

# Lecture 6 Macromolecules transport Part I

## Outline

- I. Cell compartments
- II. Transport between nucleus and cytosol
- III. Transport to mitochondria and chloroplast
- IV. Transport to peroxisome
- V. Transport to endoplasmic reticulum

# I. The major intracellular compartments of an animal cell

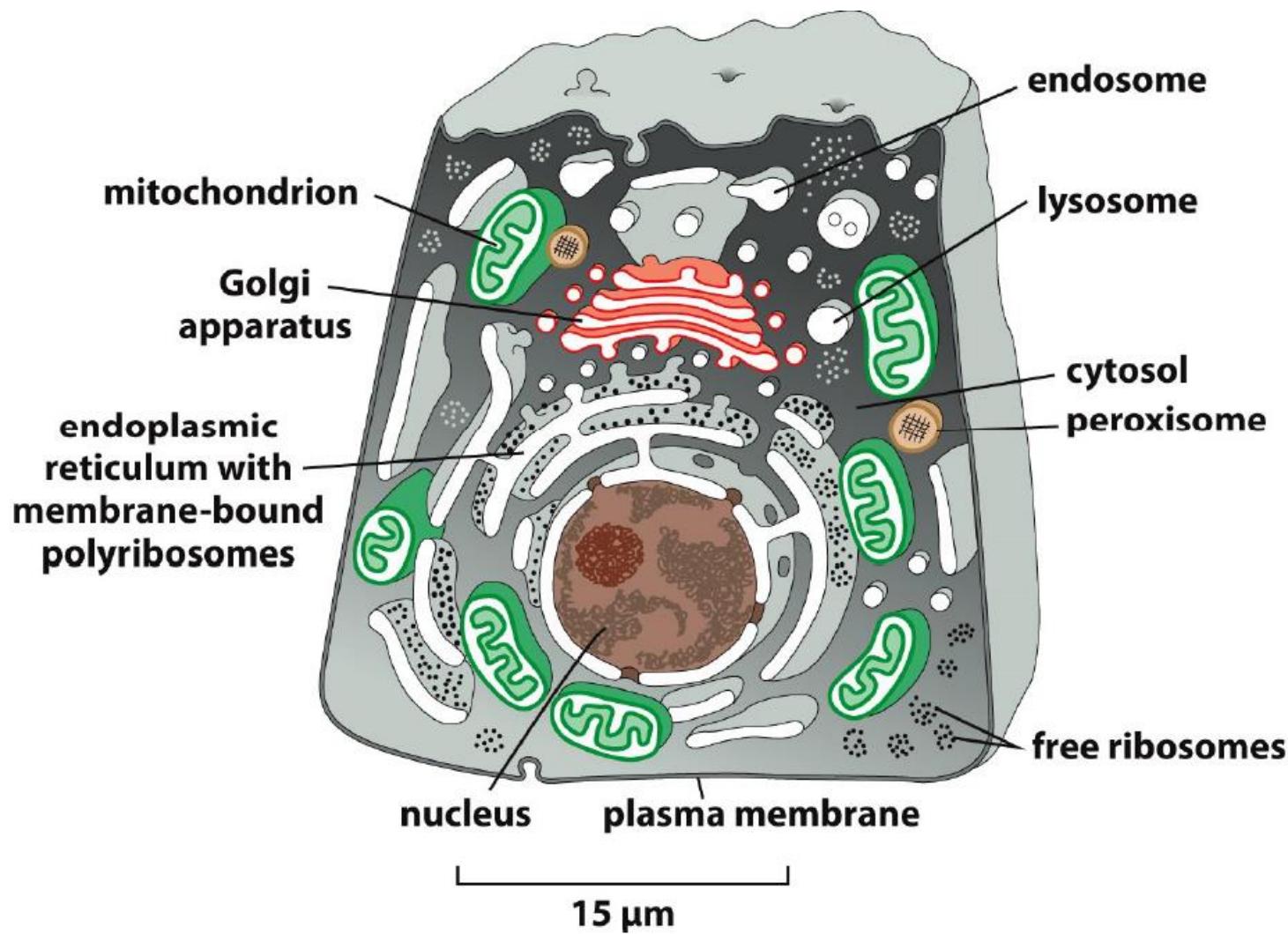
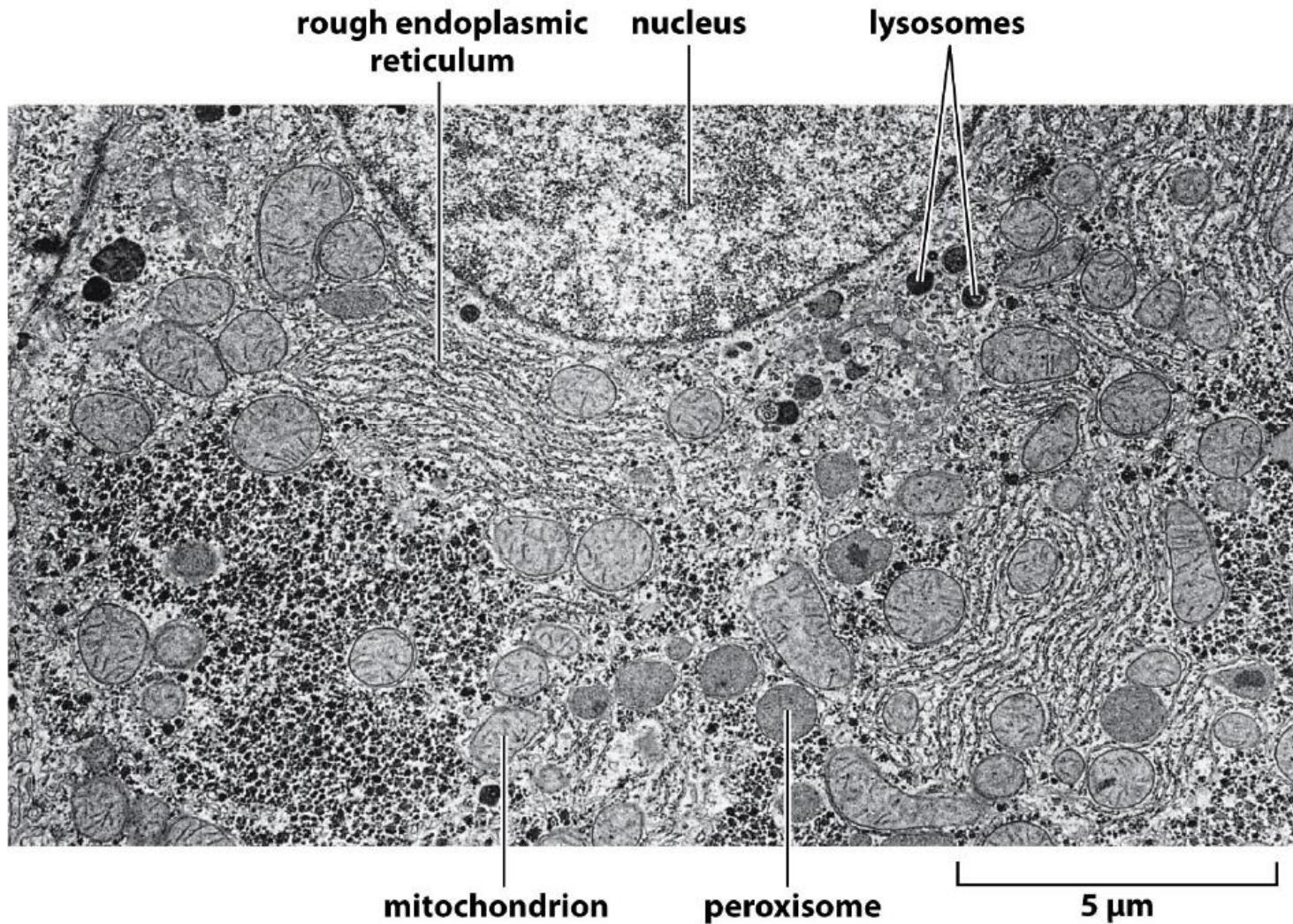


Figure 12-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

# A cross section of liver cell under TEM



Q: Why do eukaryotic cells have such extensive inner membrane system?

A: The existence of extensive inner membrane system alleviates the small surface/volume ratio that is not good for its vital functions

**TABLE 12-1 Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)**

| Intracellular compartment                | Percentage of total cell volume |
|--|---------------------------------|
| Cytosol                                  | 54                              |
| Mitochondria                             | 22                              |
| Rough ER cisternae                       | 9                               |
| Smooth ER cisternae plus Golgi cisternae | 6                               |
| Nucleus                                  | 6                               |
| Peroxisomes                              | 1                               |
| Lysosomes                                | 1                               |
| Endosomes                                | 1                               |

Relative volumes for each Compartments in a liver cell

# Percentage of total cell membrane in two eukaryotic cell types

| Membrane Type              | Percentage of total cell membrane |                           |
|----------------------------|-----------------------------------|---------------------------|
|                            | Liver hepatocyte*                 | Pancreatic exocrine cell* |
| Plasma membrane            | 2                                 | 5                         |
| Rough ER membrane          | 35                                | 60                        |
| Smooth ER membrane         | 16                                | <1                        |
| Golgi apparatus membrane   | 7                                 | 10                        |
| Mitochondria               |                                   |                           |
| Outer membrane             | 7                                 | 4                         |
| Inner membrane             | 32                                | 17                        |
| Nucleus                    |                                   |                           |
| Inner membrane             | 0.2                               | 0.7                       |
| Secretory vesicle membrane | Not determined                    | 3                         |
| Lysosome membrane          | 0.4                               | Not determined            |
| Peroxisome membrane        | 0.4                               | Not determined            |
| Endosome membrane          | 0.4                               | Not determined            |

\*These two cells are of very different sizes: the average hepatocyte has a volume of about  $5000 \mu\text{m}^3$  compared with  $1000 \mu\text{m}^3$  for the pancreatic exocrine cell. Total cell membrane areas are estimated at about  $110,000 \mu\text{m}^2$  and  $13,000 \mu\text{m}^2$ , respectively.

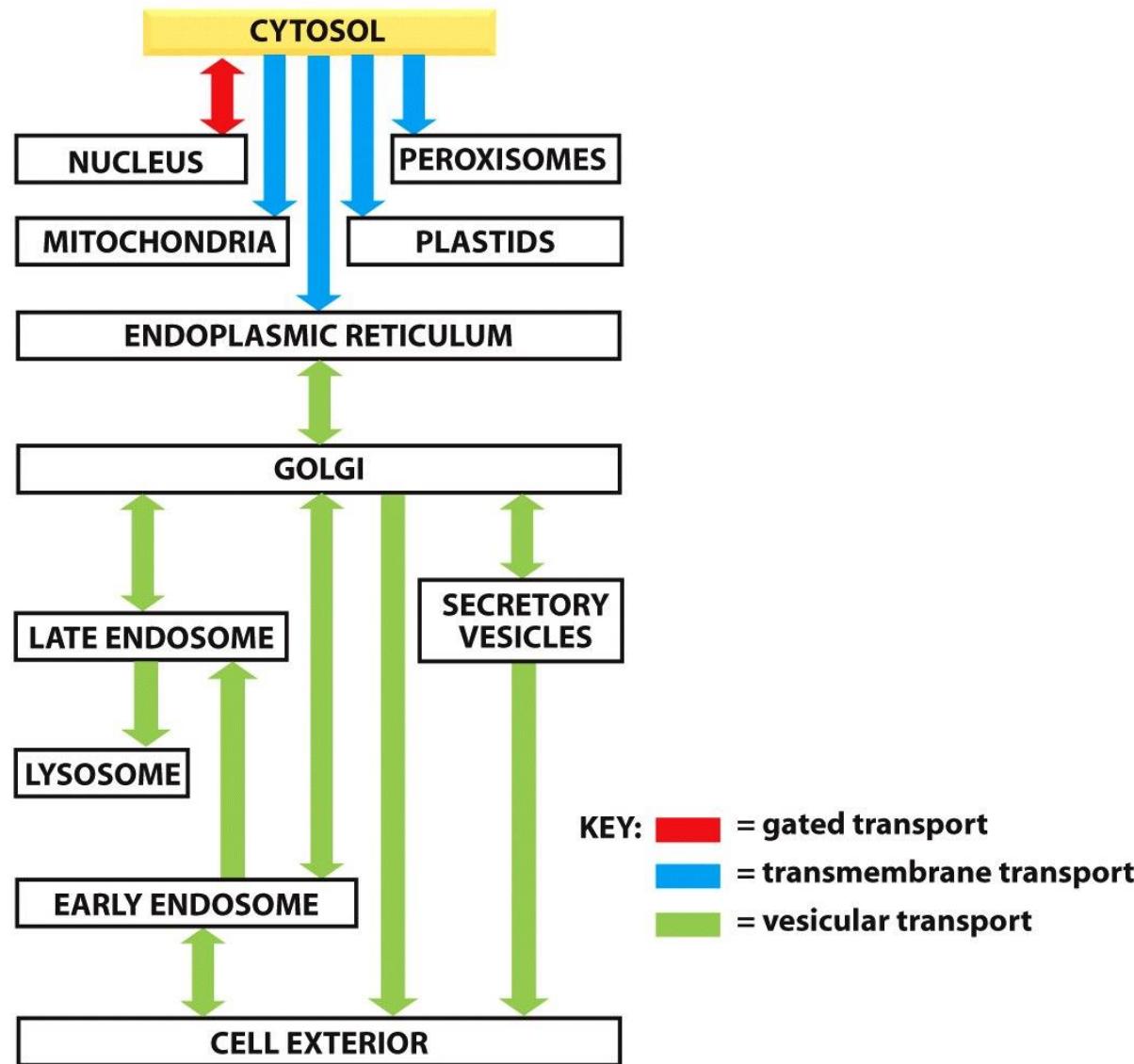
Each organelle contains its unique set of proteins that are characteristic of its distinct functions.

So what decides the specific proteins to be transported into each of them?

## Destiny of synthesized protein

- All proteins are synthesized in the cytosol
- Most protein without sorting signal stay in cytosol
- Protein with sorting signal will be transported to its designated sites by three ways:
  1. gated transport
  2. transmembrane transport
  3. vesicular transport

# Three types of transport for macromolecules in cellular compartments



- ♥ **Gated transport**: proteins move between cytosol and nucleus through nuclear pore complex (NPC) with selectivity/specifity
- ♥ **Transmembrane transport**: unfolded protein snakes through transmembrane protein translocators by the guidance of sorting signals.
- ♥ **Vesicular transport**: membrane-enclosed transport intermediates proteins from one compartment to the other. With the guidance of sorting signals and recognized by the sorting receptors.

# Signal sequence directs protein sorting

Three types :

- 1) **N-terminal signal sequence**, usually 15-60 aa long, can be removed by **signal peptidase**
- 2) **Internal stretches** of amino acids.
- 3) **Signal patch**, a specific 3-dimentional arrangement of atoms on protein's surface.

# Some typical signal sequences

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    | <sup>+</sup> H <sub>3</sub> N-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         | <sup>+</sup> H <sub>3</sub> N-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-Ser-Lys-Leu-COO <sup>-</sup> |
| Import into peroxisomes     |  |
| Import into ER              | <sup>+</sup> H <sub>3</sub> N-Met-Met-Ser-Phe-Val-Ser-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 <sup>-</sup>  |

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. <sup>+</sup>H<sub>3</sub>N indicates the N-terminus of a protein; COO<sup>-</sup> indicates the C-terminus.

## II. Transport between the nucleus and the cytosol

Transport is through nuclear pore complex (NPC)  
It occurs bi-directionally and with selectivity.

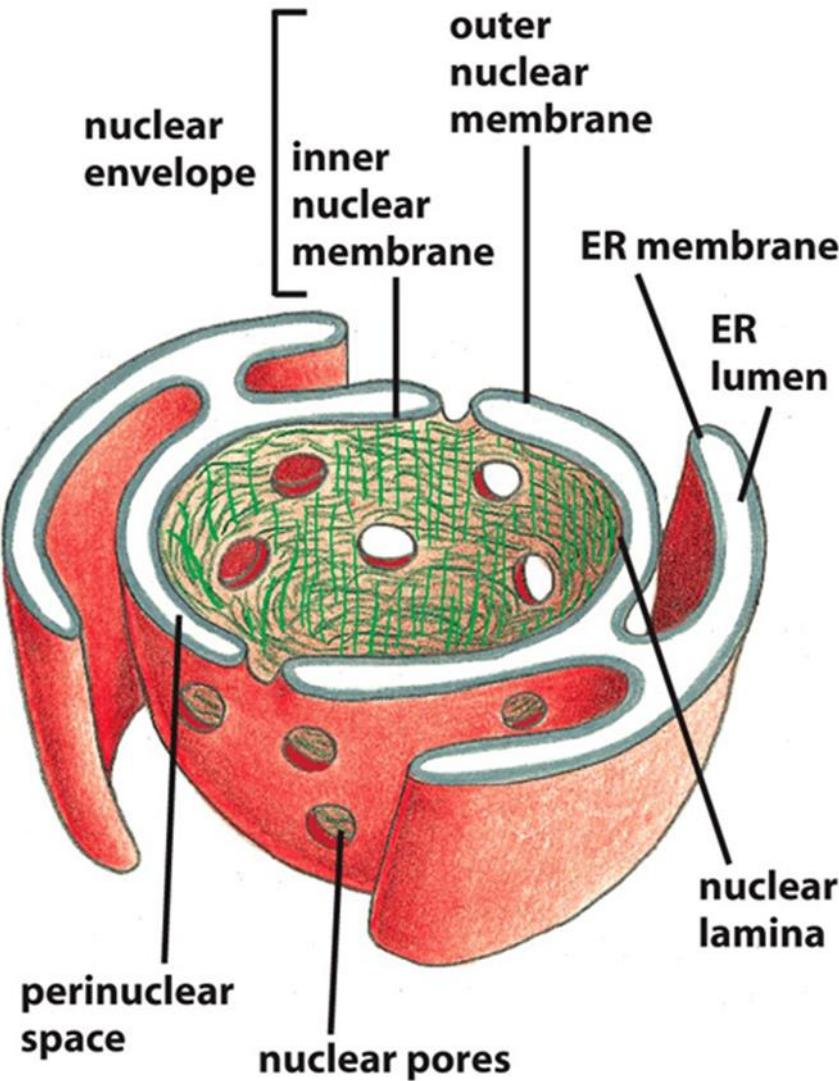
Transport in :

- histones
- DNA/RNA polymerases
- gene regulatory proteins
- RNA processing proteins
- ribosomal proteins, etc.

Transport out:

- mature RNAs
- tRNAs
- pre-ribosome subunits
- proteins, etc

# The structure of nuclear envelope



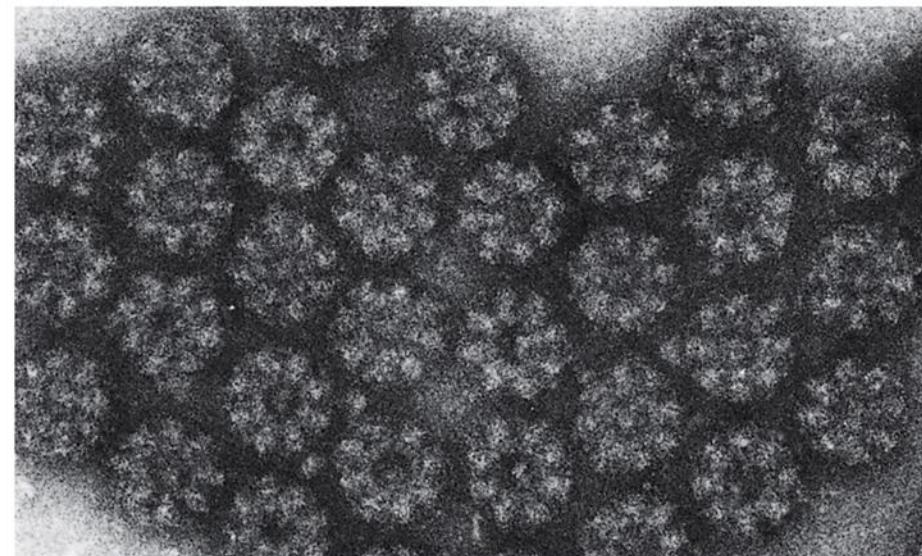
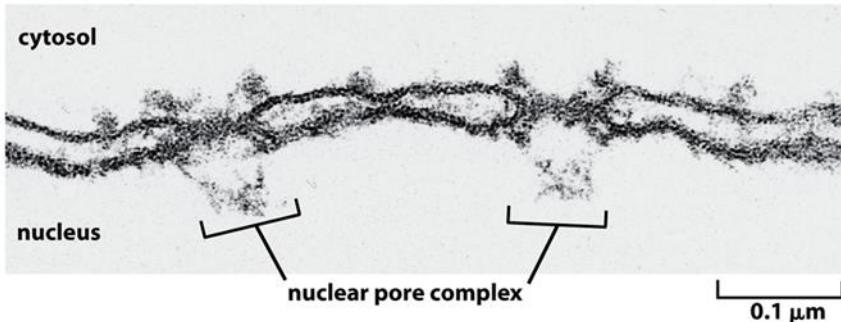
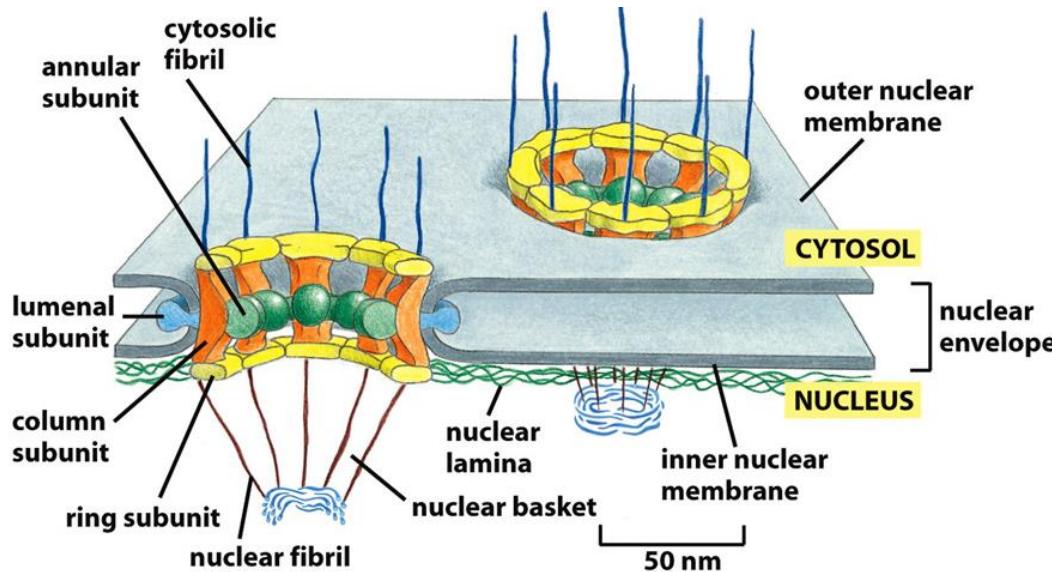
Inner membrane:

anchor sites for chromatin  
and for the nuclear lamina.

Outer membrane:

continuation of ER with  
ribosomes

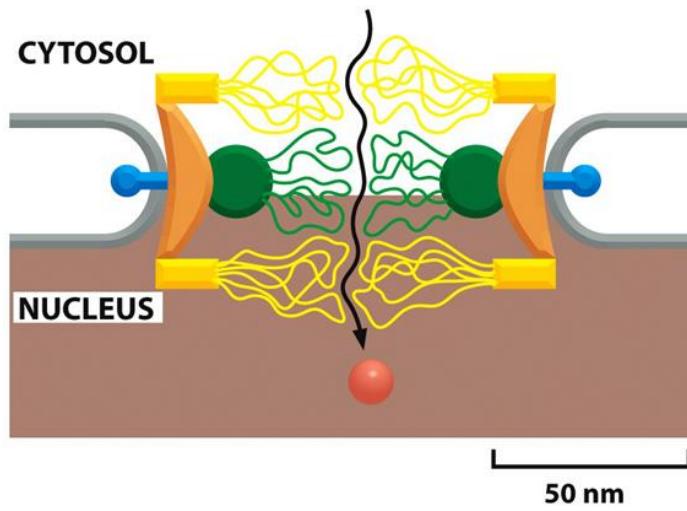
# Nuclear pore complex (NPC)



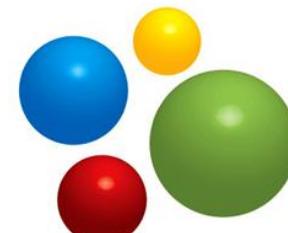
0.1 μm

# Features of nuclear-cytosol transport

1. typical 3000-4000NPC/cell
2. octagonal symmetry.
3. each NPC is a protein complex containing ~30 different proteins
4. Transport at amazing speed: ~500 macromolecules/sec, in bidirectional manner
  - small molecules (<5kD) passive diffusion freely
  - big molecules (>60KD) can not enter by passive diffusion.



size of molecules  
that enter nucleus  
by free diffusion



size of macromolecules  
that enter nucleus  
by active transport

# Nuclear import and export need special proteins and sequences

- **Nuclear import:**
  - ♥ nuclear localization signal
  - ♥ nuclear import receptors
- **Nuclear export:**
  - ♥ nuclear export signal
  - ♥ nuclear export receptor

protein that has both nuclear import and export signals  
is called **shuttling protein**

# Nuclear localization signal (NLS) directs proteins transported into the nucleus

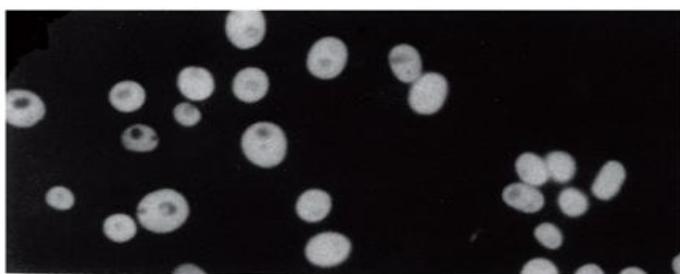
- One or two short sequences that are rich in K and R
- Can be anywhere in the amino acid sequence.
- Can form loops or patches on the protein surface

Mutation of NLS key amino acids will lose its nuclear localization



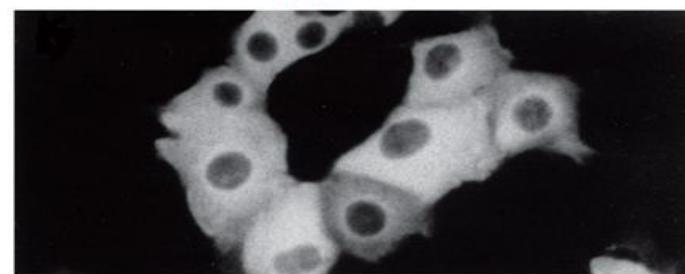
(A) LOCALIZATION OF T-ANTIGEN CONTAINING ITS NORMAL NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Lys — Lys — Arg — Lys — Val —



