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STRUCTURE AND FUNCTION OF ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is a network of membrane enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm. The entire endoplasmic reticulum is enclosed by a continuous membrane and is the largest organelle of most eukaryotic cells. Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume. These vacuoles or cavities often remain concentrated in the endoplasmic portion of the cytoplasm; therefore, known as endoplasmic reticulum, a name derived from the fact that in the light microscope it looks like a "net in the cytoplasm." The name "endoplasmic reticulum" was coined in 1953 by Porter, who in 1945 had observed it in electron micrographs of liver cells.

OCCURRENCE: The occurrence of the endoplasmic reticulum varies from cell to cell. The erythrocytes (RBC), egg and embryonic cells lack endoplasmic reticulum.

About half the total area of membrane in a eukaryotic cell encloses the labyrinthine spaces of the endoplasmic reticulum (ER). The rough ER has many ribosomes bound to its cytosolic surface. Regions of the ER that lack bound ribosomes are called smooth ER.

- The spermatocytes have poorly developed endoplasmic reticulum.
- The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER).
- The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.
- The presence of both SER and RER in the hepatocytes (liver cells) is reflective of the variety of the roles played by the liver in metabolism.

ER AND ENDOMEMBRANE SYSTEM

The endoplasmic reticulum is the main component of the endomembrane system, also called the cytoplasmic vacuolar system or cytocavity network. This system comprises following structures:

(1) The nuclear envelope, consisting of two non-identical membranes, one opposed to the nuclear chromatin and other separated from the first membrane by a perinuclear space (both forming a cisternae), the two membranes being in contact at the nuclear pores;

(2) The endoplasmic reticulum; and

(3) the Golgi apparatus, which is mainly related to some of the terminal processes of cell secretion.

GERL (or Golgi, ER and lysosome) refers to a special region of endomembrane system, which is more related to the Golgi apparatus and is involved in the formation of lysosomes. The entire endomembrane system represents a barrier separating cytoplasmic compartments.

The membrane of each component of this system has two faces: (i) the cytoplasmic or protoplasmic face and (ii) the luminal face . The luminal face borders the perinuclear cisternae, the cavities of ER and SER, and the Golgi elements. It also corresponds to the interior of the secretory granules, the lysosomes and peroxisomes and also to faces of mitochondrial membranes confronting to outer mitochondrial chamber.

MORPHOLOGY:

The endoplasmic reticulum may occur in the following three forms:

1. Lamellar form or cisternae: The cisternae are long, flattened, sac-like, unbranched tubules having the diameter of 40 to 50 µm. They remain arranged parallely in bundles or stacks. RER usually exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.
2. Vesicles. The vesicles are oval, membrane-bound vacuolar structures having the diameter of 25 to 500 µm. They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.
3. Tubules. The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 µm and occur almost in all the cells. Tubular form of ER is often found in SER and is dynamic in nature, i.e., it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

TYPES OF ENDOPLASMIC RETICULUM

Two types of endoplasmic reticulum have been observed in same or different types of cells which are as follows:

1. **Agranular or Smooth Endoplasmic Reticulum**

This type of endoplasmic reticulum possesses smooth walls because the ribosomes are not attached with its membranes. The smooth type of endoplasmic reticulum occurs mostly in those cells, which are involved in the metabolism of lipids (including steroids) and glycogen. The smooth endoplasmic reticulum is generally found in adipose cells, interstitial cells, glycogen storing cells of the liver, conduction fibres of heart, spermatocytes and leucocytes. The muscle cells are also rich in smooth type of endoplasmic reticulum and here it is known as sarcoplasmic reticulum. In the pigmented retinal cells it exists in the form of tightly packed vesicles and tubes known as myeloid bodies.

Glycosomes. Although the SER forms a continuous system with RER, it has different morphology. In liver cells it consists of a tubular network that pervades major portion of the cytoplasmic matrix. These fine tubules are present in regions rich in glycogen and can be observed as dense particles, called glycosomes, in the matrix. Glycosomes measure 50 to 200 nm in diameter and contain glycogen along with enzymes involved in the synthesis of glycogen (Rybicka, 1981). Many glycosomes attached to the membranes of SER have been observed by electron microscopy in the liver and conduction fibre of heart.

