Cell Biology
Lecture 7

Intracellular Compartments and Protein Sorting, Part 2

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15.11.2023

Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 12
Intracellular Compartments and Protein Sorting

Pages: 641-654, 669-688

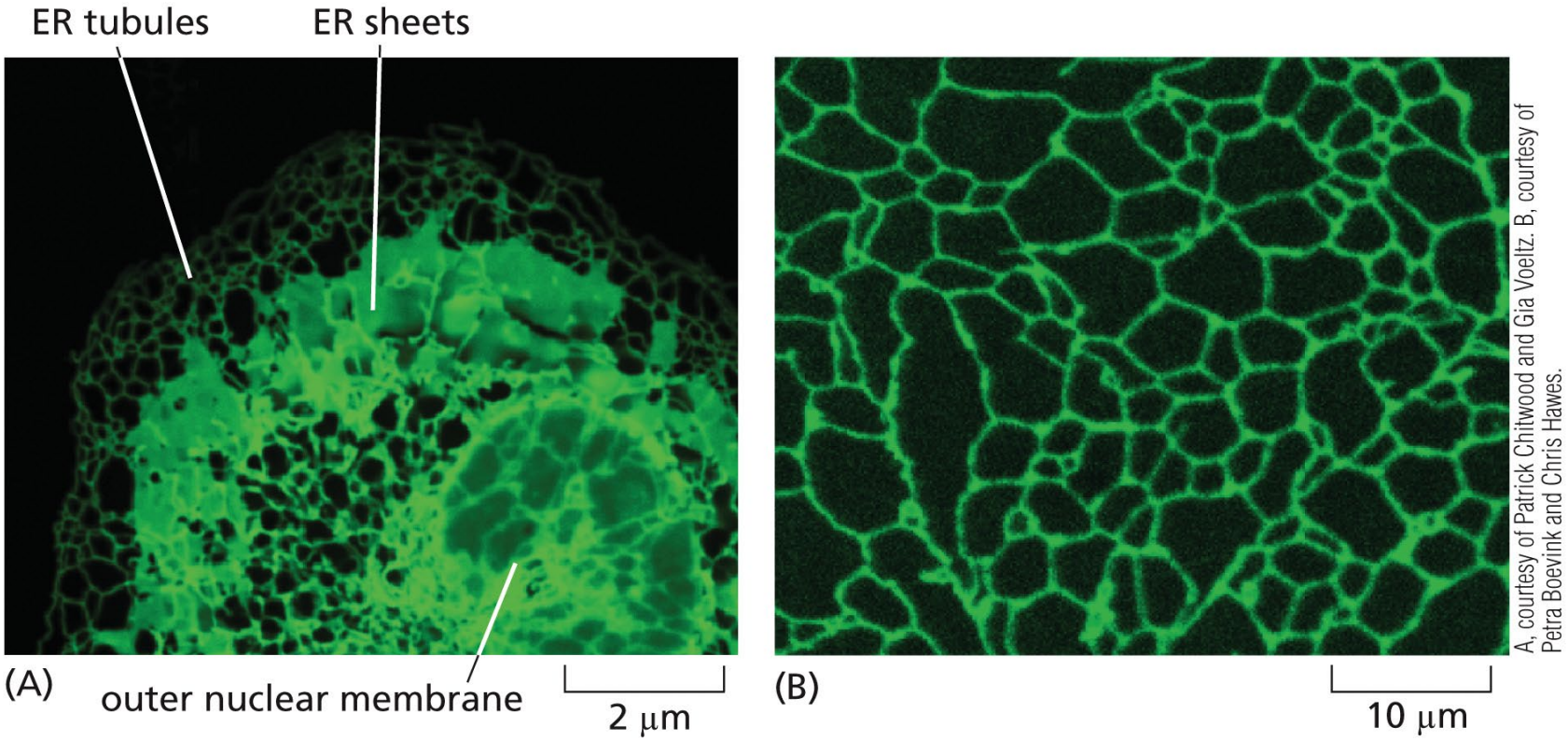
Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

LEARNING OUTCOMES

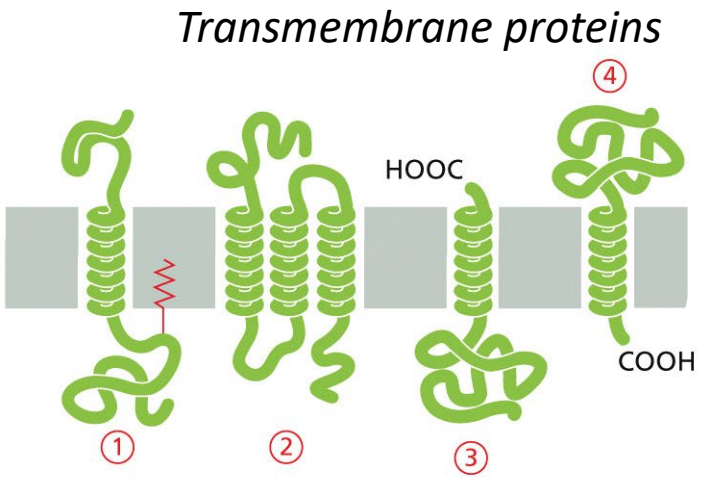
- Can describe the mechanisms of the translocation of transmembrane proteins into ER
- Can understand and described the role of ER on protein folding and processing, along with he molecular level mechanisms, and how it can be applied to control the correct folding of proteins

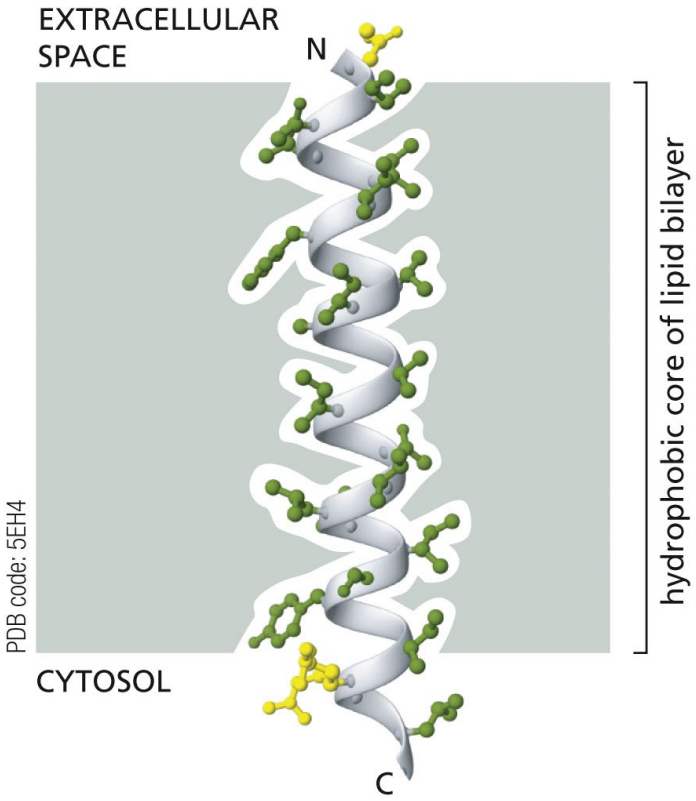
THE ENDOPLASMIC RETICULUM



Transmembrane Proteins

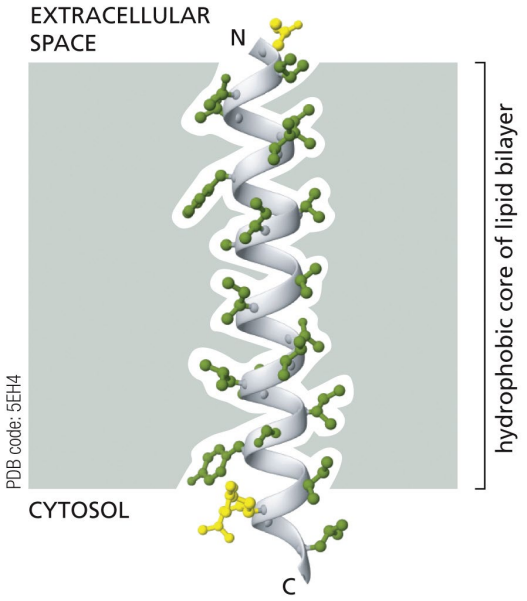
- Membrane-spanning region(~20-30 aa) composed largely of nonpolar side-chains

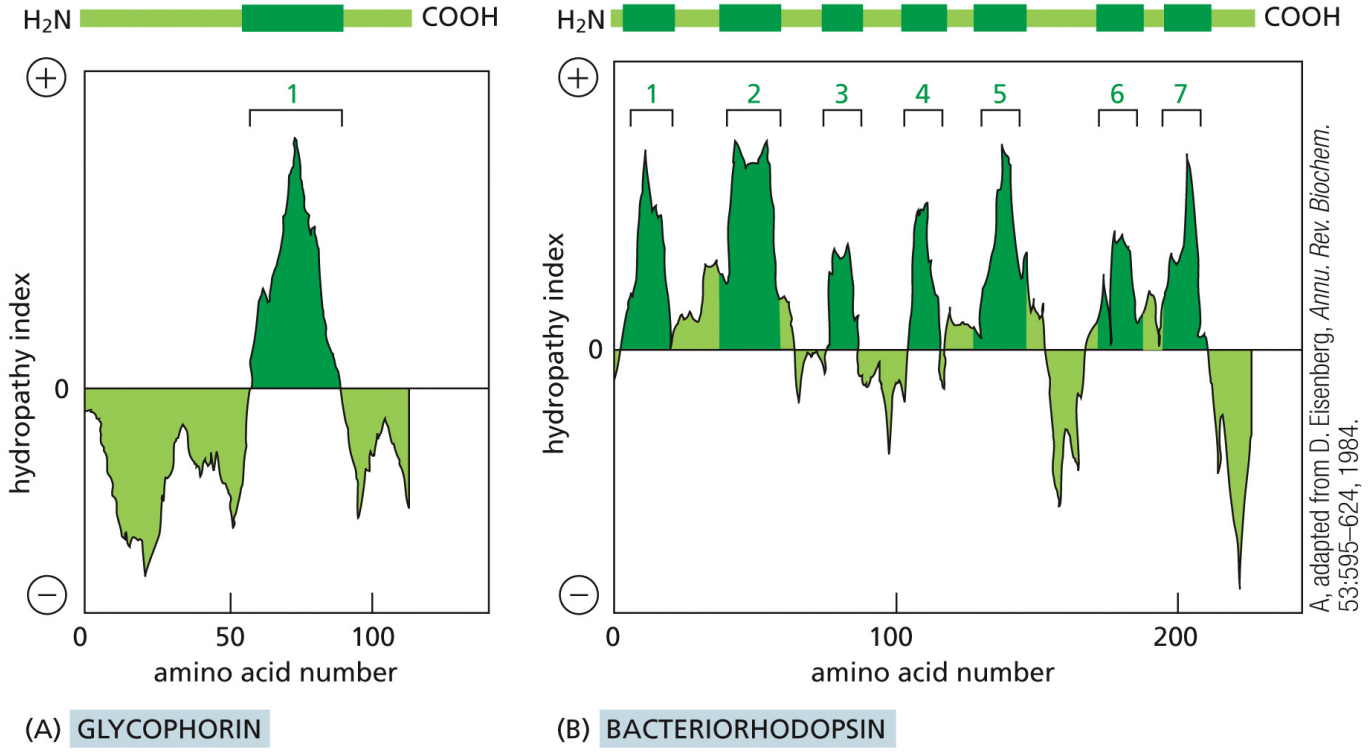




Membrane Proteins

Topology of proteins can be predicted from the primary amino acid sequence.



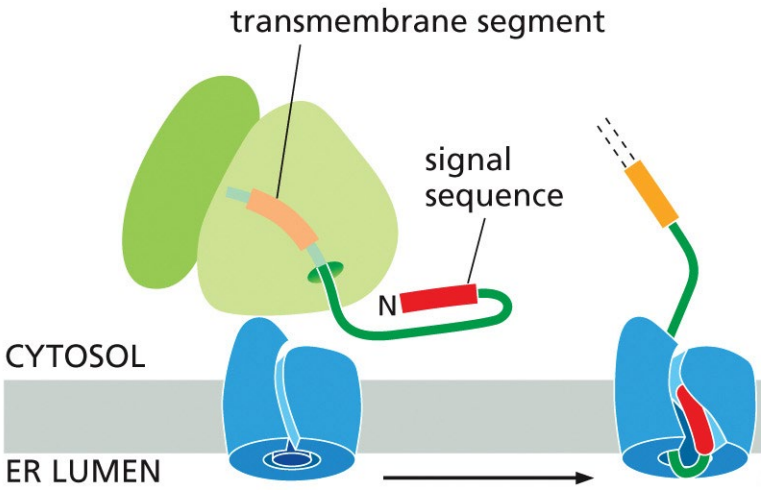


INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- *Three possibilities* to integrate membrane proteins into membranes
- All based on **hydrophobic segments** in transmembrane proteins that are recognized like **signal** sequences
- All (except for those going to mitochondria) inserted into membrane **during translocation to ER lumen**

INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

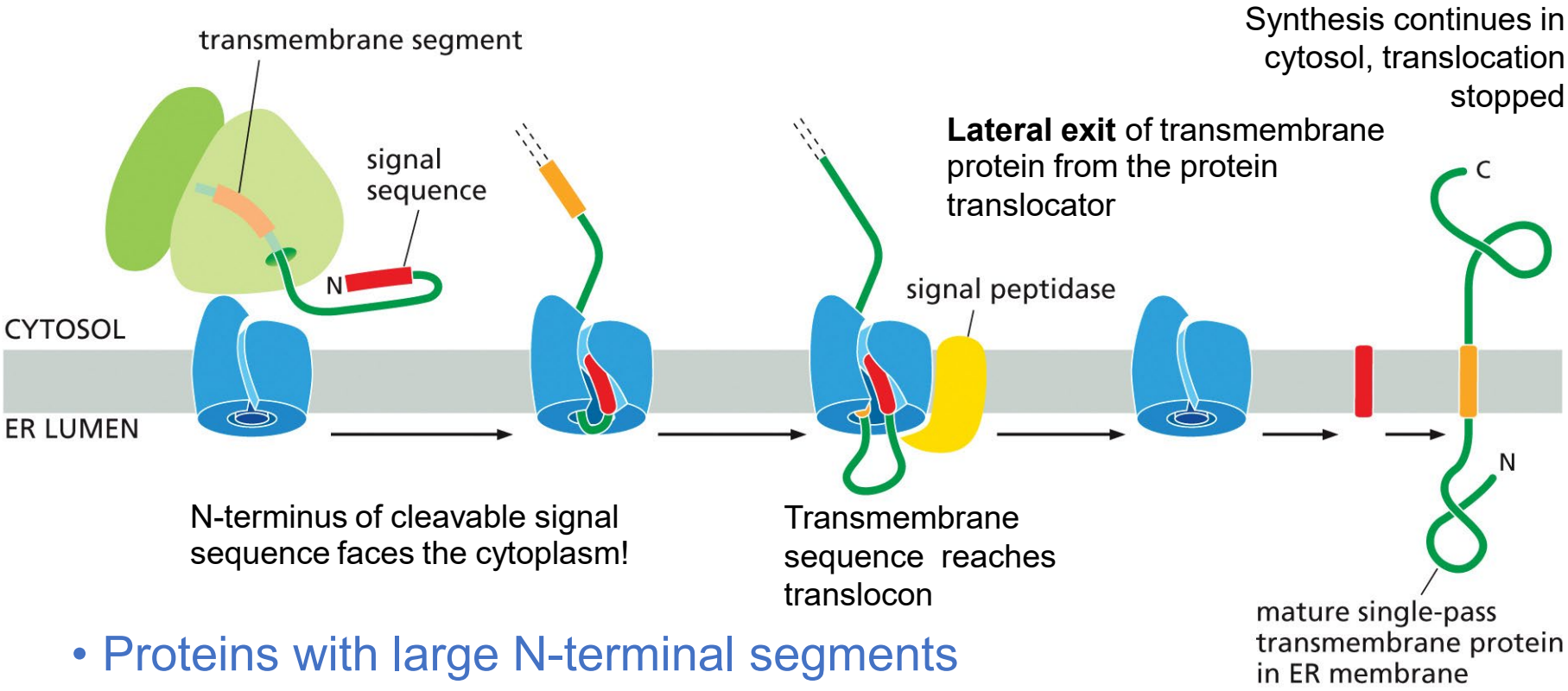
- Strategy 1: *ER signal sequence* + *transmembrane sequence*
- N-terminus in ER lumen (-> N-terminus outside the cell)



N-terminus of cleavable signal sequence faces the cytoplasm!

INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Strategy 1: *ER signal sequence* + *transmembrane sequence*
- N-terminus in ER lumen (-> N-terminus outside the cell)

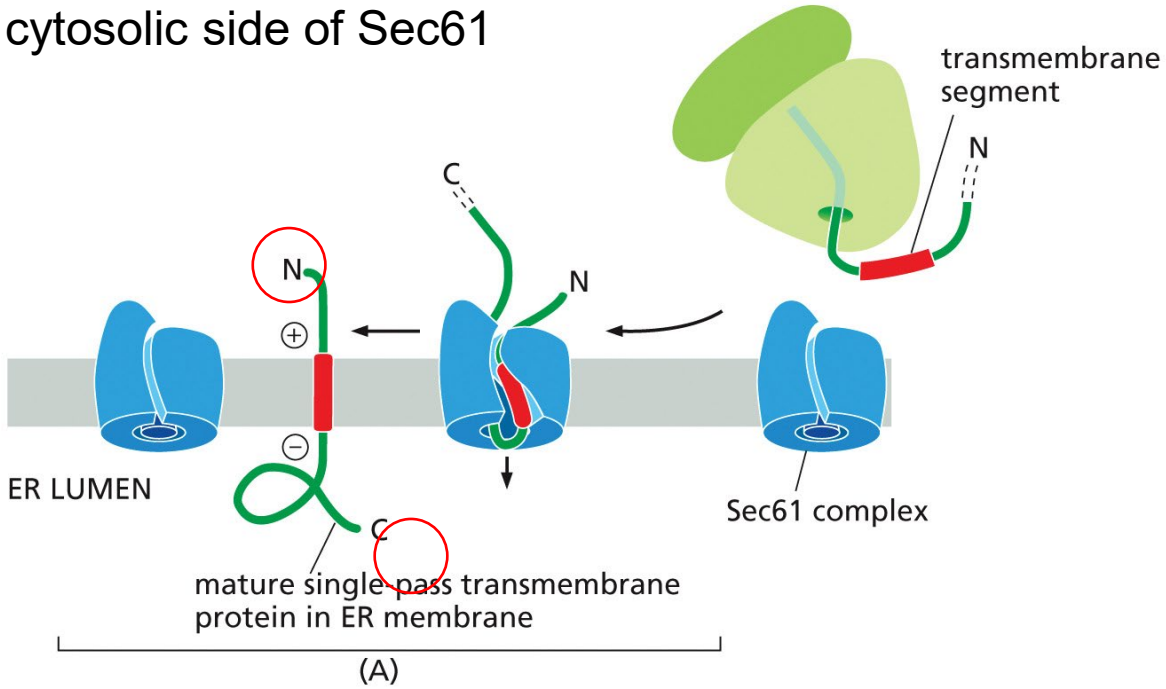


- Proteins with large N-terminal segments

INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Strategies 2A and B: *hydrophobic transmembrane segments are recognized like signal sequences*
- Recognition sequences not in the N-terminus but in the middle

A: *N-terminal* domain is retained on the cytosolic side of Sec61

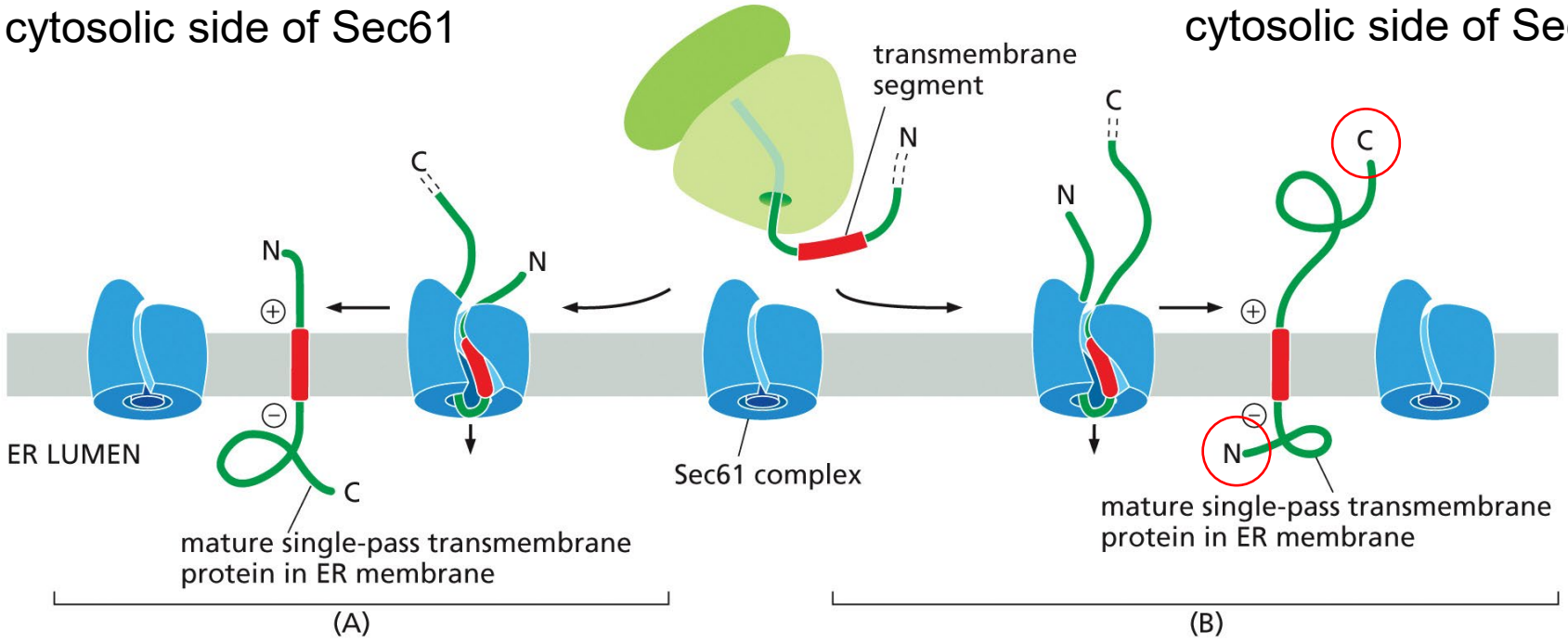


INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Strategies 2A and B: *hydrophobic transmembrane segments are recognized like signal sequences*
- Recognition sequences not in the N-terminus but in the middle

A: *N-terminal* domain is retained on the cytosolic side of Sec61

B: *C-terminal* domain is retained on the cytosolic side of Sec61

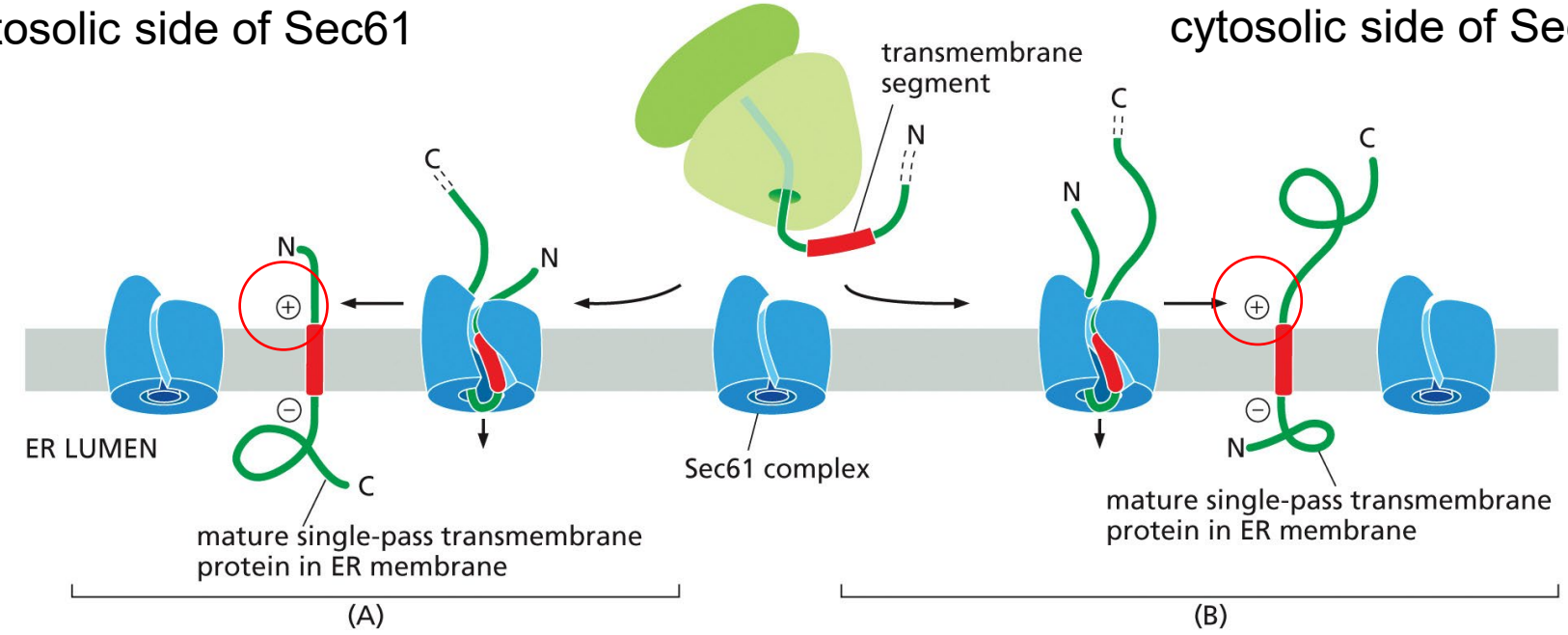


INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Orientation in which the internal transmembrane segment binds the translocation apparatus defines the orientation of the protein

A: *N-terminal* domain is retained on the cytosolic side of Sec61

B: *C-terminal* domain is retained on the cytosolic side of Sec61

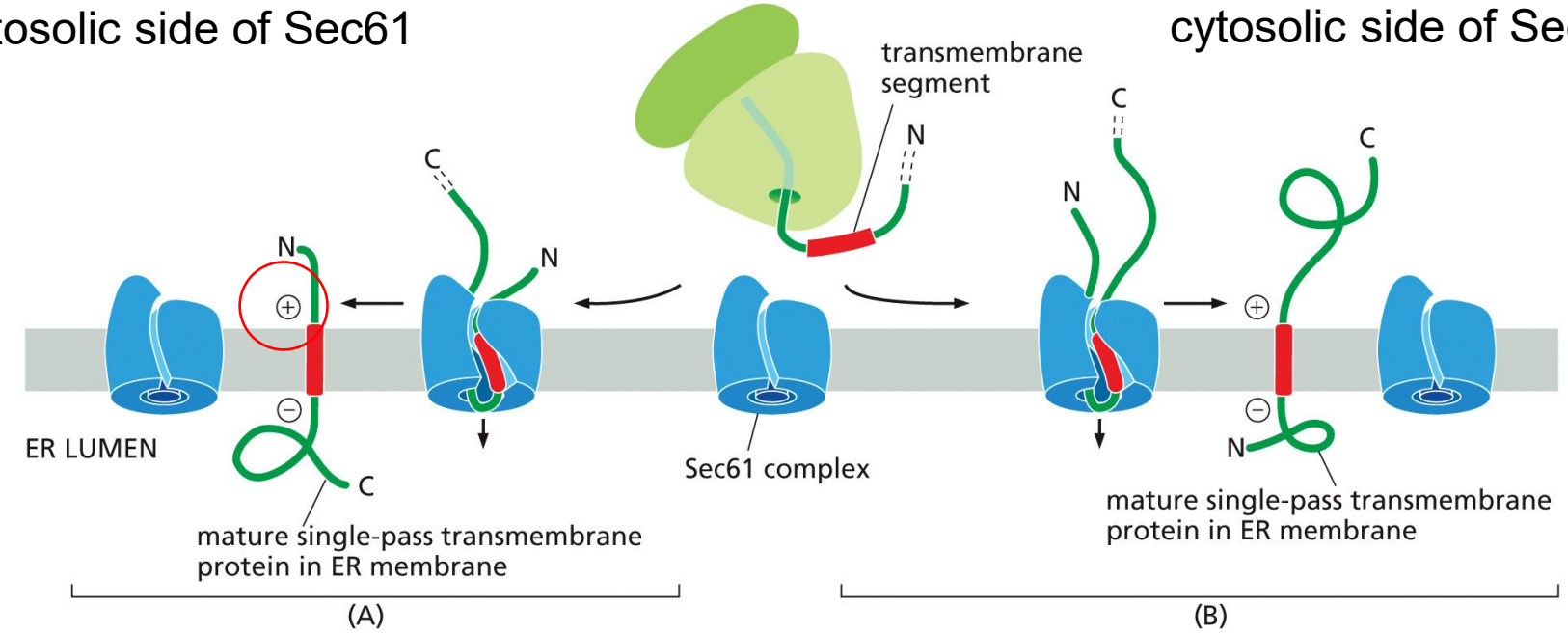


INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Favored for proteins whose N-terminal domains are very long or folded
- Flanking amino acids of transmembrane segments have a net positive charge on the N-terminal side.

A: *N-terminal* domain is retained on the cytosolic side of Sec61

B: *C-terminal* domain is retained on the cytosolic side of Sec61



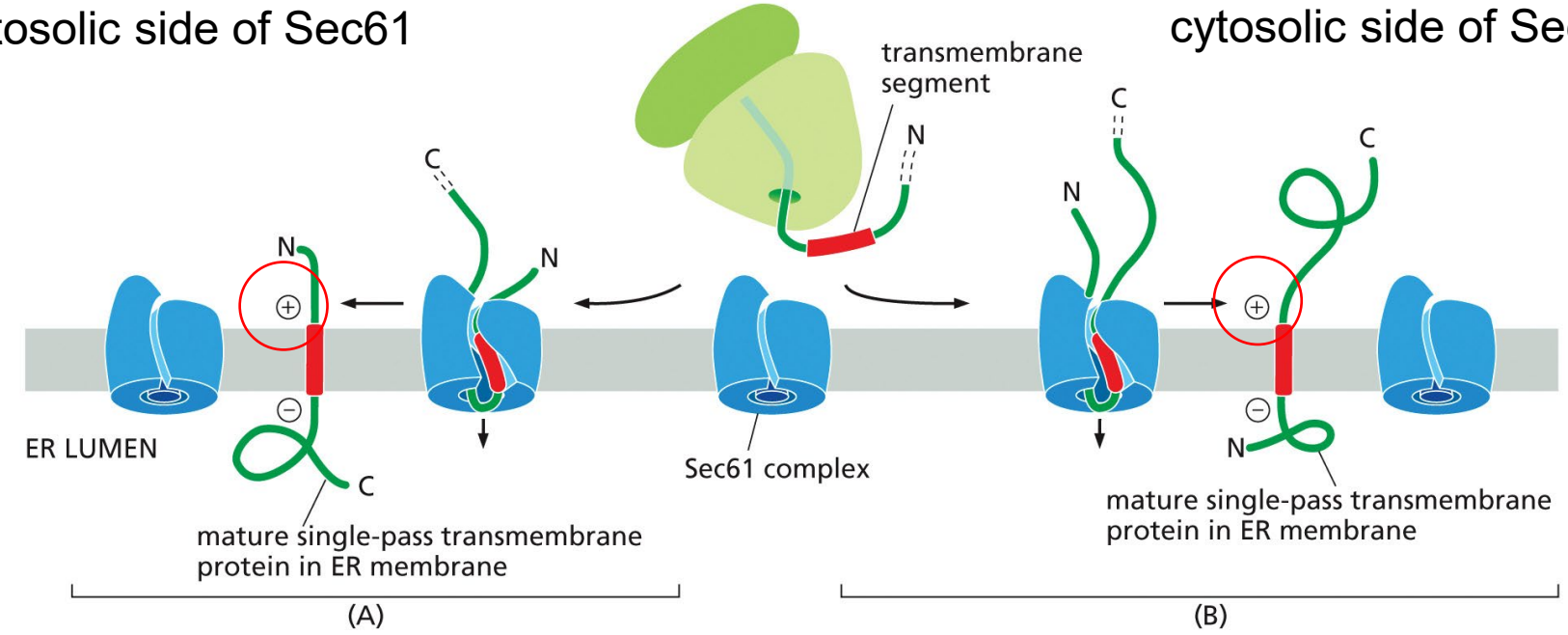
INTEGRATION OF TRANSMEMBRANE PROTEINS INTO MEMBRANES

- Favored for proteins whose N-terminal domains are very long or folded
- Flanking amino acids of transmembrane segments have a net positive charge on the N-terminal side.

