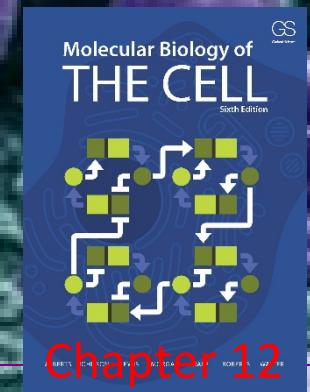


## Lecture 6

# Transport of macromolecules Part I

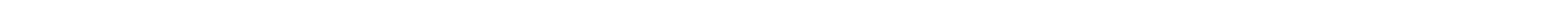
## Outline

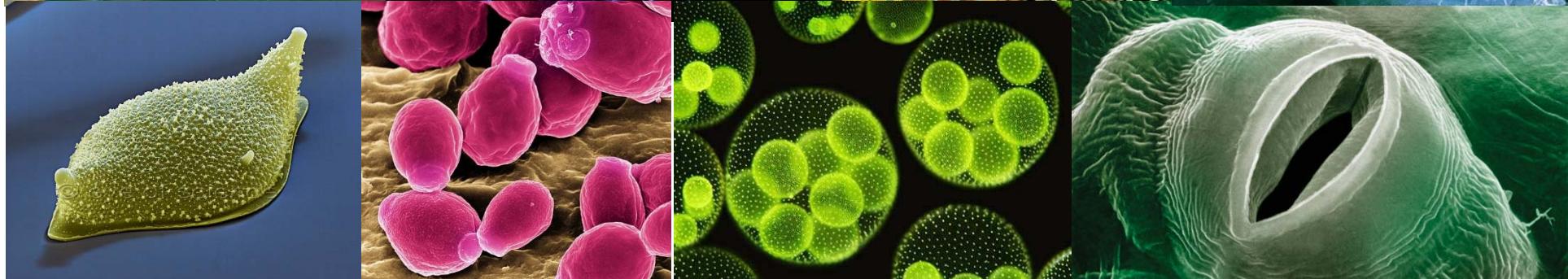
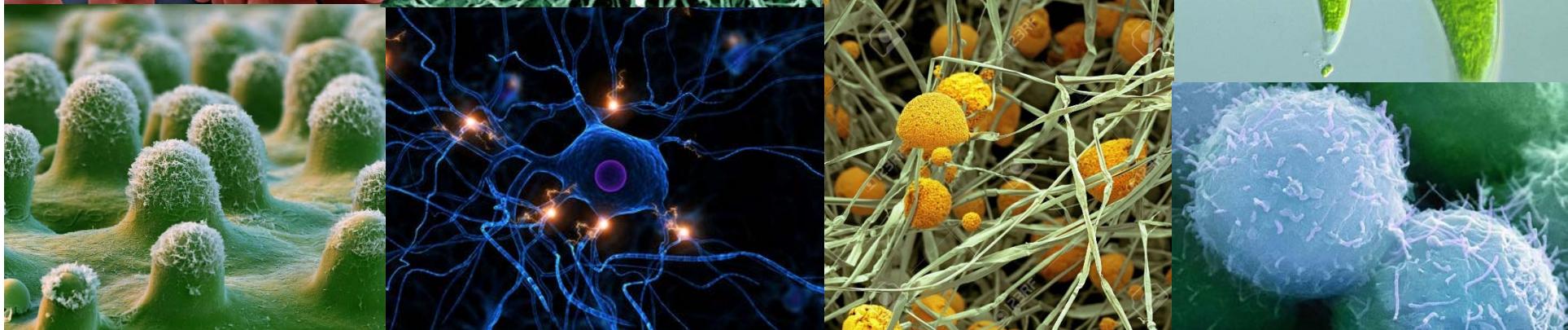
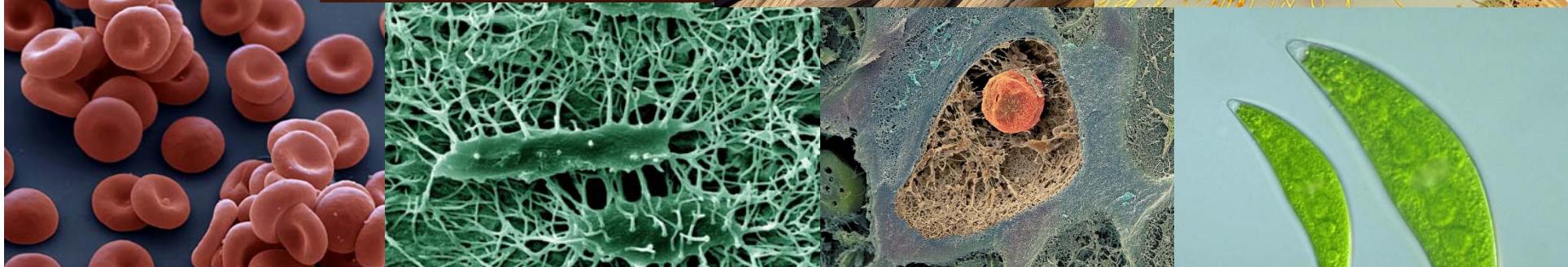
- I. Cell compartments
- II. Transport between nucleus and cytosol
- III. Transport to mitochondria and chloroplast
- IV. Transport to the peroxisome
- V. Transport to the endoplasmic reticulum



## I. The major intracellular compartments of a typical cell

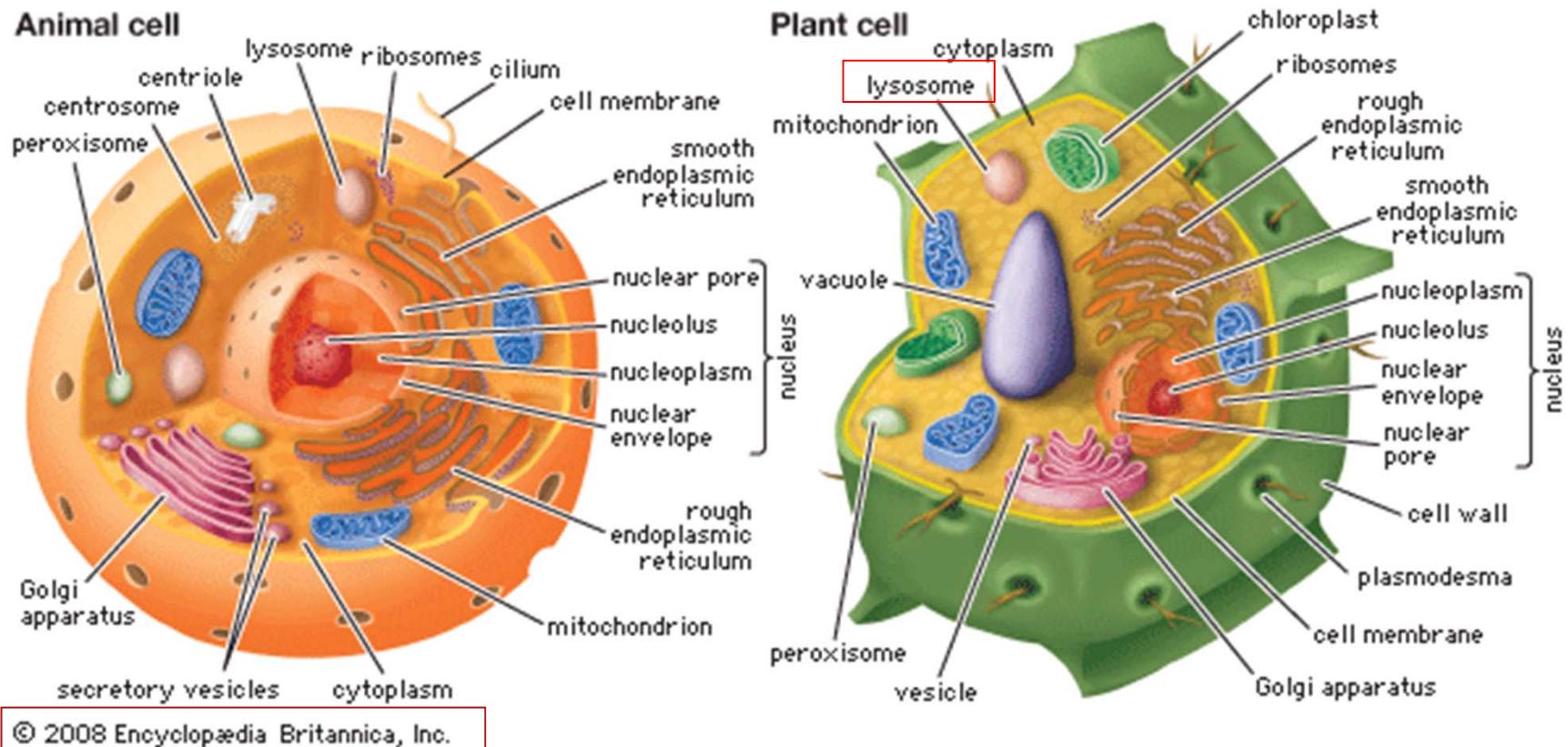
What cell are we talking about?





# I. The major intracellular compartments of a cell

## Typical animal cell and plant cell



© 2008 Encyclopædia Britannica, Inc.

Sorry to say that but this is not a good example:

- no **endosomes** mentioned, neither in plants nor in the animal cell
- plants do not have a **lysosome!**

# I. The major intracellular compartments of a cell

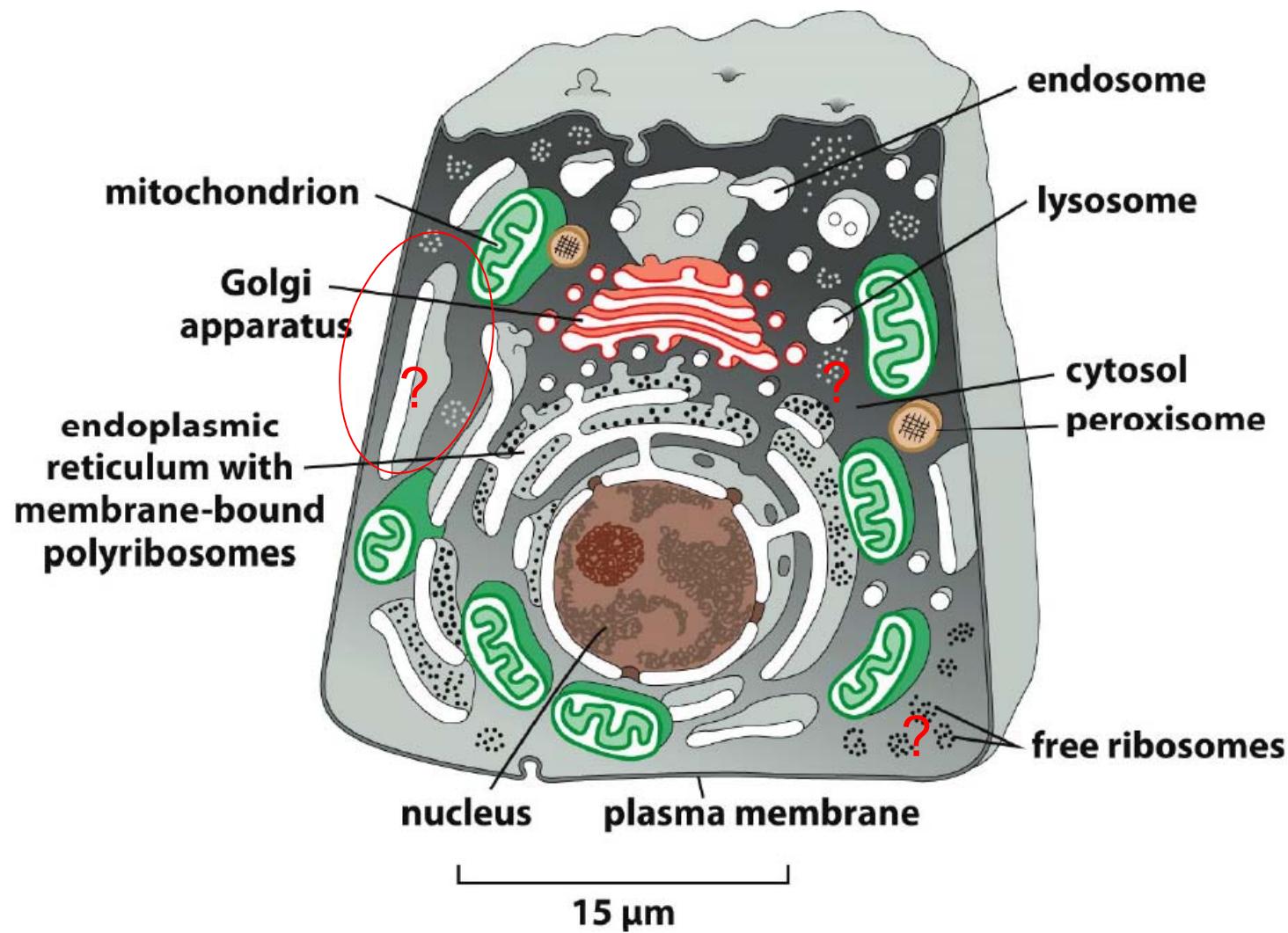


Figure 12-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

# A cross section of liver cell under TEM

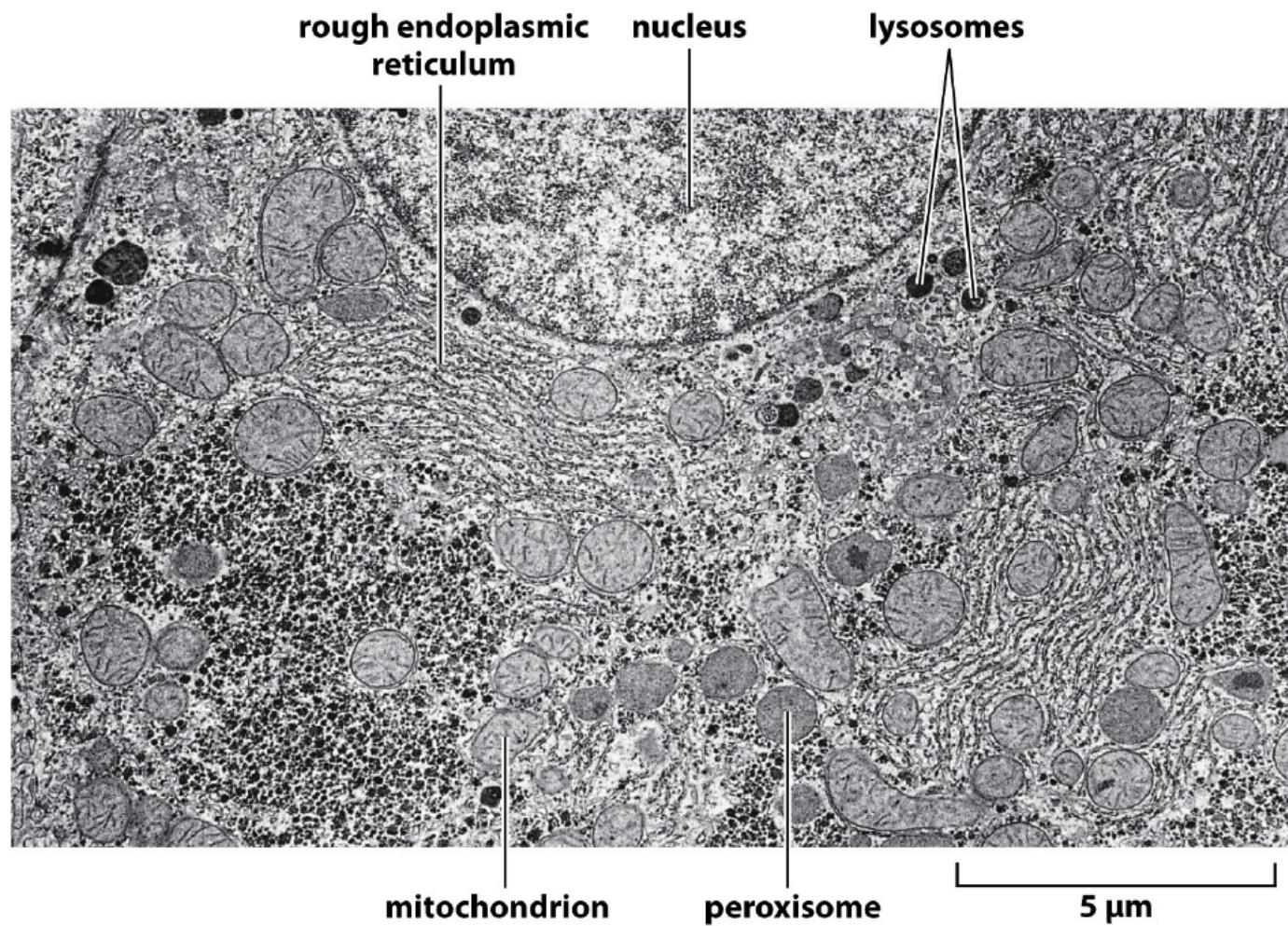
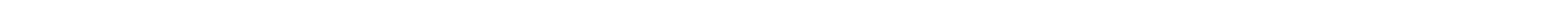


Figure 12-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

**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

Volume of compartments represents **50%** of the total volume of the cell

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

## 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.

Table 12-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Hepatocyte is **large** cell ( $5000 \mu\text{m}^3$ ) **compared to small** Pancreatic cells ( $1000 \mu\text{m}^3$ )

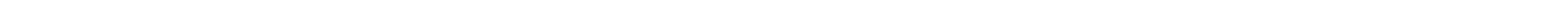
Important: **SURFACE area of ER compared to the SURFACE area of the PM**

**RATIO in Hepatocyte : PM 2% versus ER (51 (Rough (35) + smooth (16)) = abt. 25**

**RATIO in Pancreatic: PM 5% versus ER (60 (Rough (60) + smooth (<1)) = abt. 12**

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

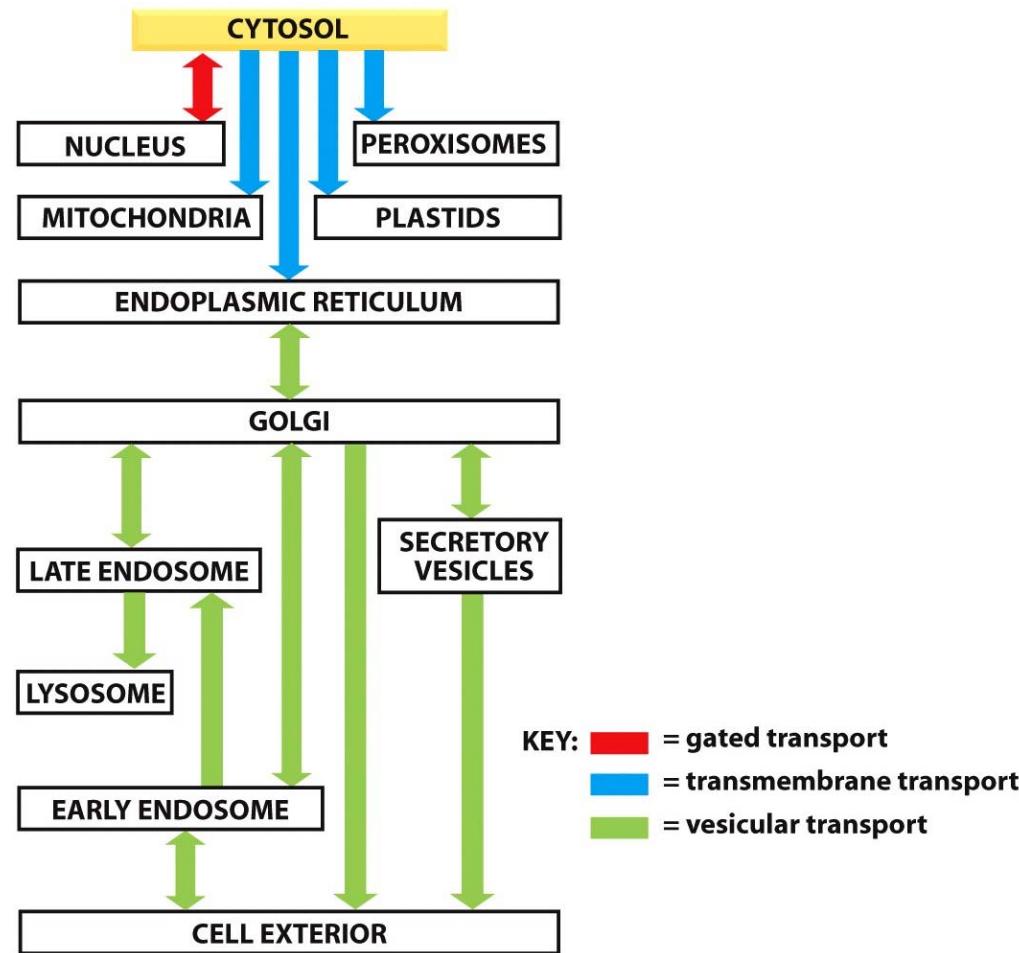
What decides whether a specific protein is to be transported to/into each of them?



## Destiny of synthesized protein depends on signals

- (Almost) all proteins are synthesized in the **cytosol**
  - Most protein **without sorting signal** stay in the cytosol
  - Protein **with sorting signal** can be transported to its designated sites by three different ways:
    1. **gated transport**
    2. **transmembrane transport**
    3. **vesicular transport**
-

# Three types of transport for macromolecules in/to cellular compartments



## Three types of transport for macromolecules in/to cellular compartments

- **Gated transport:**

Proteins move between cytosol and nucleus through **nuclear pore complex (NPC)** with selectivity/specificity

- **Transmembrane transport:**

**Unfolded protein** snakes through **transmembrane protein translocators** by the guidance of sorting signals

- **Vesicular transport:**

**Membrane-enclosed transport carriers** guide 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 different types of signal direct sorting :

- **N-terminal signal sequence:**  
usually 15-60 aa long, can be removed upon “arrival” by a  
**signal peptidase**
  - **Internal stretches** of amino acids.
  - **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-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 the **nuclear pore complex (NPC)**
- This occurs **bi-directionally** and with **selectivity (signals)**.

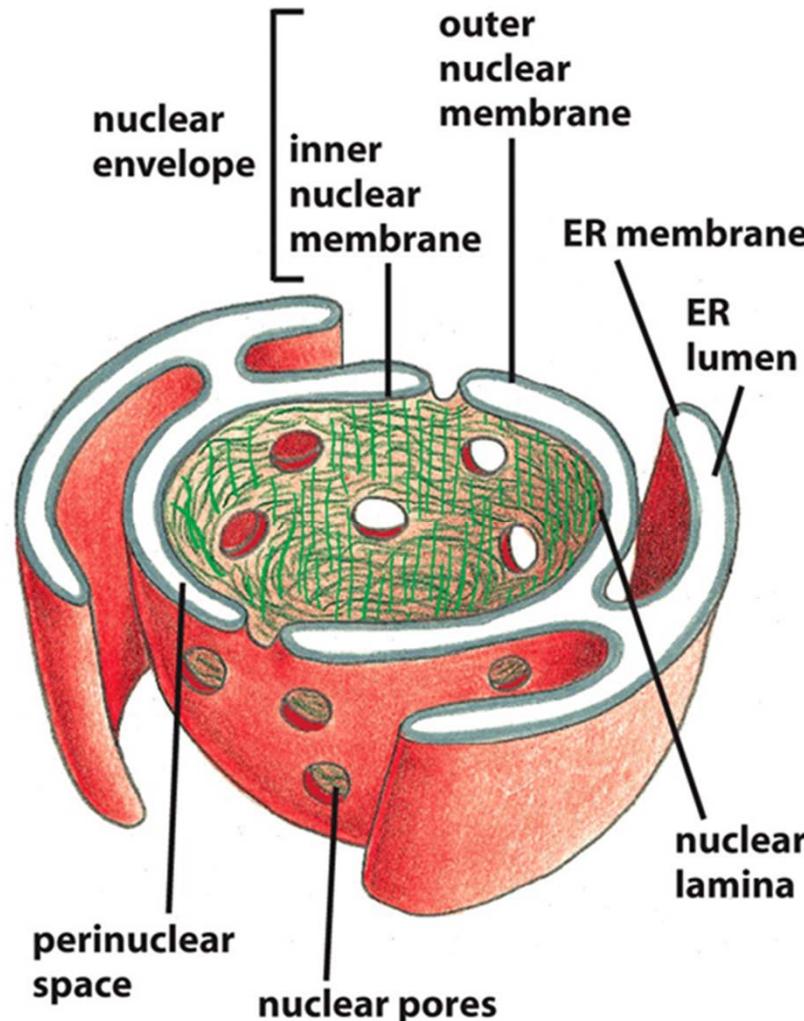
Molecules that are transported (**imported**) **in** the nucleus:

- histones
- DNA/RNA polymerases
- gene regulatory proteins (transcription factors)
- RNA processing proteins
- ribosomal proteins, etc.

Molecules that are transported (**exported**) **out** of the nucleus:

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

# The structure of nuclear envelope



## Inner membrane:

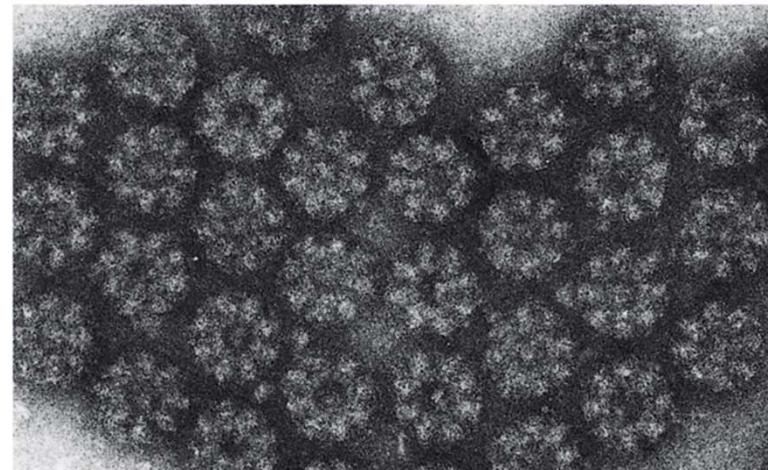
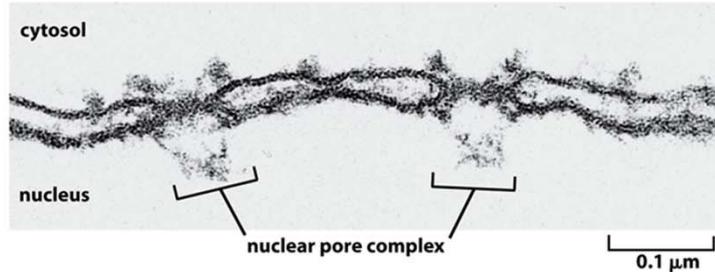
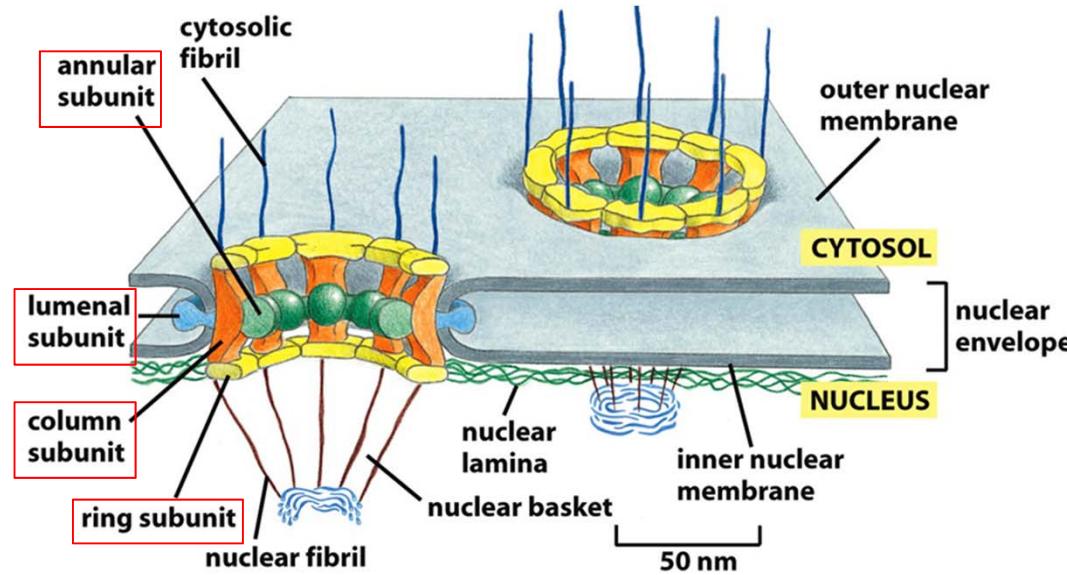
anchor sites for chromatin  
and for the nuclear lamina.

