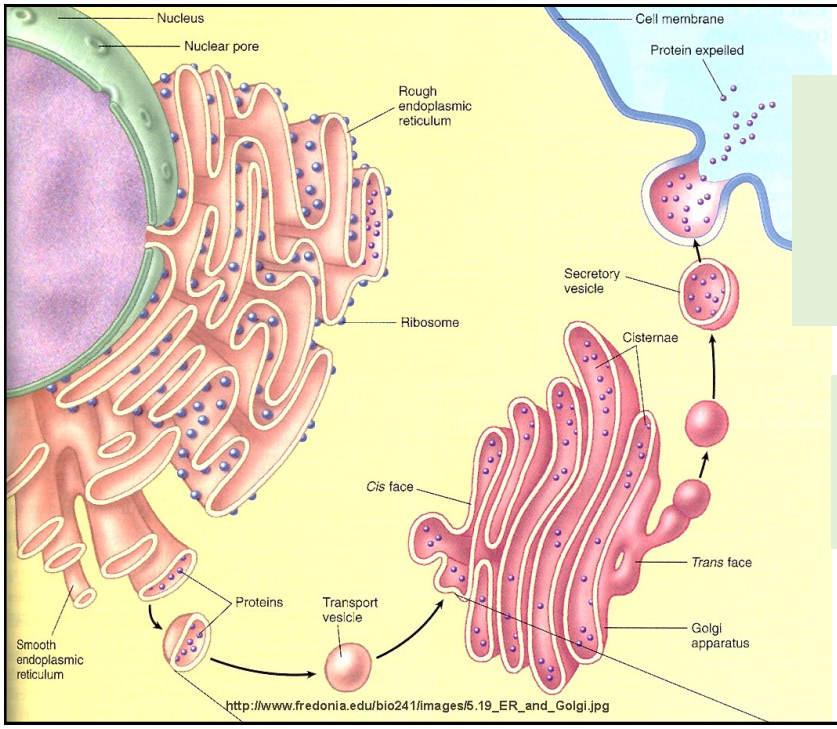


Protein Targeting and Degradation

Dr. Sukanya Luang

Objective

- Describe the important process of protein targeting to the mitochondria, endoplasmic reticulum, and nucleus
- Describe the defect of protein targeting and protein degradation in some diseases

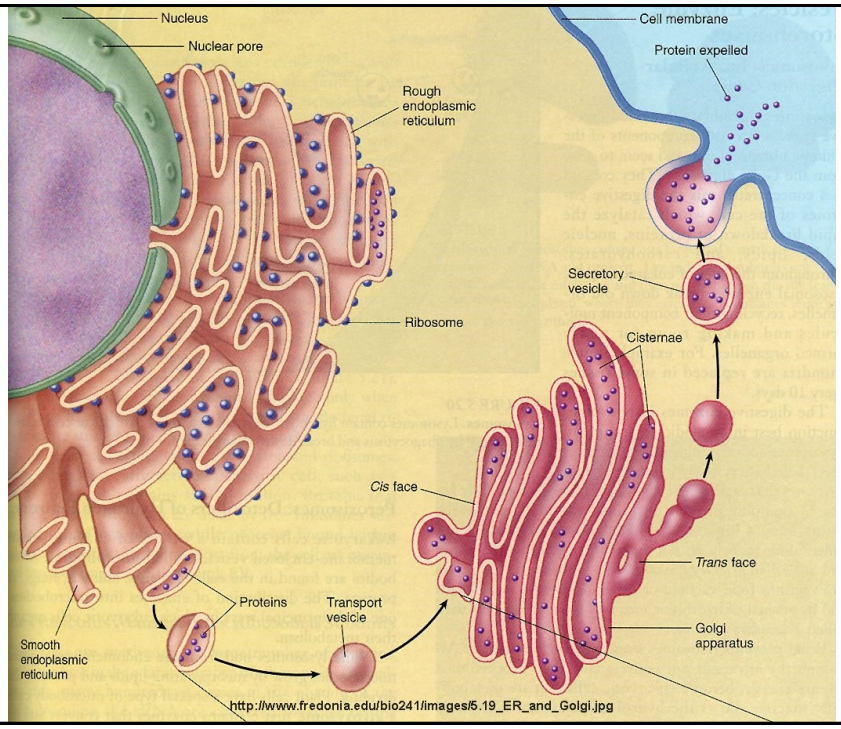
Protein Targeting

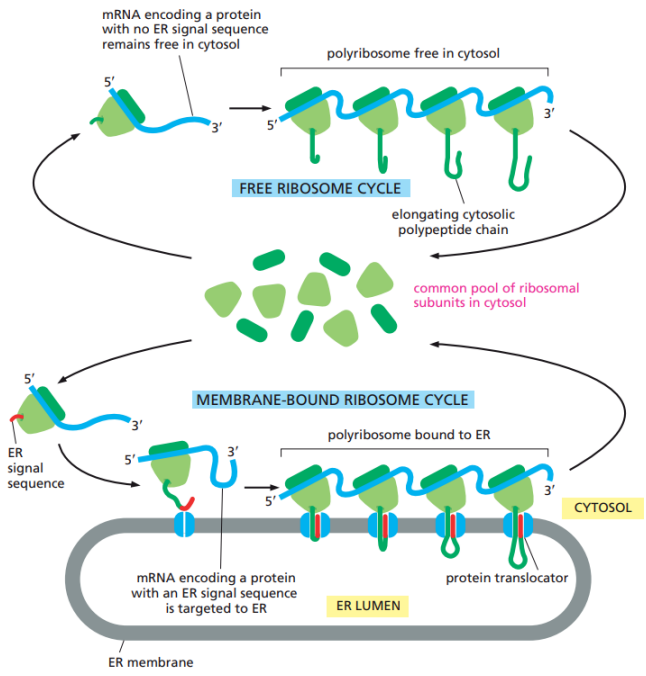
The delivery of newly synthesized protein to their proper cellular destination for function

- Receptors – plasma membrane
- DNA polymerase – nucleus
- Catalase – peroxisomes
- Insulin – secrete outside the cell

Protein Targeting

- Protein targeting is necessary for proteins that are destined to work outside the cytoplasm.
- The delivery process is carried out based on information contained in the protein itself.
- Correct sorting is crucial for the cell: errors can lead to diseases.





