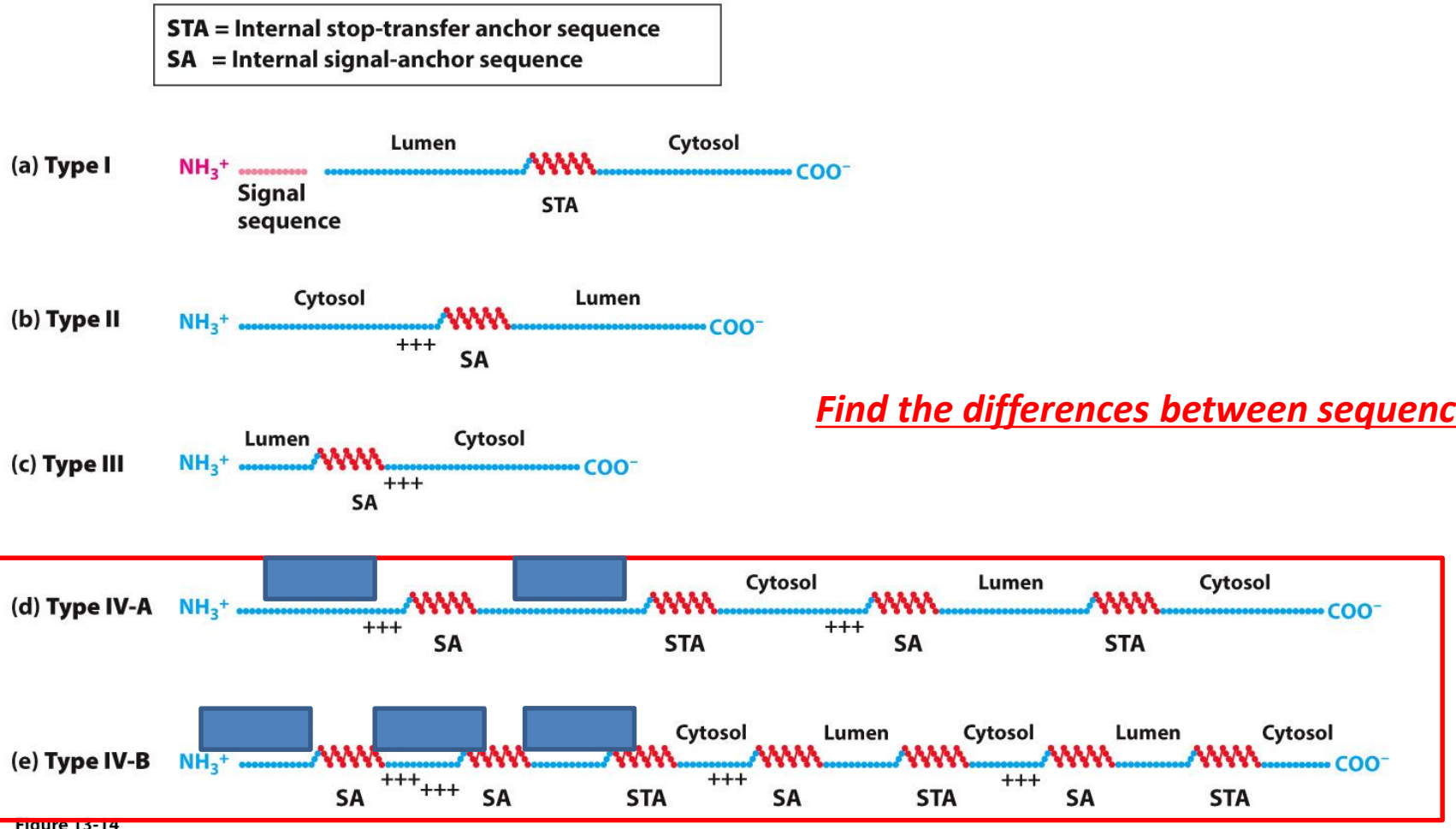


Figure 13-13
Molecular Cell Biology, Eighth Edition

- C-terminal tail-anchored proteins –
  - No N-terminal signal sequence
  - Hydrophobic C-terminus – not available for membrane insertion until protein synthesis is complete and the protein has been released from the ribosome

Then how ?

Topogenic sequences determine the orientation of ER membrane proteins.

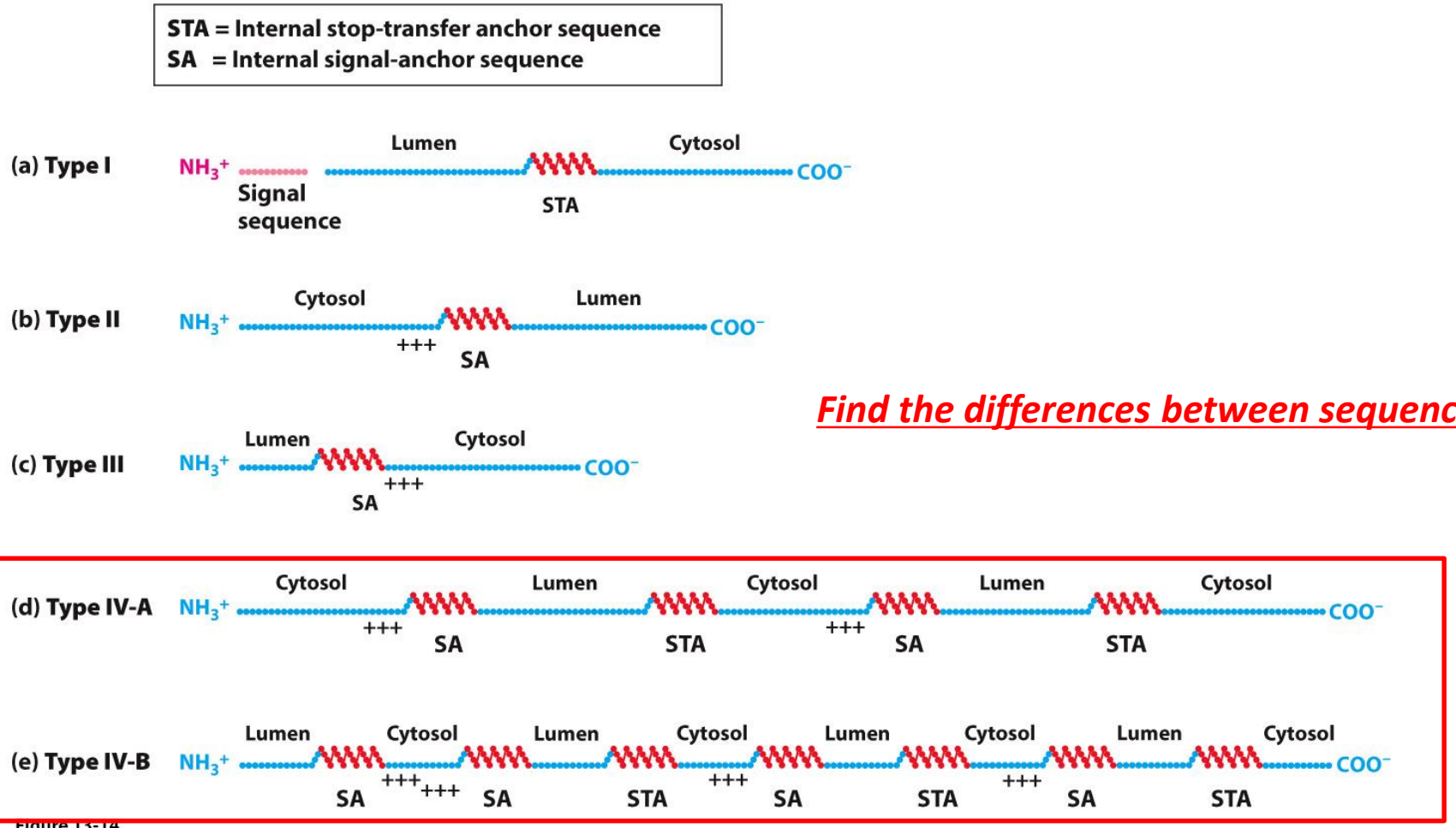


Find the differences between sequences!

Figure 13-14
Molecular Cell Biology, Eighth Edition

Where is the N-terminal? Outside or inside cell?

Topogenic sequences determine the orientation of ER membrane proteins.



Find the differences between sequences!

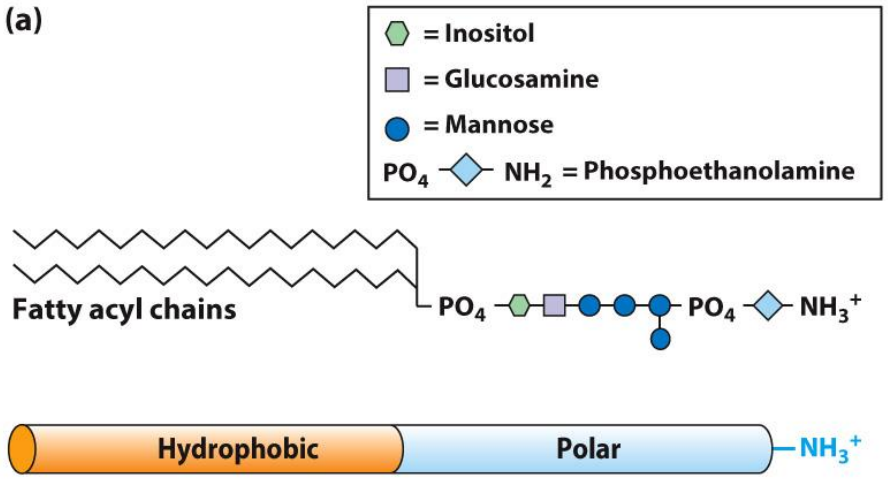
Where is the N-terminal? Outside or inside cell?

Figure 13-14
Molecular Cell Biology, Eighth Edition

GPI-anchored proteins.

(a) Glycosylphosphatidylinositol (GPI) molecule

Why is it necessary?



- (b) GPI-anchored protein formation:
  - Protein – synthesized and inserted into the ER membrane like a type I transmembrane protein
  - Specific transamidase –
    - Cleaves the precursor protein within the exoplasmic-facing domain, near the stop-transfer anchor sequence (red)
    - Covalently links new C-terminus to the terminal amino group of a preformed GPI anchor

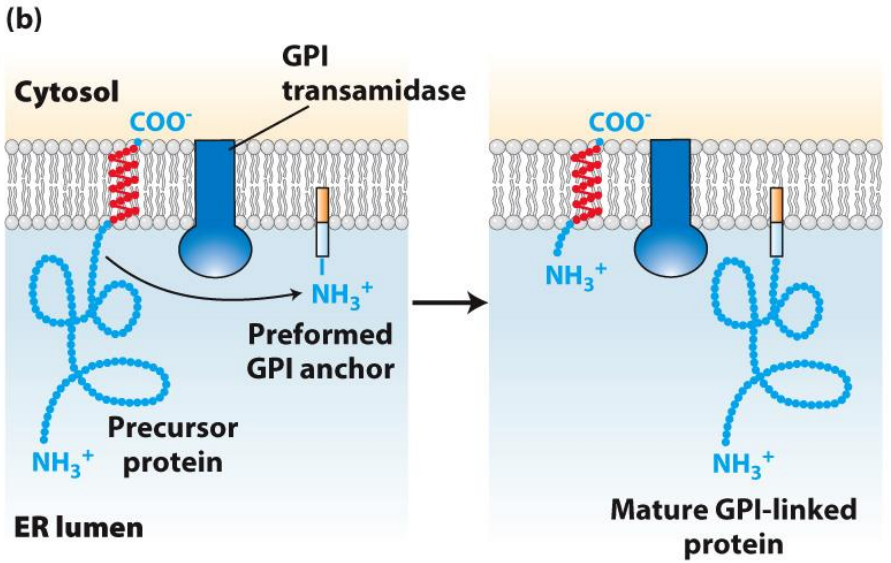


Figure 13-15
Molecular Cell Biology, Eighth Edition

Hydropathy profiles.

