Molecular Cell Biology, 6th Ed.

By Lodish, Darnell, et. al.

Chapter 13:

Moving Proteins into Membranes and Organelles

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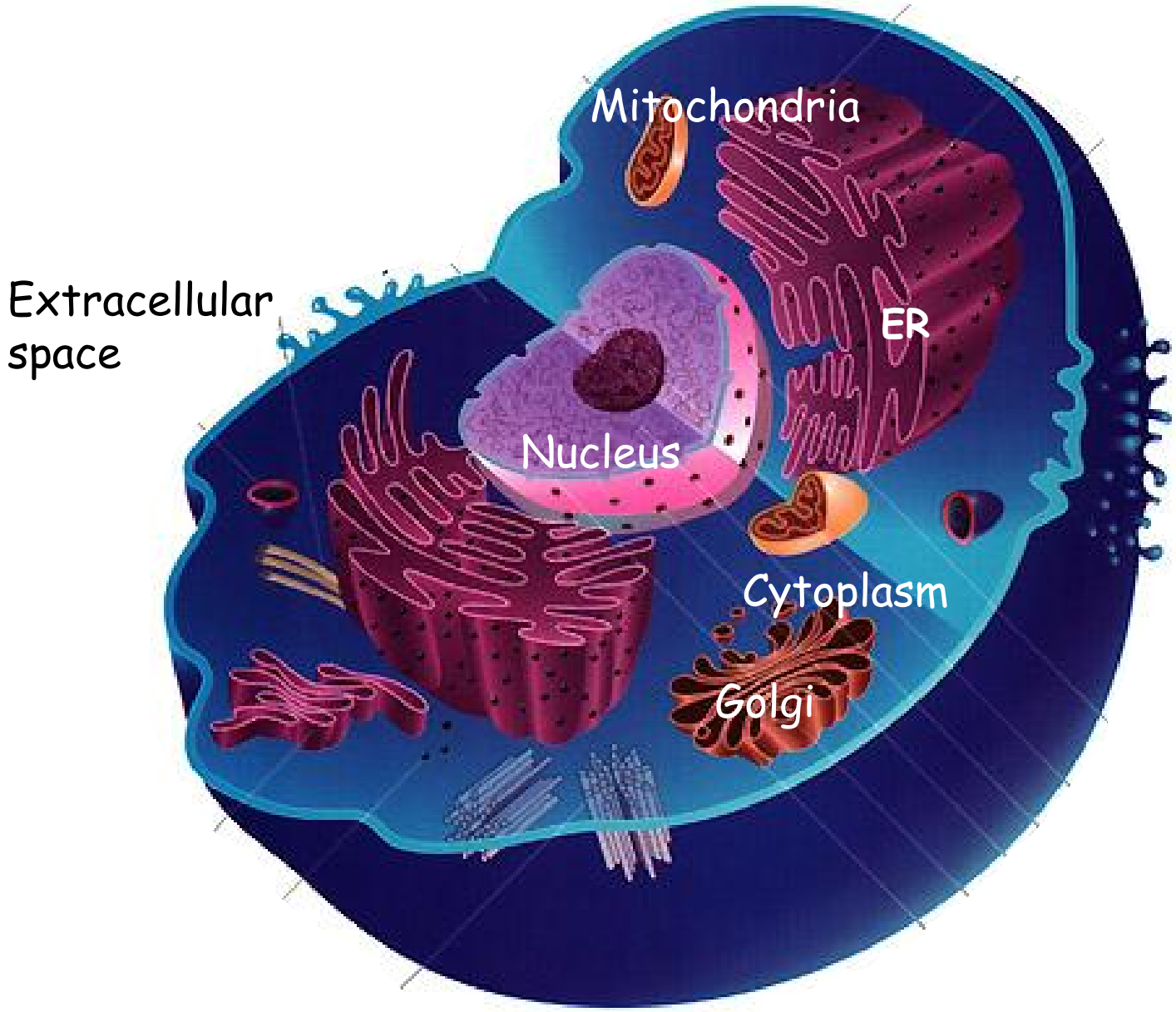
學習目標

- How does translocation of secretory proteins across the ER membrane occur?
- How does insertion of proteins into the ER membrane occur?
- How are proteins modified, folded in the ER?
- How are proteins sorted into various intracellular organelles?
  - Mitochondria
  - Peroxisomes
  - Nucleus

What determines the identity of an organelle?



Protein synthesis is a cytosolic process.

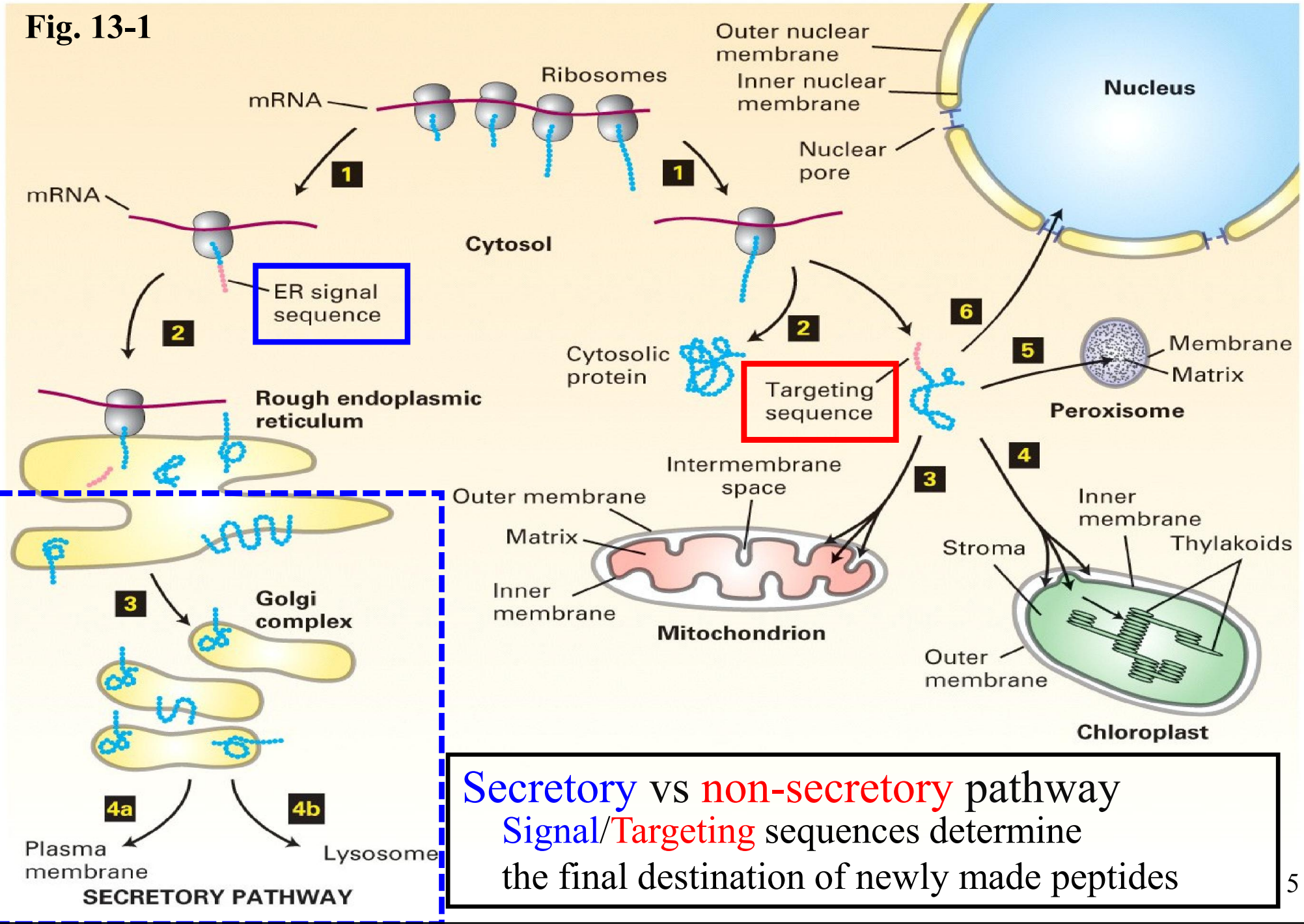
Then, how are proteins segregated into different membrane-bound organelles?

A protein's fate in the cytosol
(protein 'sorting' or 'targeting')

1. Protein sorting via the ER-bound ribosomes
   → **secretory** pathway (Ch14)
   → Proteins to be sorted to
      - Lumen & membrane of ER, Golgi, Lysosome
      - Plasma membrane
      - *outside* of the cell
2. Protein sorting via the free ribosomes
   → **non-secretory** pathway
   → Proteins to be sorted to other organelles

   - e.g. mitochondria, peroxisomes, nucleus, chloroplasts, …etc.

Protein sorting in the cytosol establishes organelle identity



Secretory vs non-secretory pathway

Signal/Targeting sequences determine the final destination of newly made peptides

Requirements for protein sorting? The "Signal hypothesis"

1. a signal sequence ('address' or 'zip code')
   - Usual 16-30 a.a. in length, in the N-terminus of the protein
   - Usually contains several (+) charged a.a. and a hydrophobic core (6-12 a.a.)
2. a receptor
   - Recognizes the signal and directs it to the correct membrane; cytosolic protein (e.g. SRP)
3. a translocation machinery
   - Translocation channel on ER membrane
4. energy
   - Unidirectionally transfers the protein to its new place
   - Requires GTP or ATP

Press Release: The 1999 Nobel Prize in Physiology or Medicine

NOBELFÖRSAMLINGEN KAROLINSKA INSTITUTET
THE NOBEL ASSEMBLY AT THE KAROLINSKA INSTITUTE

11 October 1999

The Nobel Assembly at Karolinska Institutet has today decided to award the Nobel Prize in Physiology or Medicine for 1999 to

Günter Blobel

for the discovery that

"proteins have *intrinsic signals* that govern their *transport* and *localization* in the cell"

Summary

A large number of proteins carrying out essential functions are constantly being made within our cells. These proteins have to be transported either out of the cell, or to the different compartments - the organelles - within the cell. How are newly made proteins transported across the membrane surrounding the organelles, and how are they directed to their correct location?

These questions have been answered through the work of this year's Nobel Laureate in Physiology or Medicine, Dr Günter Blobel, a cell and molecular biologist at the Rockefeller University in New York. Already at the beginning of the **1970s** he discovered that **newly synthesized proteins have an intrinsic signal that is essential for governing them to and across the membrane of the endoplasmic reticulum**, one of the cell's organelles. During the next twenty years Blobel characterized in detail the molecular mechanisms underlying these processes. He also showed that **similar "address tags", or "zip codes", direct proteins to other intracellular organelles**.

Signal hypothesis

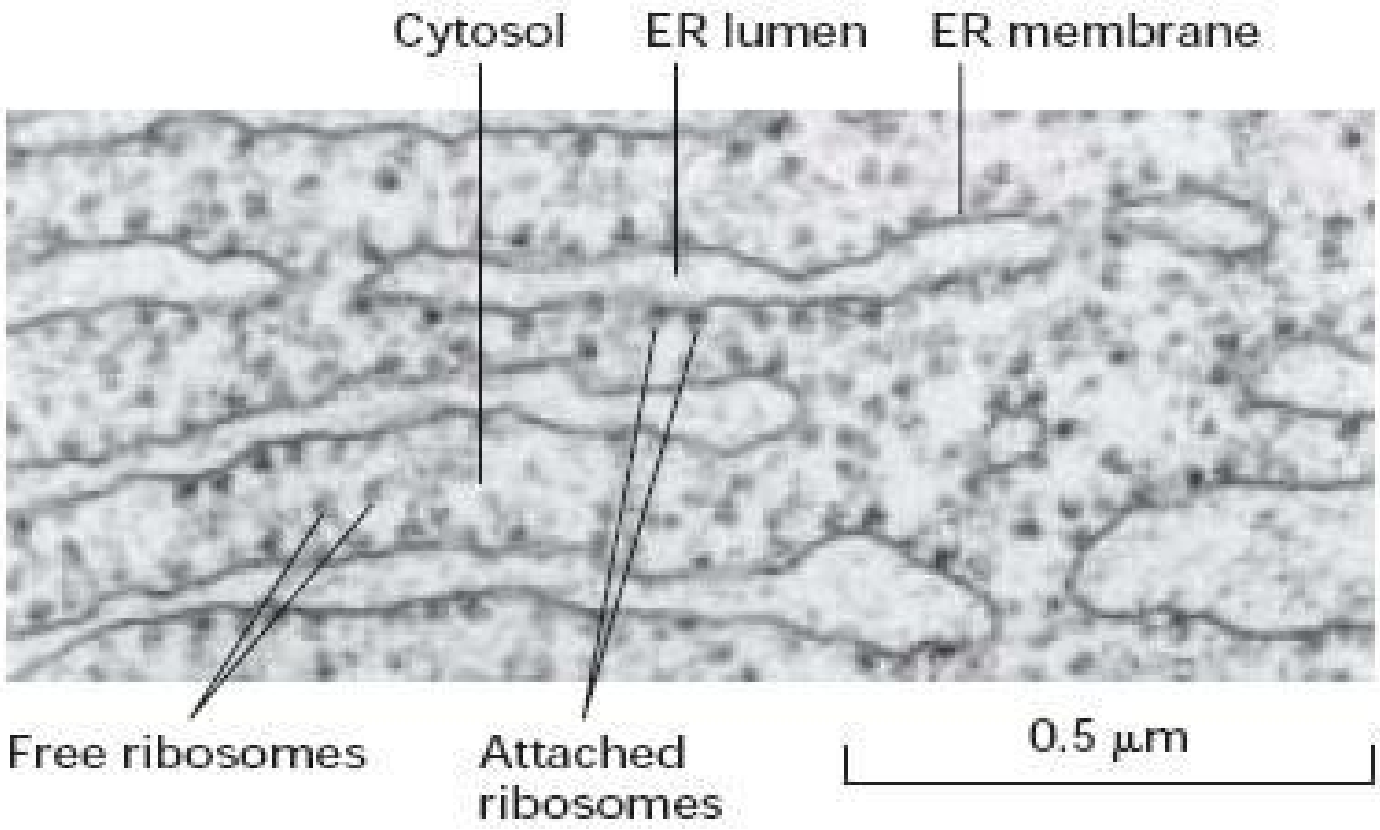
- Secreted proteins contain an N-terminal signal sequence that directs emerging polypeptide and ribosome to ER membrane

- Polypeptide moves into cisternal space of ER through channel in ER membrane as it is being synthesized (i.e. **co-translationally**)
  - "**Co-translational translocation**"

Fundamental points of every protein-targeting event

1. The *nature* of the signal sequence? And, how does it differ from others?
2. The *receptor* for the signal sequence?
3. The *structure* of the translocation channel allowing transfer of proteins across the lipid bilayer?
4. The *source of energy* driving unidirectional transfer of protein across the membrane?

13.1
Translocation of secretory proteins across ER membrane



▲ **FIGURE 13-2 Electron micrograph of ribosomes attached to the rough ER in a pancreatic acinar cell.** Most of the proteins synthesized by this type of cell are to be secreted and are formed on membrane-attached ribosomes. A few membrane-unattached (free) ribosomes are evident; presumably, these are synthesizing cytosolic or other nonsecretory proteins. [Courtesy of G. Palade.]

胰臟腺泡細胞 → makes secretory proteins

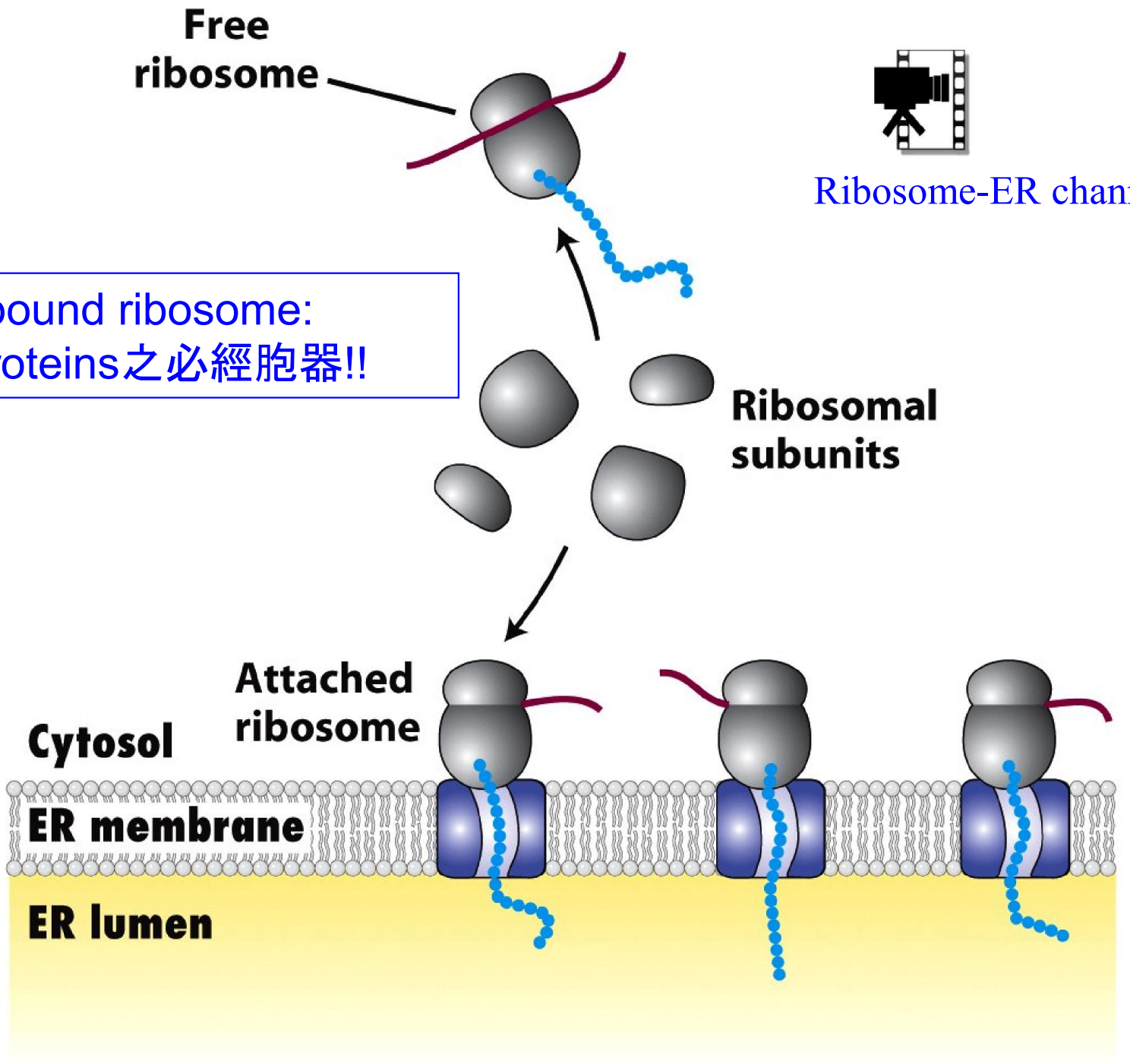


Figure 13-2b
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Ribosome-ER channel

Rough ER & bound ribosome:
為secretory proteins之必經胞器!!

Secretory proteins enter ER lumen shortly after synthesis

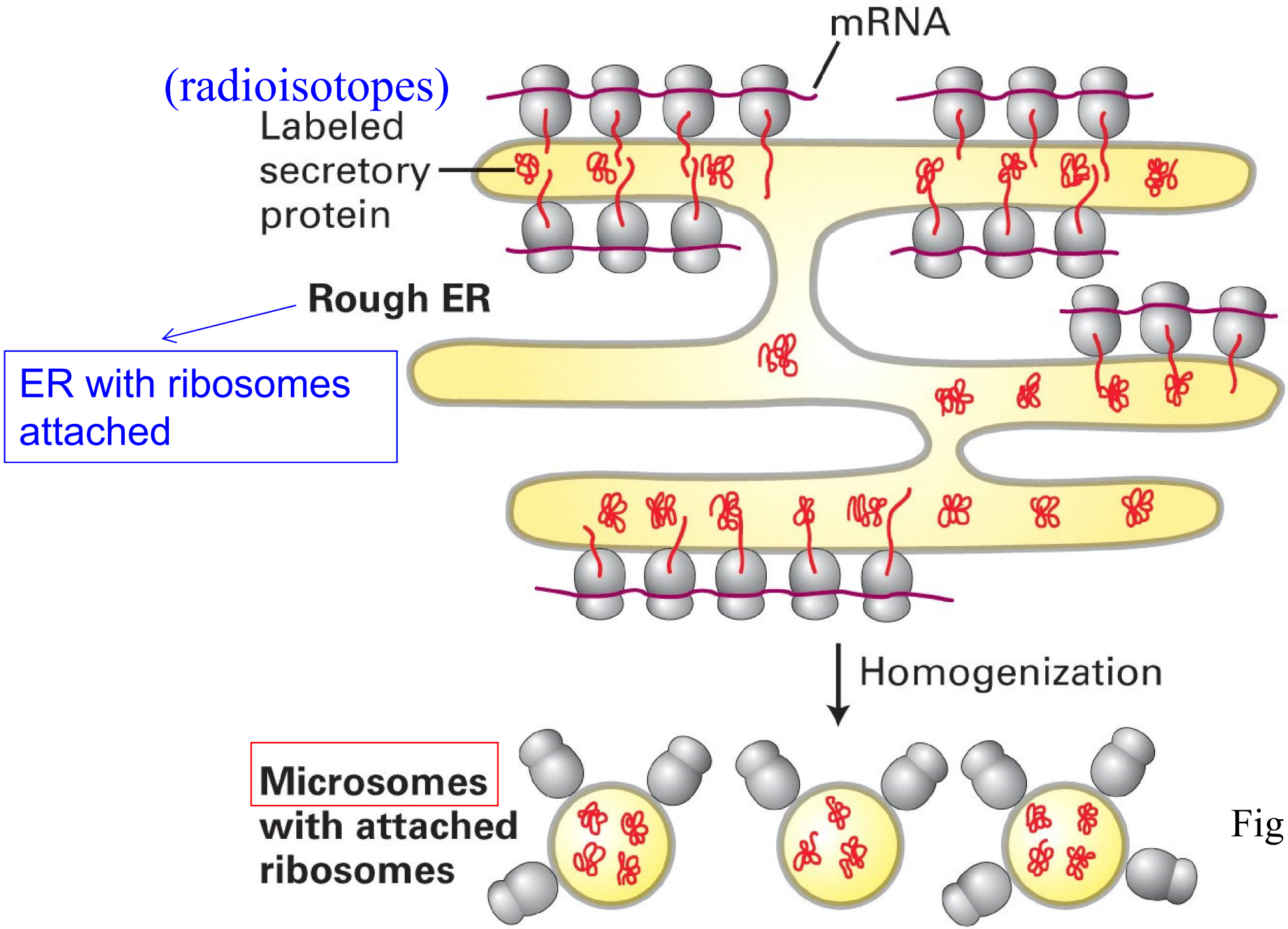
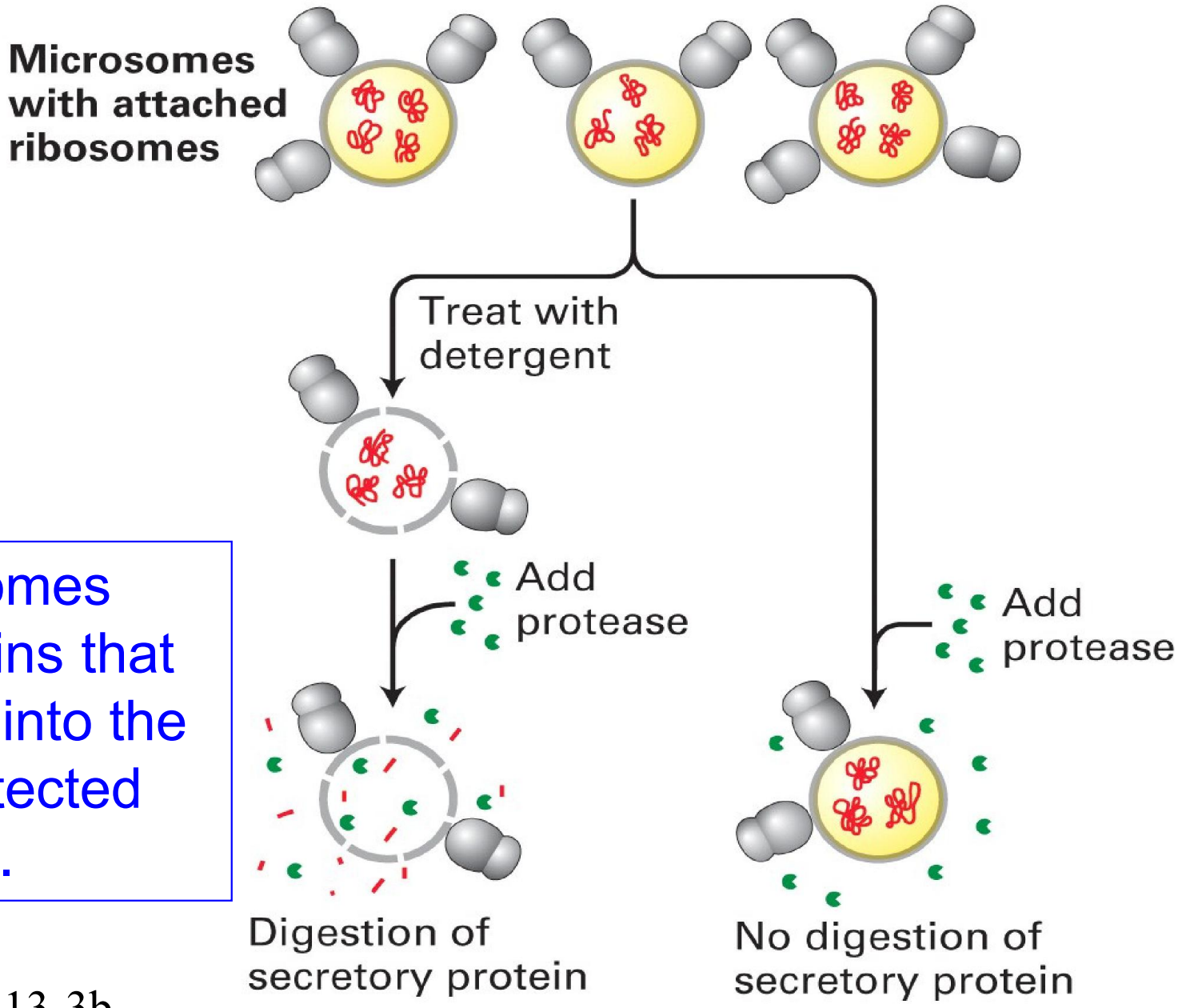


Fig 13-3a

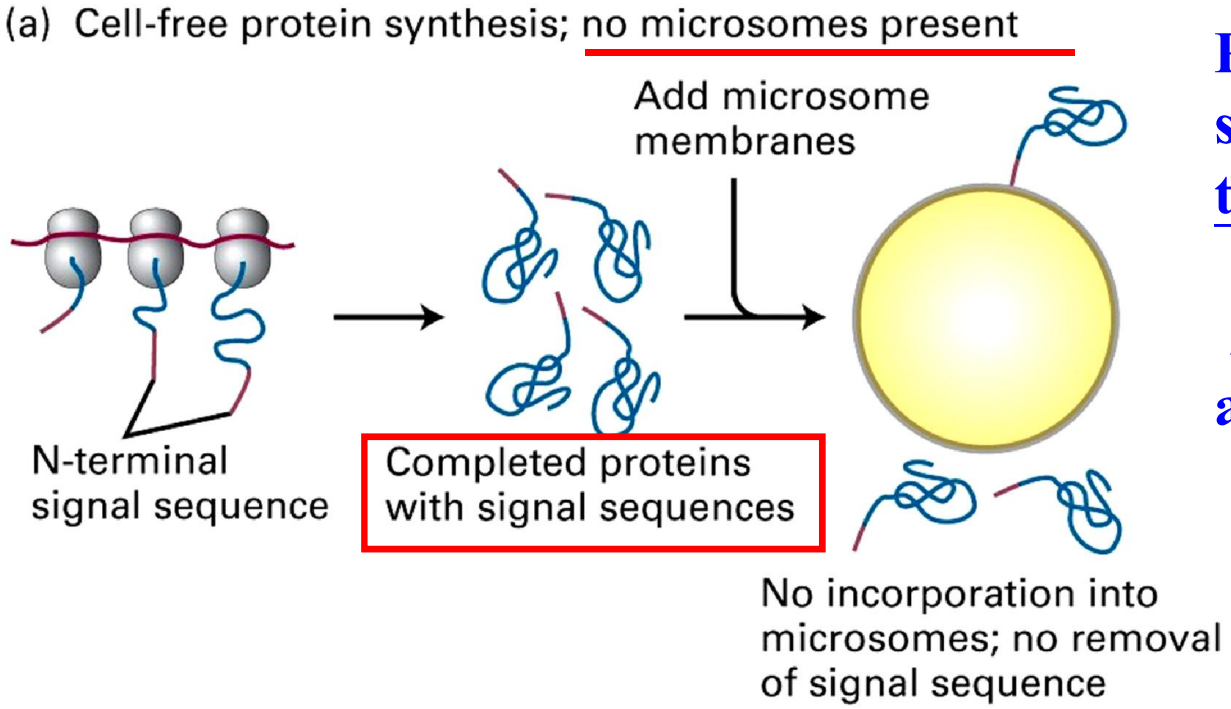
Secretory proteins enter ER lumen shortly after synthesis (cont'd)



ER-bound ribosomes synthesize proteins that are translocated into the ER and thus protected against protease.