## Outer membrane:

continuation of ER  
with ribosomes: rough ER  
without ribosomes: smooth ER

# Nuclear pore complex (NPC)

4 building blocks:

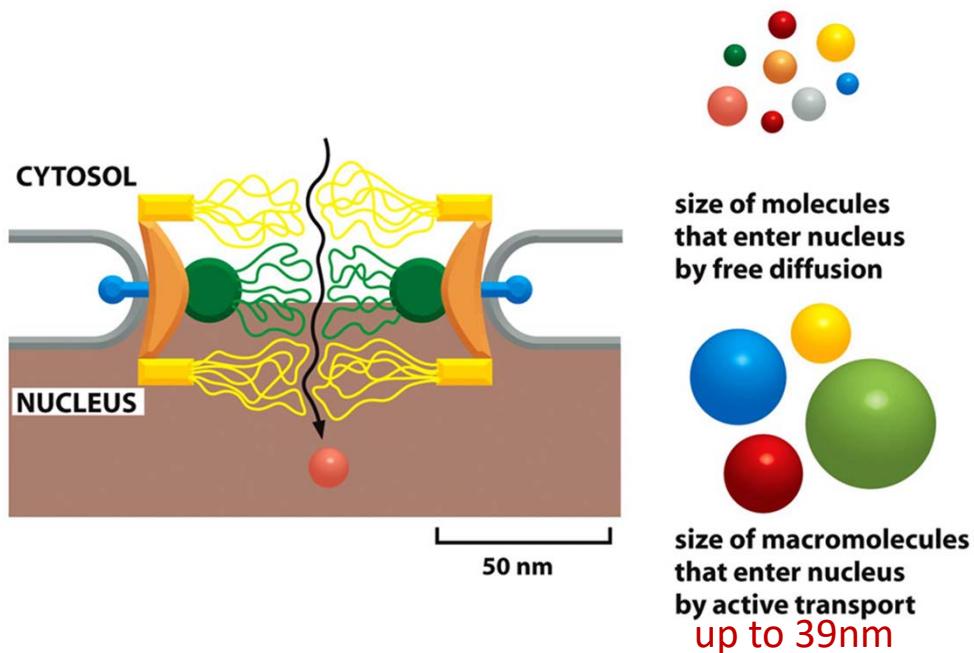


negative stained NPCs after  
detergent extraction

0.1  $\mu\text{m}$

## 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.



## 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**

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

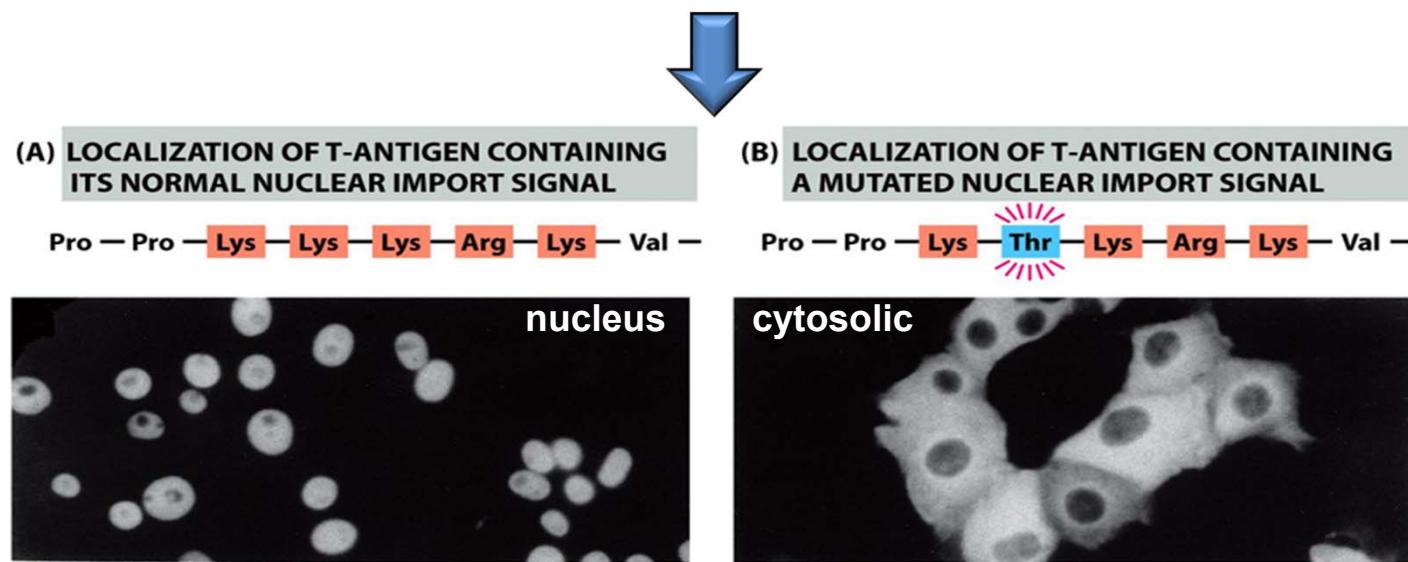
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## A nuclear localization signal (NLS) directs proteins into the nucleus

### Nuclear sorting signals:

- 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 (**structure**)

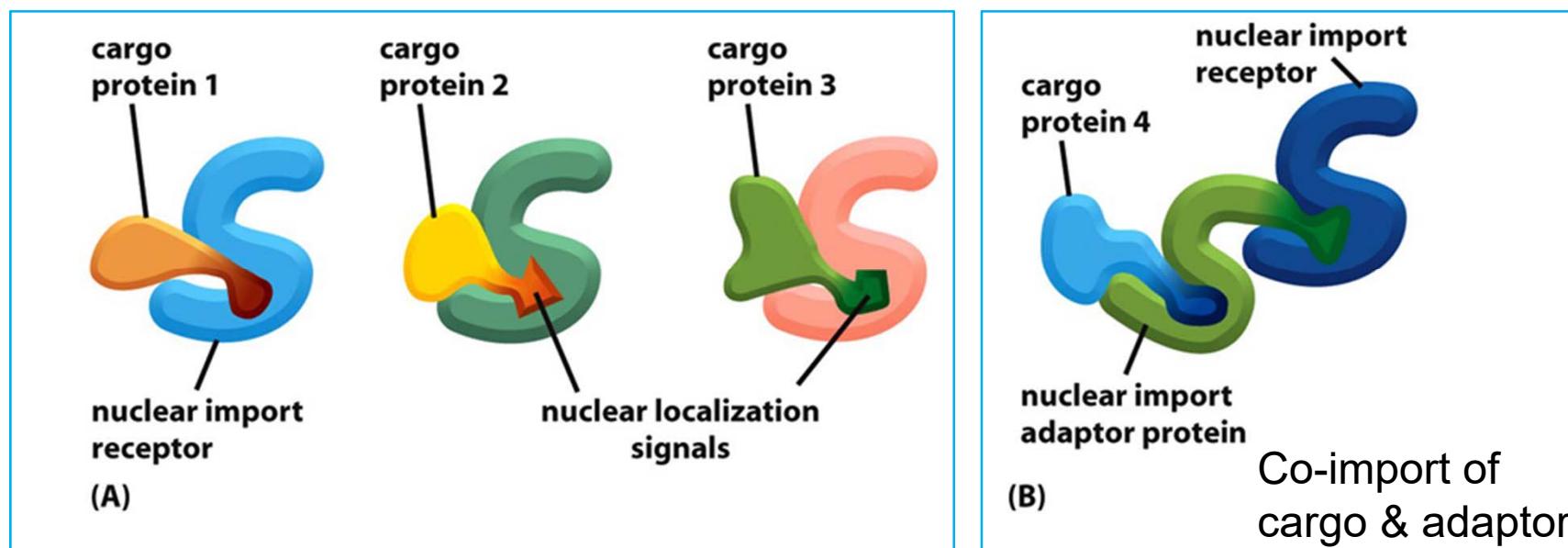
Mutation of key amino acids in the NLS destroy the signal;  
the mutant protein will lose its nuclear localization and remains in the cytosol!!!



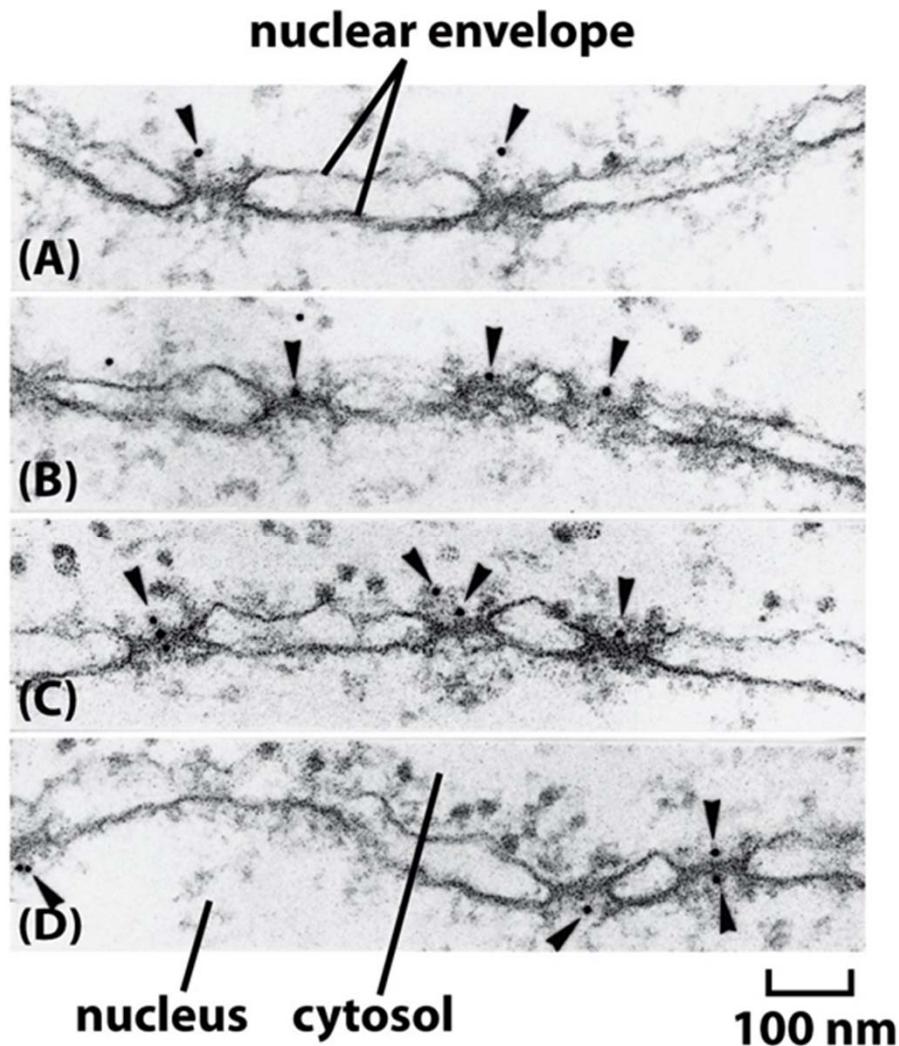
# Nuclear import receptors

## Features:

- Soluble cytosolic proteins
- Bridging NLS-cargo and FG-repeats of nucleoporin proteins
- Some receptors use **adaptor proteins** for “bridging”: the adaptors recognize NLS-cargo proteins and have also an own NLS to interact with the receptor



# Visualizing active import through NPCs



Gold particles were coated  
with NLS-peptides  
(time course experiment)

Coated gold particles were injected  
into cells. Cells were fixed at  
different time points after injection  
to **track gold particle movement**  
using electron microscopy

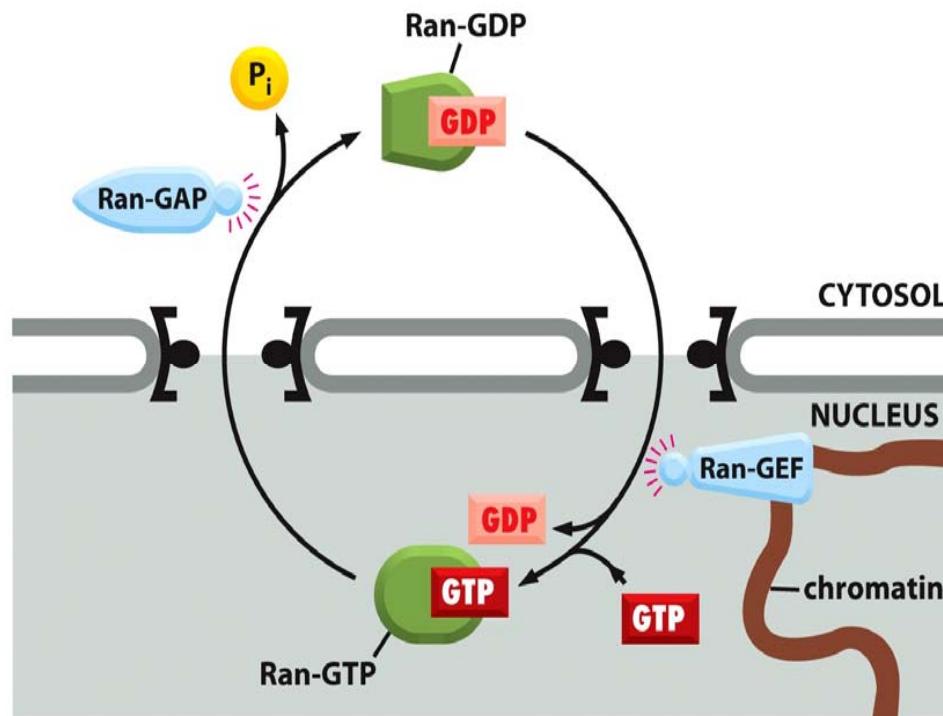
Coated gold particle can be seen to:  
1. interact with NPC  
2. get through the NPC

## Nuclear export

### Facts about nuclear export signals:

- Not many facts are known
  - Nuclear export signals relatively **poorly characterized**
  - Some have a Leucine rich sequence
  - Nuclear export receptors facilitates export.
  - Nuclear export receptors are structurally related to nuclear import receptors
-

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



The “GTPase cycle”:

**Two forms of RAN:**

- Ran-GDP (cytosol)
- Ran-GTP (nucleus)

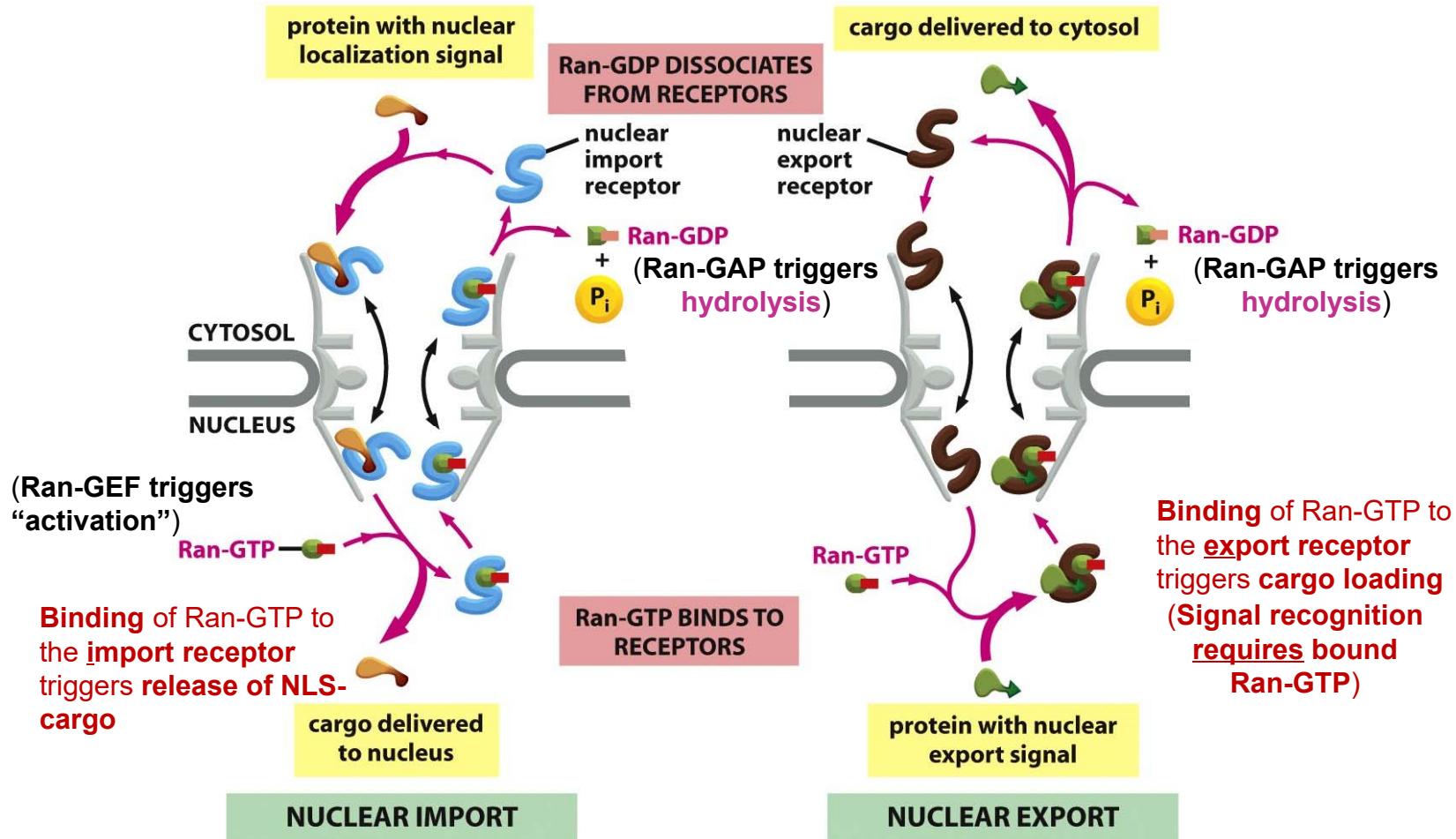
**Transport occurs according to gradient cytosol/nucleus**

Cytosolic Ran-GDP is “activated” by a Ran-GEF (Guanine nucleotide exchange factor) in the nucleus. This converts Ran-GDP to Ran-GTP

In the cytosol, Ran-GTP is stimulated by Ran-GAP (GTPase-activating protein) to hydrolyse its GTP (conversion to Ran-GDP)

**The “GTPase cycle” is a very important basic common important mechanism !!!**

# Ran controls both, nuclear import and nuclear export by interacting with the respective receptor:



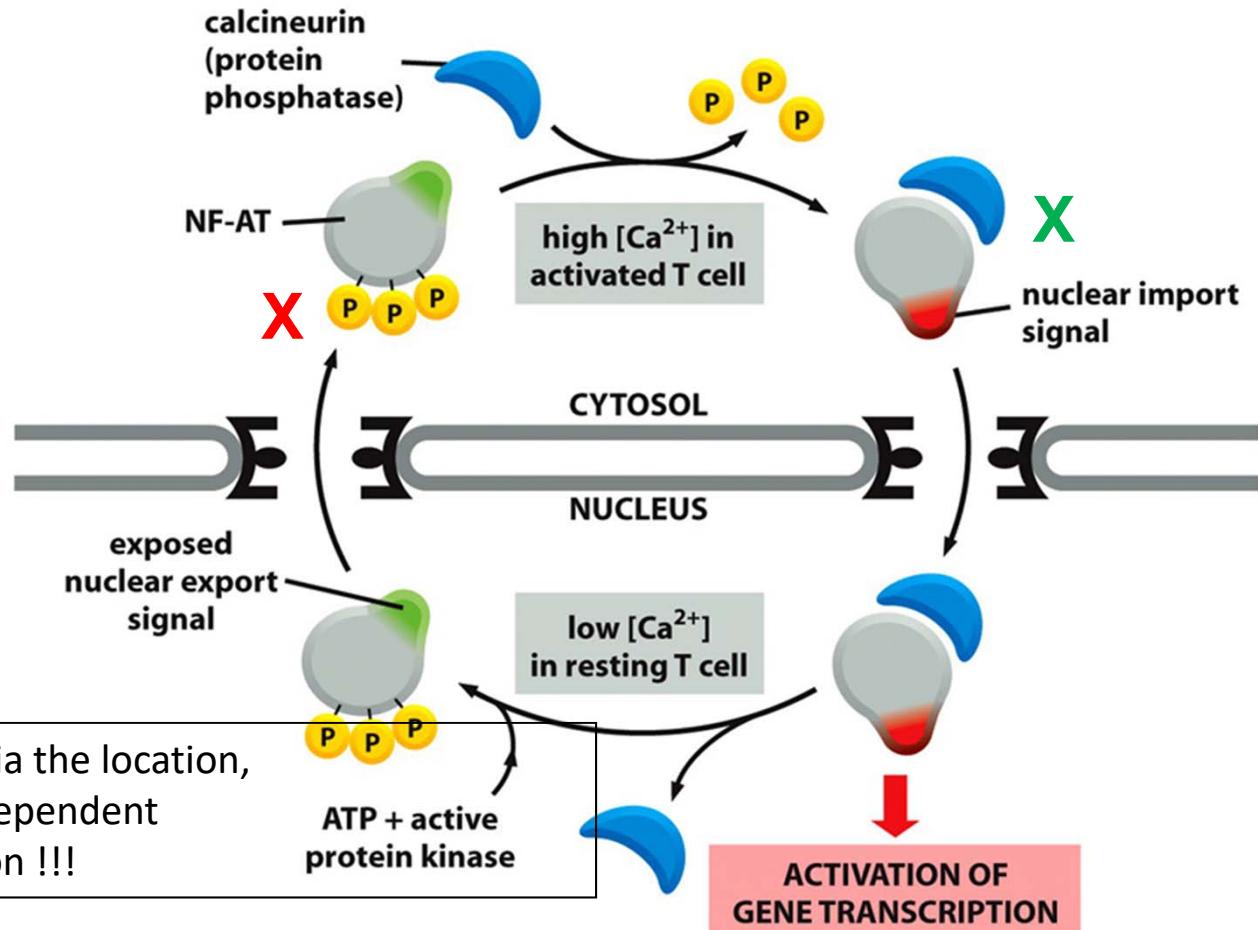
## Ran-independent nuclear transport through protein phosphorylation

The control of nuclear import of the transcription factor nuclear factor of activated T cells (NF-AT) during T cell activation due to altered Ca<sup>2+</sup> levels:

- high Ca<sup>2+</sup> activates, declining/low Ca<sup>2+</sup> levels deactivate

For example:

Nuclear **import (X)**  
and nuclear **export (X)**  
signal can be masked  
by its phosphorylation  
states.

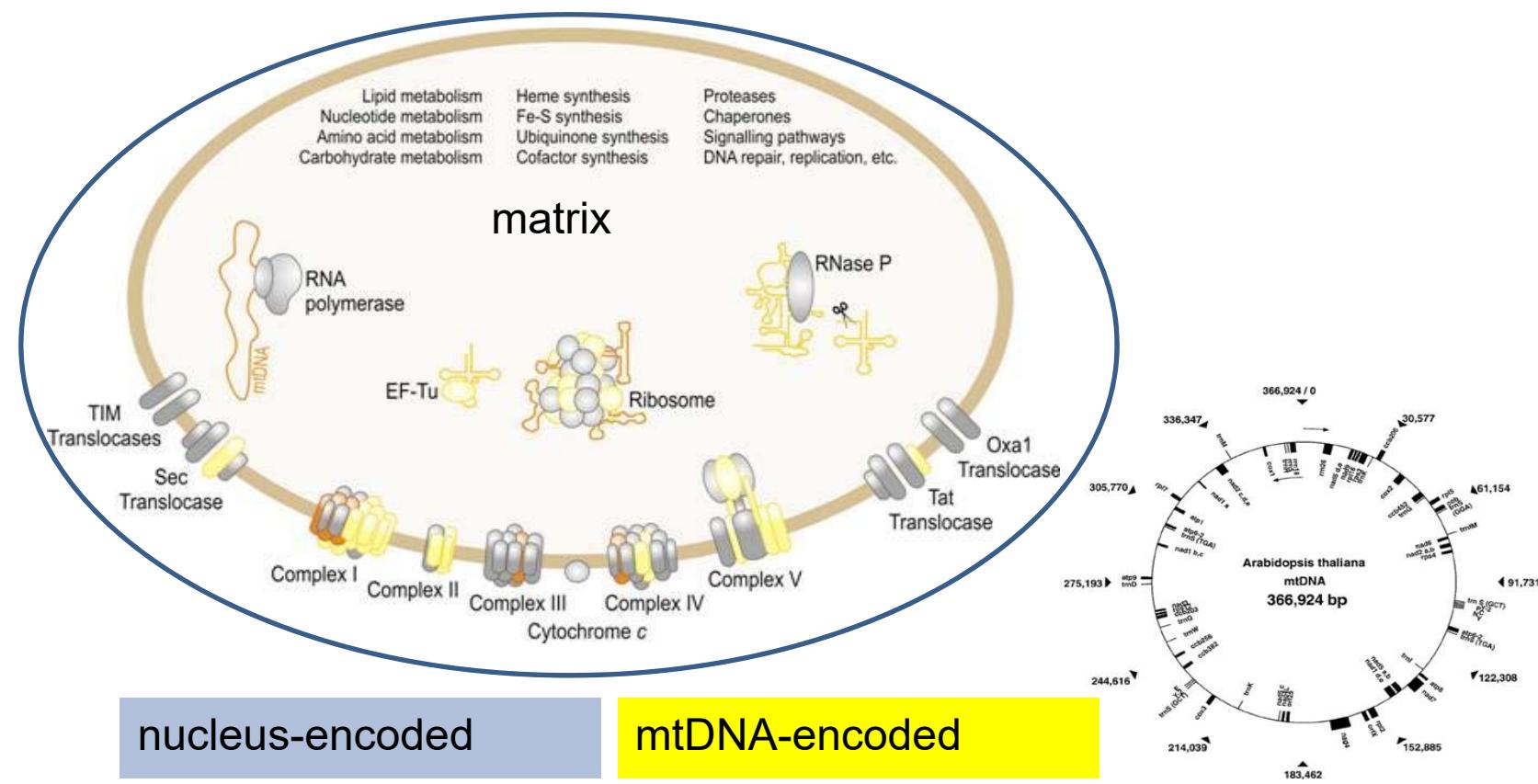


Regulation over time, not via the location,  
due to **altered Ca<sup>2+</sup> levels** dependent  
on the physiological situation !!!

### III. Transport in mitochondria and chloroplasts

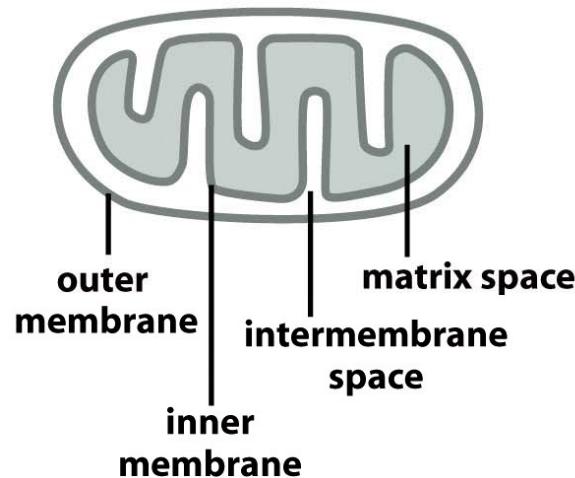
Why is targeting to mitochondria and chloroplasts important?

Even though mitochondria and chloroplast have their own protein synthesis machinery, the majority of proteins are encoded in the nucleus and are therefore synthesized in the cytosol and are then imported....

