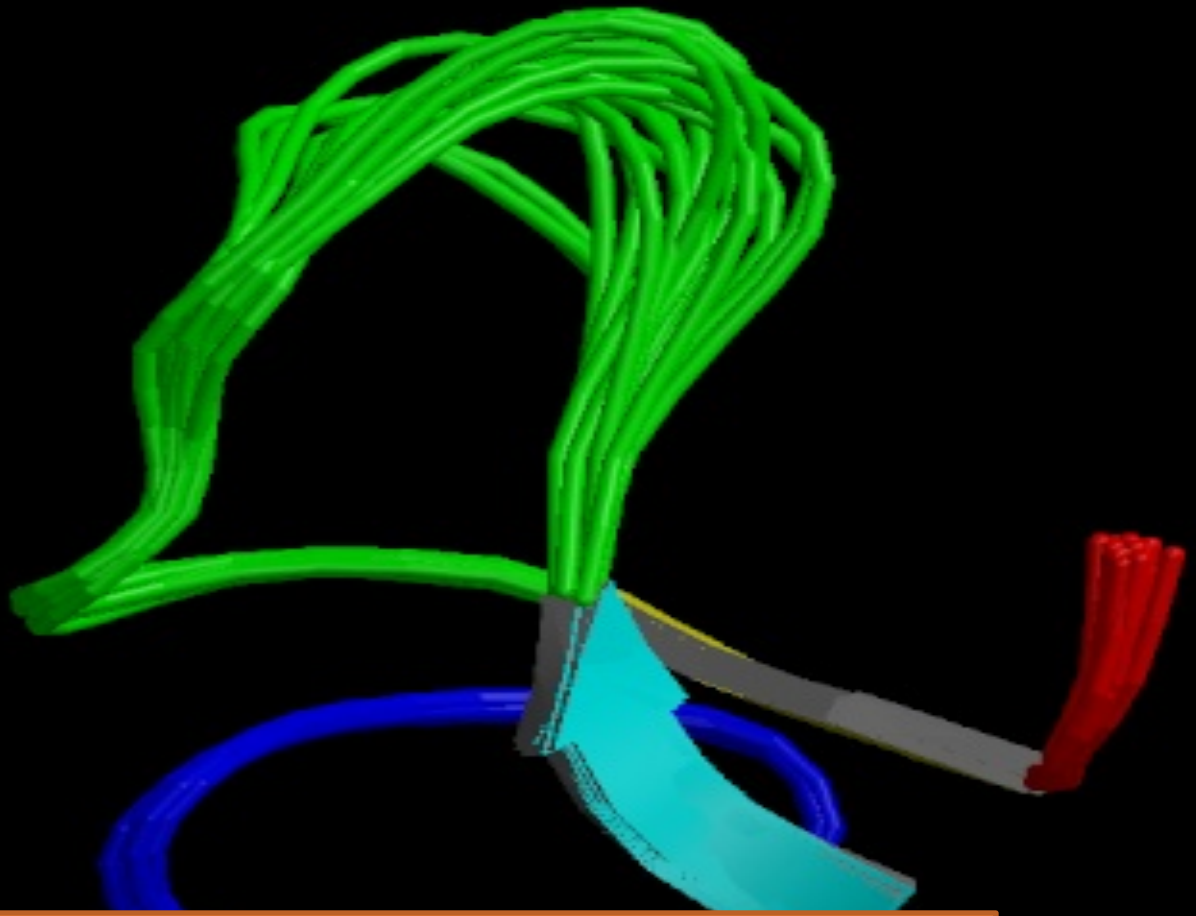
Course: Protein Structure, Function and Engineering



Lecture 10

Protein Secretion and Targeting (ER, GC, EC)

M. Fayyaz ur Rehman

Last Lecture

- Synthesis of Protein
- Posttranslational Modification
  - Modification of Individual Amino Acid
  - Glycosylation (N-linked & O-linked)
  - Proteolytic Processing
  - Prosthetic Group Addition
  - Disulphide Bond Formation
  - Lipophilic Modification
  - Selenoproteins
  - Vitamin K Dependent Modification
  - Sulfation

Organelles are defined by the proteins and lipids they contain

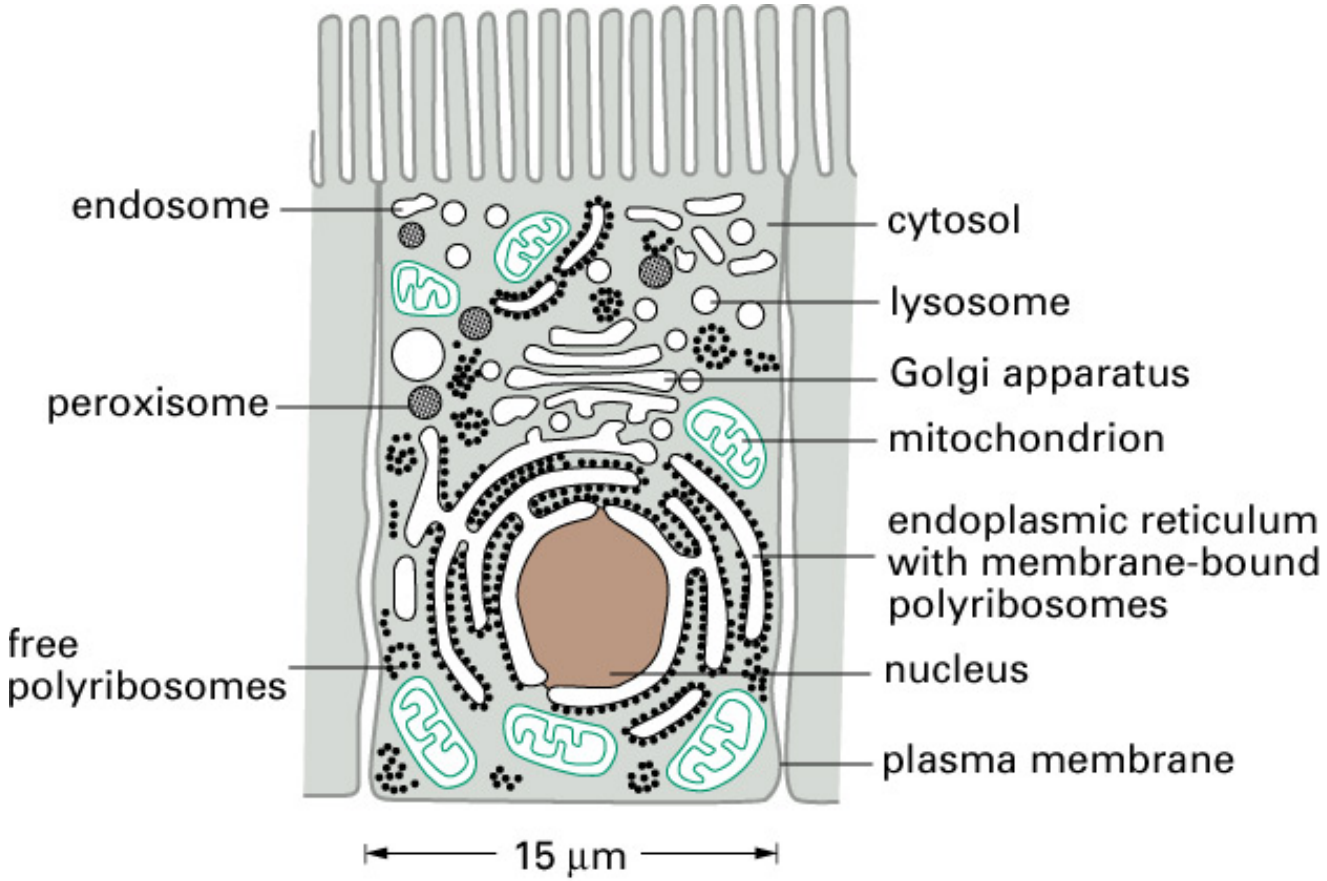
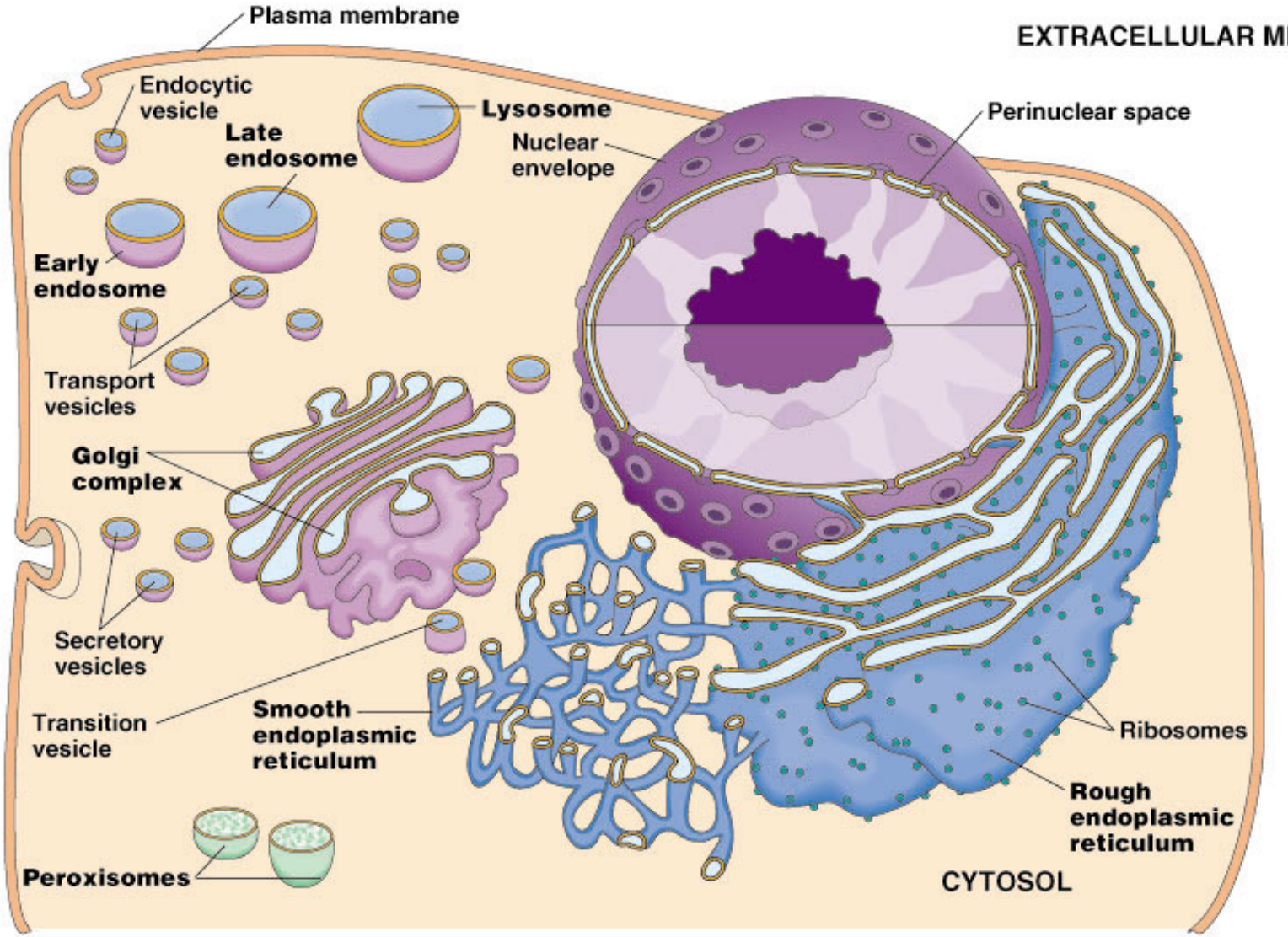
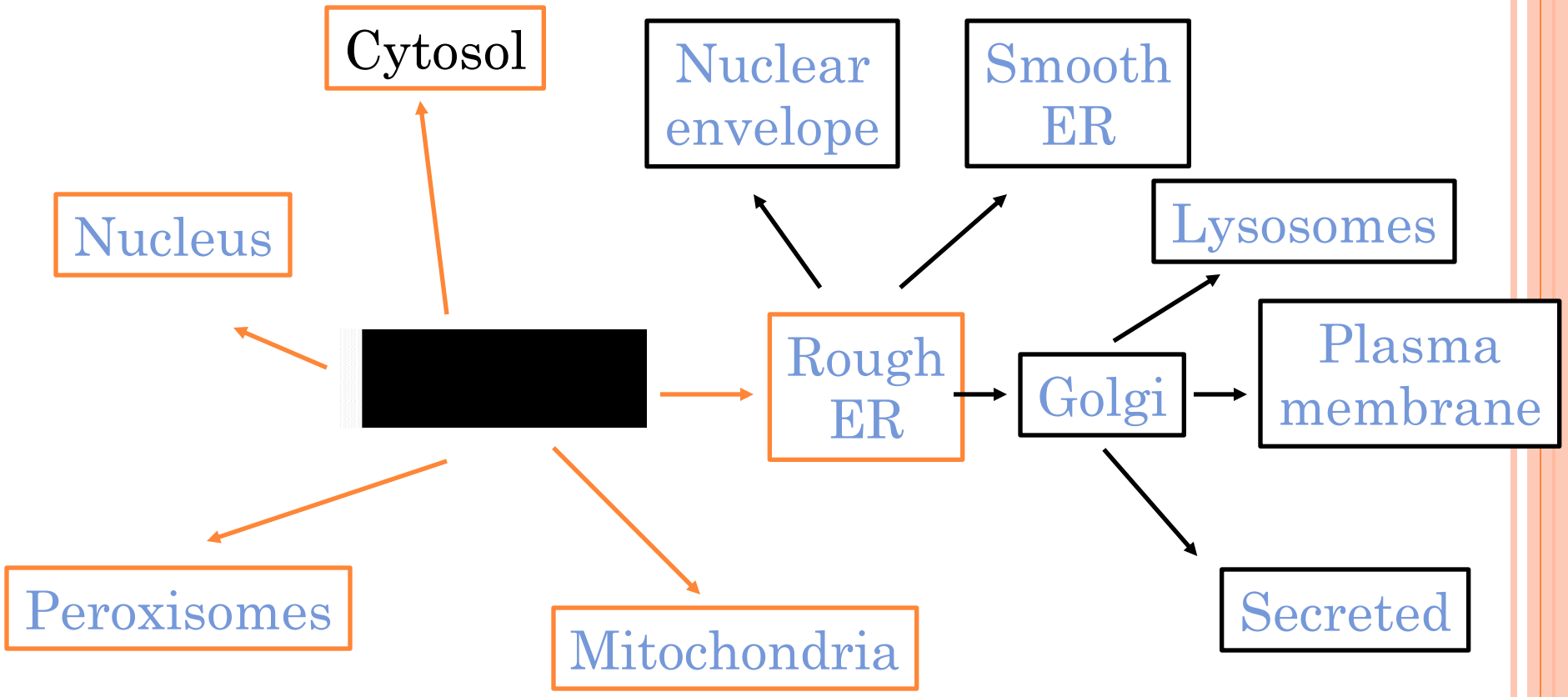


Figure 12–1. Molecular Biology of the Cell, 4th Edition.

