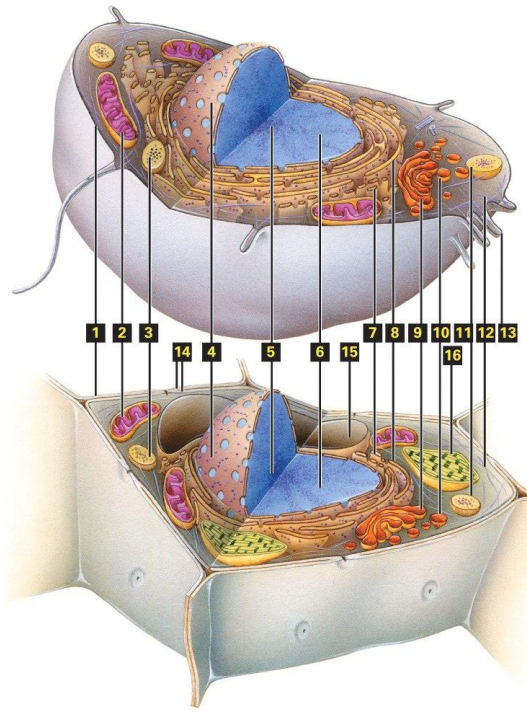
INTRACELLULAR COMPARTMENTS AND PROTEIN SORTING

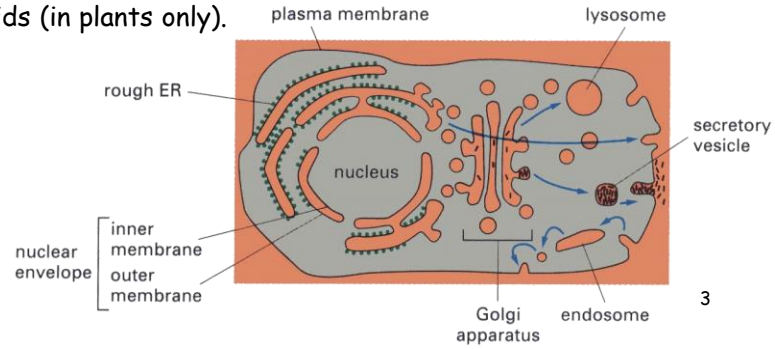
1



2

Four distinct families of intracellular compartments in eucaryotic cells:

1. the nucleus and the cytosol, which communicate with each other through *nuclear pore complexes* and are thus topologically continuous (although functionally distinct);
2. All organelles that function in the secretory and endocytic pathways: ER, Golgi apparatus, endosomes, and lysosomes, the numerous classes of transport intermediates such as transport vesicles that move between them, and possibly peroxisomes;
3. mitochondria; and
4. plastids (in plants only).



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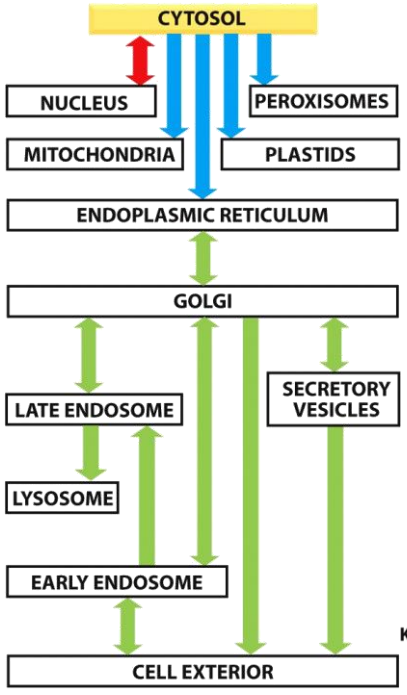
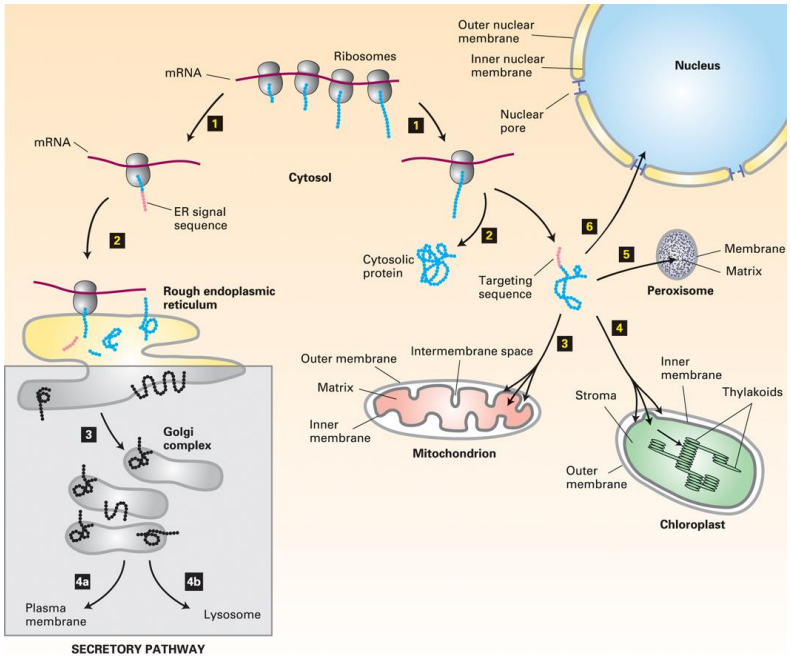


- **Sorting signals (amino acid sequence)** → direct their delivery to locations outside the cytosol.
- 3 mechanism of protein transport :

1. **Gated transport** : proteins move between the cytosol and the nucleus through nuclear pore complexes➔ Selective gates transport
2. **transmembrane transport**: transmembrane *protein translocators* directly transport specific proteins across a membrane from the cytosol into a space that is topologically distinct.
3. **vesicular transport**, membrane-enclosed transport intermediates—ferry proteins from one compartment to another.

4

Overview of major protein-sorting pathways in eukaryotes.



Correct cell address - signal sequences

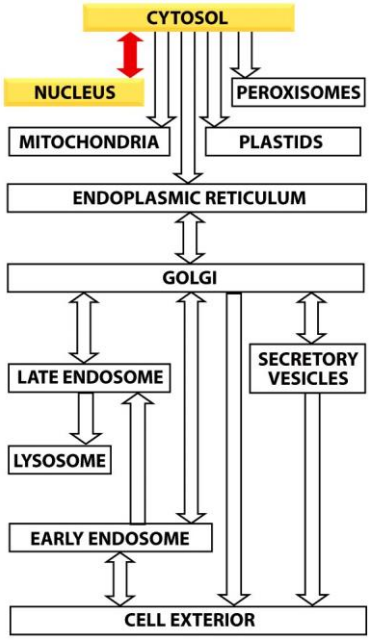
- protein sorting signals reside in a stretch of amino acid sequence, 15–60 residues long
- Signal sequences →
  - often in N-terminus protein ➔ removed by signal peptidases after sorting process
  - Multiple internal a.a. seq ➔ signal patch
- information required to construct an organelle : at least one distinct protein that preexists in the organelle membrane → passed from parent cell to progeny cell in the form of the organelle itself

