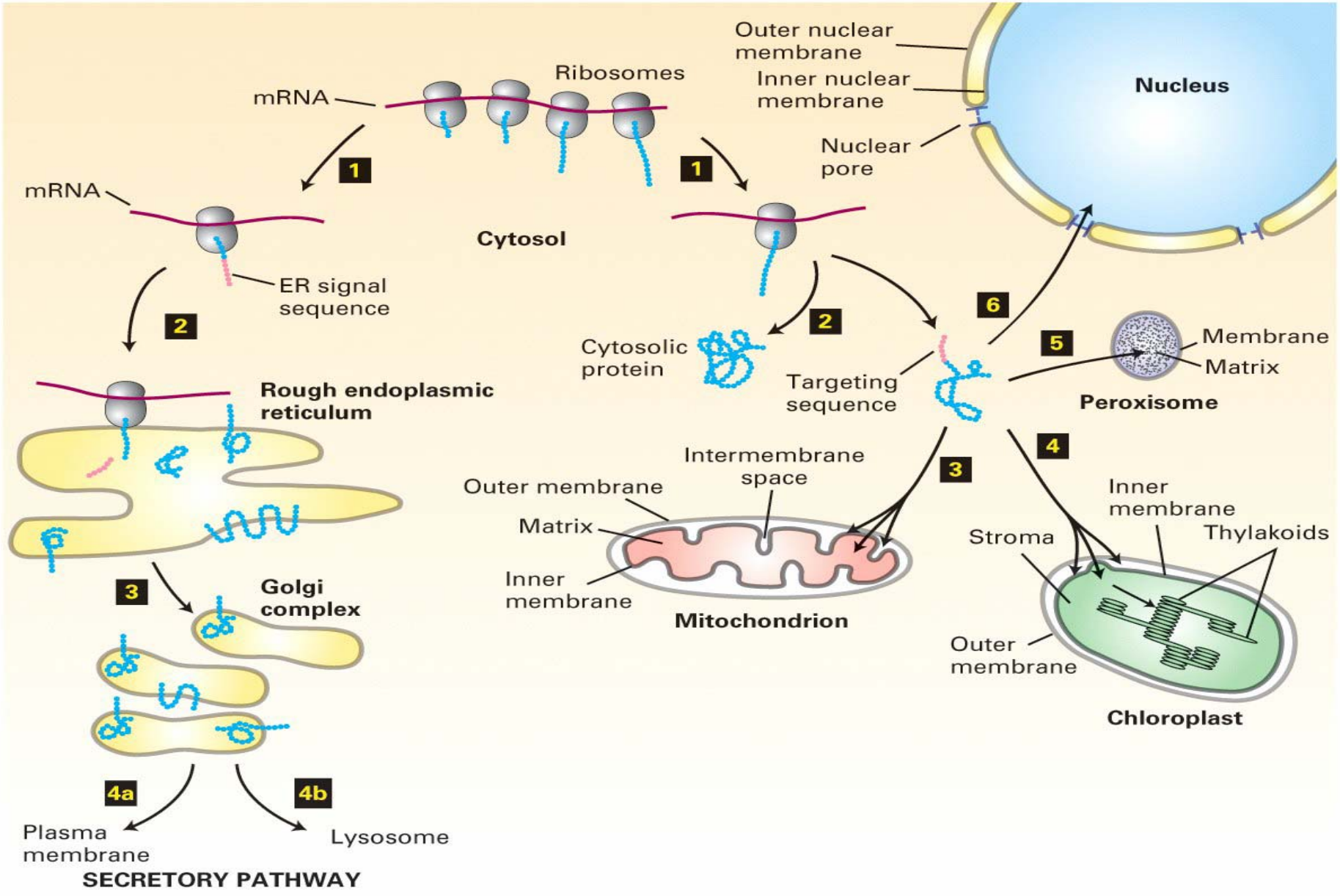
Major Protein-sorting pathways in eukaryotic cells



Key elements for the Protein targeting events

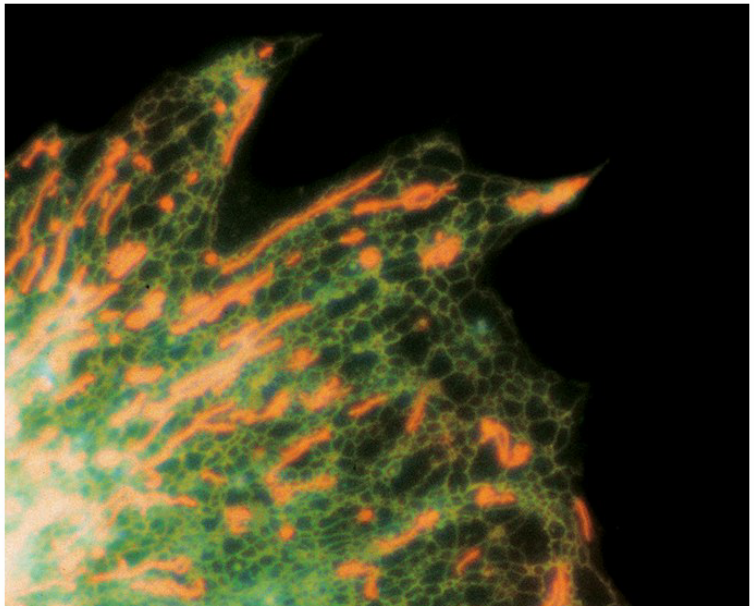
1. Signal sequence: the amino acid sequence of a protein that provides the information to target the protein to a particular organelle.

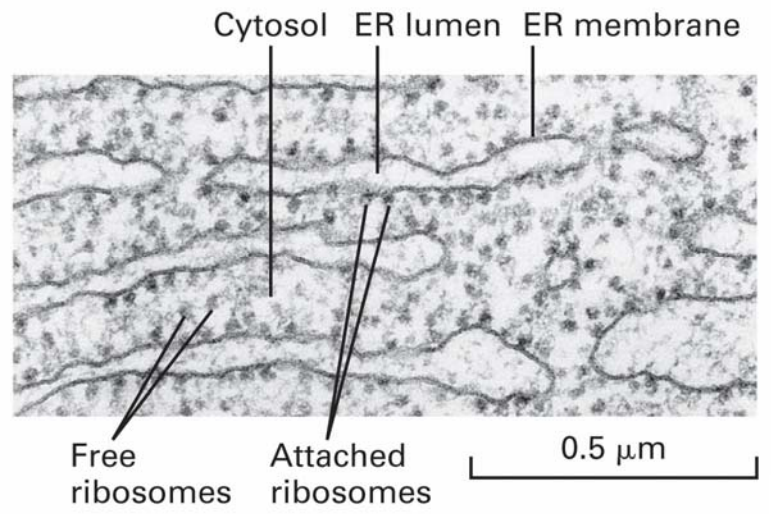
2. Receptors for the signal sequences.