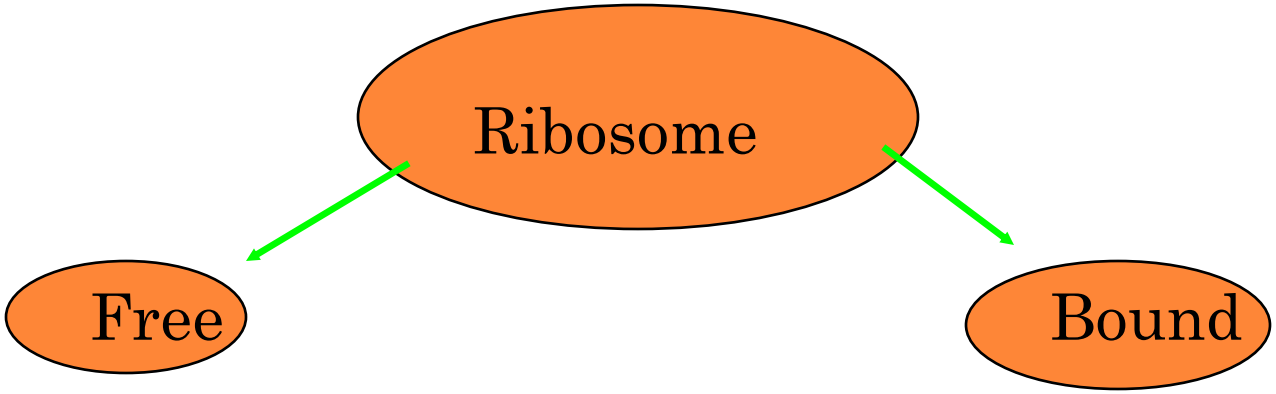
How do the 30,000 Or so proteins find their Correct Cellular Locations?



Protein sorting



Protein Targetting

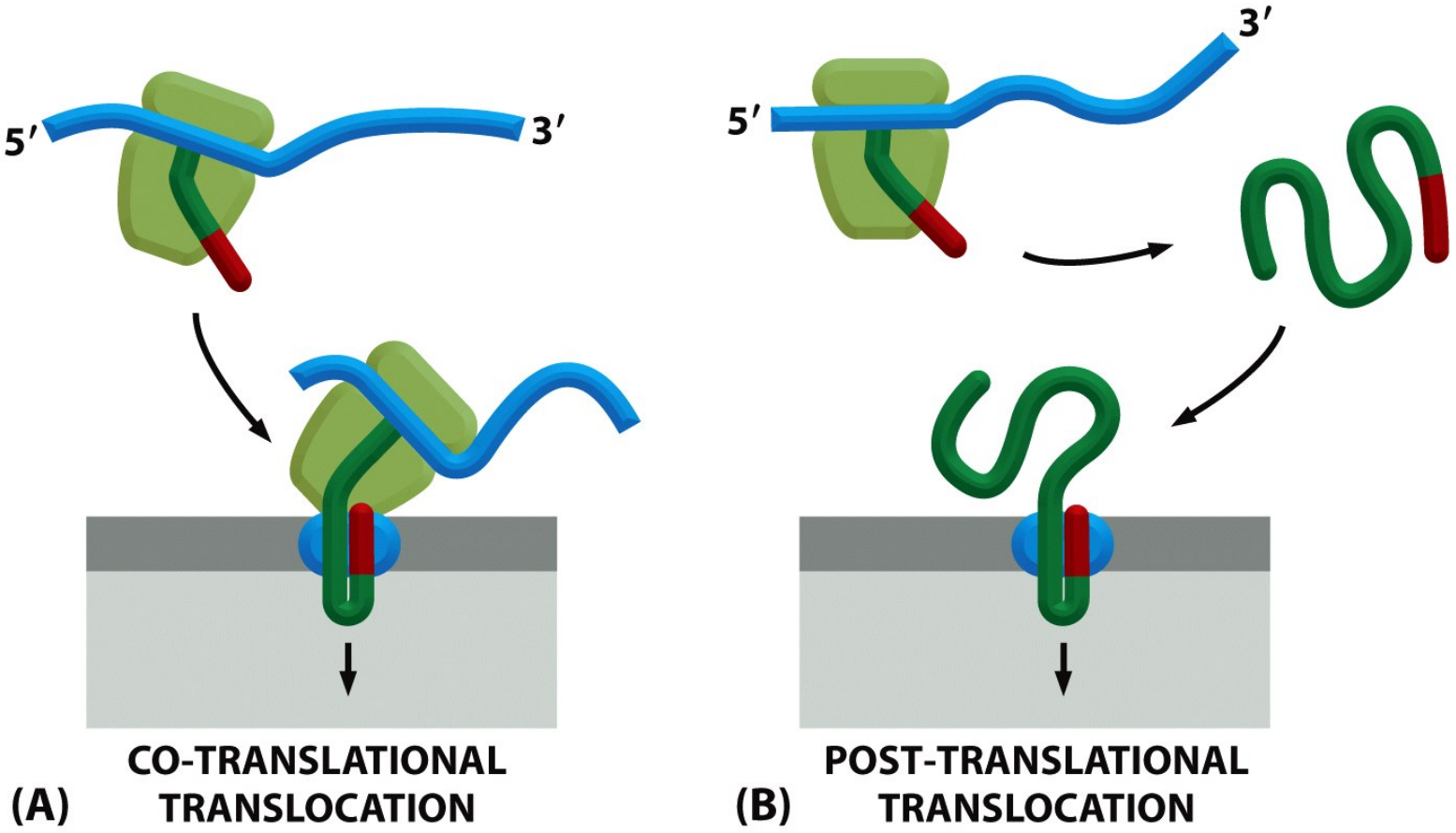


Soluble Proteins

Lysosomal
Secretory
Plasma Memb.

Ways to get proteins into organelles or membranes

- Co-translational import
  - Put proteins into organelles or membranes *during* the actual process of translation
  - Examples: extracellular proteins, cell membranes, lysosomal enzymes
- Post-translational import
  - Make proteins in the *cytoplasm*, and *subsequently* import them into the organelle of choice
  - Examples: the nucleus, the mitochondrion
- Each is directed by "signals" embedded in the amino acid sequence of the newly synthesized protein


5′
3′
5′
3′
(A)
CO-TRANSLATIONAL
TRANSLOCATION
(B)
POST-TRANSLATIONAL
TRANSLOCATION

The signal sequence

- 13-36 residues long
- The N terminus always contain a positively charged amino acid
- The central portion is a stretch of hydrophobic amino acids
- Some proteins have internal signal sequence

Table 12–3 Some Typical Signal Sequences

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

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

Signal Sequences (or Patches) function as addresses

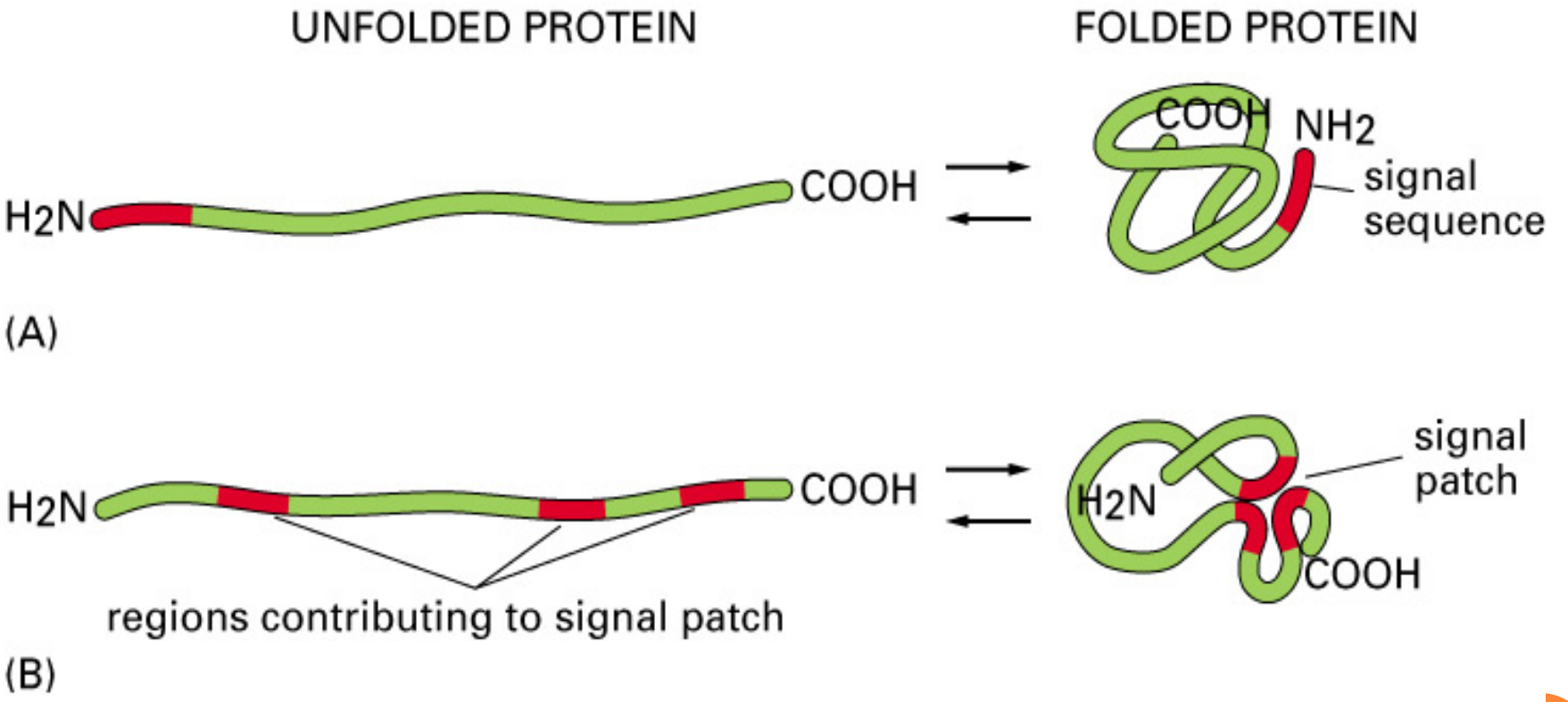


Figure 12–8. Molecular Biology of the Cell, 4th Edition.

