Protein Sorting

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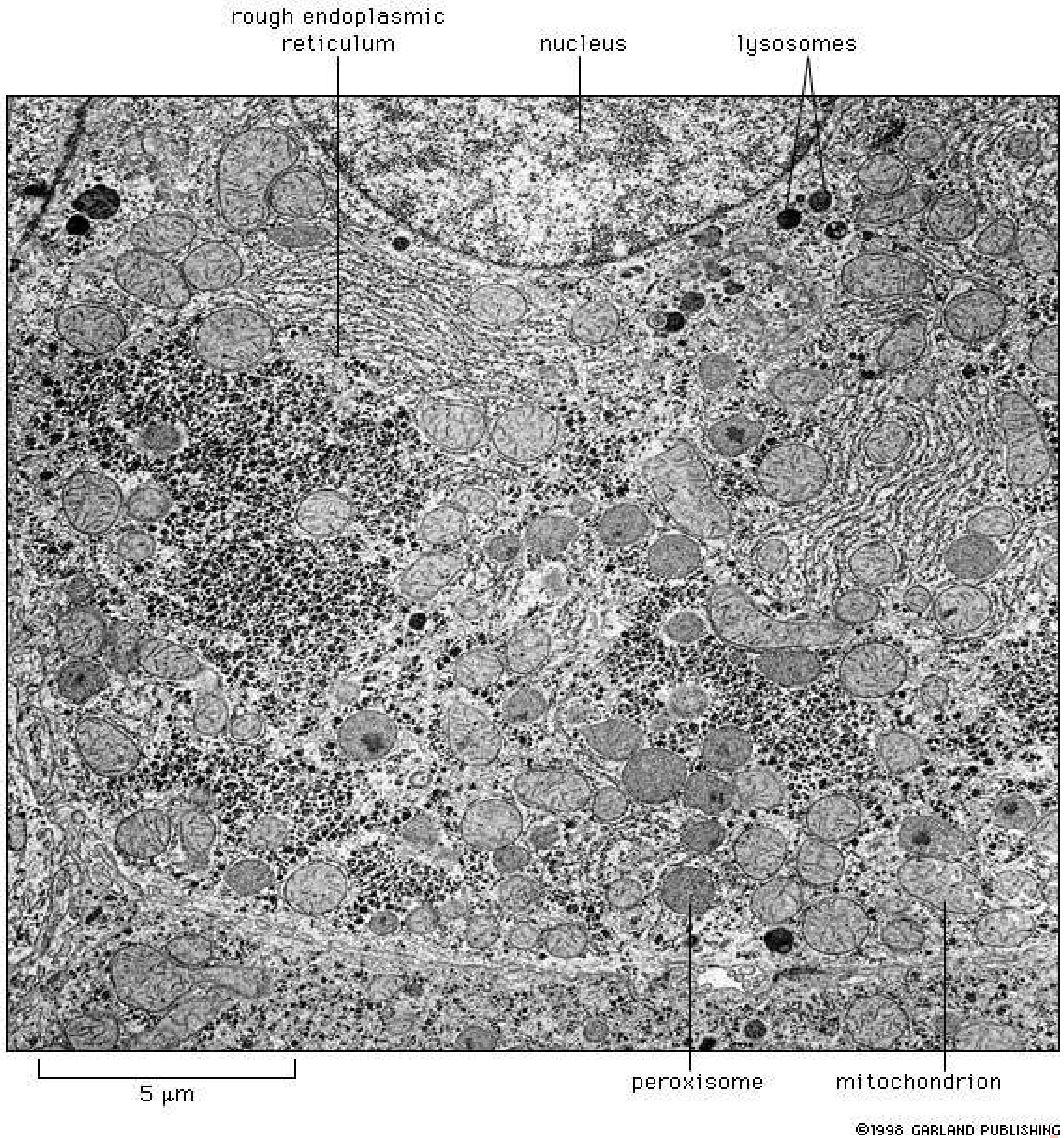
An electron micrograph (Eukaryotic cell)

Prokaryotic cell consists of a single compartment, the cytosol, enclosed by the plasma membrane.

Eukaryotic cell is subdivided by internal membranes - creating enclosed compartments where sets of enzymes can operate without interference.



The major intracellular compartments of an animal cell

- Each compartment contains a unique set of proteins.
- They are made in the cytosol and transferred selectively to the compartment in which they are used .
- **<u>Protein sorting</u>** depends on signals built into the amino acid sequence of the proteins.

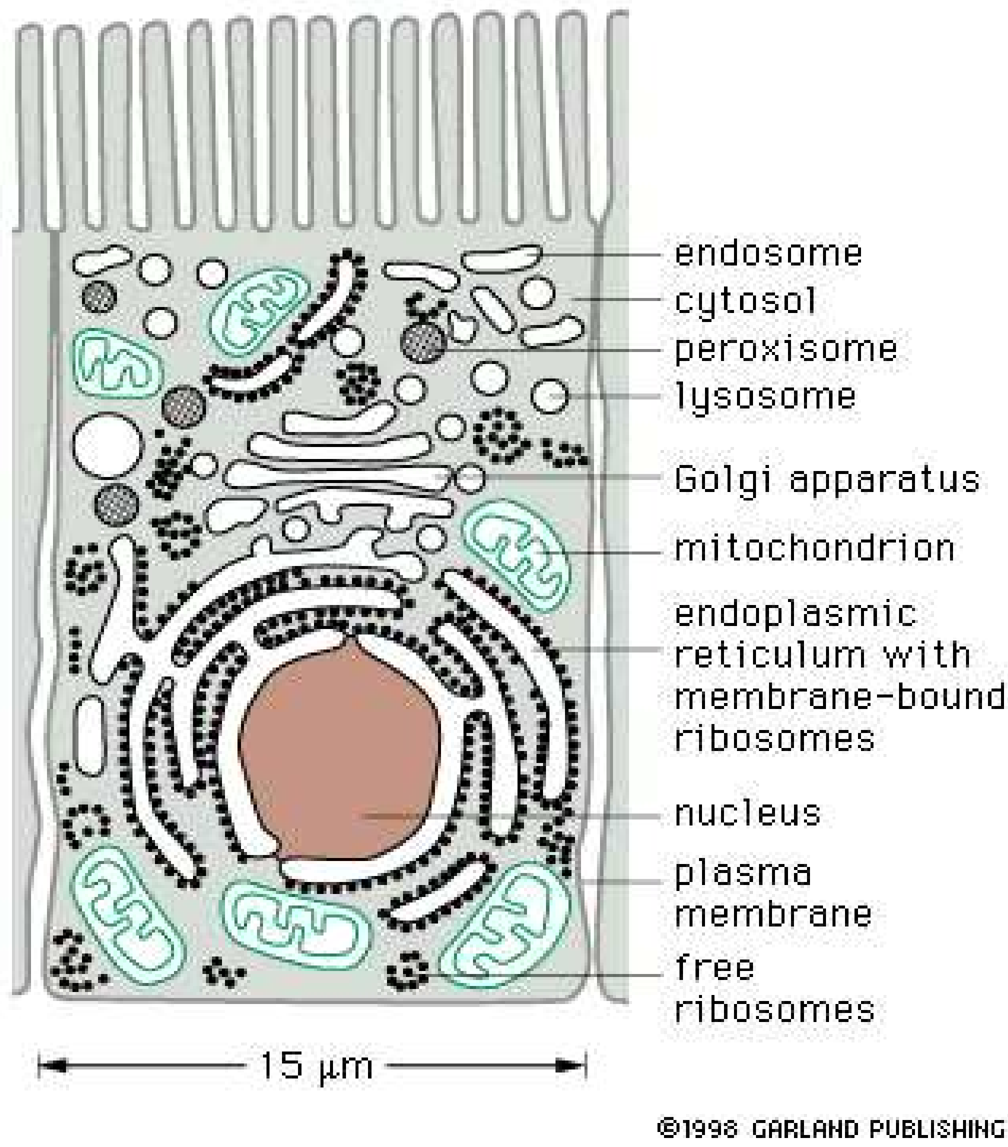


Table 14-2 The Relative Volumes Occupied by the Major Membrane-bounded Organelles in a Liver Cell (Hepatocyte)

Intracellular Compartment	Percent of Total Cell Volume	Approximate Number per Cell
Cytosol	54	1
Mitochondria	22	1700
Endoplasmic reticulum	12	1
Nucleus	6	1
Golgi apparatus	3	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

Table 14-1 The Main Functions of the Membrane-bounded Compartments of a Eucaryotic Cell

Compartment	Main Function
Cytosol	contains many metabolic pathways (Chapters 3 and 4); protein synthesis (Chapter 7)
Nucleus	contains main genome (Chapter 8); DNA and RNA synthesis (Chapters 6 and 7)
Endoplasmic reticulum (ER)	synthesis of most lipids (Chapter 11); synthesis of proteins for distribution to many organelles and to the plasma membrane (this chapter)
Golgi apparatus	modification, sorting, and packaging of proteins and lipids for either secretion or delivery to another organelle (this chapter)
Lysosomes	intracellular degradation (this chapter)
Endosomes	sorting of endocytosed material (this chapter)
Mitochondria	ATP synthesis by oxidative phosphorylation (Chapter 13)
Chloroplasts (in plant cells)	ATP synthesis and carbon fixation by photosynthesis (Chapter 13)
Peroxisomes	oxidation of toxic molecules