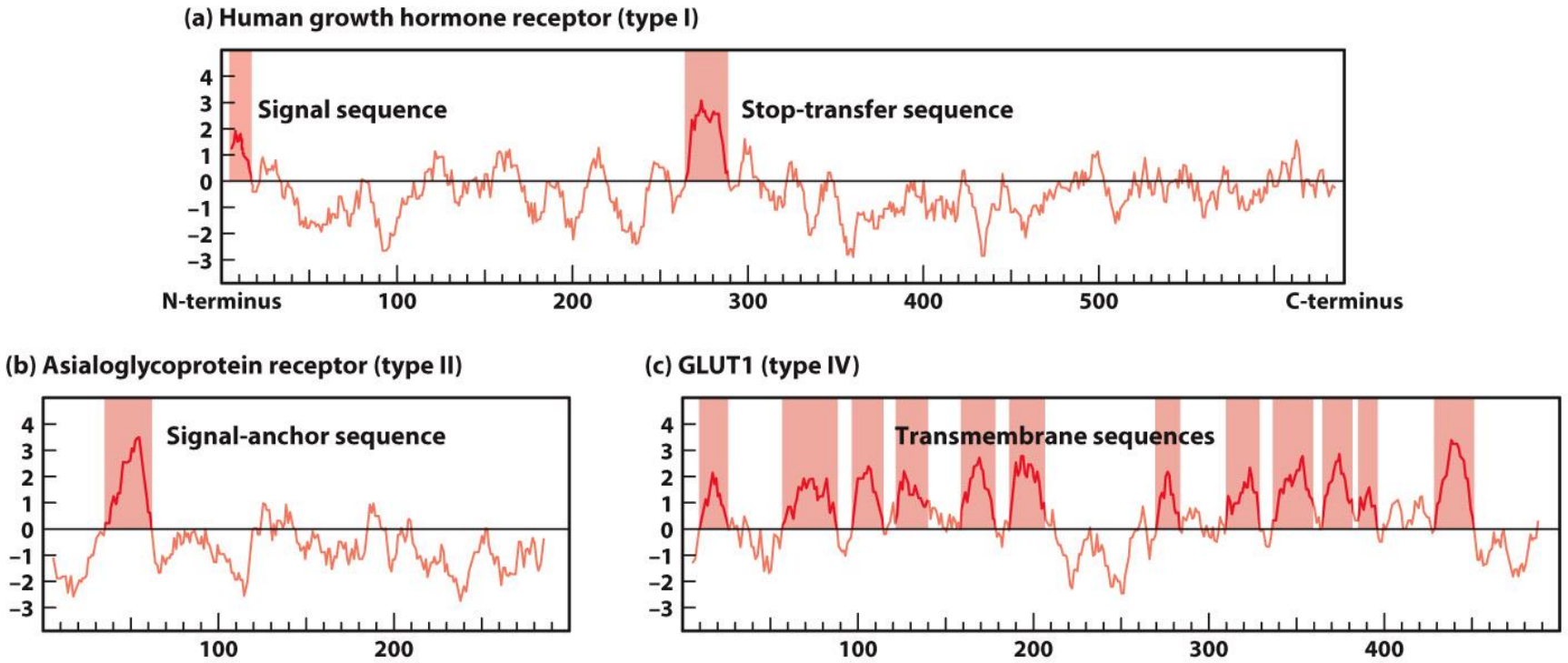


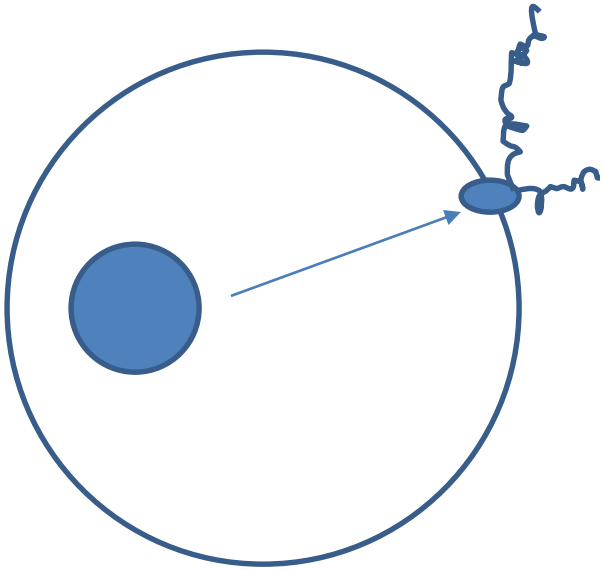
Figure 13-16
Molecular Cell Biology, Eighth Edition

Moving Proteins into Membranes and Organelles

13.3 Protein Modifications, Folding, and Quality Control in the ER

- Proteins acquire oligosaccharides, disulfide bonds, and prolyl isoform modification via ER enzymes.
- ER chaperones and lectins work to ensure proper folding of new proteins or transport of unassembled/misfolded proteins back to the cytosol, where they are degraded in the ubiquitin-proteasome pathway.
- Only properly folded proteins and assembled subunits are transported from the rough ER to the Golgi complex in vesicles.

Polysaccharides addition



Glycoprotein

Where should the polysaccharides be added?

Biosynthesis of the oligosaccharide precursor.

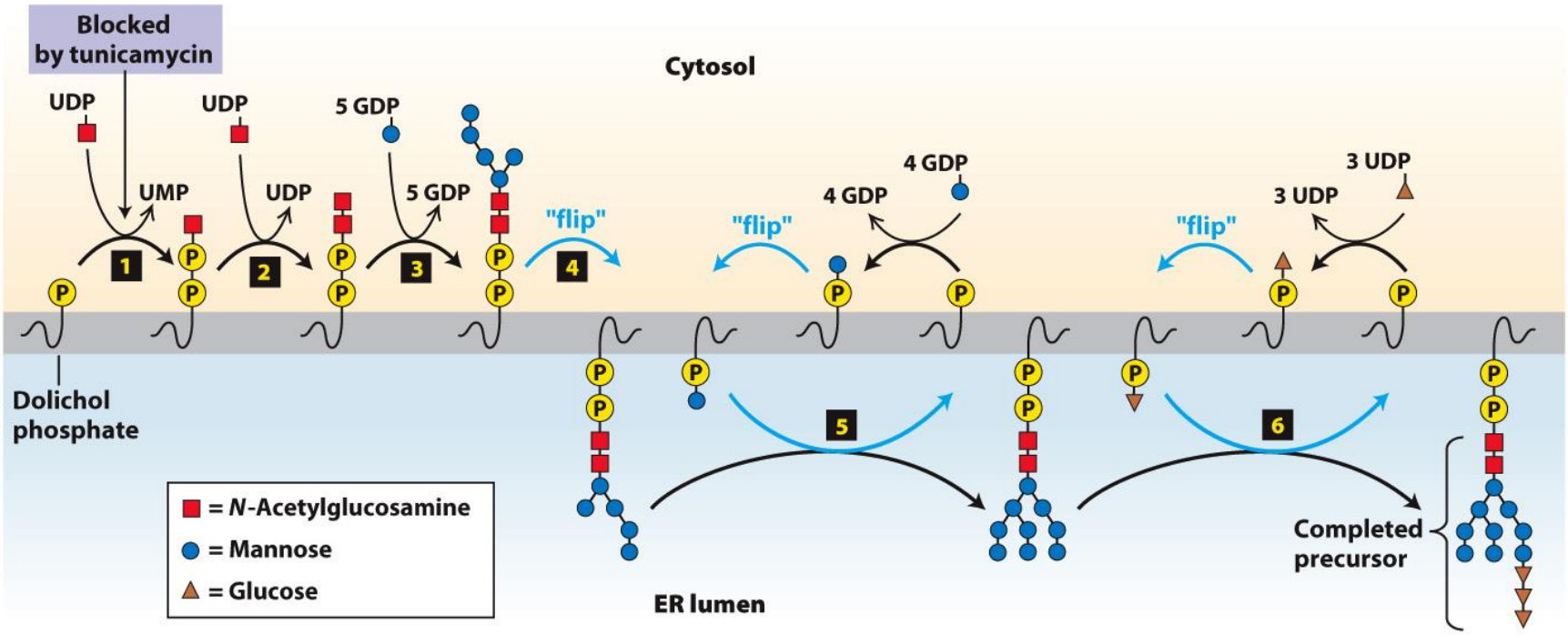


Figure 13-17
Molecular Cell Biology, Eighth Edition

- Steps 1–3: Two *N*-acetylglucosamine (GlcNAc) and five mannose residues – high-energy pyrophosphate linkages added one at a time on the ER membrane cytosolic face
    - (Tunicamycin blocks enzyme 1– inhibits synthesis of all N-linked oligosaccharides in cells)
- Step 4: The seven-residue dolichol pyrophosphoryl intermediate is flipped to the ER membrane luminal face.
- Steps 5–6: Additional sugars by ER enzymes –
    - Sugar transferred from a nucleotide precursor to a carrier dolichol phosphate and flipped to the luminal face
    - Sugar – transferred from dolichol to the growing oligosaccharide
    - “Empty” carrier dolichol – flipped back to the cytosolic face

Addition and initial processing of *N*-linked oligosaccharides

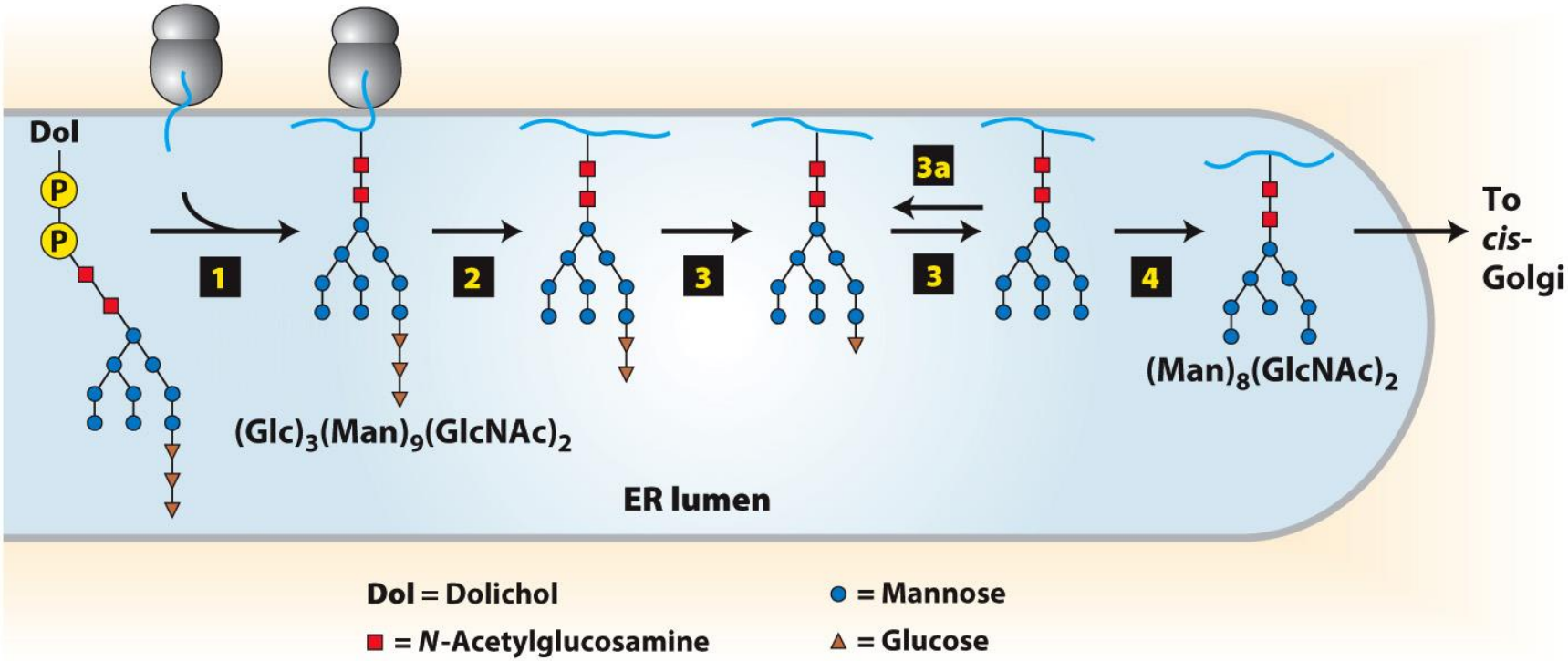


Figure 13-18
Molecular Cell Biology, Eighth Edition

- Step 1: $Glc_3Man_9(GlcNAc)_2$ precursor – transferred from the dolichol carrier to asparagine residues in a tripeptide sequences (Asn-X-Ser/Asn-X-Thr) on a nascent protein, as soon as the asparagine crosses to the luminal side of the ER
- Steps 2–4: Glucose residues removed.
- Step 3: Glucose residue removed.
- Step 3a: Re-addition of one glucose residue – plays a role in the correct folding of many proteins in the ER
- Step 4: One mannose residue – can be modified on asparagine residues by the same mechanism

Action of protein disulfide isomerase (PDI).

Protein disulfide isomerase or PDI is an enzyme in the endoplasmic reticulum in eukaryotes and the periplasm of bacteria that catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold.

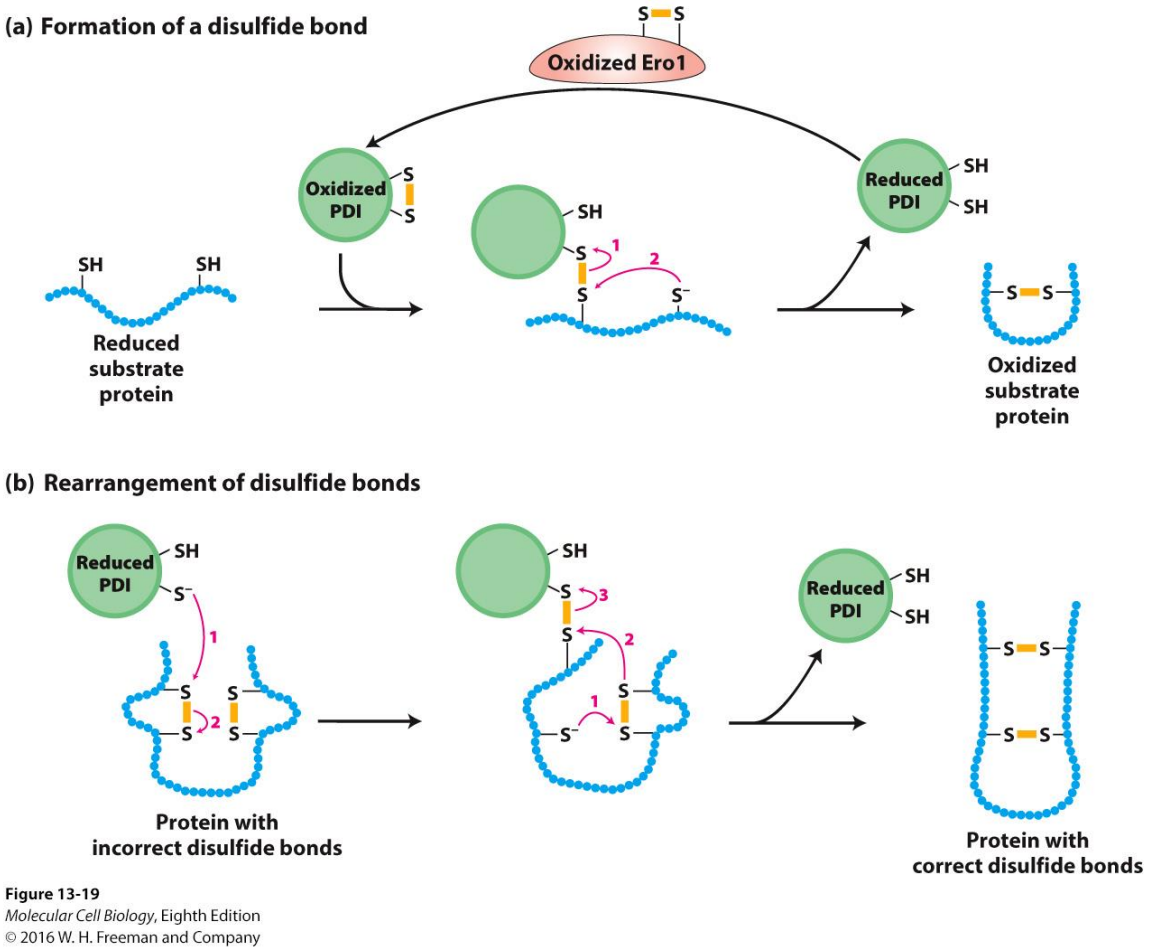


Figure 13-19
Molecular Cell Biology, Eighth Edition

Hemagglutinin folding and assembly.