Signal Recognition Particle

- **Ribonucleoprotien particle, 325 kD**

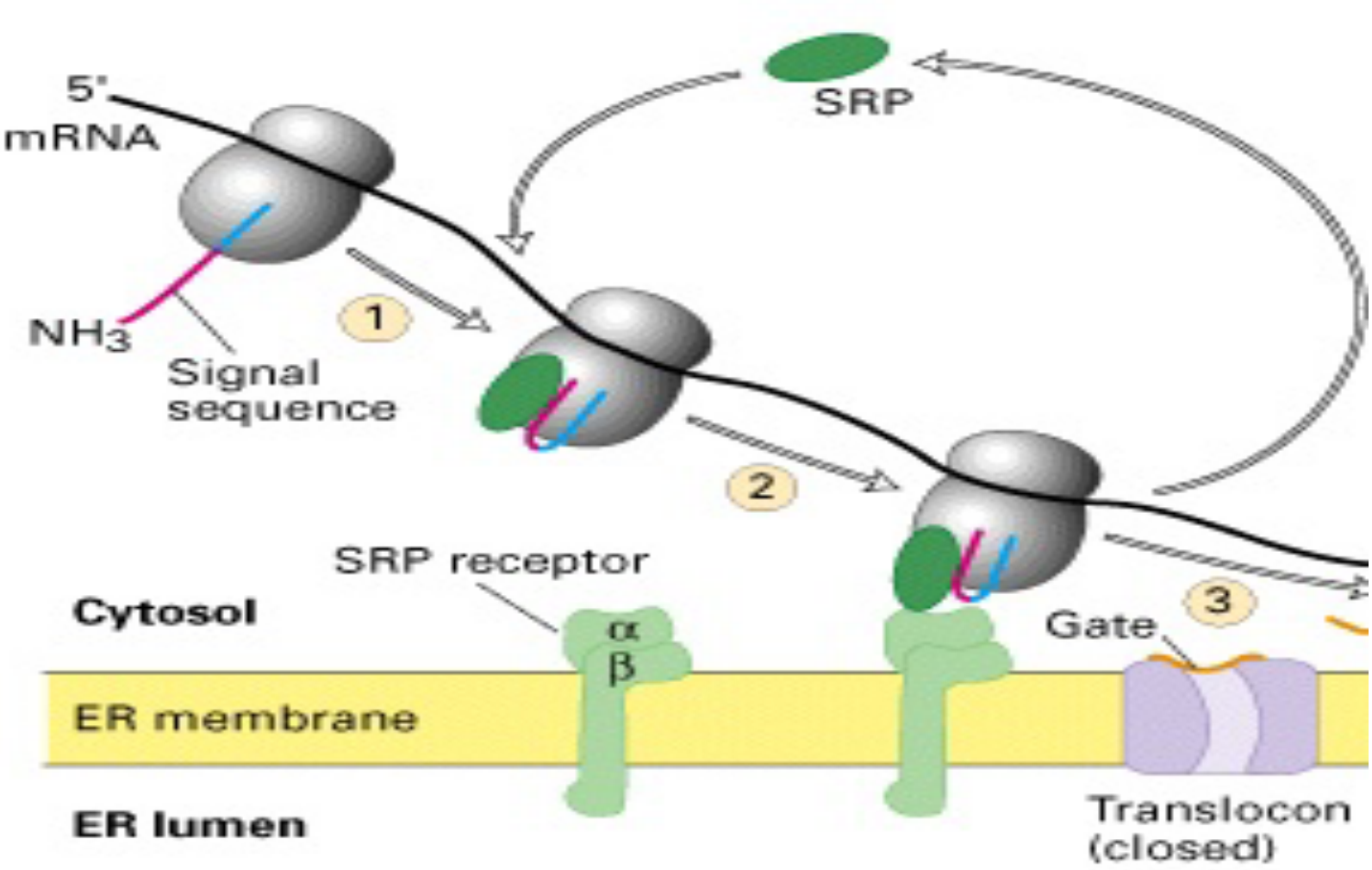
  RNA – 300 nucleotide

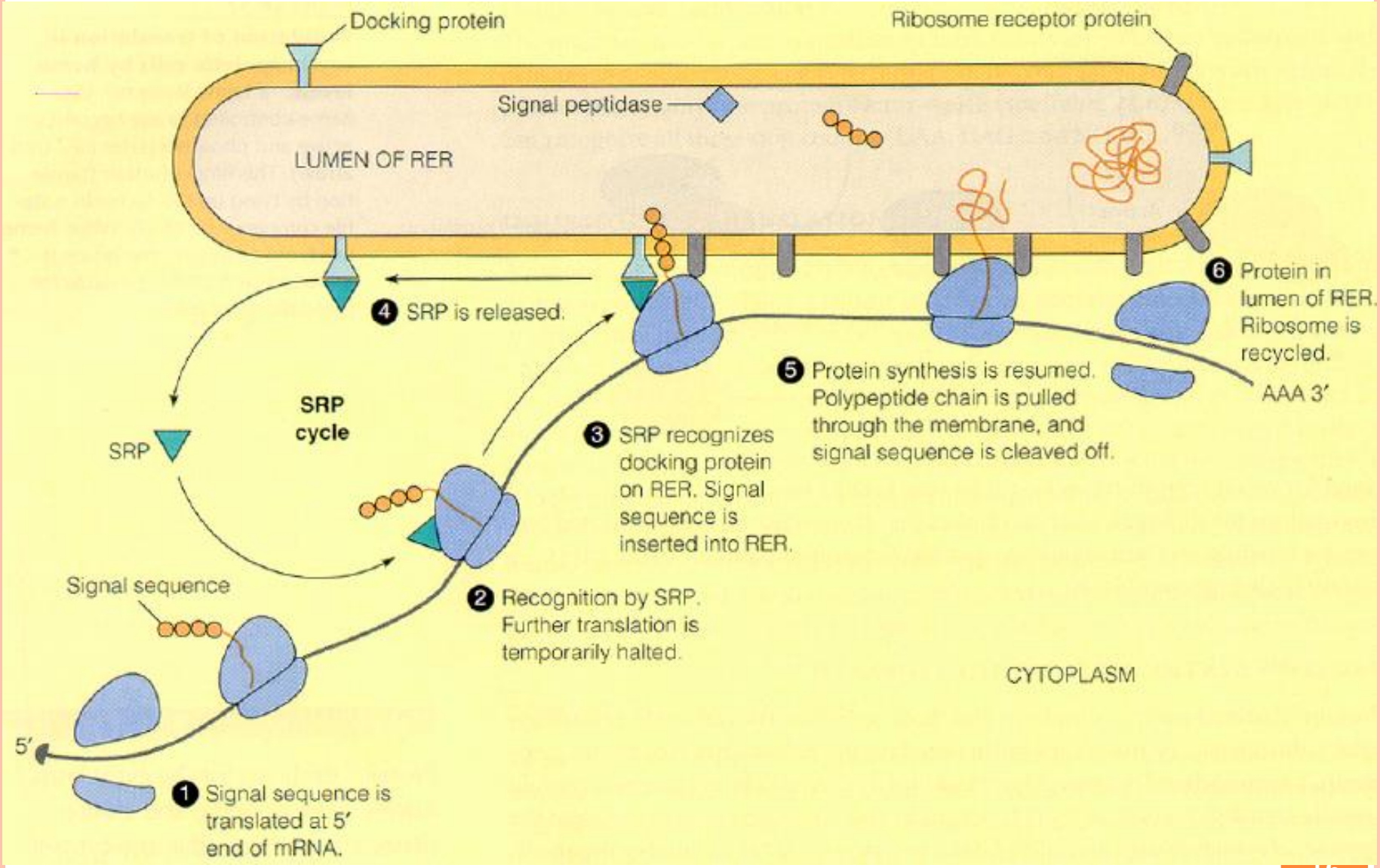
  6 polypeptides- 9, 14, 19, 54, 68 & 78 kD

54 kD polypeptide binds to the signal sequence

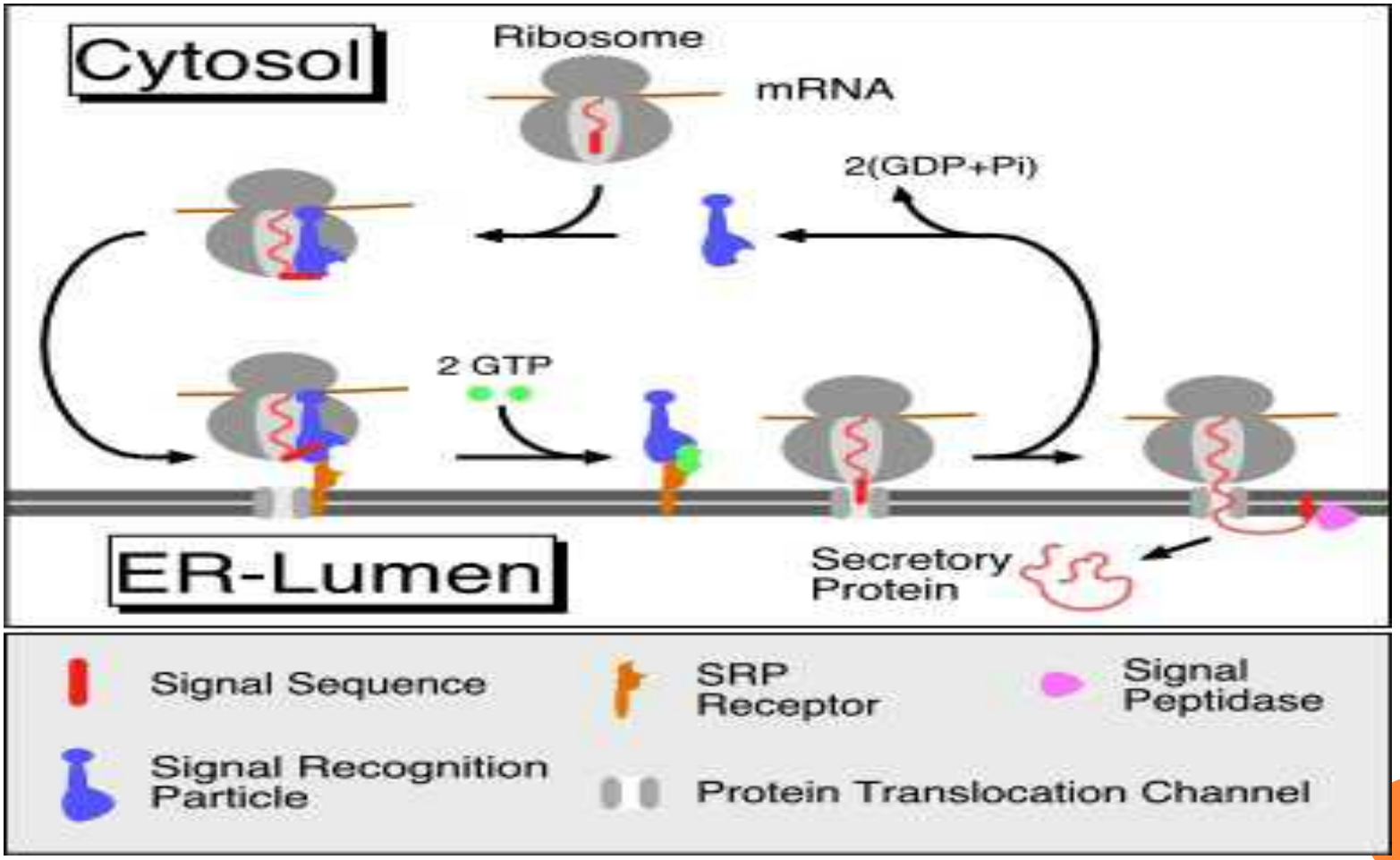
The SRP Receptor

- Made of 2 subunits
- A 69 kD alpha subunit and a 30 kD ß sub unit. Alpha sub unit has positively charged amino acids.
- Binding of SRP and SRP receptor is by ionic interactions.

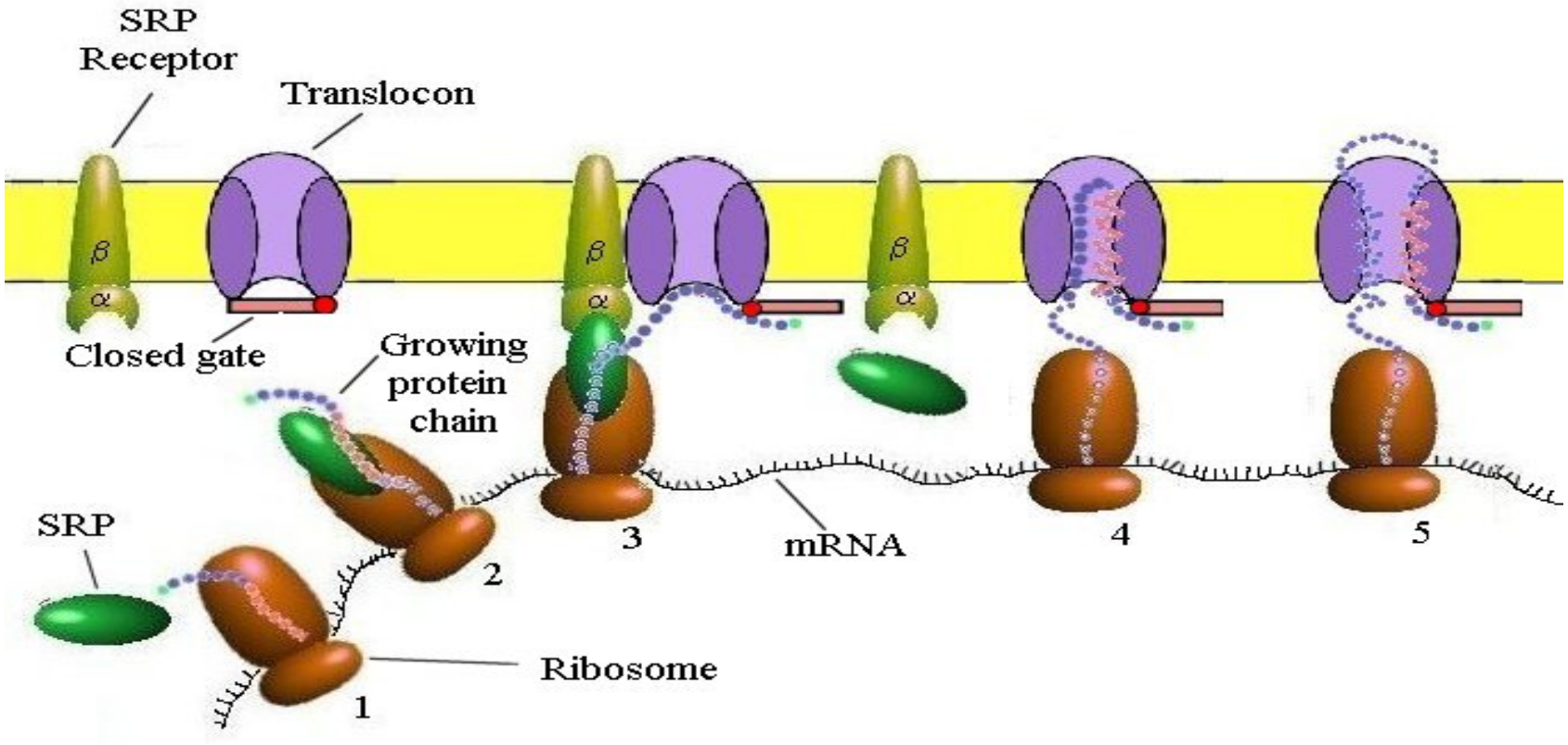

5'
mRNA
SRP
NH3
Signal
sequence
1
2
3
SRP receptor
Cytosol
α
β
Gate
ER membrane
ER lumen
Translocon
(closed)


Docking protein
Ribosome receptor protein
Signal peptidase
LUMEN OF RER
4 SRP is released.
6 Protein in lumen of RER. Ribosome is recycled.
5 Protein synthesis is resumed. Polypeptide chain is pulled through the membrane, and signal sequence is cleaved off.
AAA 3′
SRP cycle
SRP
3 SRP recognizes docking protein on RER. Signal sequence is inserted into RER.
Signal sequence
2 Recognition by SRP. Further translation is temporarily halted.
CYTOPLASM
5′
1 Signal sequence is translated at 5′ end of mRNA.

The GDP-GTP Cycle



The translocation process



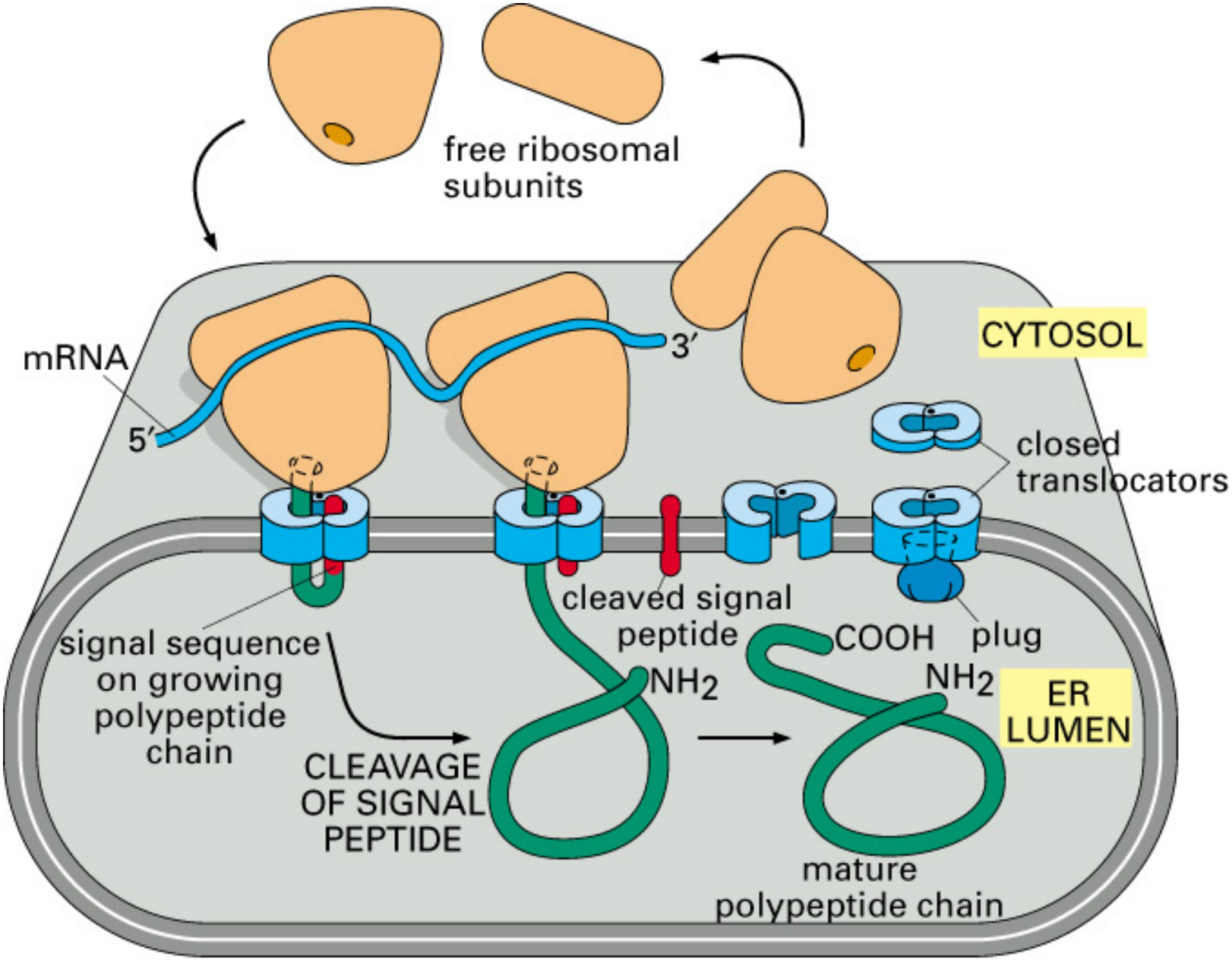


Figure 12–40. Molecular Biology of the Cell, 4th Edition.

