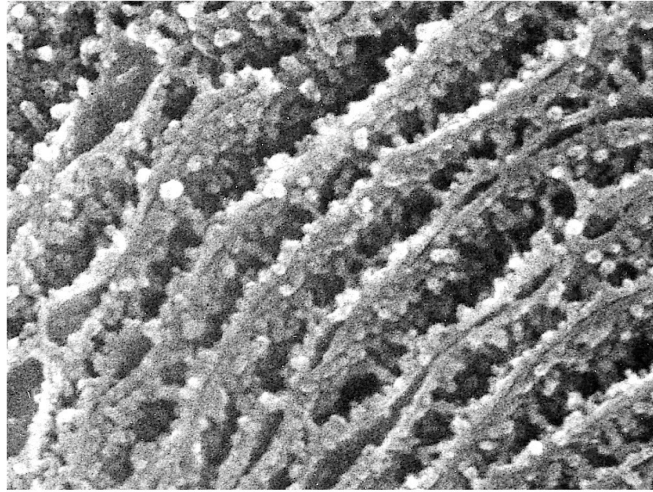


Protein Sorting: Endoplasmic Reticulum



RER in pancreatic acinar cell

1

Movement of Proteins Between Compartments

1. **Gated transport:** nucleus
2. **Transmembrane transport:** mitochondria and ER
3. **Vesicular transport:** secretory pathway

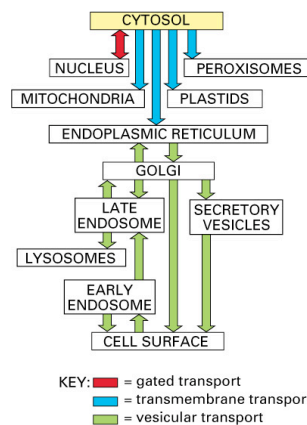


Figure 12-6. Molecular Biology of the Cell, 4th Edition.

2

Protein Sorting to the ER

- ER is a network of branching tubules.
- A membrane separates ER lumen from cytosol and mediates transfer of proteins.
- Major site of protein and lipid biosynthesis.
- Transmembrane vs. water-soluble proteins.**
- Cotranslational translocation** (primarily).
- Many of the cell's proteins must first pass through the ER.

co-translational: the ribosome that is synthesizing the protein is attached directly to the ER membrane, enabling one end of the protein to be translocated into the ER while the rest of the polypeptide chain is being assembled.

the ER captures selected proteins from the cytosol as they are being synthesized. these proteins are of 2 types: transmembrane proteins, which are only partly translocated across the ER membrane and become embedded in it, and water-soluble proteins, which are fully translocated across the ER membrane and are released into the ER lumen.

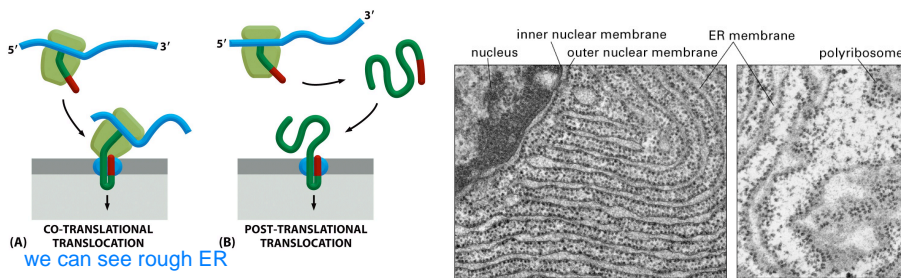


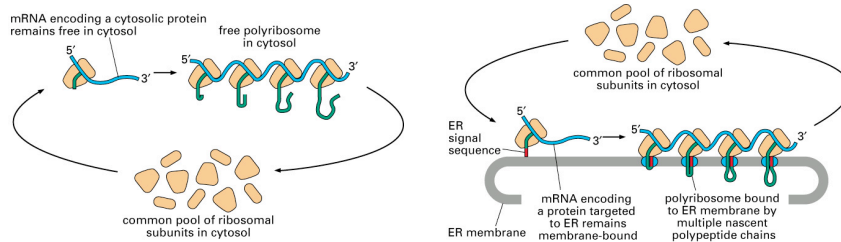
Figure 12-35 Molecular Biology of the Cell (© Garland Science 2008)