- N-terminal flanking region translocates across the membrane through the Sec61 channel
- Favored for transmembrane segments whose flanking amino acids have a net positive charge on the C-terminal side.

A: *N-terminal* domain is retained on the cytosolic side of Sec61

B: *C-terminal* domain is retained on the cytosolic side of Sec61



THE INSERTION OF A MULTIPASS TRANSMEMBRANE PROTEIN INTO THE ER MEMBRANE

- Hydrophobic segments of multipass transmembrane proteins are interpreted contextually to determine their orientation

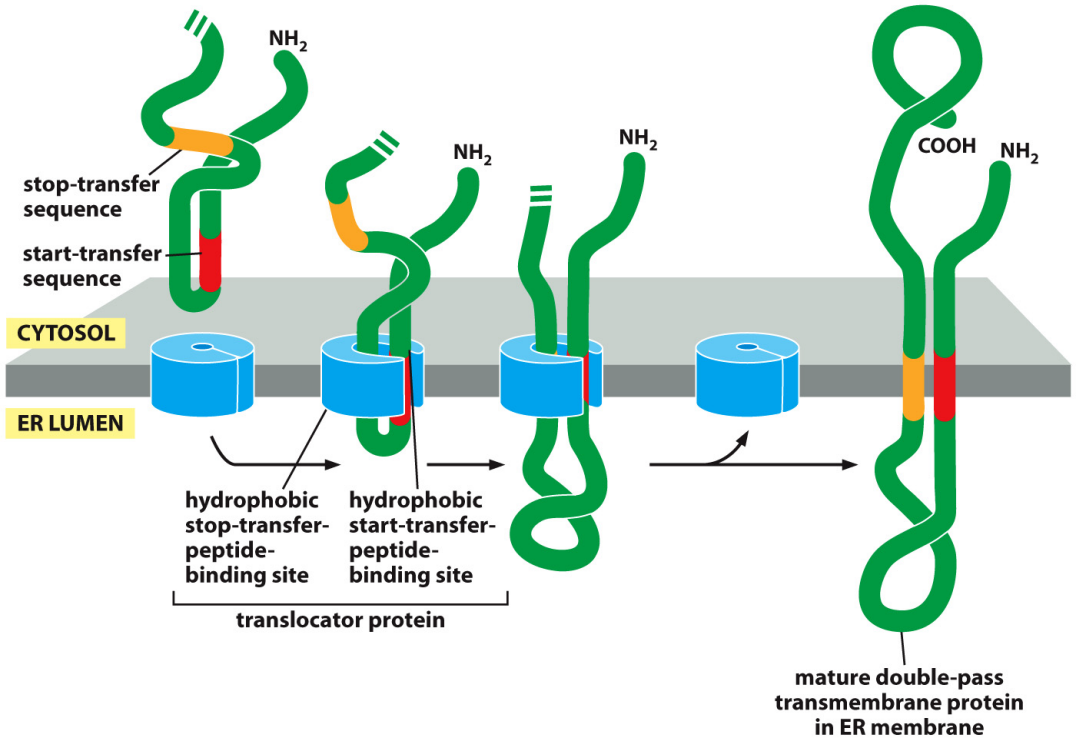
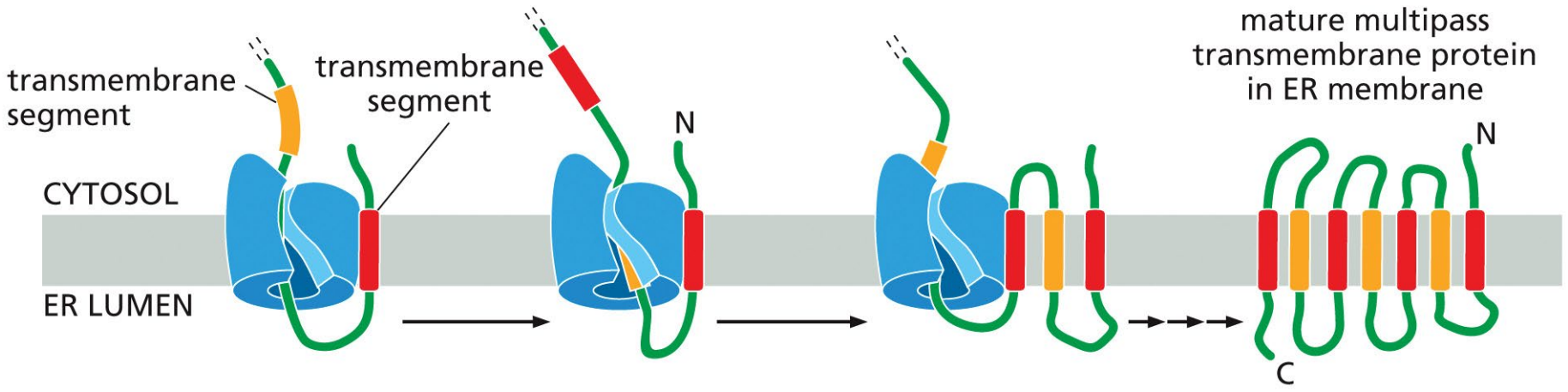


Figure 12-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

THE INSERTION OF A MULTIPASS TRANSMEMBRANE PROTEIN INTO THE ER MEMBRANE

- The orientation of this first transmembrane segment is defined just as for single-pass membrane proteins
- The next transmembrane segment inserts in an orientation opposite to that of the first transmembrane segment
- This proceeds until all transmembrane segments have been inserted into the membrane.



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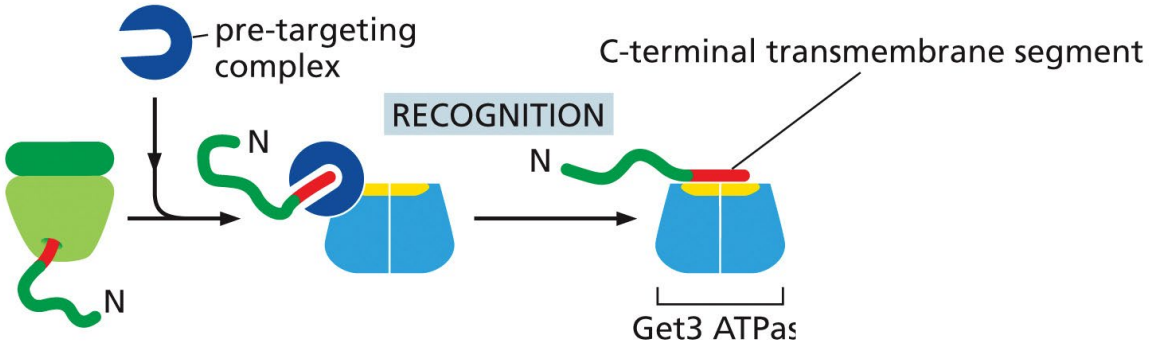
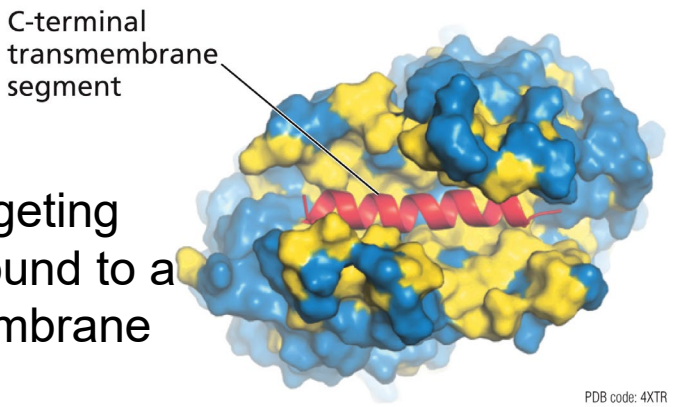
ANCHORING C-TERMINAL TAIL INTO ER MEMBRANE

- Integrated into the ER membrane by a post-translational mechanism
- A soluble pre-targeting complex captures the hydrophobic C-terminal transmembrane segment *(red)* after it emerges from the ribosomal exit tunnel and loads it onto the Get3 targeting factor.

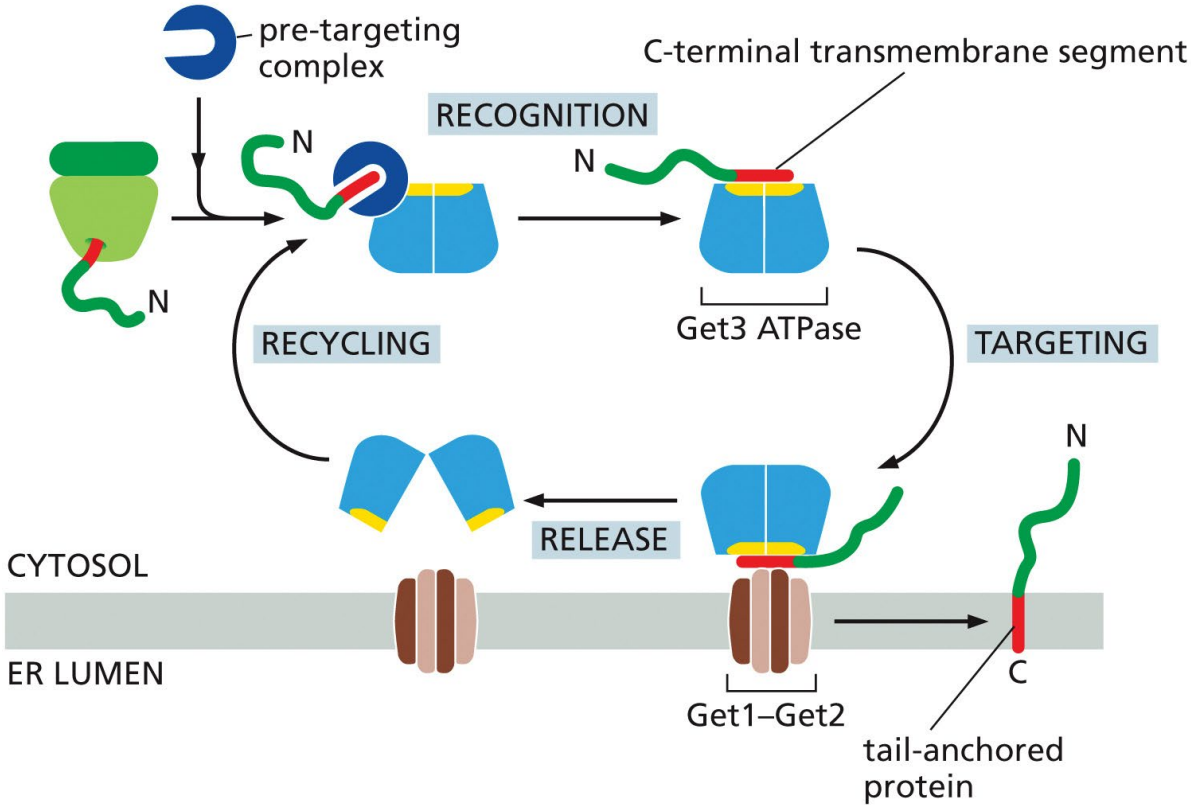




Get3 targeting factor bound to a transmembrane segment

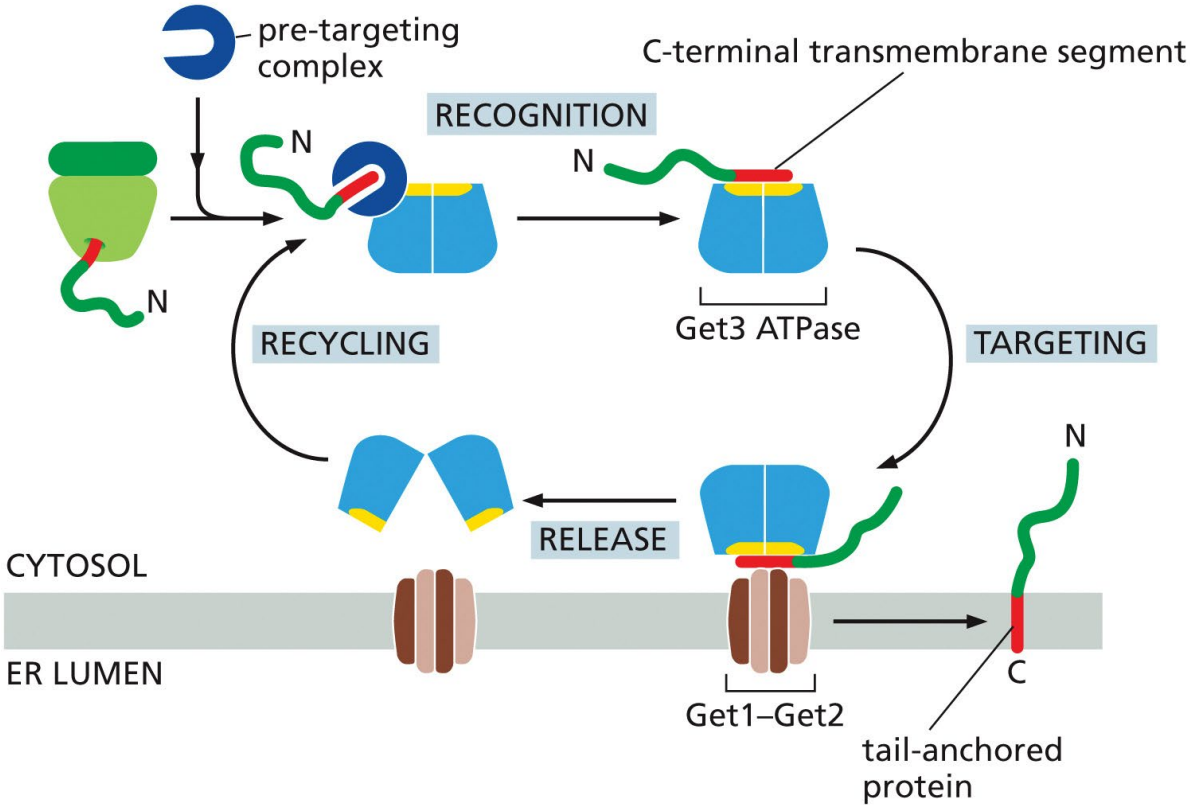
ANCHORING C-TERMINAL TAIL INTO ER MEMBRANE

- The complex is targeted to the ER membrane to Get1–Get2 receptor complex
- Get1–Get2 functions as a membrane protein insertion machine.



