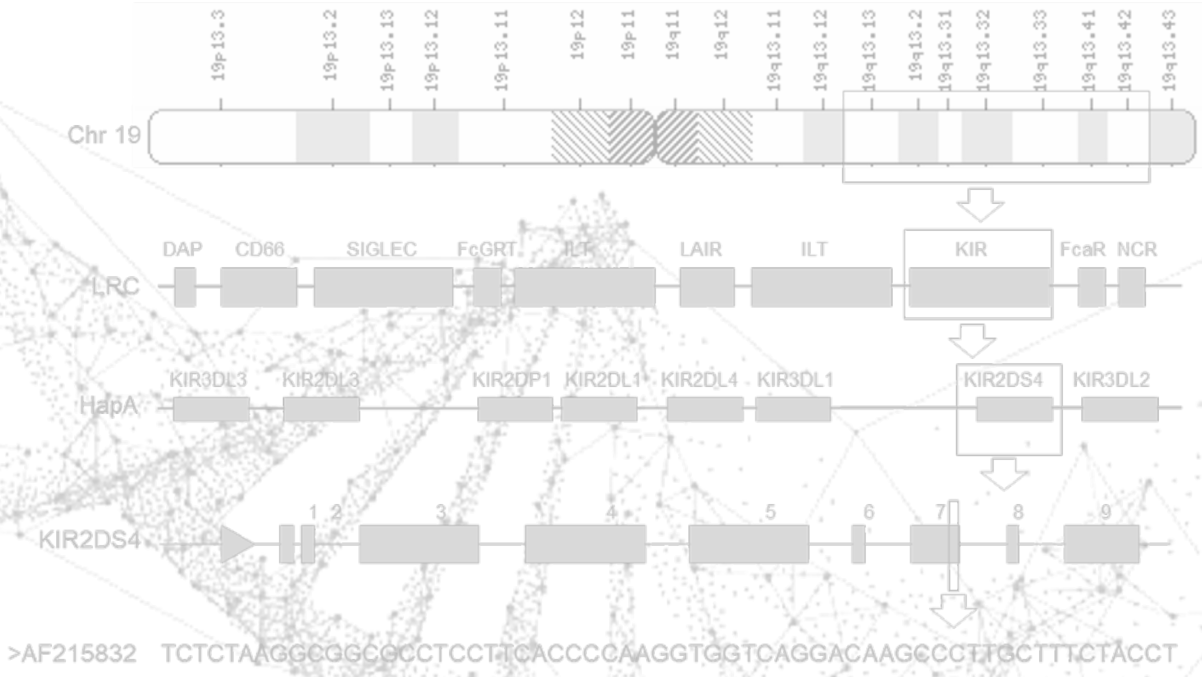


Protein Localization

San Luis Potosi State University (UASLP) Mexico
Molecular Biology Course, Faculty of Medicine post-graduate program

Dr. Christian A. García-Sepúlveda
Viral & Human Genomics BSL-3 Laboratory

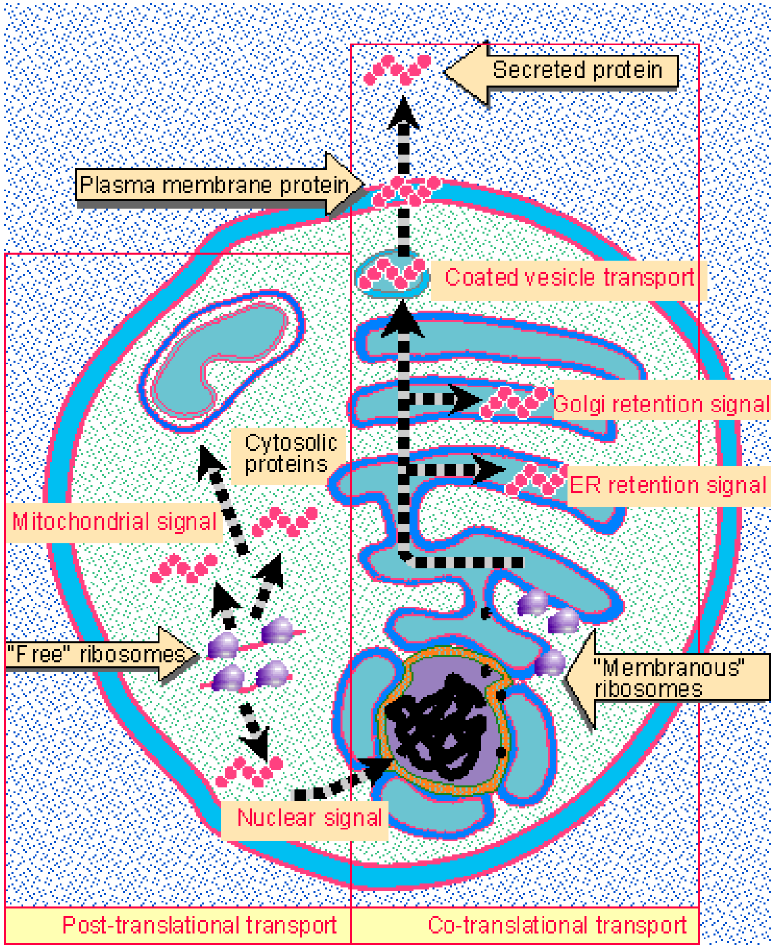
Last updated October 08, 2025 v2

Protein fate

Proteins can be classified into two general classes with regard to localization: those that are not associated with membranes; and those not-associated with membranes.

Each class can be subdivided further, depending on whether the protein associates with a particular structure in the cytosol or type of membrane.

Proteins can be localized co-translationally or post-translationally.



Protein fate

Co-translationally localized proteins

Proteins localized co-translationally associate with the ER membrane during synthesis, ribosomes are "membrane-bound".

The proteins pass into the ER along the Golgi and then through the plasma membrane, unless they have signals that cause retention at one of the steps on the pathway.

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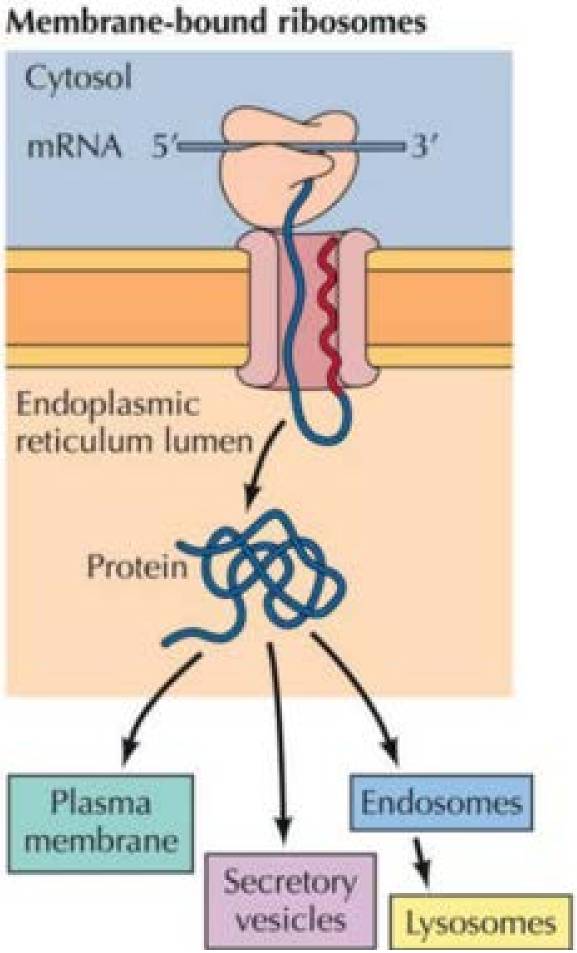


They may also be directed to other organelles, such as endosomes or lysosomes.

Cytosolic proteins

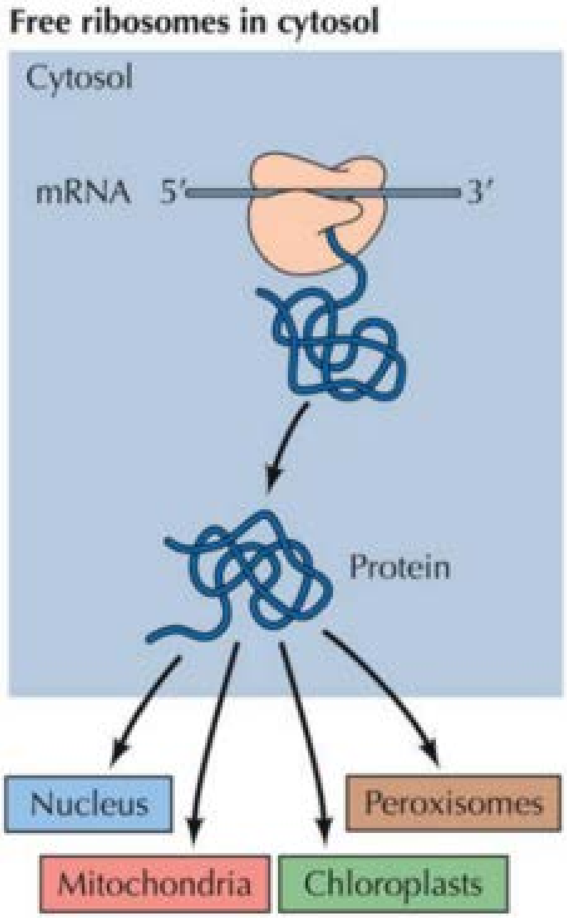
Some proteins remain free in the cytosol in quasi-soluble form; others associate with macromolecular cytosolic structures (filaments, microtubules, centrioles, etc).

This class also includes nuclear proteins (which pass into the nucleus through large aqueous pores).

Cytosolic (or "soluble") proteins carry out functions in the cytosol.

The ribosomes on which these proteins are synthesized are sometimes called "free ribosomes".

The "default" for a protein released from "free" ribosomes is to remain in the cytosol.

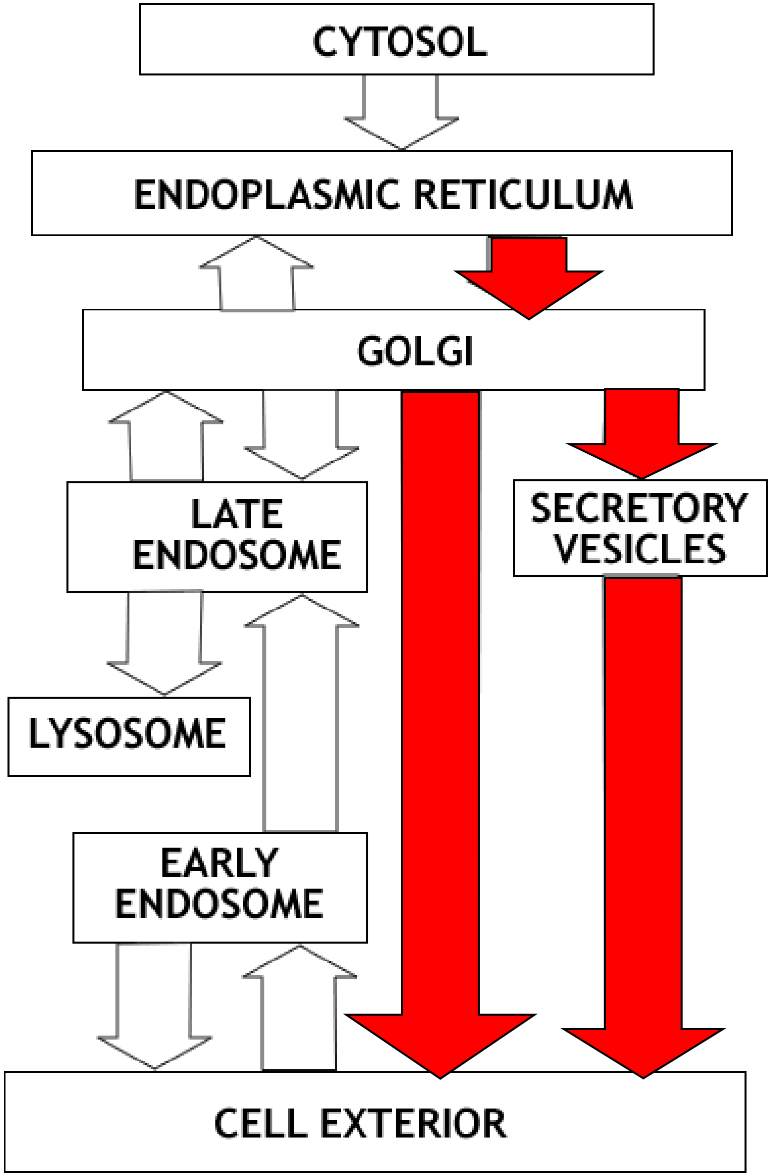


To be targeted to a specific location requires an appropriate signal, typically a sequence motif that causes it to be assembled into a macromolecular structure or recognized by a transport system.

Reticuloendothelial (endomembranous) system

There are three major subdivisions of the endomembrane system

- The secretory pathway
- The lysosomal pathway and
- The endocytotic pathway



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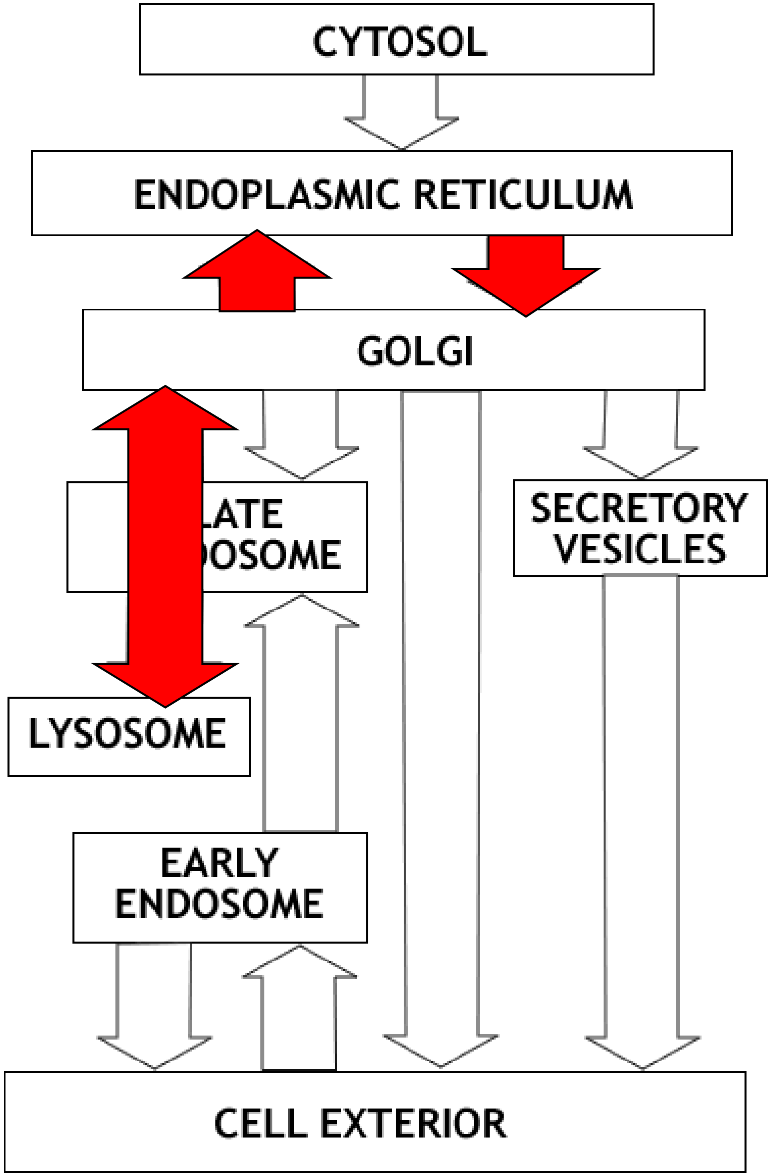

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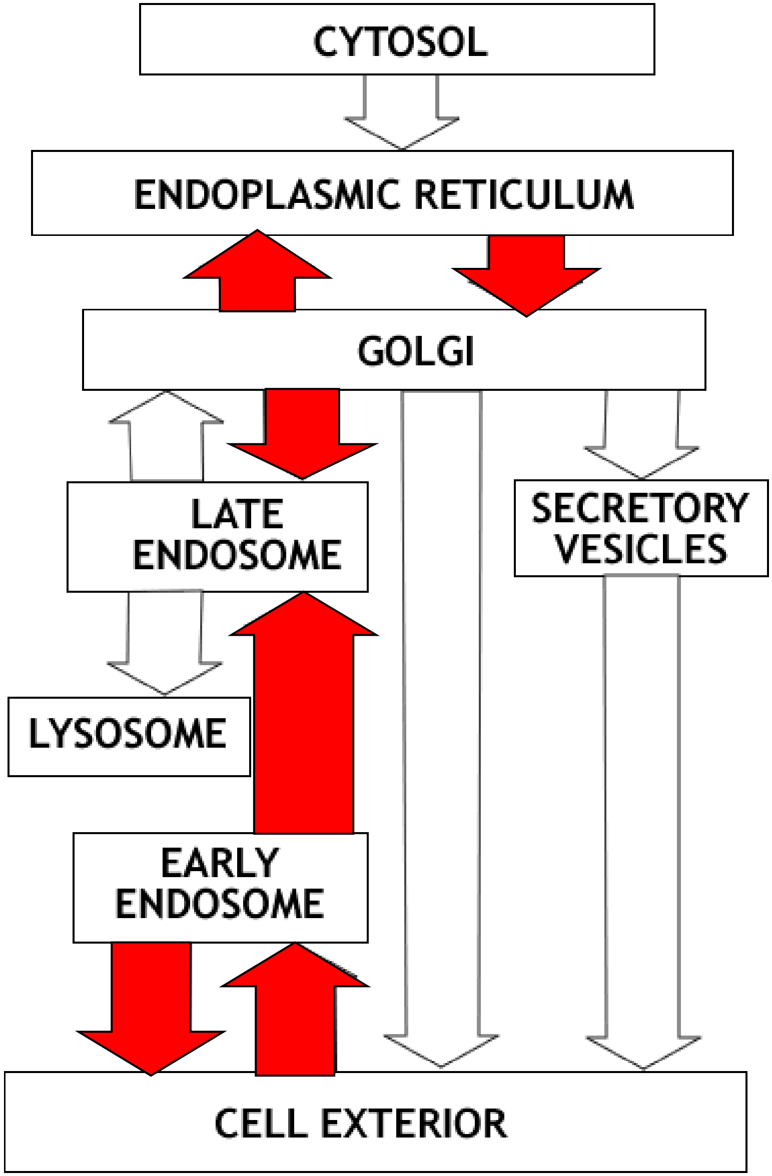
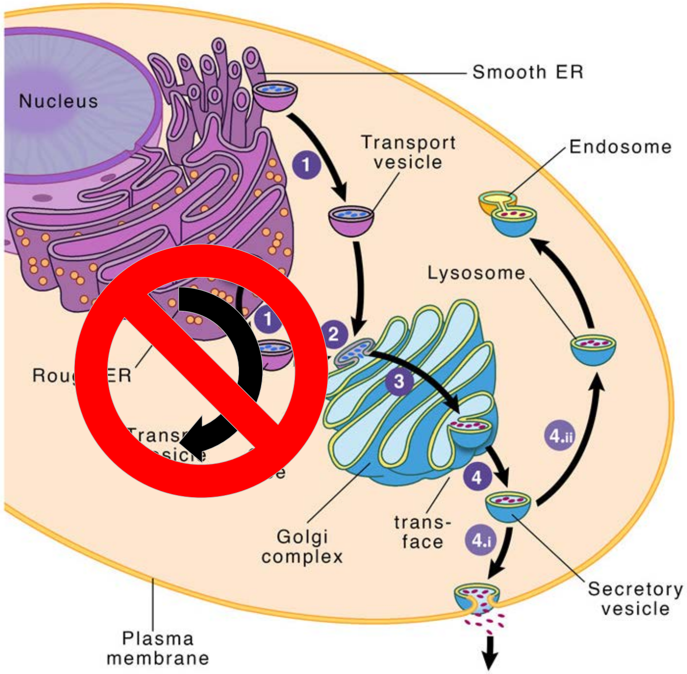


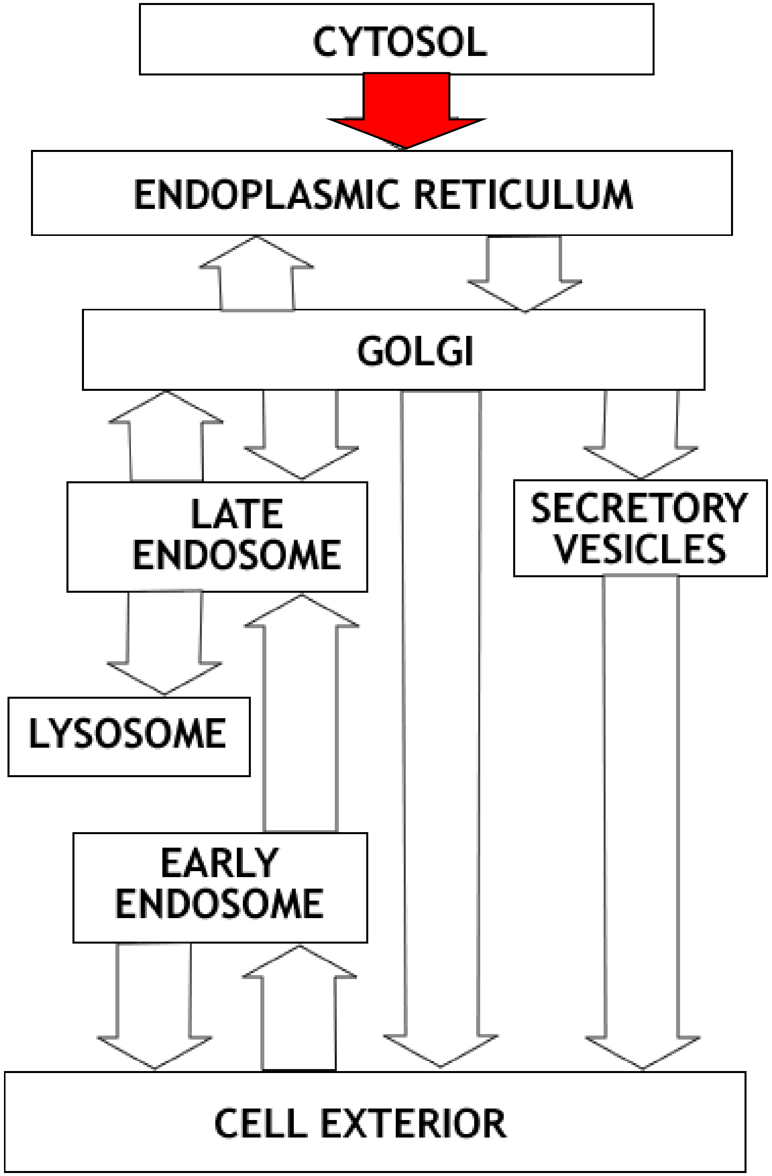
Reticuloendothelial (endomembranous) system

Once proteins enter the endoplasmic reticulum they never return to the cytosol compartment.

They are carried by vesicle transport to the other compartments of the system.