Role of the Ribosome

- Ribosomes are protein complexes that associate with mRNA and catalyze synthesis of protein.
- Ribosomes are either *free* (cytosolic) or *membrane-bound* (i.e. rough ER).
- Ribosome with polypeptide chain will be directed to ER membrane only if the appropriate signal sequence is present (otherwise it will remain in cytosol).

actually they are same ribosome from the same common pool. free can become membrane-bound

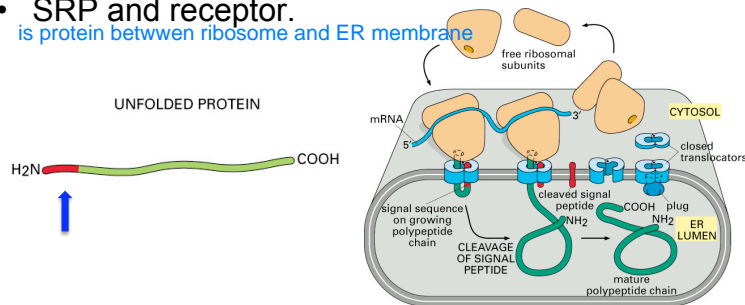
a common pool of ribosomes synthesizes the proteins that stay in the cytosol and those that are transported into the ER. the ER signal sequence on a newly formed polypeptide chain binds to SRP, which translates ribosome to the ER membrane. the mRNA molecule remains permanently bound to the ER as part of a polyribosome, while the ribosomes that move along it are recycled; at the end of each round of protein synthesis the ribosomal subunits are released and rejoin the common pool in the cytosol.



Signal Sequence

- As described for mitochondrial translocation, a signal sequence guides polypeptide chain to ER.
- Cleaved by *signal peptidase*.
- However, it's a little more complicated than that.
- SRP and receptor.

is protein between ribosome and ER membrane



SRP = signal recognition particle

Figure 12-40. Molecular Biology of the Cell, 4th Edition.

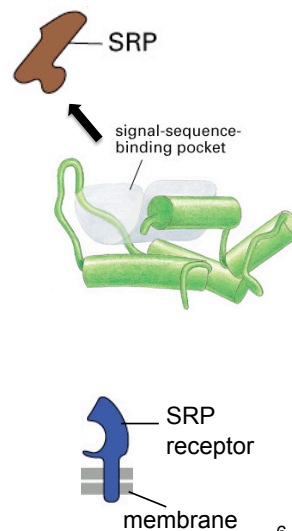
the signal sequence in the growing polypeptide chain is thought to trigger the opening of the pore in the protein translocator: after the signal sequence is released from the SRP and the growing chain has reached a sufficient length, the signal sequence binds to a specific site inside the pore itself, thereby opening the pore. an ER signal sequence is therefore recognized twice: first by an SRP in teh cytosol and then by a binding site in the pore of the protein translocator.

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cycles between the ER membrane and the cytosol and binds to the signal sequence, and an SRP receptor in the ER membrane

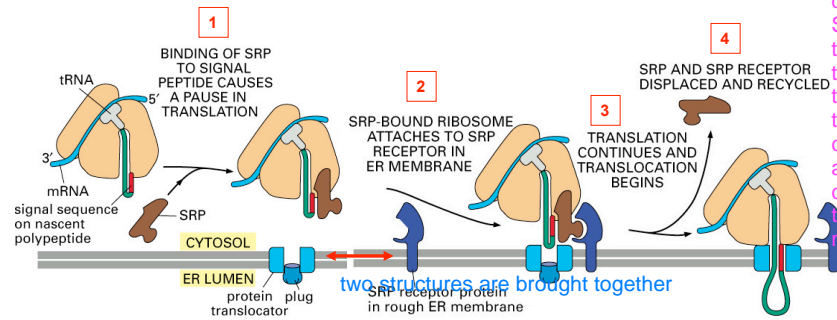
Signal Recognition Particle

- SRP cycles between ER membrane and cytosol.
- Reversibly binds to ribosome and SRP receptor.
- SRP binding site is a **methionine-rich "pocket"** that accommodates many signal sequence types.
- SRP receptor is in ER membrane.



