WHAT IS ENDO-MEMBRANE SYSTEM?

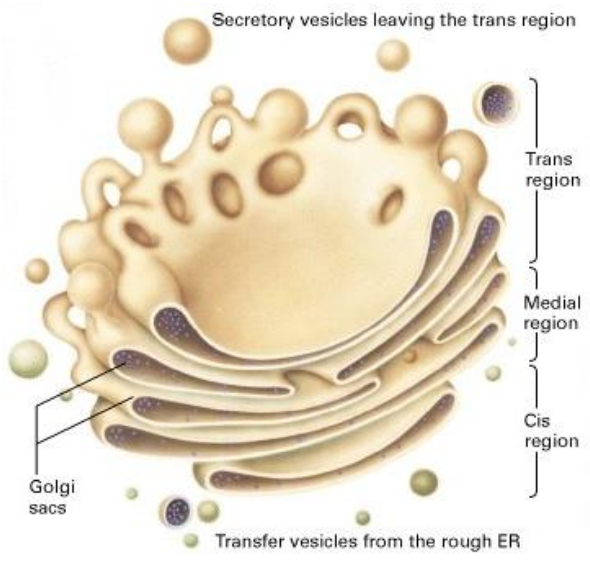
The organelles of the **endo-membrane system** are part of a dynamic, integrated network in which materials are shuttled back and forth from one part of the cell to another. Commonly, materials are shuttled between organelles—from the **Golgi complex** to the **plasma membrane in small, membrane-bounded transport vesicles that bud from a donor membrane compartment**. **Transport vesicles move through the cytoplasm in a directed manner, often pulled by motor proteins that operate on tracks made of microtubules and microfilaments of the cytoskeleton.** When they reach their destination, the vesicles fuse with the membrane of the acceptor compartment, which receives the vesicle's soluble cargo as well as its membranous wrapper. Repeated cycles of budding and fusion shuttle a diverse array of materials along numerous pathways that traverse the cell.

THE GOLGI APPARATUS

1. The Golgi apparatus, also known as the Golgi complex or Golgi body, is an organelle found in most eukaryotic cells.
2. Part of the cellular endomembrane system, the Golgi apparatus packages proteins inside the cell before they are sent to their destination; it is particularly important in the processing of proteins for secretion.
3. The Golgi apparatus is integral in modifying, sorting, and packaging these macromolecules for cell secretion (exocytosis) or use within the cell.
4. It primarily modifies proteins delivered from the rER but is also involved in the transport of lipids around the cell, and the creation of lysosomes.
5. The Golgi plays an important role in the synthesis of proteoglycans, which are molecules present in the extracellular matrix of animals.
6. It is also a major site of carbohydrate synthesis. In plant cells, the Golgi apparatus serves as the site at which the complex polysaccharides of the cell wall are synthesized

STRUCTURE

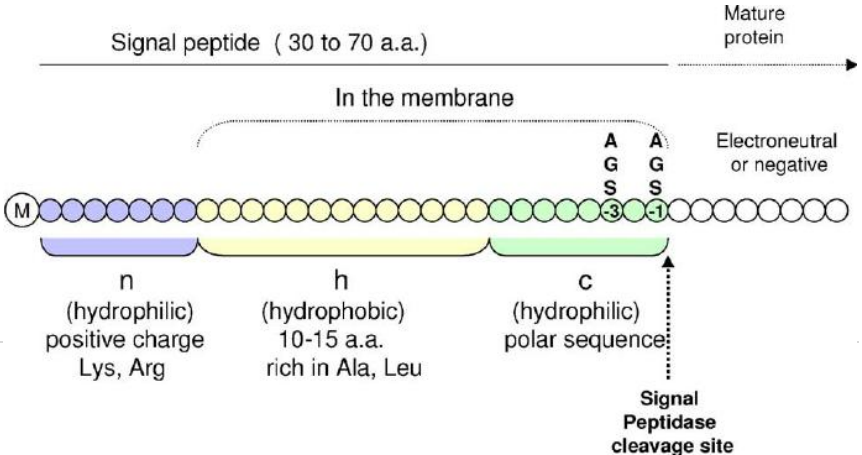
1. The Golgi is composed of stacks of membrane-bound structures known as cisternae and associated vesicles.
2. Each cisterna comprises a flat, membrane enclosed disc that includes special Golgi enzymes which modify or help modify cargo proteins that travel through it.
3. The cisternae stack has four functional regions: the *cis*-Golgi network, medial-Golgi, endo-Golgi, and *trans*-Golgi network.
4. Vesicles from the ER are transported to the ER-Golgi intermediate compartment and then enter the Golgi apparatus at the *cis* Golgi network.
5. From the *cis* Golgi network, vesicles progress to the medial and trans compartments of the Golgi stack, where most metabolic activities of the Golgi apparatus take place.
6. The modified proteins, lipids and polysaccharides then move to the *trans* Golgi network, which acts as a sorting and distribution center directing to endosomes, lysosomes, the plasma membrane and the cell exterior.
7. Each region in the cisternae stack contains different enzymes which selectively modify the contents depending on where they reside.
8. The cisternae also carry structural proteins important for their maintenance as flattened membranes which stack upon each other.



to

WHAT IS SIGNAL PEPTIDE?

