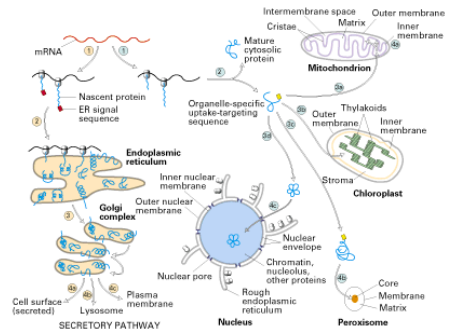


Biogenesis Posttranslational modifications

The synthesis of proteins has two divergent branches



Secretory pathway – a way to the surface

- › Translation on ribosomes attached to ER with simultaneous translocation to the ER
- Posttranslational modifications in the ER
- Transfer to Golgi
- Modifications in Golgi
- Exocytosis

Posttranslational modifications

- Proteins have to be modified to assume their final structure and function
- Ribosome and translocon “shelter” about 70 amino acids
- As soon as new polypeptide gets longer it is subjected to ER modifying enzymes
- Polypeptide (future protein) does not have to be finished to be modified!!!

Posttranslational modifications in the ER

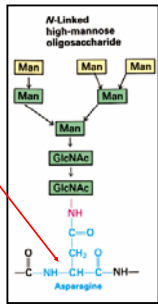
- Removal of signal peptide
- Addition of carbohydrates – glycosylation
- Addition of GPI anchors
- Folding
 - Disulfide bond formation
- Formation of multimeric proteins
- **Only properly modified proteins are transported from ER to the final destination**

Protein glycosylation

- Most plasma-membrane proteins contain one or more carbohydrate chains
 - Reduce aggregation
 - Influence folding
- Glycosylation occurs both in the ER and Golgi
 - N-linked glycosylation in ER
 - O-linked glycosylation in Golgi

N-linked oligosaccharides

- Sugars added as a large preformed oligosaccharide from a carrier **dolichol**
- Always added to asparagine as soon as Asn appears inside ER
- Addition occurs co-translationally

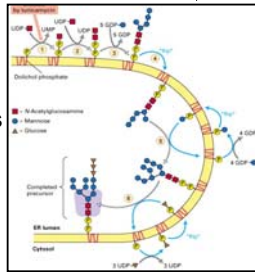


Processing of N-linked oligosaccharides in the ER

- Next additions, deletions and rearrangements of sugars
- Finished in Golgi

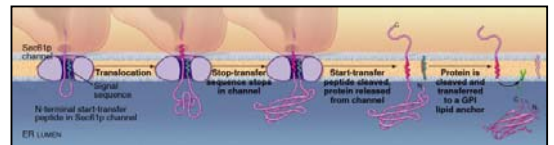
Dolichol

- Long chain lipid (75-95 carbons) strongly bound to the membrane
- Addition of sugars happens initially in the cytosol
- The molecule “flips” addition of sugars continues inside ER
- Oligosaccharide is transferred to Asn, dolichol stays in the membrane



Addition of GPI anchor

- Some proteins are cleaved at special consensus motifs and transferred into GPI (glycosylphosphatidylinositol) anchor

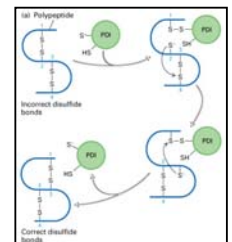


Disulfide bonds

- Oxidative linkage of sulfhydryl groups on Cys
- Help stabilize tertiary and quaternary structure
- Proper pairing of disulfide bonds is essential for normal activity
- Form inside ER (never inside the cytoplasm)

Rearrangement of S-S bonds

- Disulfide bonds can be rearranged in the cell
- Performed by protein disulfide isomerases (PDIs)



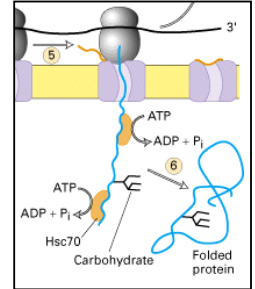
Protein folding

- Secretory proteins generally fold in the ER
- ER contains several proteins that facilitate folding
 - Chaperones
 - Lectins (calnexin)
 - Peptidylprolyl isomerases



Chaperones facilitate folding in the ER

- Similar mechanism to cytoplasmic chaperones
- Major one is BiP a homolog of Hsp70



Formation of multimeric proteins

- Occurs prior to export to Golgi
- Oligomerization occurs by self-assembly
- Involves chaperones that protect hydrophilic surfaces until contact is possible (subunits may be products of different genes and maybe translated with different speed)