6

Ribosomes are Directed to the ER Membrane



Components: ribosome, tRNA, mRNA, growing polypeptide with SS

the SRP and its receptor are thought to act in concert. the SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. the SRP receptor in the ER membrane, which is composed of 2 different polypeptide chains, binds the SRP-ribosome complex and directs it to the translocator. the SRP and SRP receptor are then released, leaving the ribosome bound to the translocator in the ER membrane. the translocator then inserts the polypeptide chain into the membrane and transfer it across the lipid bilayer.. the translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all time

7

Sec61 Protein Translocator

- Sec61 complex forms an aqueous pore in ER membrane.
- Aligns with nascent polypeptide.
- Signal sequence triggers opening of pore after its release from SRP.

Sec 61 forms the walls of the pore

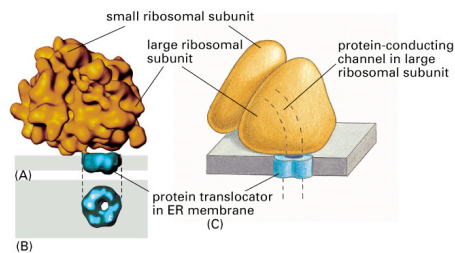


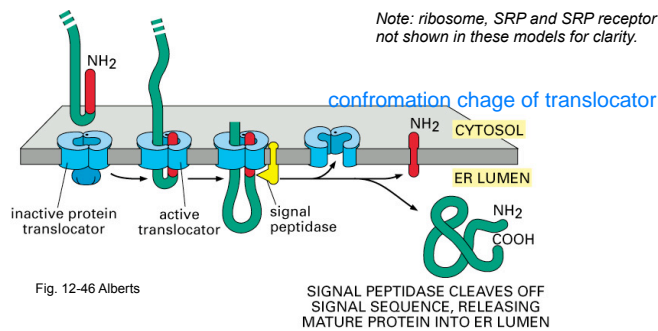
Figure 12-43. Molecular Biology of the Cell, 4th Edition.

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Translocation of Soluble Proteins

- Signal sequence is a “start-transfer signal”.
- Signal sequence is cleaved by a peptidase at a specific cleavage site.
- Release of polypeptide (to lumen) and SS (to membrane).

on binding an ER signal sequence (which acts as a start-transfer signal), the translocator opens its pore, allowing the transfer of the polypeptide chain across the lipid bilayer as a loop. after the protein has been completely translocated, the pore closes, but the translocator now opens laterally within the lipid bilayer, allowing the hydrophobic signal sequence to diffuse into the bilayer, where it is rapidly degraded



9

Single-Pass Transmembrane Proteins

(3 Models)

- A “stop-transfer signal” will bind to the translocon and prevent further translocation.
- Released into the membrane as **an α helix.**

an N-terminal signal sequence initiates translocation, just as for a soluble protein, but an additional hydrophobic segment in the polypeptide chain stops the transfer process before the entire polypeptide chain is translocated. this stop-transfer signal anchors the protein in the membrane after the ER signal sequence has been released from the translocator and has been cleaved off. the lateral gating mechanism transfers the stop-transfer sequence into the bilayer, and it remains there as a single alpha-helical membrane-spanning segment, with the N-terminus of the protein on the luminal side of the membrane and the C-terminus on the cytosolic side

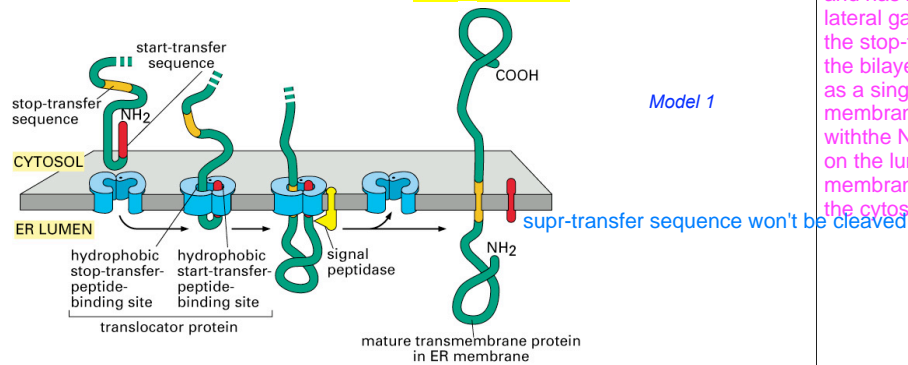
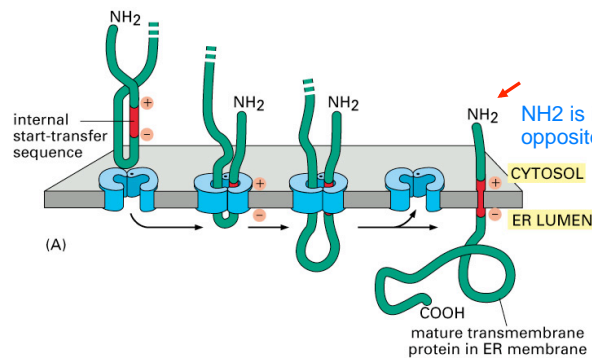