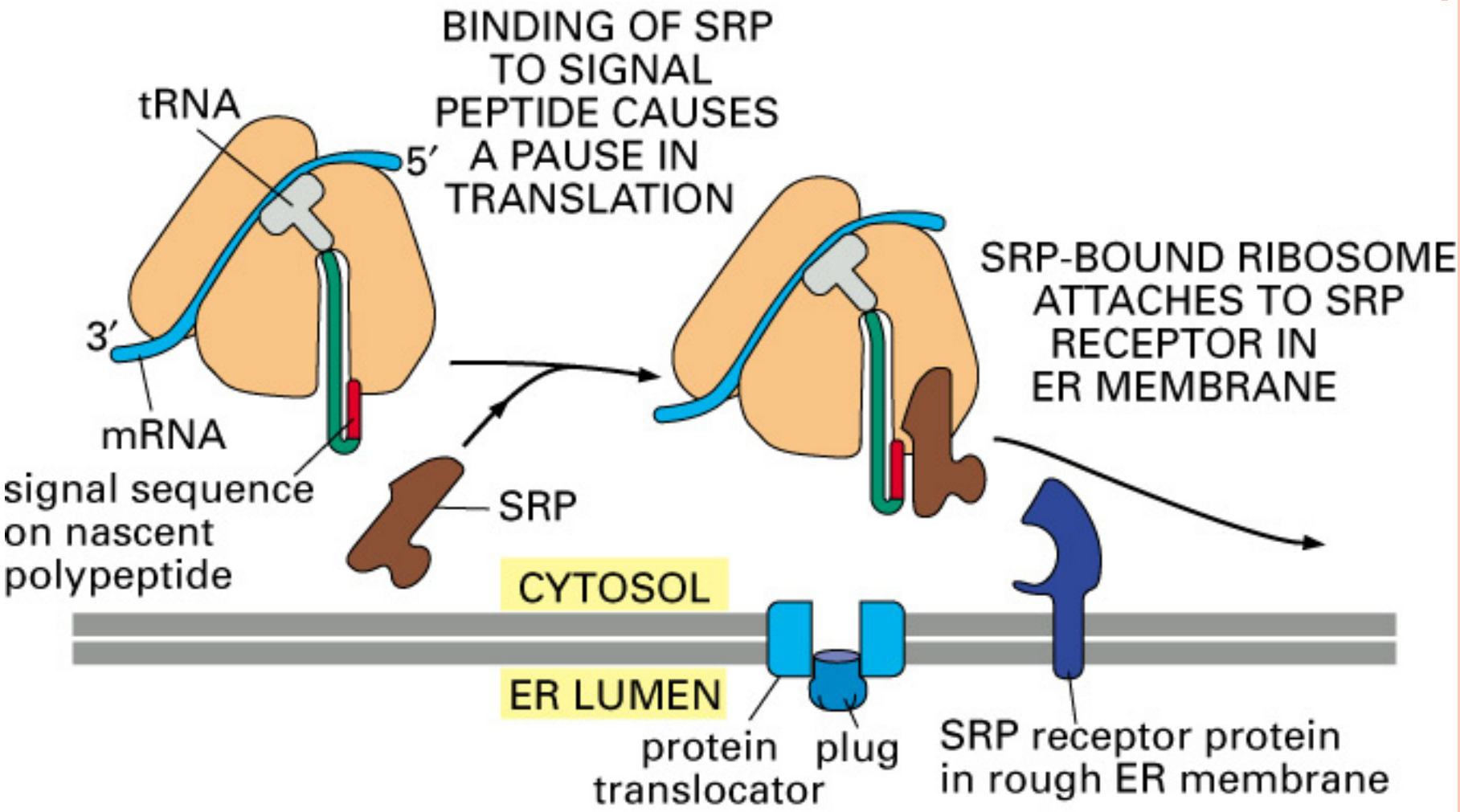


Figure 12–42 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

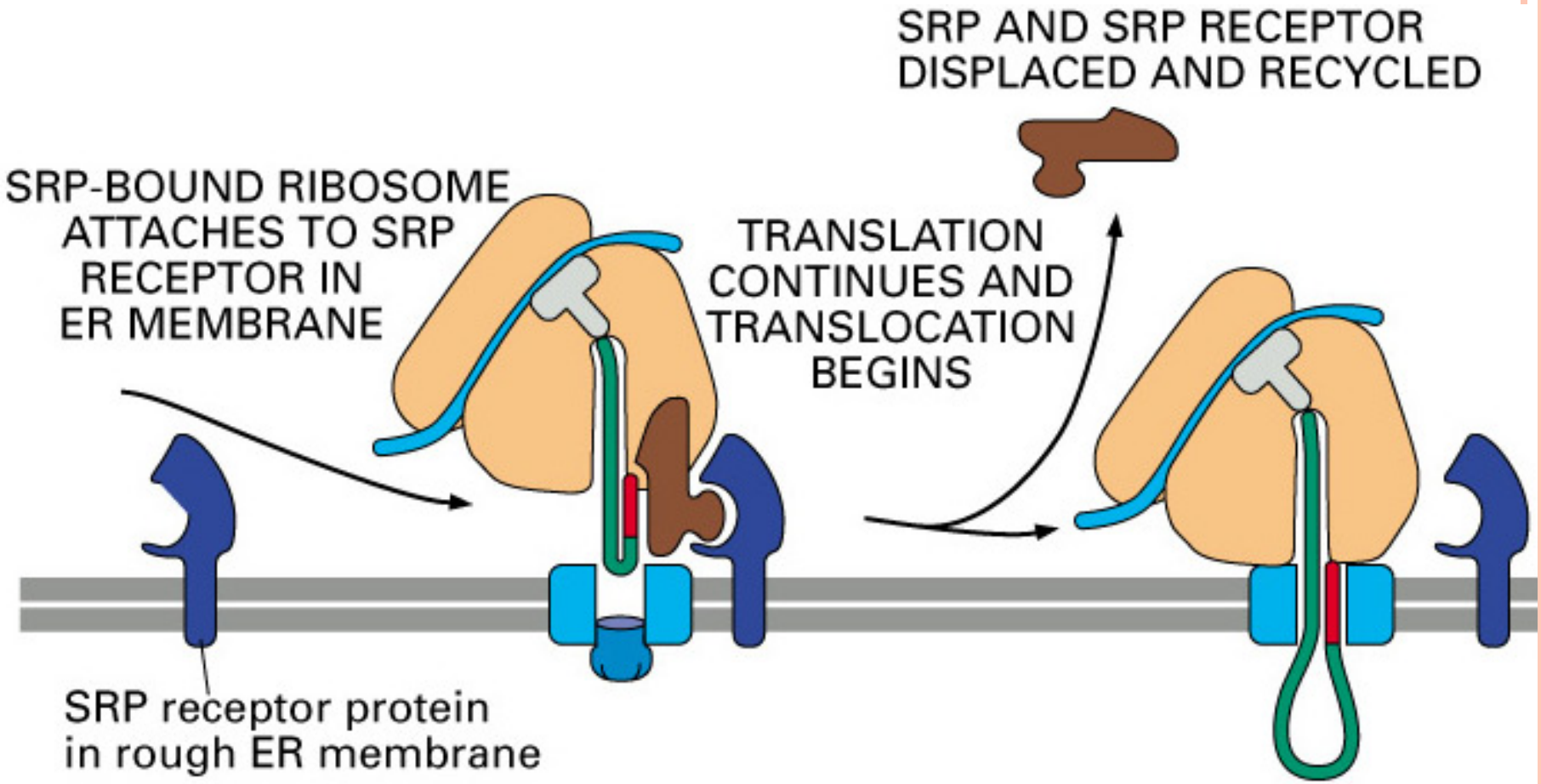


Figure 12–42 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

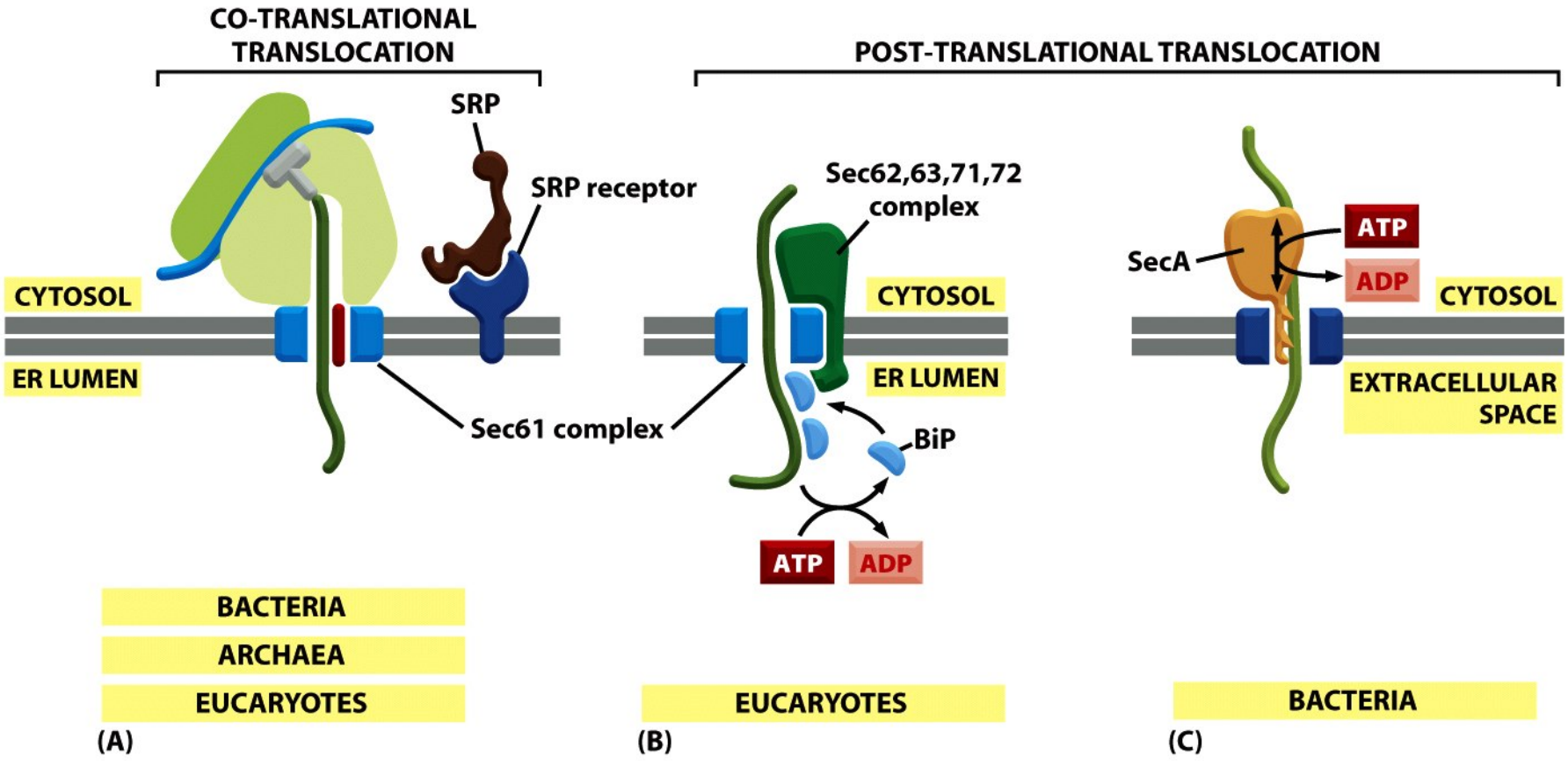
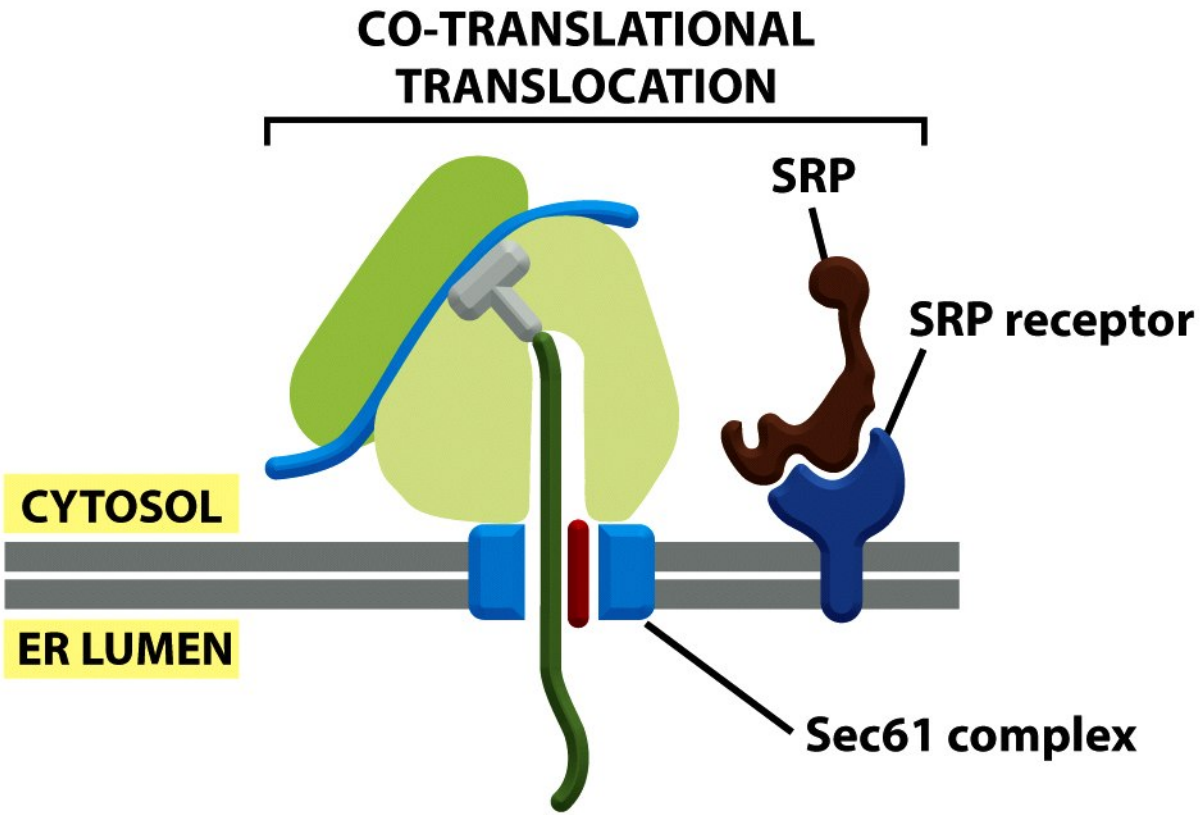


Figure 12-44 *Molecular Biology of the Cell* (© Garland Science 2008)



BACTERIA

ARCHAEA

EUCARYOTES

Figure 12-44a *Molecular Biology of the Cell* (© Garland Science 2008)

