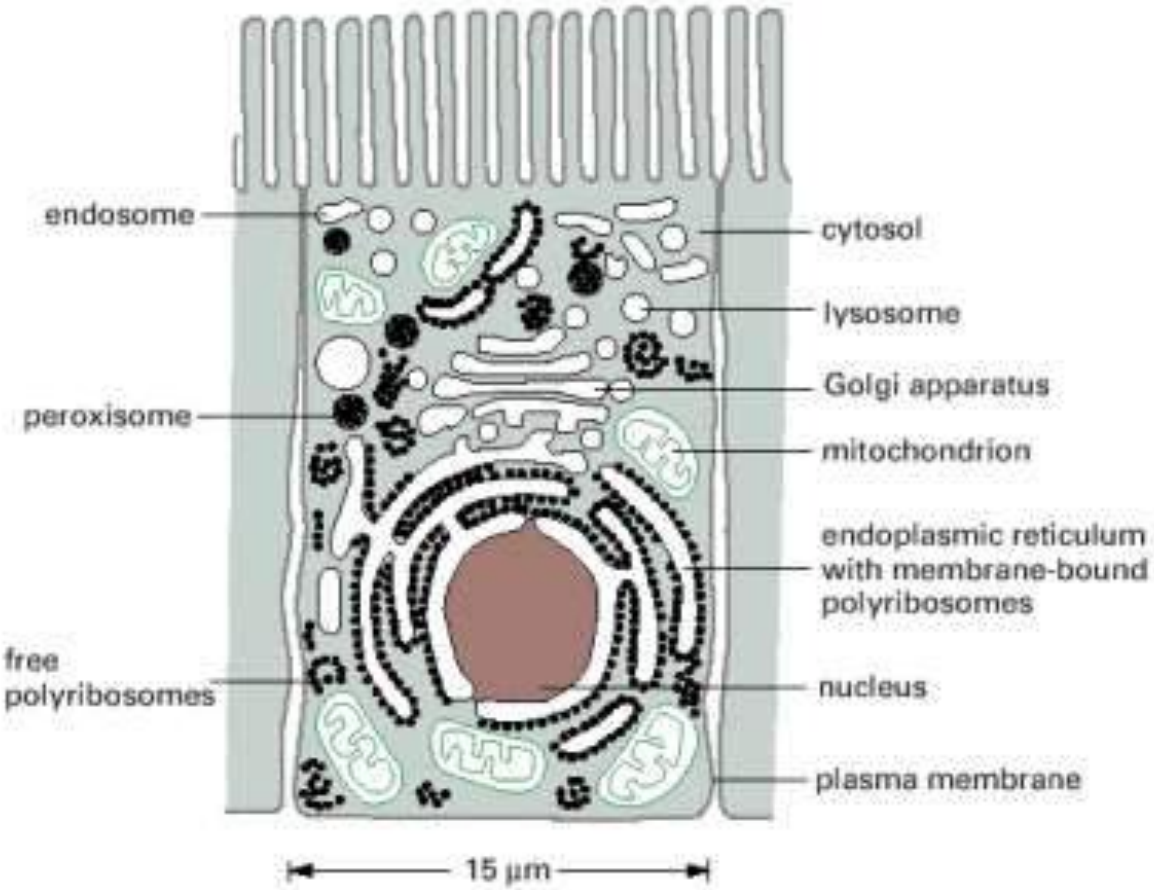
Intracellular Compartments and Protein Sorting

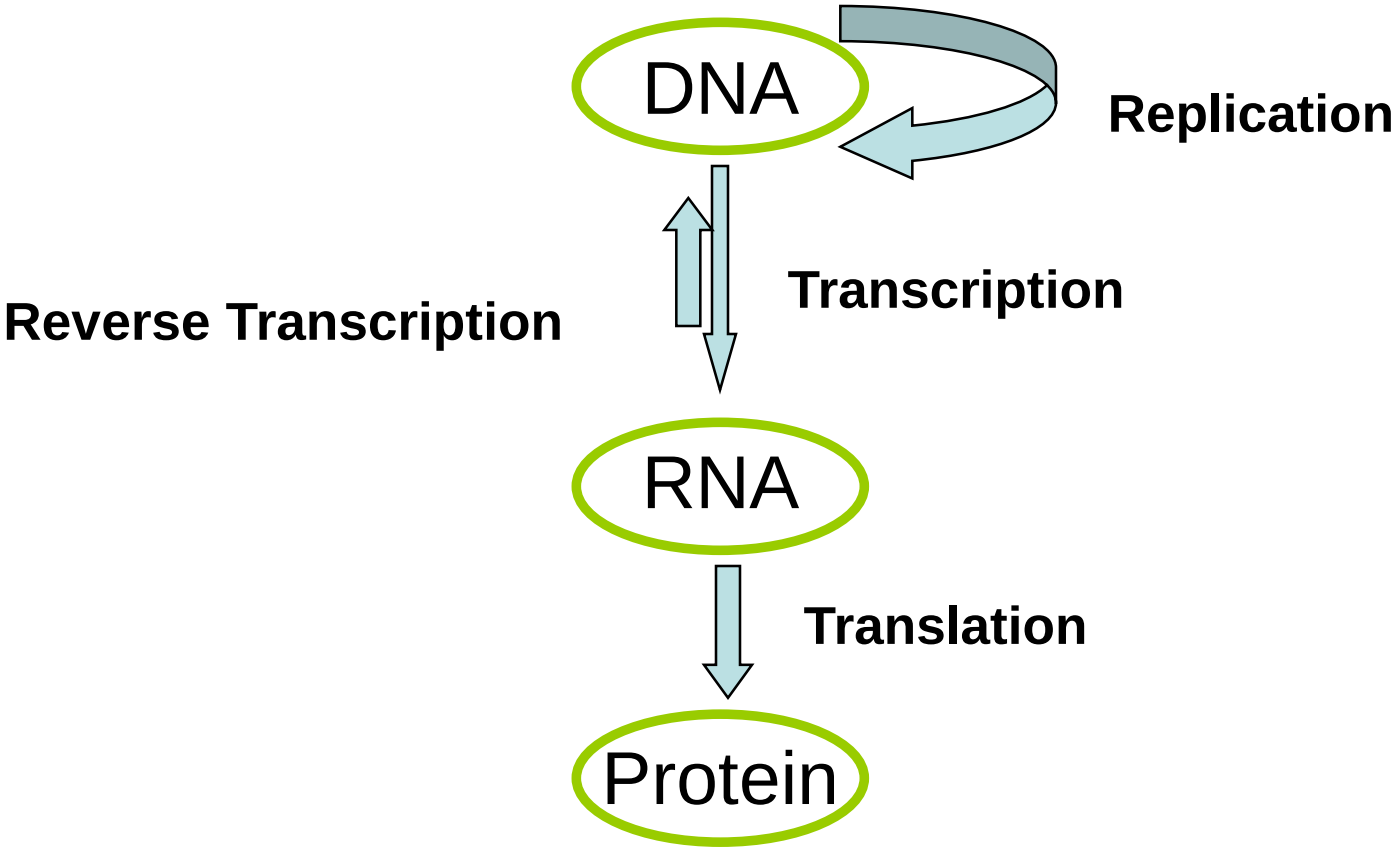
The major intracellular compartments of an animal cell



Relative Volumes Occupied by the Major Intracellular Compartments

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

The Central Dogma



Protein Biosynthesis

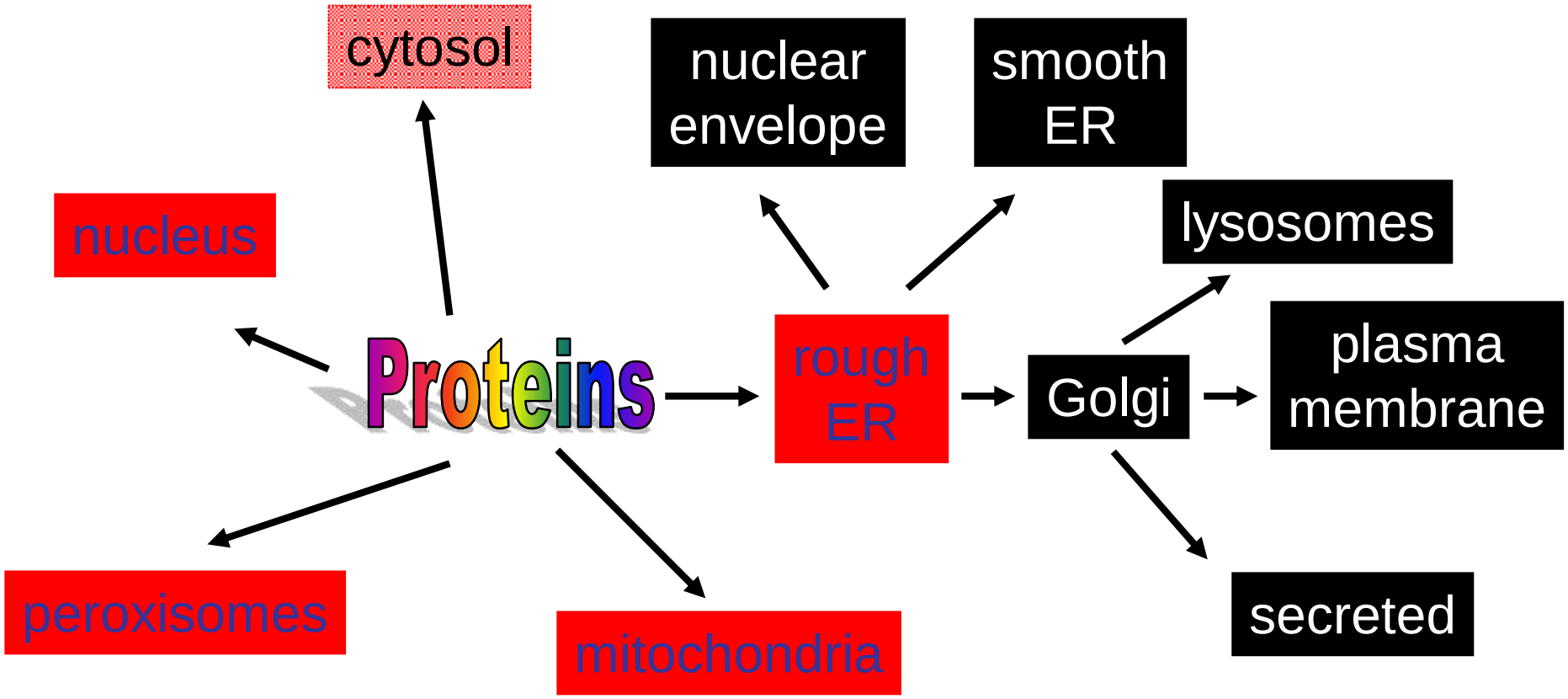
- **Major Requirements are**

  Ribosomes
  Amino Acids
  m RNA
  t RNA

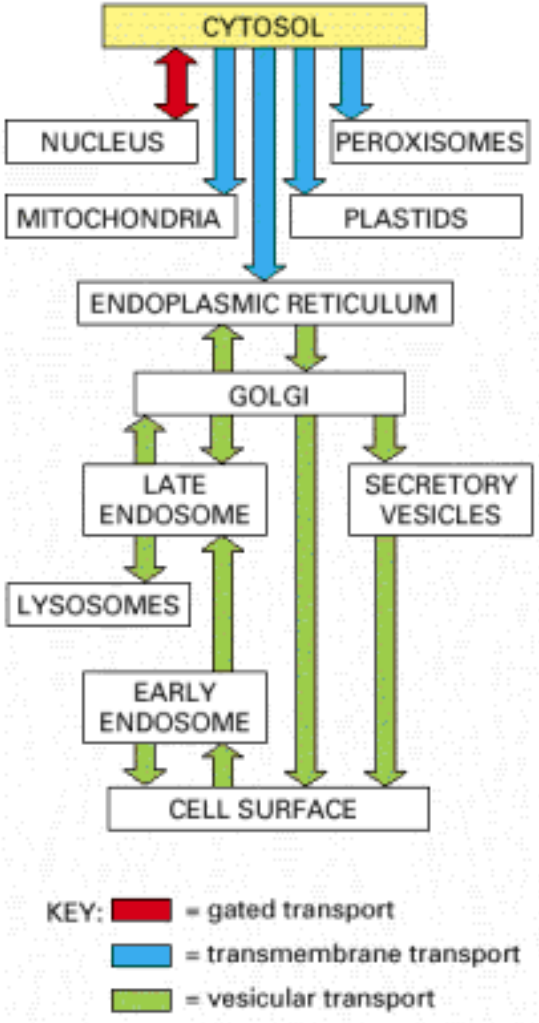
tRNA being the translational adapter is the most important molecule.

Peptide bond formation is thermodynamically unfavourable and therefore amino acids are charged

