Day 11 (Cell Biology)

Only properly folded proteins can leave ER.

- to exit from ER, proteins must be properly folded and assembled
- misfolded or incomplete → transiently bound to chaperones → escorted to cytosol → degraded in proteosomes
- failures surprisingly common → most of the newly synthesised subunits of T cell receptor and acetylcholine receptor are normally degraded.
- drawbacks of stringent control — mutated, but potentially active Cl^- transporter destroyed in cystic fibrosis

Vesicular tubular clusters mediate transport from ER to Golgi Apparatus.

- after shedding coat, membranes fuse with one another, — homotypic and heterotypic.

Homotypic fusion - fusion of membranes from same compartment
↳ set of matching SNAREs reqd.

e.g.,
Vesicular tubular clusters - formed when ER-derived vesicles fuse with one another
↳ function as transport containers that bring material from ER to Golgi

- As soon as they form, clusters bud off transport vesicles
- COPI coated;
  formed of coatomers — composed of components that make up inner and outer layers as preassembled units
- function as retrieval pathway carrying back escaped ER resident proteins, cargo receptors and SNAREs.
- COPI assembly starts only few seconds after COPII is shed
  - mechanism unknown

Retrieval Pathway

- retrieval pathway to ER uses sorting signals (ER retrieval signals)
- Resident ER memb proteins have retrieval sequences like KKXX at their extreme C-Terminal
- Lumen proteins have KDEL motif, to return to ER. To leave ER, retrieval sequence must be cleaved off.
  ↳ If the KDEL sequence is removed from a protein, it is slowly secreted from the cell.
- affinity of the KDEL receptor increases in Golgi due to pH sensitive interactions.

The Golgi Apparatus

- collection of flattened, membrane enclosed compartments called cisternae (often connected by tubular connections)
- localised near nucleus and centrosome, connected by microtubules

- generates the heterogenous oligosaccharide str. seen in mature proteins

⇓

proteins in cis Golgi network (CGN) ← multistage processing unit

↓

medial cisterna

↓

trans cisterna (glycosylation is completed)

- resident proteins are all memb-bound
- 2 broad classes of N-linked oligosaccharides

complex (formed when original N-linked sugar is trimmed & new sugars are added)

high-mannose (trimmed but no new sugars are added)

Whether a given oligosaccharide remains high mannose depends on its position in the protein

↓

if it is inaccessible to processing enzymes because sugars are tightly linked to surface → likely to remain in high-mannose form.

Proteoglycans are assembled in Golgi

- often sugar is added to hydroxyl group of serine and threonine (O-linked glycosylation)
  - ↳ use sugar nucleotides in lumen of Golgi to add sugars

to protein one at a time.

- heaviest O-linked glycosylation conferred on mucins & on proteoglycan core proteins to form proteoglycans.
  1. polymerization of one or more glycosaminoglycan chains onto serines of a core protein.

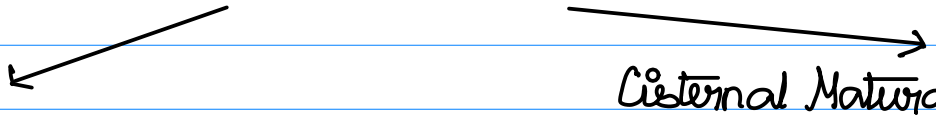
Many proteoglycans are secreted and become components of ECM, while others remain anchored to extracellular surface of memb., and others are secreted as mucus

- sugars incorporated into glycosaminoglycans are heavily sulfated in the Golgi apparatus immediately after these polymers are made (adding negative charge)

↓

sulfation depends on sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) which is transported from cytosol into lumen of Trans-Golgi network

Transport Through the Golgi Apparatus Occurs by Multiple Mechanisms



Vesicle Transport Mechanism

Cisternal Maturation Mechanism

Cisternal Maturation Mechanism

- new cis cisternae continually form as vesicular tubular clusters arrive from the ER & fuse with Golgi transport vesicles

In this way, a cisterna full of cargo moves through the Golgi stack while different subsets of Golgi resident proteins transit backwards in COPI-coated vesicles from later to earlier cisternae.

- Golgi matrix proteins (golgins) help org. stack.

Transport from Golgi Apparatus to Cell Exterior & Endosomes

Proteins that mature from Trans Golgi Network → go either via exocytosis
or to endosomes → lysosomes
late endosesome → endolysosome → lysosome

How does the Golgi Network sort proteins b/w these pathways?

Mannose-6-Phosphate receptor sorts lysosomal hydrolases

- vesicles that leave the TGN for endosomes incorporate lysosomal proteins & exclude others.

How?

M6P groups are added exclusively to the N-linked oligosacch of these hydrolases as they pass through lumen of CGN

↓

Transmembrane M6P receptors in TGN bind to them + adaptor proteins assembling clathrin coats on cytosolic side

↓

Hydrolases packaged into clathrin coated vesicles and delivered to early endosomes.

M6P binds to receptor at pH 6.5-6.7 in TGN and releases it at pH 6 in endosomes.
Receptors are retrieved by retromers in vesicles for reuse.

For this sorting system to work, M6P must be added only to hydrolases — hydrolase N-linked sugars are detected by accumulation of cluster of amino acids called signal patch.

Lysosomal Storage Disease

- caused by genetic defects affecting lysosomal hydrolases
  ↓
  result in accumulation of undigested substrates in lysosomes

Most severe form- inclusion-cell disease (I-cell disease)

- most of the hydrolytic enzymes are missing
- due to a single gene defect
- all hydrolases missing from lysosome found in blood — sorting problem (secreted instead)
- due to defective or missing GlcNAc phosphotransferase
  ↓
  Lysosomal enzymes not phosphorylated ⇒ not sorted by M6P receptors ⇒ secreted by default

- M6P-independent pathway sorts memb. proteins of

lysosomes from TGN to late endosomes, those proteins are normal in I-cell disease

Formation of Intralumenal Vesicles

As endosomes mature, patches of their membrane invaginate into the endosome lumen & pinch off to form intralumenal vesicles. Hence multivesicular bodies.

Sorting into intralumenal vesicles requires ubiquitin tags:

One or more ubiquitin tags added to cytosolic domain of memb. proteins

↓

Tags help guide proteins into clathrin-coated vesicles in the plasma memb

↓

Recognised again in endosomal memb. by ESCRT protein complexes

↓

sorting into intralumenal vesicles

* Viruses such as HIV bud directly out of cell by hijacking ESCRT machinery