Type I: N-terminal signal sequence

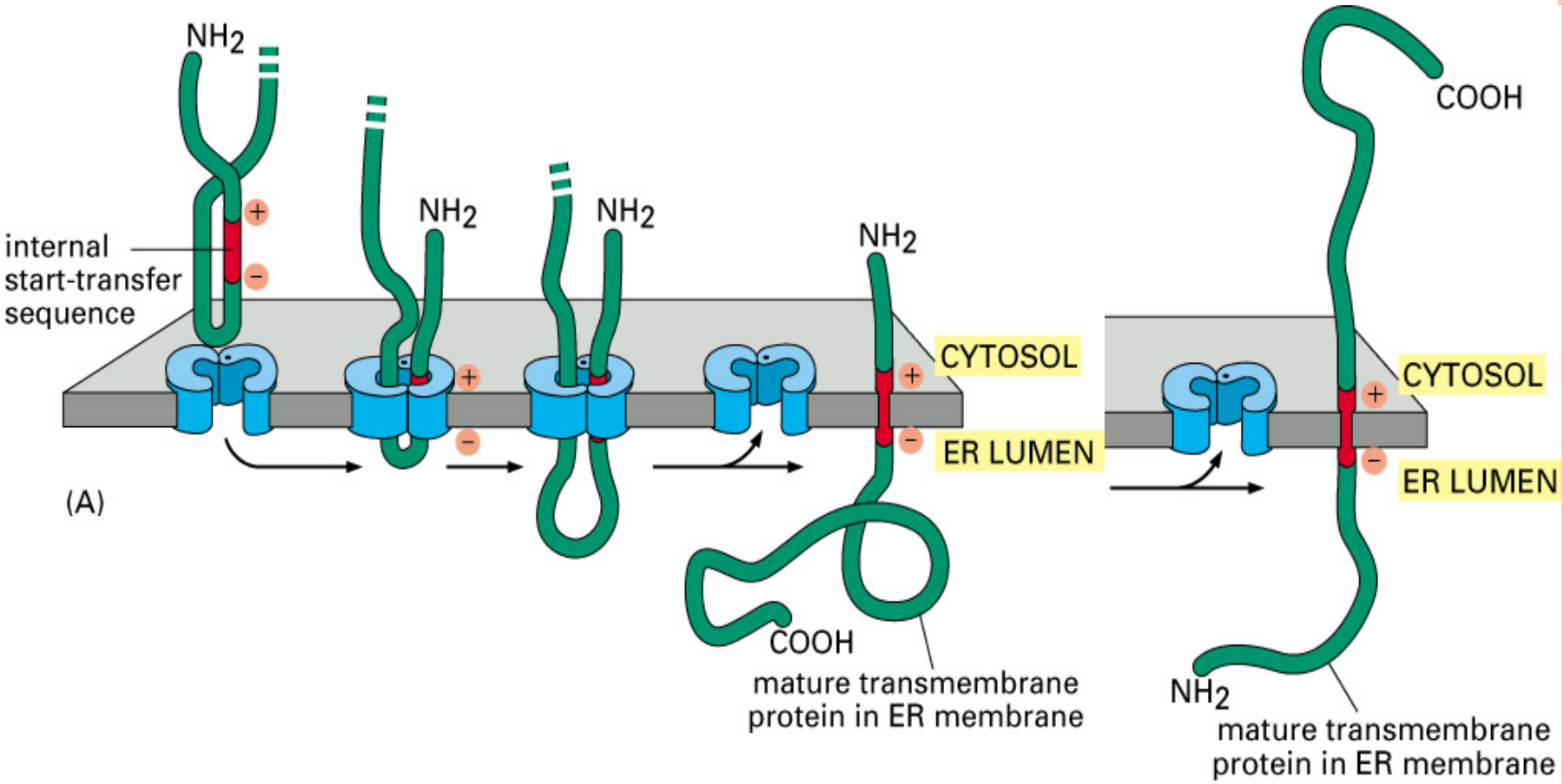
Type II: C-terminus in ER

Type III: N-terminus in ER but no N-terminal address

Type IV: Multipass

Type II

Type III



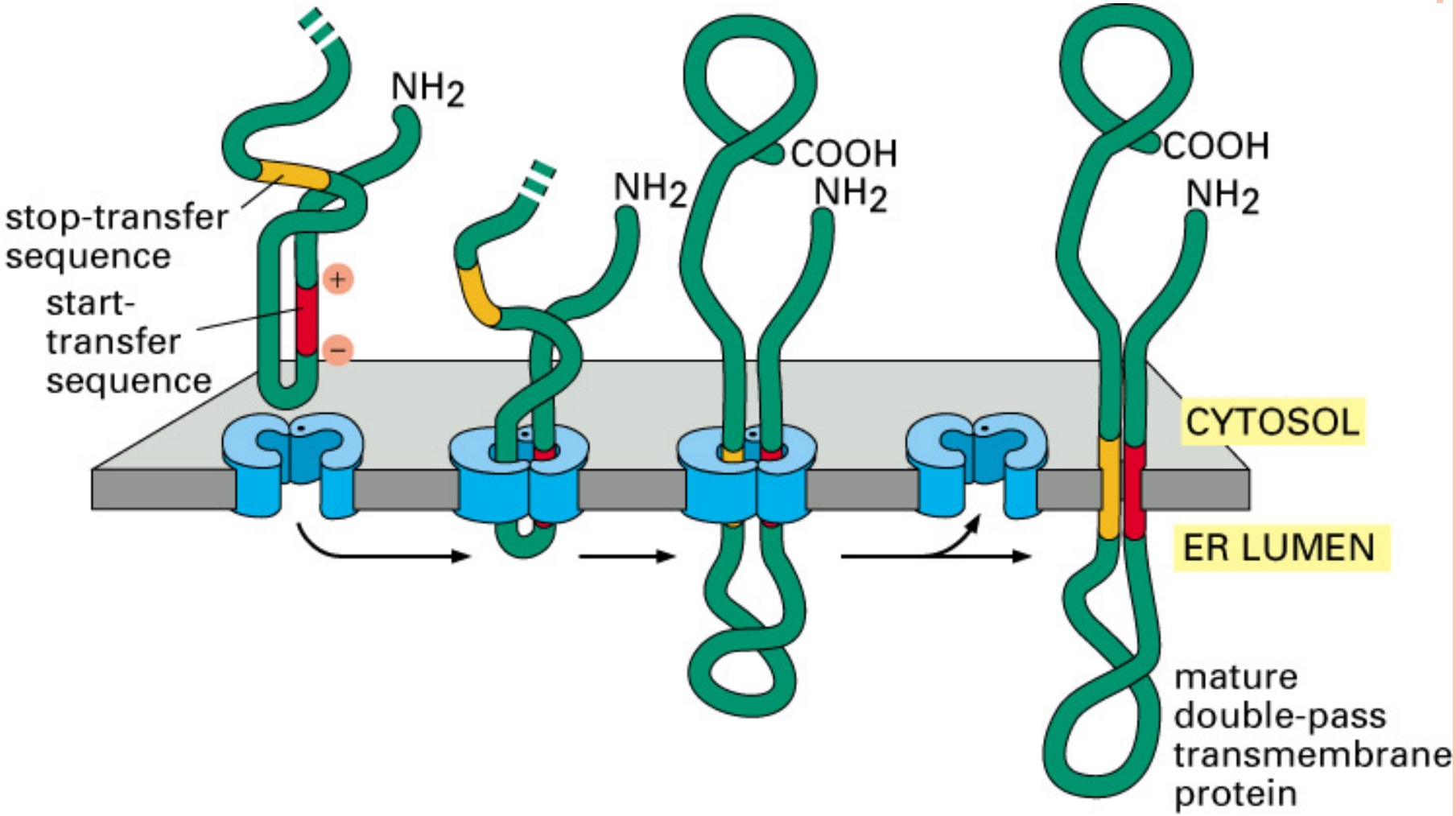
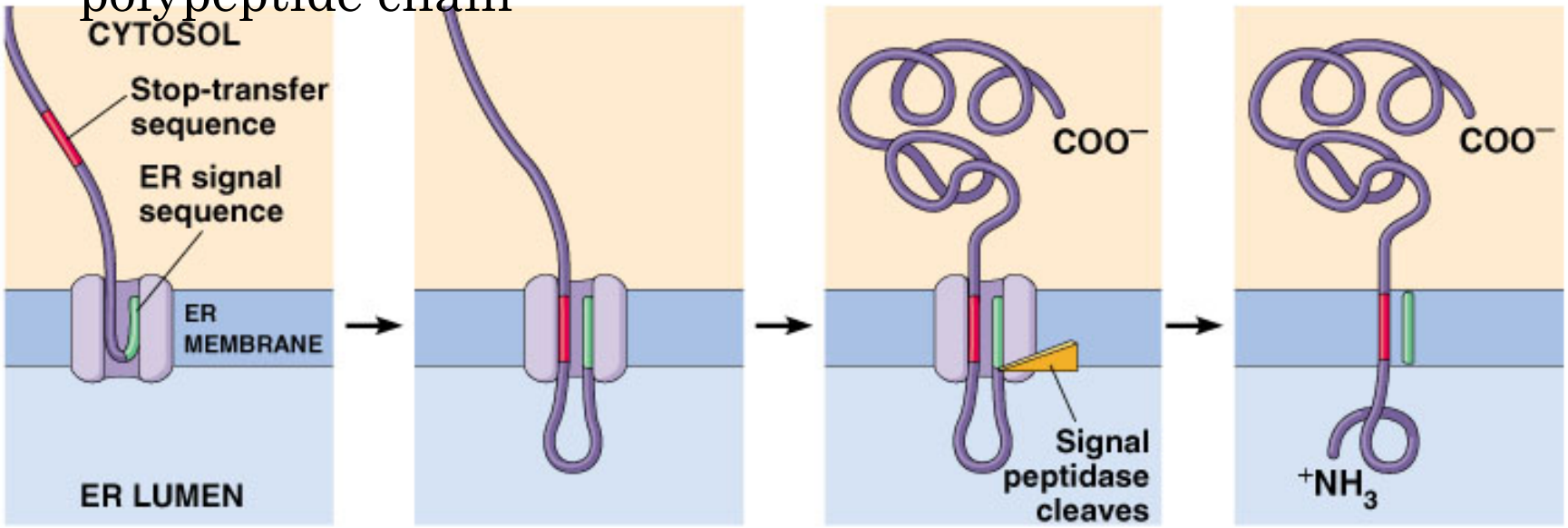


Figure 12–49. Molecular Biology of the Cell, 4th Edition.

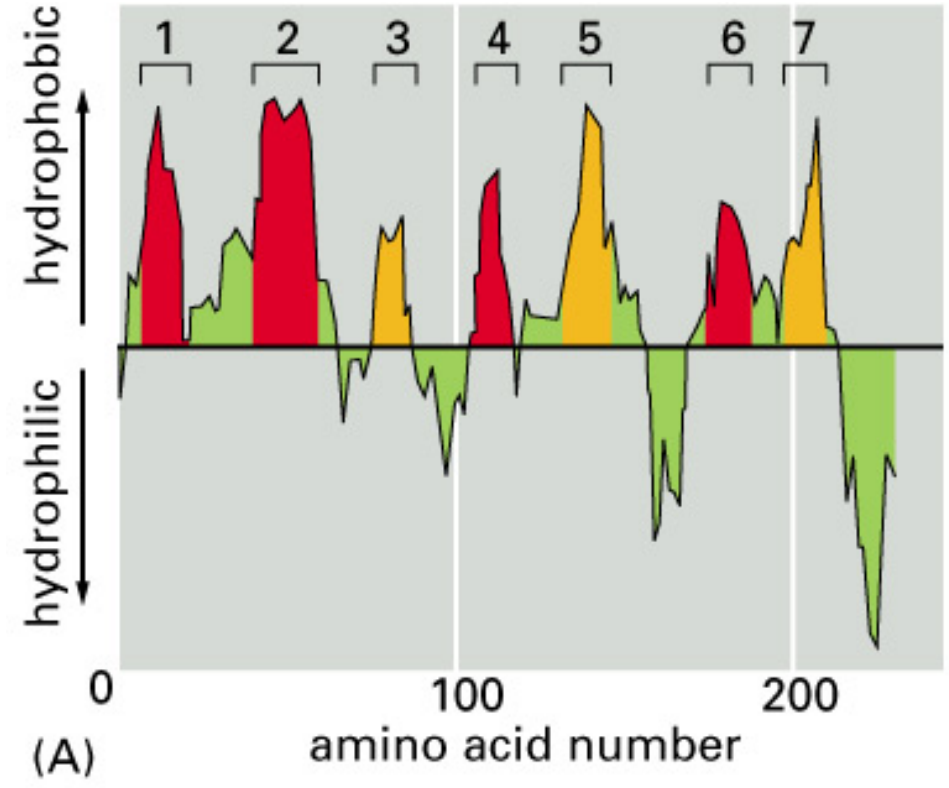
Stop-transfer or "topogenic" sequences

- Sequences of 20 hydrophobic amino acids bind inside the pore
- Move laterally out of the pore and into the membrane to build a transmembrane protein.
- There can be multiple topogenic sequences in a single polypeptide chain



(a) Polypeptide with an internal stop-transfer sequence and a terminal ER signal sequence

Hydropathy Plots can Identify Integral Membrane Domains



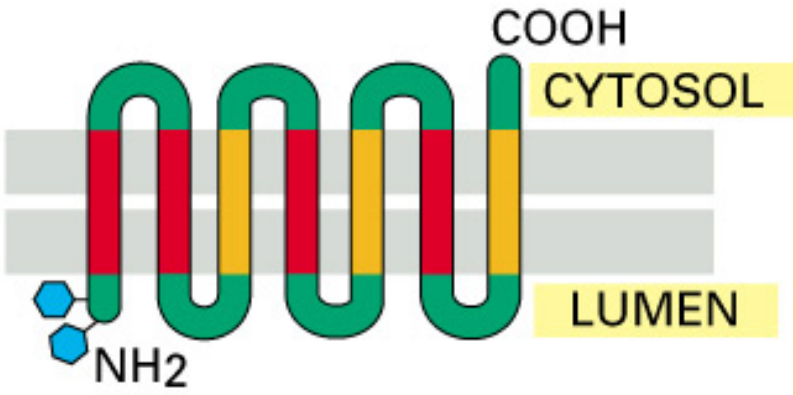


Figure 12–50. Molecular Biology of the Cell, 4th Edition.