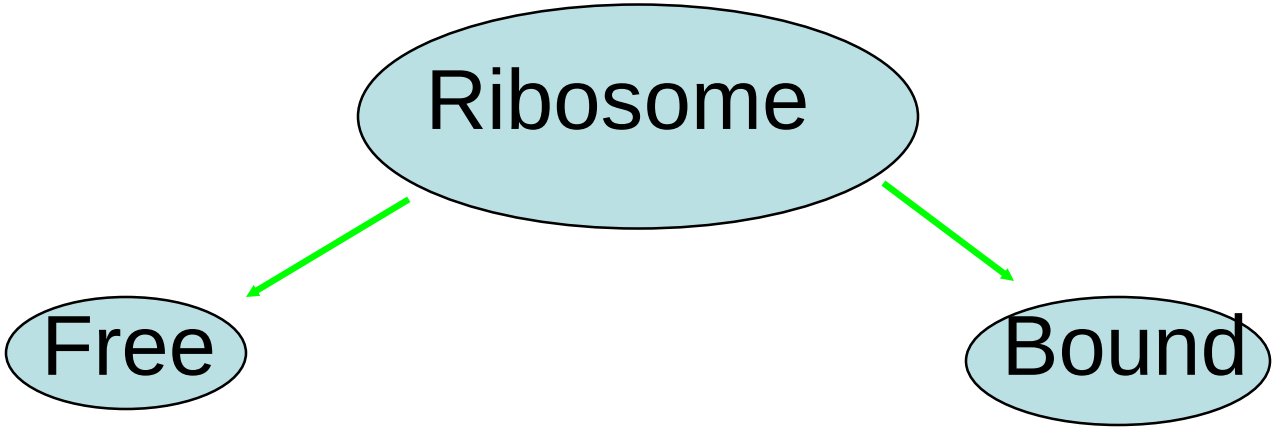
Protein sorting



Protein traffic



Protein Targetting



Soluble Proteins

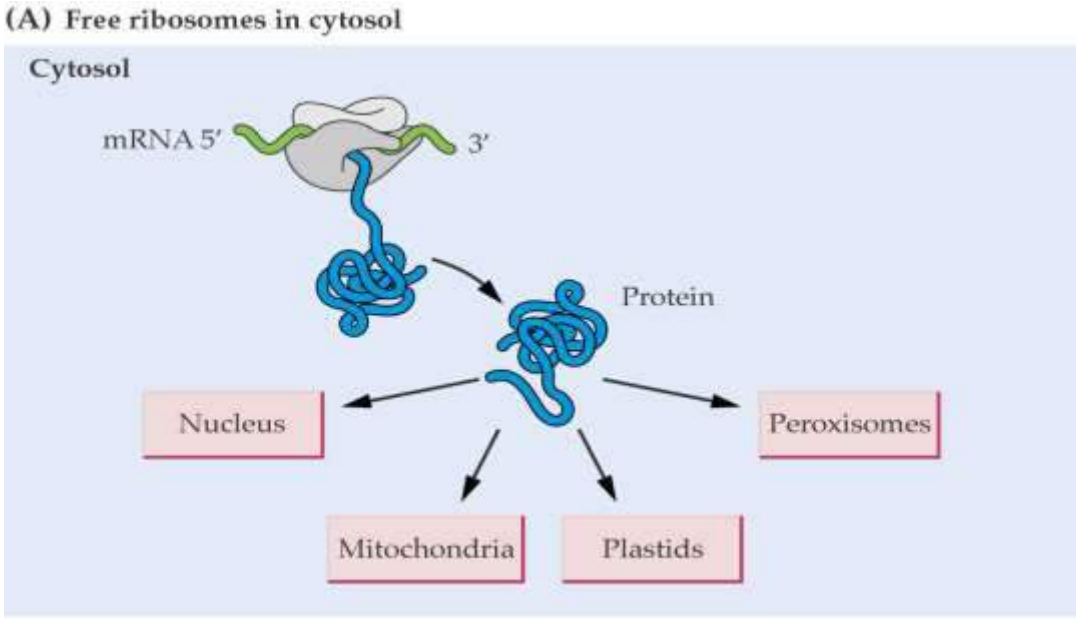
Lysosomal

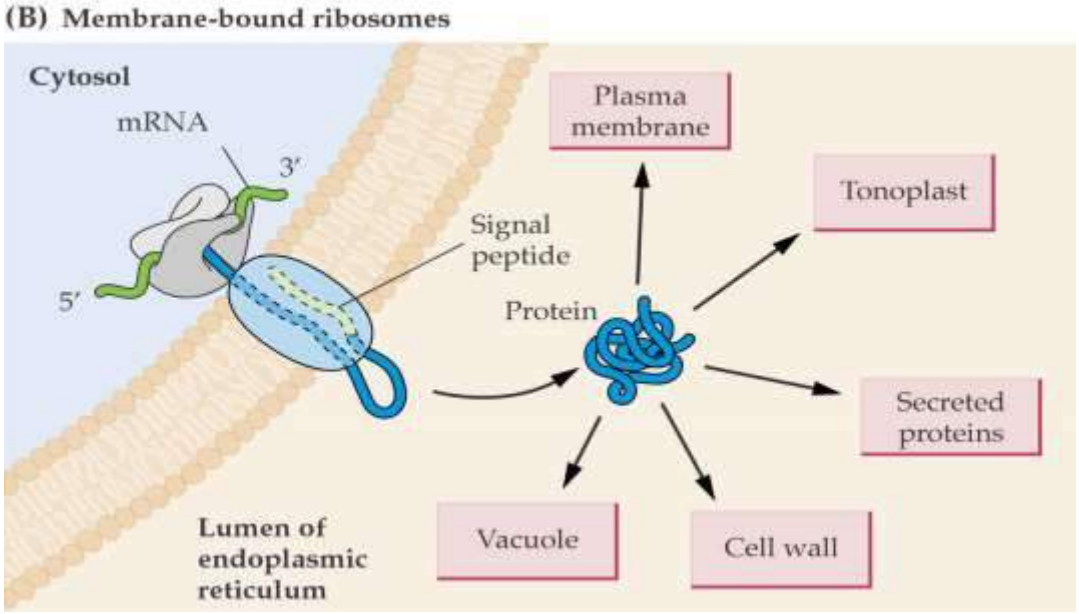
Secretory

Plasma Memb.

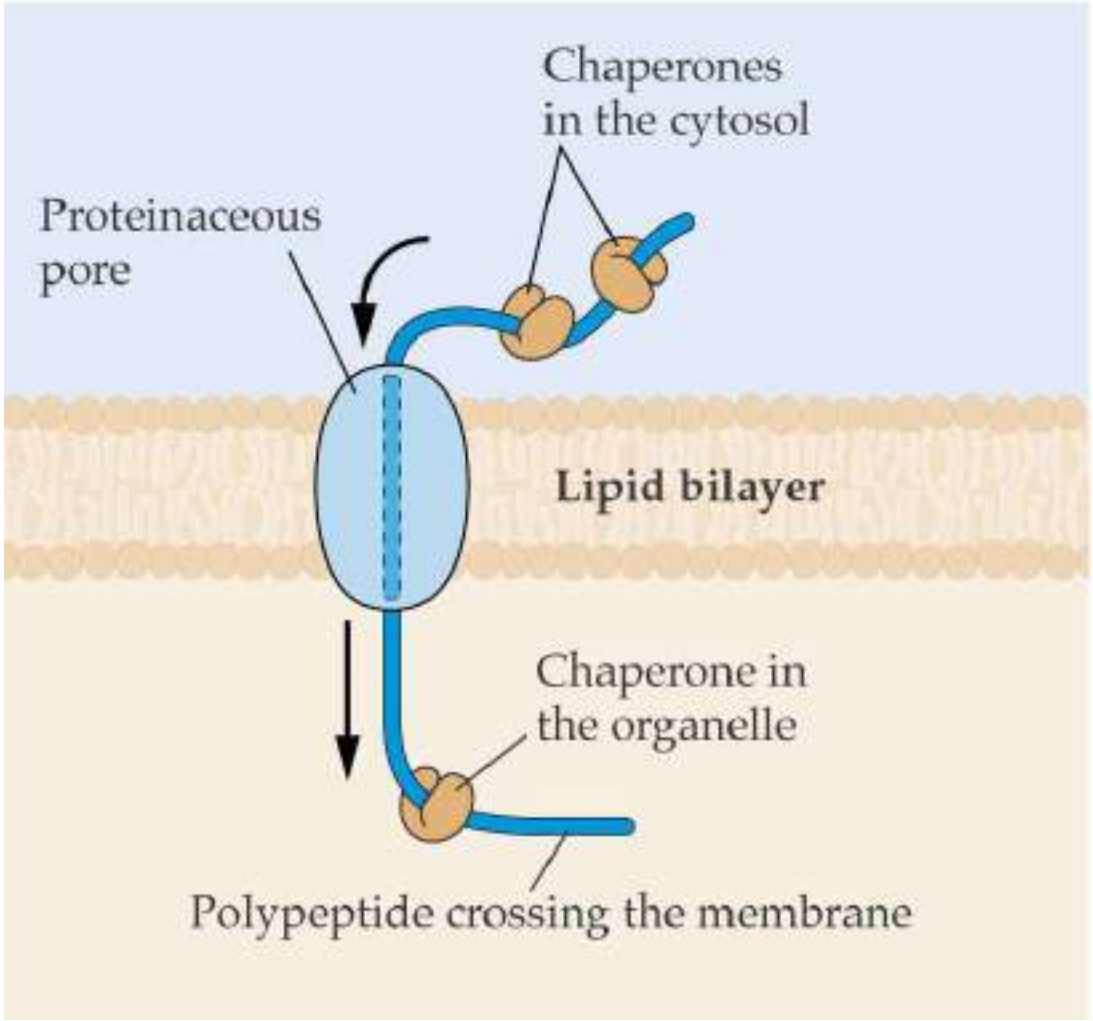
Two types of cytosolic ribosomes: free and membrane-bound.

They synthesize proteins with different destinations.





2. Chaperonins play roles in membrane transport on both sides of the membrane.



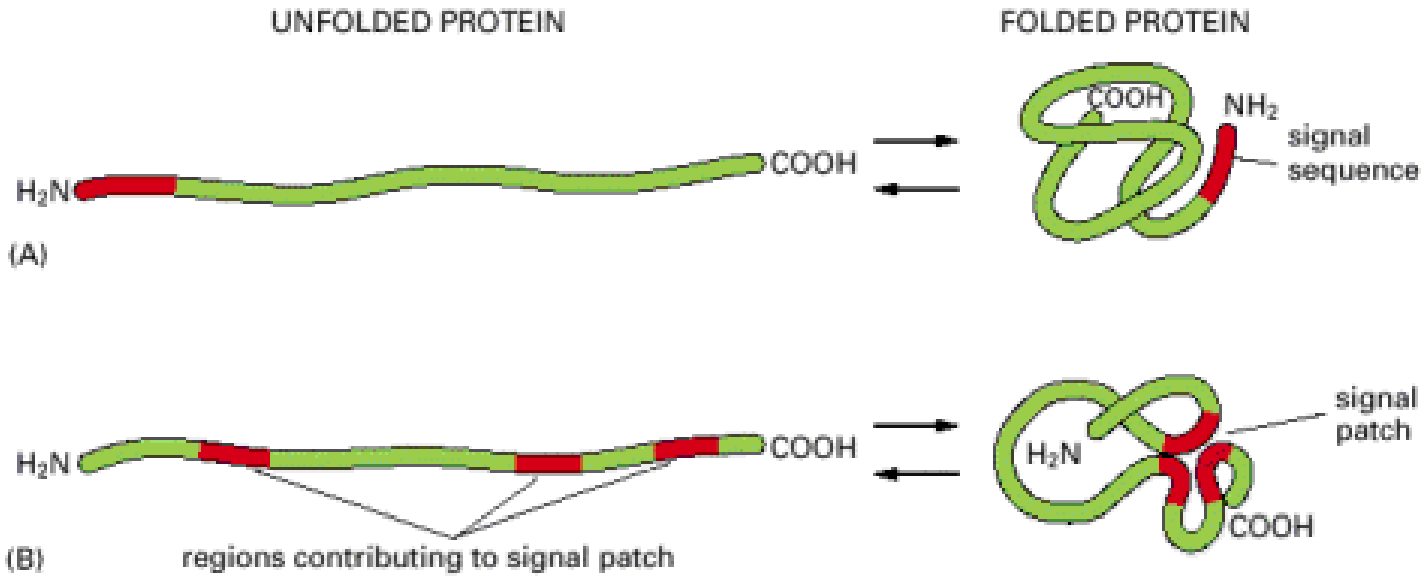
Peptide domains for targeting to different organelles

Organelle	Targeting Domain
ER	Signal peptide (SP)
Chloroplast	Transit peptide (TP)
Mitochondrion	Pre-sequence
Nucleus	Nuclear localization signal (NLS)
Peroxisome	Peroxisomal targeting signal(s) (PTS1 and PTS2)
Vacuole	Vacuolar sorting signal (VSS)

Protein traffic

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

Sorting sequences



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.

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

Signal Recognition Particle

Ribonucleoprotien particle, 325 kD

RNA – 300 nucleotide

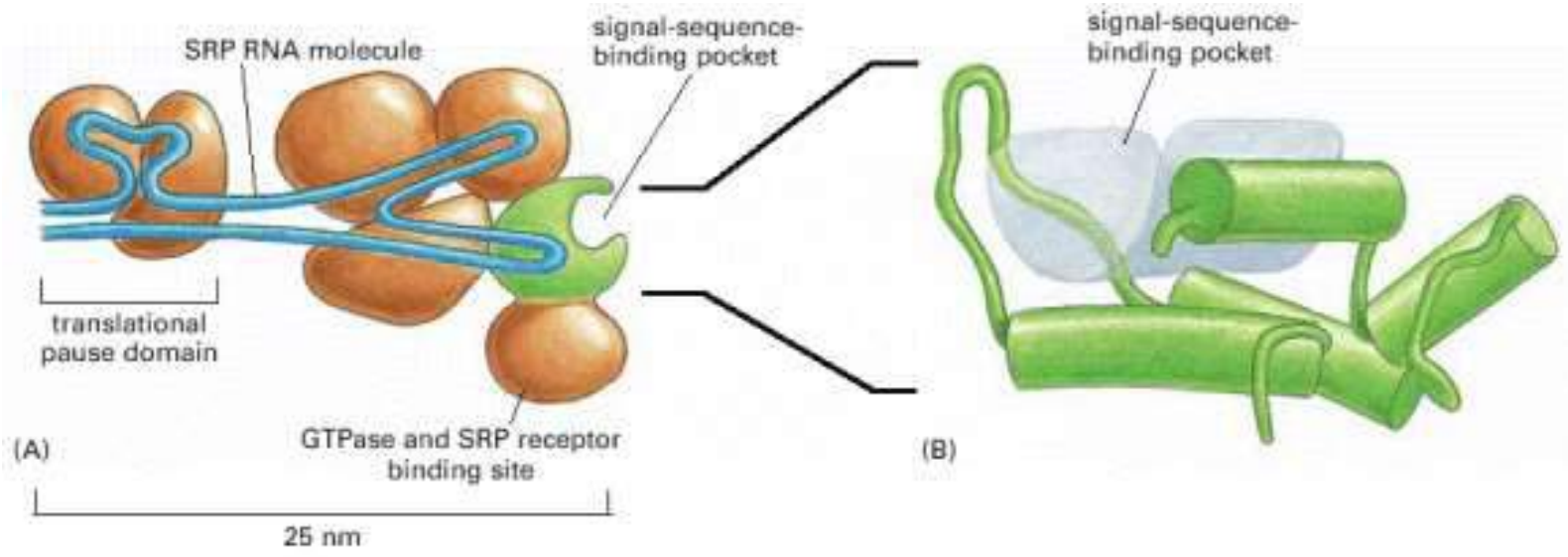
6 polypeptides- 9, 14, 19, 54, 68 & 78 kD

