Cytoplasmic organelles I

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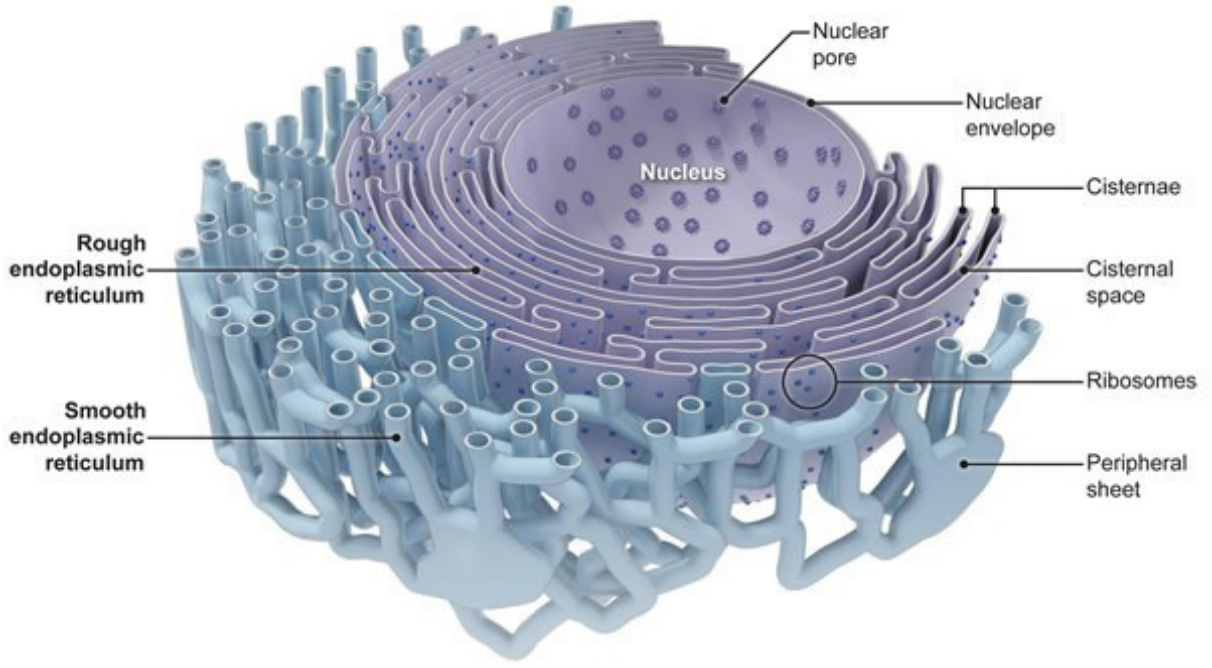
THE ENDOPLASMIC RETICULUM

With electron microscopy, the membranes of the endoplasmic reticulum were first seen in 1945 by Keith R. Porter, Albert Claude, and Ernest F. Fullam. Later, the word *reticulum*, which means "network", was applied by Porter in 1953 to describe this fabric of membranes.

All eukaryotic cells have an single membrane bound organelle- endoplasmic reticulum (ER). Its membrane typically constitutes more than half of the total membrane of an average animal cell. The ER is organized into a netlike labyrinth of branching tubules (sheets) and flattened sacs(cisternae) that extends throughout the cytosol. The tubules and sacs interconnect, and their membrane is continuous with the outer nuclear membrane. Thus, the ER and nuclear membranes form a continuous sheet enclosing a single internal space, called the ER lumen or the ER cisternal space, which often occupies more than 10% of the total cell volume.

To meet different functional demands, distinct regions of the ER become highly specialized and different cell types can therefore possess characteristically different types of ER membrane. Cells begin to import most proteins into the ER lumen before complete synthesis of the polypeptide chain known as co-translational process. In co-translational transport, the ribosome that is synthesizing the protein is attached directly to the ER membrane.These membrane-bound ribosomes coat the surface of the ER, creating regions termed rough endoplasmic reticulum, or rough ER.

Regions of ER that lack bound ribosomes are called smooth endoplasmic reticulum, or smooth ER.Areas of smooth ER from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus are called transitional ER.



Structure of Endoplasmic reticulum

Function :

The ER has a central role in protein biosynthesis, protein folding and glycosylation. The ER membrane is the site of production of all the transmembrane proteins for all cellular organelle and plasma membrane..In addition, almost all of the proteins that will be secreted to the cell exterior—plus those destined for the lumen of the ER, Golgi apparatus, or lysosomes—are initially delivered to the ER lumen.

ER synthesize lipids for most of the cell's organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, secretory vesicles, and the plasma membrane. The ER membrane also makes most of the lipids for mitochondrial and peroxisomal membranes.