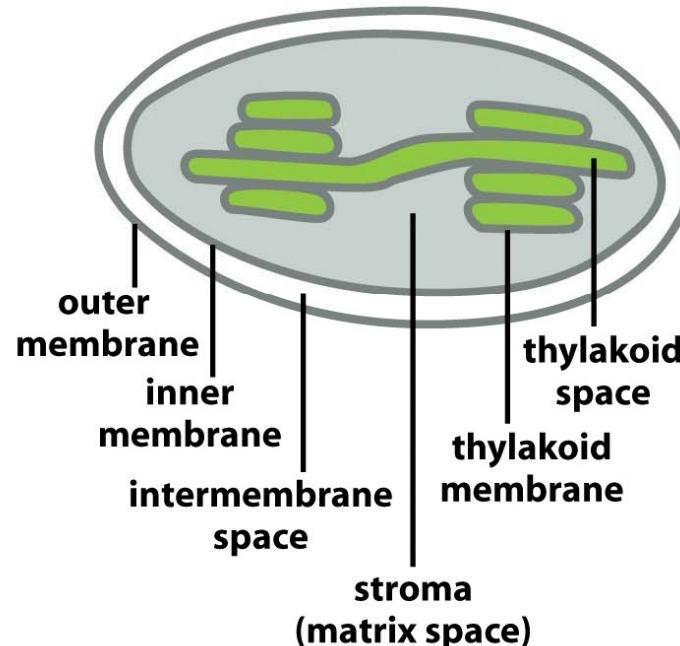


# Membranes in Mitochondrion and chloroplast

(A) MITOCHONDRION



(B) CHLOROPLAST



Mitochondria have two independent reaction rooms:

- IMS (intermembrane space)
- matrix

Targeting to mitochondria includes targeting to:

OM, IMS, IM, matrix

Chloroplasts have three independent reaction rooms:

- IMS (intermembrane space)
- stroma
- thylakoids

Targeting to chloroplasts includes targeting to:

OM, IMS, IM, stroma, TM, thylakoid lumen

## Import in mitochondria and plastids requires sorting signals...

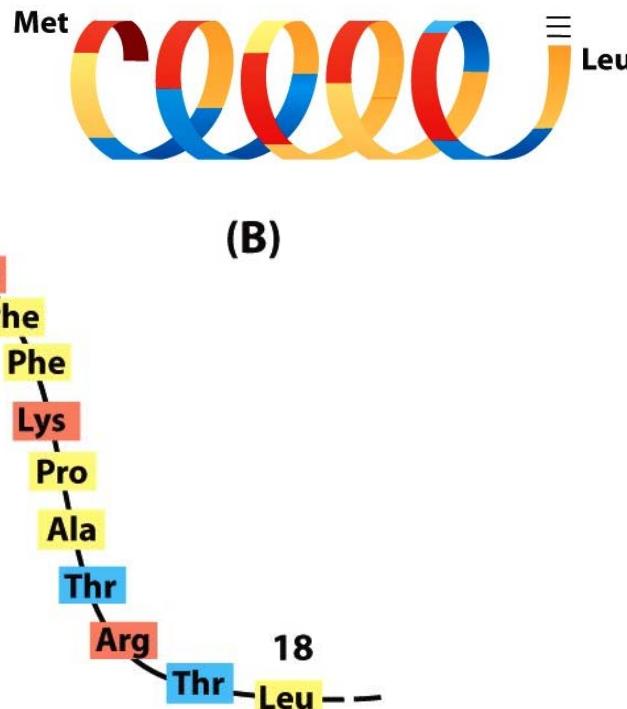
- Mitochondria and chloroplast precursor proteins are fully synthesized in the cytosol before their **import**.
    - Chaperones keep the proteins in an unfolded “import-competent” state and guide them to the TOM/TOC complexes
    - their import is referred to as “**transmembrane transport**”
  - **An N-terminal peptide** directs many precursor proteins to matrix, then, this signal peptide it is cleaved upon arrival.
  - **An internal peptide** directs all precursor proteins into the **outer membrane**, many to be in the **inner membrane** and **intermembrane (thylakoids)**
-

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

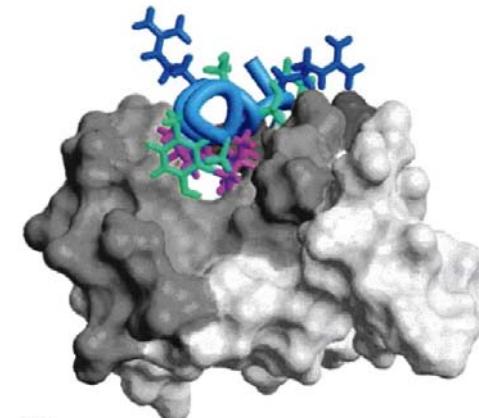
The signal: one side of the helix is **hydrophilic** the other side is **hydrophobic**



Positively charged aa on one side,  
Nonpolar aa on other side



(A)



(C)

Signal sequence of the alcohol dehydrogenase (blue), bound to an import receptor (gray). The amphiphilic  $\alpha$ -helix binds with its hydrophobic face to a hydrophobic groove in the receptor

# Different protein translocators facilitate mitochondria protein transport across the membranes

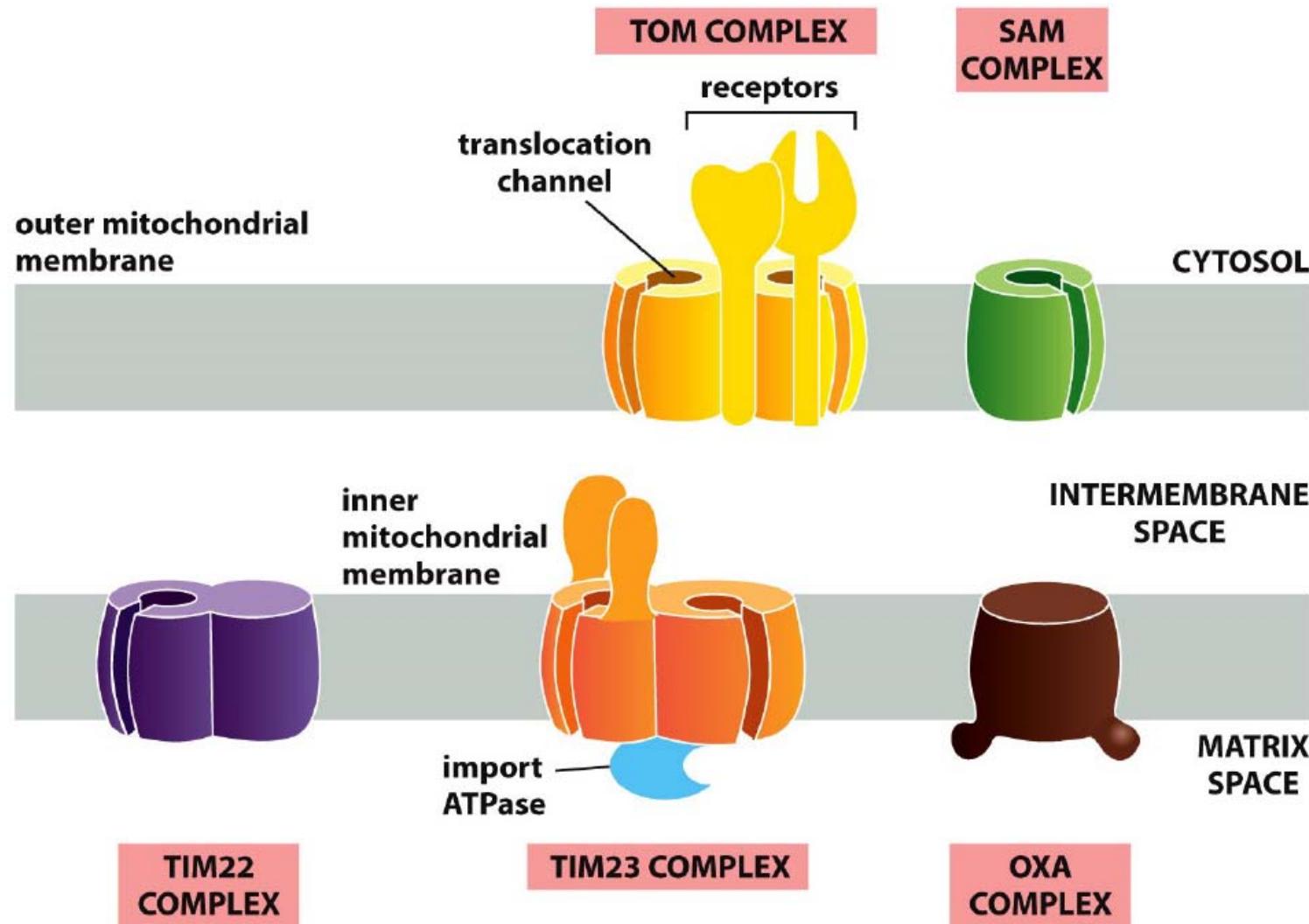
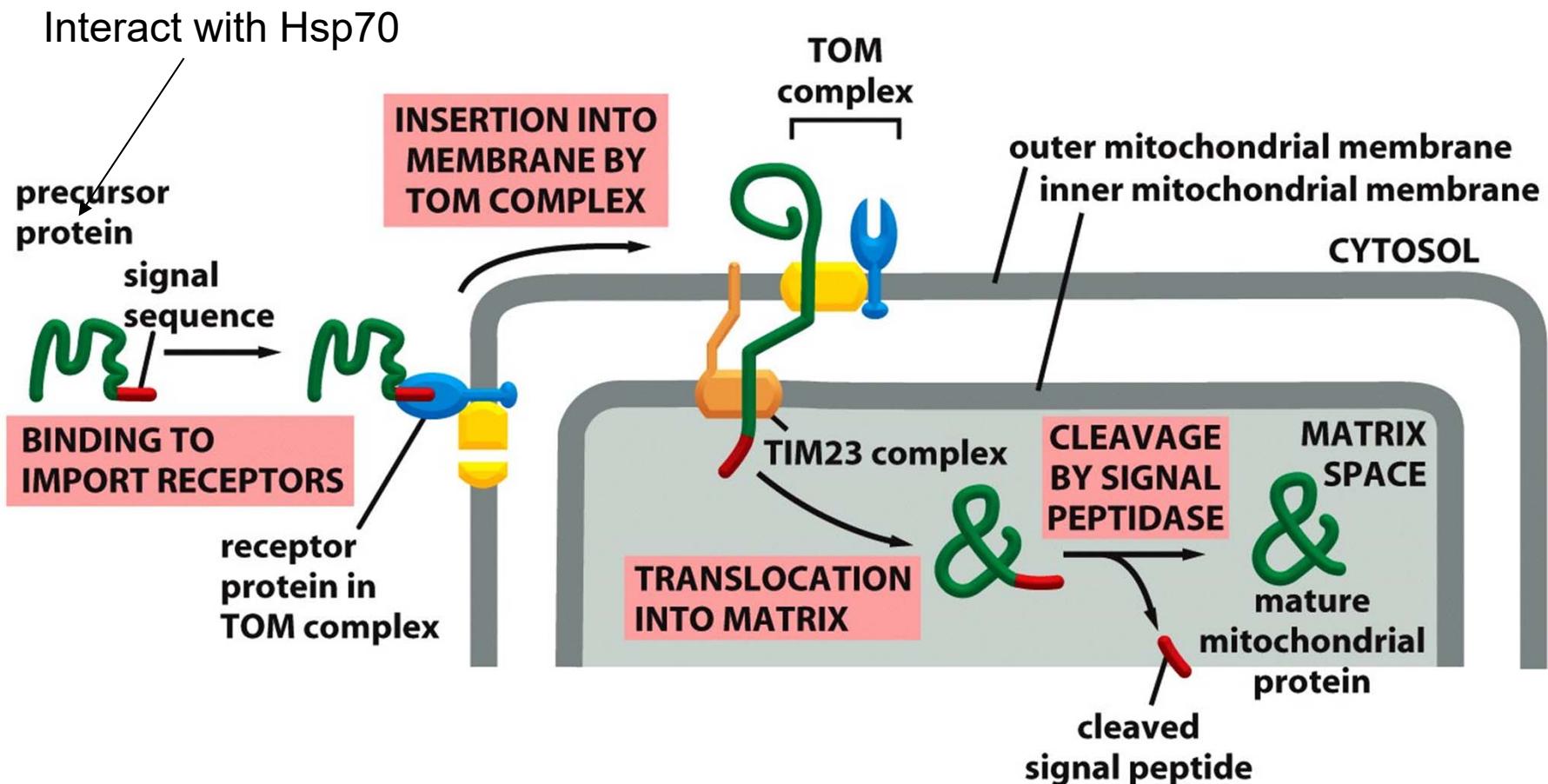


Figure 12-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

# All translocators are multimeric proteins

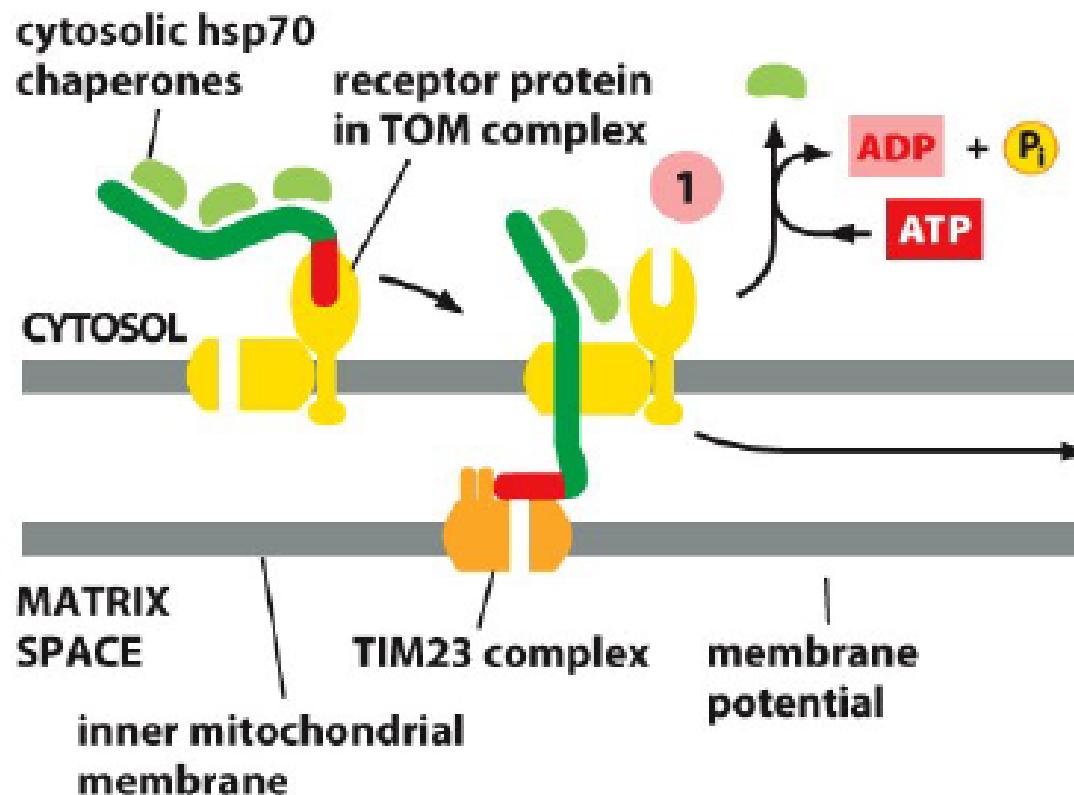
- **TOM** complex (**T**ranslocase **o**uter membrane **m**itochondria)
    - transfers proteins across the **outer** mitochondria membrane.
  - **TIM23** complex (**T**ranslocase **i**nner membrane **m**itochondria)
    - transfer proteins across the **inner** mitochondria membrane.
- TOM & TIM23, both** function as receptors for signal peptides and can form a transmembrane channels spanning **both** membranes
- **SAM** complex (**s**orting and **a**ssembly complex **m**itochondria)
    - helps to **insert/fold** properly the **β-barrel proteins** in **outer** membrane.
  - **OXA** complex (**O**xidase **a**ssembly **m**achinery)
    - helps to insert **mitochondria-synthesized** proteins and others transported proteins **from the matrix** into the **inner membrane**

# Protein import in mitochondria: Targeting to the matrix via TOM & TIM23



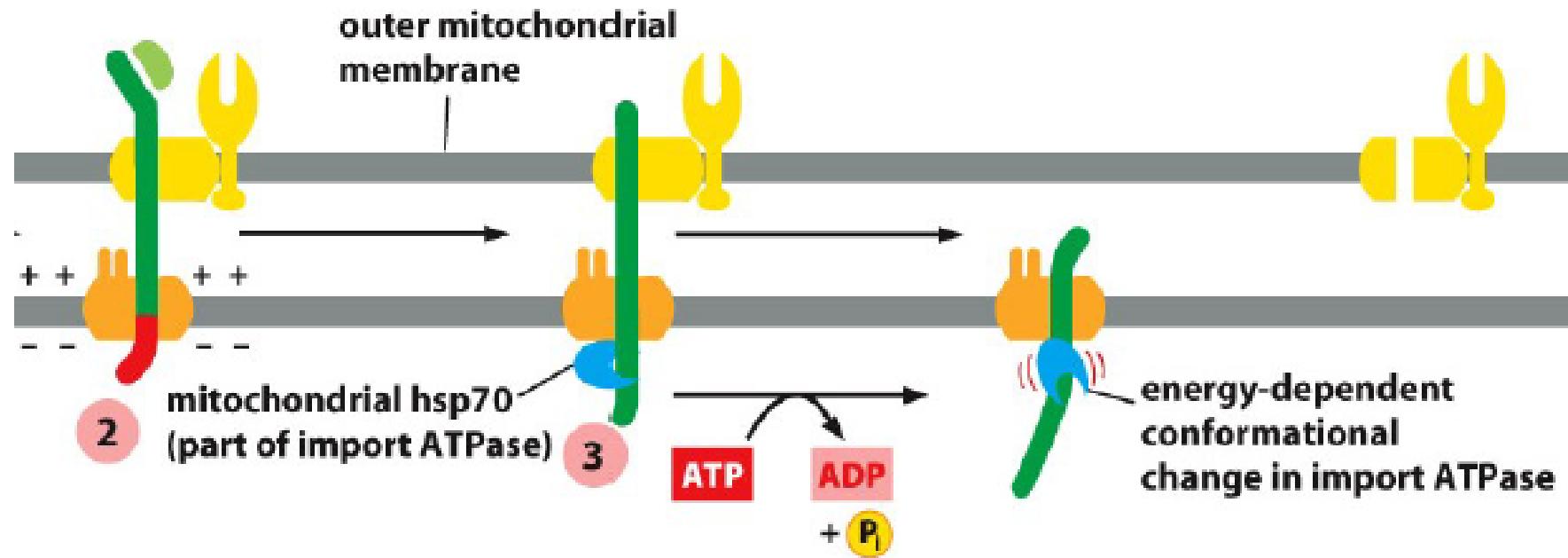
The precursor of the **matrix** protein is passing **both** membranes at the same time;  
the signal sequence works for **TOM** and **for TIM23**

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



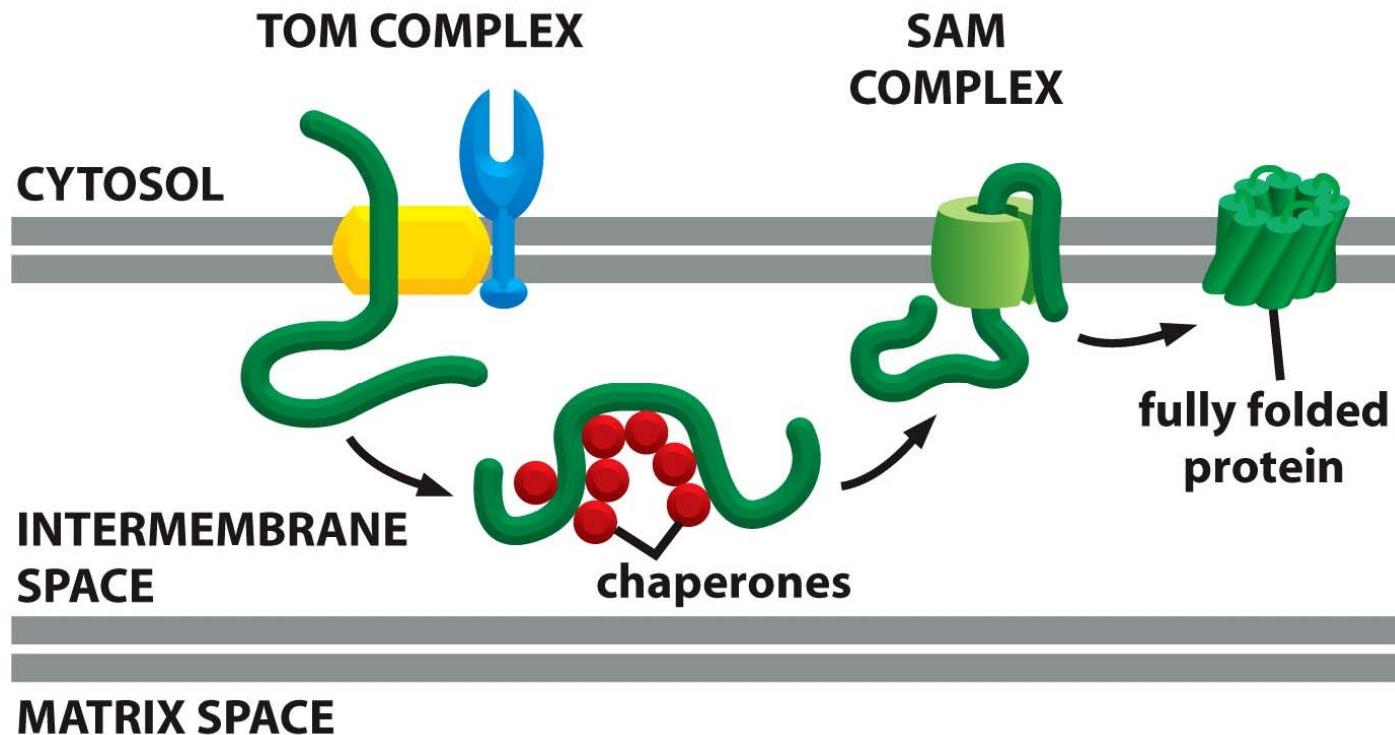
1. ATP is consumed by the chaperones (cytosolic hsp70) for “releasing” the precursor protein  
(Chaperones hydrolase ATP via an ATPase domain)

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



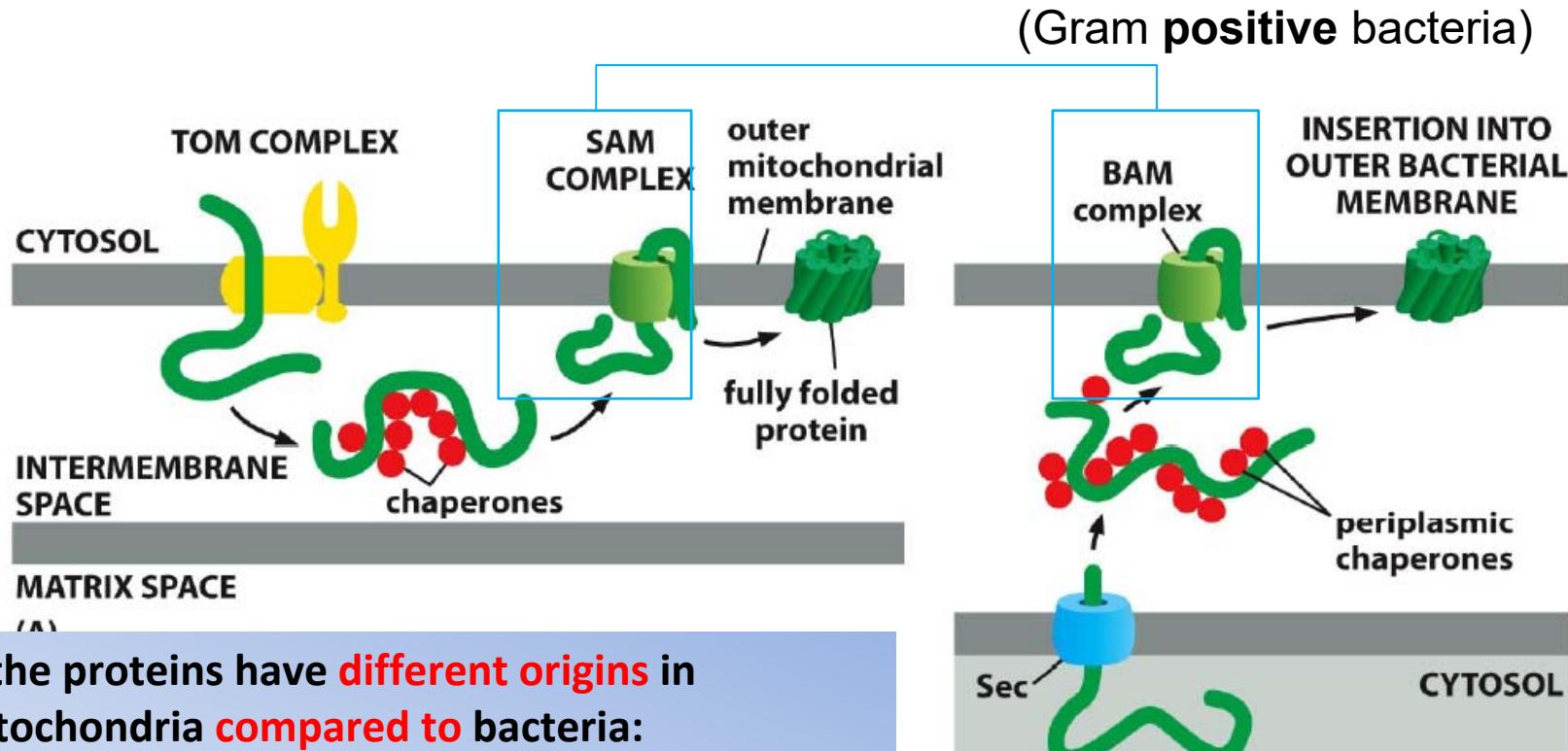
2. Translocation of the (positively charged) signal sequence depends on the membrane potential
3. Mitochondrial hsp70 consumes ATP for “pulling” (binding&release cycles)

Outer membrane porins ( $\beta$ -barrel proteins) are inserted by the help of SAM via the intermembrane space (IMS)



1. **Unfolded  $\beta$ -barrel proteins are translocated via TOM to the intermembrane space (IM) where they are stabilized by chaperones**
2. **Then, they are inserted in to the outer membrane via the SAM complex, which also aids folding (finally)**

## Bacteria and mitochondria use similar mechanisms to insert porins ( $\beta$ -barrel proteins) into the outer membrane, but...



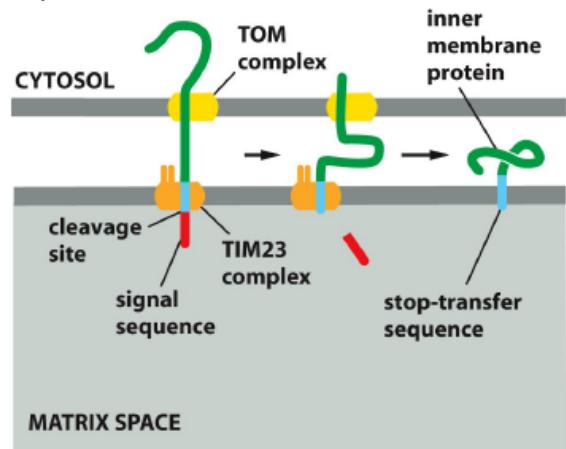
... the proteins have **different origins** in mitochondria **compared to bacteria**:

**Mitochondria:** proteins reach the IMS from the “outside” via TOM

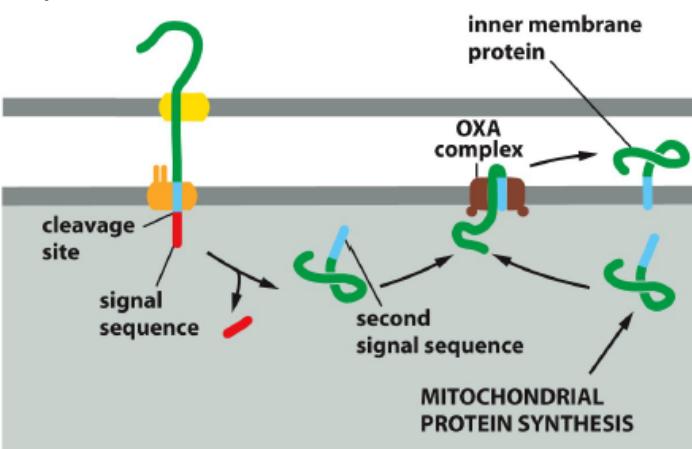
**Bacteria:** proteins reach the peri-bacterial space from “inside” (via Sec complex)

# Different ways to import proteins into the inner membrane (IM) and in the intermembrane space (IMS)

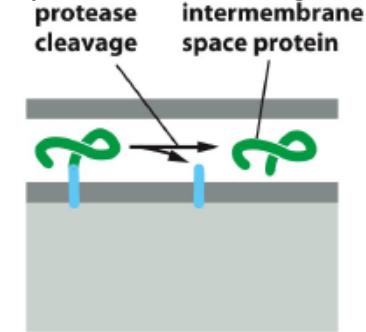
## 1) TOM & TIM23...