54 kD polypeptide binds to the signal sequence

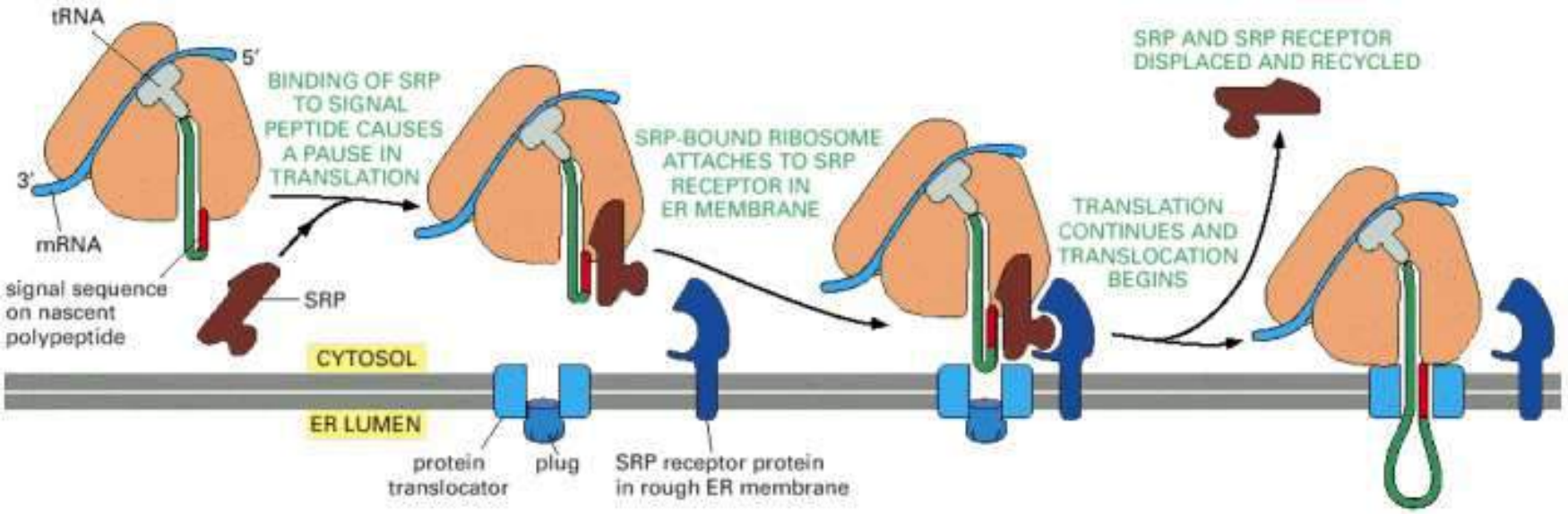
The SRP Receptor

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

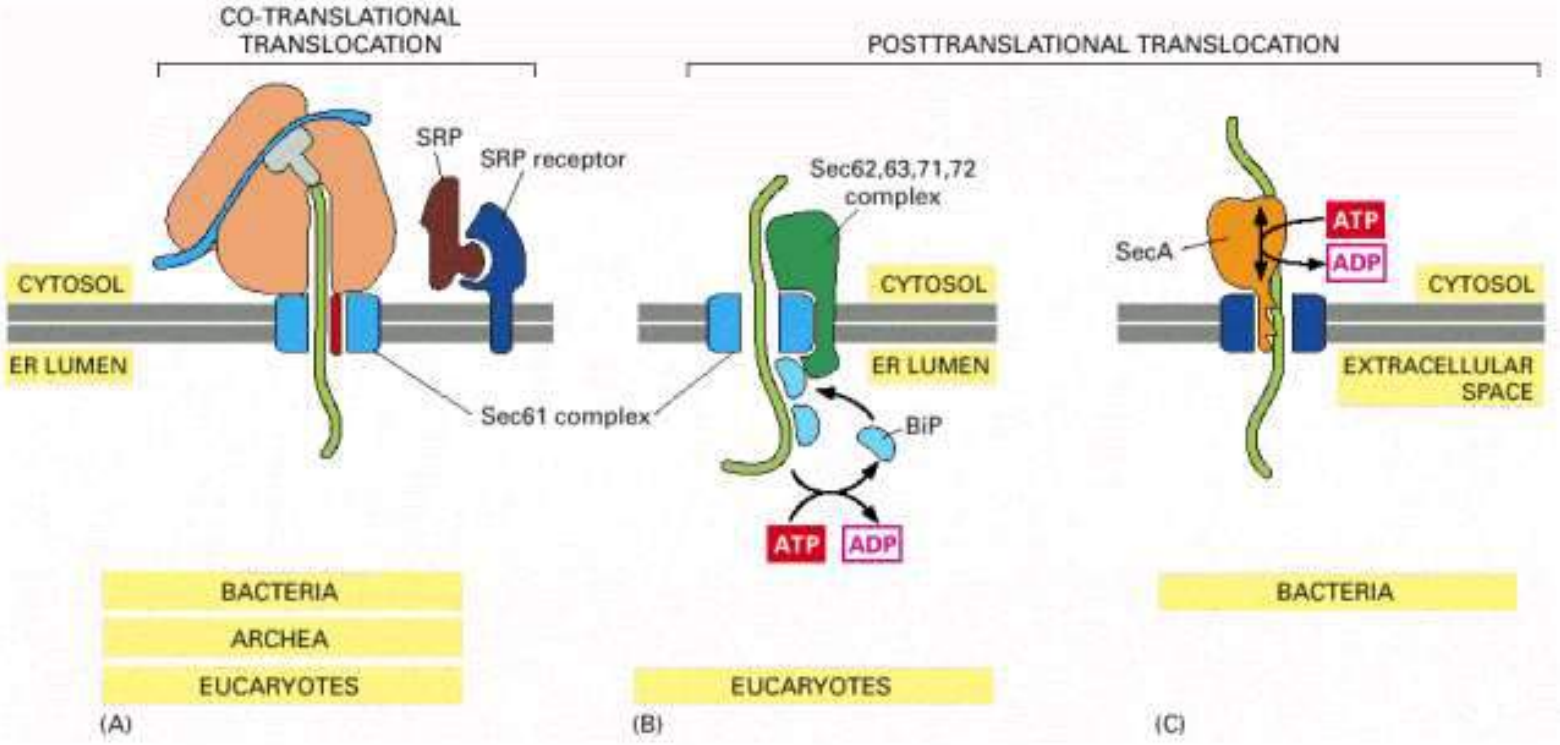
The signal-recognition particle (SRP)



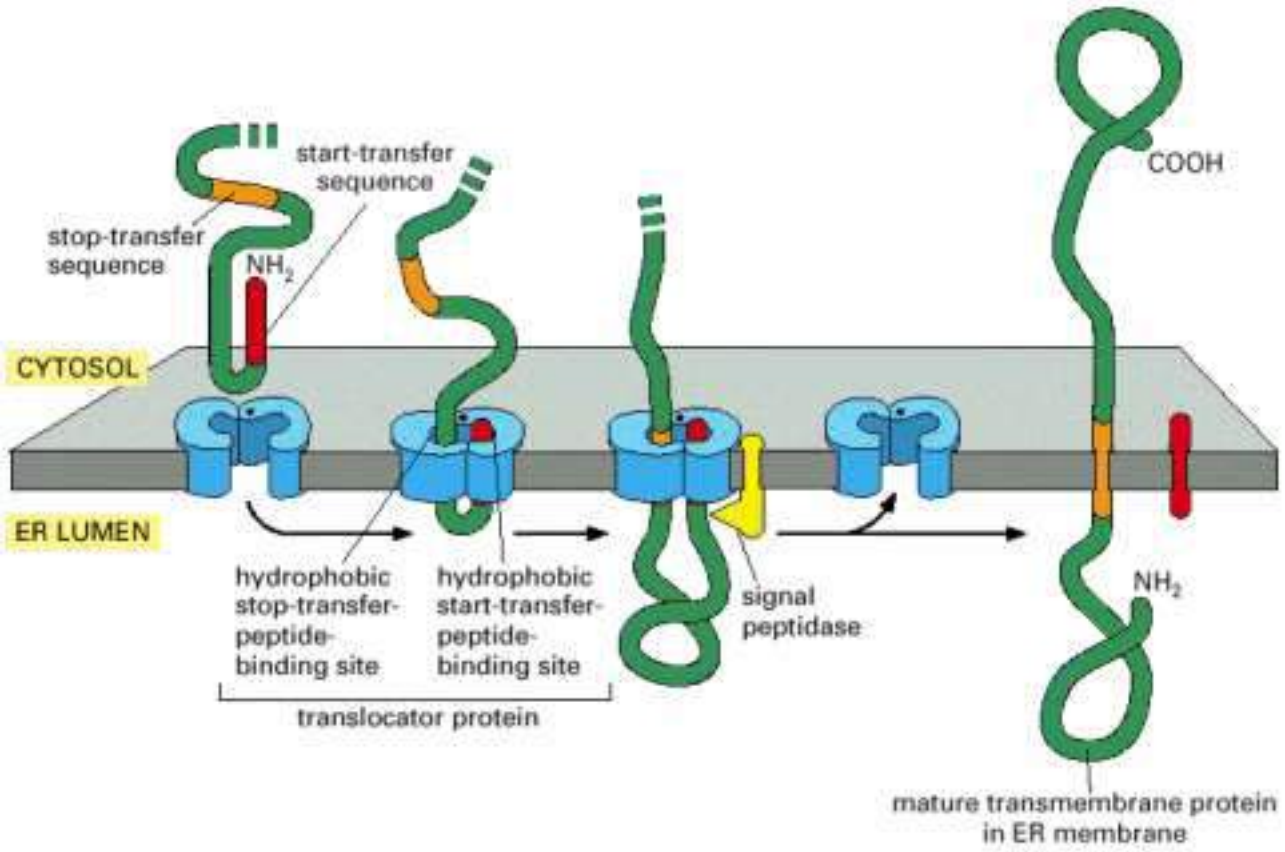
SRP direct ribosomes to the ER membrane



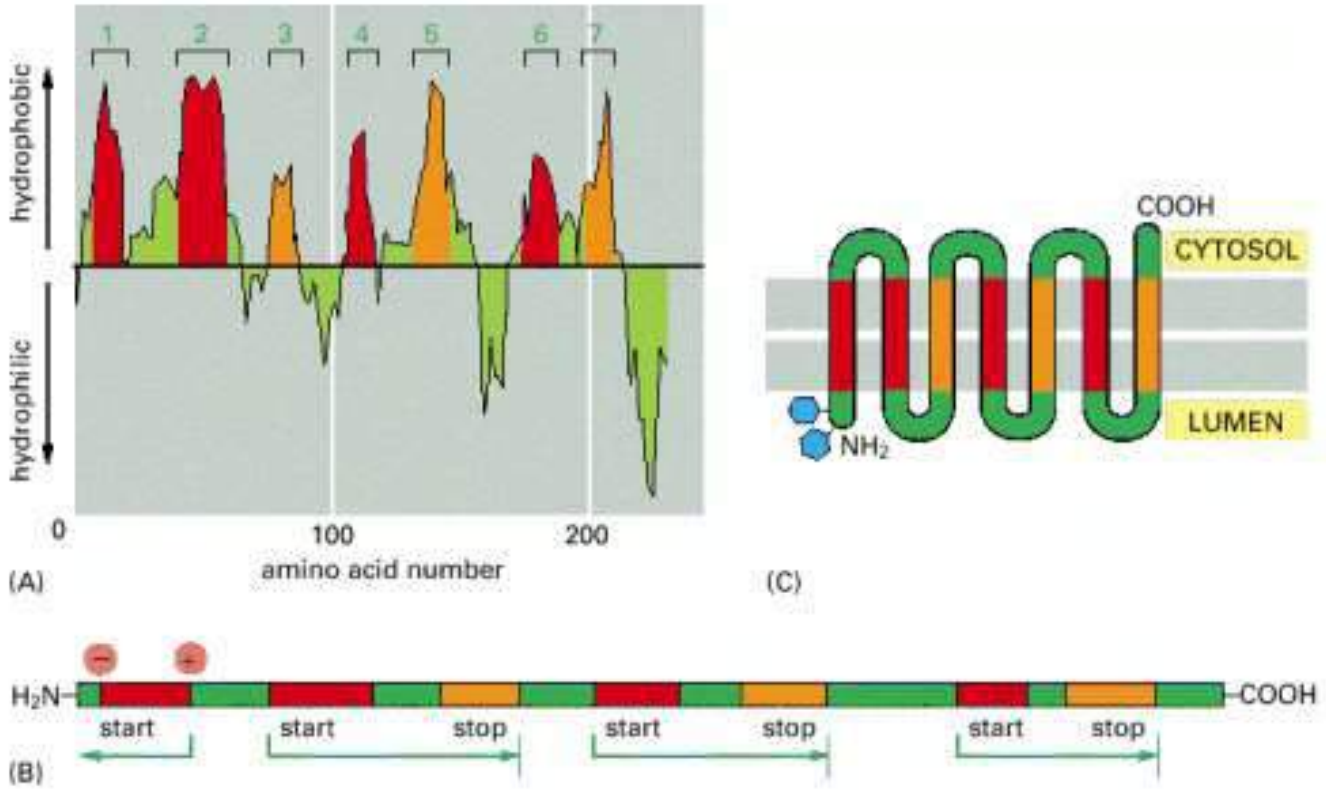
Protein translocation

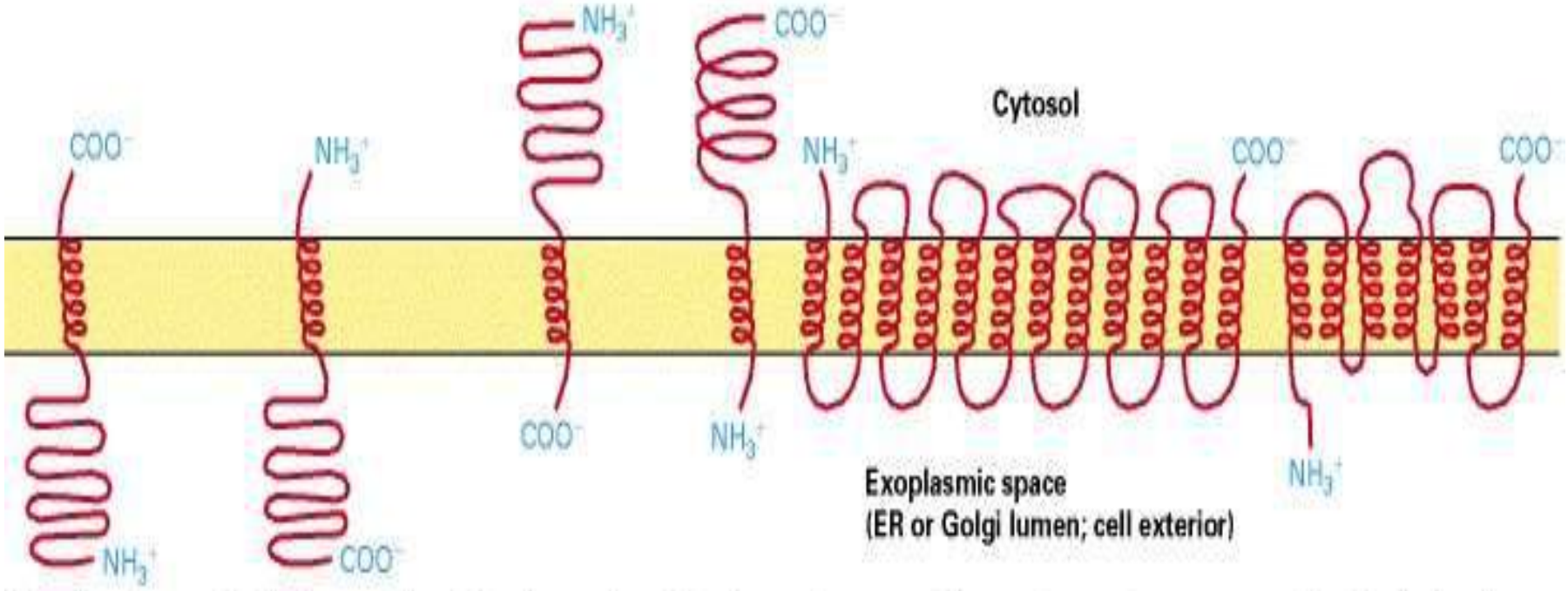


Single-pass transmembrane protein



Multipass membrane protein rhodopsin





Glycophorin
LDL receptor
Influenza HA protein
Insulin receptor

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

Cytochrome b_5

Cytochrome P_{450}

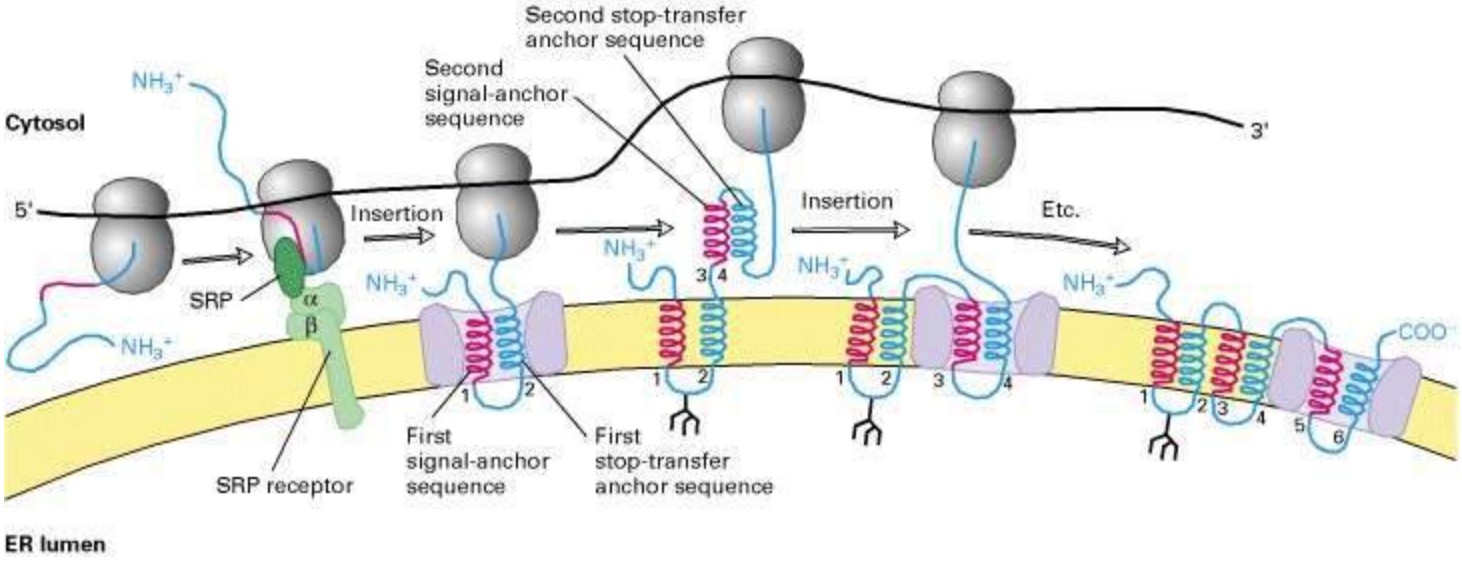
Glucose transport protein (GLUT 1)

Bacteriorhodopsin
β-adrenergic receptor
Rhodopsin

Topogenic sequences direct membrane proteins synthesized on the rough ER to assume their appropriate orientation in the ER membrane. This orientation is retained during transport of a membrane protein to its final destination.

Topogenic sequences include N-terminal cleaved signal sequences; stop-transfer membrane-anchor sequences; internal uncleaved signal-anchor sequences; and GPI-attachment sequences.

Many proteins have several membrane-spanning α helices. Each α-helical segment in such multipass proteins functions as an internal uncleaved signal anchor sequence or a stop-transfer membrane-anchor sequence depending on its location in the polypeptide chain.