Table 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

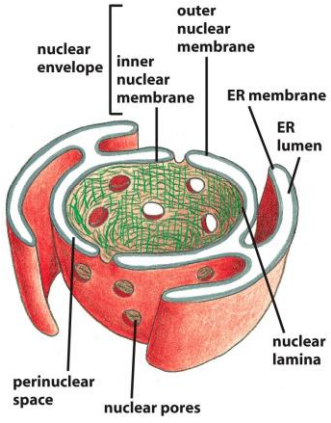
Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in *green*. Similarly, important hydrophobic amino acids are shown in *white* and hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

Table 12-3 *Molecular Biology of the Cell* (© Garland Science 2008)



Transport to nucleus

- Transportation through NPC (nuclear pore complex)/nucleoporin



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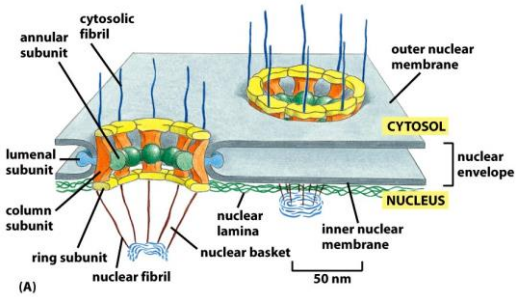
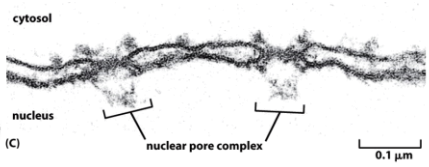
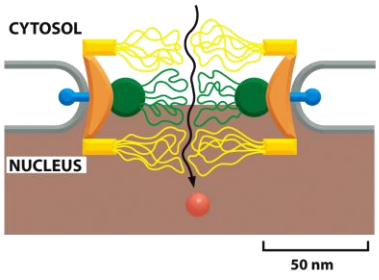




5000 daltons or less



size of molecules that enter nucleus by free diffusion

larger than 60,000 daltons

size of macromolecules that enter nucleus by active transport

Figure 12-9 *Molecular Biology of the Cell* (© Garland Science 2008)

Nuclear Localization Signals Direct Nuclear Proteins to the Nucleus

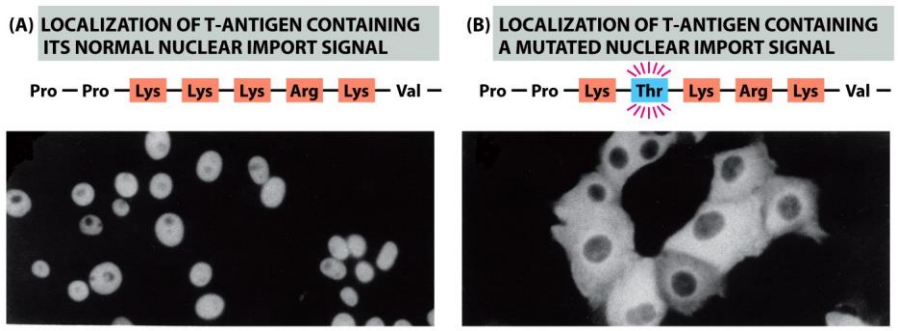
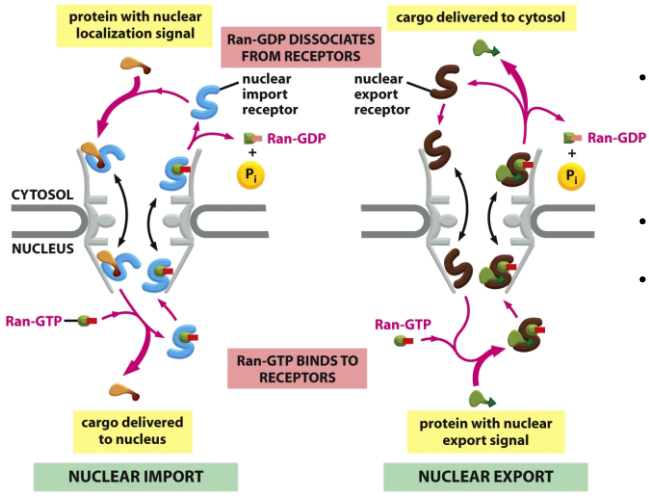
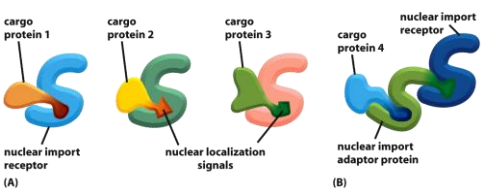


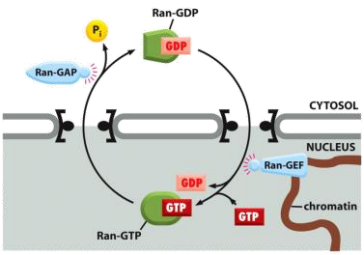
Figure 12-11 *Molecular Biology of the Cell* (© Garland Science 2008)

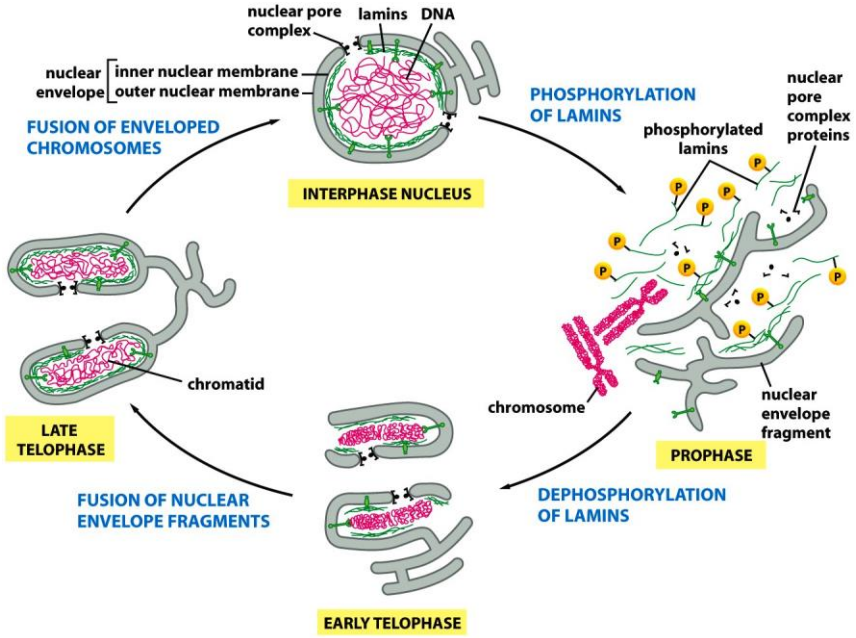


- Nuclear Import Receptors Bind to Both Nuclear Localization Signals and NPC proteins
- Nuclear export vs nuclear import
- Ran GTPase : molecular switch → regulate nuclear transportation

Figure 12-15 *Molecular Biology of the Cell* (© Garland Science 2008)