3. Translocation channels that allow transfer of proteins across the membrane bilayer.

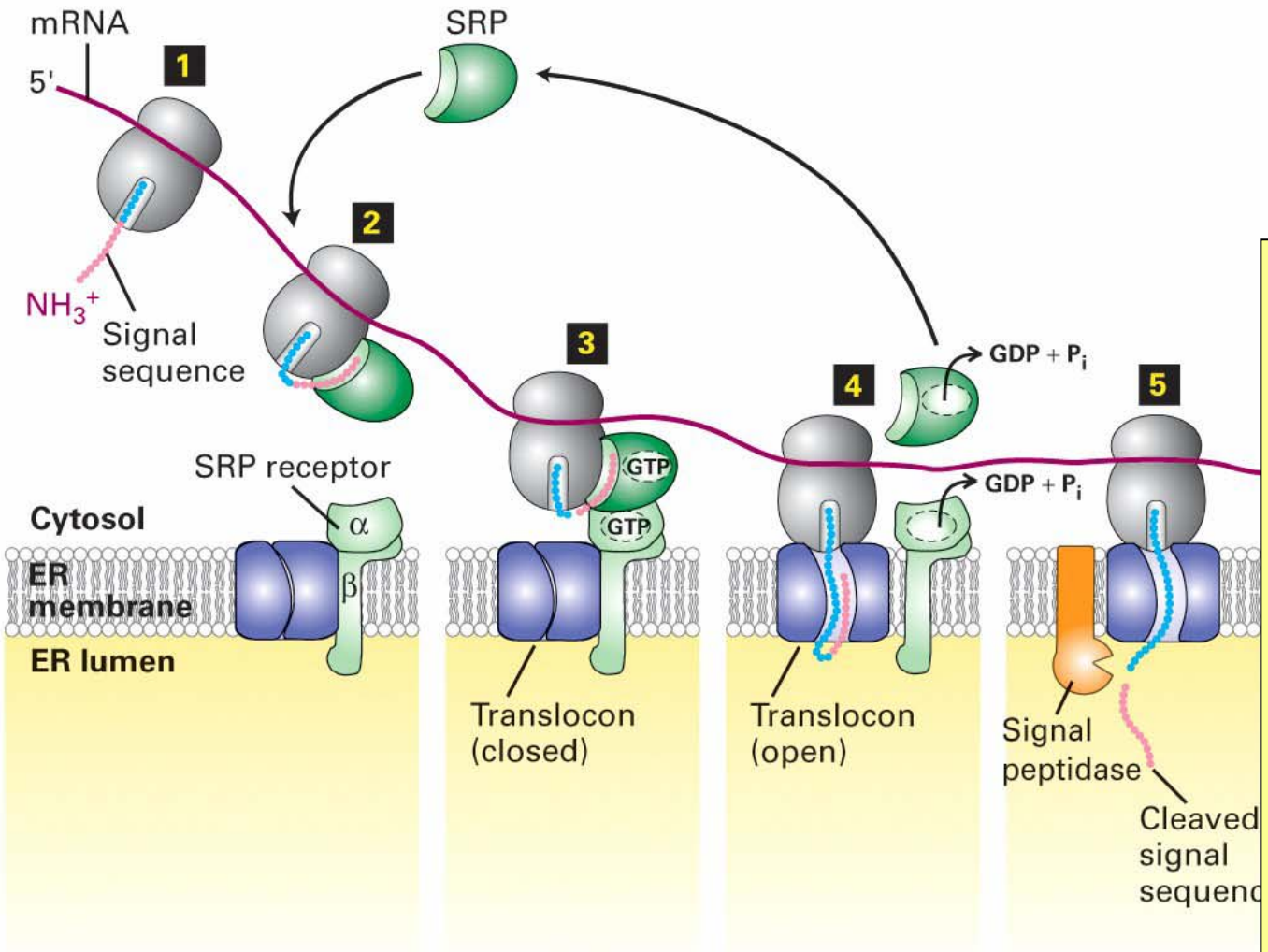
4. Energy that drives unidirectional transfer across the membrane.

Where are the endoplasmic reticulum, Golgi, and mitochondria?





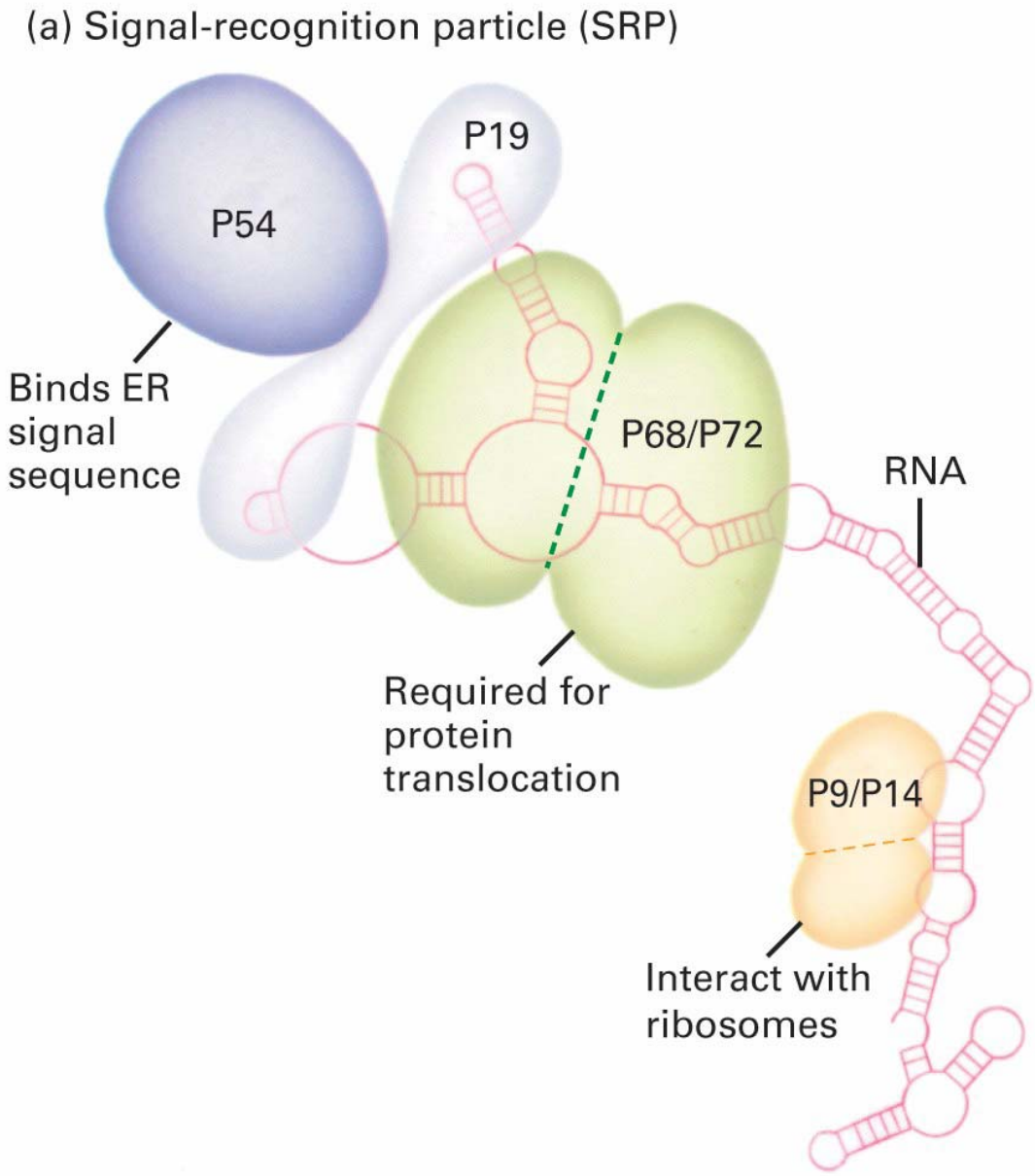
Synthesis of secretory proteins and their cotranslational translocation across the ER membrane



Wenxia:

1. ER signal sequence emerges
2. The binding by a signal-recongition particles (SRP)
3. SRP delivers the ribosome/nascent polypeptide complex to the SRP receptor in the ER membrane, and GTP binding

