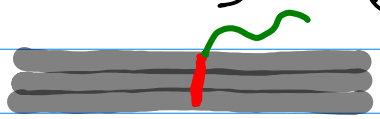
Cell Biology (Day 9)

① Some proteins are integrated into the memb. via post-translational mechanism.

- many important cytosol-facing memb proteins are anchored in the memb. by a single α-helix (transmembrane) very close to the C-terminus.



↳ Tail anchored proteins

- include a large no. of SNARE protein subunits that guide vesicular traffic

- while translational, ribosome reaches termination codon while protein sequence destined to become α-helix is still in ribosome-exit tunnel

↓

recognition by SRP is not possible, & protein is released from ribosome to cytosol

↓

Hydrophobic segment is recognised by specialised chaperone complex that transfers it to a targeting factor called Get 3

↓

Get 3 has flexible hydrophobic methionine pocket to recognise diverse hydrophobic segments

↓

2 proteins Get 1 and Get 2 function as translocator that inserts hydrophobic segments into lipid bilayer

② GPI Anchor

- For some proteins destined for plasma membrane, glycophosphatidylinositol (GPI) anchor — covalently linked to C-Terminus → for attachment to membrane

- initially made with N-Terminal sequence for guidance to ER & hydrophobic segment close to C Terminus

↓

Hydrophobic segment recognised by Transamidase enzyme — cleaved off and GPI anchored protein

↓

because they are attached to memb. by only GPI anchor, they can released from cell in soluble form

- GPI anchors also participate in directing some proteins into lipid rafts — laterally segregating them from other memb. proteins.

③ Protein Glycosylation

- 3 types of glycosylation: N-linked, O-linked, GPI-anchored

- 90% are N-linked (in ER)
- O-linked (Golgi body)
- GPI-anchored (ER)

(4) N-linked oligosaccharide

- glycosylation — one of the major funcs of the ER
- half of proteins processed in ER - glycoproteins
- some proteins in cytosol & nucleus - N-acetylglucosamine added to a serine or threonine of protein
- During most common form of glycosylation,

oligosaccharide (14 sugars - 2 N-acetylglucosamine, 9 mannose, 3 glucose)
transferred as complete unit to proteins

↳ N-linked, or, asparagine-linked because transferred to NH_2 subchain of asparagine

- lipid molecule dolichol anchors precursor oligosaccharide in ER membrane

↓

Transferred to asparagine by oligosaccharyl transferase

> Memb-bound enzyme-associates with Sec61-translocator - active side exposed on lumenal side, modifies newly made proteins immediately after asparagine enters ER lumen

- oligosaccharide precursor built sugar by sugar on dolichol
- found on 90% of all glycoproteins

Formation:

(a) Sugars first activated in cytosol by formation of nucleotide (UDP or GDP)-sugar intermediates

(b) they donate their sugar first to dolichol & then to the partially assembled oligosaccharide in an orderly sequence

(c) Partway through this process, Lipid-linked oligosaccharide is flipped — with help of a transporter, from the cytosolic to the lumenal side of ER membrane

(5) Role of glycosylation

1. Help in protein folding
2. Stability
3. Reduces proteolysis
4. Directs protein-protein interaction & recognition
5. Directs protein trafficking

(6) Oligosaccharides as tags to mark state of protein folding

- some proteins require N-linked glycosylation for proper folding in ER
- precise location of oligosaccharide on protein surface does not matter

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2 ER chaperone proteins — calnexin & calreticulin (lectins) & bind to oligosaccharides on incompletely folded proteins and retain them in ER

- prevent incompletely folded proteins from irreversibly aggregating

- promote association of incomplety folded proteins with another ER chaperone

How are incompletely folded proteins distinguished from properly folded ones?

Two enzymes: ER glucosidase and glucosyl Transferase
(glucose trimming) (glucose addition)

ER glucosidase removes terminal glucose from oligosaccha
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Protein no longer bound to calnexin or calreticulin
↓
glucosyl Transferase adds terminal glucose if improperly folded
↓
Reassociation with ER chaperones calnexin & calreticulin
↓
Cycle continues until protein properly folded

⑦ Improperly folded proteins:

- some proteins fail to achieve properly folded state
↓
exported back from ER to cytosol (Translocator necessary)
↓
degraded in proteasomes

How to select misfolded proteins?

- N-linked oligosaccharides act as a measure of how long protein has spent in ER

Mannosidase slowly trims core mannose.

↳ If succesful, protein degraded.

Protein that can escape ER before mannosidase can act on it → escapes degradation.

For passing through translocator, protein must be unfolded. Ensured by chaperones which prevent aggregation and disulfide isomerases that break incorrect disulfide bonds

- E3 ubiquitin ligase enzyme adds polyubiquitin tags to unfolded proteins to mark for destruction.

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N-glycanase deglycosylates

↓

Degraded in proteasomes.

⑧ Unfolded Protein Response

- accummulation of misfolded proteins in cytosol → heat shock resp.
  in ER

↓

Unfolded protein response

→ Transcription of genes that collectively improve protein folding capacity of ER

↓

code for chaperones, machinery for protein translocation and degradation, proteins transporting out of ER & ER expansion