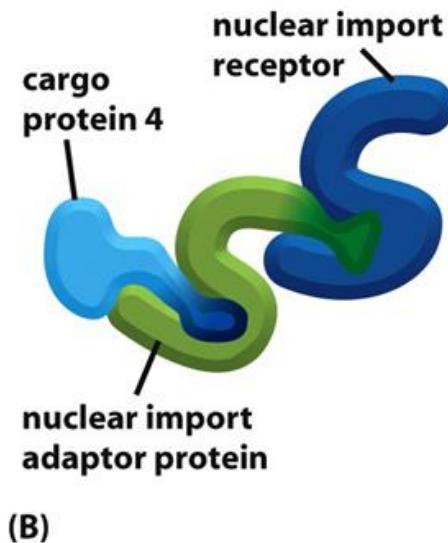
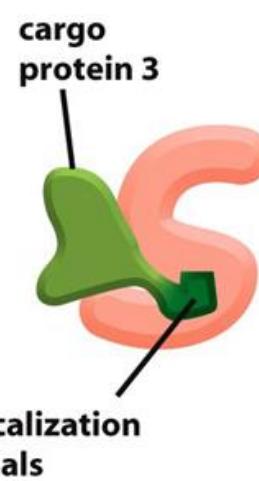
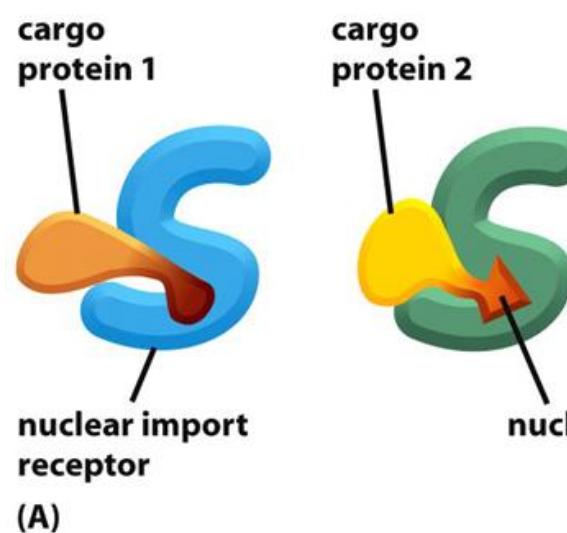
(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Thr — Lys — Arg — Lys — Val —

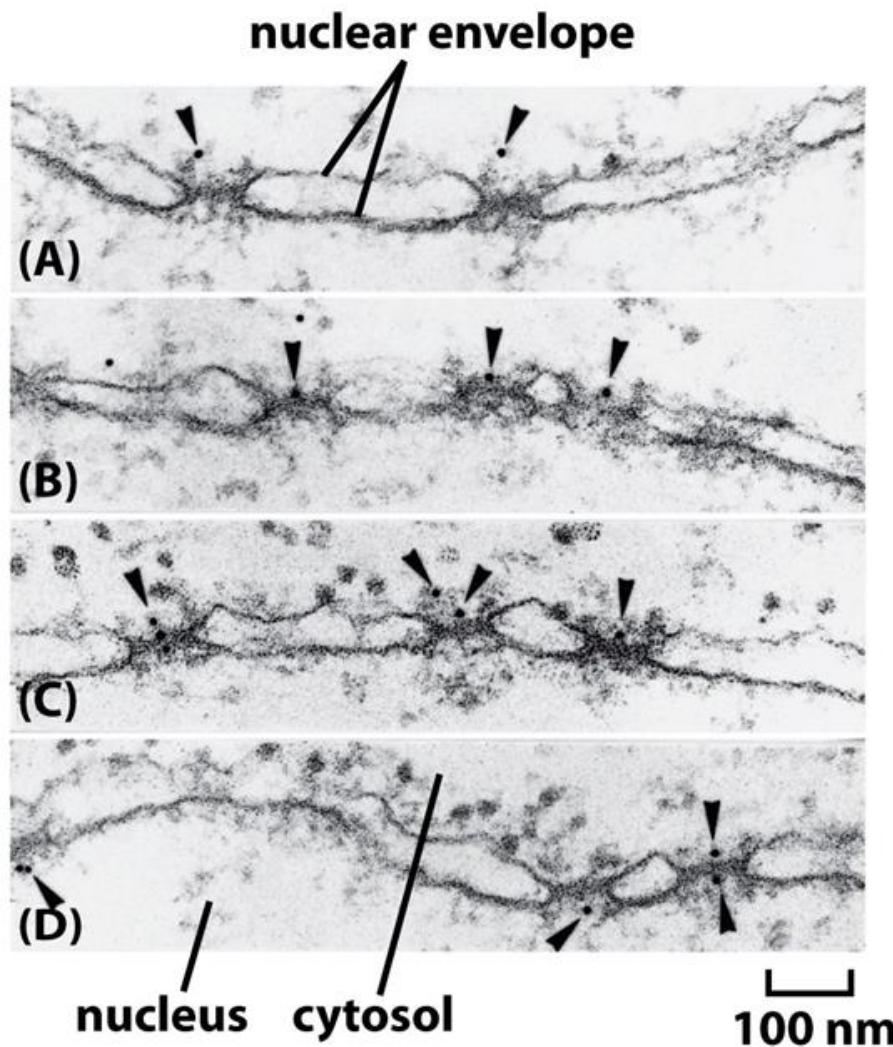


# Nuclear import receptors

- Cytosolic protein
- Bridging NLS-cargo and FG-repeats of nucleoporin proteins
- Some use adaptors for the bridging



# Visualizing active import through NPCs



Coat Gold particle with NLS

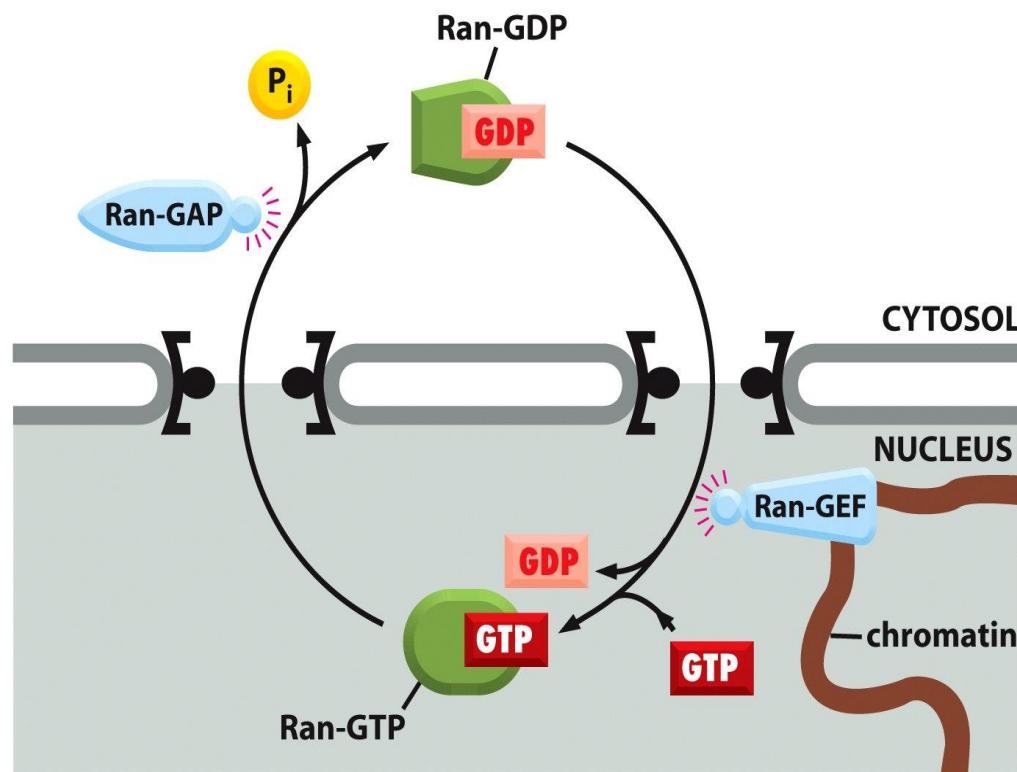
Track gold particle movement  
under electron microscope

Gold particle can be seen:  
1. interacts with NPC.  
2. gets through NPC.

## Nuclear export

- ♥ Nuclear export signals relatively poorly characterized
- ♥ Some have Leucine rich sequence.
- ♥ Nuclear export receptor facilitates export, which is structurally related to nuclear import receptor.

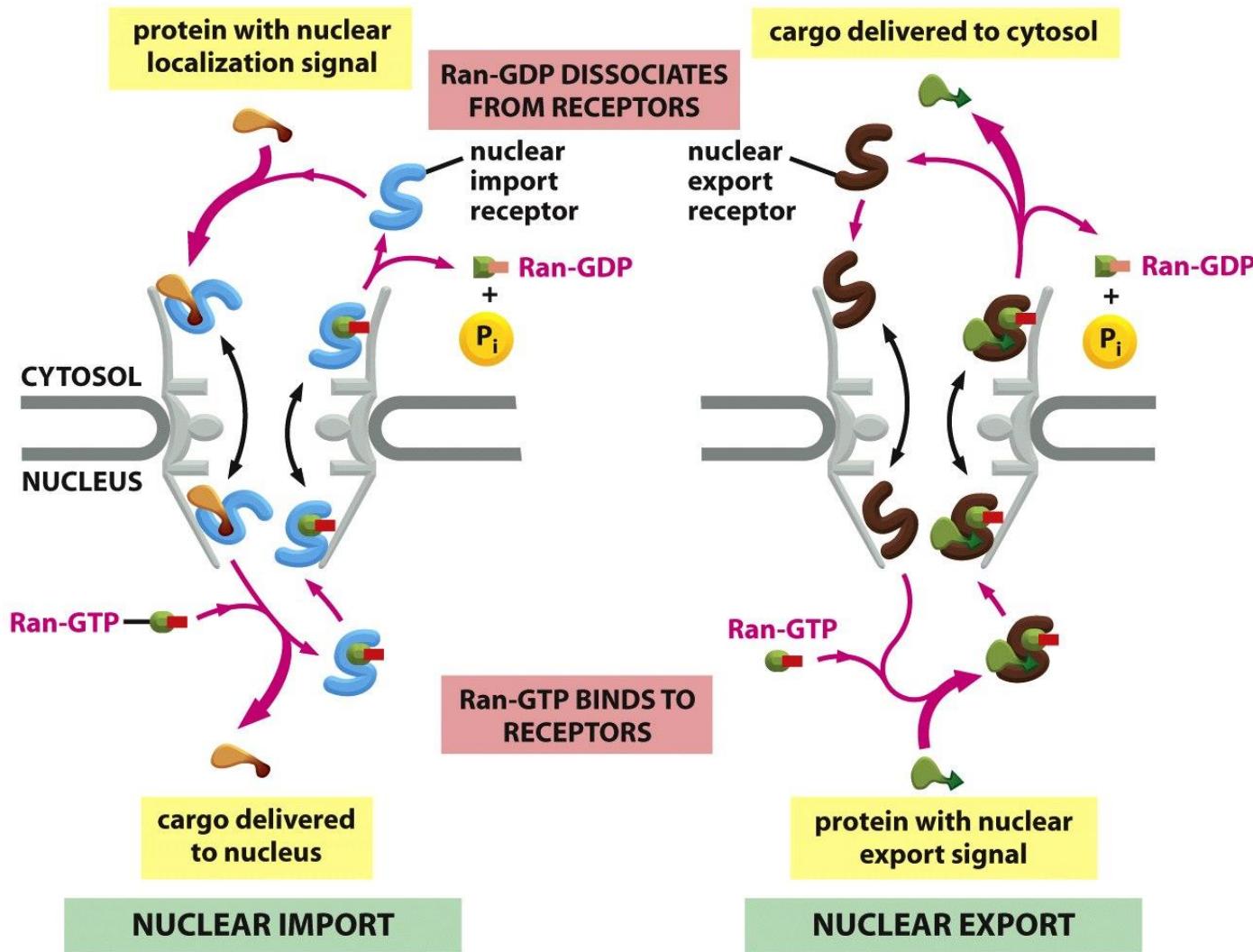
# Nuclear import/export is energy-consuming and is controlled by Ran GTPase



Ran-GAP(GTPase-activating protein)  
Locates in cytosol, converts Ran-GTP  
To Ran-GDP

Ran-GEF (Guanine exchange factor)  
Locates in nucleus, converts Ran-GDP  
To Ran-GTP

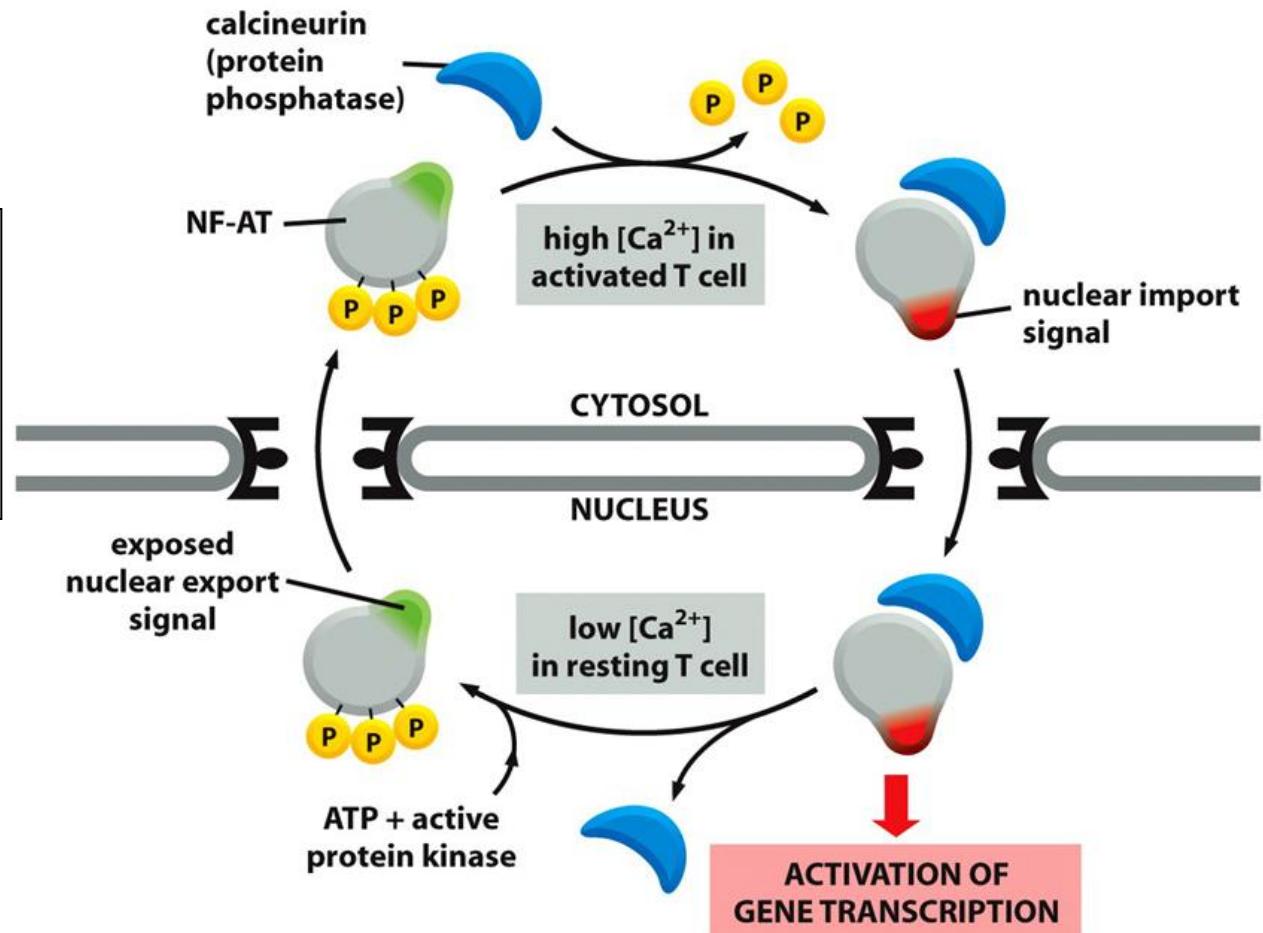
# Ran controls both nuclear import and nuclear export



# Ran-independent nuclear transport-through protein phosphorylation

For example:

Nuclear import and nuclear export signal can be masked by its phosphorylation states.

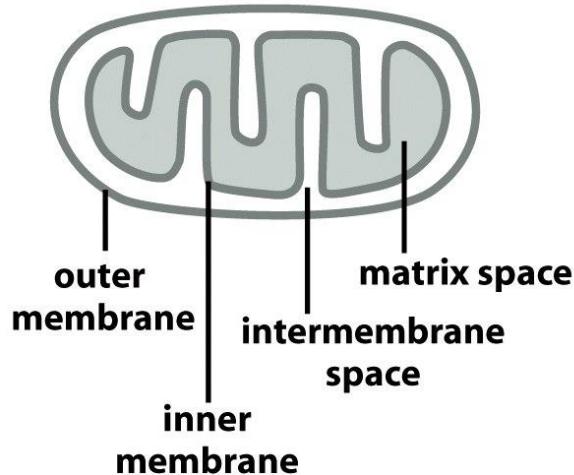


### III. Transport in mitochondria and chloroplast

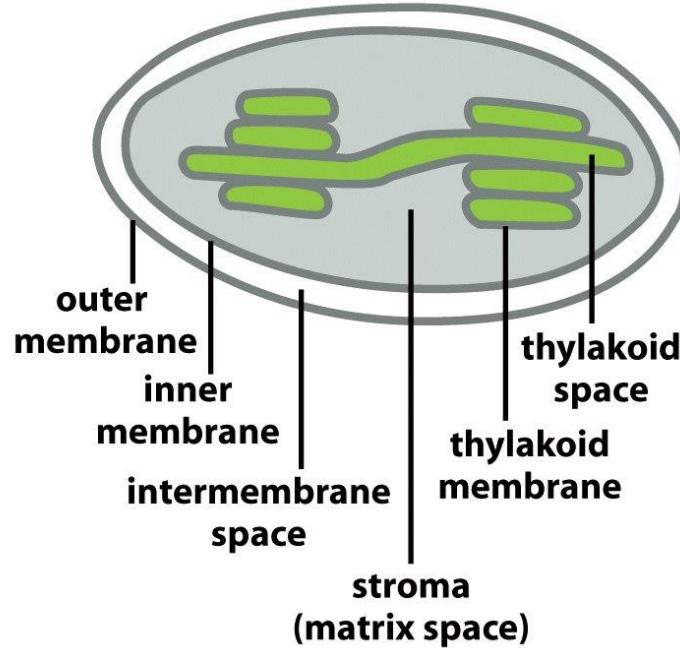
- Even though mitochondria and chloroplast have their own protein synthesis, majority of protein synthesis is completed in the cytosol and then they are transported in.

# Membrane in Mitochondrion and chloroplast

(A) MITOCHONDRION



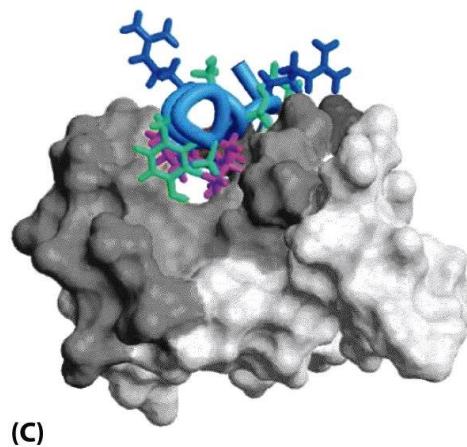
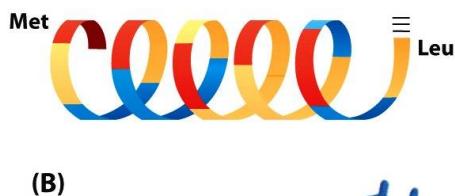
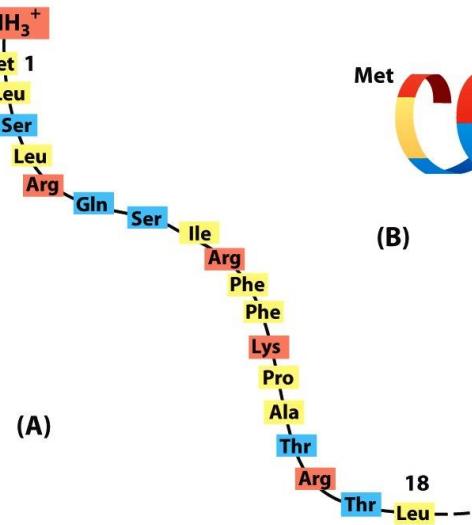
(B) CHLOROPLAST



# Signal peptide for mitochondrion

- ♥ Mitochondria precursor proteins are fully synthesized before transporting
- ♥ N-terminal peptide directs many precursor proteins to be in the matrix, then quickly removed.
- ♥ Internal peptide directs all precursor proteins to be in the outer membrane, many to be in the inner membrane and intermembrane

# Signal sequence of mitochondria--- an amphiphilic $\alpha$ -helix



One side: hydrophilic  
The other side: hydrophobic

# Protein translocators--- facilitate mitochondria protein across membrane

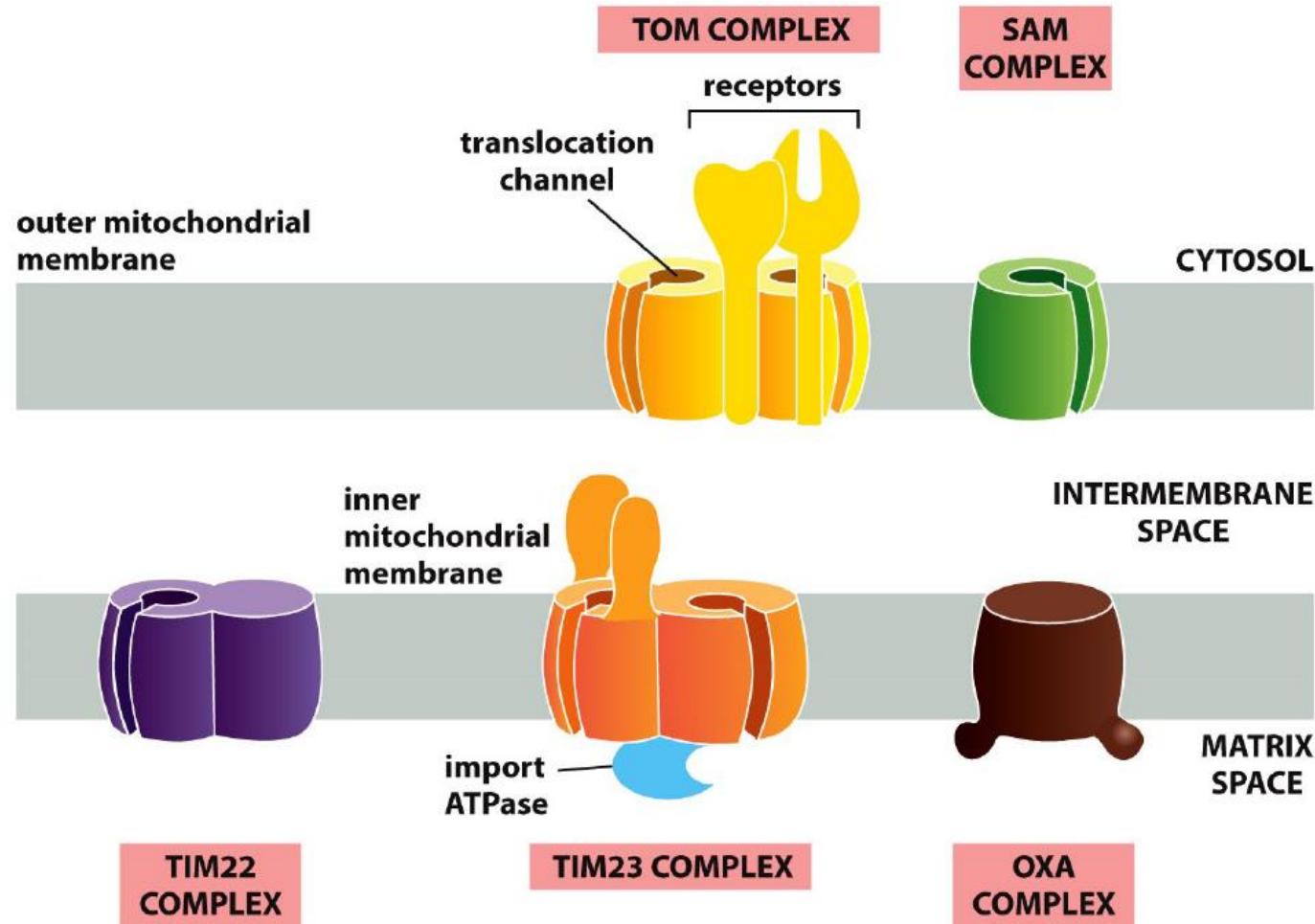


Figure 12-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

# All translocators are multimeric proteins

TOM complex---transfer proteins across the outer mitochondria membrane.

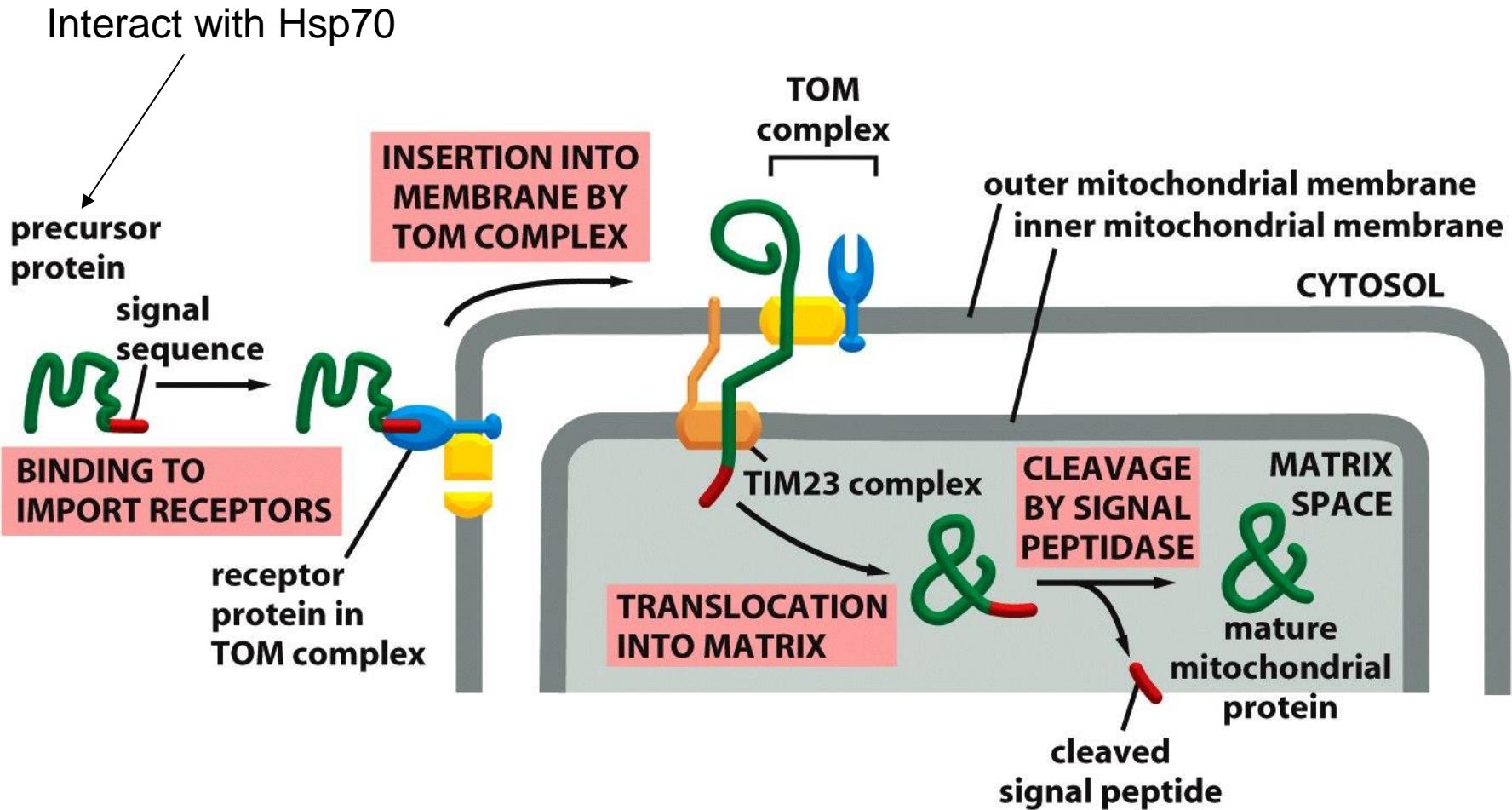
TIM complex--- transfer proteins across the inner membrane.