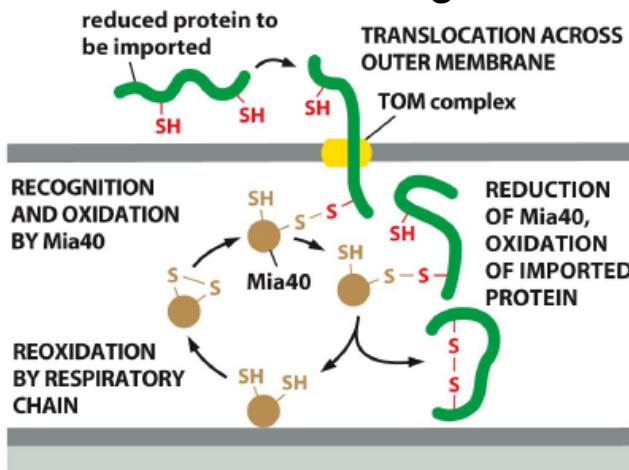
## 2) TOM & TIM + OXA



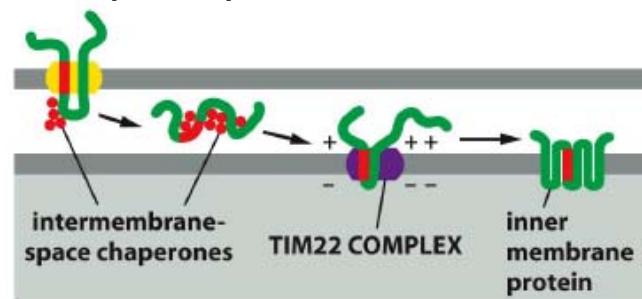
## 2) ...+ cleavage



## 4) TOM & Mia40: folding in the IMS



## 5) TOM & TIM22 insertion of multipass proteins in the IM



# Import and targeting of proteins into the inner membrane (IM)

Option1:

TOM & TIM23 & signal sequence & hydrophobic stop-transfer sequence

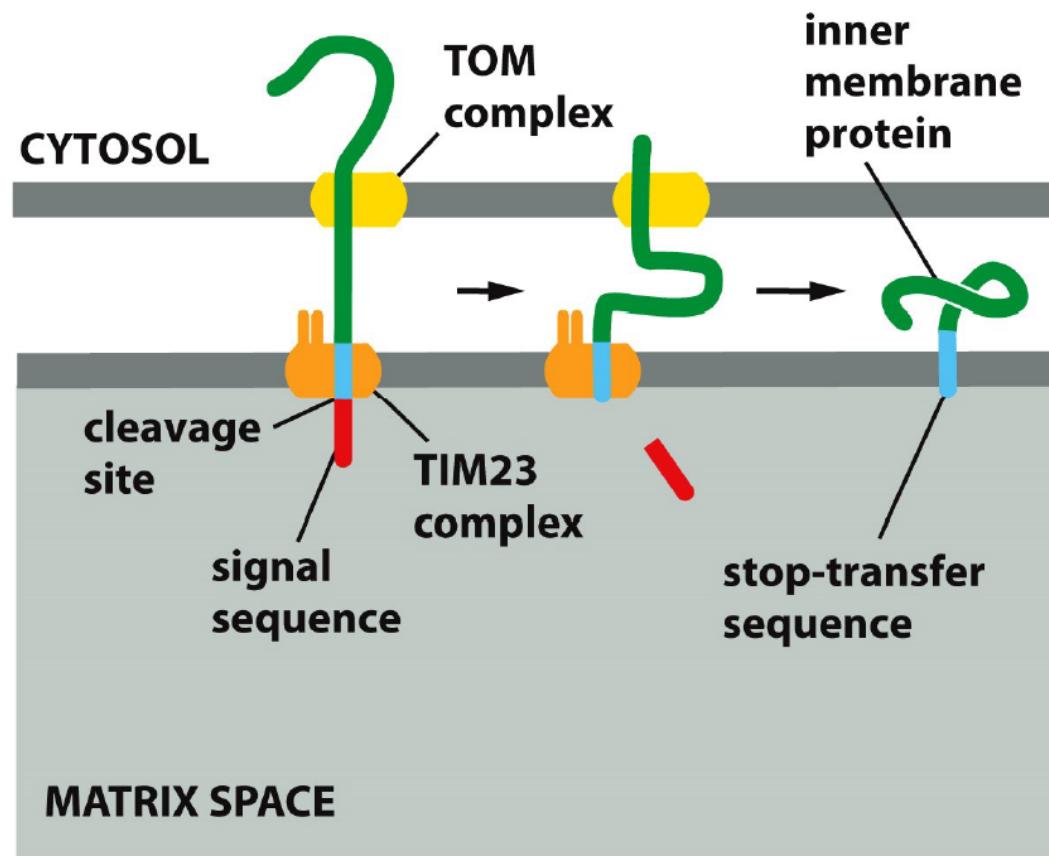
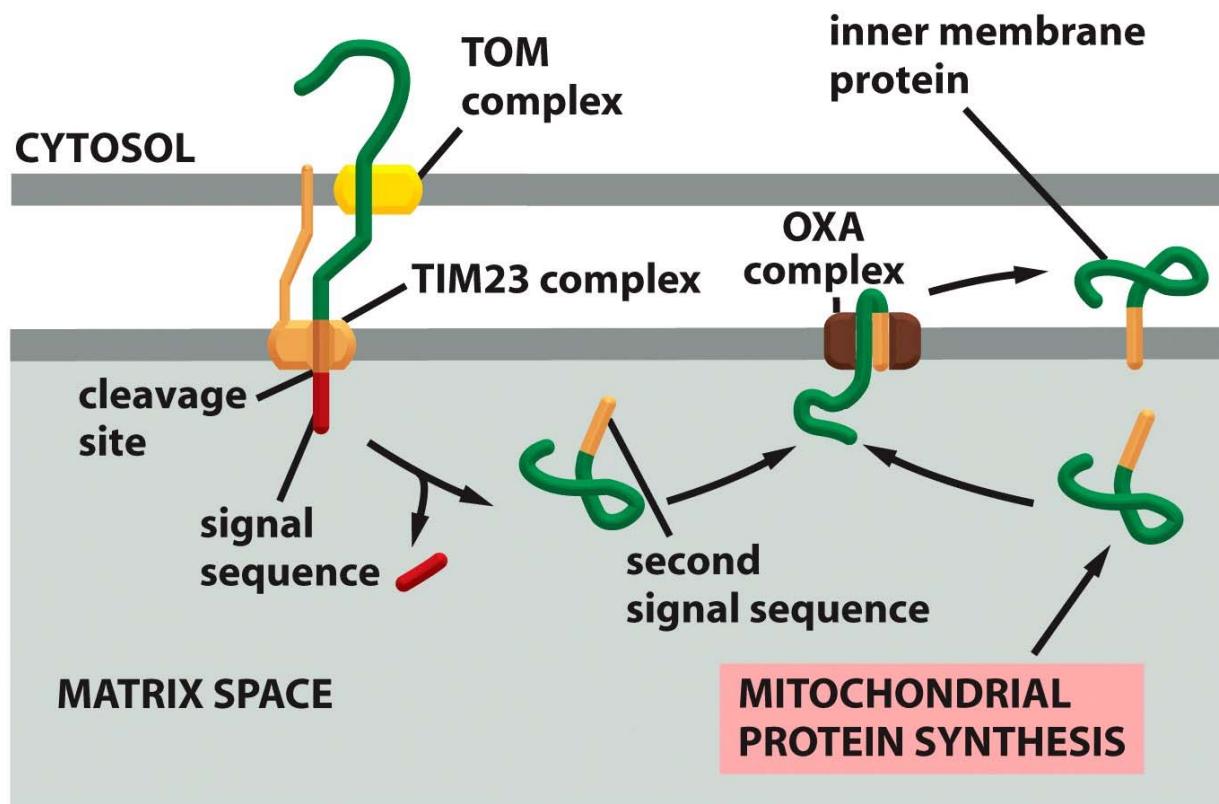


Figure 12-25a Molecular Biology of the Cell 6e (© Garland Science 2015)

## Import and targeting of proteins into the inner membrane (IM)

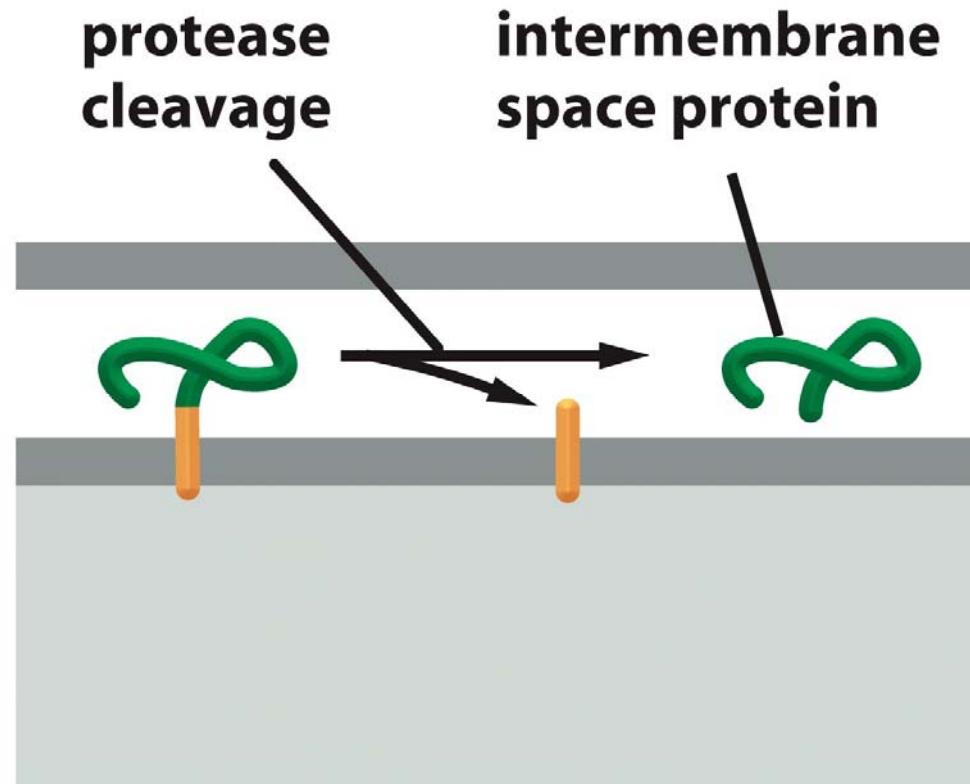
Option2:

TOM & TIM23 & OXA & signal sequence & second signal sequence



## Protein import into inner membrane (IM) and into the intermembrane space (IMS)

Targeting of a soluble protein into the intermembrane space (IMS):  
**(TOM & TIM23) & IM-localizing protease/peptidase**



# Protein import into inner membrane (IM) and into the intermembrane space (IMS)

Targeting a soluble protein to the IMS via TOM and folding in the IMS by Mia40

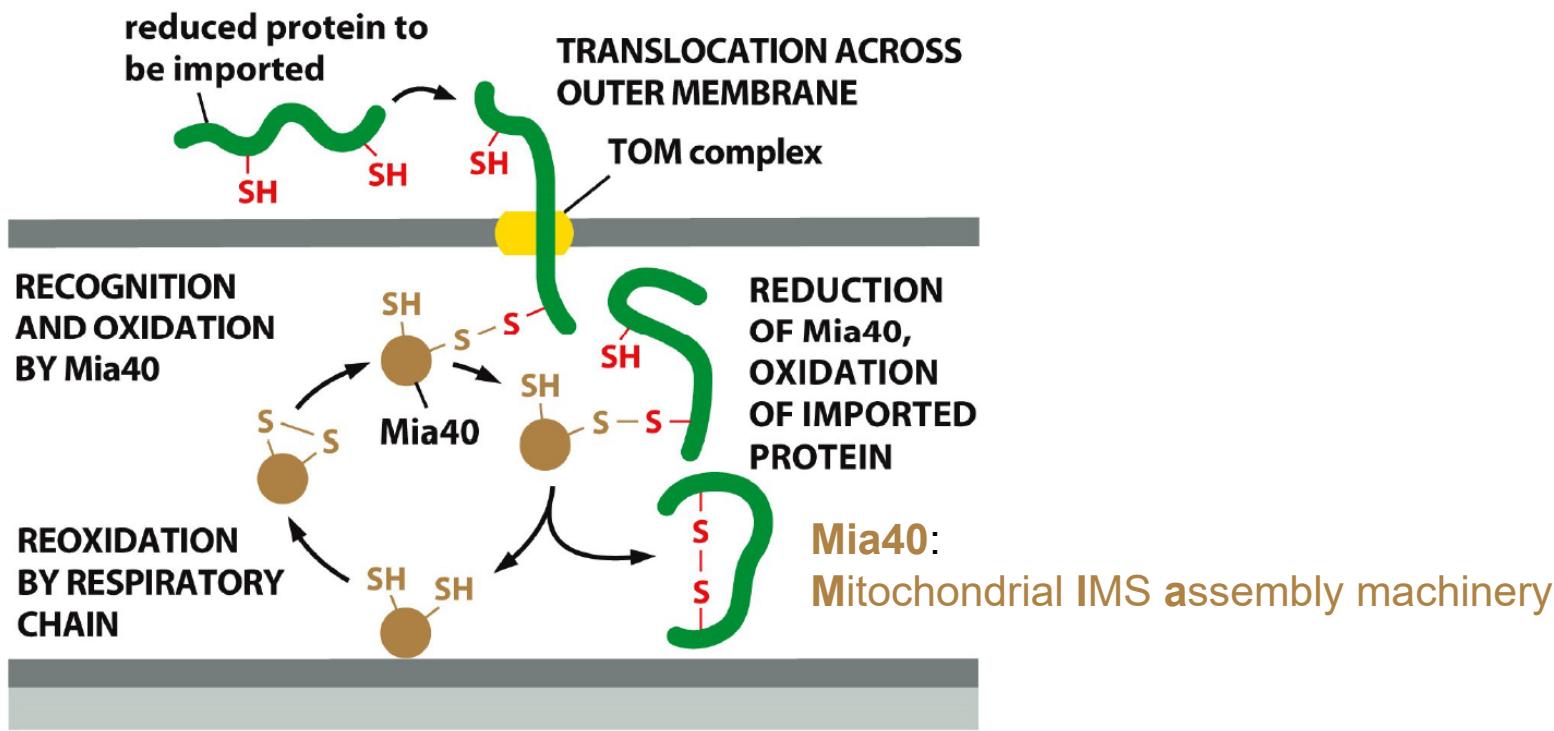
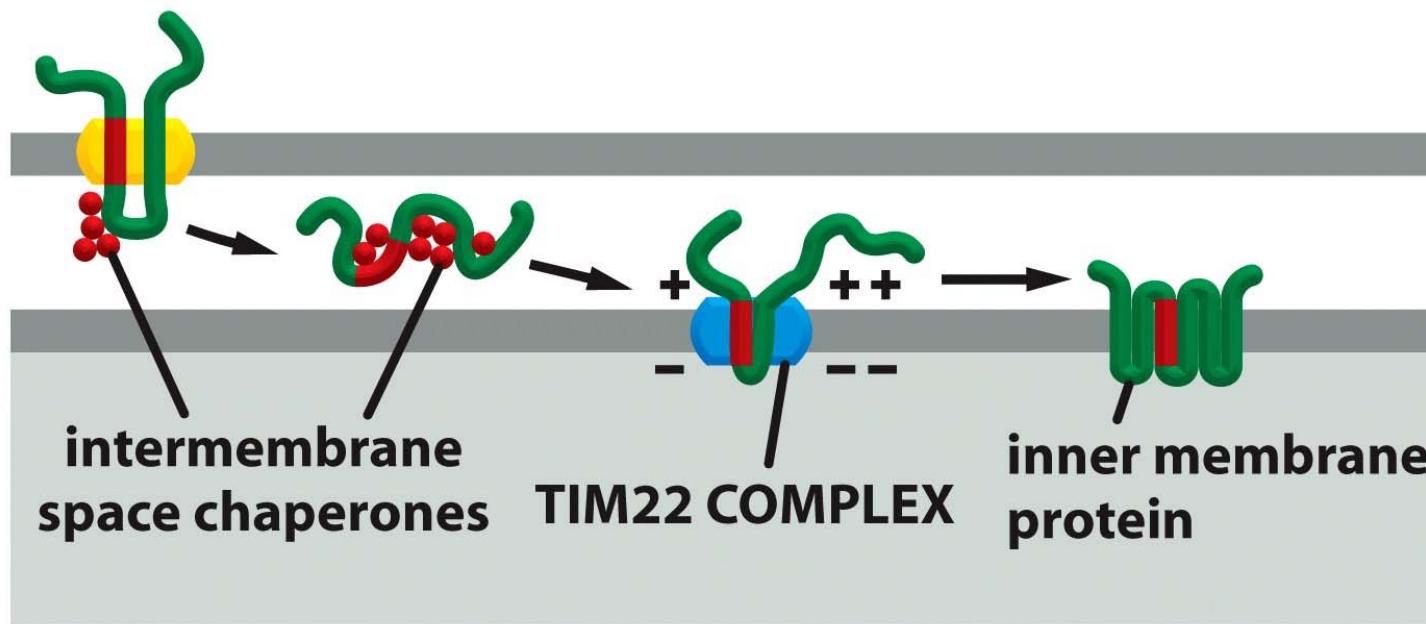


Figure 12-25d Molecular Biology of the Cell 6e (© Garland Science 2015)

Mia40 forms a **covalent intermediate** through an **intermolecular disulfide bond**, which helps folding/pulling of the transported protein through the TOM complex. Mia40 is reduced in the process, and is then reoxidized by the electron transport chain...

## Import and targeting of proteins into the inner membrane (IM)

Targeting of a multipass IM protein via TOM & chaperones & TIM22



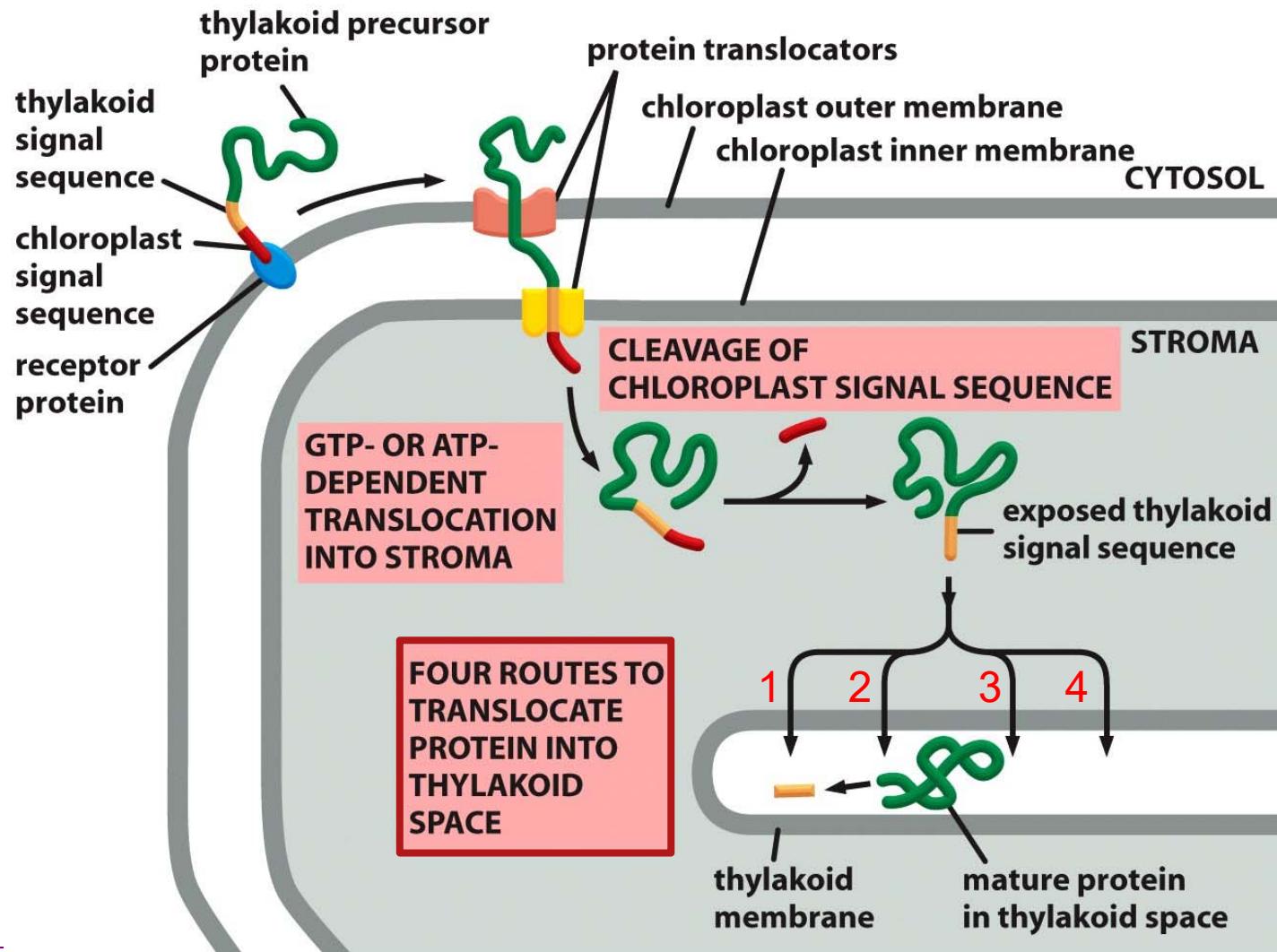
Multipass IM proteins that function as metabolite transporters contain internal signal sequences and sneak through the TOM complex as a loop.

IMS chaperones bind the precursor in the intermembrane space and guide the protein to the TIM22 complex, which is specialized for the insertion of multipass IM proteins.

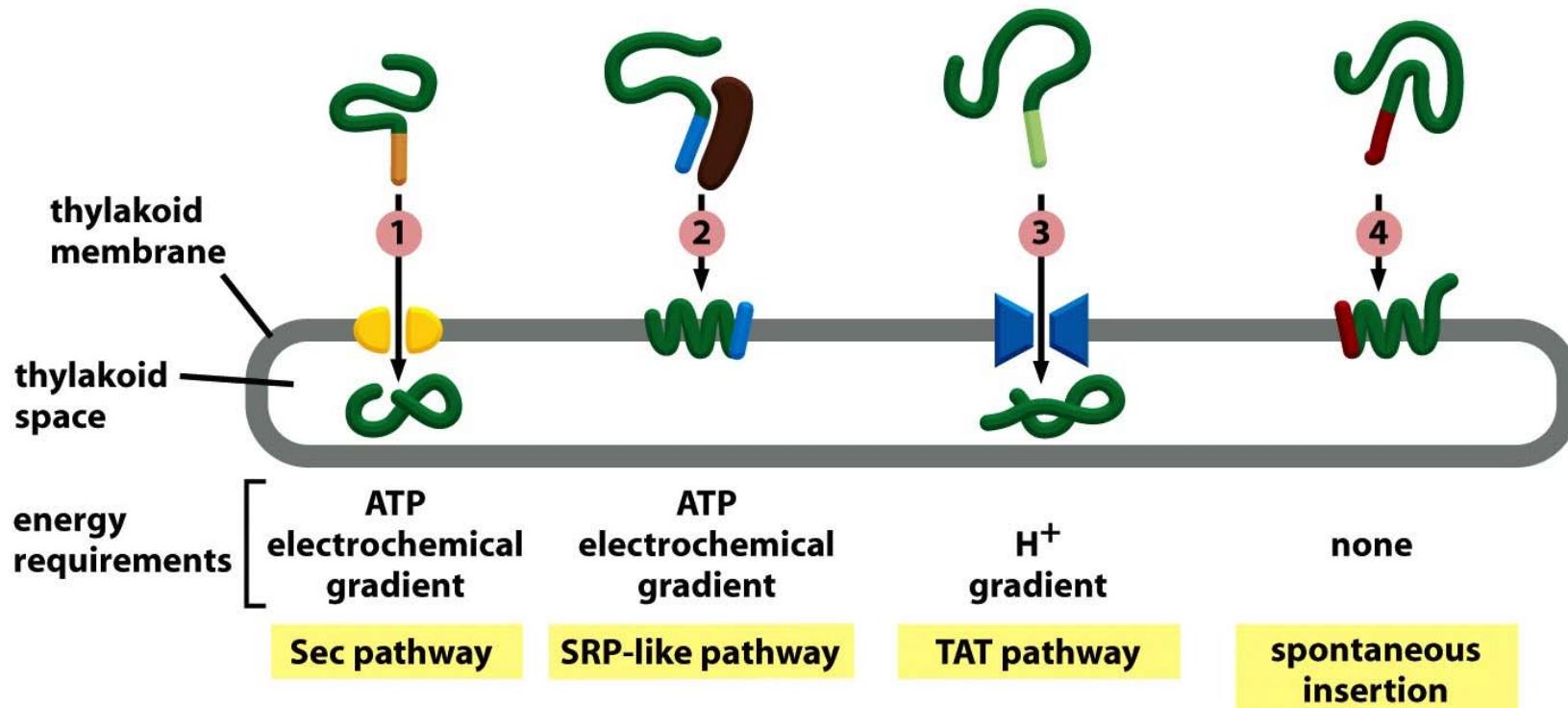
## Membrane protein transport in chloroplast differs from that of mitochondria

- **Two signal peptides** directs proteins to the thylakoid, the **first** to reach the **stroma**, the **second** to reach the **thylakoids (thylakoid-specific)**.
- Import receptors are chloroplast–specific:  
**TOC:** Translocase outer membrane chloroplast  
**TIC:** Translocase inner membrane chloroplast
- Not driven by electrochemical energy, import is driven by ATP and GTP hydrolysis, instead.

# Translocation of chloroplast precursor proteins into the thylakoid space



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



1. **Sec pathway**, so called because it uses components that are homologs of Sec proteins which mediate protein translocation across the bacterial plasma membrane (discussed)
2. **SRP-like pathway**, so called because it uses a chloroplast homolog of the **signal-recognition particle (SRP)**
3. **TAT (twin arginine translocation) pathway**, so called because **two arginines are critical** in the signal sequences that direct proteins into this pathway, which **depends on the H<sup>+</sup> gradient across the thylakoid membrane**
4. **Spontaneous insertion pathway** that seems **not to require any protein translocator**.

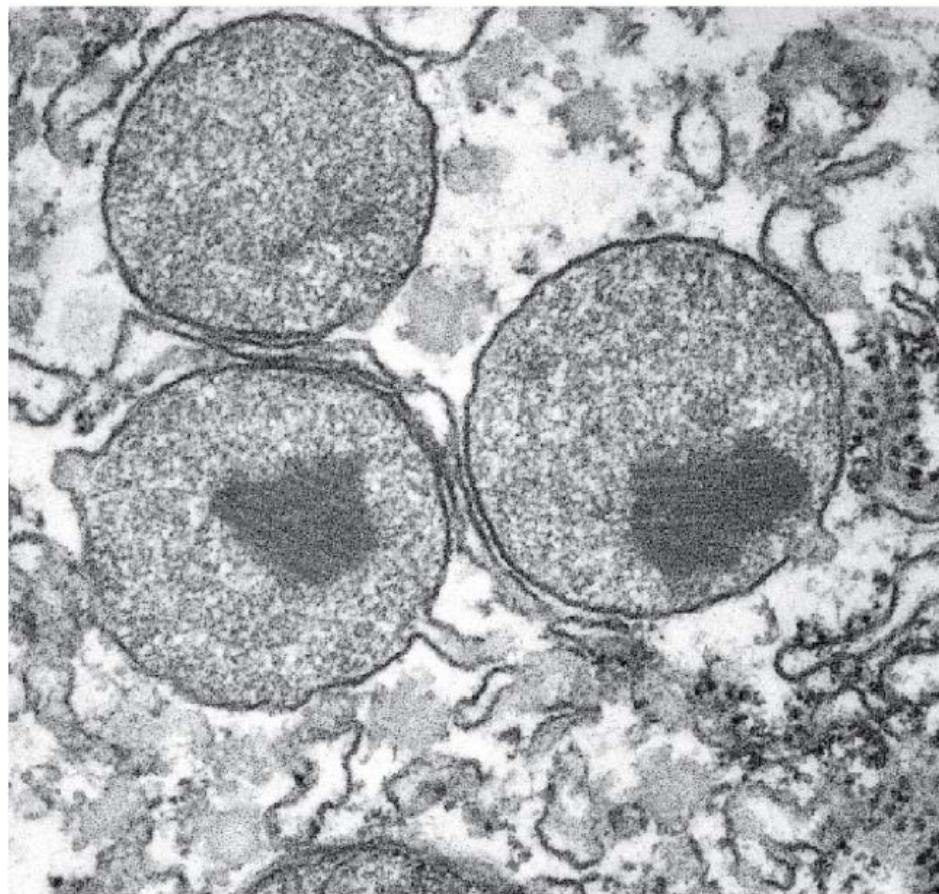
## IV. Transport in peroxisomes

### Peroxisomes: an overview

- All eukaryotic cells **have** peroxisomes
- **Acquire** most protein **from cytosol**, some **from ER**
- Contains **catalase** and **oxidase**.
- Use **O<sub>2</sub>** to **produce** hydrogen peroxide (**H<sub>2</sub>O<sub>2</sub>**)
- **Catalase** uses 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).
- **Plants contain two types of peroxisomes:**
  - **Leaf peroxysomes: catalyze photorespiration**
  - **Glyoxysomes in seeds: catalyze β-oxidation of fatty acids (glyoxylate cycle) to produce carbohydrates**



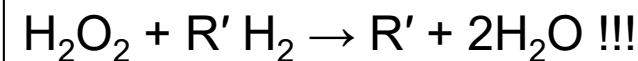
## Peroxisomes in rat liver cell under EM



Electron micrograph of peroxysomes  
with **paracrystalline, electron-dense**  
**inclusions (enzyme urate oxidase)**

200 nm

**Peroxisomes oxidize a variety of other substrates:**  
including **phenols, formic acid, formaldehyde, and alcohol** by the **“peroxidative” reaction**



This type of oxidative reaction is particularly important **in liver** and **kidney cells**, where **peroxisomes detoxify various toxic molecules** that enter the bloodstream. About **25% of the ethanol** we drink is **oxidized to acetaldehyde** in this way!