Signal-recognition particles



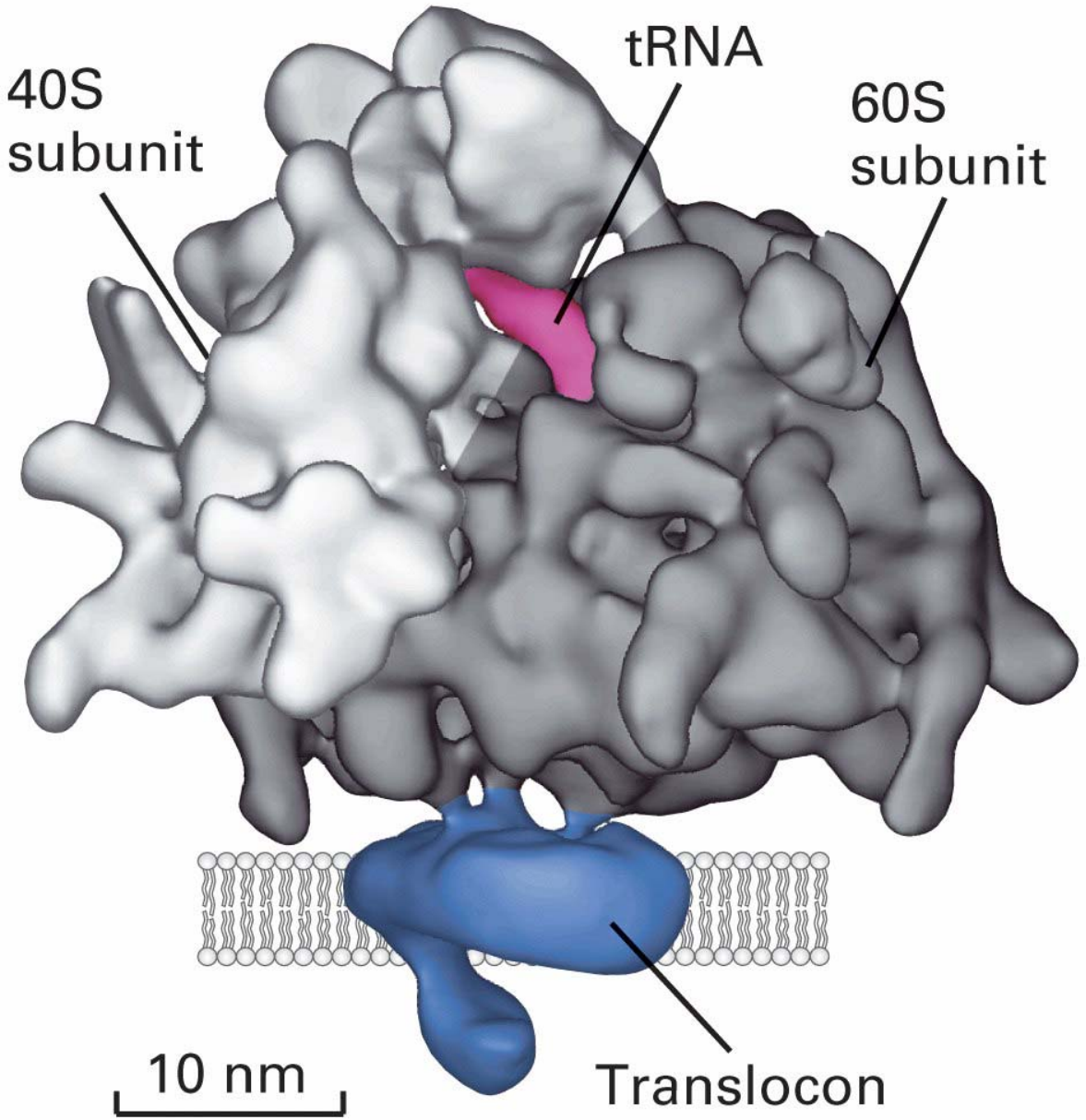
Wenxia:

1. Six subnits
2. All proteins binding to 300-nucleotide RNA except p54
3. P54 bind to ER signal sequences and have hydrophobic binding groove

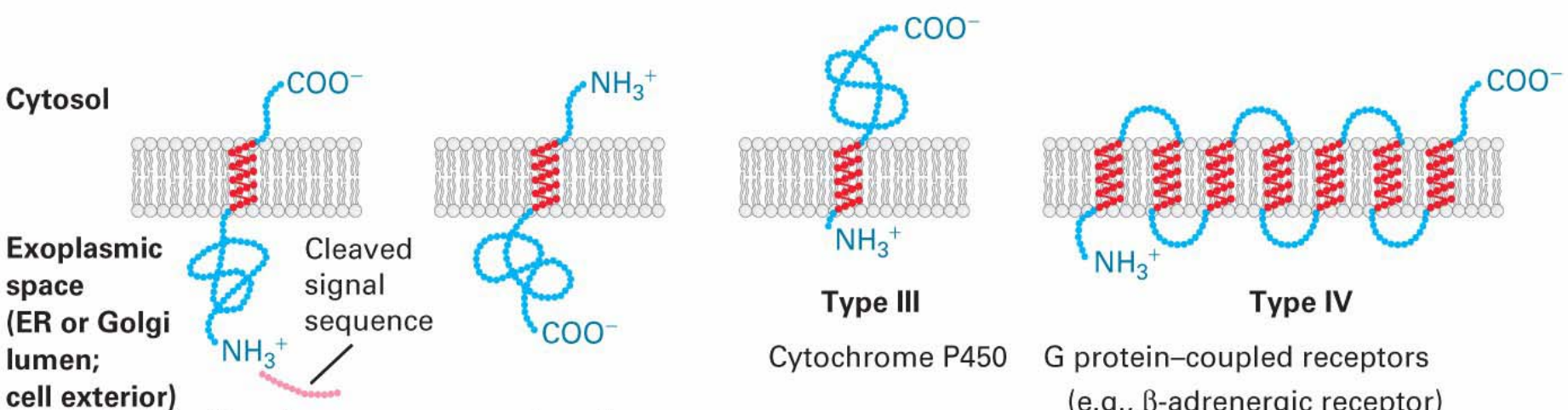
Translocon

Wenxia:

1. First found in yeast by mutations
2. Sec61 complex, consisting of 3 proteins
3. Sec61a contacts translocating polypeptide chain
4. A cylinder 5-6 nm high, 8.5 nm in diameter, and pore 2 nm diameter
5. Open and close of translocon is regulated



Major topological classes of integral membrane proteins synthesized on the rough ER



Type I

Glycophorin
LDL receptor
Influenza HA protein
Insulin receptor
Growth hormone receptor

Type II

Asialoglycoprotein receptor
Transferrin receptor
Sucrase–isomaltase precursor
Golgi galactosyltransferase
Golgi sialyltransferase
Influenza HN protein