Protein translation

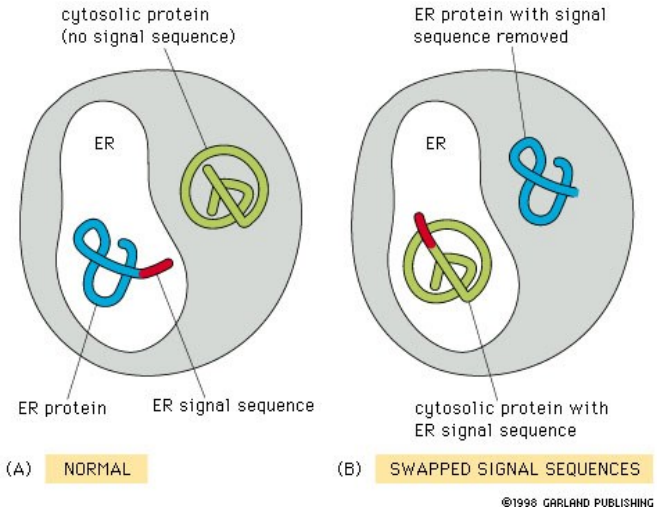
How are proteins directed to the correct cell address?

- Signal peptide: 15–60 residues long
- Often present at the N-terminus of polypeptide chain

Function of signal	Example of signal sequence
Import into ER	$^{+}H_3$N-Met-Met-Ser-Phe-Val-Ser-Lue-Lue-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Lue-COO^{-}
Import into mitochondria	$^{+}H_3$N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Lue-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids
Negatively charged amino acids
An extended block of hydrophobic amino acids

Signal peptide



The two basic targeting pathways

Post-translational targeting:

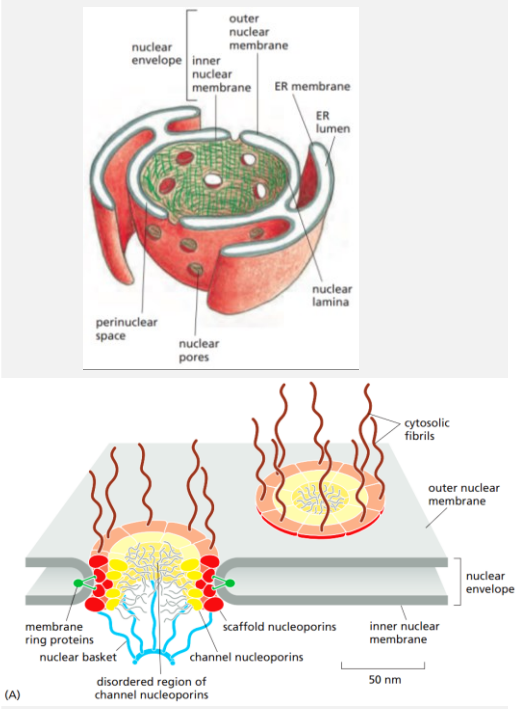
- Nucleus
- Mitochondria
- Chloroplasts
- Peroxisomes

Co-translational targeting (Secretory pathway):

- ER
- Golgi apparatus
- Lysosomes
- Plasma membranes
- Secreted protein

Post-translational targeting

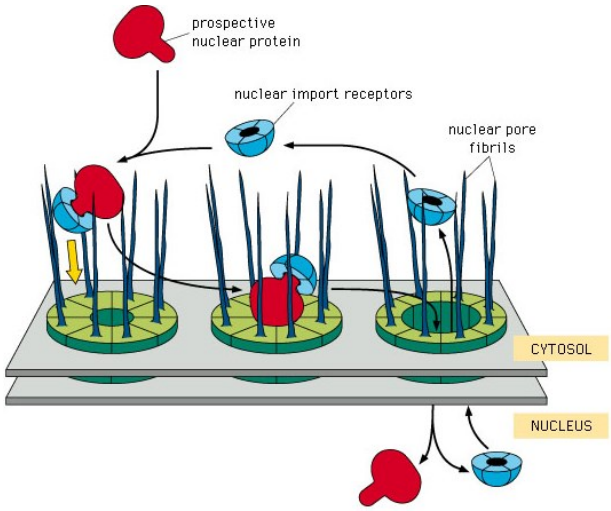
- This pathway occurs after the process of protein translation.
- Even though most proteins are co-translationally translocated, some are *translated in the cytosol and later transported to their destination.*
- These proteins go to a mitochondrion, a chloroplast, or peroxisome.