FOUR DISTINCT FAMILIES

1) The nucleus and the cytosol, which communicate through *nuclear pore complexes* and are thus topologically continuous (although functionally distinct);

2) All organelles that function in the secretory and endocytic pathways, including the ER, Golgi apparatus, endosomes, lysosomes, the numerous classes of transport intermediates such as transport vesicles, and possibly peroxisomes;

3) The mitochondria;

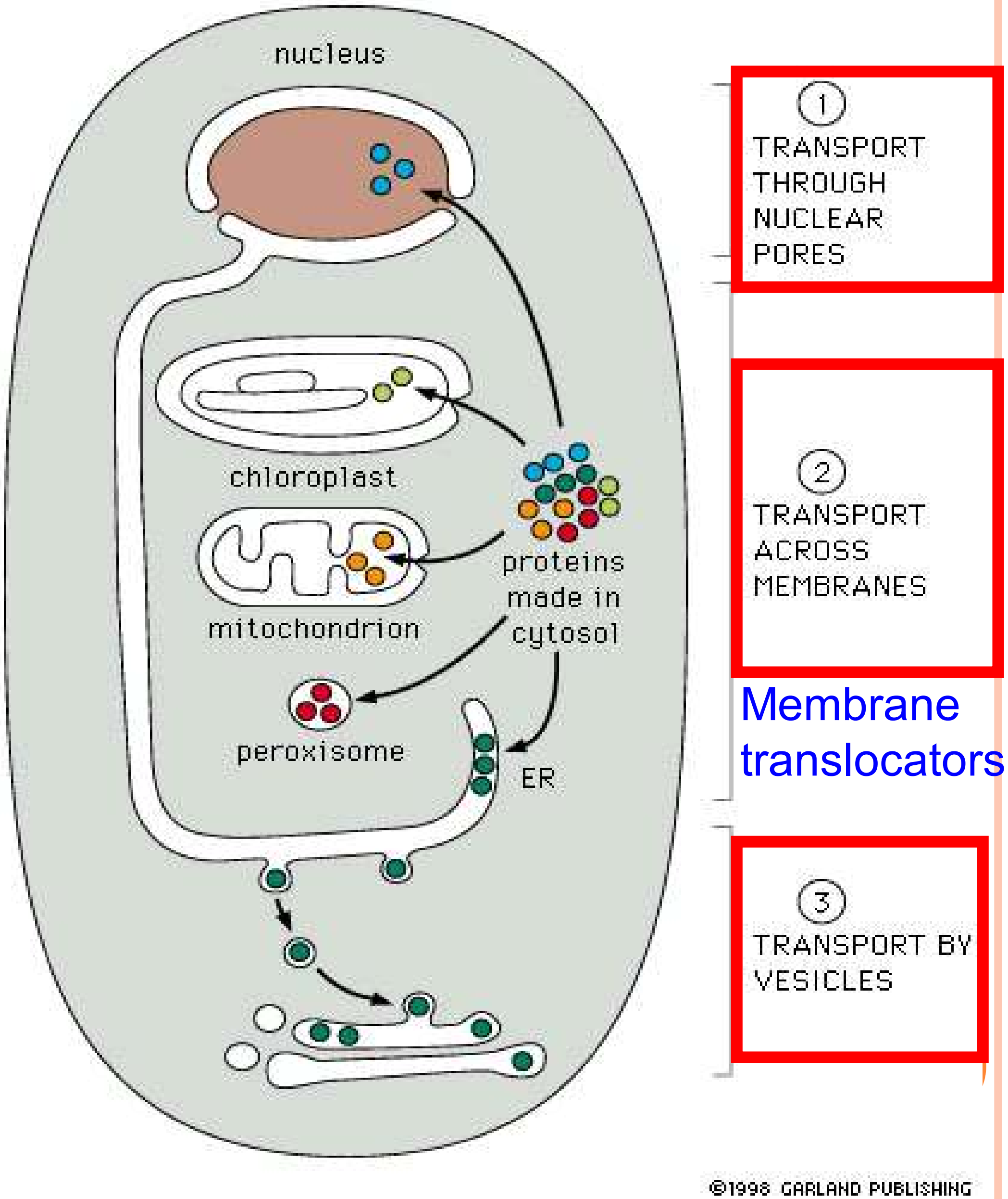
4) The plastids (in plants only).

PROTEIN SORTING

Membrane-bound organelles must be enlarged for cell division and maintained during the life of the cell.

Lipids and proteins must be sythesized and directed appropriately.

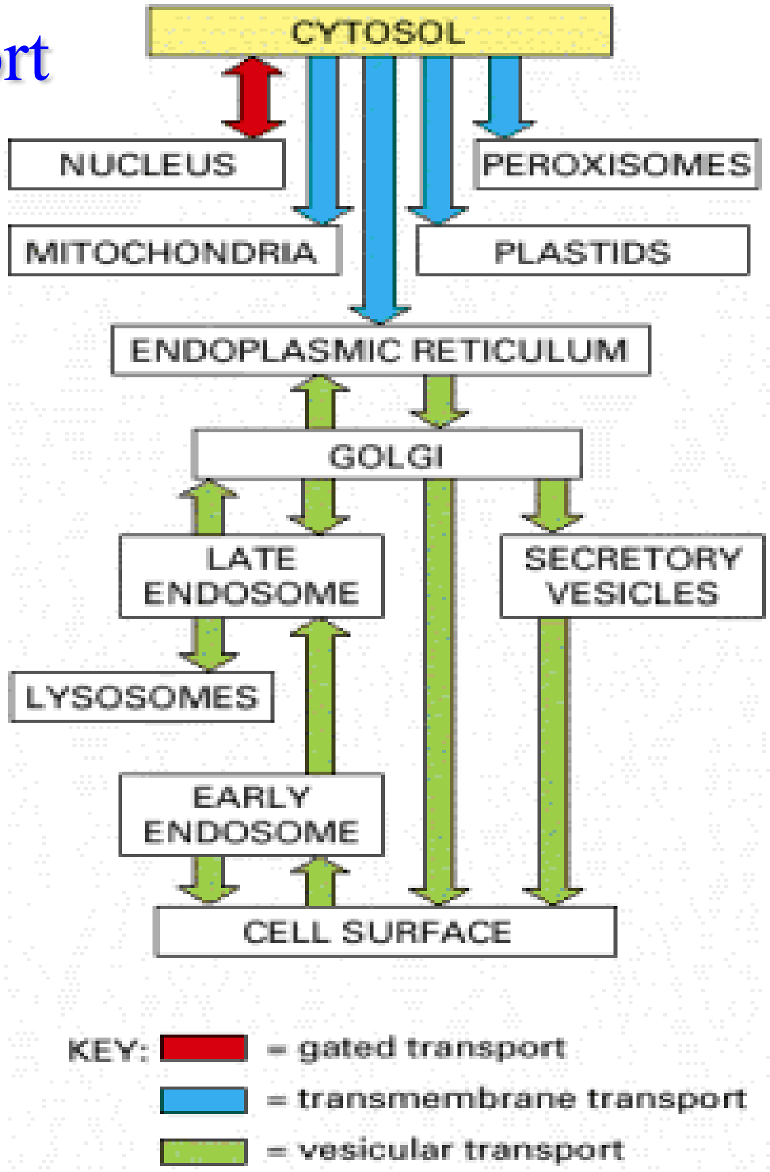
Nuclear envelope, ER, and Golgi break up into small vesicles which later coalase in the daughter cells.



Protein Traffic

Three basic modes of transport

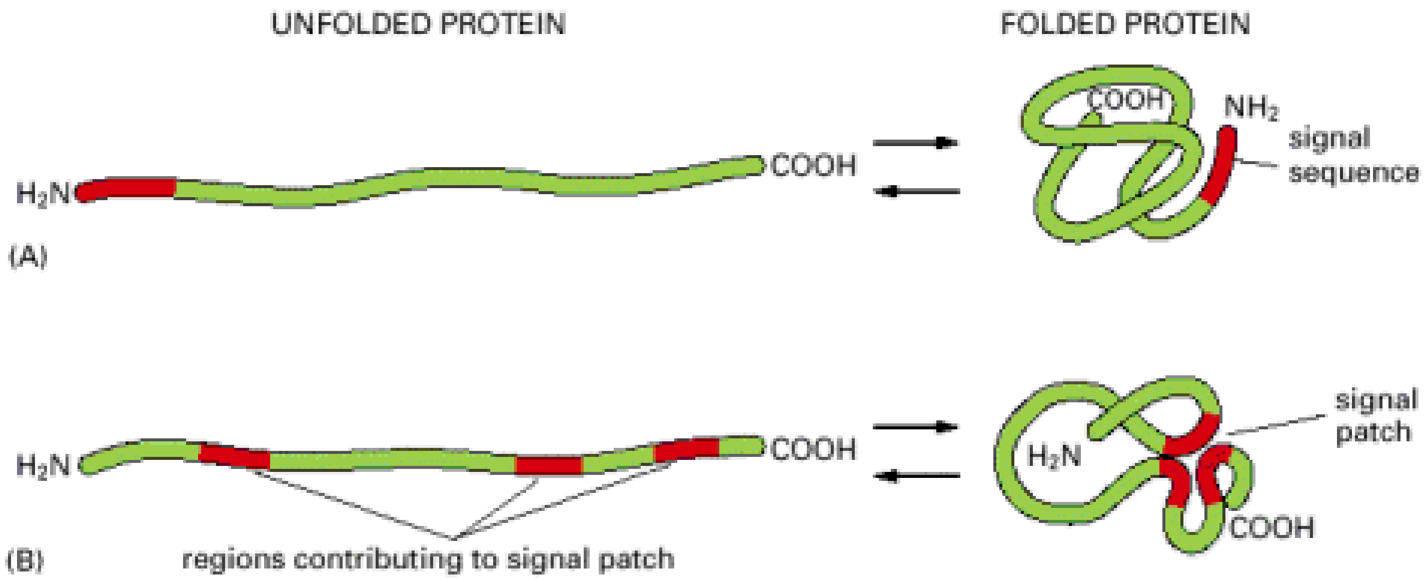
- Gated transport
- Transmembrane transport
- Vesicular transport
    - membrane-enclosed transport intermediates