The ER is an impressive factory

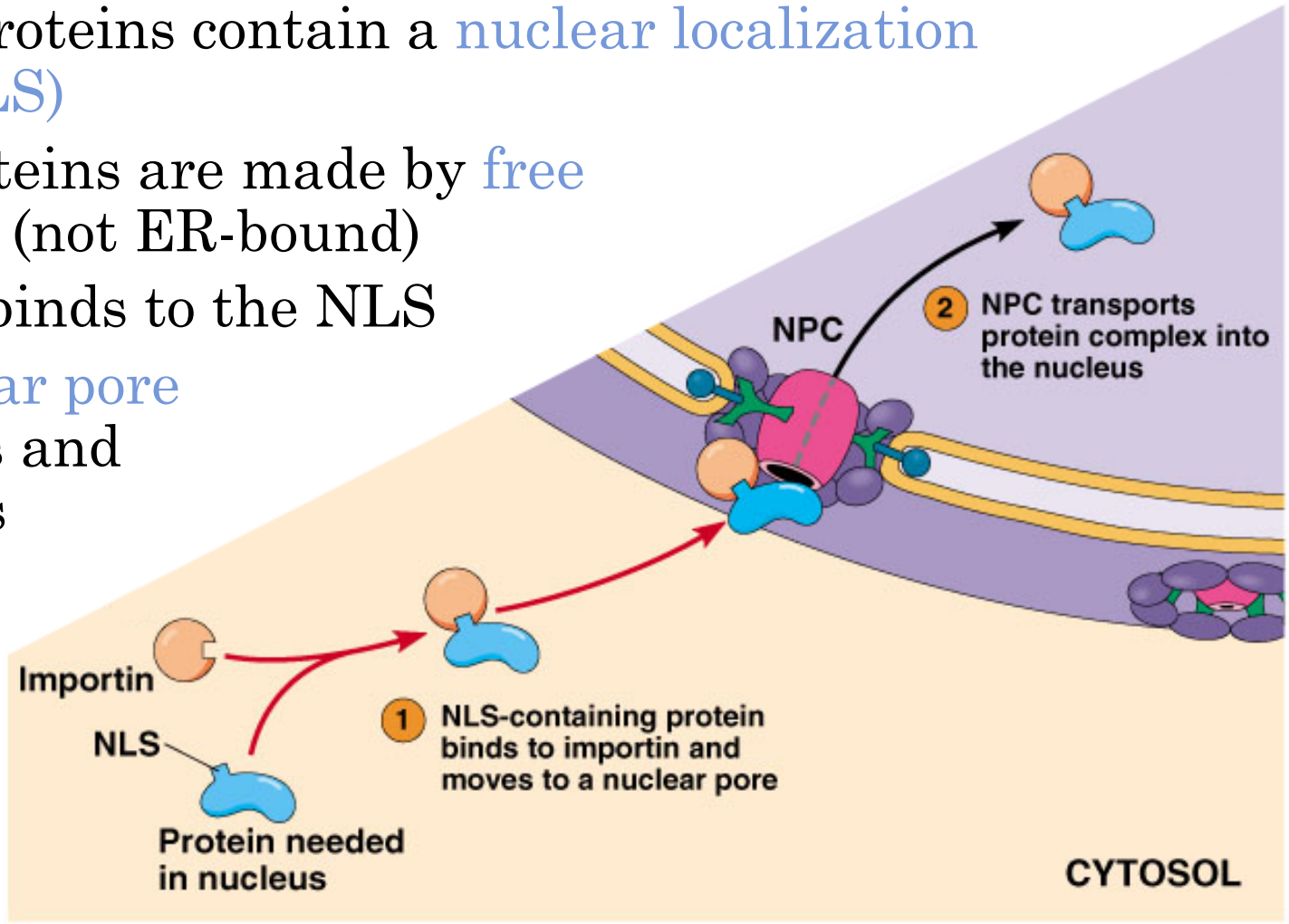
- **Lipid synthesis**
- **Secretory protein synthesis**
- **Integral membrane protein synthesis**
- **Protein folding**
- **Post-translational modification**
- **Protein degradation**

Inside ER Lumen

- **Proteins are not folded immediately**
- **Chaperon proteins keep them unfolded**
- **Chaperons have slow ATPase activity**
- **ADP Chaperons have high affinity for unfolded proteins**
- **BiP (binding proteins) is a major chaperon**
- **78 kD hsp family protein**
- **ER lumen also contains proteins and factors required for folding**

Post-translational import into the nucleus

- Nuclear proteins contain a nuclear localization signal (NLS)
- These proteins are made by free ribosomes (not ER-bound)
- Importin binds to the NLS
- The nuclear pore recognizes and transports importin and the protein



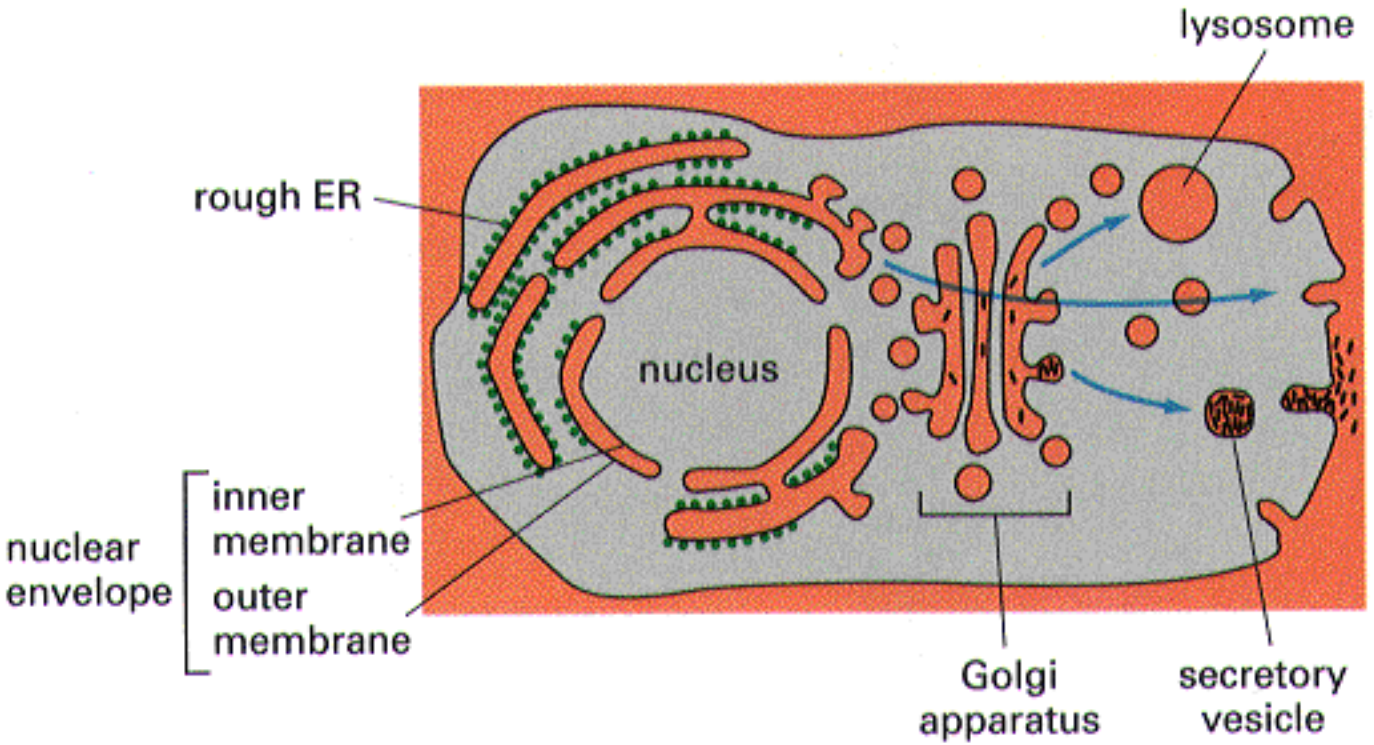
Golgi Apparatus

- **Major sorting centre - GPO of cell**

  Made of 6 cisternae
  Cis (importing end)
  Medial
  Trans (exporting end)

- **Transport vesicle mediate transfer b/w ER and golgi**
- **Small GTP binding proteins, coat proteins etc play a key role in vesicular transport**

Topology of eukaryotic organelles

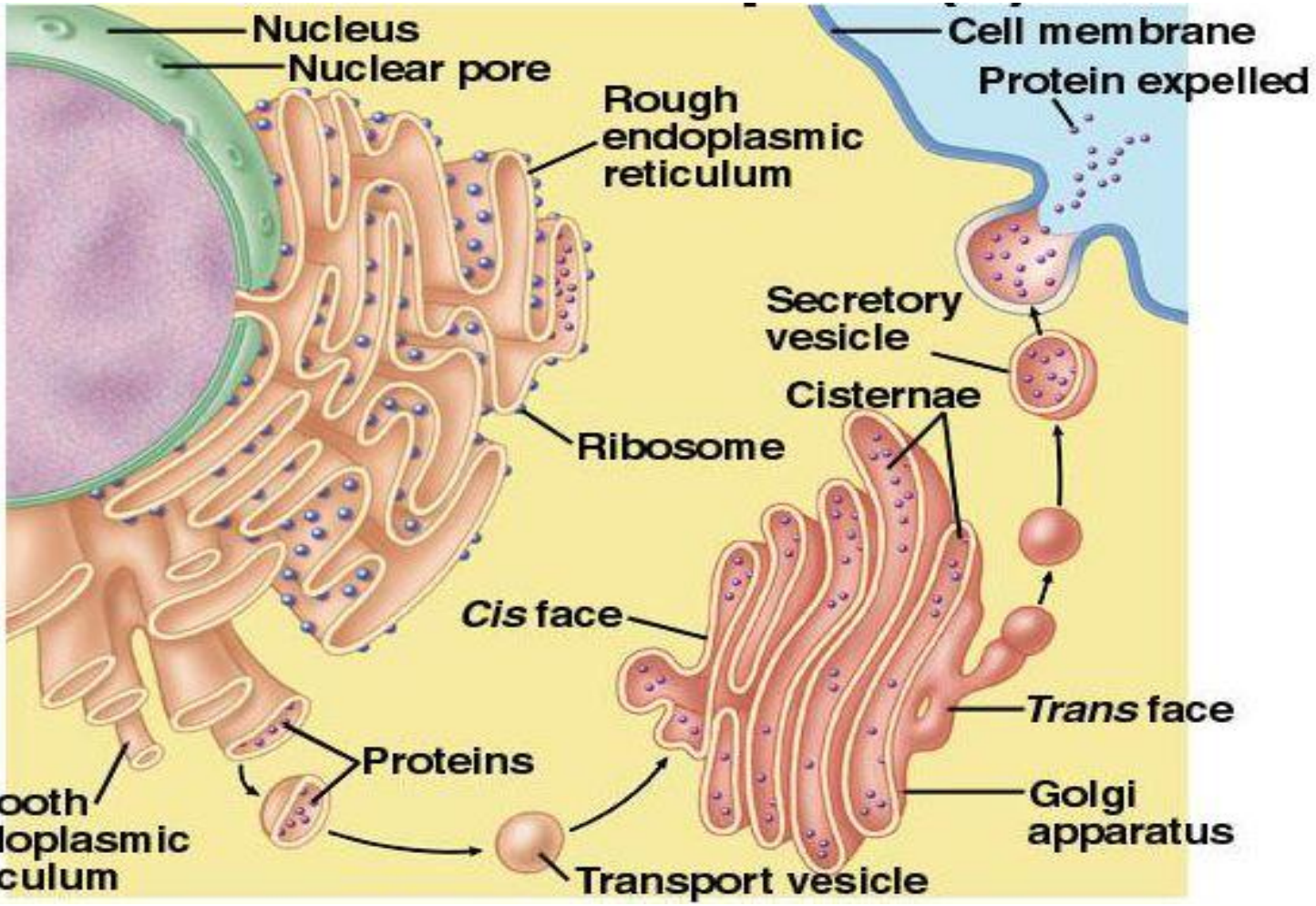


Lysosomal Targeting

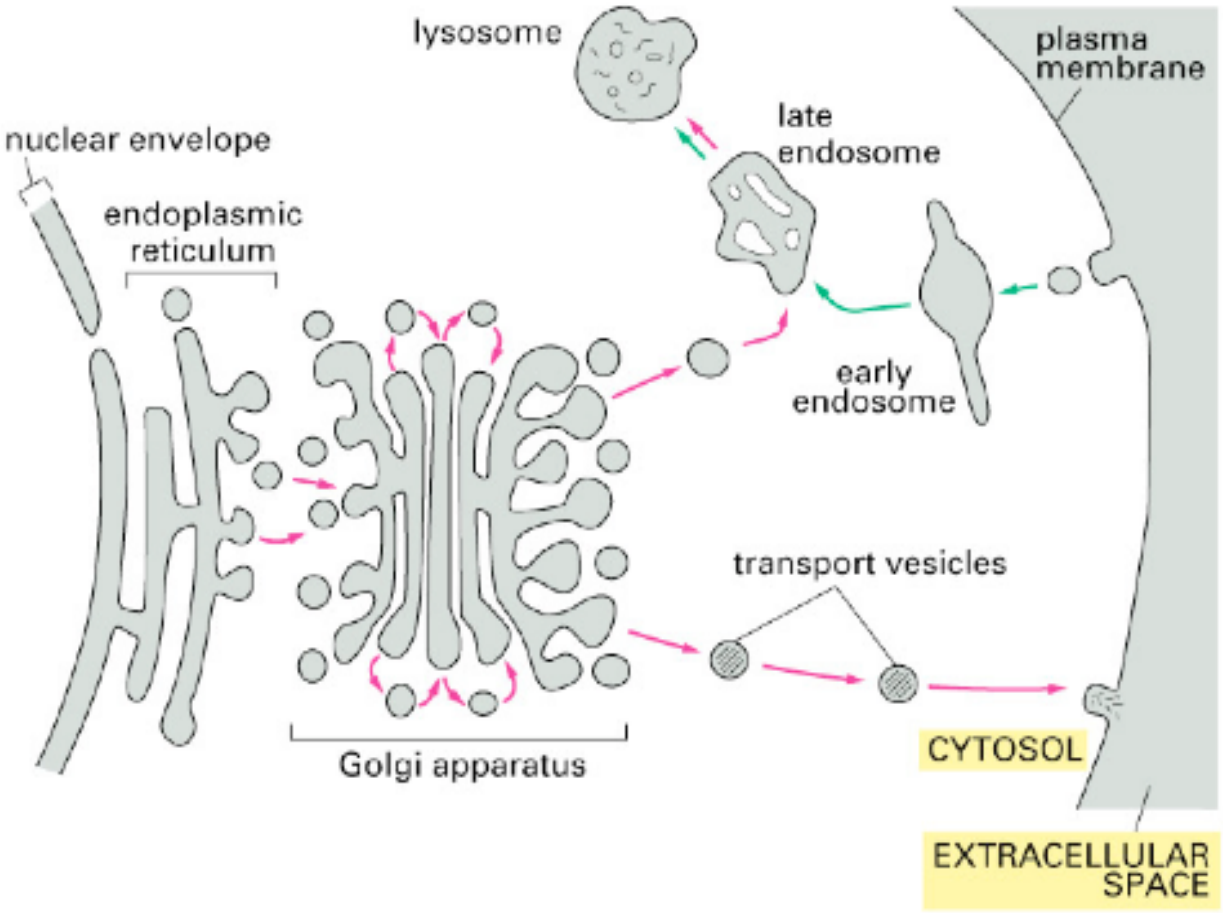
- Man-6 P is the marker, added in cis golgi
- Added by 2 step enzyme catalysed reaction

Phosphotransferase
Phosphodiesterase

- Man-6 P receptors in trans golgi
- Fuses with pre lysosomal vesicles, acidic pH release proteins from receptors
- I Cell disease- severe psychomotor retardation