Protein import into nucleus (Gated transport)

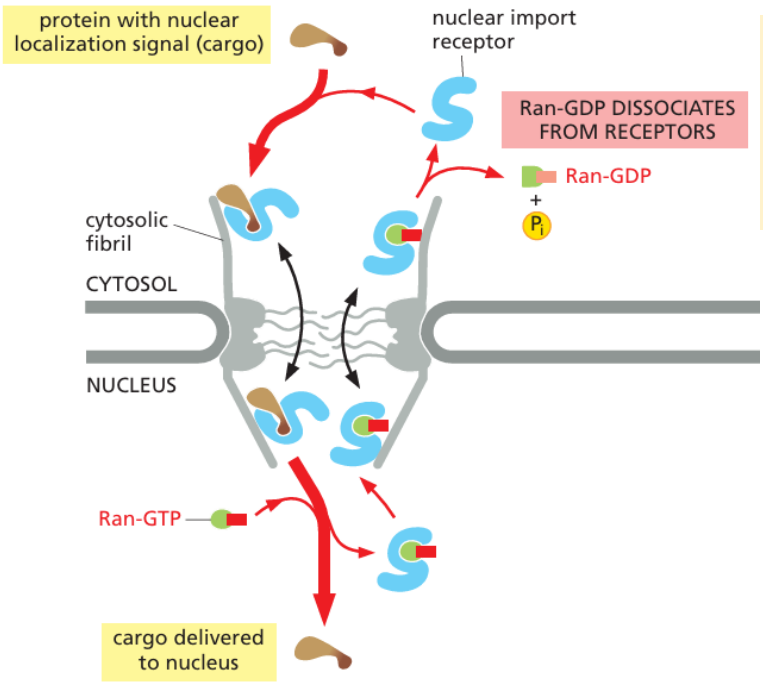
- Nuclear pore complexes (NPCs) are protein-based channel in the nuclear envelope.
- Regulate the movement of molecules between nucleus and cytoplasm
- Some molecules are simply small enough to pass through the pores (carbohydrates, lipids, ribosome)
- Large molecules must be recognized by different signal sequences (nuclear localization signal :NLS) before being allowed to diffuse through the nuclear pores (proteins, mRNA, tRNA)

Protein import into nucleus (Gated transport)



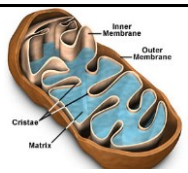
- Protein with nuclear localization signal (NLS) bind to the nuclear import receptors (NIR)
- NLS-NIR bind to the nuclear pore
- Translocation of the NIR-NLS-protein complex into the nucleus
- NIR proteins dissociate from the NLS-protein by Ran GTP interact with NIR
- NIR-Ran GTP return to cytosol

https://www.zoology.ubc.ca/~berger/B200sample/unit_8_protein_processing/protein_targetting/lect26.htm

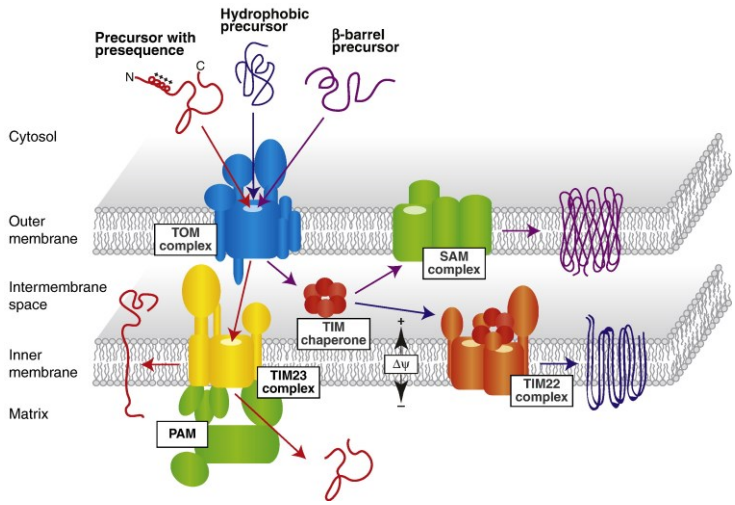


Protein import into nucleus

(Gated transport)



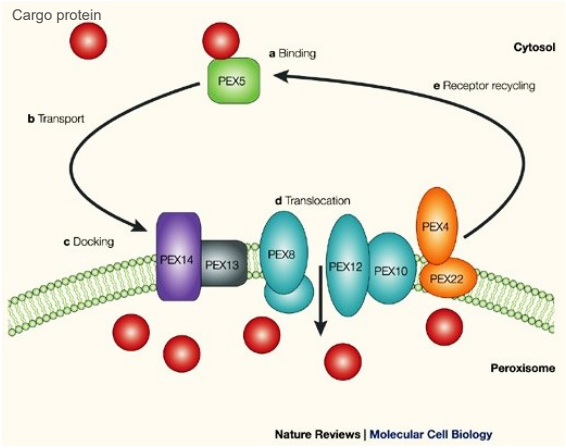
Protein import into mitochondria (Protein translocation)



- TOM: Translocase of the outer mitochondrial membrane
- TIM: Translocase of the inner mitochondrial membrane
- SAM: Sorting and assembly machinery
- Chaperone: proteins that assist the conformational folding or unfolding of large proteins or macromolecular protein complexes.
- Unfold protein => fold protein

Gebert, N., et al., (2011) Biochimica et Biophysica Acta (BBA)-Biomembranes, pp.1002-1011.

Protein targeting to peroxisome (Protein translocation)



PEX, peroxin

- Peroxisome contains the reducing enzymes (catalases, oxidases, etc.)
- Peroxisomal translocation signal
  - PTS1 (-SKL at C-terminus, very conserved)
  - PTS2 (-R/KLX$_5$Q2HL at N-terminus, just few proteins)
- Proteins (Cargo) are synthesized and fold in cytosol