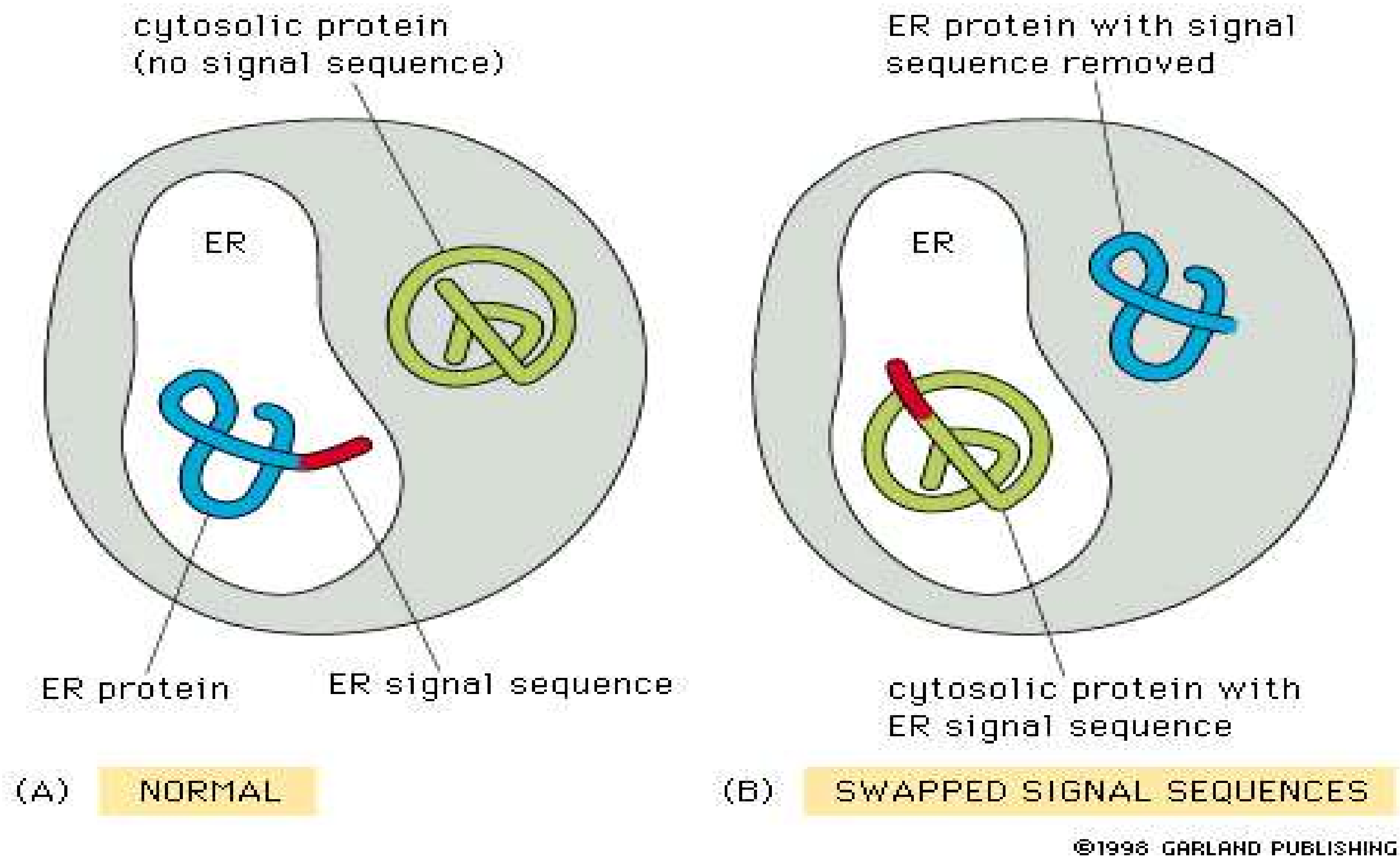
PROTEIN SORTING

- Synthesis of most proteins begins on ribosomes in the cytosol.
- Each protein contains a **sorting signal** which directs the protein to the organelle in which it is required.
- Lacking a sorting signal, proteins remain in the cytosol.
- Signal sequences specifying the same destination can vary greatly even though they have the same function.

SORTING SEQUENCES



Signal sequences are both necessary and sufficient to direct a protein to a particular organelle.

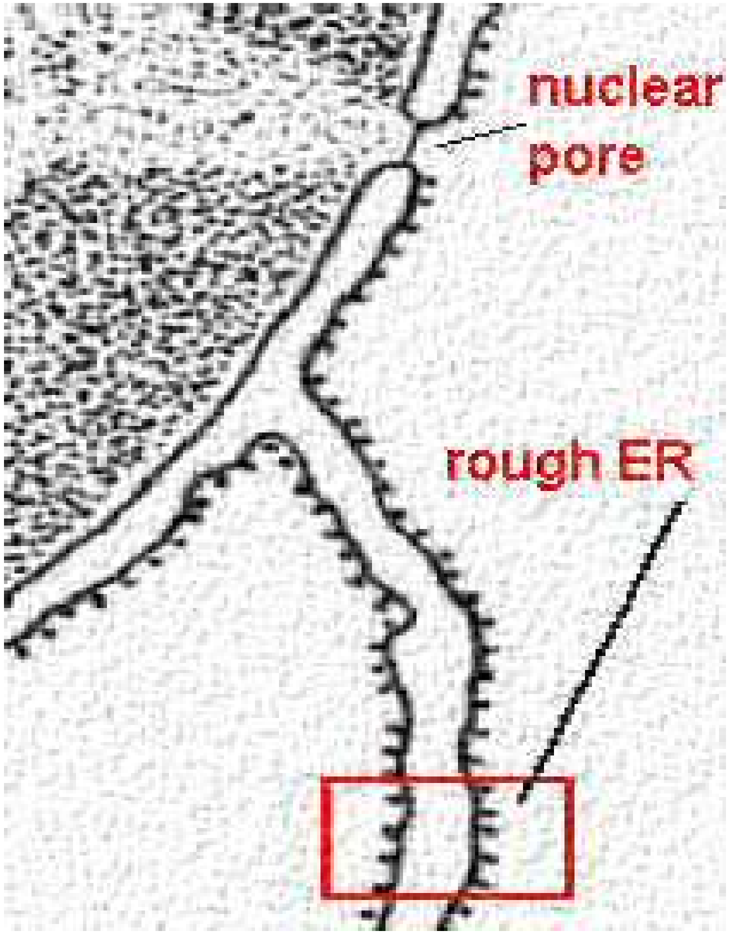


Some Sorting sequences

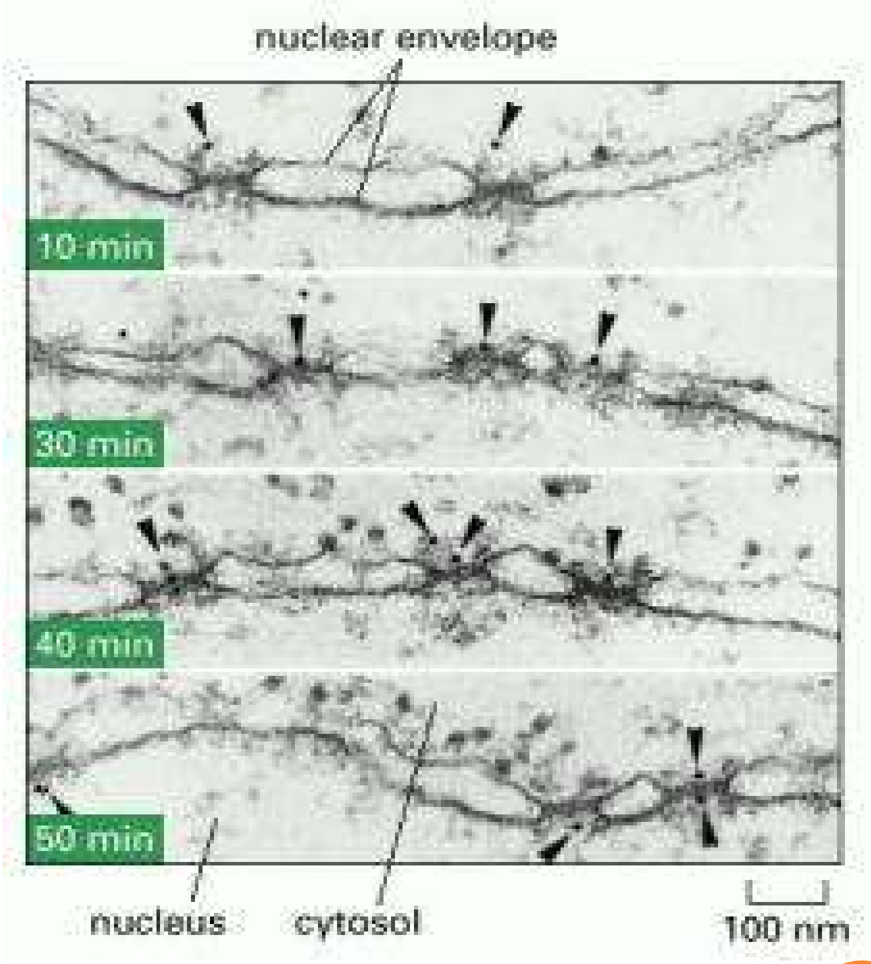
FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
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 *yellow* and hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^- indicates the C-terminus.