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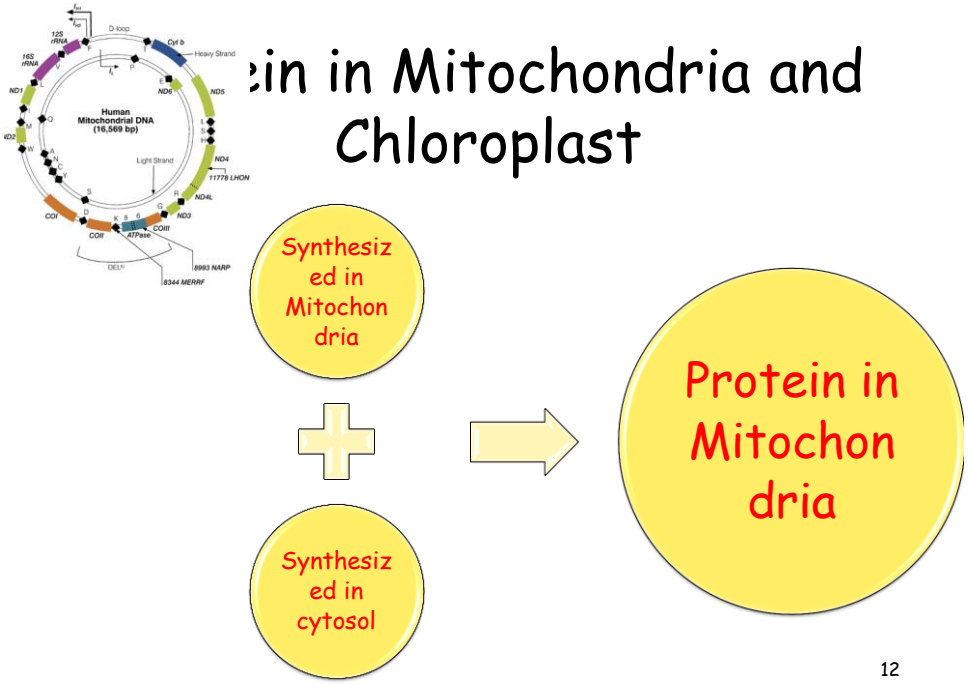

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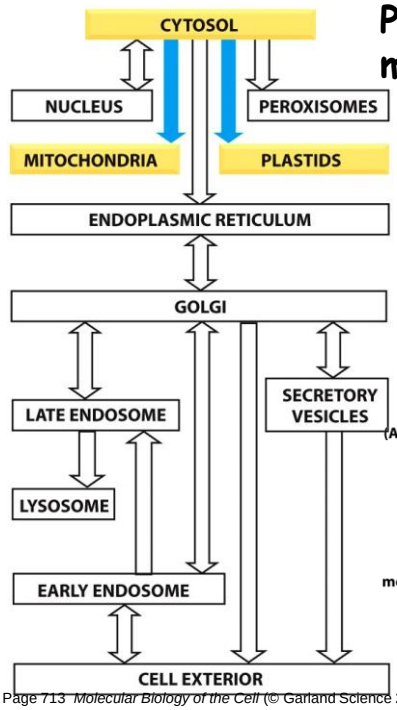

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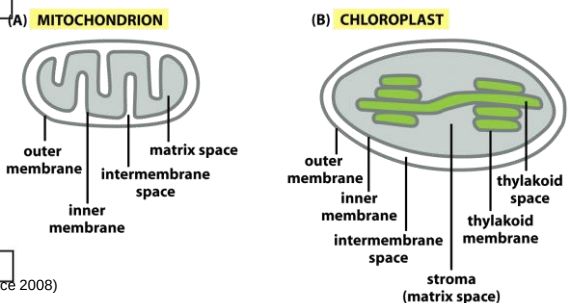
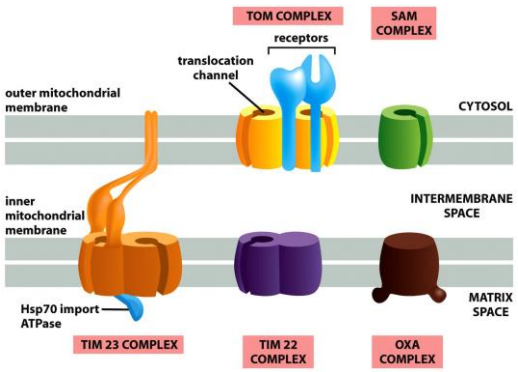
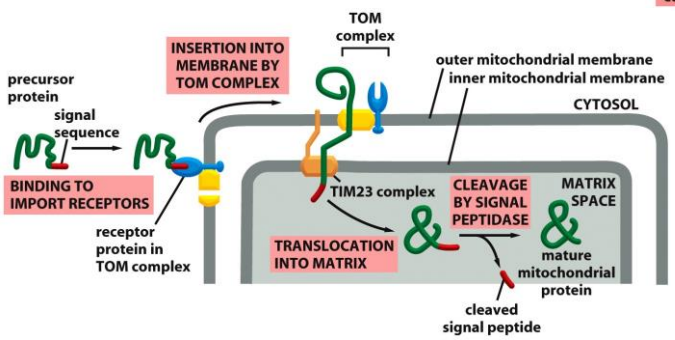
Protein transport into mitochondria and chloroplast

- mitochondrial proteins : synthesized as **mitochondrial precursor proteins** in the cytosol and then translocated into mitochondria by a *post-translational* mechanism.

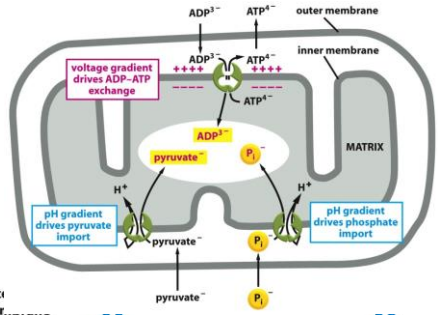


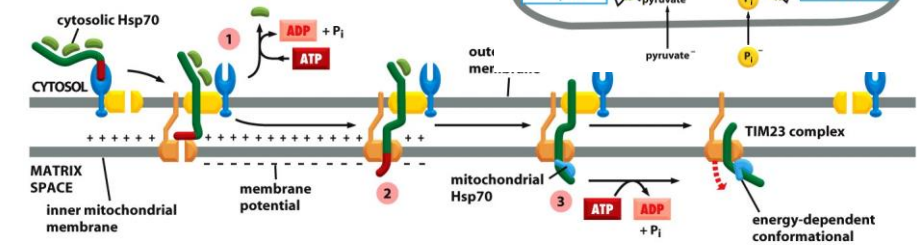
- protein translocators mediate protein translocation across mitochondrial membranes: TOM and TIM (TIM23 and TIM22) complex, SAM, OXA
- Protein :
  - in outer membrane,
  - Intramembrane space
  - In inner membrane
  - matrix