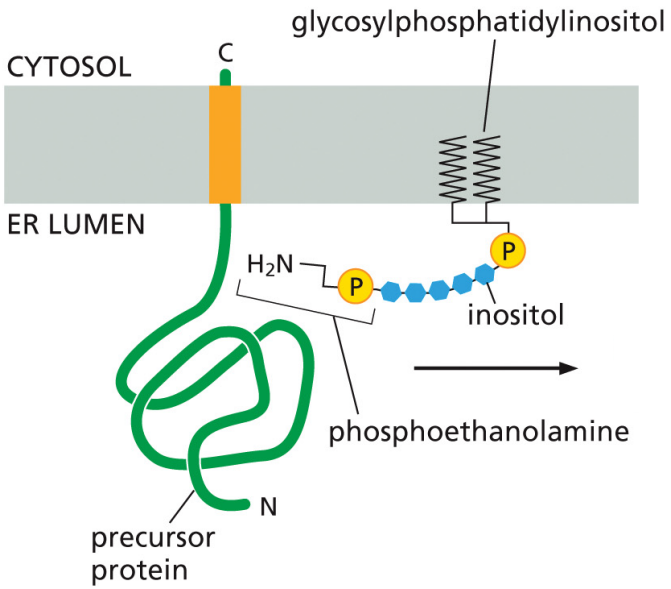
ANCHORING C-TERMINAL TAIL INTO ER MEMBRANE

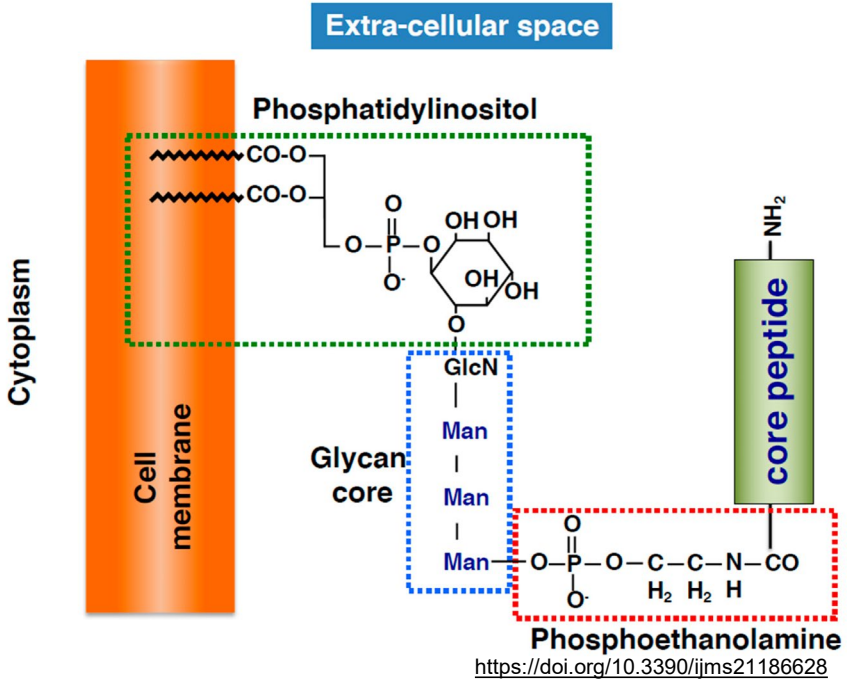
- Get3 is released and recycled back to the cytosol.
- Cycle is conceptually similar to protein targeting by SRP
  - Both Get3 and SRP bind and hydrolyze nucleoside triphosphates to provide directionality to the targeting cycle. ATP is used by Get3, and GTP is used by SRP.



ATTACHMENT WITH GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHOR

- First steps similar to a single-pass transmembrane protein
- After the completion of protein synthesis, the precursor protein remains anchored in the ER membrane by a hydrophobic C-terminal sequence of 15–20 amino acids; the rest of the protein is in the ER lumen.

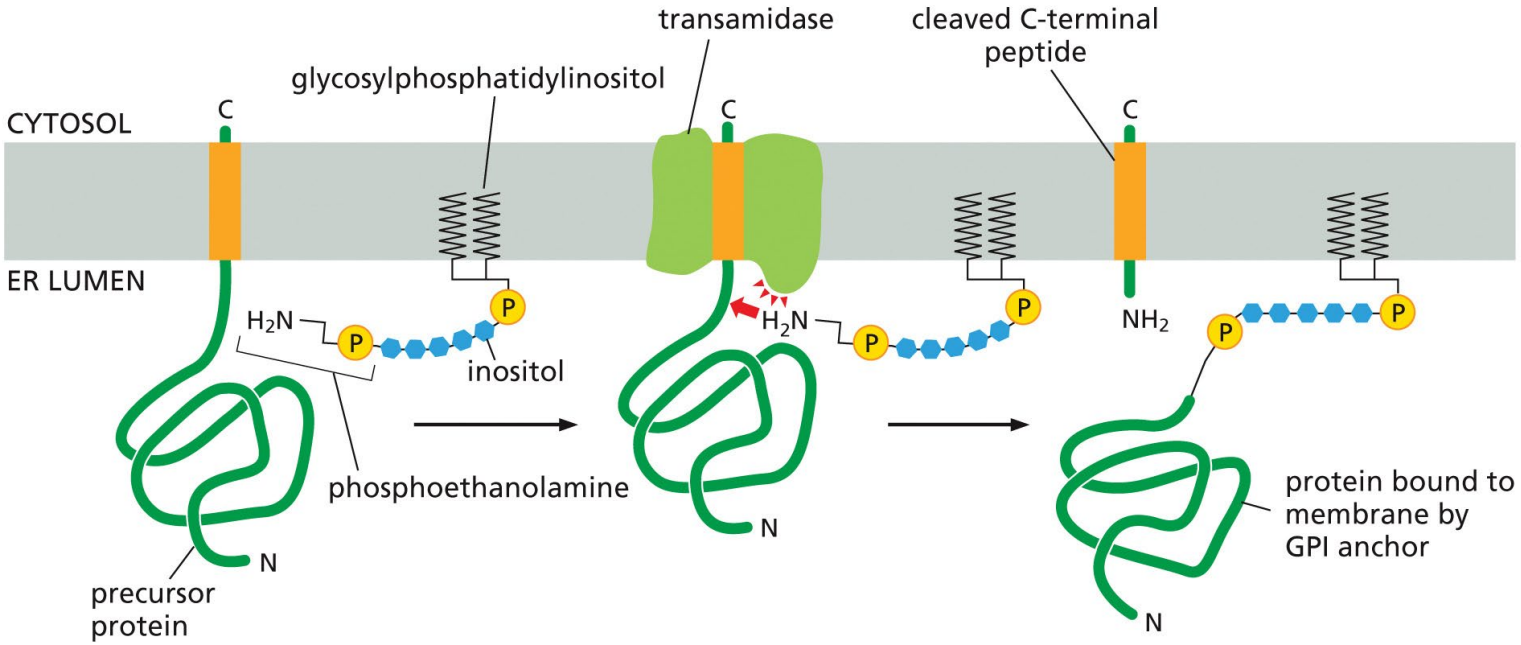




https://doi.org/10.3390/ijms21186628

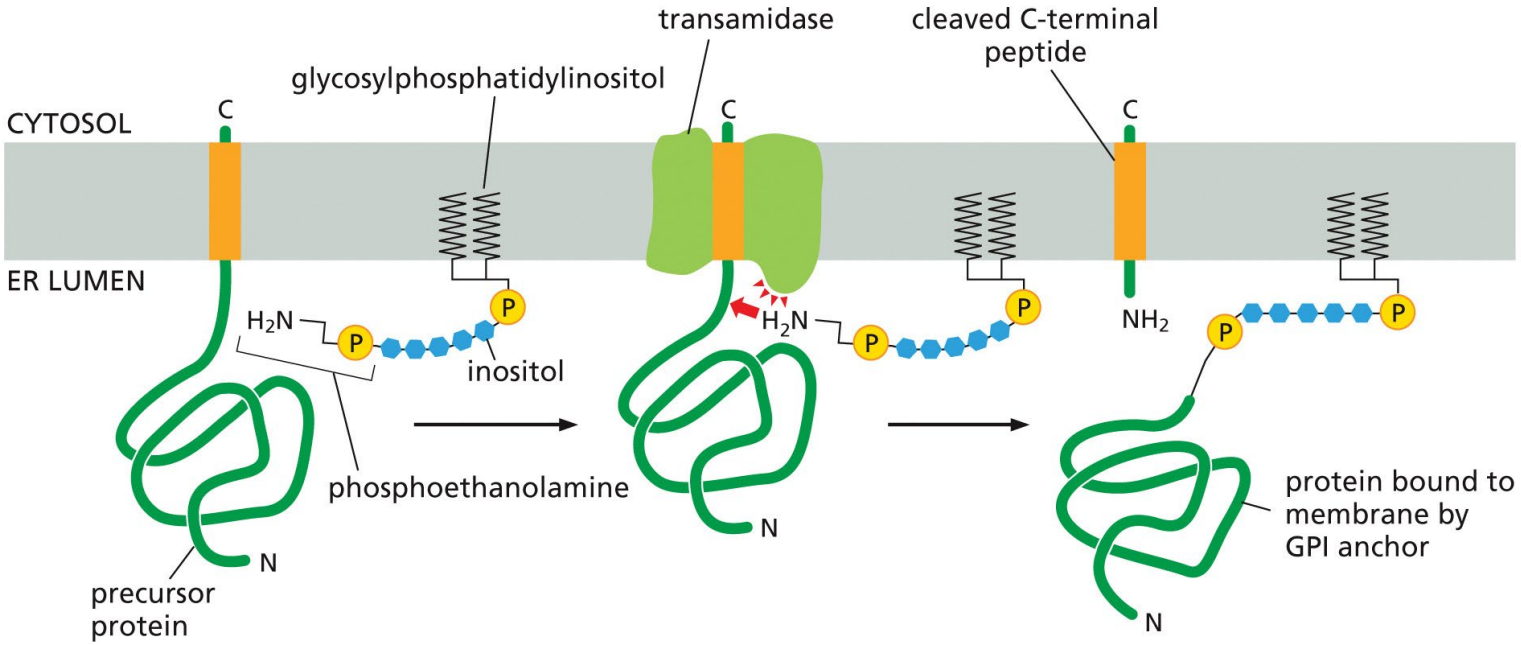
ATTACHMENT WITH GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHOR

- Within less than a minute*, a transamidase enzyme* in the ER cleaves the protein from its membrane-bound C-terminus and simultaneously attaches the new C-terminus to an amino group on a preassembled GPI intermediate



ATTACHMENT WITH GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHOR

- The signal that specifies this modification is contained within the hydrophobic C-terminal sequence and a few amino acids adjacent to it on the lumenal side of the ER membrane; if this signal is added to other proteins, they too become modified in this way.

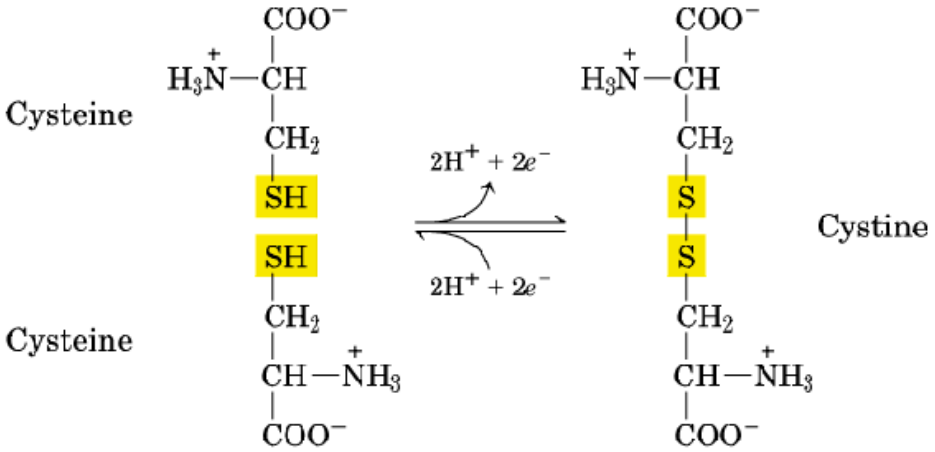


TRANSLOCATED POLYPEPTIDE CHAINS **FOLD AND ASSEMBLE** IN THE LUMEN OF THE ROUGH ER

- ER proteins (=protein that remain in ER), include many that help other proteins to fold
- **Chaperone protein (BiP)** and protein disulfide isomerase (PDI) are important examples
- BiP belongs to hsp70 chaperone family
- Uses ATP to shuttle high affinity – low affinity states
- Prevents, for example, aggregation of β-sheet regions, by binding to non-folded regions

TRANSLOCATED POLYPEPTIDE CHAINS **FOLD AND ASSEMBLE** IN THE LUMEN OF THE ROUGH ER

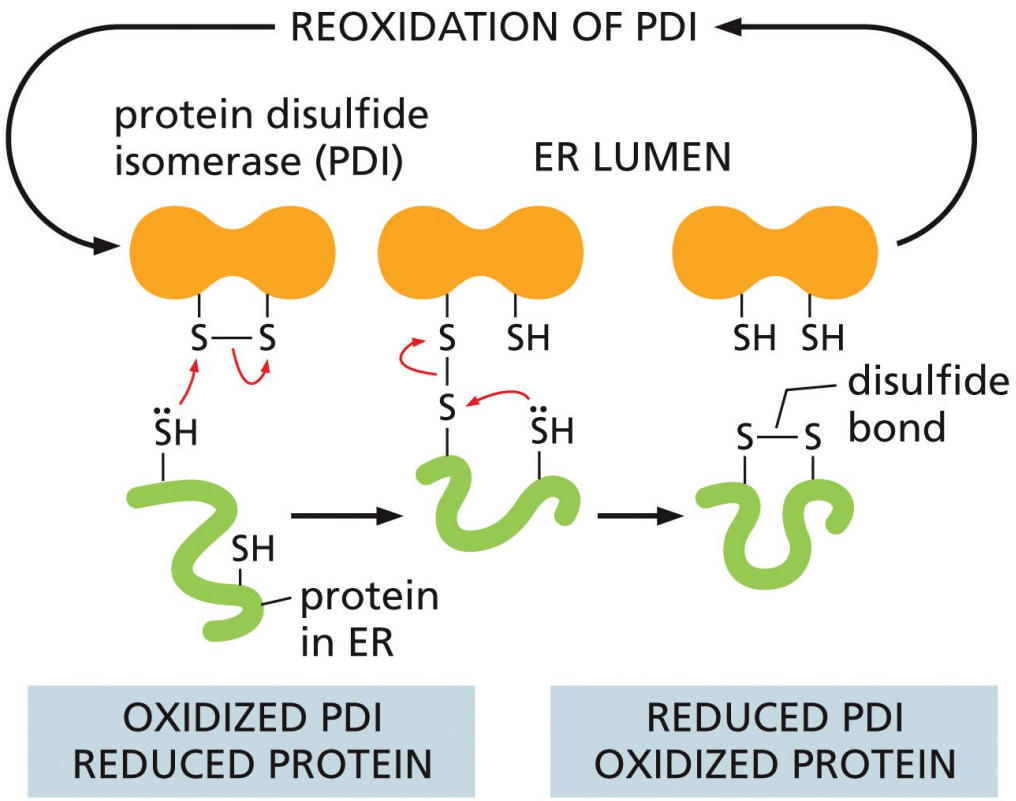
- ER proteins (=protein that remain in ER), include many that help other proteins to fold
- Chaperone protein (BiP) and **protein disulfide isomerase (PDI)** are important examples
- Almost all cysteines in secreted proteins and protein staying in organelles form disulfide bonds
- Free sulfhydryl (SH) groups are oxidized to incorporate disulfide (S—S) bonds during protein folding



PROTEIN DISULFIDE ISOMERASE (PDI)

- PDI contains an intramolecular disulfide bond that accepts electrons from a free sulfhydryl group