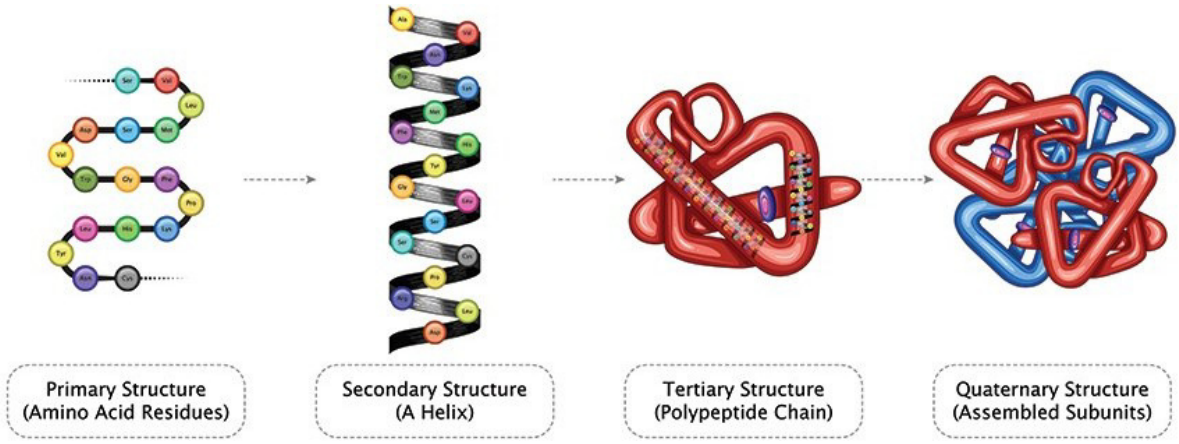
ER serves as an intracellular Ca 2+ store that is used in many cell signaling responses.

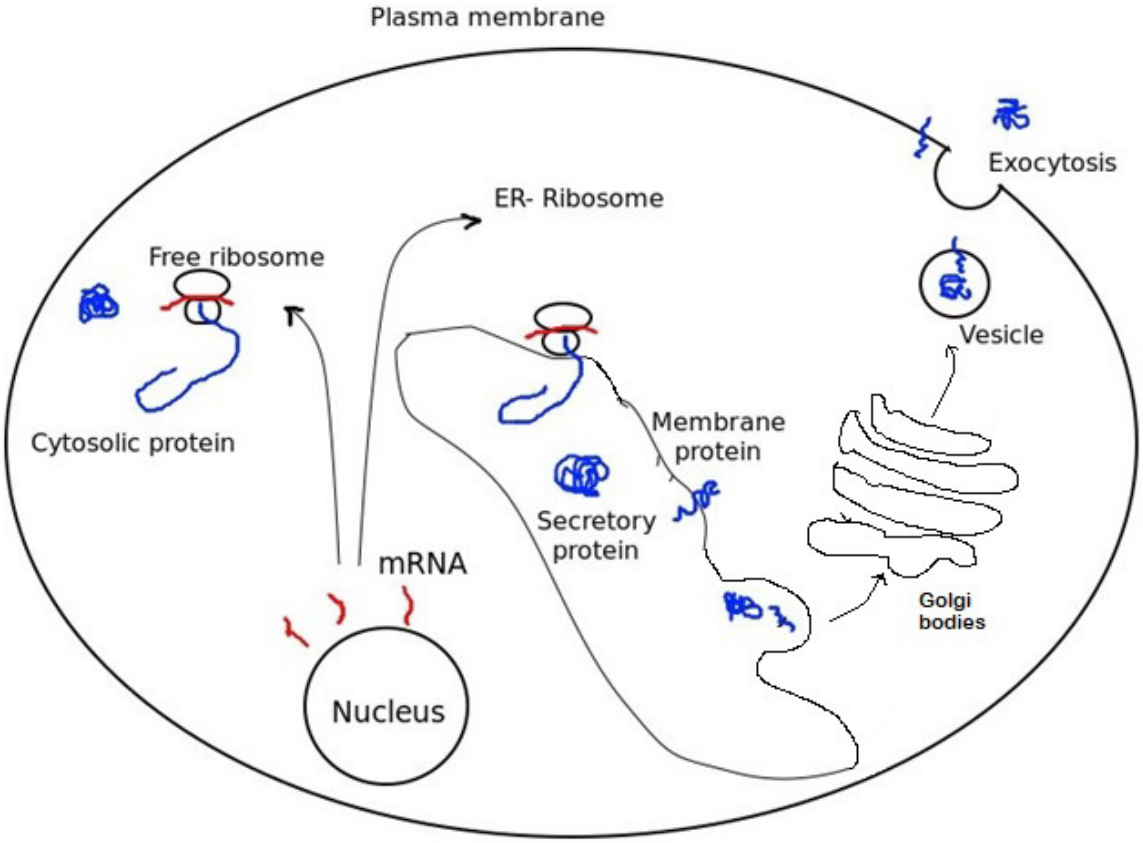
Cells that specialize in lipid metabolism, such as cells that synthesize steroid hormones from cholesterol; the expanded smooth ER accommodates the enzymes that make cholesterol and modify it to form the hormones.