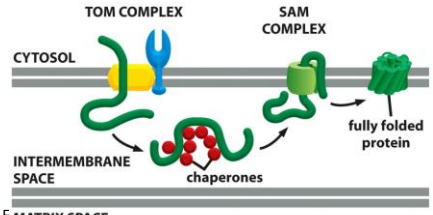




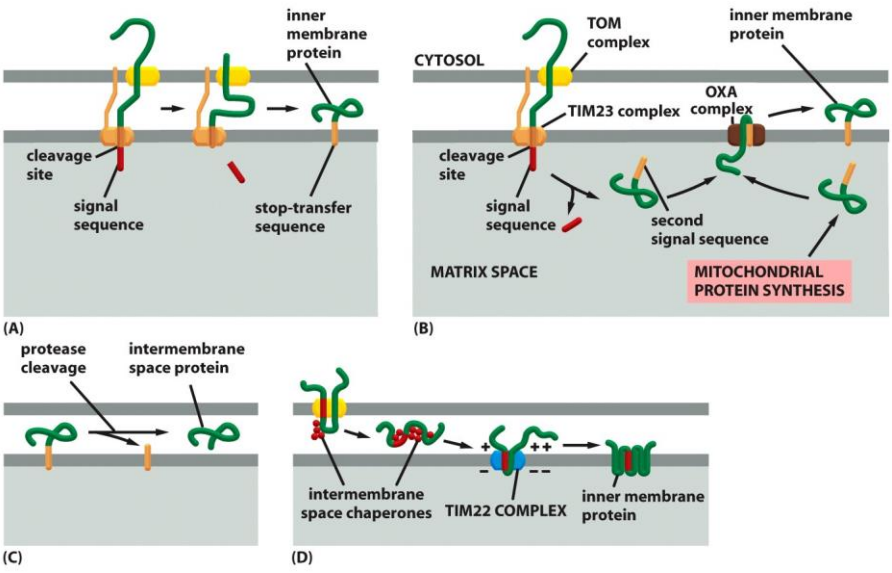
- **ATP Hydrolysis and a Membrane Potential Drive Protein Import Into the Matrix Space**







Integration of porins into the outer mitochondrial membrane.



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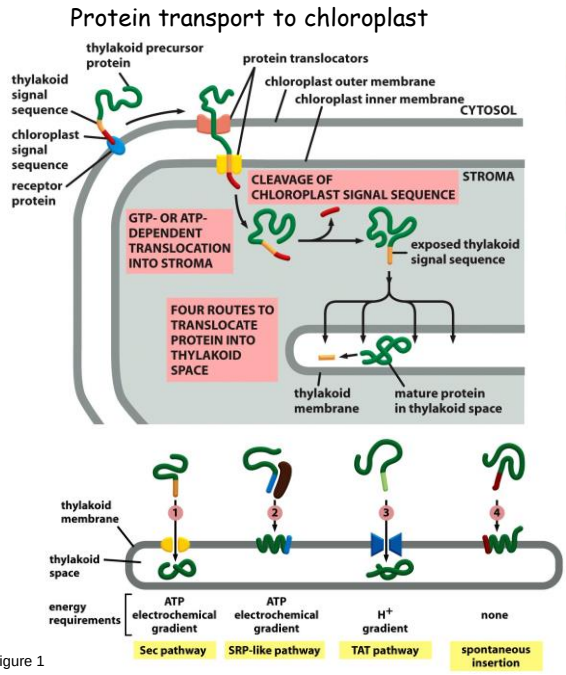
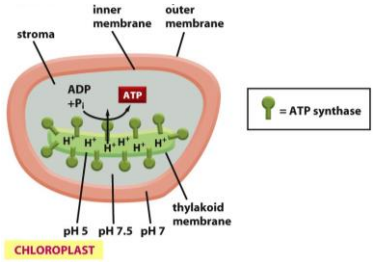
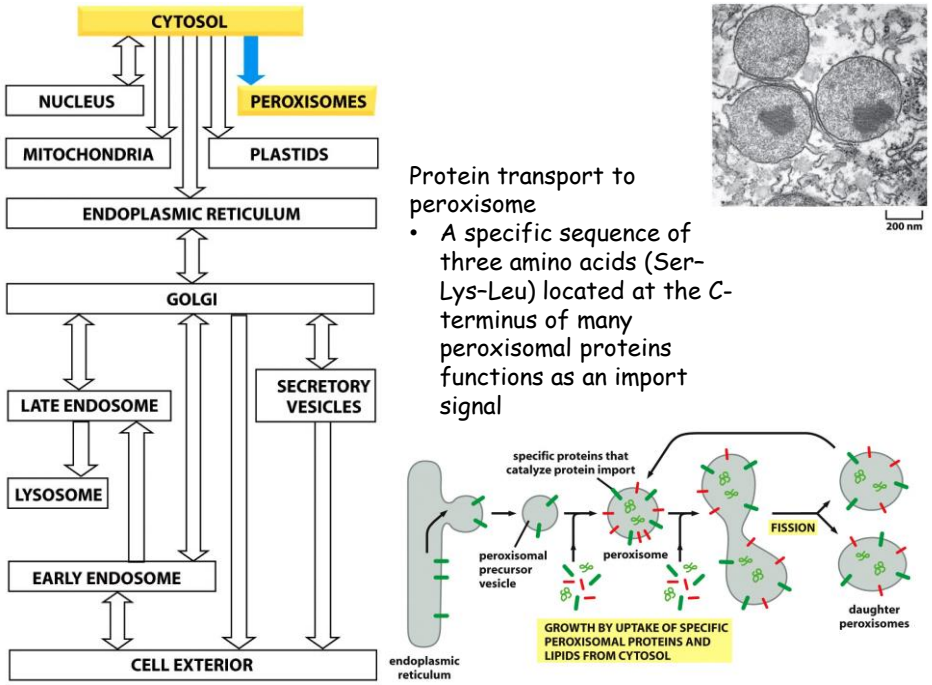




Protein in:
- Chloroplast outer membrane
- Chloroplast inner membrane
- Stroma
- Thylakoid membrane
- Thylakoid space

4 pathway protein transport to thylakoid

Figure 1



Protein transport to peroxisome
- A specific sequence of three amino acids (Ser–Lys–Leu) located at the C-terminus of many peroxisomal proteins functions as an import signal

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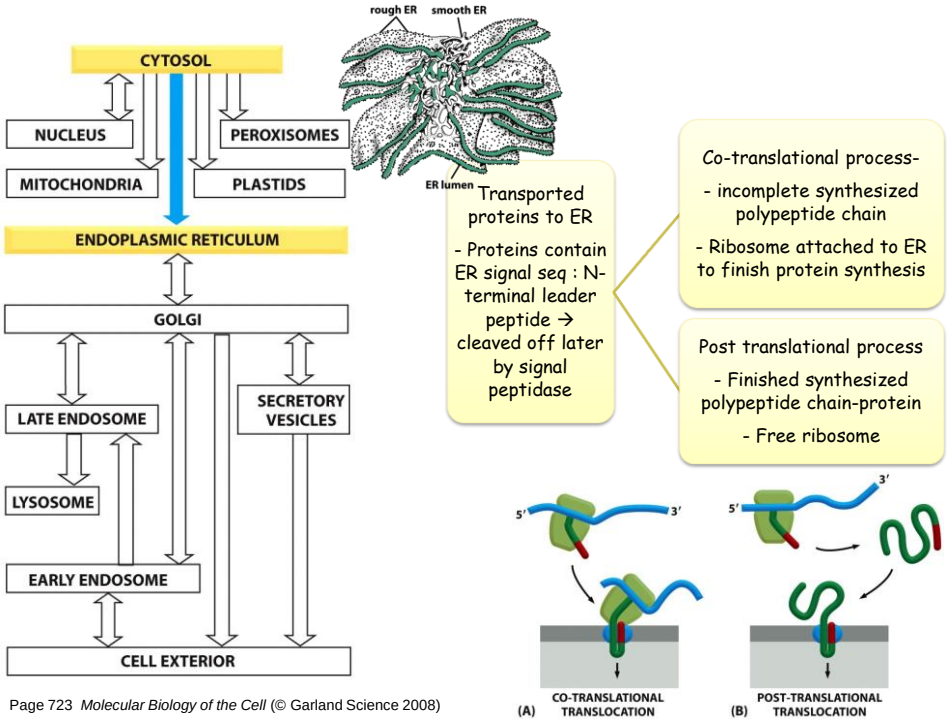