Fig 13-3b

Translation and translocation of secretory proteins are a *coupled* process



Requires a 16-30 a.a. signal sequence located at the N-terminus of the nascent peptide

1. **One or more (+)-charged a.a.**
2. **Stretch of 6-12 hydrophobic a.a. (core)**

- **Microsomes MUST be added before the first 70 a.a. are linked together**
- **Called "Co-translational translocation"**

Fig 13-4

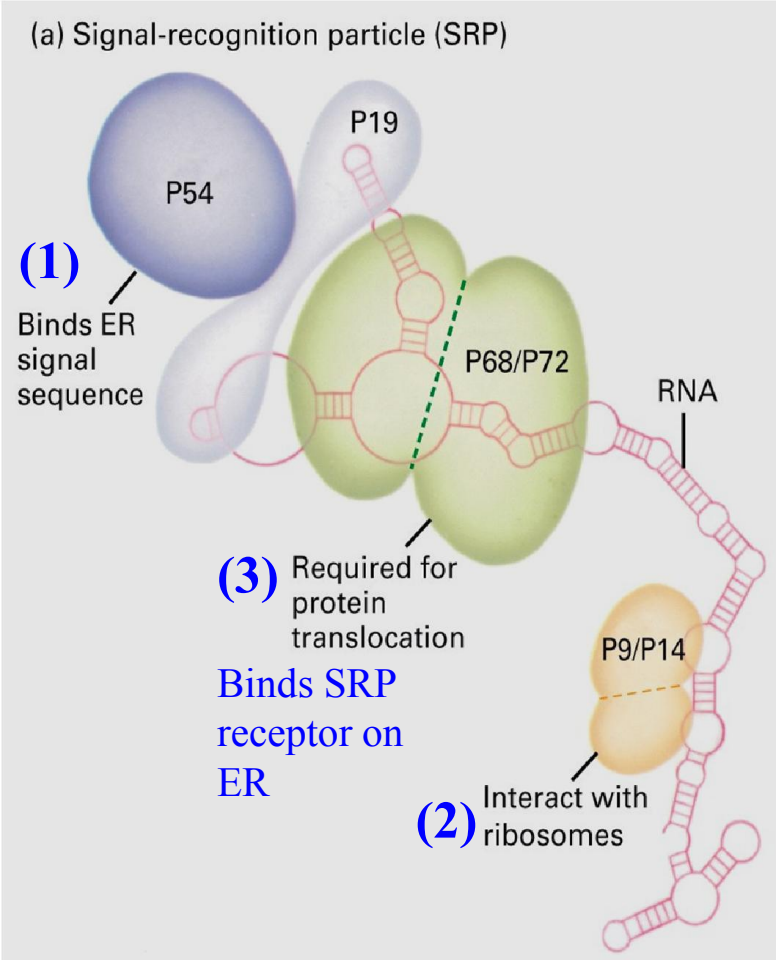
Initiation of co-translational translocation in ER

- Secretory proteins are synthesized in association with ER membrane
- How to target such proteins there?
  1. Signal-recognition particle (SRP)
     - Located in cytosol
     - A ribonucleoprotein (a hexamer + RNA)
  2. SRP receptor (SRPR)
     - Located on ER membrane
     - Very close to the membrane translocon
- Both SRP and SRPR are **GTPases** !!

Signal-recognition particle (SRP)

Lodish 5th, Fig 16.5a

- Composed of
  - 6 protein subunits
    - P54, P19, P68/P72, P9/P14
  - a 300-bp RNA molecule

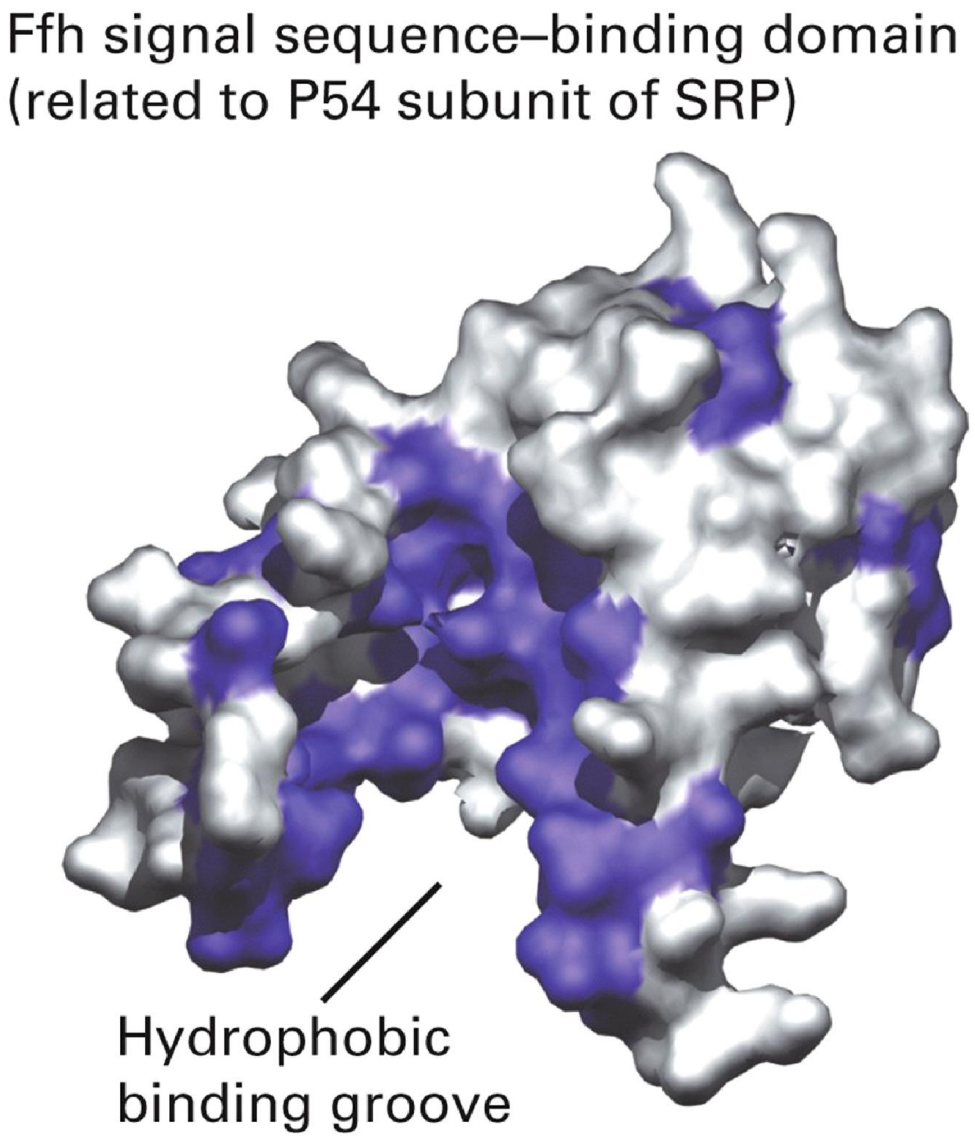


- Binds 3 components **simultaneously**
  1. ER signal on nascent peptide (via P54)
  2. Large ribosomal unit (via P9/14)
  3. SRP receptor's α subunit (via P68/72)

- SRP *inhibits* the synthesis of the complete protein
  - Will direct the nascent to the ER membrane first
  - Release of SRP then allows peptide elongation to continue

Bacterial Fft protein (homolog of P54 of SRP)

Fig 13-5a

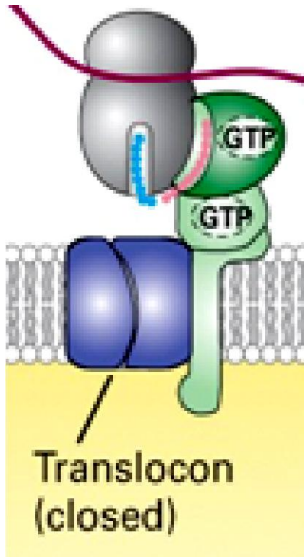


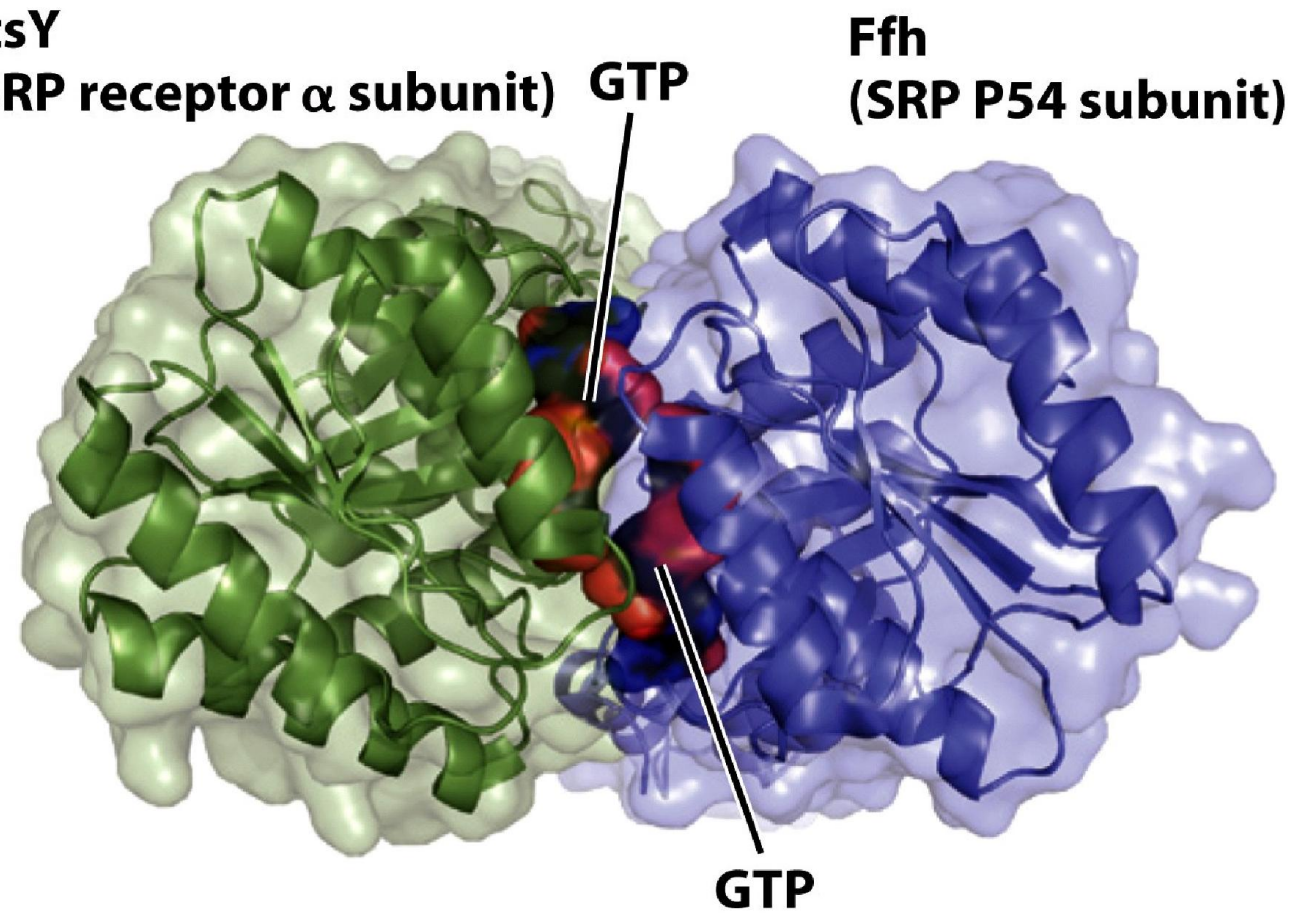
(purple: hydrophobic a.a.)

- Homolog to the P54 of SRP
  - Responsible for binding the ER signal sequence
- Interaction between hydrophobic a.a. in Ffh (or SRP) with signal sequence helps *stabilize* the nascent protein onto the SRP!!

Bacterial FtsY (GTP-bound) protein (homolog of SRPR α subunit)

Fig 13-5b





- FtsY-GTP, Ffh-GTP
  - Total **2** GTPs bound per one [Ffh-FtsY] (SRP-SRPR) complex
- Two active sites for GTP hydrolysis are formed ONLY after the Ffh-FtsY complex is formed!!

Synthesis and co-translational translocation of secretory protein into ER

Secreted protein

SRP-receptor (2 subunits)

β is transmembrane,

α is peripheral (w/ GTPase activity; e.g. FtsY)

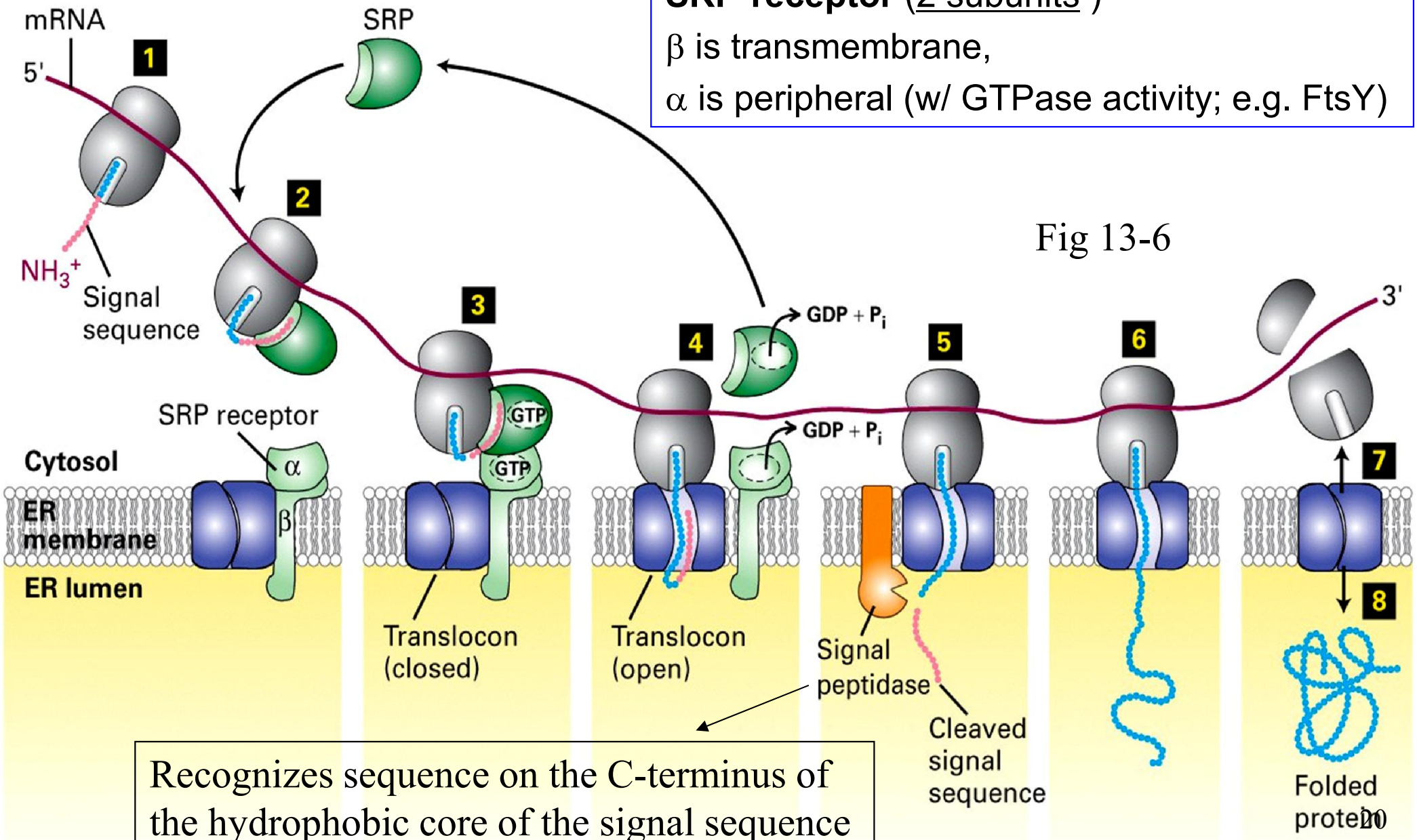
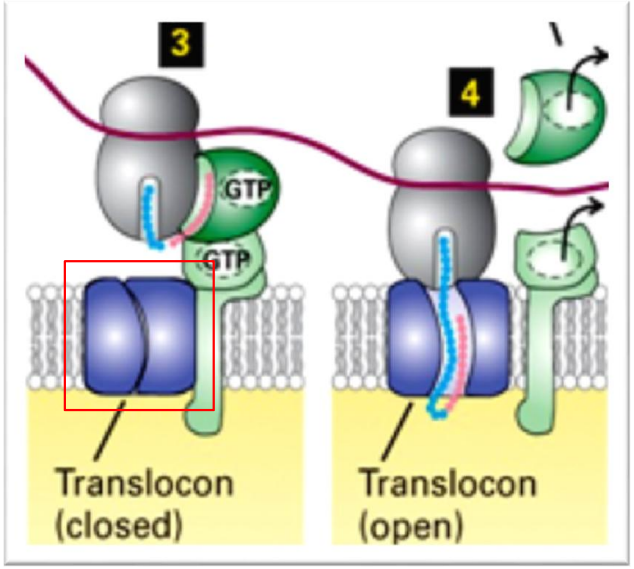


Fig 13-6

Recognizes sequence on the C-terminus of the hydrophobic core of the signal sequence

Translocon (轉位子)
(called the 'Sec61 complex' in yeasts)



- ER translocation only requires
  - SRP receptor and translocon
- Translocon
  - a protein channel complex on ER membrane
  - (yeast) Sec61 complex (α, β, γ)
- Growing peptide enters translocon into ER lumen
  - First contacts the Sec61α subunit!

Chemical cross-linking of lysyl-tRNA linked to Sec61α subunit of translocon

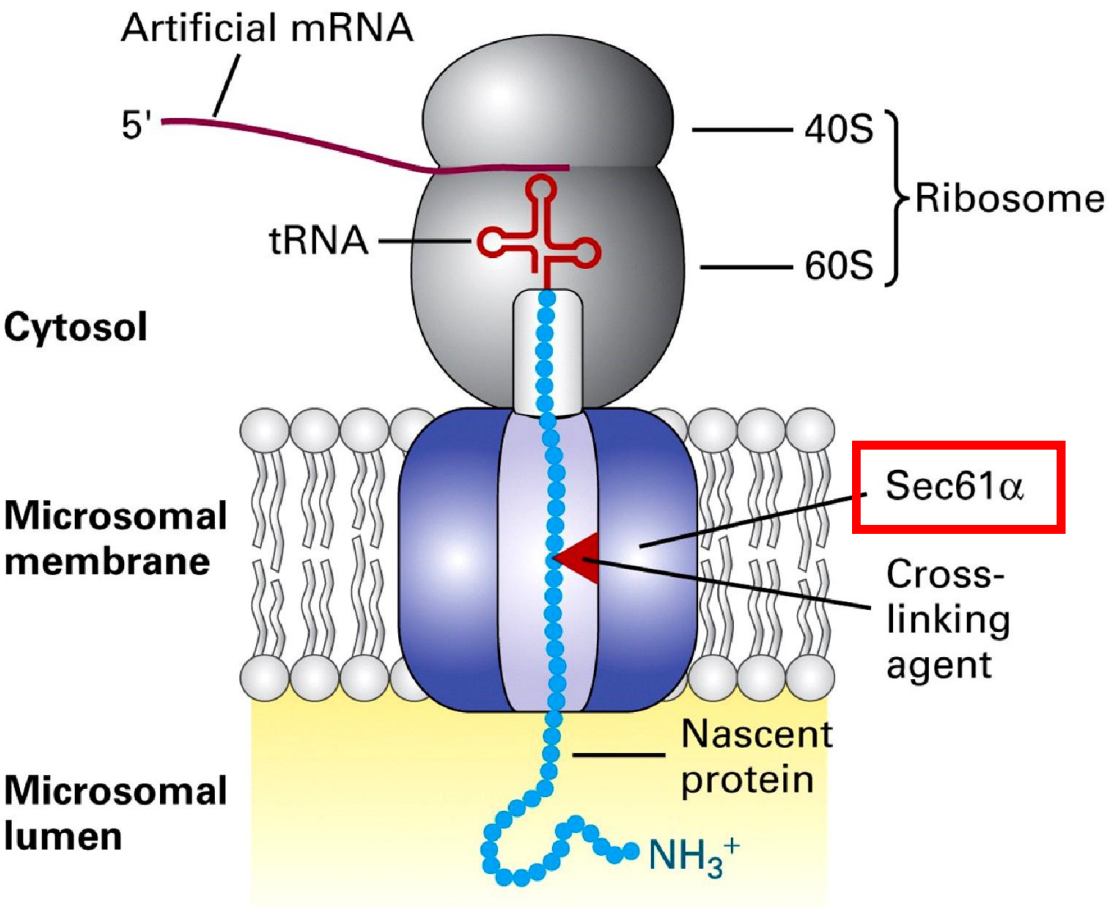


Fig 13-7

- Artificial mRNA (no stop codon)

  - peptide stuck on ribosome after synthesis is completed
- Chemically modified lysyl-tRNA added
  - Cross-linking reagent added on Lys side chain
  - Light-activated
- Upon treatment with light, lysine will cross-link to protein located in close proximity.
  - is always the Sec61α protein

Structure of a bacterial Sec61 translocon

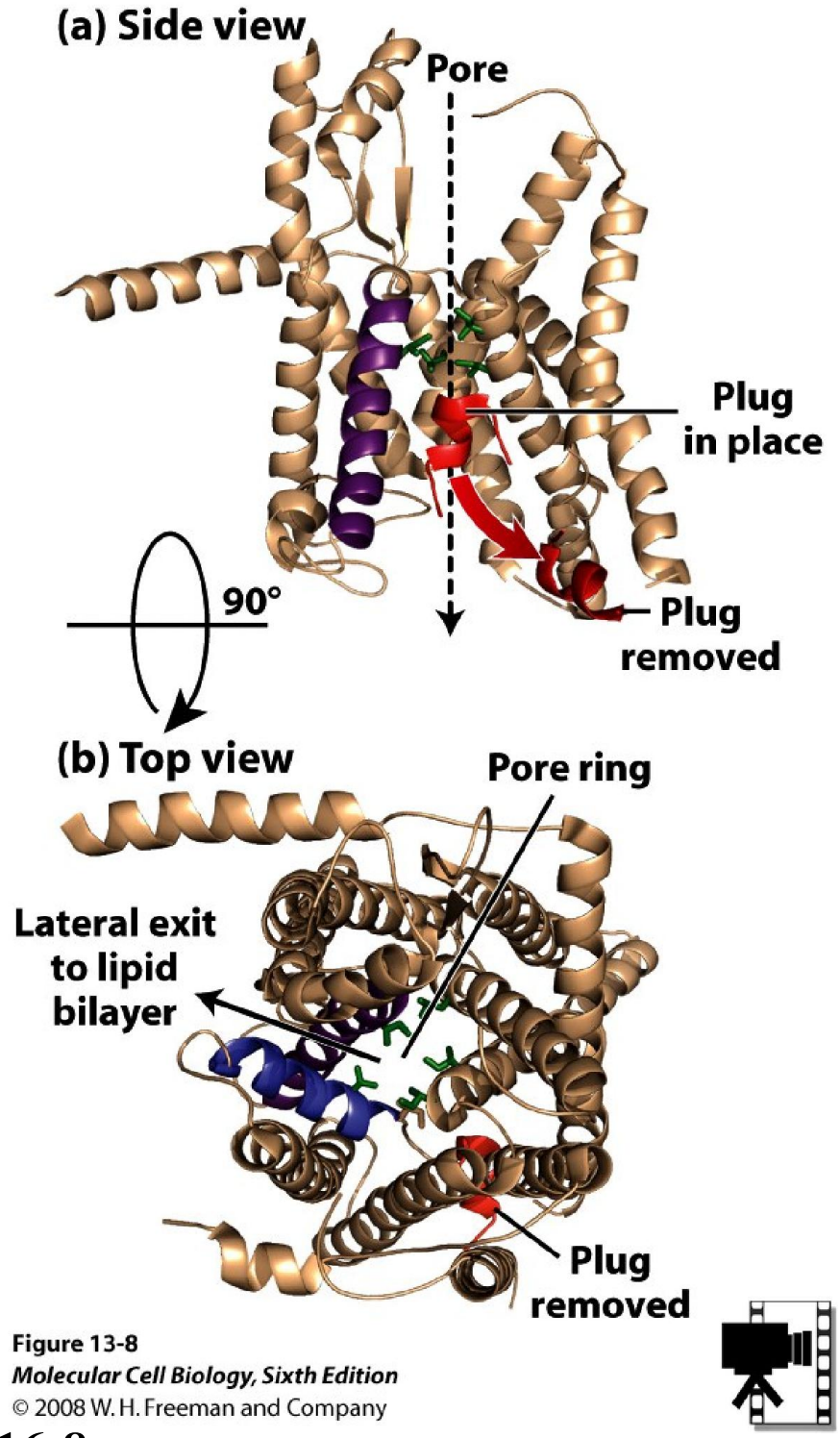


Figure 13-8
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Fig 16-8

- Also knows as the **SecY complex**
  - 10 transmembrane α helices
- Detergent treatment
  - To purify Sec61 complexes
- Subsequently add ribosomes and artificial lipid bilayers
- Translocon is re-formed in close connection with ribosomes

Translocon is a '**gated channel**'
→Two forms: 'open' vs. 'closed'
→Only open when ribosome-nascent peptide is bound.