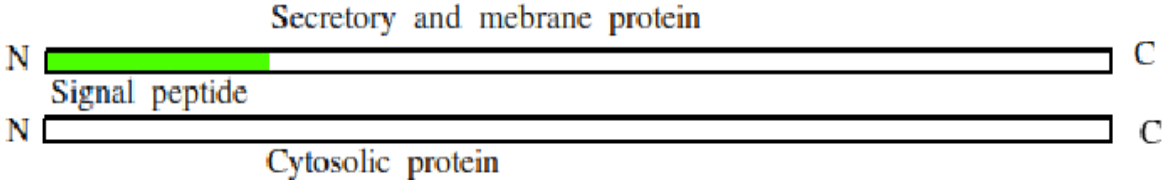
It is the principal site of production of lipoprotein particles, which carry lipids via the bloodstream to other parts of the body.

ER also contains enzymes that catalyze a series of reactions to detoxify both lipid-soluble drugs and various harmful compounds produced by metabolism. The most extensively studied of these detoxification reactions are carried out by the cytochrome P450 family of enzymes.

Muscle cells have an abundant, modified smooth ER, called the sarcoplasmic reticulum. The release and reuptake of Ca 2+ by the sarcoplasmic reticulum trigger myofibril contraction and relaxation, respectively, during each round of muscle contraction.







Overview of secretory pathway

Signal hypothesis:

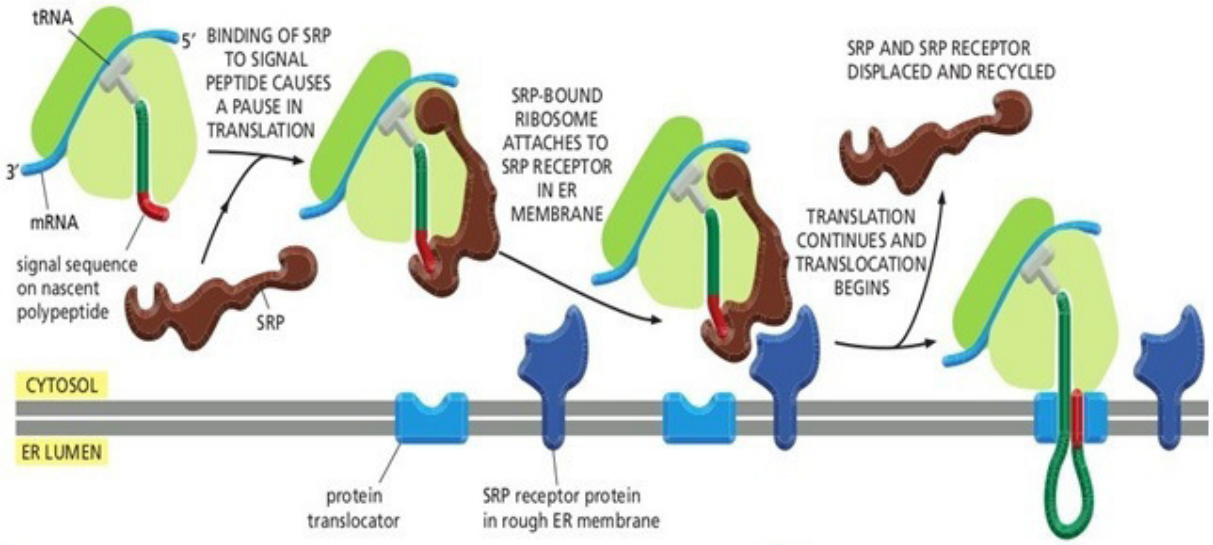


Figure : **How ER signal sequences and SRP direct ribosomes to the ER membrane.** <TTCC> The SRP and its receptor are thought to act in concert. The SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which is composed of two different polypeptide chains, binds the SRP–ribosome complex and directs it to the translocator. In a poorly understood reaction, the SRP and SRP receptor are then released, leaving the ribosome bound to the translocator in the ER membrane. The translocator then inserts the polypeptide chain into the membrane and transfers it across the lipid bilayer. Because one of the SRP proteins and both chains of the SRP receptor contain GTP-binding domains, it is thought that conformational changes that occur during cycles of GTP binding and hydrolysis (discussed in Chapter 15) ensure that SRP release occurs only after the ribosome has become properly engaged with the translocator in the ER membrane. The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times.

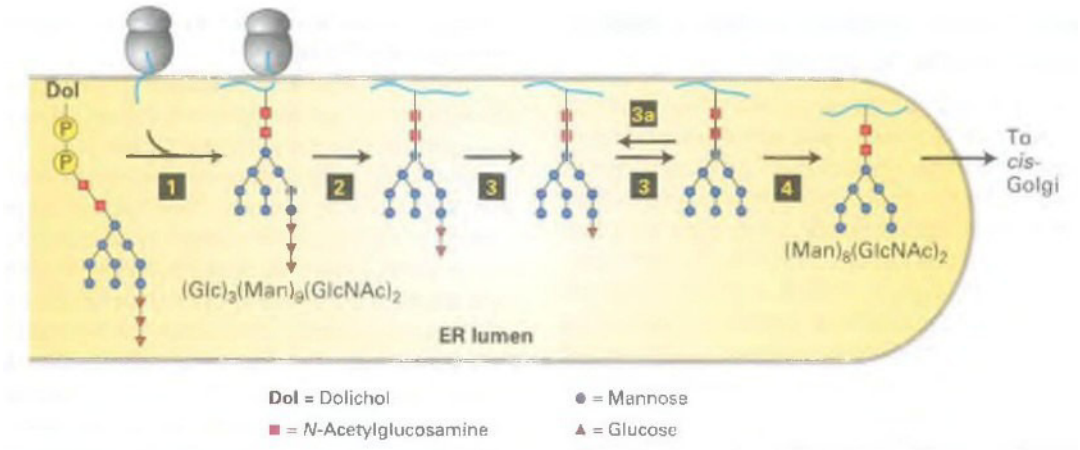
Glycosylation of Proteins Synthesized in the Rough ER