Gould and Collins, 2002, Nature Review Molecular Cell Biology

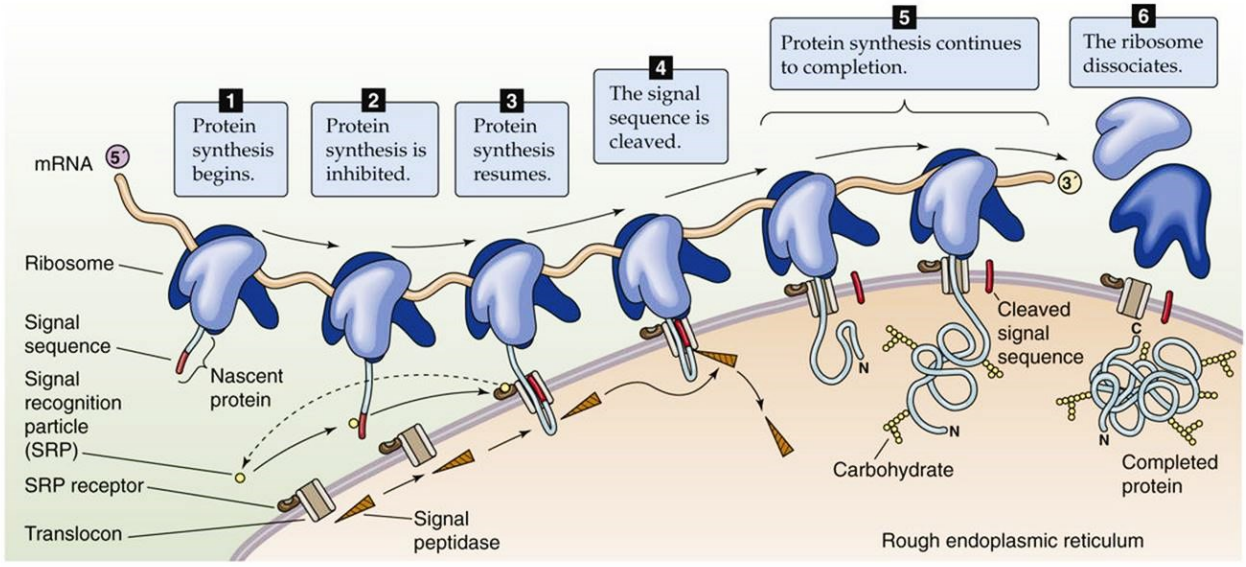
Co-translational targeting (Secretory pathway)

- This pathway occurs during translation which is not completed fully.
- Synthesized protein is transferred to an SRP receptor on the ER
- The nascent protein is inserted into the translocation complex.
- Two kinds of proteins are transferred from the cytosol to the ER:

(1) Water-soluble proteins are completely translocated across the ER membrane and are released into the ER lumen

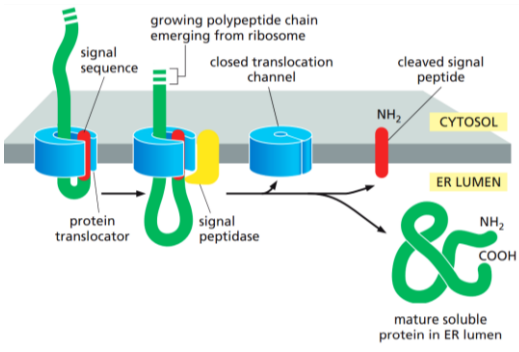
(2) Prospective transmembrane proteins are only partly translocated across the ER membrane and become embedded in it.

Co-translational targeting (Secretory pathway)

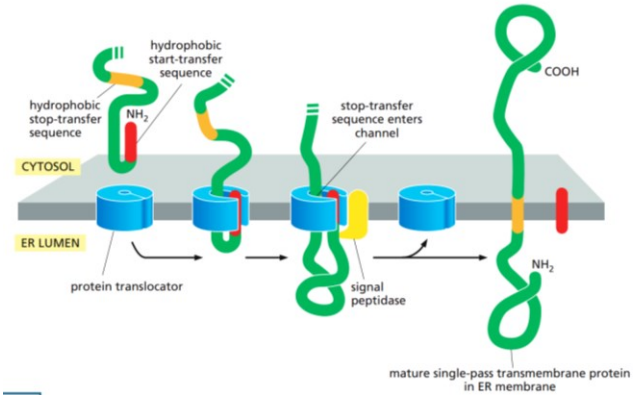


https://www.weareeaton.com/the-d-proteins-essential-proteins-for-cell-membranes/

A soluble protein crosses the ER membrane and enters the ER lumen.

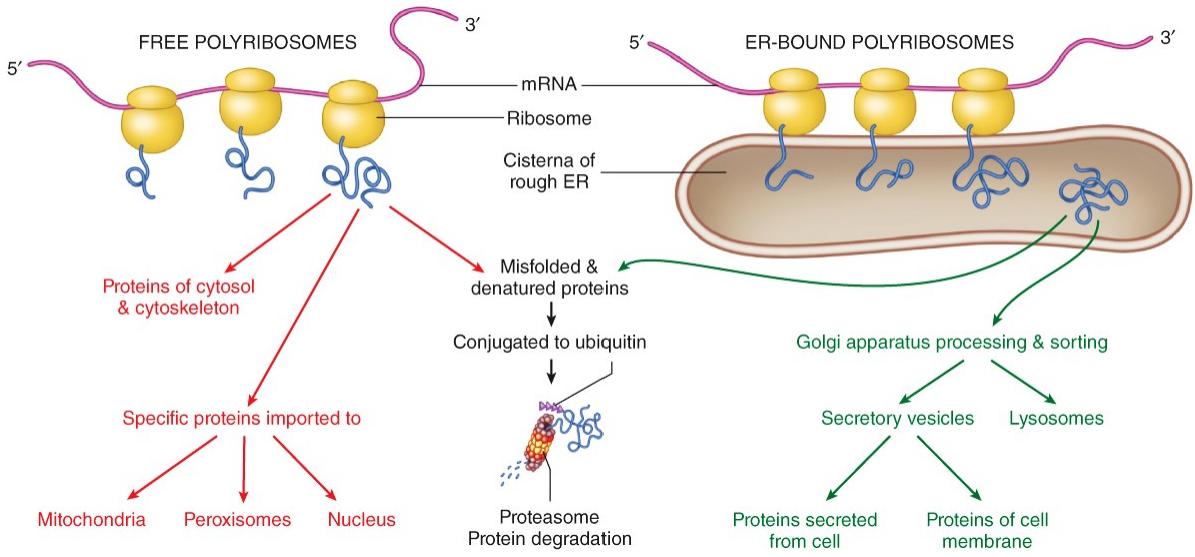


A single-pass transmembrane protein is retained in the lipid bilayer.



