Day 8 (Cell Biology)

Signal Recognition Particle (SRP)

- ER signal sequence is guided to ER membrane by:
  1. Signal recognition particle
  2. SRP receptor

- SRP cycles b/w ER membrane and cytosol and binds to the signal sequence
- SRP → complex particle comprising 6 different polypeptide chains bound to a single small RNA molecule
- found in all cells - old and conserved

How do SRP bind to so many sequences specifically?

→ signal-sequence-binding-pocket : large hydrophobic pocket lined by methionines

↙ unbranched flexible chains; plastic to accommodate hydrophobic sequences

- rod-like structure that wraps around large ribosomal subunit - with one end binding to the ER signal sequence, as it emerges as part of the newly made polypeptide chain emerging from the ribosome

&

the other part blocks the elongation factor binding site at the interface b/w large and small ribosomes (A site factor)

↓

blocks protein synthesis as soon as the signal sequence emerges

↓

gives ribosome enough time to bind to the ER membrane

↓

ensures the protein is not released into cytosol

↓

also allows protein to fold.

↓

Once formed, SRP-ribosome complex binds to the SRP receptor embedded in RER membrane

↓

this interaction binds the SRP–ribosome complex to a protein Translocator

↓

Translocator frees SRP-ribosome complex & SRP receptor & transfers polypeptide chain across the membrane

Cotranslational transfer process creates two separate population of ribosomes

- Membrane-bound (engaged in the synthesis of proteins that are concurrently translocated into ER)
- Free (unattached to any membrane, synthesize all other proteins encoded by the nuclear genome)

Single mRNA molecule – many ribosomes

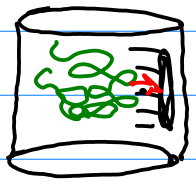
↳ polyribosome complex

Polypeptide chain passes through aqueous pore in translocator

- Translocater forms aqueous pore through which polypeptide chain passes through membrane
- core of Translocator — Sec 61 complex
  (determined by X-ray crystallography)

Sec 61 complex

- dynamic gated structure that opens only transiently when a polypeptide chain passes through
  ↳ does not allow ions to pass through



- pore can also open laterally → allows hydrophobic signal sequence to move into hydrophobic membrane & also allows membrane proteins into membrane

- in eukaryotic cells, on ribosomes, 4 Sec 61 complexes form large Translocator assembly
- the Translocator forms tight diaphragm around the Translocating chains that prevents the escape of other molecules

In single membrane proteins, a single internal ER Signal Sequence remains in the lipid bilayer as a membrane-spanning α-helix.

Signal sequence binds to Translocator protein complex

↓

opens the pore — double recognition allows only specific proteins to pass through

↓

Signal sequence binds to translocator as Start Transfer Signal

- Sec61 compler forms walls of translocator complex.
- While bound in the Translocation pore, signal sequence is in contact with Sec61 and hydrophobic membrane.
- When the nascent polypeptide chain grows long enough, an ER signal peptidase cleaves of the signal sequence, and releases them into the lipid core and signal sequences are degraded by proteases

How are single-pass transmembrane proteins become inserted into the ER?

- Signal sequence functions as Start-Transfer signal, similarly, additional hydrophobic segment in polypeptide chain functions as Stop Transfer Protein
- Stop Transfer Signal stops the transfer process before the entire polypeptide chain is translocated
- Anchors the pridein in the membrane after Start Transfer Signal hasbeen released into membs lipid