Nuclear Envelope



Protein import through Nuclear pores



MITOCHONDRIAL PROTEIN IMPORT

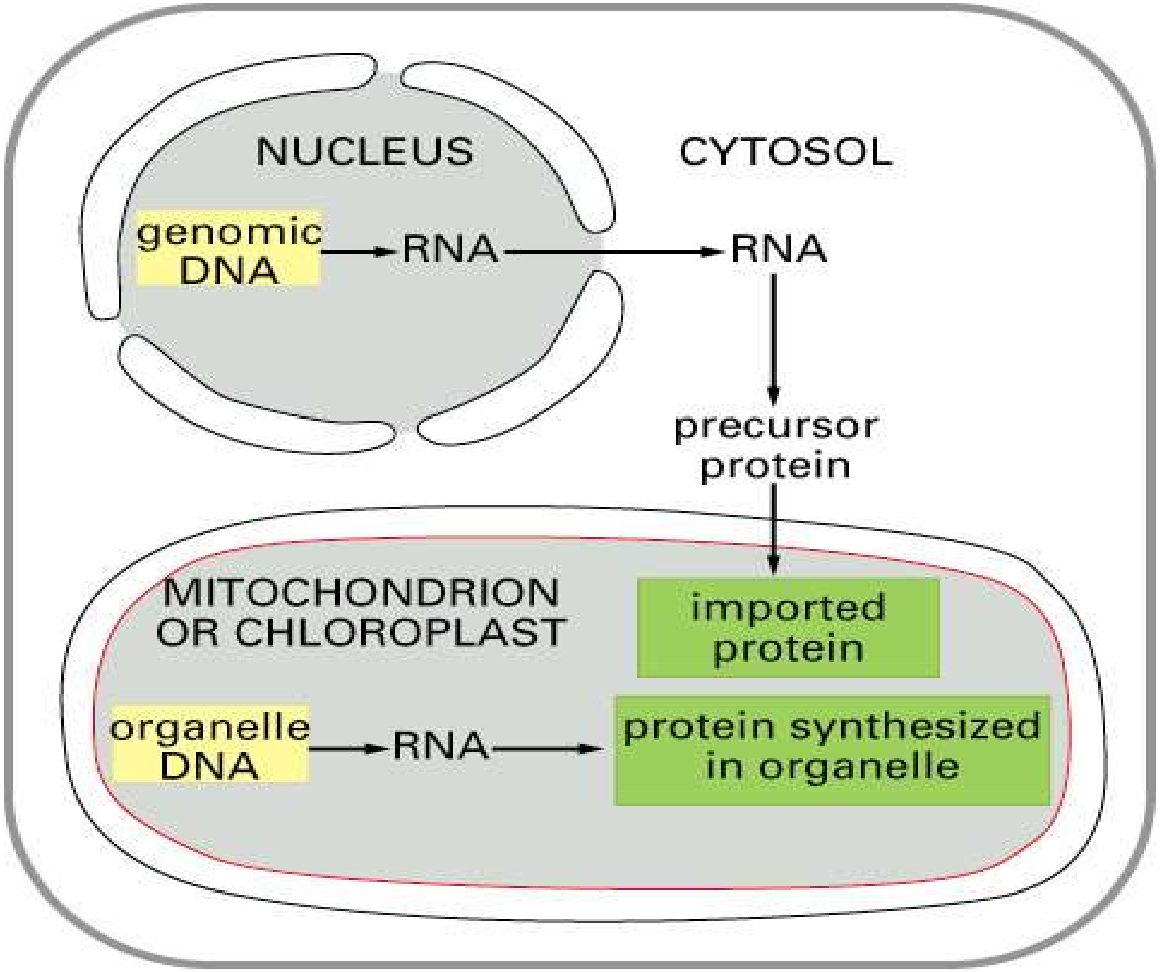
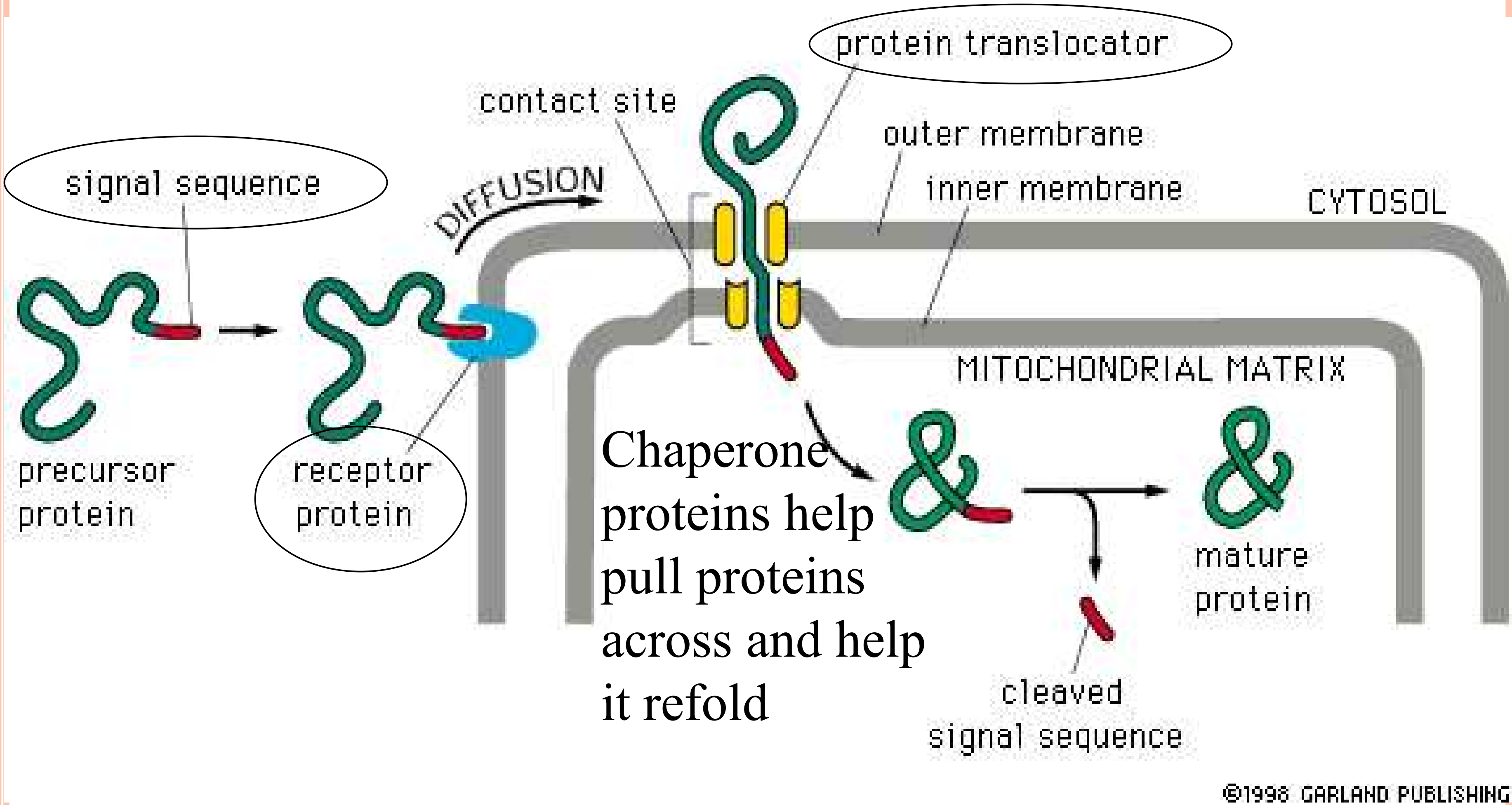
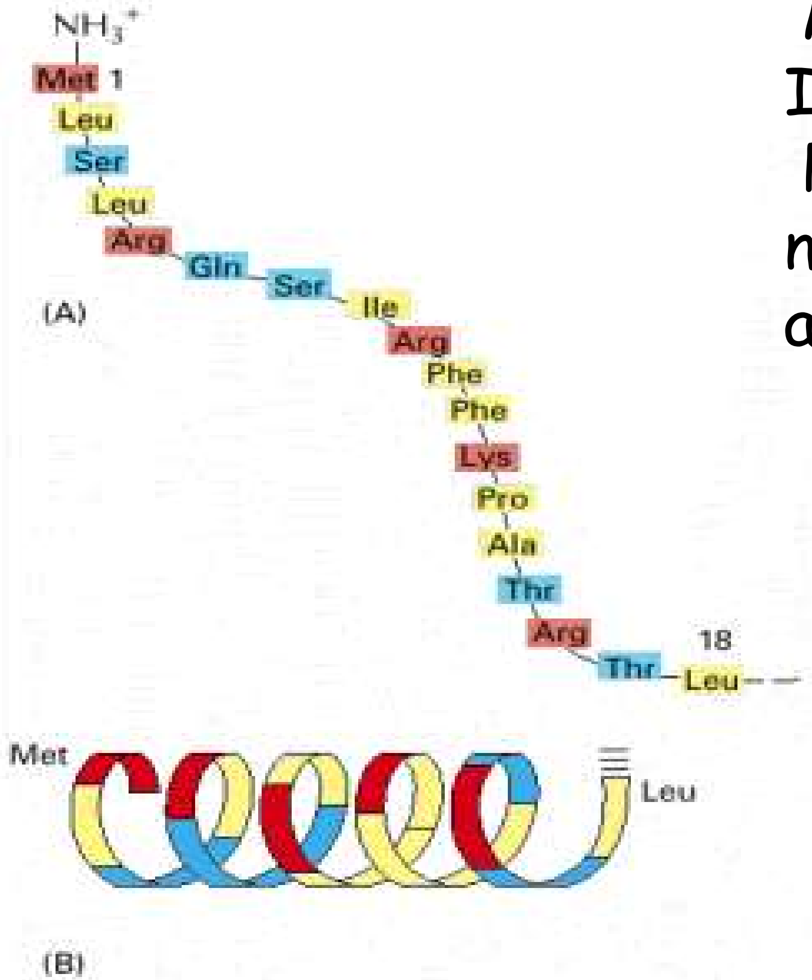