Ribosome-ER channel

Translocation of some proteins occurs *post*-translationally

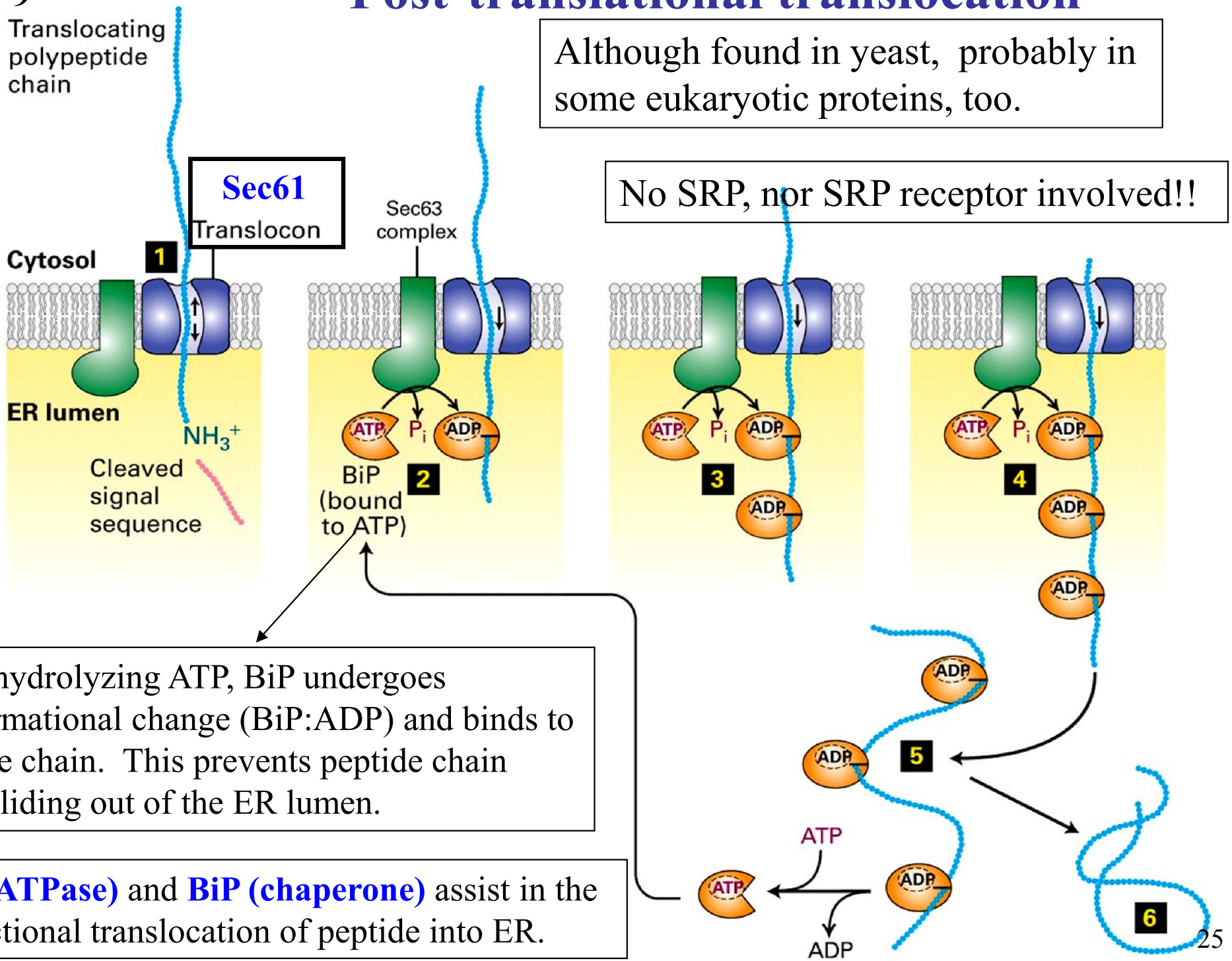
- Some **yeast** secretory proteins are transported into ER lumen *only after* translation is completed
- SRP/SRP receptor not involved!
- Requirements
  - Translocon (Sec61 complex)
  - Sec63 complex (membrane-bound ATPase)
  - BiP (chaperones) → two forms (BiP-ATP vs. BiP-ADP)
    - Substrate-binding domain: binds to nascent peptide
    - Nucleotide-binding domain: (old name: "ATPase domain", where Sec63 acts upon with bound ATP) binds to ATP
  - ATP hydrolysis (Note: not GTP hydrolysis!!)

Fig 13-9

Post-translational translocation

Although found in yeast, probably in some eukaryotic proteins, too.

No SRP, nor SRP receptor involved!!



After hydrolyzing ATP, BiP undergoes conformational change (BiP:ADP) and binds to peptide chain. This prevents peptide chain from sliding out of the ER lumen.

Sec63 (ATPase) and **BiP (chaperone)** assist in the unidirectional translocation of peptide into ER.

13.2
Insertion of proteins into the ER membrane (integral proteins)

同義詞
- Integral proteins
- Membrane proteins
- Non-secretory proteins (較少用)

How do integral proteins insert into ER membrane?

- Final orientation of membrane proteins is established during their biosynthesis
- Different topogenic sequences direct the insertion of orientation of different membrane protein classes

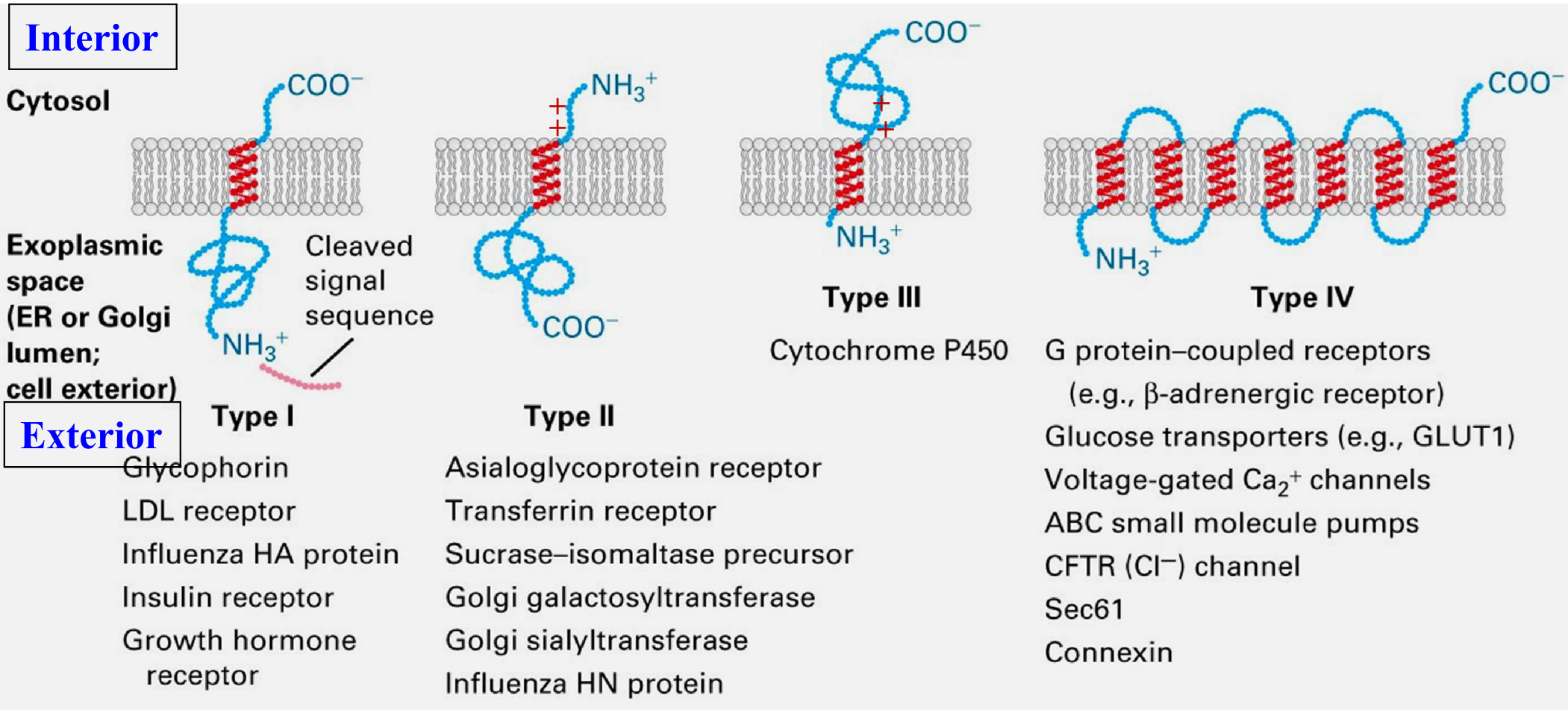
Topology of membrane proteins

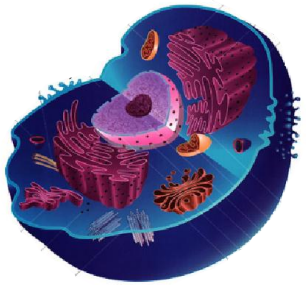
(拓樸學；局部解剖學)

- Topology (of a membrane protein)
  - Spanning numbers across the membrane
  - Orientation (location of N- & C-terminus)
- Key determining elements
  - 20-25 hydrophobic a.a.
  - Forms an α-helix
    - Can interact with the hydrophobic interior of lipid bilayer

      → to help *anchor* the protein into the membrane
- 4 classes of protein topology
  - I, II, III, IV (Fig. 13-10)

Types of membrane proteins



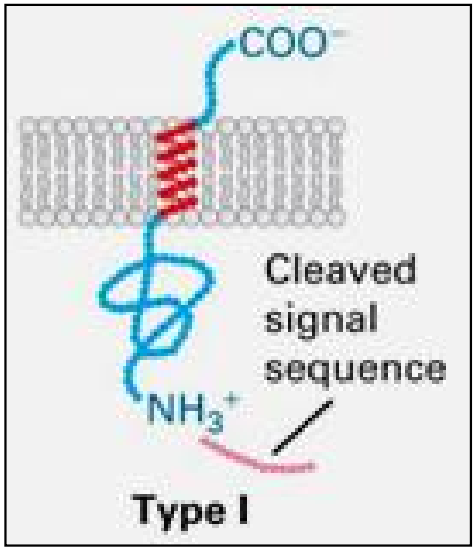


Single-pass proteins (Types I-III)

Multi-pass protein (Type IV)

Fig 13-10

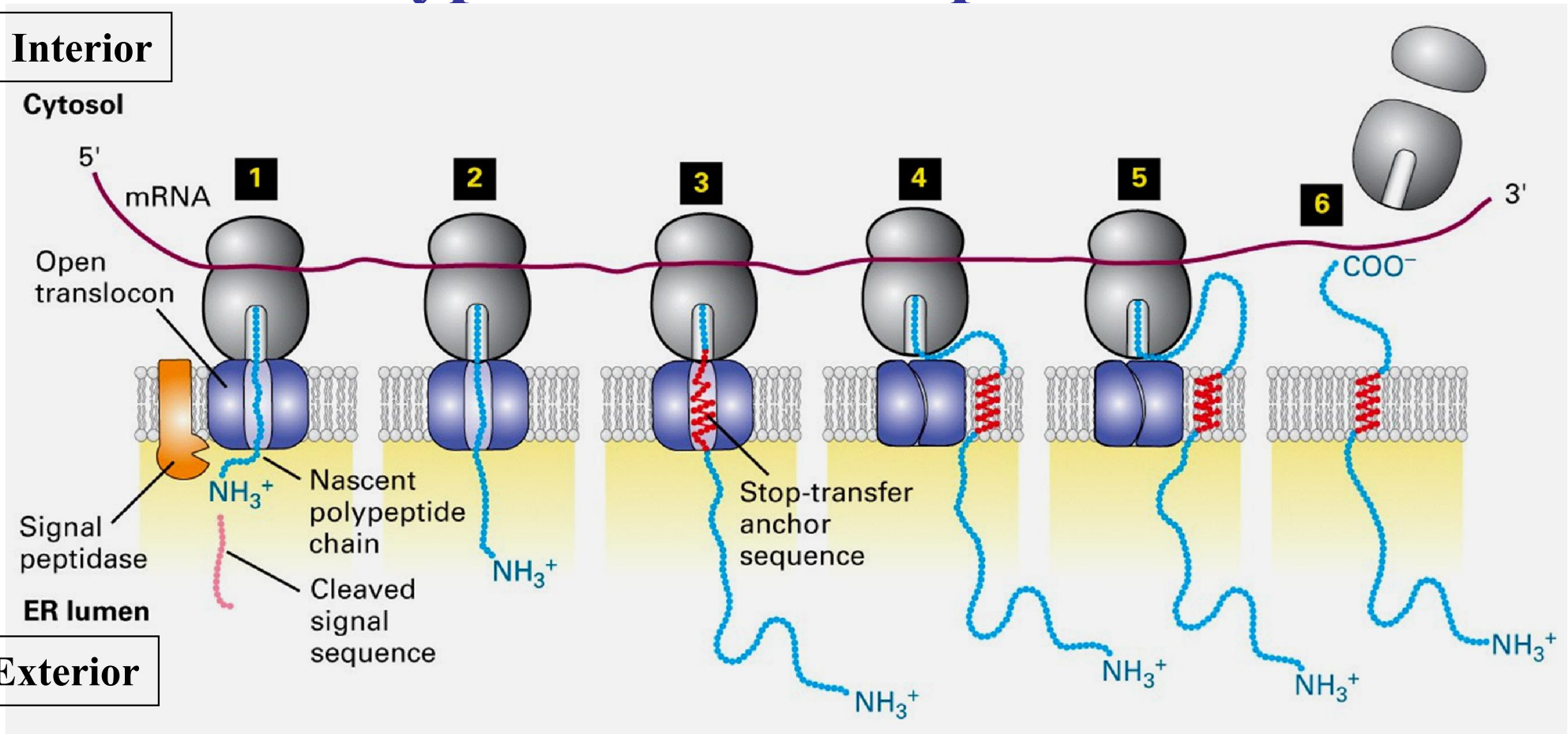
Type I membrane proteins



- The only type possessing
  - An N-terminal signal sequence
    - for ER targeting
    - initiates co-translational translocation
      - Via assistance of SRP/SRP receptor
    - Is cleaved upon entering ER lumen
  - An internal hydrophobic sequence
    - ~ 16-22 a.a. long
    - Called "stop-transfer anchor (STA) sequence", close to the C-terminus
    - Forms α-helix for membrane spanning
    - Stops transfer of nascent peptide chain while crossing the ER membrane
    - Helps *anchor* peptide chain in the phospholipid bilayer
      - Can move laterally in the lipid bilayer (less freely than the GPI-anchor)

Type I membrane proteins

Fig 13-11



Type I: 1. cleavable N-terminal signal sequence (SS),
2. stop-transfer sequence (STA) in the C-terminal portion of the protein
3. most part of protein is facing toward the exoplasmic side (ER lumen)

Type III: 1. *same orientation as type I*; but, NO signal sequence!!
2. often (+) charge C-terminal to the hydrophobic domain

Types II & III membrane proteins

- No cleavable N-terminal signal sequence
- Posses a single "signal-anchor (SA) sequence"
  - Functions as both signal & anchor
  - *Internal* and hydrophobic
    - (c.f.) Type I: *N-terminal* and hydrophobic
  - After being inserted, can move laterally within the bilayer
    - Similar to the STA sequence in type I protein
  - This internal SA sequence is NOT cleaved during the co-translational translocation
- Orientation determines type II vs III topology

Type II

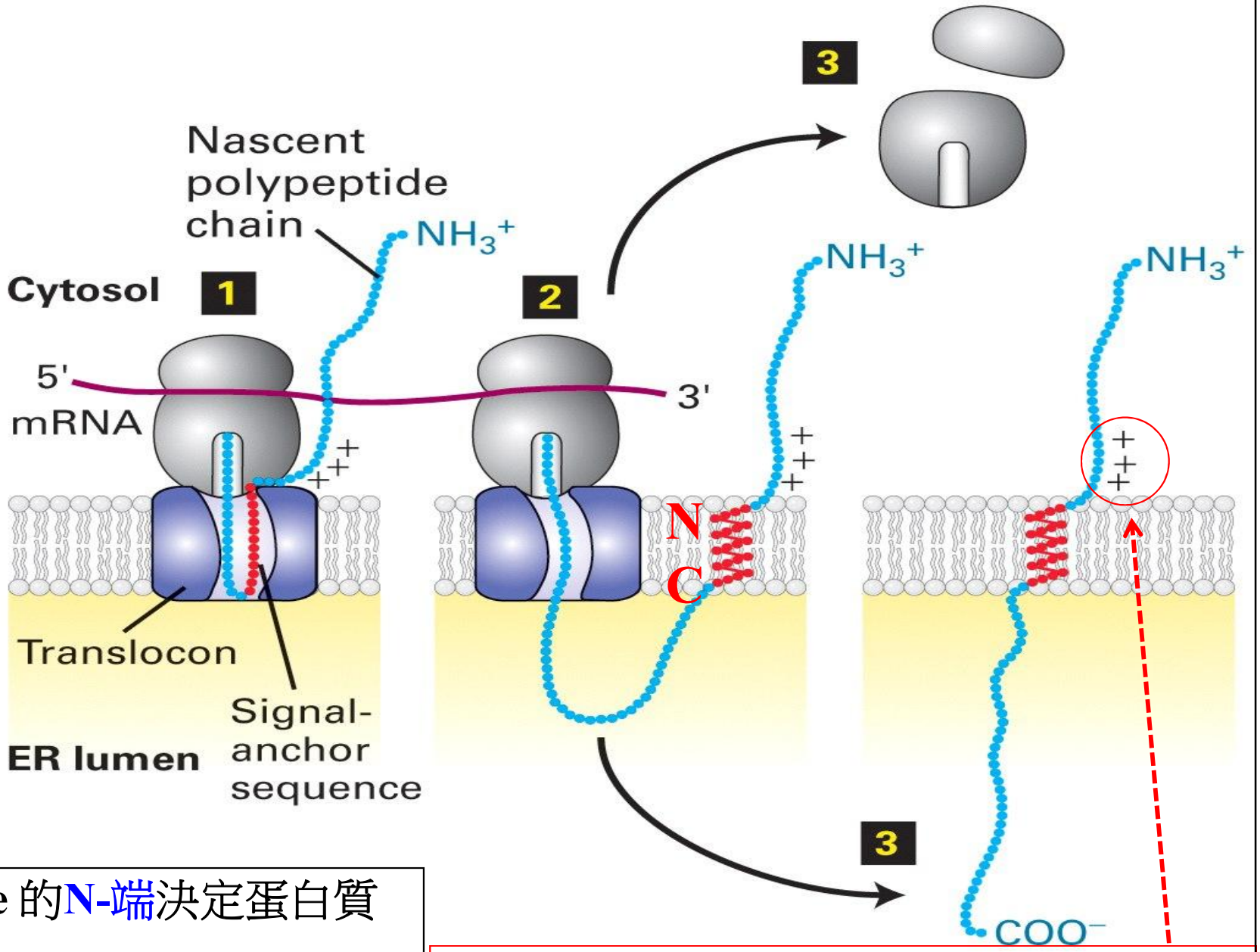


Fig 13-12

Signal sequence 的N-端決定蛋白質的不同type

- **Faces cytosol → type II**
- **Faces ER lumen → type III**

In both types II and III,
(+) charged a.a. are always found
to be adjacent to the signal-anchor sequence
and face the cytosolic side.

Type III

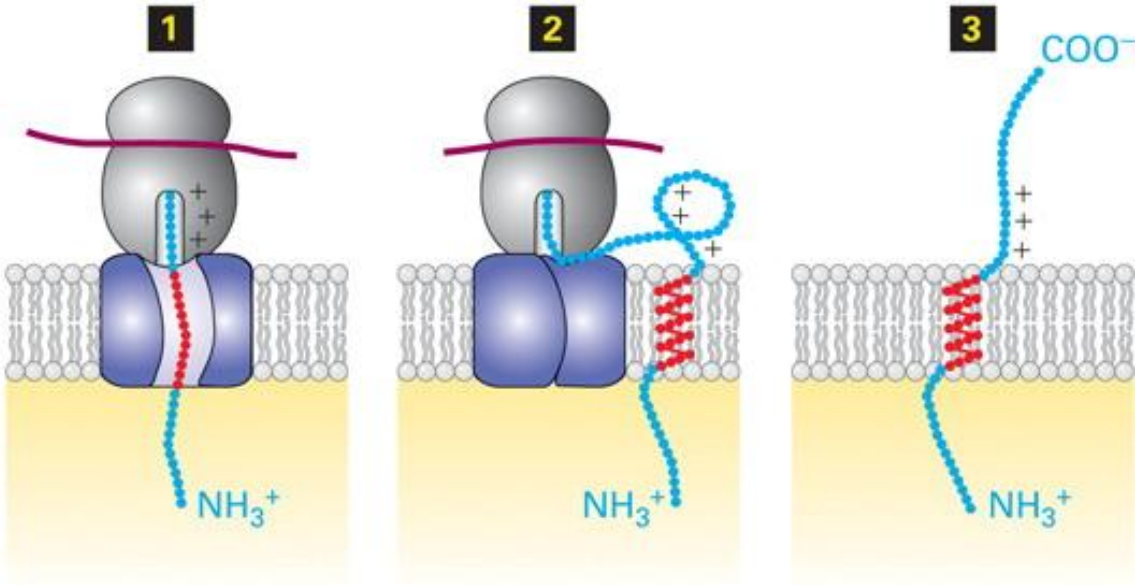


Fig 13-12b

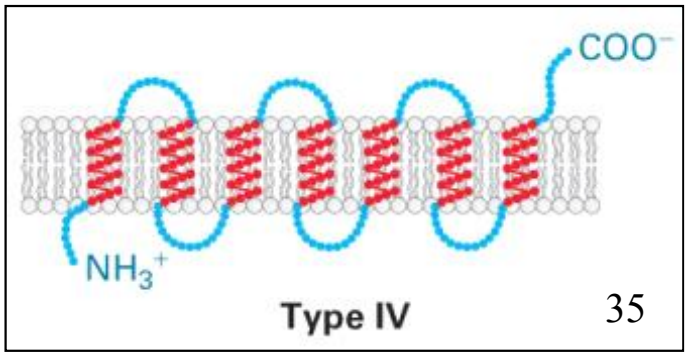
Signal sequence 的N-端決定蛋白質的不同**type**

- **Faces cytosol → type II**
- **Faces ER lumen → type III**

In both types II and III,
(+) charged a.a. are always found
to be adjacent to the signal-anchor sequence
and face the cytosolic side.

Type IV membrane proteins

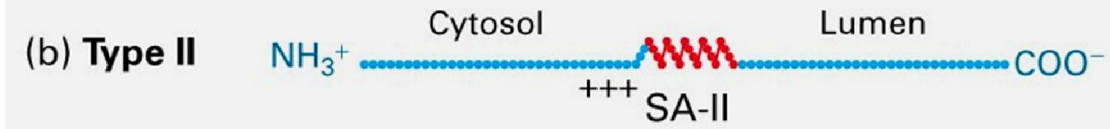
- Orientation depends on the numbers of topogenic sequences (**STA sequences**)
  - Even
    - Both ends of peptide chain will face the same side (either ER lumen or cytosol)
  - Odd
    - Both ends will face opposite sides



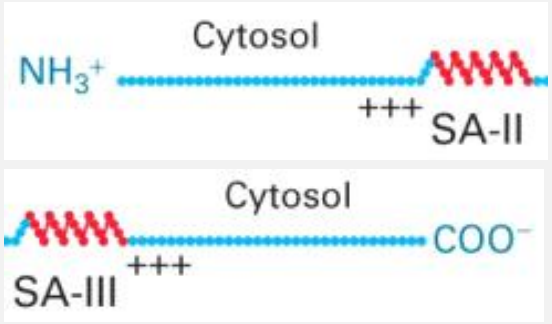
Topogenic sequences in types I-IV proteins

Fig 13-13

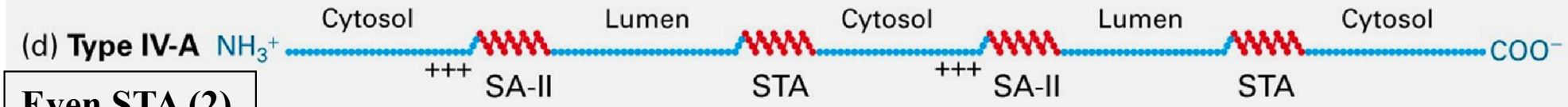
STA = Internal stop-transfer anchor sequence
SA-II = Internal signal-anchor sequence
SA-III = Internal signal-anchor sequence



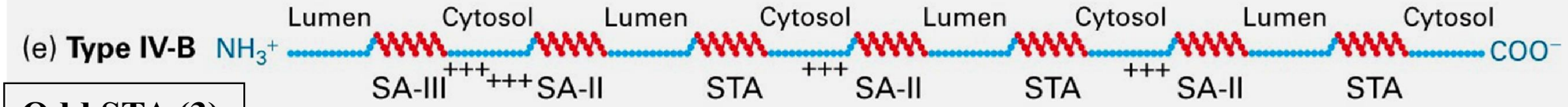
SA-II vs SA-III



Type IV-A = (Types II + I)n



Even STA (2)



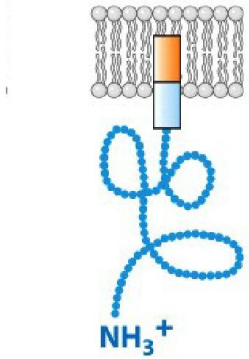
Odd STA (3)

Type IV-B = Type III + (Types II + I)n

Other integral protein type (phospholipid-anchored proteins)

- Some cell-surface proteins are attached to the phospholipid bilayer via the covalent linkage to an amphipathic molecule
  - Glycosylphosphatidylinositol (GPI)
- These proteins are initially synthesized as type I protein
  - Anchored on ER membrane via type I STA sequence first.
  - GPI transaminidase cleaves off the STA sequence
  - Protein then transferred to preformed GPI (anchoring)

Yeast GPI-anchored proteins



GPI-linked protein

Plasminogen activator receptor

Fasciclin II

Fig 13-10

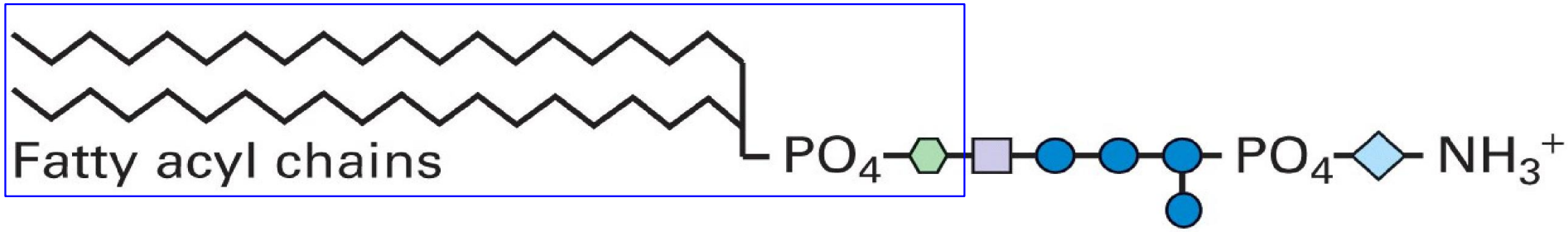
= Inositol

= Phosphatidylinositol (PI)

= Glucosamine

= Mannose

PO_4 — NH_2 = Phosphoethanolamine



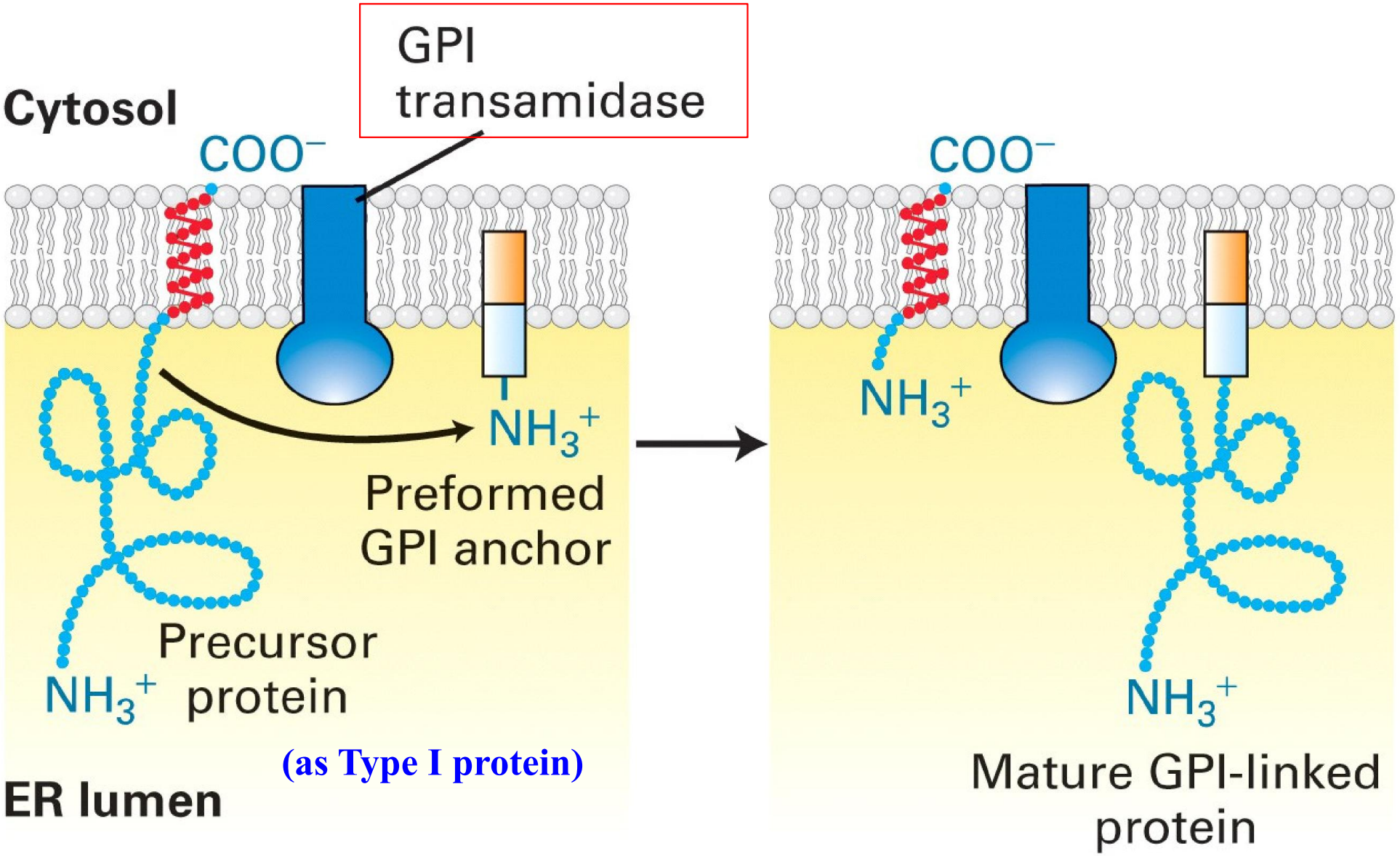
(faces cytosol)
(embedded in the membrane)

(faces ER lumen)

Hydrophilic

links to C-terminus of protein

Fig 13-14a



- Cleaved off stop-transfer-anchor (STA) sequence
- Transferred to GPI via GPI transamidase

Fig 13-14b

Why is GPI-anchoring important?