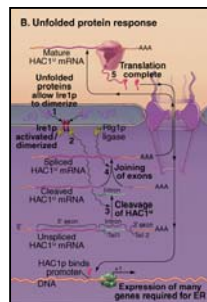
Folding – quality control

- Only properly folded proteins can be transported from rough ER to Golgi
- Misfolded or unassembled proteins are retained in the ER bound to chaperones or lectins
- They are degraded or transported back to cytosol for degradation



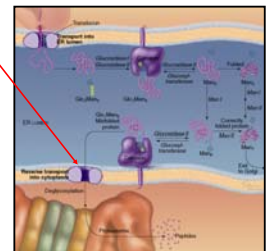
Unfolded protein response a.k.a. ER overload response

- Increase of transcription of chaperones and other folding catalysts
- Activated by transmembrane protein (IRE1) in the inner nuclear membrane
- IRE1 is dimerized by unfolded proteins and activates transcription factors for chaperones



Cytoplasmic degradation pathway

- Misfolded or unfolded proteins are transported back through translocon and degraded in the ubiquitin-proteasome pathway



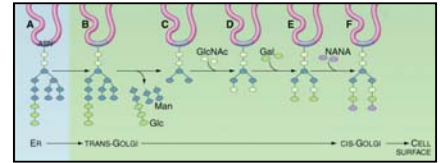
Protein processing in Golgi

- Properly folded and modified proteins will move to Golgi
- More post-translational modifications
 - Further N-linked oligosaccharide processing
 - O-linked glycosylation
 - Proteolytic cleavage
- Sorting and packaging for delivery
- Proteins are transported to the surface in secretory vesicles



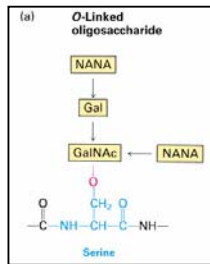
Modifications of N-linked oligosaccharides in Golgi complex

- Modifications to N-linked oligosaccharides are completed in Golgi
- Oligosaccharides promote folding and stability of glycoproteins



O-linked oligosaccharides

- Sugars added one at a time
- Sugars are sequentially transferred from nucleotide precursors by glycosyltransferases



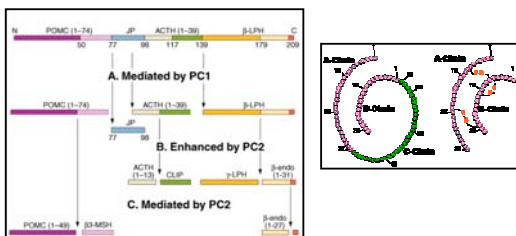
Proteolytic cleavage

- Late in maturation
- Can be as simple as removal of signal sequence or
- More complicated to activate the inactive precursor



Proteolytic cleavage

- Prohormone convertases



Organelle biogenesis

- Cell synthesize new membranes only by expansion of existing membranes
- Lipids and cholesterol can only be synthesized in association with membranes
 - Phospholipids – ER
 - Sphingolipids – Golgi
 - Some other (cardiolipin) – mitochondria



Synthesis of phospholipids

- All enzymes reside in the ER membrane with the active sites facing the cytosol (where the substrates are found)
- Synthesis occurs ONLY on the cytosolic surface



Synthesis of membrane lipids

1. Two fatty acids are added to glycerol 3-phosphate to produce phosphatidic acid (acyl transferases)
2. Phosphatase and phosphotransferase attach head groups

This step enlarges lipid bilayer

This step determines the chemical nature of the bilayer



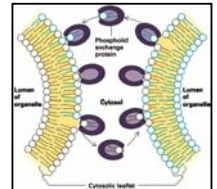
Translocation of newly formed phospholipids between leaflets

- Special molecules catalyze the translocation between leaflets (flip-flop)
- Phospholipid translocators
 - Are head group specific
 - Flippase - choline specific translocator



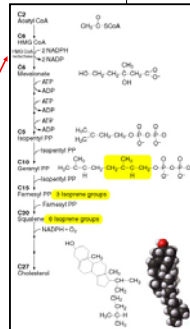
Phospholipids move from ER to other organelles

- Assisted by the phospholipid exchange protein
 - Specific for a phospholipid
- Extracts phospholipid from the membrane and transports to the destination place buried inside and protected from aqueous environment



Cholesterol synthesis

- Synthesized if not delivered
- In the ER membranes
- 22 steps
- Regulated step is HMG-CoA reductase
 - Regulated by cholesterol levels in blood
 - Target for cholesterol lowering drugs



Way to the surface

- Properly folded, properly modified proteins are secreted to the surface in secretory vesicles in the process of exocytosis