This flow of vesicles is highly regulated.




CYTOSOL

ENDOPLASMIC RETICULUM

GOLGI

LATE ENDOSOME

SECRETORY VESICLES

LYSOSOME

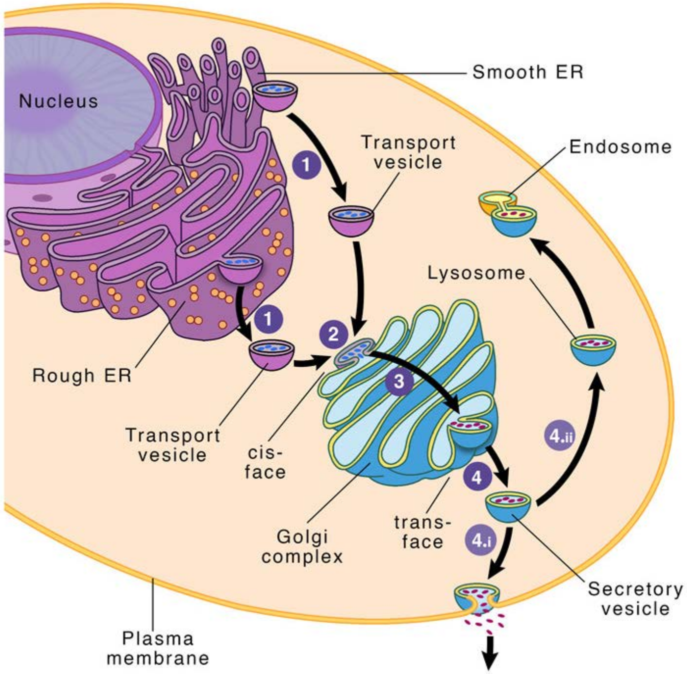
EARLY ENDOSOME

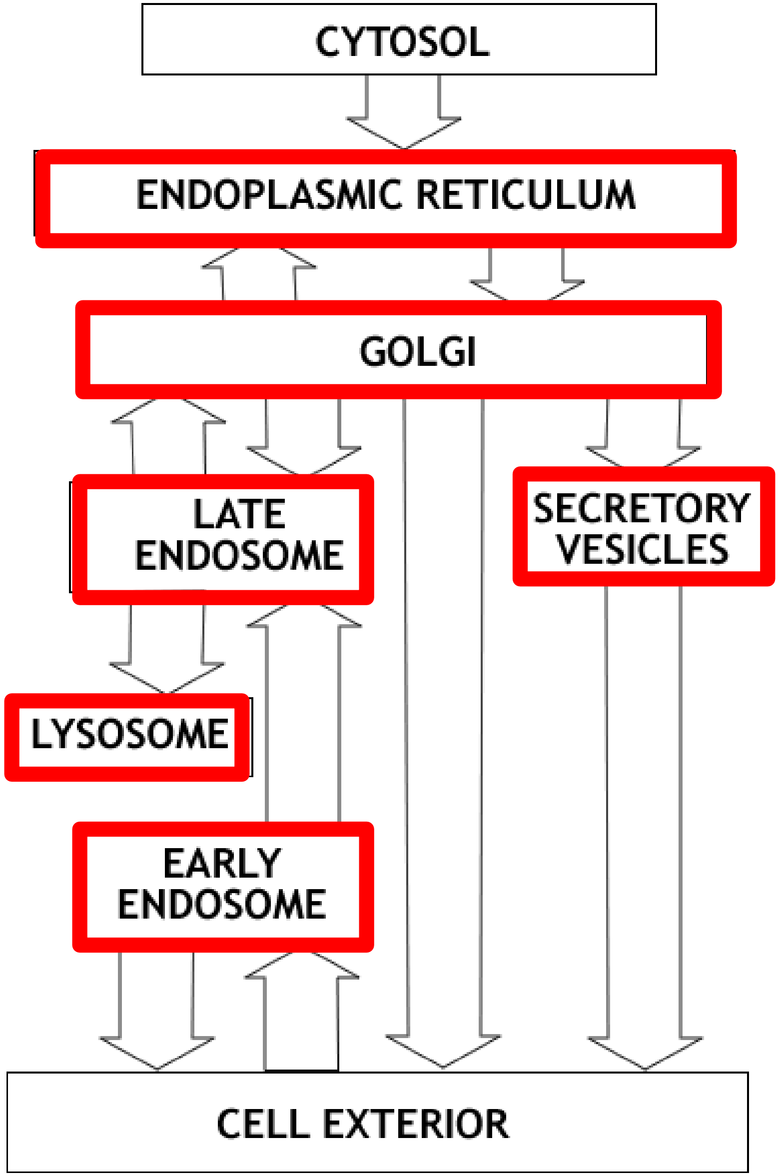
CELL EXTERIOR

Reticuloendothelial (endomembranous) system

Consists of compartments:

- Endoplasmic Reticulum
- Golgi apparatus
- Lysosomes
- Endosomes and
- Secretory Vesicles.





CYTOSOL

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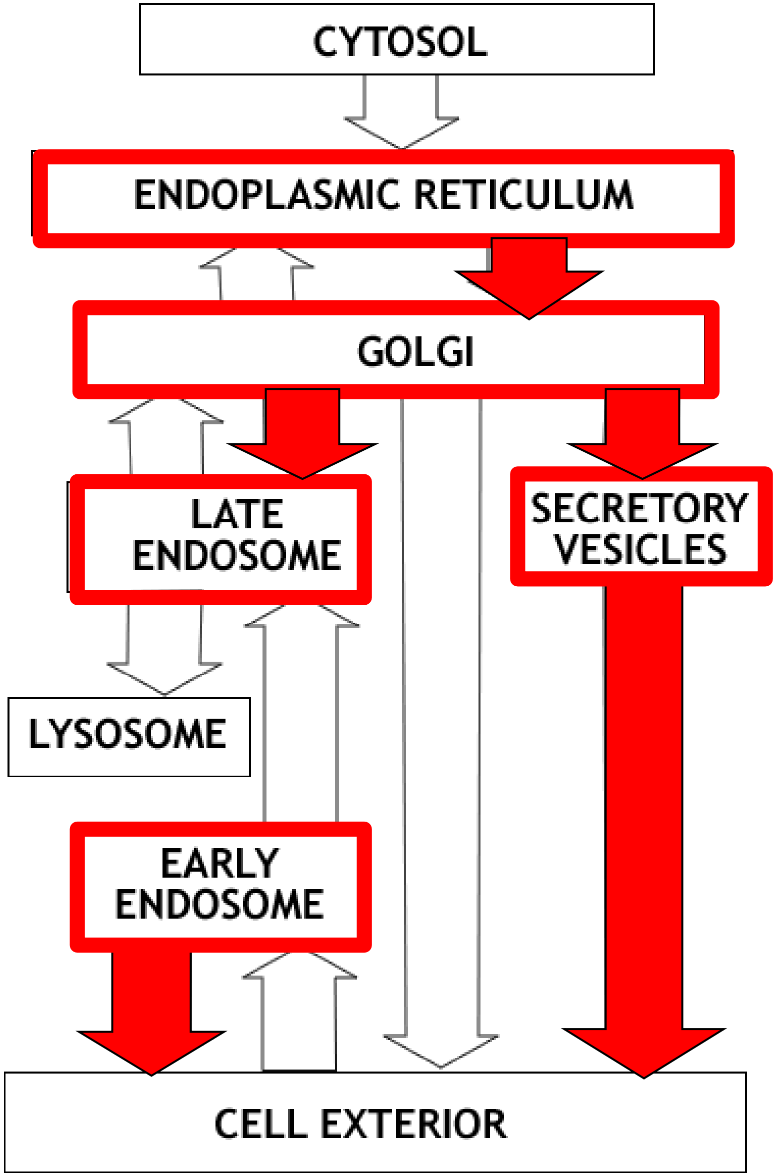
Reticuloendothelial (endomembranous) system

Compartments involved in the processing of proteins for:

- export from the cell
- for lysosomes (destruction)
- for proteins entering the cell from the cell surface.



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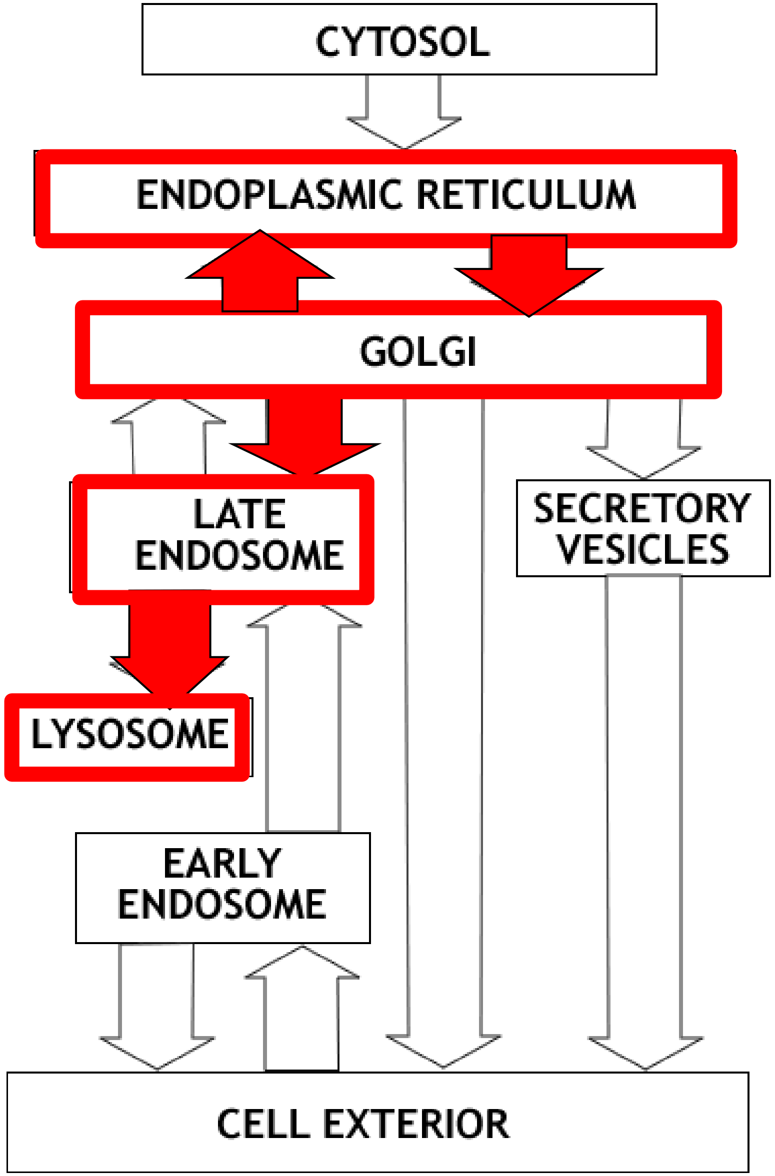

Reticuloendothelial (endomembranous) system

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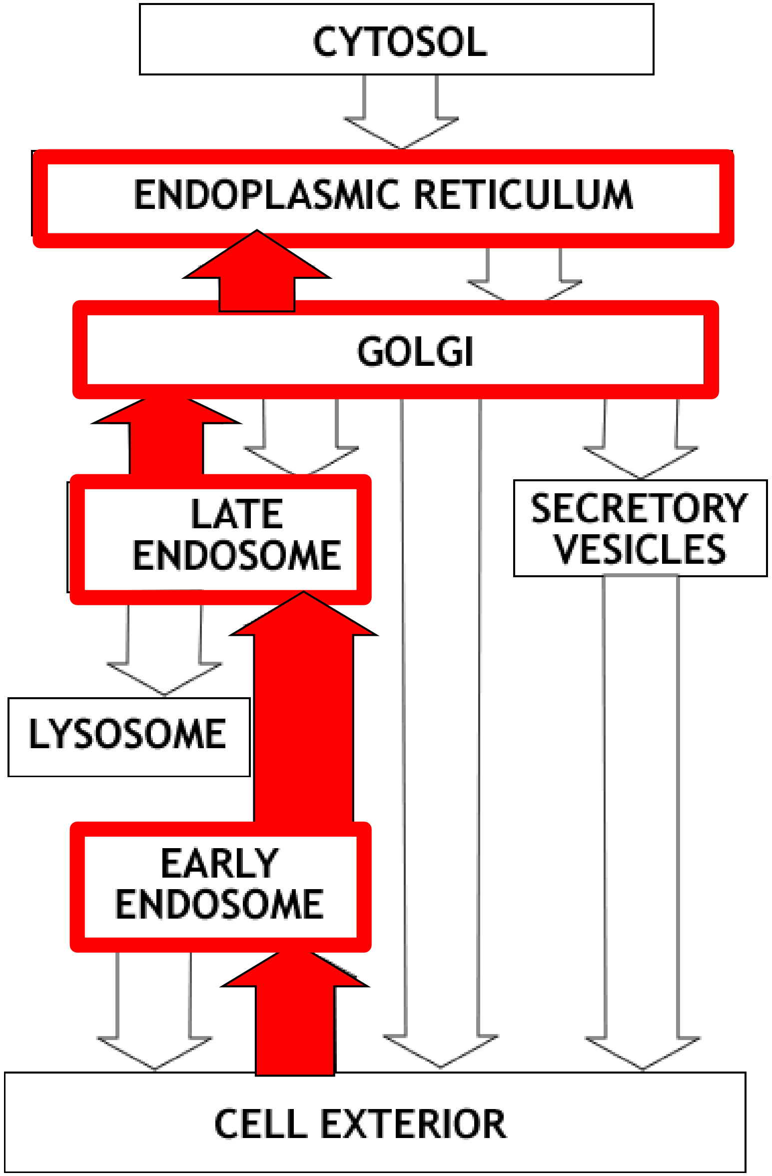
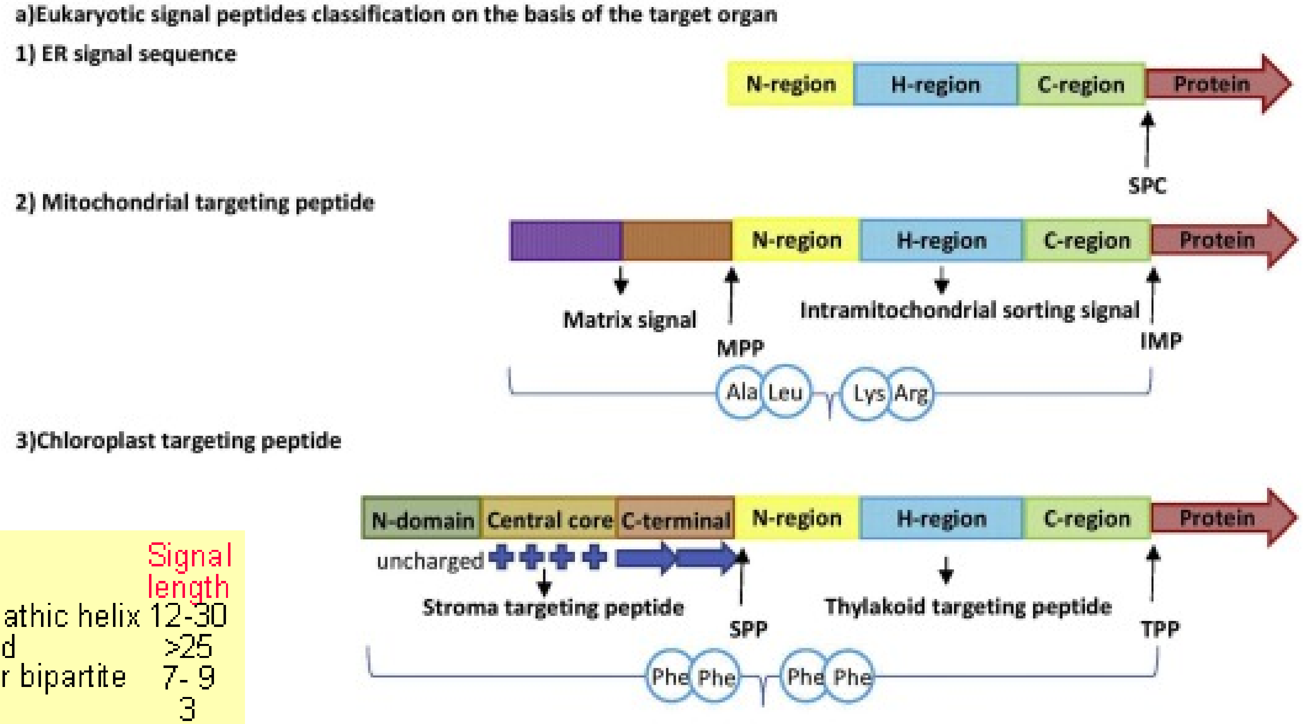


Signal peptides or sequences

Nuclear localization signals (short sequences within proteins) enable the proteins to pass through nuclear pores.

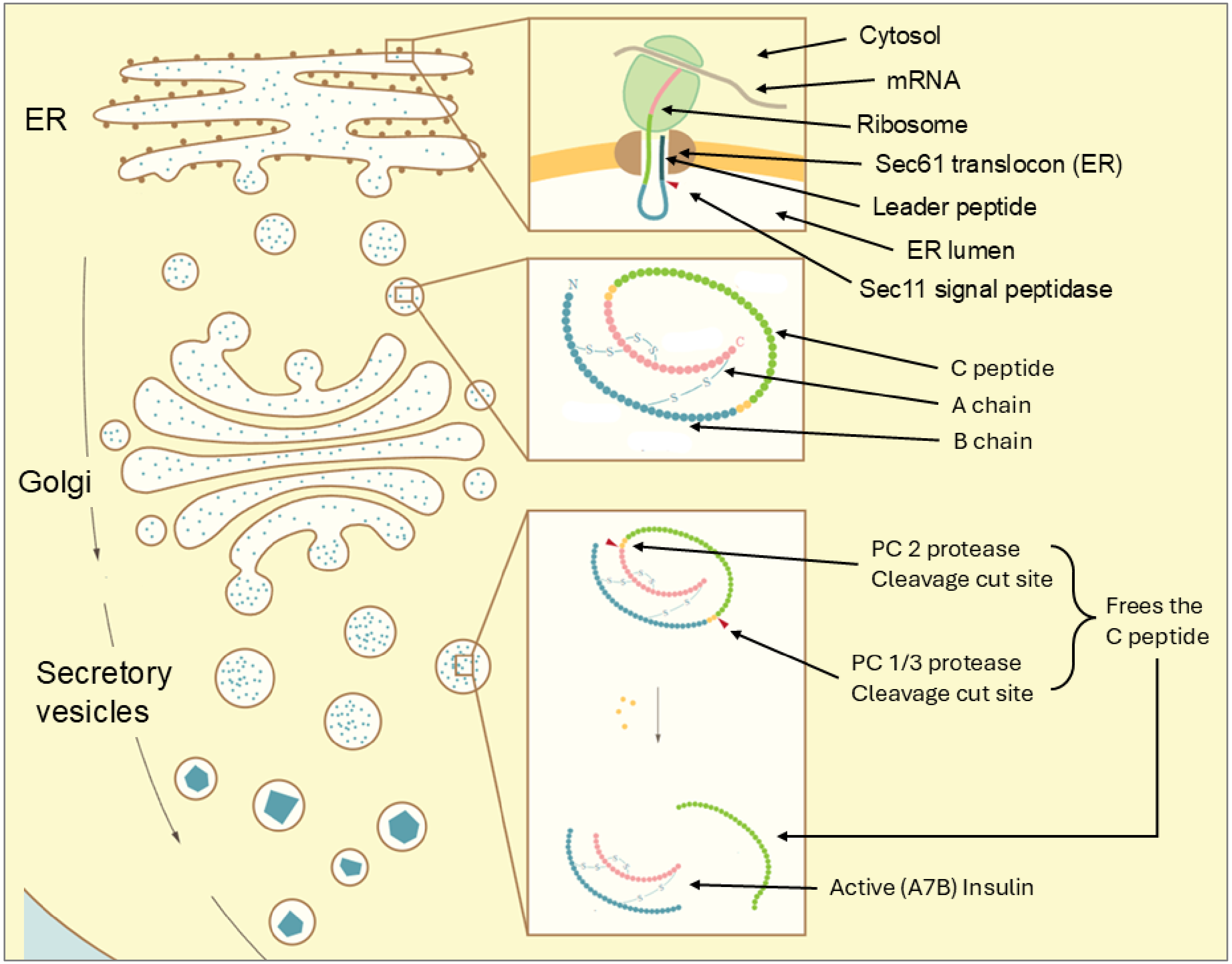
One type of signal that determines transport to the peroxisome is a very short C-terminal sequence.



Organelle	Signal location	Type	Signal length
Mitochondrion	N-terminal	Amphipathic helix	12-30
Chloroplast	N-terminal	Charged	>25
Nucleus	Internal	Basic or bipartite	7- 9
Peroxisome	C-terminal	SKL	3

Insulin processing example

Insulin translation, proinsulin and active insulin as an example of signal sequences.



Co-translational localization of proteins to ER

For all proteins entering the secretory or lysosomal pathways, the first step is ER targeting.

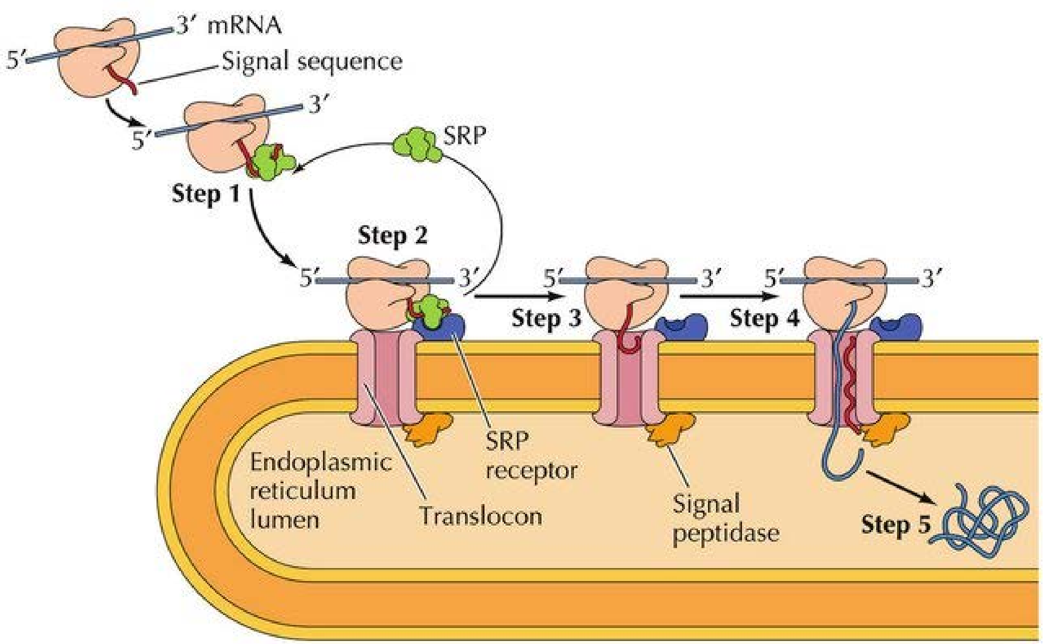
ER targeting relies on a signal encoded in the N terminal portion of the protein.