## Two types of peroxisomes in plant cells: peroxysomes and glyoxysomes

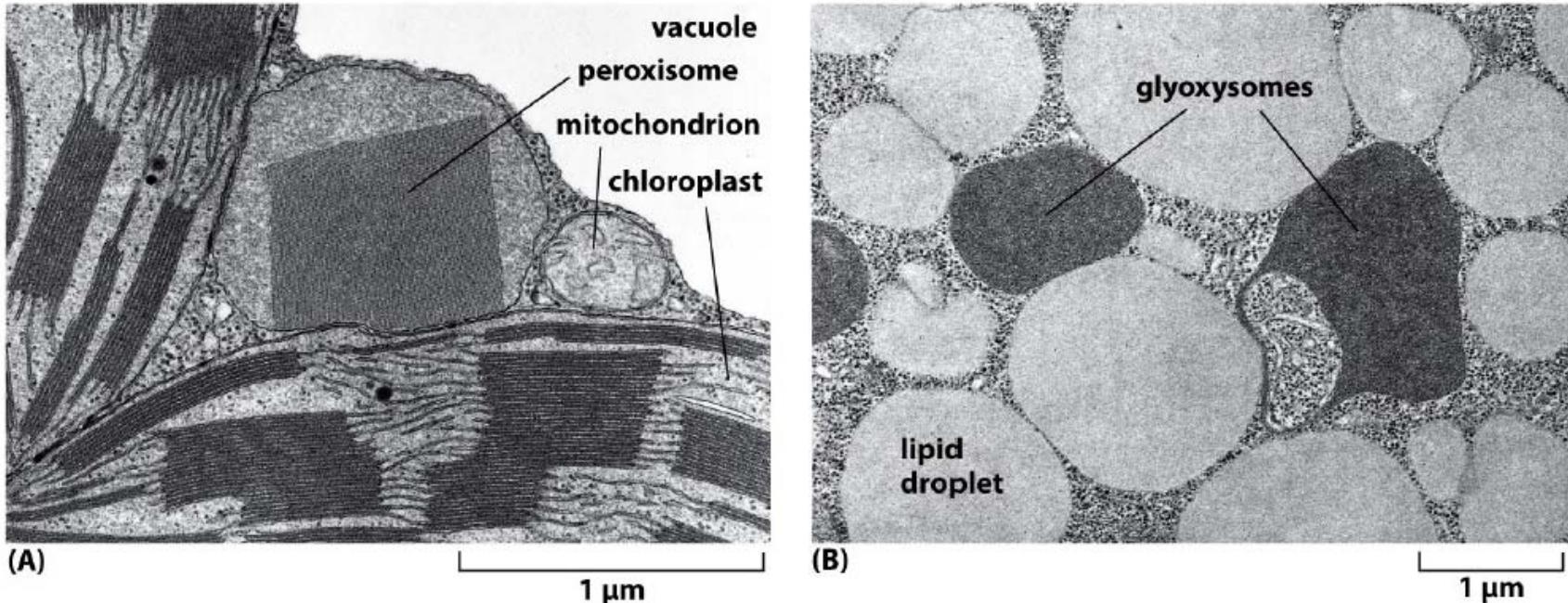
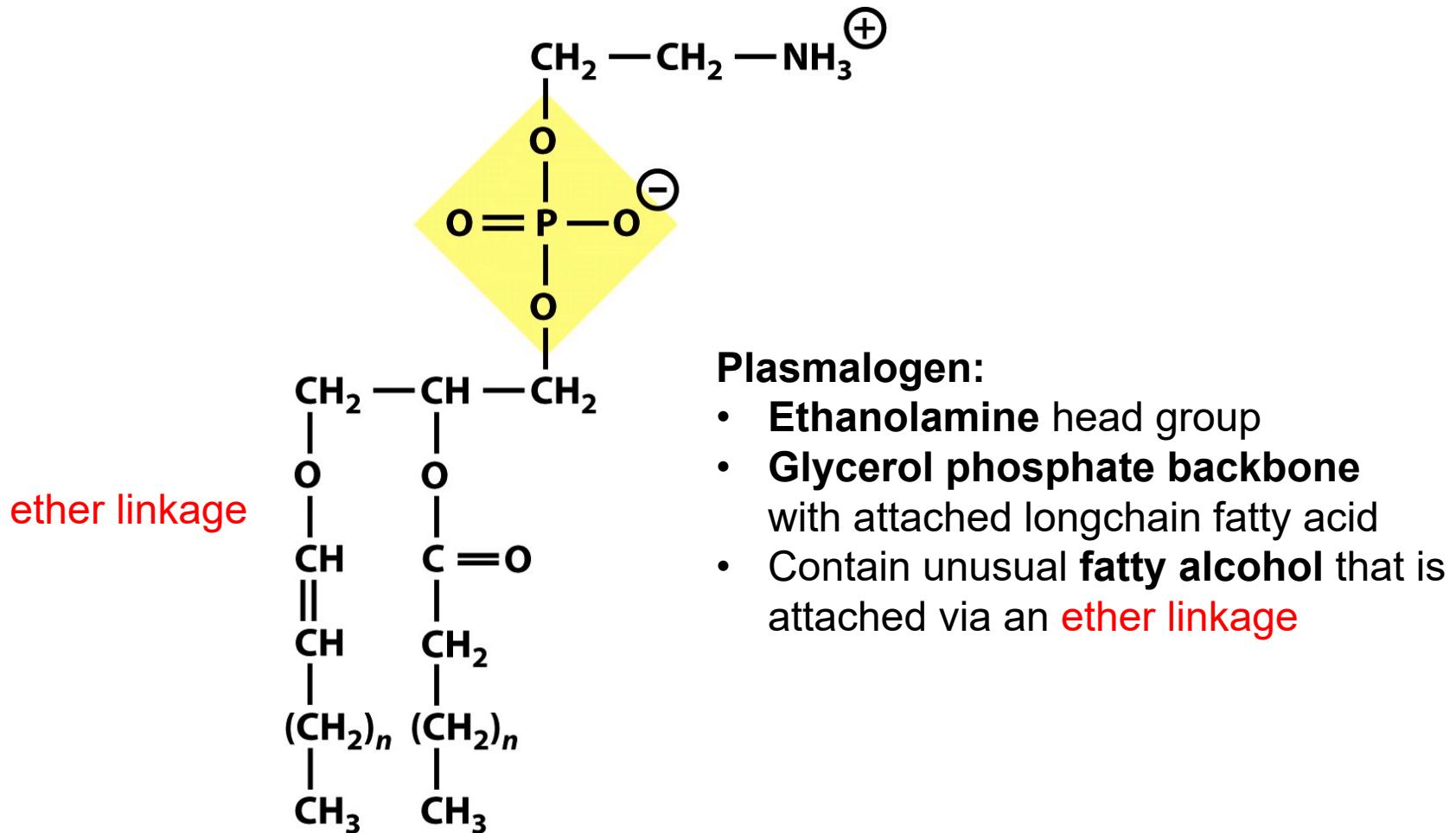


Figure 12-29 Molecular Biology of the Cell 6e (© Garland Science 2015)

**Peroxisome with a paracrystalline core in a tobacco leaf mesophyll cell:**  
close association with chloroplasts to  
**facilitate the exchange of materials**  
between these organelles during  
**photorespiration**

**Peroxisomes in a fat-storing cotyledon cell**  
of a tomato seed 4 days after germination:  
**Peroxisomes (glyoxysomes), associated**  
with the lipid droplets that store fat, reflecting  
their central role in **fat mobilization** and  
**gluconeogenesis** during **seed germination**

## Peroxisome is important to synthesize plasmalogen



Important substance for **sheath myelin** to **protect/insulate** axons of nerve cells.

## How new peroxisomes arise: may apply to other subcellular organelles (Golgi?)

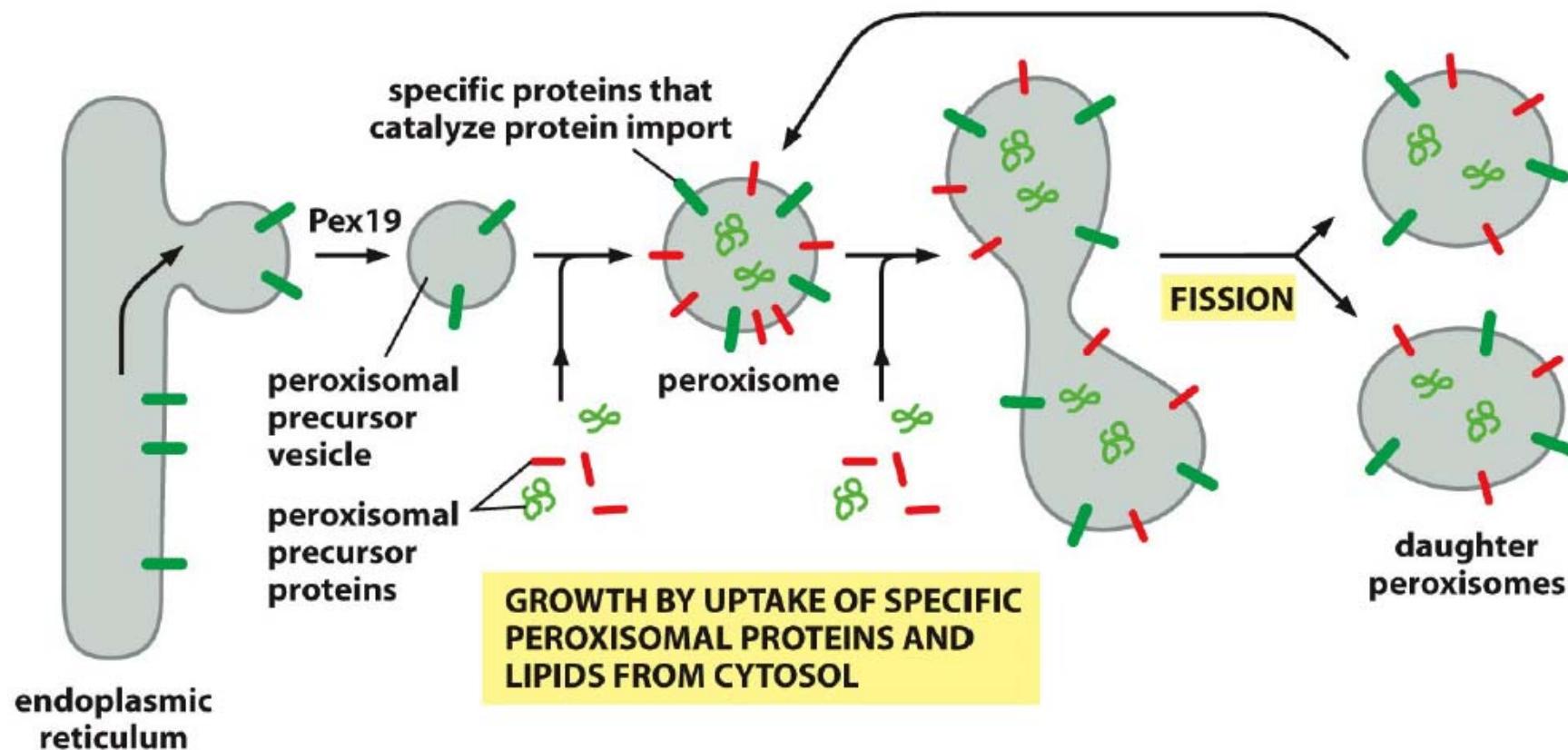


Figure 12-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Transport in peroxisomes

- **Short C-terminal signal peptide (Ser–Lys–Leu )** directs protein import into peroxisome.
- **Cytosolic receptor protein** recognize signal sequence.
- Several **peroxins dock** on cytosolic surface as a membrane translocator
- **Import requires ATP.**

# The Zellweger syndrome is a defect in peroxisomal protein import

- Disease is named after Hans Zellweger (1909–1990), a Swiss-American pediatrician
- inherited genetic disease due to mutation in peroxin Pex5
- 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 into the Endoplasmic reticulum

The ER possesses different domains:  
smooth ER and rough ER are functionally and structurally different

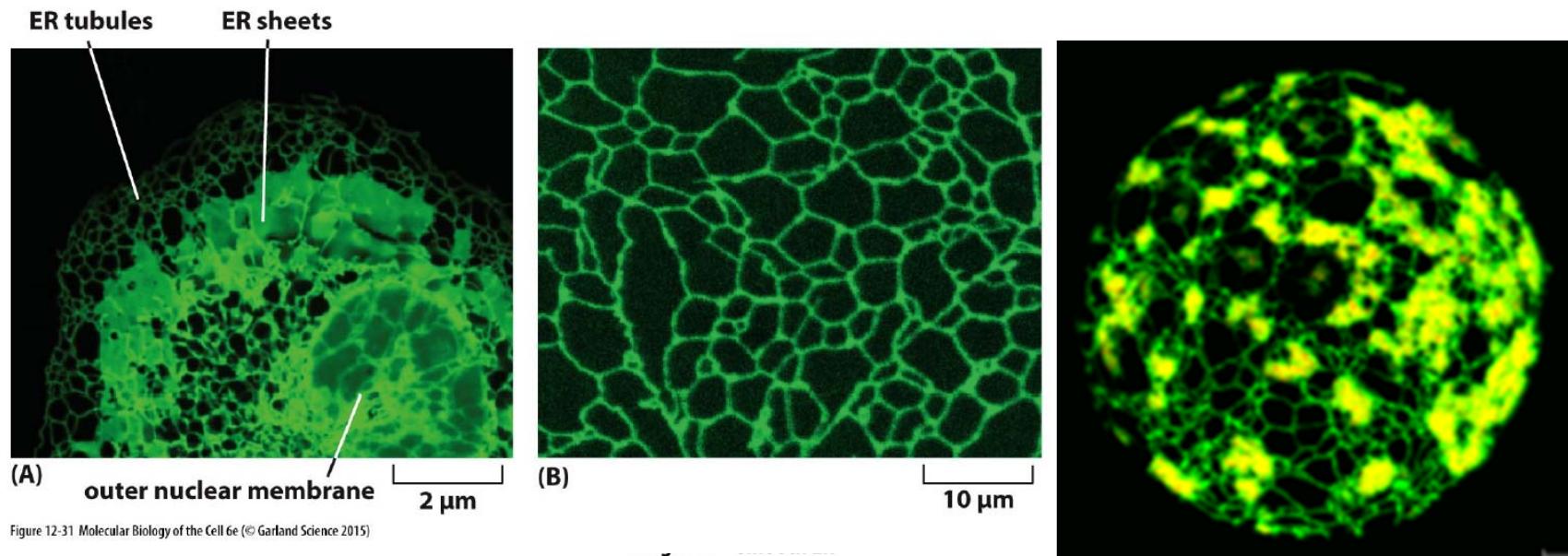
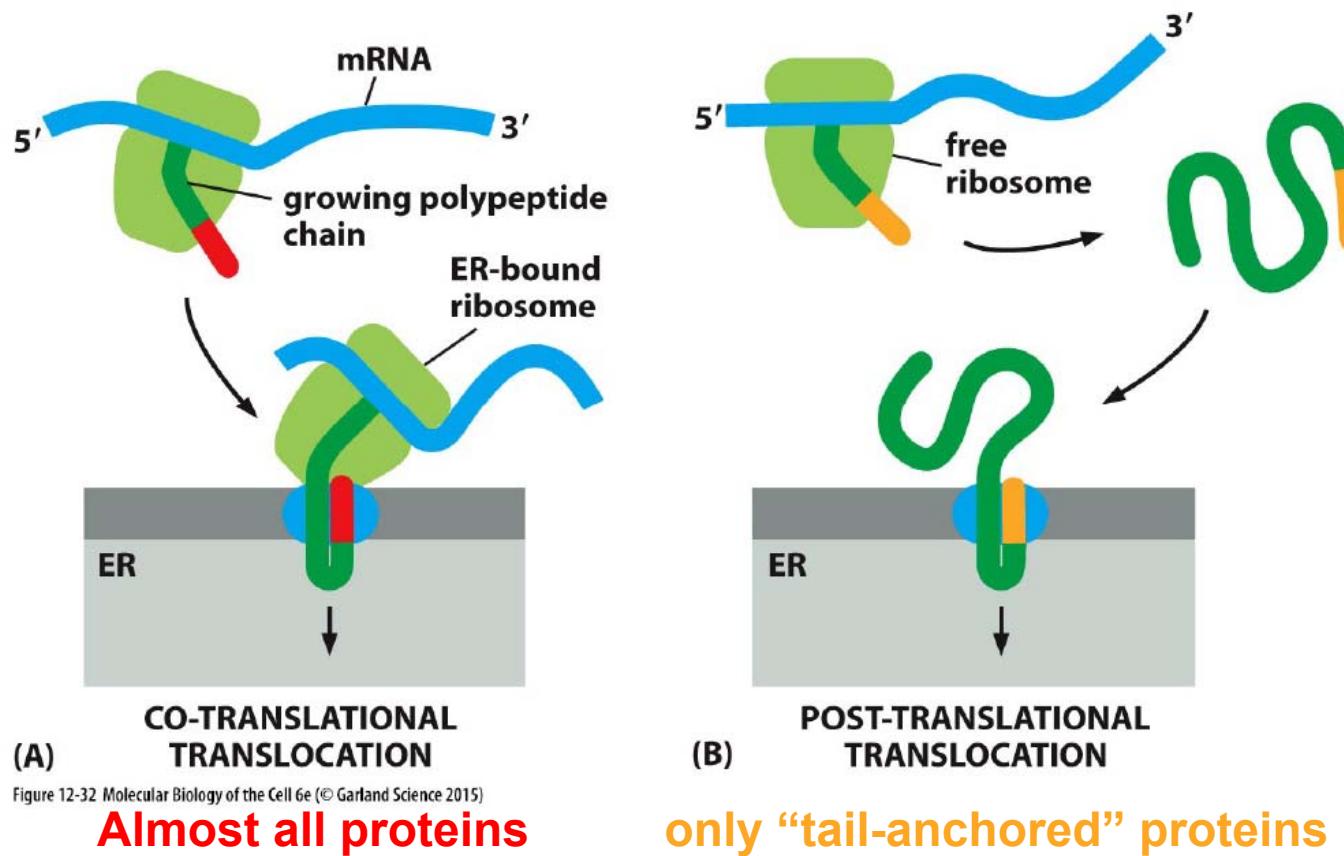


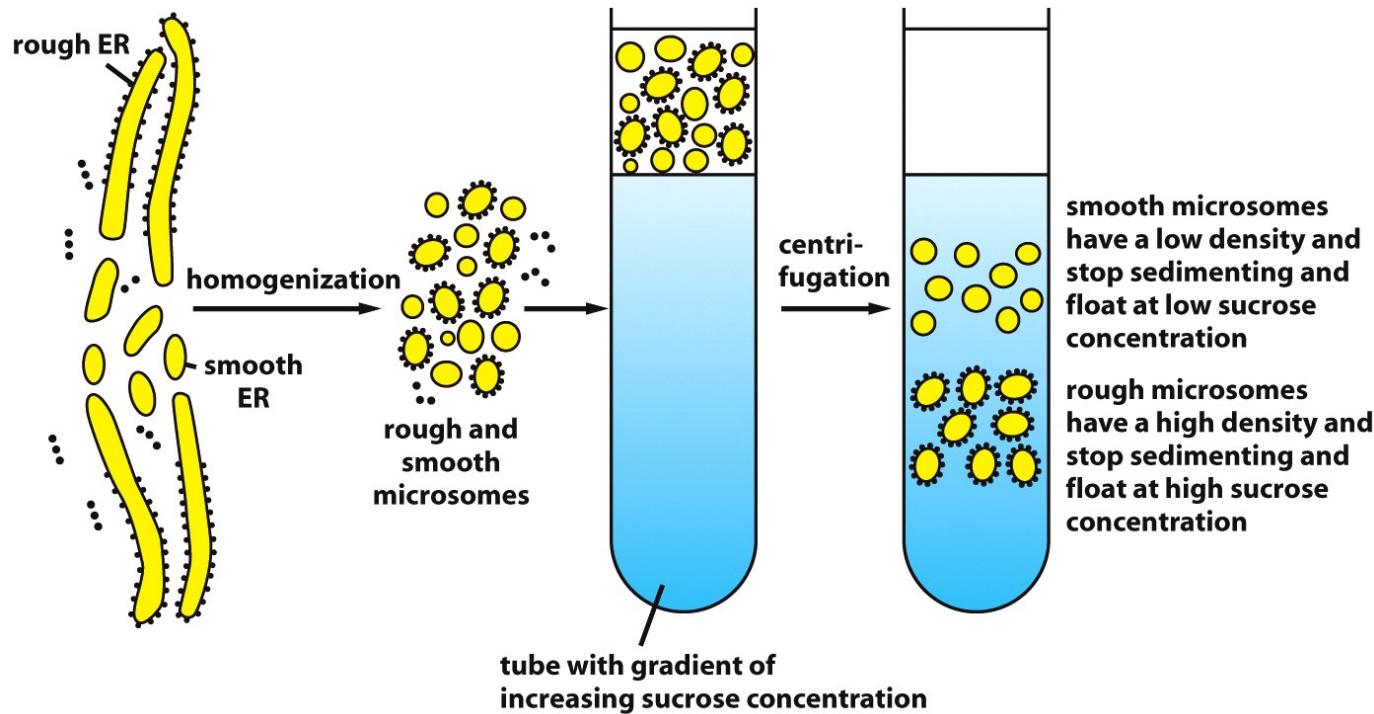
Figure 12-31 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Transport into the ER: co-translational translocation

- **Co-translational:** (Almost all) proteins in ER (not all)
- In contrast to the post-translational transport to mitochondria, chloroplasts, nuclei and peroxisomes



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

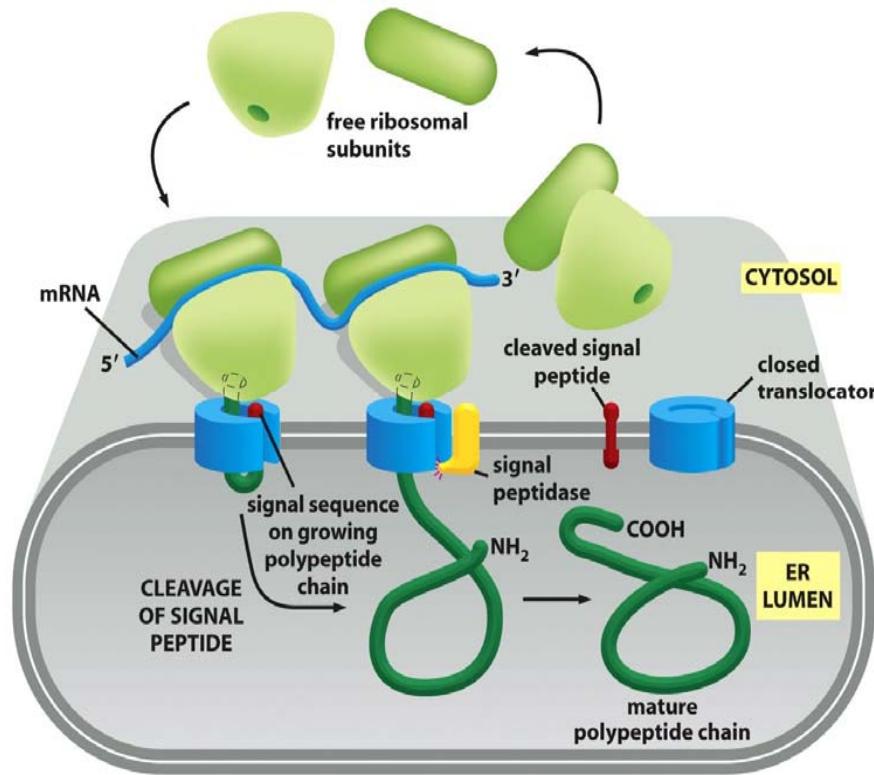


**Rough microsomes:** only from rough ER

**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. (**physiologically intact**)

## Signal sequences were first discovered on ER proteins



**Cell free system** with and without microsomes reveals that the protein in the ER is larger without microsome because it has an extra signal peptide...

... which is not cleaved without the “microsomal” signal-peptidase!

## How is the signal peptide recognized?

How is a protein targeted to ER and transported?

- A **signal recognition particle** (SRP) stops the translation and guides the complex to the translocation pore...

# The signal Recognition Particle (SRP)

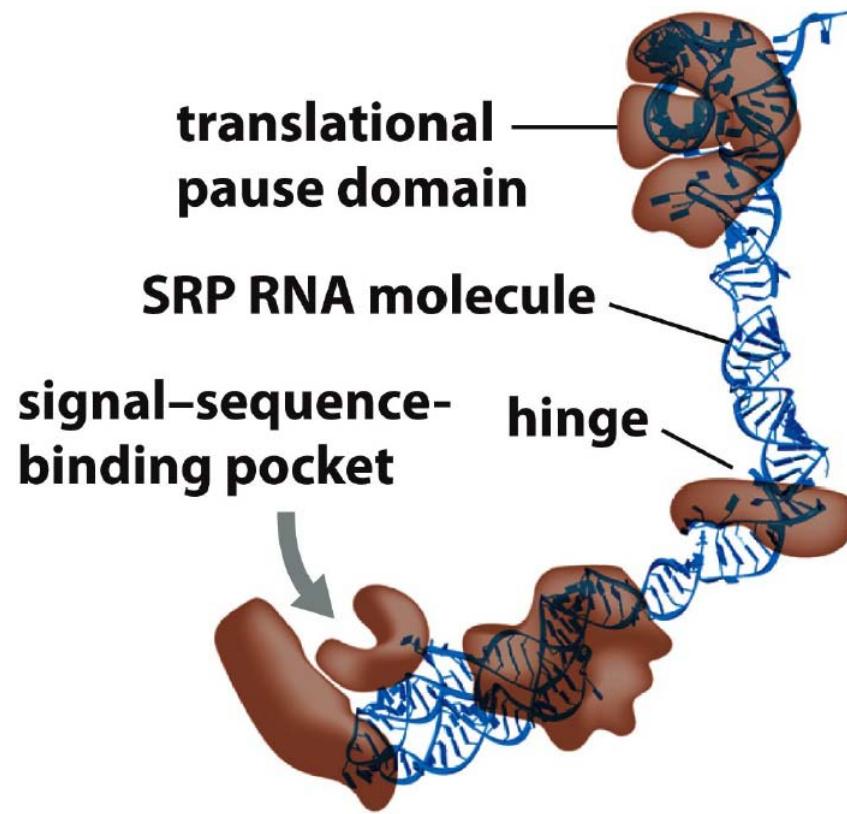


Figure 12-36a Molecular Biology of the Cell 6e (© Garland Science 2015)

## SRP:

- **6 subunits and a small RNA molecule**
- **“shuttles” between ER and “cytosol”**

## The SRP binds to signal peptide and ribosome

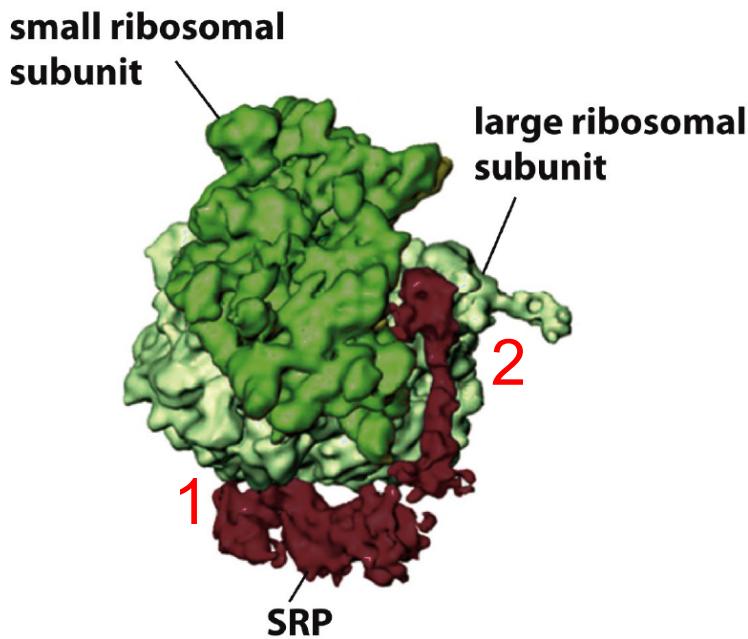
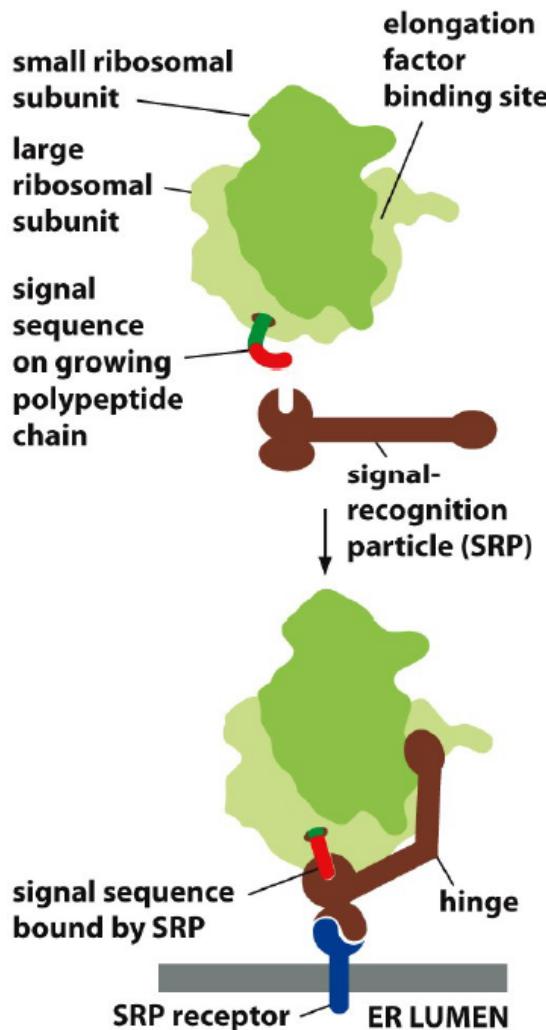


Figure 12-36b Molecular Biology of the Cell 6e (© Garland Science 2015)

**The three-dimensional structure of SRP bound to a ribosome (cryoEM):**

1. SRP binds to the large ribosomal subunit so that its **signal-sequence-binding pocket** is positioned **near the growing polypeptide chain exit site**.
2. Its **translational pause domain** is positioned at the interface between the ribosomal subunits, where it interferes with elongation factor binding.