Nucleus
Nuclear pore
Rough endoplasmic reticulum
Ribosome
Cell membrane
Protein expelled
Secretory vesicle
Cisternae
Cis face
Trans face
Golgi apparatus
Proteins
Smooth endoplasmic reticulum
Transport vesicle

MECHANISM 3: VESICULAR TRANSPORT



TRANSPORT VESICLES

- Continually bud off from and fuse to other membrane compartments producing a constant flux of material
- Carry soluble proteins (in the lumen) and lipids & membrane proteins (in the bilayer) between compartments
- Are transported along microtubules by motor proteins

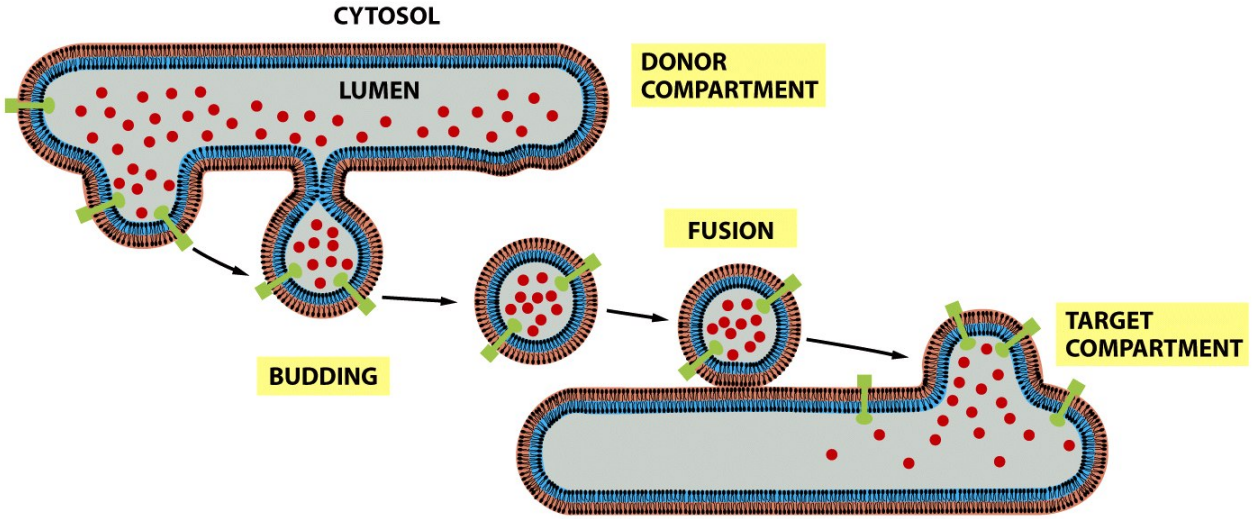
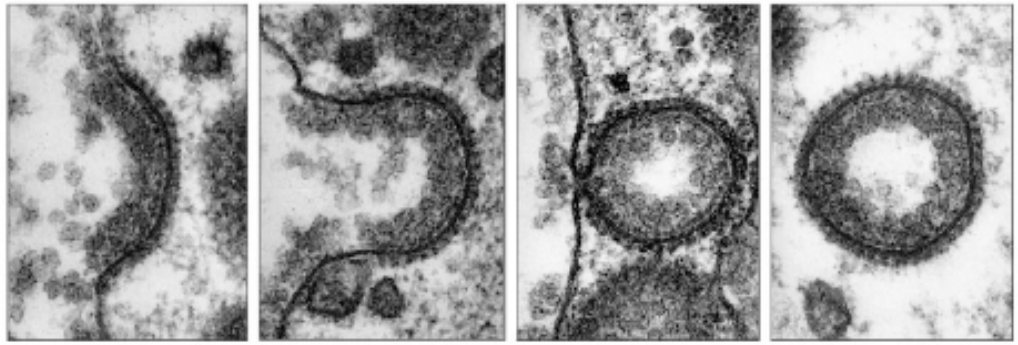
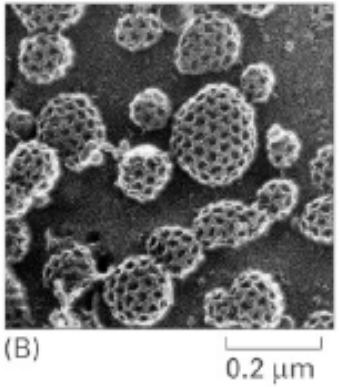


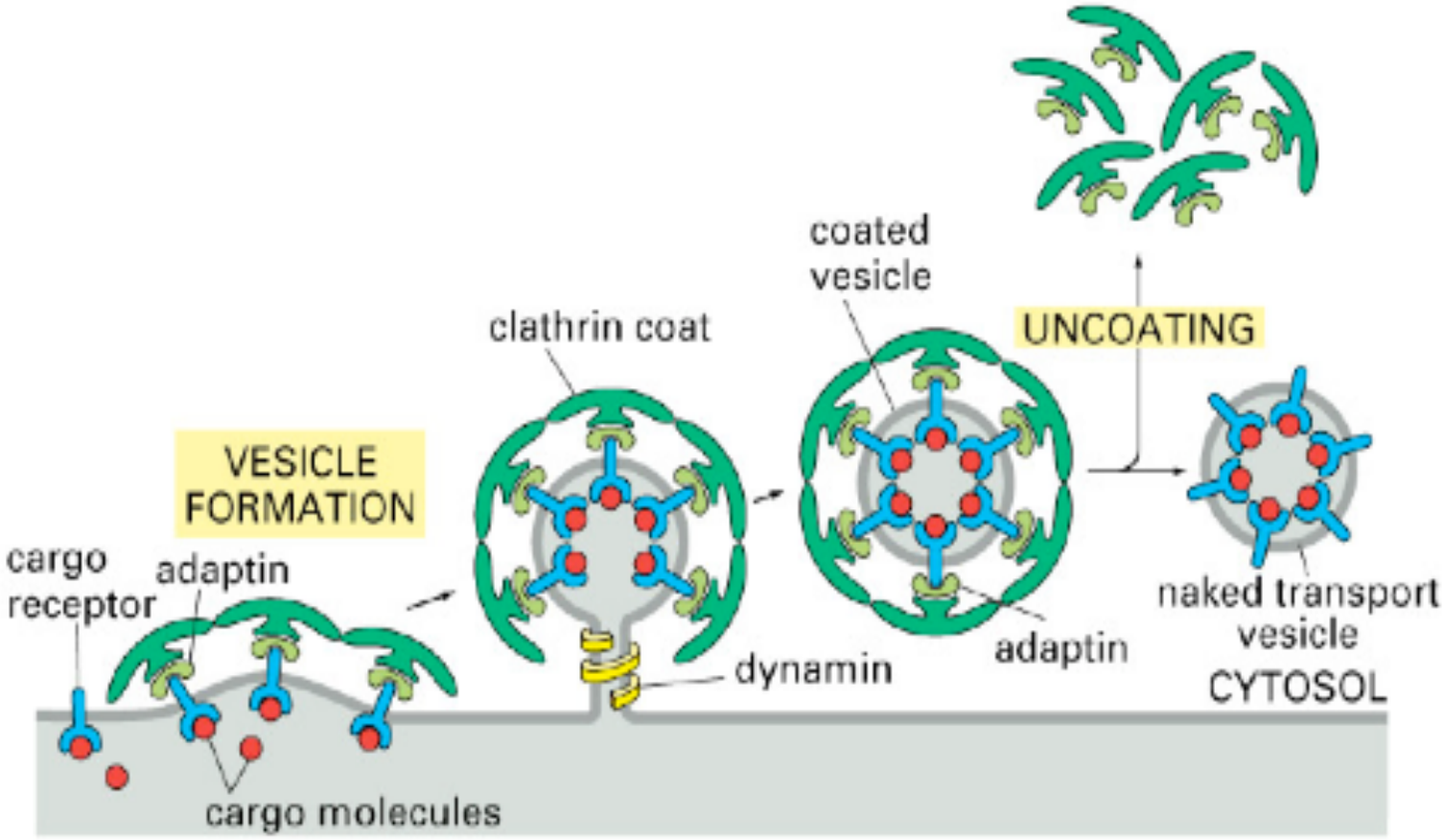
Figure 13-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Vesicle budding is driven by assembly of a protein coat

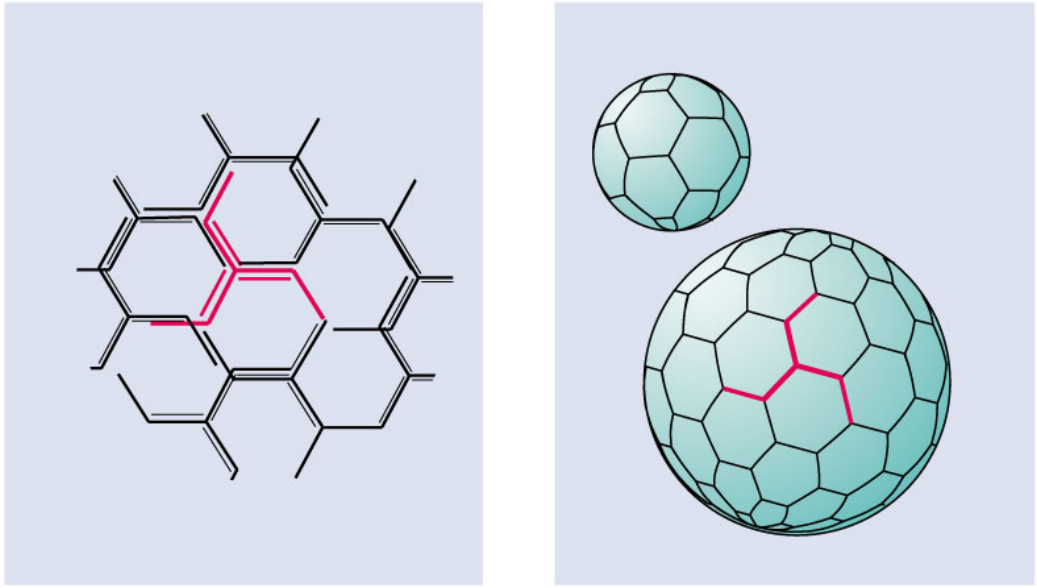




Clathrin-coated vesicles transport selected cargo molecules

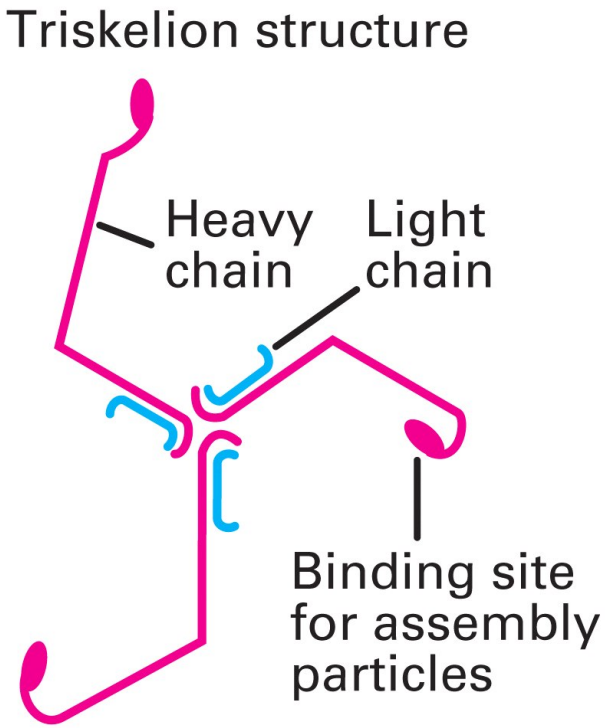


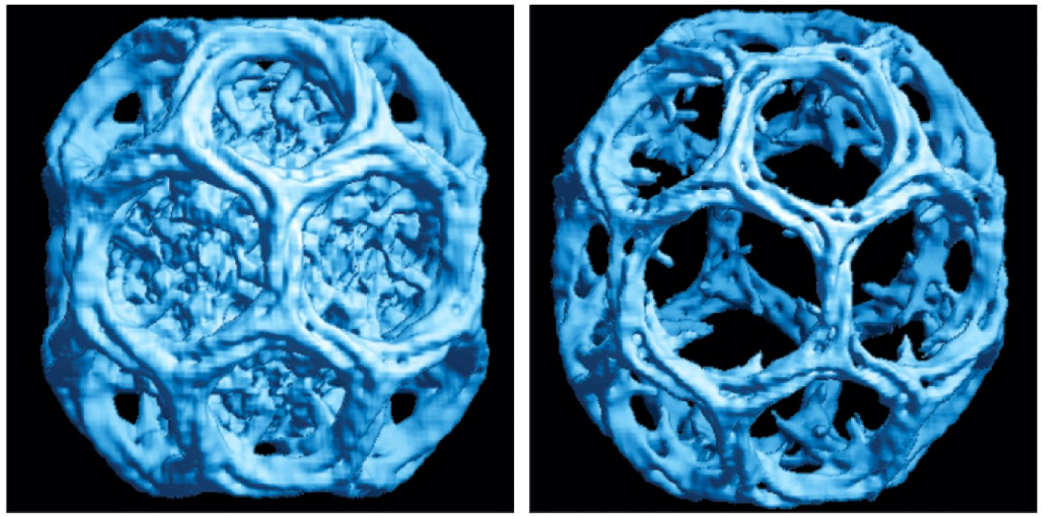
Structure of a clathrin-coated vesicle



- Clathrin
  - Forms triskelions
  - Consists of three heavy chains and three light chains.
- Coats also contain adaptor proteins that link membrane receptors to the clathrin coat.
- Assembly causes the budding of a coated vesicle, completely enclosed by clathrin
- This is often called "receptor-mediated endocytosis"

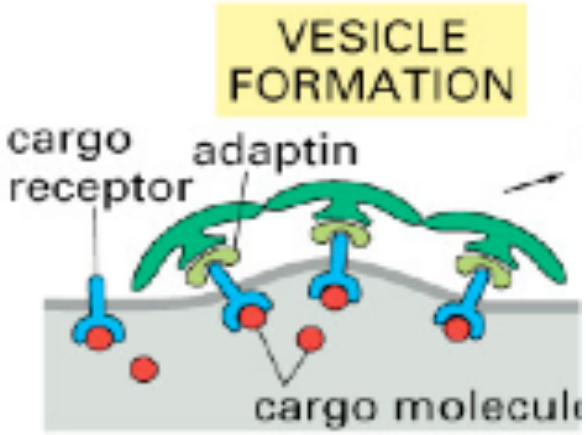
Complexes of clathrin form a basket around vesicles and help them to pinch from membranes





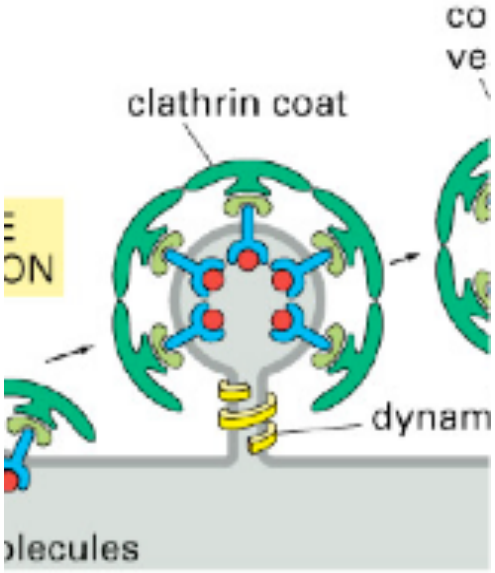
Step 1

- Cargo molecules (red) bind to transmembrane cargo receptors
- Cytoplasmic domains of receptors bind to adaptin (light green)which recruits clathrin
- Clathrin clusters cargo/receptor/adaptin complexes and induces curvature to the membrane - clathrin-coated pit