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

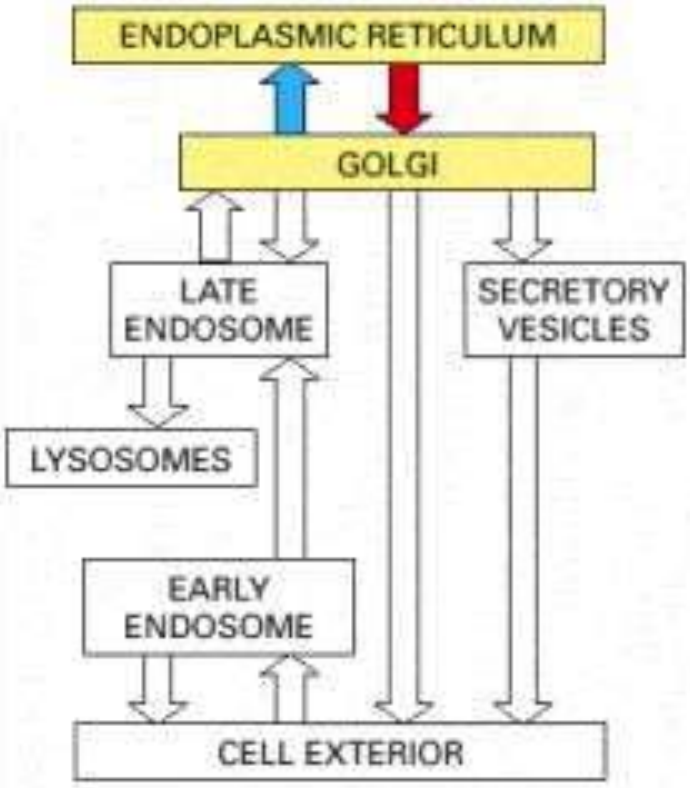
Golgi Apparatus

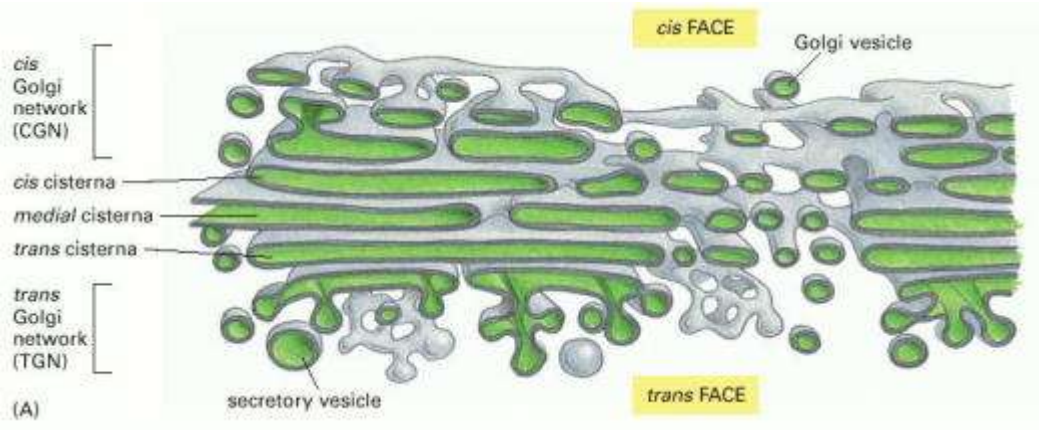
- **Major sorting centre - GPO of cell**

  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**

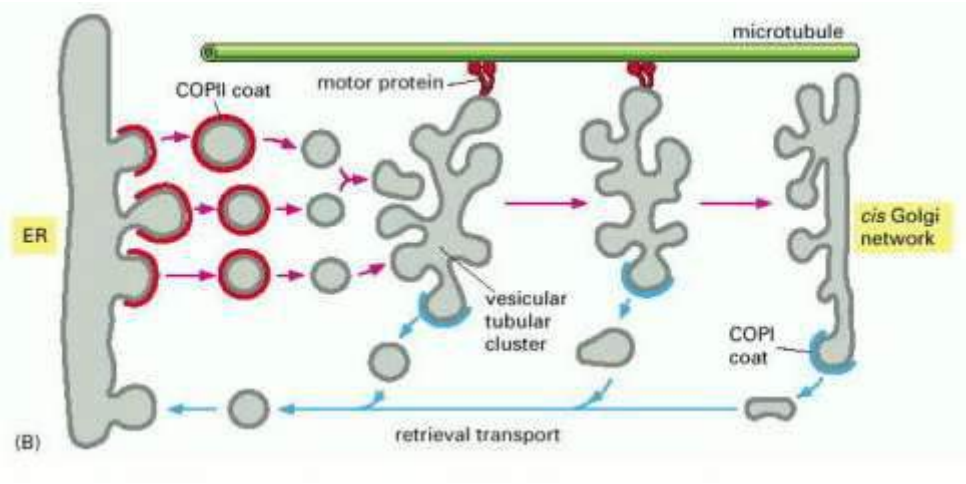
The Golgi Apparatus





- Because of its large and regular structure, the Golgi apparatus was one of the first organelles described by early light microscopists.
- It consists of a collection of flattened, membrane-enclosed cisternae, somewhat resembling a stack of pancakes. Each of these Golgi stacks usually consists of four to six cisternae
- Each Golgi stack has two distinct faces: a *cis* face (or entry face) and a *trans* face (or exit face). Both *cis* and *trans* faces are closely associated with special compartments, each composed of a network of interconnected tubular and cisternal structures.

Transport of proteins from ER to Golgi



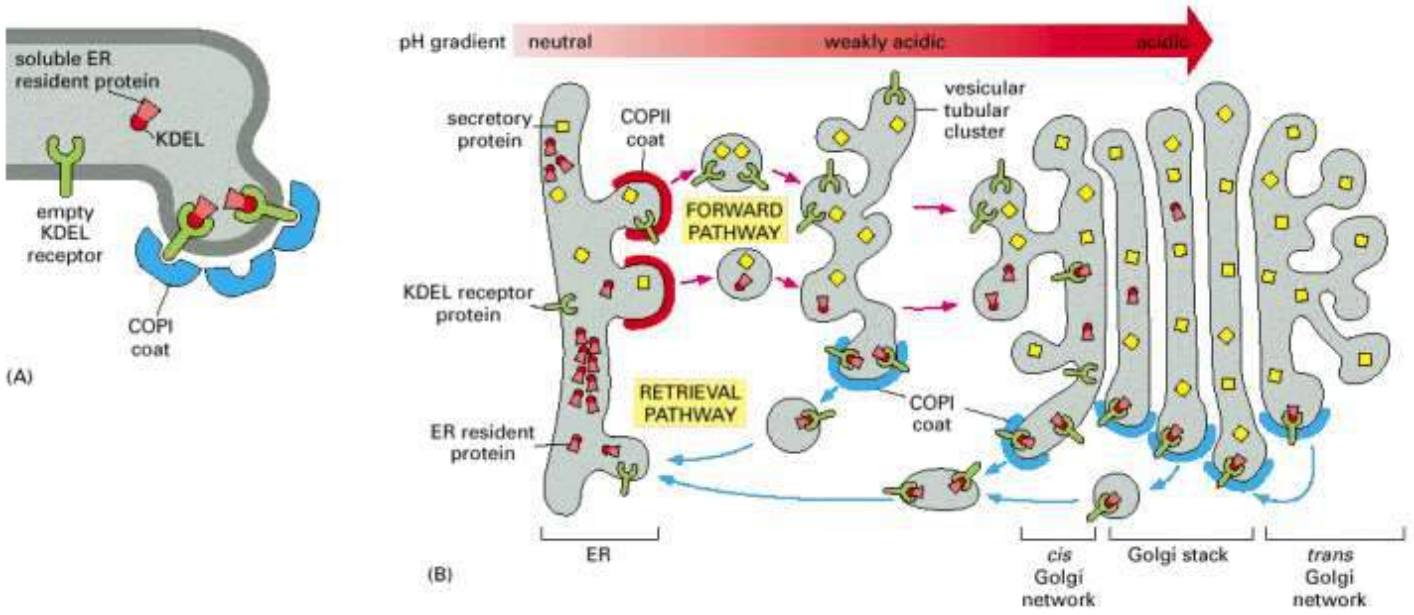
• Proteins destined for the Golgi, lysosome, PM, or extracellular fluid are packaged into vesicles at specialized sites referred to as ER EXIT SITES.

• ER exit sites are studded with receptors which bind to proteins destined to leave the ER. Proteins leaving the ER contain specific amino acid sequences which are bound by these receptors.

• Binding the receptor induces vesicle budding and the transport of the vesicle to the *cis*-Golgi network. It is important to note that only properly folded proteins are transported.

• Following vesicle budding, vesicles fuse to form a vesicular tubular cluster which is then transferred to the Golgi.

The ER retrieval pathway



• During the vesicular transport of proteins from the ER to the Golgi, proteins from the ER can be accidently packaged within the vesicles destined for the Golgi. Proteins resident to the ER are recovered by the ER RETRIEVAL PATHWAY (RETROGRADE TRANSPORT). ER proteins are packaged in COPI vesicles and transferred back to the ER. Membrane proteins are easily packaged into the vesicle by a KKXX sequence.

• Soluble proteins, such as Bip, also contain retrieval signals however the mechanism is slightly different. This signal consists of Lys-Asp-Glu-Leu (KDEL sequence)

• Soluble ER proteins which have escaped the lumen of the ER are retrieved by KDEL receptors.

• The affinity of KDEL receptors for KDEL sequences is dependent on the pH of each organelle. While the KDEL receptor has a high affinity for the KDEL sequence at the more acidic pH of the Golgi lumen, the neutral pH of the ER lumen decreases the affinity of the receptor for the protein prompting its release.

• Thus the Retrieval Pathway is pH dependent

Golgi are involved in the sorting and post-translational modification of proteins

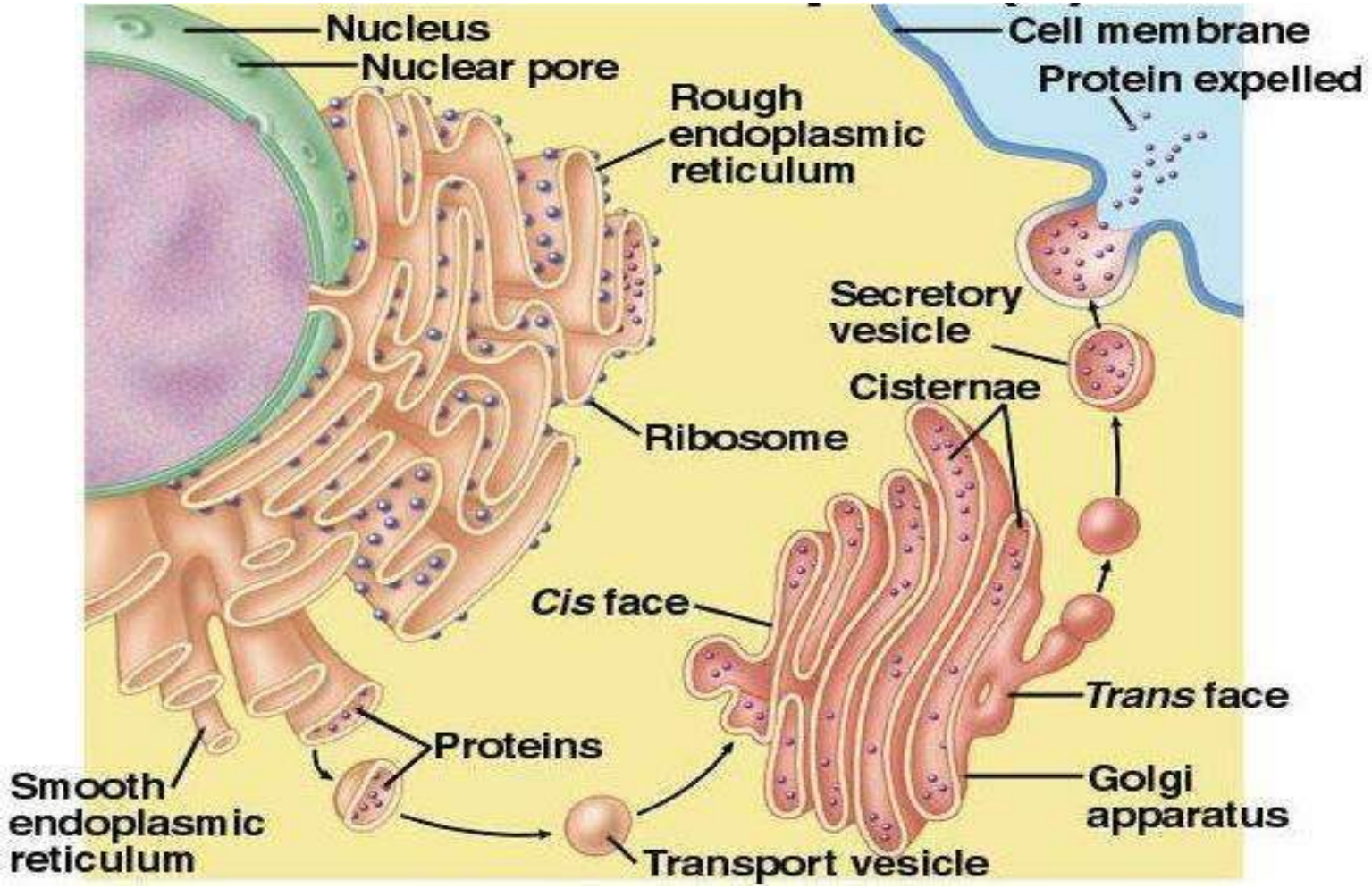
- During the passage of proteins through the Golgi compartments, various covalent modifications take place in order to provide the specific structure and function to the protein
- Modification of existing glycosyl groups, *O*-glycosylation, sulfation (addition of sulfates to OH on tyrosine), and phosphorylation all take place within the various Golgi compartments.
- For simplicity, the primary focus of this lecture series will be the modification of proteins by glycosylation
- As mentioned previously, the ER *N*-glycosylates various proteins with oligosaccharides The Golgi then modifies these oligos providing either a COMPLEX OLIGOSACCHARIDE and HIGH MANNOSE CONTAINING OLIGOSACCHARIDE.
- The high mannose oligo is produced by removing glucose and N-acetylglucosamine moeities while the complex oligo is produced adding addition monosaccharides consecutively.
- Some proteins require additional oligosaccharides to provide a specific function. The Golgi also modifies proteins by *O*-glycosylation. Serines are used for this type of post-translational modification.