Figure 12-47. Molecular Biology of the Cell, 4th Edition.

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- Internal signal (start) sequence is recognized and initiates translocation.
- α helix not cleaved but will remain in membrane.
- **Final orientation of the protein is determined by the polarity of the SS.
- (-) end of SS always enters the translocon first.



if the signal sequence is internal, rather than at the N-terminal end of the protein, the SRP also binds to it. the SRP brings the ribosome making the protein to the ER membrane and serves as a start-transfer signal that initiates the protein's translocation. after release from the translocator, the internal start-transfer sequence remains in the lipid bilayer as a single membrane-spanning alpha helix

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- Entry of (-) end of SS causes reorientation of the growing polypeptide chain

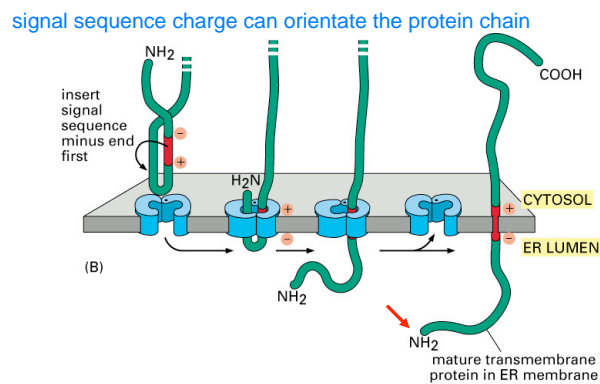


Figure 12-48 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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Double-Pass Membrane Proteins

- Internal signal sequence initiates translocation.
- Stop-transfer signal stops translocation.
- Both sequences are α helices and are released to the membrane.

it is thought that an internal signal sequence serves as a start-transfer signal in these proteins to initiate translocation, which continues until the translocator encounters a stop-transfer sequence

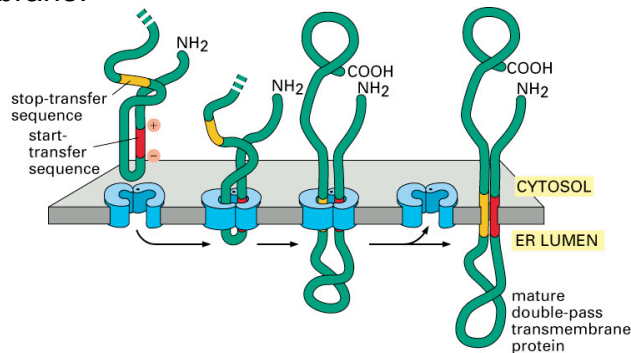


Figure 12-49. Molecular Biology of the Cell, 4th Edition.

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Multipass Membrane Insertion

- Insertion of further hydrophobic protein segments is **intermittently reinitiated by additional start and stop transfer signals.**
- Segments bind to the same translocon and are released as α helices to the membrane.
- e.g. insertion of **rhodopsin** into the ER membrane.

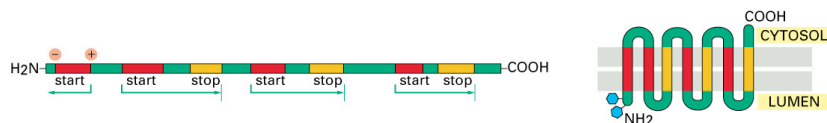
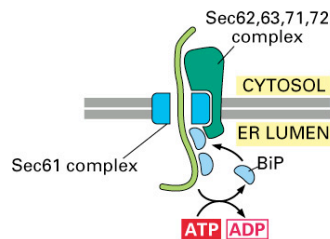


Fig. 12-50 Alberts

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Posttranslational Translocation

- Not all proteins are translocated to the ER lumen by a cotranslational mechanism.
- Those formed by free ribosomes are translocated by a posttranslational mechanism.
- In eukaryotes, accessory proteins (receptors) associate with Sec61 and span the ER membrane.
- Molecular chaperones (BiP) bind to polypeptides and pull chain through, as in mitochondrial ratchet model.