Type III

Cytochrome P450

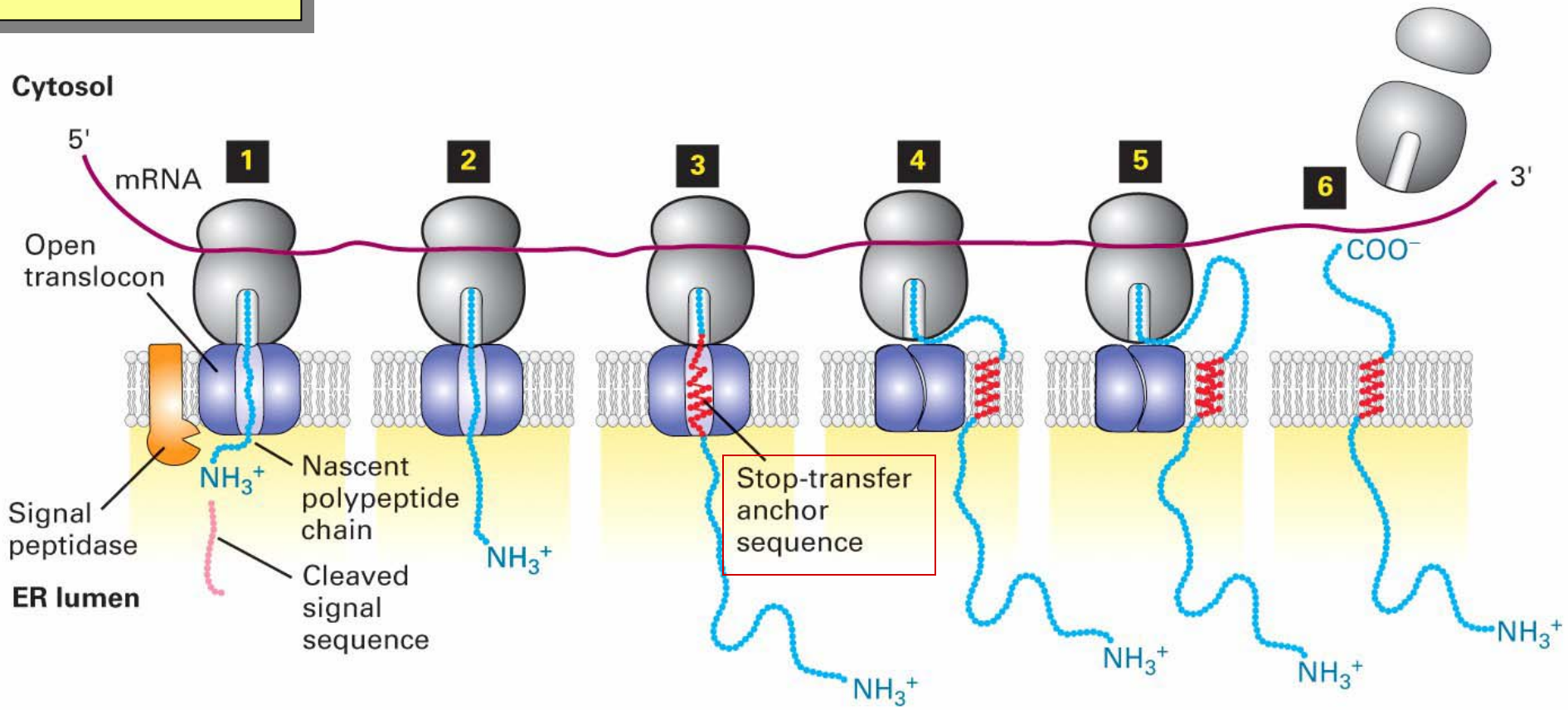
Type IV

G protein–coupled receptors (e.g., β-adrenergic receptor)
Glucose transporters (e.g., GLUT1)
Voltage-gated Ca_2^+ channels
ABC small molecule pumps
CFTR (Cl^-) channel
Sec61
Connexin

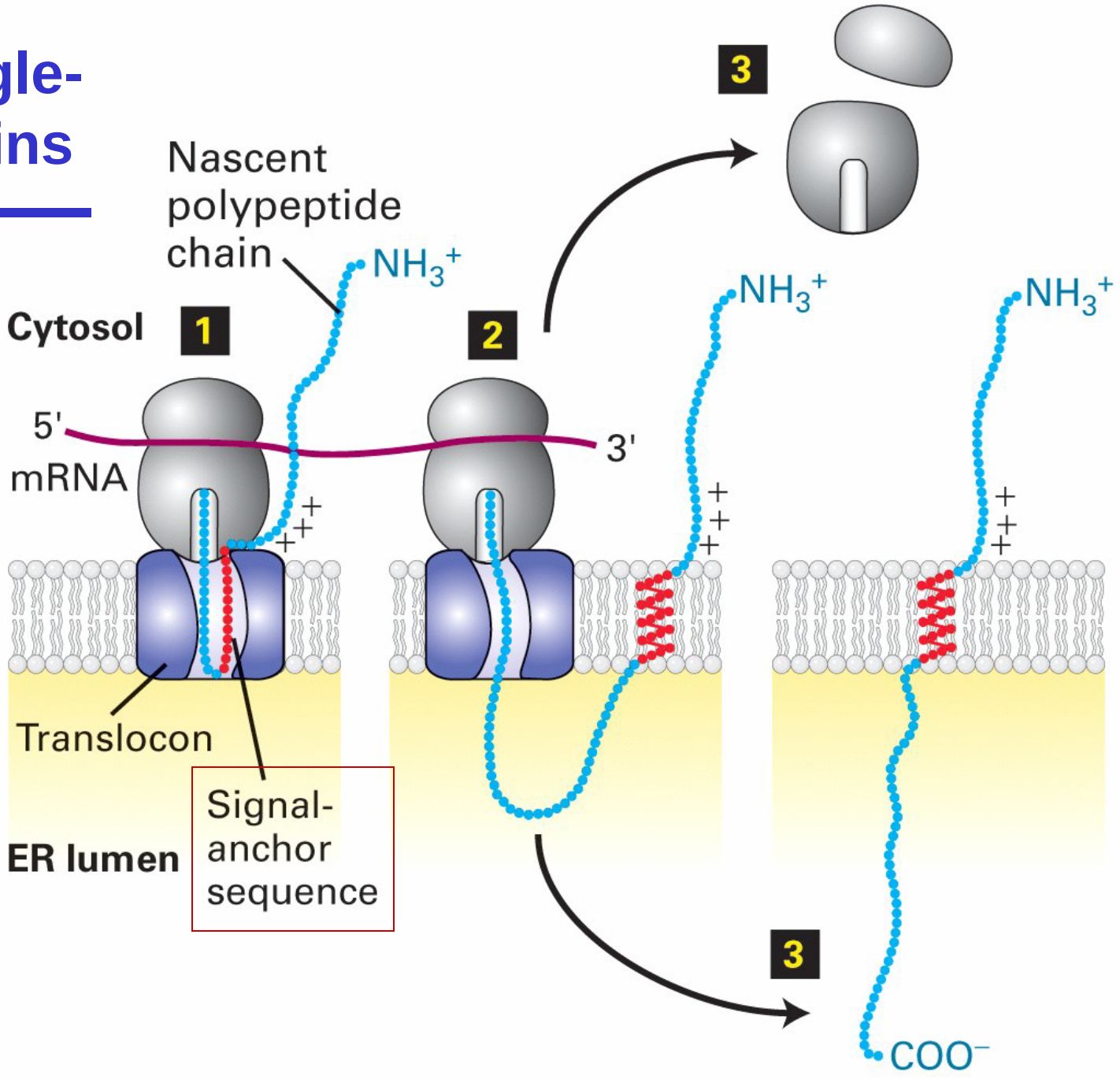
Wenxia:

Hydrophobic transmembrane segment is the stop-transfer anchor sequence

sis and insertion into the ER membrane of type I single pass proteins



Type II single-pass proteins

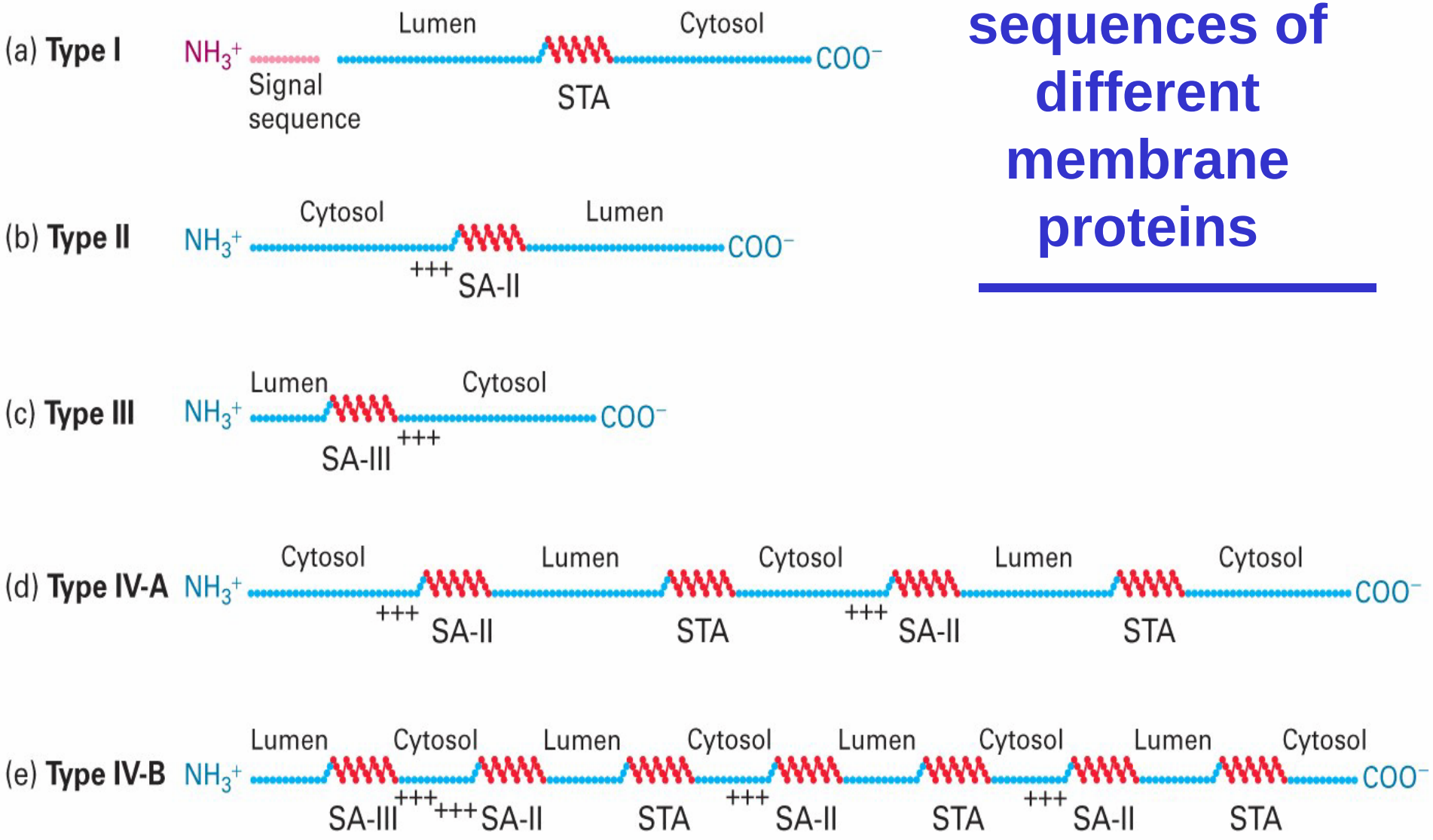


Experiments:

Mutating the positive charged amino acids change the orientation of the protein.

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

Arrangement of topogenic sequences of different membrane proteins



GPI anchored proteins

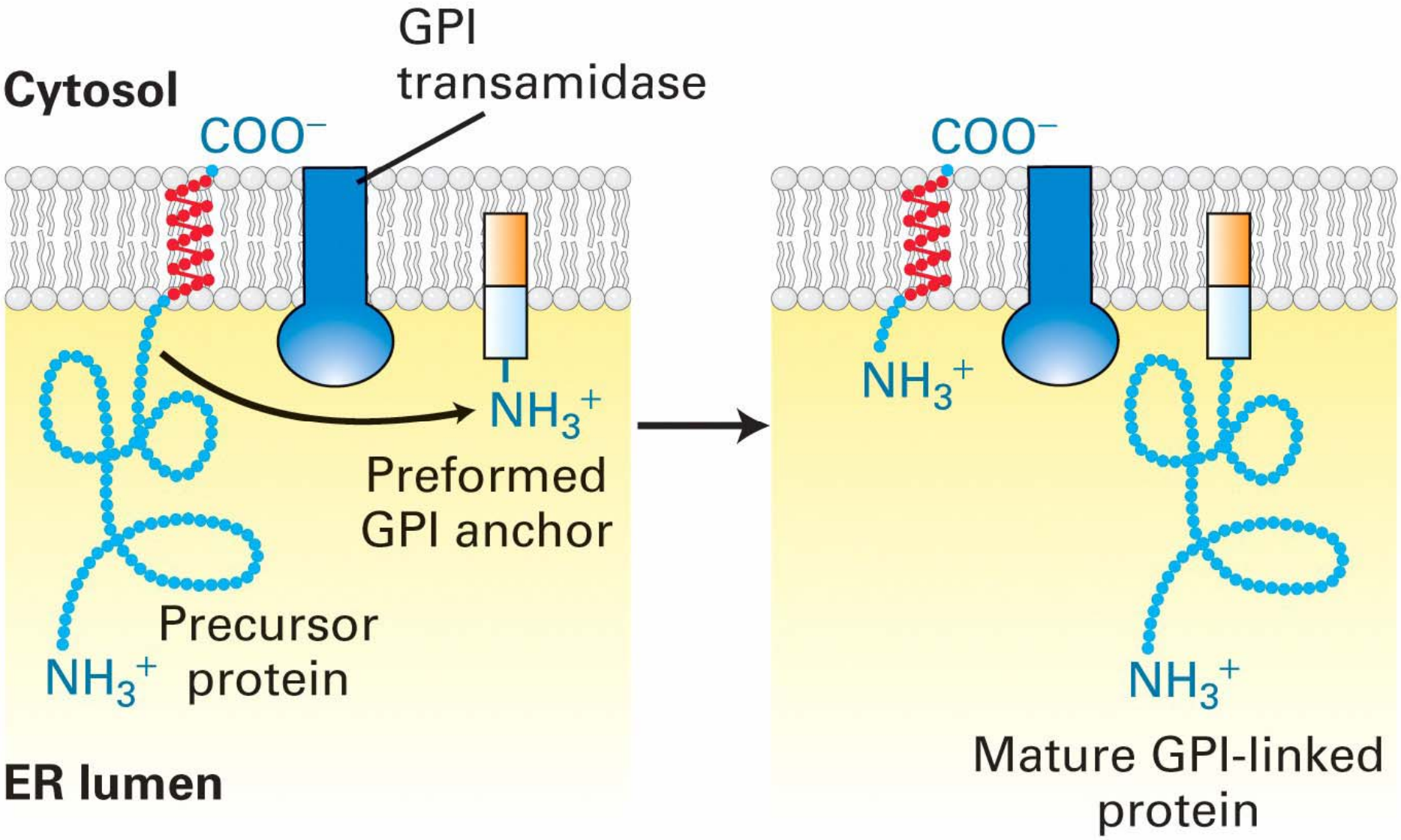
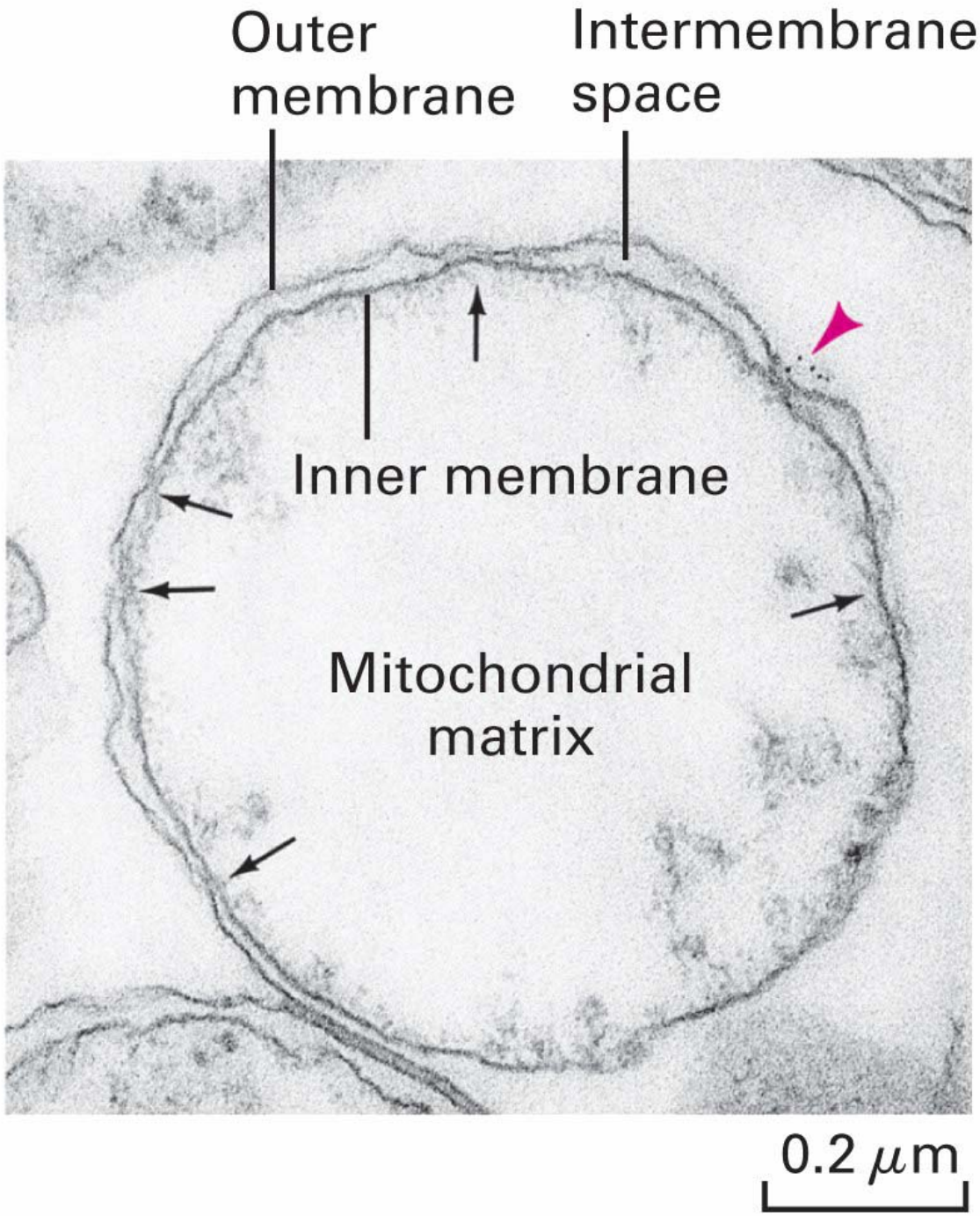