The signal is recognized by a Signal Recognition Particle (SRP).

The SRP enables the ribosome to dock to the corresponding translocon.

Nascent polypeptide is inyected into ER and signal sequence is cleaved by Signal Peptidase.

Protein synthesis continues to completion until the ribosome is undocked & dissociated.



ER localization signal sequence

Signal peptide directs proteins to ER for proper folding, modification, and secretion.

15-30 amino acids at N-terminus with hydrophobic core.

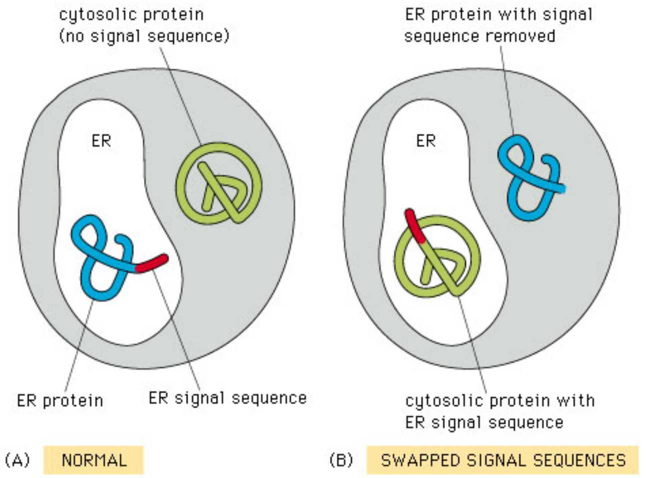
Transplanting signal to cytosolic proteins redirects them into ER lumen.

Enables experimental manipulation of protein localization and trafficking.

Allows creation of secreted versions of normally cytoplasmic proteins.

Demonstrates sufficiency of signal peptide for ER targeting independent of protein context.

Used in biotechnology to engineer protein secretion for therapeutic production.



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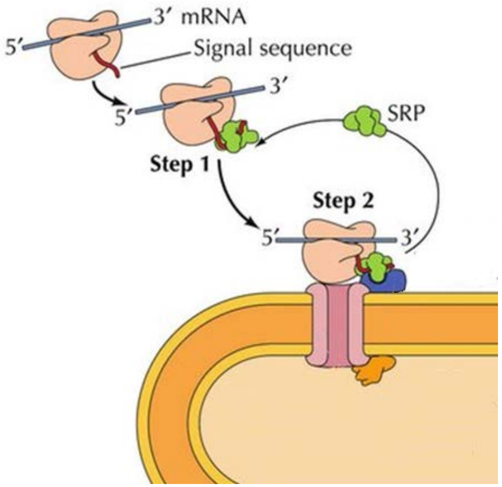
ER localization signal sequence



Abundant, cytosolic, universally conserved ribonucleoprotein (RNA + 6 protein subunits).

Recognizes and binds to ER signal sequences as they emerge from ribosomes.

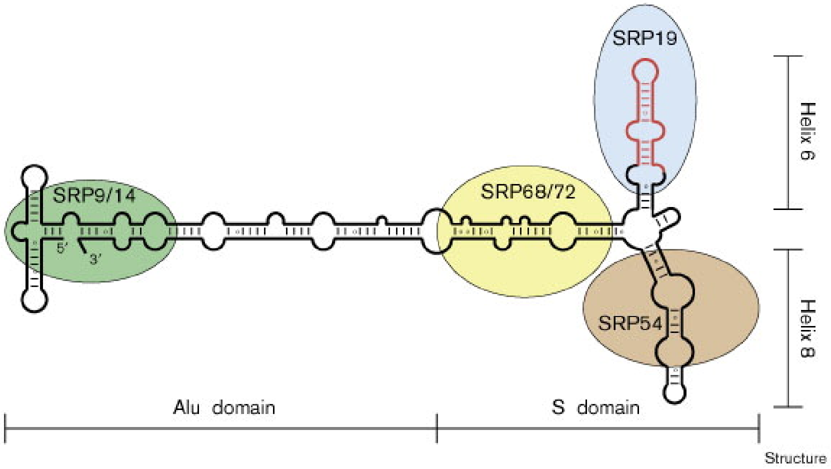
Targets specific proteins to the ER (eukaryotes) or to plasma membrane (prokaryotes).

SRP contain 7SL RNA, evolutionarily related to Alu sequences.

Alu elements are short interspersed nuclear elements (SINEs) derived from 7SL RNA.

7SL RNA in SRP is approximately 300 nucleotides and serves as the structural scaffold.

Alu sequences are the most abundant repetitive elements in human genome (over 1 million copies).



Post-translational localization of proteins to ER

Proteins translocate into ER until hydrophobic domain encountered.

Alpha helices act as stop transfer signals, anchoring protein in membrane.

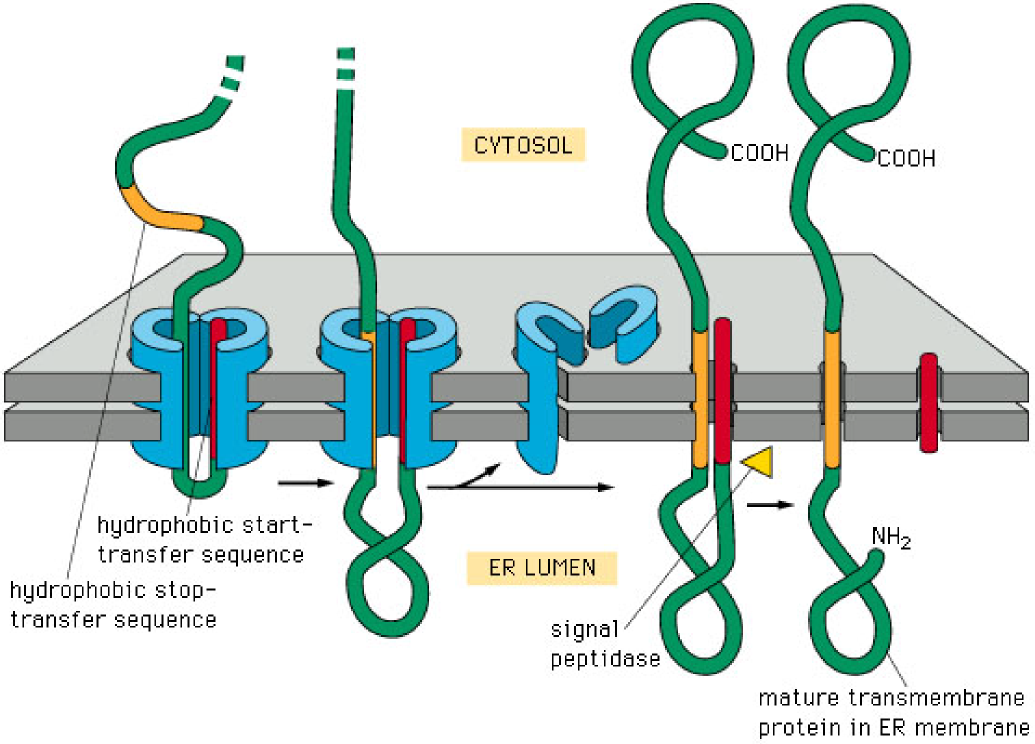
The signal peptide remains attached to the membrane.

Signal peptide is cleaved off by a signal peptidase.

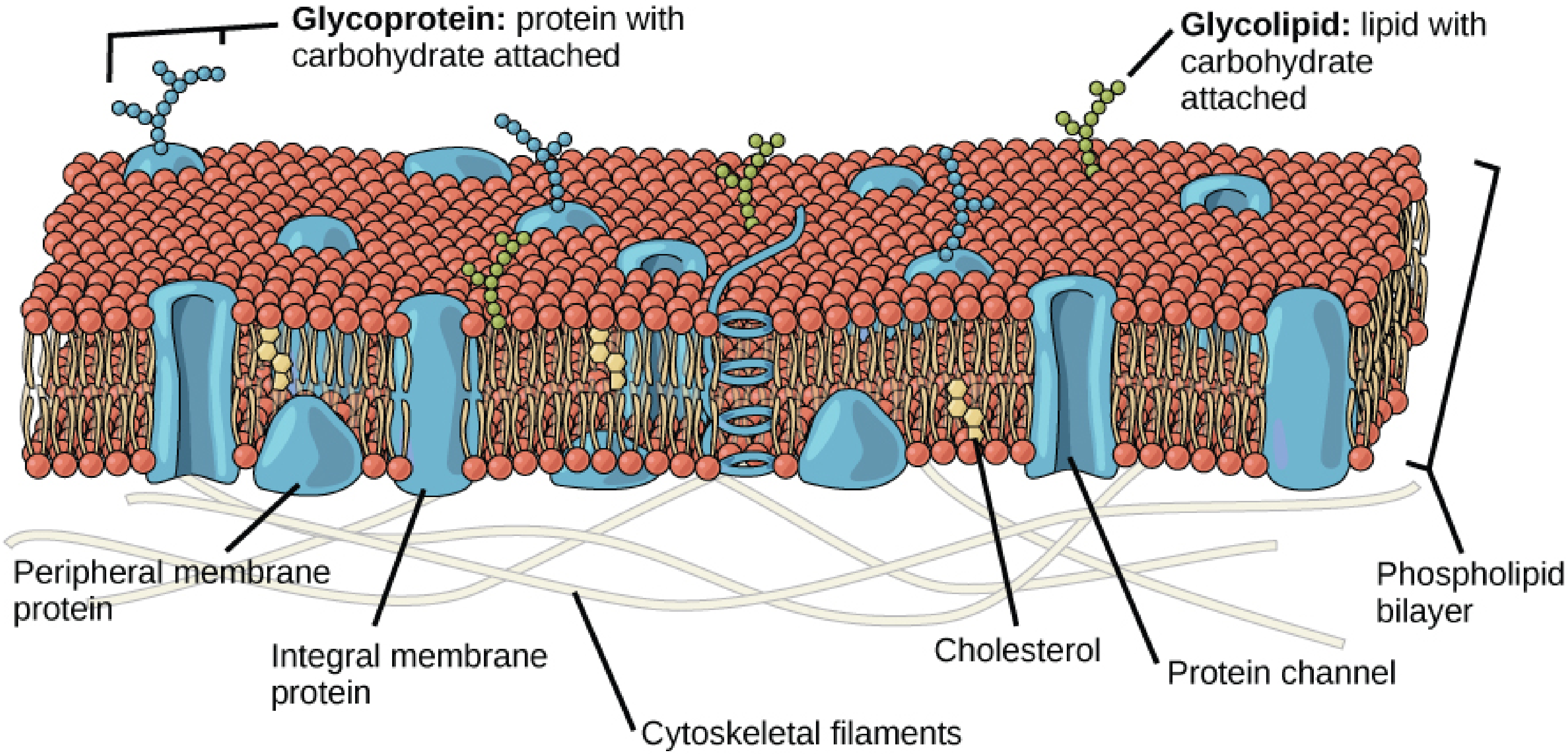
Orientation established at initial insertion into ER membrane.

Orientation remains fixed throughout all transport to final destination.

Cytosolic side stays cytosolic at all stages.



Membrane proteins



Transmembrane protein capture and orientation

Proteins translocate into ER until hydrophobic domain encountered.

Alpha helices act as stop transfer signals, anchoring protein in membrane.

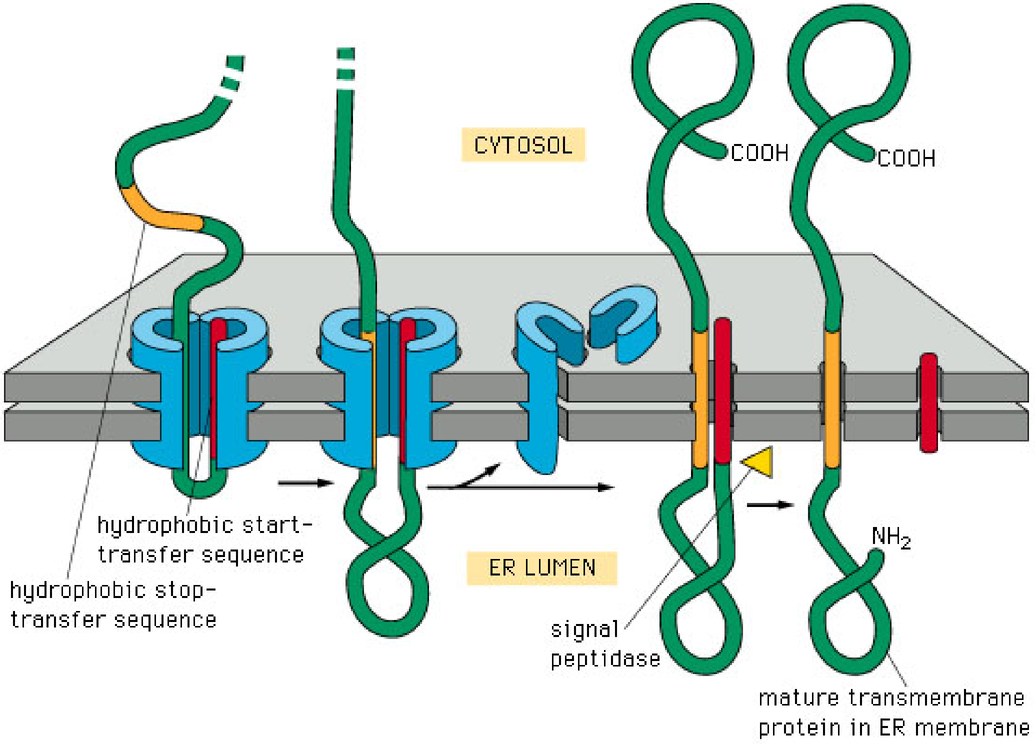
The signal peptide remains attached to the membrane.

Signal peptide is cleaved off by a signal peptidase.

Orientation established at initial insertion into ER membrane.

Orientation remains fixed throughout all transport to final destination.

Cytosolic side stays cytosolic at all stages.

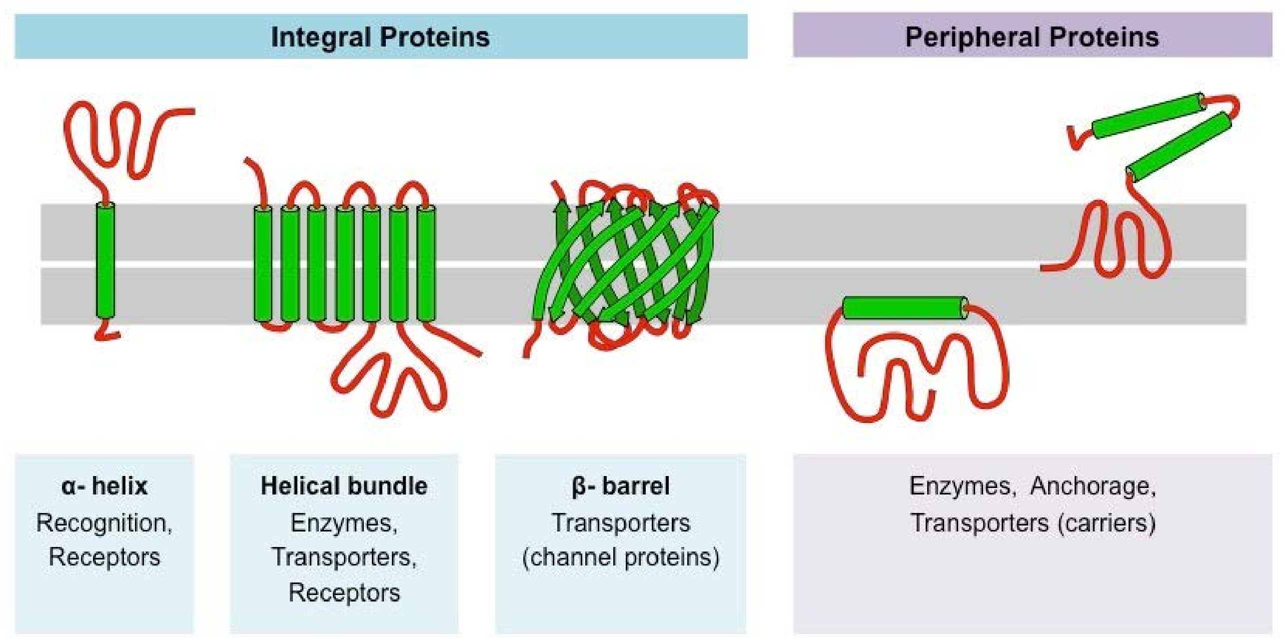


Membrane proteins

Phospholipid bilayers embedded with either permanently or temporarily bound proteins.

Integral proteins: permanently attached to the membrane, typically transmembrane.

Peripheral proteins: temporarily attached by non-covalent interactions and associate with one surface of the membrane



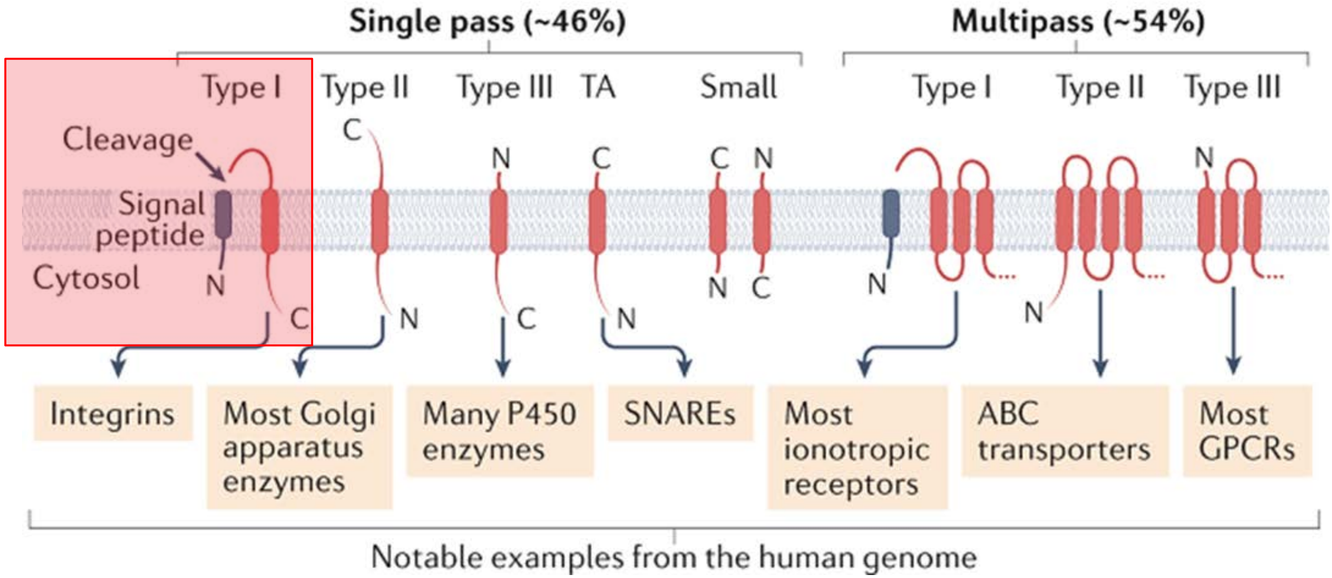
BioNinja webpage

Single pass type I transmembrane proteins

Single pass, N-terminus in extracellular or luminal space.

Leader sequence in N-terminus

Leader sequence is cleaved inside the ER lumen.

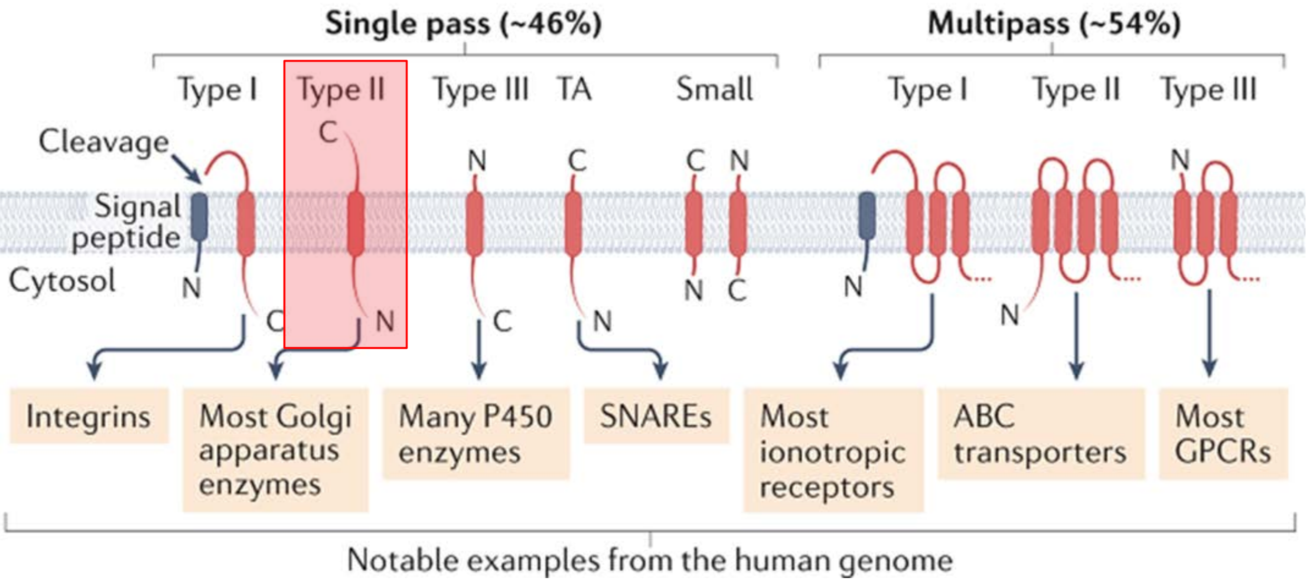


Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

Single pass type II transmembrane proteins

Single pass, C-terminus in extracellular or luminal space.

Leader sequence absent but protein introduced C-terminus first.



[Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb](#)

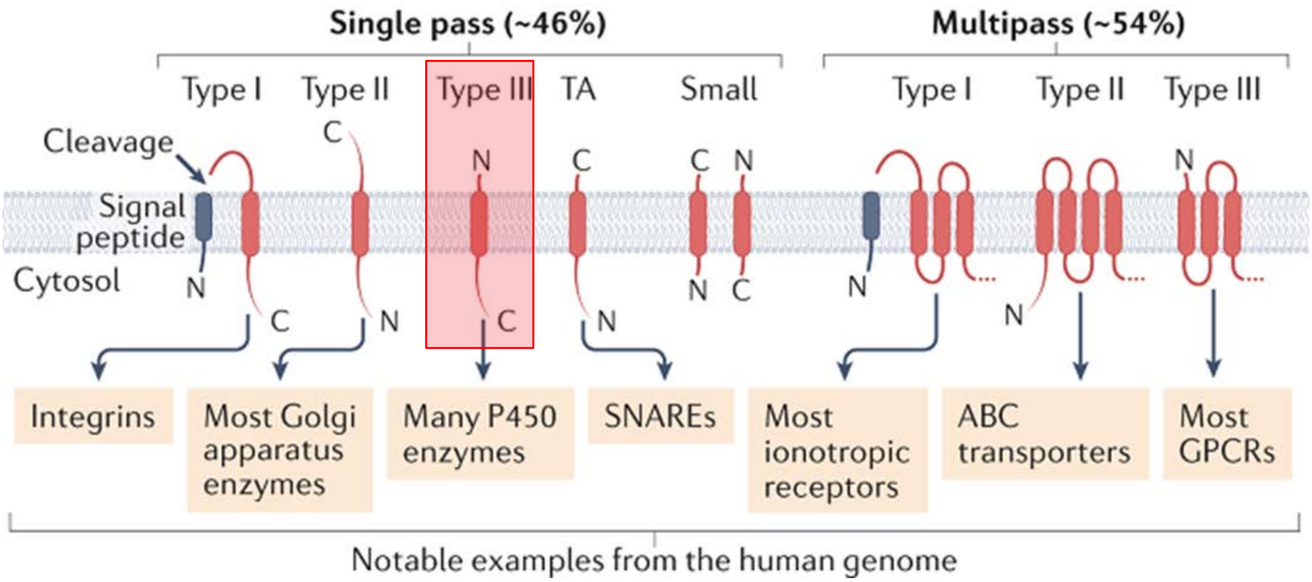
Single pass type III transmembrane proteins

Single pass, N-terminus in extracellular or luminal space.

