Overview: Protein synthesis and sorting in the secretory pathway

2 classes of proteins:
- Membrane
- Exoplasmic soluble

Synthesis and sorting of secreted and membrane proteins

Outline: 3 parts
1. Synthesis of secreted and membrane proteins
   b. How are proteins targeted to ER? Recognition and binding. SRP, SRP receptor
   c. How do proteins cross the membrane? Transport via translocon
   d. Energy source? ATP and chaperones. What regulates the process?
2. Modification of proteins to their mature and active form. E.g. glycosylation
   Improperly folded proteins are exported from ER and degraded in the cytosol
3. Mechanism and regulation of sorting and vesicular transport.
   Soluble & membrane-bound proteins have different targeting signals
   Movement of vesicle/tubules from ER -> Golgi -> Vac or PM.
   Vesicle budding, vesicle transport, and membrane fusion

17-16. Big picture - synthesis of secreted proteins
17-18, 17-19. Protein cross ER via a translocon powered by GTP
17-20. Energy from GTP is required for binding and protein insertion
   GTP/GDP switch affinity of SRP and of SRP-receptor.

A mutation here would give ?? Phenotype in yeast.
Freeze fracture picture of reconstituted translocon

17-21. Membrane proteins with 1 or more TM. How are they inserted in the ER?

17-22. Stop transfer sequence anchors type I protein to membrane

17-24. Insertion of transporter with multiple TM segments 1 signal/anchor sequence & stop-anchor sequences

Post-translational Modifications in the ER
1. Formation of S-S bonds
2. Folding
3. Addition and processing of carbohydrates
4. Proteolytic cleavages
5. Assembly into multimeric proteins

Misfolded proteins are discarded.

Approaches to study synthesis and insertion
1. Cell Biology-
   b. Transfection: Follow GFP-protein dynamics in living cells
3. Genetic approach: Identify players and their roles in living cells using yeast mutants defective in sorting and secretion
4. Combination of cell biol., biochem, and molecular genetics
Follow synth of proteins after In vivo labeling

17-12. Lodish. Determine Location of protein after synthesis.
- Cells labeled with 14C-Leu
- Isolate RER
- Test location of protein:
  Protease sensitivity

In vivo targeting and trafficking

Follow movement of GFP-tagged protein in a living cell.

Genetic Approach

17-14. Yeast mutants that are defective in secretion at nonpermissive temperature
- Class A
  - Transport, including ER
  - Accumulation in ER
- Class B
  - Building of invertase in ER
  - Transient accum. in cis G and cis G + ER
- Class C
  - Accumulation in cis G
- Class D
  - Accumulation in cis G + ER
- Class E
  - Accumulation in cis G + ER
  - Transient accum. in cis G + ER + cell surface

How do ER proteins stay in the ER?
ER retention signal-KDEL

17-29. ER-resident proteins are retrieved from the Golgi by KDEL receptors

invertase