A **signal peptide** (or s*ignal sequence, targeting signal, localization signal, localization*



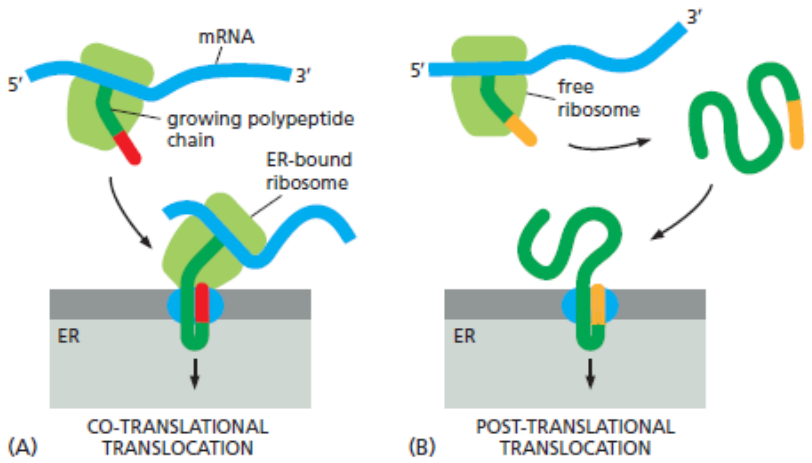
sequence, transit peptide, leader sequence or leader peptide) is a stretch of hydrophobic amino acids, peptide located at the N-terminus of the majority of newly synthesized proteins that are destined towards the secretory pathway.

The **signal peptide** at their N-terminus that directs the emerging polypeptide and ribosome to the ER membrane. The polypeptide moves into the cisternal space of the ER through a protein-lined, aqueous channel in the ER membrane.
It was proposed that the polypeptide moves through the membrane as it is being synthesized, that is a co-translational occurrence.

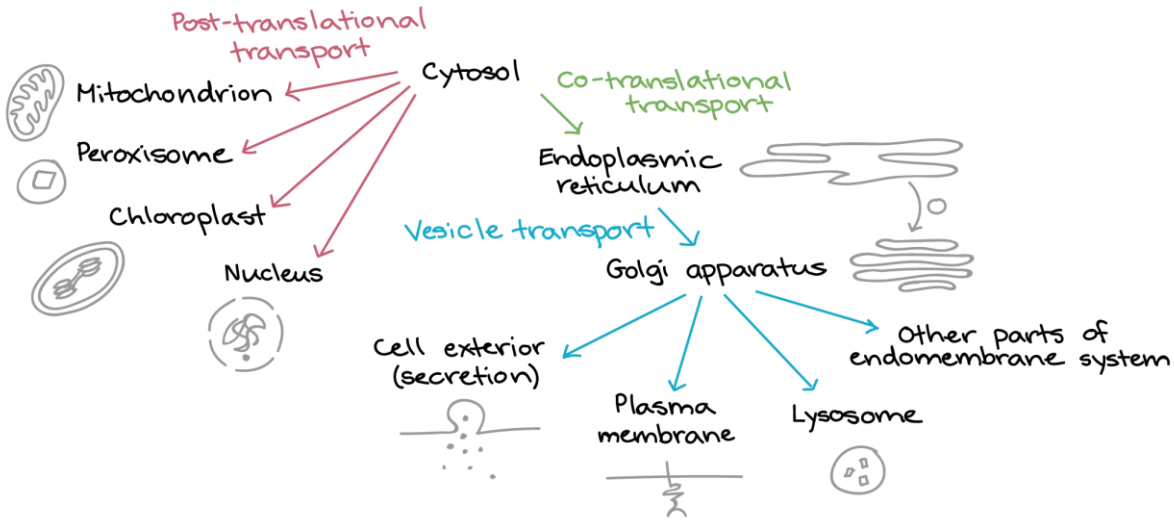
PROTEIN TARGETING: In mammalian cells, most proteins are transferred into the endoplasmic reticulum while they are being translated on membrane-bound ribosomes. In all eukaryotes, there are two pathways by which proteins can be translocated into the endoplasmic reticulum i.e., a ***co-translational pathway*** and a ***post-translational pathway***.

In the ***co-translational pathway***, transport occurs while the polypeptide chain is being synthesized on a membrane-bound ribosome, whereas in the ***post-translational pathway***, the polypeptide chain is completed in the cytoplasm before being transported into the endoplasmic reticulum. In prokaryotes, ribosomes don't seem to be tightly bound to the membrane and most proteins may be transported post-translationally. Both translocation modes require that polypeptides destined for translocation be specifically targeted to the membrane.

In the ***co-translational pathway***, first ribosomes are associated with the endoplasmic reticulum. Proteins which are destined for secretion are targeted to the endoplasmic reticulum by a ***signal sequence*** at the amino terminus of the growing polypeptide chain. These signal sequences are short stretches of hydrophobic amino acids which are usually cleaved from the polypeptide chain during its transfer into the lumen of the endoplasmic reticulum.

