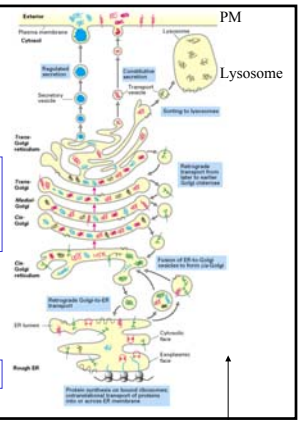
17-13. Overview: Protein synthesis and sorting in the secretory pathway



2 classes of proteins:
-Membrane
-exoplasmic soluble

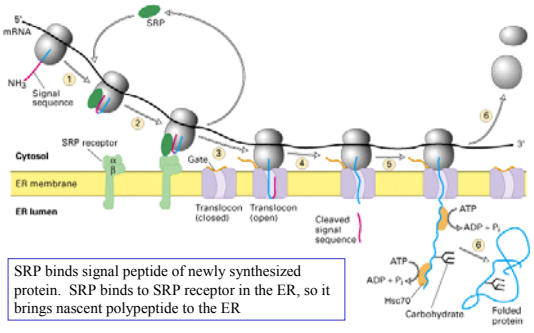
Synthesis and sorting of secreted and membrane proteins
[Lodish 2000, ch 17; Alberts 2002, ch. 12
Outline: 3 parts

1. Synthesis of secreted and membrane proteins
a. Overview. Expt. Approach
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

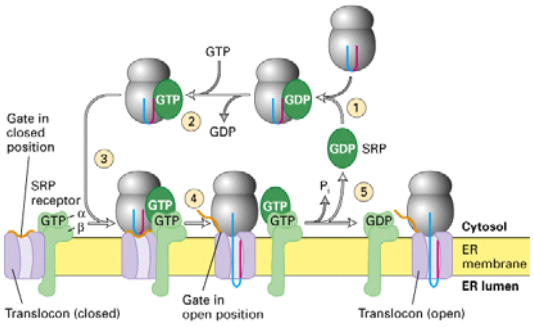
17-16. Big picture- synthesis of secreted proteins



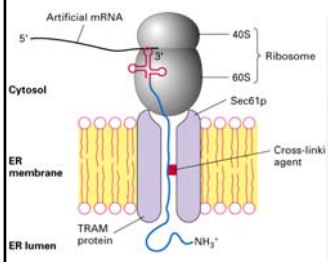
SRP binds signal peptide of newly synthesized protein. SRP binds to SRP receptor in the ER, so it brings nascent polypeptide to the ER

17-20. Energy from GTP is required for binding and protein insertion

GTP/GDP switch affinity of SRP and of SRP-receptor.



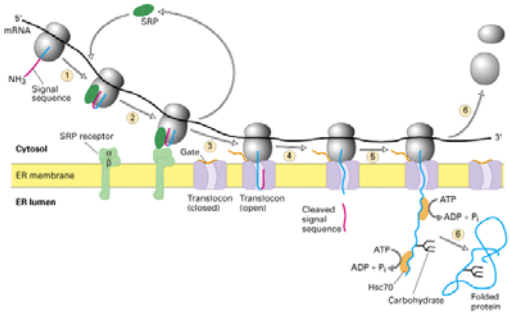
17-18, 17-19. Protein cross ER via a translocon powered by GTP



A mutation here would give ?? Phenotype in yeast.

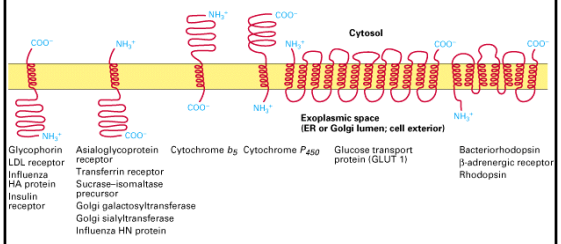
Freeze fracture picture of reconstituted translocon

17-16. Driving force for transport. Chaperones and ATP.
Chaperones prevent misfolding and are essential for translocation.



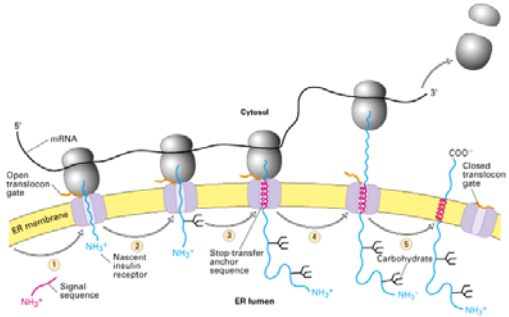
17-21. Membrane proteins with 1 or more TM.