Figure 14–51. Molecular Biology of the Cell, 4th Edition.

Most mitochondrial and chloroplast proteins are encoded by nuclear genes and imported from the cytosol. Proteins unfold to enter mitochondria and chloroplasts. The protein is translocated simultaneously across both the inner and outer membranes at specialized sites where the two membranes are in contact with each other



A SIGNAL SEQUENCE FOR MITOCHONDRIAL PROTEIN IMPORT



Mitochondrial Import signal Polar (+) and non-polar stripe alpha-helix

PROTEIN TRANSLOCATORS IN THE MITOCHONDRIAL MEMBRANE

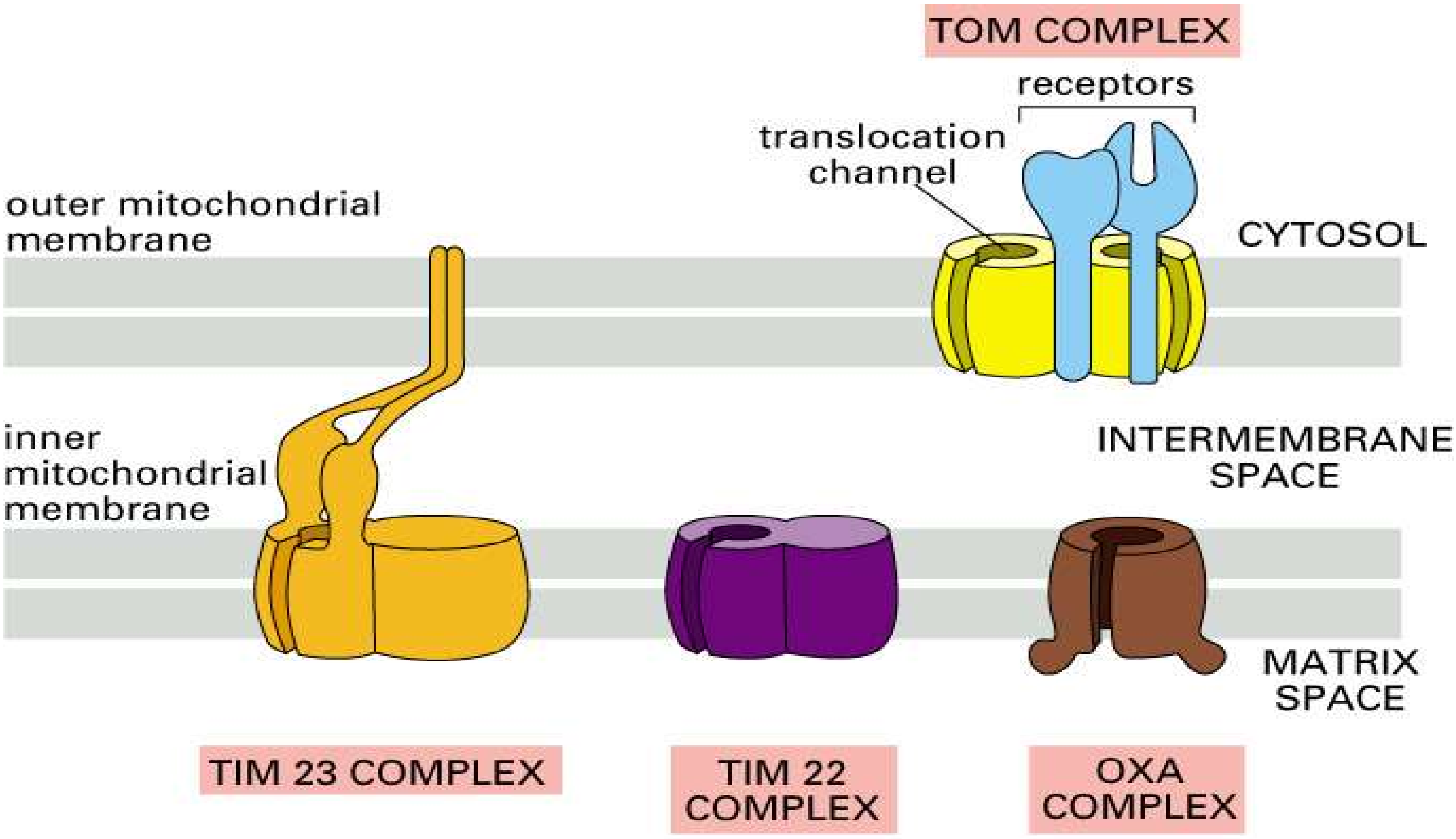


Figure 12–24. Molecular Biology of the Cell, 4th Edition.

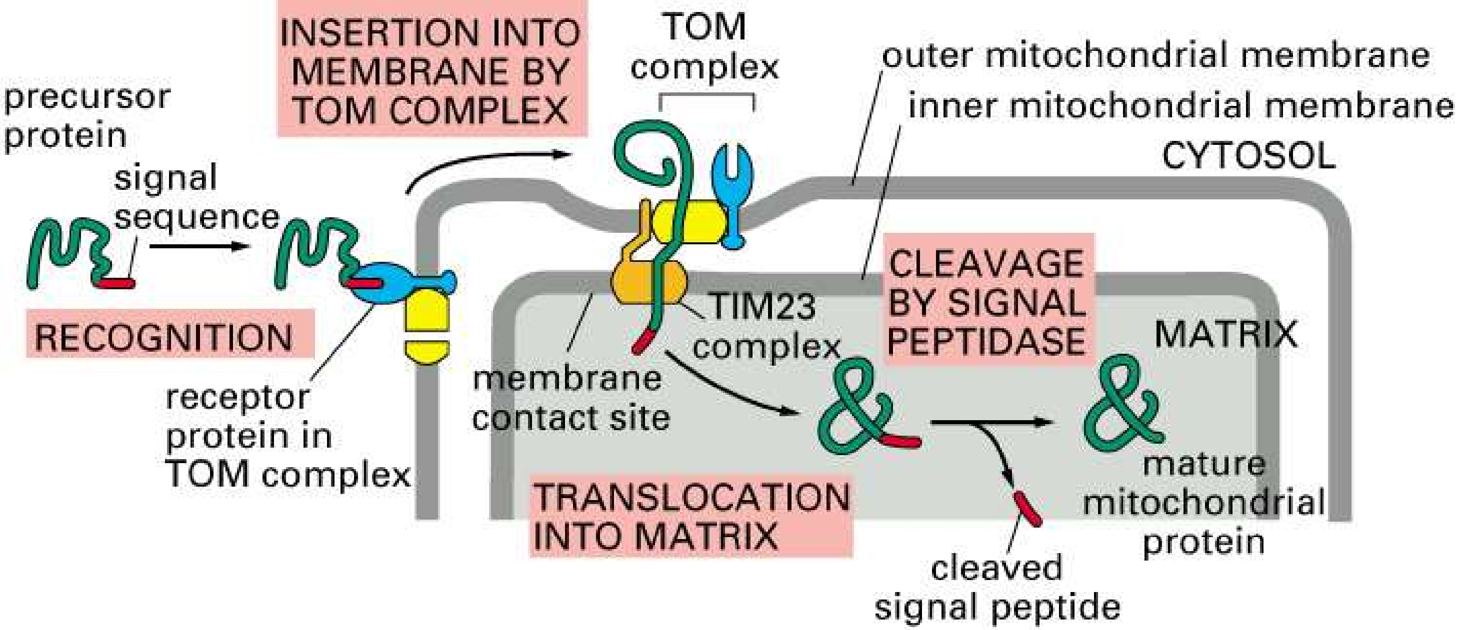


Figure 12–26. Molecular Biology of the Cell, 4th Edition.