Both functions as receptors for signal peptides and form transmembrane channels

SAM complex---help to fold properly the  $\beta$ -barrel proteins in outer membrane.

OXA complex--- help to insert mitochondria-synthesized inner membrane protein and others transported from the matrix.

# Protein import in mitochondria



# ATP and membrane potential drive protein import into the matrix space

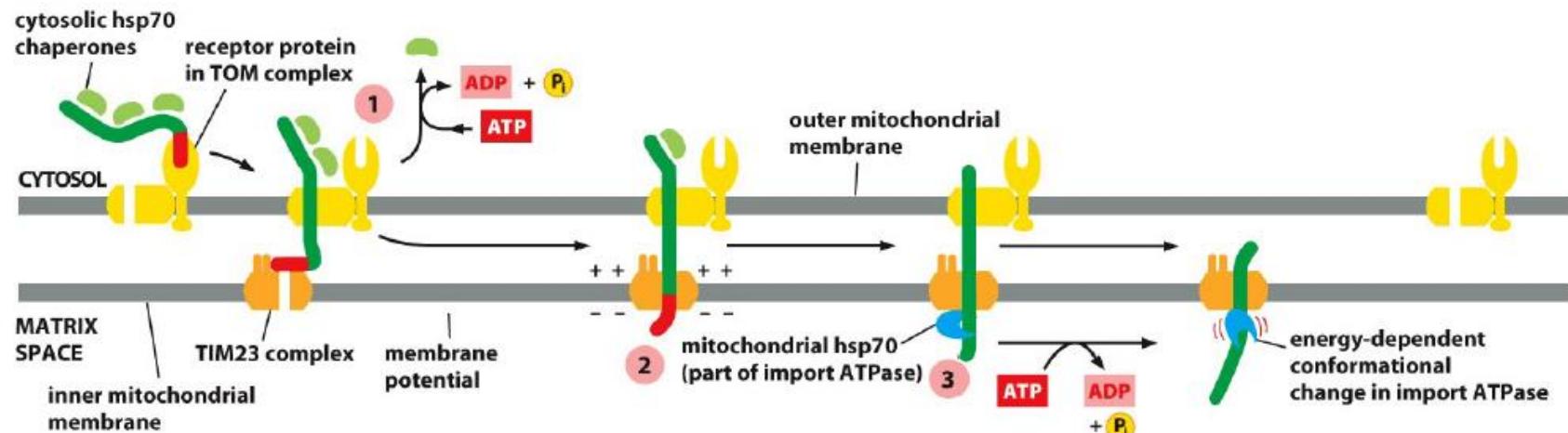
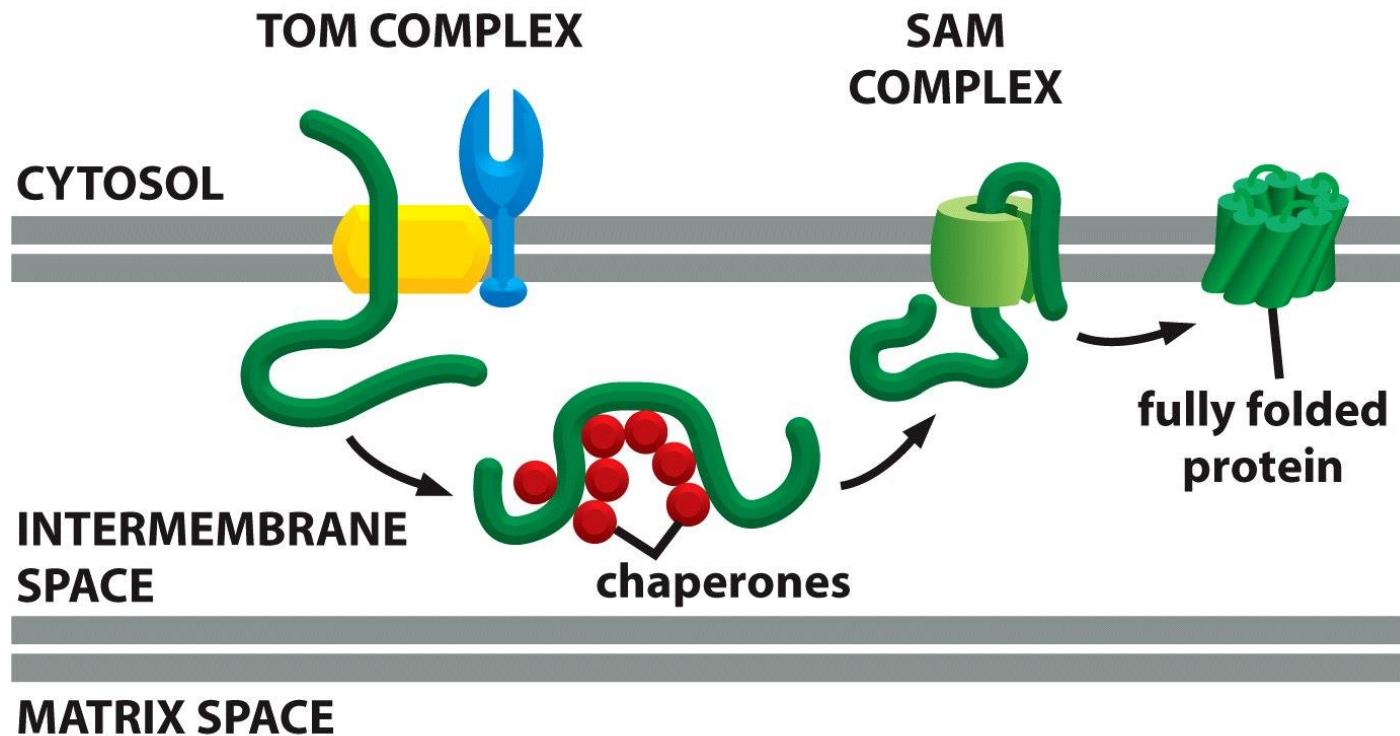


Figure 12-23 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Outer membrane porins are inserted by the help of SAM in intermembrane space



# Bacteria and mitochondria use similar mechanisms to insert their porins into their outer membrane

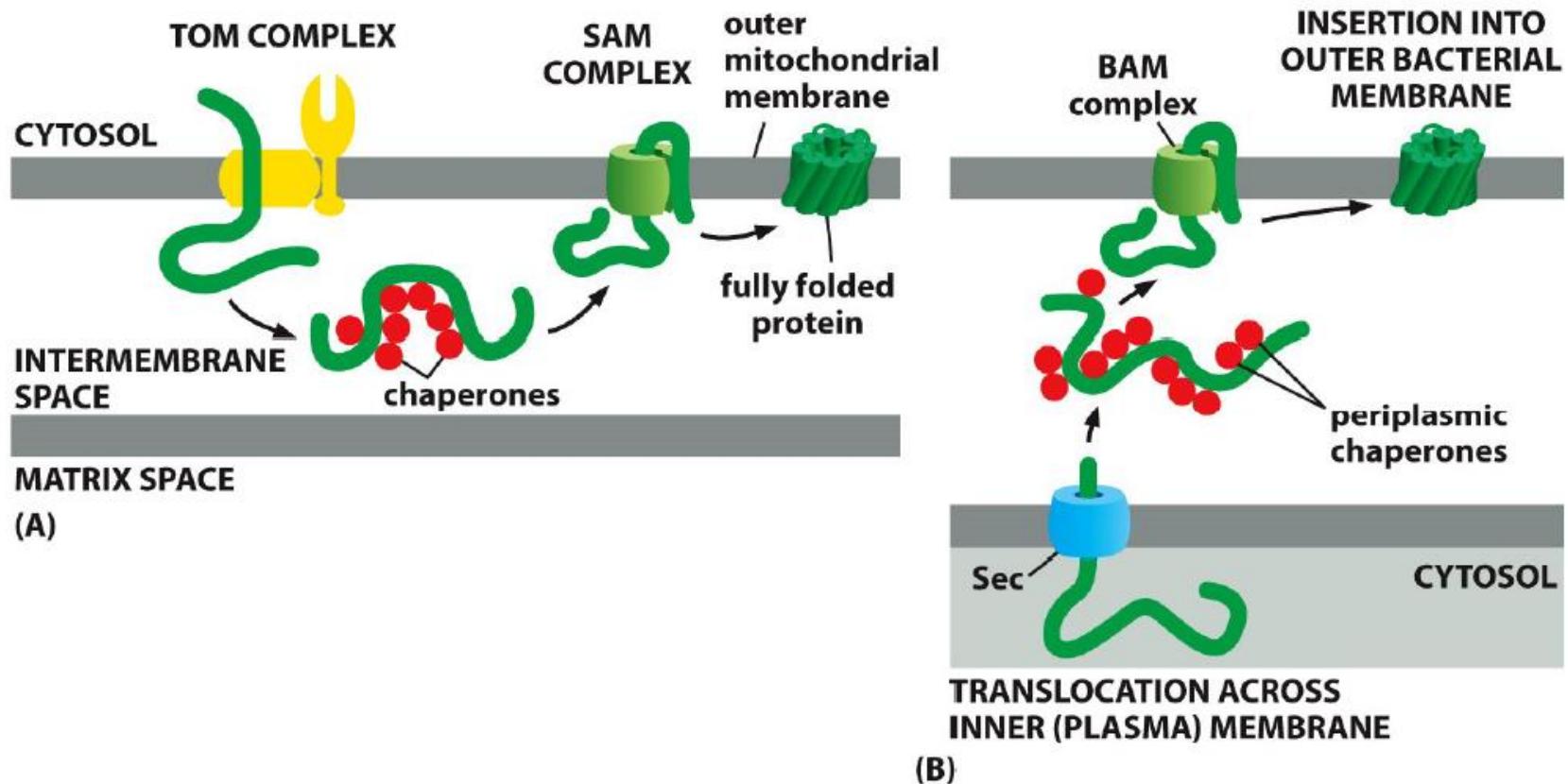
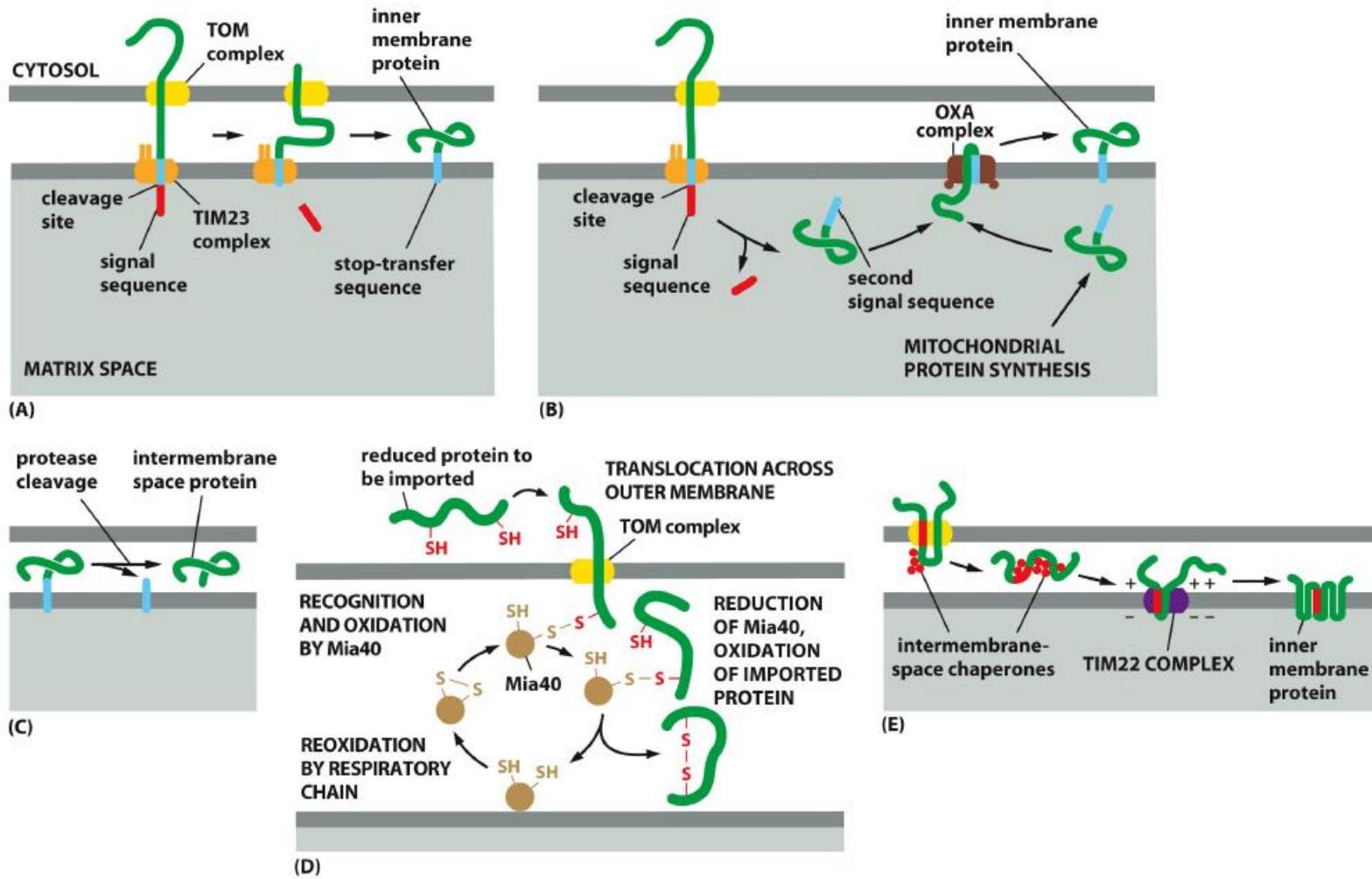


Figure 12-24 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Protein imported into inner membrane and intermembrane space



# Protein imported into inner membrane and intermembrane space

Type 1:

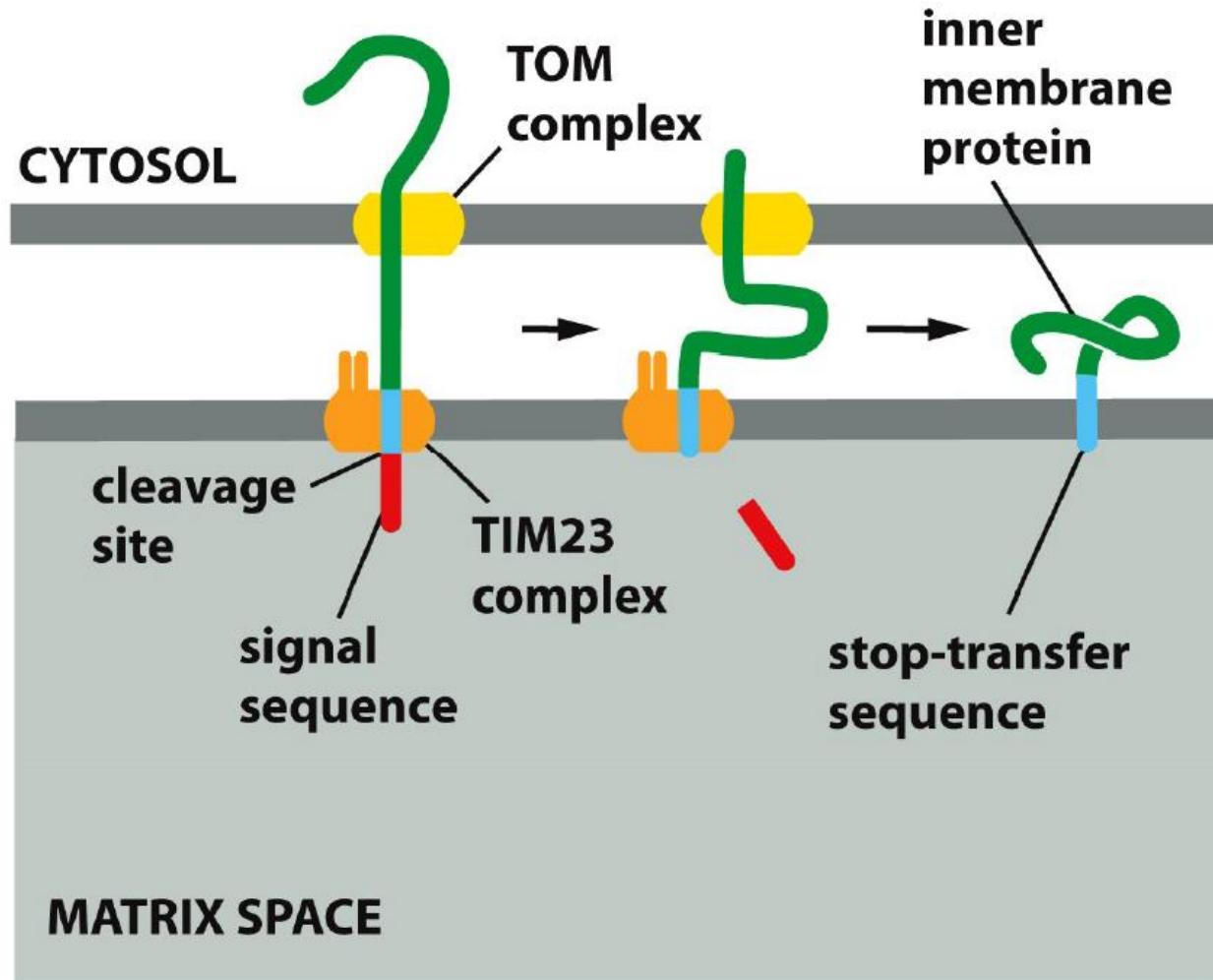
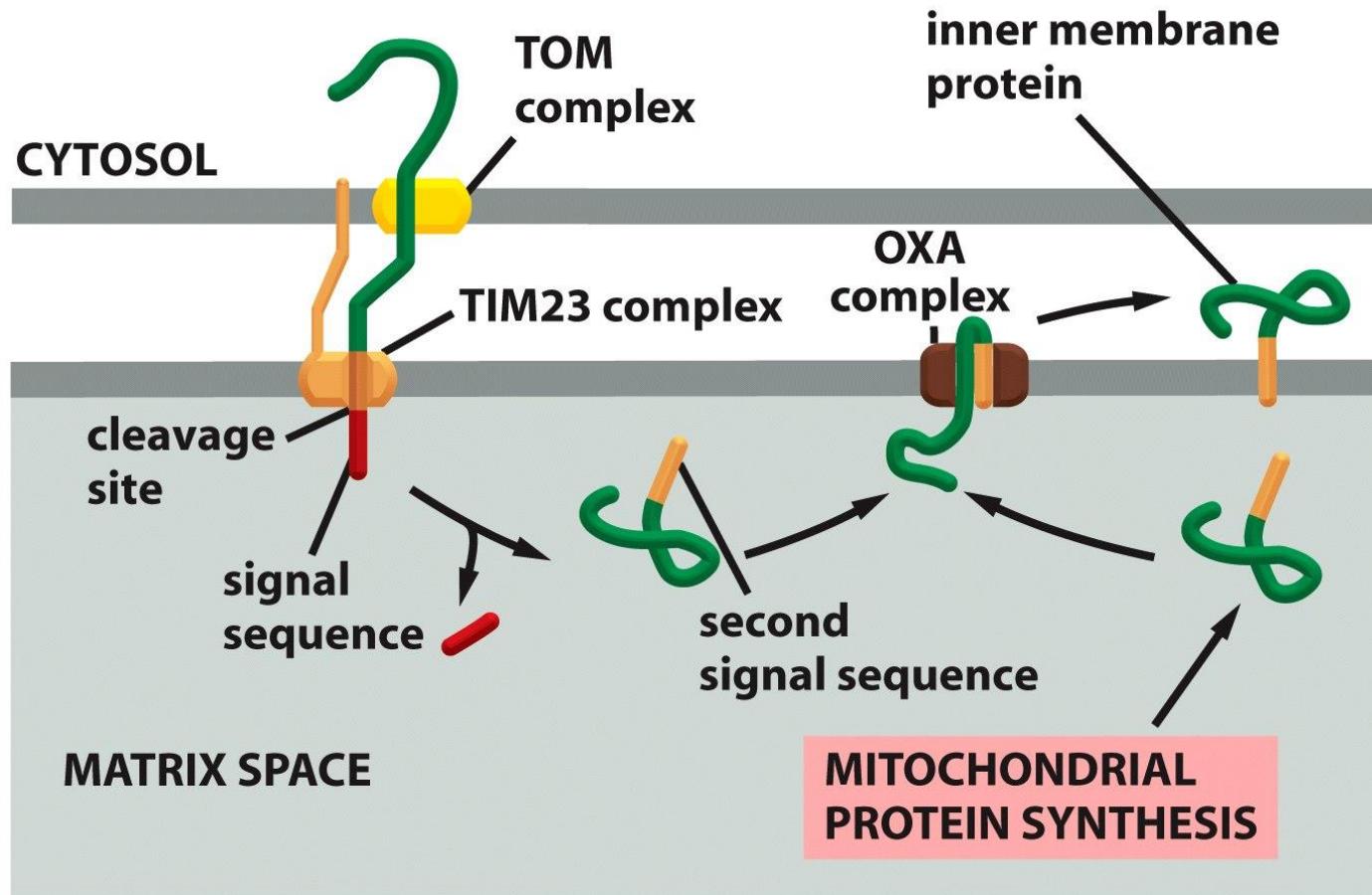


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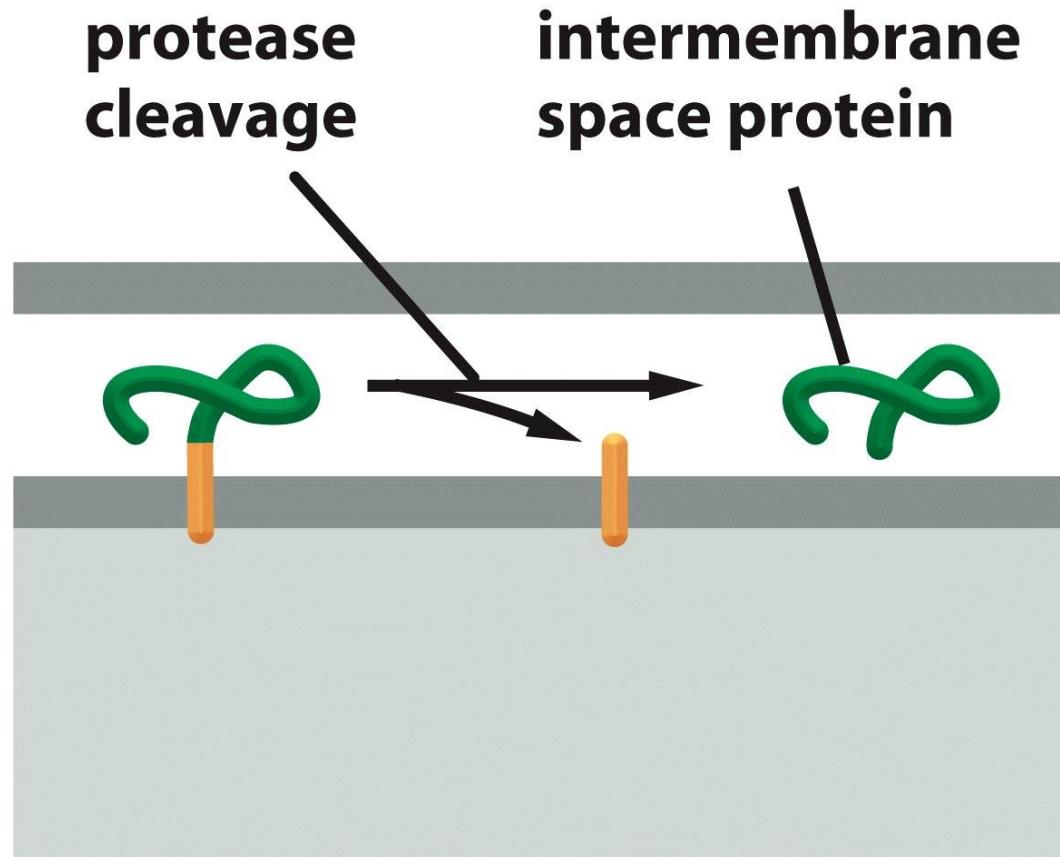
# Protein imported into inner membrane and intermembrane space

Type 2:



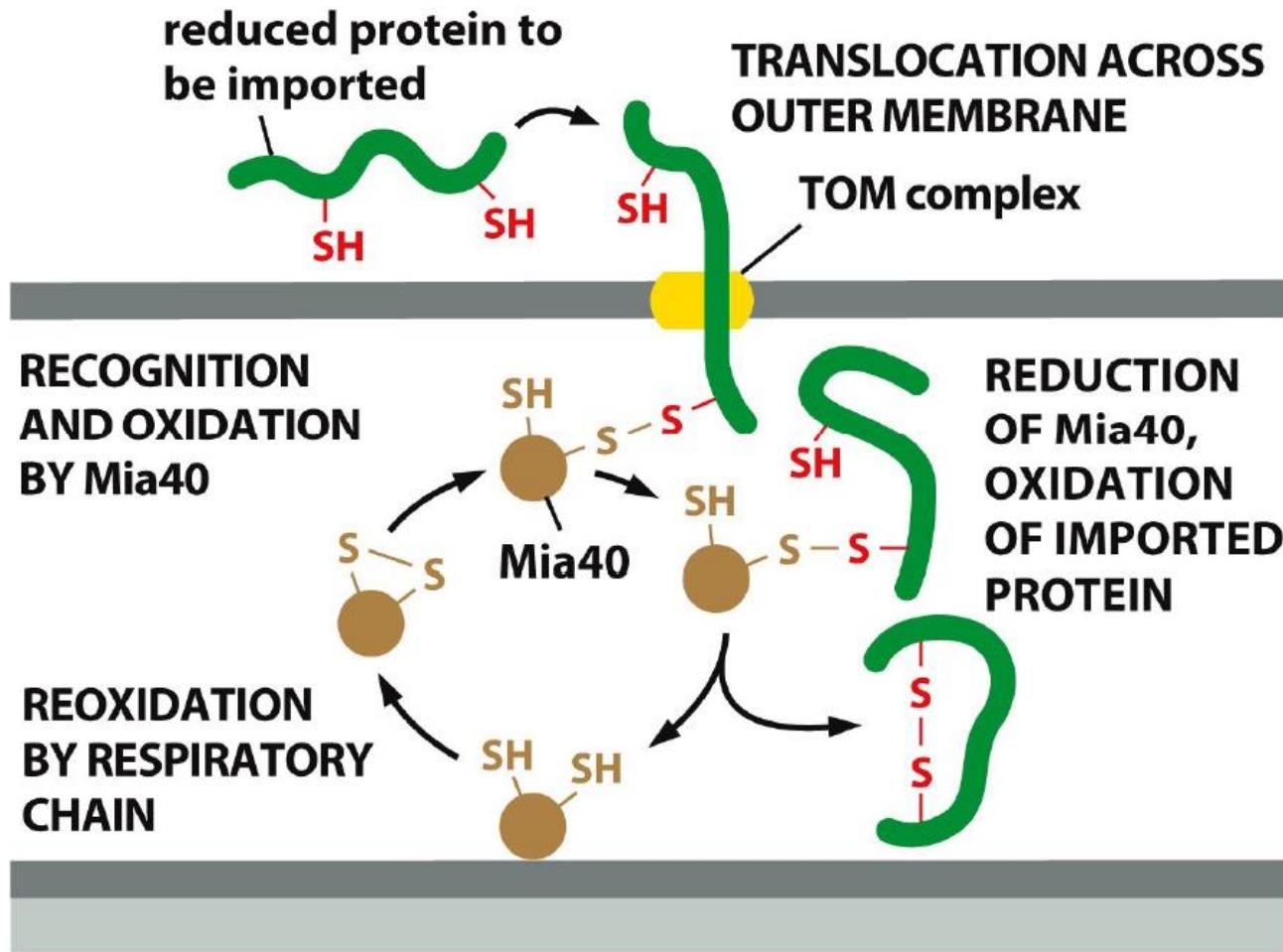
# Protein import into inner membrane and intermembrane space