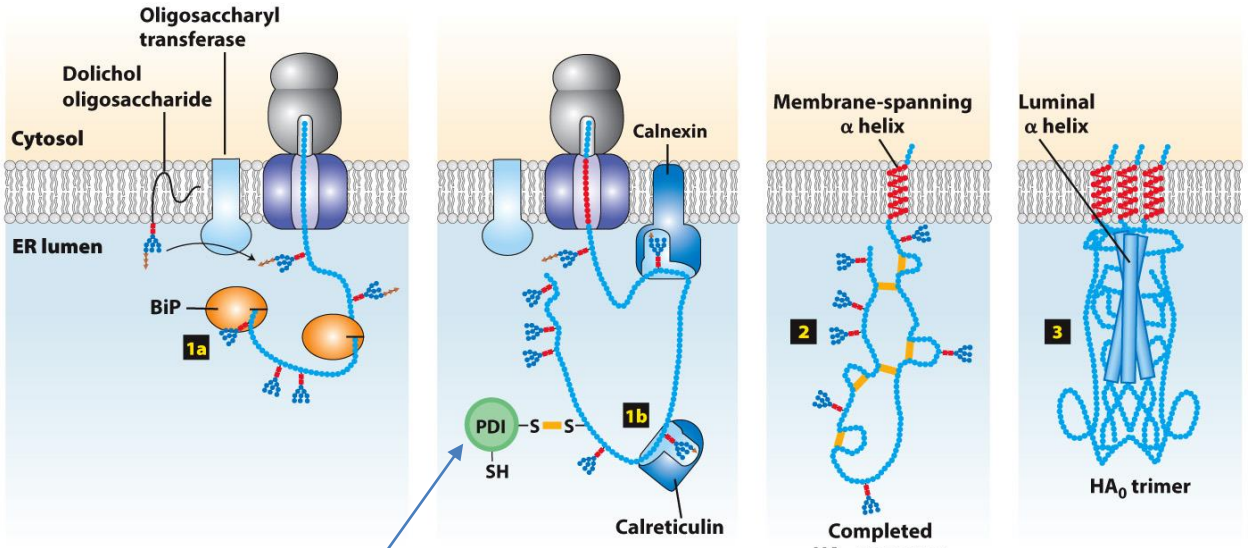


Figure 13-20a
Molecular Cell Biology, Eighth Edition
© 2016 W. H. Freeman and Company

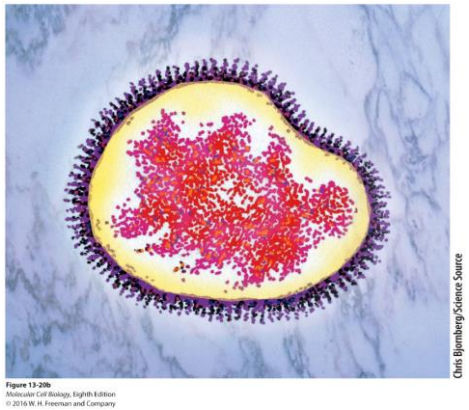


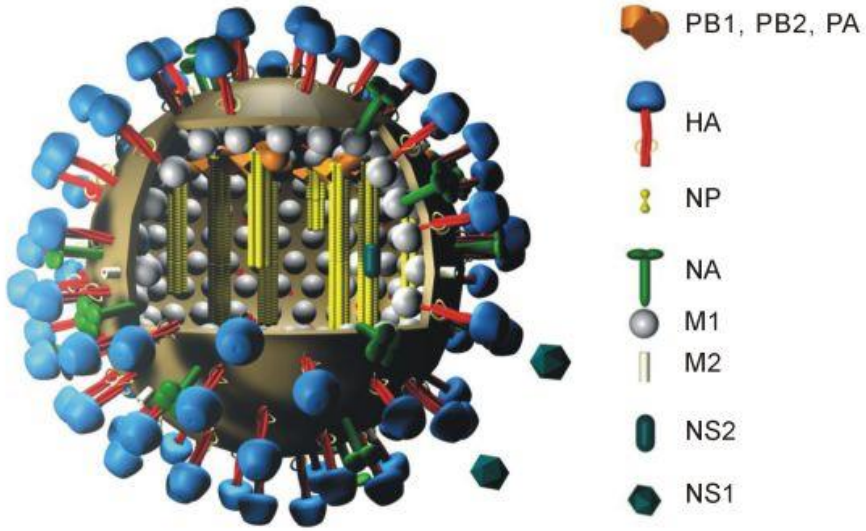
Figure 13-20b
Molecular Cell Biology, Eighth Edition
© 2016 W. H. Freeman and Company

Chris Bjornberg/Science Source

Calreticulin and Calnexin?

- Step 2:
  - **PDI** – catalyzes formation of six disulfide bonds per monomer
  - Properly folded monomer releases folding chaperones.

Influenza



The unfolded-protein *response.*

- Unfolded-protein response – presence of unfolded proteins in the rough ER increases transcription of genes that encode ER chaperones and other folding catalysts
- Ire1:
  - ER membrane transmembrane
  - Luminal domain – binds excess BiP (not associated with unfolded proteins)
  - Cytosolic domain – specific RNA endonuclease
- Mechanism:
  - Step 1:
    - Accumulating unfolded proteins in the ER lumen bind available BiPs.
    - Causes release of BiPs from monomeric Ire1s
    - Ire1 lacking BiP – dimerizes; activates its cytosolic endonuclease activity
  - Step 2: Ire1 endonuclease activity in the cytosol cuts an unspliced mRNA precursor encoding the transcription factor Hac1. (Unspliced mRNA precursor in the cytosol is unusual.)
  - Step 3: The two Hac1 exons join to form a functional Hac1 mRNA. (Pre-mRNA processing generally occurs in the nucleus.)
  - Step 4 : Hac1 is translated into Hac1 protein, which moves into the nucleus and activates transcription of genes encoding several protein-folding chaperones.

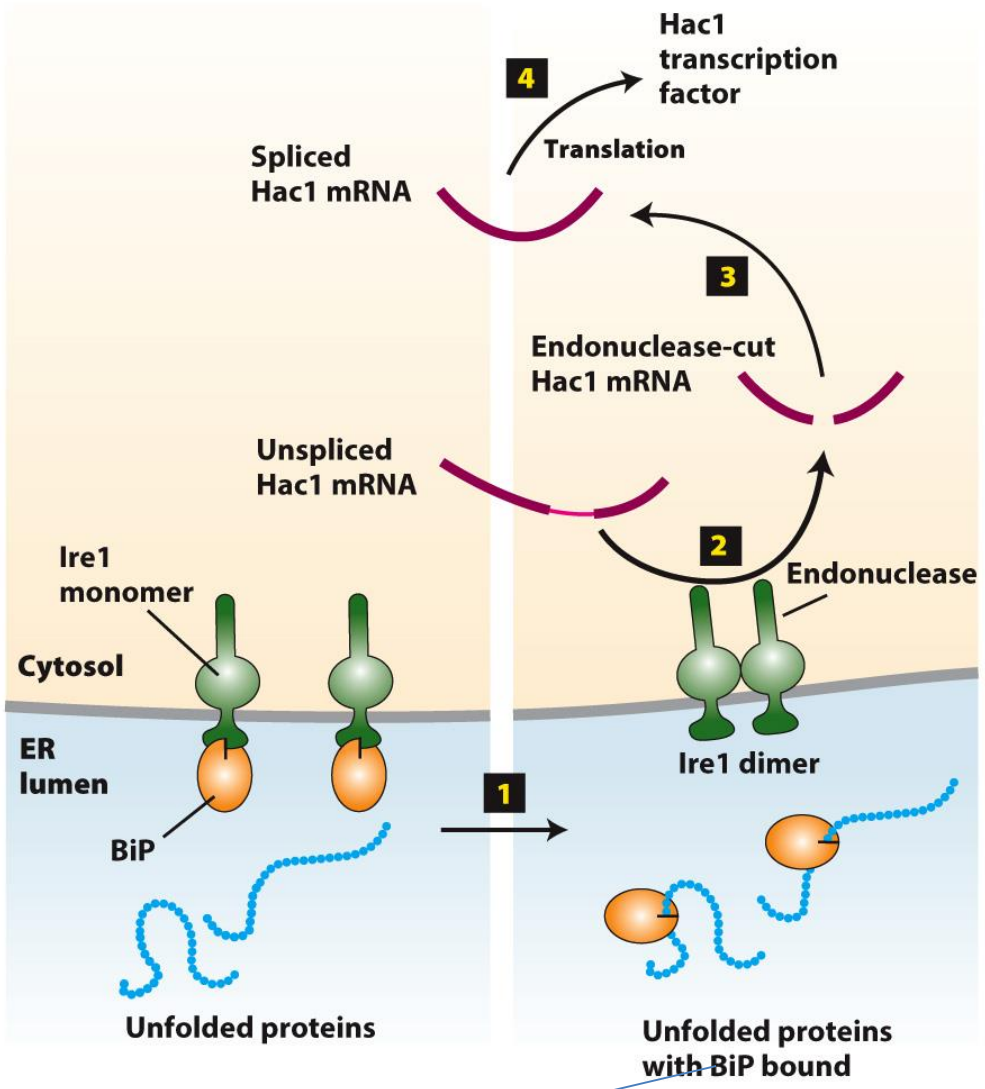


Figure 13-21
Molecular Cell Biology, Eighth Edition

Binding immunoglobulin protein

BiP is a **HSP70 molecular chaperone** located in the lumen of the endoplasmic reticulum (ER) that binds newly synthesized proteins as they are translocated into the ER, and maintains them in a state competent for subsequent folding and oligomerization.

Modifications of *N*-linked oligosaccharides are used to monitor folding and for quality control.

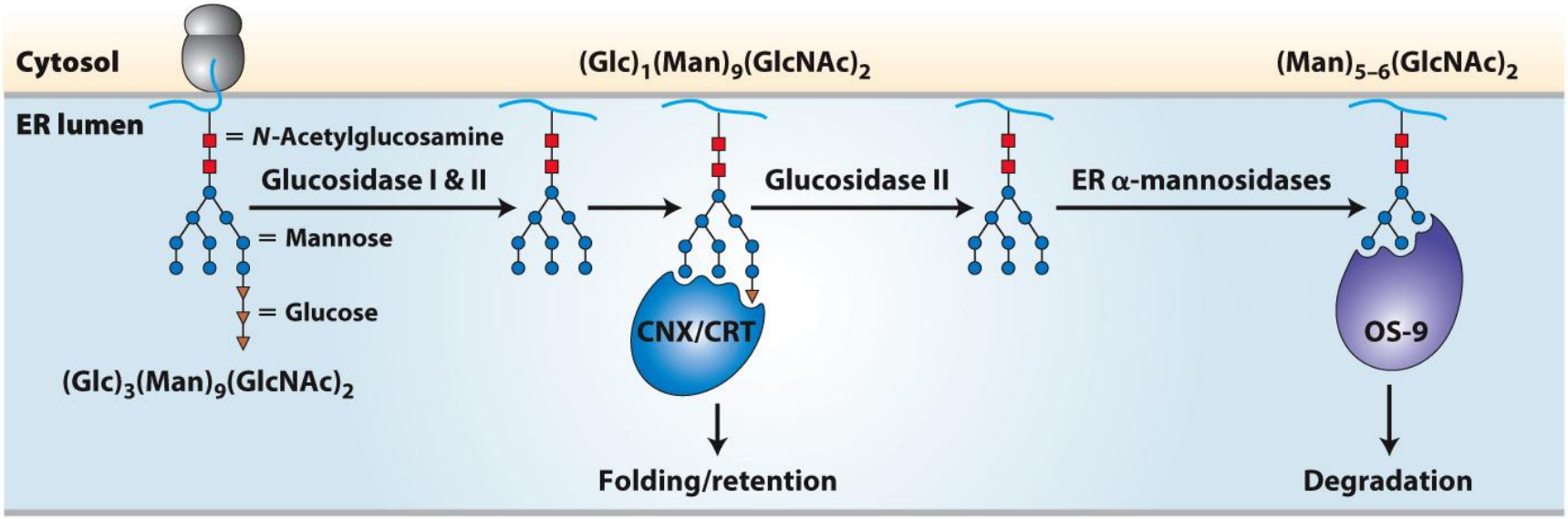


Figure 13-22
Molecular Cell Biology, Eighth Edition

- Dislocation: Misfolded secretory proteins – recognized by specific ER membrane proteins and targeted for transport from the ER lumen into the cytosol for degradation
- Mechanism:
  - Calnexin (CNX) and calreticulin (CRT) –
    - Bind *N*-linked oligosaccharides in which a glucose is re-added to form $Glc_1Man_9(GlcNAc)_2$ (after removal of three glucoses during normal ER processing)
    - Retain protein in the ER for folding chaperone activity until properly folded
  - Proteins that cannot fold properly –
    - Retained in the ER for longer times.
    - Eventually undergo mannose trimming by ER α-mannosidases to form $Man_{5–6}(GlcNAc)_2$
    - OS-9 binding to $Man_{5–6}(GlcNAc)_2$ leads to dislocation of the misfolded protein out of the ER, ubiquitinylation, and degradation by cytosolic proteasomes.

Moving Proteins into Membranes and Organelles

13.4 Targeting of Proteins to Mitochondria and Chloroplasts

- Mitochondrial and chloroplast proteins encoded by nuclear genes are synthesized on cytosolic ribosomes, and maintained in an unfolded state by chaperones.
- N-terminal targeting sequences direct post-translational transport of the unfolded proteins through translocons into the organelles.
- Multiple internal targeting sequences direct protein targeting inside the organelles to different membrane and lumen destinations.