Alberts, Bruce. Essential Cell Biology, 4th edition

The two basic targeting pathways



Compartmental translocation of proteins

1) Gated transport (via nuclear pore complex in the nuclear envelope)

Proteins and RNA molecules move between the cytosol and the nucleus

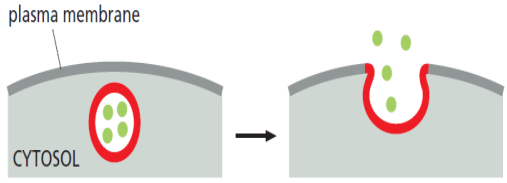
2) Protein translocation

Transmembrane *protein translocators* directly transport specific proteins across a membrane from the cytosol to the target location (mitochondria, peroxisomes, chloroplast)

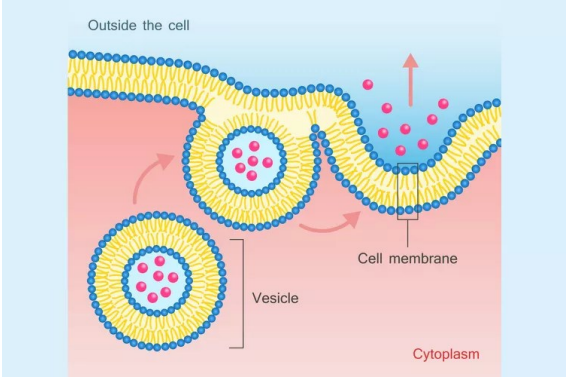
3) Vesicular transport

Vesicular transport

Exocytosis



https://www.thoughtco.com/what-is-exocytosis-4114427

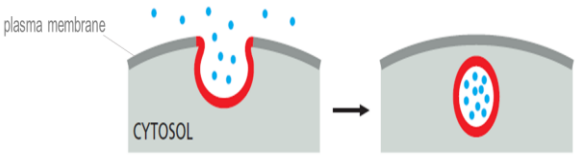


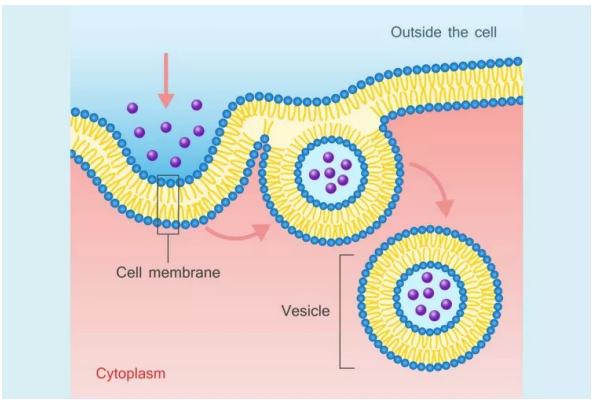
https://www.thoughtco.com/what-is-exocytosis-4114427

The fusion of secretory vesicles with the plasma membrane and results in the discharge of vesicle content into the extracellular space and the incorporation of new proteins and lipids into the plasma membrane.

Vesicular transport

Endocytosis

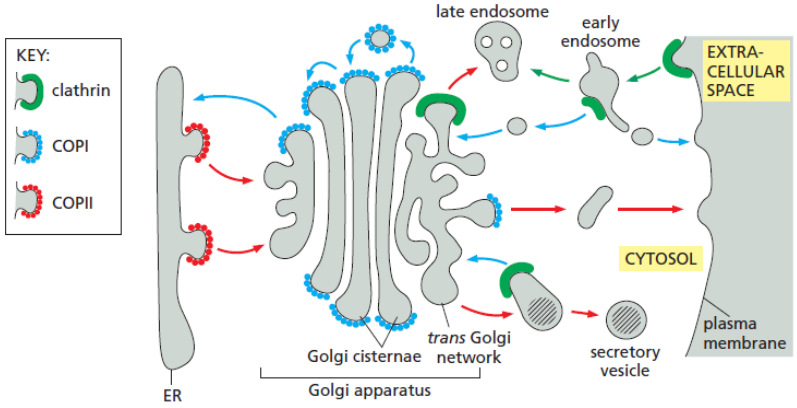




https://www.thoughtco.com/what-is-endocytosis-4163670

The material to be internalized is surrounded by an area of cell membrane, which then buds off inside the cell to form a vesicle containing the ingested material.