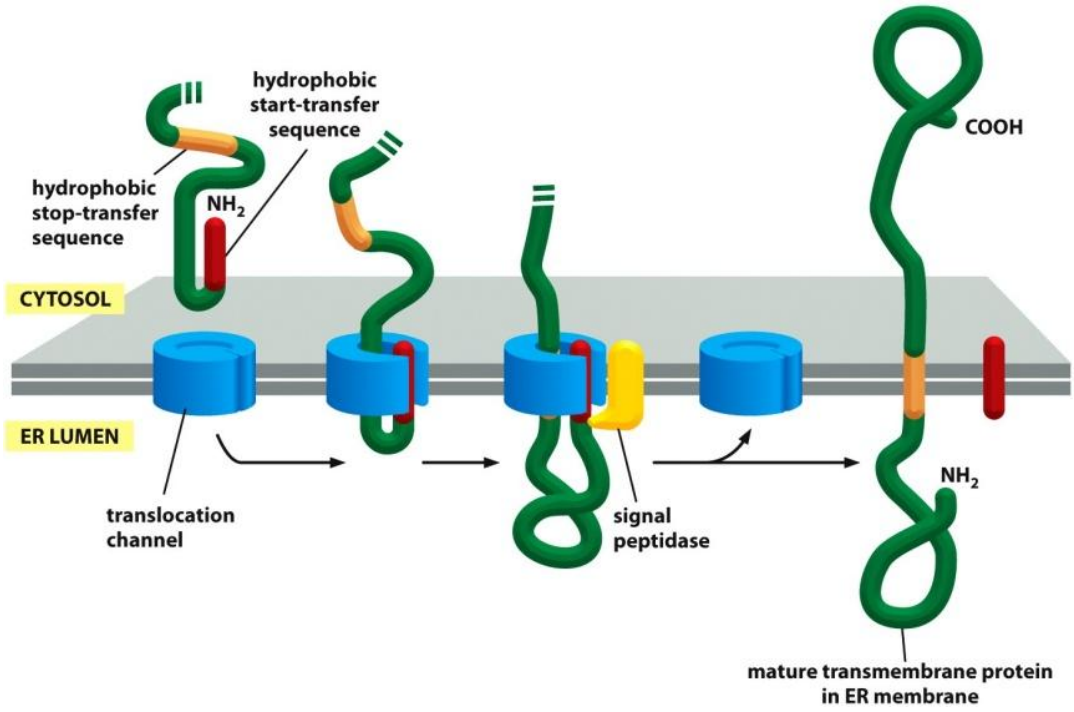


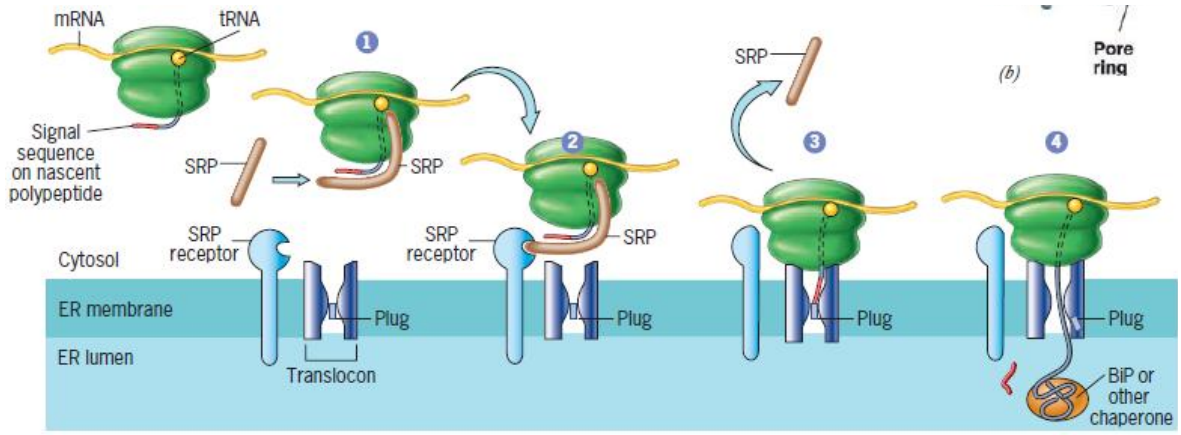
Co-translational and post-translational protein translocation.
(A) Ribosomes bind to the ER membrane during co-translational translocation. (B) By contrast, cytosolic ribosomes complete the synthesis of a protein and release it prior to post-translational translocation. In both cases, the protein is directed to the ER by an ER signal sequence (*red* and *orange*).