Targeting Sequences That Direct Proteins from the Cytosol to Organelles

- Organelle targeting sequences are similar in their location and general function, but differ in sequence characteristics.

TABLE 13-1 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 (nucleoplasm)	Varies	No	Multiple different kinds; a common motif includes a short segment rich in Lys and Arg residues

*Different or additional sequences target proteins to organelle membranes and subcompartments.

Table 13-1
Molecular Cell Biology, Eighth Edition

Import of mitochondrial precursor proteins is assayed in a cell-free system.

Uptake or not?
How to test?

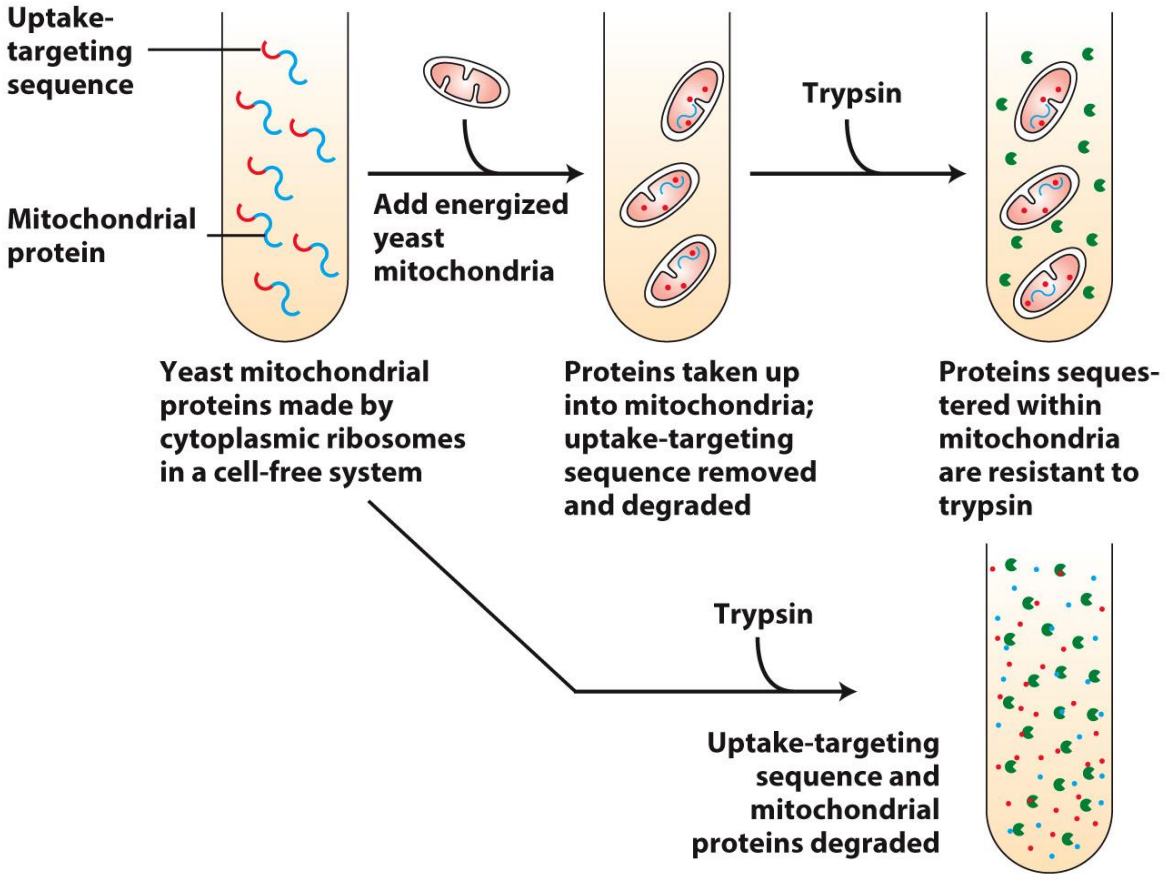
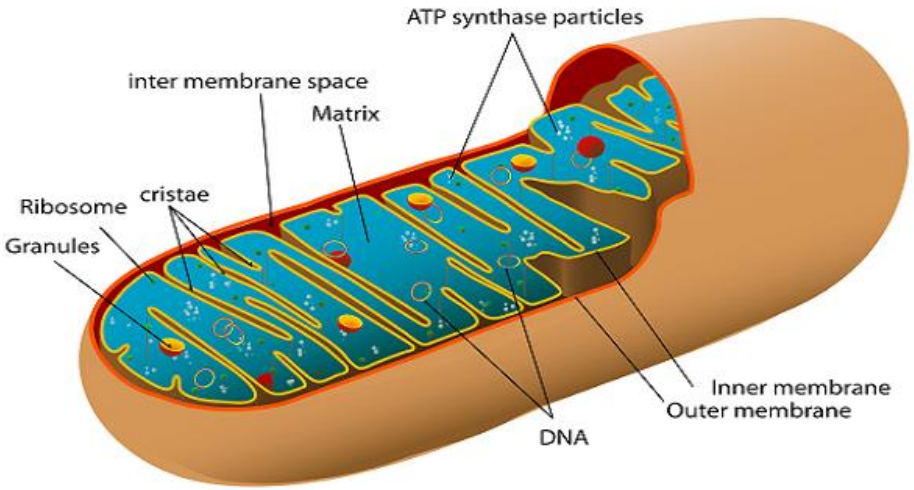


Figure 13-23
Molecular Cell Biology, Eighth Edition

Protein import into the mitochondrial matrix.



- Precursor proteins synthesized on cytosolic ribosomes
- Step 1: Maintained in an unfolded state by bound chaperones, such as cytosolic Hsp70
- Step 2: Matrix-targeting sequence binds to outer membrane import receptor near a rare “contact site” with the inner membrane.
- Step 3: MTS inserted into outer membrane translocon (Tom – translocon of the outer membrane)
- Step 4: Translocating protein moves through Tom and inserts into inner membrane translocon (Tim – translocon of the inner membrane)
- Step 5:
  - Protein translocates through Tim.
  - Binding by matrix Hsp70 helps drive import into the matrix.
  - MTS removed by a matrix protease
- Step 6: Hsp70 ATP hydrolysis releases newly imported protein.
- Step 7: Protein folds into its mature, active conformation within the matrix.

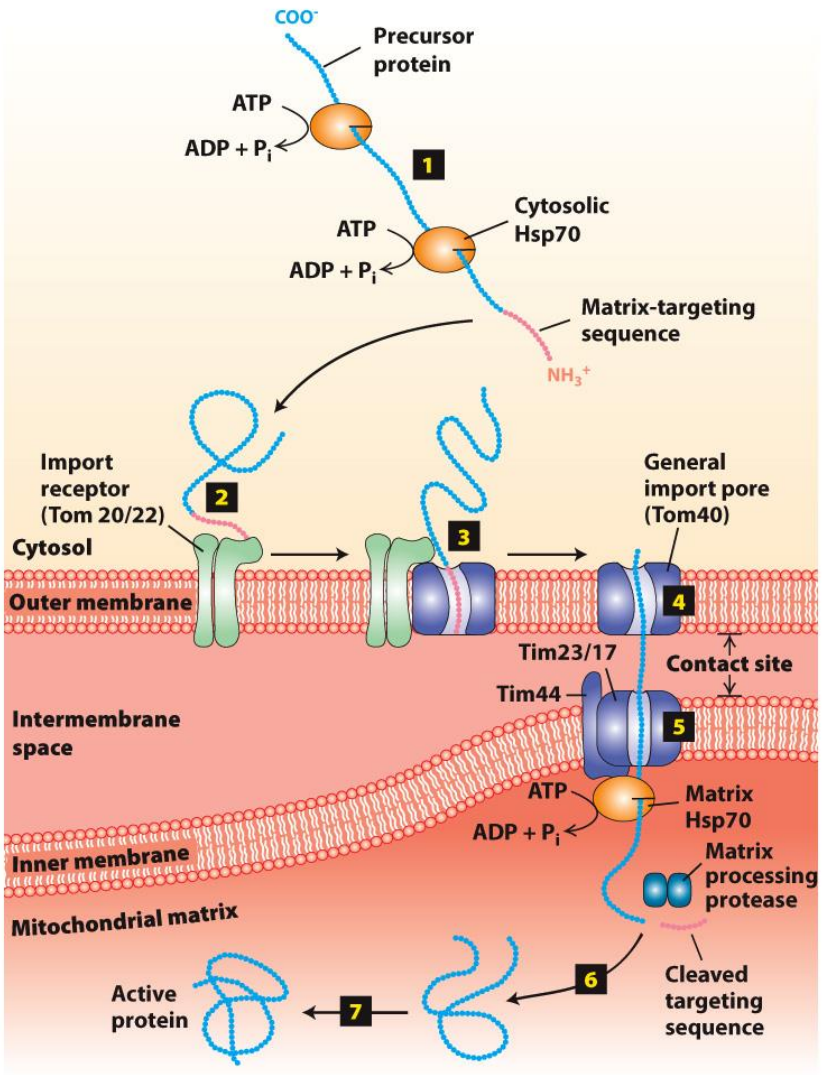
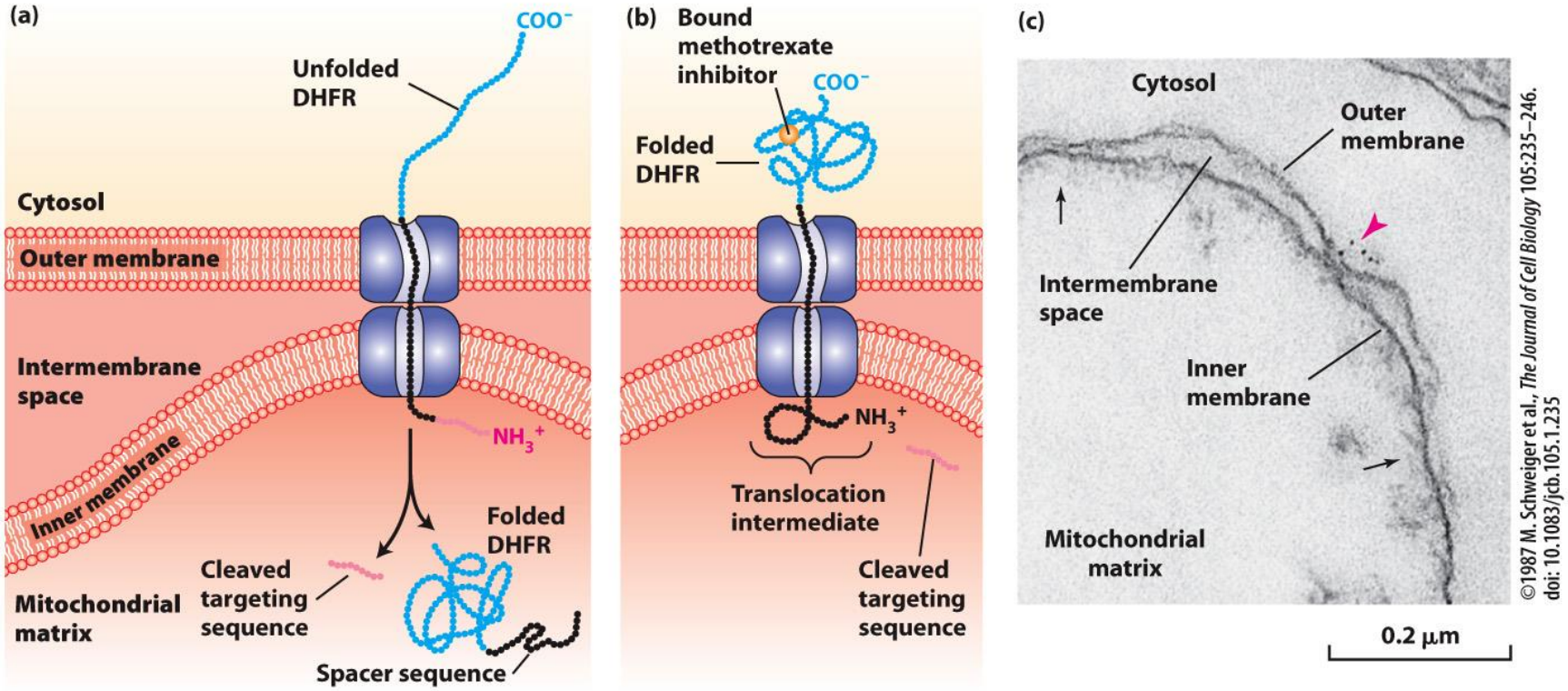


Figure 13-24
Molecular Cell Biology, Eighth Edition

Experiments with chimeric proteins elucidate mitochondrial protein import processes.

How to show the translocation by experiment?

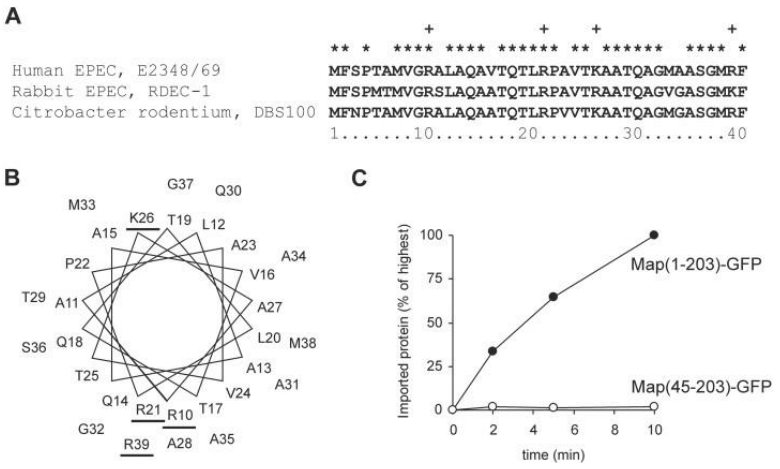


- (a) (–) Methotrexate –
  - DHFR segment is unfolded by chaperones.
  - Chimeric protein – translocated through translocons across both membranes into the matrix of an energized mitochondrion
  - Matrix-targeting signal is removed.
- (b) (+) Methotrexate –
  - Locks DHFR in the folded state
  - Translocation is blocked – forming a stable translocation intermediate – Tom/Tim transfer only unfolded proteins
  - If the spacer sequence is long enough to extend across Tom-Tim, MTS is cleaved.
- (c) Localization of translocation – bound to the translocation intermediate at a contact site between the inner and outer membranes

Targeting sequences in imported mitochondrial proteins.