2**. Granular or Rough Endoplasmic Reticulum**:

The granular or rough type of endoplasmic reticulum possesses rough walls because the ribosomes remain attached with its membranes. Ribosomes play a vital role in the process of protein synthesis. The granular or rough type of endoplasmic reticulum is found abundantly in those cells which are active in protein synthesis such as pancreatic cells, plasma cells, goblet cells, and liver cells. The granular type of endoplasmic reticulum takes basophilic stain due to its RNA contents of ribosomes. The region of the matrix containing granular type of endoplasmic reticulum takes basophilic stain and is named as ergastoplasm, basophilic bodies, chromophilic substances or Nissl bodies by early cytologists. In RER, ribosomes are often present as polysomes held together by mRNA and are arranged in typical “rosettes” or spirals.

RER contains two transmembrane glycoproteins (called ribophorins I and II of 65,000 and 64,000 dalton MW, respectively), to which are attached the ribosomes by their 60S subunits.

FUNCTIONS OF ROUGH ENDOPLASMIC RETICULUM

The role of the endoplasmic reticulum in protein processing and sorting was first demonstrated by George Palade and his colleagues in the 1960s. It plays a major role in the production, processing, and transport of proteins and lipids. The ER produces transmembrane proteins and lipids for its membrane and many other cell components including lysosomes, secretory vesicles, the Golgi apparatus, the cell membrane, and plant cell vacuoles.

1. **Protein targeting:**
   The entrance of proteins into the ER thus represents a major branch point for the traffic of proteins within eukaryotic cells. Proteins destined for secretion or incorporation into the ER, Golgi apparatus, lysosomes, or plasma membrane are initially targeted to the ER.
   Co-translational translocation: Proteins can be translocated into the ER either during their synthesis on membrane-bound ribosomes. Approximately one-third of the proteins encoded by a mammalian genome are synthesized on ribosomes attached to the cytosolic surface of the RER membranes. These include **(a) secreted proteins, (b) integral membrane proteins, and (c) soluble proteins that reside within compartments of the endomembrane system, including the ER, Golgi complex, lysosomes, endosomes, vesicles, and plant vacuoles.**
   Post-translational translocation: Proteins can be translocated into the ER after their translation has been completed on free ribosomes in the cytosol. Proteins destined to remain in the cytosol or to be incorporated into the nucleus, mitochondria, chloroplasts, or peroxisomes are synthesized on free ribosomes and released into the cytosol when their translation is complete.

   CO-TRANSLATIONAL PATHWAY:

   a. **The association of ribosomes with the ER:** Ribosomes are targeted for binding to the ER membrane by the amino-acid sequence of the polypeptide chain being synthesized, rather than by intrinsic properties of the ribosome itself. (Free and membrane bound ribosomes are functionally indistinguishable, and all protein synthesis initiates on ribosomes that are free in the cytosol.) Ribosomes engaged in the synthesis of proteins that are destined for secretion are then 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 that are usually cleaved from the polypeptide chain during its transfer into the ER lumen. David Sabatini and Gunter Blobel first proposed in 1971 that the signal for ribosome attachment to the ER might be an amino acid sequence near the amino terminus of the growing polypeptide chain. This hypothesis was known as SIGNAL HYPOTHESIS. The signal sequences span about 20 amino acids, including a stretch of hydrophobic residues usually located at the amino terminus of the polypeptide chain. ER signal sequences vary greatly in amino acid sequence, but each has eight or more nonpolar amino acids at its center.
   b. **(SRP) directs the ER Signal Sequence to a Specific Receptor in the Rough ER Membrane:** The ER signal sequence is guided to the ER membrane by at least two components:
      - ✓ a signal-recognition particle (SRP): It consists of six different polypeptide chains bound to a single small 7s RNA molecule. It cycles between the ER membrane and the cytosol and binds to the signal sequence. The signal-sequence-binding site is a large hydrophobic pocket lined by methionines. Because methionines have unbranched, flexible side chains, the pocket is sufficiently plastic to accommodate hydrophobic signal sequences of different sequences, sizes, and shapes.