MECHANISM OF PROTEIN TARGETING AT ER

1. The signal sequences cover about 20 amino acids, including a stretch of hydrophobic residues, usually at the amino terminus of the polypeptide chain.
2. As soon as the signal sequences of the growing polypeptide chain emerge from the ribosome, they are recognized and bound by a **signal recognition particle (SRP)** consisting of six polypeptides and a small **cytoplasmic RNA (srp RNA**).
3. Then the complex containing the growing polypeptide chain, ribosome, and SRP is specifically targeted to the endoplasmic reticulum membrane by an interaction with a membrane-bound receptor, the SRP receptor or docking protein.
4. In the next step, the SRP is released from both the ribosome and the signal sequence, where GTP plays a key role. The ribosome then binds to a protein translocation complex in the membrane of the endoplasmic reticulum, and the signal sequence is inserted into a membrane channel or translocon.
5. The translocons are complexes of three transmembrane proteins, known as Sec61 proteins. Transfer of the ribosome from the SRP to the translocon allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the membrane of the endoplasmic reticulum as translation proceeds.
6. As translocation proceeds, the signal sequence is cleaved by the signal peptidase and the polypeptide is released into the lumen of the endoplasmic reticulum.
7. Finally, GTP hydrolysis leads to the dissociation of the SRP from its receptor, and a new targeting cycle can begin.
8. The actual transfer of the polypeptide through the membrane does not require the SRP or its receptor and commences only after their disengagement. Two basic functions are done by the SRP, where first it targets the polypeptide chain to the Endoplasmic reticulum membrane by interacting both with the signal sequence and with the translocation apparatus and secondly it keeps the bound signal sequence segregated from the rest of the polypeptide chain and thereby prevents aberrant, premature folding.
9. Some proteins in mammals and many proteins in yeast are transported through post-translational pathway. These proteins are synthesized on free cytosolic ribosomes and these proteins do not require a signal recognition particle (SRP) for their transport. Their signal sequences are recognized by distinct receptor proteins associated with the translocon in the endoplasmic reticulum membrane. The polypeptide chains are remained in an unfolded conformation by the cytosolic Hsp70 chaperones.





PROTEINS INSERTED INTO THE MEMBRANE OF ER

1. The proteins which are to be incorporated into the plasma membrane or in the membrane of an organelle are initially inserted into the ER membrane instead of into its lumen.
2. The proteins from the ER membrane travel the same pathway as secretory proteins. But along this pathway these proteins are transported as membrane components.
3. Integral membrane proteins are embedded in the membrane by the hydrophobic regions that span the phospholipid bilayer.
4. The membrane spanning portions of the integral membrane proteins are usually α-helical regions consisting of 20-25 hydrophobic amino acids.
5. Hydrogen bonding between the peptide bonds gets maximized by the formation of an α-helix and the hydrophobic amino acid side chains interact with the fatty acid tails of the phospholipids in the bilayer.
6. After insertion into the ER membrane the transmembrane proteins are oriented with their carboxy-termini exposed to the cytosol.
7. Before insertion of these transmembrane proteins into the ER membrane, the normal amino-terminal signal sequence of these proteins is cleaved by ***signal peptidase*** during translocation of the polypeptide chain across the ER membrane through the translocon.
8. Proteins are translocated across the endoplasmic reticulum membrane as unfolded polypeptide chains during the progression of translation.
9. These polypeptides fold into their three-dimensional conformation within the endoplasmic reticulum by the help of molecular chaperones.
10. Those proteins that cannot be correctly folded are diverted from the secretory pathway and marked for degradation.