Has an internal signal sequence that is not cleaved during insertion.

Are inserted into the membrane in a reverse orientation compared to Type I proteins.

Mechanism involves signal recognition particle (SRP) or tail-anchored protein insertion.



[Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb]

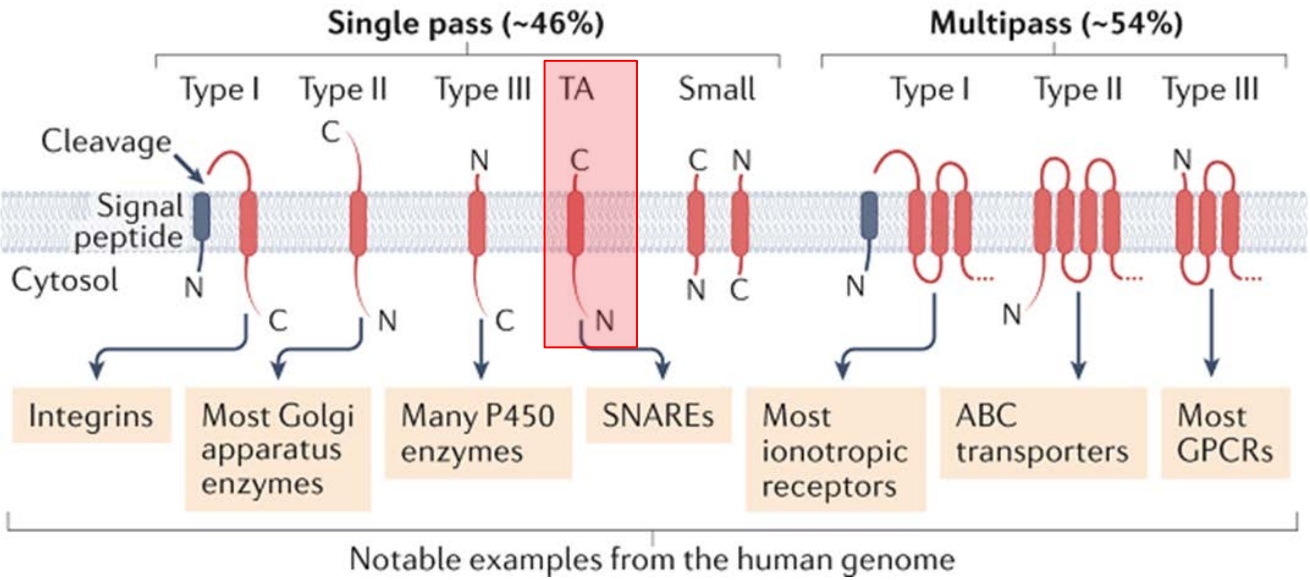
Single pass TA type transmembrane proteins

Single-pass proteins with N-terminus in cytoplasm (TA= Tail-anchored).

Transmembrane domain located near C-terminus (within 30-50 amino acids of end).

Cannot use conventional SRP co-translational insertion pathway.

Inserted post-translationally via specialized mechanisms.



Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

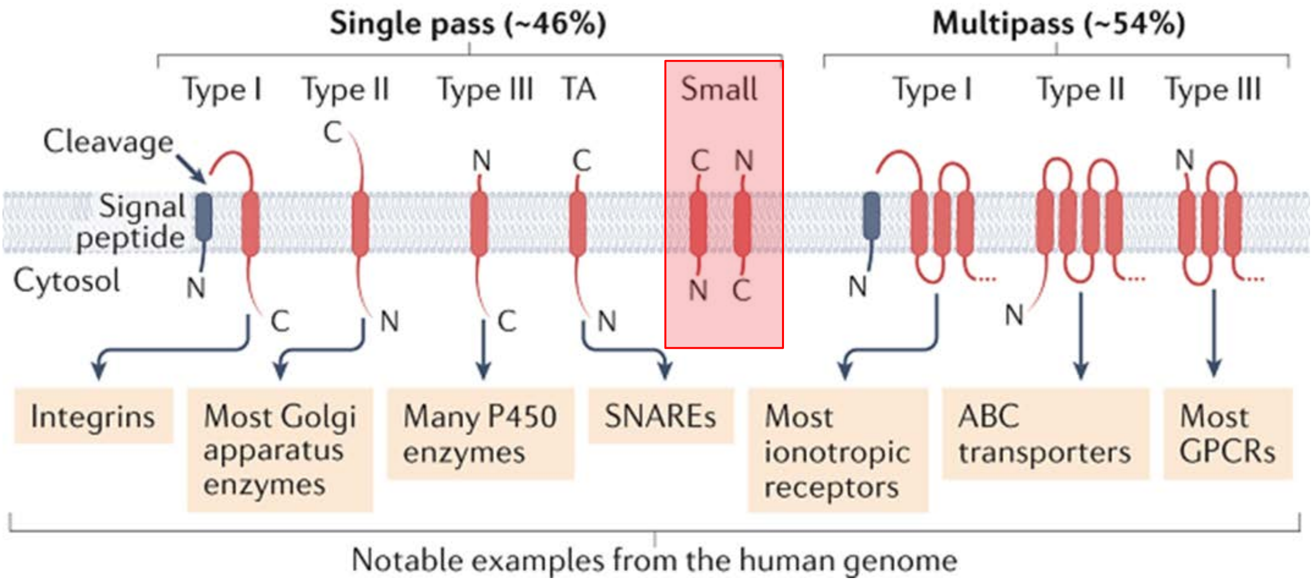
Single pass TA type transmembrane proteins

Typically less than 100-150 amino acids in total length.

Contain one hydrophobic transmembrane domain (approximately 20-25 amino acids).

Short cytoplasmic and extracellular/luminal domains.

Often function as regulatory proteins, signaling molecules, or subunits of larger complexes



Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

Multipass Type I transmembrane proteins

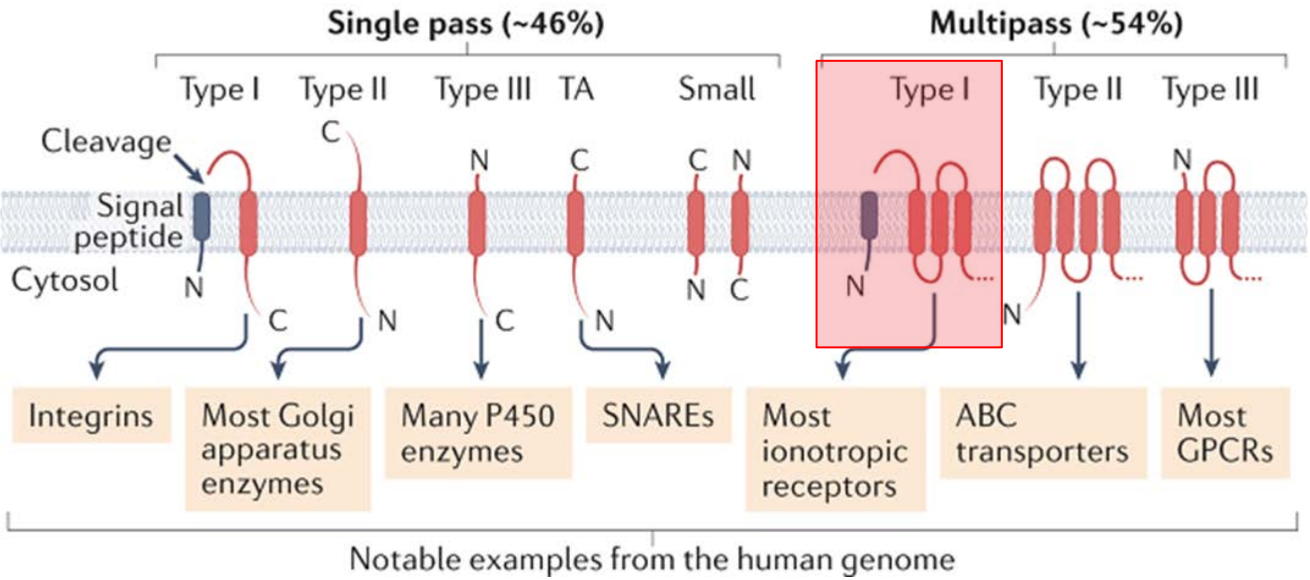
Contain multiple (2 or more) transmembrane domains spanning the membrane.

N-terminus is extracellular/luminal, C-terminus is cytoplasmic.

Inserted co-translationally via the SRP pathway.

First transmembrane domain acts as signal-anchor sequence.

Other domains inserted sequentially as stop-transfer and signal-anchor sequences.



Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

Multipass Type II transmembrane proteins

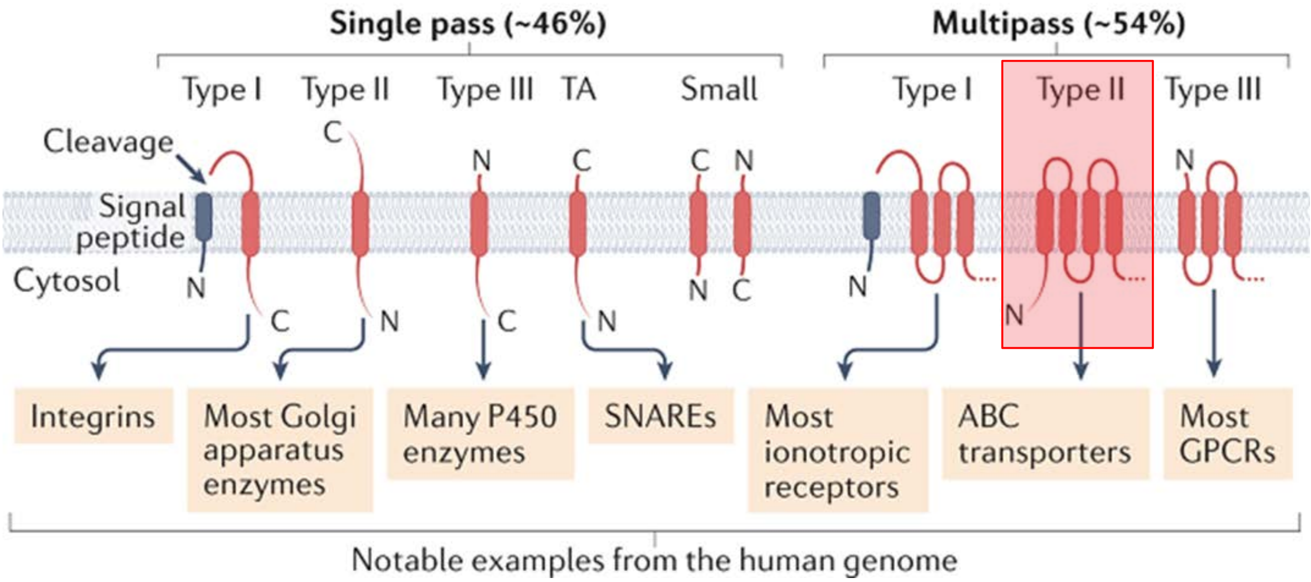
Contain multiple (2 or more) transmembrane domains spanning the membrane.

N-terminus is cytoplasmic, C-terminus is extracellular/luminal.

Inserted co-translationally via the SRP pathway.

First transmembrane domain acts as an internal signal-anchor.

Subsequent transmembrane domains inserted sequentially as the protein is synthesized.



Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

Multipass Type III transmembrane proteins

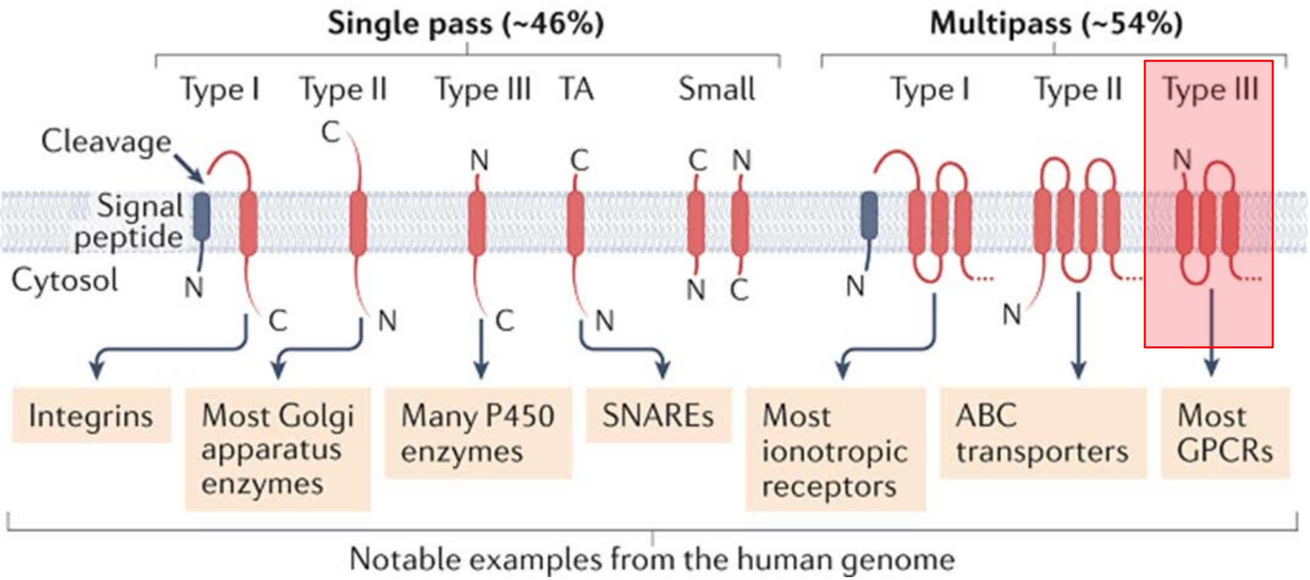
Contain multiple (2 or more) transmembrane domains spanning the membrane.

Transmembrane segments create an even number of membrane crossings.

Inserted co-translationally via the SRP pathway.

Complex topology with multiple signal-anchor and stop-transfer sequences.

Loop regions alternate between cytoplasmic and extracellular/luminal compartments.



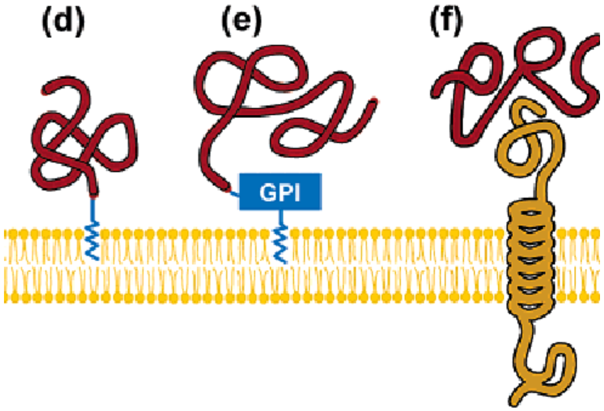
Hegde RS, Keenan RJ. Nat Rev Mol Cell Biol. 2022 Feb

Type IV transmembrane proteins

Lipid chain-anchored membrane proteins (d) and GPI-anchored membrane proteins (e):

Associated with the bilayer only by means of one or more covalently attached fatty acid chains.

Peripheral membrane proteins (f) linked to membrane through other proteins.



[Chou KC, Cai YD, J Chem Inf Model. 2005 Mar-Apr](#)

Functional roles of membrane proteins

Membrane proteins can serve a variety of key functions: **JETRAT**

Junctions – Serve to connect and join two cells together

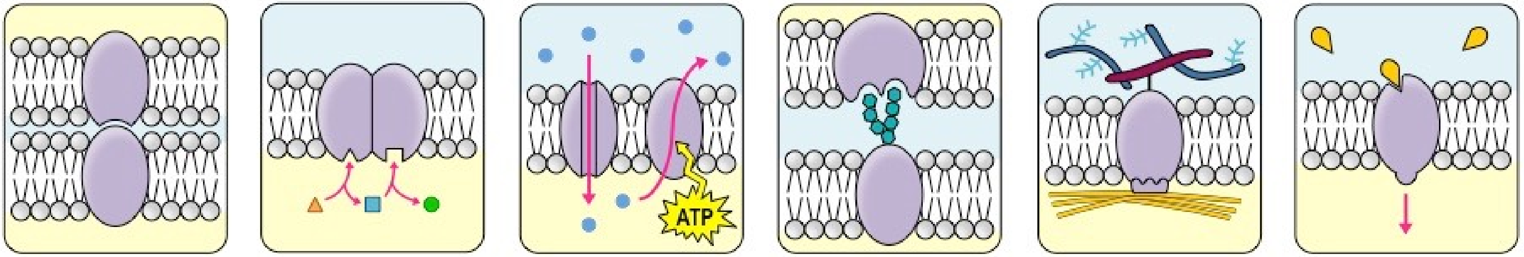
Enzymes – Fixing to membranes localises metabolic pathways

Transport – Responsible for facilitated diffusion and active transport

Recognition – May function as markers for cellular identification

Anchorage – Attachment points for cytoskeleton and extracellular matrix

Transduction – Function as receptors for peptide hormones



Intercellular Joinings

Enzymatic Activity

Transport (Active / Passive)

Cell-Cell Recognition

Anchorage / Attachment

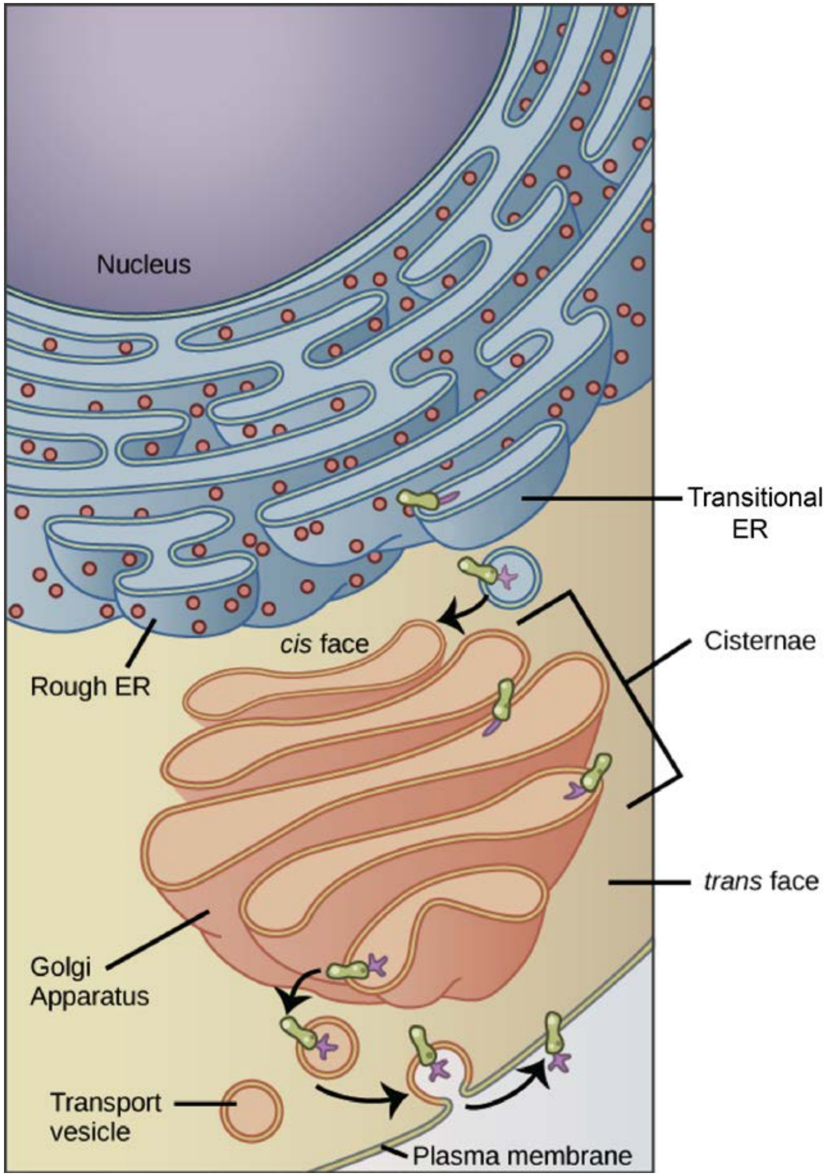
Signal Transduction

BioNinja webpage

Protein trafficking

The "default pathway" takes a protein through the ER, into the Golgi, and on to the plasma membrane.