The SRP is a rod-like structure, which wraps around the large ribosomal subunit, with one end binding to the ER signal sequence as it emerges from the ribosome as part of the newly made polypeptide chain; the other end blocks the elongation factor binding site at the interface between the large and small ribosomal subunits. This block halts protein synthesis as soon as the signal peptide has emerged from the ribosome. The transient pause presumably gives the ribosome enough time to bind to the ER membrane before completion of the polypeptide chain, thereby ensuring that the protein is not released into the cytosol.

- ✓ an SRP receptor in the ER membrane: A transmembrane protein, a heterodimer of α and β polypeptide chains.

When a signal sequence binds, SRP exposes a binding site for the SRP receptor. The binding of the SRP to its receptor brings the SRP–ribosome complex to an unoccupied protein translocator in the same membrane. The SRP and SRP receptor are then released, and the translocator transfers the growing polypeptide chain across the membrane. The signal sequence is inserted into a membrane channel or translocon. This entire process is coordinated by GTP binding to both the SRP and the SRP receptor, with hydrolysis of GTP to GOP leading to dissociation of SRP from both the receptor and the ribosome-mRNA complex.

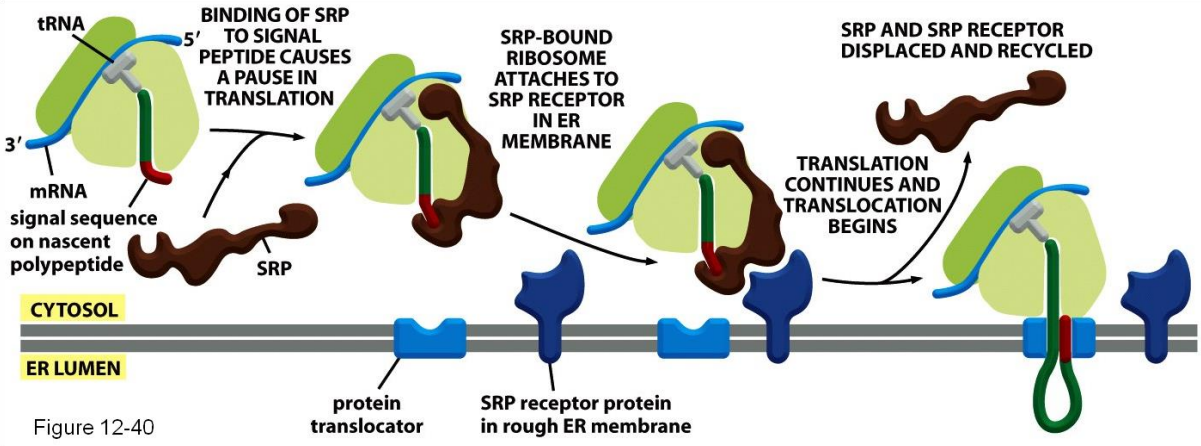


Figure 12-40

c. Translation resumes and polypeptide chain grows: The core of the translocator, called the Sec61 complex, is built from three subunits that are highly conserved from bacteria to eukaryotic cells. The channel is gated by a short α helix that is thought to keep the translocator closed when it is idle and to move aside when it is engaged in passing a polypeptide chain.

[In eukaryotic cells, four Sec61 complexes form a large translocator assembly that can be visualized on ER-bound ribosomes after detergent solubilization of the ER membrane. It is likely that this assembly includes other membrane complexes that associate with the translocator, such as enzymes that modify the growing polypeptide chain, including oligosaccharide transferase and the signal peptidase. The assembly of a translocator with these accessory components is called the translocon.]