Vesicular transport



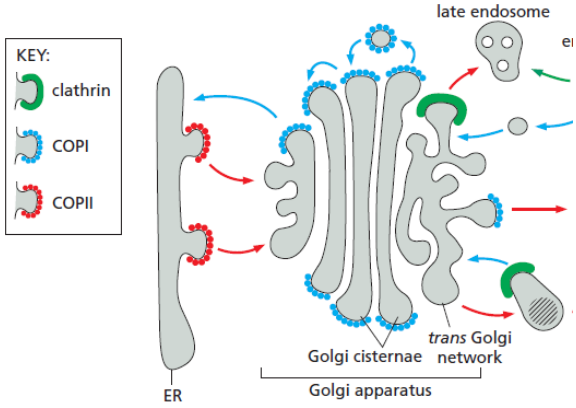
Three main vesicular frameworks found across eukaryotic life

Coat proteins:

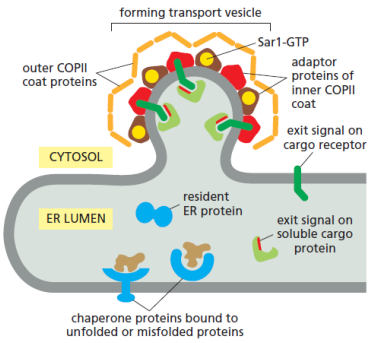
- Clathrin (plasma mb to lysosome)
- COPI (Golgi apparatus to ER)
- COPII (ER to Golgi apparatus)

ER → Golgi apparatus →
- Plasma membrane protein
- Secreted protein
- Golgi or ER protein

Transport the protein from ER to Golgi apparatus



- Materials are packaged into COPII-coated transport vesicles
- Vesicles bud from the ER exit site (membrane lacks bound ribosomes) and transport to Golgi apparatus



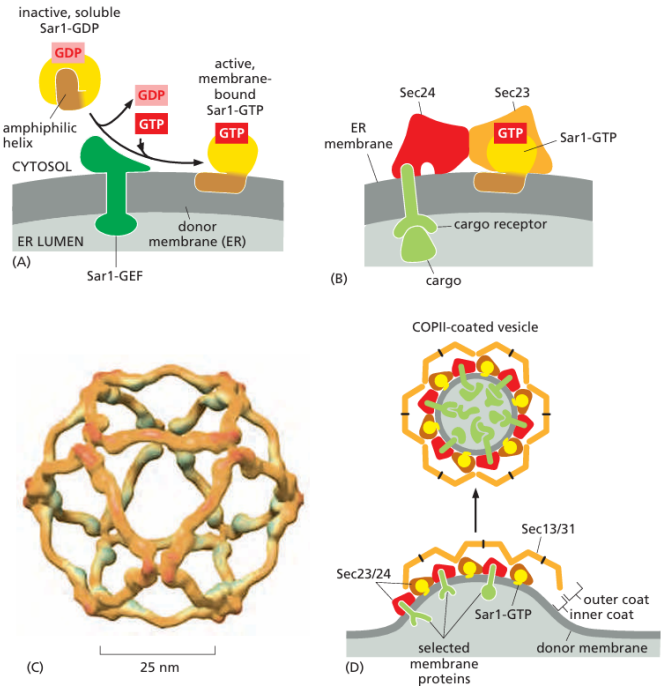
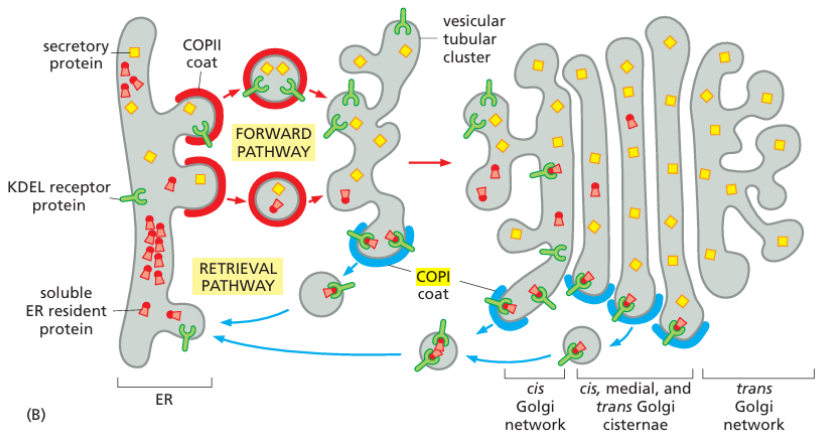


Figure 13–14 Formation of a COPII-coated vesicle. (A) Inactive, soluble Sar1-GDP binds to a Sar1-GEF in the ER membrane, causing the Sar1 to release its GDP and bind GTP. A GTP-triggered conformational change in Sar1 exposes an amphiphilic helix, which inserts into the cytoplasmic leaflet of the ER membrane, initiating membrane bending (which is not shown). (B) GTP-bound Sar1 binds to a complex of two COPII adaptor coat proteins, called Sec23 and Sec24, which form the inner coat. Sec24 has several different binding sites for the cytosolic tails of cargo receptors. The entire surface of the complex that attaches to the membrane is gently curved, matching the diameter of COPII-coated vesicles. (C) A complex of two additional COPII coat proteins, called Sec13 and Sec 31, forms the outer shell of the coat. Like clathrin, they can assemble on their own into symmetrical cages with appropriate dimensions to enclose a COPII-coated vesicle. (D) Membrane-bound, active Sar1-GTP recruits COPII adaptor proteins to the membrane. They select certain transmembrane proteins and cause the membrane to deform. The adaptor proteins then recruit the outer coat proteins which help form a bud. A subsequent membrane fusion event pinches off the coated vesicle. Other coated vesicles are thought to form in a similar way. (C, modified from S.M. Stagg et al., *Nature* 439:234–238, 2006. With permission from Macmillan Publishers Ltd.)