The covalent addition of oligosaccharides to proteins is one of the major biosynthetic functions of the ER. About half of the soluble and membrane-bound proteins that are processed in the ER—including those destined for transport to the Golgi apparatus, lysosomes, plasma membrane, or extracellular space—are glycoproteins that are modifed in this way. Many proteins in the cytosol and nucleus are also glycosylated, but not with oligosaccharides: they carry a much simpler sugar modifcation, in which a single N-acetylglucosamine group is added to a serine or threonine of the protein.

During the most common form of protein glycosylation in the ER, a preformed precursor oligosaccharide (composed of N-acetylglucosamine, mannose, and glucose, and containing a total of 14 sugars) is transferred en bloc to proteins. Because this oligosaccharide is transferred to the side-chain NH2 group of an asparagine in the protein, it is said to be **N-linked or asparagine-linked**.

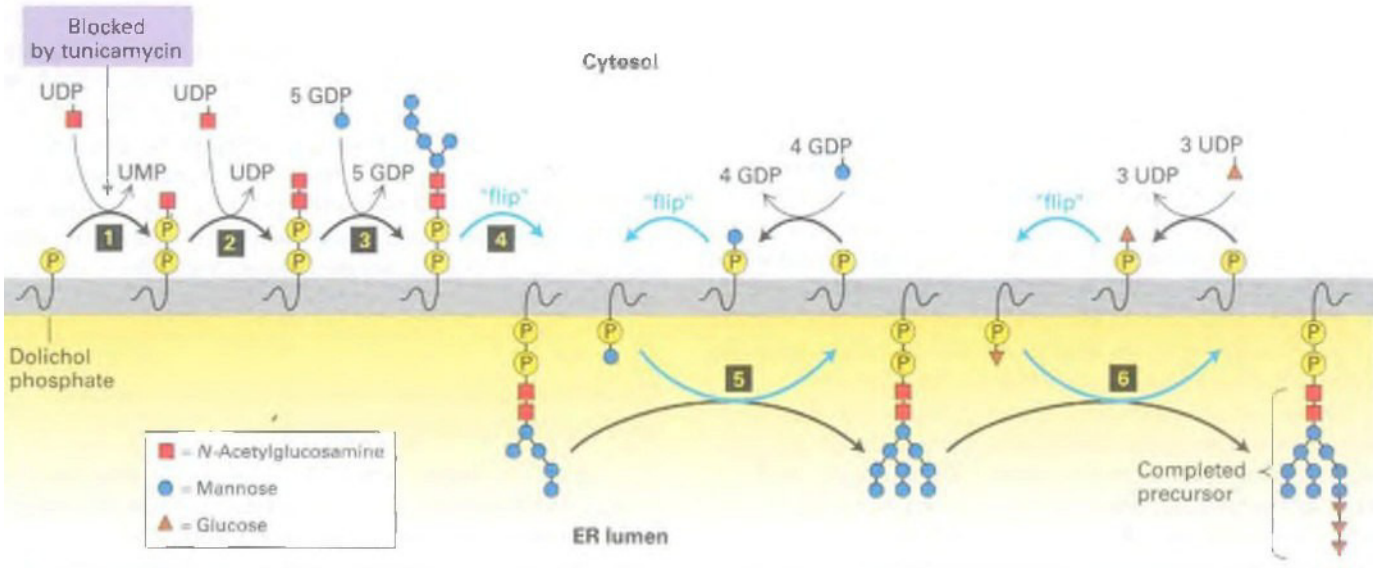
The transfer is catalyzed by a membrane-bound enzyme complex, an ***oligosaccharyl transferase***, which has its active site exposed on the lumenal side of the ER membrane; this explains why cytosolic proteins are not glycosylated in this way.

A special lipid molecule called dolichol anchors the precursor oligosaccharide in the ER membrane. The precursor oligosaccharide is transferred to the target asparagine in a single enzymatic step immediately after that amino acid has reached the ER lumen during protein translocation. The precursor oligosaccharide is linked to the **dolichol** lipid by a high-energy pyrophosphate bond, which provides the activation energy that drives the glycosylation reaction. One copy of oligosaccharyl transferase is associated with each protein translocator, allowing it to scan and glycosylate the incoming polypeptide chains efficiently.



Glycosylation of protein in ER lumen

The precursor oligosaccharide is built up sugar by sugar on the membrane-bound dolichol lipid and is then transferred to a protein. The sugars are first activated in the cytosol by the formation of nucleotide (UDP or GDP)-sugar intermediates, which then donate their sugar (directly or indirectly) to the lipid in an orderly sequence. Part way through this process, the lipid-linked oligosaccharide is flipped, with the help of a transporter, from the cytosolic to the lumenal side of the ER membrane.