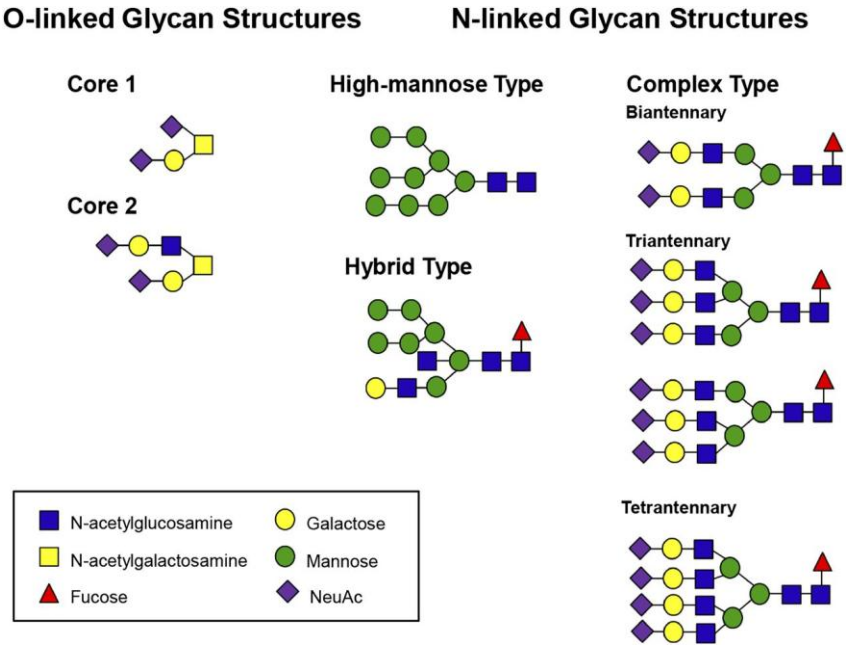
TRANSPORT OF PROTEINS FROM ER

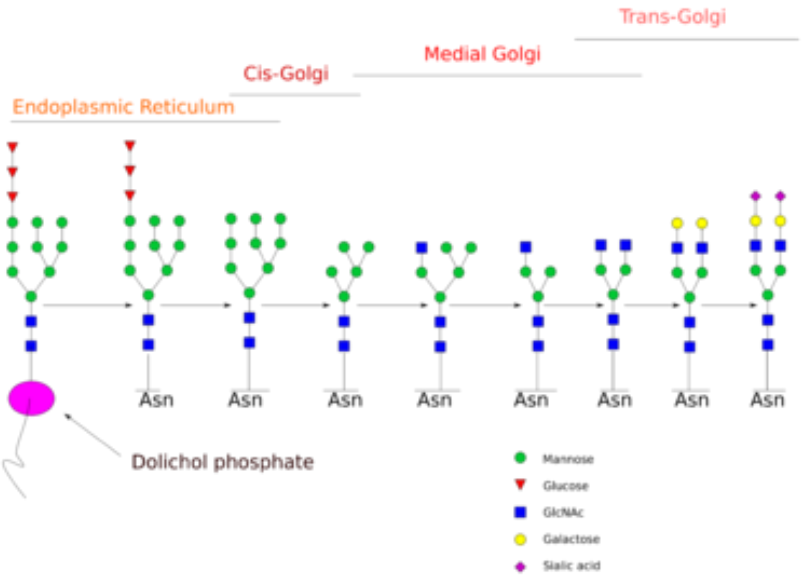
1. **Transport of proteins from ER to Golgi bodies occurs through vesicles along the secretory pathway**.
2. Vesicles are very small, membrane-enclosed sacs that carry cargo within the cell.
3. Vesicles typically transport large molecules that cannot pass through a membrane on their own or by using other transport molecules.
4. Vesicles gather their cargo in a process called budding as they move with their cargo through the cell and then deliver their cargo by fusing with another membrane enclosed compartment or with the cell's plasma membrane.
5. Vesicles bud-off from the ER and in the process capture the molecules within the lumen of the endoplasmic reticulum.
6. **Proteins from the lumen of one organelle carried as budding transport vesicle, released into the lumen of the recipient organelle following vesicle fusion.**
7. Vesicles that bud from the transitional ER, carry their cargo first to the ER-Golgi intermediate compartment and then to the Golgi apparatus.
8. **From Golgi apparatus, vesicular transport occurs to lysosomes or the plasma membrane.**
9. The membrane proteins also undergo the same pathway and their topological orientation is maintained as they travel from one membrane-bound organelle to another.

10. In the transport pathway not all the proteins are transported but some proteins are retained within the ER. Many proteins are retained in the ER lumen as a result of the presence of the targeting sequence Lys-Asp-Glu-Leu (KDEL sequence) at their carboxy terminus.
11. These signals cause resident ER proteins to be selectively retrieved from the ER-Golgi intermediate compartment or the Golgi complex and returned to the ER through a recycling pathway.
12. Proteins bearing these sequences bind to specific recycling receptors in the membranes of these compartments and then selectively transported back to the ER.

N-LINKED AND O-LINKED GLYCOSYLATION

1. **Most proteins are modified in the ER by addition of polysaccharides and the process is known as glycosylation**. **There are two types of glycosylation i.e. N-linked glycosylation and O-linked glycosylation**. **The enzymes involved in the glycosylation process resides in the lumen of the ER.**
2. Initially in glycosylation, a pre-fabricated oligosaccharide unit consisting of N-acetyl-glucosamine, mannose and glucose residues is transferred from a glycolipid which is further attached with **dolichol phosphate** (a phospholipid with an extremely long hydrophobic chain), which serves as a membrane anchor for the oligosaccharide chain.
3. The oligosaccharide chain is attached to an asparagine (N) residue through its free amino group.
4. The N-linked oligosachharides are generally undergo modification in the Golgi apparatus.





5. Subsequent to the addition of the 14 sugar oligosaccharide chain to the protein, there are a long series of modifications of the polysaccharide chain, where Some of these modifiacations take place in the ER, others in the *cis* Golgi network, other in the *cis* Golgi, others in medial Golgi and yet others in the *trans* Golgi and trans Golgi network.
6. Each of these steps is carried by a different enzyme that is localized in a particular region of the Golgi.

IN CASE OF PLASMA MEMBRANE

7. The proteins which are destined for secretion or for the plasma membrane are first modified by the removal of three additional mannose residues which is followed by the sequential addition of an N-acetylglucosamine, the removal of two more mannoses, and the addition of a fructose with two more N-acetylglucosamines. Finally there is an addition of two galactoses and three sialic acid residues is made to it.

IN CASE OF LYSOSOMES

8. The processing of N-linked oligosaccharides of lysosomal proteins differs from that of plasma membrane and secreted proteins. The lysosomal proteins are modified by mannose phosphorylation. First, there is addition of N-acetylglucosamine phosphates to specific mannose residues, and this happens probably while the protein is still in the *cis* Golgi network. After this N-acetylglucosamine group is removed, leaving mannose-6-phosphate residues on the N-linked oligosaccharide. ***The phosphorylated mannose residues are specifically recognized by a mannose-6-phosphate receptor in the* trans *Golgi network, which directs the transport of these proteins to lysosomes.***

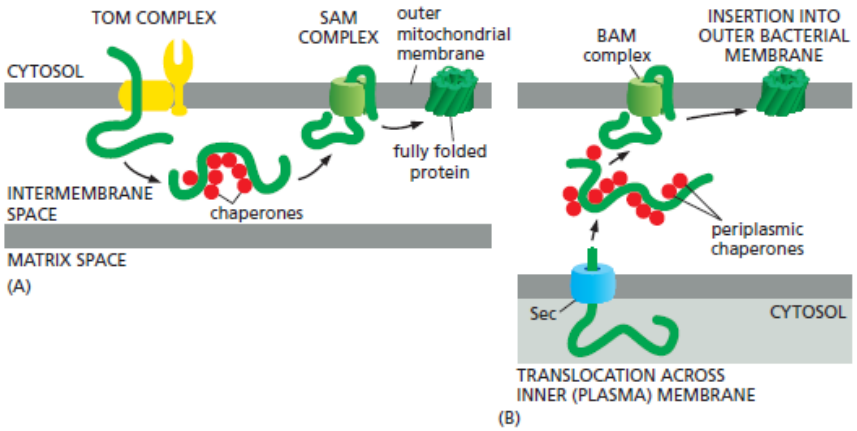
O-LINKED GLYCOSYLATION

9. Proteins can be modified by ***O-linked glycosylation***, which involves the addition of carbohydrates to the side chains of acceptor ***serine*** and ***thereonine*** residues with OH endwithin specific sequences of amino acids. These modifications take place in the Golgi apparatus by the sequential addition of single sugar residues.