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



Signal sequence binding site has multiple hydrophobic methionine residues

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

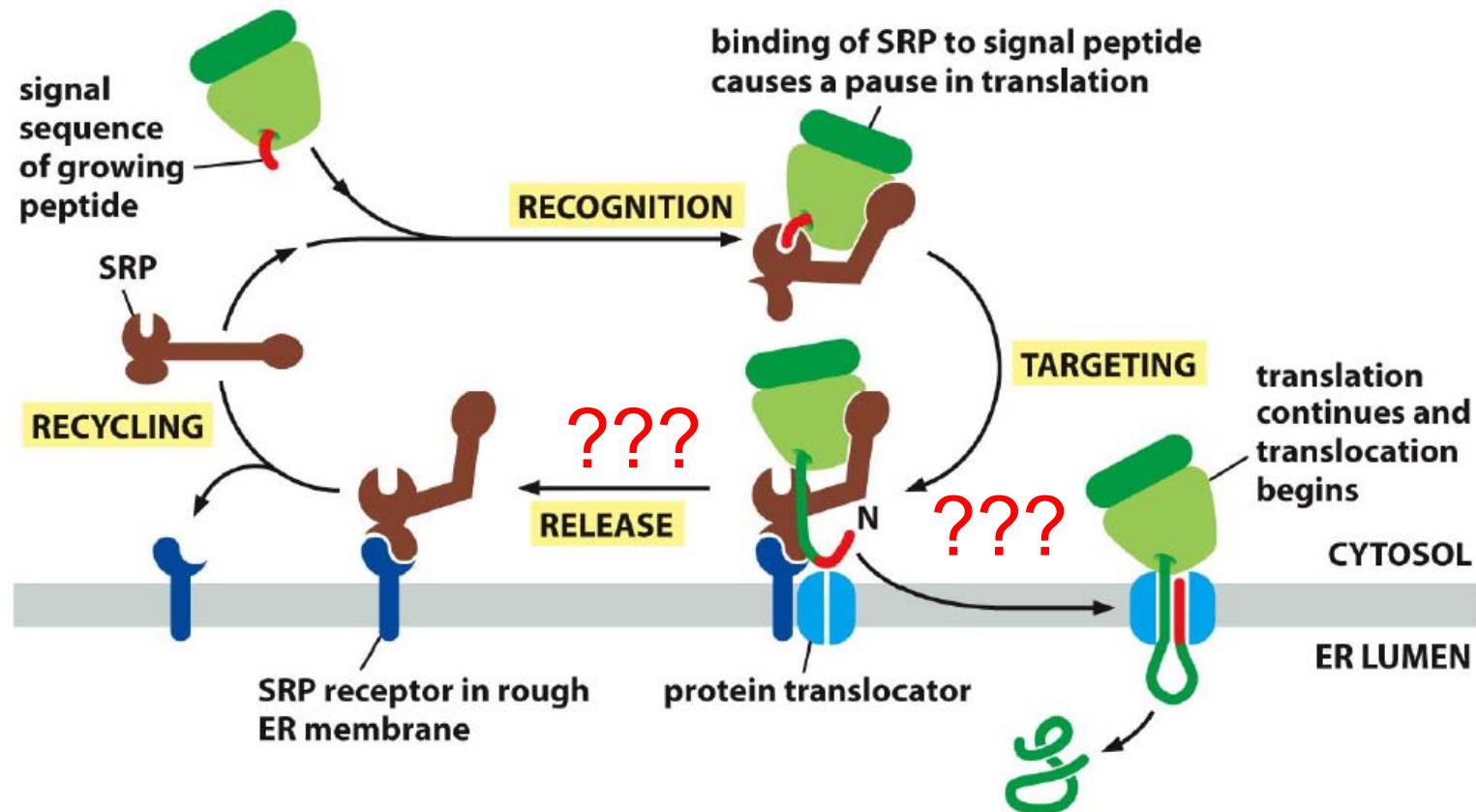
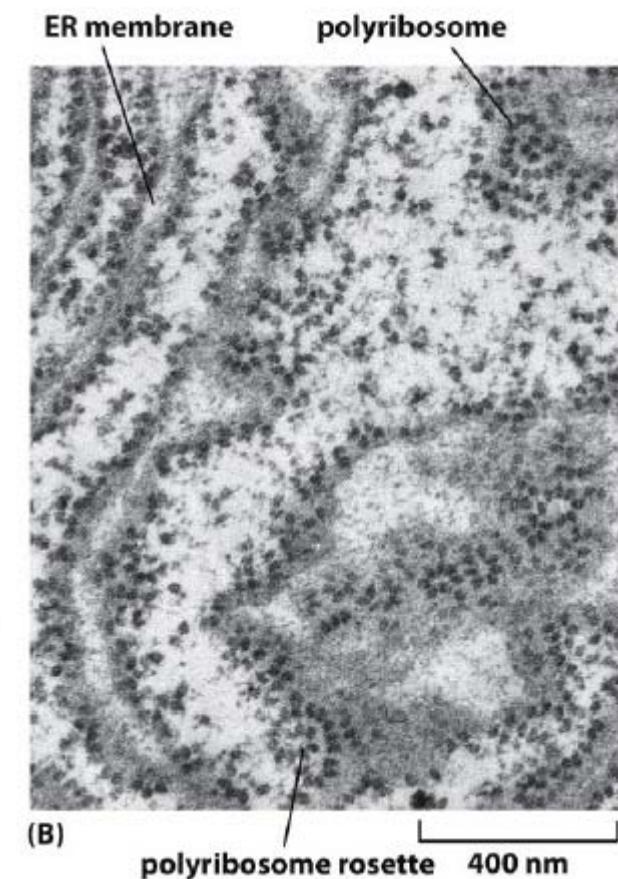
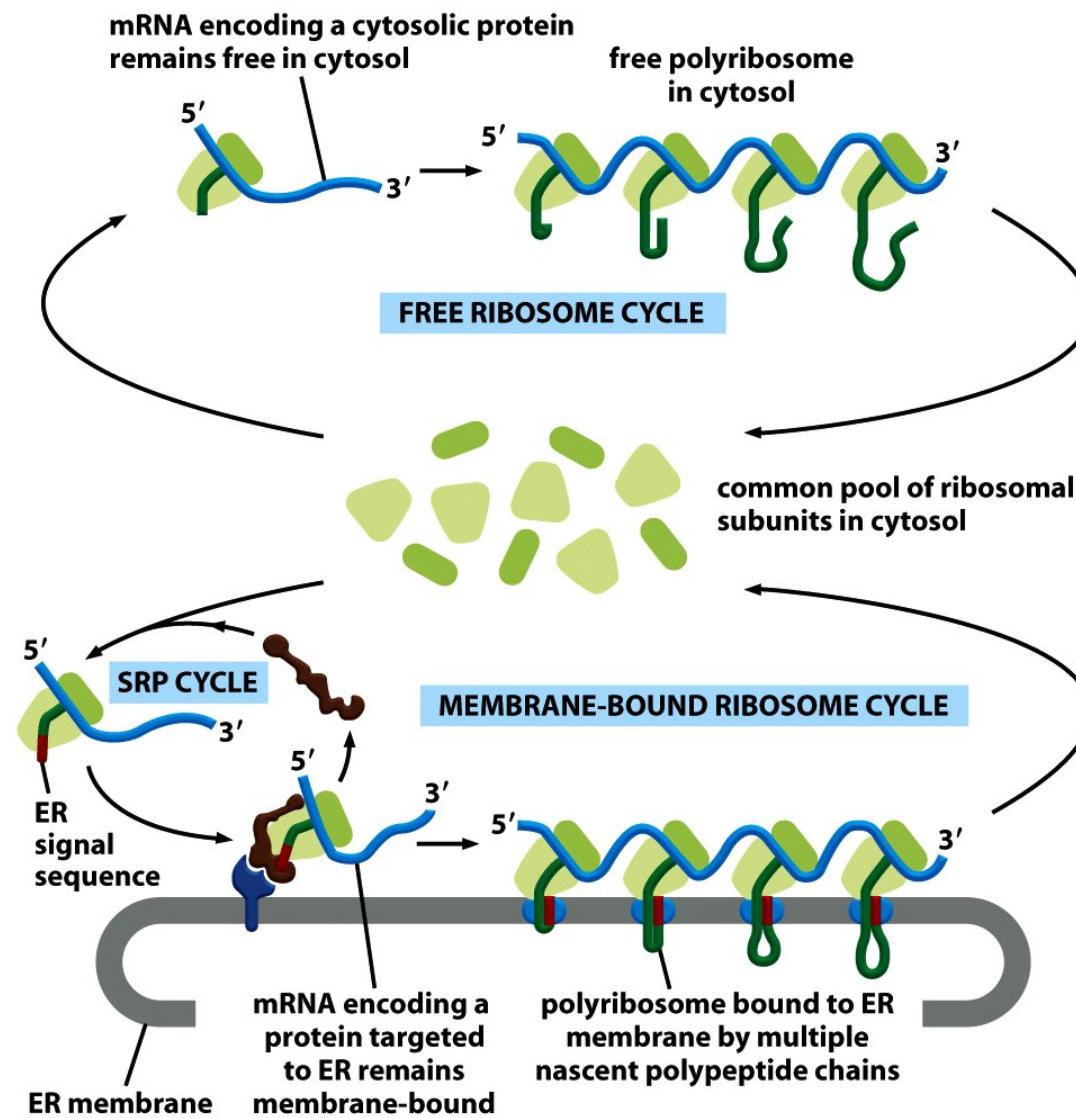


Figure 12-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Free and membrane-bound ribosomes



## Protein translocator facilitates protein transferring: The Sec61 complex

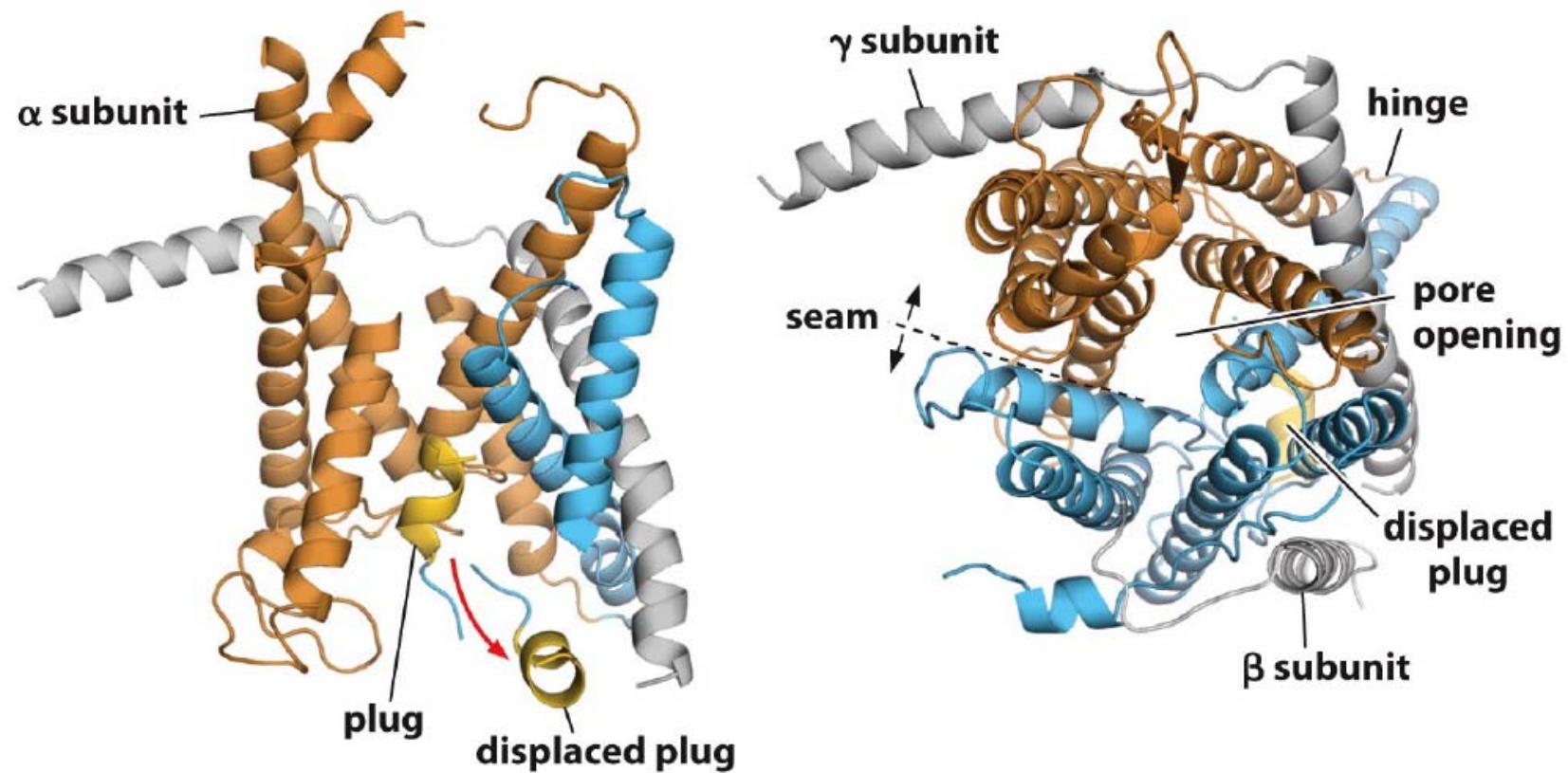


Figure 12-39a Molecular Biology of the Cell 6e (© Garland Science 2015)

## Protein translocator facilitates protein transferring: The Sec61 complex

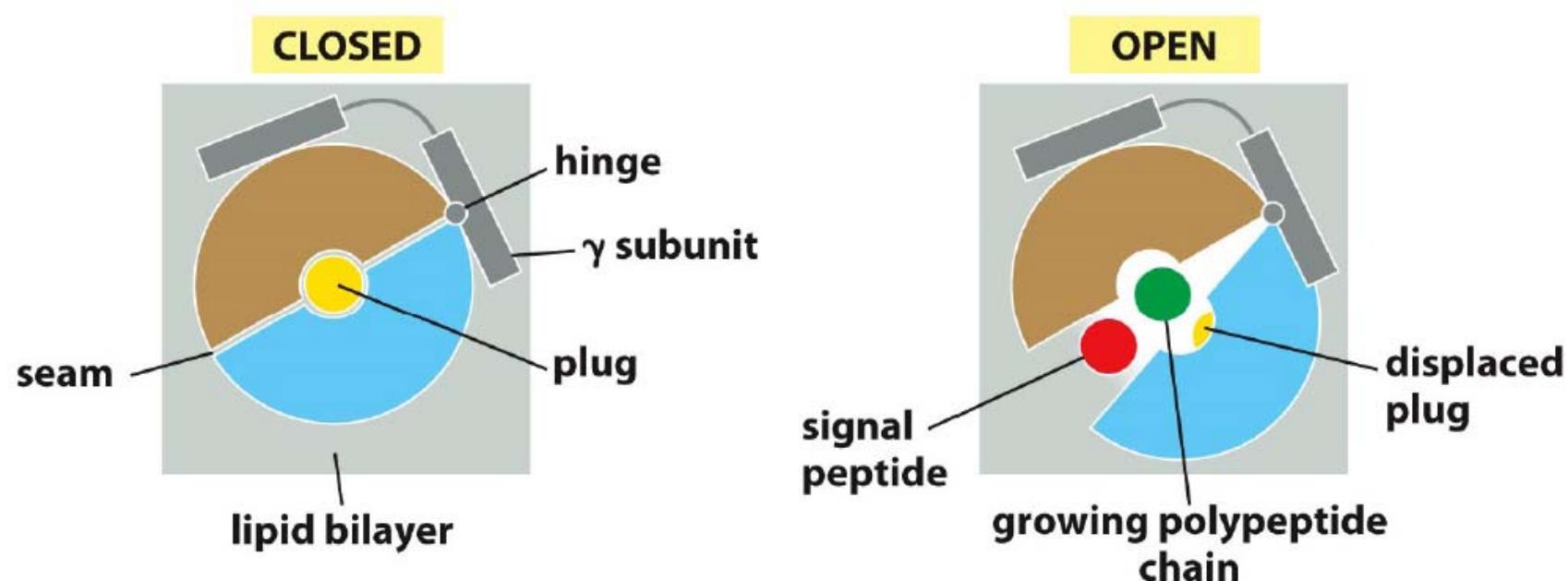
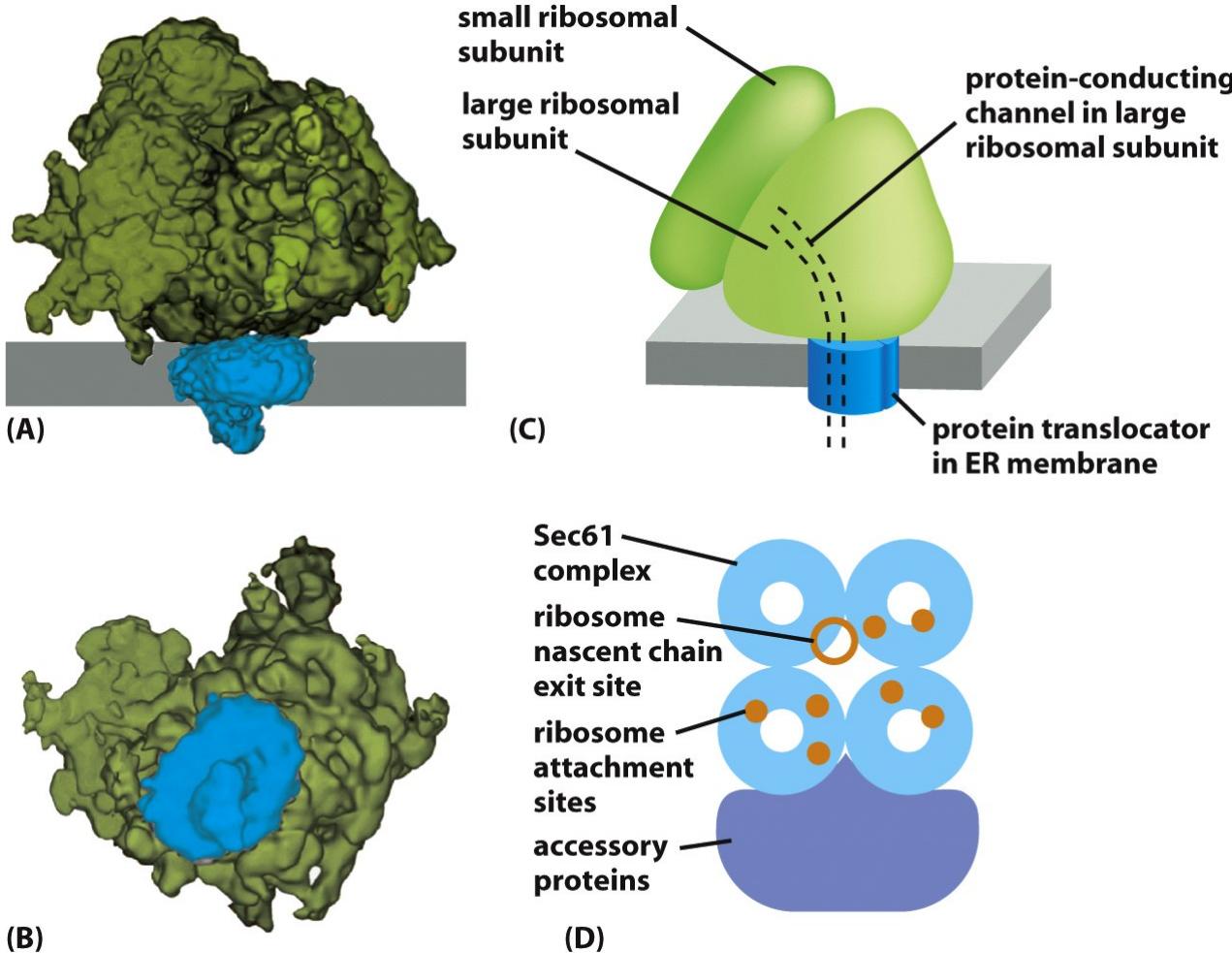


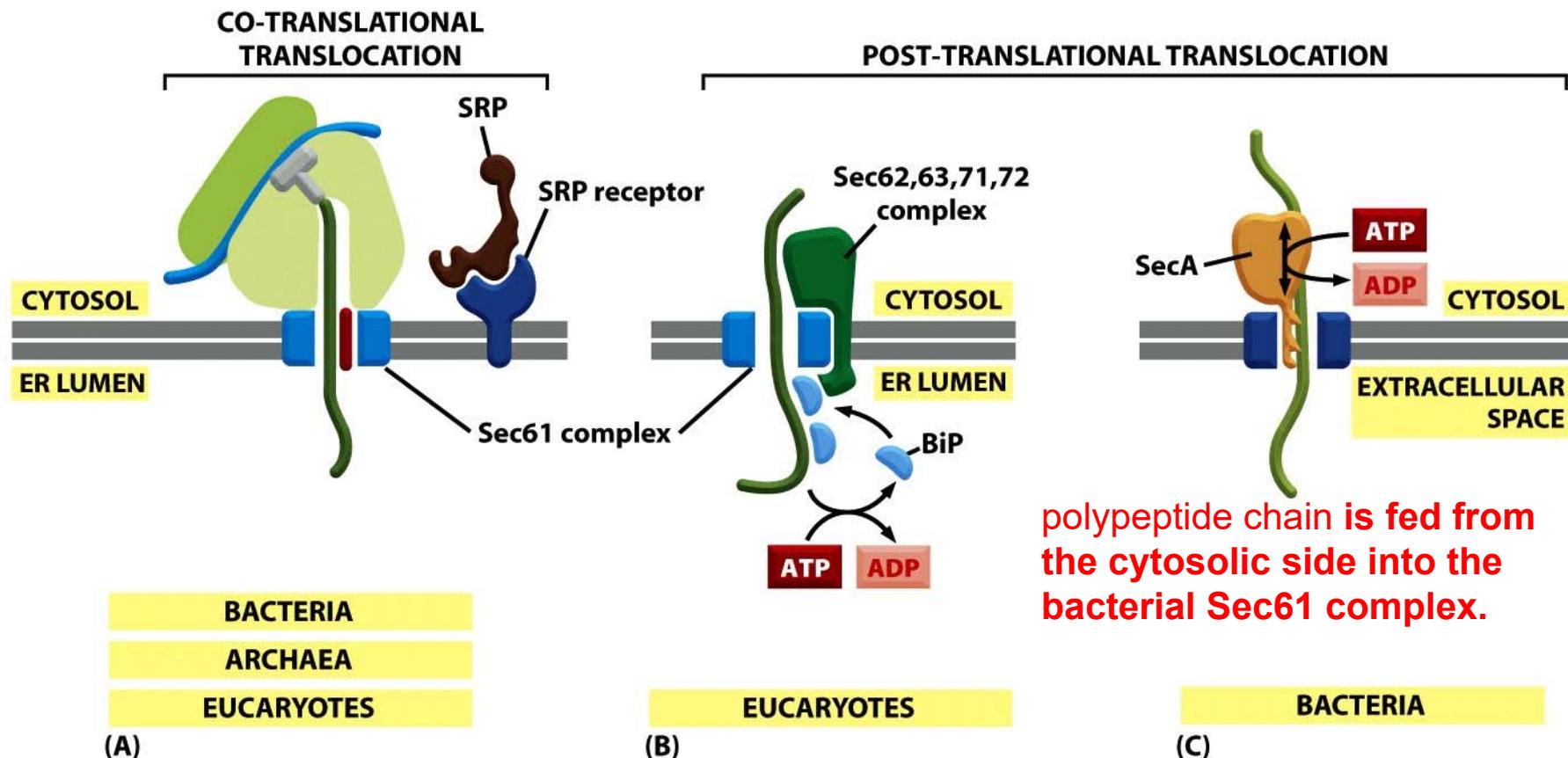
Figure 12-39b Molecular Biology of the Cell 6e (© Garland Science 2015)

Models of the closed and open states of the translocator are shown in top view, illustrating how a signal sequence (or a transmembrane segment) could be released into the lipid bilayer after opening of the seam.

# A ribosome bound to the Sec61 complex



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

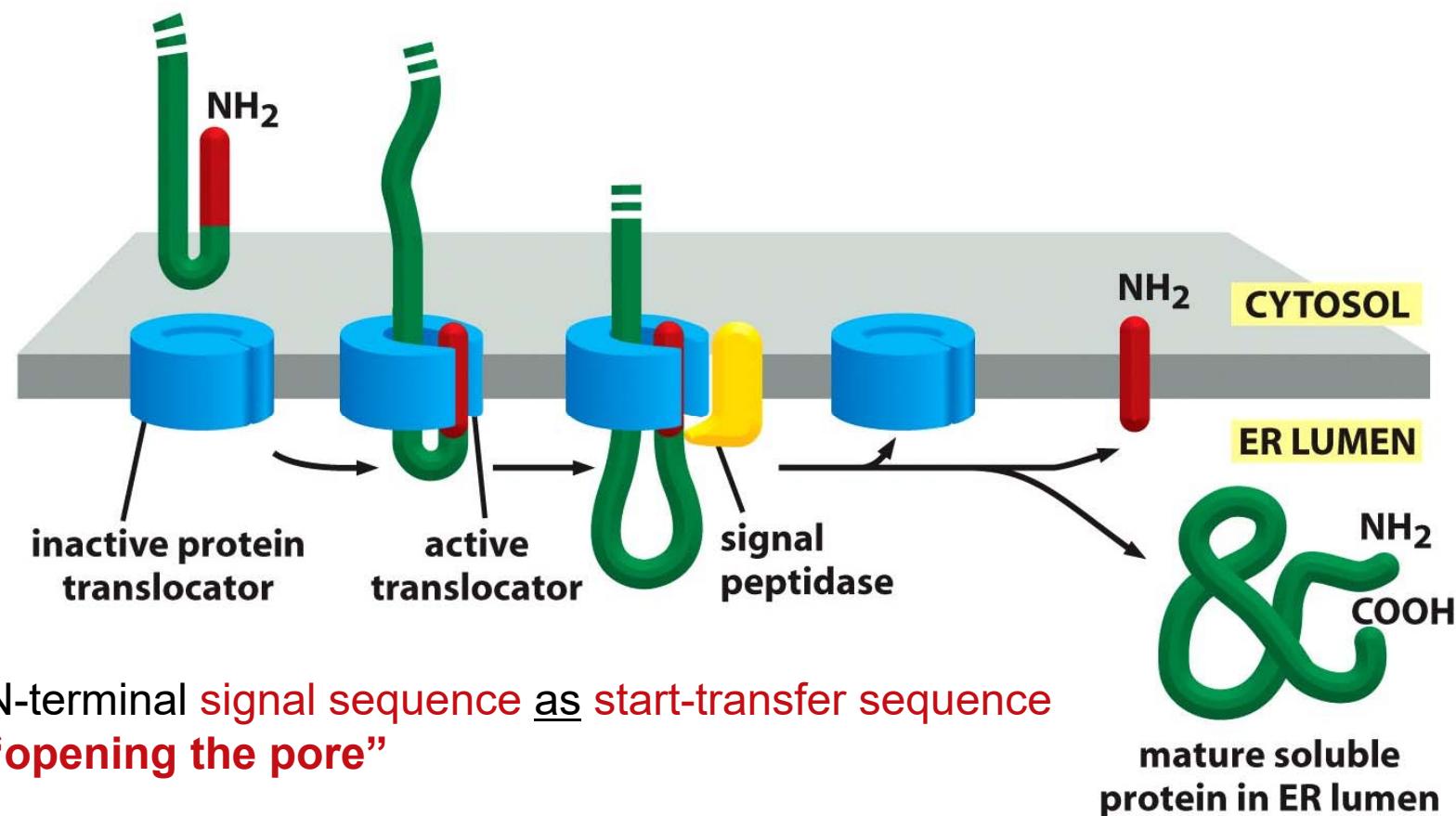


Translocation does not always require ongoing protein elongation

## Different ways for the translocation of ER proteins dependent on their topology:

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

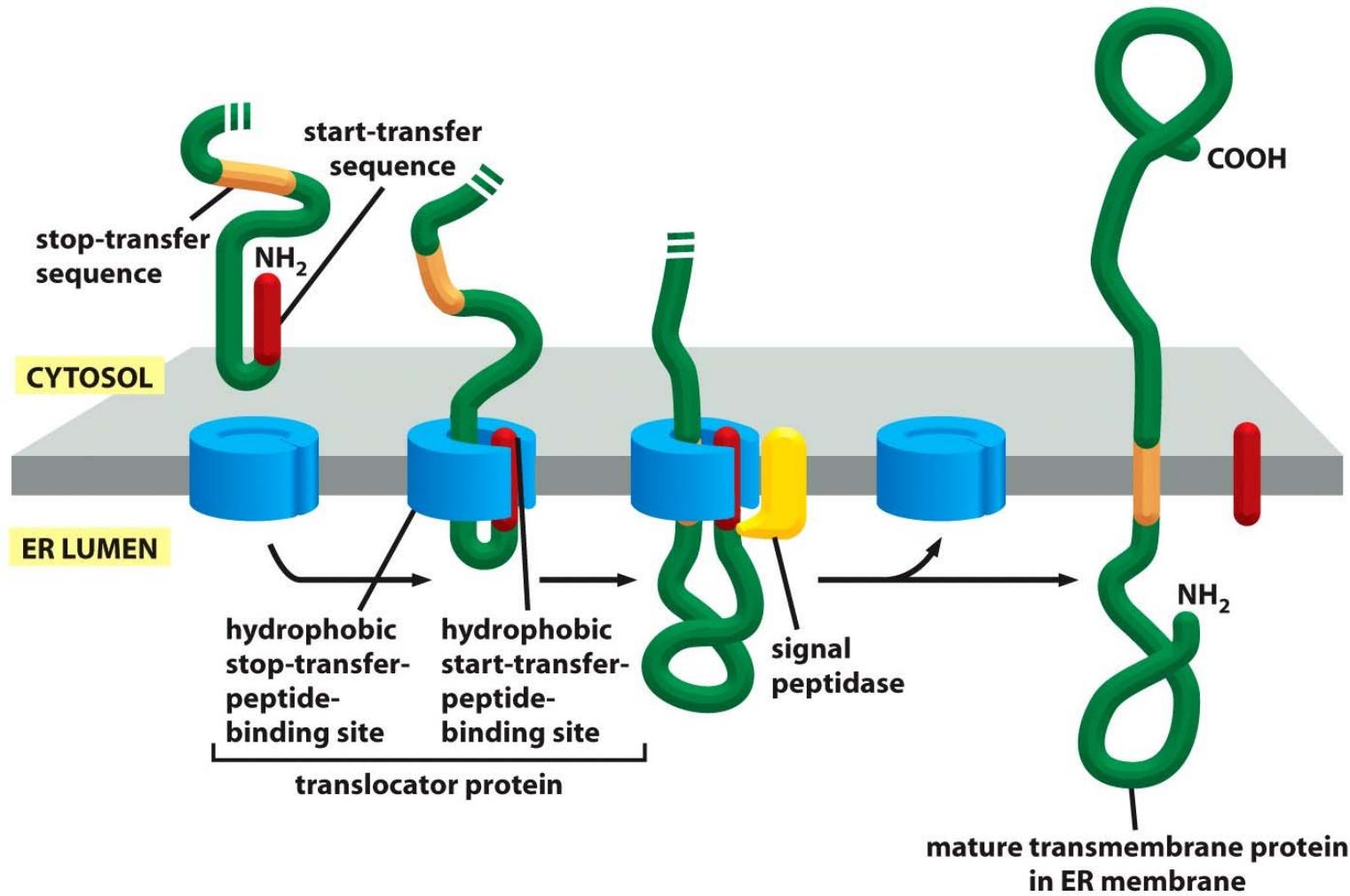
# How a soluble protein is translocated across the ER membrane