Synthesis of oligosaccharide chain for glycosylation

All of the diversity of the N-linked oligosaccharide structures on mature glycoproteins results from the later modification of the original precursor oligosaccharide. While still in the ER, three glucoses and one mannose are quickly removed from the oligosaccharides of most glycoproteins. This oligosaccharide "trimming," or "processing," continues in the Golgi apparatus. The N-linked oligosaccharides are by far the most common oligosaccharides, being found on 90% of all glycoproteins.

Less frequently, oligosaccharides are linked to the hydroxyl group on the side chain of a serine, threonine, or hydroxylysine amino acid. A first sugar of these **O-linked oligosaccharides** is added in the ER and the oligosaccharide is then further extended in the Golgi apparatus.

Molecular Mechanisms of Vesicle Budding and Fusion

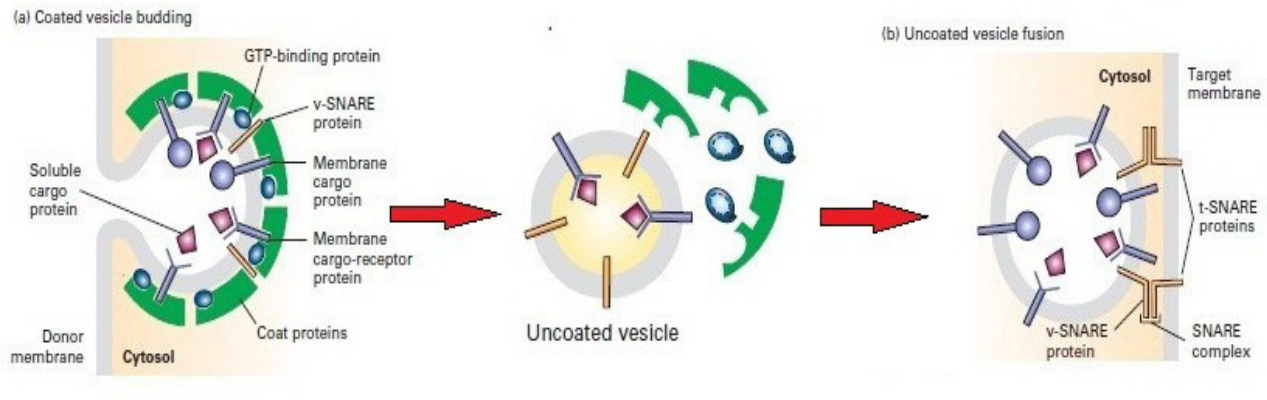
Small membrane-bounded vesicles that transport proteins from one organelle to another are common elements in the secretory and endocytic pathways. These vesicles bud from the membrane of a particular *"parent" (donor) organelle* and fuse with the membrane of a particular *"target" (destination) organelle*. Although each step in the secretory and endocytic pathways employs a different type of vesicle, but each of the different vesicular transport steps is simply a variation on a common theme. The basic mechanisms underlying vesicle budding and fusion are as follows –

1. The budding of a vesicle from its parent membrane is driven by the polymerization of soluble protein complexes on the membrane to form a proteinaceous vesicle coat .The vesicle buds eventually pinch off from the membrane to release a complete vesicle.

2. Interactions between the cytosolic portions of integral membrane proteins or receptor of soluble cargo and the vesicle coat gather the appropriate cargo proteins into the forming vesicle. Thus the coat gives curvature to the membrane to form a vesicle and acts as the filter to determine which proteins are admitted into the vesicle.

3. The three well-characterized types of transport vesicles— COPI, COPII, and clathrin-coated vesicles—are distinguished by the proteins that form their coats and the transport routes they mediate.

- **COPII** vesicles transport proteins from the ER to the Golgi.
- **COPI** vesicles mainly transport proteins in the retrograde direction between Golgi cisternae and from the *cis*-Golgi back to the ER.
- **Clathrin**-coated vesicles transport proteins from the plasma membrane (cell surface) and the *trans*-Golgi network to late endosomes.



Vesicle budding and fusion

4. Small GTP-binding proteins (ARF or Sar1) belonging to the GTPase superfamily control polymerization of coat proteins. After vesicles are released from the donor membrane, hydrolysis of GTP bound to ARF or Sar1 triggers disassembly of the vesicle coats.

5. A second set of GTP-binding proteins, the Rab proteins, label specific vesicle types and enable their docking to the appropriate membrane. Activated Rab·GTP in a vesicle can bind to a specific type of effector protein. One type of effector is a filamentous that enables tethering of the vesicle to the target membrane. Another type of effector is a motor protein that enables vesicles to move along cytoskeletal filaments to their correct destination.

6. Shortly after a vesicle buds off from the donor membrane, the vesicle coat disassembles to uncover a vesicle-specific membrane protein, a v-SNARE. Likewise, each type of target membrane in a cell contains t-SNARE membrane proteins. Each v-SNARE in a vesicular membrane specifically binds to a complex of cognate t-SNARE proteins in the target membrane, inducing fusion of the two membranes. After fusion is completed, the SNARE complex is disassembled in an ATP-dependent reaction mediated by other cytosolic proteins.