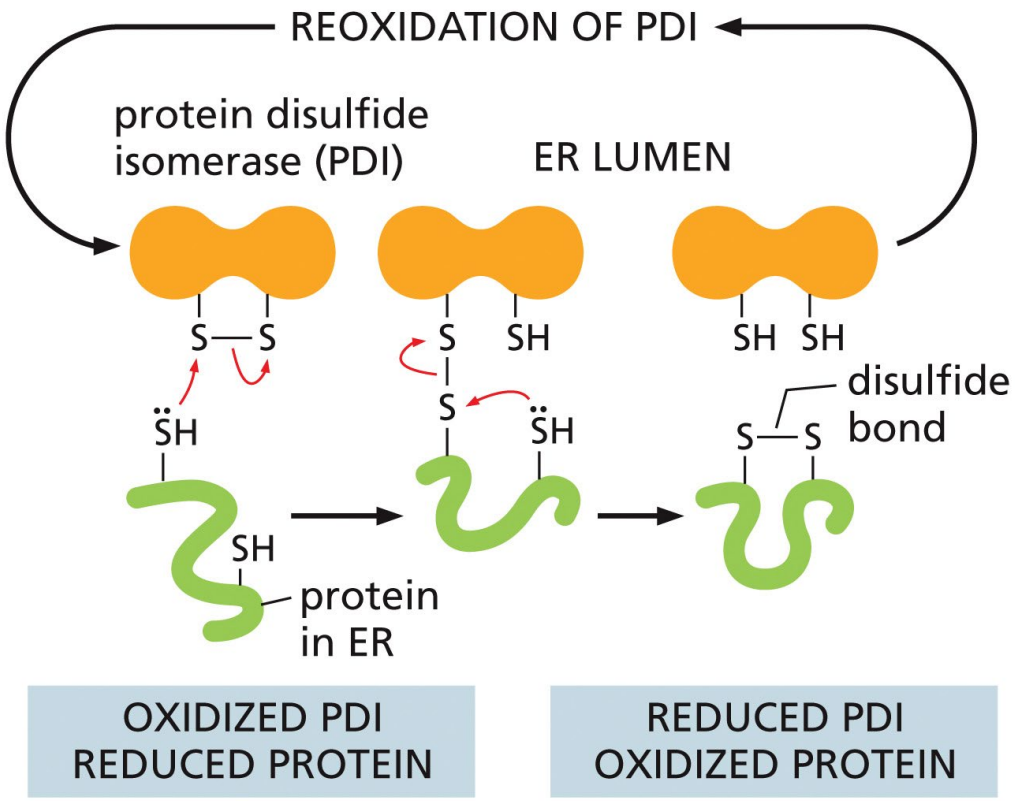
→ An intermolecular mixed disulfide bond between PDI and its substrate.
→ A second free sulfhydryl group in the substrate then donates its electrons to the mixed disulfide bond, resulting in an oxidized substrate and reduced PDI.



PROTEIN DISULFIDE ISOMERASE (PDI)

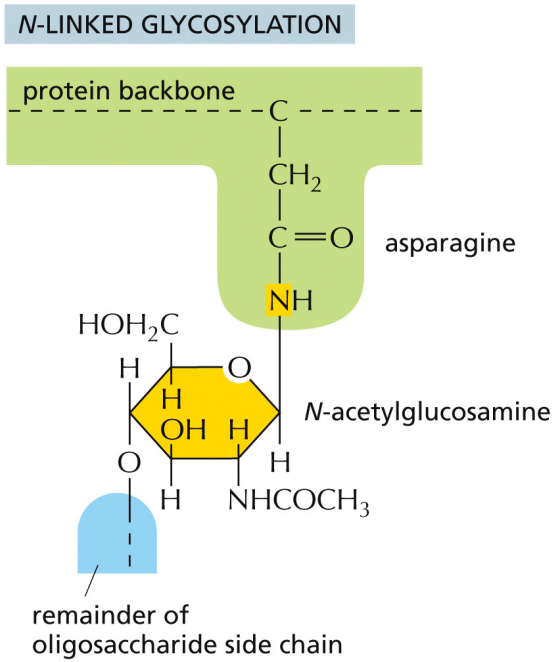
- PDI contains an intramolecular disulfide bond that accepts electrons from a free sulfhydryl group

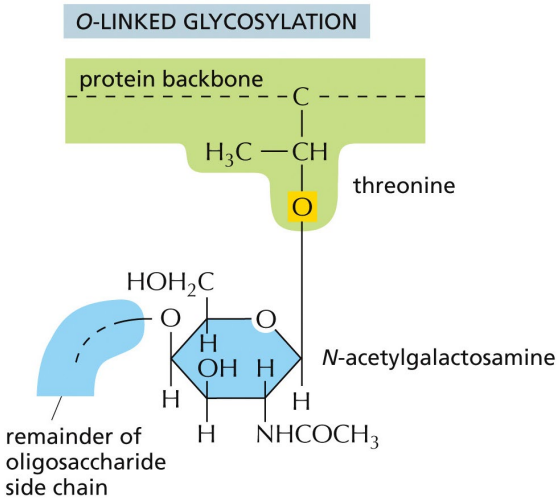
Reoxidation of PDI is carried out by other ER enzymes



N- AND O-LINKED GLYCOSYLATION

- N-linked glycosylation
  - 90% glycosylated proteins
  - Asn-X-Ser/Thr
  - Core glycan added in ER and modified in ER and Golgi to add diversity
- O-linked glycosylation
  - Ser or Thr or Hydroxylysine
  - In Golgi





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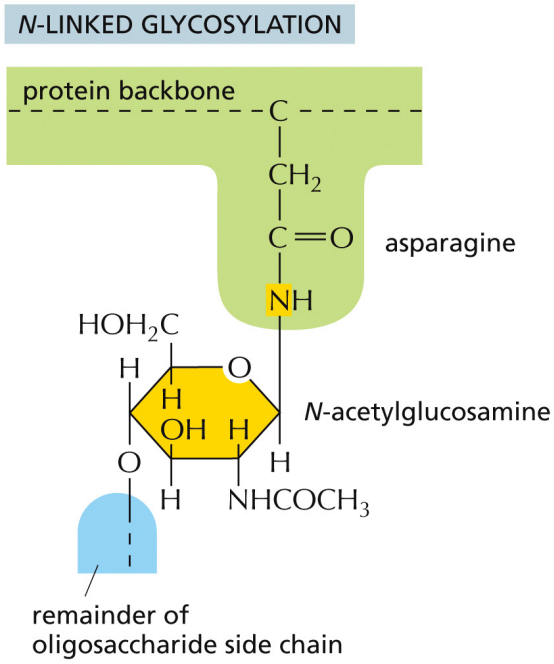
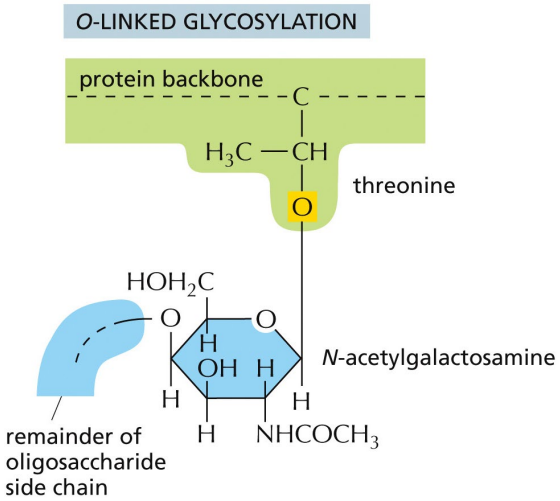
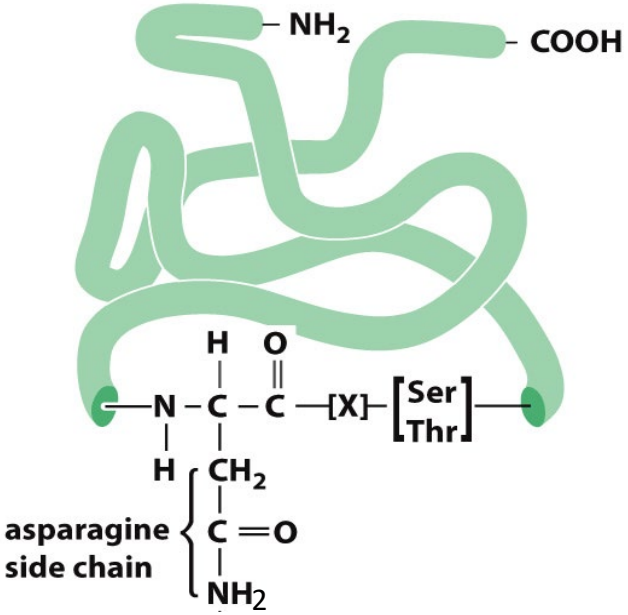




N-GLYCOSYLATION

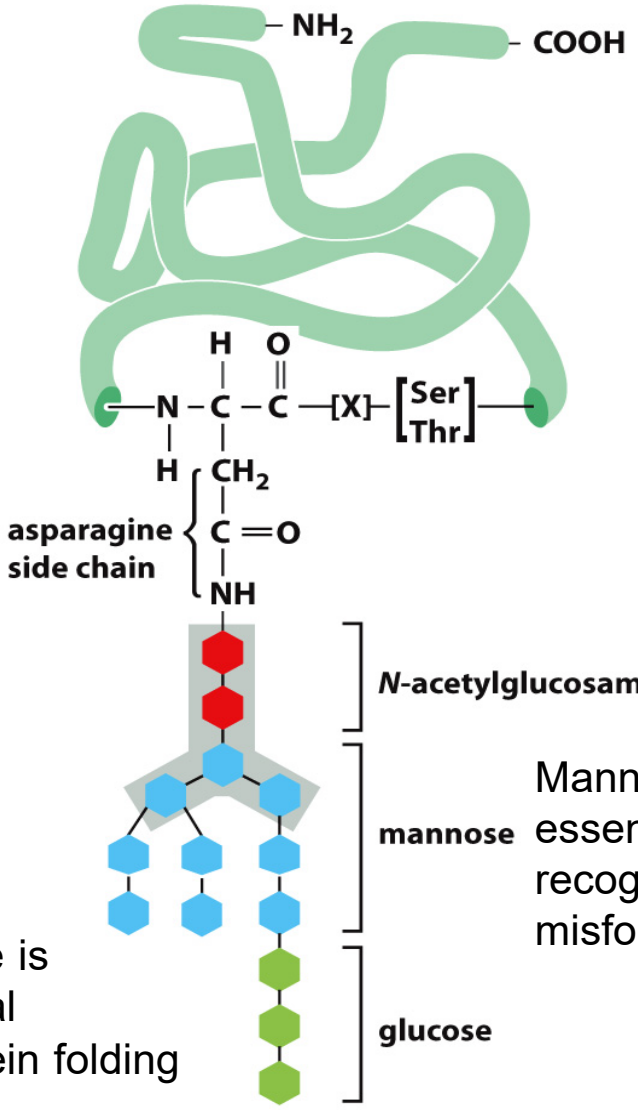
- Most proteins synthesized in the rough ER are glycosylated by the addition of a common *N-linked oligosaccharide*
- **N-linked glycosylation** = glycosylation of asparagine amino acids in the sequences **Asn-X-Ser** and **Asn-X-Thr** (where X is any amino acid except proline)

- Glycosylation at inappropriate sites would interfere with protein folding – in glycoproteins selective pressure against glycosylation sites (that are not needed)



N-GLYCOSYLATION

- Precursor oligosaccharide added in a typical case of N-glycosylation
- 14 sugars: N-acetylglucosamine + mannose + glucose
- Extensive oligosaccharide trimming takes place in the Golgi apparatus
- For many glycoproteins, only the core sugars survive (the five sugars in the *gray box*)



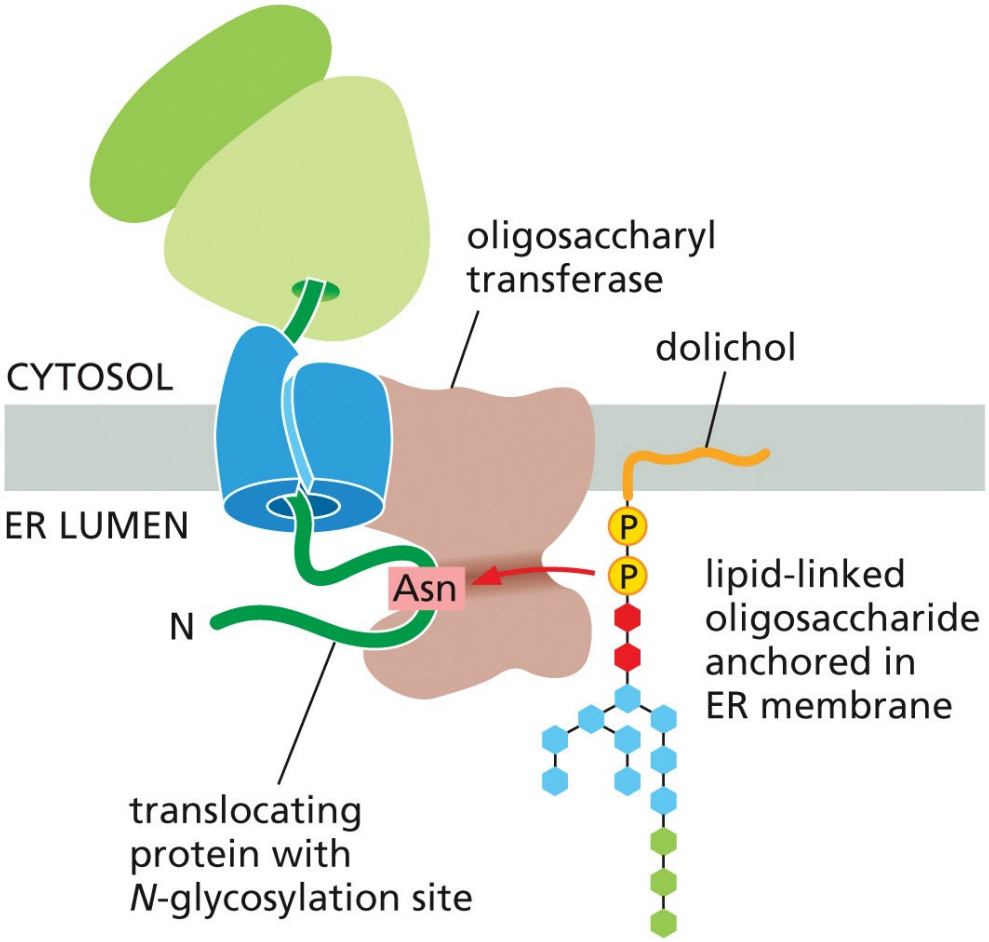
Mannose is essential for recognition of misfolded proteins

Glucose is essential for protein folding

Figure 12-47a Molecular Biology of the Cell 6e (© Garland Science 2015)

N-GLYCOSYLATION

- A transmembrane **oligosaccharyl transferase enzyme complex** catalyzes N-glycosylation
- One copy of this enzyme is *associated with each protein translocator* in the ER membrane
- N-glycosylation happens when protein is translocated to ER



OLIGOSACCHARYL TRANSFERASE

- Contains 13 transmembrane a helices and a large ER lumenal domain
- ER luminal domain contains **binding sites** for the **nascent protein** and **dolichol–oligosaccharide**.