How are they inserted in the ER?

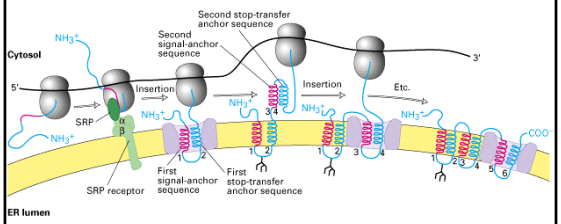


A B How do A or B get into the membrane?

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



After synthesis, some proteins are then modified in the ER to their mature, active state. How?

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.

Unfolded proteins are exported and degraded

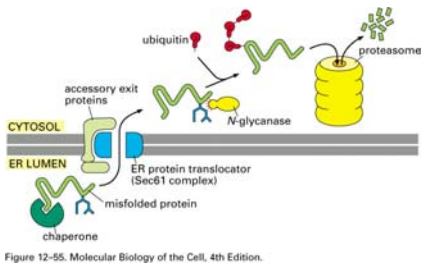


Figure 12-55. Molecular Biology of the Cell, 4th Edition.

Approaches to study synthesis and insertion

1. **Cell Biology-/**

a. In vivo: Label protein **in cells** & follow its fate: Pulse-chase
-follow protein by microscopy- EM
-follow protein by cell fractionation, Immuno-ppte

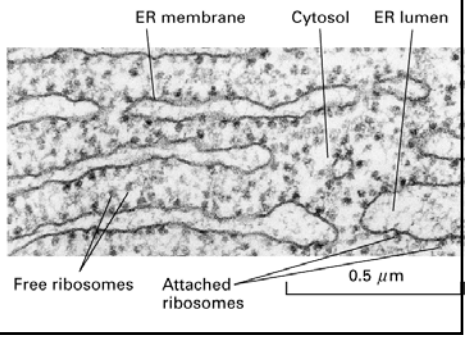
b. Transfection: Follow **GFP-protein** dynamics in **living cells**

2. **Biochemistry**. **In vivo & in vitro** expts: synthesize and label protein in vivo or in vitro using isolated ER membranes and test its fate . Identify players and understand their roles.

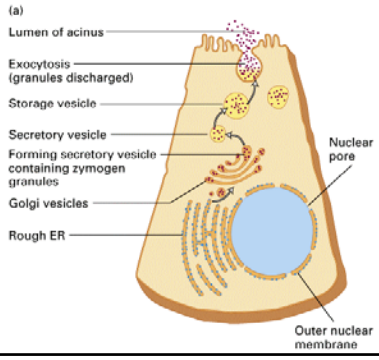
3. **Genetic approach**: Identify players and their roles in living cells using yeast mutants defective in sorting and secretion

4. Combination of cell biol., biochem, and molecular genetics

17-11. Pancreatic cell synthesizes and secretes digestive enzymes



5-48. Pancreatic Acinar cell secretes digestive enzymes



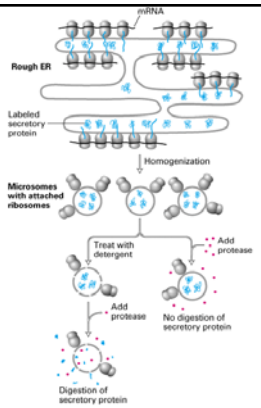
Follow synth of proteins after In vivo labeling

17-12. Lodish. Determine Location of protein after synthesis.

•Cells labeled with 14C-Leu

•Isolate RER

•Test location of protein: Protease sensitivity

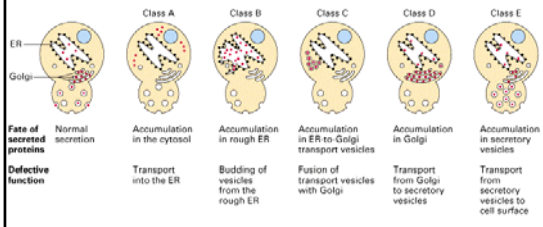


In vivo targeting and trafficking

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

Genetic Approach

17-14. Yeast mutants that are defective in secretion at nonpermissive temperature



invertase

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