Lysosomal Targeting

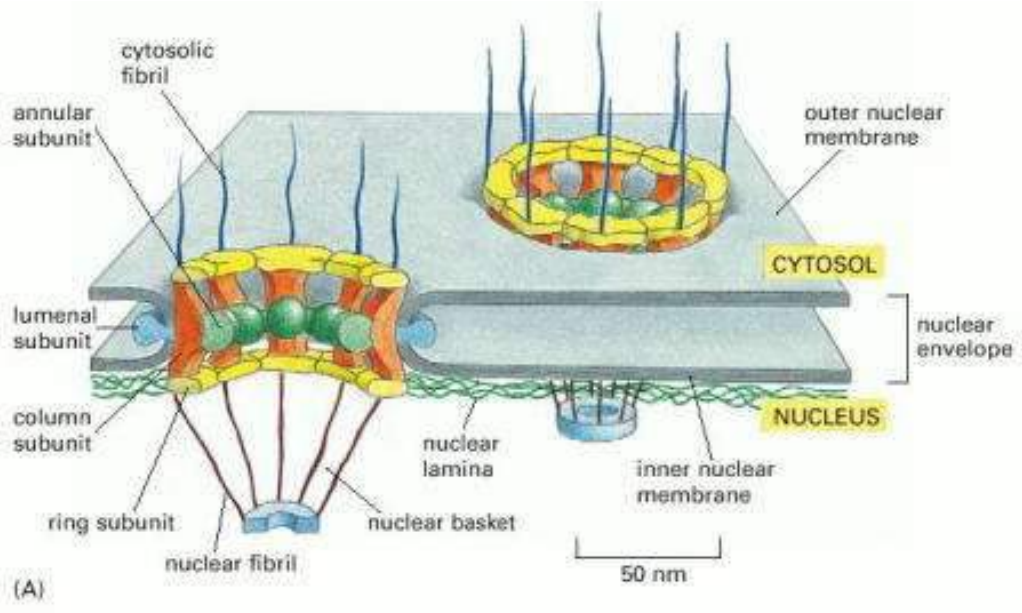
- Man-6 P is the marker, added in cis golgi
- Added by 2 step enzyme catalysed reaction

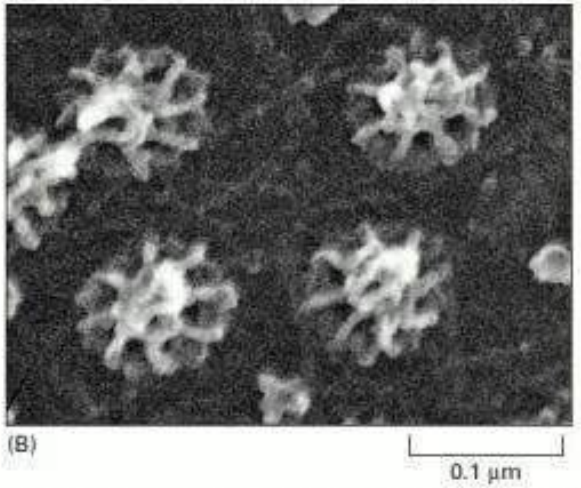
Phosphotransferase
Phosphodiesterase

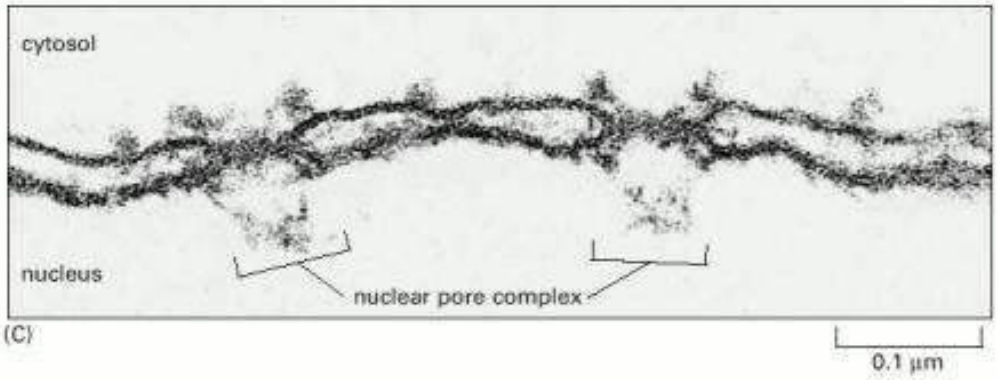
- Man-6 P receptors in trans golgi
- Fuses with pre lysosomal vesicles, acidic pH release proteins from receptors
- I Cell disease- severe psychomotor retardation

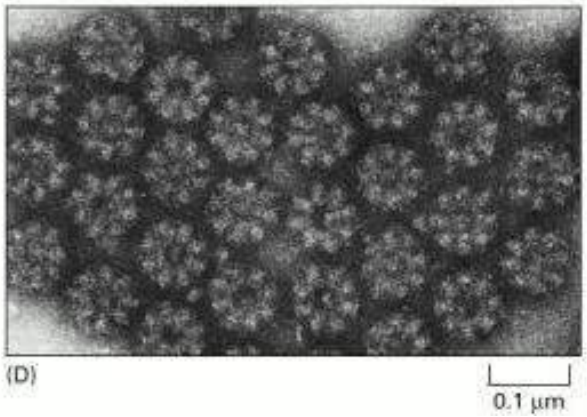

Nucleus
Nuclear pore
Rough endoplasmic reticulum
Ribosome
Cell membrane
Protein expelled
Secretory vesicle
Cisternae
Cis face
Trans face
Golgi apparatus
Proteins
Smooth endoplasmic reticulum
Transport vesicle

Nuclear pore complexes





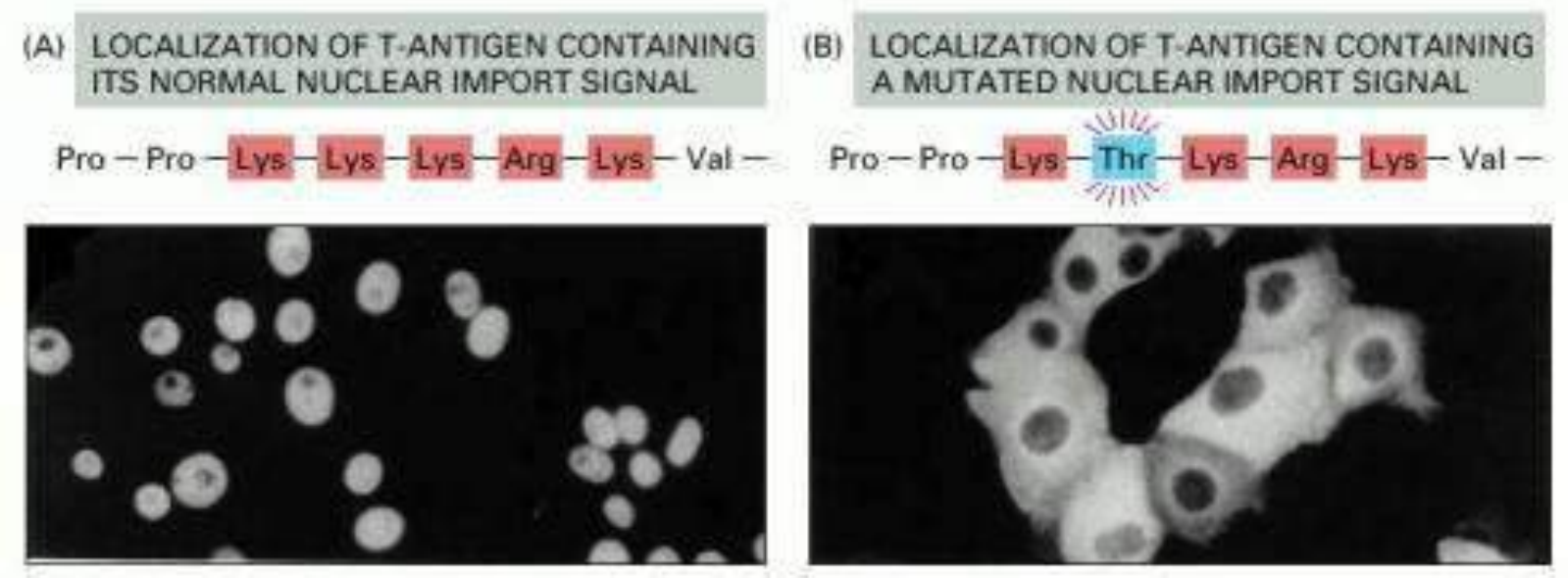




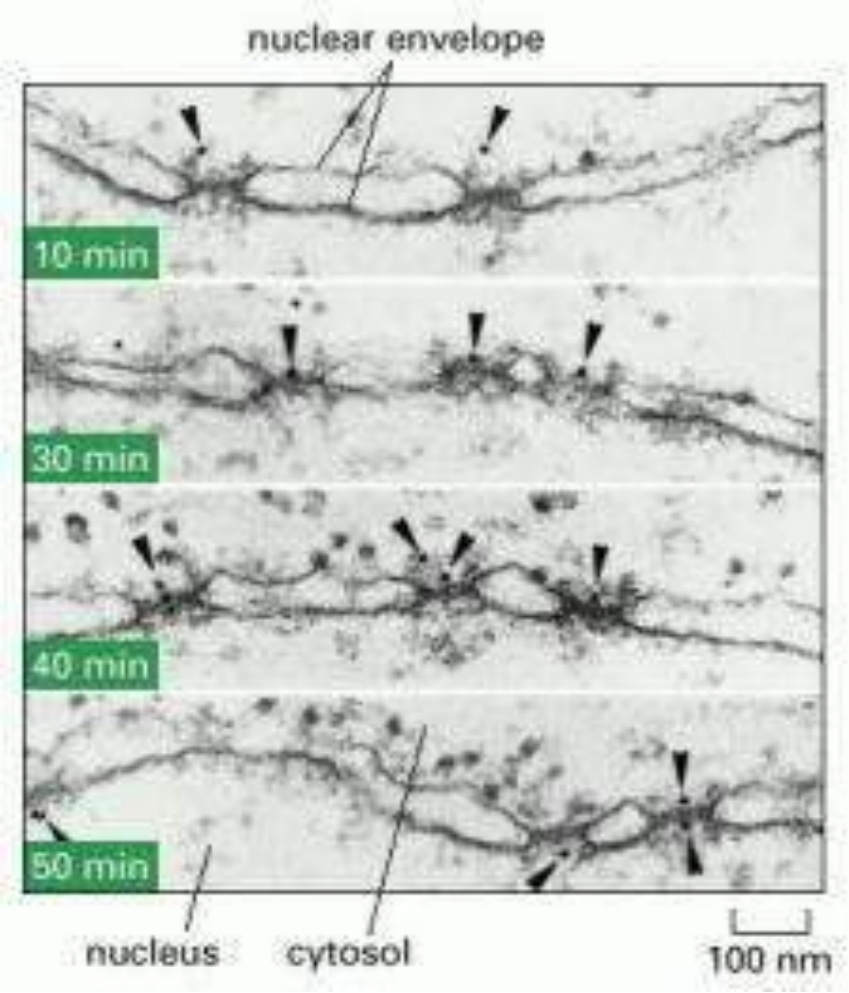
Nuclear lamina

- Consists of "intermediate filaments", 30-100 nm thick.
- These intermediate filaments are polymers of lamin, ranging from 60-75 kD.
- A-type lamins are inside, next to nucleoplasm; B-type lamins are near the nuclear membrane (inner). They may bind to integral proteins inside that membrane.
- The lamins may be involved in the functional organization of the nucleus.

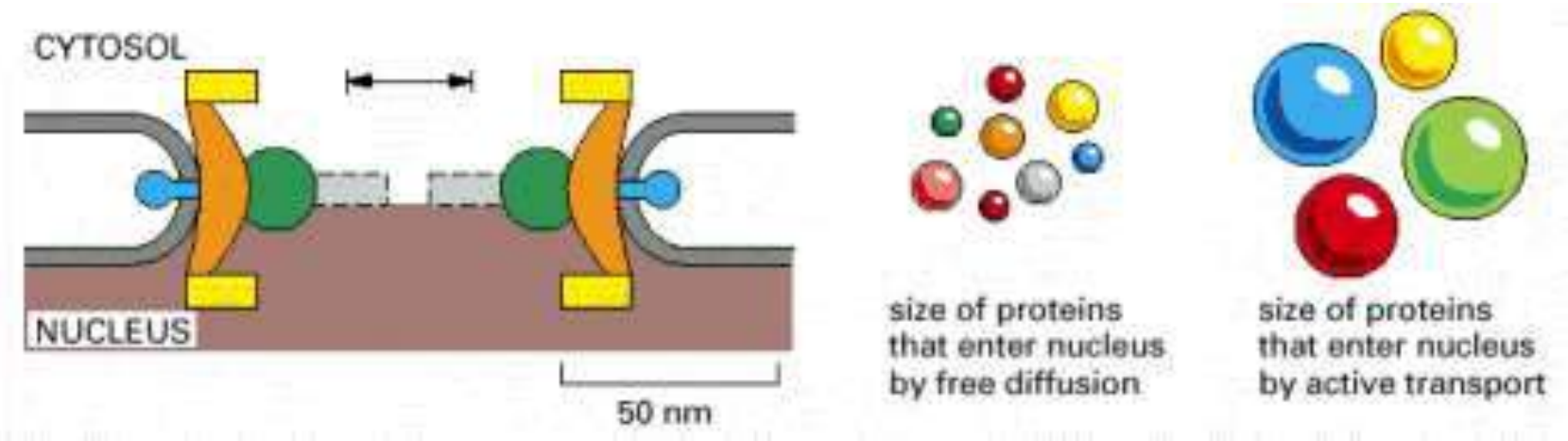
Nuclear localization signals (NLSs)