Transfer of the ribosome-mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the ER membrane as translation proceeds. An hsp70-like chaperone protein (called BiP, for binding protein) are deposited on

the polypeptide chain as it emerges from the pore into the ER lumen. Proteins that are transported into the ER by a post-translational mechanism are first released into the cytosol, where they bind to chaperone proteins to prevent folding.

d. Cleavage of signal sequence and release of polypeptide chain: Thus the process of protein synthesis directly drives the transfer of growing polypeptide chains through the translocon and into the ER. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

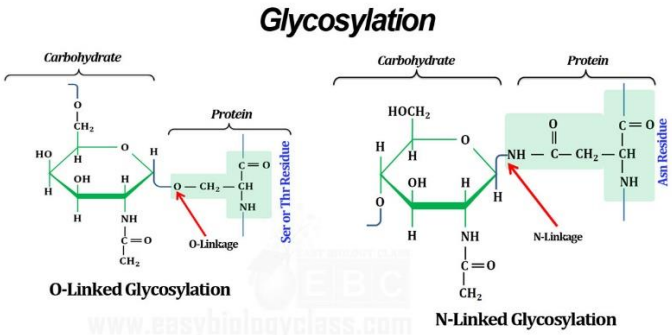
2. Glycosylation of proteins:

Most plasma membrane and secretory proteins contain one or more carbohydrate chains. The covalent addition of oligosaccharides and subsequent processing of carbohydrates is the principal chemical modification to most such proteins known as *glycosylation*. Some glycosylation reactions occur in the lumen of the ER; others, in the lumen of the *cis*-, *medial*-, or *trans*-Golgi cisternae. Thus the presence of certain carbohydrate residues on proteins provide useful markers for following their movement from the ER and through the Golgi cisternae.

Types of glycosylation: There are mainly two types of glycosylation depending on the different types of sugar residues mainly found in each type.

a. N-linked glycosylation: In all *N*-linked oligosaccharides, *N*-acetylglucosamine (GlcNAc) is linked to the amide nitrogen of asparagine. Typical *N*-linked oligosaccharides, always contain mannose as well as *N*-acetylglucosamine and usually have several branches each terminating with a negatively charged sialic acid residue. Most cytosolic and nuclear proteins are not glycosylated;

b. O-linked glycosylation: It occurs in Golgi apparatus. *O*-linked oligosaccharides are linked to the hydroxyl group of serine or threonine via *N*-acetylgalactosamine (GalNac) or (in collagens) to the hydroxyl group of hydroxylysine via galactose. *O*-linked oligosaccharides are generally short, often containing only one to four sugar residues.

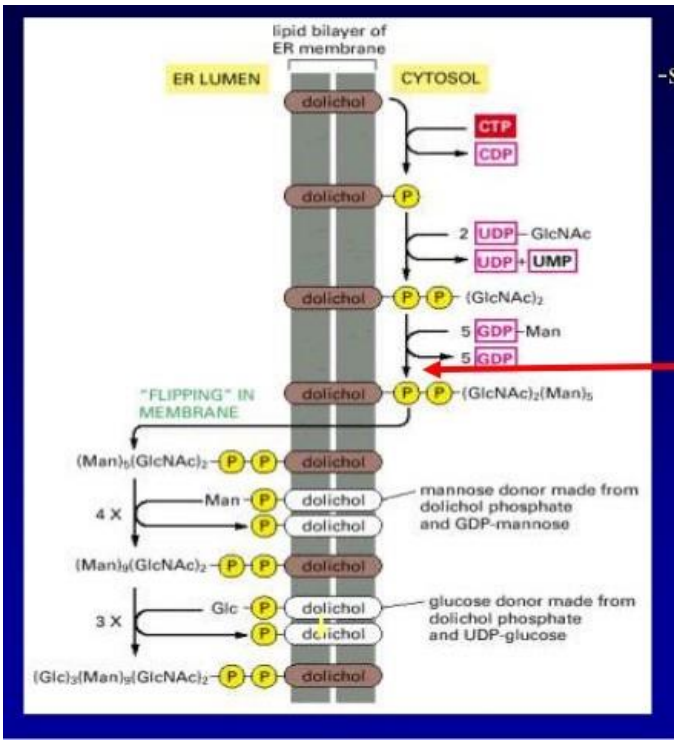


N-LINKED GLYCOSYLATION

N-linked protein glycosylation in the endoplasmic reticulum (ER) is a conserved two-phase process in eukaryotic cells. It involves **i) the assembly of an oligosaccharide on a lipid carrier, dolichol phosphate and ii) the transfer of the oligosaccharide to selected asparagine residues of polypeptides that have entered the lumen of the ER.** The assembly of the oligosaccharide (LLO) takes place at the ER membrane and requires the activity of several specific glycosyltransferases.