- Nuclear-encoded mitochondrial proteins – N-terminal matrix-targeting sequence
- Proteins destined for the inner membrane, the intermembrane space, or the outer membrane – have one or more additional targeting sequences

Ex) Comparison of the N-terminal sequence of AcuJ



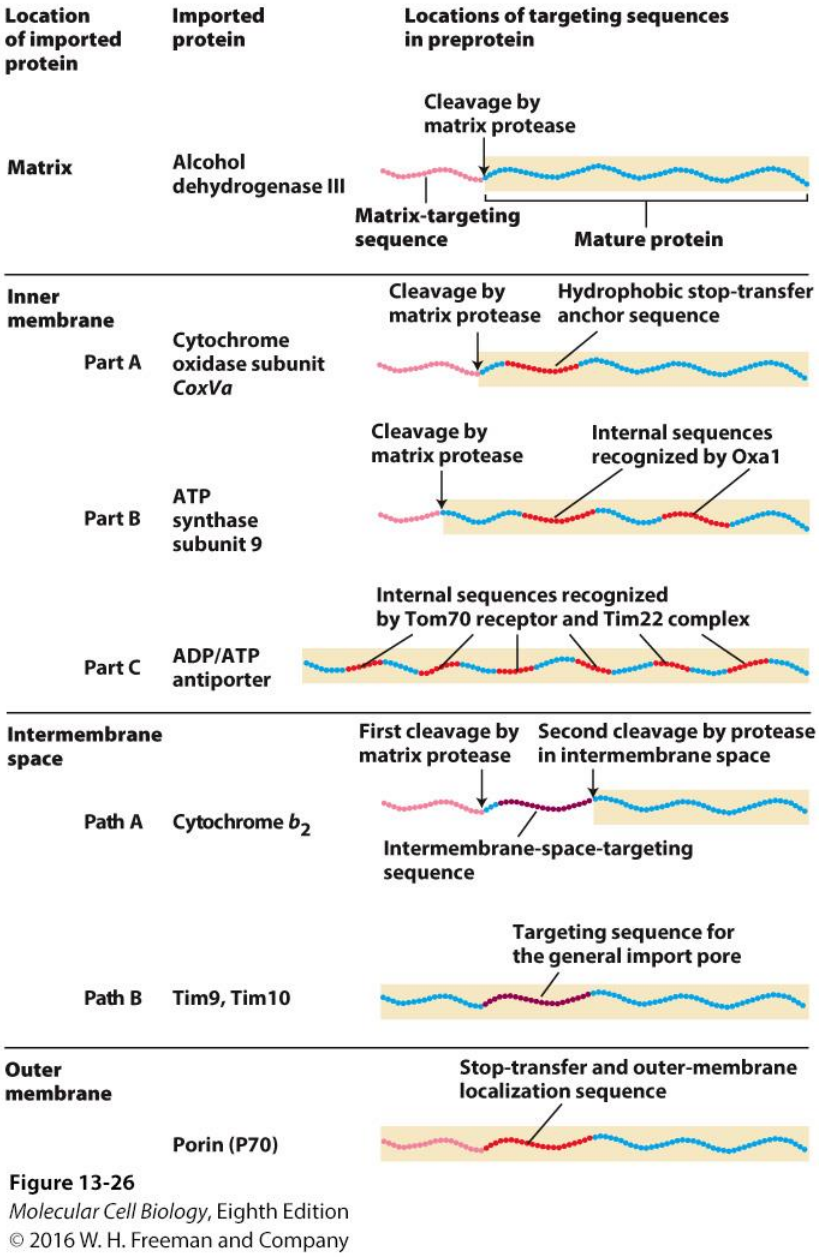
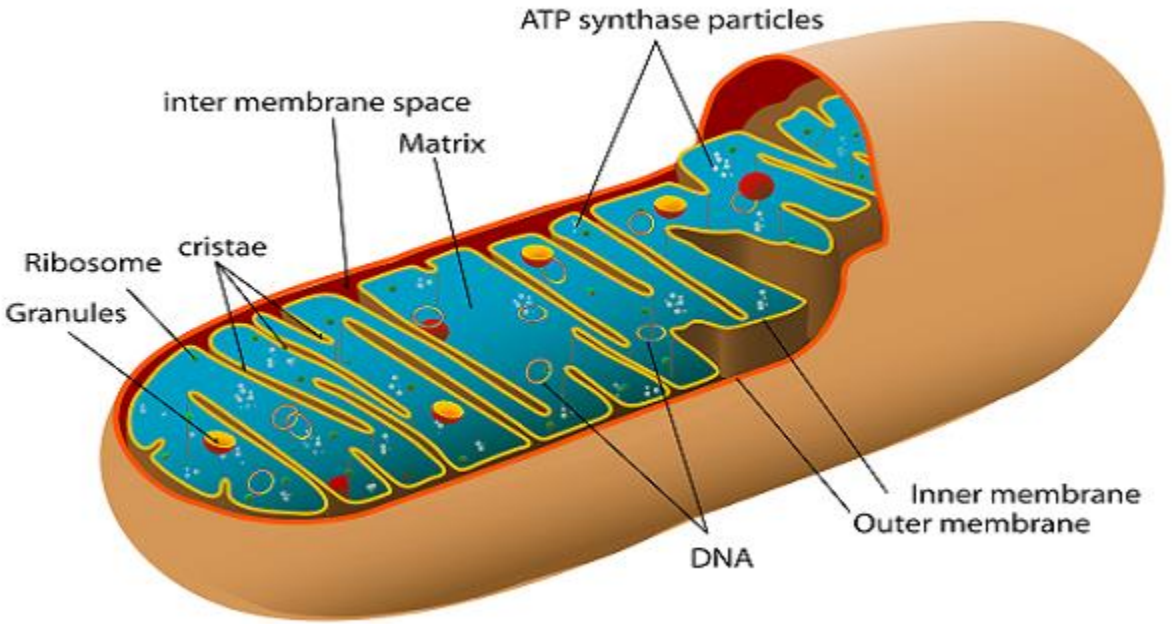


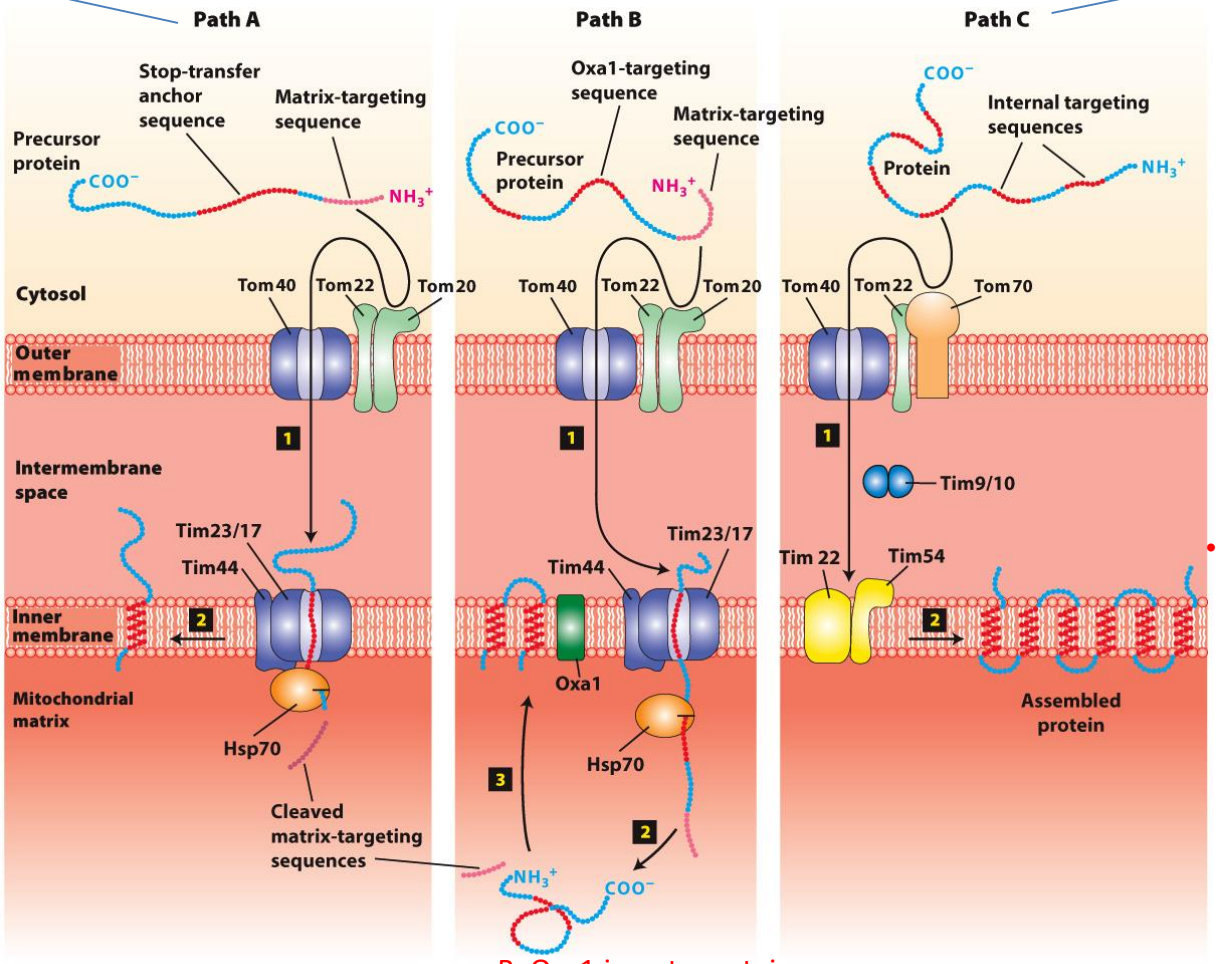
Figure 13-26
Molecular Cell Biology, Eighth Edition

Then, how to inner membrane and inner membrane space?



Three pathways to the inner mitochondrial membrane from the cytosol.

- CoxVa – matrix-targeting sequence and hydrophobic stop-transfer anchor sequence –
- ATP synthase subunit 9 – matrix-targeting sequence and internal hydrophobic domains recognized by the Oxa1 inner-membrane protein
- ATP/ADP antiporter – lack N-terminal matrix-targeting sequence; internal sequences recognized by the Tom70/Tom22 import receptor



- A.Hydrophobic stop-transfer anchor sequence – blocks transfer through Tim
- B. Oxa1 inserts protein into inner membrane
- C. Different innermembrane translocation channel (Tim22/54) – incorporates multiple hydrophobic segments of the imported protein into the inner membrane

Figure 13-27
Molecular Cell Biology, Eighth Edition

Two pathways to the mitochondrial intermembrane space.

- Path A: cytochrome b_2
- Path B: specialized pathway for delivery of the intermembrane chaperones Tim9 and Tim10

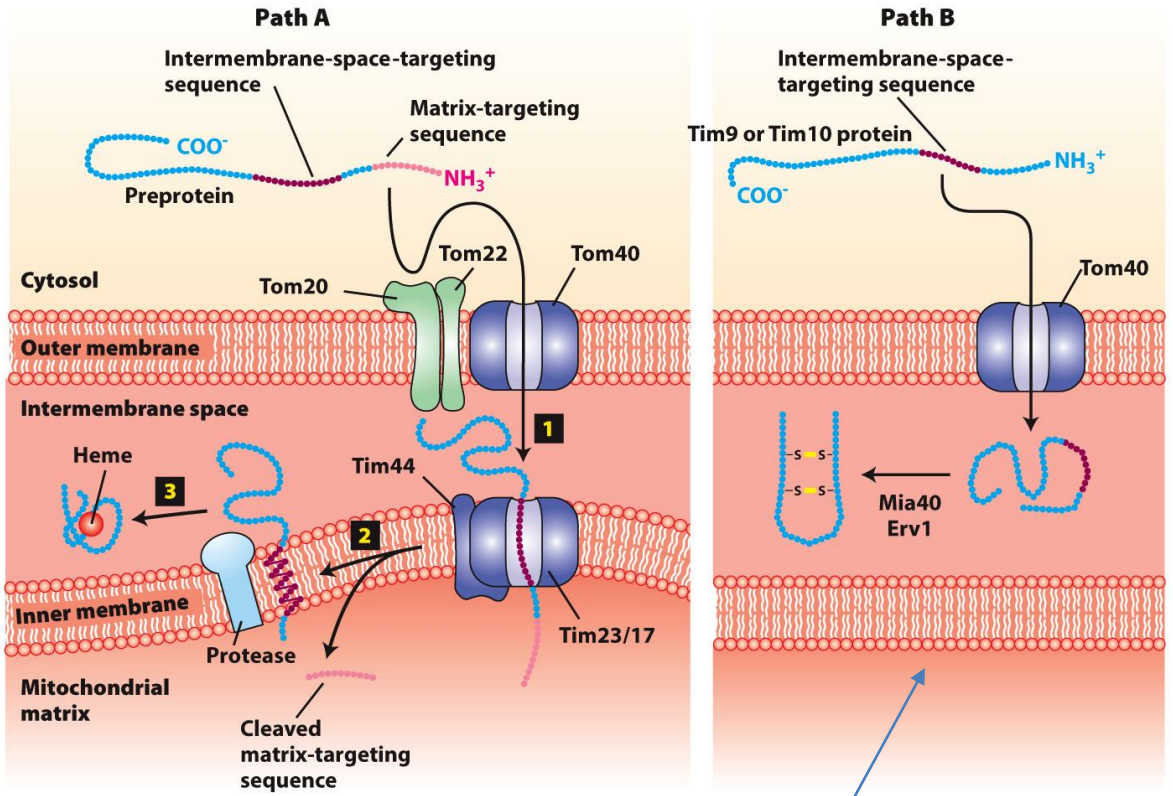


Figure 13-28
Molecular Cell Biology, Eighth Edition

- N-terminal matrix-targeting sequence – removed by the matrix protease
- Stop transfer sequence –
  - Blocks translocation of the protein across the inner membrane
  - Causes release from Tim into the inner membrane (similar to inner membrane protein Path A)
- Inner membrane protease – cleaves protein on the intermembrane side of the stop-transfer sequence

- No N-terminal matrix-targeting sequence or Tim targeting sequence
- Tom40 – translocates proteins directly to the intermembrane space
- Proteins fold and Erv1 forms two disulfide bonds – prevents reverse translocation through Tom40

Moving Proteins into Membranes and Organelles

13.5 Targeting of Peroxisomal Proteins

- Luminal peroxisomal proteins are synthesized on free cytosolic ribosomes, contain a targeting sequence, and are incorporated into the peroxisome post-translationally.
- Folded luminal proteins are imported by a system involving a cytosolic receptor protein and translocation machinery on the peroxisomal membrane.
- Peroxisomal membrane proteins contain different targeting sequences than peroxisomal matrix proteins and are imported by a different pathway.