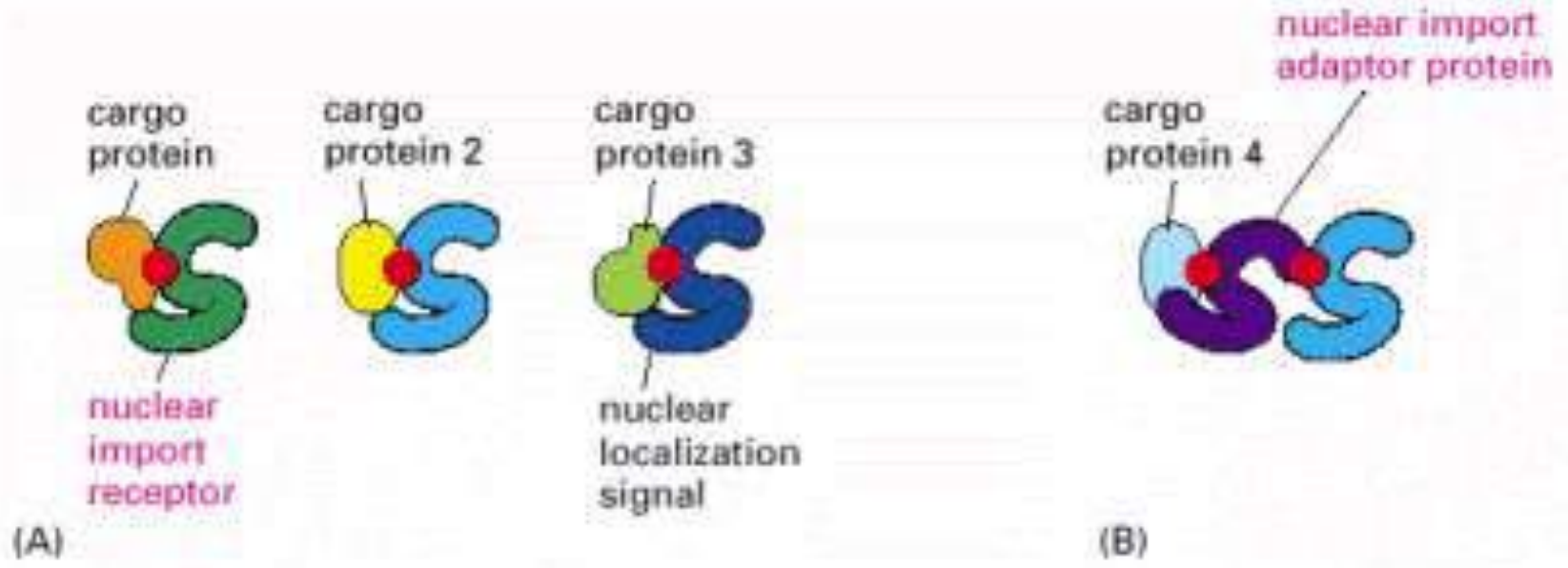
Protein import through nuclear pores

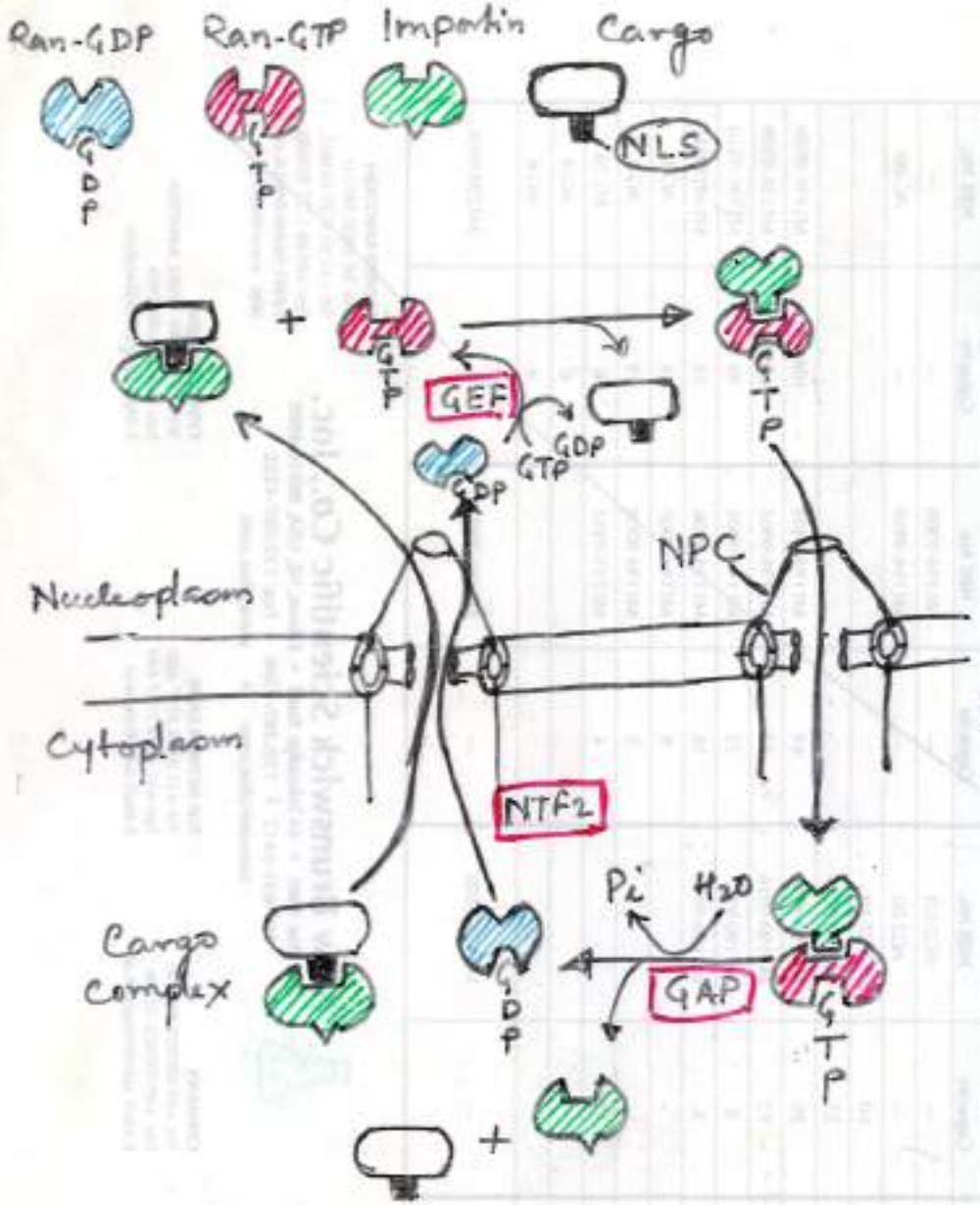


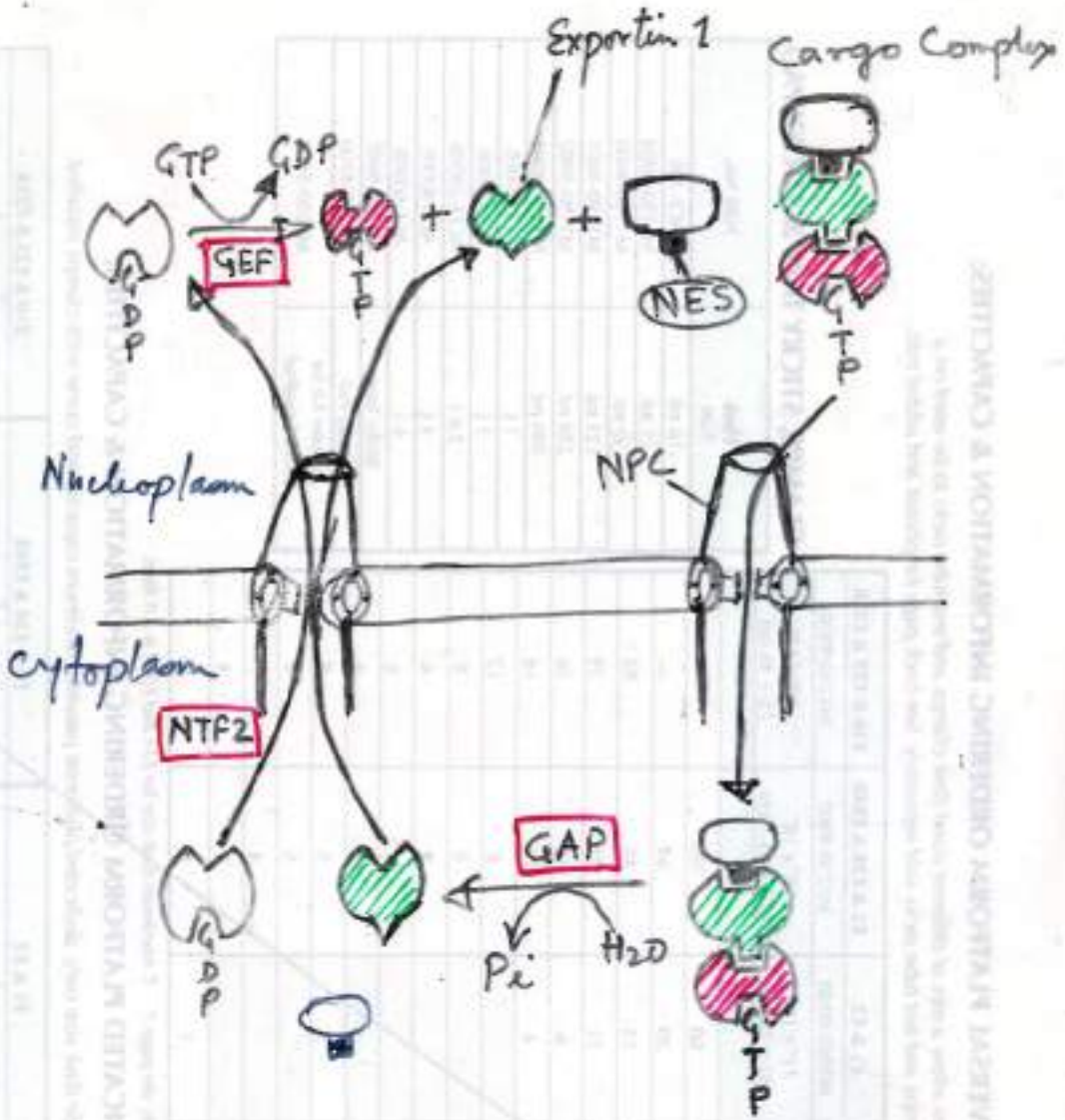
Possible paths for free diffusion through the nuclear pore complex



Nuclear Import / Export Receptors







GAP : GTPase accelerating protein (Ran-GAP)

GEF : Guanine nucleotide-exchange factor (Ran-GEF)

NTF2 : Nuclear Transport factor 2

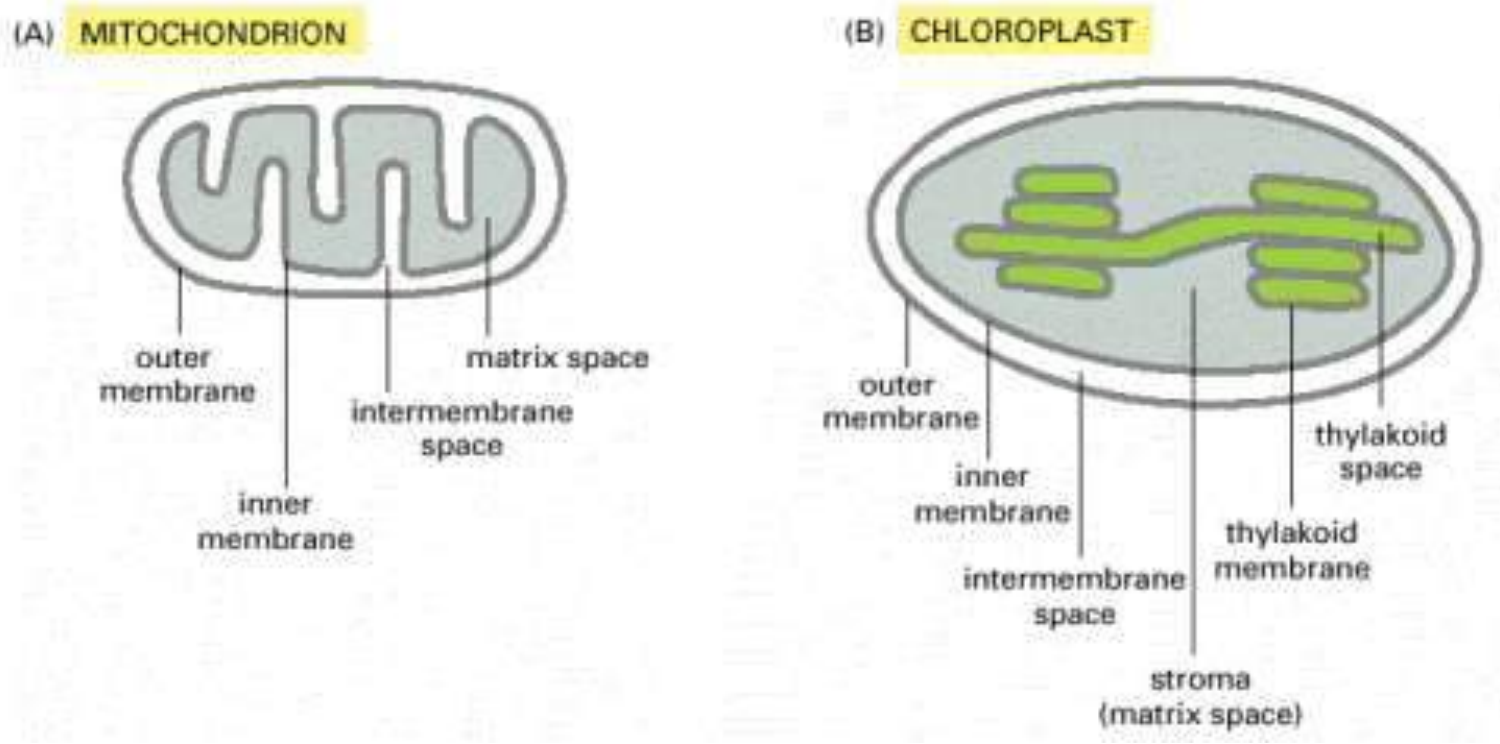
Nucleoporins – FG-nucleoporins

F- Phenylalanine

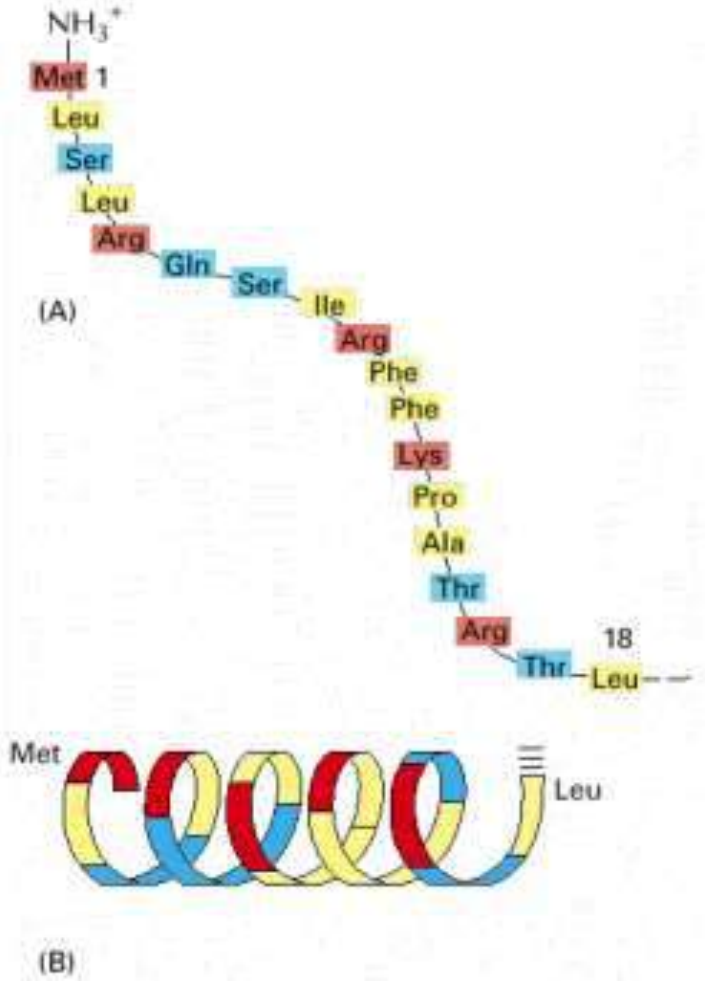
Targeting and assembly of proteins destined for chloroplasts and mitochondria

- How are proteins targeted to chloroplasts and mitochondria from the cytoplasm?
- How do they get through the membranes?

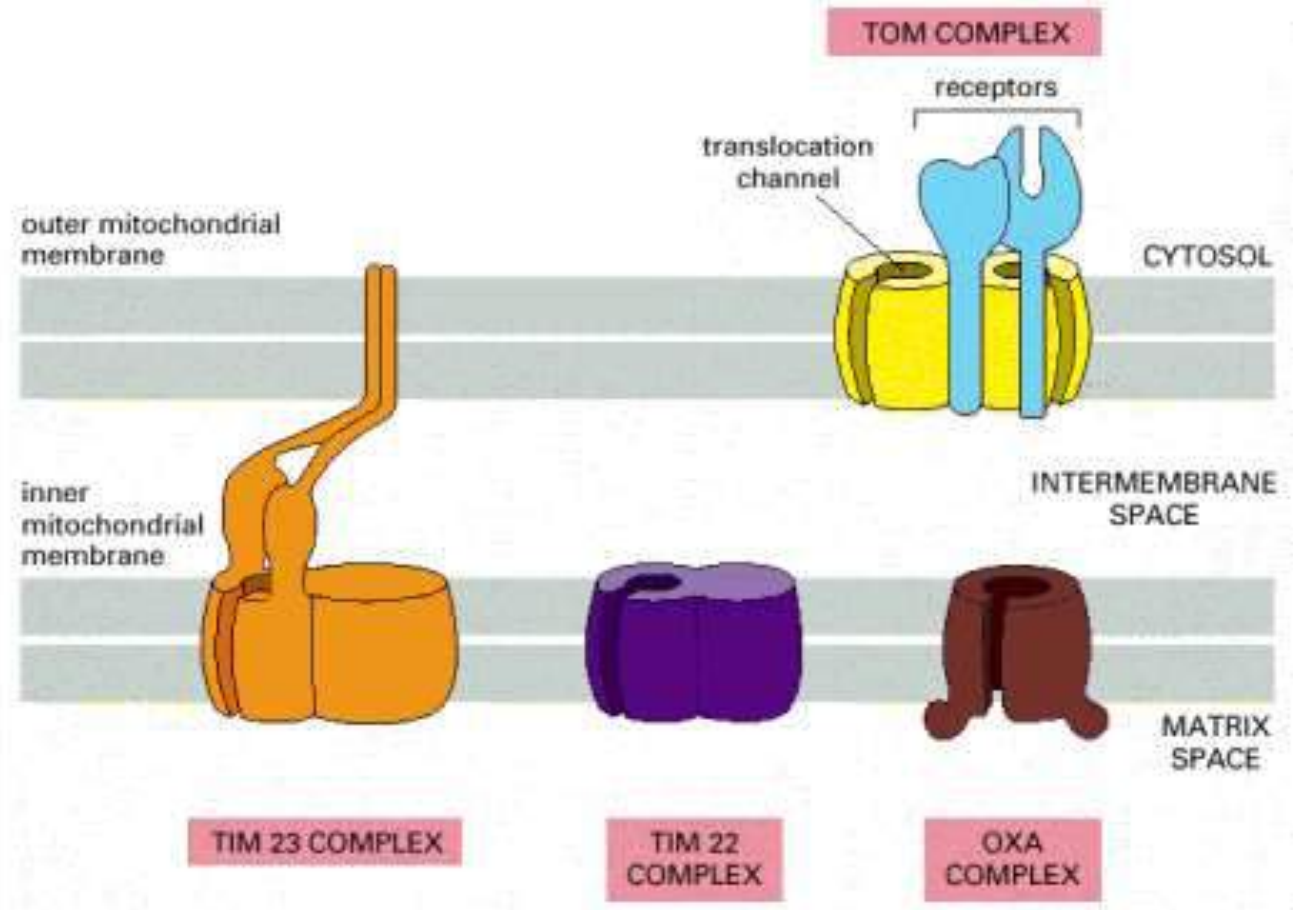
The subcompartments of mitochondria and chloroplasts