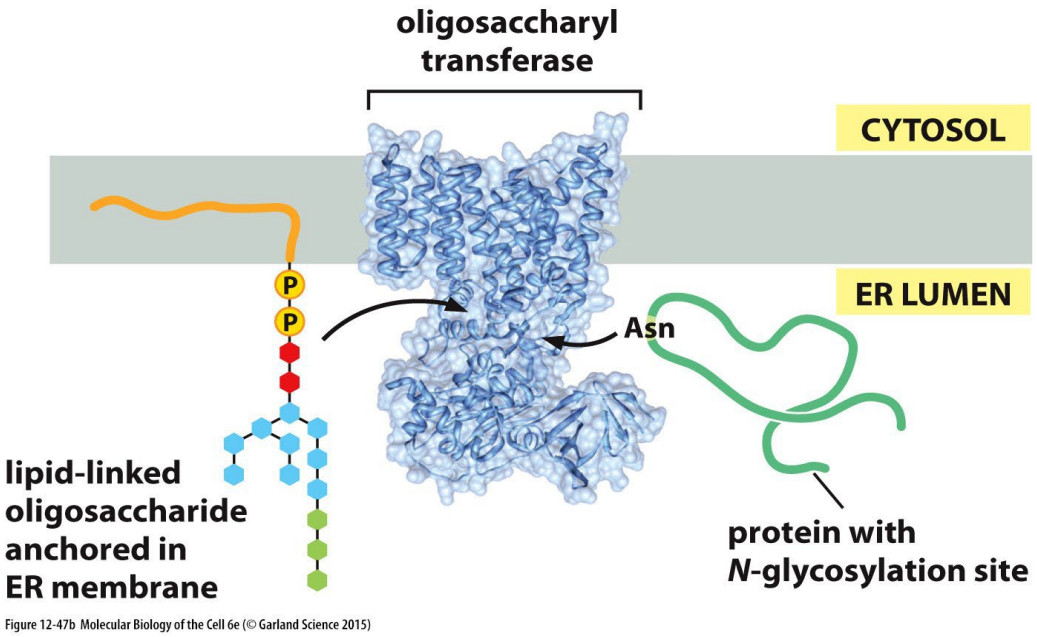
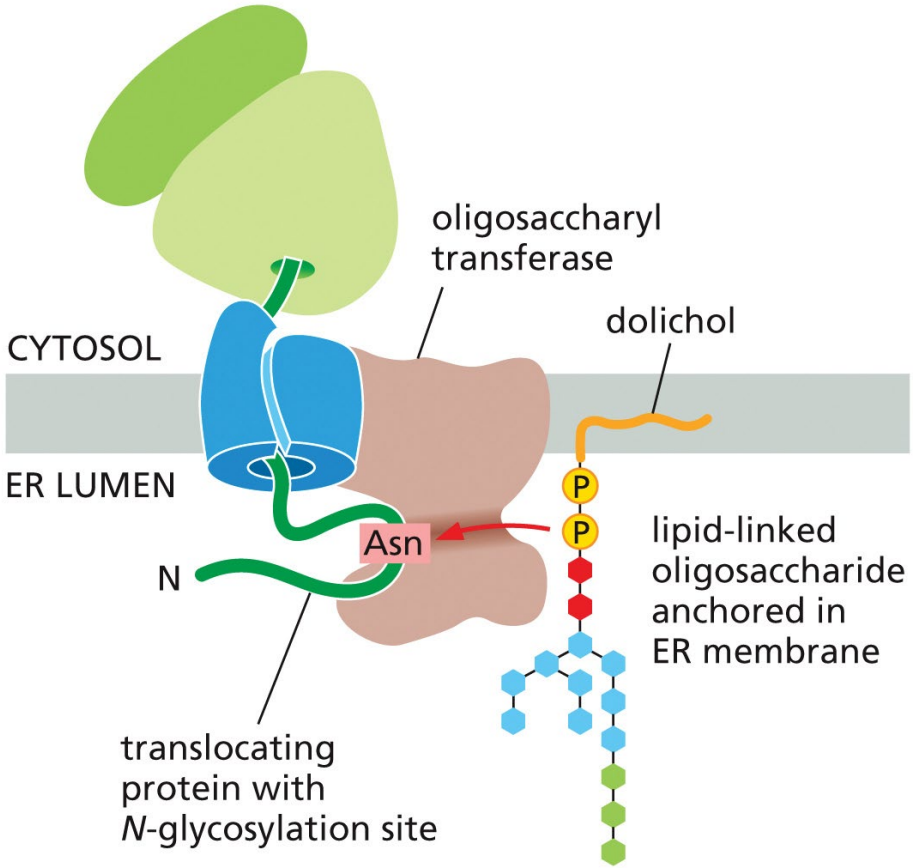


Figure 12-47b Molecular Biology of the Cell 6e (© Garland Science 2015)

N-GLYCOSYLATION

- The **asparagine binds a tunnel** that penetrates the enzyme interior
- The *amino group of the asparagine* is twisted out of the plane that stabilizes the otherwise poorly reactive amide bond, *activating* it for reaction with the dolichol–oligosaccharide.
- The precursor **oligosaccharide is transferred** from a dolichol lipid anchor **to the asparagine**

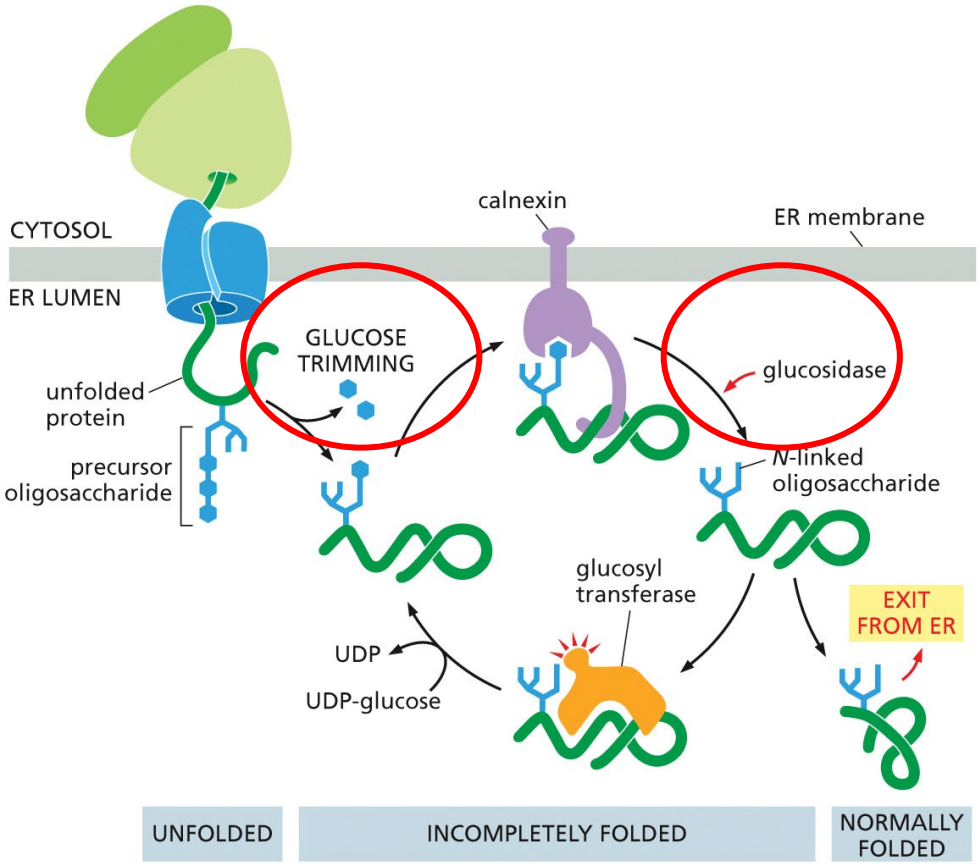


CONTROL FOR UNFOLDED/MISFOLDED PROTEINS

- Oligosaccharides are used as tags to mark the state of protein folding
  - Glucoses: folding state
  - Mannoses: timer
- Unfolded protein response
  - Three sensors: IRE1, PERK, and ATF6

OLIGOSACCHARIDES AS TAGS FOR PROTEIN FOLDING STATE

- Glucosidase removes glucoses from the precursor oligosaccharide



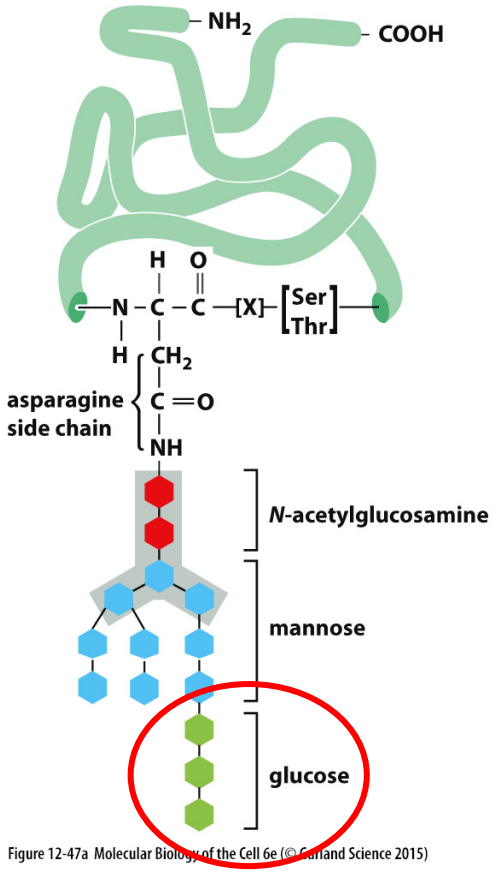
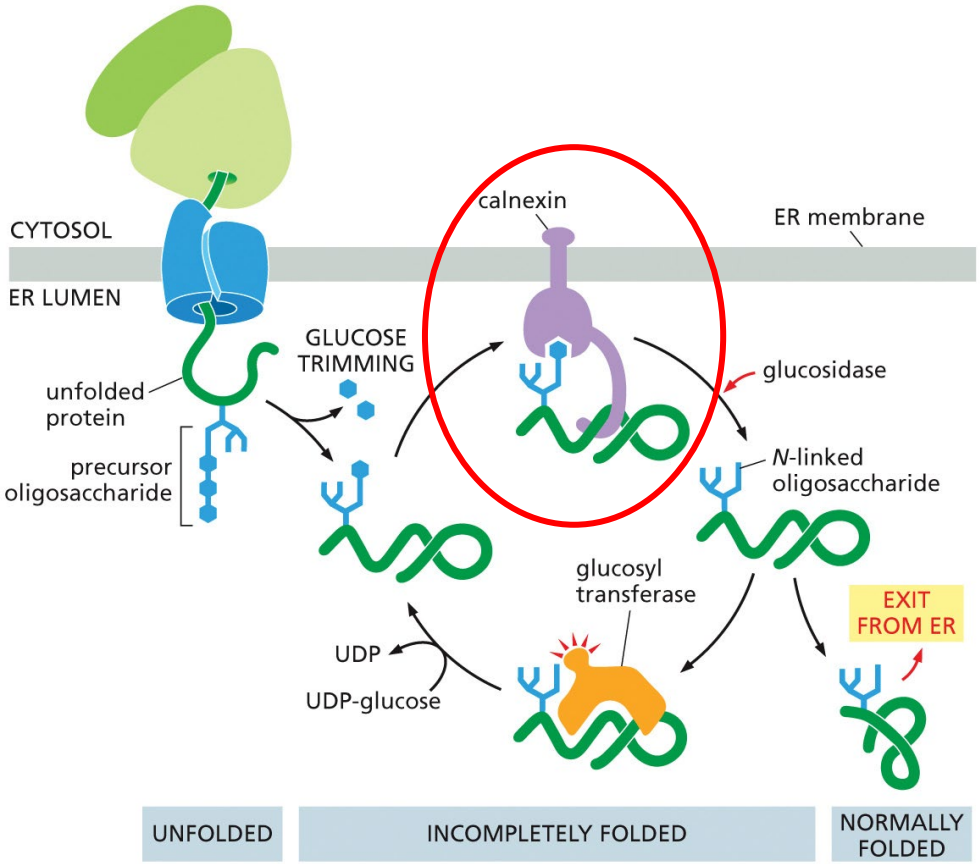


Figure 12-47a Molecular Biology of the Cell 6e (© Garland Science 2015)

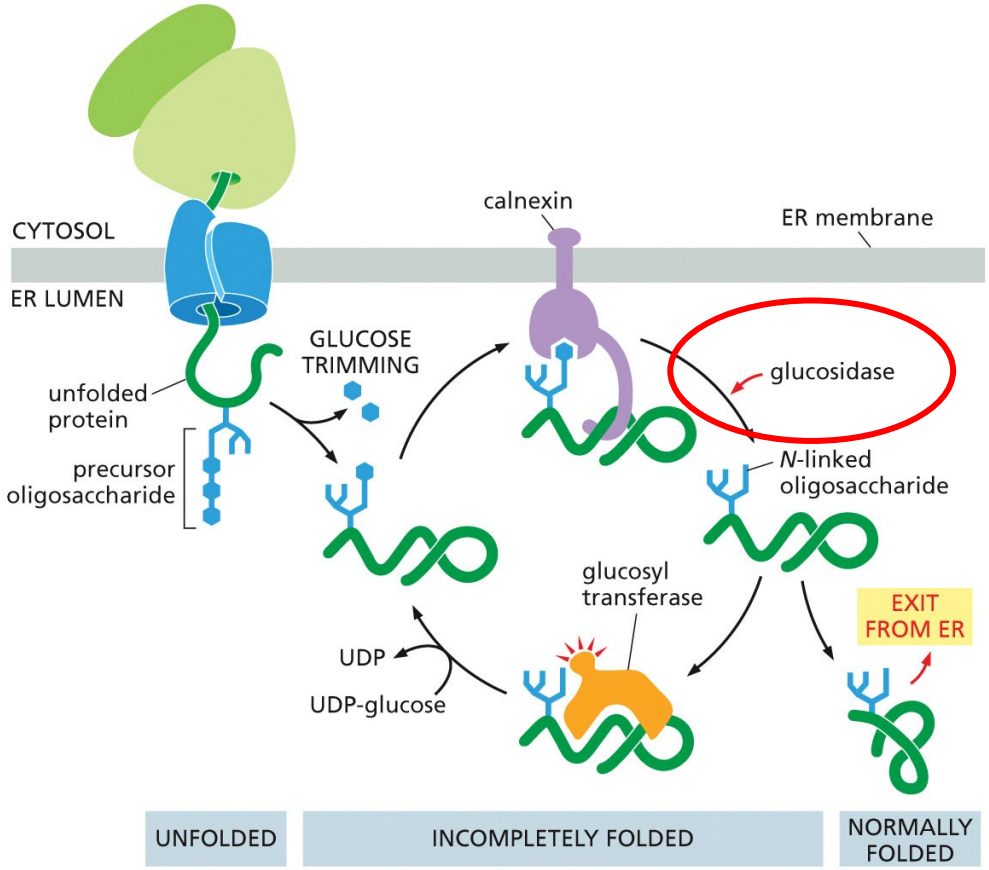
OLIGOSACCHARIDES AS TAGS FOR PROTEIN FOLDING STATE

- Membrane-bound chaperon **calnexin** recognizes unfolded proteins with *one terminal glucose* on *N*-linked oligosaccharides, **trapping the protein in the ER**