Multipass Membrane Protein

→ polypeptide chain passes back and forth repeatedly across the membrane

→ internal sequence serves as Start Transfer Signal

↓

Translocation continues until Stop Transfer Signal

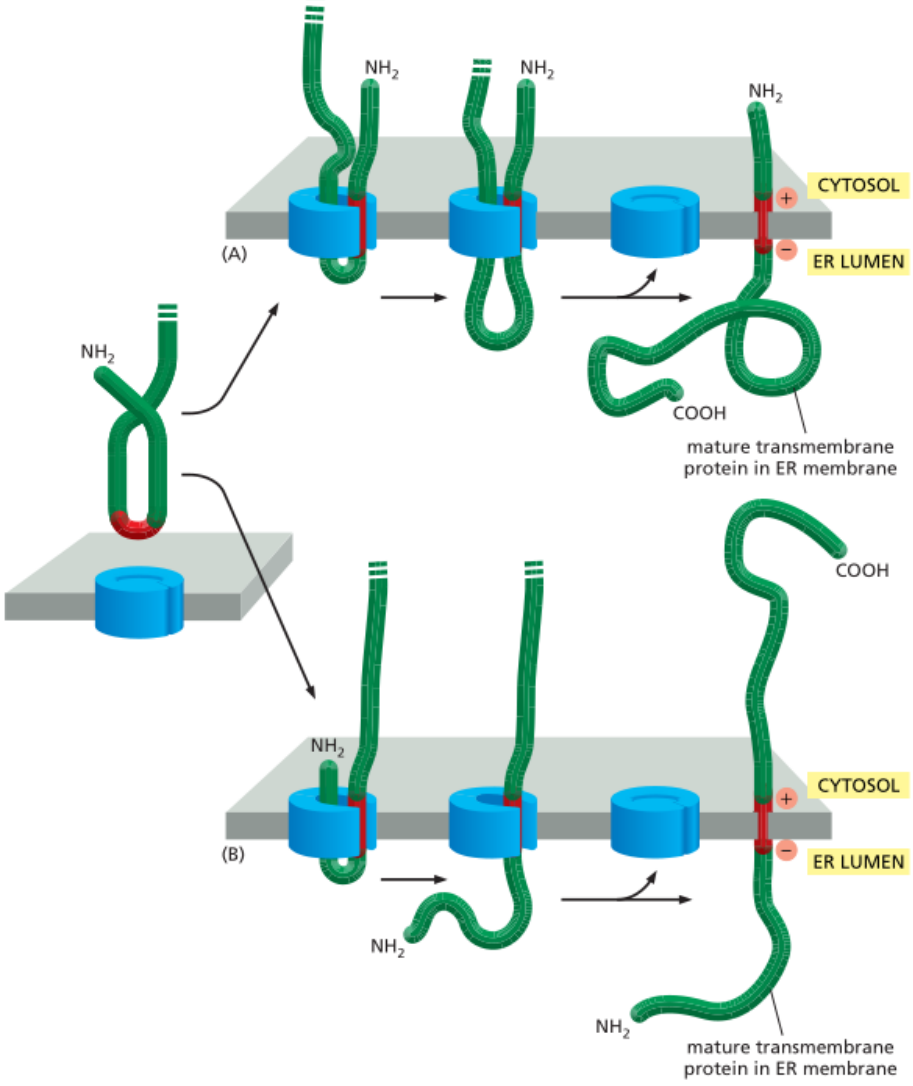


Figure 12–47 Integration of a single-pass transmembrane protein with an internal signal sequence into the ER membrane. An internal ER signal sequence that functions as a start-transfer signal can bind to the translocator in one of two different ways, leading to a membrane protein that has either its C-terminus (pathway A) or its N-terminus (pathway B) in the ER lumen. Proteins are directed into either pathway by features in the polypeptide chain flanking the internal start transfer sequence: if there are more positively charged amino acids immediately *preceding* the hydrophobic core of the start-transfer sequence than there are following it, the membrane protein is inserted into the translocator in the orientation shown in pathway A, whereas if there are more positively charged amino acids immediately *following* the hydrophobic core of the start-transfer sequence than there are preceding it, the membrane protein is inserted into the translocator in the orientation shown in pathway B. Because translocation cannot start before a start-transfer sequence appears outside the ribosome, translocation of the N-terminal portion of the protein shown in (B) can occur only after this portion has been fully synthesized.

Note that there are two ways to insert a single-pass membrane-spanning protein whose N-terminus is located in the ER lumen: that shown in Figure 12–46 and that shown in (B) here.