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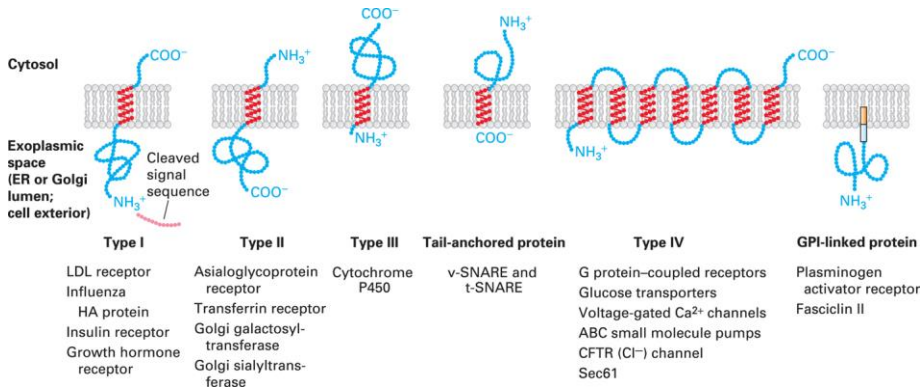
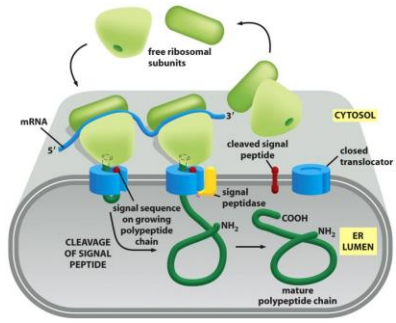
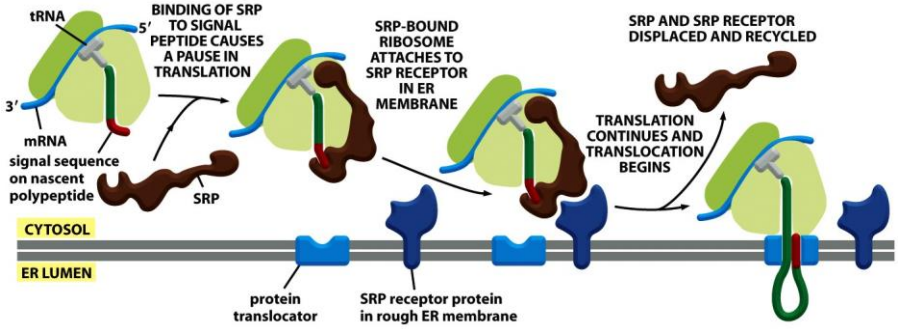
Figure 13.10 **ER membrane proteins.**



- Signal peptide will be recognized by signal-recognition particle (SRP), which will direct ribosome to a translocator on the ER membrane, ie sec complex
- In mature protein, Signal peptide will be cleaved off by signal peptidase





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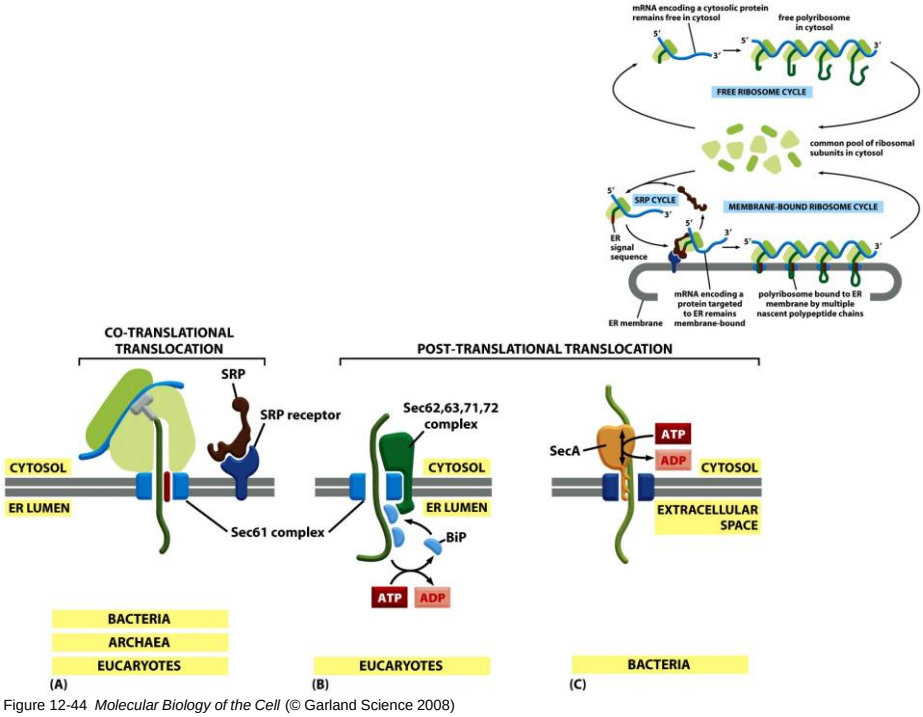

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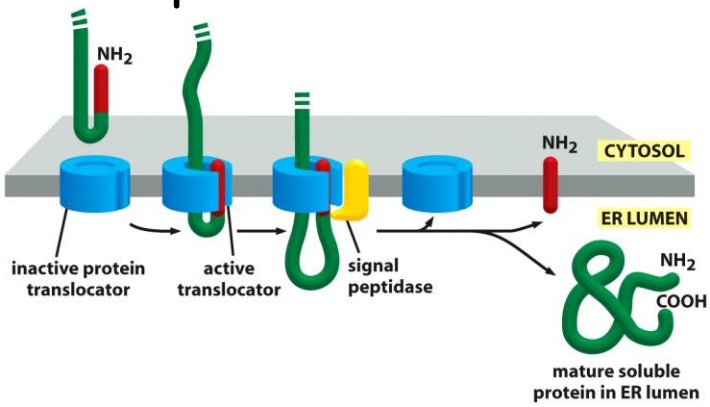
Soluble protein in ER lumen



Soluble protein is translocated across the ER membrane- need a signal peptide → recognized by:

- SRP
- A binding site in the pore of the protein translocator

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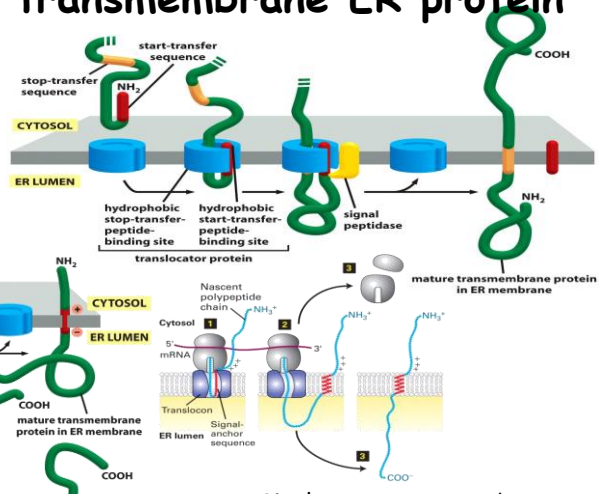
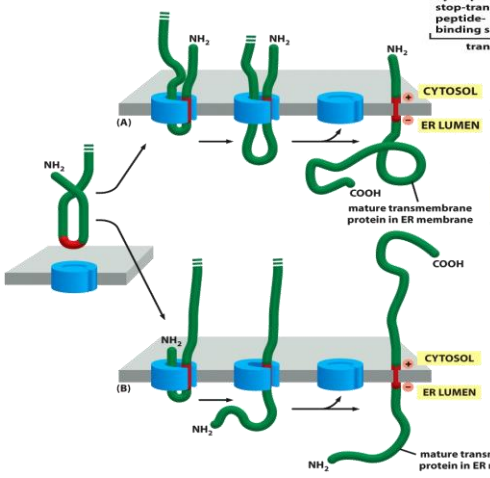
Single pass transmembrane ER protein

a single-pass transmembrane protein → need:

- Signal peptide – start transfer seq
- Stop transfer signal

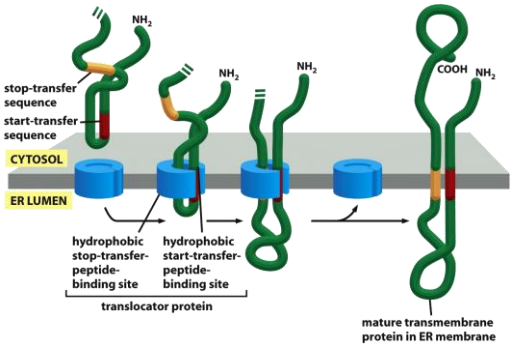




Single pass transmembrane with an internal signal peptide
Internal signal peptide is recognized by

- SRP and
- protein translocator

Multipass transmembrane proteins



Have several internal signal sequences: start-transfer signal seq and stop transfer signal seq

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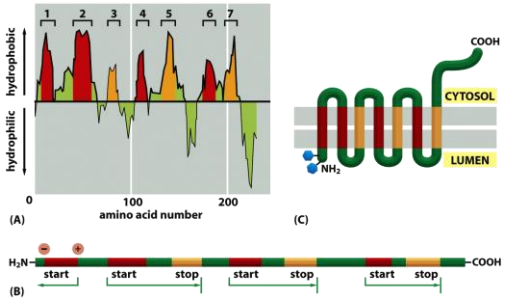
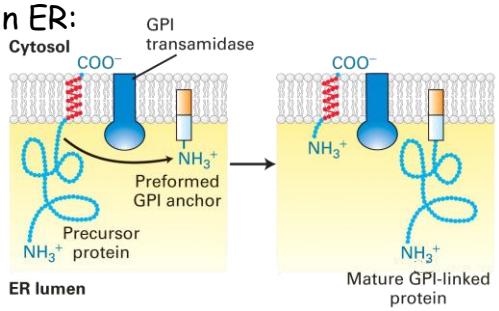


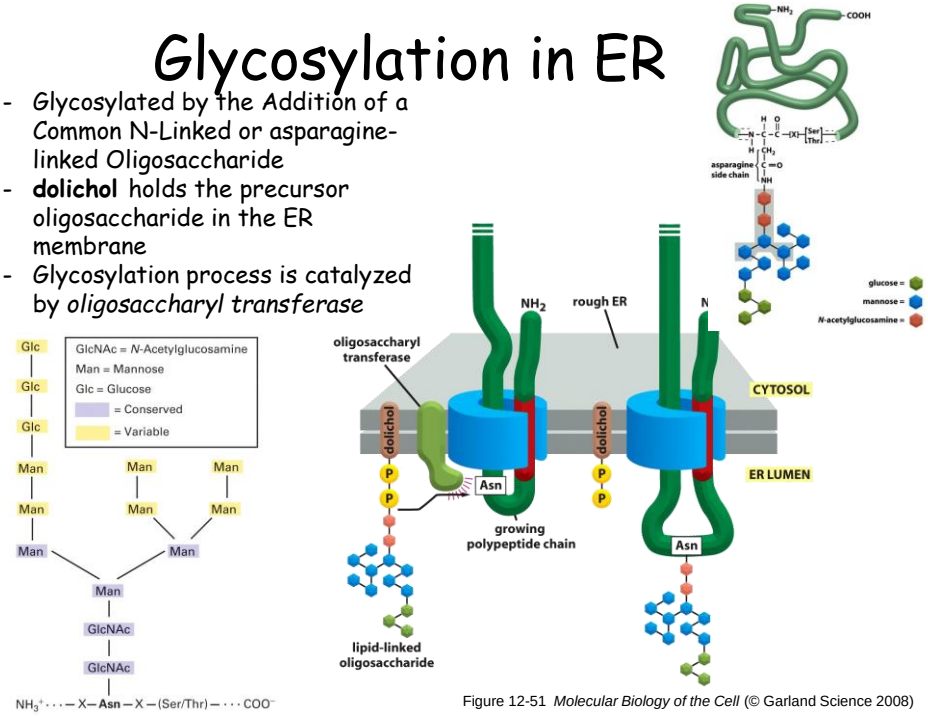
Translocated Polypeptide Chains Fold and Assemble in the Lumen of the Rough ER

- **ER resident proteins** contain an **ER retention signal** of four amino acids at their C-terminus
- ER resident protein:
  - *protein disulfide isomerase (PDI)*➔ catalyzes the oxidation of free sulfhydryl (SH) groups on cysteines to form disulfide (S-S) bonds
  - chaperone protein **BiP** ➔ recognizes incorrectly folded proteins
- Posttranslational process in ER:
  - N-linked Glycosylation
  - Bind to GPI
  - Form disulfide bonds & Protein folding



Glycosylation in ER

- Glycosylated by the Addition of a Common N-Linked or asparagine-linked Oligosaccharide
- **dolichol** holds the precursor oligosaccharide in the ER membrane
- Glycosylation process is catalyzed by *oligosaccharyl transferase*