- GPI-anchored proteins can freely move in the phospholipid bilayer
- GPI anchor can assist in targeting the attached proteins to the apical domain of the plasma membrane (e.g. Fig 14-25)

Deducing topology of a membrane protein via its sequence

- Must understand the function of the protein to some extent
  - e.g. unknown receptor for cell-to-cell interaction,…etc.
- Use computer to generate a hydropathy profile of the protein of interest
  - Assign hydropathic index of each a.a.
  - Hydrophobic a.a. → assigned (+) index
    - e.g. a.a. with hydrocarbon side chains (Phe, Met)
  - Hydrophilic a.a. → assigned (-)
    - e.g. a.a. with charges (Arg, Asp, Lys)
- To identify longer protein segments (usually ~20 a.a.) having sufficient overall hydrophobicity

Hydropathy profiles for the deduction of topogenic sequences

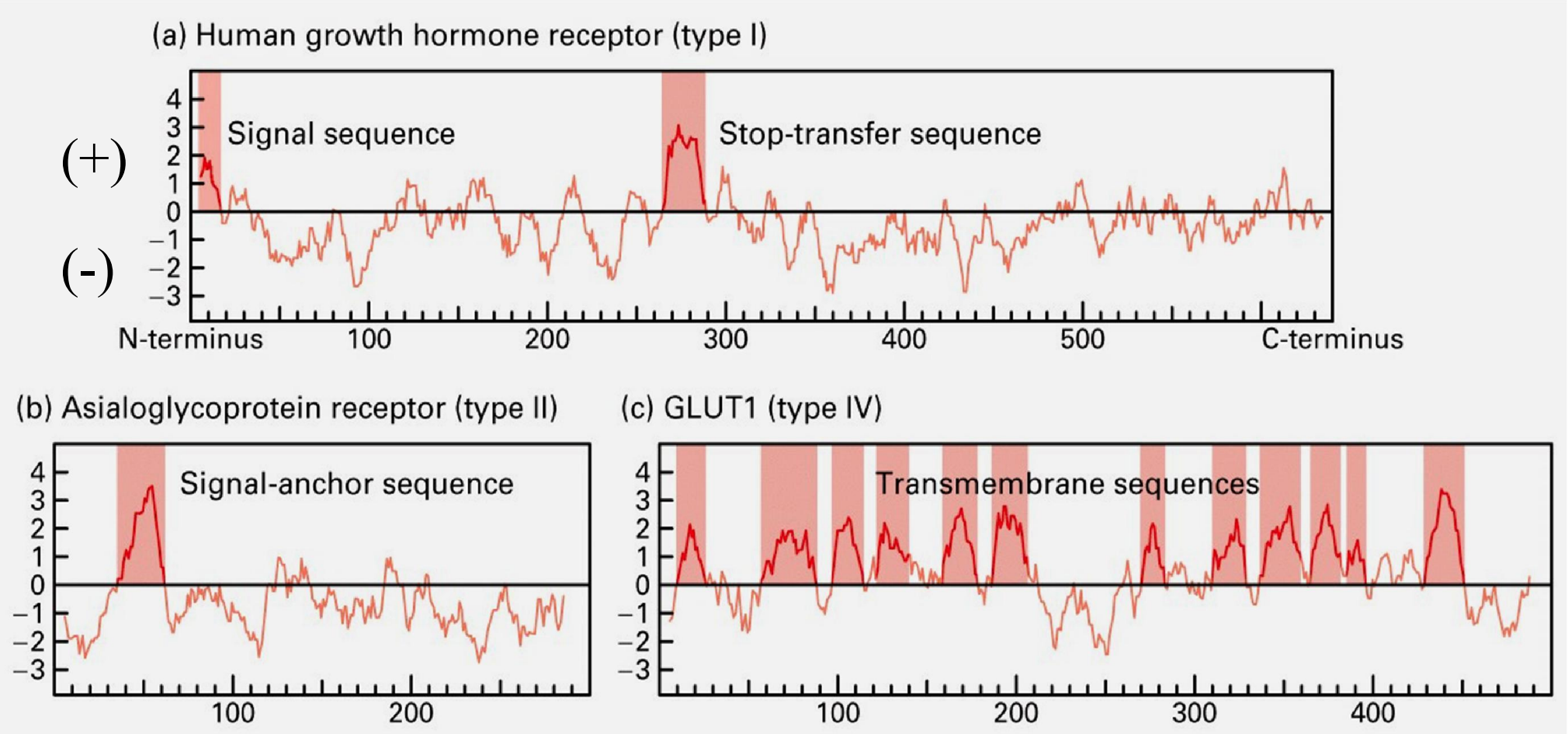
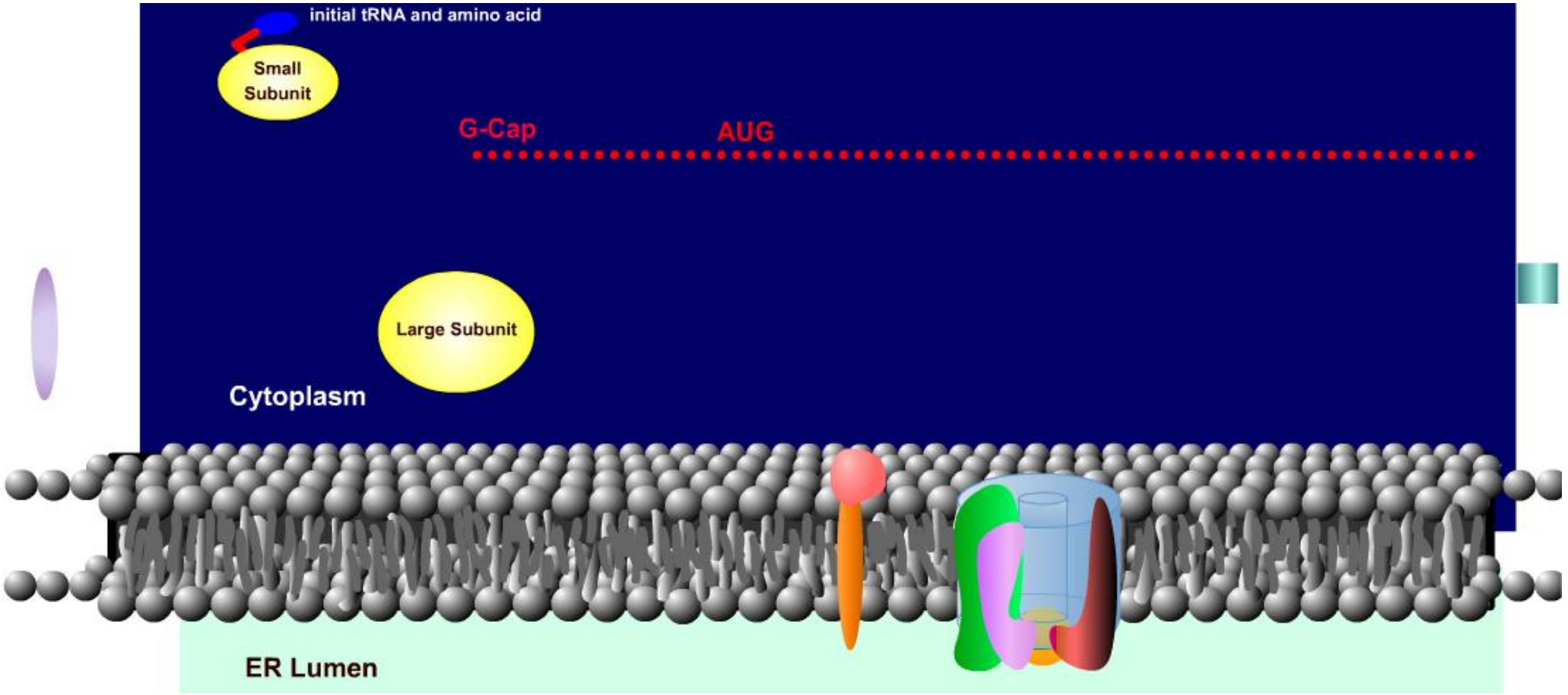


Fig 13-15

Animation of translocation of membrane protein into ER



- http://bama.ua.edu/~hsmithso/class/bsc_495/er/er-memb-controlled.swf

SWF 檔案

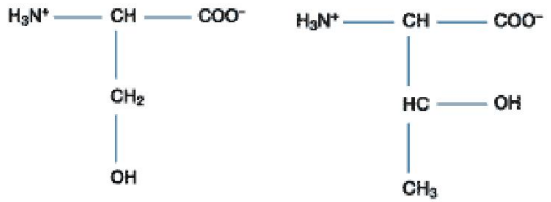
13.3
Protein modification, folding, and quality control in the ER

Protein modification (post-translational process)

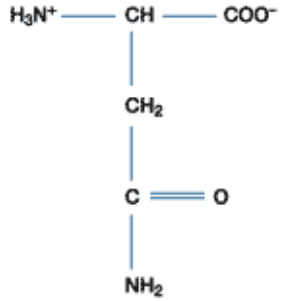
- Glycosylation (ER, Golgi)
  - Addition and processing of carbohydrates
- Formation of S-S disulfide bond (only in ER)
- Proper folding, formation of multimeric protein (ER)
- Specific cleavage (ER, Golgi, secretory vesicles)
  - e.g. removal of signal peptide (type I protein)

ONLY properly assembled proteins can leave rough ER and be transported to Golgi!!

1. Glycosylation (two types)



- O-linked (less common)
  - -CHO added to the -OH group of Ser or Thr
  - Often contains 1-4 oligosaccharides
  - by glycosyltransferase (in Glogi lumen)

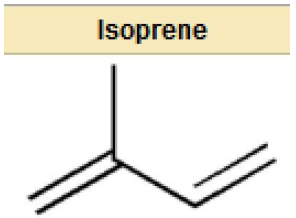


- N-linked (more common)
  - -CHO added to the -NH2 group of Asn
  - More large and complex (branches) than the O-linked type
  - Initiated in ER, but can later be modified in ER or Golgi

N-linked Glycosylation

- Begins with preformed precursor (same among various species)
  - Plants, animals, single-celled eukaryotes
  - Branched oligosaccharide (14-residue precursor; Fig 13-16)
    - 2 GlcNAc (N-acetylglucosamine)
    - 9 Man (Mannose)
    - 3 Glc (Glucose)
  - 5 residues are conserved (**core**) → 3 Man + 2 GlcNAc
  - Modified in ER and Golgi
- Core precursor then transferred to ER-embedded ***dolichol*** to begin the glycosylation process

Dolichol: long chain of unsaturated organic isoprene molecules terminating with alcohol functional group (-OH)

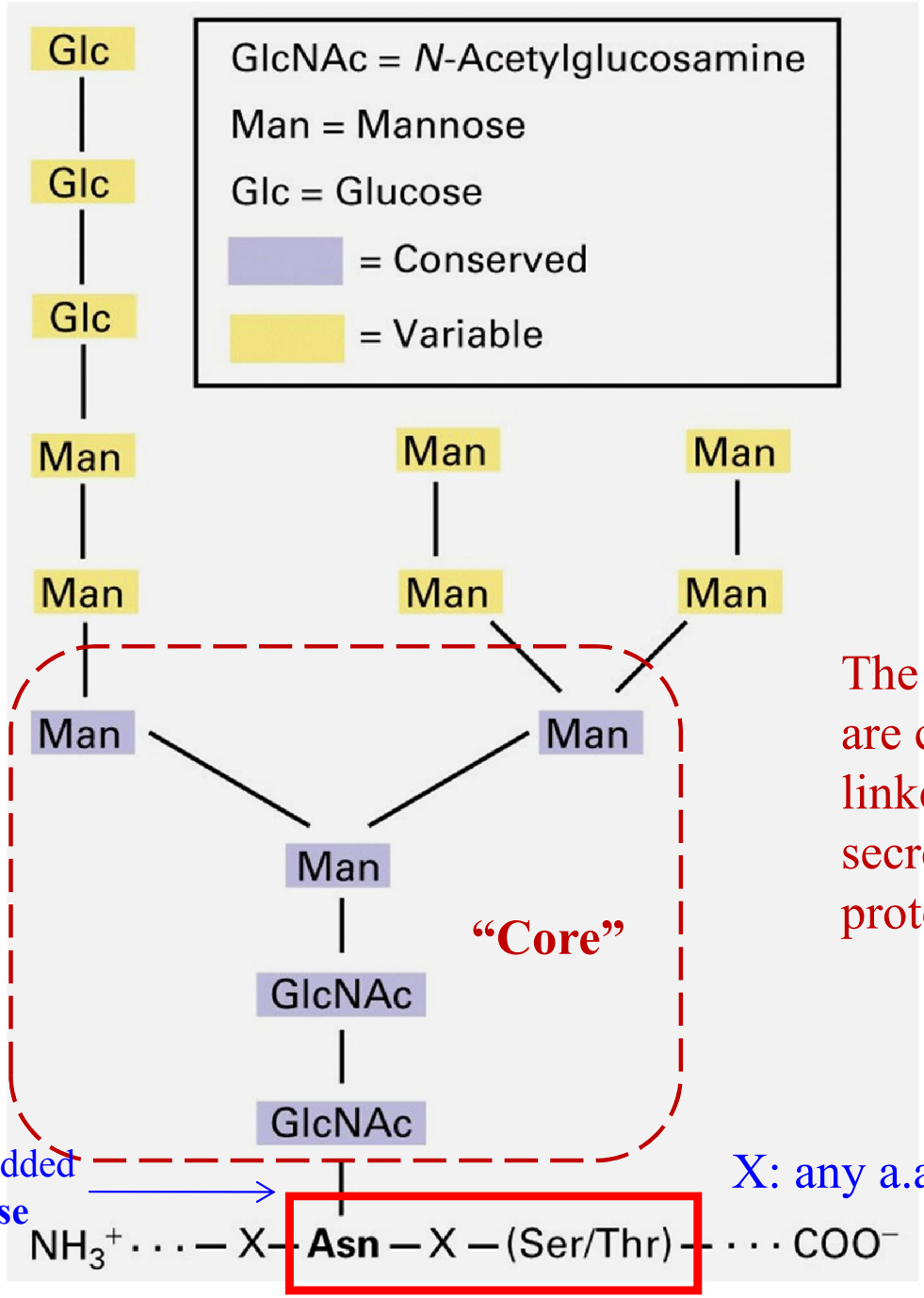


Precursor of N-linked oligosaccharide

3 Glc

9 Man

2 GlcNAc



The 5 core oligosaccharides are conserved in ALL N-linked glycoproteins on either secretory or membrane proteins!!!

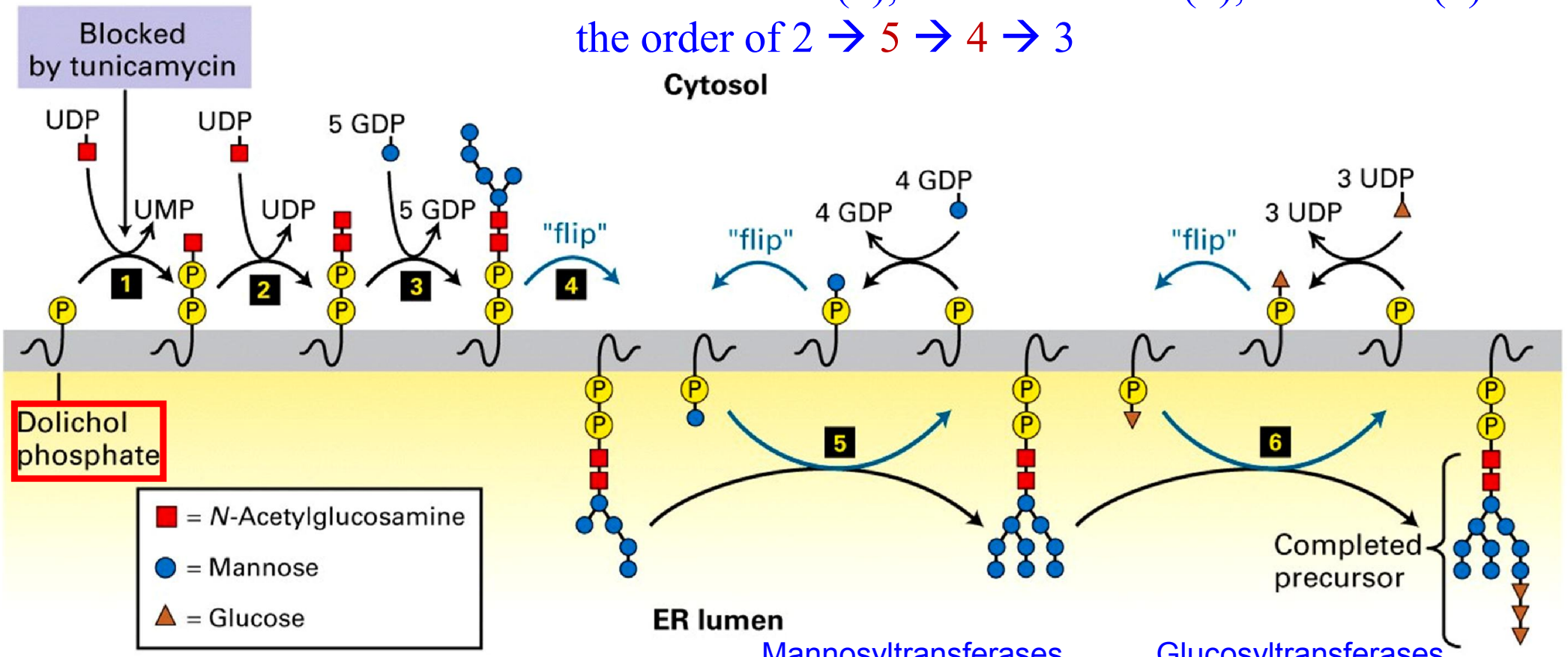
The 14-residue precursor is added by **oligosaccharyl transferase** (Fig. 13-18)

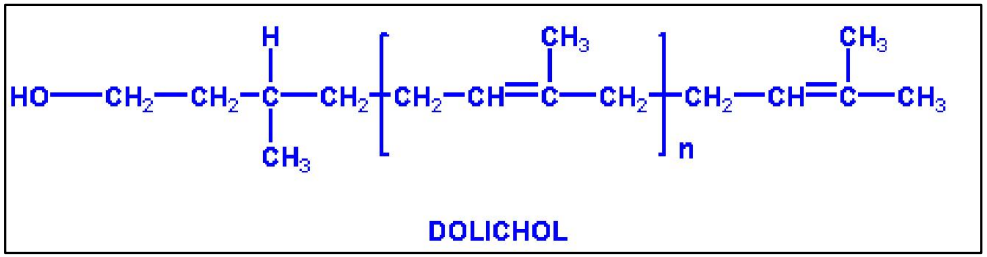
X: any a.a. except Proline

Fig 13-16

Biosynthesis of dolichol pyrophosphoryl oligosaccharide precursor

Materials: (ALL are from the cytosol!!)
GlcNAc-UDP (2), Mannose-GDP (9), Glc-UDP (3) in the order of 2 → 5 → 4 → 3

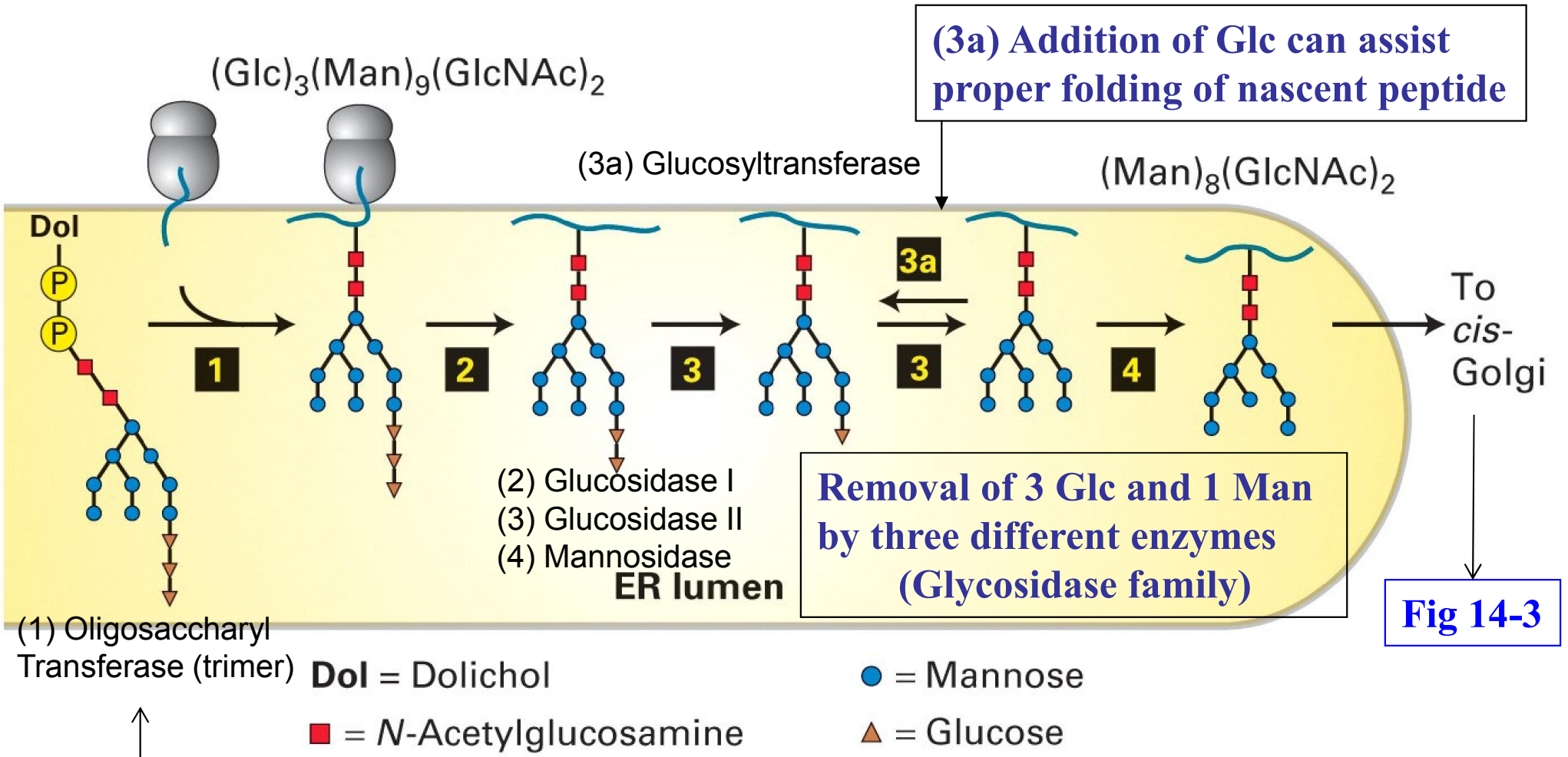




(both in the **Glycosyltransferase** family)

Fig 13-17

Addition/Initial process of N-linked oligosaccharide in ER



Recognition sequence for oligosaccharyl transferase:
1. Asn-X-Ser 2. Asn-X-Thr
(X: any a.a. except Proline)

Fig 13-18

Importance of glycosylation

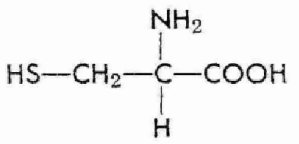
1. Promotes proper folding of proteins
2. Helps stabilize secreted proteins
3. Helps aid in cell-to-cell recognition/adhesion (for membrane-bound proteins)

2. Formation of disulfide bond

- ER is an oxidative environment which promotes the formation of disulfide S-S bonds.
  - -SH + -SH → S-S

- NOTE: S-S bonds are ONLY formed in the ER!!!

- Disulfide bond (S-S)

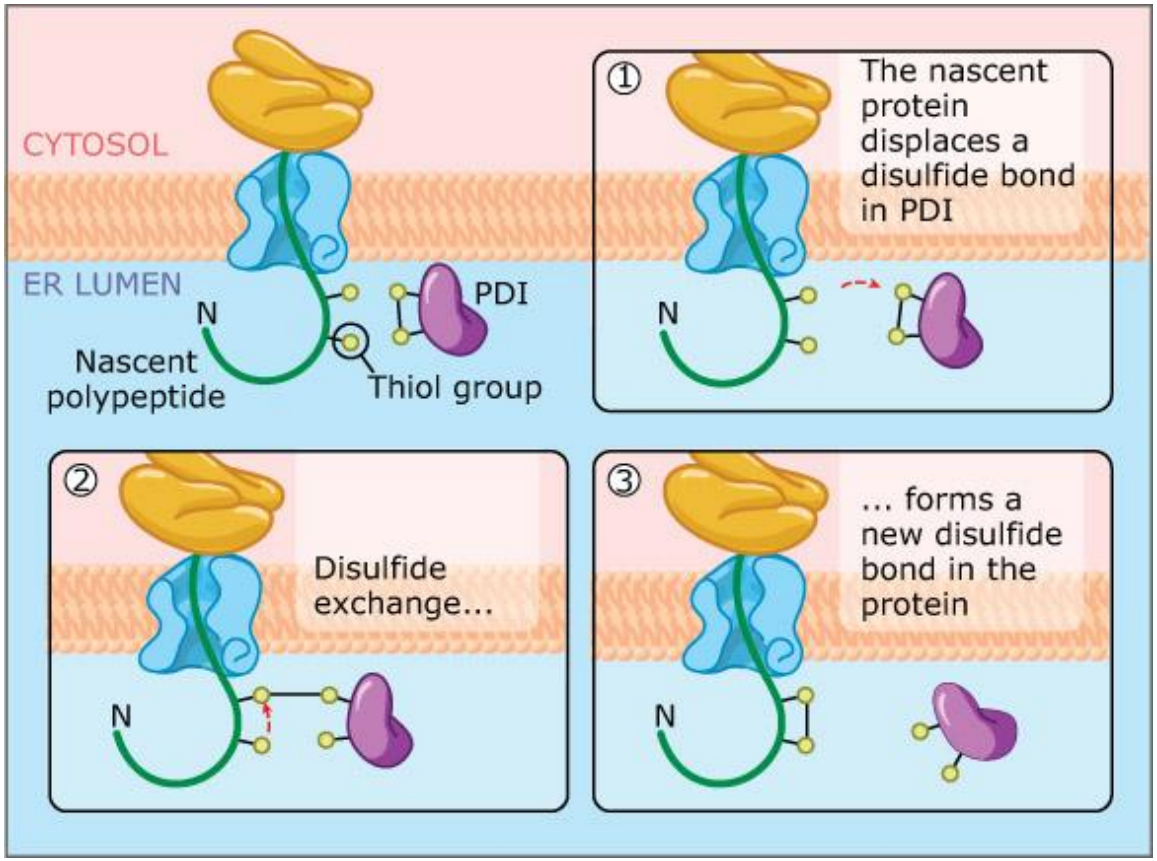


  - Covalent bond
  - Formed by oxidative linkage of 2 –SH groups on Cys residues
    - Requires **protein disulfide isomerase (PDI)**
  - Helps stabilize the 3° and 4° structure of proteins
  - Euk. (in ER); Prok. (in periplasmic space = exoplasmic domain)

Protein disulfide isomerase ensures the formation of the correct disulfide bonds as proteins fold

- Protein disulfide isomerases catalyze disulfide bond formation and rearrangement in the ER.