Type 3: intermembrane space proteins



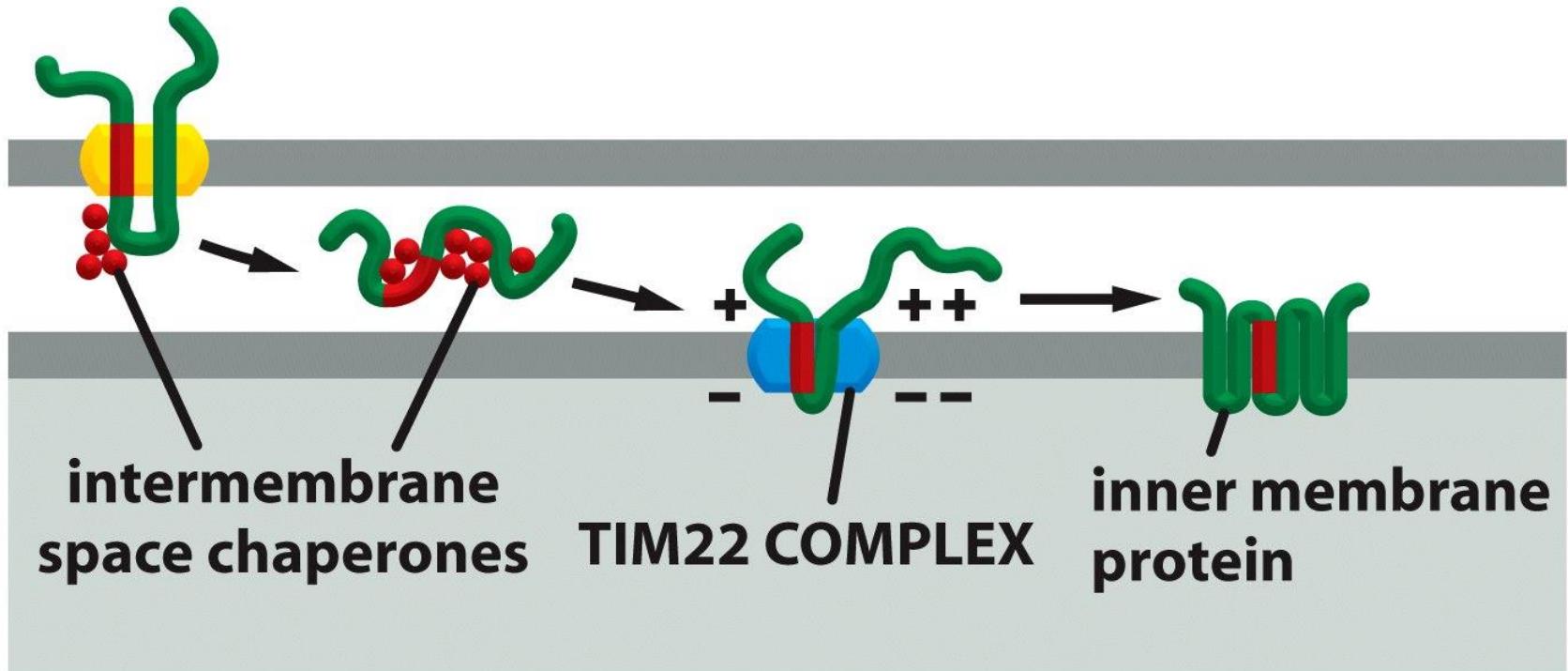
# Protein import into inner membrane and intermembrane space

Type 4: intermembrane space proteins



# Protein import into inner membrane and intermembrane space

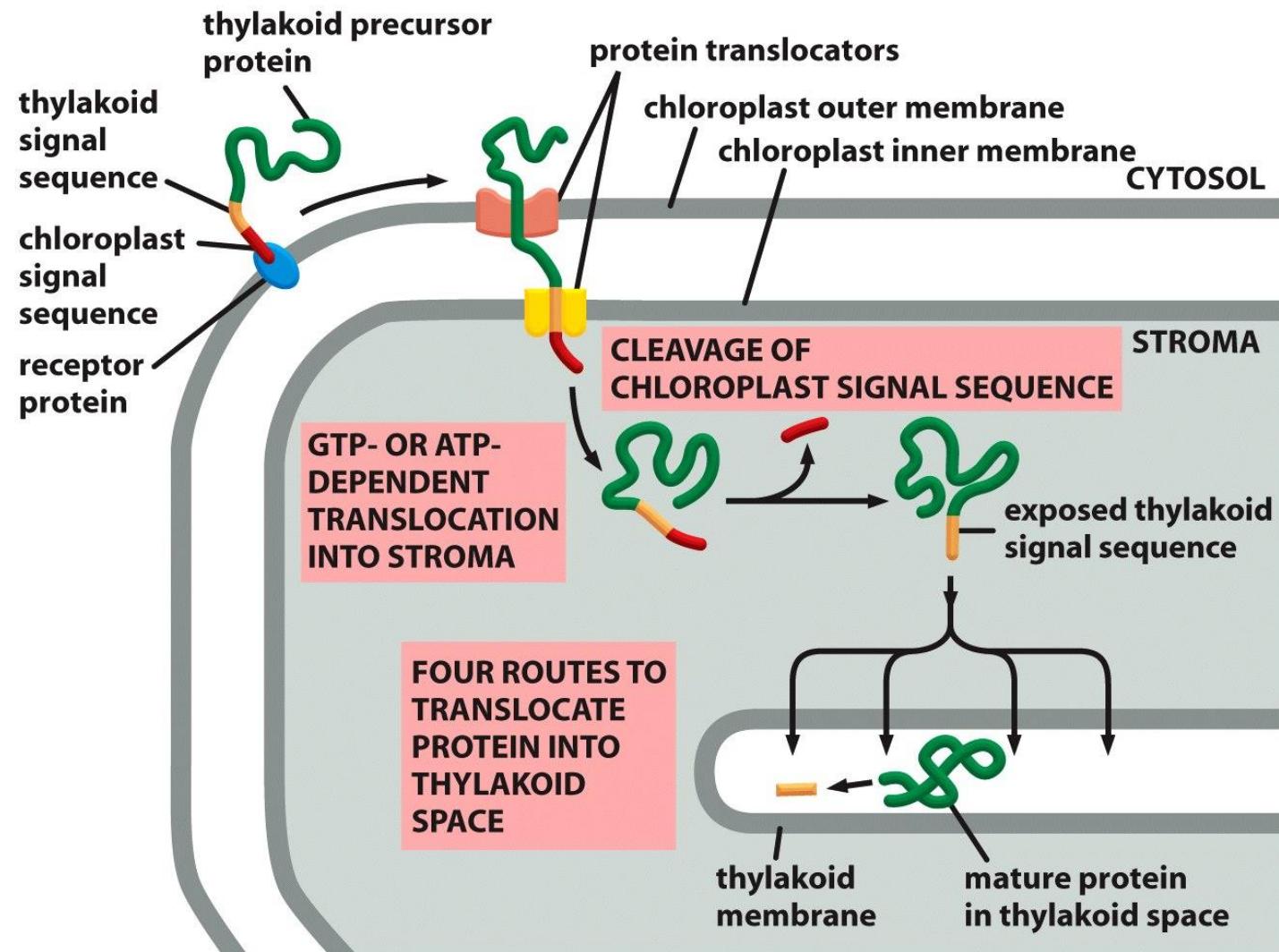
Type 5: inner membrane transport proteins



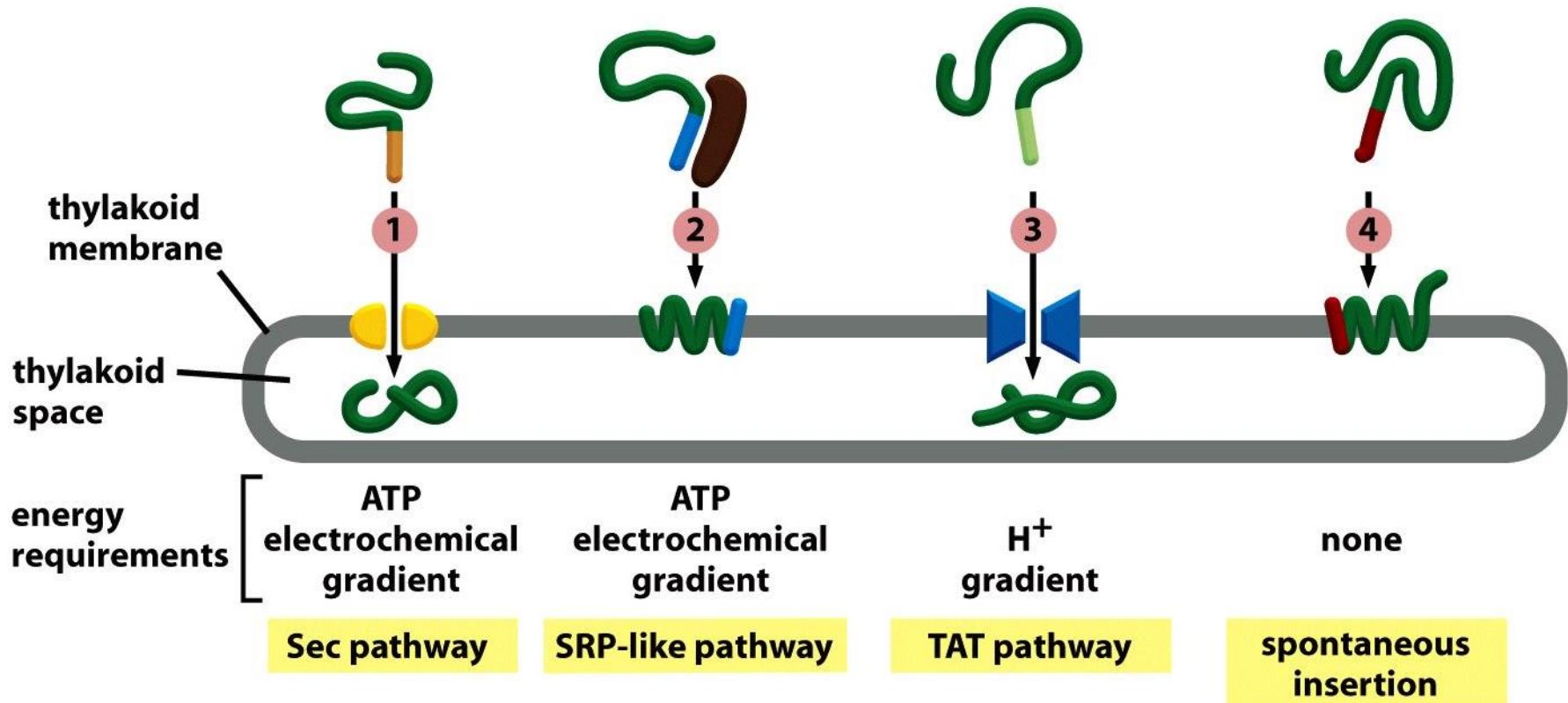
## Membrane protein transport in chloroplast

- Two signal peptides directs proteins to the thylaloid, one is thylakoid-specific.
- Import receptors are chloroplast–specific.
- No electrochemical energy-driven, consumption of ATP and GTP instead.

# Translocation of chloroplast precursor proteins into the thylakoid space



# Translocation into the thylakoid space or thylakoid membrane by 4 routes

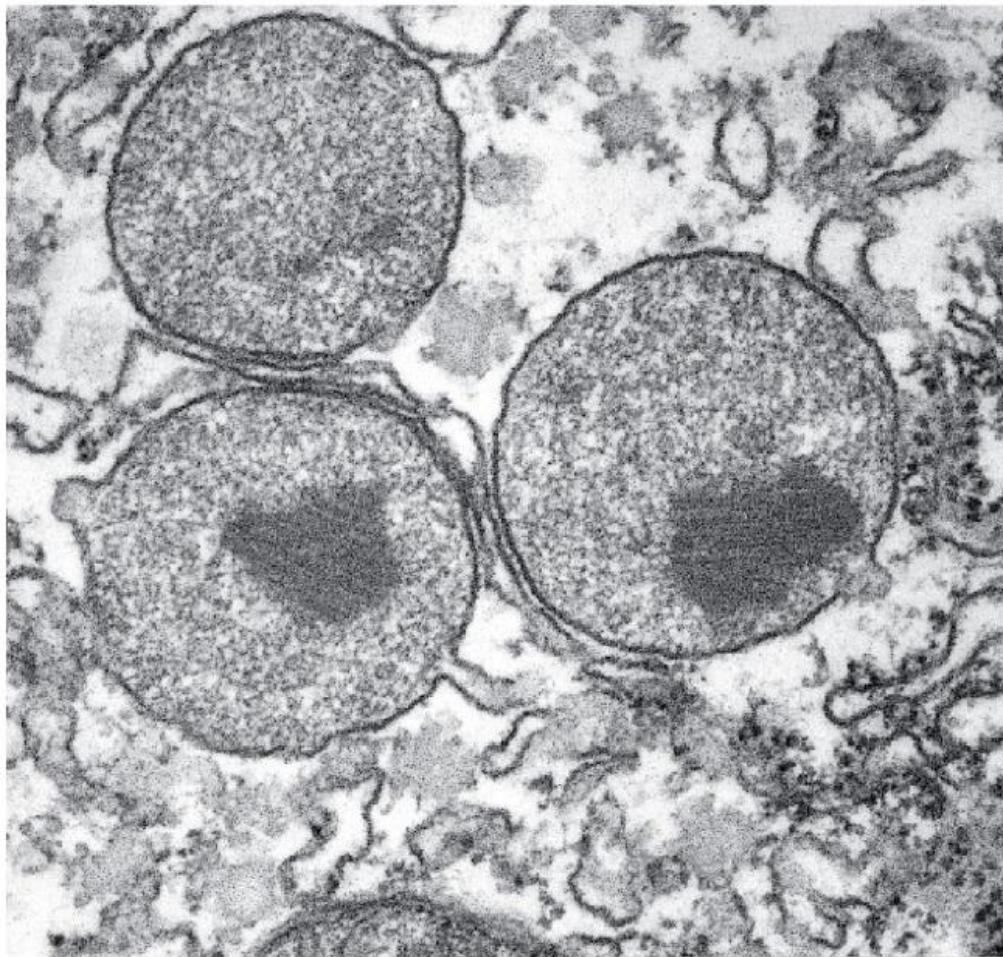


## IV. Transport in peroxisomes

### overview of peroxisomes:

- All eukaryotic cells have peroxisomes
- Contains catalase and oxidase.
- Acquire most protein from cytosol, some from ER.
- Use O<sub>2</sub> to produce hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)
- Catalase use H<sub>2</sub>O<sub>2</sub> to oxidize substances, such as alcohol
- Oxidize fatty acid by β- oxidation, producing acetyl-CoA.
- Catalyze the first reaction for the formation of plasmalogens ( important substance for myelin sheath).

# Peroxisomes in rat liver cell under EM



200 nm

# Peroxisomes in plant cells

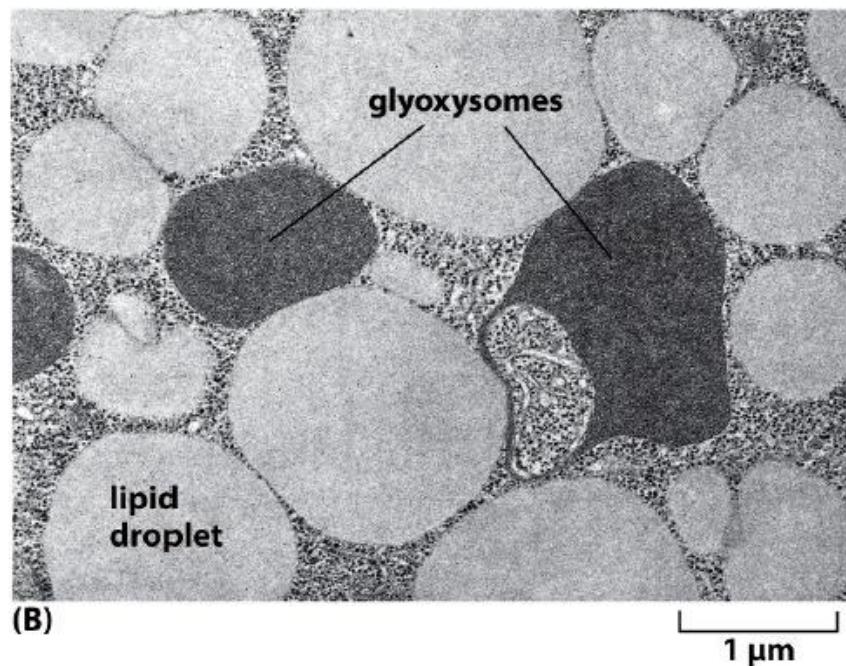
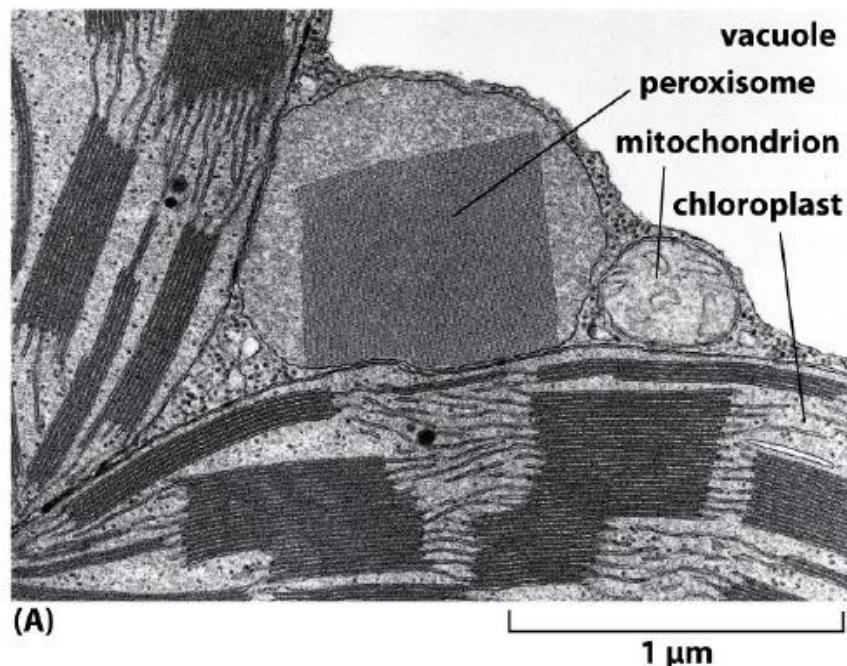
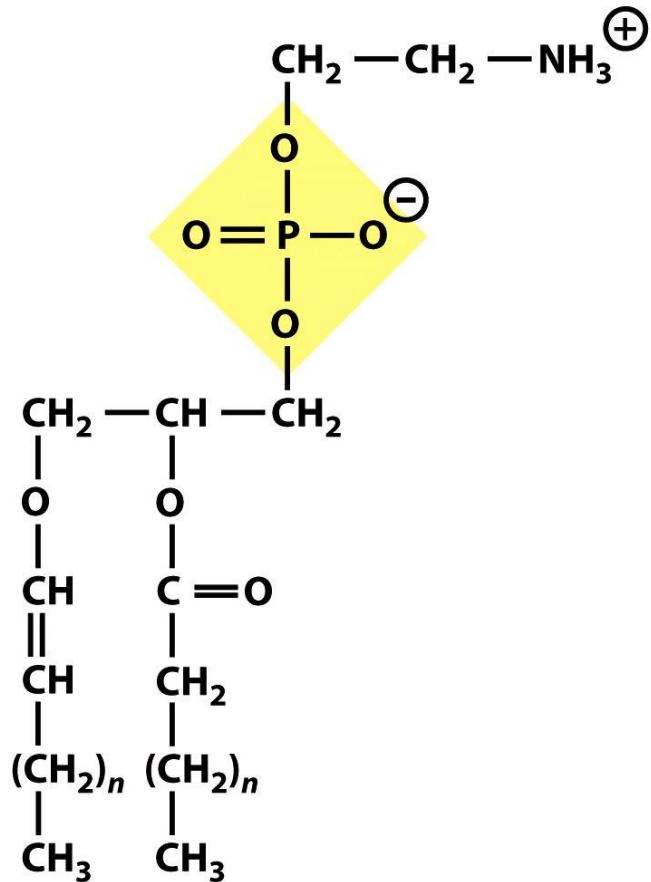


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# Peroxisome is important to synthesize plasmalogen



Important substance for sheath myelin to protect nerve cells.

# How new peroxisomes arise

--- may apply to other subcellular organelles

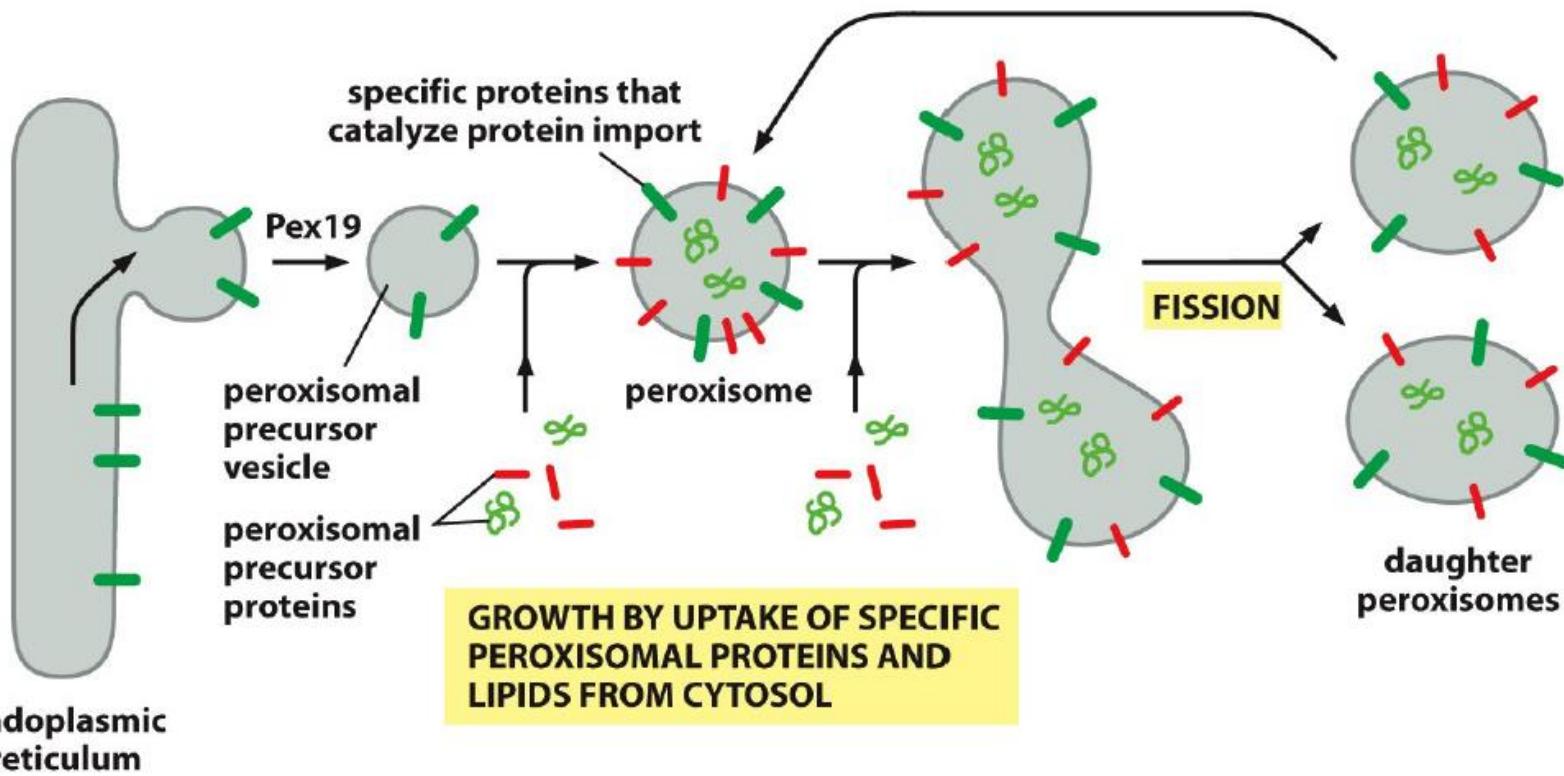


Figure 12-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Transport in peroxisomes

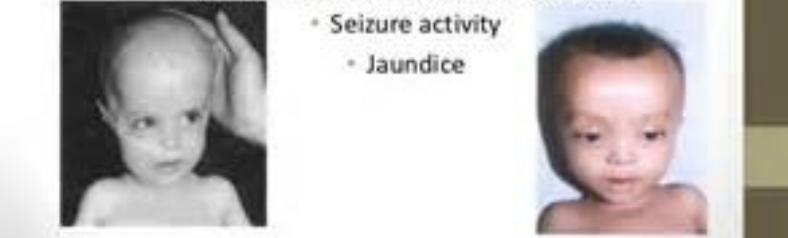
- Short signal peptide directs protein import into peroxisome.
- Cytosolic receptor protein recognize signal sequence.
- Several **Peroxins** dock on cytosolic surface as a membrane translocator
- Need ATP.