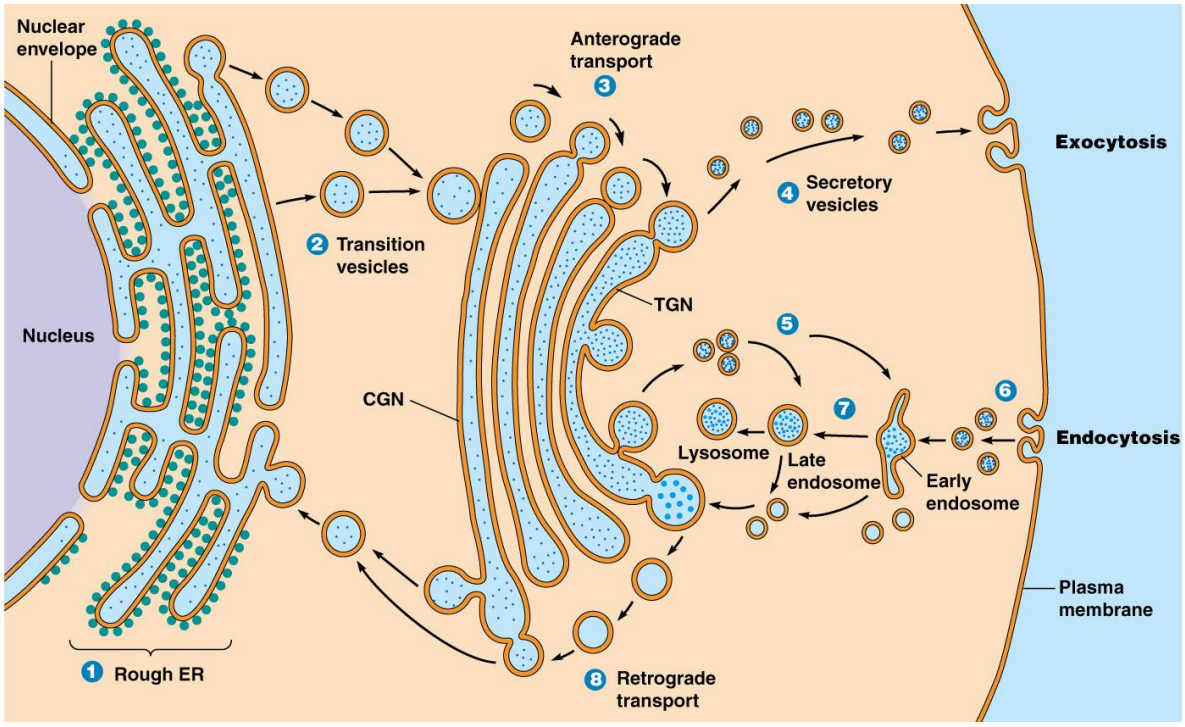
TRANSPORT OF PROTEINS FROM THE GOLGI APPARATUS

10. Proteins, lipids and polysaccharides are transported from the Golgi apparatus to their final destinations through the ***secretory pathway***. Proteins are sorted into different kinds of transport vesicles, which bud from the *trans* Golgi network and deliver their contents to the appropriate cellular locations.
11. Proteins that function within the Golgi apparatus must be retained within that organelle, rather than being transported along the secretory pathway.

12. The proteins retained within the Golgi complex are associated with the Golgi membrane. The signals responsible for retention of some proteins within the Golgi apparatus have been localized to their transmembrane domains, which prevent the protein from being packaged in the transport vesicles that leave the *trans* Golgi network.
13. Some proteins are carried from the Golgi apparatus to the plasma membrane by a constitutive secretory pathway and some proteins are transported to the cell surface by a distinct pathway of regulated secretion or are specifically targeted to other intracellular destinations, such as lysosomes in animal cells or vacuoles in yeast.
14. Proteins are sorted into the regulated secretory pathway in the *trans* Golgi network, where they are packaged into specialized secretory vesicles.
15. These immature secretory vesicles are larger than the transport vesicles, often fuses with each other while further processing their protein contents. The sorting of proteins into the regulated secretory pathway appears to involve the recognition of signal patches shared by multiple proteins that enter this pathway.
16. In the process of selective transport of proteins to lysosomes, the lumenal lysosomal proteins are marked by mannose-6-phosphates that are formed by modification of their N-linked oligosaccharides shortly after entry into the Golgi apparatus. A specific receptor in the membrane of the *trans* Golgi network then recognizes these mannose-6-phosphate residues. The resulting complexes of receptor with the lysosomal enzymes are packaged into transport vesicles destined for lysosomes.
17. The cisternal maturation model proposed for transport of proteins through the Golgi apparatus deals with the fact that the cisternae of the Golgi apparatus move by being built at the *cis* face and destroyed at the *trans* face. Vesicles from the endoplasmic reticulum fuse with each other to form a cisterna at the *cis* face, consequently this cisterna would appear to move through the Golgi stack when a new cisterna is formed at the *cis* face. This model is supported by the fact that structures larger than the transport vesicles, such as collagen rods, were observed microscopically to progress through the Golgi apparatus.
18. The vesicular transport model views the Golgi as a very stable organelle, divided into compartments in the *cis* to *trans* direction. Membrane bound carriers transport material between the endoplasmic reticulum and the different compartments of the Golgi. Experimental evidence includes the abundance of small vesicles also known as shuttle vesicles in proximity to the Golgi apparatus. To direct the vesicles, actin filaments connect packaging proteins to the membrane to ensure that they fuse with the correct compartment.
19. The cisternal maturation model and the vesicular transport model may actually work in conjuction with each other and sometimes referred to as the combined model.