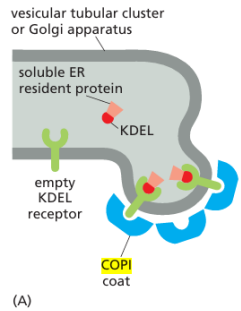
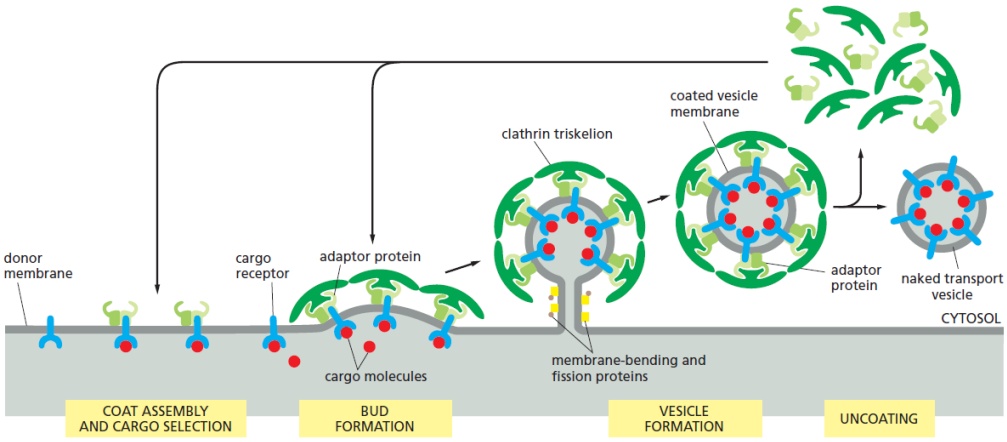
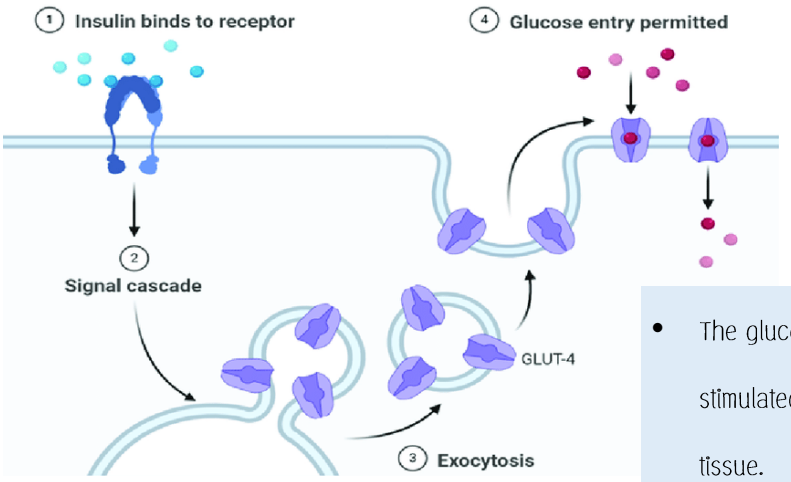


Figure 13–25 Retrieval of soluble ER resident proteins. ER resident proteins that escape from the ER are returned by vesicle transport. (A) The KDEL receptor present in both vesicular tubular clusters and the Golgi apparatus captures the soluble ER resident proteins and carries them in COPI-coated transport vesicles back to the ER. (Recall that the COPI-coated vesicles shed their coats as soon as they are formed.) Upon binding its ligands in the tubular cluster or Golgi, the KDEL receptor may change conformation, so as to facilitate its recruitment into budding COPI-coated vesicles. (B) The retrieval of ER proteins begins in vesicular tubular clusters and continues from later parts of the Golgi apparatus. In the environment of the ER, the ER resident proteins dissociate from the KDEL receptor, which is then returned to the Golgi apparatus for reuse. We discuss the different compartments of the Golgi apparatus shortly.

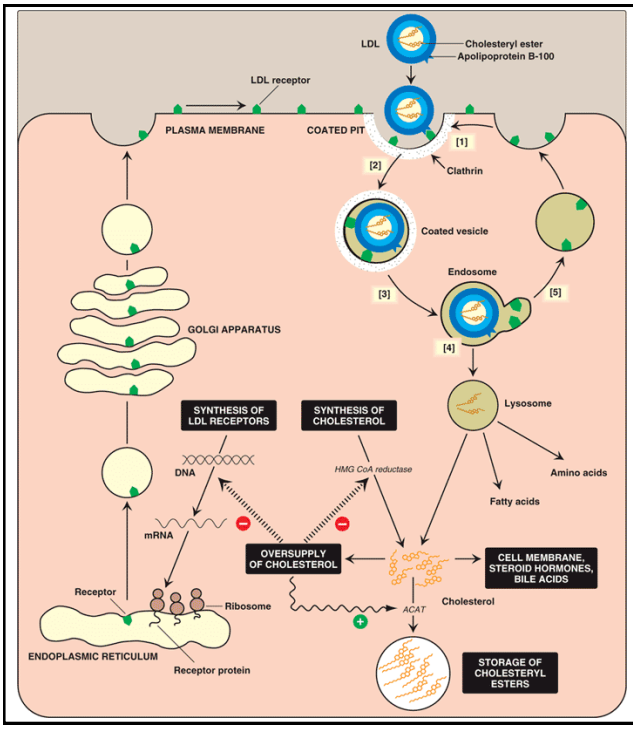
The assembly and disassembly of a clathrin coat





- The glucose transporter GLUT4 facilitates insulin-stimulated glucose uptake into muscle and adipose tissue.
- Defects in glucose uptake represent an early step in the development of type 2 diabetes mellitus

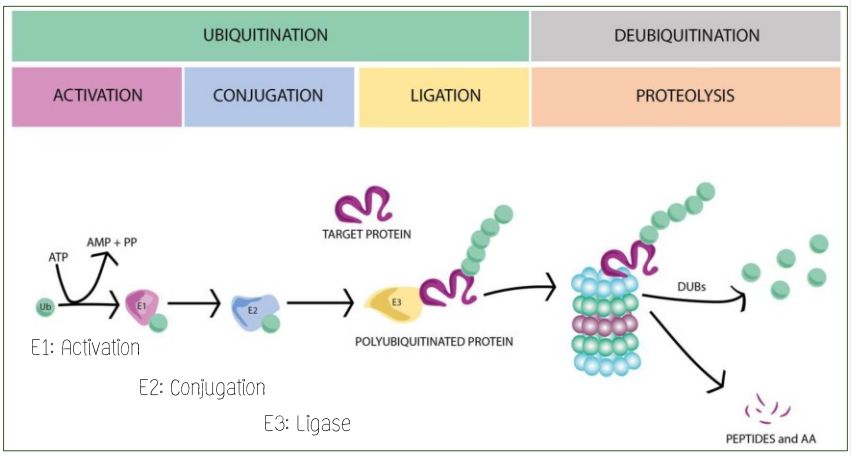
Ashish A, et al. (2020) J Diabetes Metab Disord Control. 58-61.