i) The assembly of an oligosaccharide on a lipid carrier, dolichol phosphate:

The isoprenoid lipid dolichol serves as carrier of the oligosaccharide and it localizes the biosynthetic pathway to the membrane of the ER. It anchors the precursor oligosaccharide in the ER membrane. The precursor oligosaccharide is built up sugar by sugar on the membrane-bound dolichol lipid. 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 luminal side of the ER membrane.



ii) **The transfer of the oligosaccharide to selected asparagine residues of polypeptides that have entered the lumen of the ER:**

This 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 luminal side of the ER membrane; this explains why cytosolic proteins are not glycosylated in this way. One copy of oligosaccharyl transferase is associated with each protein translocator, allowing it to scan and glycosylate the incoming polypeptide chains efficiently. While still in the ER, three glucoses and one mannose are quickly removed from the oligosaccharides of most glycoproteins.

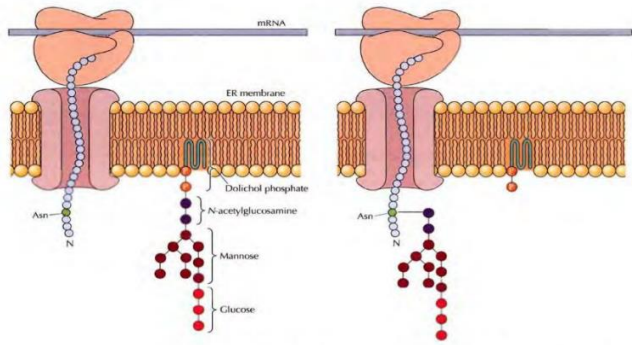
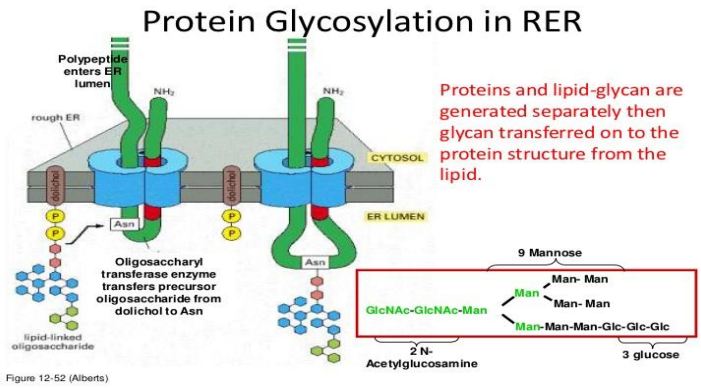


fig: Synthesis of N-linked glycoproteins

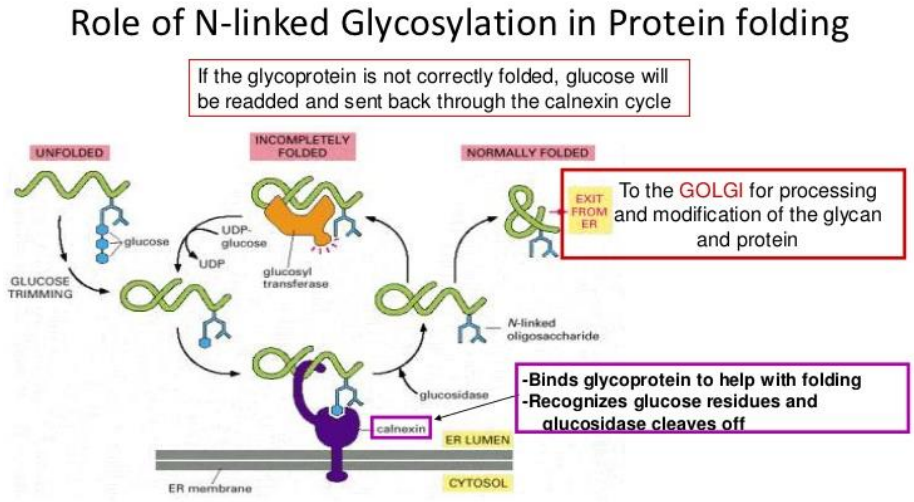


3. **Protein folding in ER**

Glycosylation plays an important role in protein folding. Two ER chaperone proteins, called **calnexin and calreticulin** are carbohydrate-binding proteins, or lectins, which bind to oligosaccharides on incompletely folded proteins and retain them in the ER. They require Ca2+ for their activities, Ca2+ being abundant in ER.