OLIGOSACCHARIDES AS TAGS FOR PROTEIN FOLDING STATE

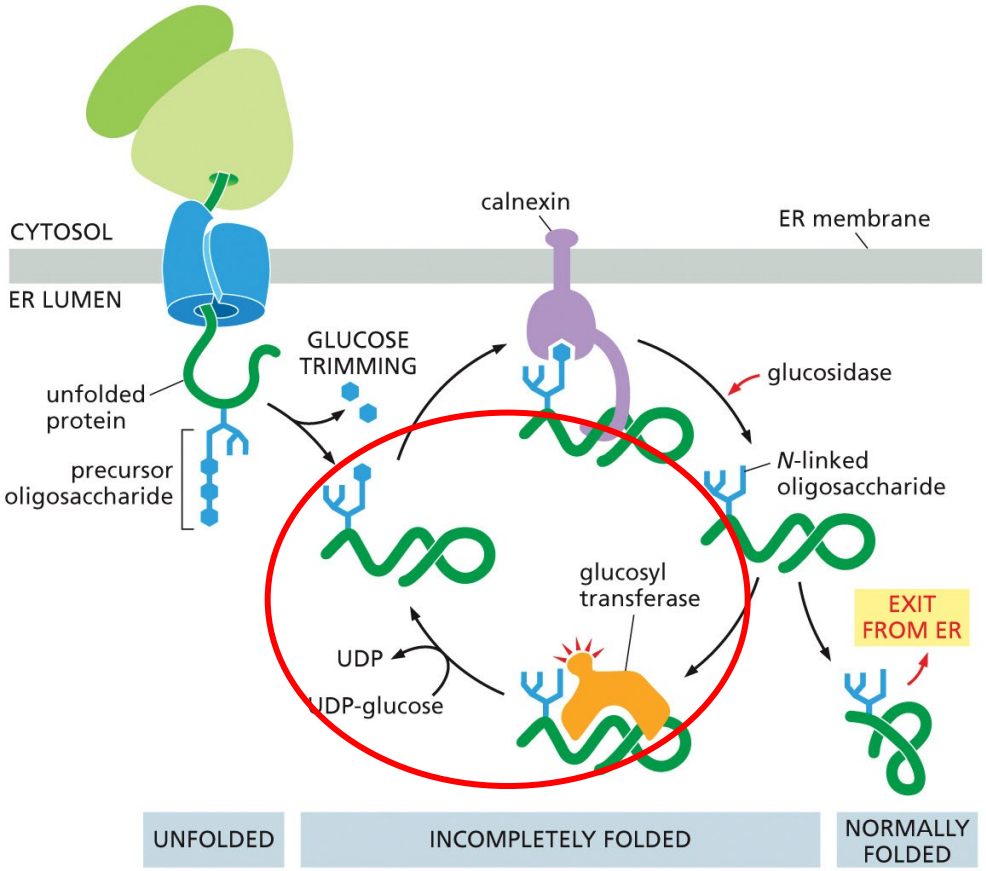
- Removal of the terminal glucose by a glucosidase releases the protein from calnexin



- If the protein folds, it can exit ER

OLIGOSACCHARIDES AS TAGS FOR PROTEIN FOLDING STATE

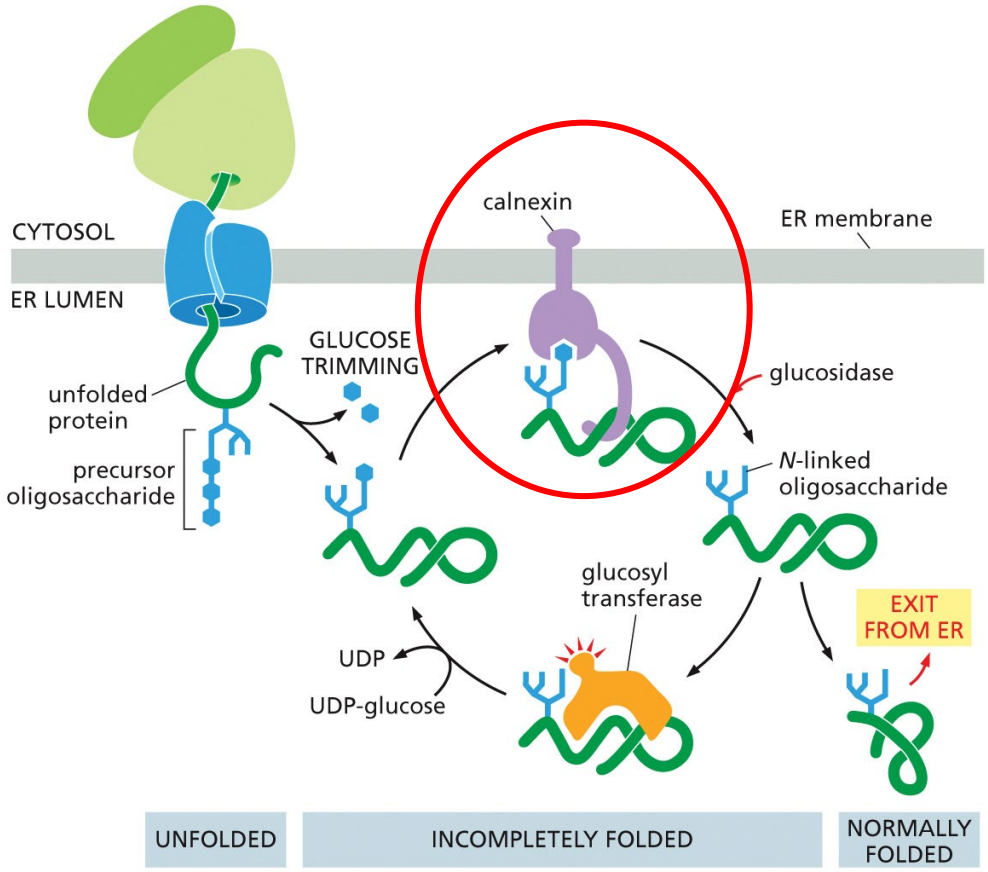
- **A glucosyl transferase** *determines whether the protein is folded properly or not*



- If the protein is still **incompletely folded**, the **enzyme transfers a new glucose** from UDP-glucose to the N-linked oligosaccharide

OLIGOSACCHARIDES AS TAGS FOR PROTEIN FOLDING STATE

- Protein bound (again) by calnexin and retained it in the ER (new cycle)



EXPORT OF IMPROPERLY FOLDED PROTEINS

- Improperly folded proteins are exported from the ER and degraded in the cytosol

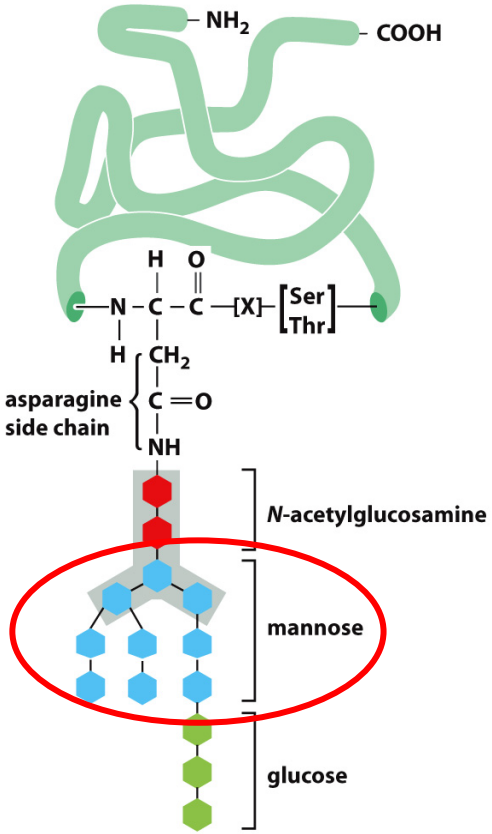


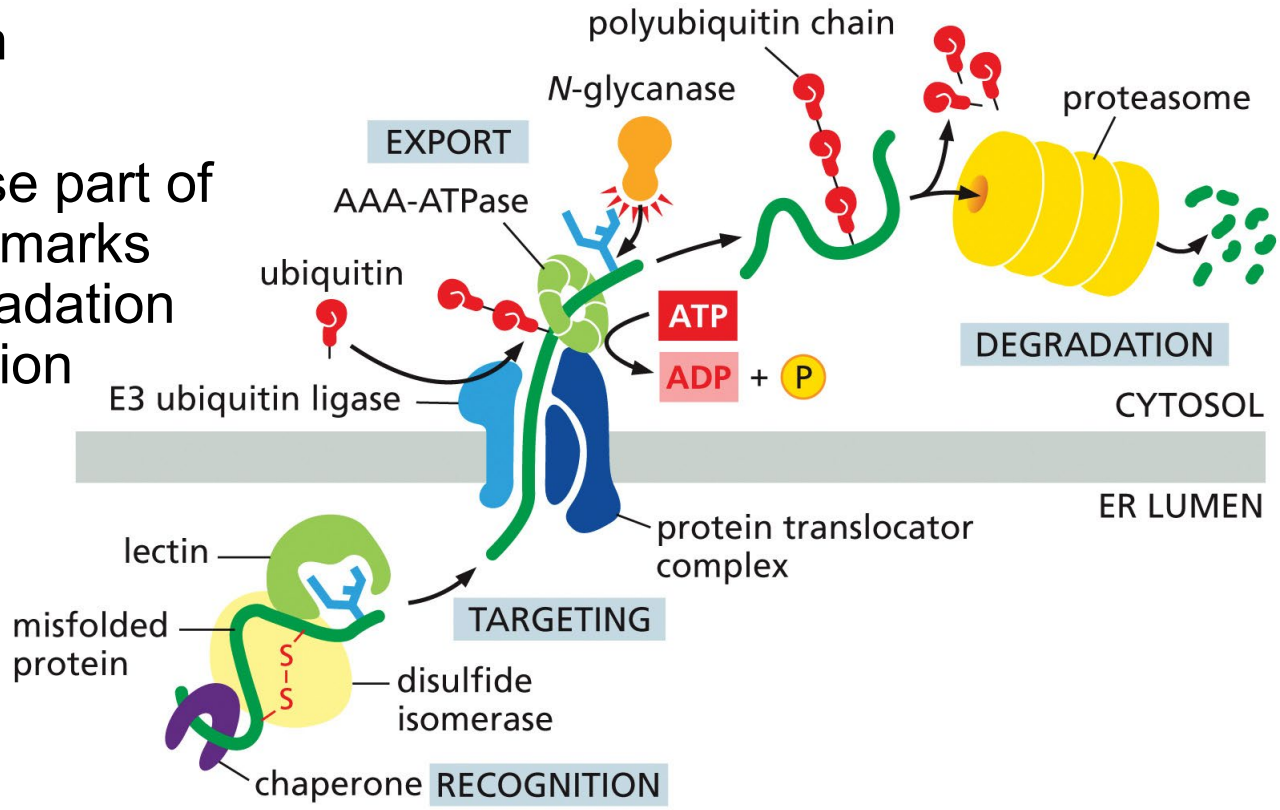
Figure 12-47a Molecular Biology of the Cell 6e (© Garland Science 2015)

- **Mannoses act as a timer** to distinguish between proteins that are unfolded but still can fold and those that are misfolded
- *Mannosidase* slowly removes mannoses
- If protein can fold faster than mannoses are removed, it can escape ER
- *If mannoses are removed faster, protein will be sent for degradation*

EXPORT OF IMPROPERLY FOLDED PROTEINS

- Improperly folded proteins are exported from the ER and degraded in the cytosol

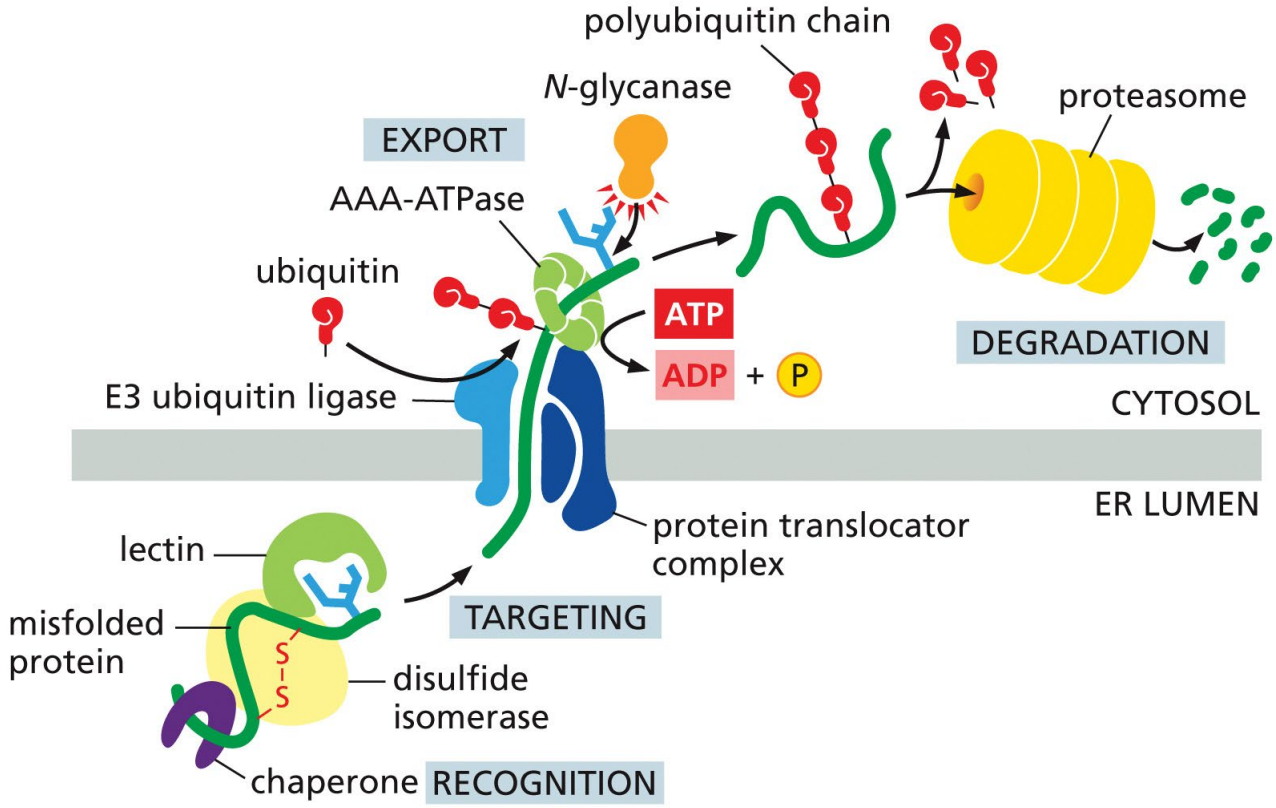
- Export by protein translocators
- E3 ubiquitin ligase part of the translocator, marks proteins for degradation during translocation



EXPORT OF IMPROPERLY FOLDED PROTEINS

- Improperly folded proteins are exported from the ER and degraded in the cytosol

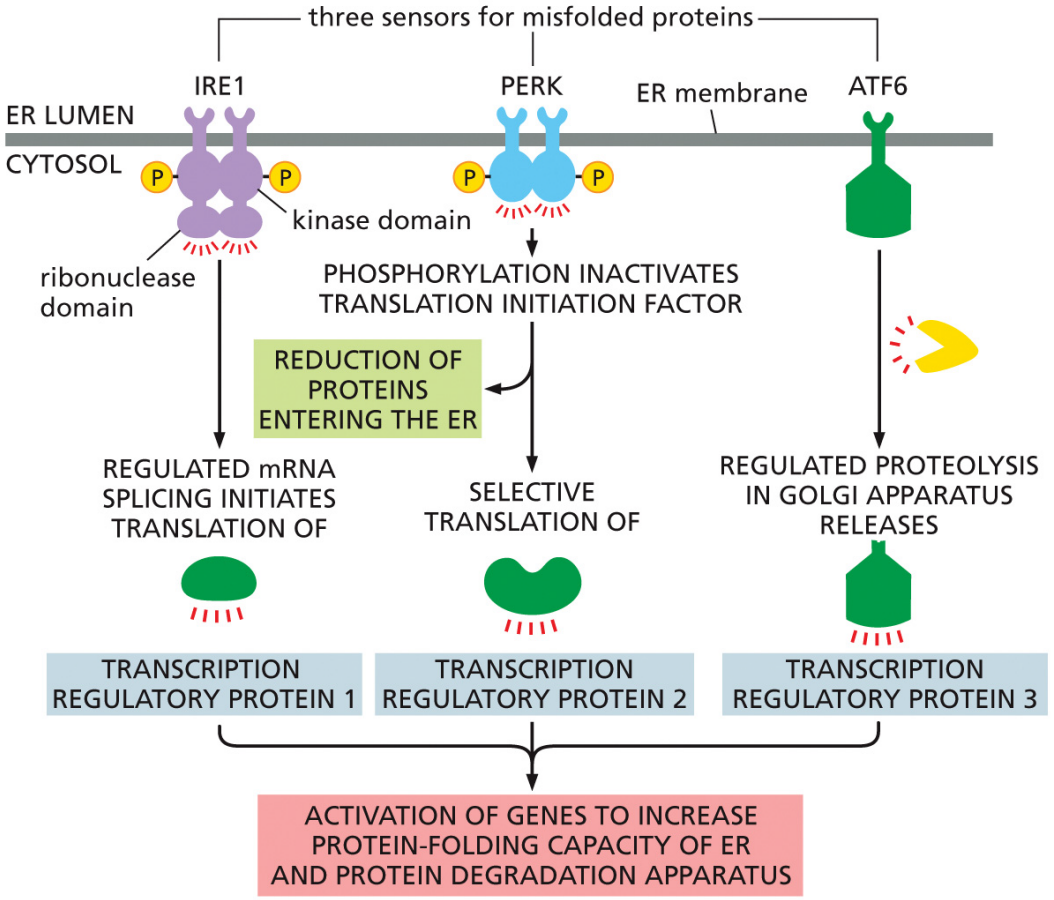
- Unfolded protein is then de-glycosylated and degraded in proteasomes