Early Stages of the Secretory Pathway / Transport from the ER through the Golgi apparatus / Vesicle-mediated protein trafficking between the ER and *cis*-Golgi.

A single principle governs all protein trafficking in the secretory and endocytic pathways which is transport of membrane and soluble proteins from one membrane-bounded compartment to another mediated by **transport vesicles** that collect *cargo proteins* in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment. There are three major events in the early stages of secretory pathways. These are –

Anterograde transport from the ER to the Golgi :

1. Anterograde transport from the ER to the Golgi, the first vesicle trafficking step in the secretory pathway, is mediated by COPII vesicles. These vesicles contain newly synthesized proteins destined for the Golgi, cell surface, or lysosomes as well as vesicle components such as v- SNAREs that are required to target vesicles to the ***cis***-Golgi membrane.

2. The budding of COP II vesicle from ER membrane is driven by the polymerization of soluble protein complexes on the membrane to form a proteinaceous vesicle coat .The COP II vesicle buds eventually pinch off from the membrane to release a complete vesicle. Formation of COPII vesicles is triggered when Sec12, a GEF in the ER membrane, catalyzes the exchange of bound GDP for GTP on cytosolic Sar1(A small GTP binding protein). This exchange induces binding of Sar1 to the ER membrane, followed by binding of a complex of Sec23 and Sec24 proteins.
3. This core coat protein complex then provides binding sites for the recruitment of a second complex of Sec13 and Sec31 proteins to complete the coat structure.

4. After the vesicle budding, another GTP binding protein - Rab , enable their docking to the Cis-golgi membrane through interaction with other effector protein.

5.After budding, COPII vesicle coat disassembles to uncover a vesicle-specific membrane protein, a v-SNARE. v-SNARE in a COP II vesicle membrane specifically binds to a complex of cognate t-SNARE proteins in the Cis-golgi membrane, inducing fusion of the two membranes.

Retrograde Transport Within the Golgi and from the Golgi to the ER :

1. Proper sorting of proteins between the ER and Golgi also requires retrograde transport within CIs-golgi compartments and from the *cis*-Golgi to the ER, which is mediated by COPI vesicles. This retrograde vesicle transport serves to retrieve v-SNARE proteins and components of the membrane itself to provide the necessary material for additional rounds of vesicle budding from the ER.

2. Analysis of COP I vesicles showed that the coat is formed from large cytosolic complexes, called *coatomers*, composed of seven polypeptide subunits. The GTP binding protein required

for coat assembly for COPI is ARF. The mechanism of docking and fusion of COPI vesicle is similar with that of COPII vesicle but using the other variant of Coat protein, GPT-binding protein, Rab and SNARE protein.

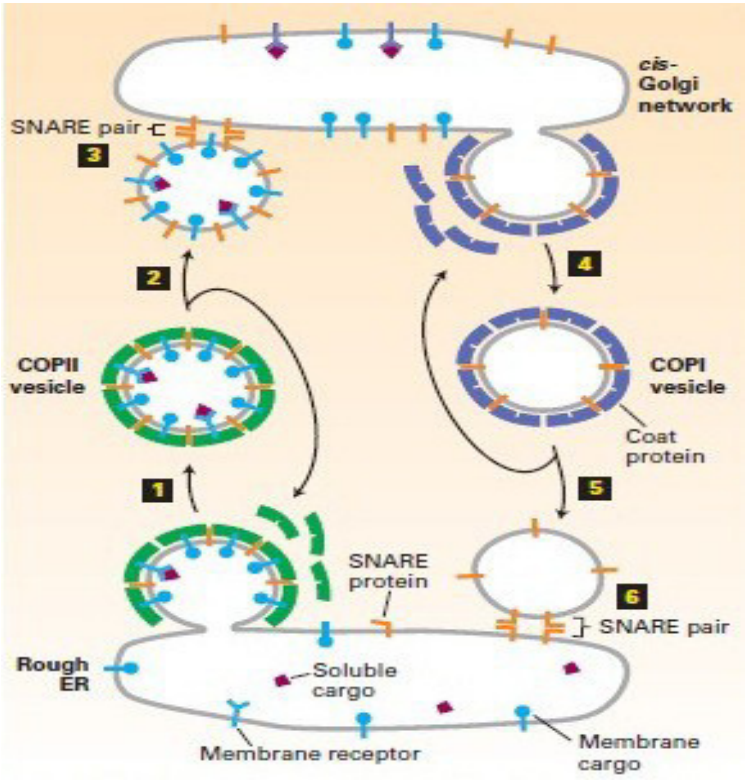
Anterograde transport through cisternal maturation :

1. The Golgi complex is organized into three compartments, often arranged in a stacked set of flattened sacs, called cisternae. The compartments of the Golgi differ from one another according to the enzymes they contain. The transport among the Golgi compartments are occurred by COPI vesicle.

2. These vesicles are now known to mediate retrograde transport, retrieving ER or Golgi enzymes from a later compartment and transporting them to an earlier compartment in the secretory pathway. Thus the Golgi appears to have a highly dynamic organization, continually forming transport vesicles, though only in the retrograde direction.