PTS1-directed import of peroxisomal matrix proteins.

- PST1-containing proteins –
  - Synthesized by cytosolic ribosomes
  - Folds – may bind cofactors – remains folded for translocation
- Step 1 : PTS1 targeting sequence binds to the cytosolic receptor Pex5.
- Step 2 : Pex5-matrix protein complex - forms a multimeric complex with the Pex14 receptor in the peroxisomal membrane. (Size of complex – adjusts to size of imported protein.)
- Step 3 : Matrix protein dissociates from Pex5 and is released into the peroxisomal matrix. (Targeting sequence is retained.)
- Steps 4 and 5 : Pex5 is returned to the cytosol by a process that involves –
  - Ubiquitinylation by the membrane proteins Pex2, Pex10, and Pex12
  - ATP-dependent removal from the membrane by the AAA-ATPase proteins Pex1 and Pex6

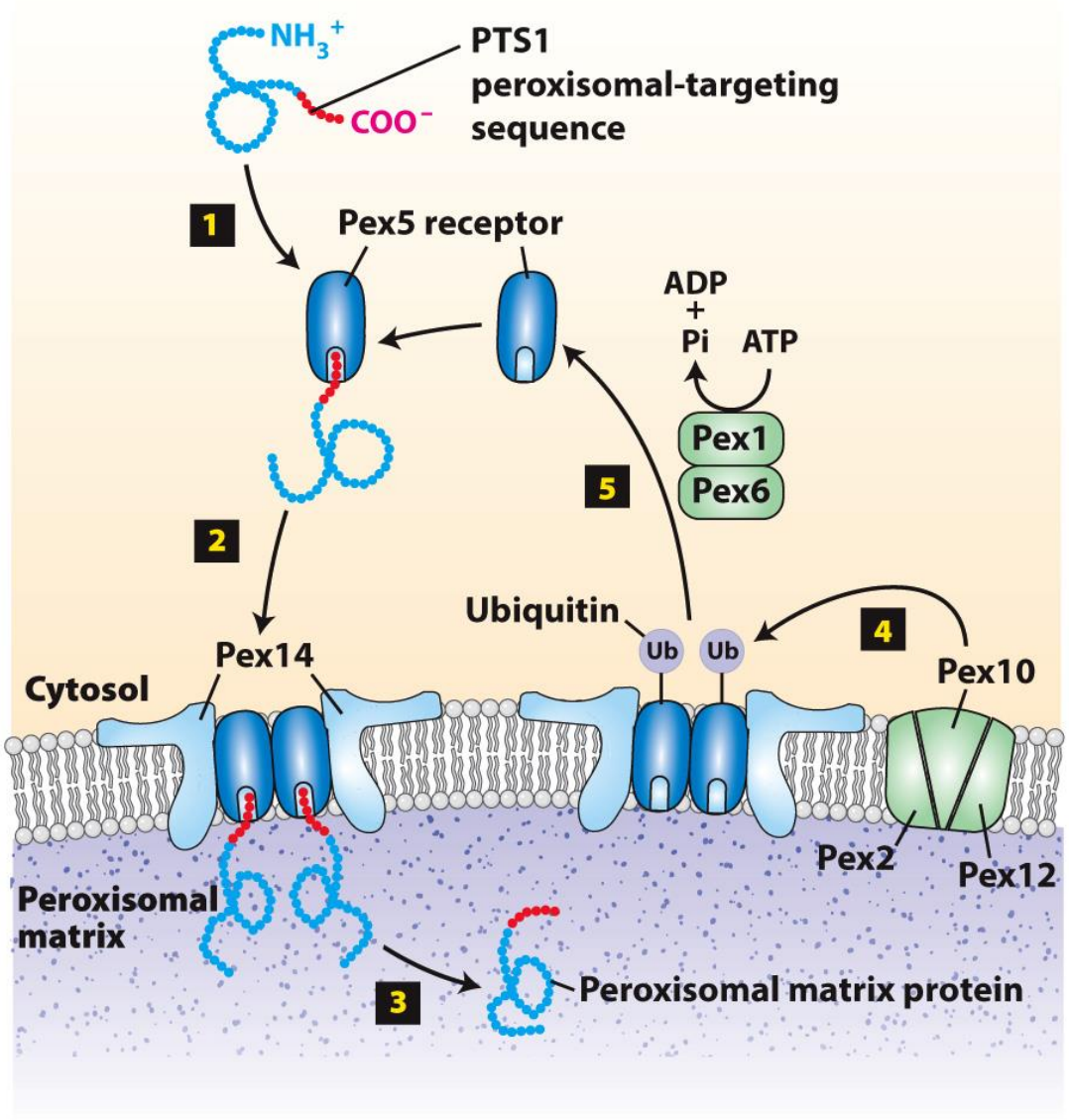


Figure 13-30
Molecular Cell Biology, Eighth Edition

Studies reveal different pathways for incorporation of peroxisomal membrane and matrix proteins.

Which protein is used for which process?

- Cells stained with fluorescent antibodies to:
  - PMP70 – peroxisomal membrane protein
  - Catalase – peroxisomal matrix protein
- (a) Wild-type cells: both peroxisomal membrane and matrix proteins in numerous peroxisomes
- (b) Cells from a Pex12-deficient patient:
  - PMP70 – localized normally in peroxisomes
  - Catalase – distributed uniformly throughout the cytosol – not imported into peroxisomes
  - Conclusion – Pex12 is required for import of matrix but not membrane proteins
- (c) Cells from a Pex3-deficient patient:
  - Both catalase and PMP70 – mislocalized to the cytosol
  - Peroxisomal membranes cannot assemble – peroxisomes do not form
  - Conclusion – Pex3 is required for import of membrane proteins

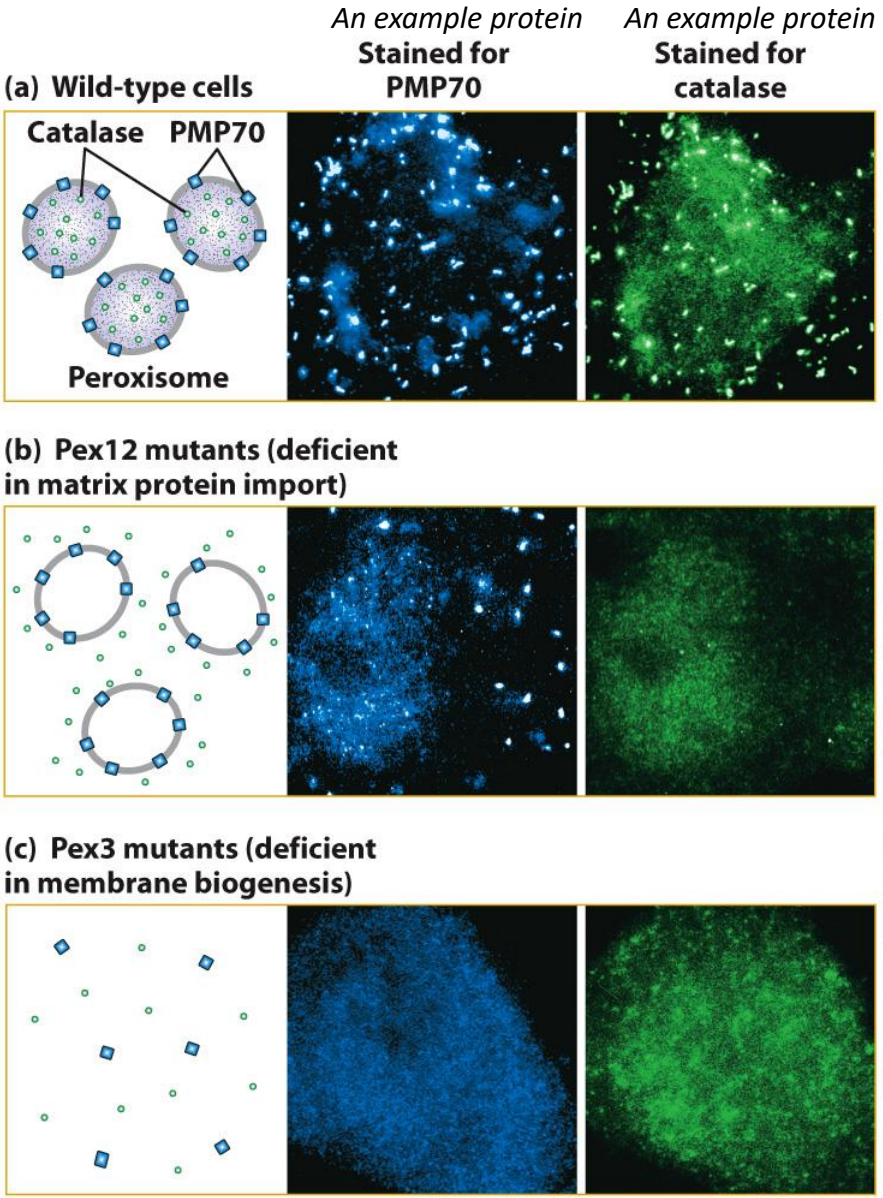


Figure 13-31
Molecular Cell Biology, Eighth Edition

Model of peroxisomal biogenesis and division.

Where does the peroxisome membrane come from?

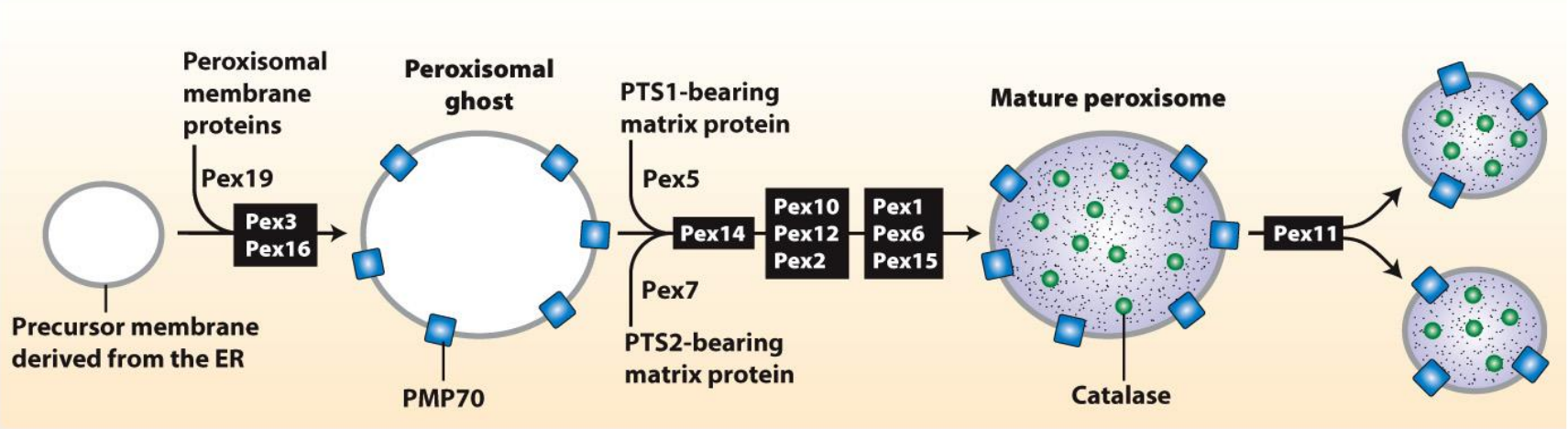


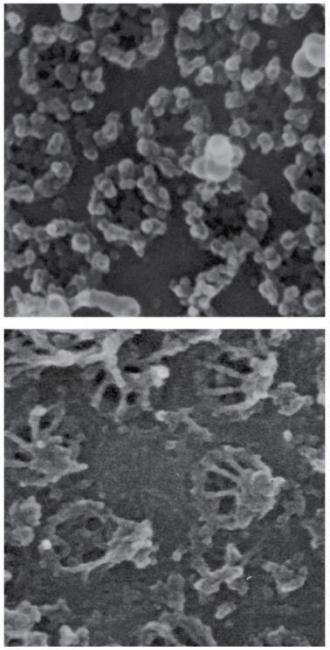
Figure 13-32
Molecular Cell Biology, Eighth Edition

- First stage – Incorporation of peroxisomal membrane proteins into precursor membranes derived from the ER:
  - Pex19 – Receptor for membrane targeting sequences
  - Pex3 and Pex16 complex – Required for proper insertion of proteins (e.g., PMP70) into the forming peroxisomal membrane
- Peroxisomal membrane protein insertion – produces a peroxisomal ghost, which imports matrix-targeted proteins
  - PTS1-bearing matrix proteins – imported via Pex5 cytosolic receptors
  - PTS2-bearing matrix proteins – imported via Pex7 cytosolic receptors

Moving Proteins into Membranes and Organelles

13.6 Transport Into and Out of the Nucleus

- Unidirectional transport of a protein larger than 40 kDa through large, complex nuclear pore complexes requires a nuclear-localization or nuclear-export signal, nuclear transport receptors, Ran G-proteins, and localized Ran-GEFs and GAPs.
- Other molecules, including mRNPs, are transported by a Ran-independent pathway.