<u>Import of the protein into the matrix is directed by an N-terminal signal sequence.</u>

•For polypeptides encoded by the nuclear genome, synthesis of the polypeptide is first completed in the cytosol. Transport occurs by a posttranslational mechanism.

•Signal sequence at the N-terminus associates with the TOM complex located in the outer mitochondrial membrane. TOM is both a receptor for the signal sequence and a translocase.

•The polypeptide is passed from TOM to TIM in the innermembrane. During the transport process, the polypeptide traverses both inner and outer membranes via the two translocators at a point known as a contact site.

•The polypeptide is imported and the signal peptide is removed by a signal peptidase.

Protein import from the cytosol into the inner mitochondrial membrane or intermembrane space

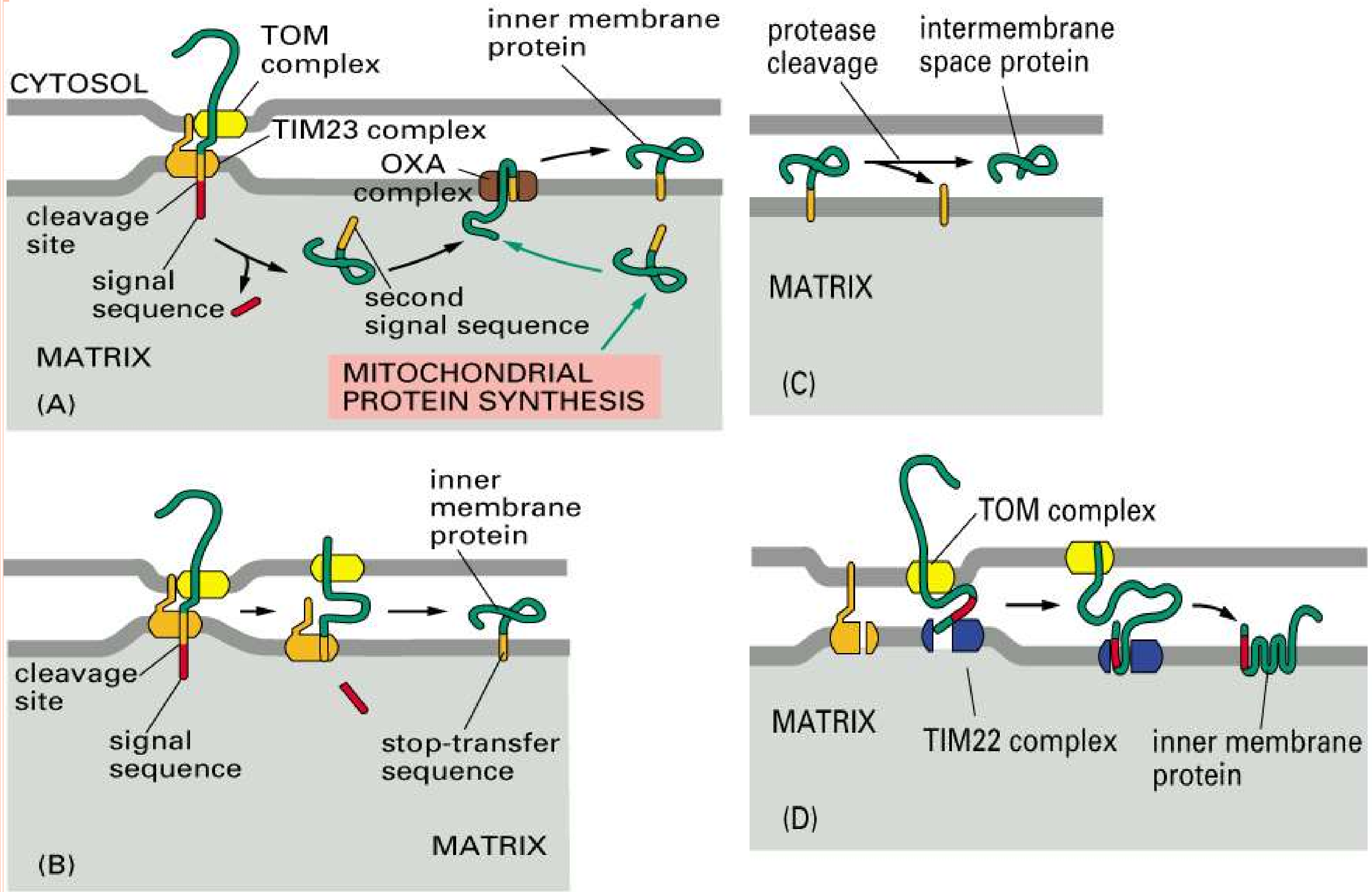


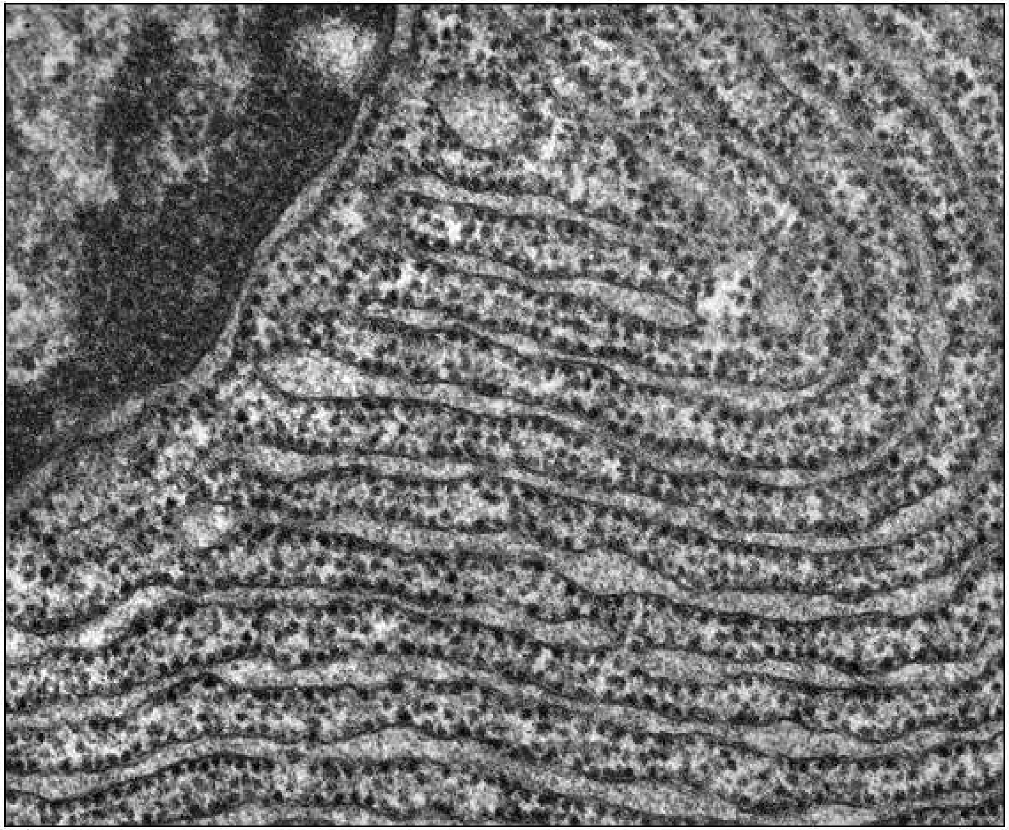
Figure 12–29 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 12–29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Comparison of mitochondrial and nuclear import.

	Nucleus	Mitochondria
Signal sequence	Short, positively charged, located anywhere	N-terminus, amphipathic alpha helix
Fate of the signal sequence	Unchanged after transport	Removed by signal peptidase
Energy	GTP hydrolysis	ATP hydrolysis and electrochemical proton gradient
Conformation of the transported protein	Folded	Unfolded

PROTEIN SORTING – ER



(B)

200 nm

ER is the most extensive membrane system in the cell.

The ER serves as an entry point for proteins destined for other organelles, as well as the ER.

Once enter inside the ER, proteins will not reenter the cytosol.