3. As this process continues, the *medial*-Golgi acquires enzymes from the *trans*-Golgi while losing *medial*-Golgi enzymes to the *cis*-Golgi and thus progressively becomes a new *trans*- Golgi compartment.

4. In this way, secretory cargo proteins acquire carbohydrate modifications in the proper sequential order without being moved from one cisterna to another via anterograde vesicle transport. This event is called cisternal maturation.



Anterograde and retrograde transport of vesicles

Later Stages of the Secretory Pathway:

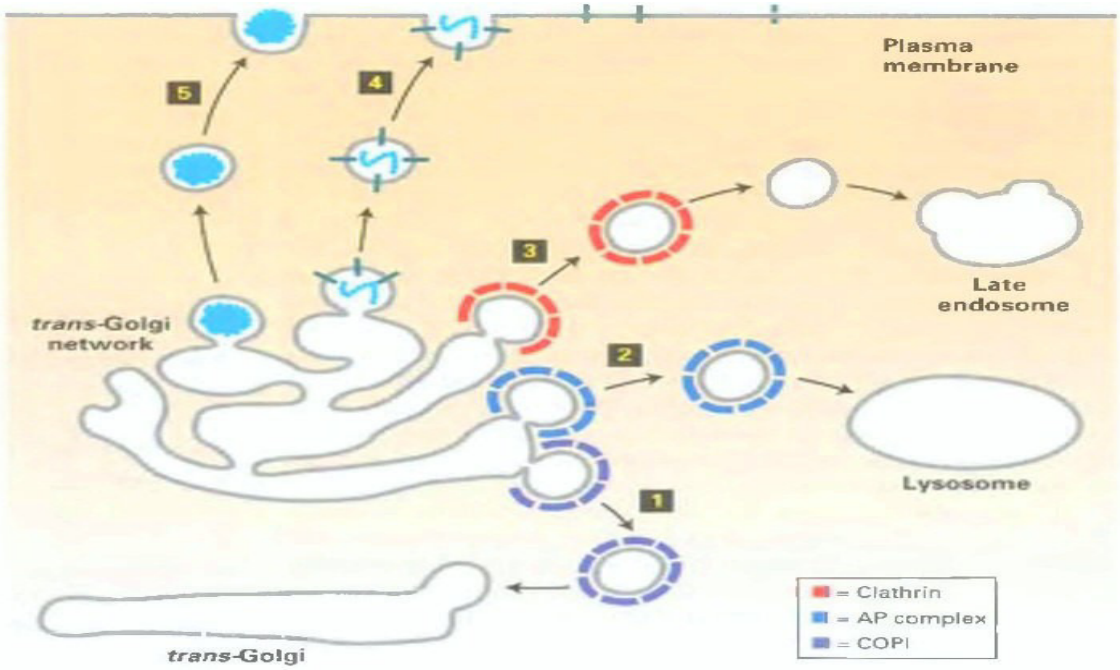
The later phase of secretory pathway involves budding of vesicles from the trans golgi network and fusion of the vesicles with lysosome or late endosome or plasma membrane to release its content. Transport vesicles destined for the plasma membrane normally leave the trans golgi nework in a steady stream as irregularly shaped tubules. The membrane proteins and the lipids in these vesicles provide new components for the cell's plasma membrane, while the soluble proteins inside the vesicles are secreted to the extracellular space. This follows two pathways-

Constitutive secretory pathway: It operates continuously. Cells secrete proteoglycans and glycoproteins of the extracellular matrix by this pathway.

Regulated secretory pathway : soluble proteins and other substances are initially stored in secretory vesicles for later release by exocytosis. Found mainly in cells specialized for secreting products rapidly on demand—such as hormones, neurotransmitters, or digestive enzymes. Regulated secreted proteins are concentrated and stored in secretory vesicles to await a neural or hormonal signal for exocytosis.

- The trans-Golgi network (TGN) is a major branch point in the secretory pathway where soluble secretory proteins, lysosomal proteins, and membrane proteins destined for the basolateral or apical plasma membrane are segregated into different transport vesicles.