TABLE 16-1 Uptake-Targeting Sequences That Direct Proteins from the Cytosol to Organelles*

Target Organelle	Location of Sequence Within Protein	Removal of Sequence	Nature of Sequence
Endoplasmic reticulum (lumen)	N-terminus	Yes	Core of 6–12 hydrophobic amino acids, often preceded by one or more basic amino acids (Arg, Lys)
Mitochondrion (matrix)	N-terminus	Yes	Amphipathic helix, 20–50 residues in length, with Arg and Lys residues on one side and hydrophobic residues on the other
Chloroplast (stroma)	N-terminus	Yes	No common motifs; generally rich in Ser, Thr, and small hydrophobic residues and poor in Glu and Asp
Peroxisome (matrix)	C-terminus (most proteins); N-terminus (few proteins)	No	PTS1 signal (Ser-Lys-Leu) at extreme C-terminus; PTS2 signal at N-terminus

*Different or additional sequences target proteins to organelle membranes and subcompartments.
See Chapter 12 for targeting sequences required for uptake of proteins into the nucleus.

Mitochondrial



Protein import into the mitochondrial matrix

Tom-translocon of outer membrane
Tim-translocon of the inner membrane

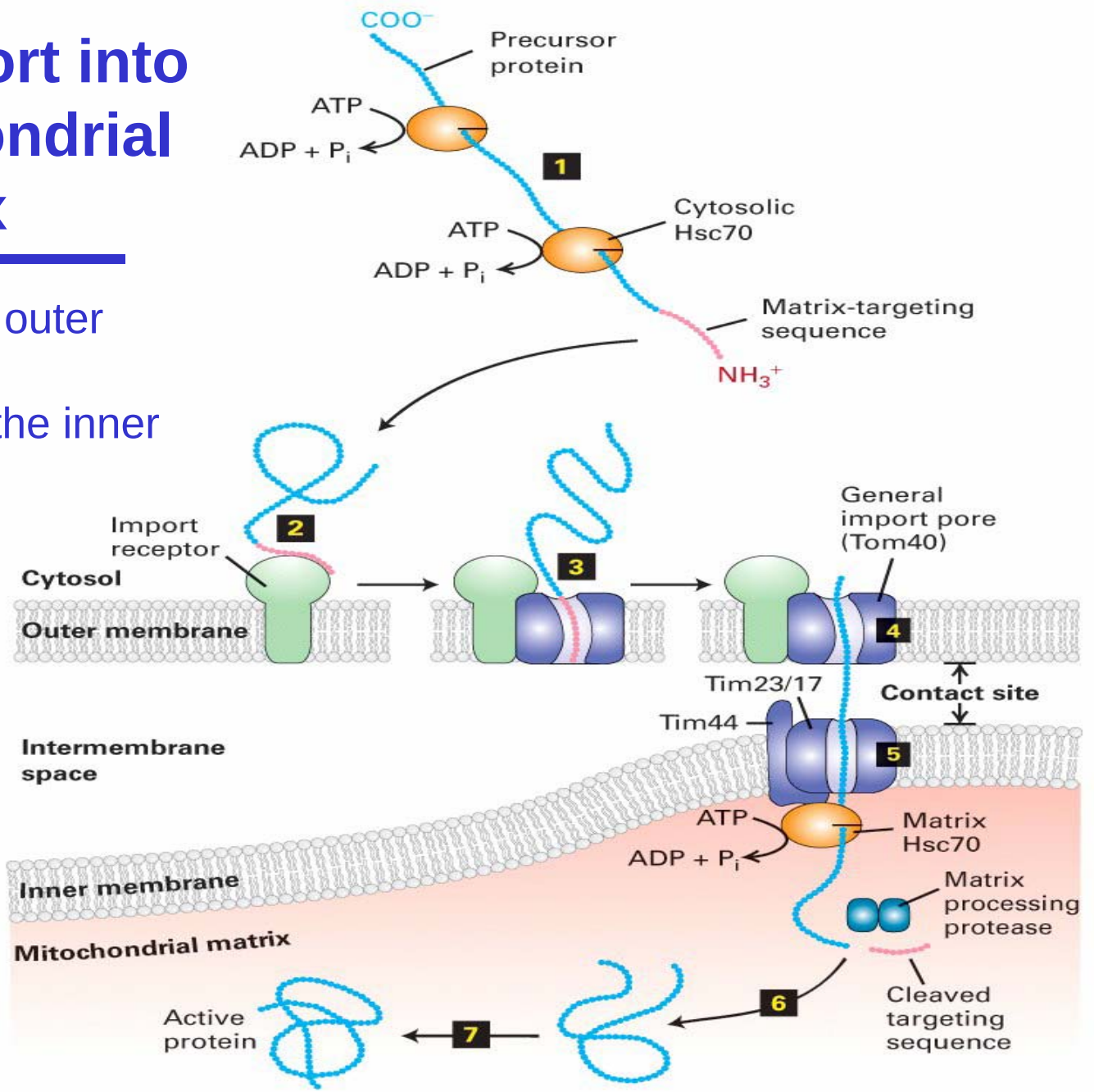
Wenxia:

Targeting signals

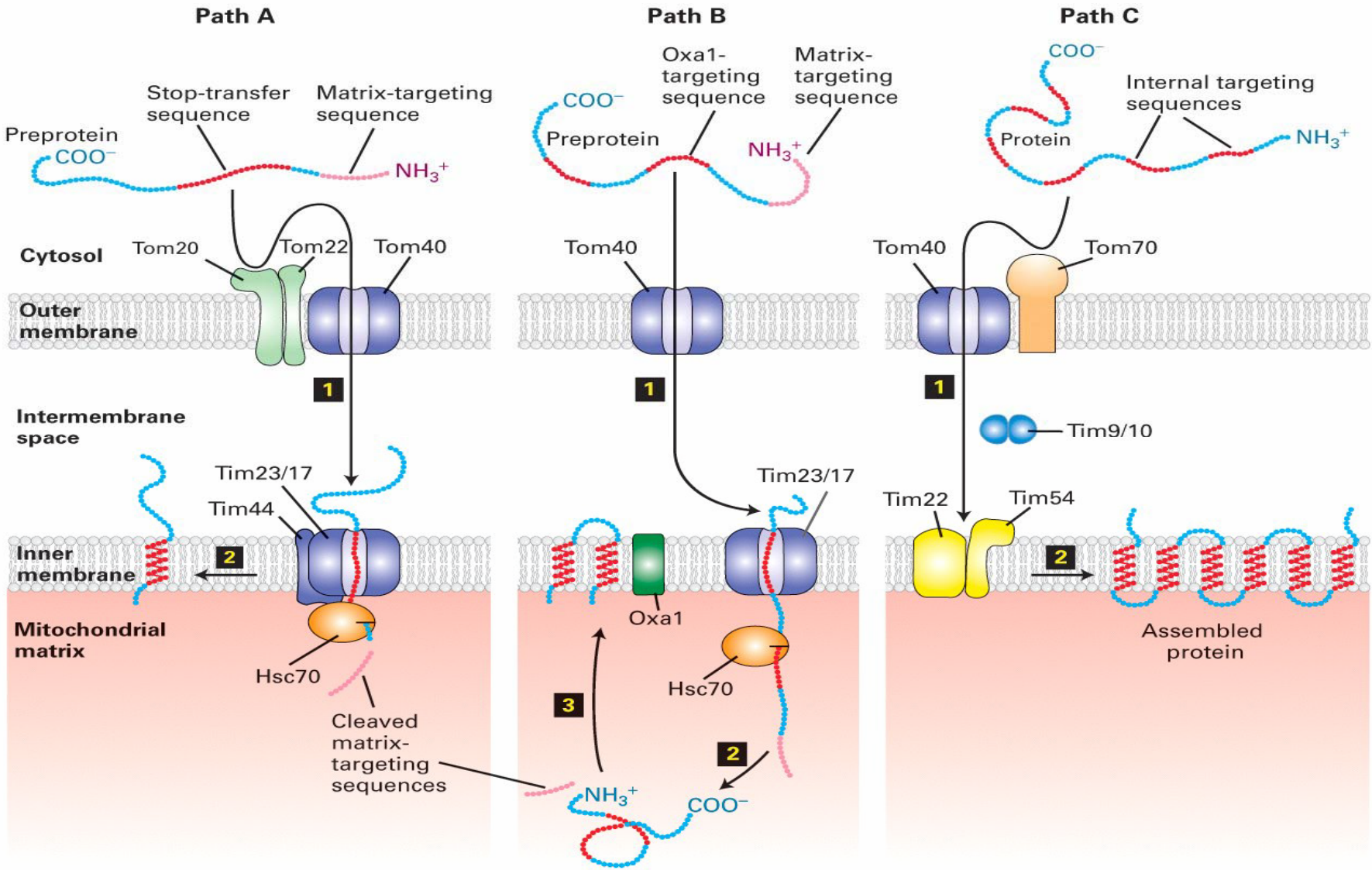
Signal receptor

Translocation channel

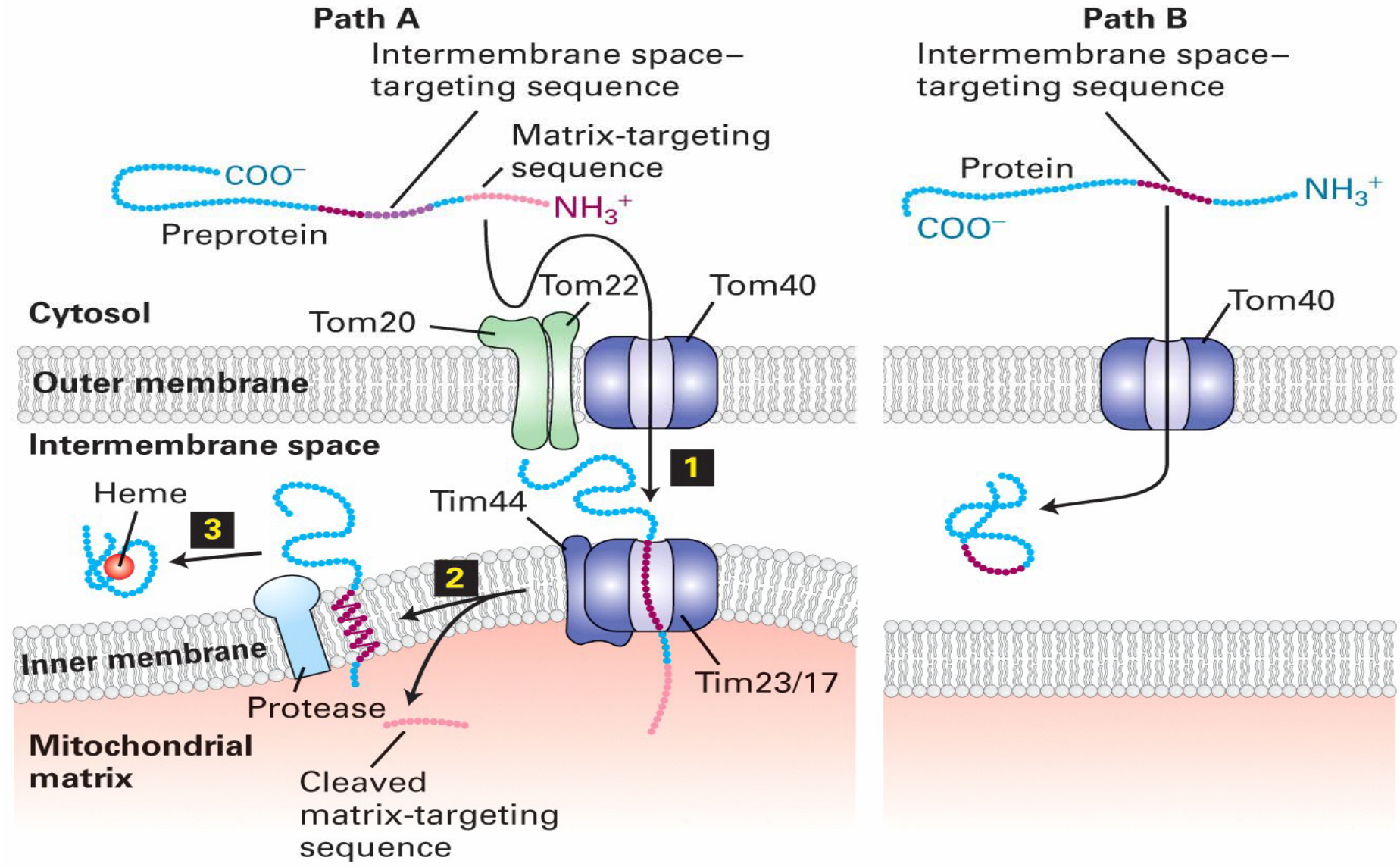
Energy



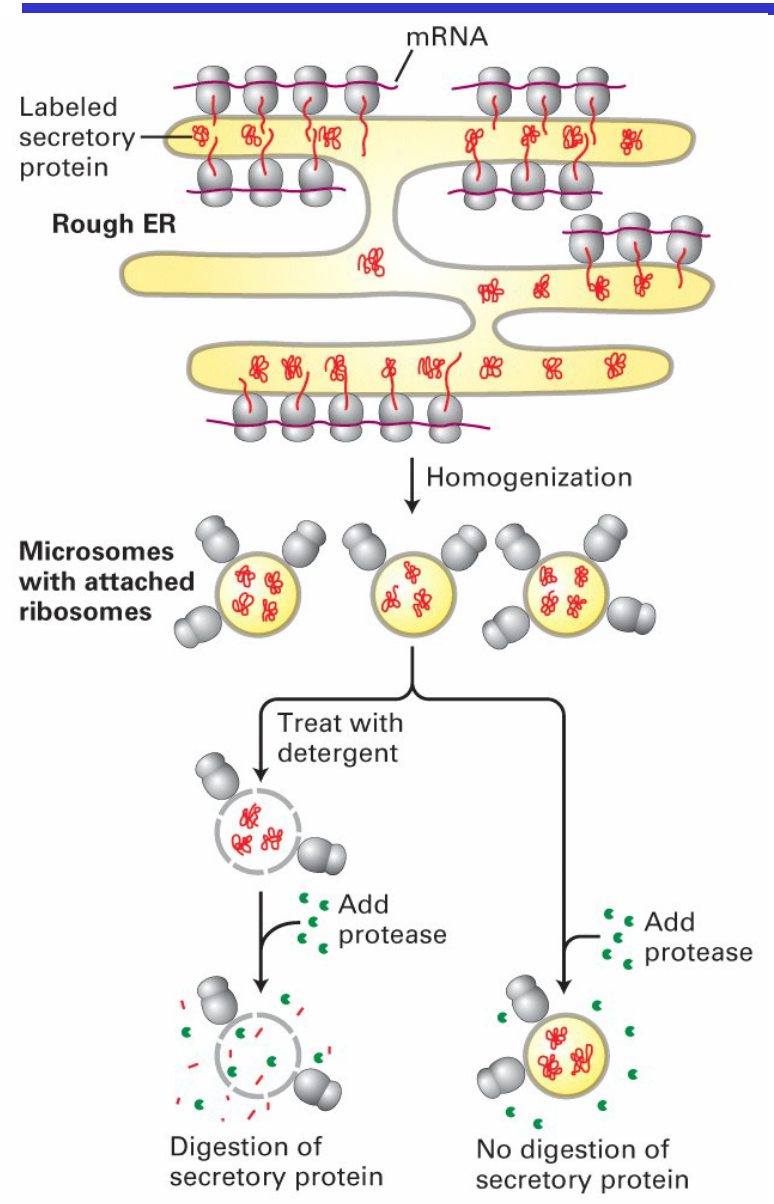
Transporting protein from the cytosol to the inner mitrochondrial membrane



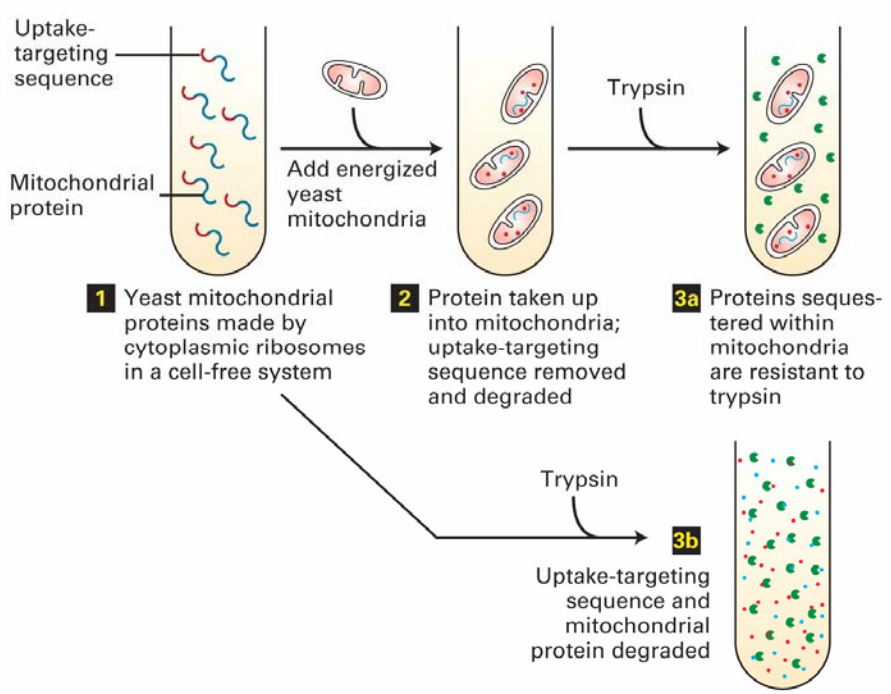
Transporting proteins from the cytosol to the mitochondrial intermembrane space