Republished with permission of Elsevier, from Doye, V. and Hurt, E., "Nucleoporins to Nuclear Pore Complexes," *Curr. Opin. Cell Biol.* 9(3):401–411, 1997; permission conveyed through the Copyright Clearance Center, Inc.

Figure 13-33a
Molecular Cell Biology, Eighth Edition

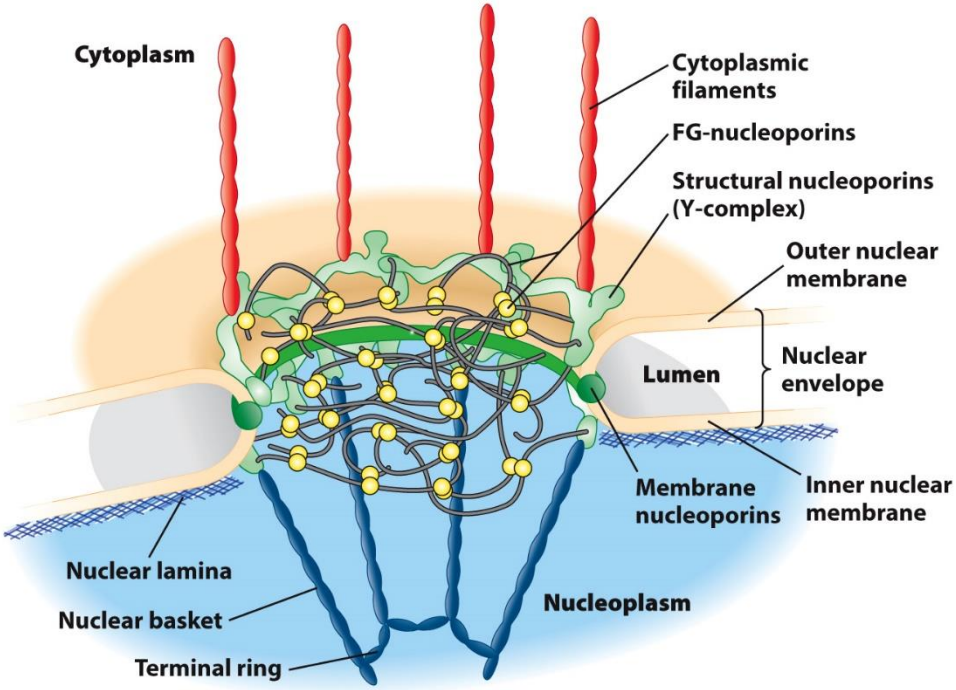


Figure 13-33b
Cell Biology, Eighth Edition
H. Freeman and Company

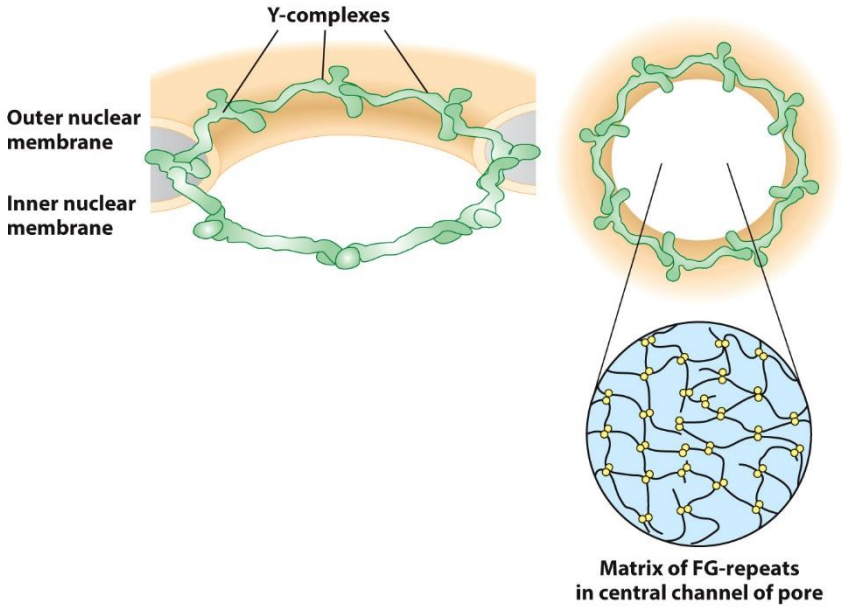


Figure 13-33c
Molecular Cell Biology, Eighth Edition

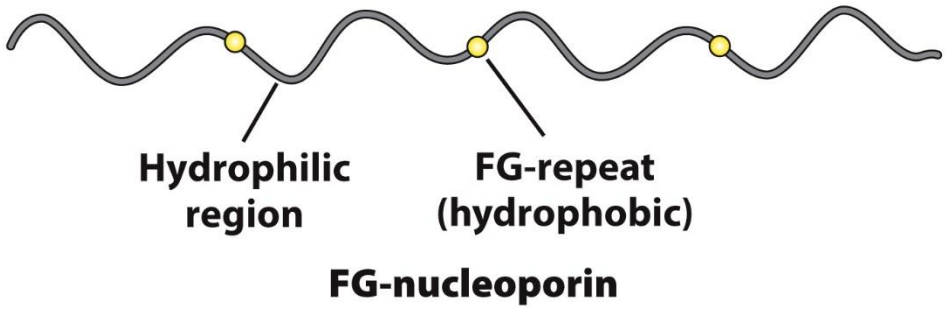


Figure 13-33d
Molecular Cell Biology, Eighth Edition

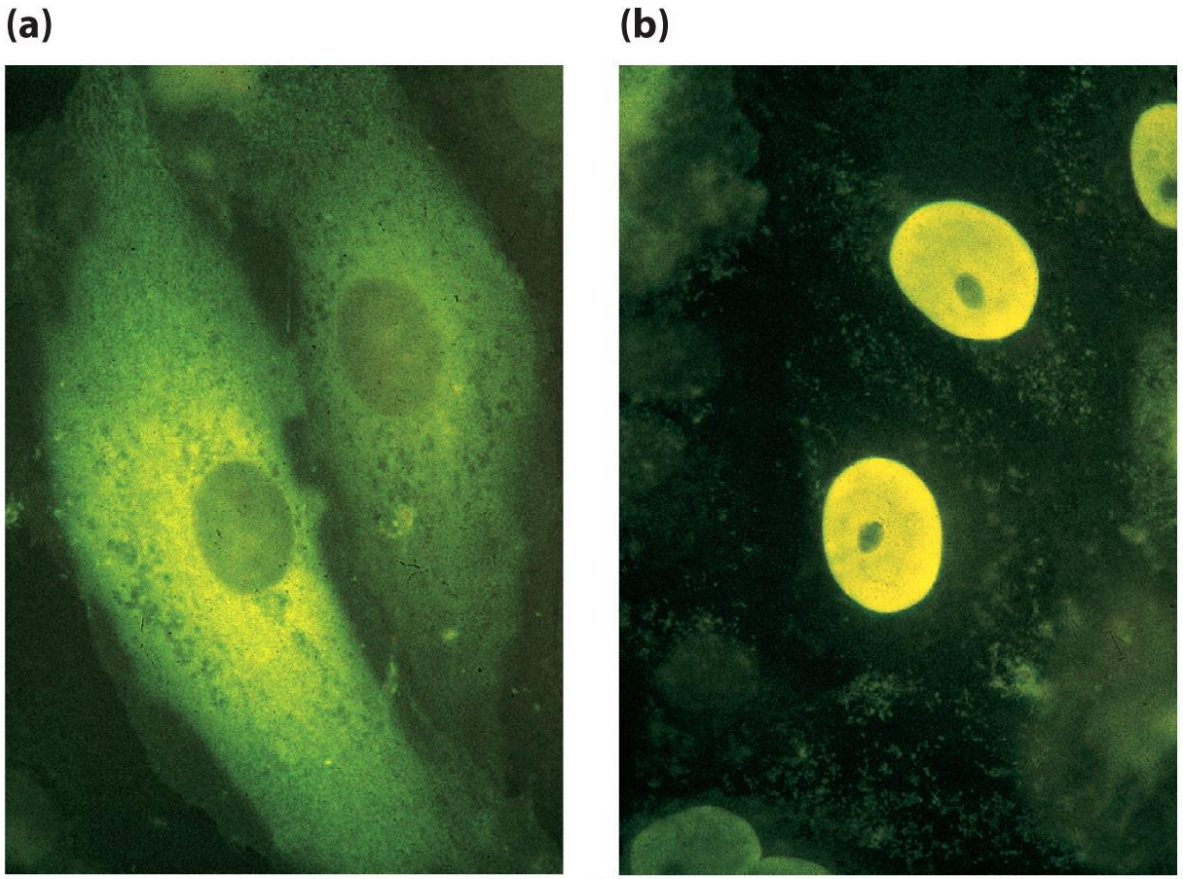


Figure 13-34
Molecular Cell Biology, Eighth Edition

Nuclear localization signal (NLS)

Cytosolic proteins are required for nuclear transport.

- Experiment:
  - Proteins containing a NLS (similar to the SV40 large T-antigen NL) – efficiently transported into isolated nuclei only if provided with an extract containing crucial soluble cytosolic components
  - (a) Digitonin-permeabilized HeLa cells
  - (b) Digitonin-permeabilized HeLa cells incubated with a fluorescent protein chemically coupled to a synthetic SV40 T-antigen NLS peptide:
    - (–) cell lysate (cytosol) – no transport (or accumulation) into nuclei
    - (+) cell lysate – transport (and accumulation) in nuclei
  - Conclusion: nuclear transport requires soluble cytosolic factor(s).

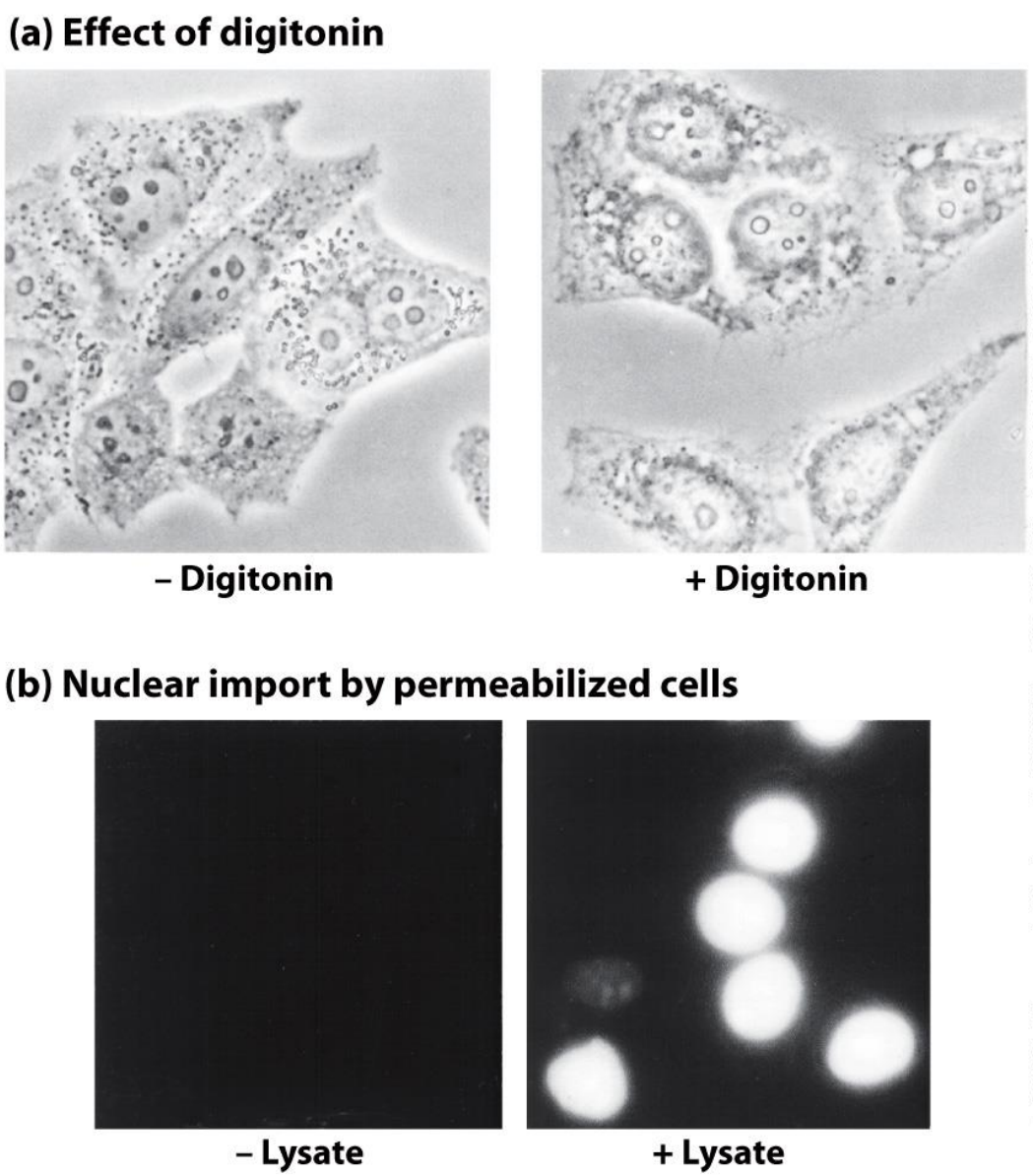


Figure 13-35
Molecular Cell Biology, Eighth Edition

Mechanism for nuclear import of proteins.