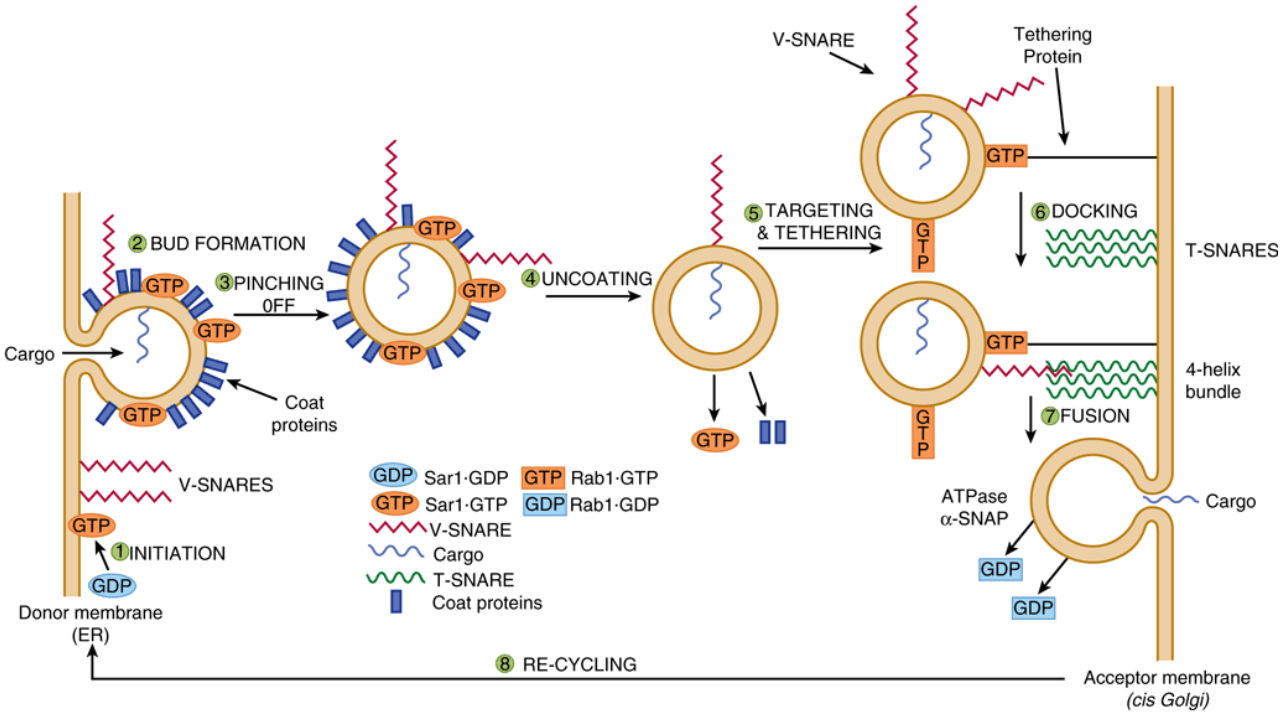


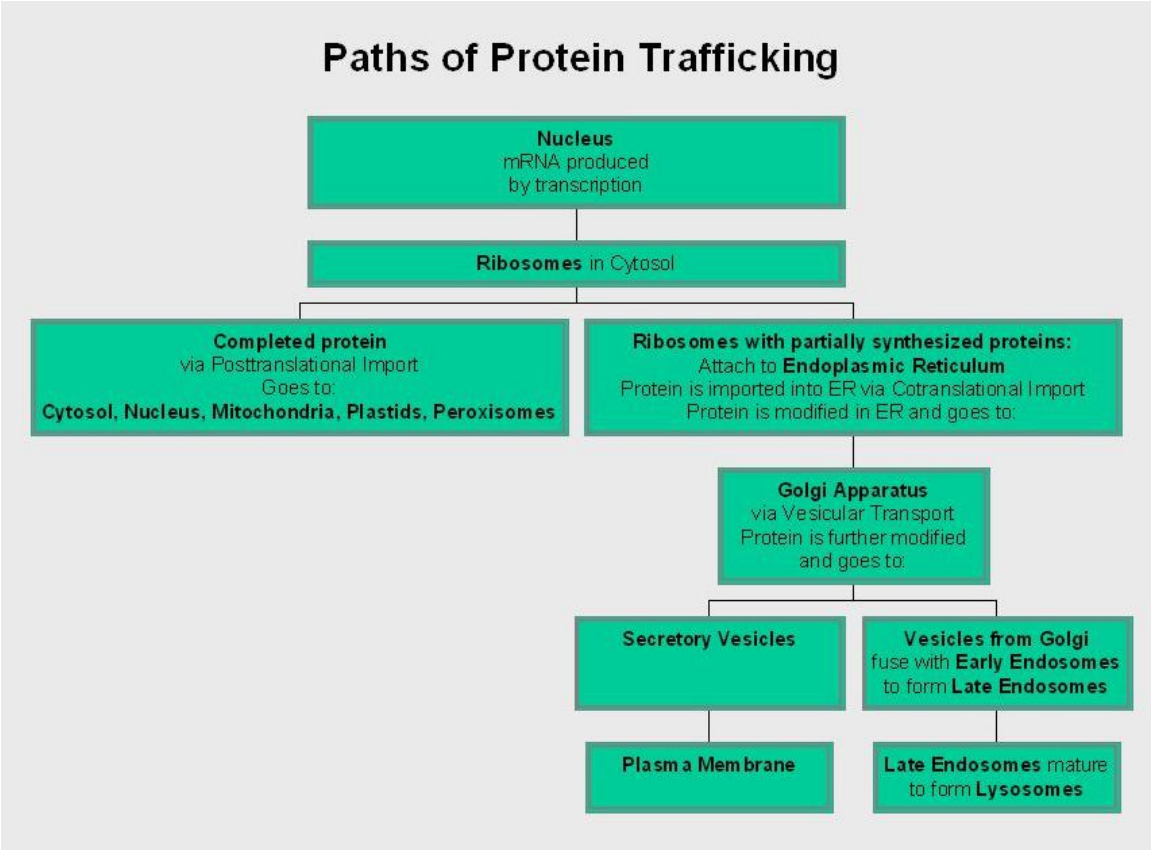
Integration of porins into the outer mitochondrial and bacterial membranes. (A) After translocation through the TOM complex in the outer mitochondrial membrane, β-barrel proteins bind to chaperones in the intermembrane space. The SAM complex then inserts the unfolded polypeptide chain into the outer membrane and helps the chain fold. (B) A structurally related BAM complex in the outer membrane of Gram-negative bacteria catalyzes β-barrel protein insertion and folding



VESICULAR TRANSPORT

1. Transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secondary pathway. The functional organization of the cell is maintained by the selectivity of the vesicular transport.
2. The vesicles that leave the rough ER are transported to the *cis* face of the Golgi apparatus, where they fuse with the Golgi membrane and empty their contents into the lumen.
3. Once inside the lumen, the molecules are modified, then sorted for transport to their next destinations.
4. Those proteins destined for areas of the cell other than either the ER or Golgi apparatus are moved towards the *trans* face, to a complex network of membranes and associated vesicles known as the trans-Golgi network (TGN).
5. This area of the Golgi is the point at which proteins are sorted and shipped to their intended destinations by their placement into one of at least three different types of vesicles, depending upon the molecular marker they carry.
6. **There are three different types of vesicles: *exocytic vesicles*** (continous vesicles), ***secretory vesicles*** (regulated vesicles) and ***lysosomal vesicles*** which take part in different types of secretion.