Calnexin and calreticulin recognize N-linked oligosaccharides that contain a single terminal glucose, and they therefore bind proteins only after two of the three glucoses on the precursor oligosaccharide have been removed during glucose trimming by ER glucosidases. When the third glucose has been removed, the glycoprotein dissociates from its chaperone and can leave the ER.

Yet another ER enzyme, a glucosyl transferase that keeps adding a glucose to those oligosaccharides that have lost their last glucose. It adds the glucose, however, only to oligosaccharides that are attached to unfolded proteins. Thus, an unfolded protein undergoes continuous cycles of glucose trimming (by glucosidase) and glucose addition (by glucosyl transferase), maintaining an affinity for calnexin and calreticulin until it has achieved its fully folded state.



4. **Quality control: Degradation of misfolded proteins**

Many proteins synthesized in the ER are rapidly degraded, primarily because they fail to fold correctly; others reside in the ER for several hours while they are properly folded. Thus an important role of the ER is to identify misfolded proteins, mark them, and divert them to a

degradation pathway. If the glycoprotein fails to fold after multiple cycles, the chaperone complex will send it into a degradation pathway that involves retro-translocation of the protein back through the translocon channel. Once in the cytosol, the oligosaccharide chains are removed, and the misfolded proteins are destroyed in proteasomes, which are protein-degrading machines. This process, known as ER-associated degradation (ERAD), ensures that aberrant proteins are not transported to other parts of the cell In the cytosol it is marked by ubiquitination and degraded in the proteasome.

The major pathway of selective protein degradation in eukaryotic cells uses ubiquitin as a marker that targets misfolded proteins in cytosol for rapid proteolysis. Ubiquitin is a 76-amino-acid polypeptide that is highly conserved in all eukaryotes (yeasts, animals, and plants). Proteins are marked for degradation by the attachment of ubiquitin to the amino group of the side chain of a lysine residue. Additional ubiquitins are then added to form a multiubiquitin chain. Such polyubiquinated proteins are recognized and degraded by a large, multisubunit protease complex, called the proteasome. Ubiquitin is released in the process, so it can be reused in another cycle. Both the attachment of ubiquitin and the degradation of marked proteins require energy in the form of ATP.

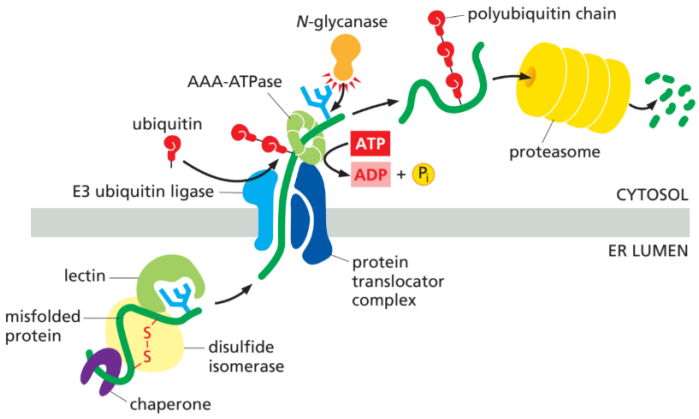


Fig: The export and degradation of misfolded ER proteins

FUNCTIONS OF SMOOTH ER

- Lipid Synthesis

SER is the major site at which membrane lipids are synthesized in eukaryotic cells. The membranes of eukaryotic cells are composed of three main types of lipids: phospholipids, glycolipids, and cholesterol. Most of the phospholipids, which are the basic structural components of the membrane, are derived from glycerol. They are synthesized in association with already existing cellular membranes on the cytosolic side of the ER membrane from water-soluble cytosolic precursors.