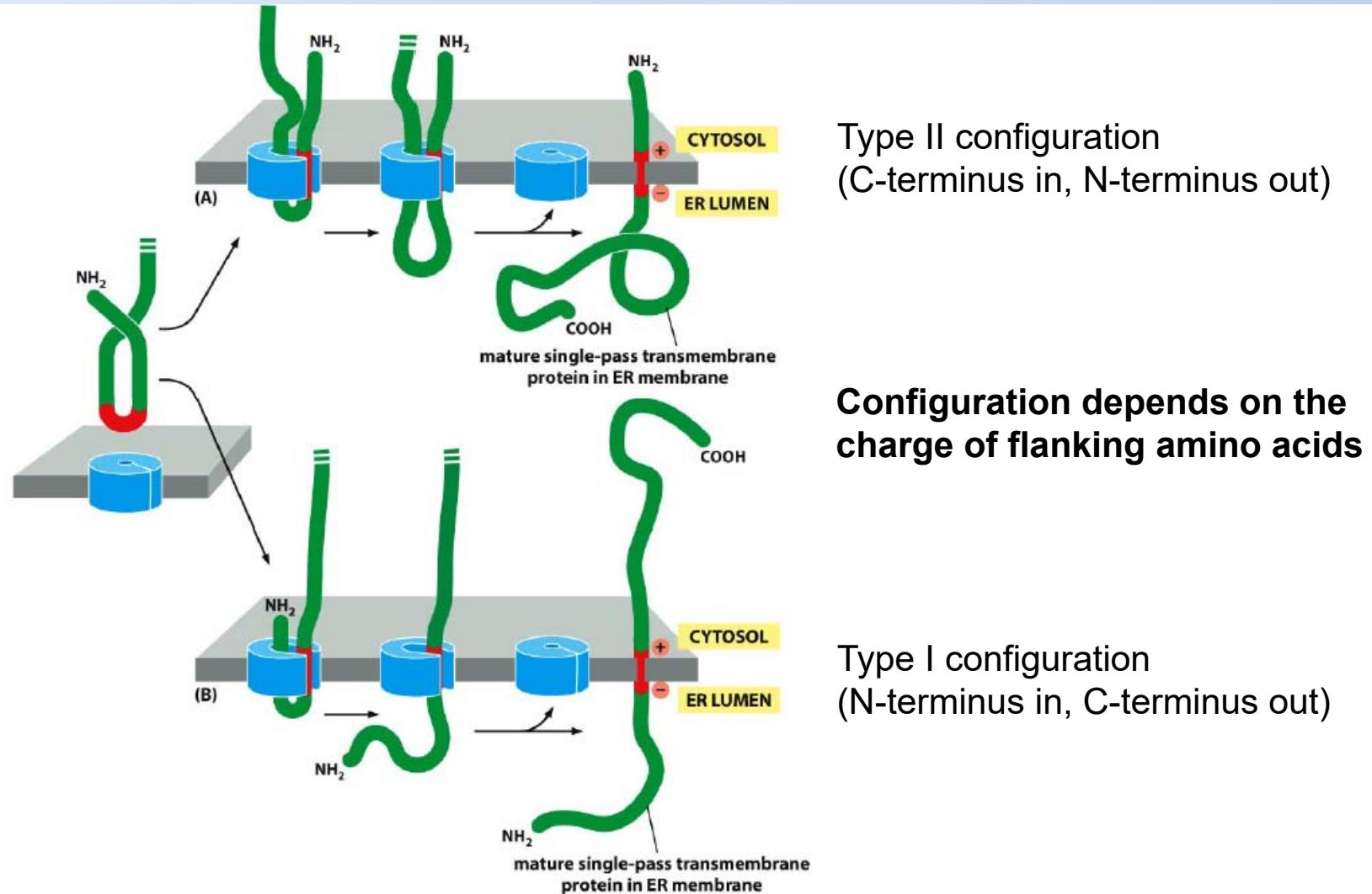
## Insertion of a single-pass transmembrane protein into the ER membrane

- ER-signal sequence (start-transfer) is recognized first by SRP in the cytosol and then by the pore of the protein translocator.
  - Two options for the insertion:
    - 1.) N-terminal start-transfer signal + stop-transfer signal
    - 2.) Internal signal sequence
-

## Insertion of a single-pass transmembrane protein via a N-terminal **start-transfer** signal and an internal **stop-transfer** signal



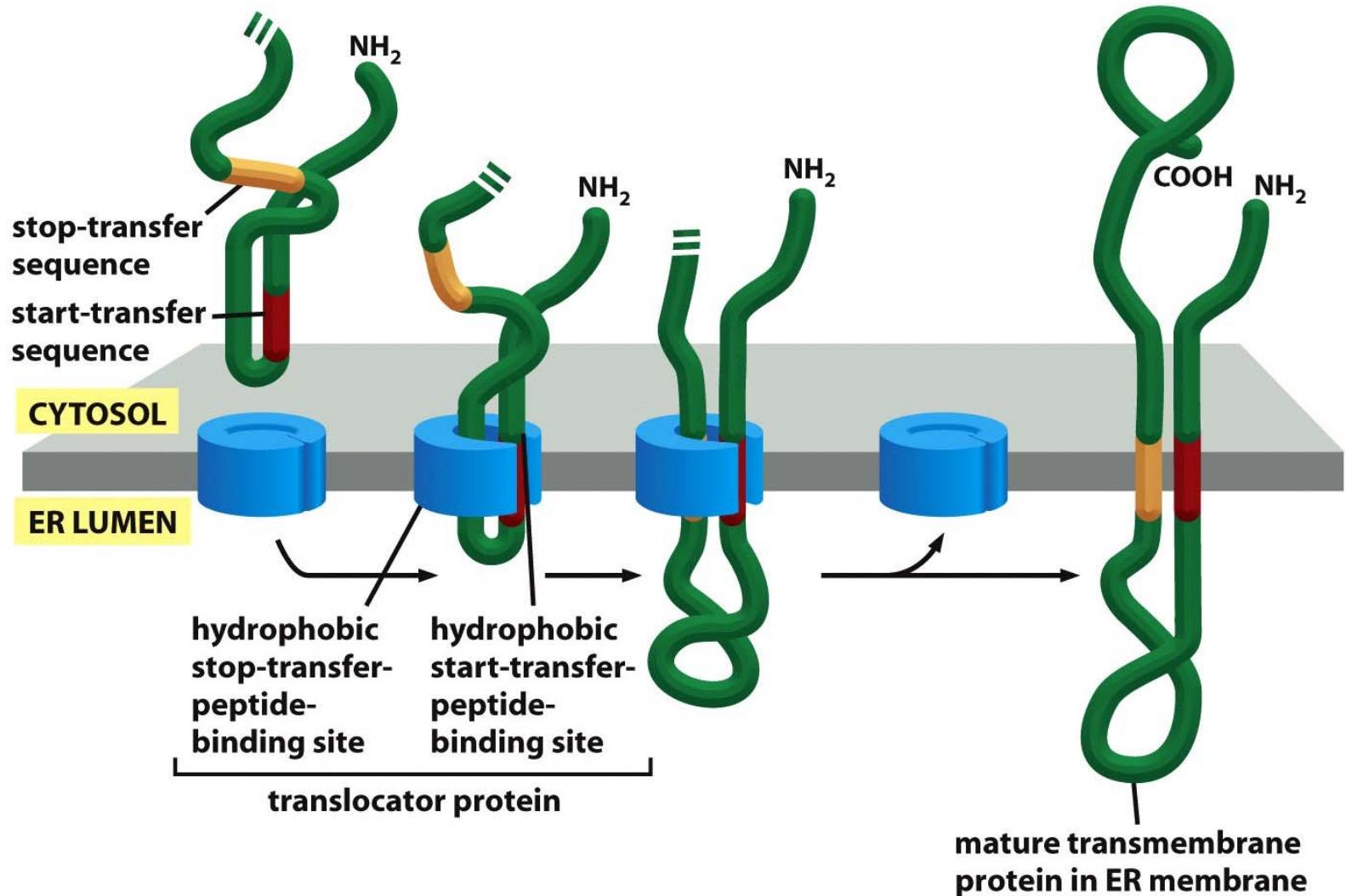
# Charge of aa that flank the internal signal sequence determine the type I/II configuration



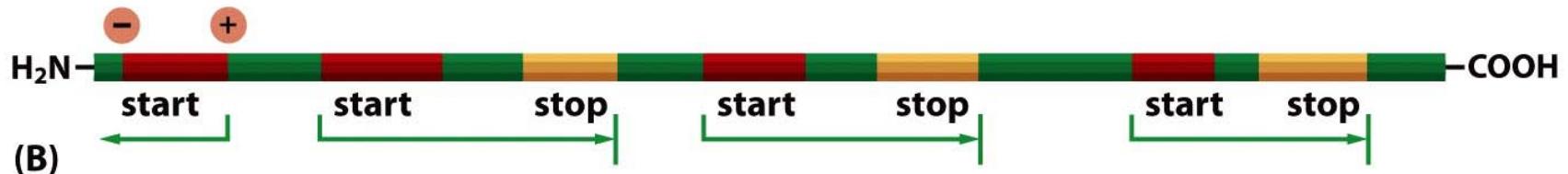
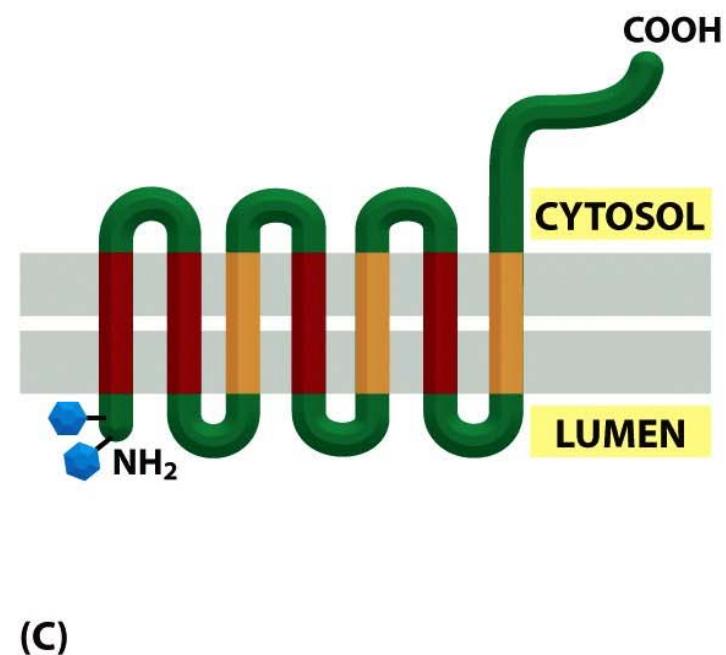
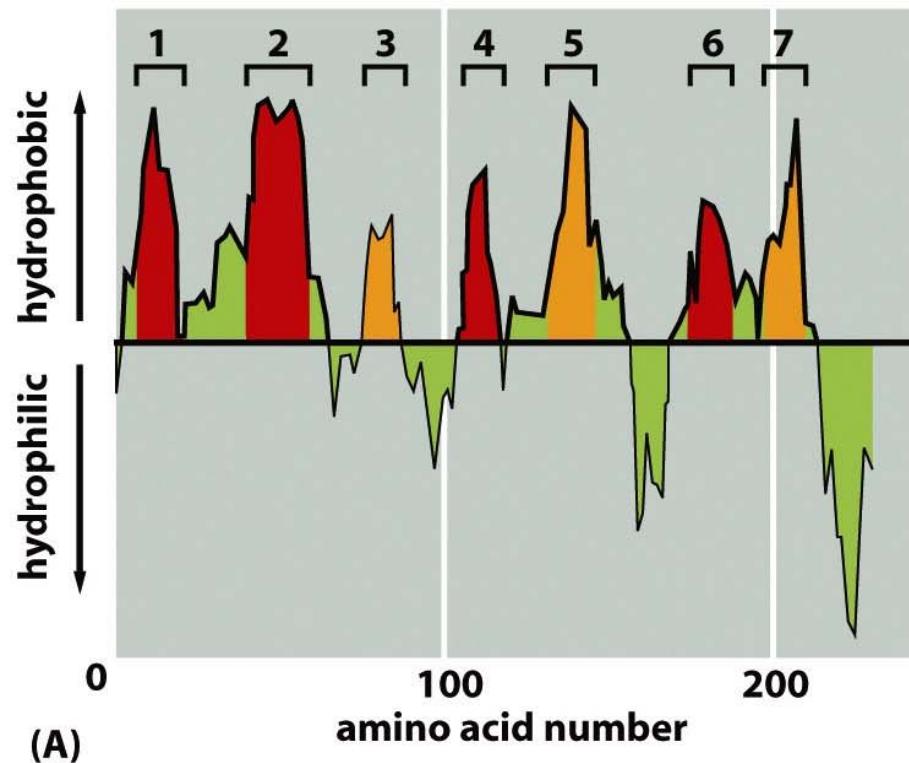
## Insertion of multi-pass transmembrane proteins

- 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 and so on and 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

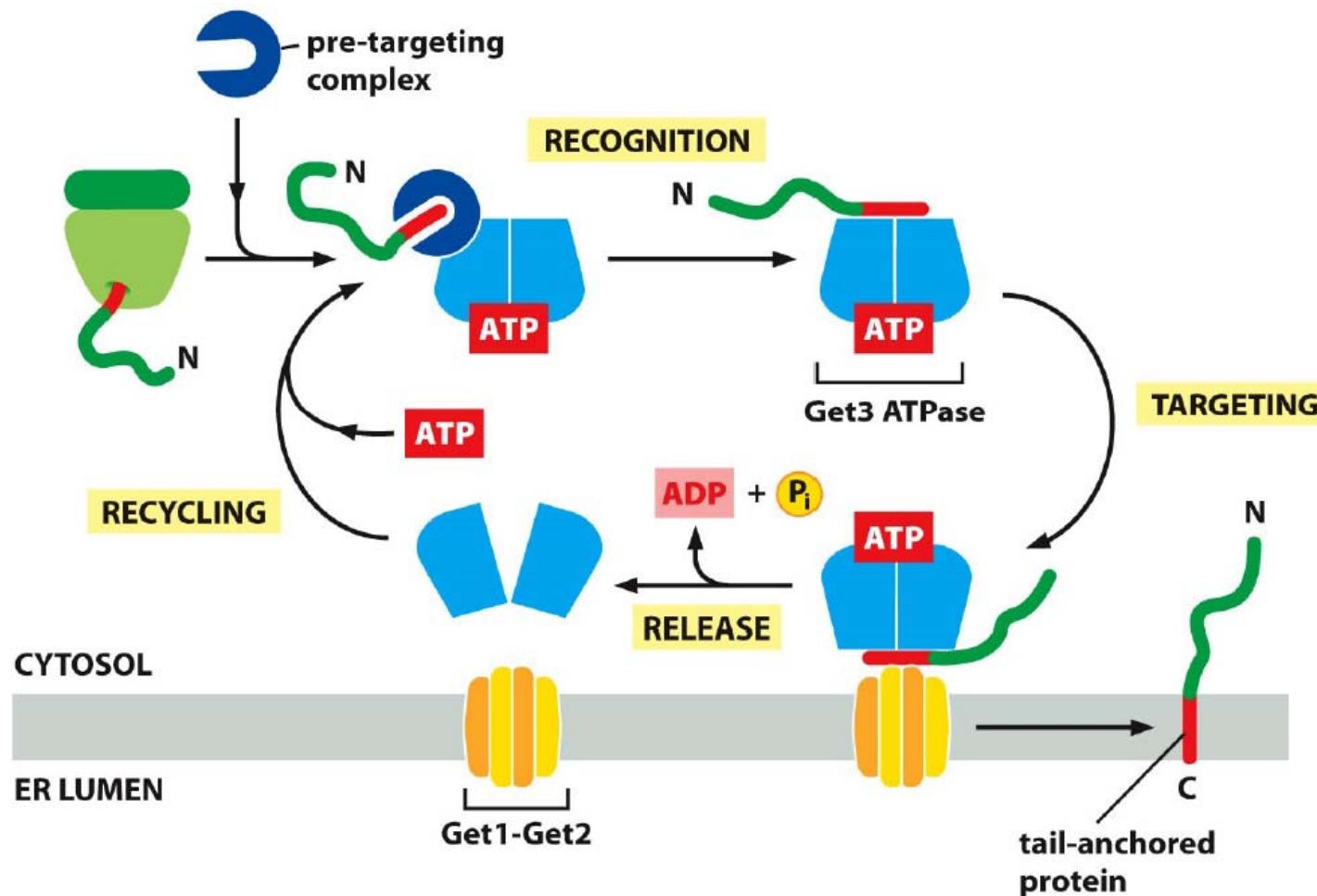
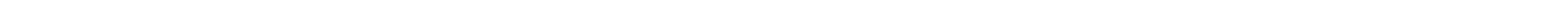


Figure 12-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Some soluble ER resident proteins carry an ER-retention/retrieval signal

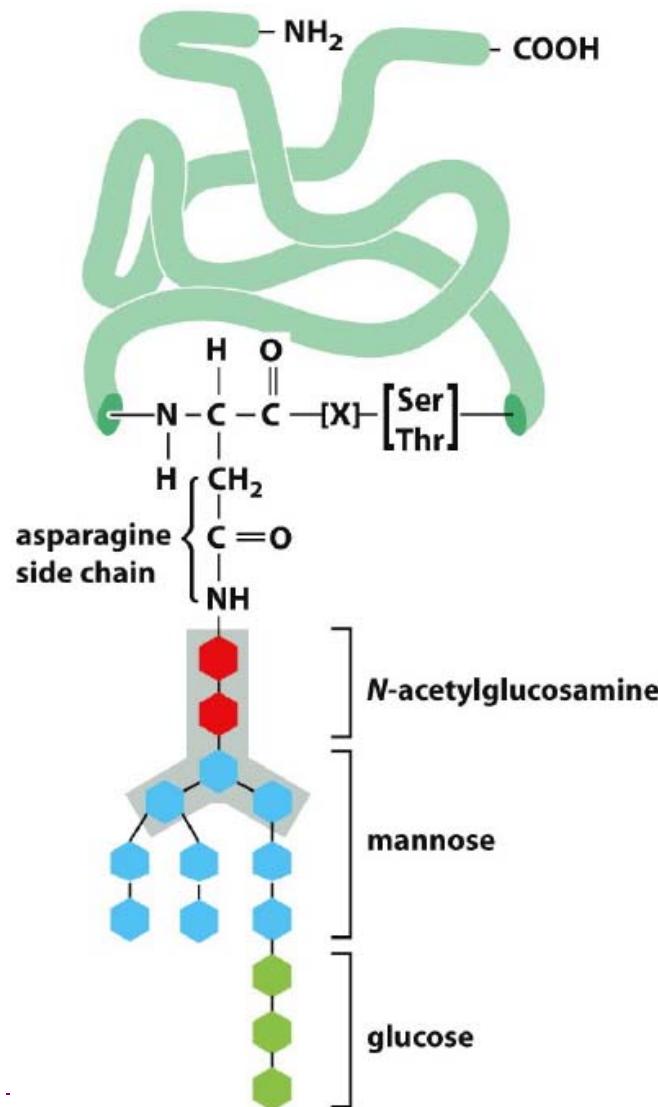
- Contain ER retention/retrieval signal of four amino acids at C-terminus (**H/KDEL**)
- Examples:
  - protein disulfide isomerase ( **PDI**)
  - “binding protein” **BiP** ( chaperone, helps to fold proteins)



## Protein folding and “quality control” in the ER

- 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: N- (asparagine)-linked glycosylation



- **Most ER-made** proteins are glycosylated
- **Cytosolic** protein is **rarely** glycosylated
- **N-linked** oligosaccharides takes up 90% of all glyco-proteins  
The precursor oligosaccharide (shown in color) is **attached only to asparagines** in the sequences **Asn-X-Ser** and **Asn-X-Thr** (where X is any aminoacid except proline)
- The other from O-linked in Golgi Apparatus (Ser, Thr, hydroxylysine)

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

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

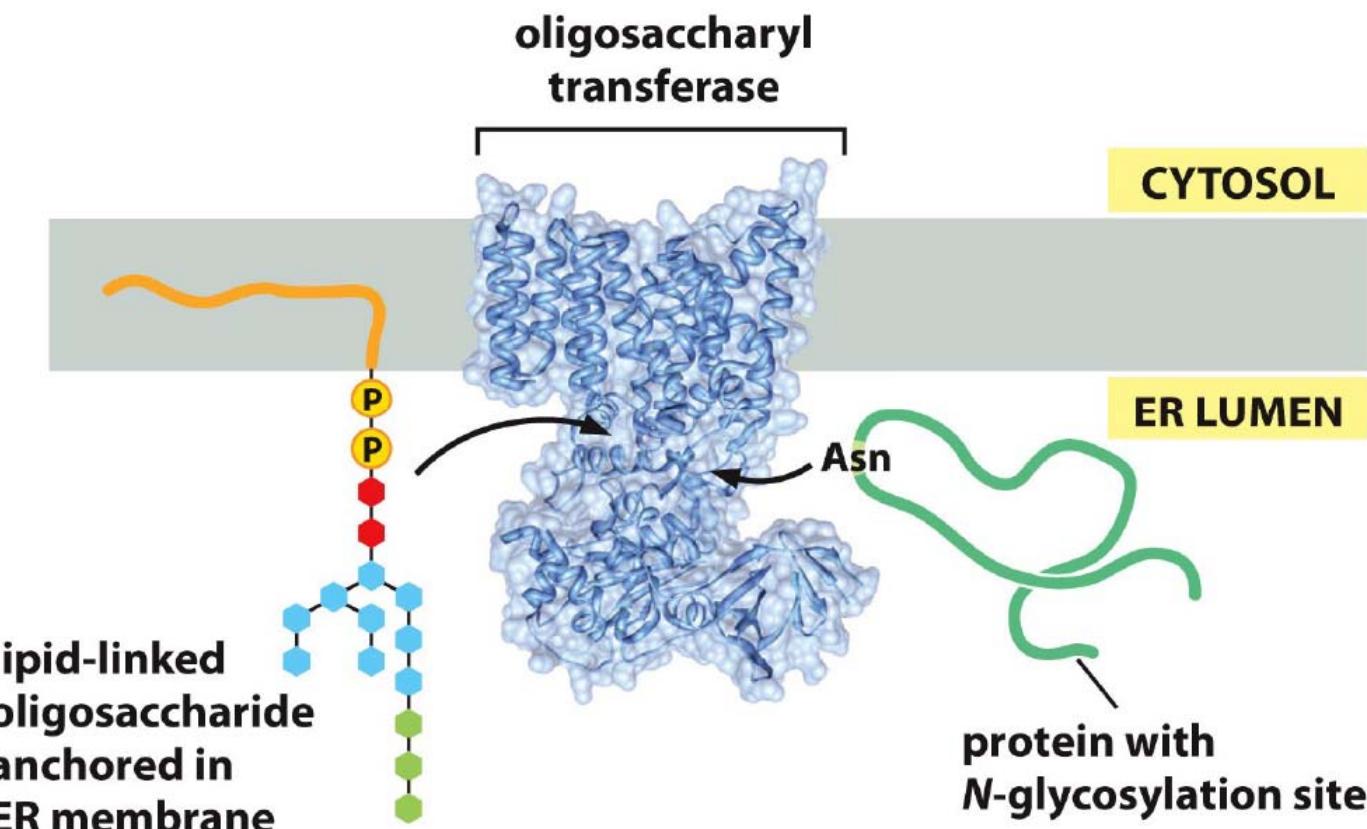
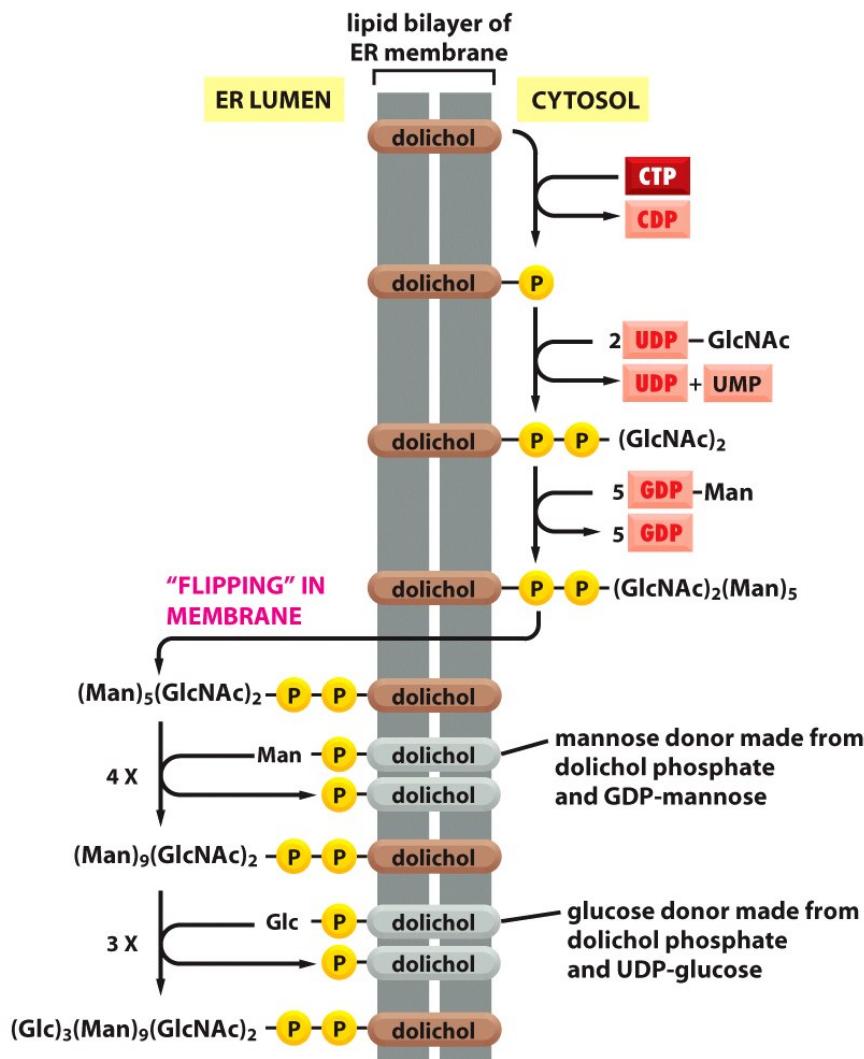


Figure 12-47b Molecular Biology of the Cell 6e (© Garland Science 2015)

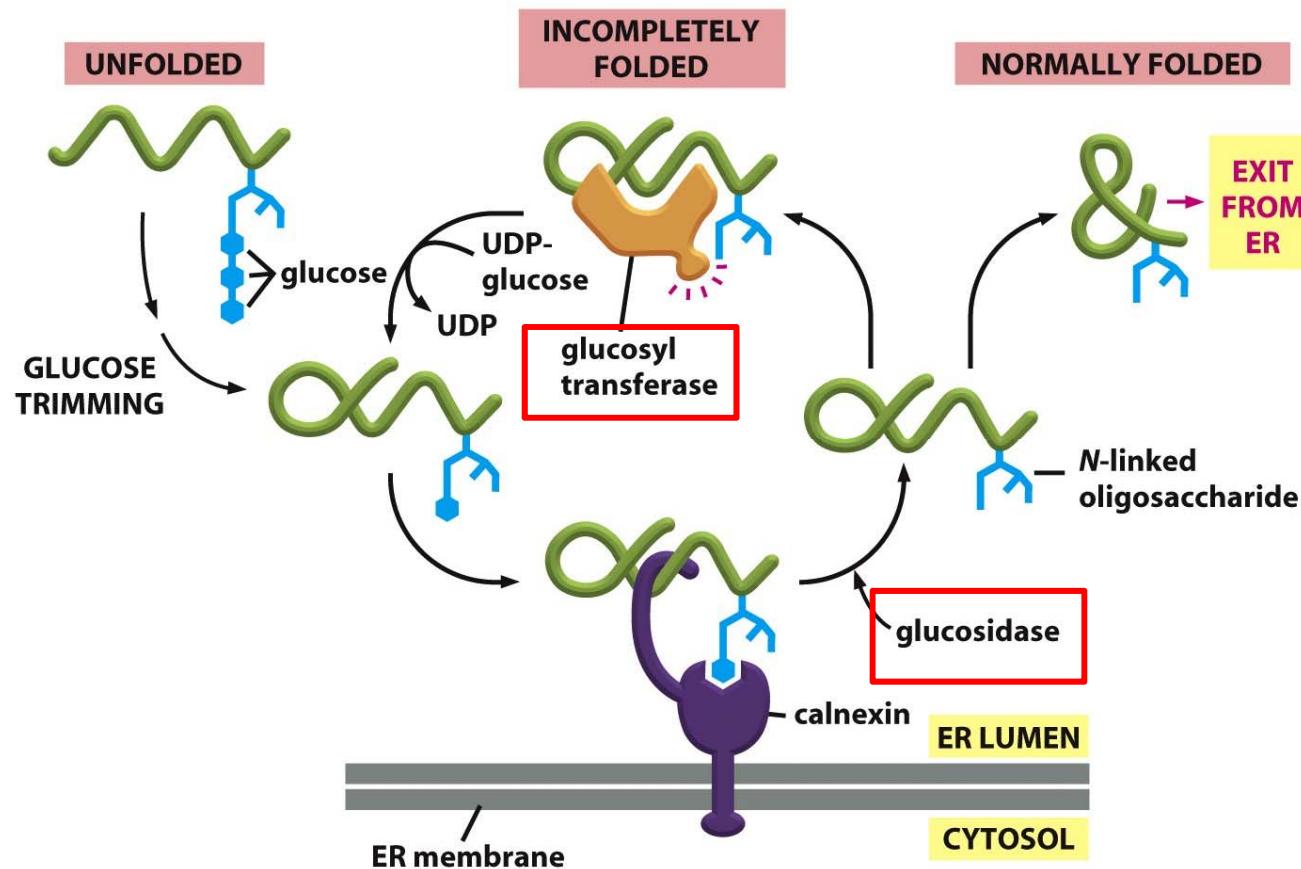
It is catalyzed by oligosaccharyl transferase, whose active sites are exposed in the ER lumen only.