UNFOLDED PROTEIN RESPONSE

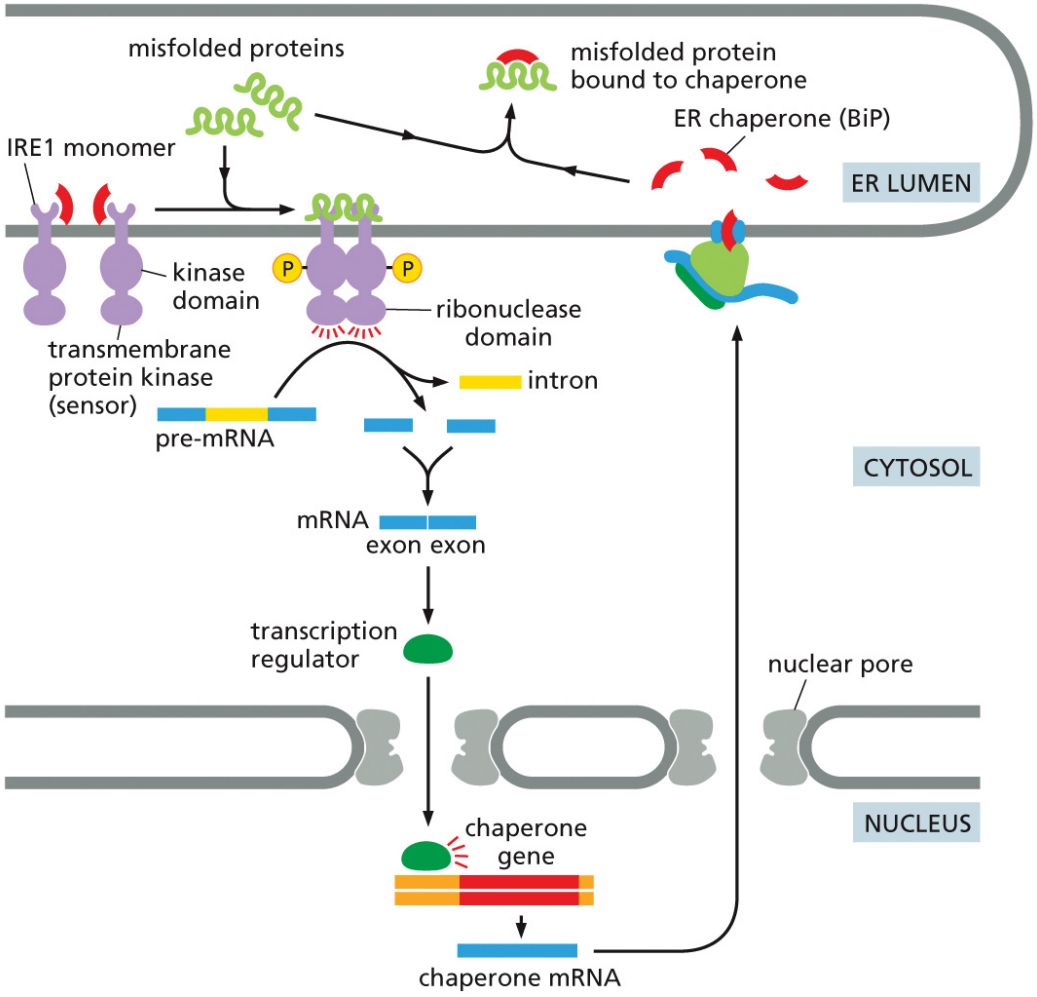
- Misfolded proteins in the ER activate an **unfolded protein response**

- 3 pathways



UNFOLDED PROTEIN RESPONSE – IRE1

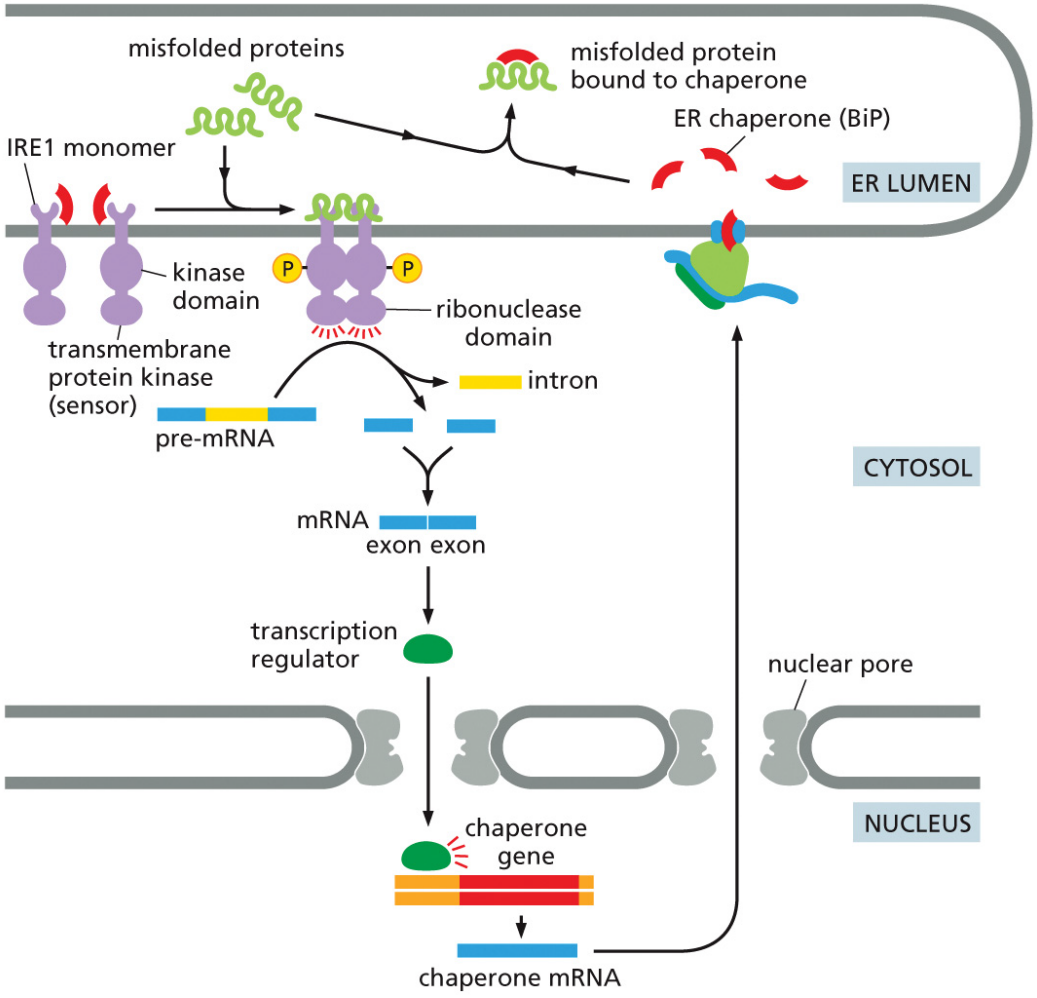
- IRE1 is maintained in an inactive state by its association with chaperone BiP.
- Elevated levels of misfolded proteins activate IRE1:
  - BiP dissociates from IRE1 to bind and protect misfolded proteins from aggregation
  - Misfolded proteins bind to the lumenal domain of IRE1 -> oligomerization



UNFOLDED PROTEIN RESPONSE – IRE1

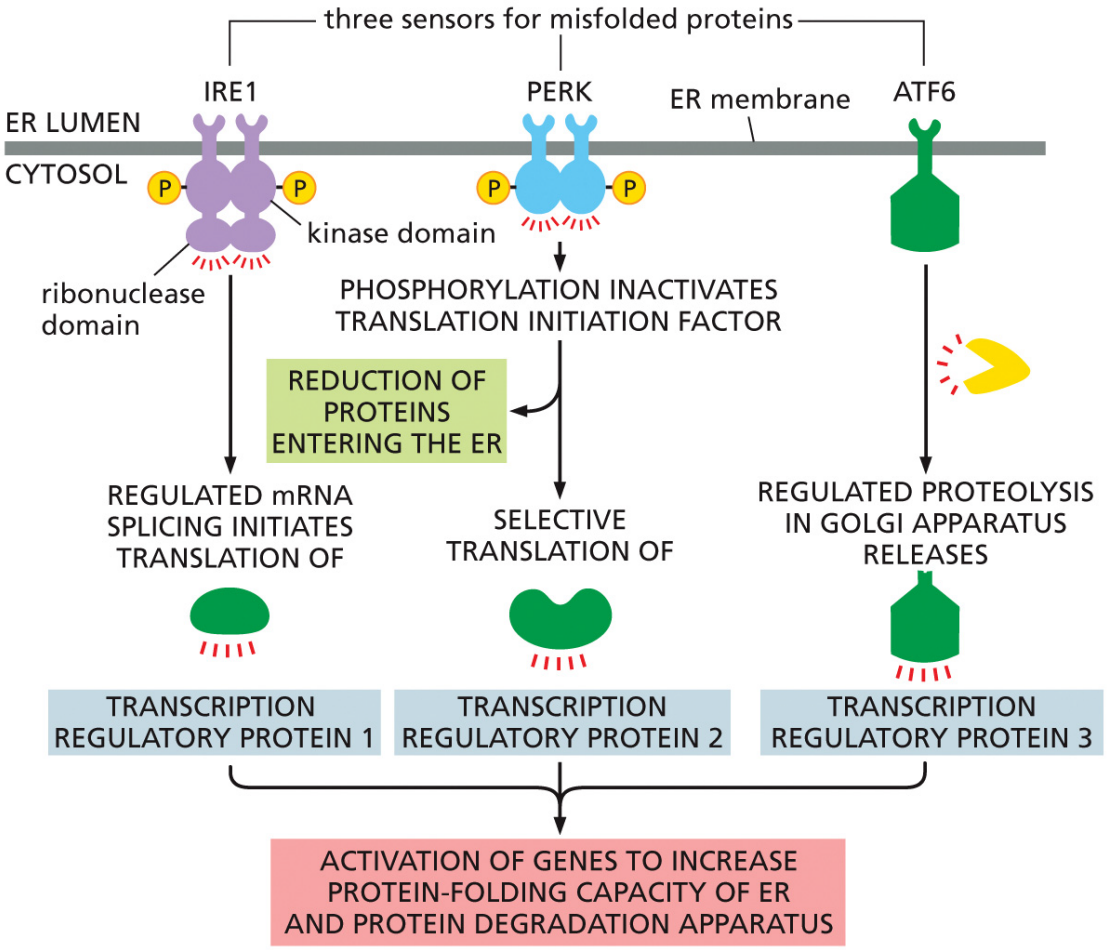
- The oligomerized IRE1 phosphorylates itself on the cytosolic side
- Ribonuclease domain activated
- Catalyzes the splicing of a pre-mRNA that codes for a transcription factor
- TF activates numerous genes in the nucleus including those coding for chaperones

Note! RNA splicing in cytosol in an exception!



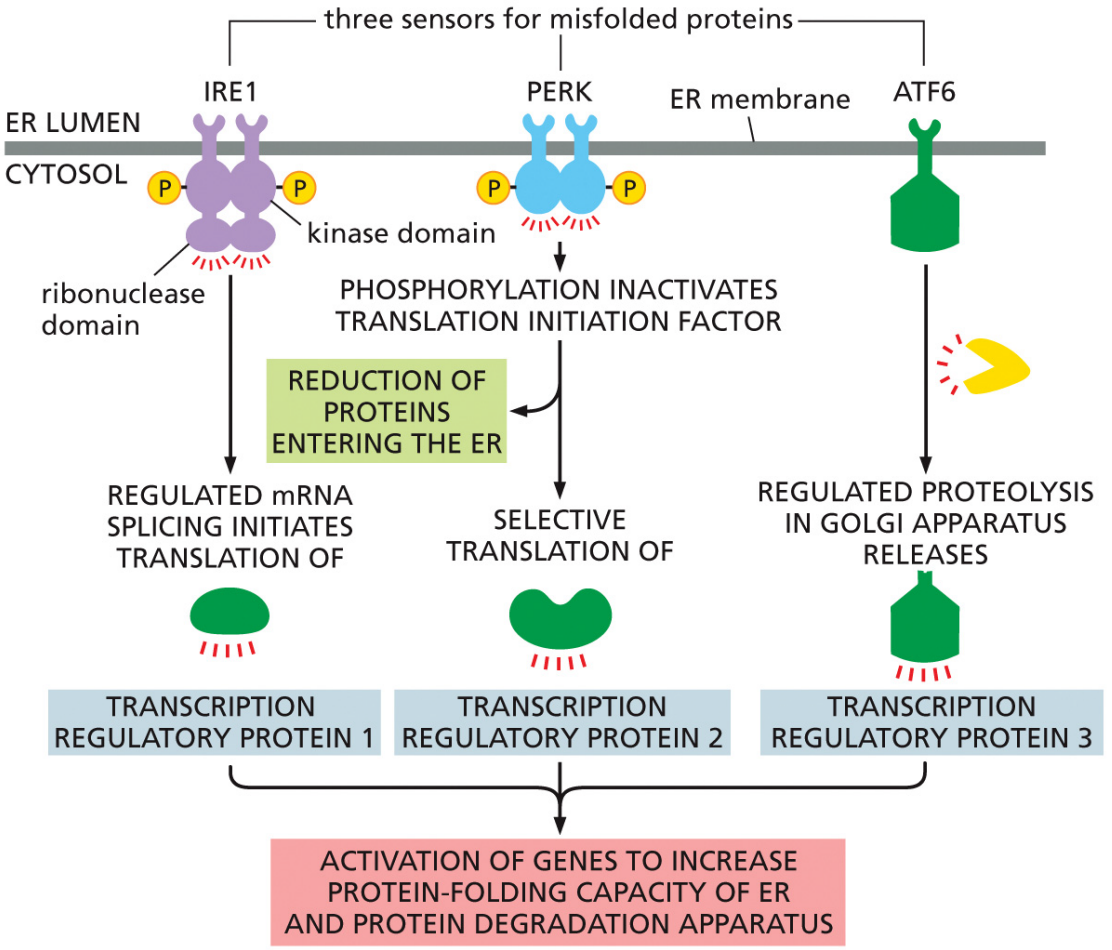
UNFOLDED PROTEIN RESPONSE

- PERK, phosphorylation inactivates translation initiation factor -> reduced number of proteins to ER



UNFOLDED PROTEIN RESPONSE

- ATF6, Transported to Golgi -> cytosolic domain cleaved off -> cytosolic domain migrates to nucleus where activates unfolded protein response genes



SUMMARY

- **Transmembrane proteins** contain hydrophobic segments that are recognized like signal sequences
- Hydrophobic segments of **multipass transmembrane proteins** are interpreted contextually to determine their orientation
- Some membrane proteins acquire a covalently attached **glycosylphosphatidylinositol (GPI) anchor**
- Translocated polypeptide chains **fold and assemble in** the lumen of the rough **ER**
- Most proteins synthesized in the rough ER are glycosylated by the addition of a common ***N*-linked oligosaccharide**
- Oligosaccharides are used as tags **to mark the state of protein folding**
- **Improperly folded proteins** are exported from the ER and degraded in the cytosol
- Misfolded proteins in the ER activate an **unfolded protein response**