# *Zellweger syndrome*

- ◆ named after Hans Zellweger (1909–1990), a Swiss-American pediatrician
- ◆ inherited genetic disease due to mutation in peroxin
- ◆ severe abnormality in brain, liver and kidney
- ◆ rarely survives till 6-month old

## Physical Symptoms

- Defects in the face, development, or eyes
  - Up slanting eyes
  - High forehead
- Skin folds along the person's nasal borders of the space between the upper and lower eyelids of their eyes
- Loss of muscle tone/extreme weakness
  - Seizure activity
  - Jaundice



## V. Transport in Endoplasmic reticulum

ER: smooth ER and rough ER are functionally and structurally diverse

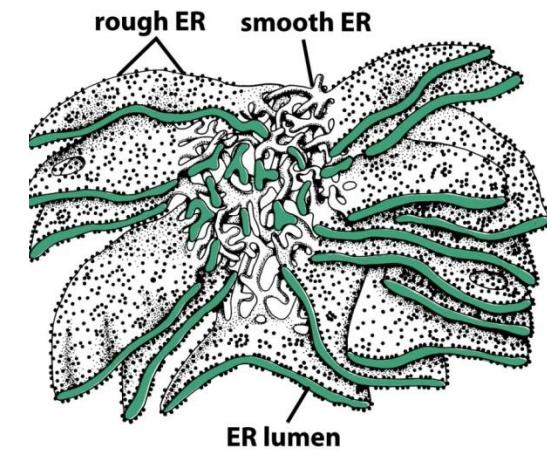
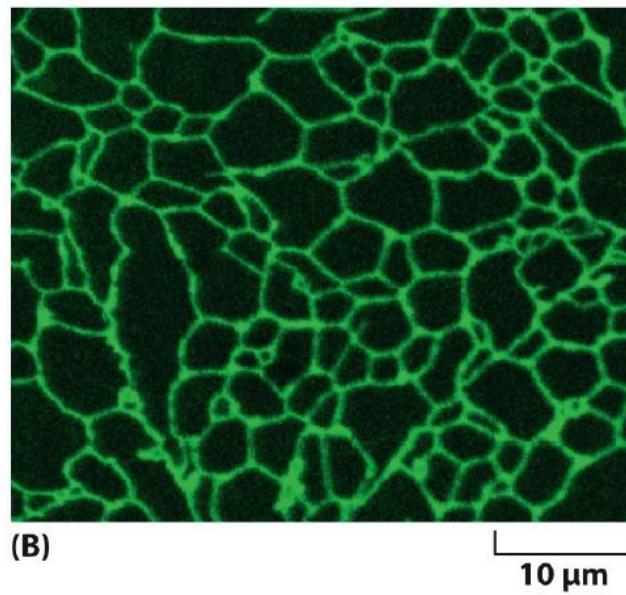
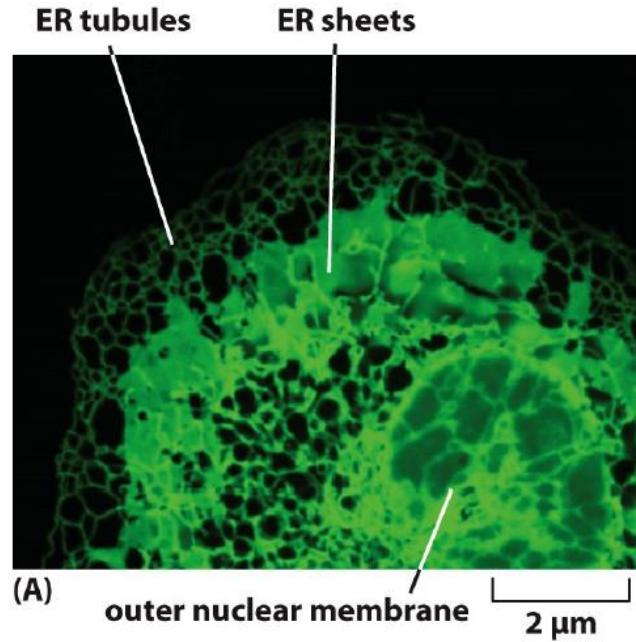


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# Transport in ER

- Co-translational: most proteins in ER (not all)
- Post-translational: mitochondria, chloroplasts, nuclei, peroxisomes

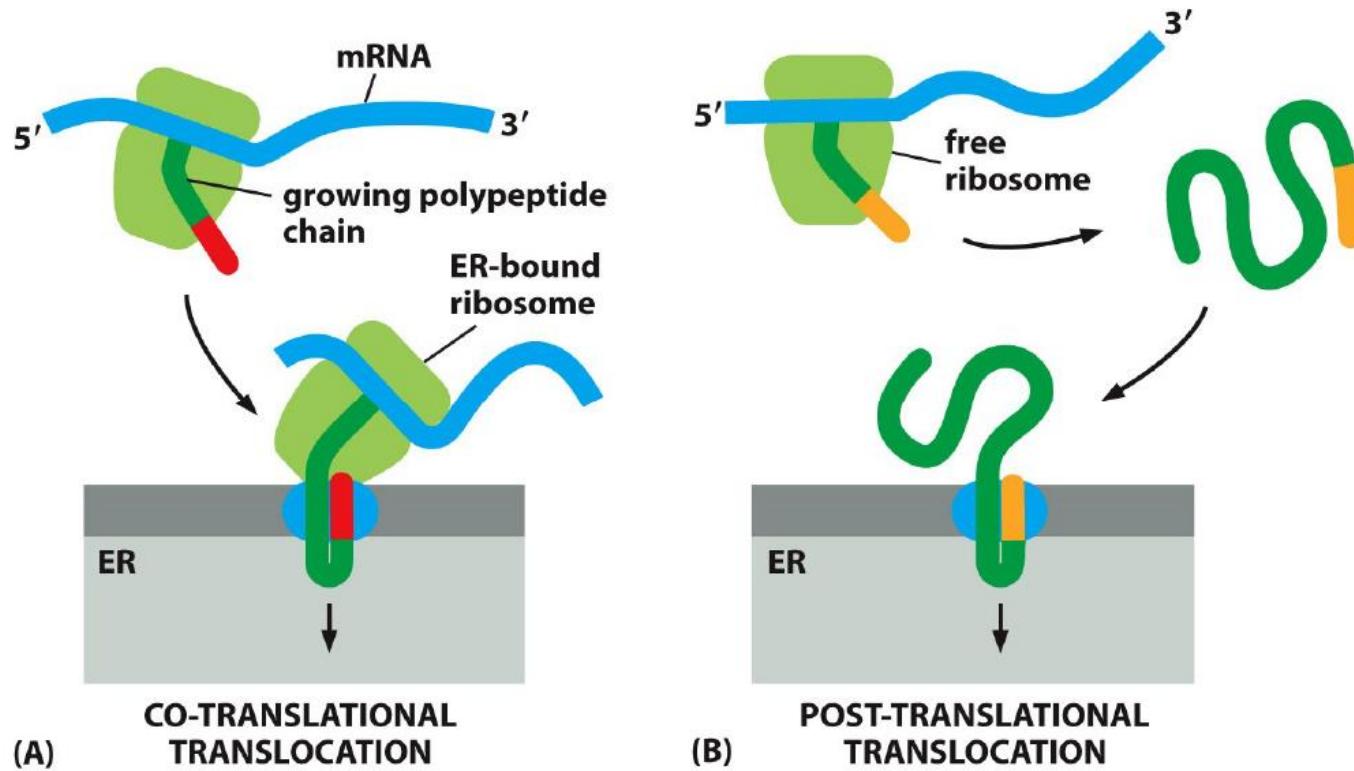
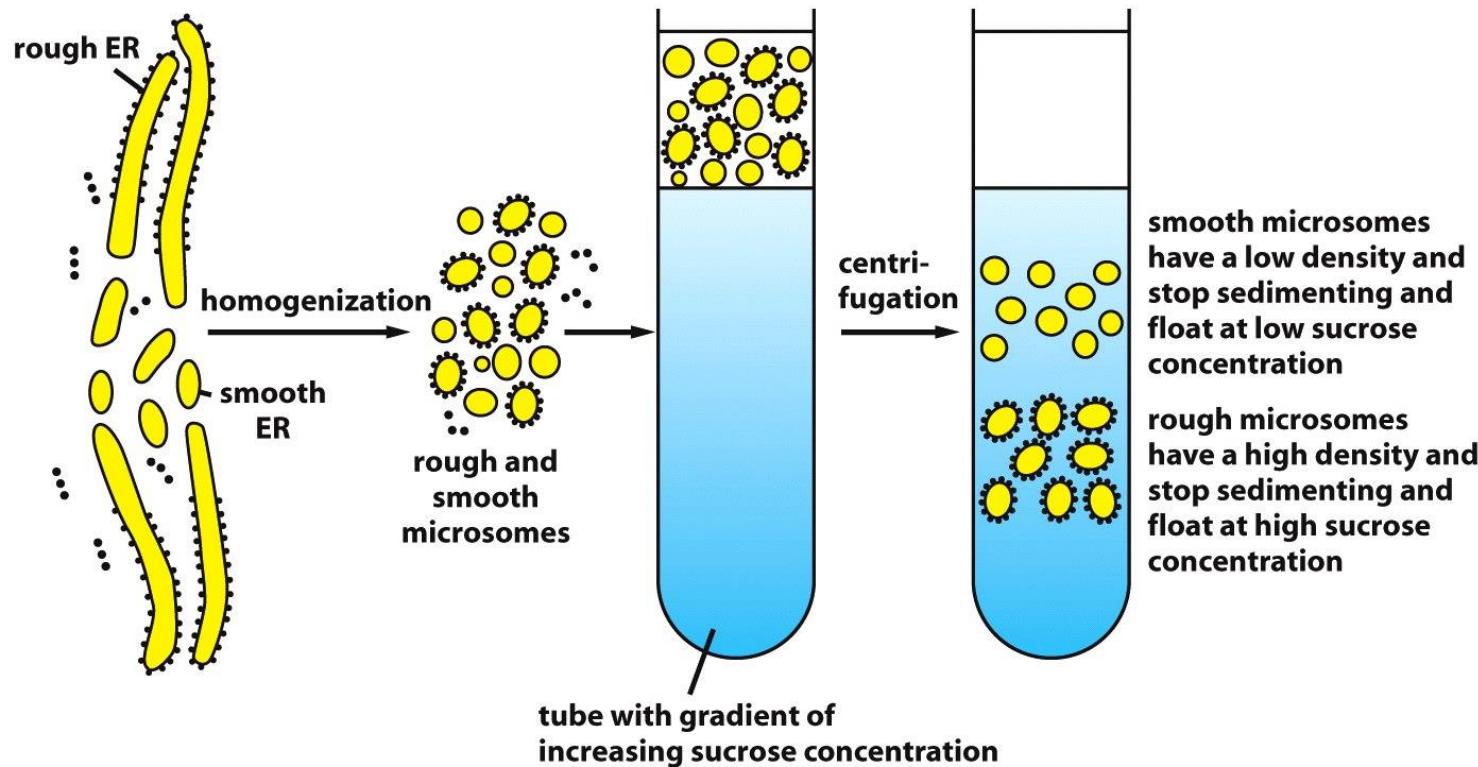


Figure 12-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Rough and smooth microsomes---enclosed mini-membrane system in cell homogenates

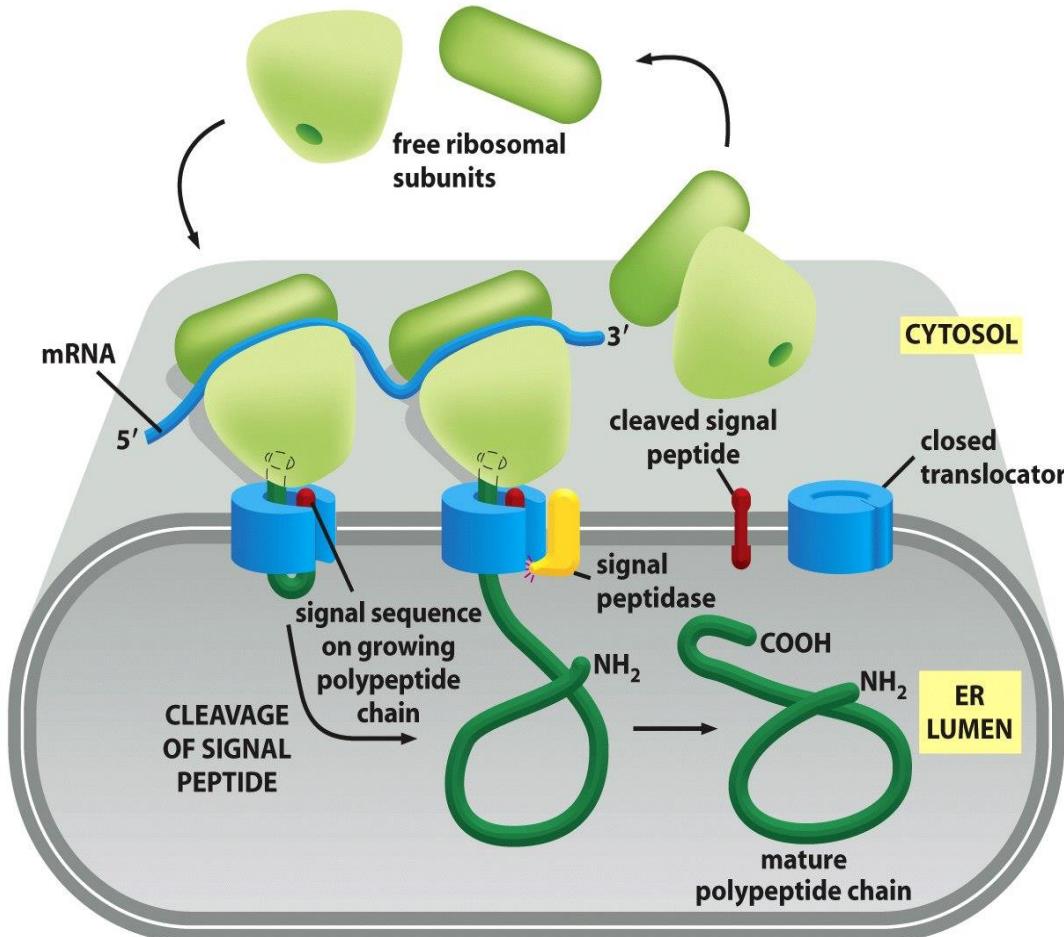


Rough microsomes: only from rough ER

Smooth microsomes: may from smooth ER, golgi apparatus, endosomes, plasma membrane, mitochondria, etc.

Microsomes: still capable of protein translocation, protein glycosylation, uptake of  $\text{Ca}^{2+}$  and release, lipid synthesis, etc.

# Signal sequences were first discovered in rough ER proteins



Cell free system with and without Microsomes show the protein in ER is larger without microsome because it has an extra signal peptide.

How is the signal peptide recognized?

How is protein targeted to ER and transported?

A signal recognition particle (SRP) directs  
the signal sequence to receptor in ER

# Signal Recognition Particle (SRP)

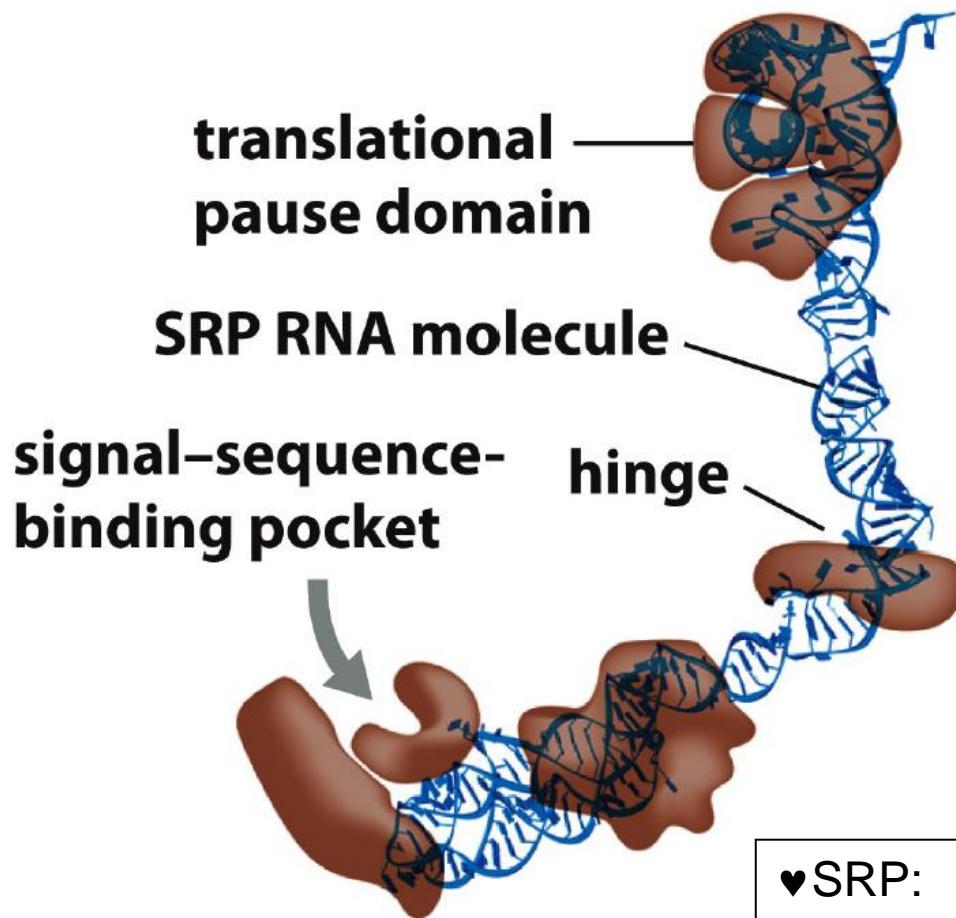


Figure 12-36a Molecular Biology of the Cell 6e (© Garland Science 2015)

♥SRP:  
cytosolic protein shuttle between ER  
and cytosol;  
6 subunits and a small RNA molecule

# SRP binds to signal peptide and ribosome

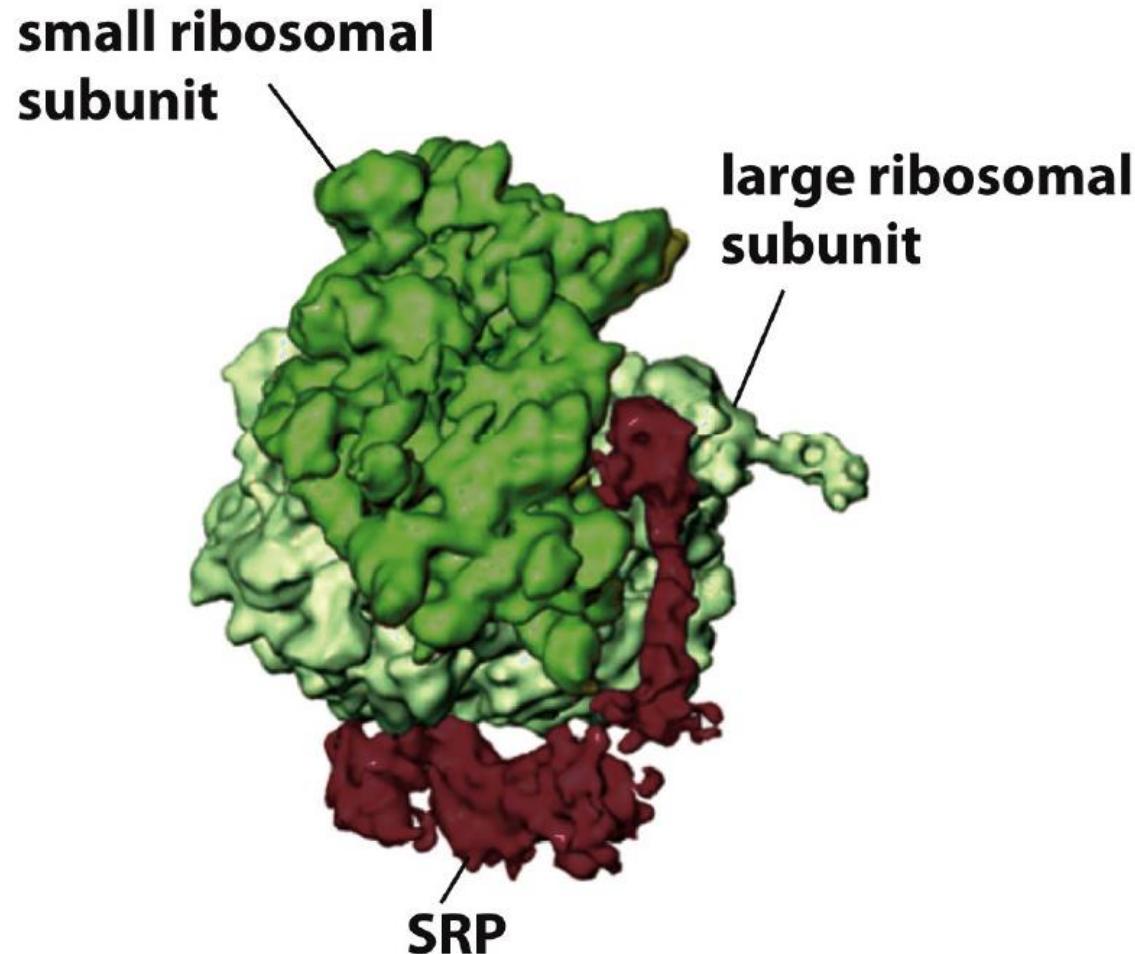
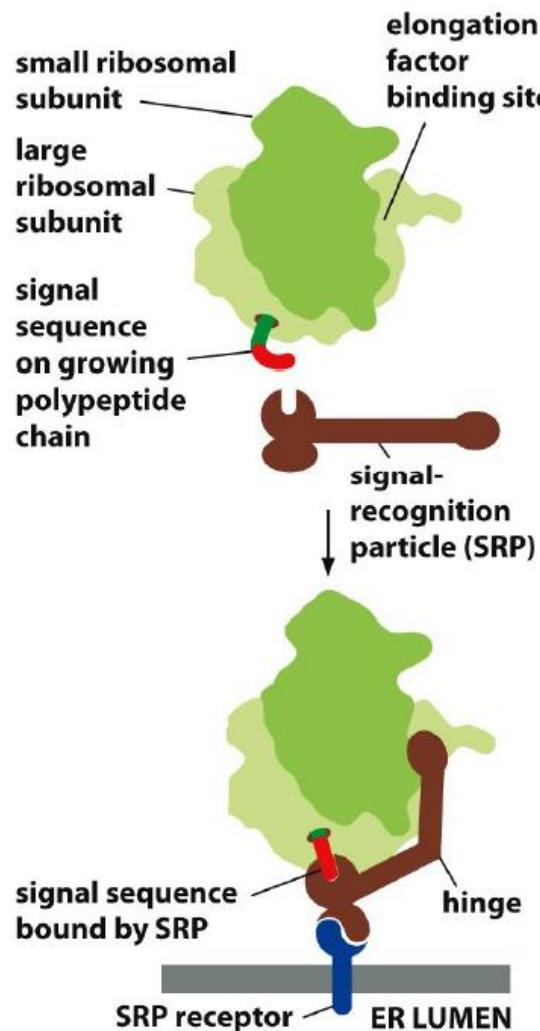


Figure 12-36b Molecular Biology of the Cell 6e (© Garland Science 2015)

# SRP directs the ER signal sequence to a specific receptor in ER membrane



Signal sequence binding site has multiple hydrophobic Met

Figure 12-36c Molecular Biology of the Cell 6e (© Garland Science 2015)

# How ER signal sequences and SRP direct ribosomes to the ER membrane

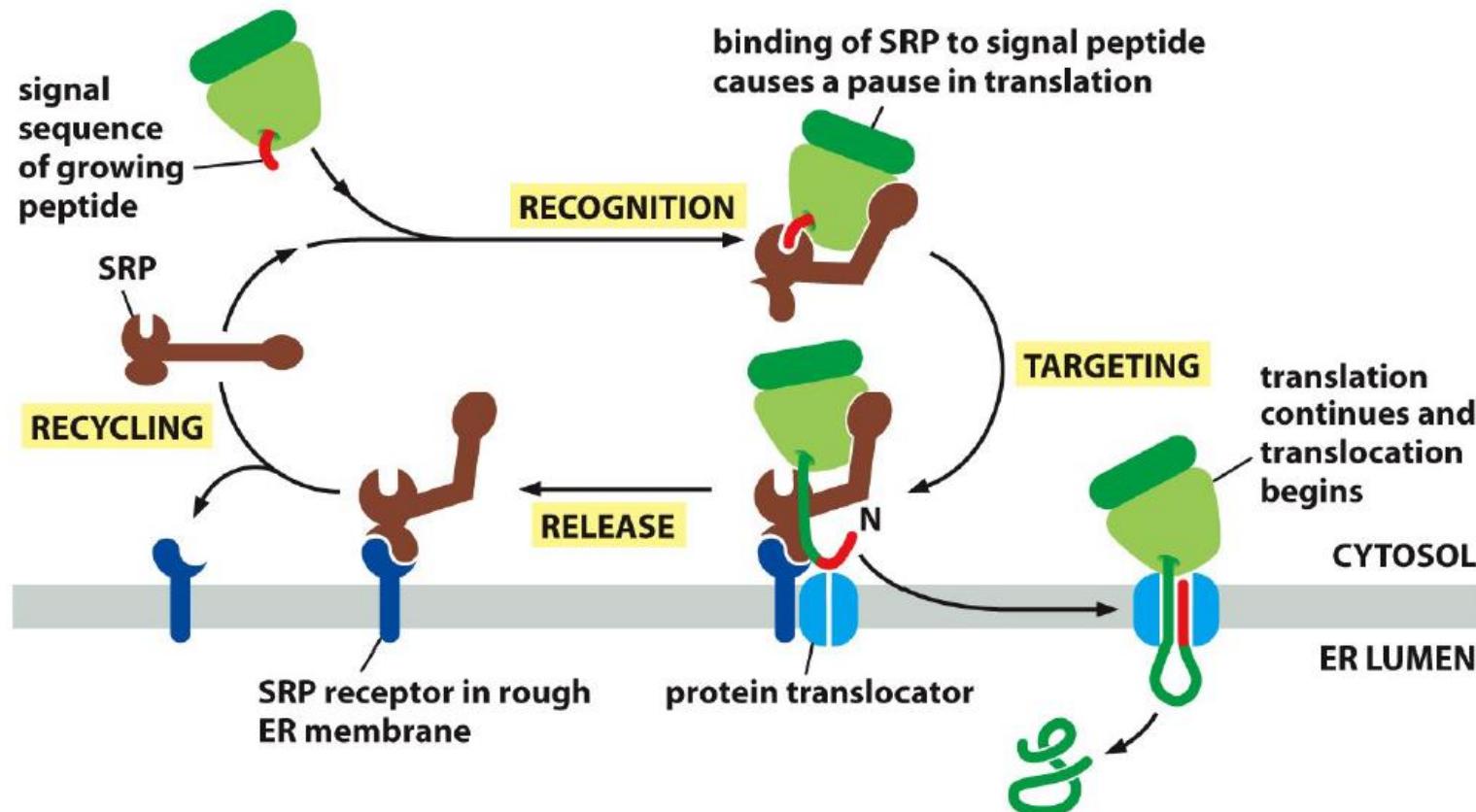
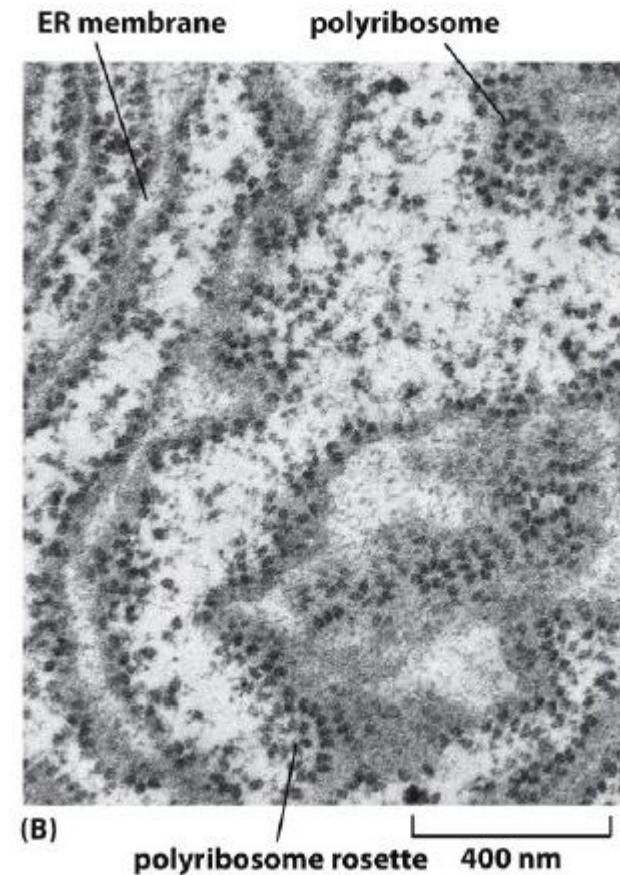
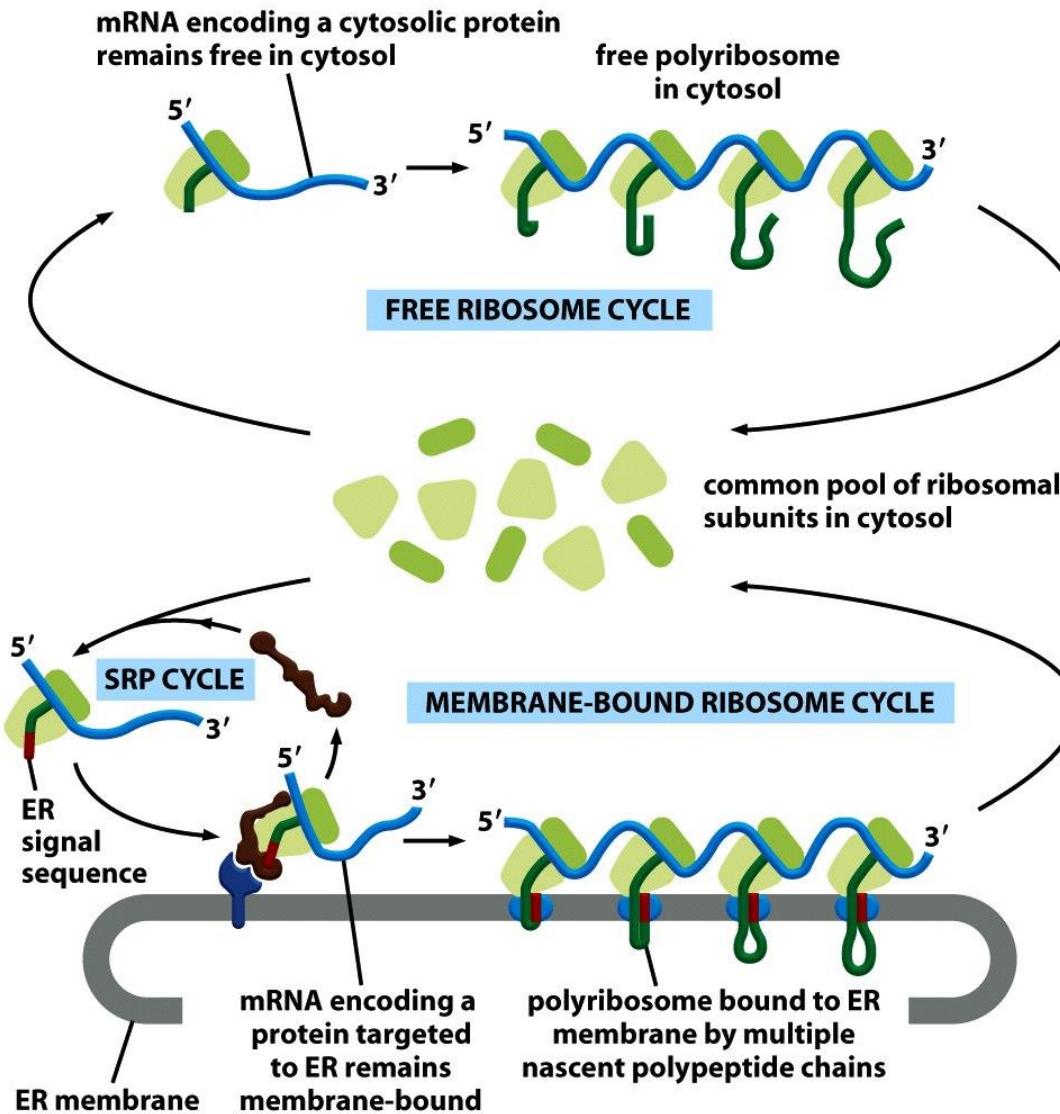