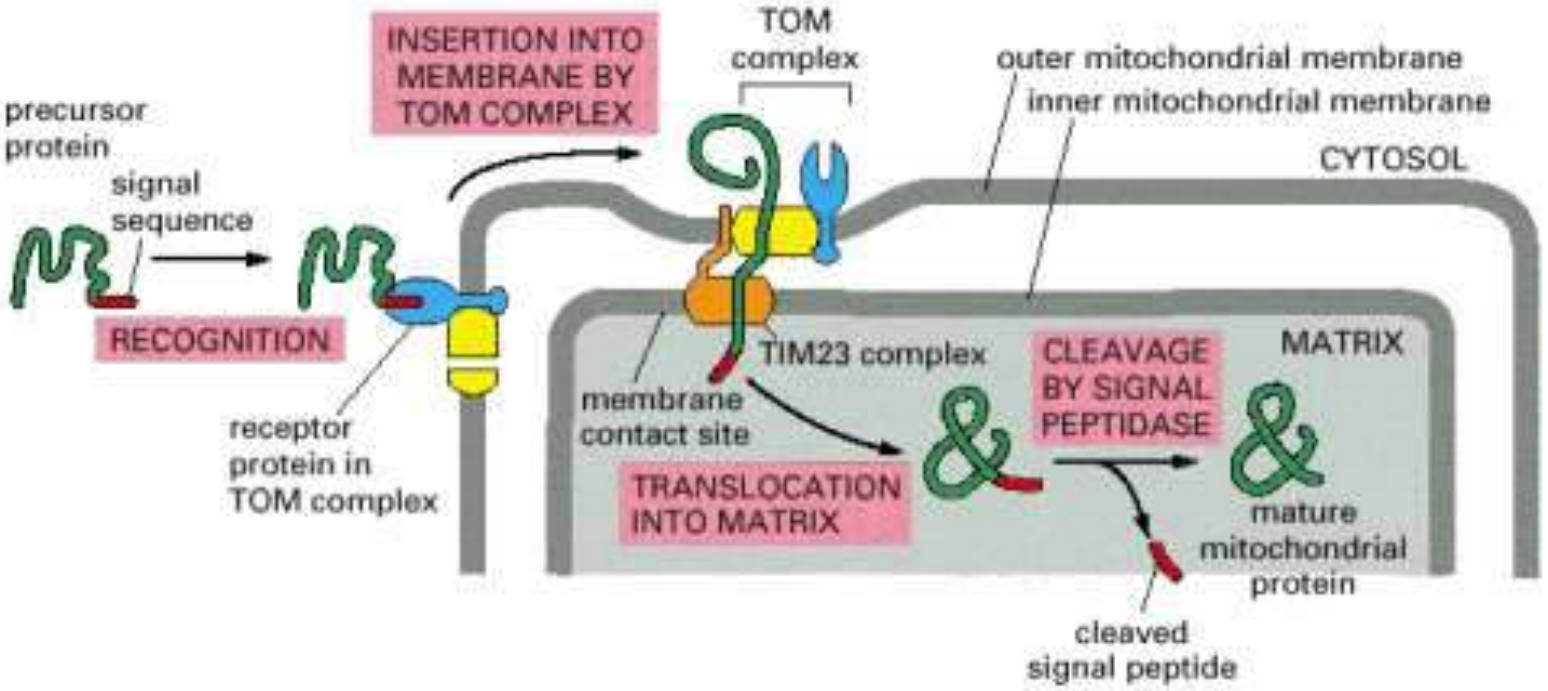
A signal sequence for mitochondrial protein import



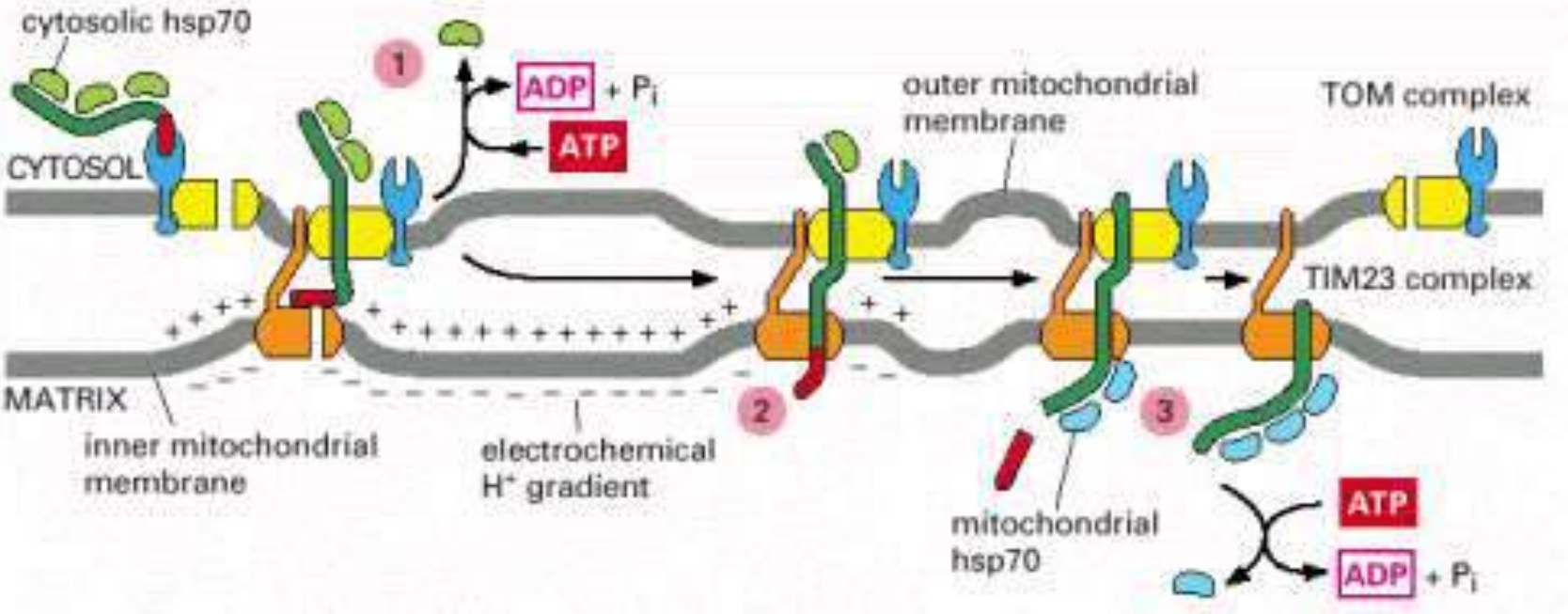
Protein translocators in the mitochondrial membrane



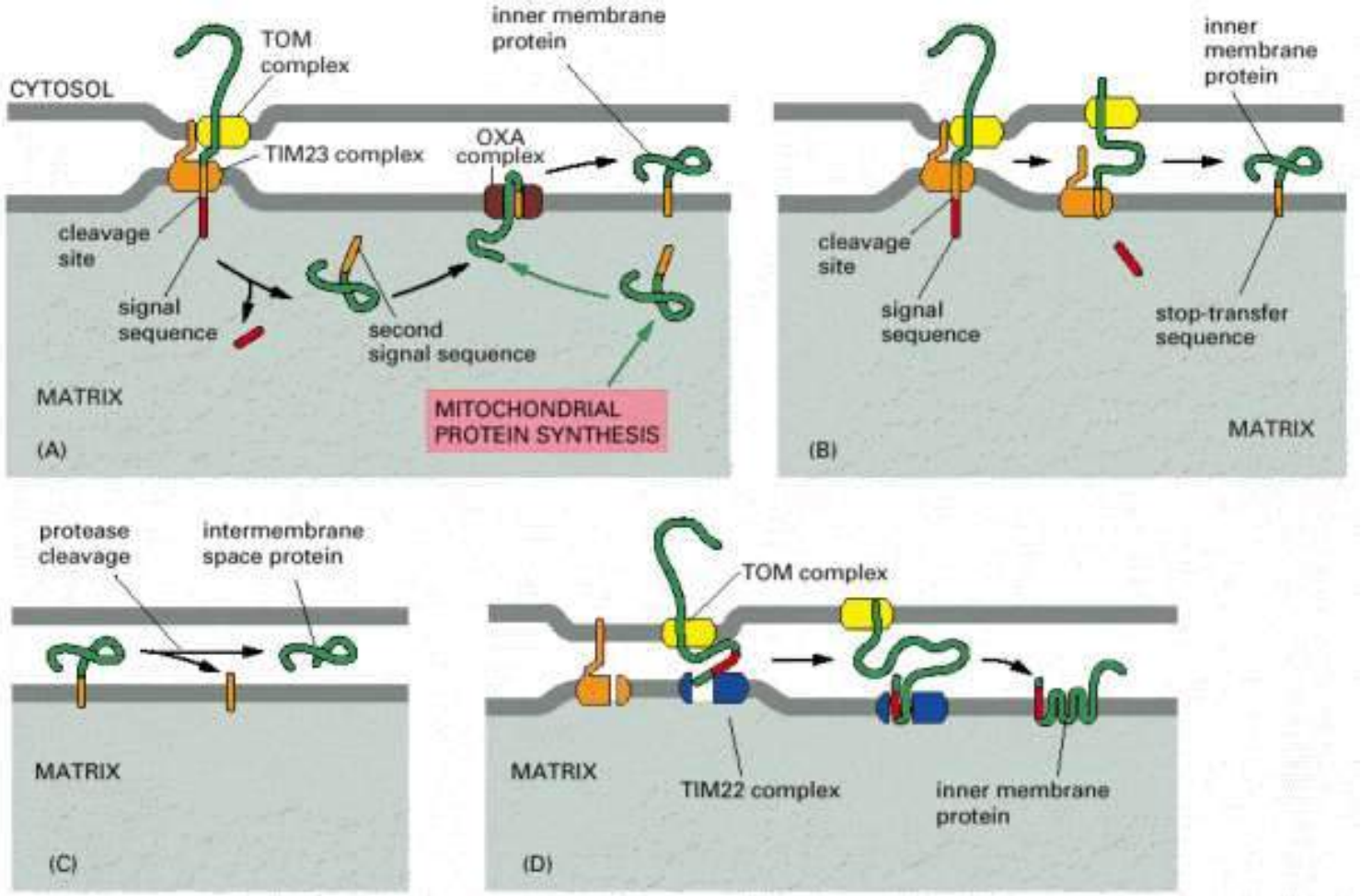
Protein import by mitochondria



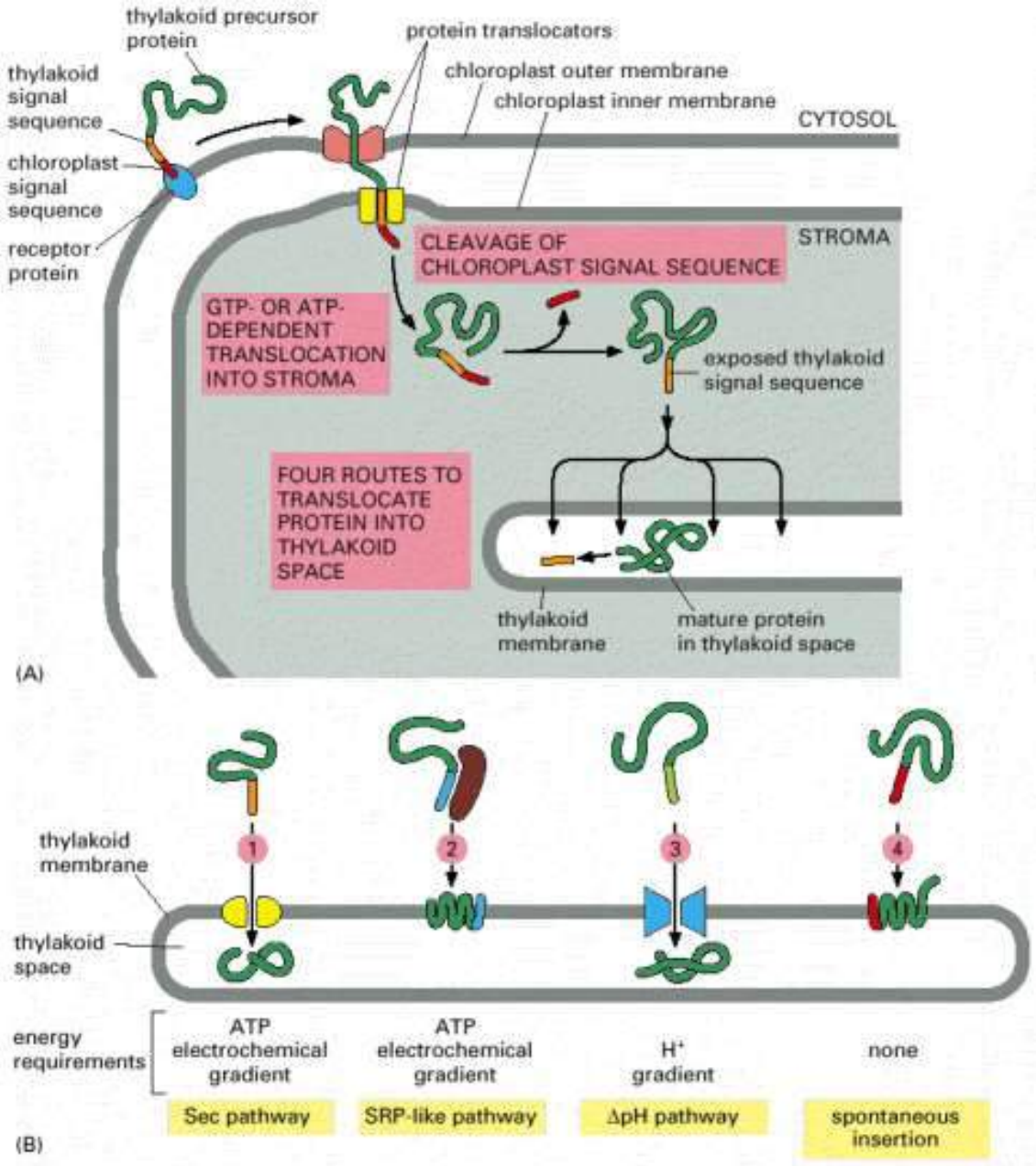
Energy required



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



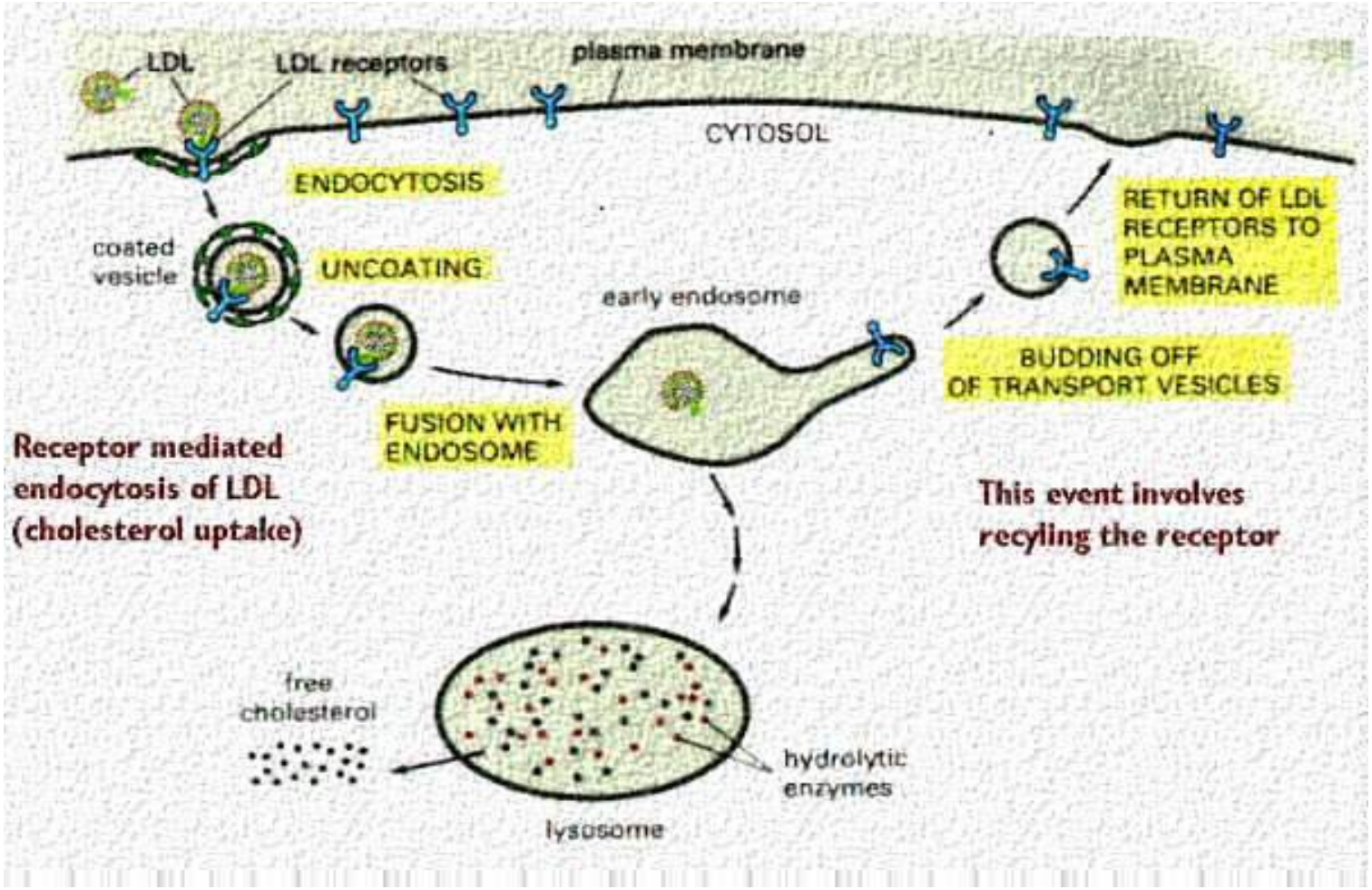
Translocation of a precursor protein into the thylakoid space of chloroplasts