- PDI's oxidation capacity is regenerated by Ero1 and its cofactor, FAD.



PDI forms disulfide bonds in nascent proteins.

PDI-mediated disulfide bond formation & rearrangement

Fig 13-19

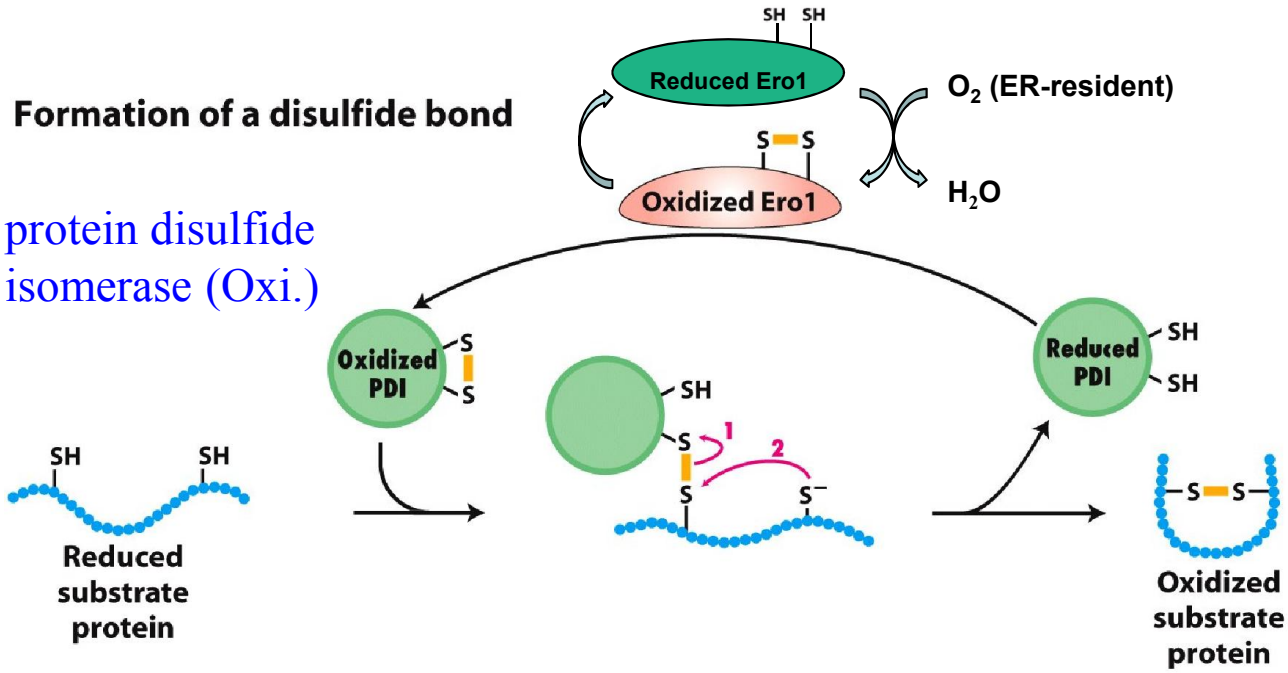


Figure 13-19a
Molecular Cell Biology, Sixth Edition

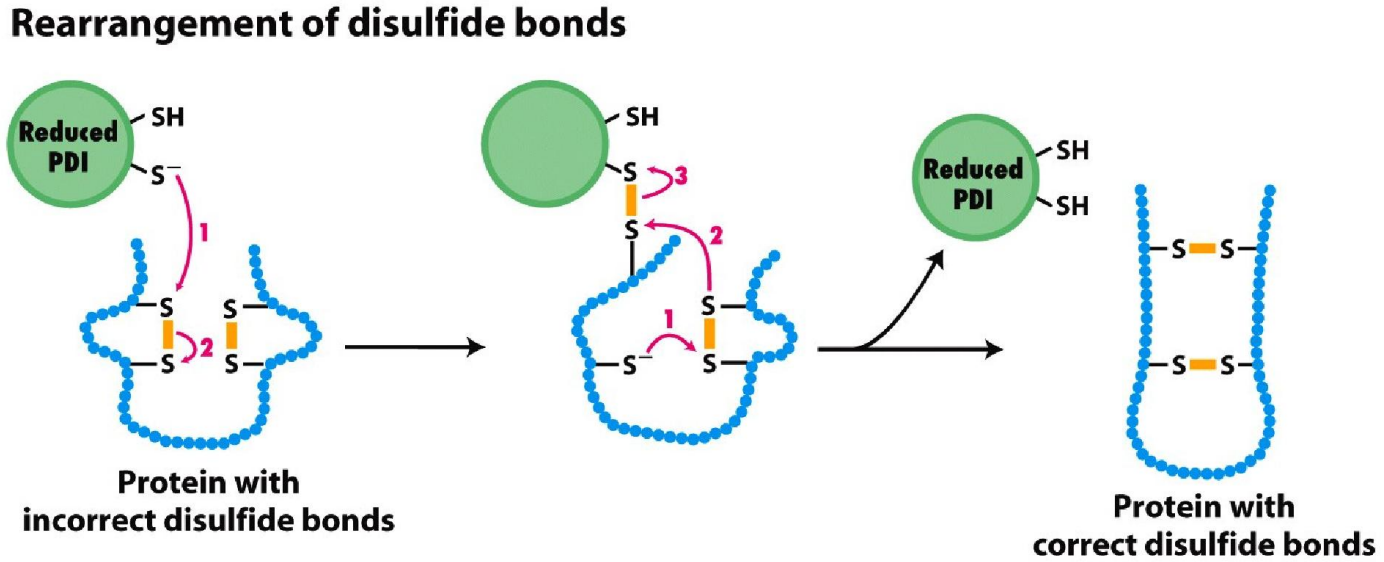


Figure 13-19b
Molecular Cell Biology, Sixth Edition

3. Protein folding and assembly

- Newly synthesized proteins in the ER generally fold into their proper conformation within minutes after their synthesis
- Requires several ER lumen proteins
  - Chaperone BiP
  - Protein disulfide isomerase (PDI) for S-S bond formation
    - Allows proteins to reach their most thermodynamically stable conformation
  - Lectins (CHO-binding proteins)
    - Calnexin (membrane-bound) & calreticulin (in ER lumen) (5th Fig. 16-21)
    - Binds to a single Glc residue on N-linked oligosaccharides (Fig. 13-18, 3a)
    - Prevents unfolded nascent chain into aggregates!!

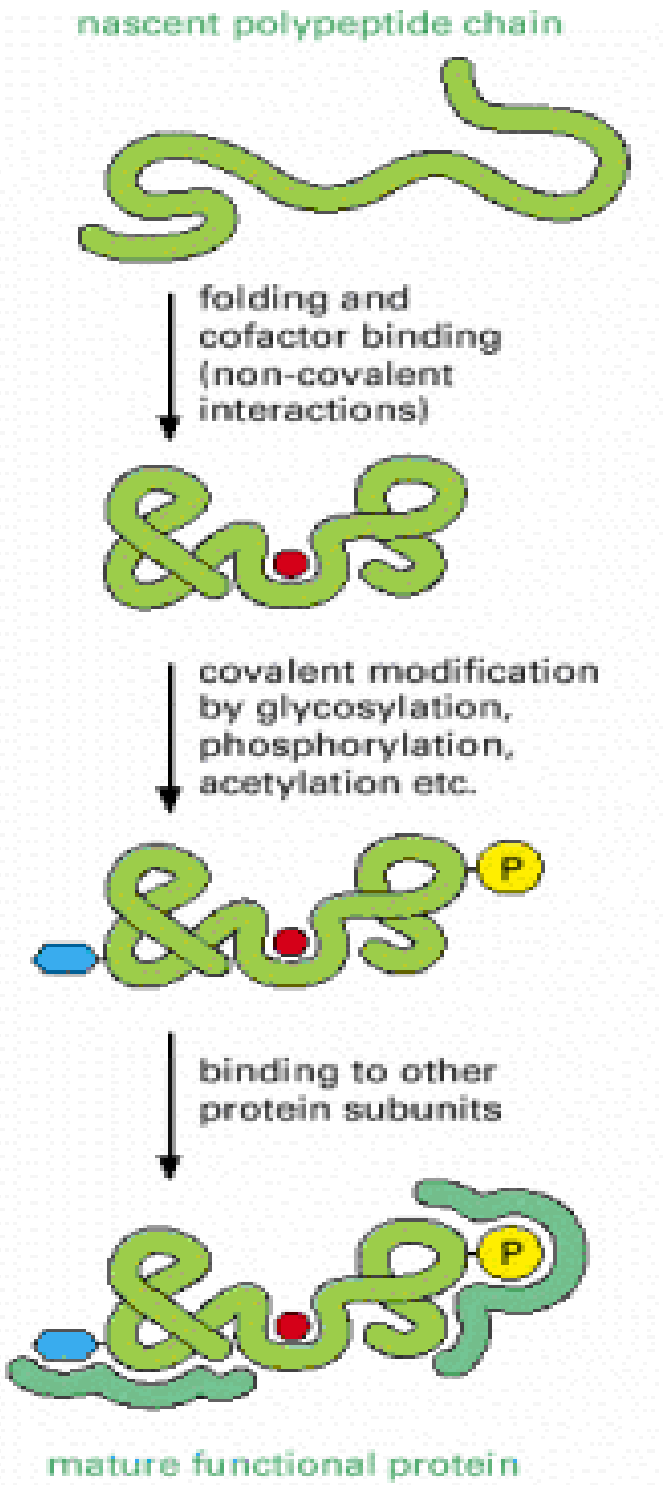
Upon arrival in the ER

Unfolded protein

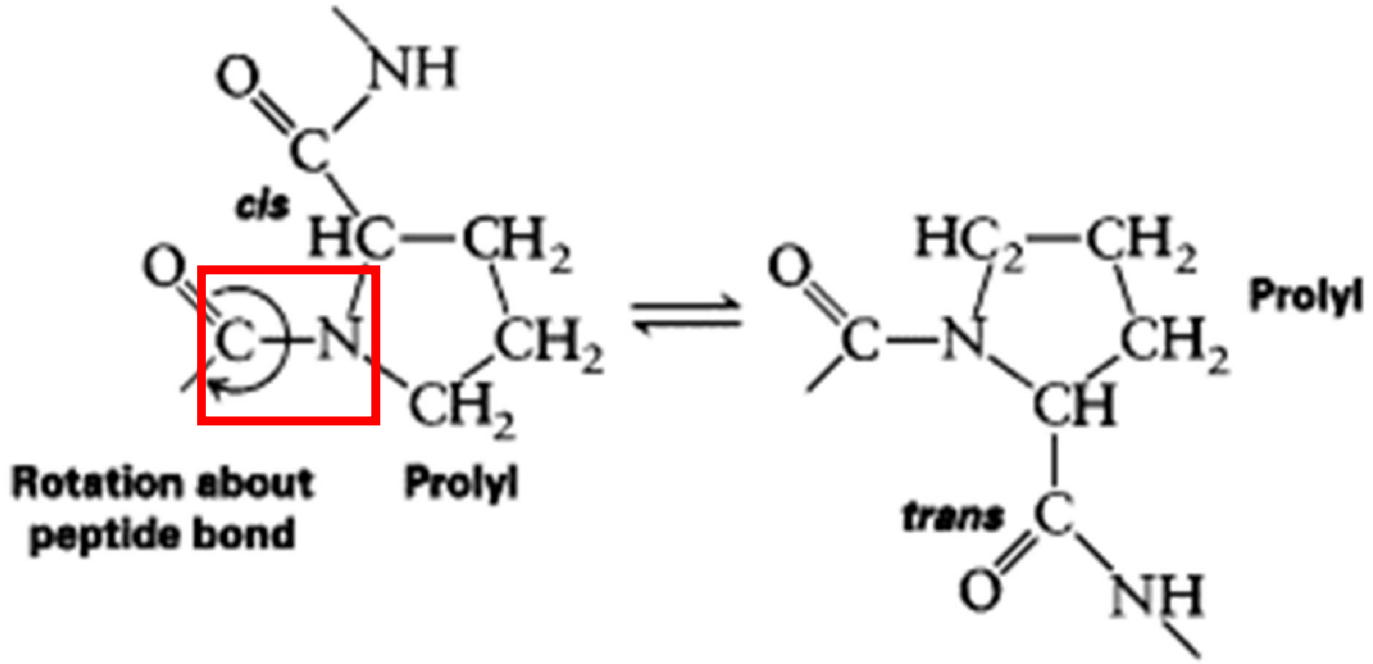
↓

Native structure/ multimeric complex

1. Chaperones (e.g. BiP)
2. Folding enzymes
   -- Protein disulfide isomerase
   -- Peptidyl-prolyl isomerase
3. Lectins
   -- Calnexin & Calreticulin



Peptidyl-prolyl-isomerase (PPI)



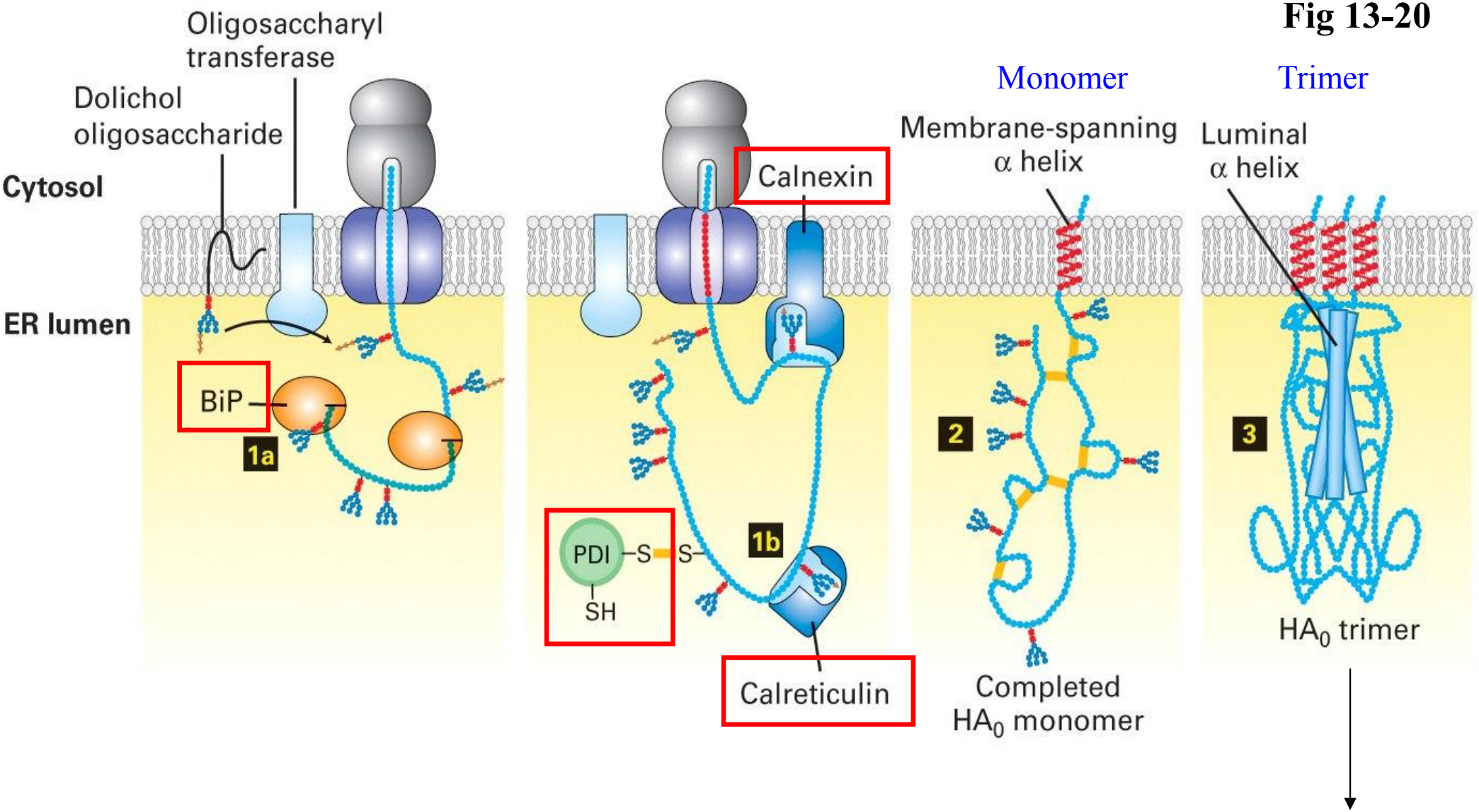
cis → *trans*

PPI catalyzes the rotation about peptide prolyl-bonds, which can be rate limiting in the folding of protein domains

Folding of multimeric protein in ER

(e.g. Hemagglutinin, trimeric)

Fig 13-20



1 HA0 trimer → 1 (HA1 + HA2) trimer

Each HA0 monomer will be cleaved into HA1 and HA2 in the Golgi

How does ER deal with *improperly* folded proteins? ("unfolded protein response")

- Only properly folded proteins are allowed to leave ER
- Unfolded (or improperly/incompletely folded) proteins are bound by chaperone BiP in the ER lumen
- **Quantity of free BiP** determines whether the unfolded protein response is to be initiated or not
  - More unfolded proteins (more BiP bound to them)

    →Less free BiP in the ER lumen

    →More **Ire1 dimers** formed

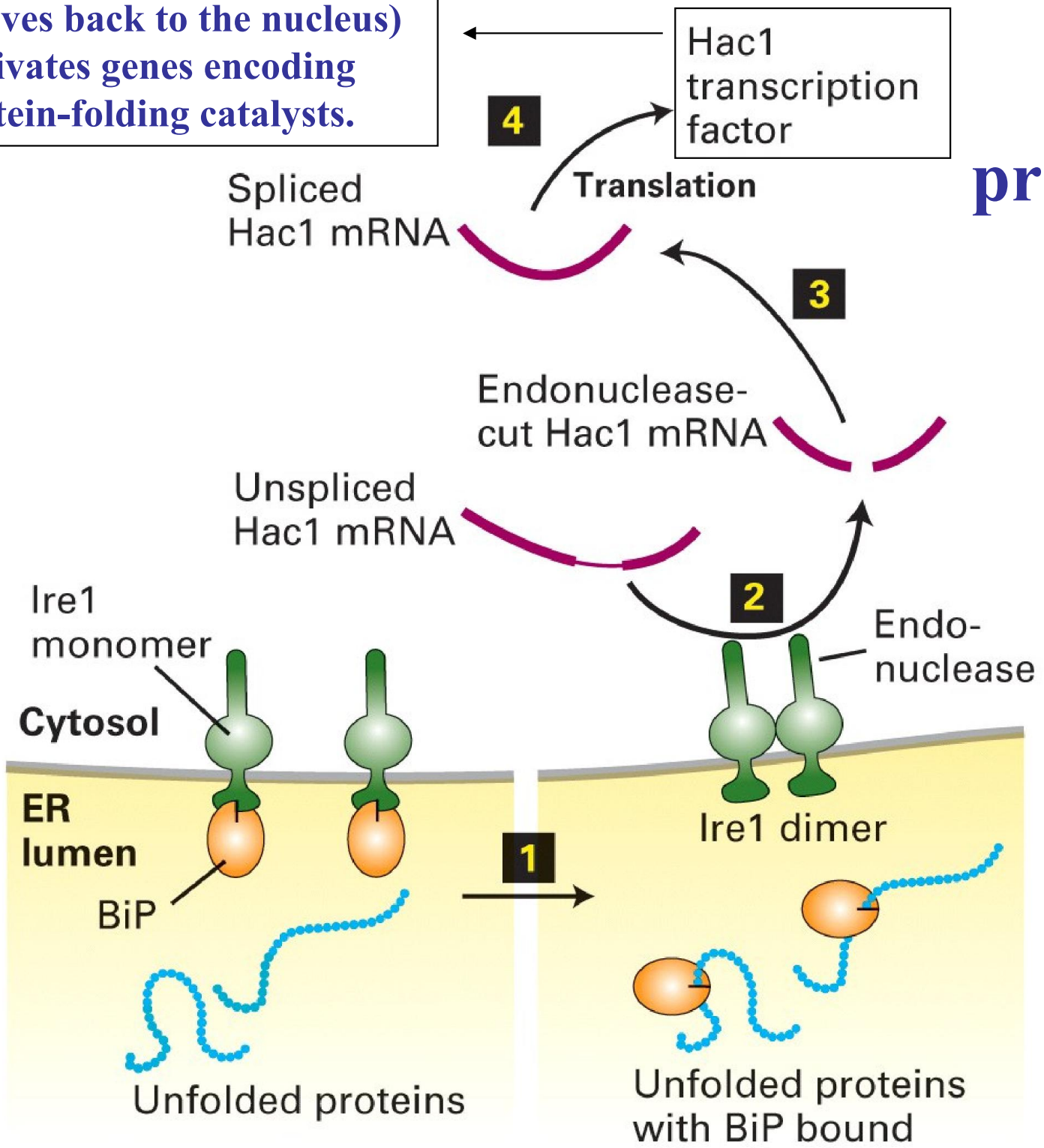
    →BiP present → formation of BiP-Ire1 complex ↑ ↑

    →Bip absent → formation of Ire1-Ire1 complexes ↑ ↑

    →Start of "unfolded-protein response"

    →Generation of **Hac1** (a transcription factor) to promote synthesis of protein-folding catalysts

Unfolded-protein response



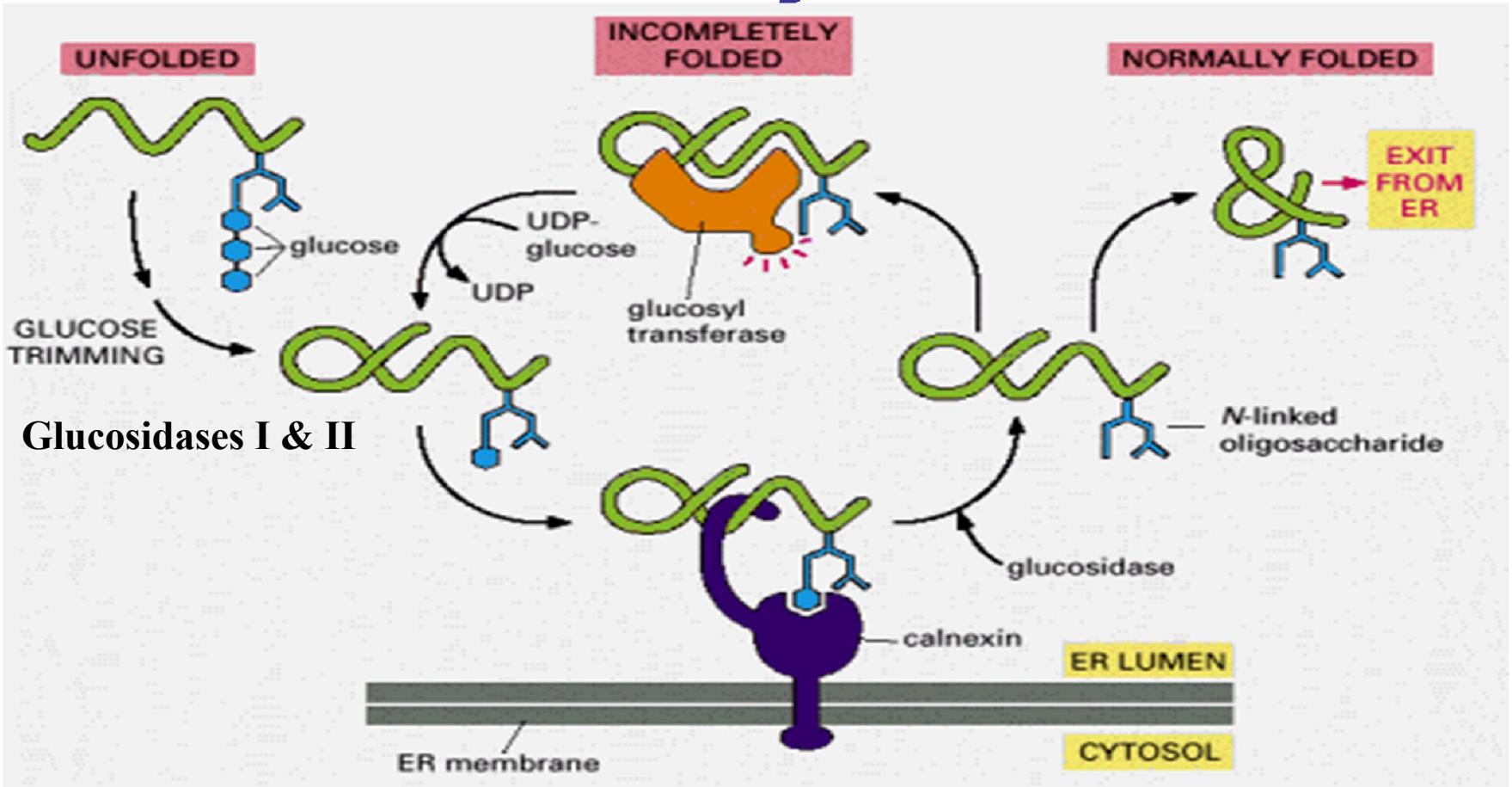
In yeasts and mammalian cells

Ire1 monomer
→Bound by BiP

Ire1 dimers
→ No BiP bound

Fig 13-21

ER Quality control



1. Glucosidase I: removes terminal Glc

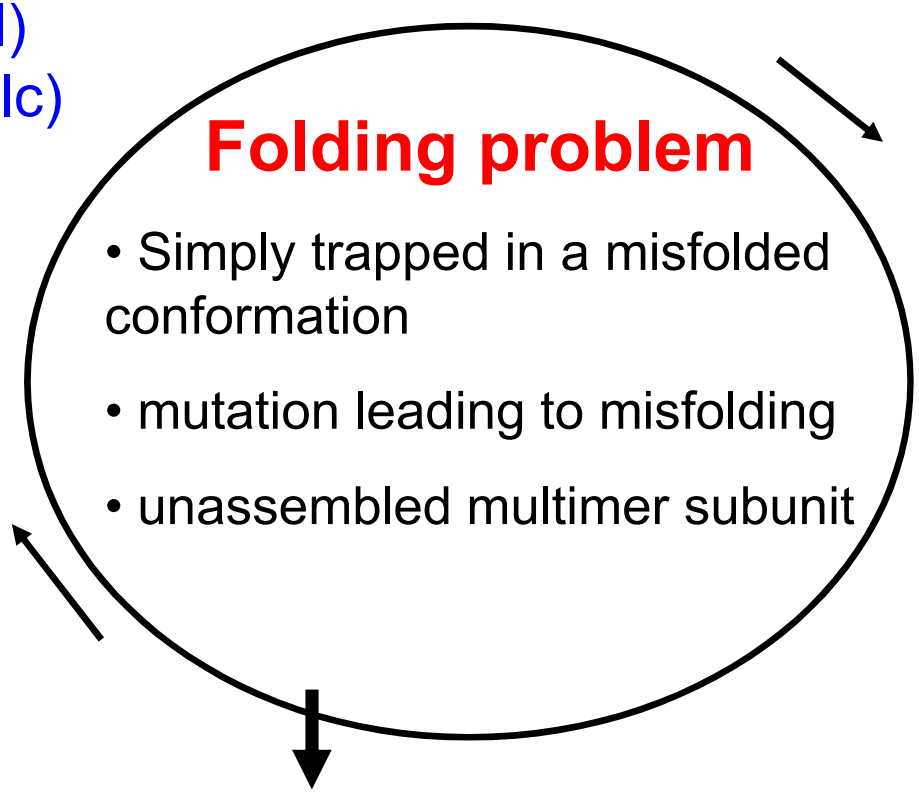
2. Glucosidase II: removes next Glc → then, 'checked' by membrane-bound lectin **calnexin**

3. Glucosidase II: removes next Glc → if folded correctly, exit from the ER → if not, addition of a new Glc (from UDP-Glc) as donor, followed by interaction with soluble lectin **calreticulin**.