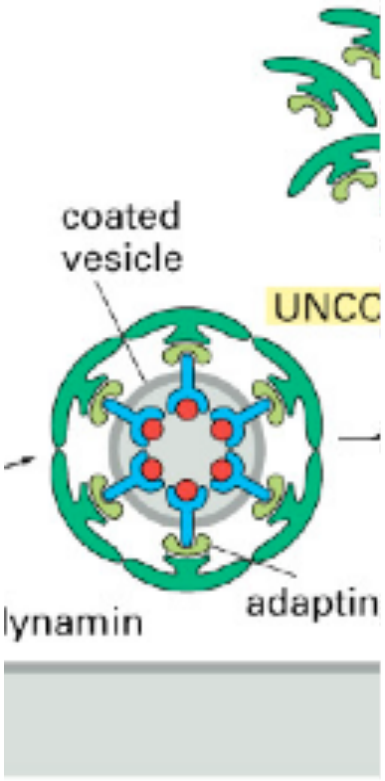
Step 2

- Additional clathrin molecules bind - increasing curvature
- Dynamin assembles a ring around each clathrin-coated pit



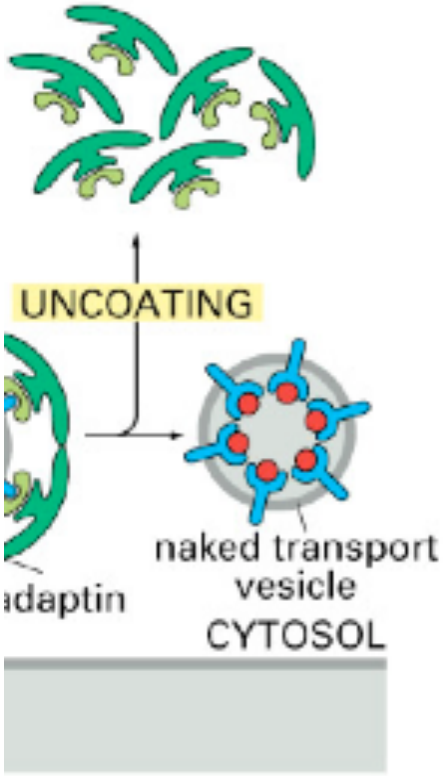
Step 3

- Dynamin rings constrict to "pinch" the membrane off
- Dynamin is a GTPase and used the energy released from GTP hydrolysis to power this reaction

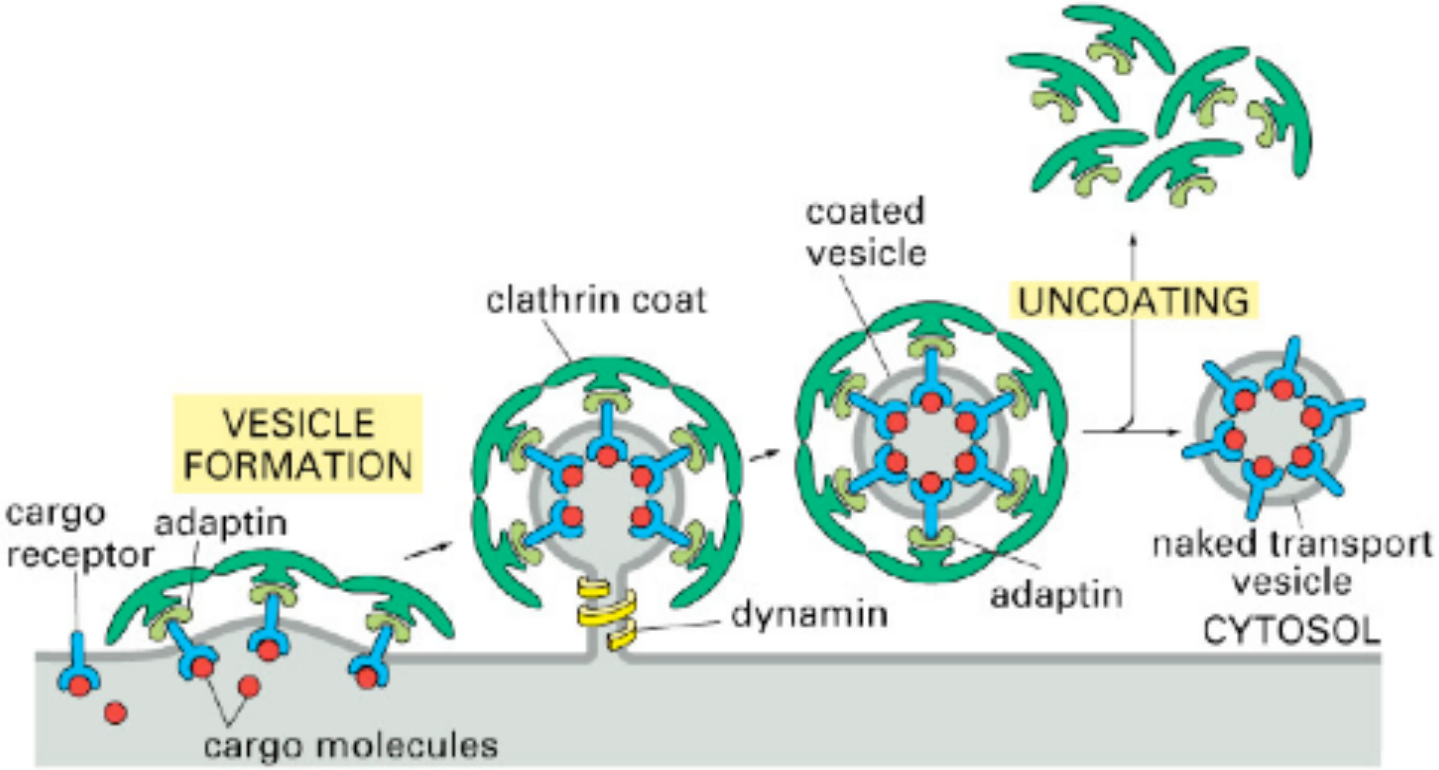


Step 4

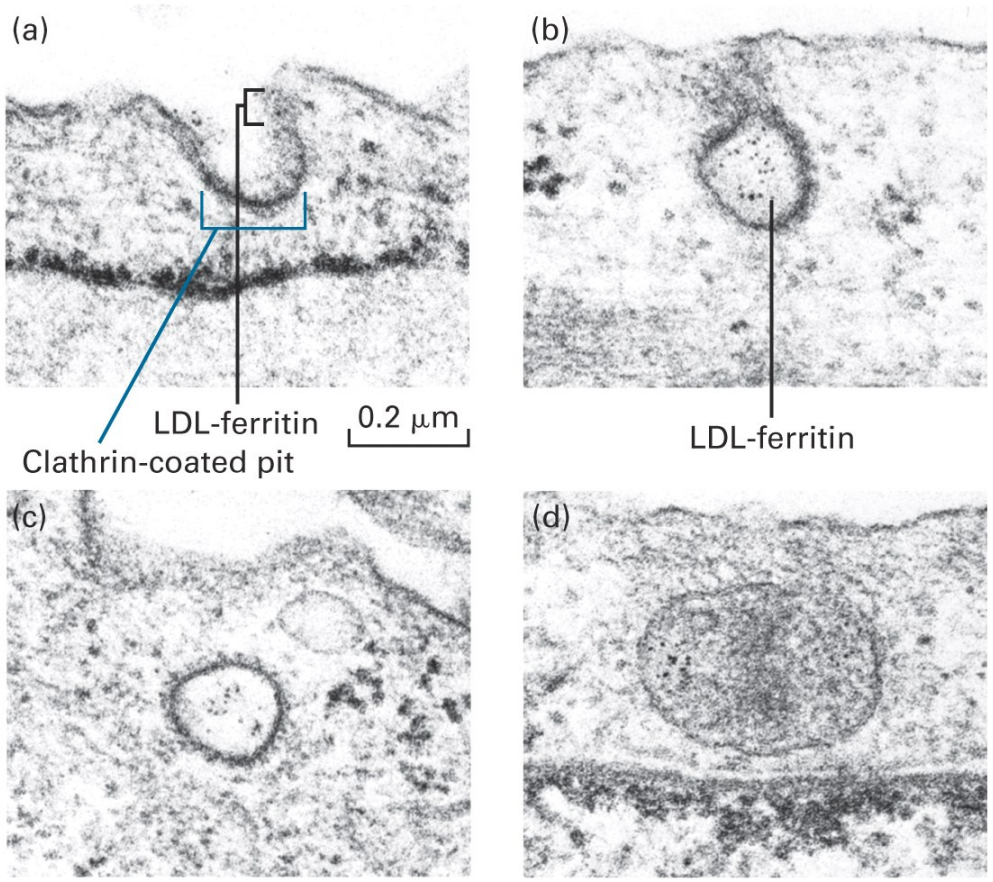
- The free vesicle sheds its coat of adaptin and clathrin
- Vesicles are transported to their destination on microtubules



Clathrin-coated vesicles transport selected cargo molecules



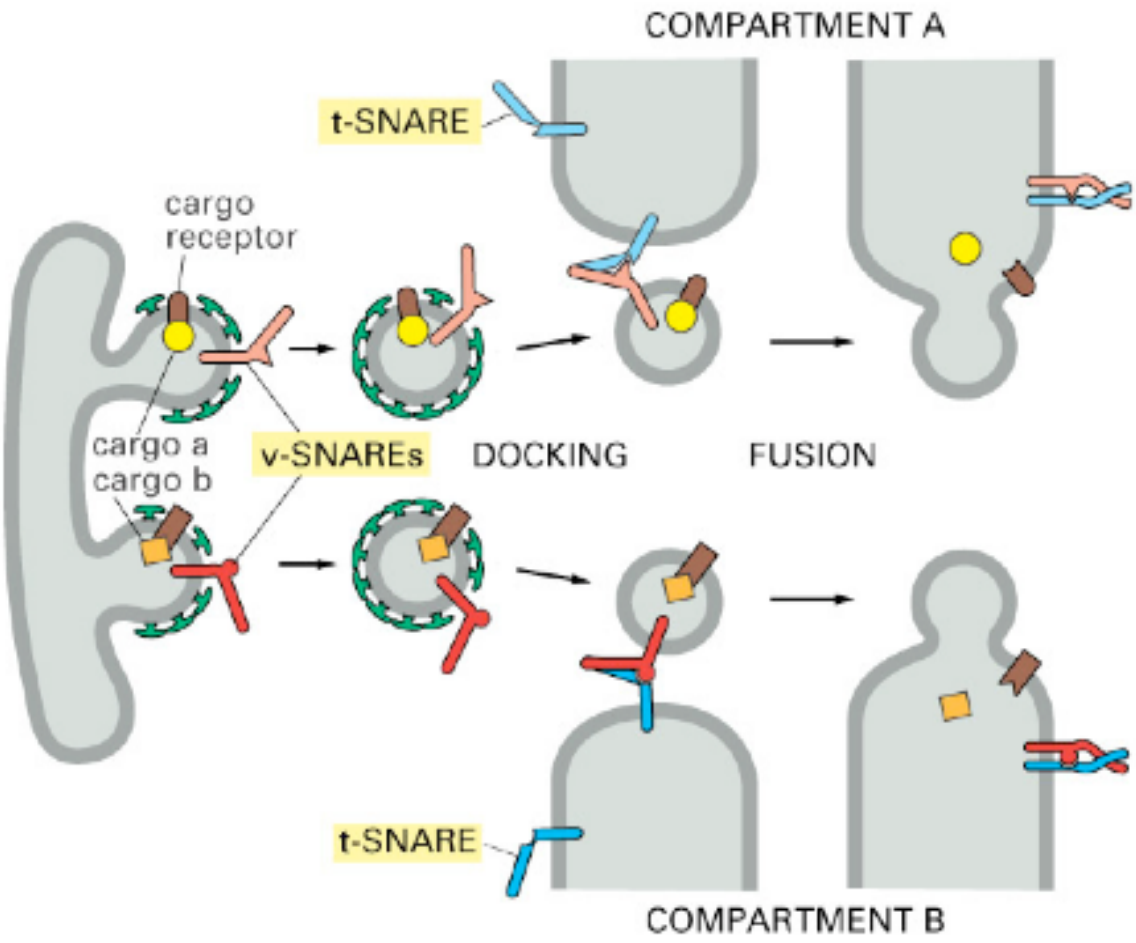
Clathrin-coated vesicles transport selected cargo molecules



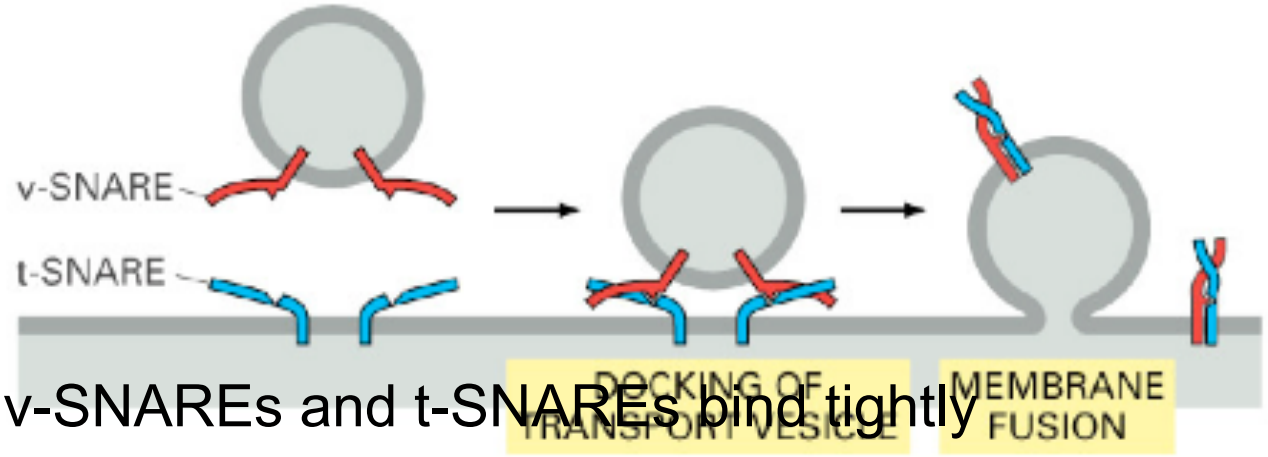
SNAREs are proteins that target vesicles to specific compartments

v-SNAREs are on vesicles

t-SNARES are on target compartments



SNARE PROTEINS ARE IMPORTANT FOR MEMBRANE FUSION



- v-SNAREs and t-SNAREs bind tightly
- Complexes bring the two membranes together to promote fusion