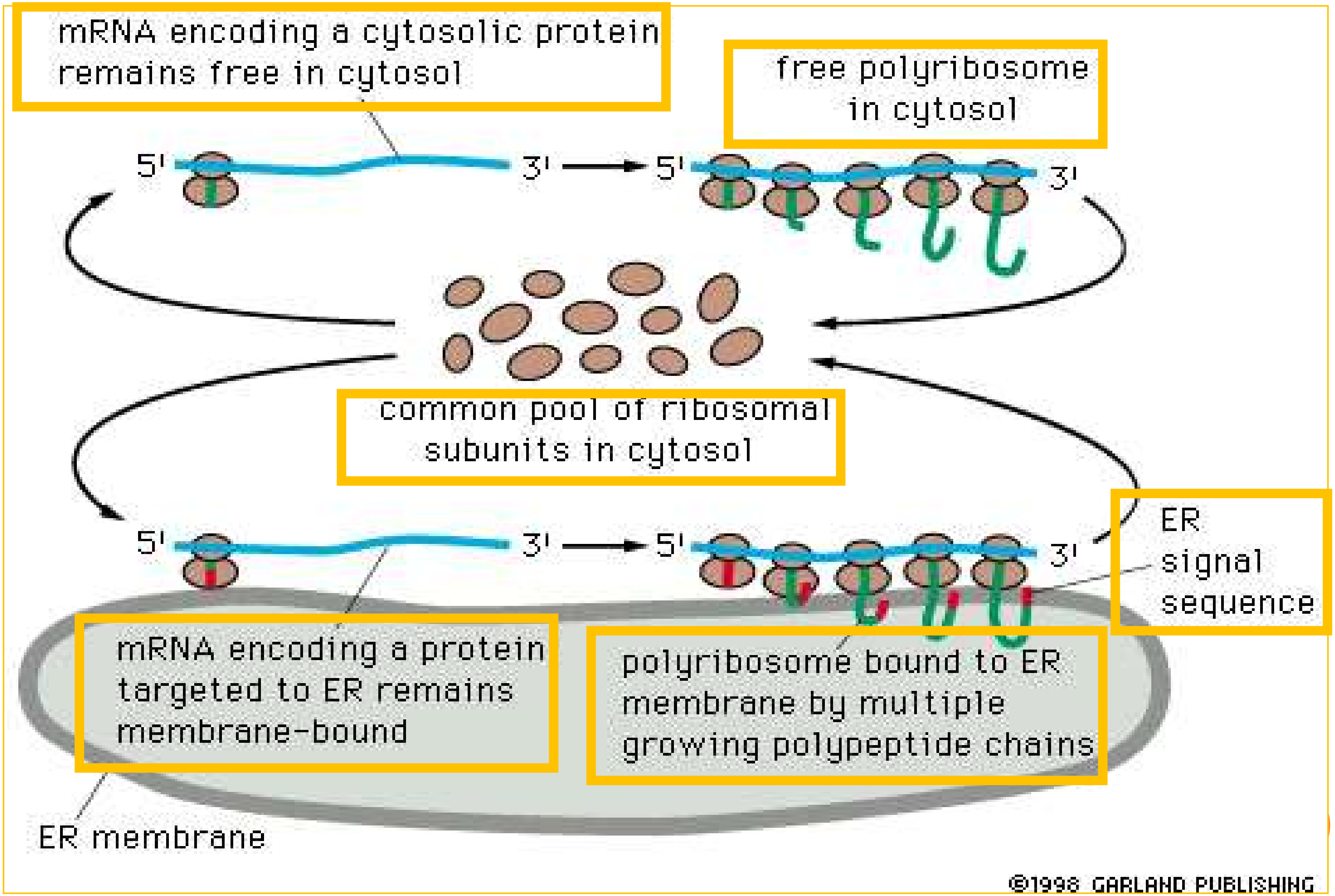
They are ferried by transport vesicles to various organelles.

PROTEIN SORTING – ER

- Two kinds of proteins are transferred from the cytosol to the ER:

1. Water-soluble proteins are completely translocated into the ER lumen and will be secreted or transported into the lumen of an organelle.
2. Trans-membrane proteins are only partly translocated and become embedded in the ER membrane in the proper orientation.
3. And they are transported into the membrane of another organelle or the plasma membrane.

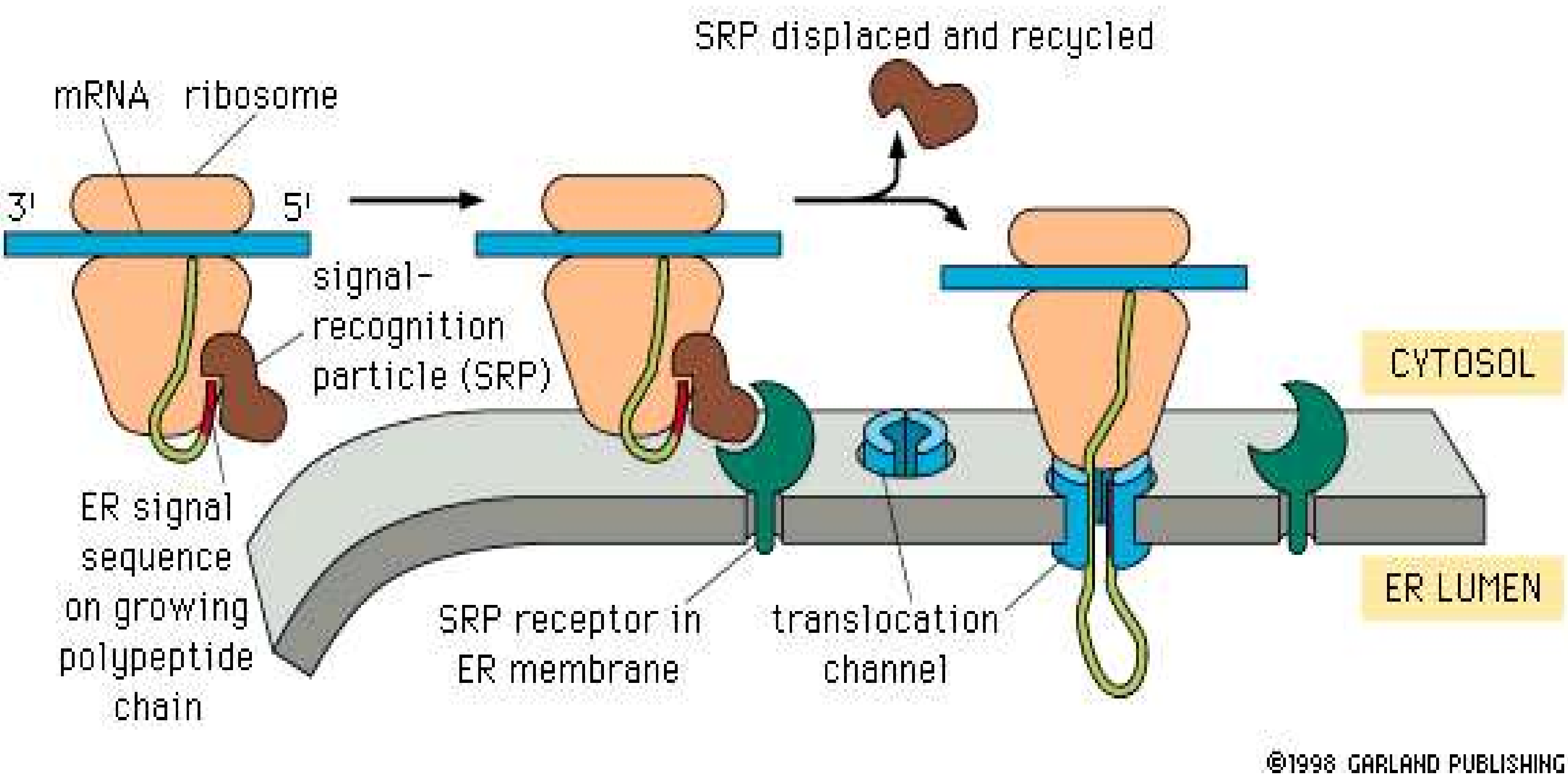
PROTEIN SORTING IN ER



SRP (signal-recognition particle) binds the ER signal sequence on the mRNA.

This slows protein synthesis.

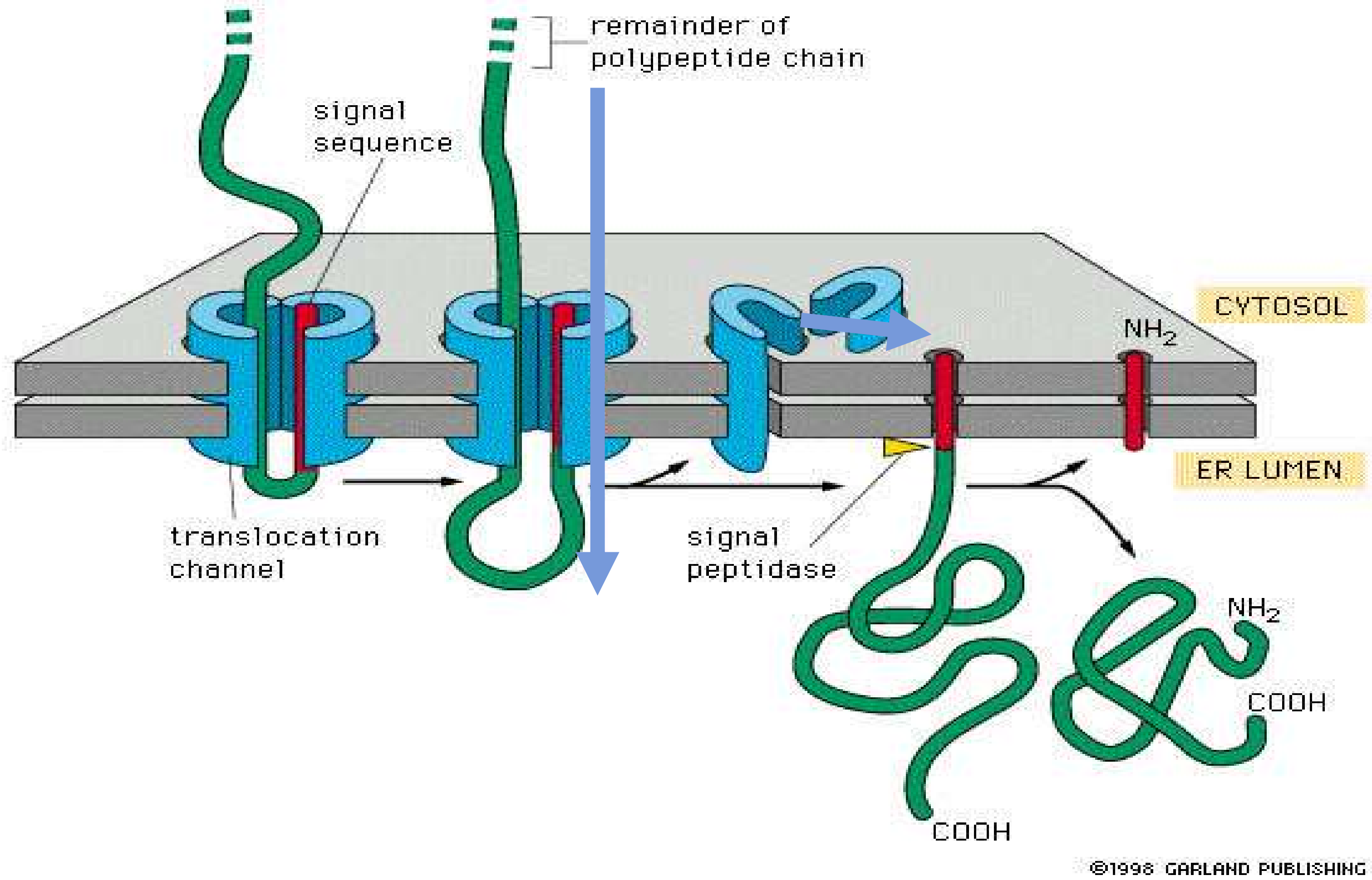
Once SRP bind its receptor in the ER membrane and docks with the translocation channel, it is released and protein synthesis resumes.