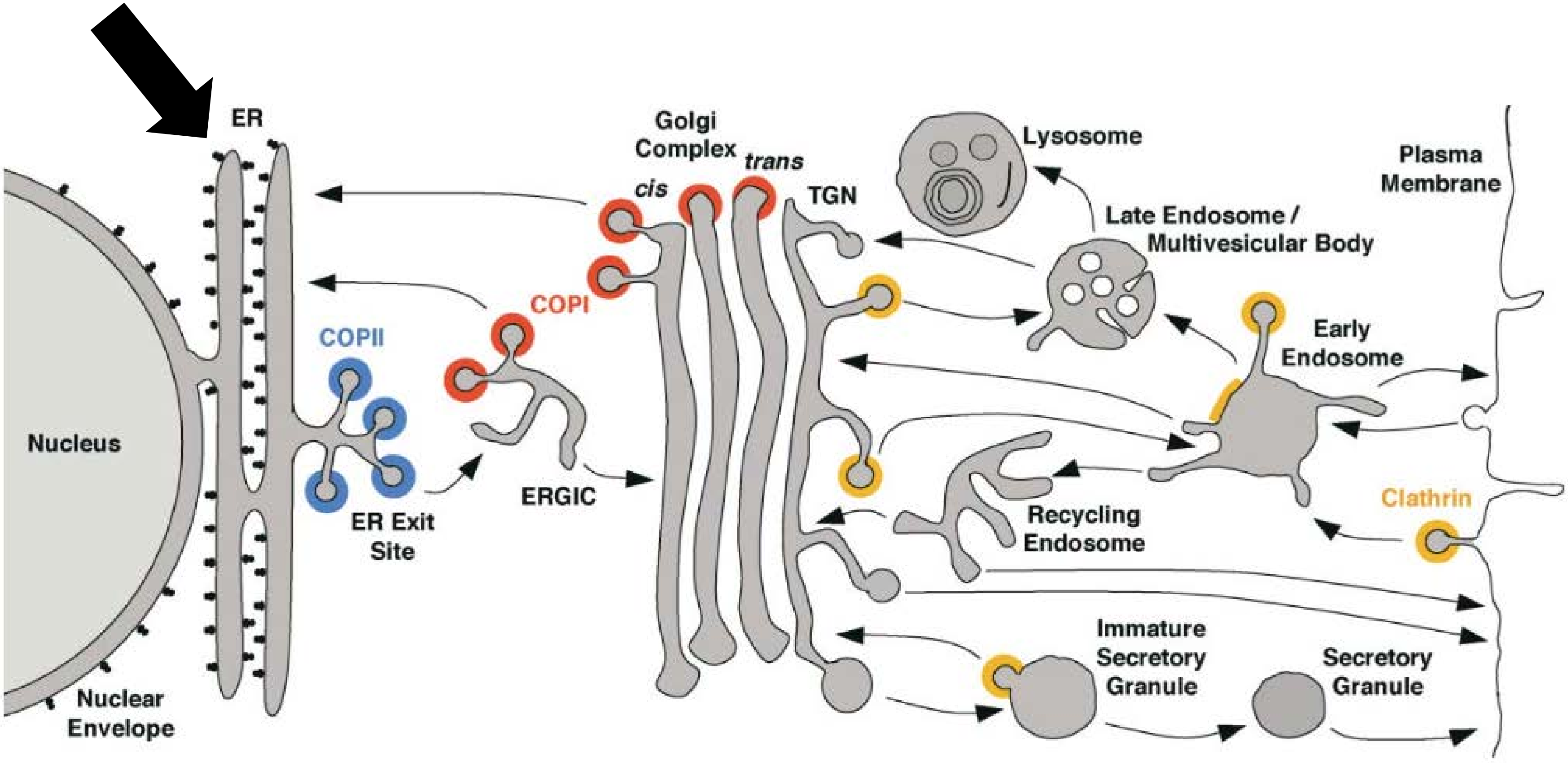
Luminal side becomes extracellular side for some proteins.



Protein trafficking

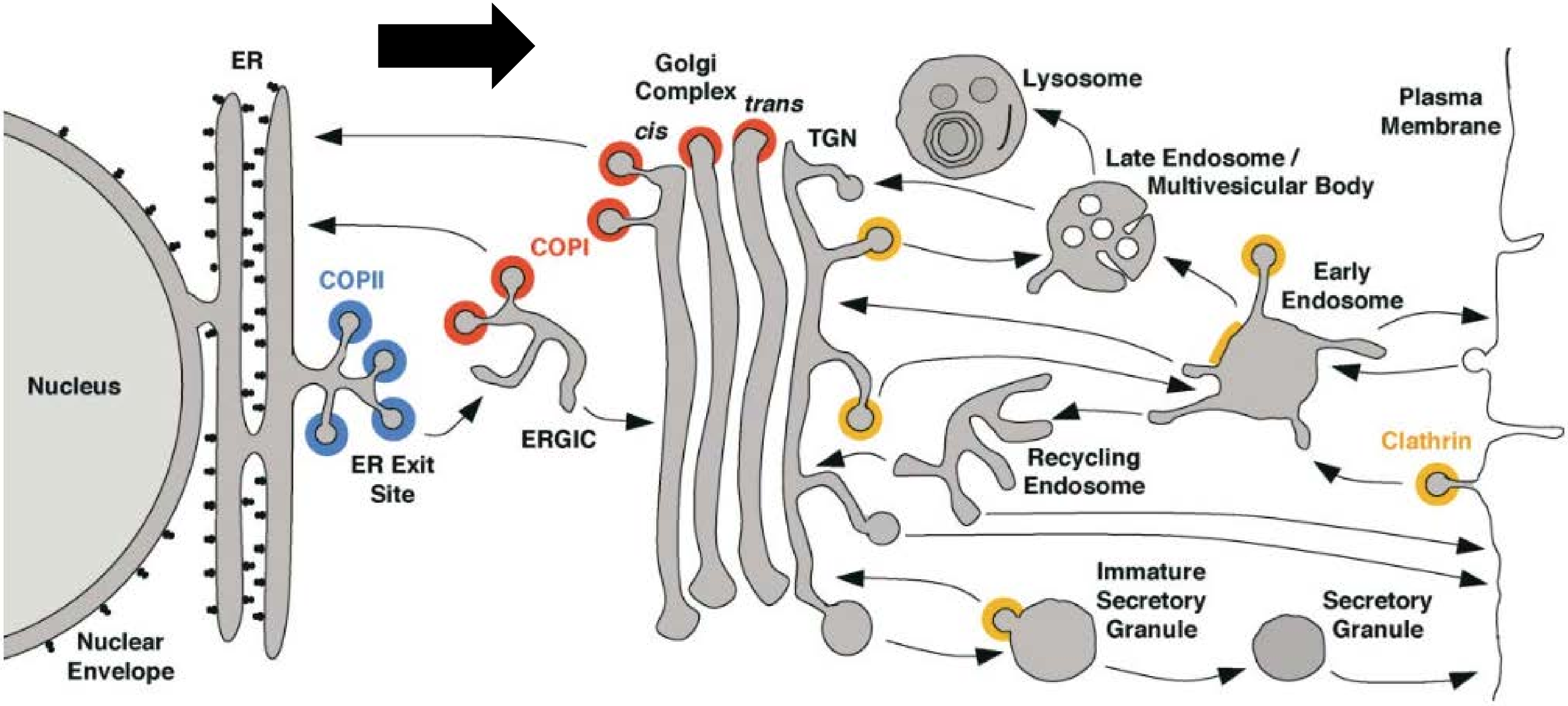
Polypeptides generated by RER membrane-bound polysomes, enter the lumen of RER.

Proteins undergo core glycosylation and by interacting with chaperones acquire their conformation.



Protein trafficking

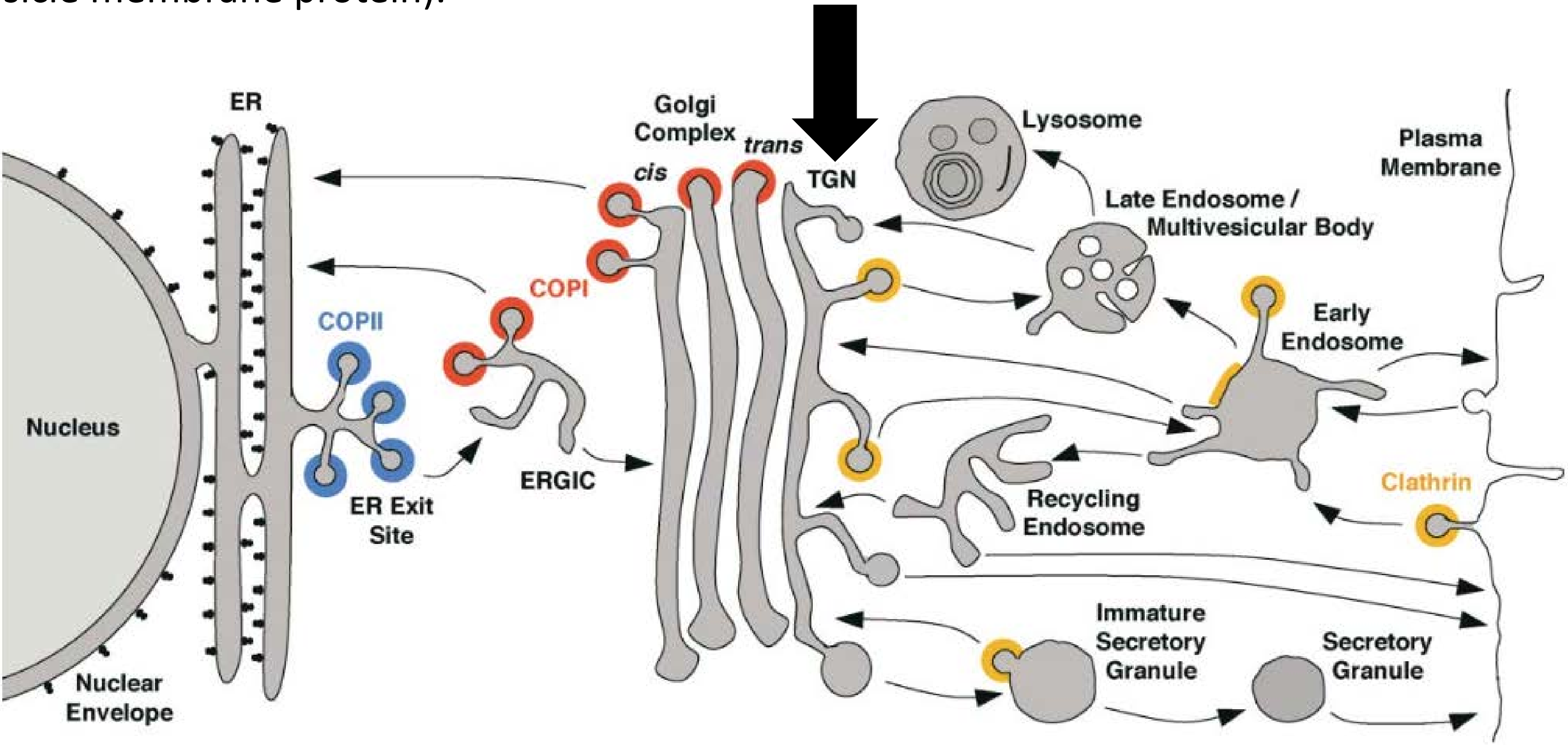
Proteins are then transported to the Golgi apparatus, where terminal glycosylation and other post-translational reactions take place.



Protein trafficking

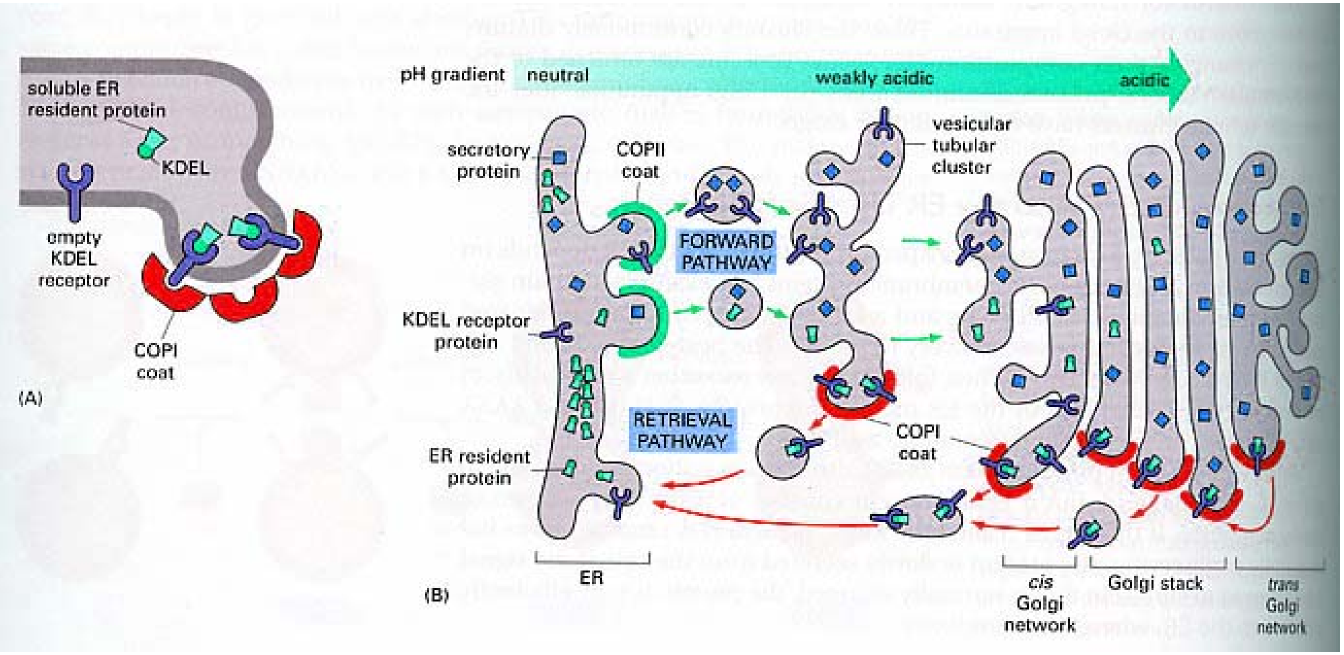
In the Trans-Golgi network (TGN), mature proteins undergo sorting processes and are packed into transport vesicles.

The vesicles carry soluble proteins (inside the vesicle) and membrane proteins (as integral vesicle membrane protein).



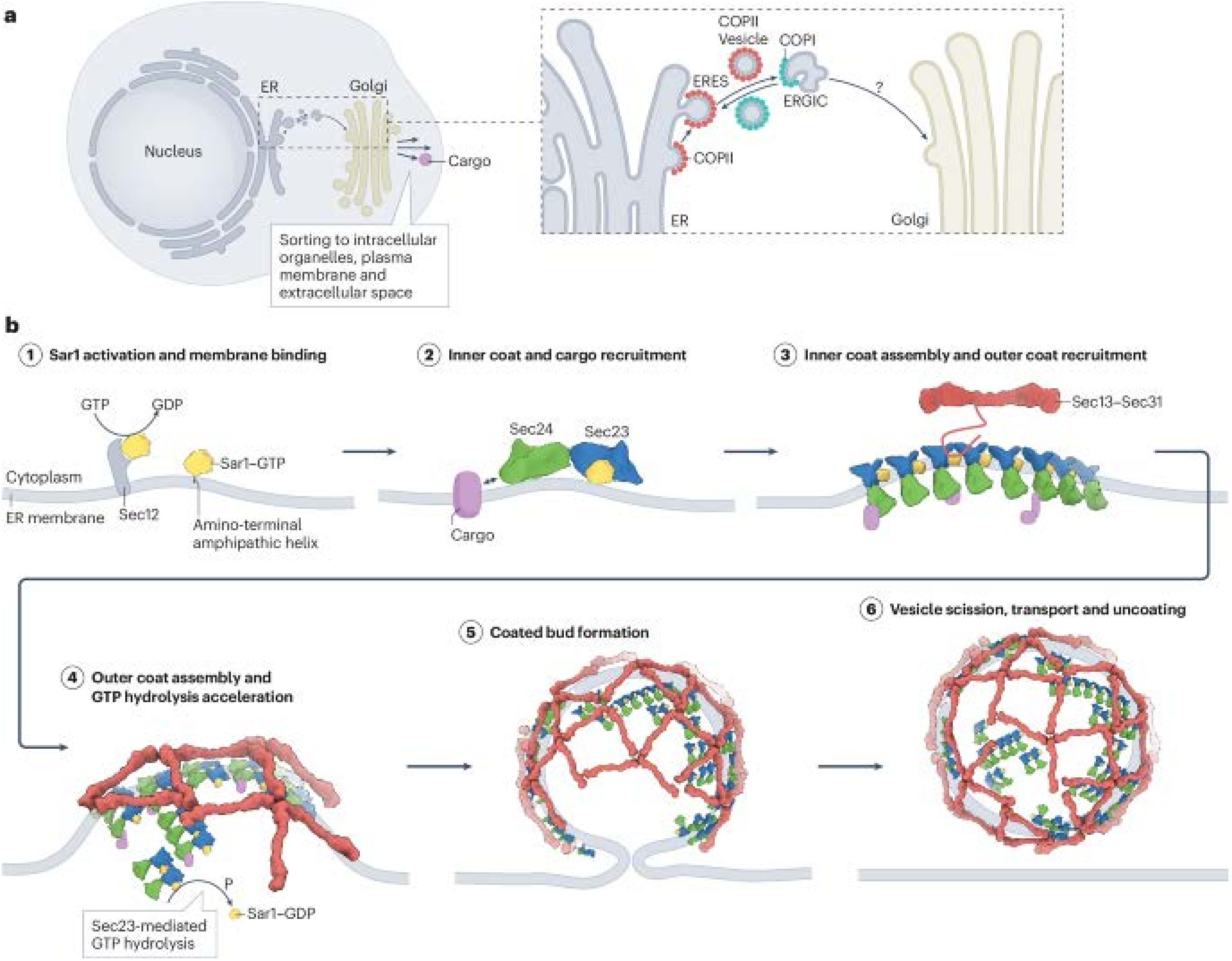
Protein trafficking

Proteins that reside in the ER possess a C-terminal tetrapeptide KDEL (Lys-Asp-Glu-Leu) which signals their return to the ER from the Golgi.



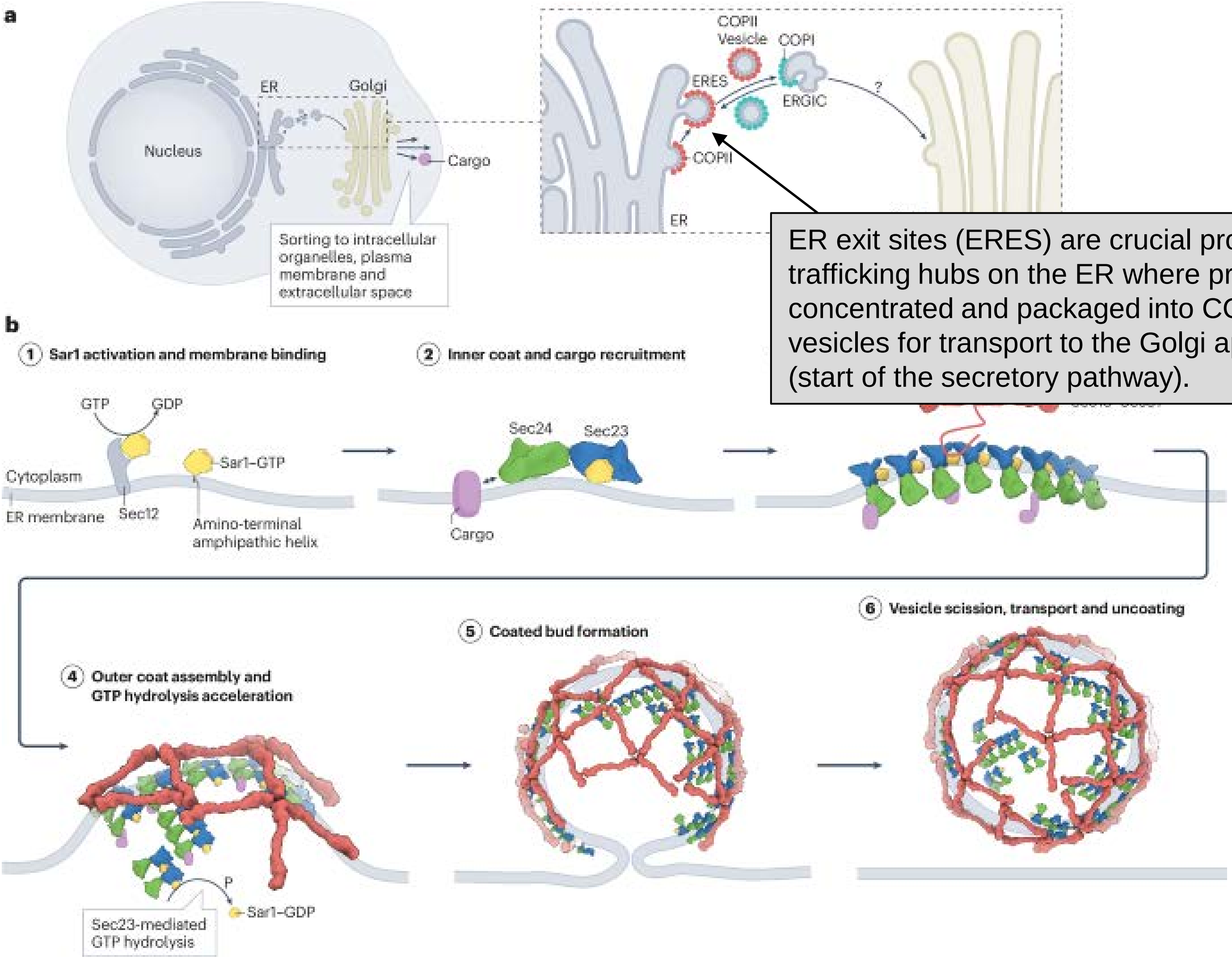
COPI is a protein that coats vesicles that transports proteins from the cis end of the Golgi complex to the RER. This type of transport is termed retrograde transport.

Protein trafficking



Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

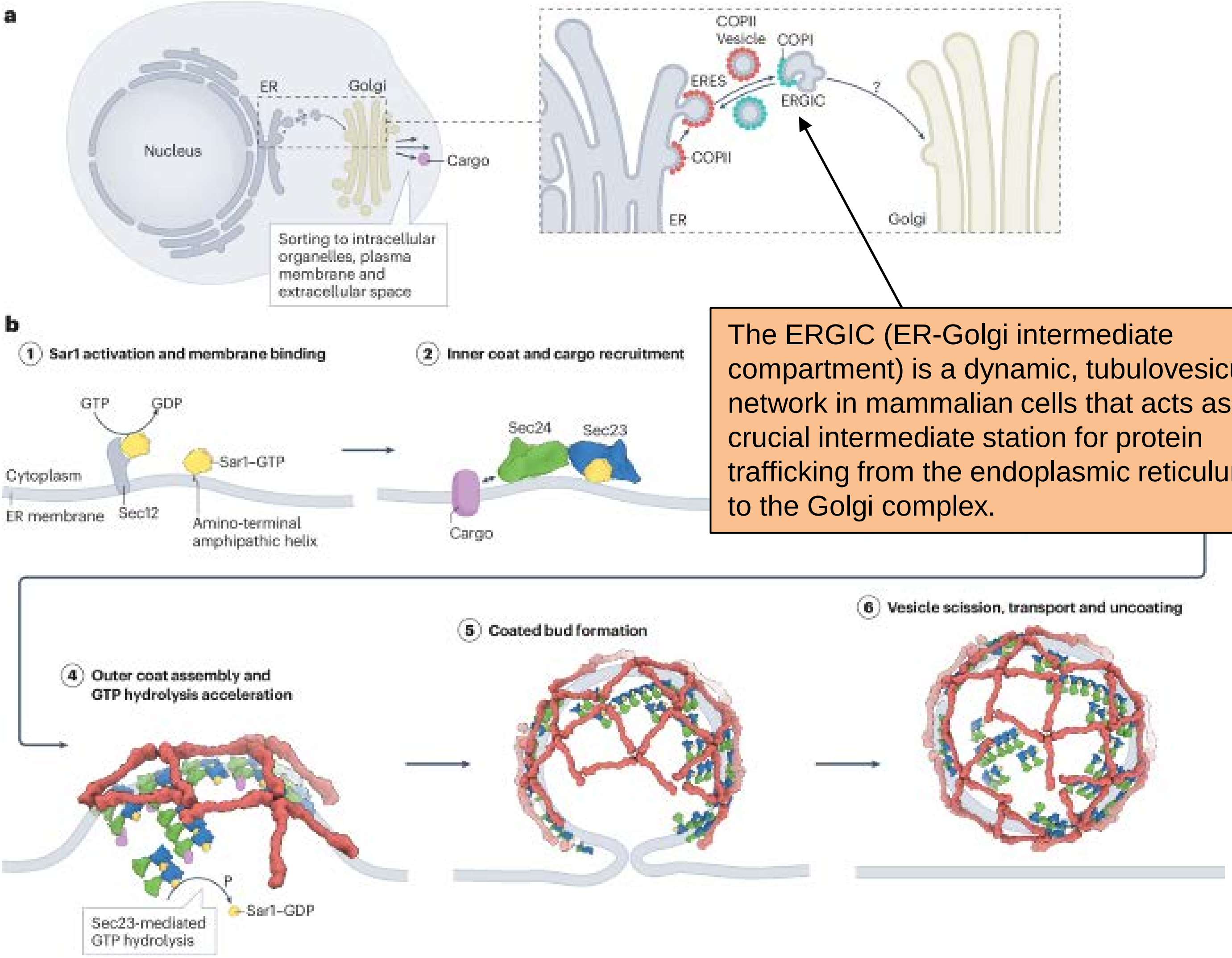
Protein trafficking



ER exit sites (ERES) are crucial protein-trafficking hubs on the ER where proteins are concentrated and packaged into COPII vesicles for transport to the Golgi apparatus (start of the secretory pathway).