Figure 12-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Free and membrane-bound ribosomes



# Protein translocator facilitates protein transferring--- Sec61 complex

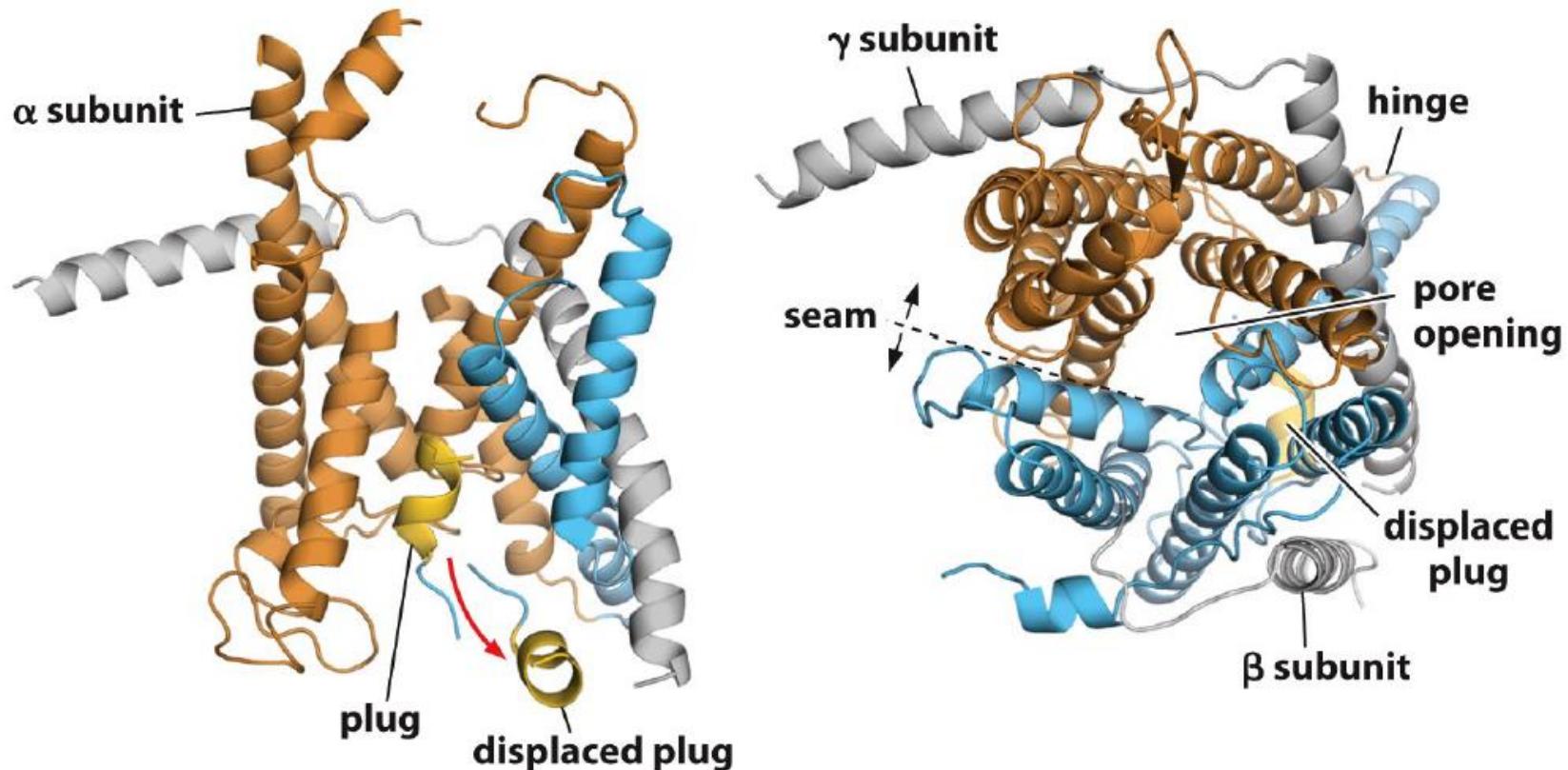


Figure 12-39a Molecular Biology of the Cell 6e (© Garland Science 2015)

# Protein translocator facilitates protein transferring--- Sec61 complex

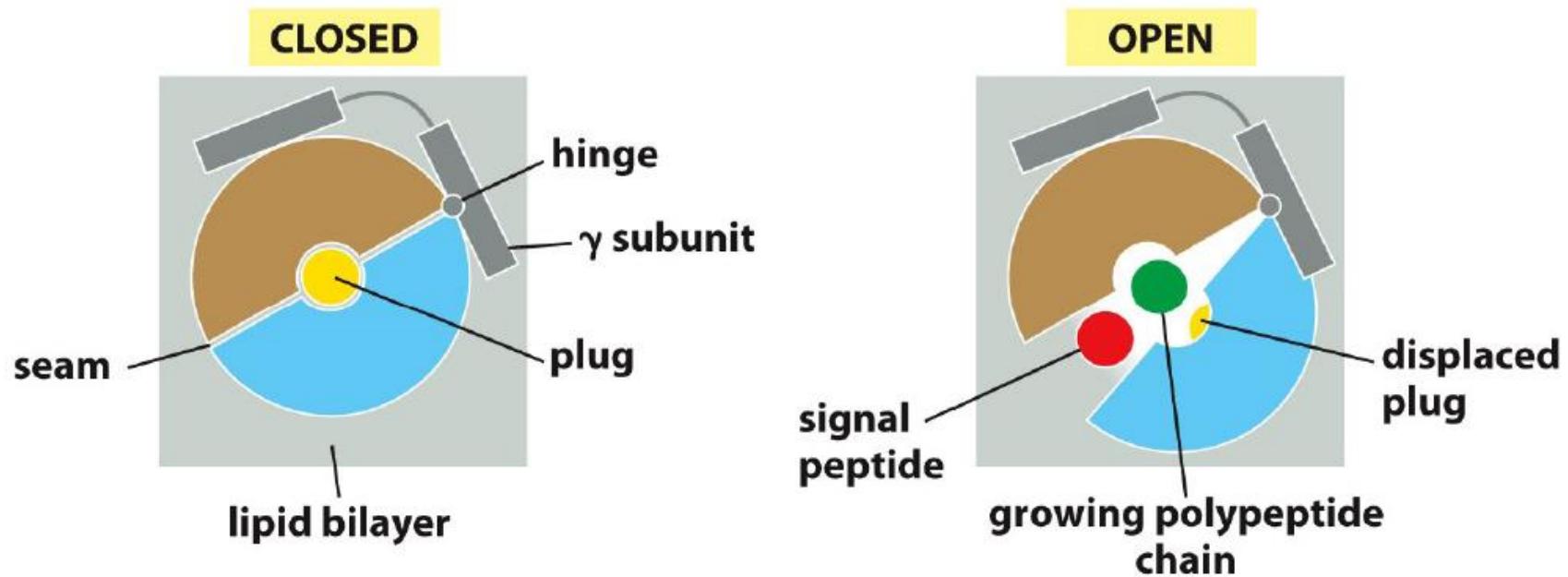
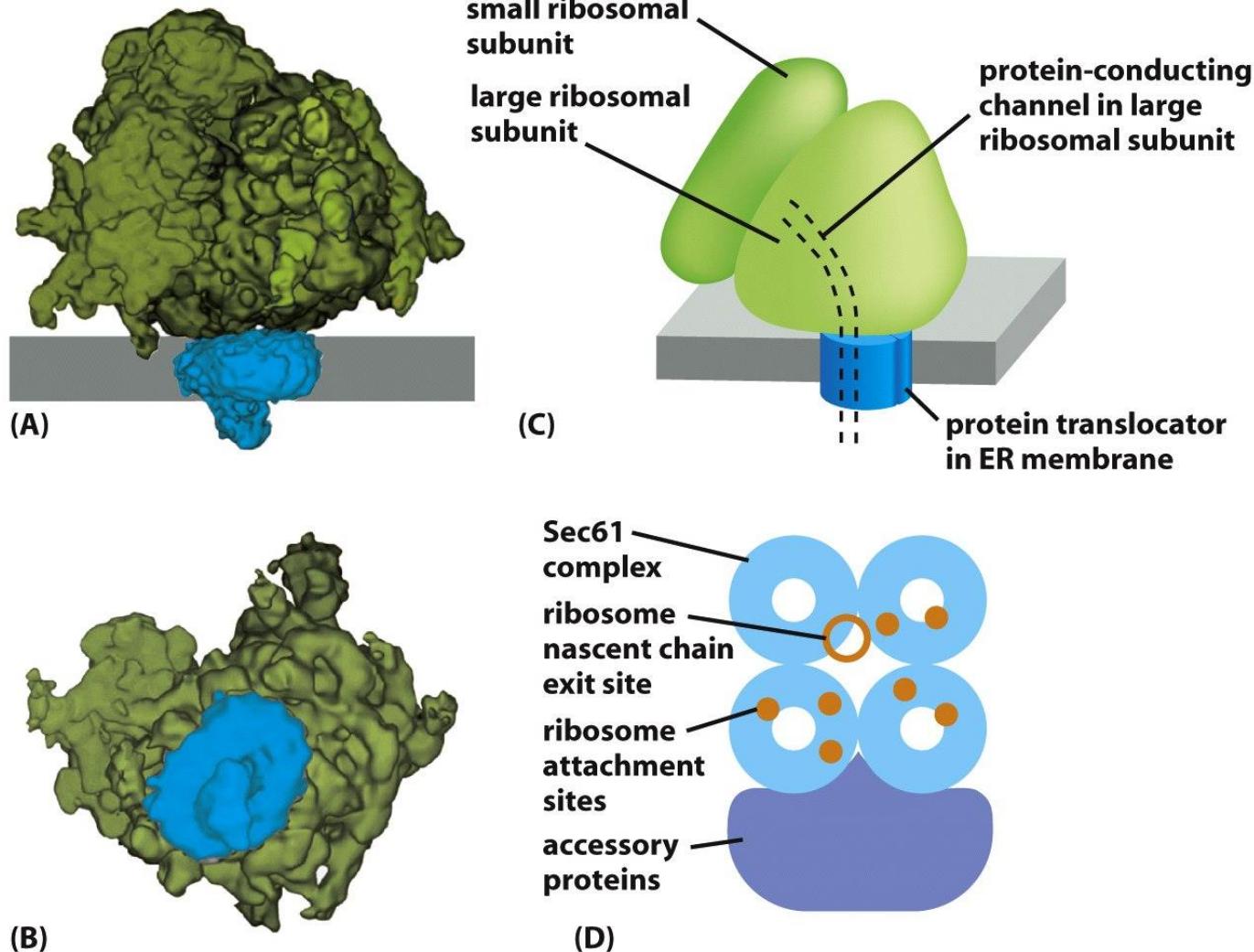
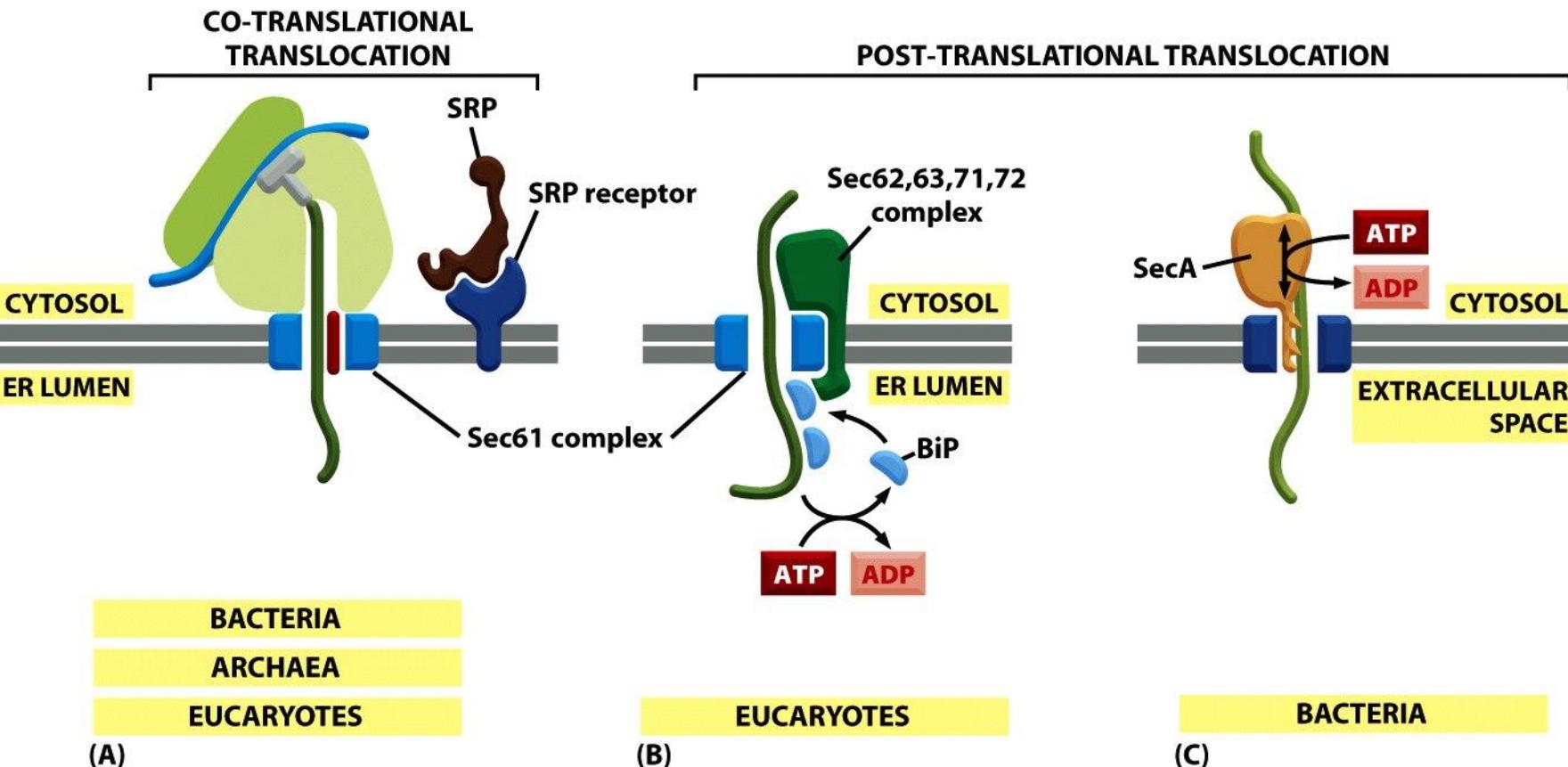


Figure 12-39b Molecular Biology of the Cell 6e (© Garland Science 2015)

# A ribosome bound to the Sec61 complex



# Three ways in which protein translocation can be driven through structurally similar translocators

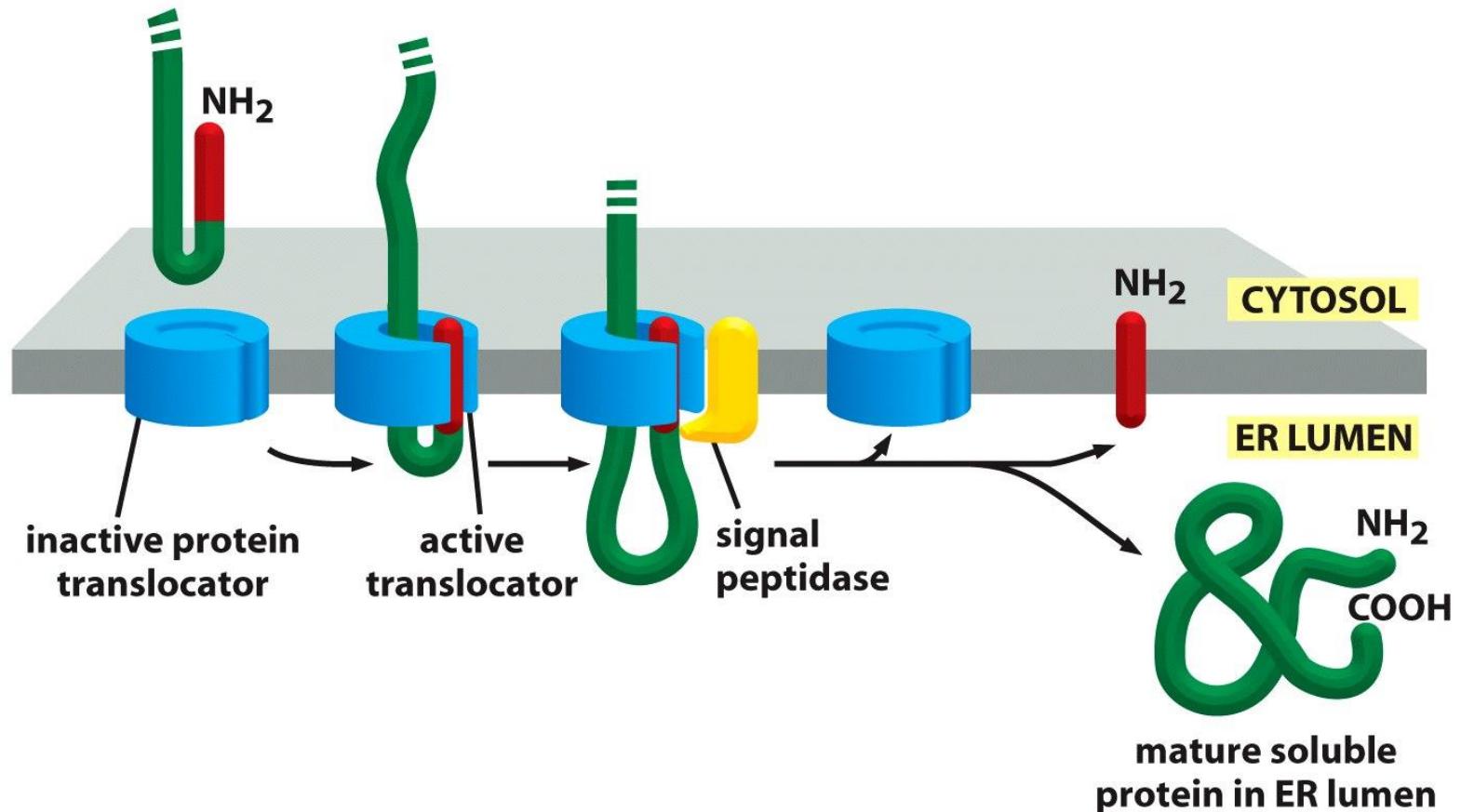


Translocation does not always require ongoing protein elongation

## Different ways for the translocation of ER proteins

- ♥ ER soluble proteins
- ♥ Single pass ER transmembrane proteins
- ♥ Multi-pass ER transmembrane proteins
- ♥ ER tail-anchored proteins
- ♥ ER residence proteins

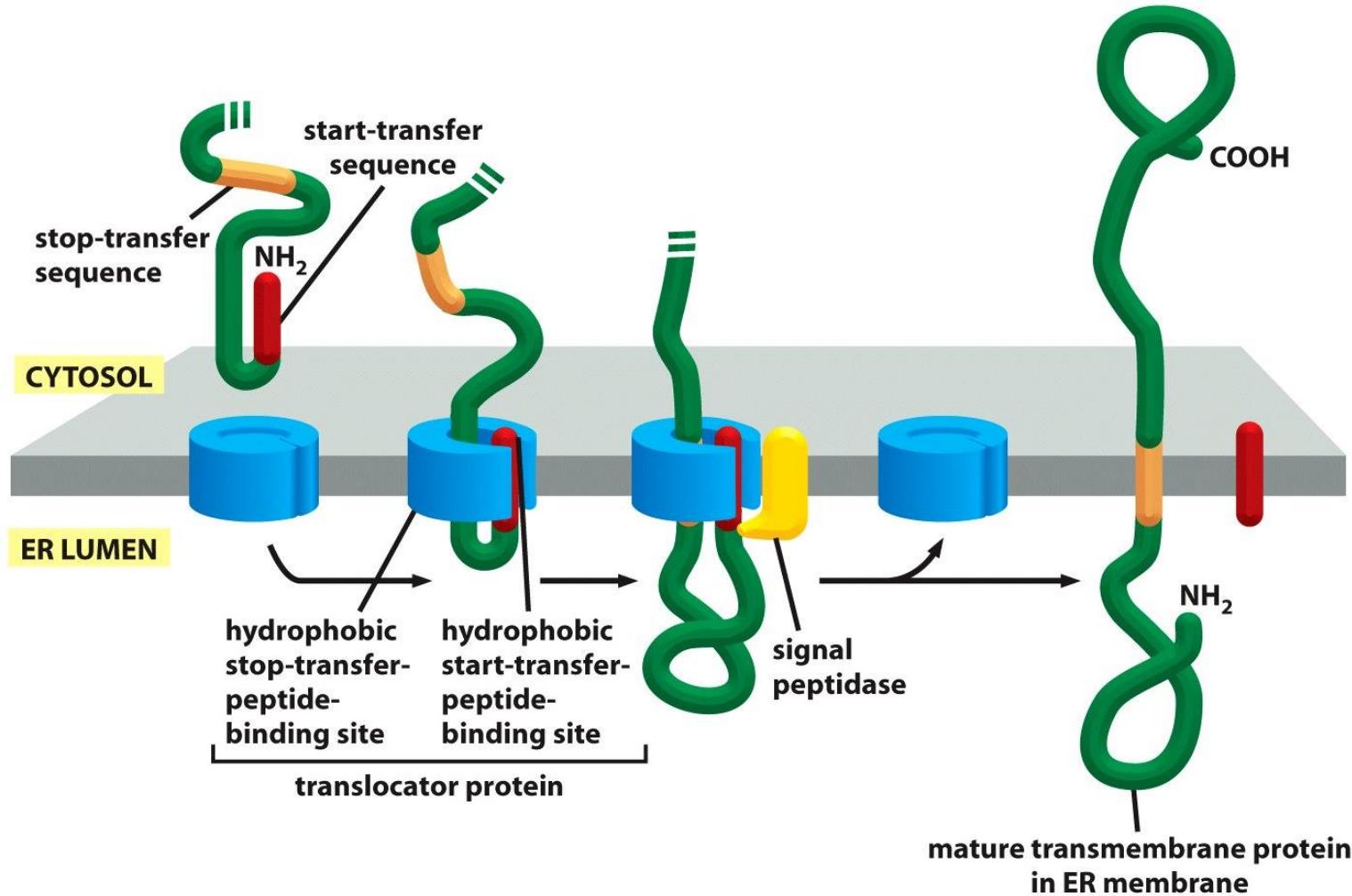
# How a soluble protein is translocated across the ER membrane



## single-pass transmembrane protein on ER membrane

- ♥ ER-signal sequence (start-transfer) is recognized first by SRP in the cytosol and then by the pore of the protein translocator.
- ♥ In two cases:
  - N-terminal start-transfer signal
  - Internal signal sequence