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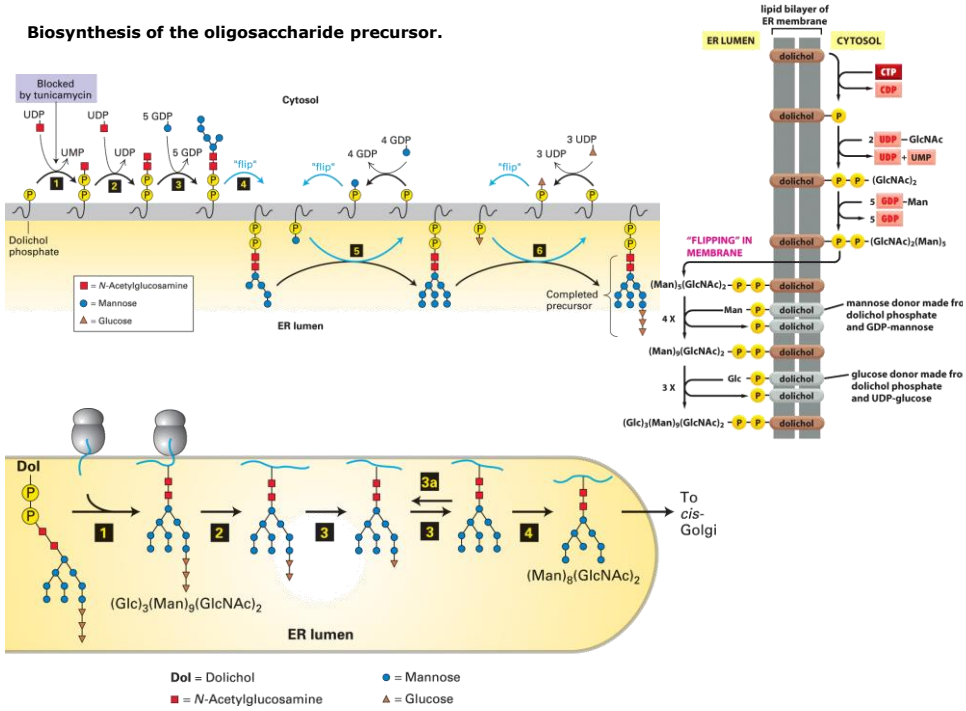
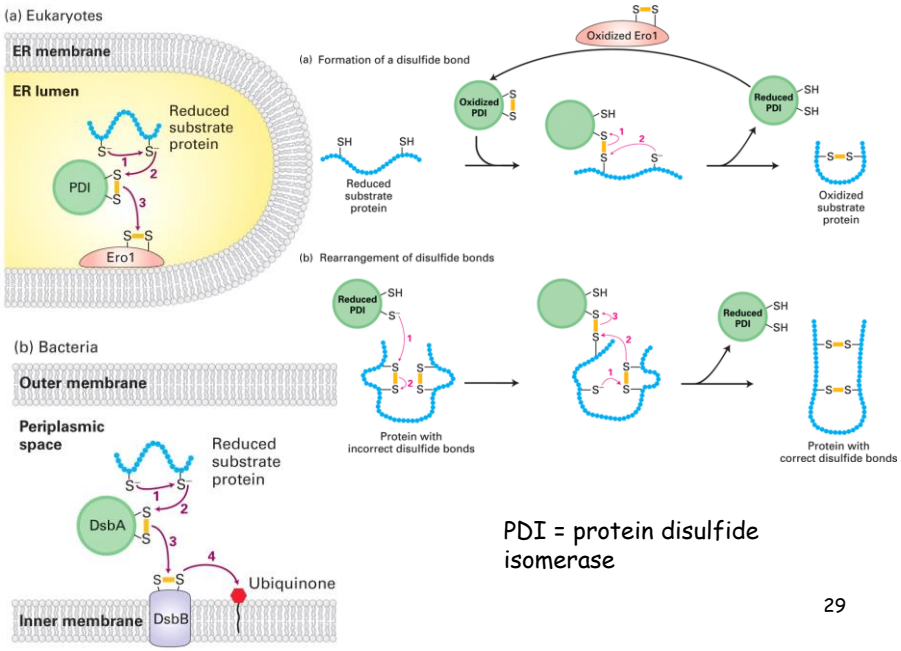


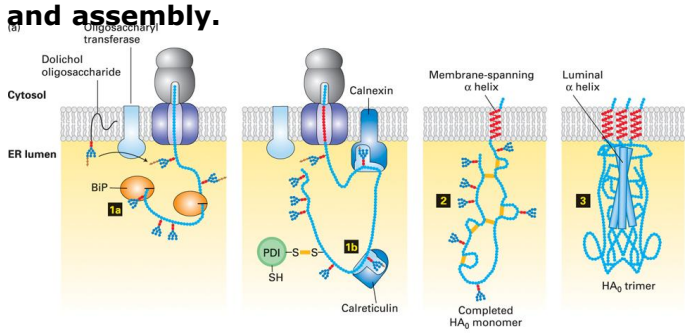
disulfide bonds formation in ER

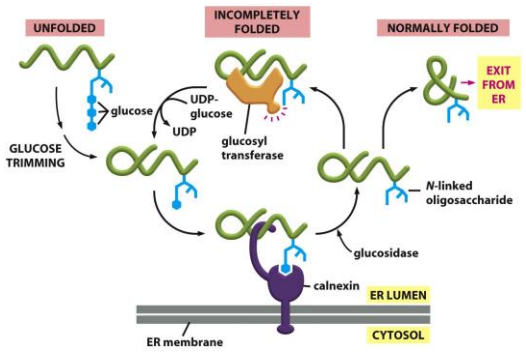


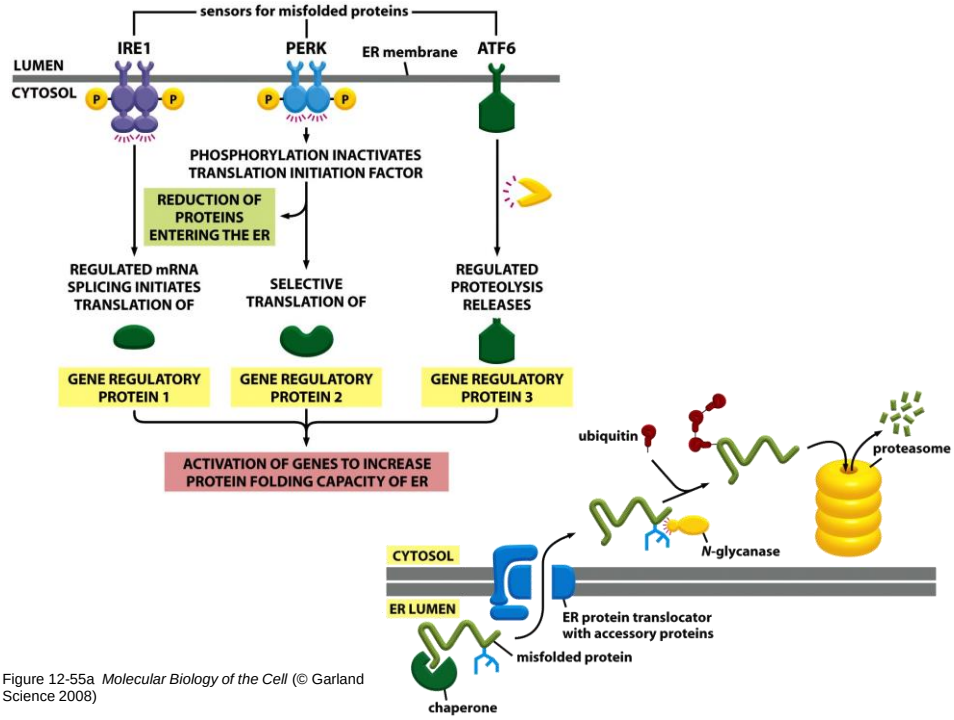
PDI = protein disulfide isomerase

29

Protein folding and assembly.