1. Fatty acids are first transferred from coenzyme A carriers to glycerol-3-phosphate by membrane-bound enzymes, and the resulting phospholipid (phosphatidic acid) is inserted into the membrane.
2. Enzymes on the cytosolic face of the ER membrane then either modify phosphatidic acid or directly catalyze the addition of different polar head groups, resulting in formation of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, or phosphatidylinositol. The synthesis of these phospholipids on the cytosolic side of the ER membrane allows the hydrophobic fatty acid chains to remain buried in the membrane while membrane-bound enzymes catalyze their reactions with water-soluble precursors

(e.g., COP-choline) in the cytosol. Because of this topography, however, new phospholipids are added only to the cytosolic half of the ER membrane

3. To maintain a stable membrane some of these newly synthesized phospholipids must therefore be transferred to the other (lumenal) half of the ER bilayer. This transfer does not occur spon taneously because it requires the passage of a polar head group through the membrane. Instead, membrane proteins called flippases catalyze the rapid translocation of phospholipids across the ER membrane resulting in even growth of both halves of the bilayer. At least some flippases are phospholipid-specific and ATP-dependent. In addition to its role in synthesis of the glycerol phospholipids, the ER also serves as the major site of synthesis of two other membrane lipids: cholesterol and ceramide.

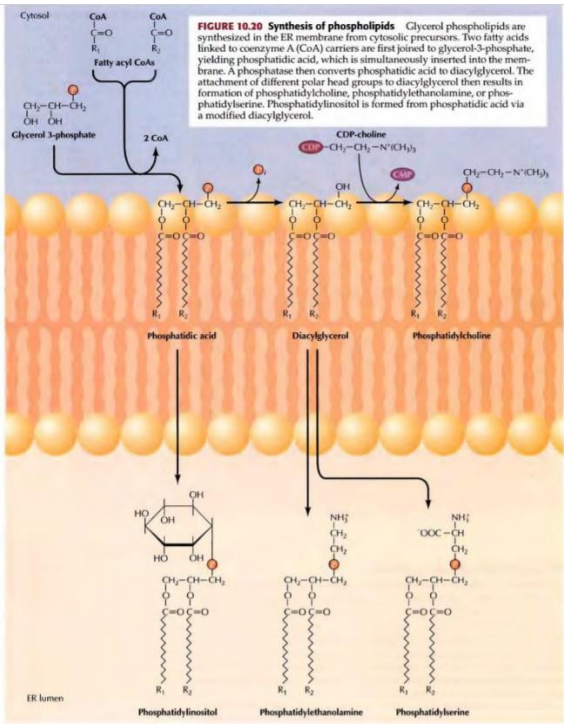


FIGURE 10.20 Synthesis of phospholipids Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors. Two fatty acids linked to coenzyme A (CoA) carriers are first joined to glycerol-3-phosphate, yielding phosphatidic acid, which is simultaneously inserted into the membrane. A phosphatase then converts phosphatidic acid to diacylglycerol. The attachment of different polar head groups to diacylglycerol then results in formation of phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine. Phosphatidylinositol is formed from phosphatidic acid via a modified diacylglycerol.

- **Synthesis of steroid hormones** in the endocrine cells of the gonad and adrenal cortex.
- **Detoxification**: In the liver of a wide variety of organic compounds, including barbiturates and ethanol, whose chronic use can lead to proliferation of the SER in liver cells. Detoxification is carried out by a collection of oxygen transferring enzymes (oxygenases), including the cytochrome P450 family. These enzymes are noteworthy for their lack of substrate specificity, being able to oxidize thousands of different hydrophobic compounds and convert them into more hydrophilic, more readily excreted derivatives. The results are not always positive. For example, the relatively harmless compound benzo[a]pyrene formed when meat is charred on a grill is converted into a potent carcinogen by the “detoxifying” enzymes of the SER. Cytochrome P450s metabolize many prescribed medications, and genetic variation in these enzymes among humans may explain differences from one person to the next in the effectiveness and side effects of many drugs.
- **Sequestering calcium ions** within the cytoplasm of cells. The regulated release of Ca2 from the SER of skeletal and cardiac muscle cells (known as the sarcoplasmic reticulum in muscle cells) triggers contraction.