# N-terminal start-transfer signal



# Internal signal sequence

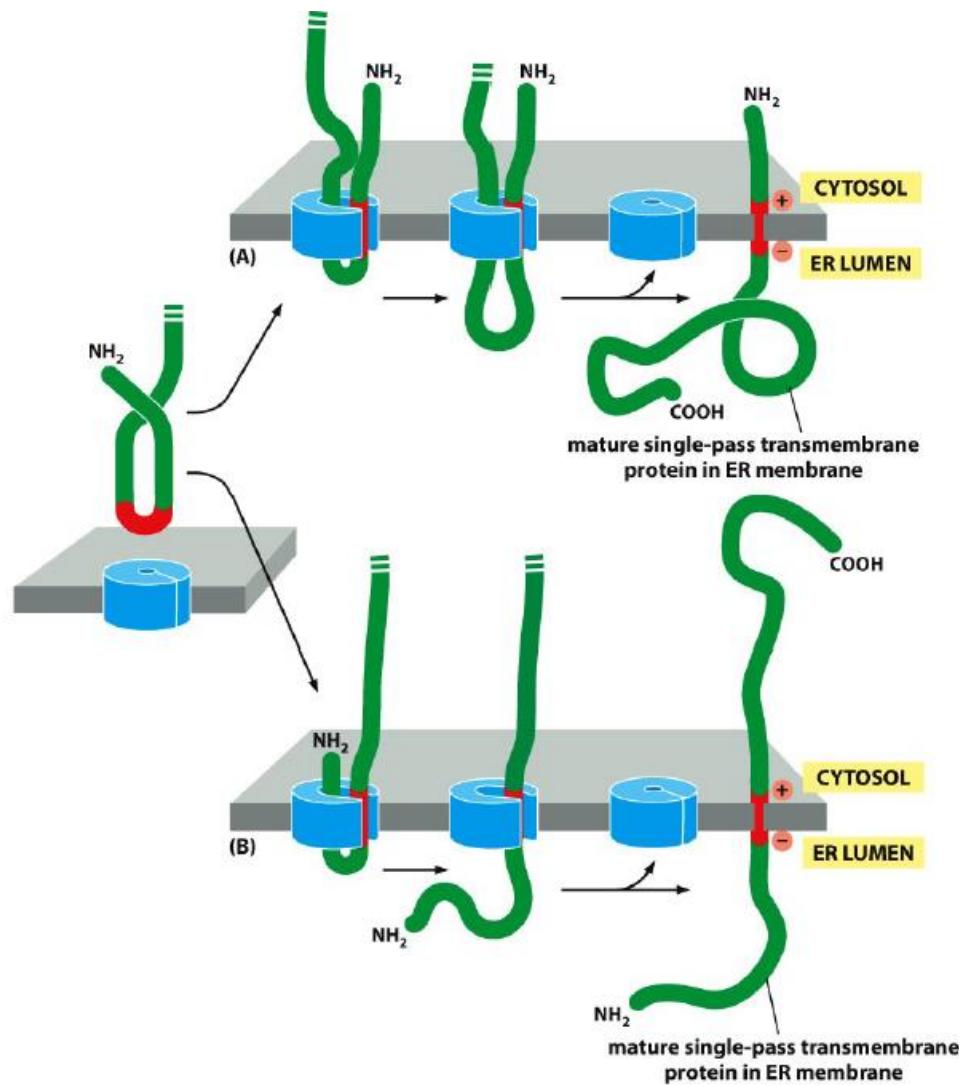
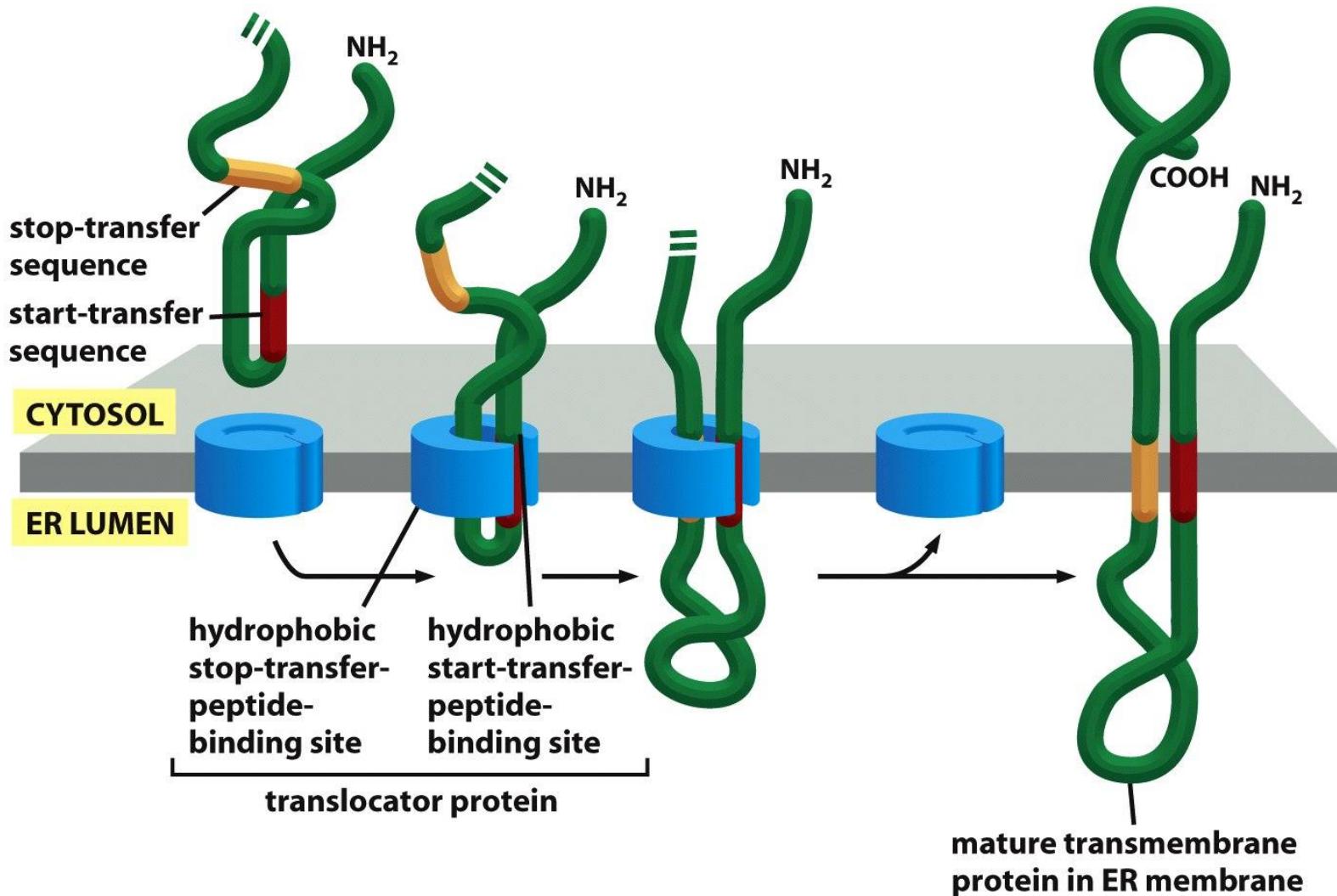


Figure 12-43 Molecular Biology of the Cell 6e (© Garland Science 2015)

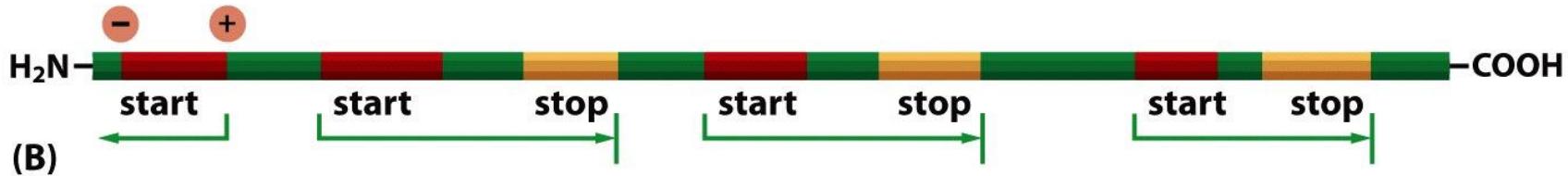
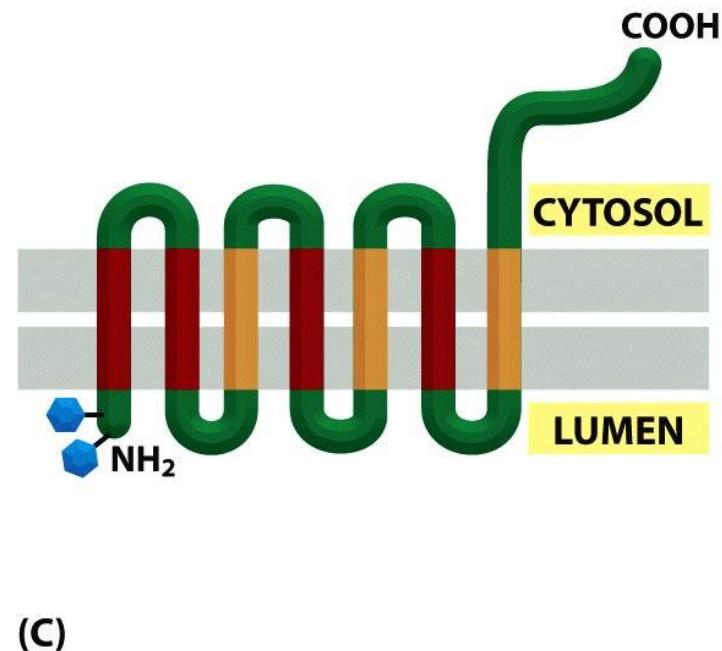
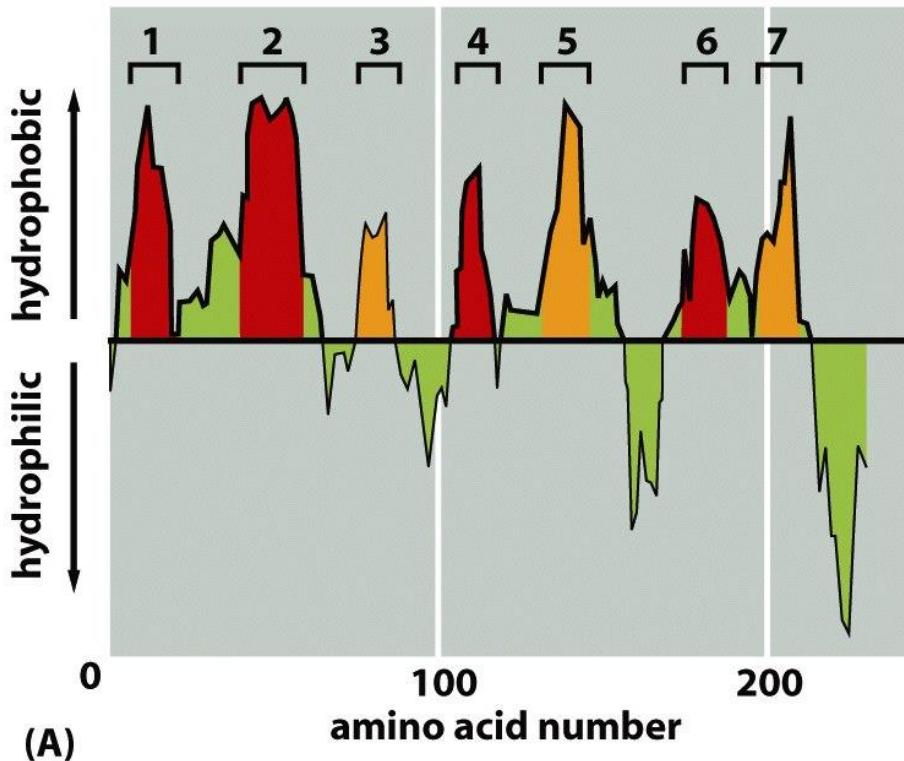
## Multi-pass transmembrane proteins

- ♥ First **internal signal sequence** serves as a start-transfer signal
- ♥ Translocation stops when encountering a stop-transfer sequence.
- ♥ Then the second start-transfer signal will direct translocation
- ♥ So on so forth...

# Integration of a double-pass transmembrane protein with an internal signal sequence into ER membrane



# The insertion of the multi-pass membrane protein rhodopsin into the ER membrane



# ER tail-anchored protein is integrated by a special mechanism

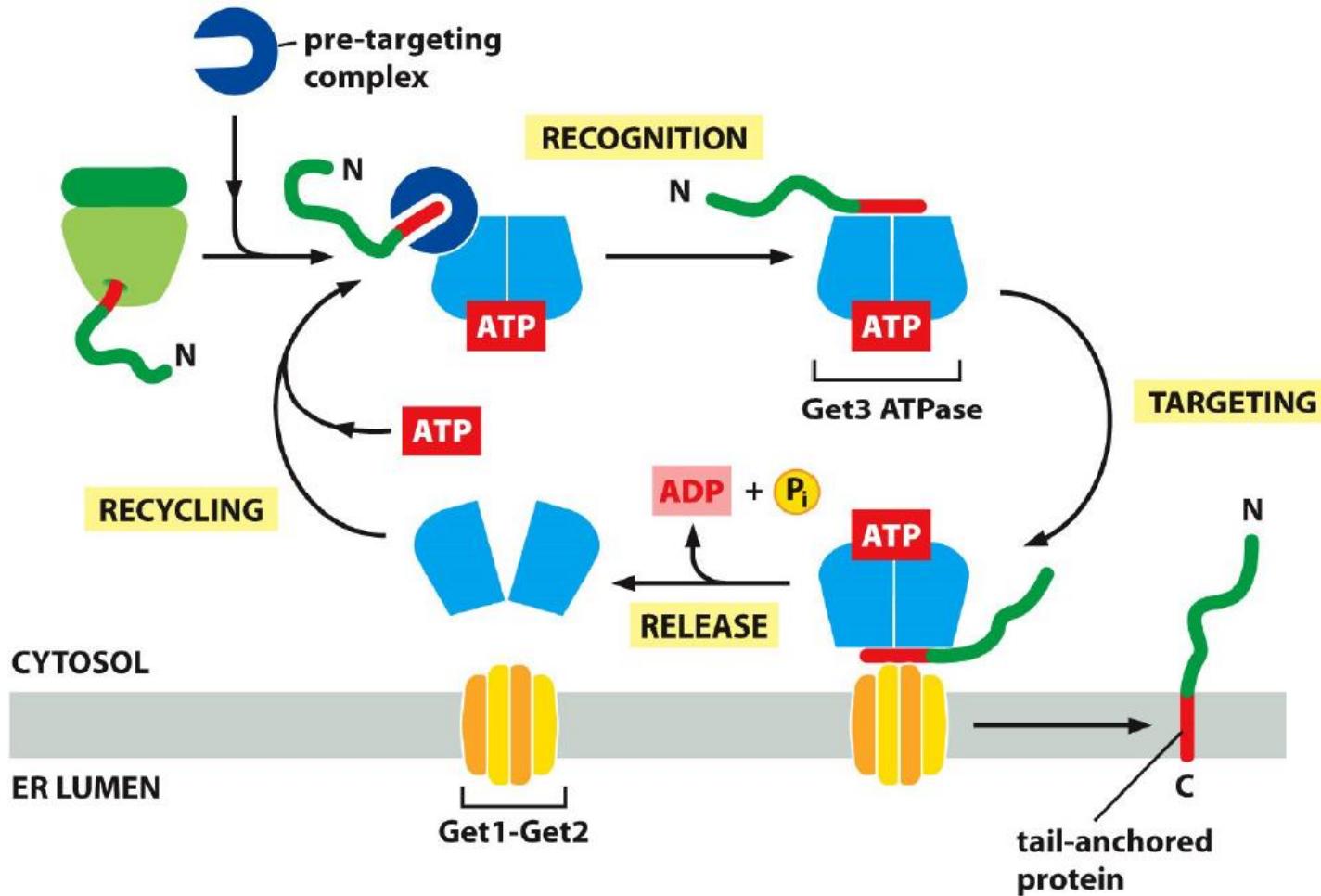


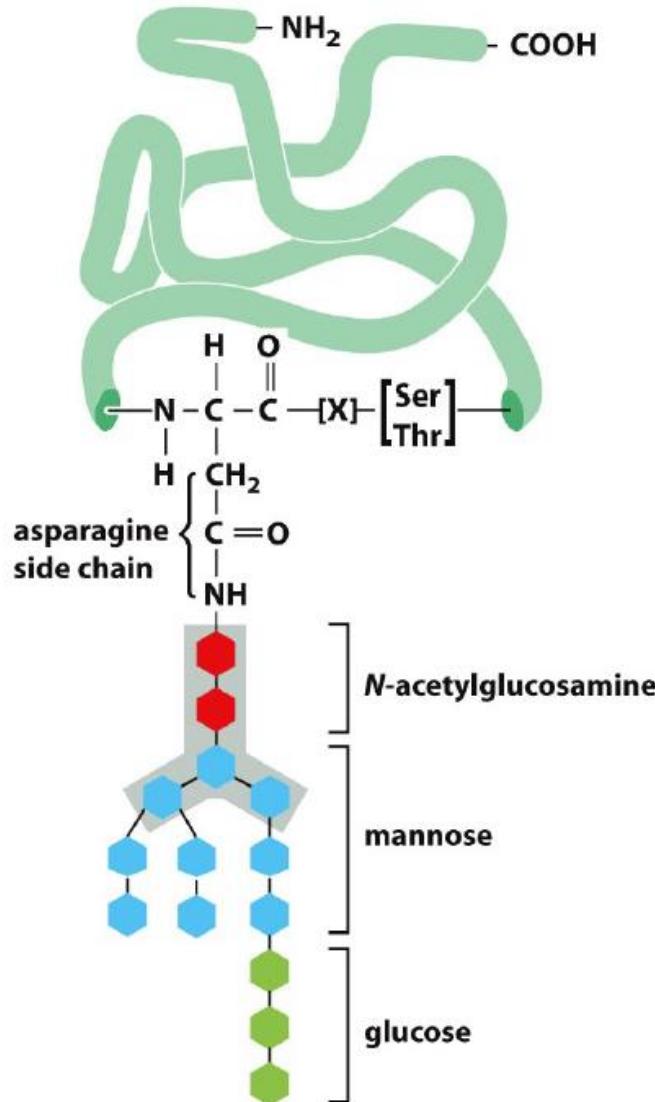
Figure 12-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Some ER resident proteins

- ♥ Contain ER retention signal of four amino acids in C-terminus
- ♥ Examples:
  - ◆ protein disulfide isomerase ( PDI)
  - ◆ BiP ( help to fold proteins)

- ♥ Translocated polypeptide chains are folded and assembled in the ER lumen
- ♥ Most proteins in rough ER are glycosylated via the addition of a common N-linked oligosaccharide

# Rough ER- glycosylation



- ♥ Most ER made protein is glycosylated
- ♥ Cytosolic protein is rarely glycosylated.

- ♥ N-linked oligosaccharide takes up 90% of all glyco-proteins
- ♥ The other from O-linked in Golgi Apparatus (Ser, Thr, hydroxylysine)

N-linked precursor oligosaccharide that is added to most proteins in the rough ER membrane

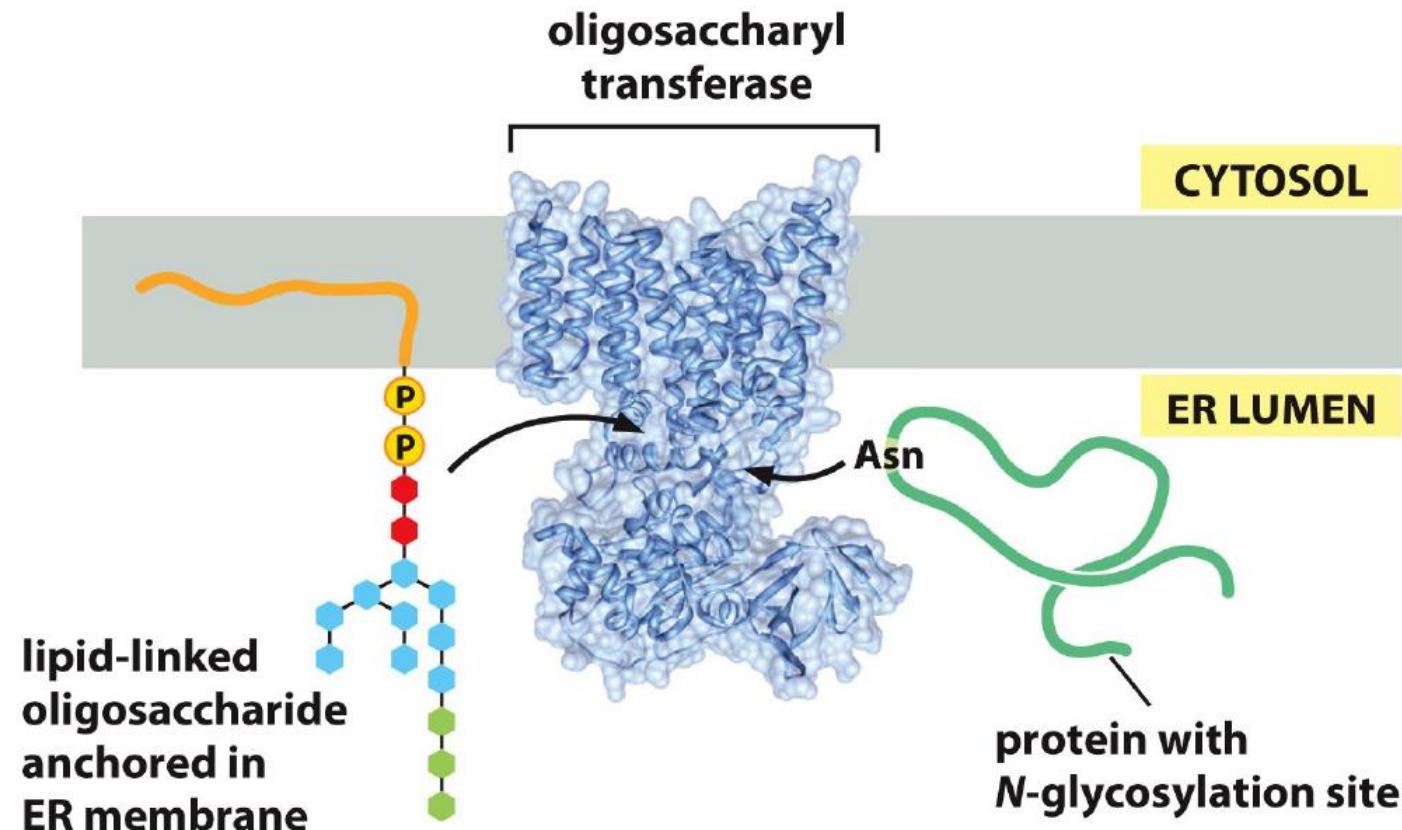
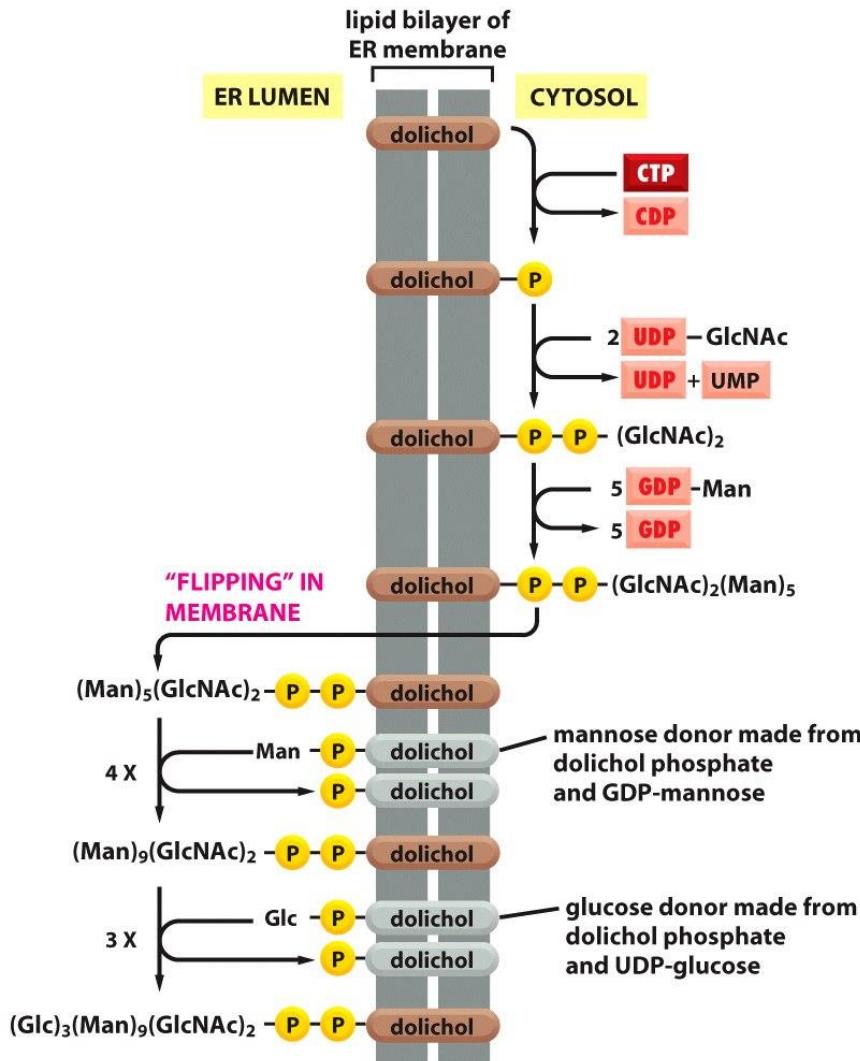


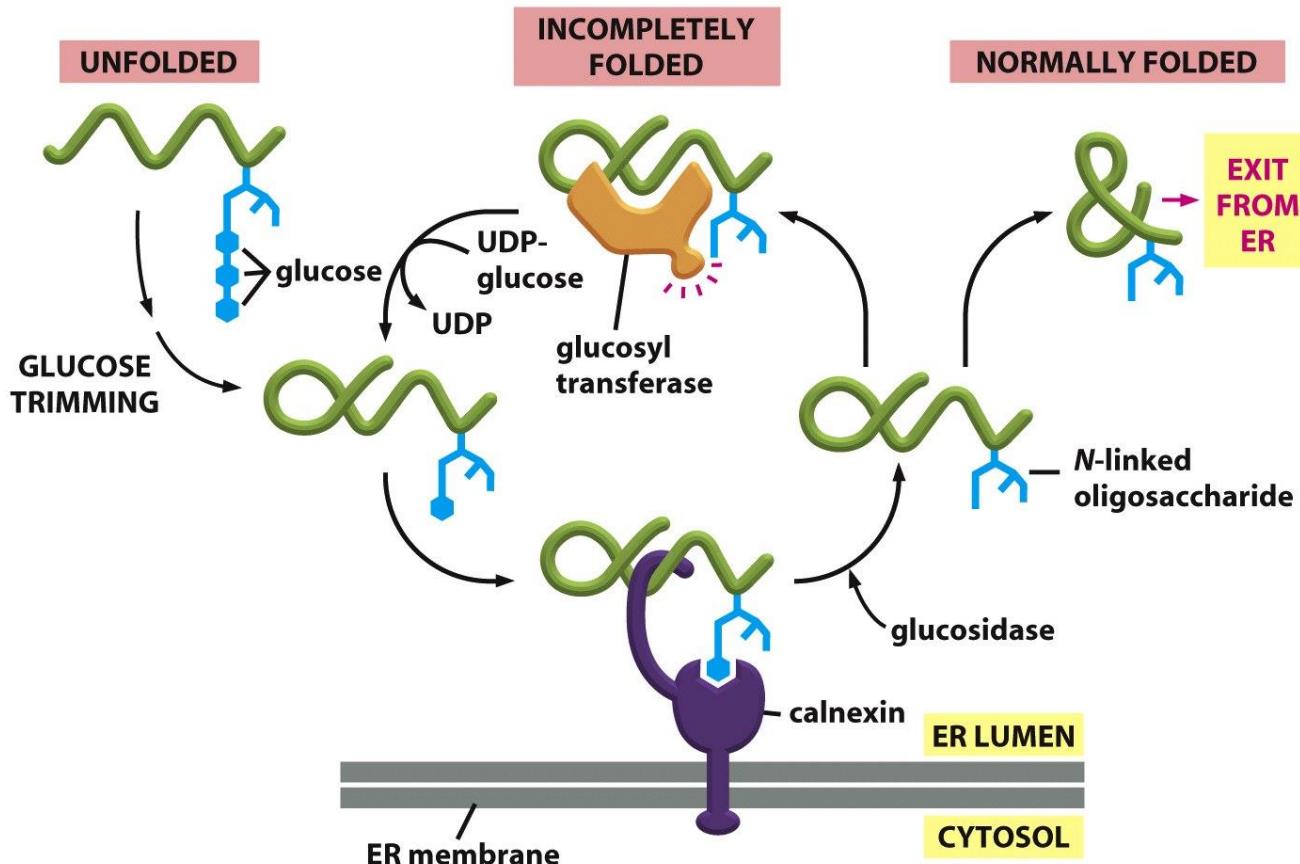
Figure 12-47b Molecular Biology of the Cell 6e (© Garland Science 2015)

It is catalyzed by oligosaccharyl transferase, whose active sites are exposed On ER lumen only.