How to analyze protein movement into membranes and organelles



The in vitro system

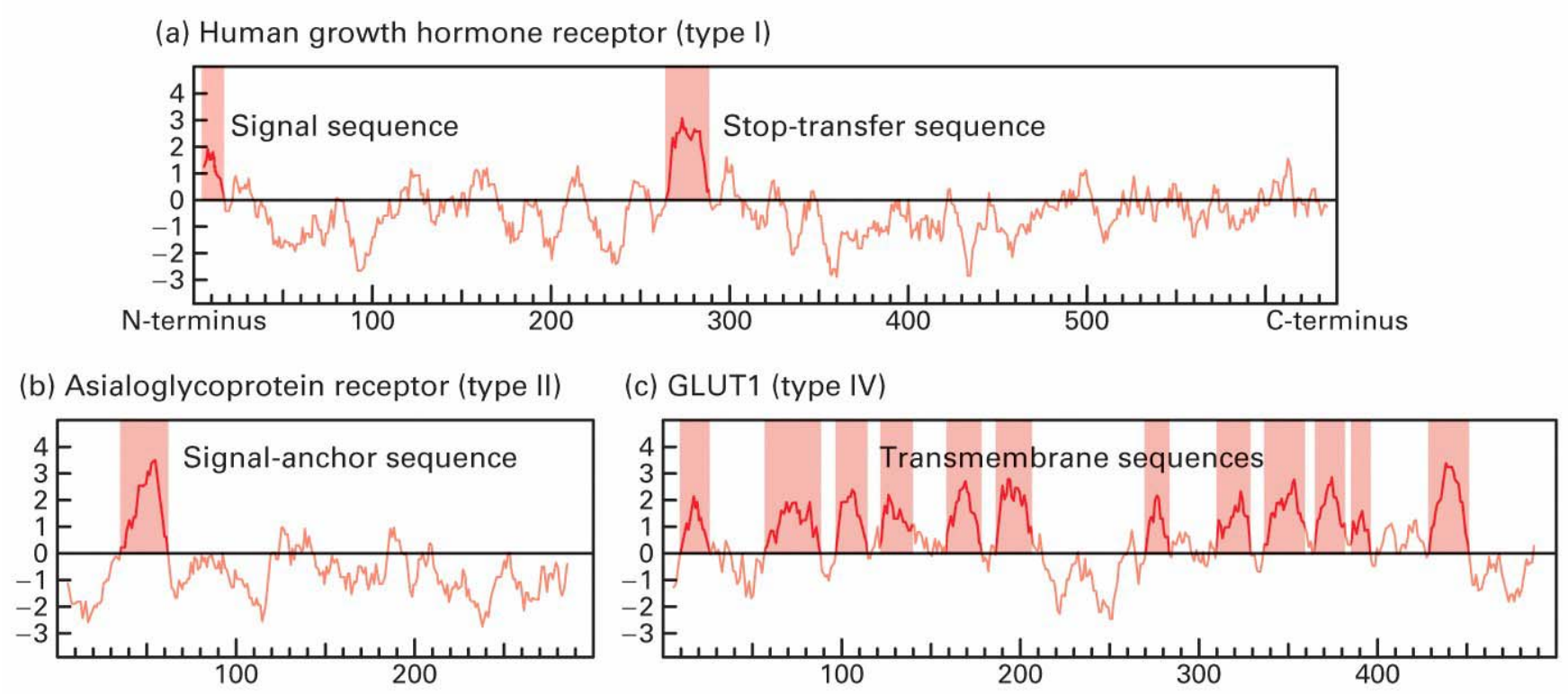


How to identify signal sequences

Genetic manipulations

1. ***Sequence homology***
2. ***Targeted mutagenesis***
3. ***Fusion proteins***

Hydropathy profiles



How to identify a translocon component that contacts nascent secretory proteins

Chemical cross-link