# Synthesis of the precursor oligosaccharide in rough ER



- Assembly: sugar by sugar onto the **carrier lipid dolichol on the cytosolic side**
- **First sugar is linked via a high energy pyrophosphate bridge** (required later on for the transfer to the asparagine side chain)
- **Flipping in the membrane** after the addition of  $(\text{Man})_5(\text{GlcNac})_2$

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



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

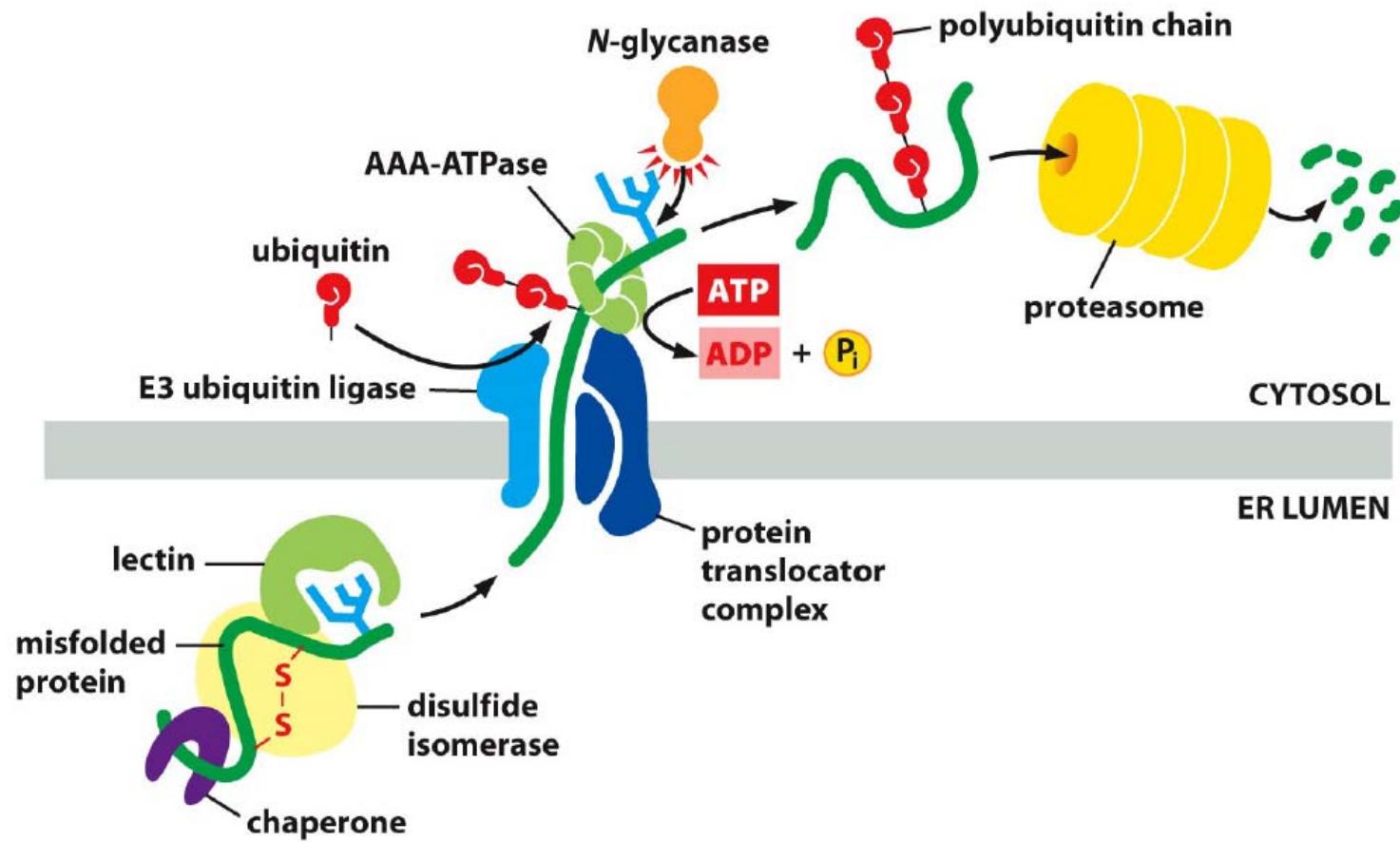
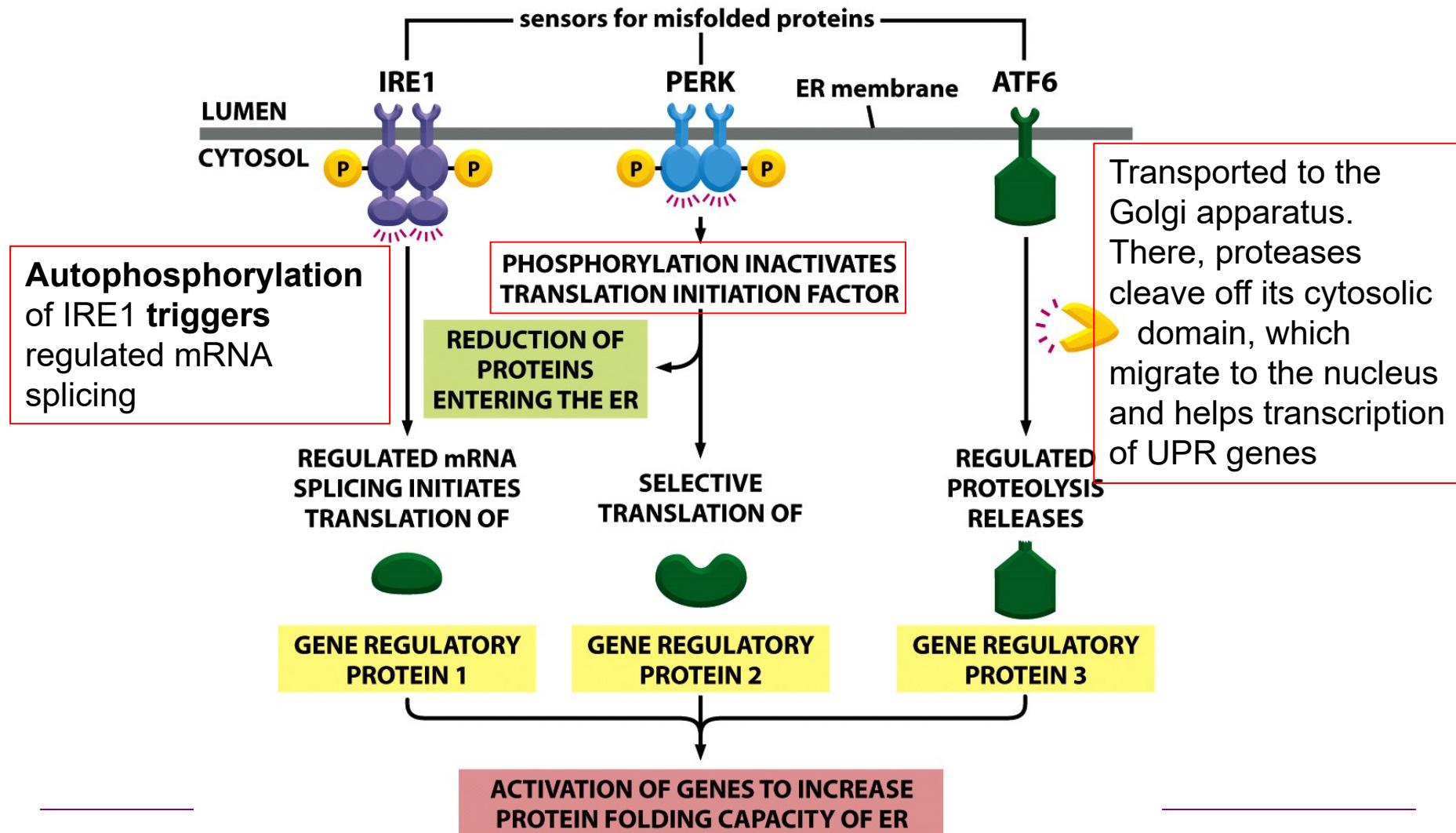


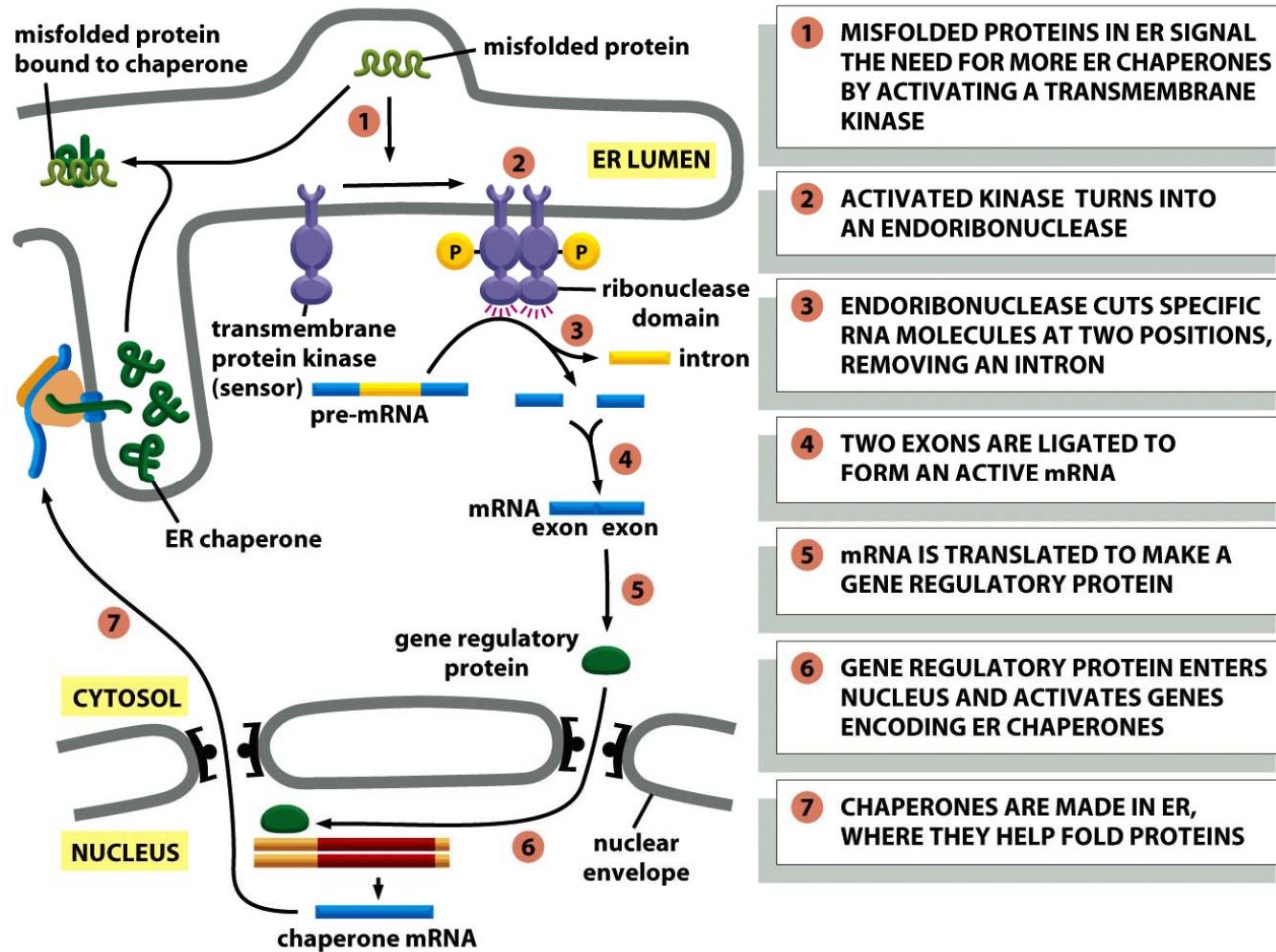
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N-linked oligosaccharide serves as a timer to determine how long misfolded protein will be degraded

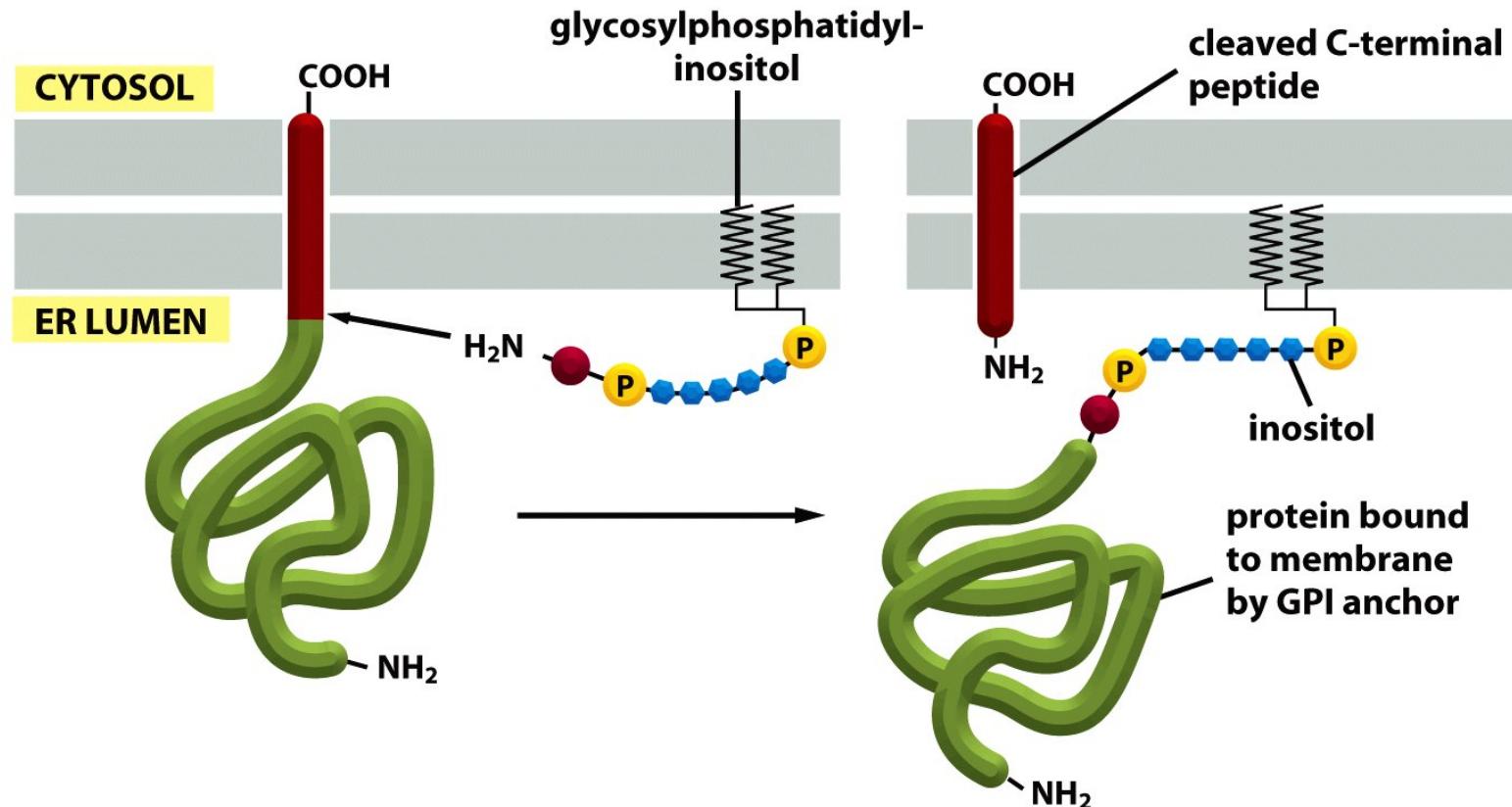
# Misfolded proteins in the ER activate an unfolded protein response



# Activation of transcription of genes involved in unfolded protein response



## How to covalently attach GPI anchor



Phosphoethanolamine: provides the amino group for the attachment of the protein by an enzymatic “cleavage-transfer” reaction

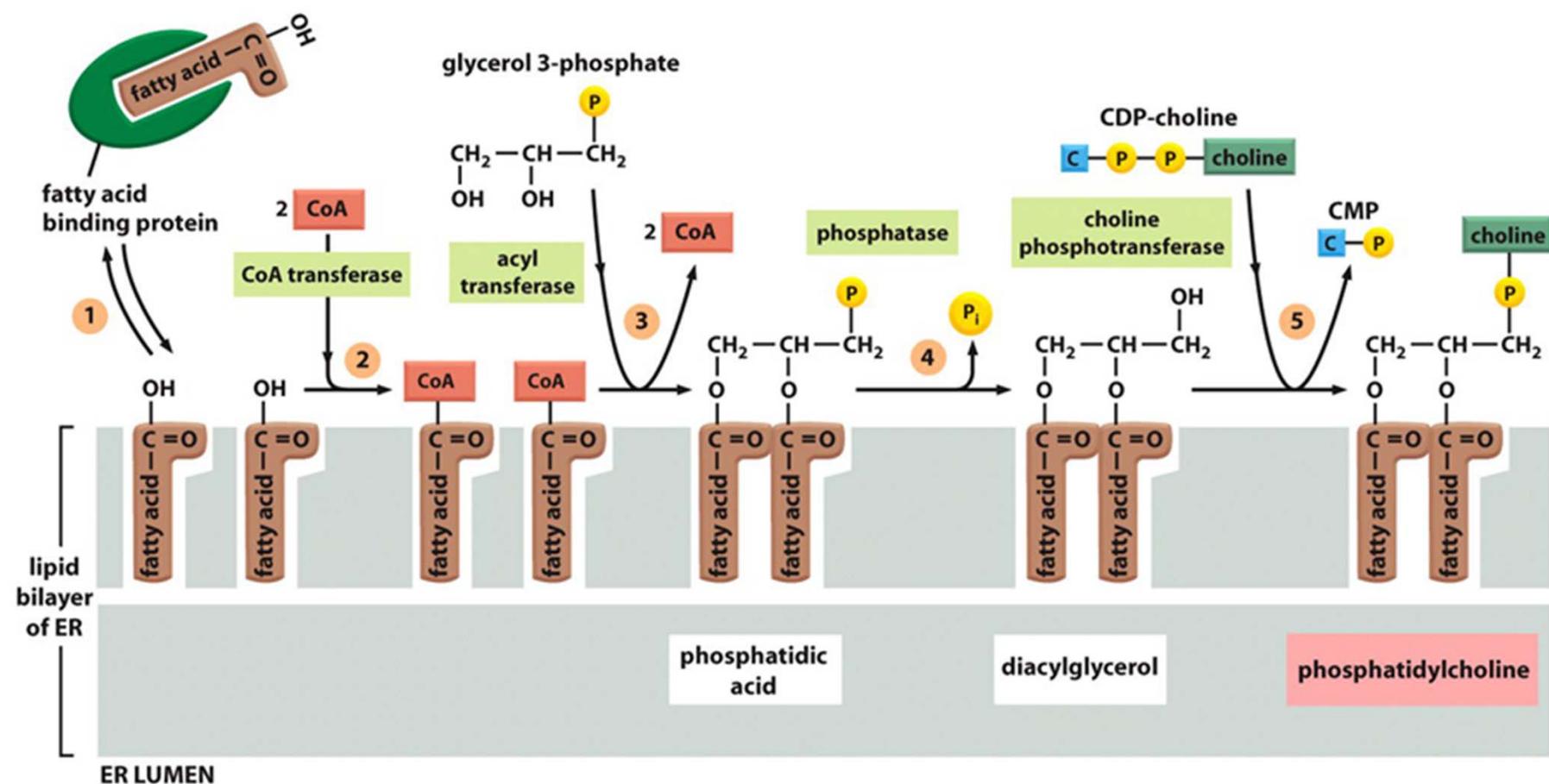
## Phospholipid synthesis in ER

- Nearly all major lipids are synthesized in ER these include:
  - Phospholipids
  - 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**.

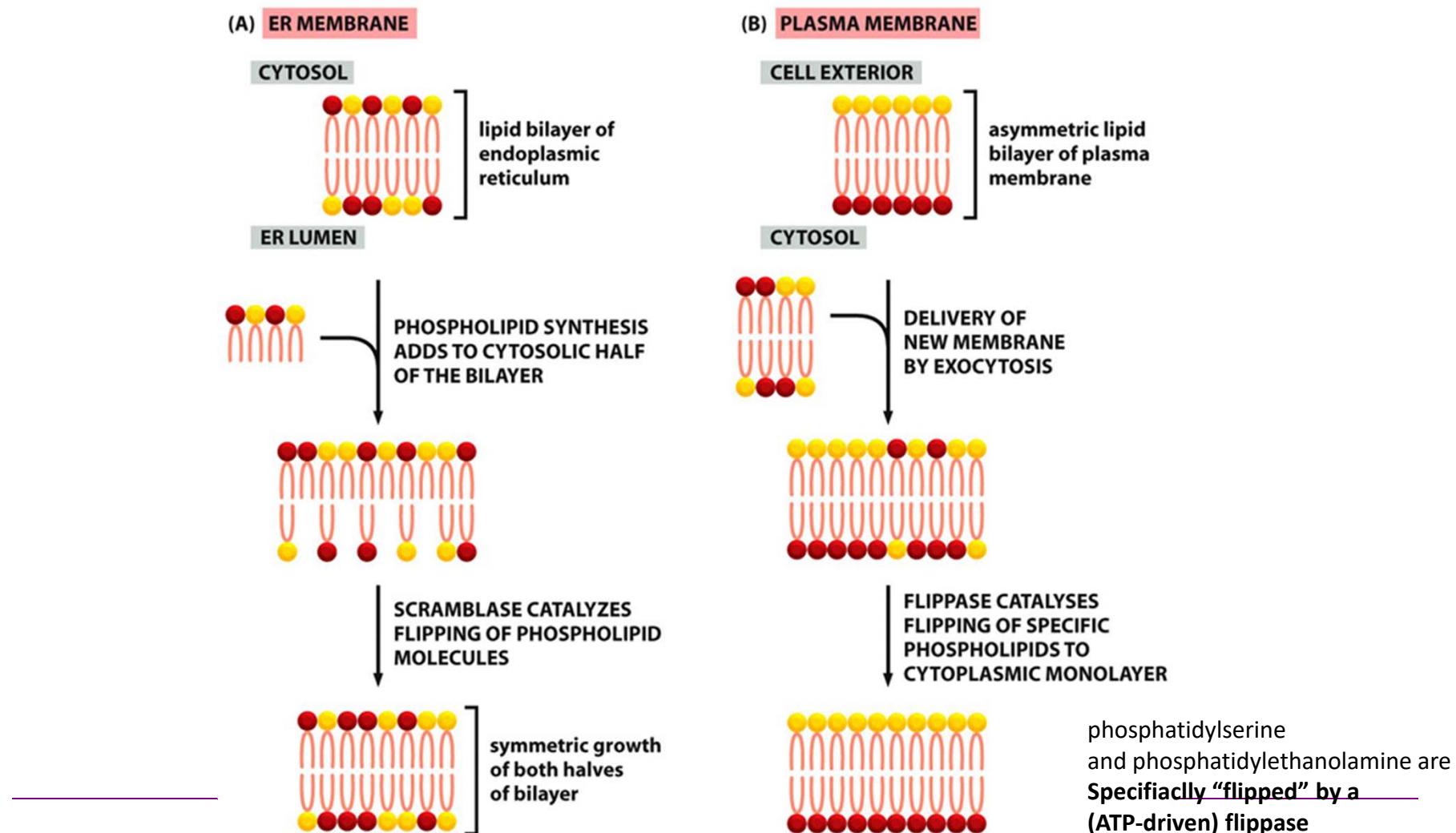
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# (1). Synthesis of phosphatidylcholine as the major lipid from two fatty acids, glycerol phosphate and choline

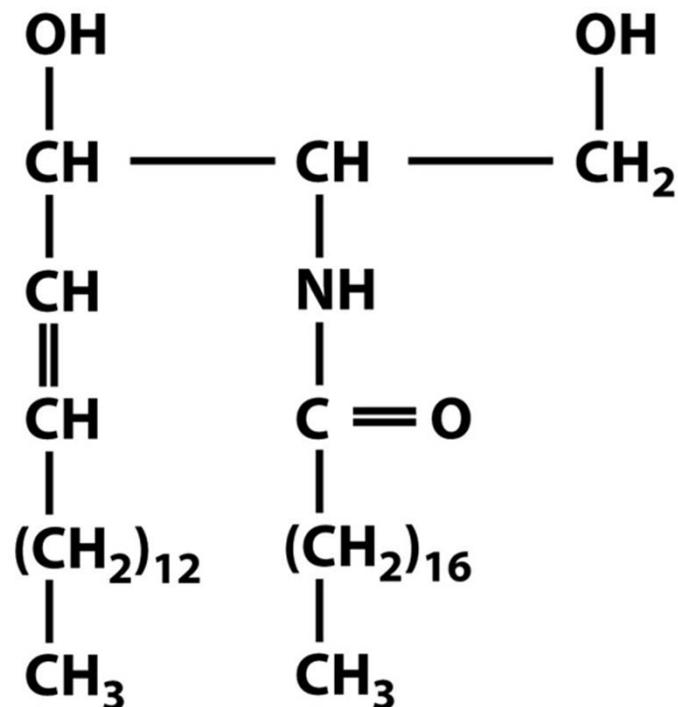
Similar mechanism for other major phospholipids



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



### (3). ER also produces sphingolipids: 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