- NLS-protein import mechanism:
  - Cytoplasm –
    - Importin (soluble nuclear transport receptor) – binds an NLS of a cargo protein to form an importin-cargo complex
    - Importin-cargo complex diffuses through the NPC by transiently interacting with FG-nucleoporins.
    - Ran-GDP diffuses into nucleus (not shown).
  - Nucleoplasm:
    - Ran-GDP activated by Guanine Exchange Factor (GEF) – releases GDP, binds GTP
    - Ran-GTP binding to the importin causes importin conformational change that releases the NLS-cargo protein.
  - System recycling –
    - Importin-Ran-GTP complex – transported back to the cytoplasm
    - A GTPase-activating protein (GAP) associated with the cytoplasmic filaments of the NPC – stimulates Ran hydrolysis of its bound GTP
    - Ran-GDP conformational change releases importin.
  - Localization of GEF in nucleus and GAP in cytoplasm ensures direction of nuclear import.

Ran (RAs-related Nuclear protein) also known as GTP-binding nuclear protein Ran is a protein that in humans is encoded by the RAN gene.

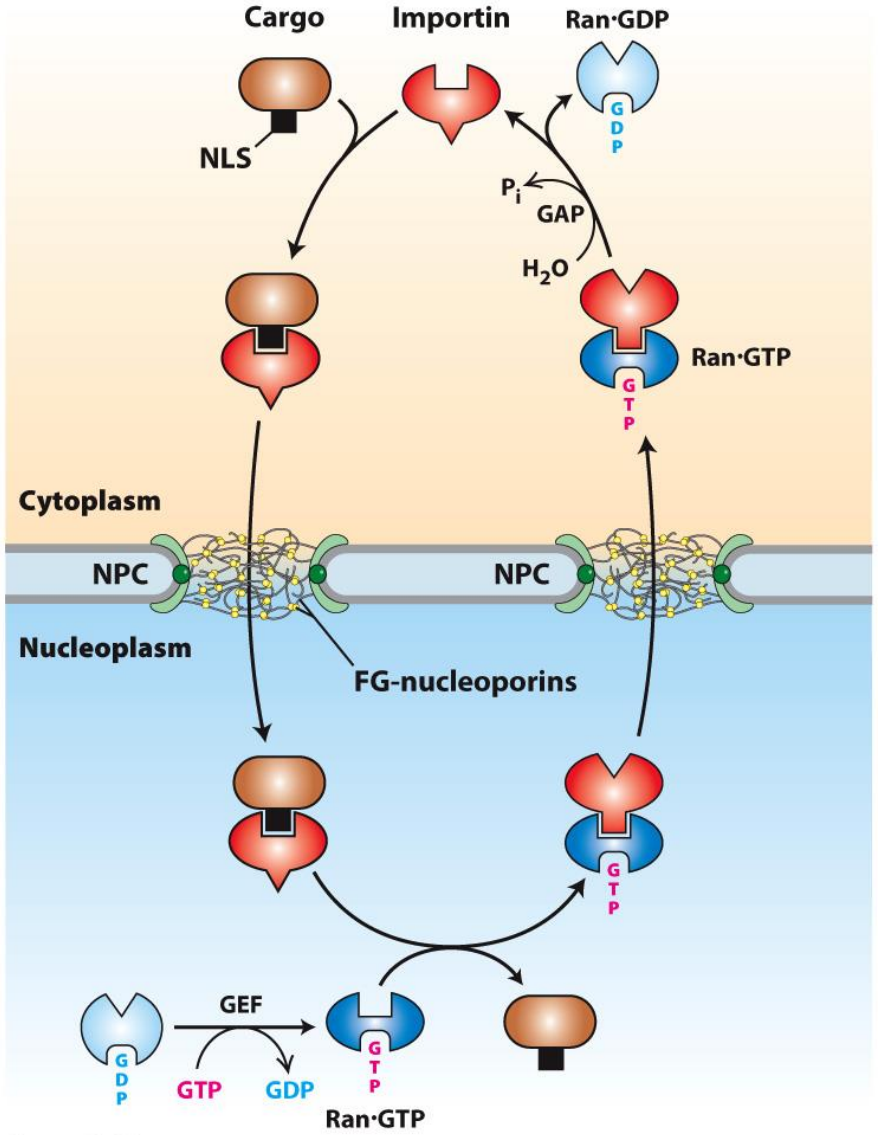


Figure 13-36
Molecular Cell Biology, Eighth Edition

Ran-dependent and Ran-independent nuclear export.

- (a) Ran-dependent mechanism for nuclear export of NES-proteins:
  - Nuclear-export signal (NES) – leucine-rich region
  - Nucleoplasm –
    - Exportin 1 – binds cooperatively to form a complex with an NES-cargo protein and Ran-GTP
    - Complex diffuses through an NPC via transient interactions with FG-repeats in FG-nucleoporins.
  - Cytoplasm –
    - Ran-GAP associated with the NPC cytoplasmic filaments stimulates Ran-GTP hydrolysis to Ran-GDP.
    - Ran-GDP conformational change releases NES-containing cargo protein into the cytosol.
  - Recycling system –
    - Exportin 1 and Ran-GDP – transported back into the nucleus
    - Ran-GEF in the nucleoplasm – converts Ran-GDP to Ran-GTP
- (b) Ran-independent mechanism for nuclear export of mRNAs:
  - Nucleoplasm –
    - Heterodimeric NXF1/NXT1 nuclear export receptor complex binds to mRNA-protein complexes (mRNPs).
    - Complex diffuses through NPC by transiently interacting with FG nucleoporins.
  - Cytoplasm – An RNA helicase (Dbp5) located on the cytoplasmic side of the NPC uses ATP energy to remove NXF1 and NXT1 from the mRNA.
  - Recycling system – The Ran-dependent import process recycles free NXF1 and NXT1 proteins back into the nucleus.

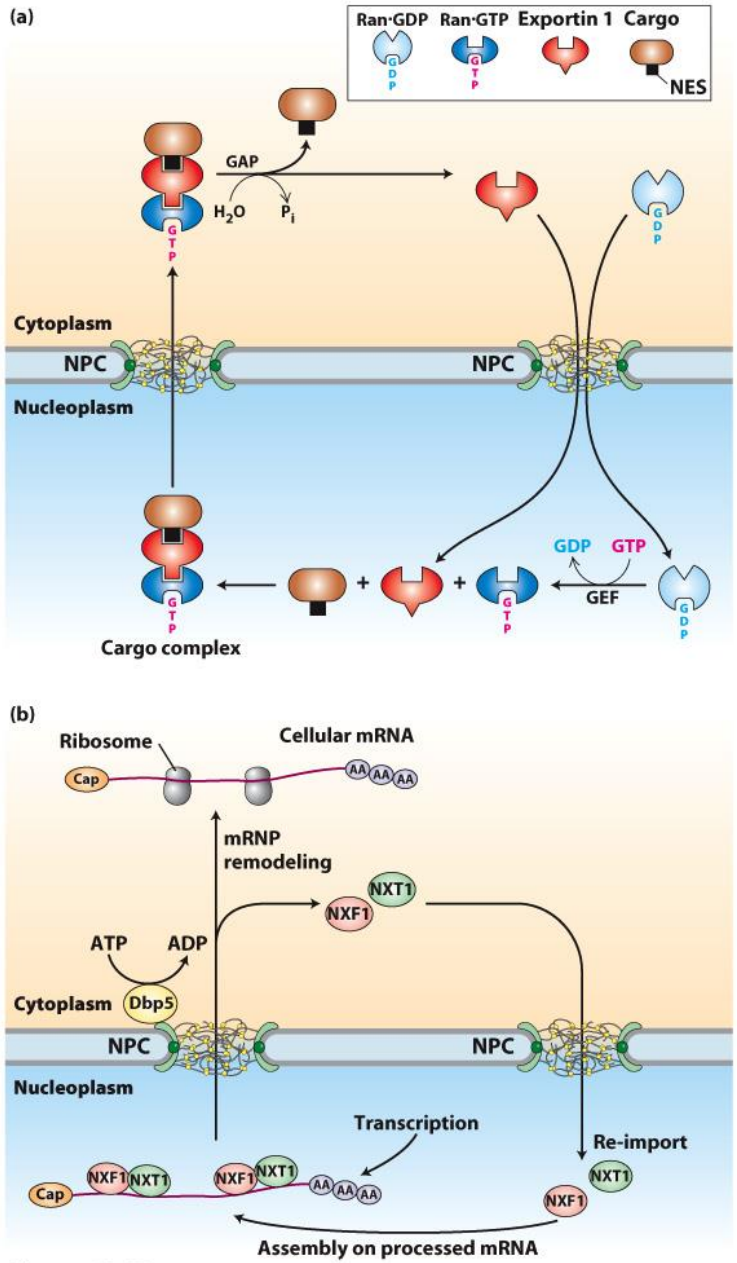


Figure 13-37
Molecular Cell Biology, Eighth Edition

Discussion with friends

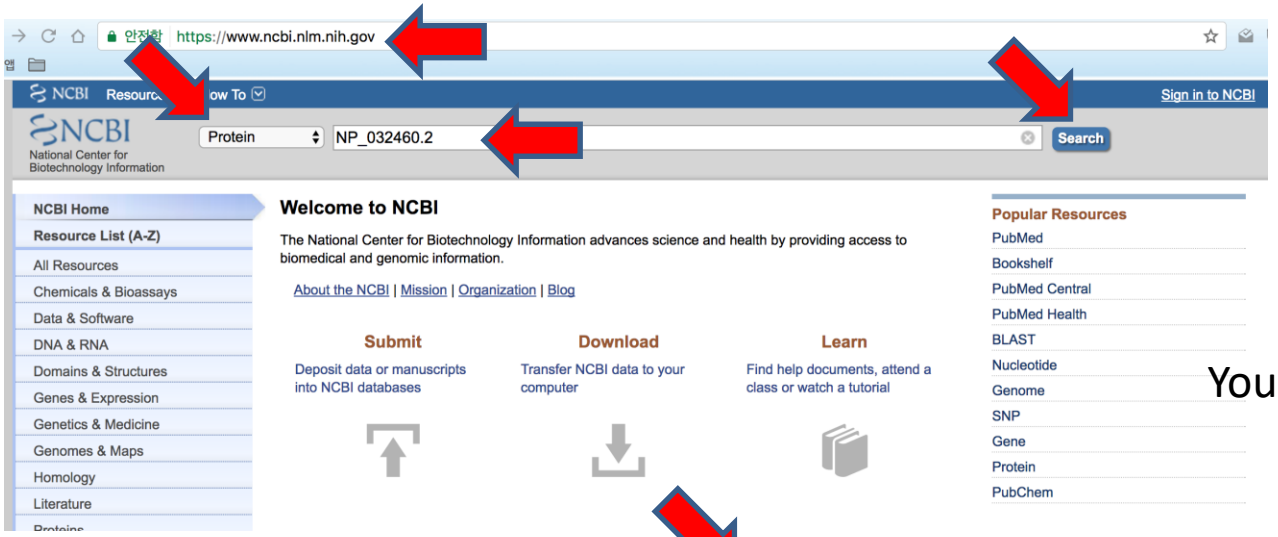
- Find the examples of nuclear localization sequence and ER targeting sequence, and explain their properties.
- Find a sequence of the ion channel, potassium voltage-gated channel (NCBI reference seq: NP_032460.2) and then analyze which region would be in the membrane using website program. Please submit the result and discuss how many transmembrane domains it would has and which region you can really find the positive charged amino acids.
- How to determine the targeting peptide sequence? For example how to determine which region of peptide is important for nuclear localization? Please see the figure 3 in this paper and explain it.

Neuroscience Letters 343 (2003) 134–138

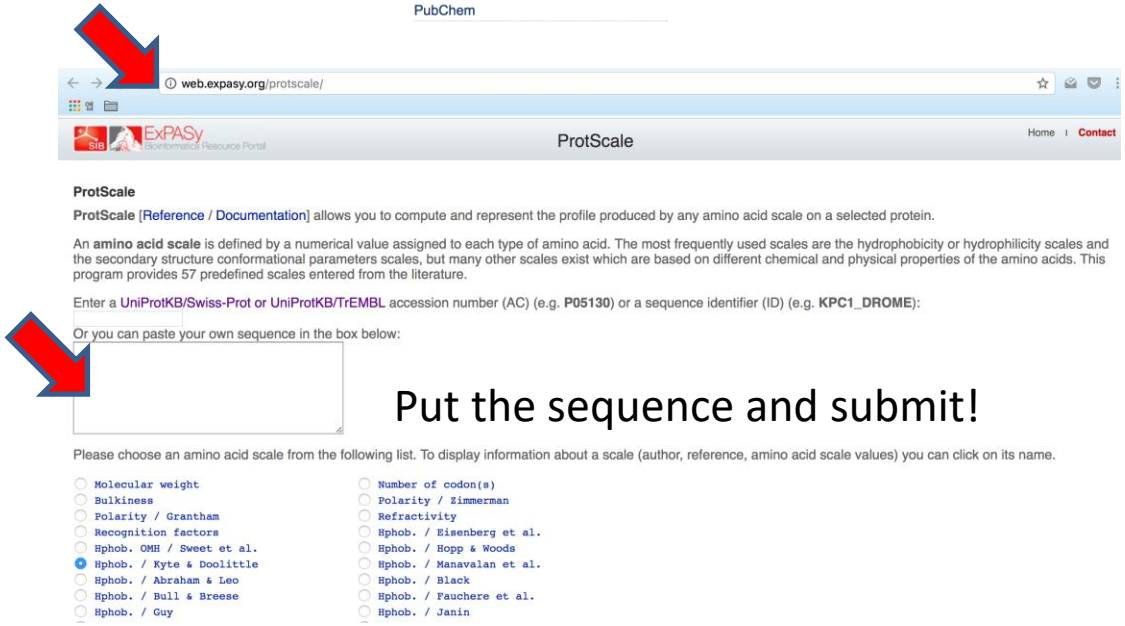
Identification of nuclear/nucleolar localization signal in Aplysia learning associated protein of slug with a molecular mass of 18 kDa homologous protein

Hyoung Kim, Deok-Jin Chang, Jin-A Lee, Yong-Seok Lee, Bong-Kiun Kaang*

How to find the protein sequence?



You can get the sequence!



Put the sequence and submit!