The receptor-mediated endocytosis of lipoprotein (low-density lipoprotein: LDL)

https://alchetron.com/LDL-receptor

Protein Degradation: UBIQUITIN-PROTEASOME SYSTEM

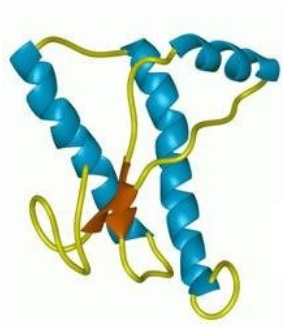


- Multiprotein complex in cytoplasm
- Protein to be degraded 1st tagged with ubiquitin
- Ubiquitin will be attached to Lys of protein
- Protease inside the proteasome cleave the polypeptide into small peptides

Bachiller S., et al., Int. J. Mol. Sci 2020, 21(17), 6429

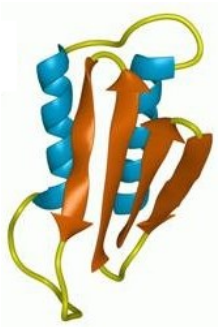
Prion protein

Normal prion



Normal cellular protein (PrP C) has an α-helical internal structure

Diseased prion



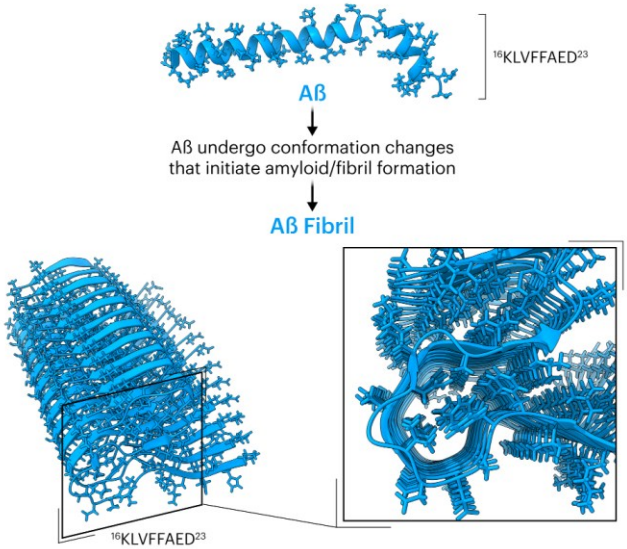
Abnormal protein has anti-parallel β-pleated strands

In the central nervous system high PrP C expression is found in the synaptic membranes of neurons, and the protein also is expressed in astrocytes.

These misfolded prion proteins are associated with several neurodegenerative diseases in humans and animals such as mad cow disease.

https://microbewiki.kenyon.edu

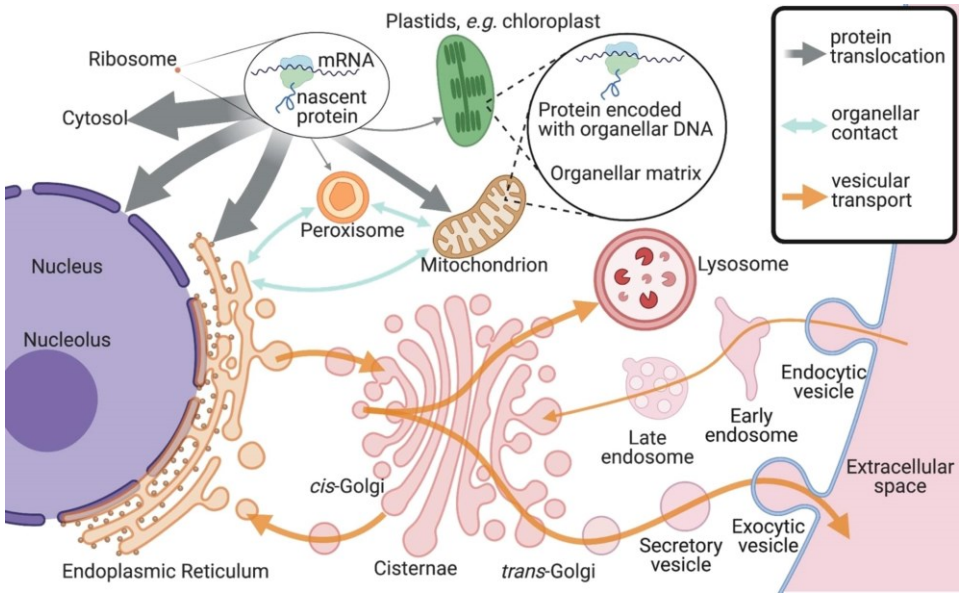
Amyloid protein



https://www.news-medical.net/whitepaper/20230725/How-can-we-monitor-Alzheimers-Progression-using-Pathological-Variant-Detection-in-beta-amyloid-Proteins.aspx

A characteristic of Alzheimer's is the accumulation of mutated beta-amyloid proteins which form plaques around neurons and disturb normal cell function in the brain.

SUMMARY



References