4. New cycle → **"buys" more time for correct folding by PDI, PPI, BiP,…etc.**

(Summary) What happens if there are folding problems?

Glucosidases I & II
(Glc removal)
(remove 3 Glc)



Glucosyltransferases
(Glc addition)
(Adds 1 Glc)

Calnexin and **BiP** bind irreversibly to misfolded proteins

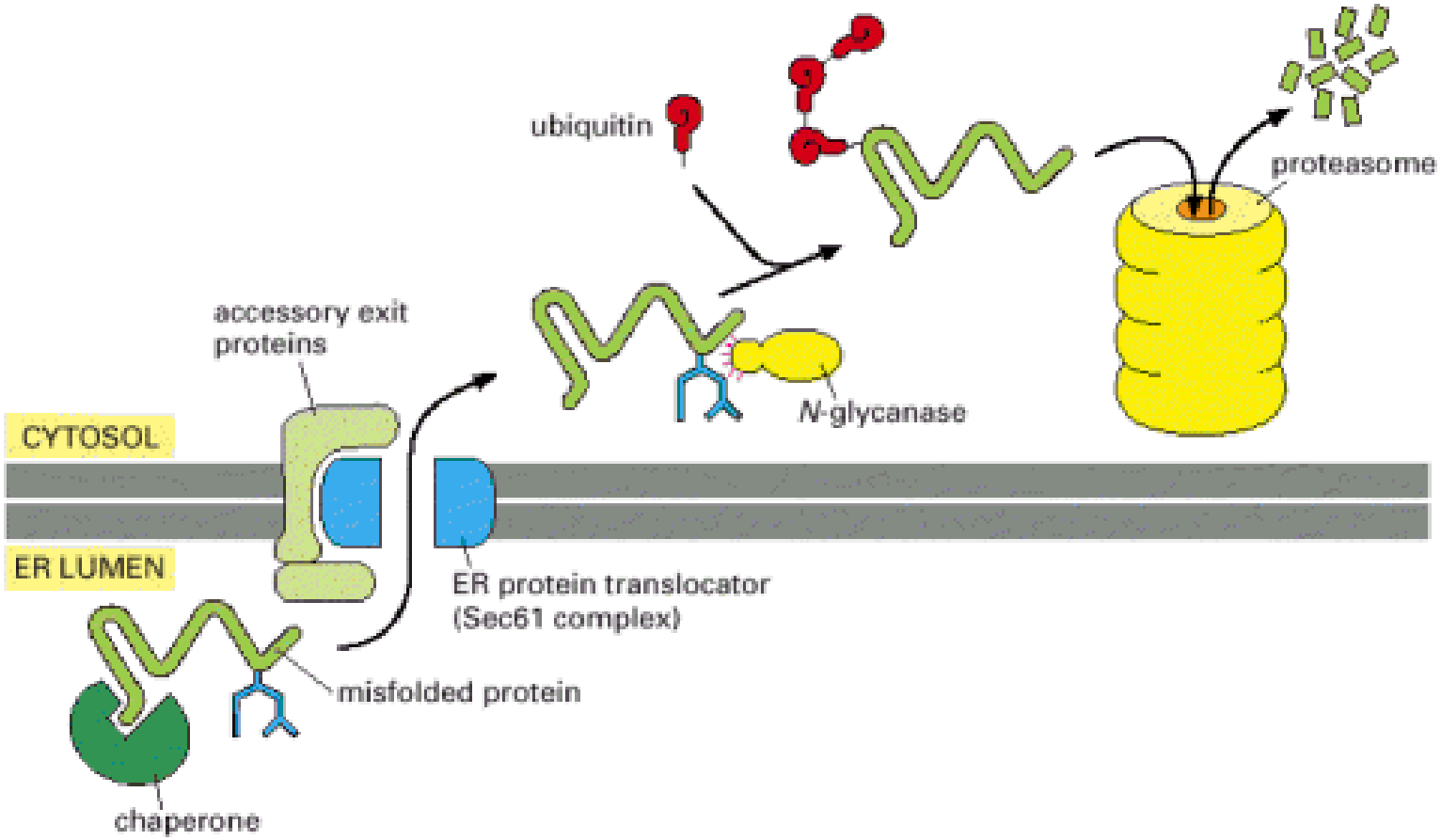
'Unfolded protein response' pathway

Increased transcription of chaperones and folding catalysts

How does ER deal with terminally *unassembled* or *misfolded* proteins?

- Unassembled or misfolded proteins (ER)
  - transported ***backwards*** through translocon (into cytosol)
  - Target for ubiquitin-mediated degradation
- Ubiquitinylating enzymes (Fig 3-13)
  - located on the cytosolic side of ER
  - Add ubiquitin onto misfolded proteins as they exit
  - 3 enzymes
    - Ubiquitin-activating enzyme
    - Ubiquitin-conjugating enzyme
    - Ubiquitin ligase

Retro-translocation of unfolded proteins from the ER



4 steps:

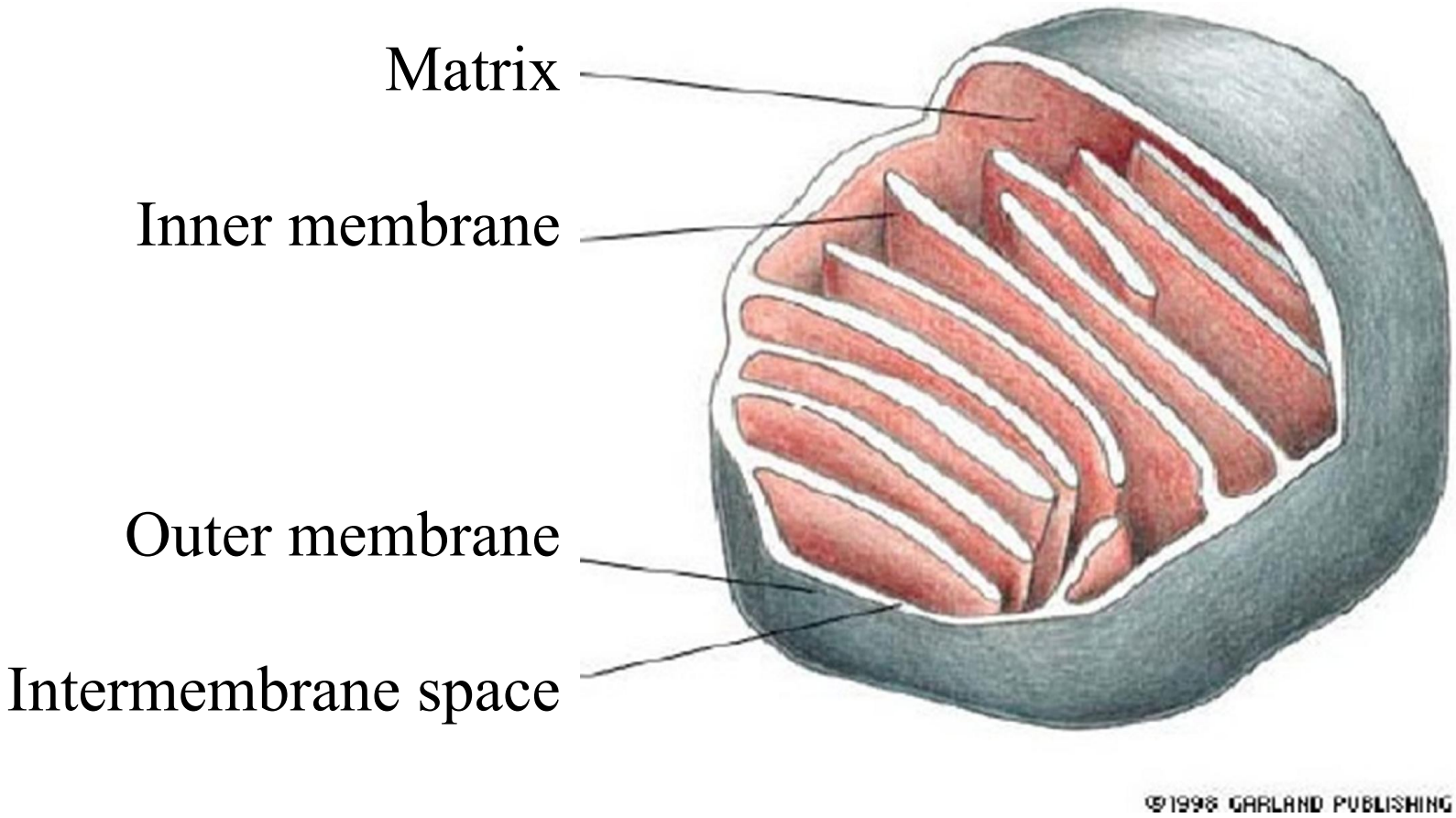
1. substrate recognition
2. transport through the channel and deglycosylation (by N-glycanase)
3. poly-ubiquitination
4. proteasome-dependent degradation

Nat Rev Mol Cell Biol. 2002 Apr;3(4):246-55.
Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. Tsai B, Ye Y, Rapoport TA.

13.4
Sorting of proteins into mitochondria and chloroplasts

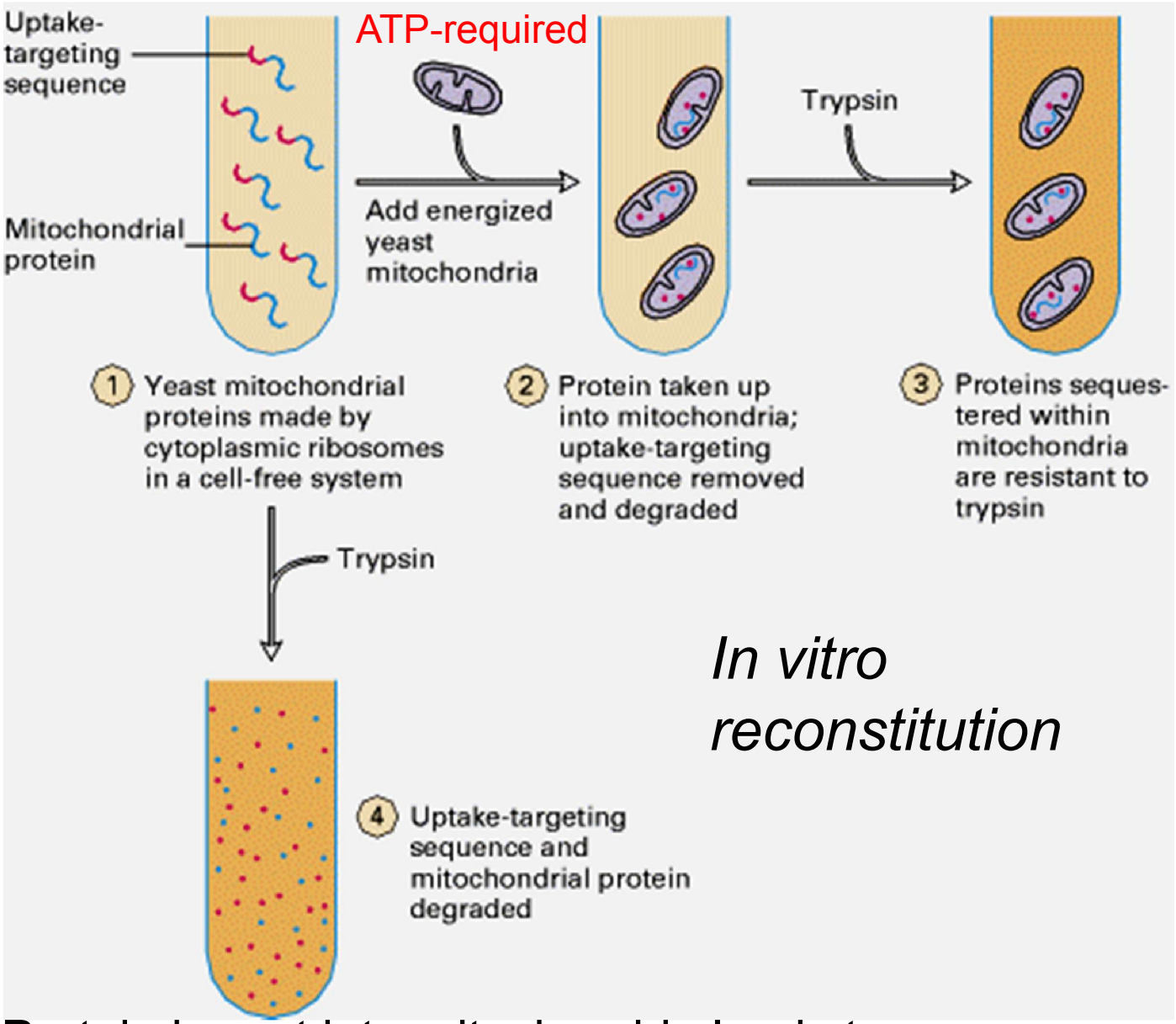
Protein sorting into mito. & chlo.

Cross section of a mitochondrion



4 possible locations of a mitochondrial protein

How can mitochondrial protein import be studied?



Protein into mito.

1. a post-translational process
2. In unfolded state

Fig 13-22

Protein import into mitochondria leads to

1. Protection against protease, and
2. Change in size due to removal of the import sequence

Protein import into mitochondria

- Most mitochondrial proteins
  - Synthesized in cytosol, and
  - Imported into mitochondria (post-translationally)
    - In unfolded state
- Requirements
  - Uptake-targeting sequence for mitochondria
    - Common motif, but *not* identical sequence among proteins
  - ATP
  - Chaperones (maintain peptide in unfolded state)
  - Small channels (receptors) on mito. membrane

Protein import into mitochondria

- Uptake-targeting sequence
  - ~20-50 a.a., N-terminal
  - Forms **amphipathic α-helix**
    - (+) a.a. on one side (Arg/Lys), hydrophobic a.a. on the other side
    - (-) a.a. → none or very few
    - Might also contain –OH a.a. (Ser/Thr)

- First, signal sequence recognized by receptor

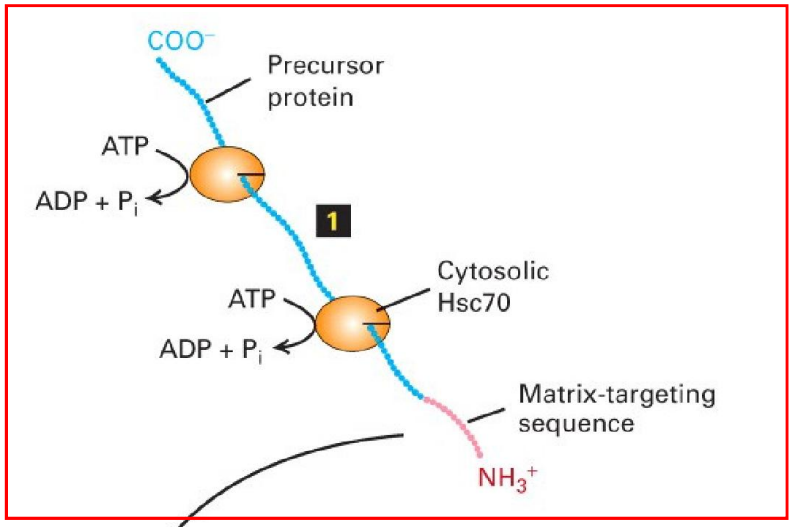
  → protein passed to translocator channel

  → enters into matrix

  → further sorting signals re-direct protein to either
    - inner membrane,
    - intermembrane space, or
    - outer membrane

Protein into mitochondrial matrix

Part I



- See next slide

Part II

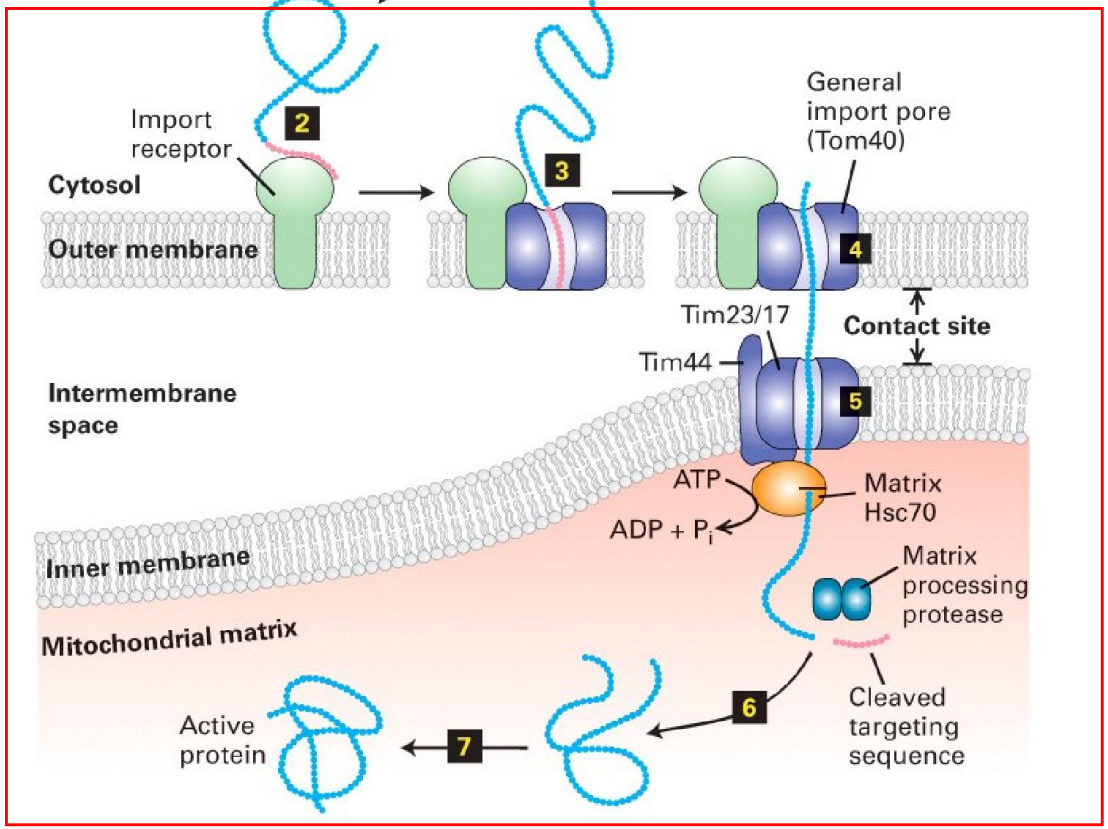
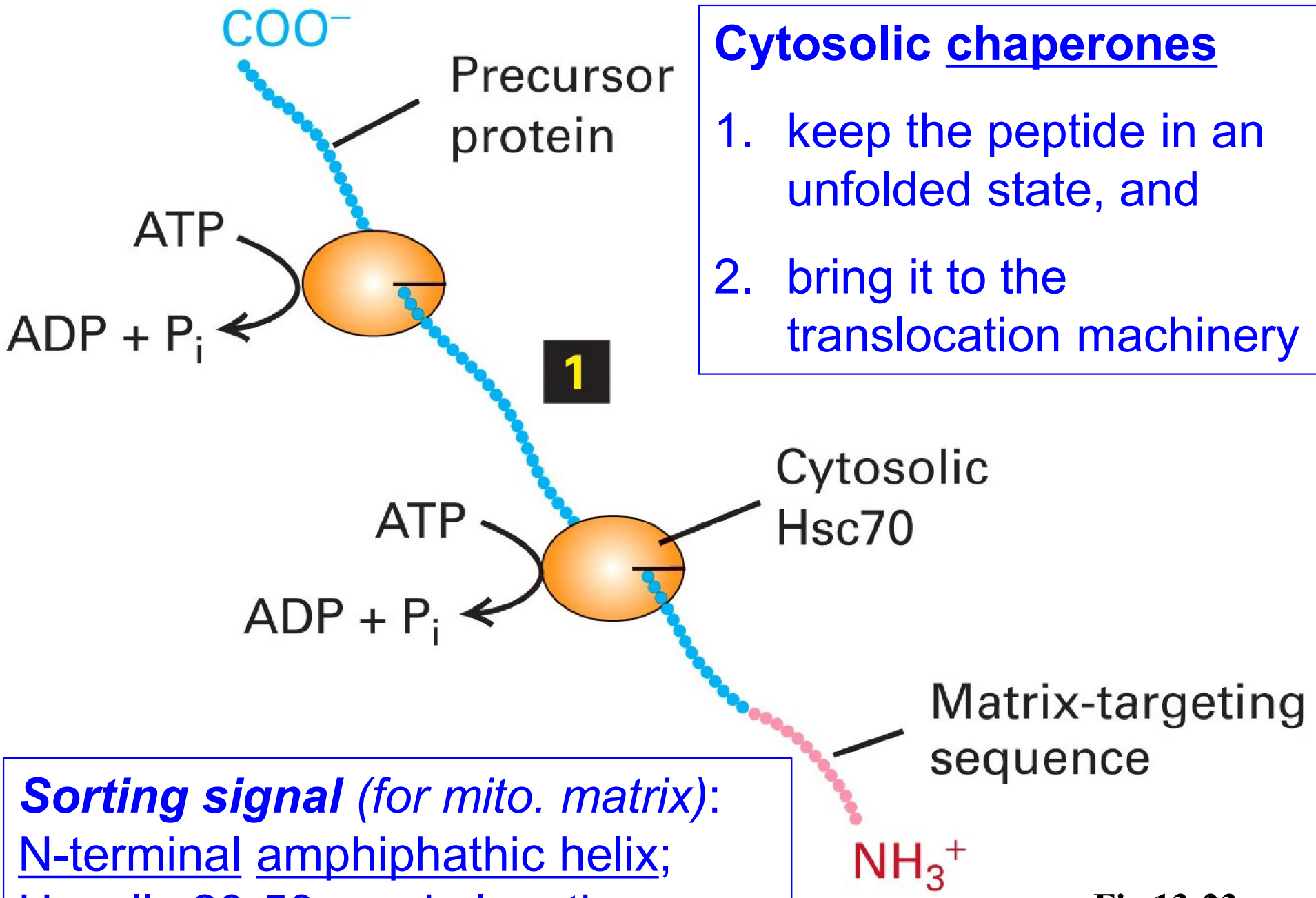


Fig 13-23

Part I



Cytosolic chaperones

1. keep the peptide in an unfolded state, and
2. bring it to the translocation machinery

Sorting signal *(for mito. matrix)*:
N-terminal amphiphathic helix;
Usually 20-50 a.a. in length

Fig 13-23

Part II

"*t*ranslocon of *o*uter *m*embrane "

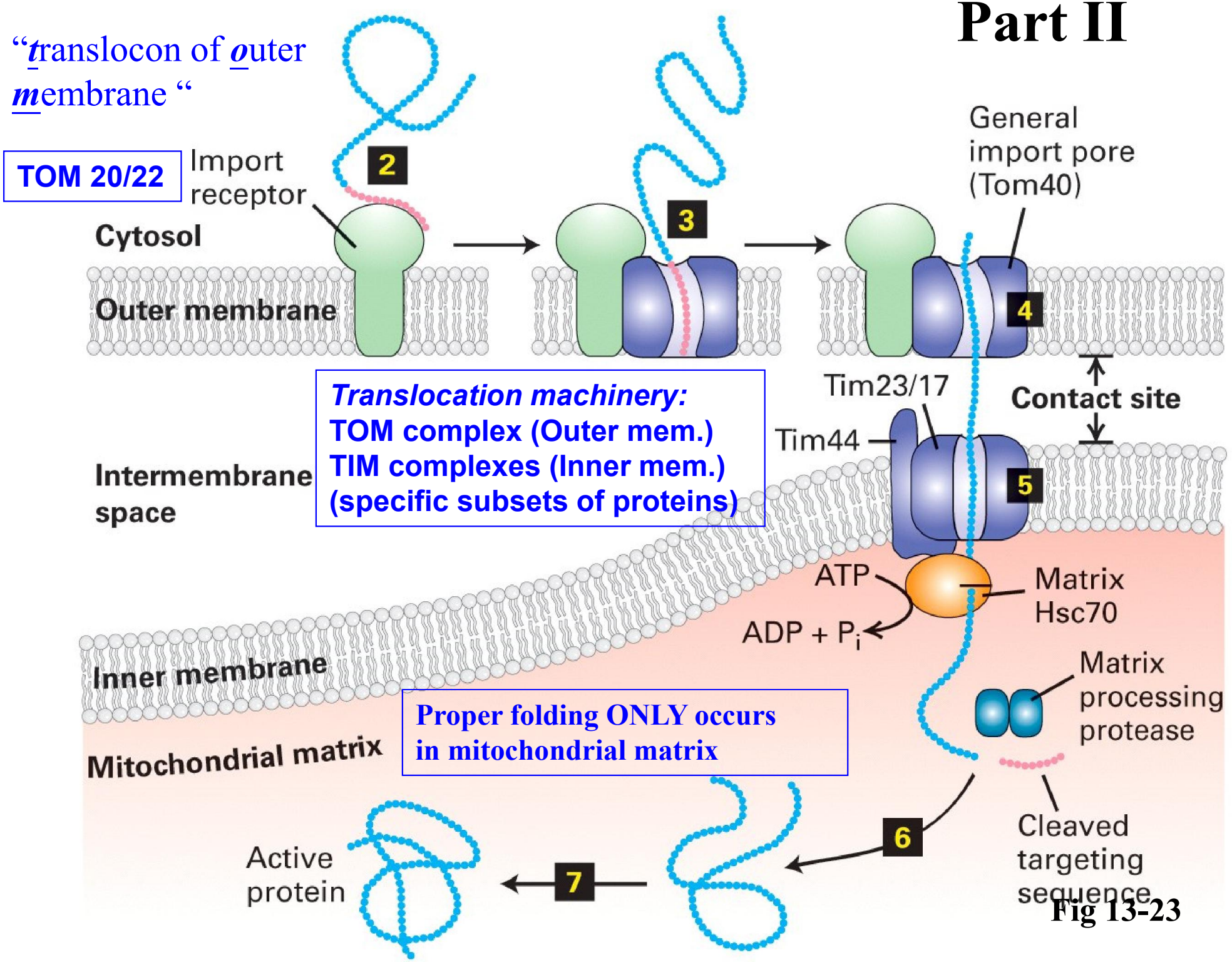
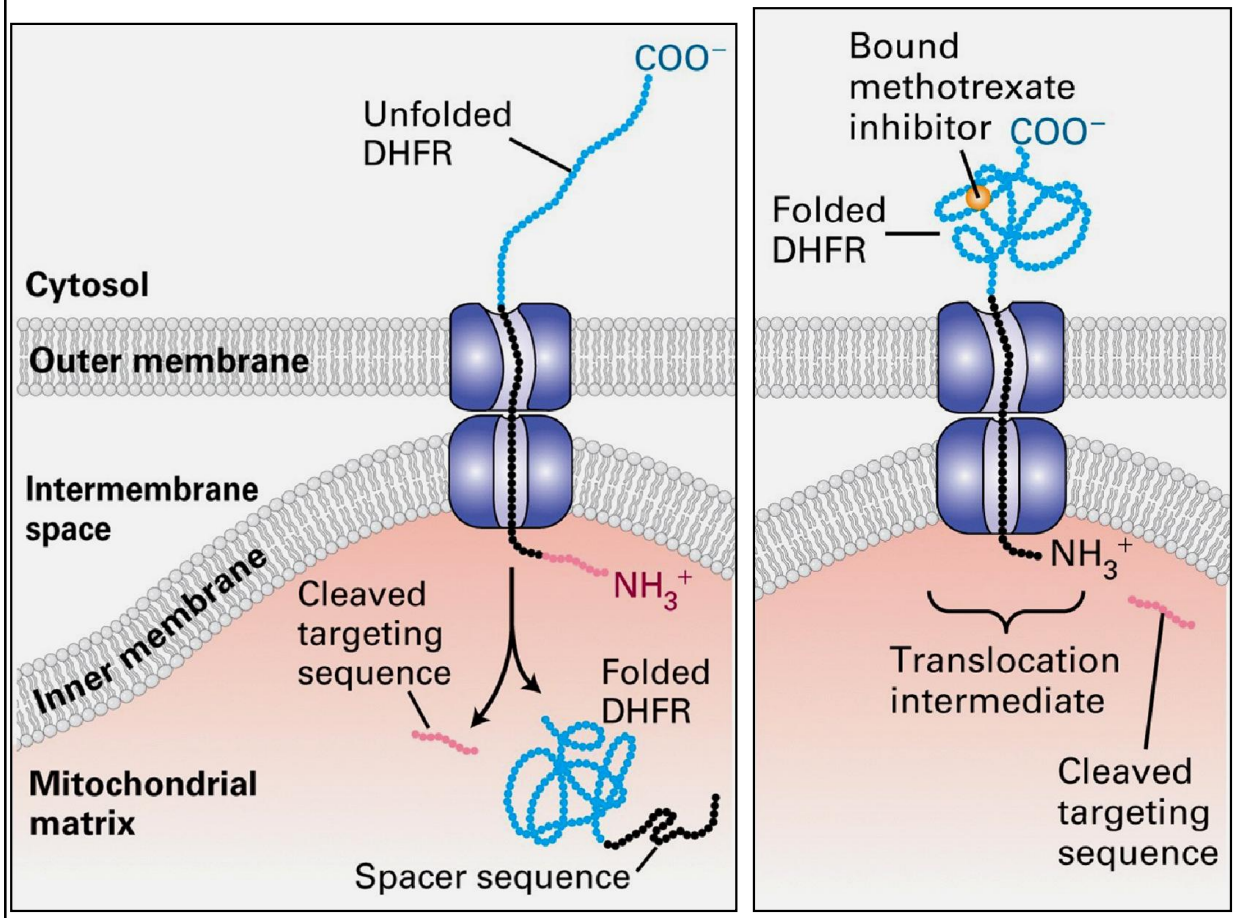