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

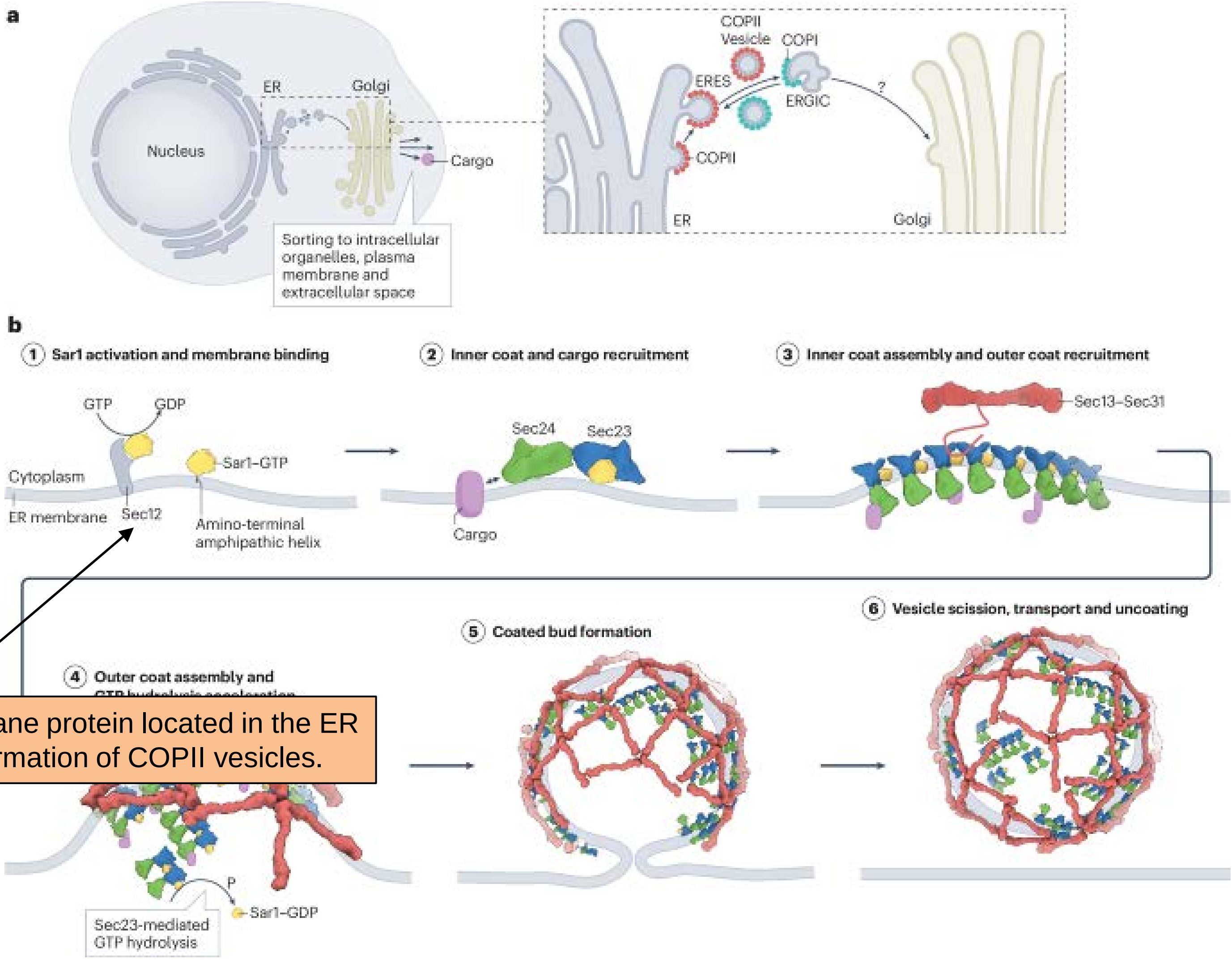
Protein trafficking



The ERGIC (ER-Golgi intermediate compartment) is a dynamic, tubulovesicular network in mammalian cells that acts as a crucial intermediate station for protein trafficking from the endoplasmic reticulum (ER) to the Golgi complex.

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

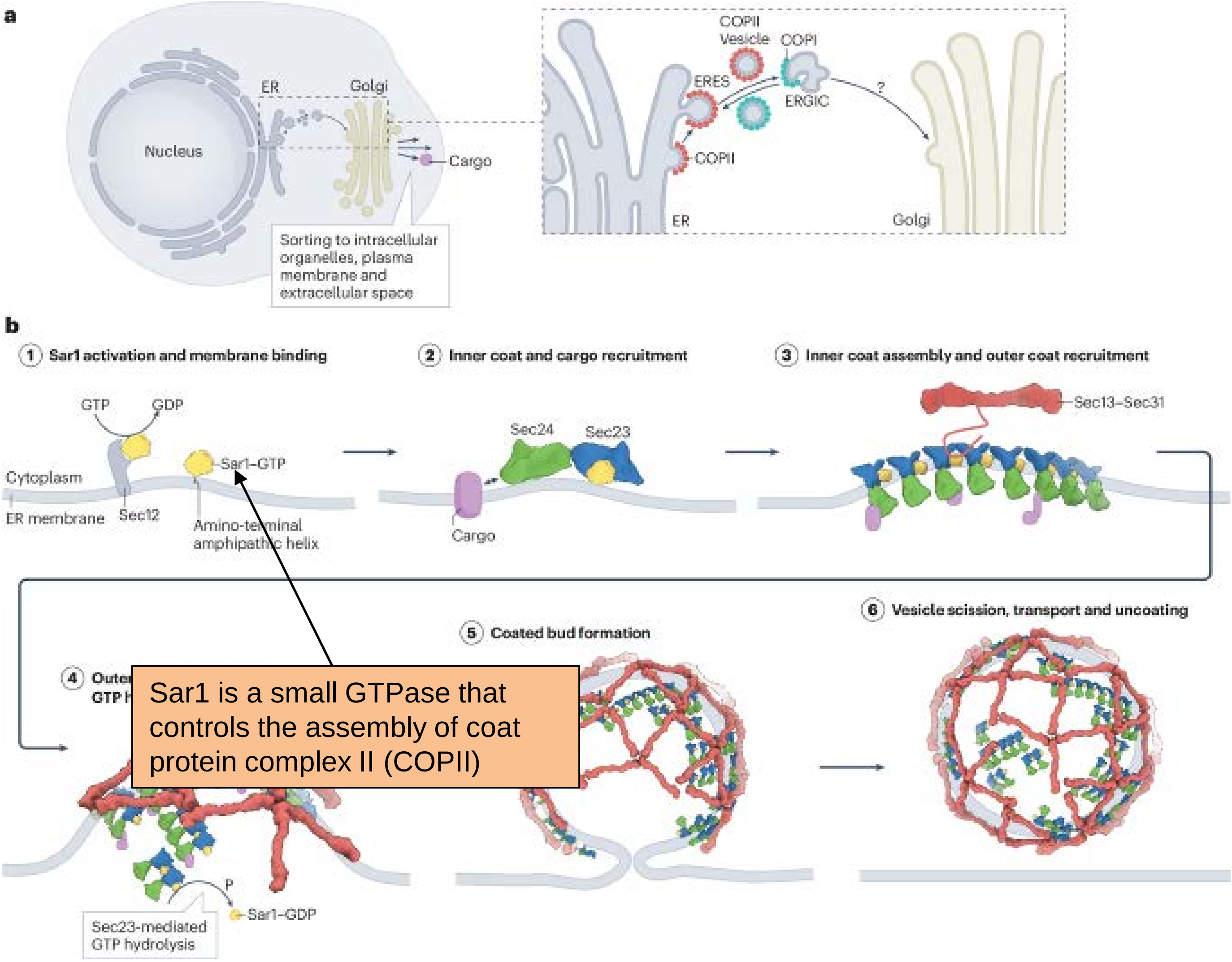
Protein trafficking



Sec12 is a membrane protein located in the ER that initiates the formation of COPII vesicles.

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

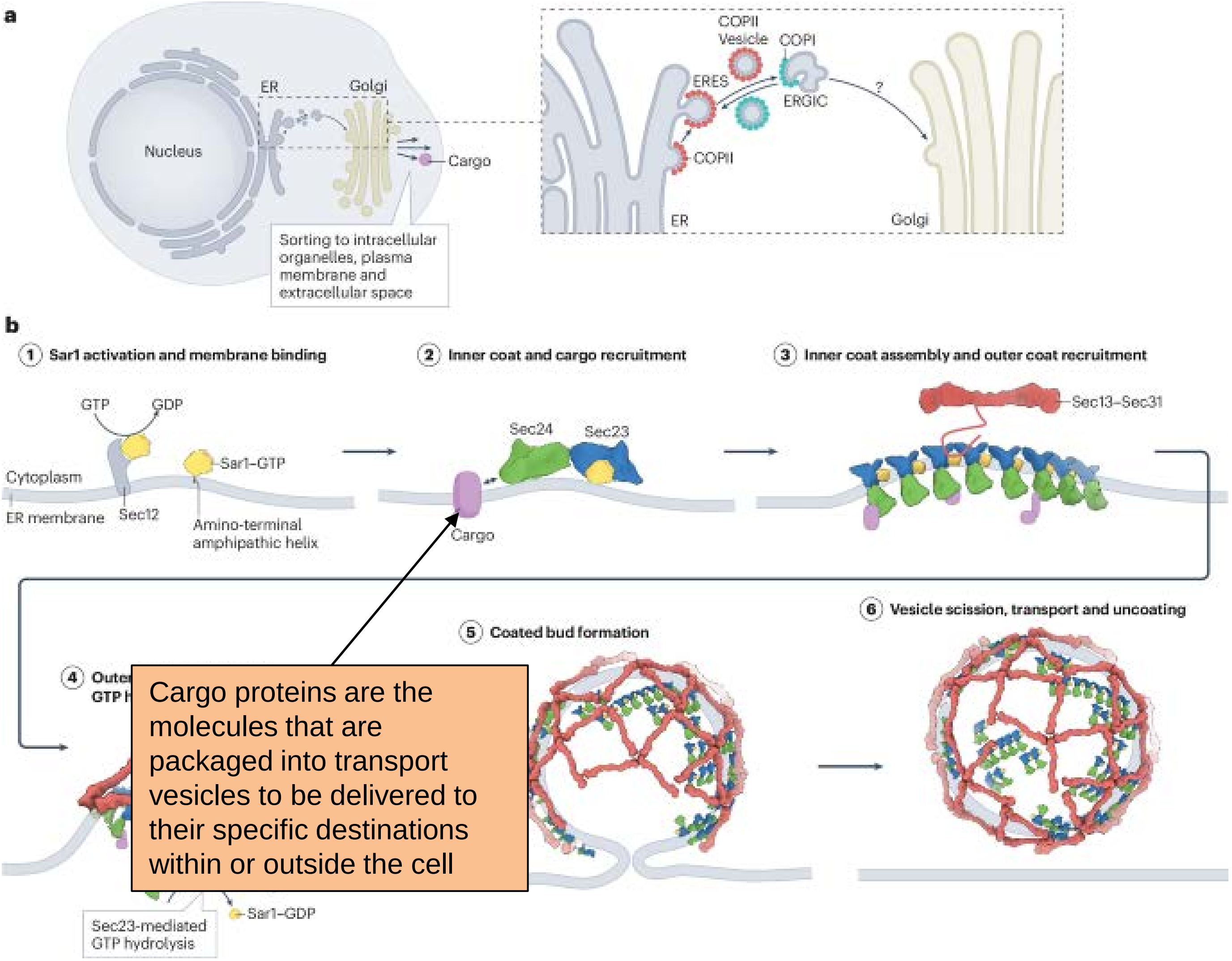
Protein trafficking



Sar1 is a small GTPase that controls the assembly of coat protein complex II (COPII)

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

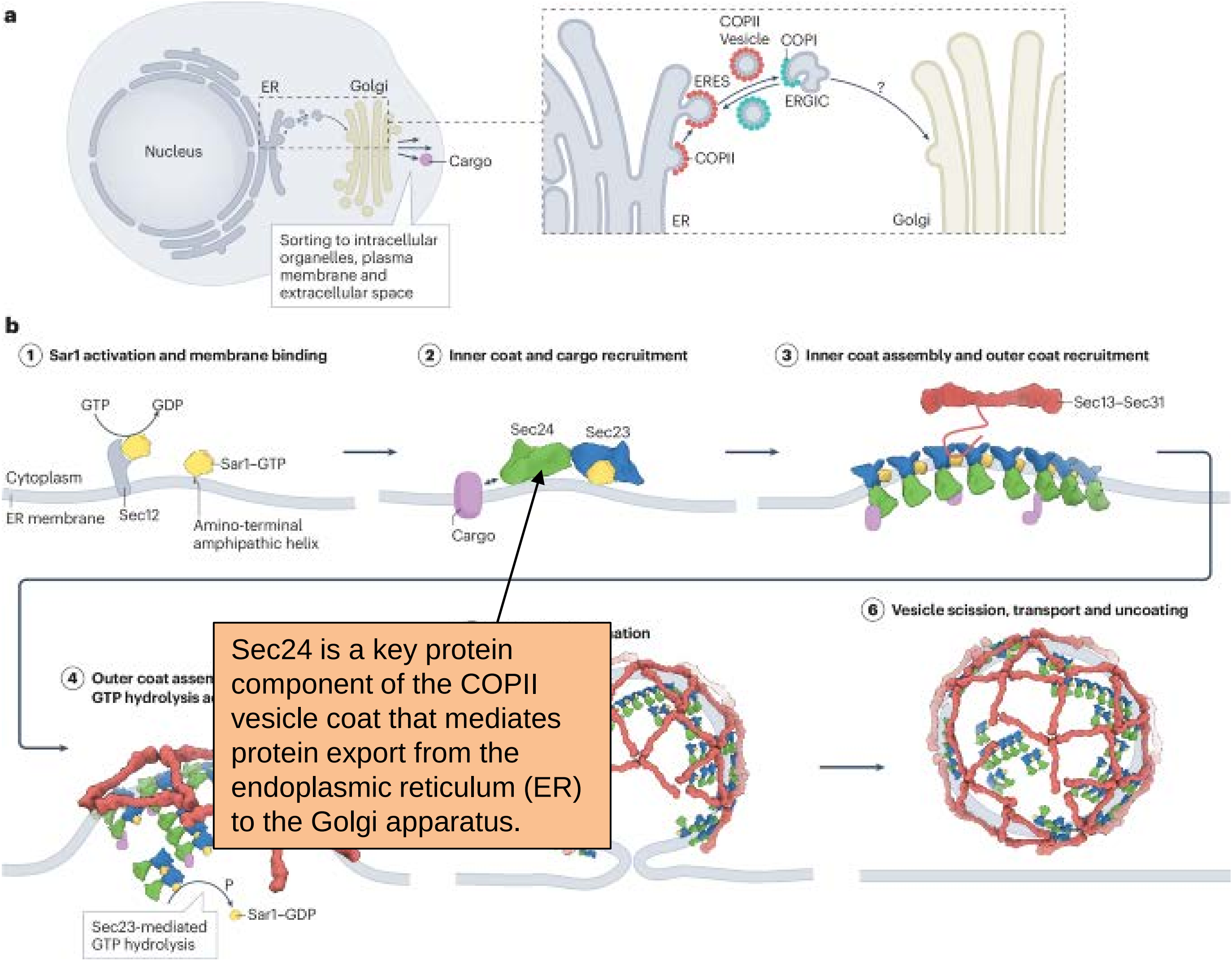
Protein trafficking



Cargo proteins are the molecules that are packaged into transport vesicles to be delivered to their specific destinations within or outside the cell

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

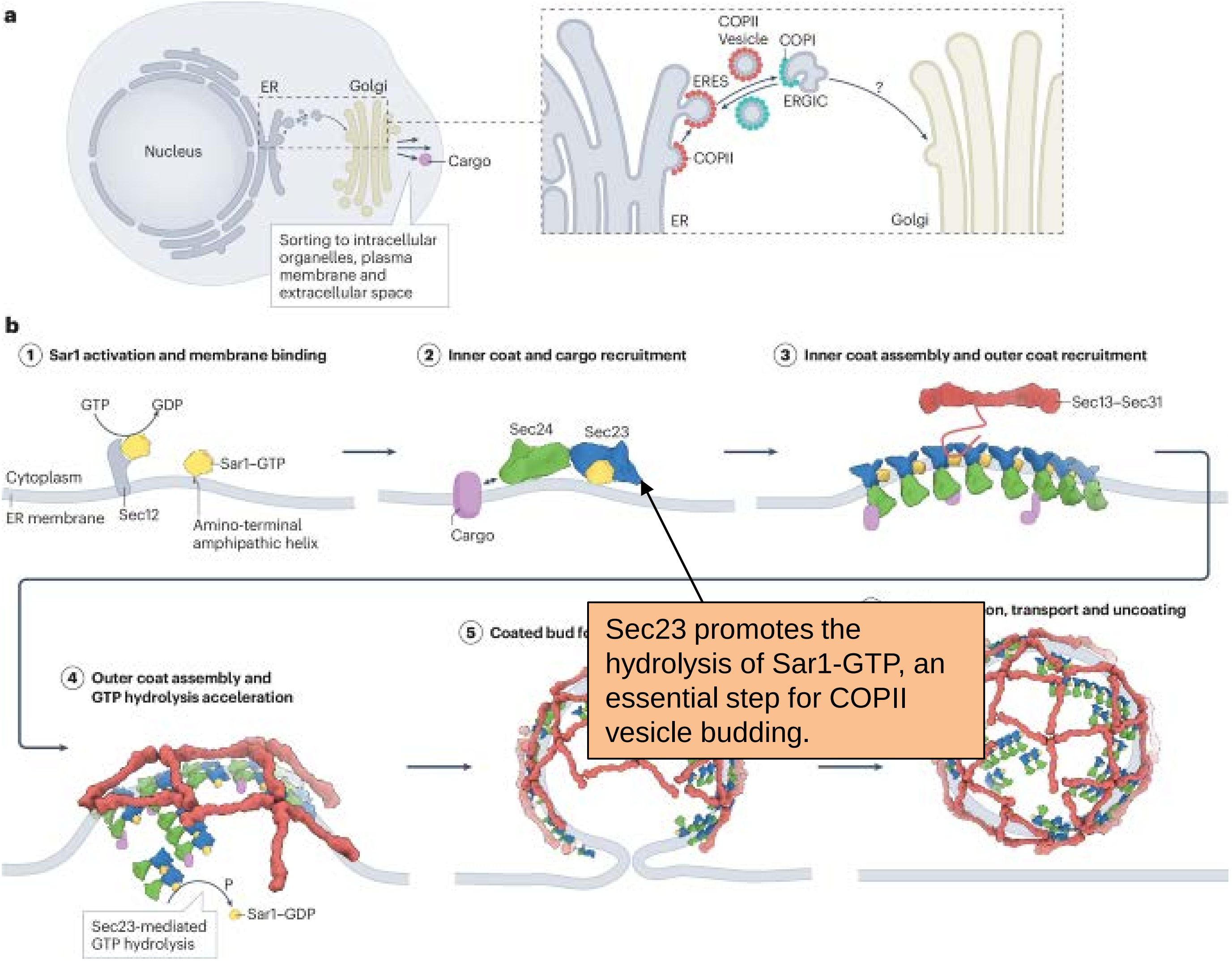
Protein trafficking



Sec24 is a key protein component of the COPII vesicle coat that mediates protein export from the endoplasmic reticulum (ER) to the Golgi apparatus.

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

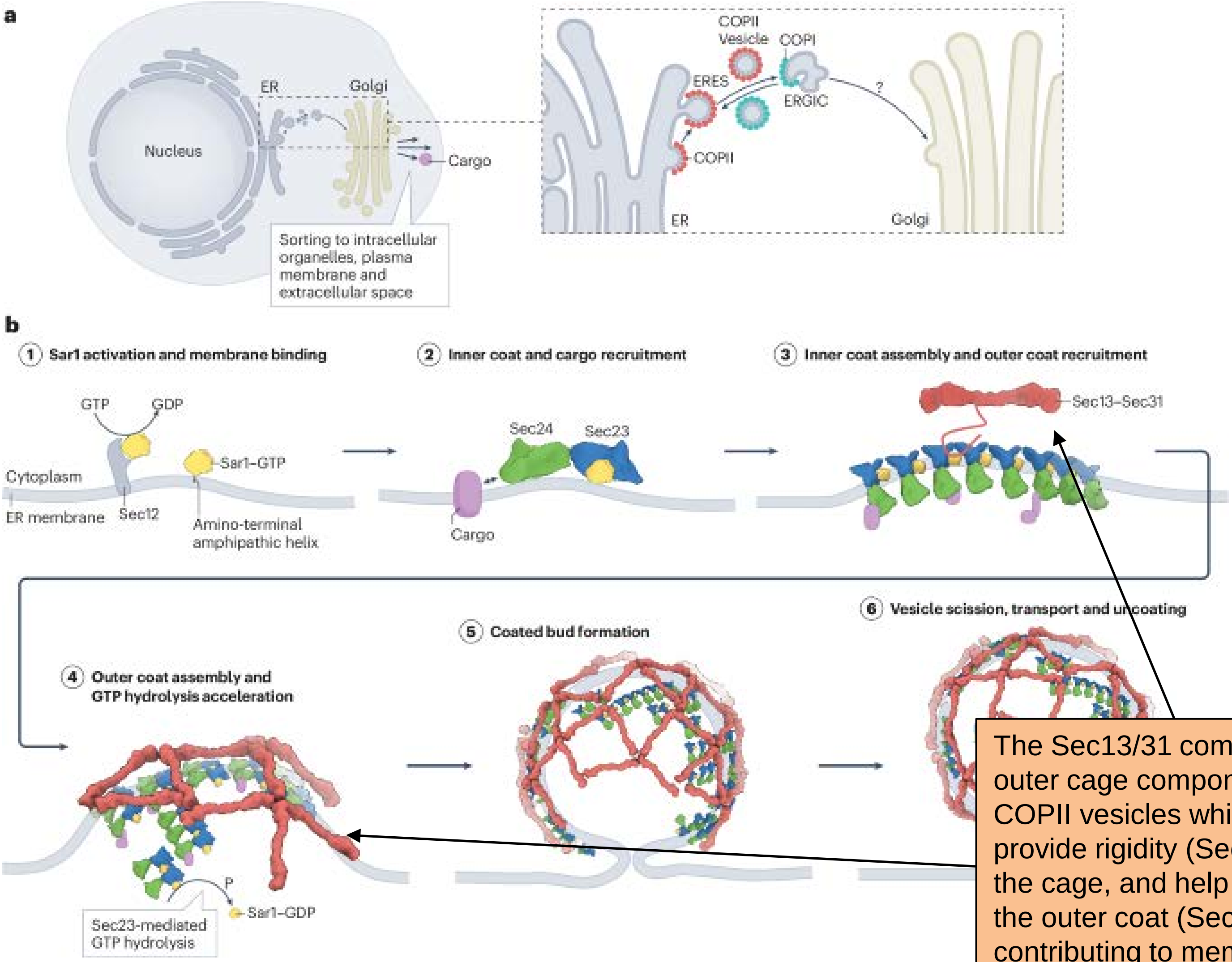
Protein trafficking



Sec23 promotes the hydrolysis of Sar1-GTP, an essential step for COPII vesicle budding.