# Synthesis of precursor oligosaccharide in rough ER



# Oligosaccharides are used as tags to mark the state of protein folding



Two lectins:  
**calnexin**  
**calreticulin**  
need  $\text{Ca}^{2+}$  binding  
for their activity.  
recognize sugar  
group and bind them,  
Until unfolded protein  
is proper folded,  
then exit ER

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

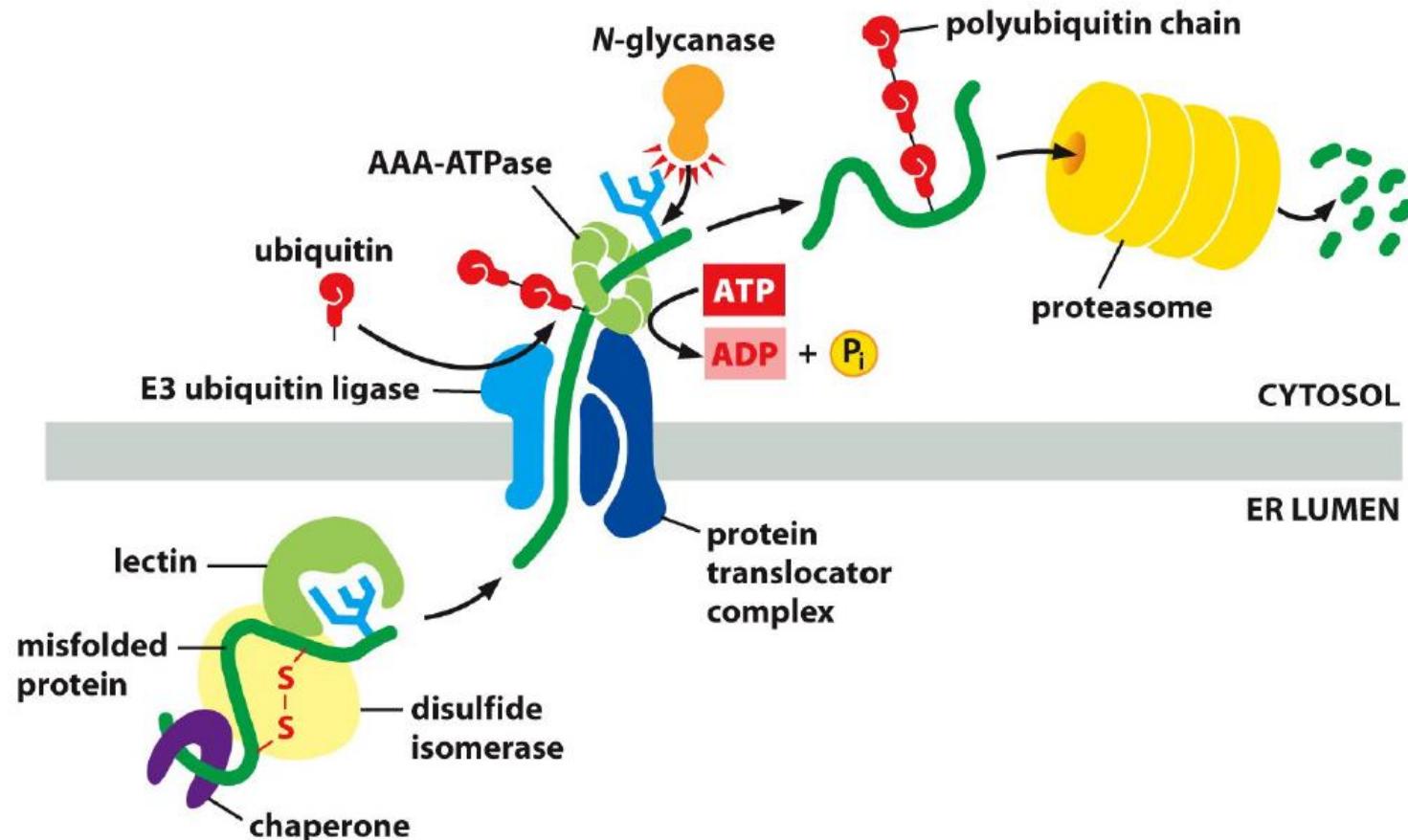
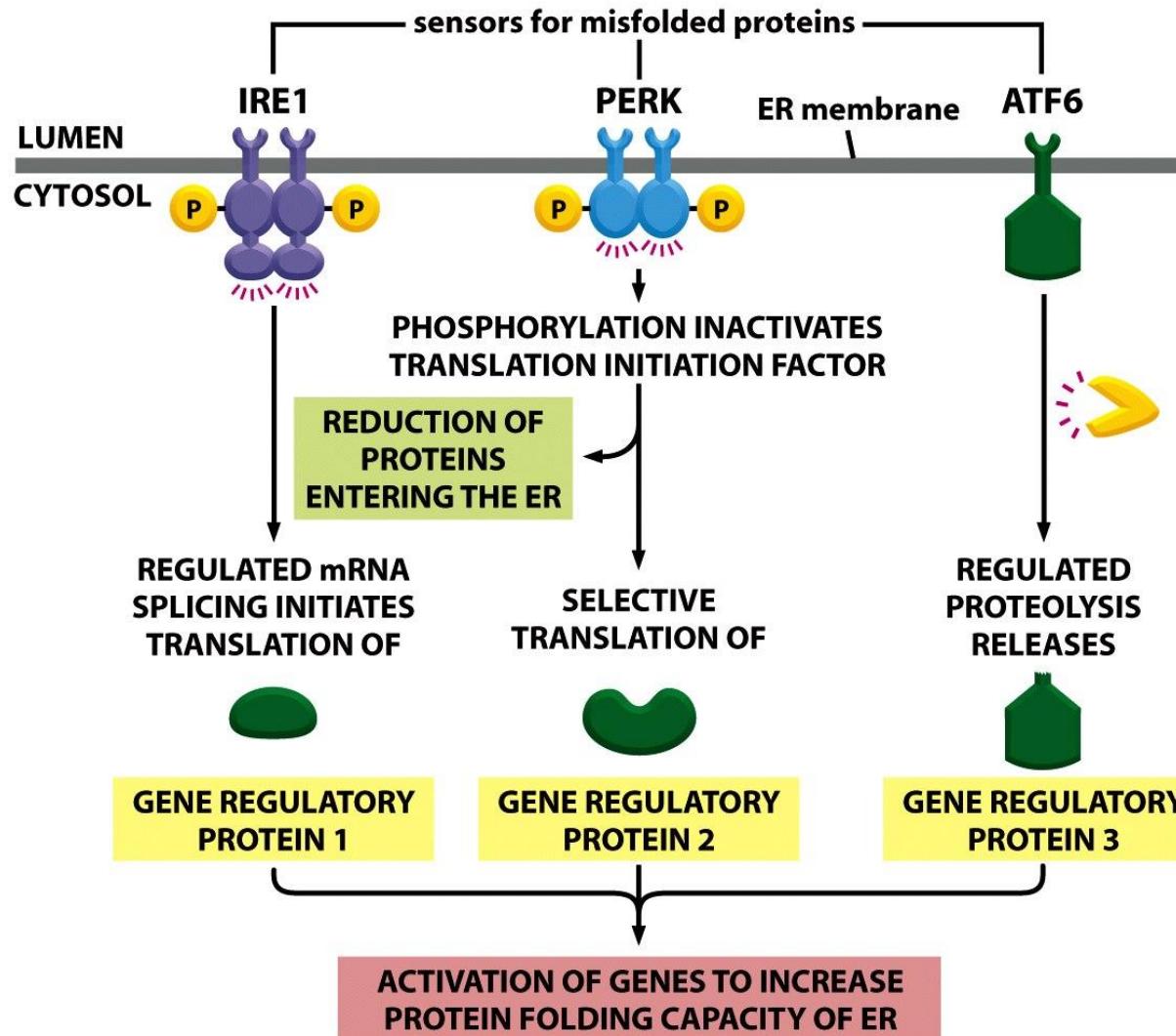


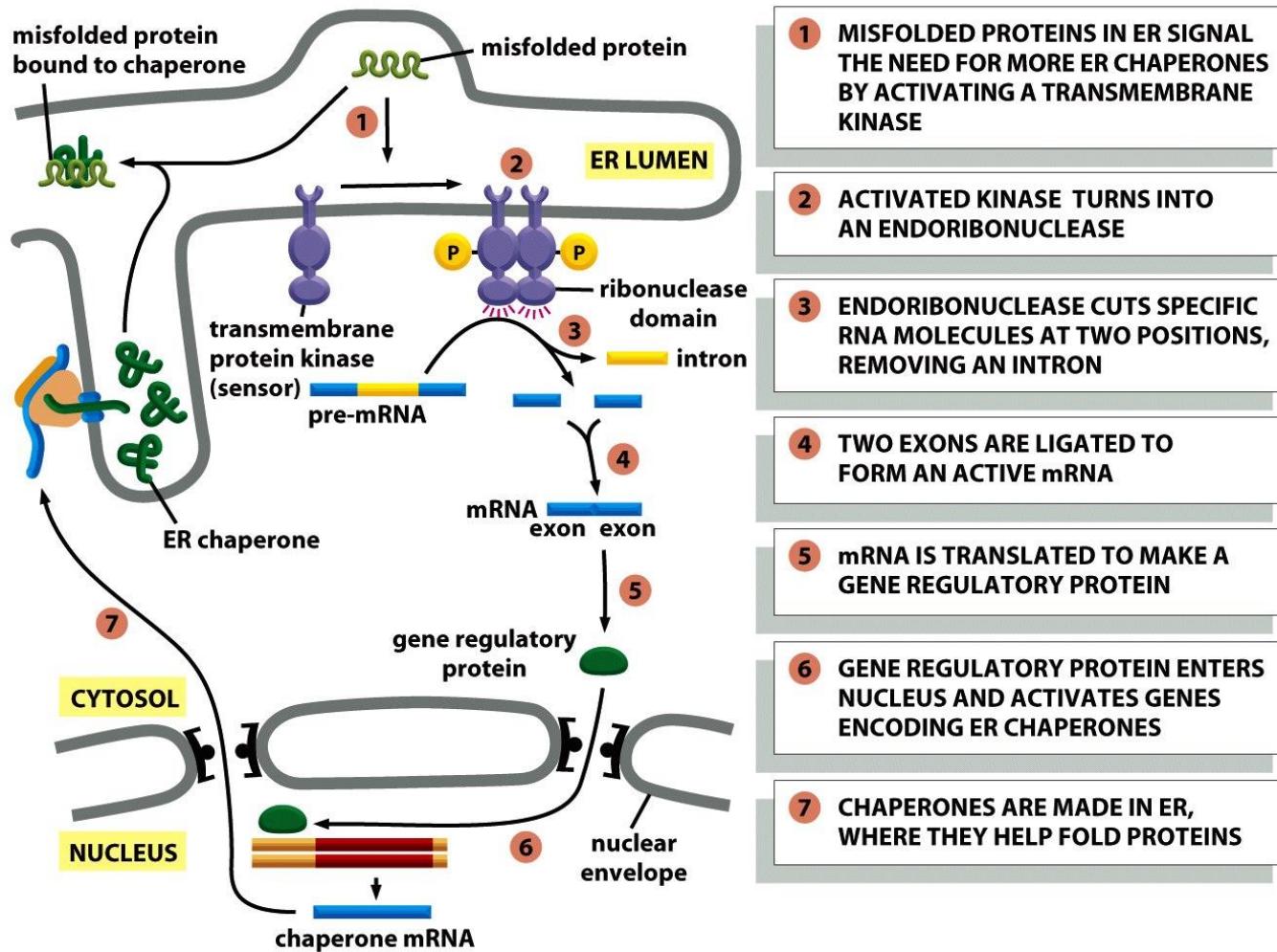
Figure 12-50 Molecular Biology of the Cell 6e (© Garland Science 2015)

N-linked oligosaccharide serves as a timer to determine how long misfolded protein will be degraded

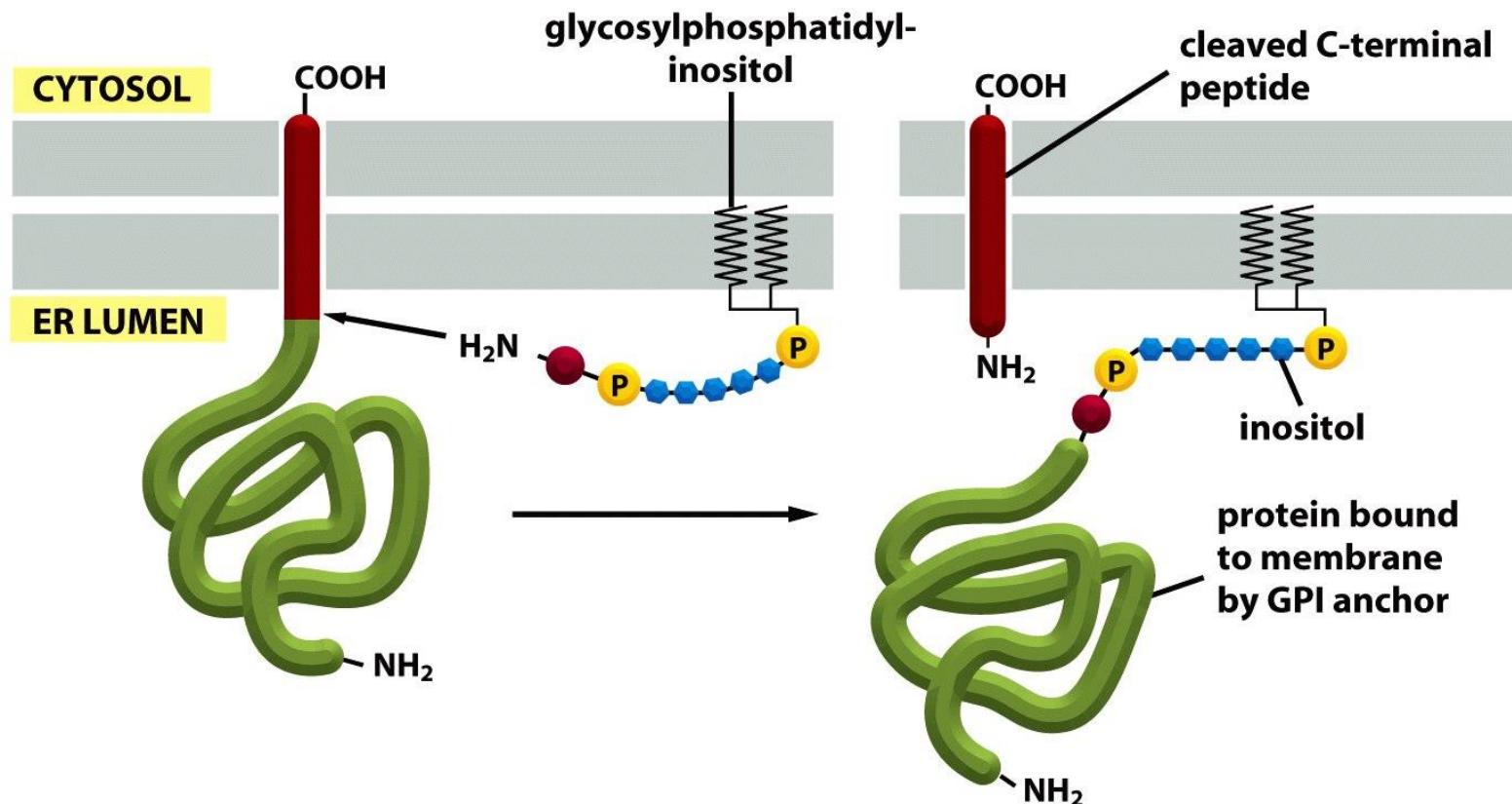
# Misfolded proteins in the ER activate an unfolded protein response



# Activation of transcription of genes involved in unfolded protein response



# How to covalently attach GPI anchor



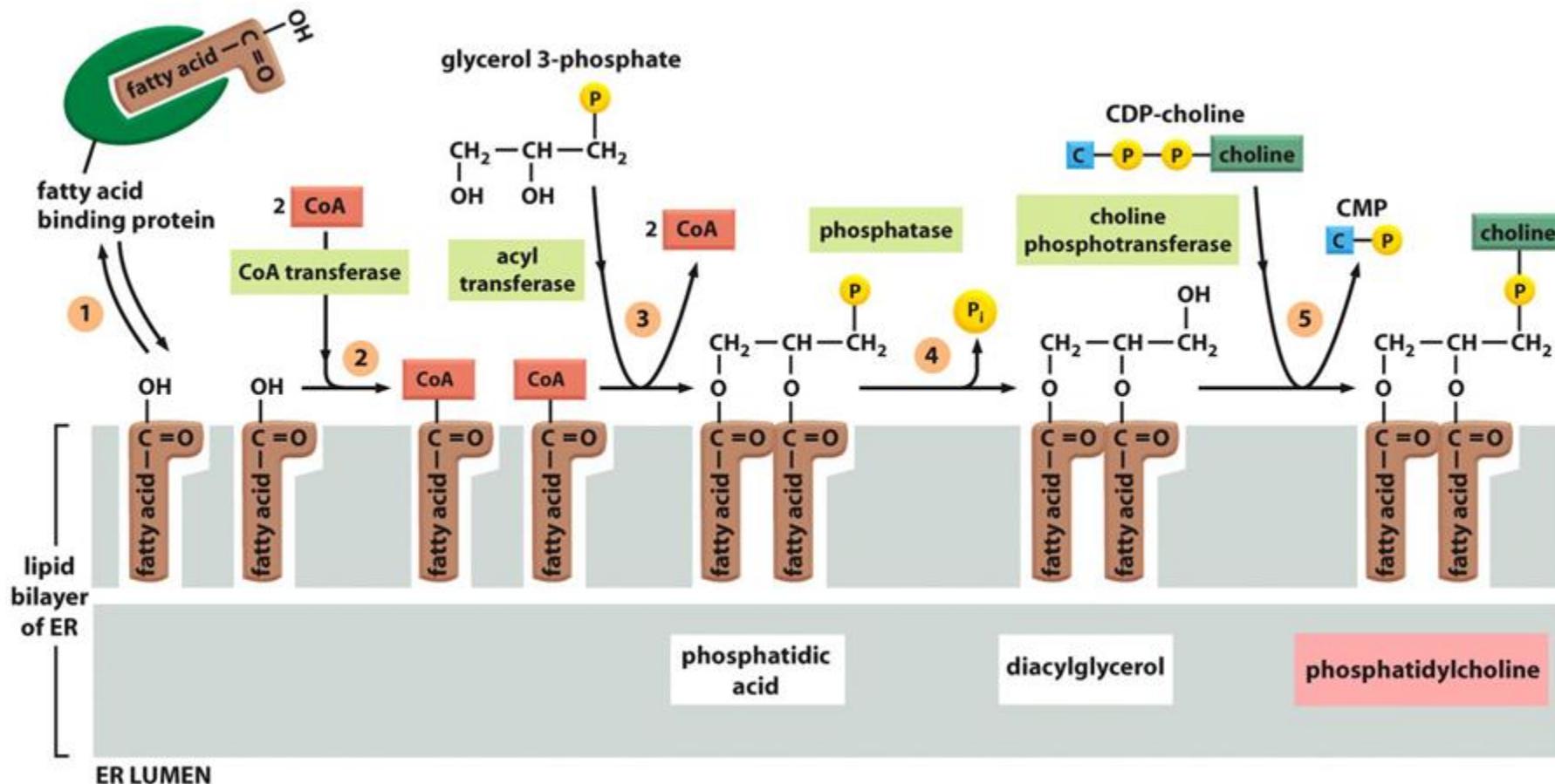
Red: phosphoethanolamine

## Phospholipid synthesis in ER

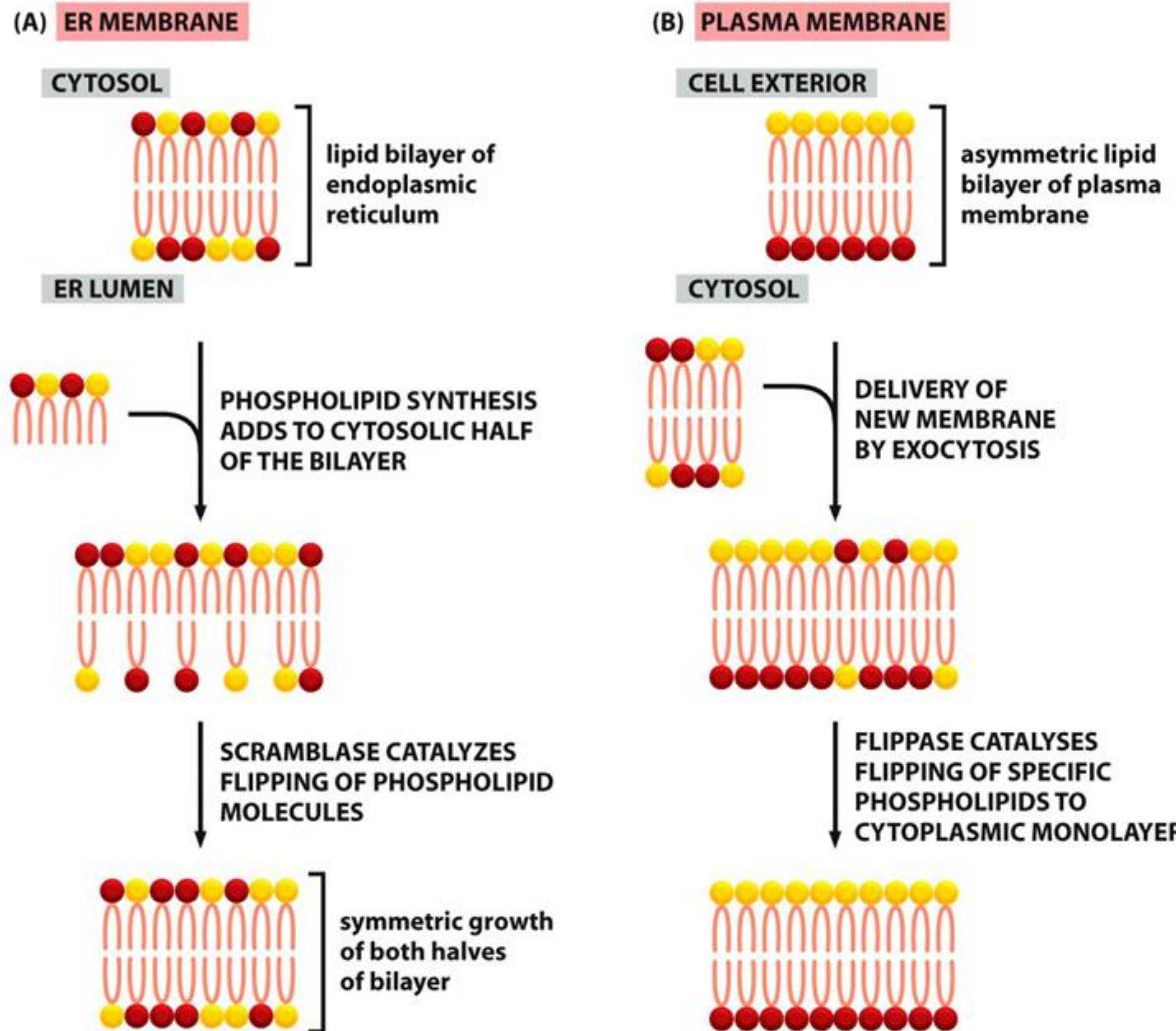
- ♥ Nearly all major lipids are synthesized in ER  
these include phospholipid, cholesterol, ceramide  
(precursor for Sphingomyelin)
- ♥ Synthesis is on the cytosolic side of ER, where key enzymes are located.
- ♥ Equal distribution for these lipids between **both leaflets of ER** after synthesis due to scramblase, which catalyzes rapid flip-flop.

# (1). Synthesis of phosphatidylcholine

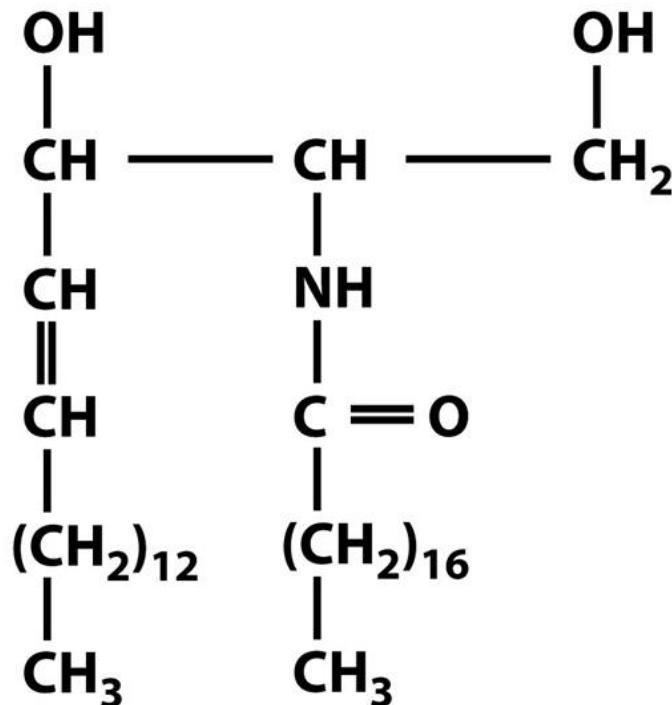
Similar mechanism for other major phospholipids



## (2). Symmetry and asymmetry in ER and plasma membrane



### (3). ER production of ceramide



**CERAMIDE**

1<sup>st</sup> step: condensing between Serine  
And a fatty acid to form sphingosine.

2<sup>nd</sup> step: a second fatty acid is added  
To form ceramide.

Further transported into lumen of Golgi for Sphingomyelin synthesis