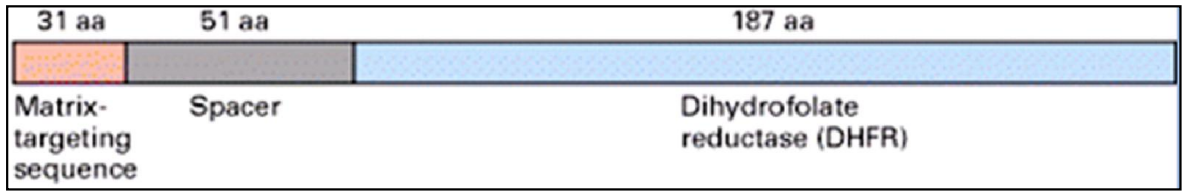
Fig 13-23

Requirement for translocation of protein into mitochondria matrix

1. Matrix-targeting sequence (N-terminal)
2. Proteins in the unfolded state
   - maintained by Hsc70
3. Energy (3 sources)
   - ATP hydrolysis by cytosolic and matrix Hsc70
   - Proton-motive force (H+ electrochemical gradient) across the inner membrane
4. Close contact between outer and inner membranes (between Tom40 and Tim23/17 channels)

Chimeric proteins reveal the mechanism of protein import into mitochondria





Chimeric proteins

Variable spacer region allows protein to be inserted in a defined manner:

1. crosslinking was used to identify component of the channel
2. proteins have to be unfolded for import into mitochondria
3. energy is required (ATP and a proton gradient)
4. import occurs where inner and outer membrane are close

Fig 13-24

Targeting sequences for mitochondrial matrix proteins

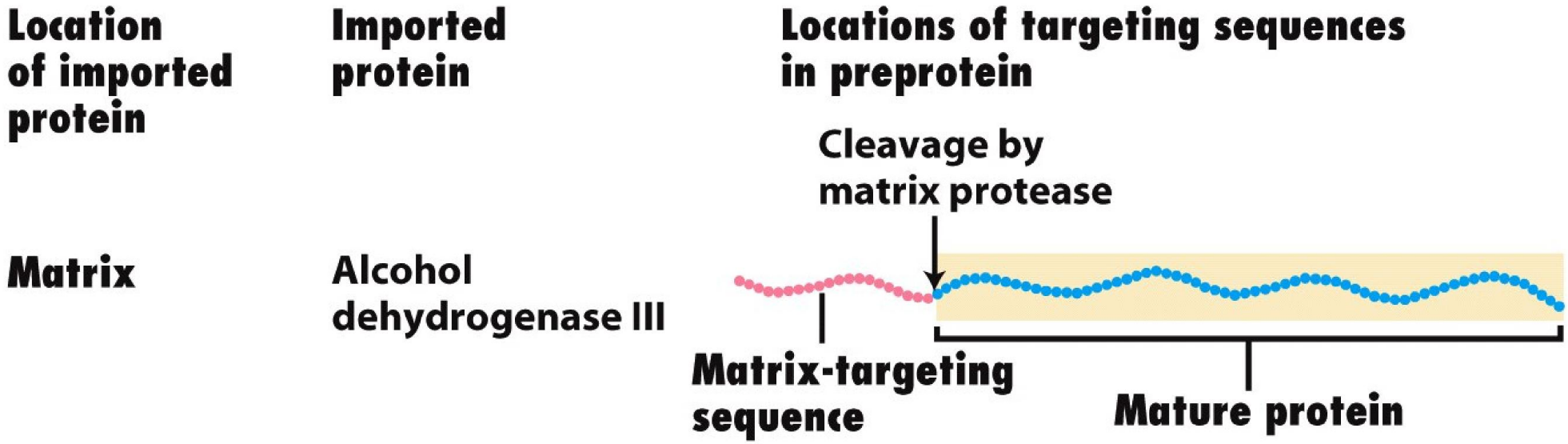


Figure 13-25 part 1
Molecular Cell Biology, Sixth Edition

Mitochon. Matrix –targeting sequence
1.N-terminal
2.α-helical conformation
3.Amphipathic

Targeting sequences for mitochondrial outer membrane proteins

Location of imported protein	Imported protein	Locations of targeting sequences in preprotein
Outer membrane	Porin (P70)	Stop-transfer and outer-membrane localization sequence

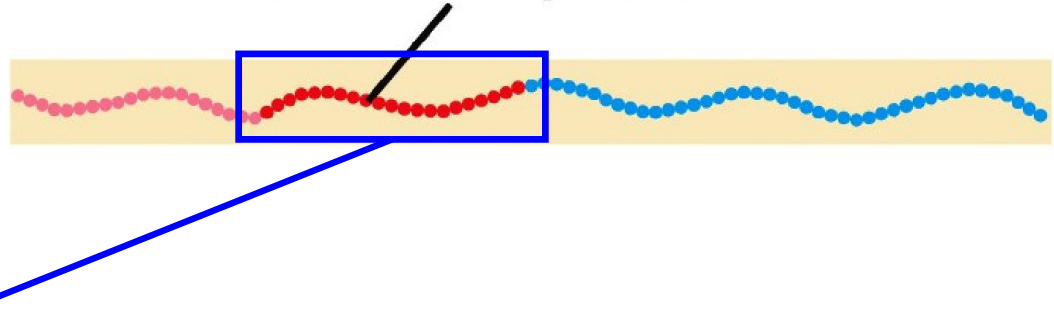


Figure 13-25 part 4
Molecular Cell Biology, Sixth Edition

Outer-membrane localization sequence will assist in the lateral movement of peptide out of Tom40 and into the outer membrane!!

Protein import into inner mitochondrial membrane

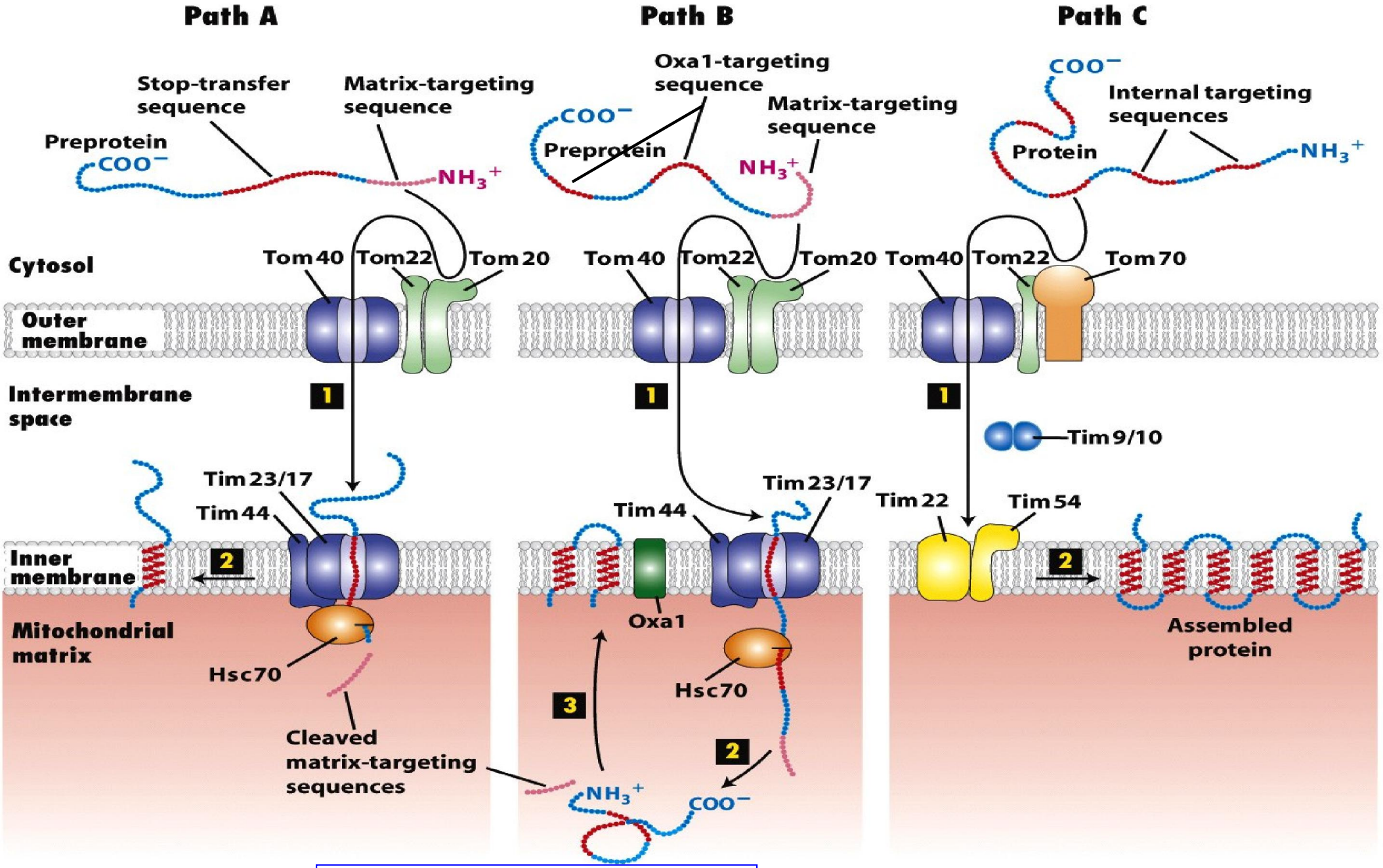


Figure 13-26
Molecular Cell Biology, Sixth Edition

BOTH possess N-terminal matrix targeting sequences

Targeting sequences for mitochondrial inner membrane proteins

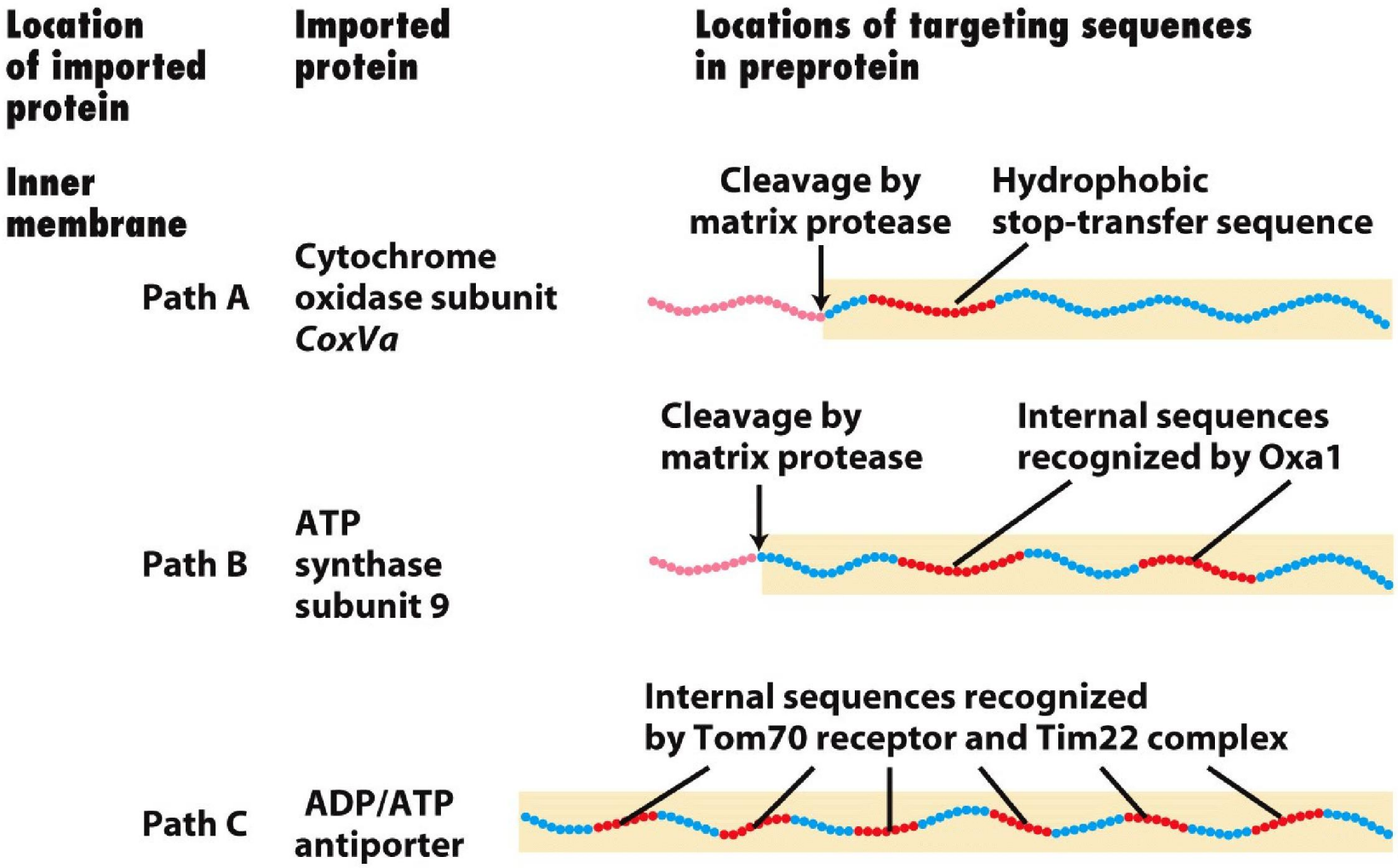


Figure 13-25 part 2
Molecular Cell Biology, Sixth Edition

Protein import into mitochondrial intermembrane space

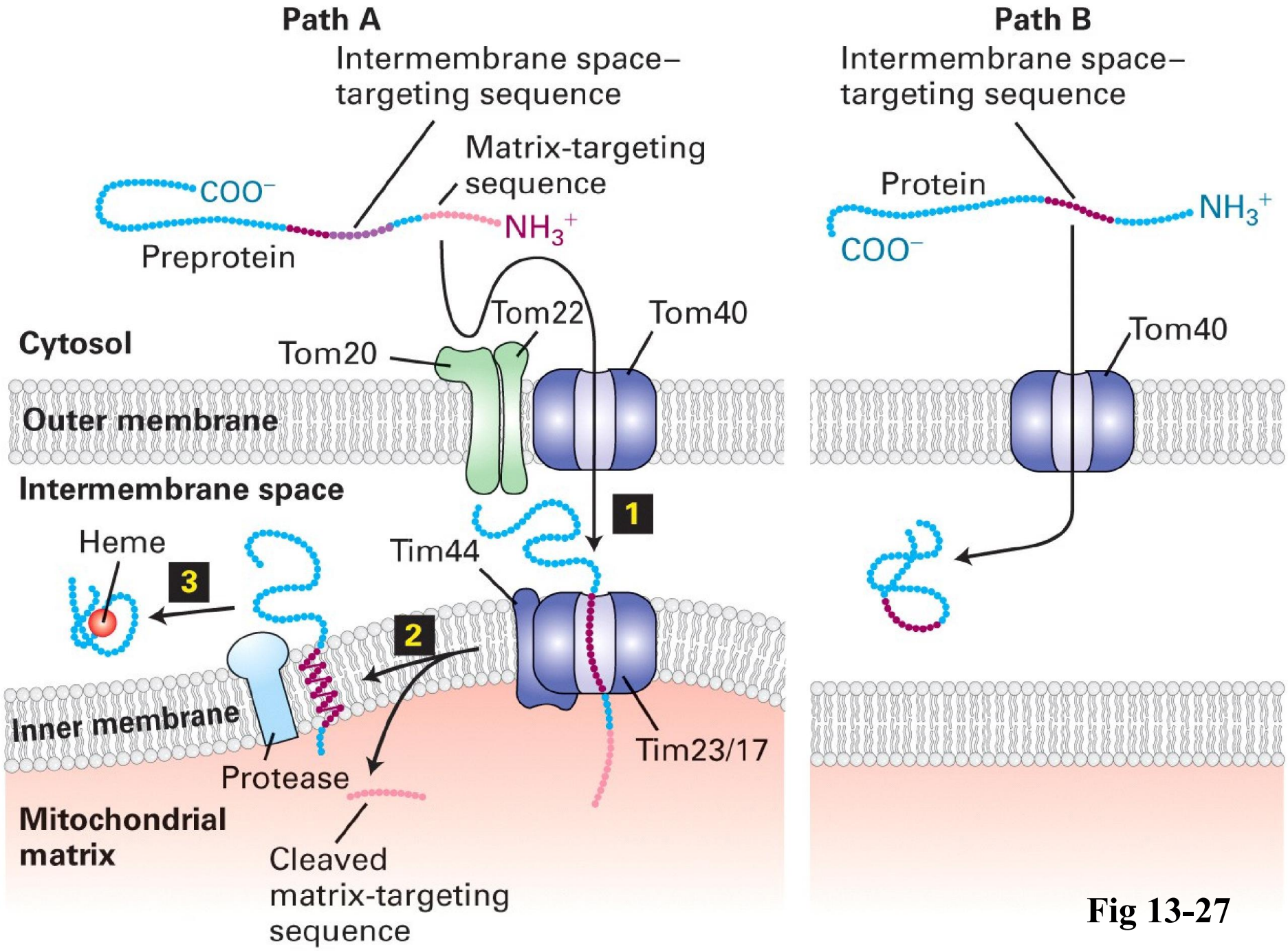


Fig 13-27

Targeting sequences for mitochondrial intermembrane-spaced proteins

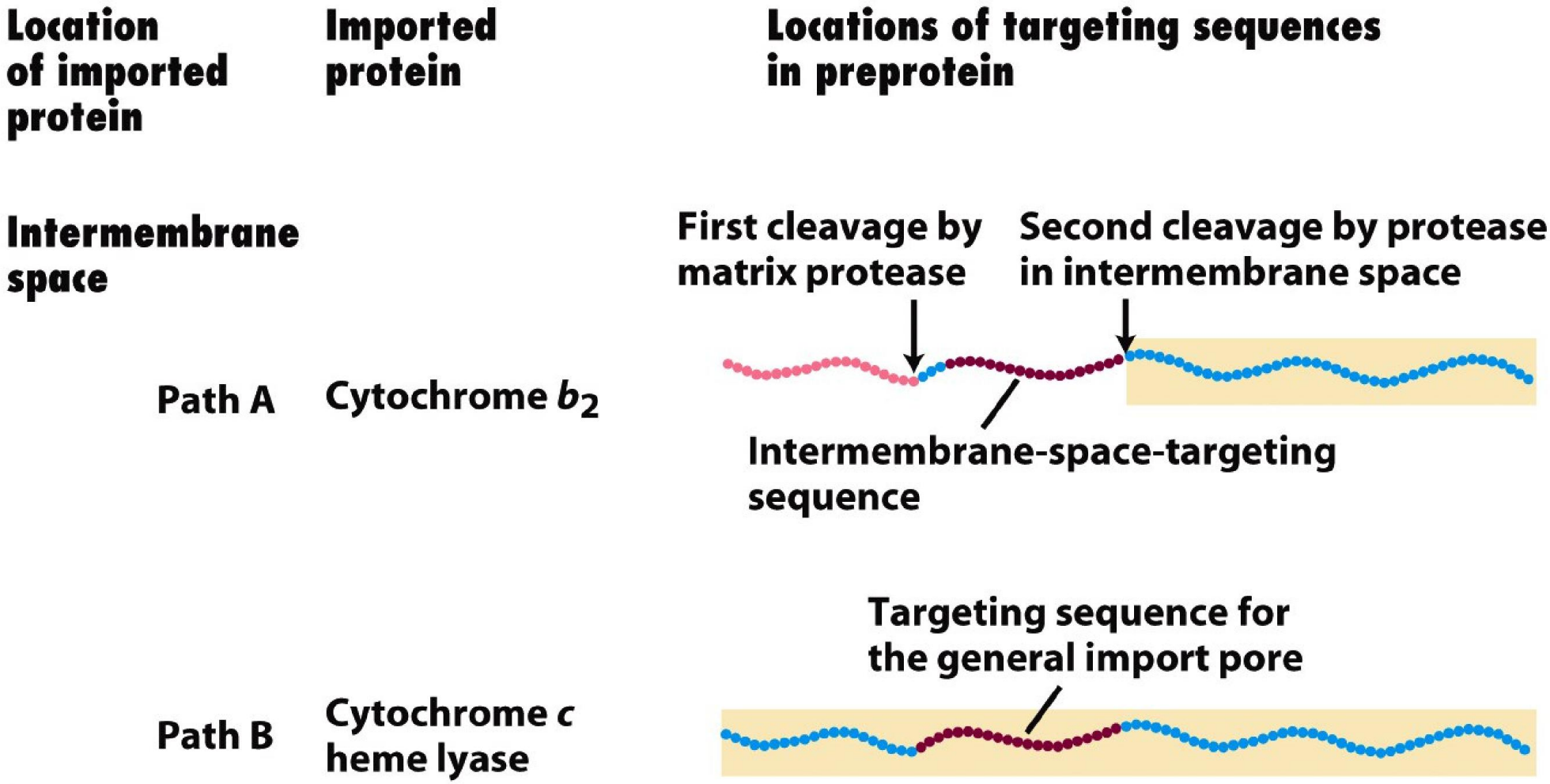


Figure 13-25 part 3
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13.5
Sorting of peroxisomal proteins

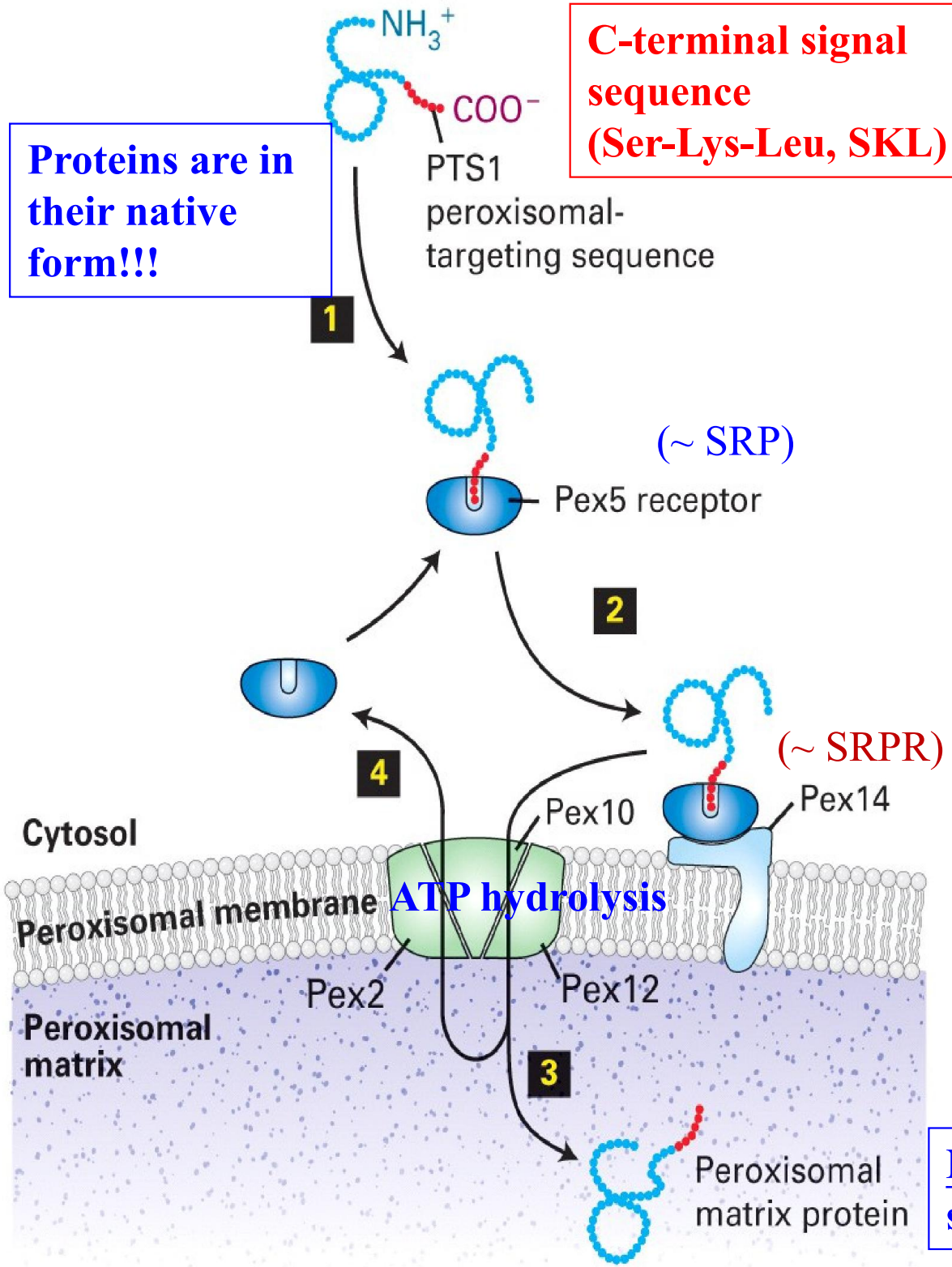
Peroxisome (過氧化小體)

- Single-membraned organelle
- No DNA, nor ribosomes
  - So, ALL peroxisomal proteins are imported from cytosol!!
- Contains various oxidizing enzymes
  - Oxidase, hydrogen peroxidase
- Most abundant in liver and kidney (vertebrates)
- New peroxisomes are made through
  - Division of pre-existing peroxisomes (by Pex11)
  - *de novo* biogeneisis from precursor Pex proteins

Protein import into peroxisomes

C-terminal signal sequence (Ser-Lys-Leu, SKL)

Proteins are in their native form!!!



The Pex5R/Pex14 pair is analogous to SRP/SRP receptor for ER translocation

The Pex/Peroxin import machinery is ONLY responsible for peroxisomal matrix proteins, not for membrane proteins

Any of Pex2, 10, 12 mutation → failure in protein transport into matrix, but not membrane

No cleavage of PTS1 signal sequence!!