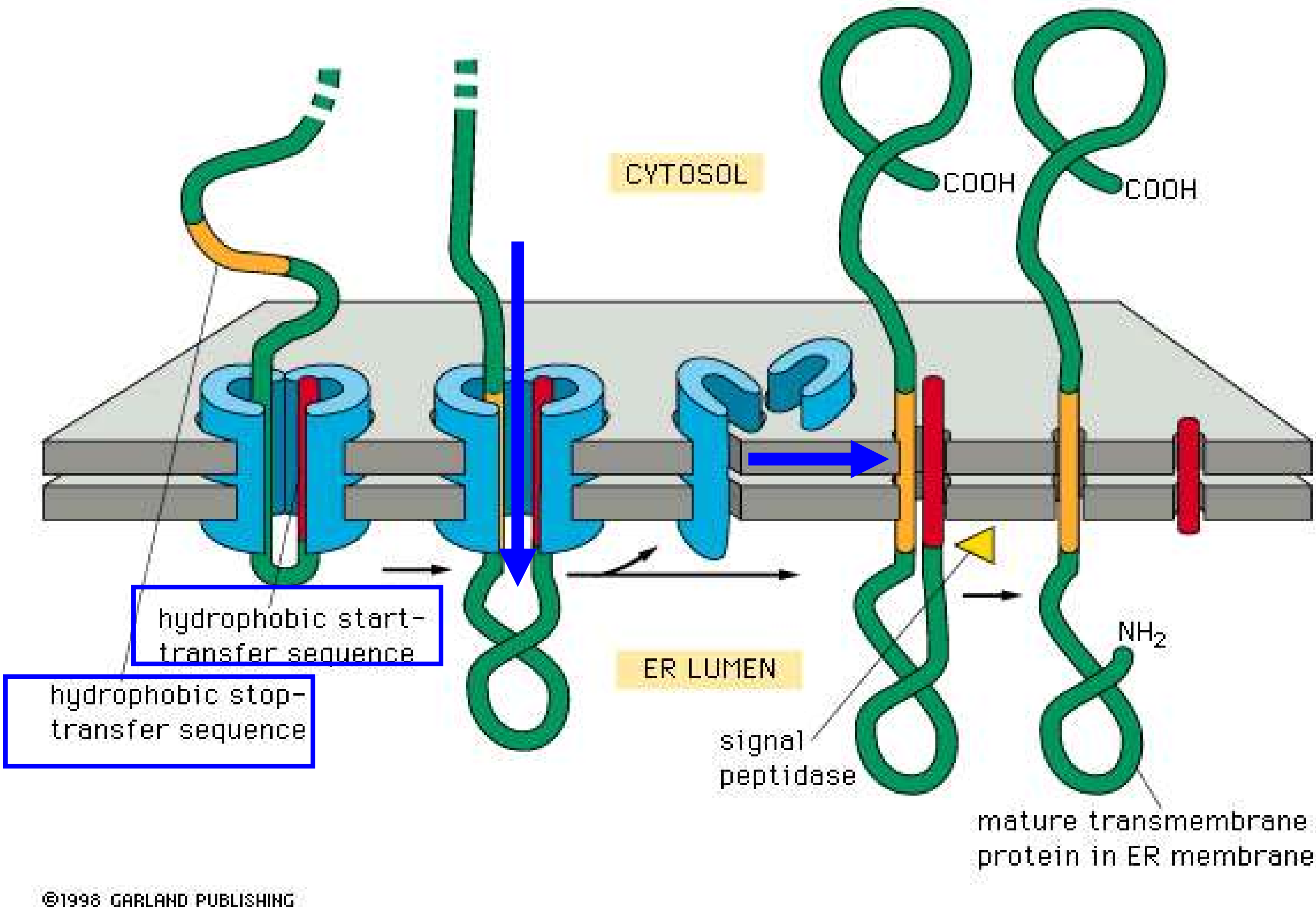
The signal sequence opens the translocation channel.

The ribosome remains attached while the rest of the protein is threaded through the membrane.

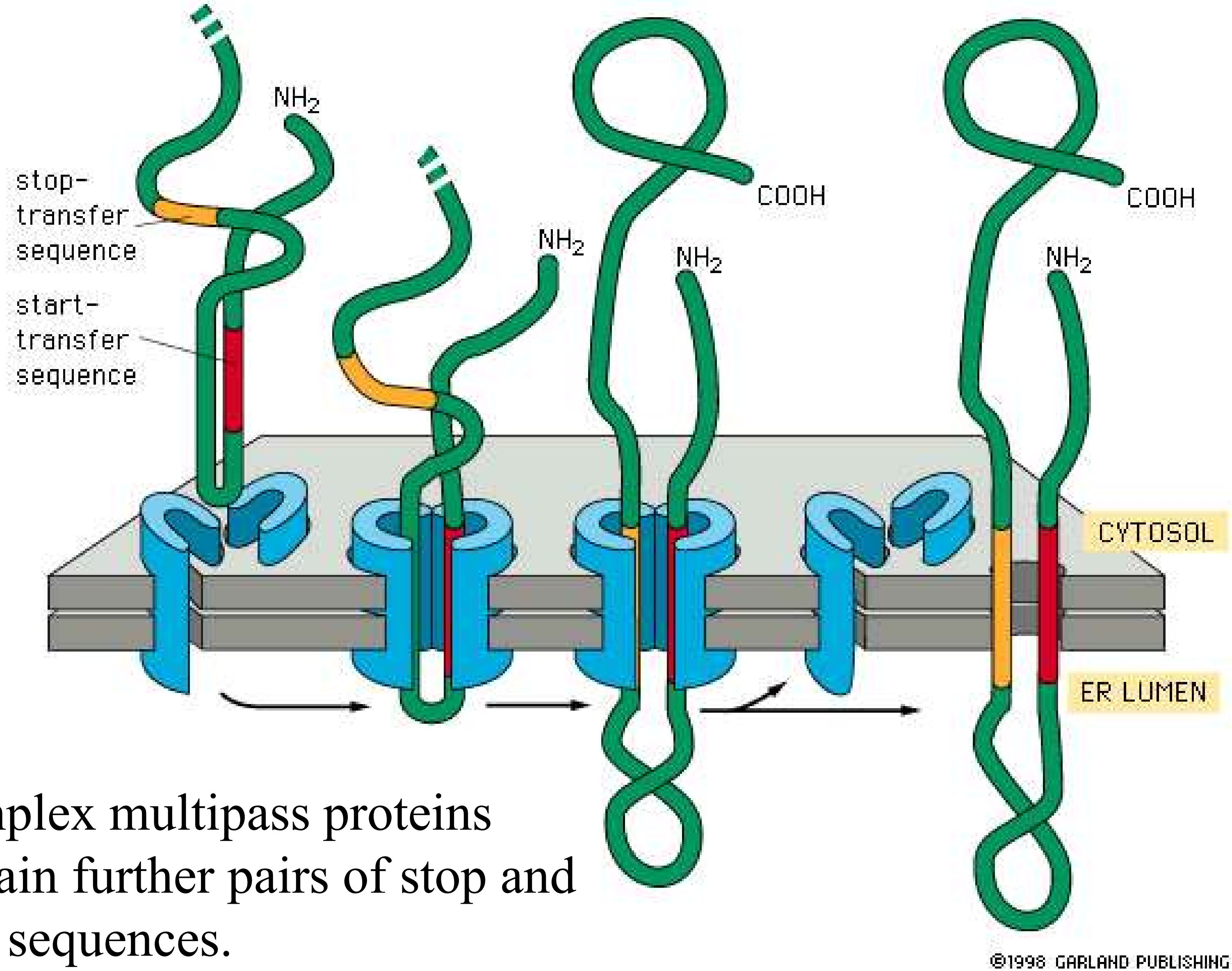
Signal peptidase cleaves the signal sequence.



Start and Stop signals determine the arrangement of a **trans-membrane protein** in the lipid bilayer.



The integration of a double pass trans-membrane protein.



Complex multipass proteins contain further pairs of stop and start sequences.