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

Protein trafficking



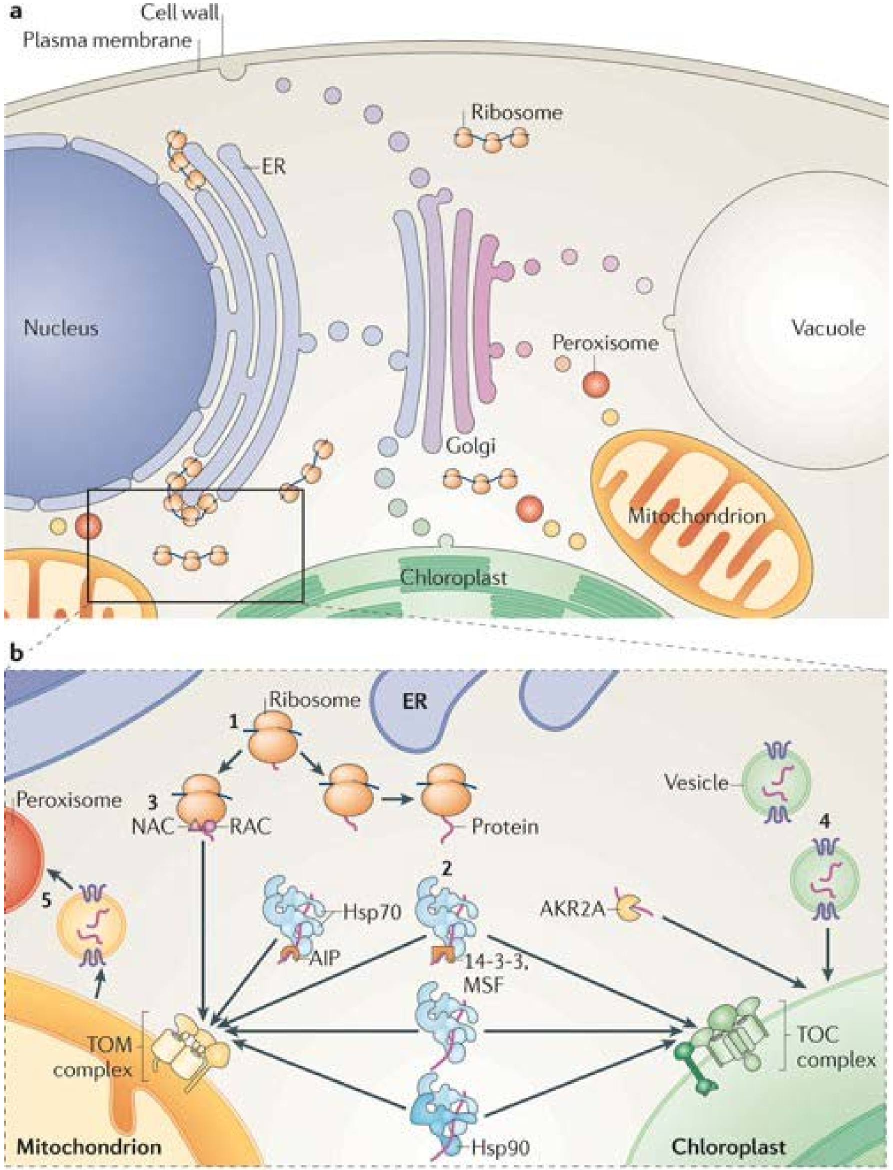
The Sec13/31 complex is an outer cage component of COPII vesicles which provide rigidity (Sec13) to the cage, and help recruit the outer coat (Sec31) contributing to membrane curvature.

Downes KW, Zanetti G. Nat Rev Mol Cell Biol. 2025 Mar

Leader sequence hierarchy (mitochondria)

Mitochondrial and chloroplast proteins are synthesized on "free" ribosomes.

- They associate with the organelle membranes by means of N-terminal sequences of ~25 amino acids that are recognized by receptors on the the organelle envelope.
- Because this process takes place after synthesis of the protein has been completed, it is called post-translational translocation.



Schleiff E, Becker T. Nat Rev Mol Cell Biol. 2011 Jan

Leader sequence hierarchy (mitochondria)

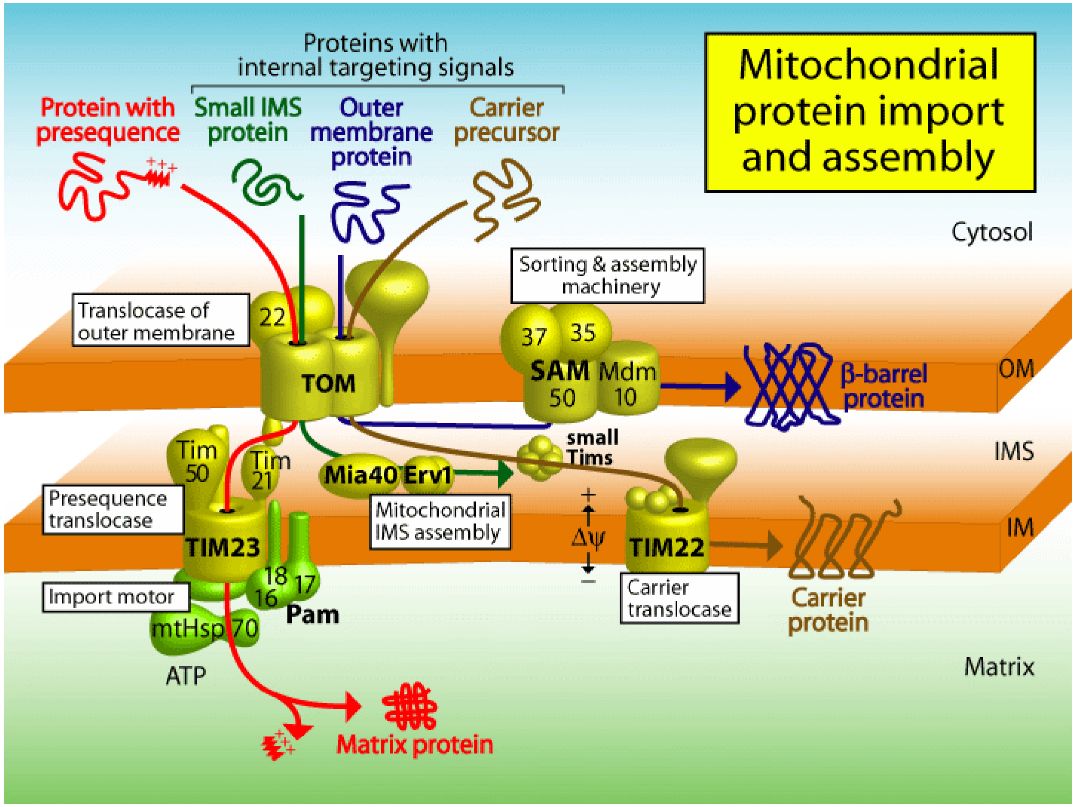
Same as for ER.

Requires specific translocons and Signal Recognition Proteins (SRPs).

As endosymbionts have two membranes, two different types of translocons are needed

TOM
TIM

Incorporated proteins can be integrated into membranes as happens for ER proteins.



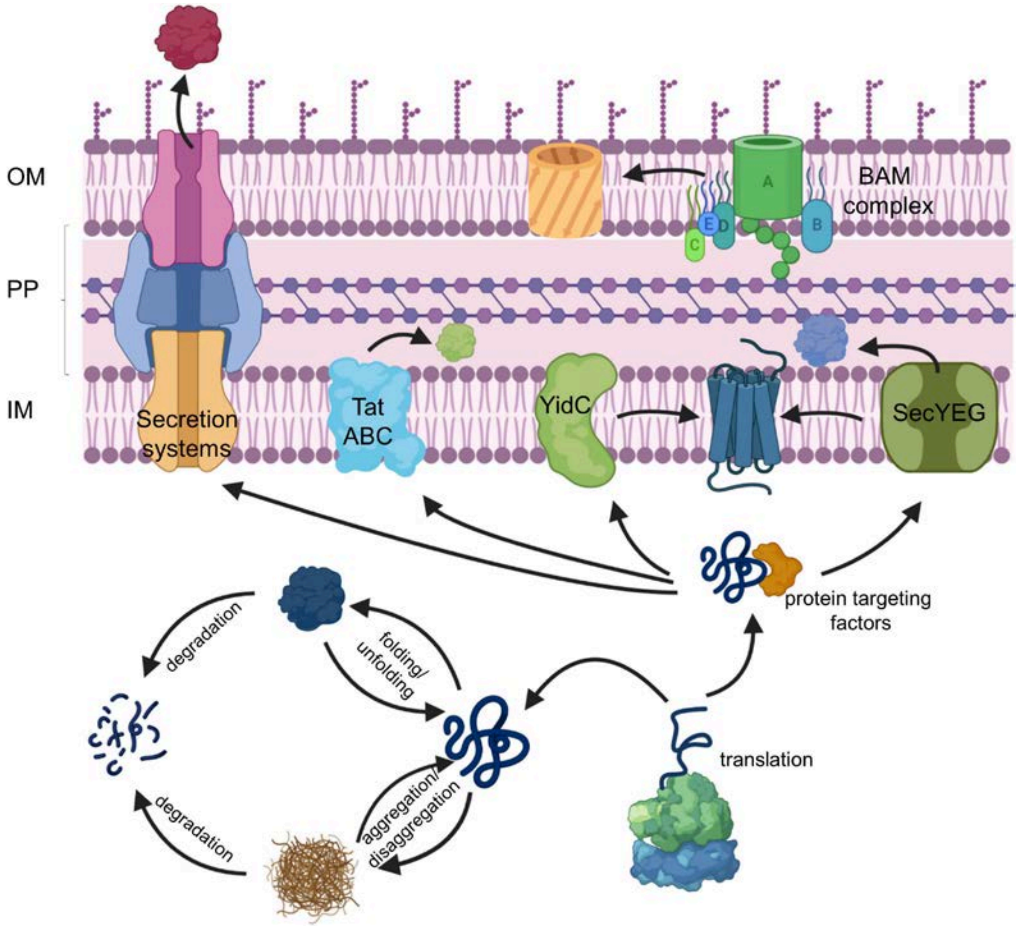
Leader sequence hierarchy (mitochondria)

Leader signals create a hierarchy that directs each protein to its specific destination.

The default pathway imports proteins completely into the matrix using two sequential signals in the leader sequence.

The first signal enables organelle recognition and passage through the outer membrane.

The second signal enables inner membrane recognition and passage into the matrix.



Proteins destined for the intermembrane space or as integral inner membrane proteins require additional signals beyond these two.

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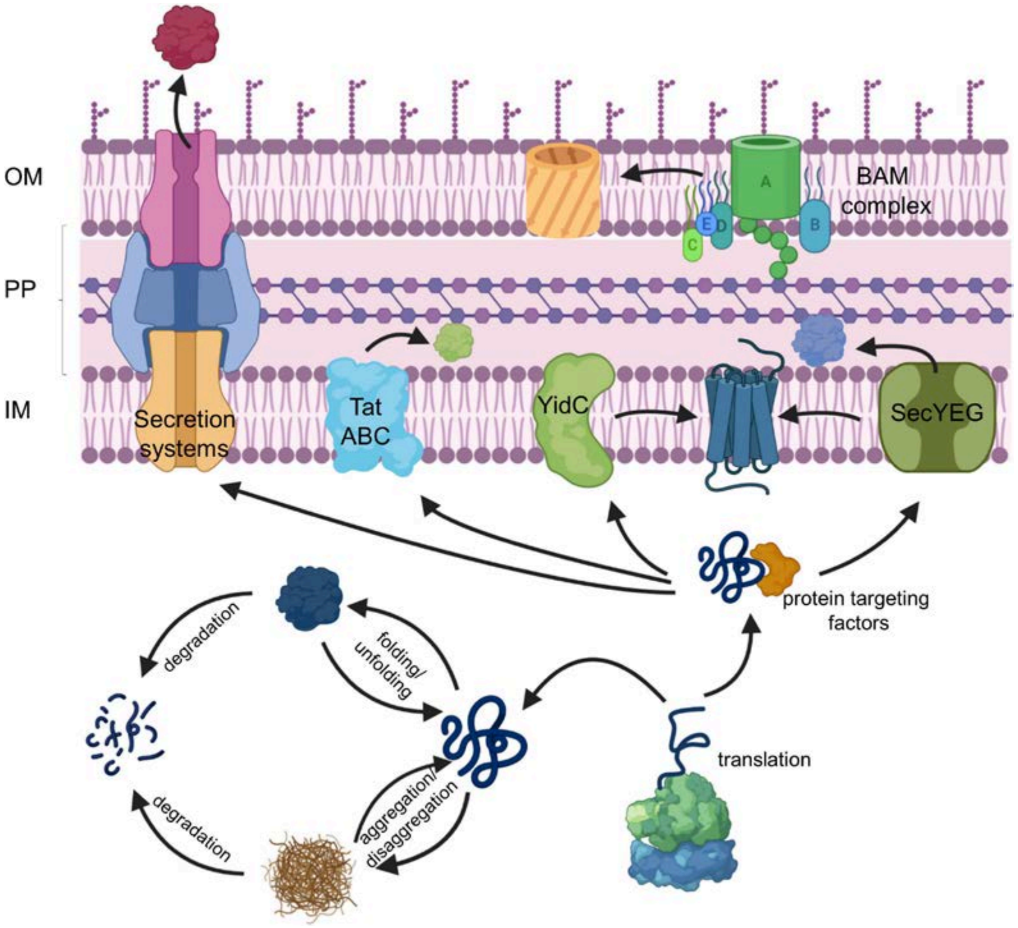
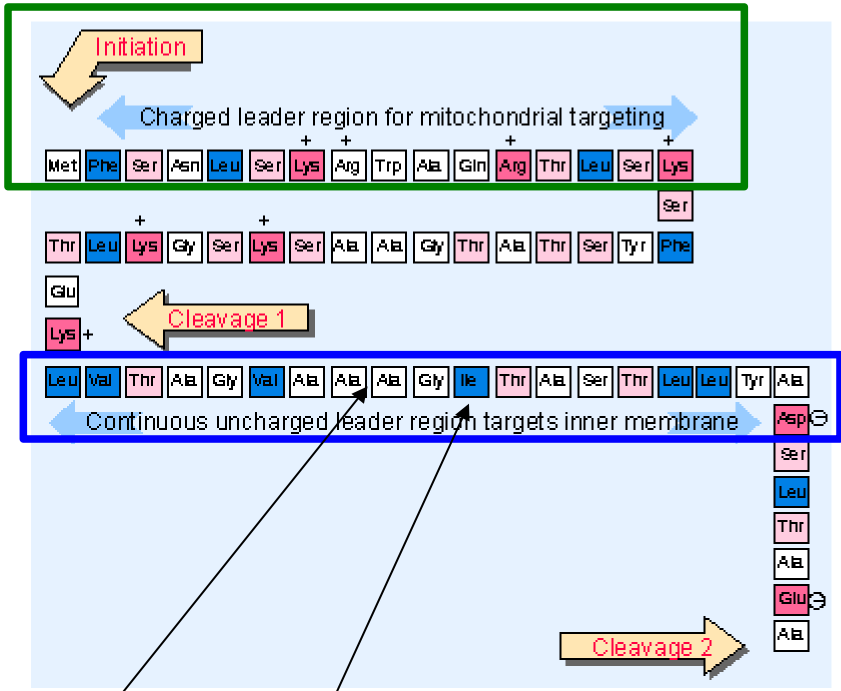


Proteins destined for the intermembrane space or as integral inner membrane proteins require additional signals beyond these two.

Leader sequence hierarchy

- This requires two signals (in the leader):
  - •Organelle recognition & outer membrane passage (first part of the leader sequence).
  - •Inner Membrane recognition & passage (second part).



Many uncharged amino acids

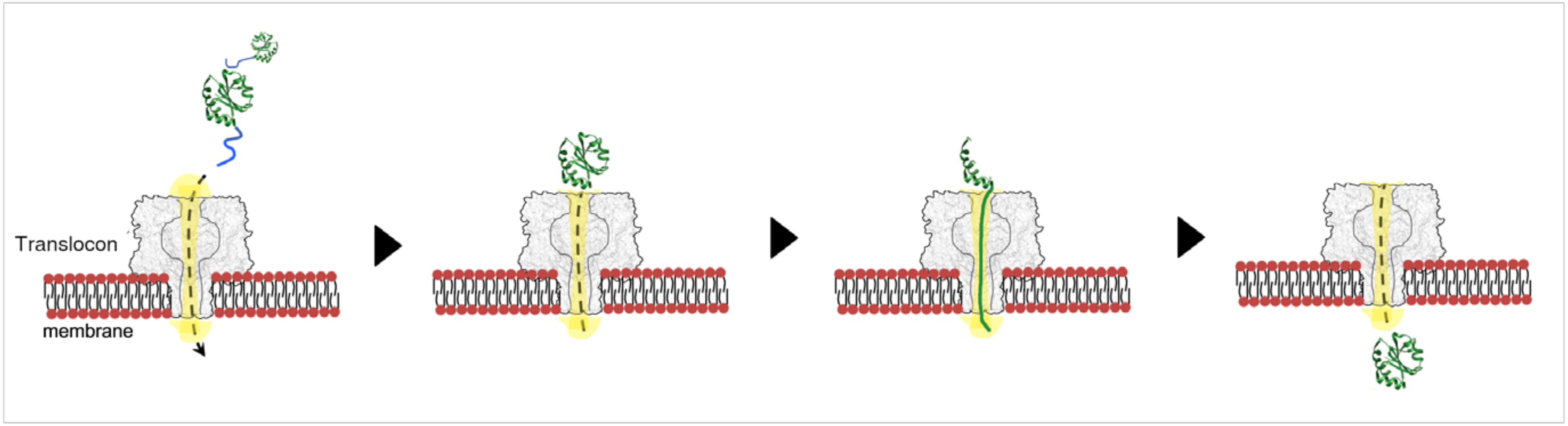
Basic amino acids

Translocons (Translocation Channels)

There is a basic problem in passing a (largely) hydrophilic protein through a hydrophobic membrane.

The energetics of the interaction are highly unfavorable.

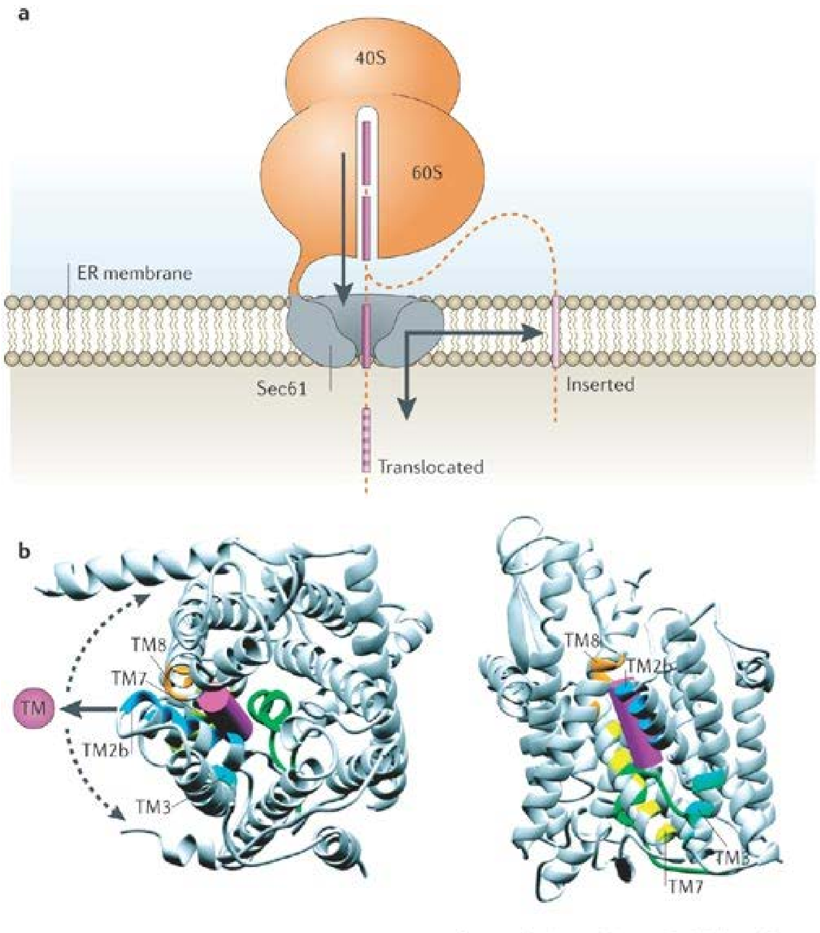
Translocating proteins move through an aqueous channel (translocon), interacting with the resident (integral) proteins rather than with the lipid bilayer.



Translocons (Translocation Channels)

- When the signal sequence enters the translocon, the ribosome attaches, forming a seal so that the pore is not exposed to the cytosol.

- Ribosome is bound by the interaction of the Signal Recognition Particle (SRP) and the SRP-receptor.