Receptor mediated endocytosis

- Transport of essential metabolites **(cholesterol, Vit B_{12}, iron etc.)**
- Modulation of activity of protein hormones
- Proteins targeted for destruction
- Entry route for many viruses and toxins

The process

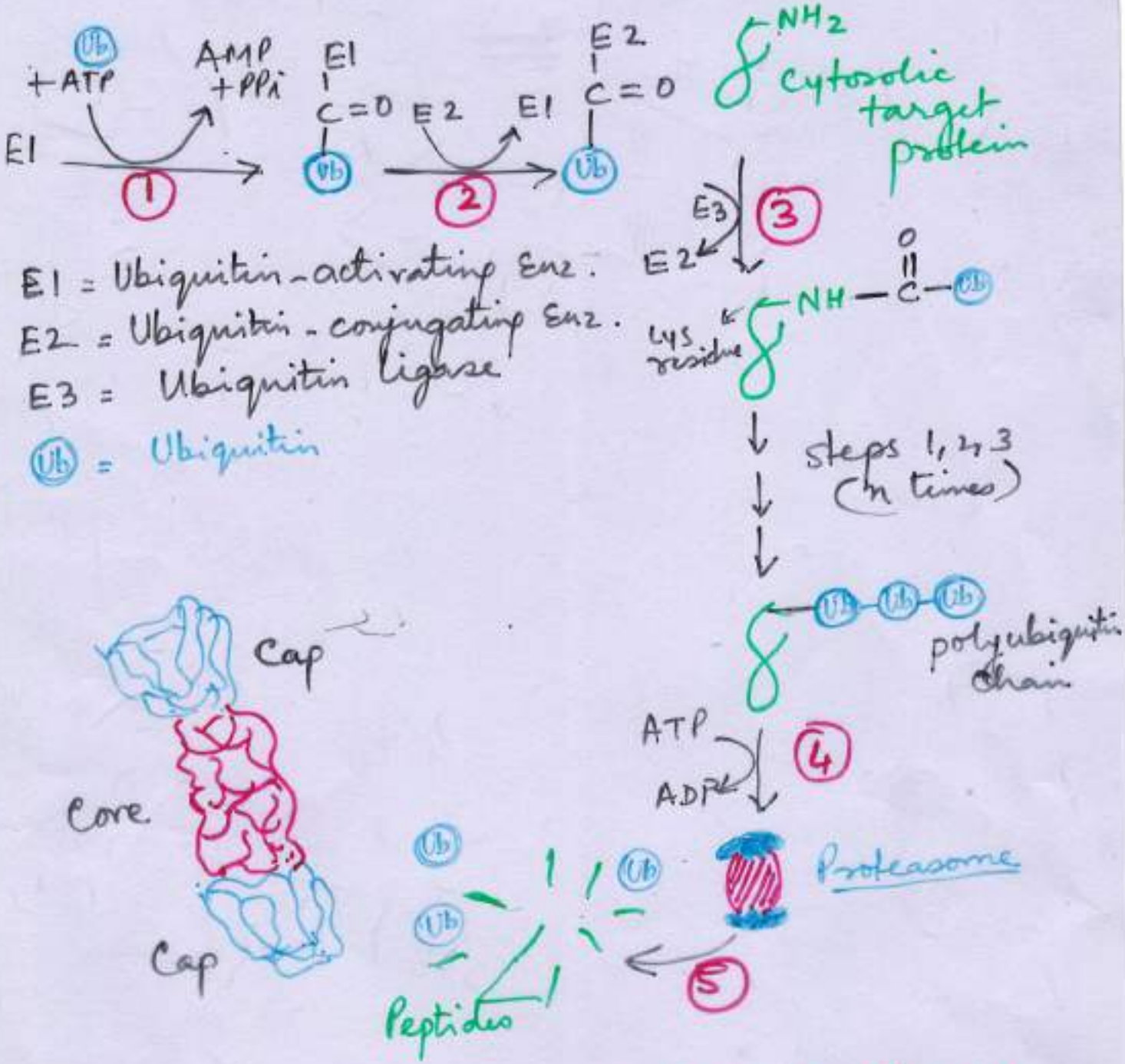

LDL
LDL receptors
plasma membrane
CYTOSOL
ENDOCYTOSIS
coated vesicle
UNCOATING
early endosome
RETURN OF LDL RECEPTORS TO PLASMA MEMBRANE
BUDDING OFF OF TRANSPORT VESICLES
FUSION WITH ENDOSOME
Receptor mediated endocytosis of LDL (cholesterol uptake)
This event involves recyling the receptor
free cholesterol
hydrolytic enzymes
lysosome

The life of a protein

- Determined by N terminal amino acid.
- Proteins with ala, met, gly, ser, val, thr *etc* at the N terminus have more half life.
- Proteins with glu, gln, asp and asn have less half life.
- The tagged proteins are turned over by a 26S protease complex.
- It leaves ubiquitin unaffected.

Protein Destruction

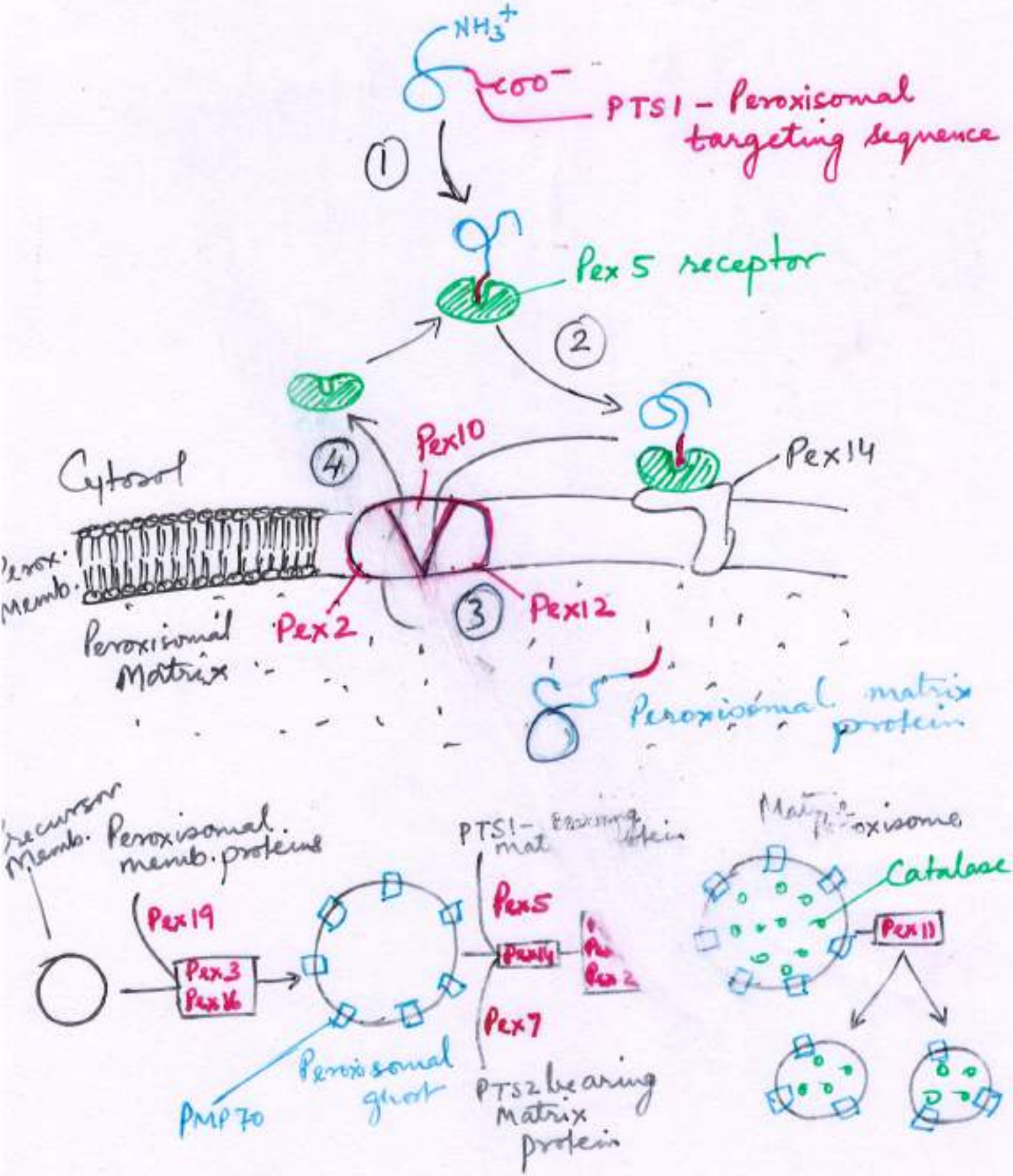
- Ubiquitin serves as a tag
- It is a small 8.5 KD protein
- Gets attached by its C terminal to lys of target protein
- Reaction catalysed by three enzymes, E1, E2 and E3.



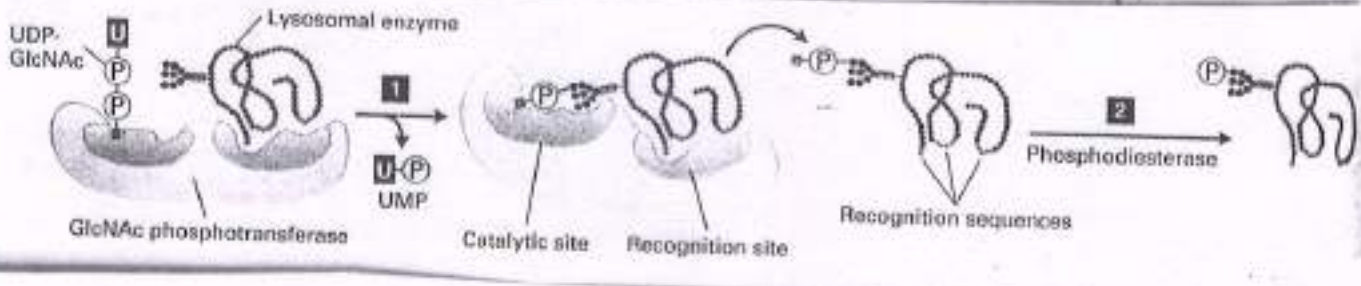
E1 = Ubiquitin-activating enz.
E2 = Ubiquitin-conjugating enz.
E3 = Ubiquitin ligase
Ub = Ubiquitin

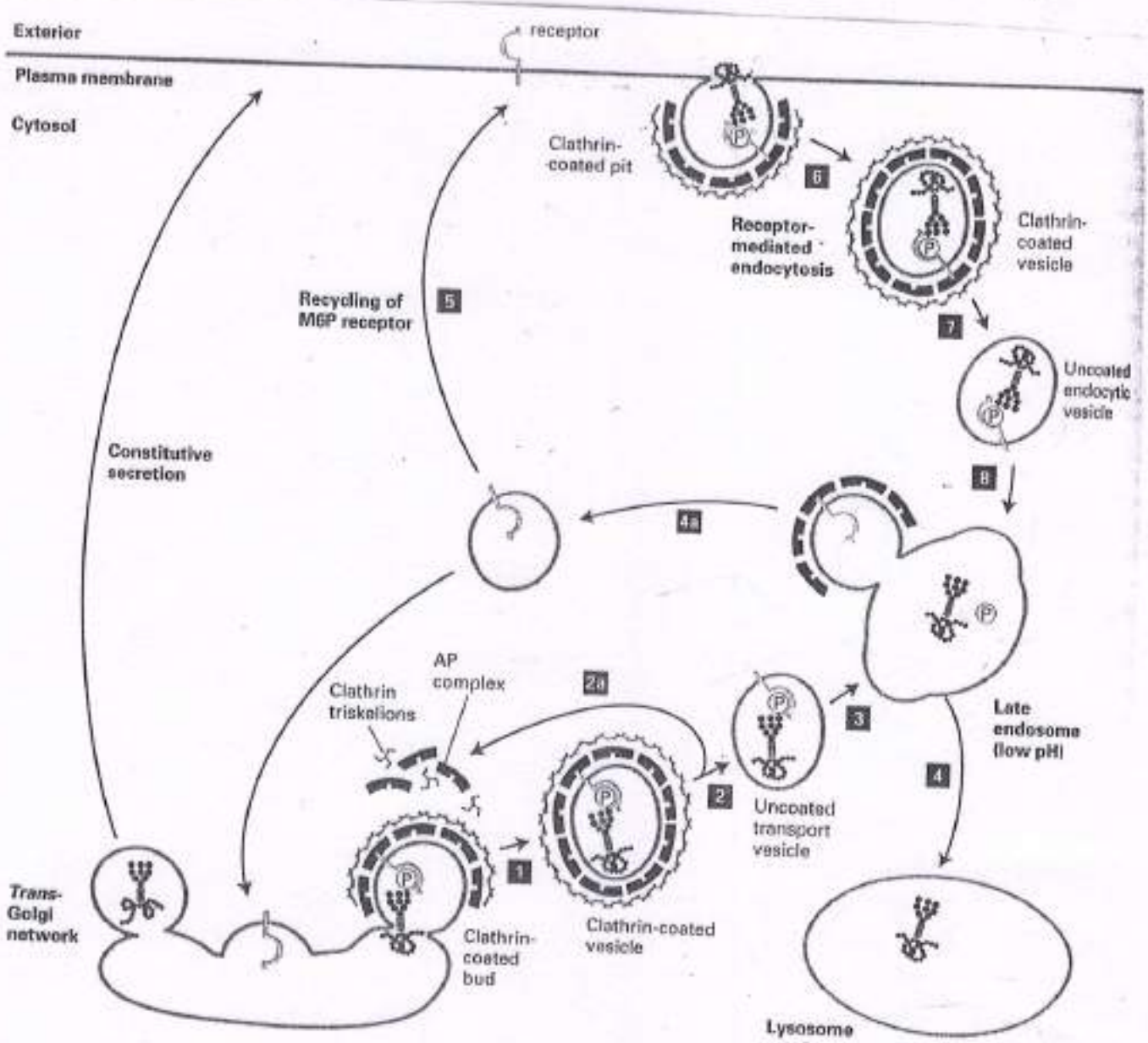
Ubiquitin-mediated proteolytic pathway.

PROTEIN DEGRADATION



Protein targeting in Peroxisomes


UDP-GlcNAc
Lysosomal enzyme
GlcNAc phosphotransferase
UMP
Catalytic site
Recognition site
Recognition sequences
Phosphodiesterase


Exterior
receptor
Plasma membrane
Cytosol
Clathrin-coated pit
Receptor-mediated endocytosis
Clathrin-coated vesicle
Recycling of M6P receptor
Uncoated endocytic vesicle
Constitutive secretion
Late endosome (low pH)
AP complex
Clathrin triskelions
Uncoated transport vesicle
Trans-Golgi network
Clathrin-coated bud
Clathrin-coated vesicle
Lysosome