7. **Exocytic vesicles** contain proteins destined for extracellular release. The vesicles bud off after packaging and release their contents into the extracellular space in a process known as **constitutive secretion**. Antibody release by activated plasma B cells is an example of constitutive secretion.
8. **Secretory vesicles** after packaging bud off and are stored in the cell until a signal is given for their release. They move towards the membrane after receiving the appropriate signal and fuse with the membrane to release their contents in a process also known as **regulated secretion**.
9. **Lysosomal vesicles** contain proteins destined for the lysosome, an organelle of degradation containing many acid hydrolases, or to lysosome-like storage organelles.


V-SNARE
Tethering Protein
GTP
2 BUD FORMATION
3 PINCHING OFF
4 UNCOATING
5 TARGETING & TETHERING
6 DOCKING
T-SNARES
Cargo
Coat proteins
4-helix bundle
7 FUSION
V-SNARES
GDP Sar1·GDP
GTP Rab1·GTP
GTP Sar1·GTP
GDP Rab1·GDP
V-SNARE
Cargo
T-SNARE
Coat proteins
1 INITIATION
ATPase
α-SNAP
Cargo
Donor membrane (ER)
8 RE-CYCLING
Acceptor membrane
(cis Golgi)


Paths of Protein Trafficking
Nucleus
mRNA produced by transcription
Ribosomes in Cytosol
Completed protein
via Posttranslational Import
Goes to:
Cytosol, Nucleus, Mitochondria, Plastids, Peroxisomes
Ribosomes with partially synthesized proteins:
Attach to Endoplasmic Reticulum
Protein is imported into ER via Cotranslational Import
Protein is modified in ER and goes to:
Golgi Apparatus
via Vesicular Transport
Protein is further modified and goes to:
Secretory Vesicles
Vesicles from Golgi fuse with Early Endosomes to form Late Endosomes
Plasma Membrane
Late Endosomes mature to form Lysosomes