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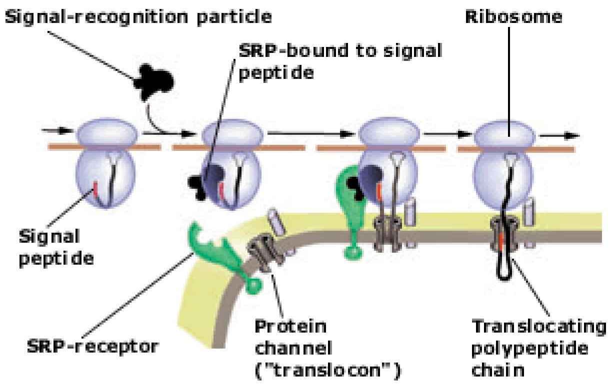
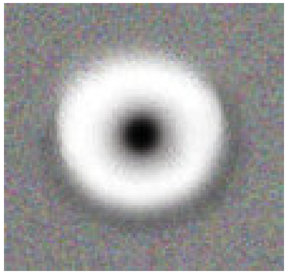
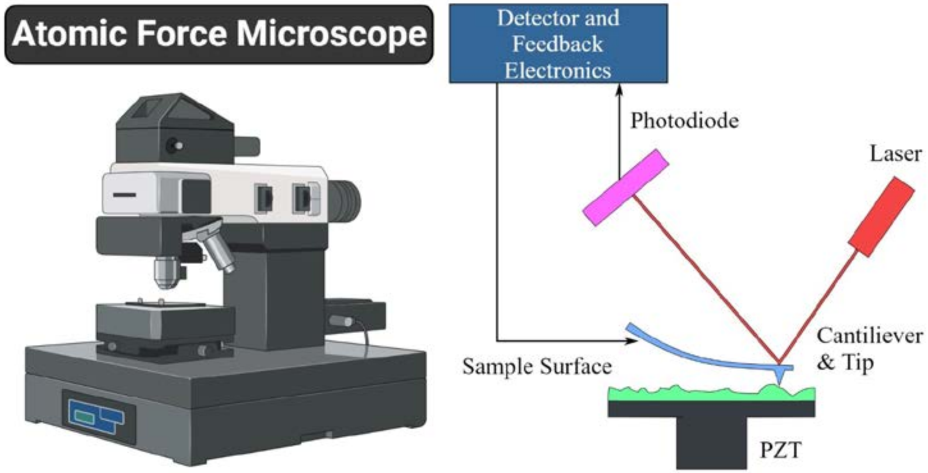


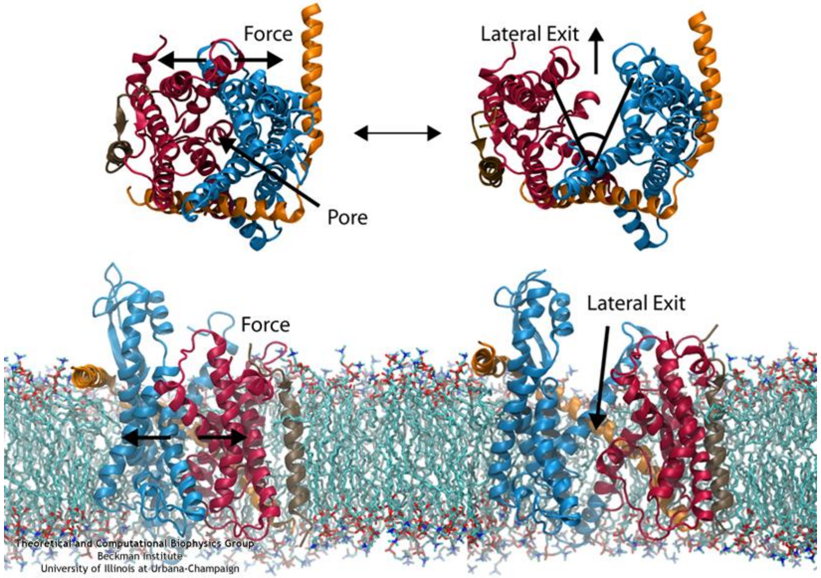
Translocons (Translocation Channels)

- Sec61 Complex is the major component of the translocon:
  - •Sec61α
  - •Sec61β
  - •Sec61γ



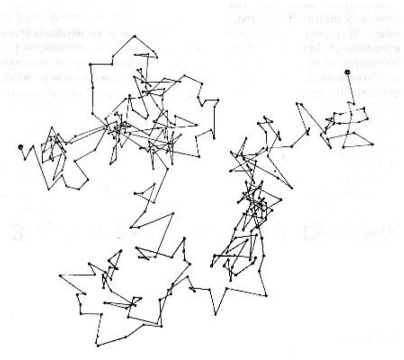
- Forms cylindrical oligomers (each of 3 to 4 heterotrimers) with a diameter of ~8.5nm and a central pore of ~2 nm.

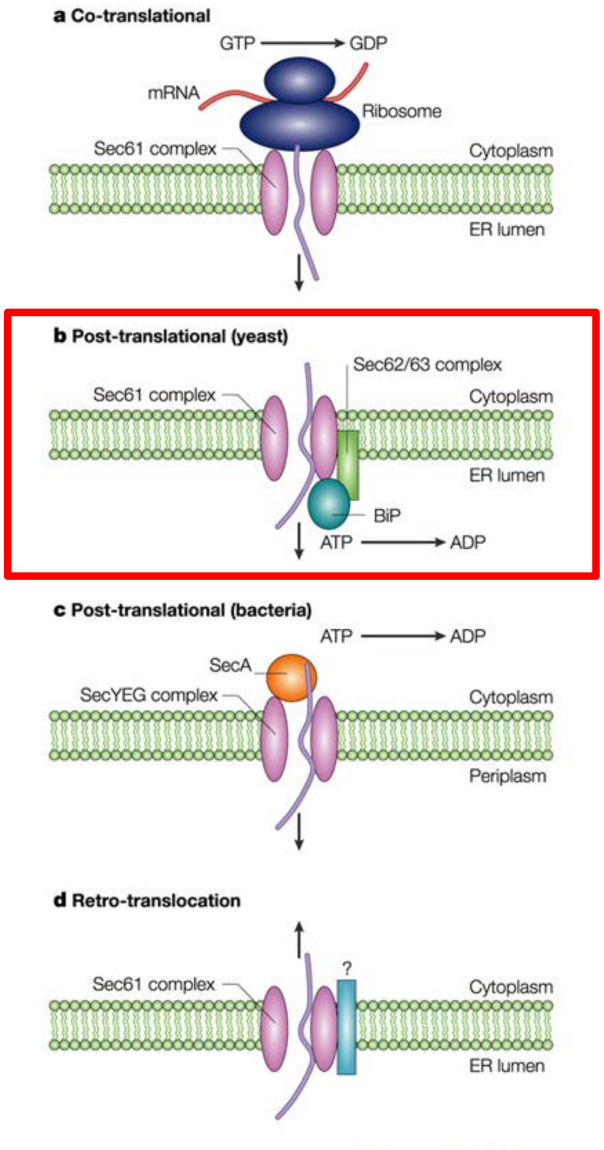




Translocons (Translocation Channels)

- A more complex translocon is required when a protein is inserted into a membrane post-translationally.
  - •Sec61 complex
  - •Four other Sec proteins
  - •Chaperone BiP (a member of the Hsp70 class)
  - •Supply of ATP
- BiP prevents protein backslash due to Brownian Motion.





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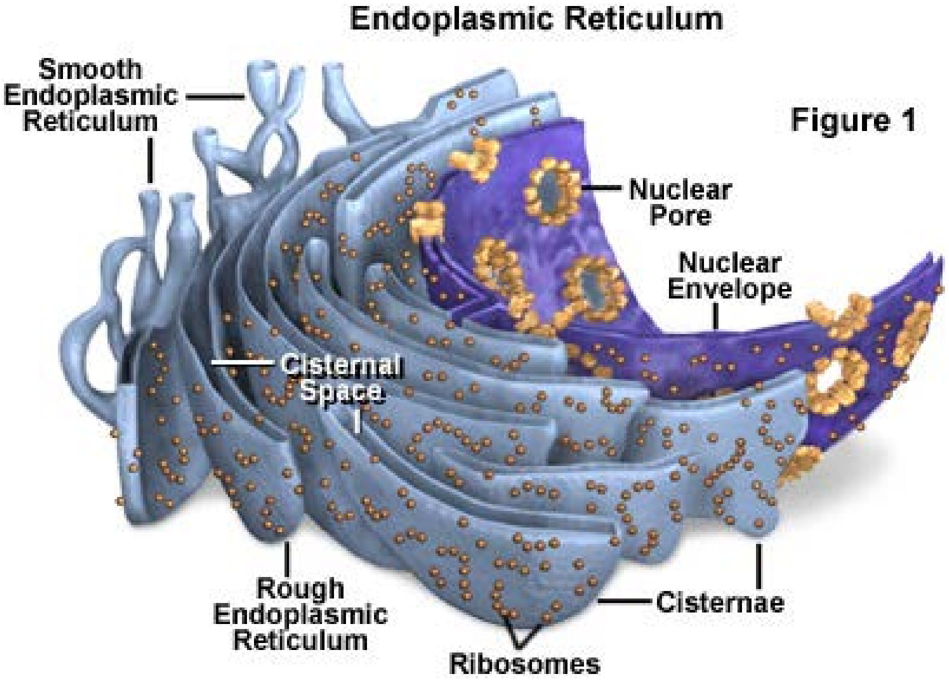
Nuclear pore complex

- The nucleus is segregated from the cytoplasm by an envelope consisting of two membranes.
- The outer membrane is continuous with the ER in the cytosol.
- The two membranes come into contact at openings called nuclear pore complexes (~3000 per cell).



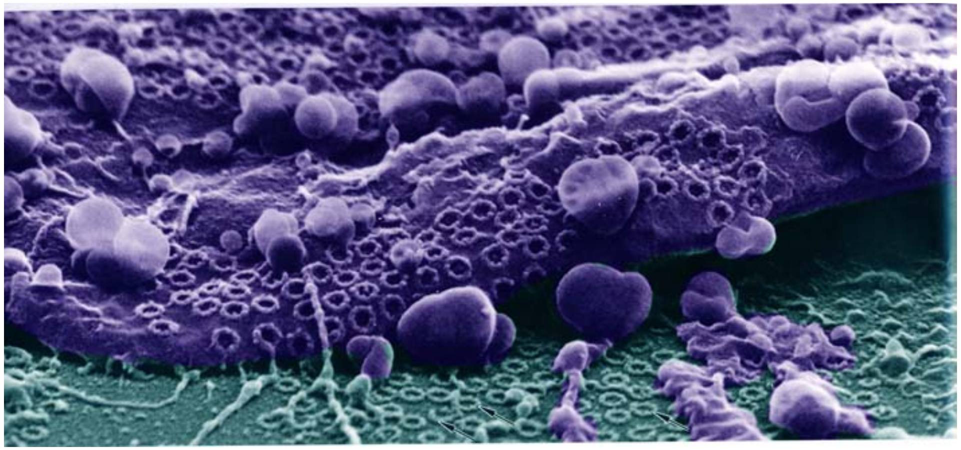
Pore provides a water-soluble channel between nucleus and cytoplasm.

Nucleus and cytosol have the same ionic milieu !

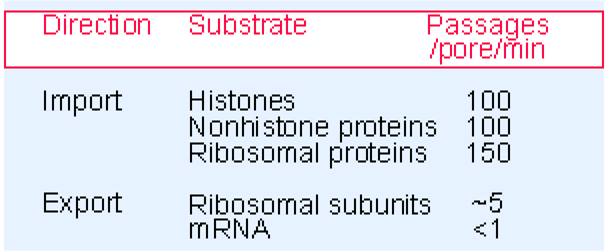
Nuclear pore complex

- Nuclear pores are used for both import and export of material.

- Proteins are synthesized in the cytosol so any protein required in the nucleus must be transported there.



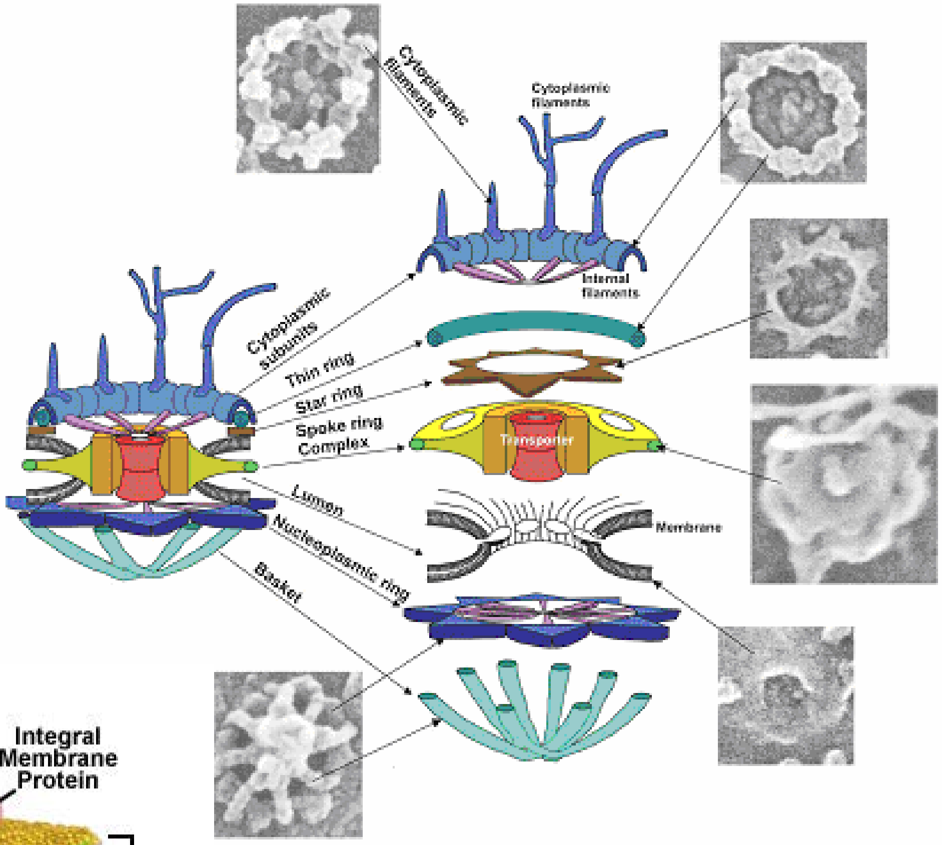
- Since all RNA is synthesized in the nucleus, the entire cytoplasmic complement of RNA (mRNA, rRNA, tRNA, and other small RNAs) must be exported from the nucleus.

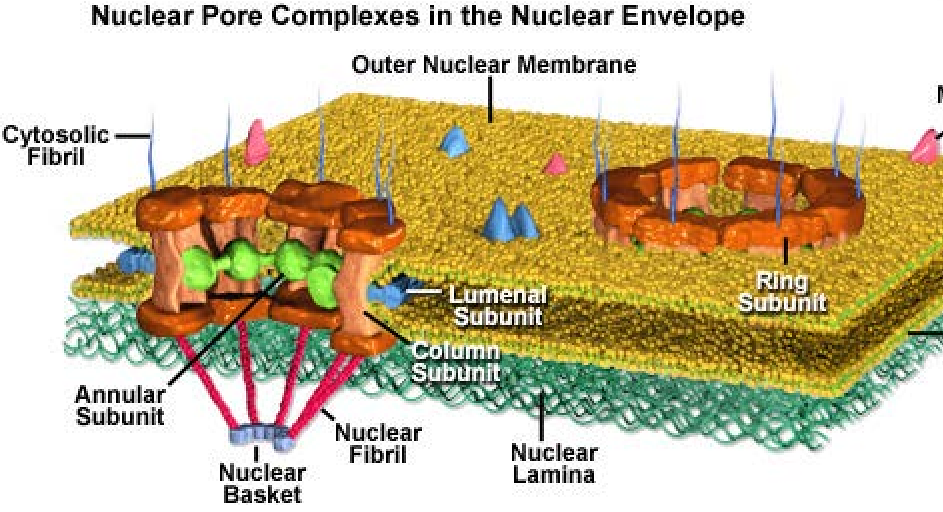


Direction	Substrate	Passages /pore/min
Import	Histones	100
	Nonhistone proteins	100
	Ribosomal proteins	150
Export	Ribosomal subunits	~5
	mRNA	<1

Nuclear pore complex

- The entire pore complex has a diameter of about 120 nm.
- Pore diameter is 50 nm wide and its "depth" is about 200 nm.
- Mammalian is 120 MDalton and contains approximately 30 different protein components.



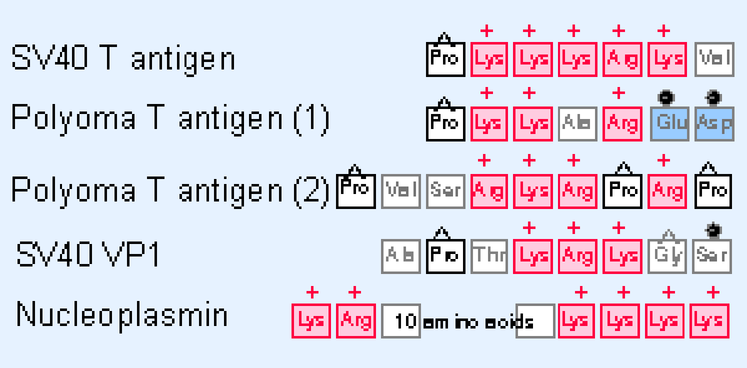


Nuclear pore complex

- Molecules of <5 kD that are injected into the cytoplasm appear virtually instantaneously in the nucleus.
  - Freely permeable to ions, nucleotides and other small molecules.
- Proteins between 5-50 kD diffuse at a rate that is inversely related to their size.
  - Presumably determined by random contacts with the pore.
  - It takes a few hours for the levels of an injected protein to equilibrate between cytoplasm and nucleus.
  - Small proteins can enter the nucleus by passive diffusion (but they may also be actively transported).
- Proteins >50 kD in size do not enter the nucleus by passive diffusion.
  - Active transport required for their passage

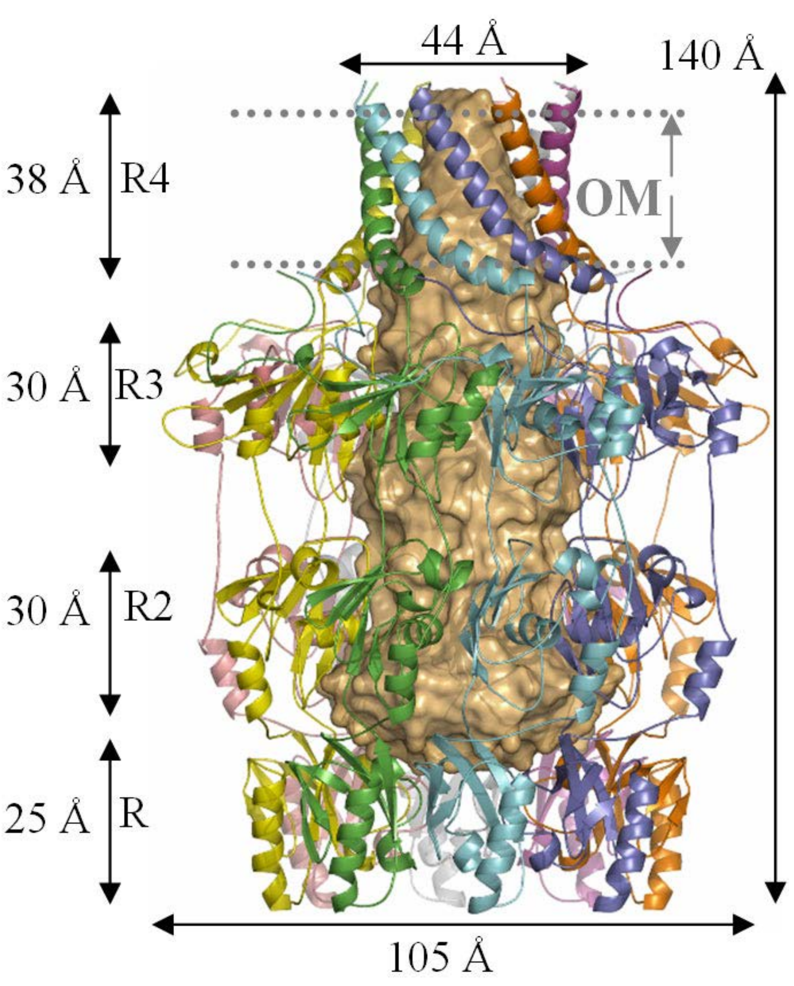
Nuclear pore complex

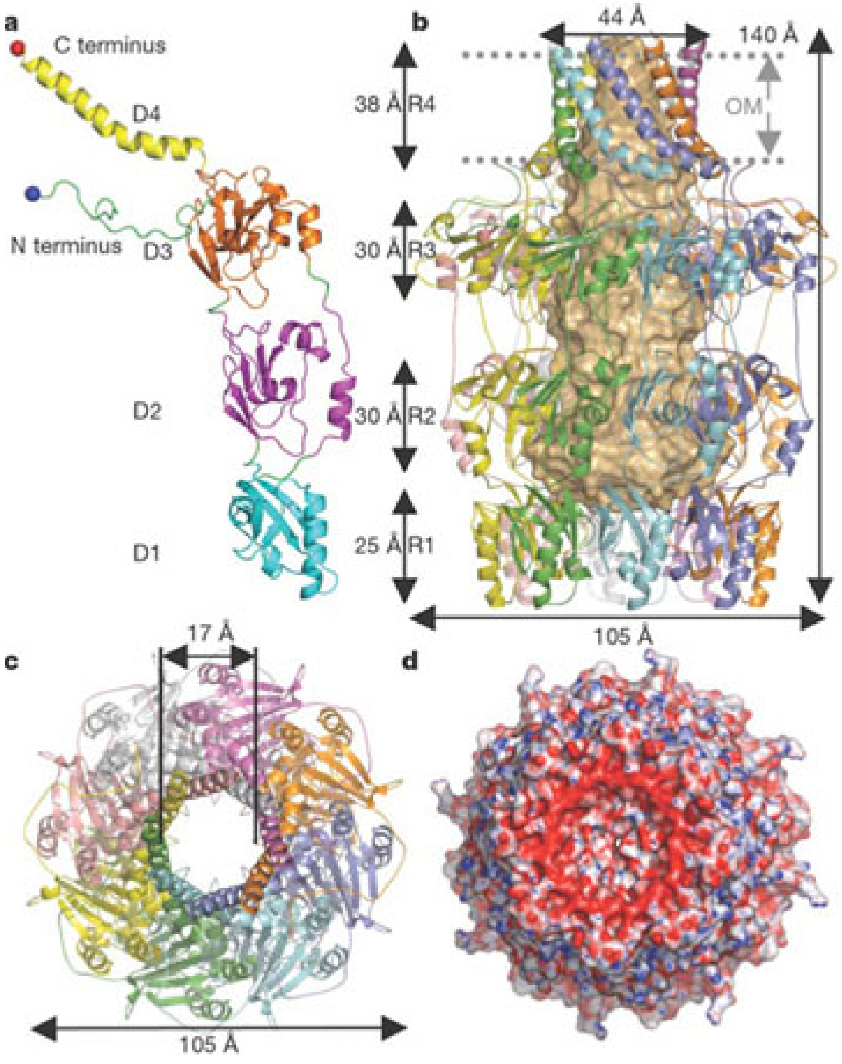
- For a protein to pass through a NPC it must have a special signal sequence.
- The most common motif responsible for import into the nucleus is the Nuclear Localization Signal (NLS).



- Its presence is necessary and sufficient to sponsor import into the nucleus.
- Mutation of the signal can prevent the protein from entering the nucleus
- There is no apparent conservation of sequence of NLS signals
  - •short, rather basic stretch of amino acids.
  - •Proline residue usually breaks α-helix upstream of basic residues.
  - •Hydrophobic residues are rare.

Nuclear pore complex





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