Biogenesis and division of peroxisome

Pex19: Receptor for membrane-targeting sequence

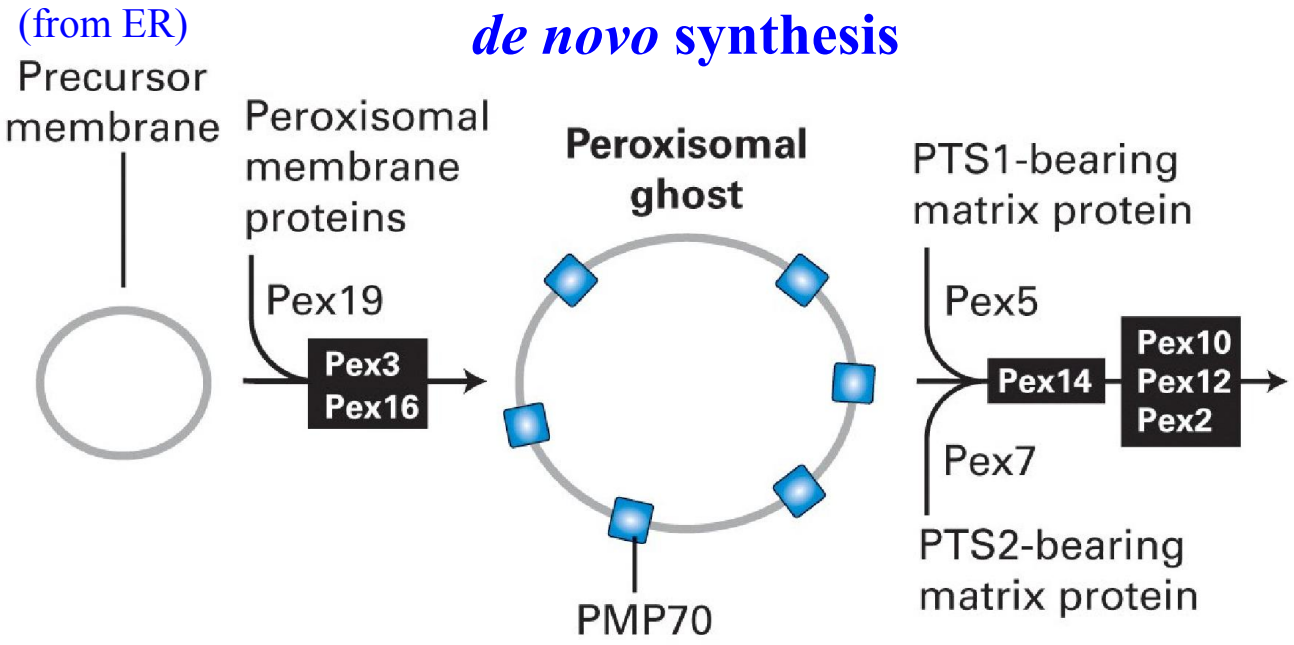
Pex3/16: Receptor complex to accept the insertion of membrane proteins

Pex5/7: Cytosolic receptors for PTS1/PTS2-bearing peroxisomal matrix proteins

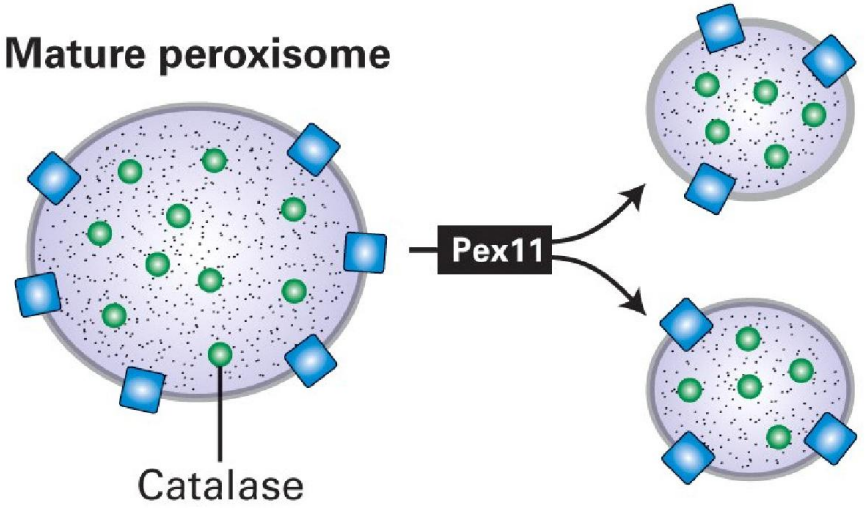
Pex14: Membrane receptor

Pex2/10/12: Expandable membrane translocon

Pex11: directs the division of perxisomes



***de novo* synthesis**

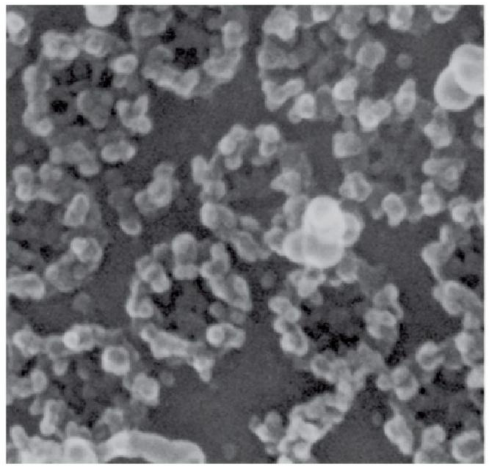


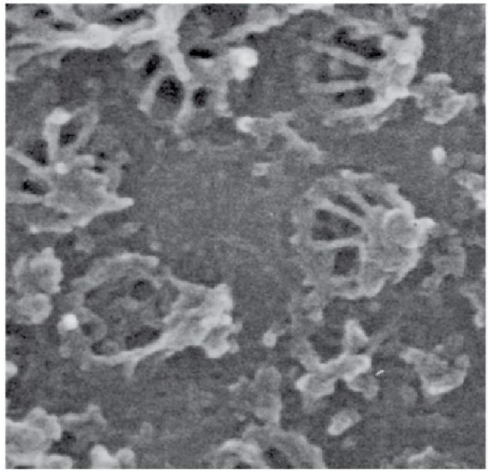
Devision of Pex11

13.5
Transport into and out of the Nucleus

The nuclear pore complex (NPC)

(cytosolic side)





(nucleoplasmic side)

Nuclear envelope of Xenopus oocyte

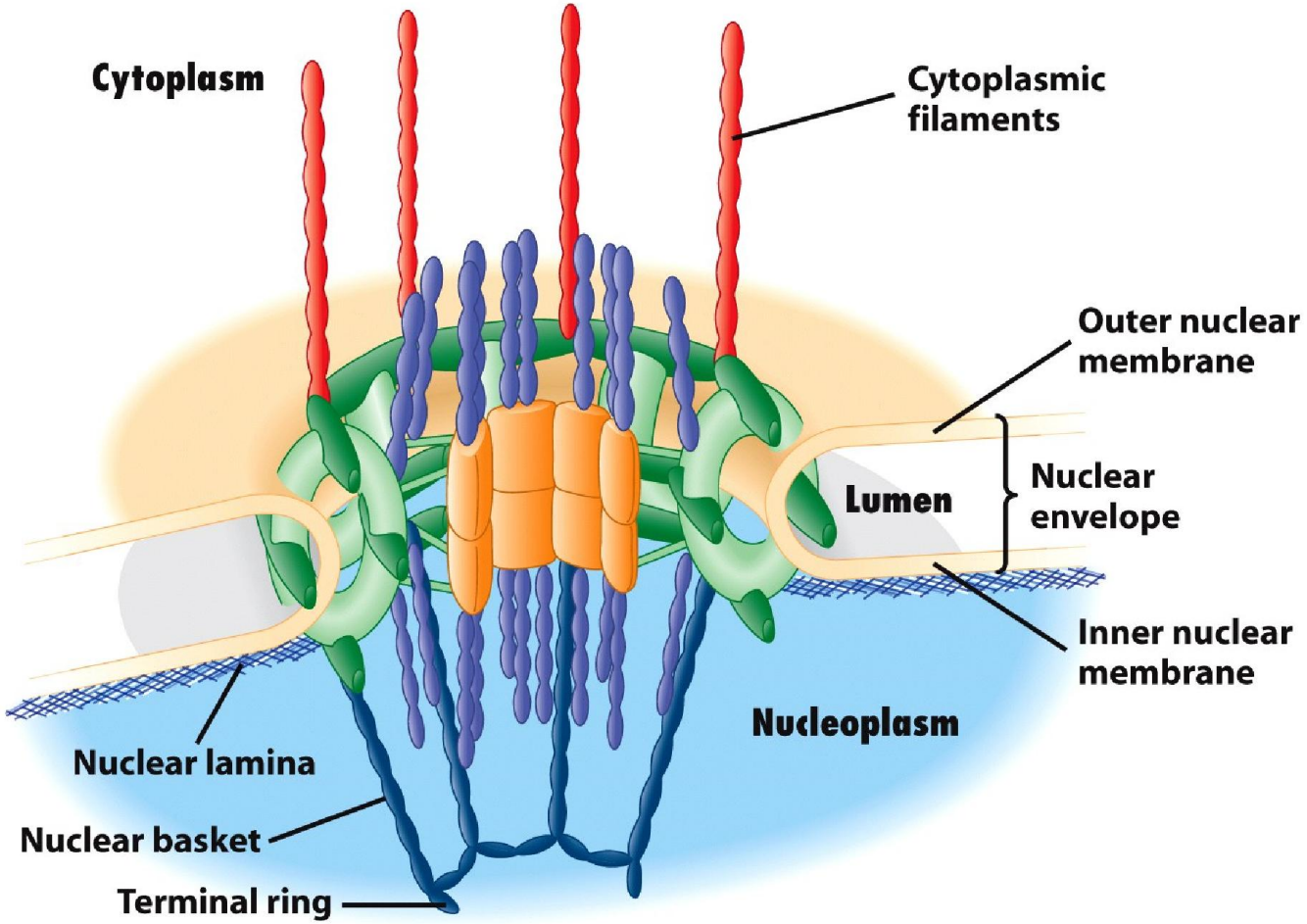


Figure 13-32b
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NPC is composed of ~30-50 different **nucleoporins**.

Nuclear localization signal (NLS) directs the transport of nuclear proteins

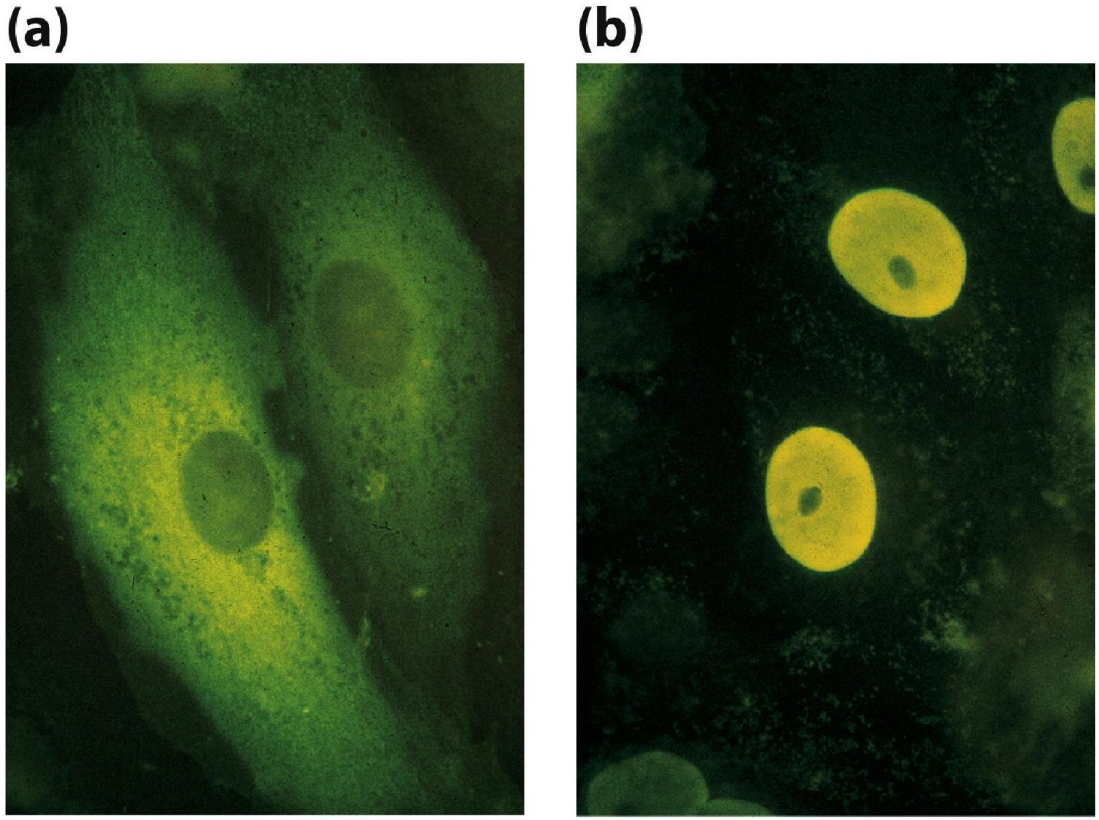

Figure 13-33
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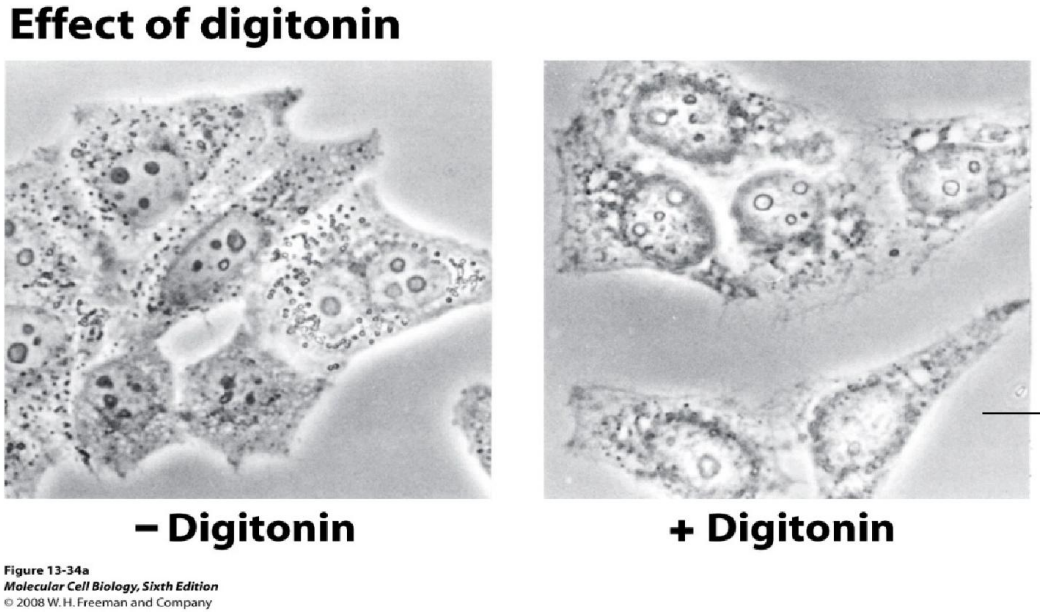
Large T-antigen

- Produced by SV40
- Contains NLS for nuclear transport
  - PKKKRKV
  - Located at C-terminus

(a) Normal pyruvate kinase (PK) → in cytosol
- Abundant cytosolic protein

(b) Chimeric PK → changed to in the nucleus
- PK attached with a SV40 NLS (large T Ag)

Cytosolic proteins are required for nuclear transport



Incubated with synthetic protein
(e.g. pyruvate kinase-SV40 large T-FITC)

Digitonin

- non-ionic detergent
- can permeabilize membrane while leaving nucleus intact

Lysates harvested

Lysates contain

- Ran (monomeric G protein)
- Importins α & β (form dimer)
  - α binds to NLS
  - β binds to nuclear pore

Nuclear import

[importin-cargo]high
[importin-RanGTP]low

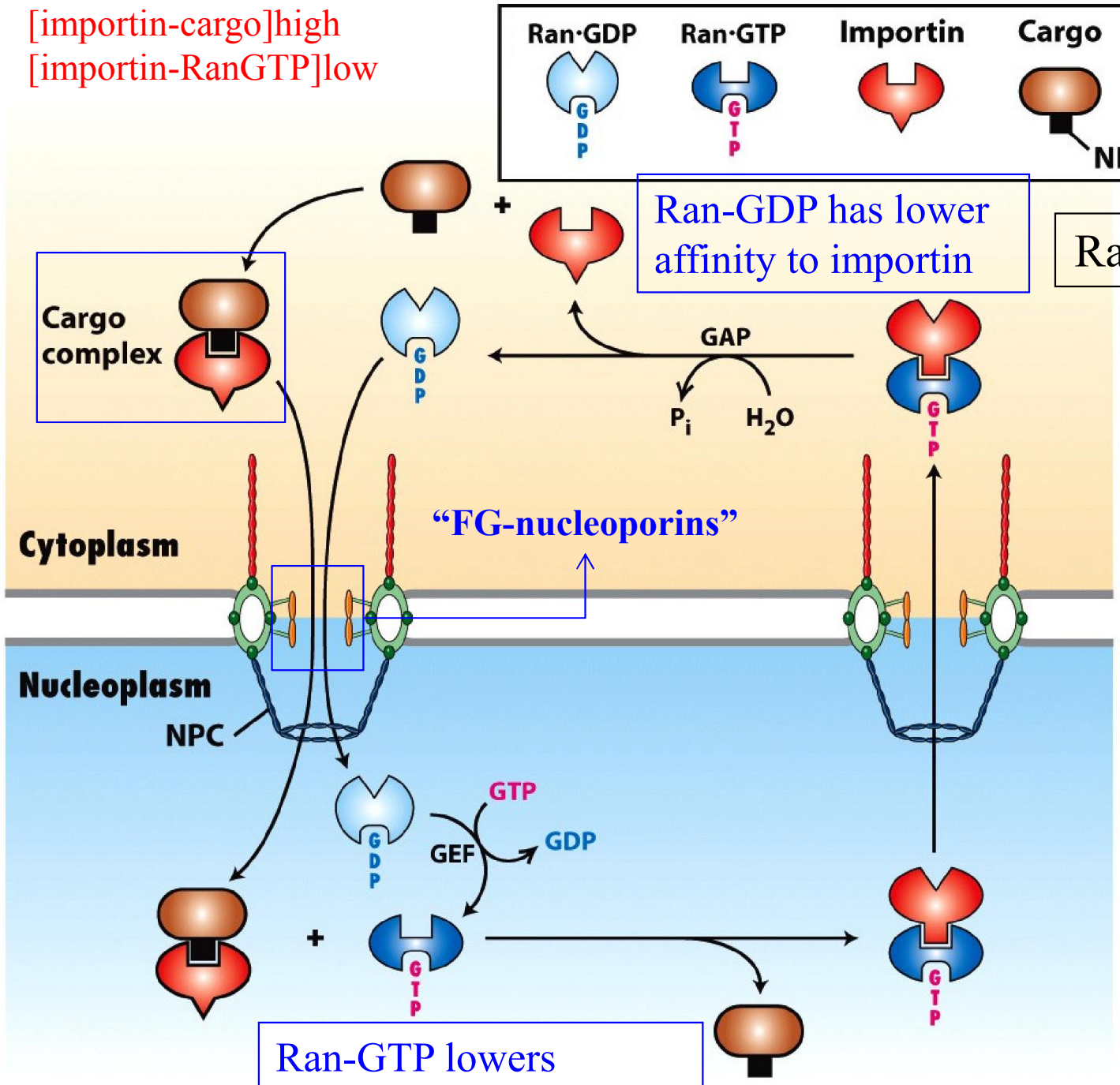


Figure 13-35
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Ran-dependent nuclear import

- GAP (cytosol)
  - GTPase-activating protein
- GEF (nucleoplasm)
  - guanine nucleotide exchange factor
- Importin
  - Shuttles in between the cytoplasmic and nucleoplasmic sides of the nuclear membrane
  - Forms bi-molecular complex (α & β)
- Import examples
  - Histones, transcription factors
  - DNA & RNA pol.

Nuclear export (1)

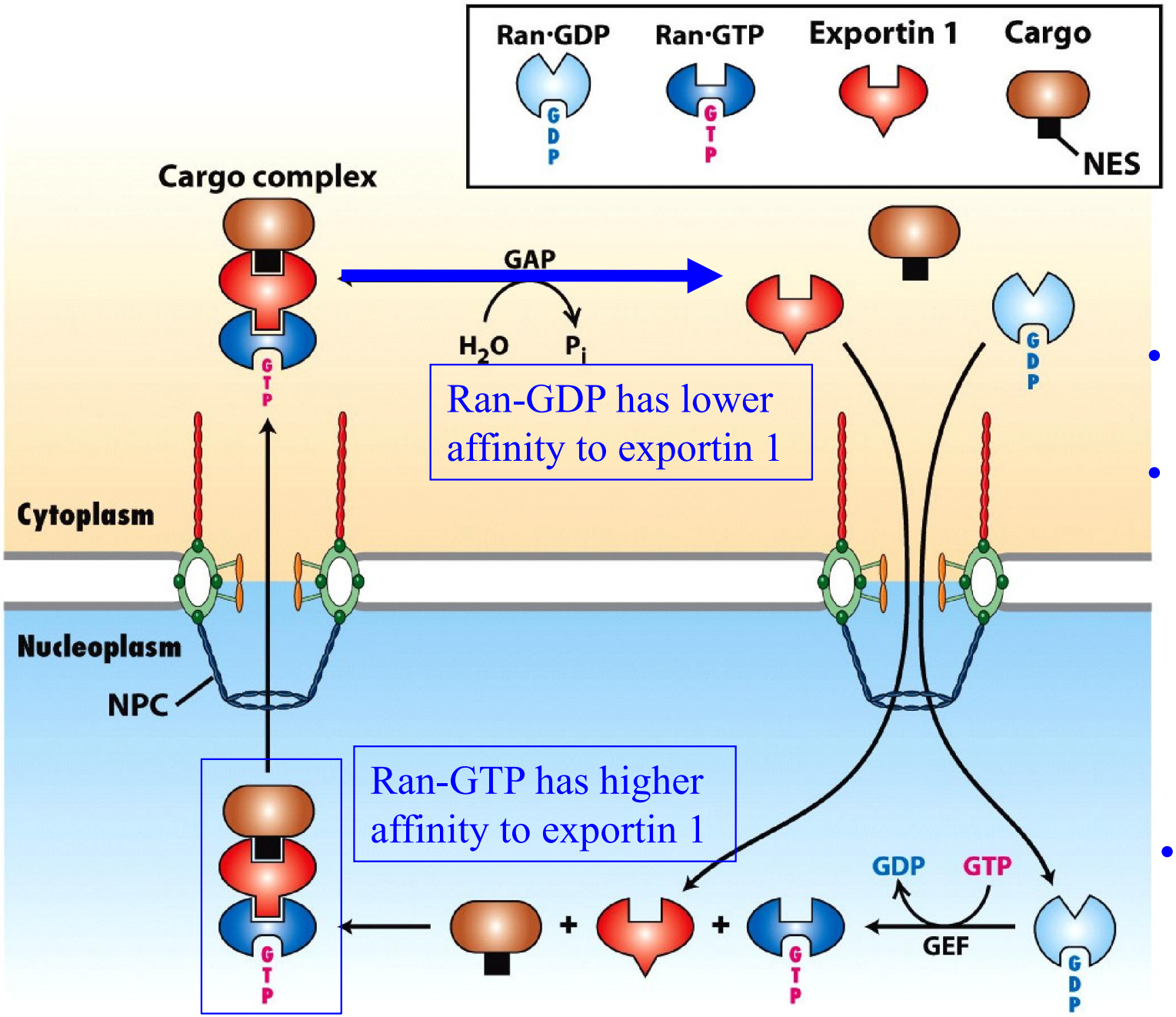

Figure 13-36a
Molecular Cell Biology, Sixth Edition

- NES
  - Nuclear-export signal
- Exportin 1
  - Binds Ran-GTP and promotes Ran-GTP binding to NES
  - Forms tri-molecular complex
- Export examples
  - Ribosomal subunits
  - tRNAs

Ran-dependent nuclear export

Nuclear export (2)

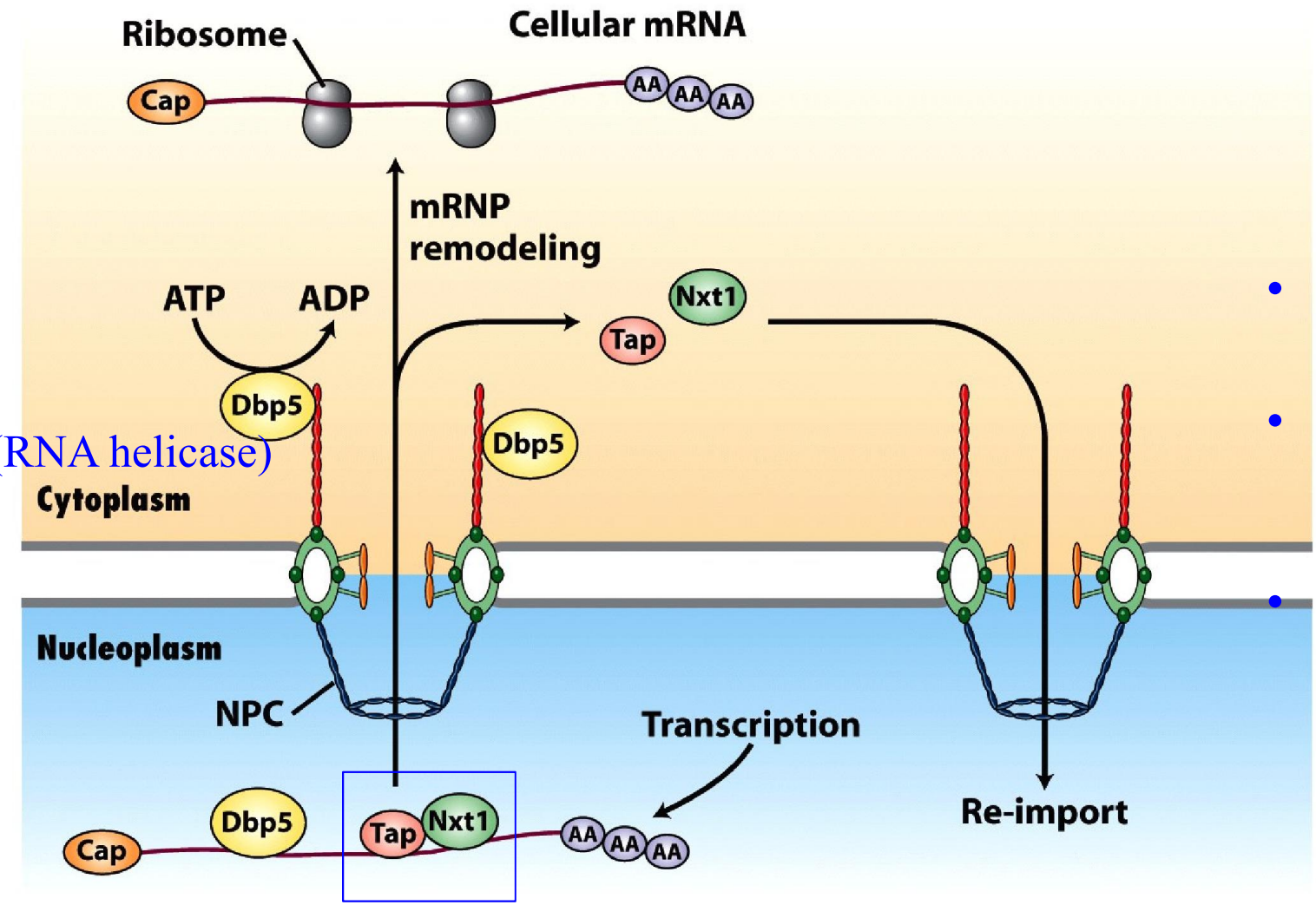

Figure 13-36b
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"mRNP (mRNA protein complex) exporter"
→Tap/Nxt1 dimer
→Multiple units bound to mRNA

- NES
  - Nuclear-export signal
- Tap/Nxt1 dimer
  - Directs mRNA towards nuclear pore
- Dbp5 (RNA helicase)
  - Hydrolyses ATP to drag mRNA into cytosol
  - Frees Tap/Nxt1 off mRNA
- Export examples
  - Most mRNAs

Ran-independent nuclear export

Summary
Organelle sorting signals

Nucleus

Internal (Near C-terminus) ***import:*** One cluster of 5 basic amino acids (P**KKKRK**V)

export: Leucine-rich: e.g. LQLPPLERLTL (rev protein of HIV-1)

Mitochondrion

N-terminal — 3 – 5 nonconsecutive Arg or Lys residues (→ amphiphatic helix) often with Ser and Thr; no Glu or Asp residues

---

Chloroplast

N-terminal — No common sequence motifs; generally rich in Ser/Thr and small hydrophobic amino acids, poor in Glu and Asp residues

---

Peroxisome

C-terminal — PTS1: Usually Ser-Lys-Leu (SKL) at extreme C-terminus

PTS2: (few proteins) located at N-terminus

ER

N-terminus — hydrophilic domain (often basic) followed by 6 to 12 hydrophobic residues

Internal — (total length: about 16 to 30 hydrophobic residues)

End of Chapter 13