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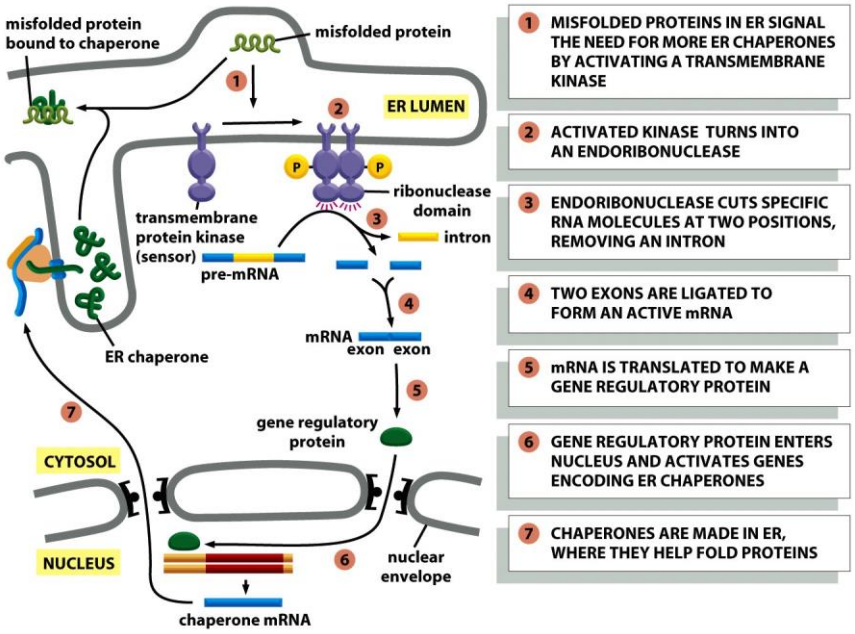
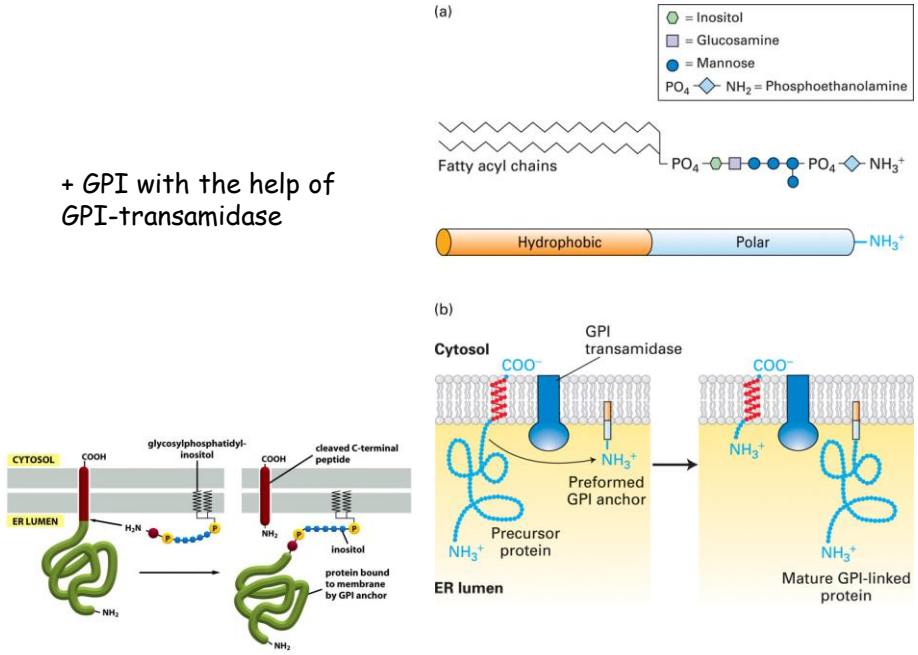


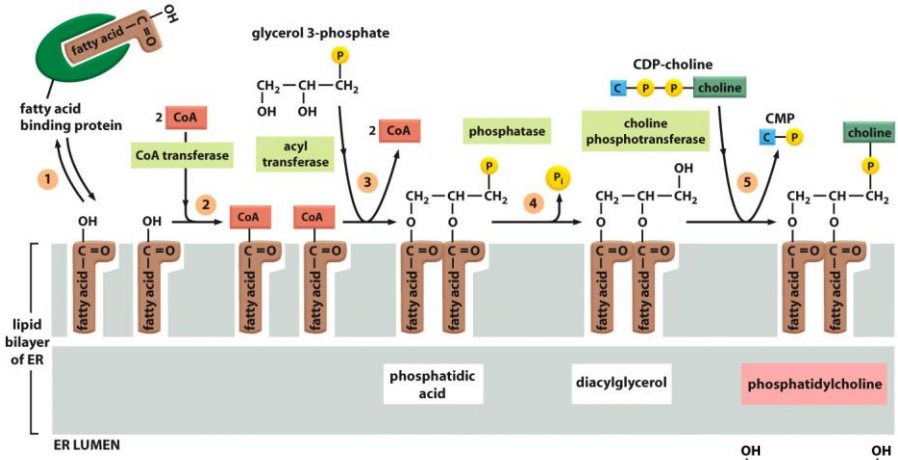
Figure 12-55b *Molecular Biology of the Cell* (© Garland Science 2008)

Figure 13.15 **GPI-anchored proteins.**

+ GPI with the help of GPI-transamidase



Lipid bilayers assembles in ER



- Phosphatidylcholine, phosphatidyl ethanolamine and phosphatidylserine, phosphatidylinositol , cholesterol & ceramide are synthesized in ER – in luminal leaflet of ER bilayer

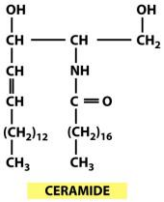


Figure 12-57 *Molecular Biology of the Cell* (© Garland Science 2008)

Phospholipid translocation in lipid bilayer synthesis

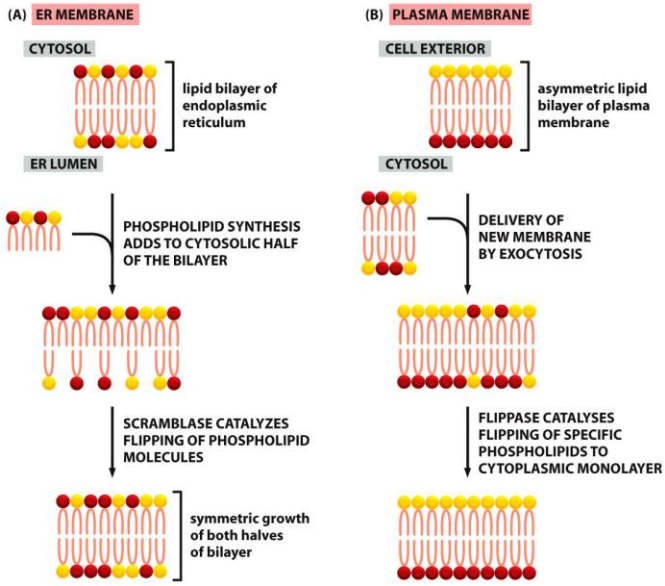


Figure 12-58 *Molecular Biology of the Cell* (© Garland Science 2008)