Note: the signal sequence is present but not illustrated in this model.

Fig. 12-45B Alberts

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Translocated Polypeptides

- Translocated polypeptide chains are folded in the ER.
- These proteins may remain, or continue to other destinations.
- 3 basic steps:
 - 1) Glycosylation *once the unfolded translocated into ER, they may remain unfolded until it's folded. or maybe injected if the protein is folded before*
 - 2) Folding
 - 3) Export and degradation of misfolded proteins

16

Protein Glycosylation

- Oligosaccharide transferred from the lipid, *dolichol*, to asparagine residue of polypeptide.
- Reaction catalyzed by oligosaccharyl transferase.
- Protein is then marked for folding.

single pass or double or multiple can contribute to protein folding

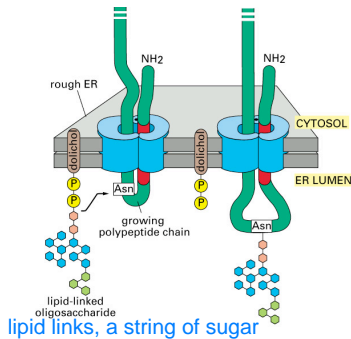


Figure 12-52. Molecular Biology of the Cell, 4th Edition.

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Role of Glycosylation in Protein Folding

- **Calnexin** binds to incompletely folded proteins in the ER.
- Glucosidase “trims”, and glucosyl transferase adds glucose.
- Cycle continues until protein folded properly.

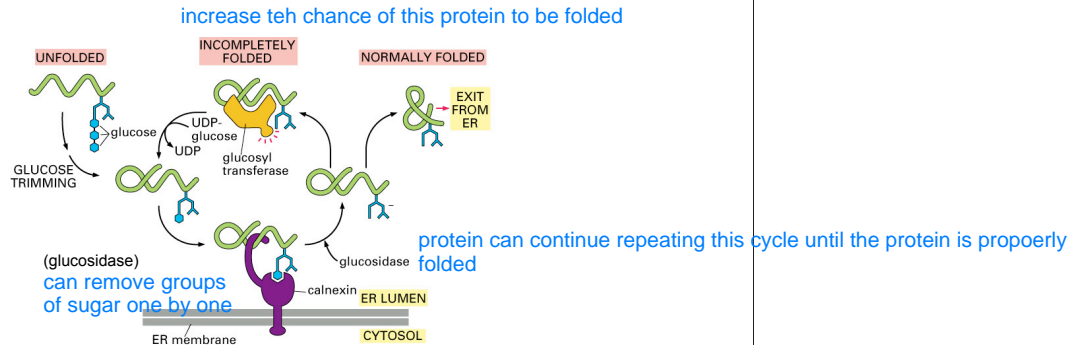


Figure 12-54. Molecular Biology of the Cell, 4th Edition.

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Misfolded Proteins

- Misfolded proteins are “dislocated” back to the cytosol through Sec61 by an unknown mechanism.
- Deglycosylation occurs.
- Ubiquitination and degradation in the cytosol.

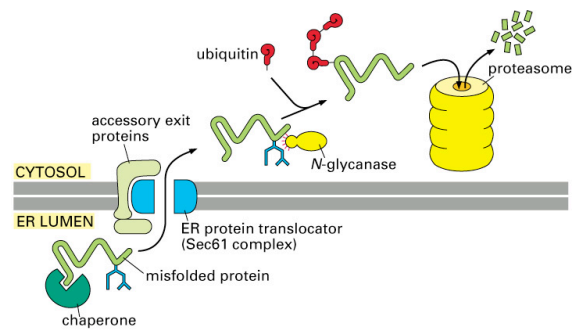


Figure 12-55. Molecular Biology of the Cell, 4th Edition.

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Things to Consider...

1. Why are molecular chaperones *usually* not needed for delivery of polypeptide chains to the ER?
2. What is the difference between *posttranslational* and *cotranslational* mechanisms of translocation?

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