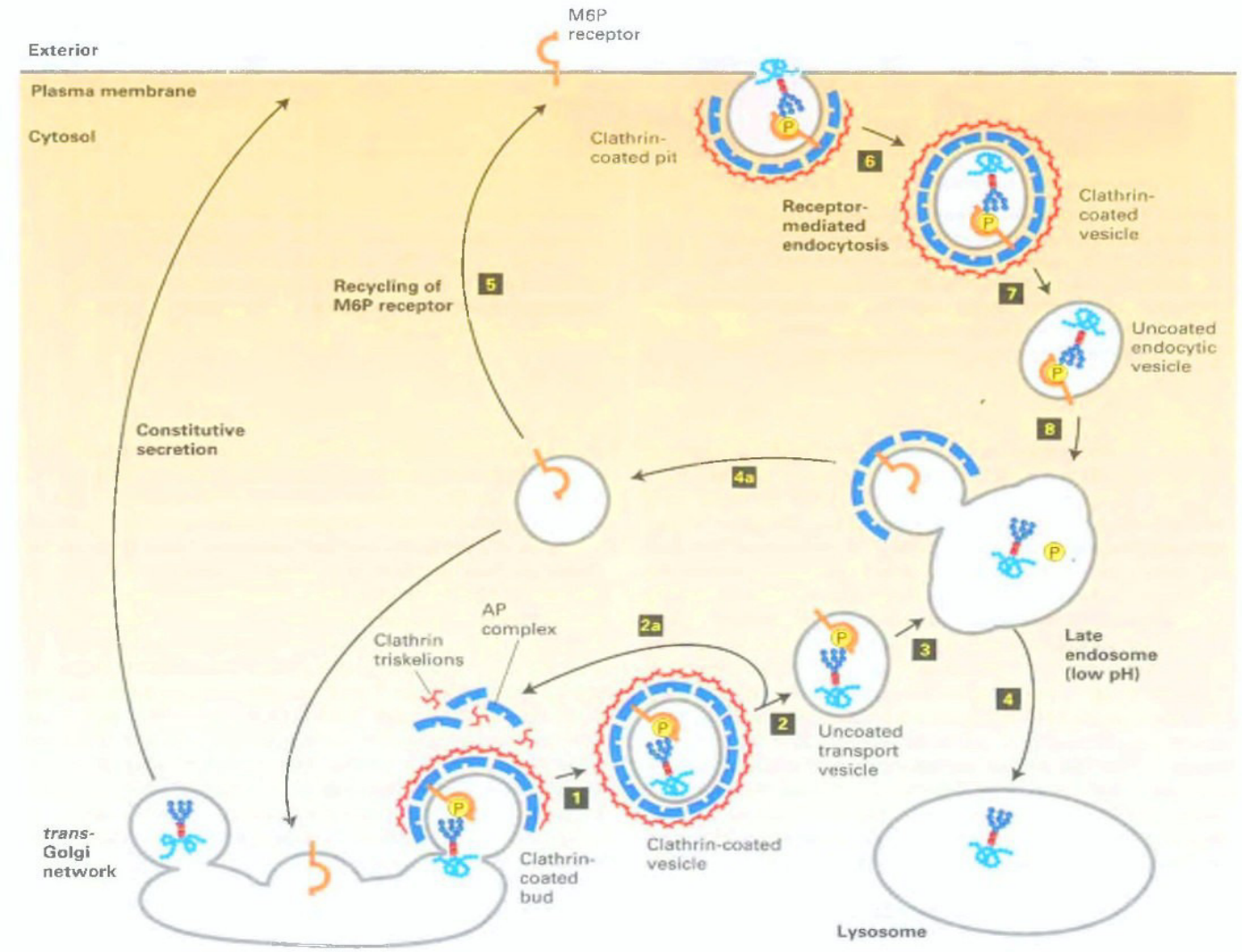
- Proteins that function in the lumen or in the membrane of the lysosome are first transported from the trans-Golgi network via clathrin-coated (red) vesicles. The clathrin molecules have a three-limbed shape, are called triskelions, from the Greek for "three-legged". These vesicles fuse with late endosomes, which deliver their contents to the lysosome. The coat on most clathrin vesicles contains additional proteins (AP complexes). Some vesicles from the frans-Golgi carrying cargo destined for the lysosome fuse with the lysosome directly bypassing the endosome. The coat proteins surrounding constitutive and regulated secretory vesicles are not yet characterized.



Later stages of secretory pathways

- In the case of clathrin/AP-coated vesicles, a cytosolic protein called dynamin is essential for release of complete vesicles. At the later stages of bud formation, dynamin polymerizes around the neck portion and then hydrolyzes GTP. The energy derived from GTP hydrolysis is thought to drive a conformational change in dynamin that stretches the vesicle neck until the vesicle pinches off.

- Soluble enzymes destined for lysosomes are modified in the cis-Golgi, yielding multiple mannose 6-phosphate (M6P) residues on their oligosaccharide chains. M6P receptors in the membrane of the trans-Golgi network bind proteins bearing M6P residues and direct their transfer to late endosomes, where receptors and their ligand proteins dissociate. The receptors then are recycled to the Golgi or plasma membrane, and the lysosomal enzymes are delivered to lysosomes.



- In polarized epithelial cells, membrane proteins destined for the apical or basolateral domains of the plasma membrane are sorted in the transs-Golgi network into different transport vesicles. The GPI anchor is the only apical-basolateral sorting signal identified so far.

- Many proteins transported through the secretory pathway undergo post-Golgi proteolytic cleavages that yield the mature, active proteins. Generally, proteolytic maturation can occur in vesicles carrying proteins from the trans-Golgi network to the cell surface, in the late endosome, or in the lysosome.

Related questions:

1. What are the types of ER? State their respective functions.
2. State the secretory pathways of proteins.
3. What is protein glycosylation?
4. What is protein sorting in Golgi apparatus?
5. What is signal hypothesis?
6. Describe the molecular mechanism of vesicle budding and fusion.
7. Describe the vesicle mediated protein trafficking between ER and cis-golgi.

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