

Biogenesis Co-translational targeting a.k.a. Secretory pathway of protein synthesis

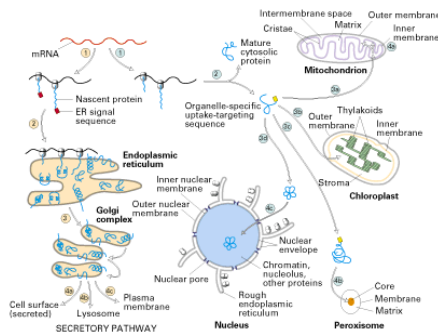


The synthesis of proteins has two divergent branches



- **Cytosolic** - for proteins that stay in the **cytosol** or are translocated post-translationally (**nucleus, mitochondria and peroxisomes**)
- **Secretory** – for proteins that are going to the surface of the cell or other organelles
 - For proteins to be secreted
 - And proteins that will become integral membrane proteins (in plasma membrane and organelles such as lysosomes)
 - Proteins are translocated co-translationally

The synthesis of proteins has two divergent branches



Secretory pathway of protein synthesis



- In secretory pathway proteins have to stop in the ER first
 - Those to be secreted are translocated to the inside of ER
 - Those to become integral membrane proteins are incorporated into the ER membranes
- Proteins are synthesized by ribosomes sitting on the cytosolic side of ER membrane (not inside the ER) !!!!! . They translocate to the ER during synthesis.

Secretory pathway of protein synthesis



- In secretory pathway translocation occurs simultaneously with translation = co-translationally
 - If proteins are going to the outside of the cell or to the membranes they can not stay in the cytosol too long!!!
 - Translation is coupled to translocation
- Targeting signal – ER targeting sequence

Way to the surface



- Start of translation at the cytosolic ribosomes
- As soon as signal sequence at the beginning of the protein emerges from the ribosomal tunnel a new polypeptide with ribosome is targeted to to the ER
 - ER becomes rough
 - Signal peptide – ER receptor interaction



Way to the surface

- Translation continues while the peptide is translocated to the inside of ER or inserted into ER membrane
- Modifications in the ER
- Transfer to Golgi
- Modifications in Golgi
- Exocytosis (secretory proteins)
- Merging with the plasma membrane (membrane proteins)



Initial steps in synthesis of secretory proteins

1. Translation starts on the cytosolic ribosomes
2. Translation continues until signal sequence emerges from the ribosomal tunnel
3. A signal recognition particle (SRP) binds to signal sequence
 - Translational arrest



Initial steps in synthesis of secretory proteins

4. SRP (with the new peptide and ribosome attached to it) directs the complex to the ER membrane
5. SRP binds to SRP receptor on ER membrane
6. Ribosome docks to translocon

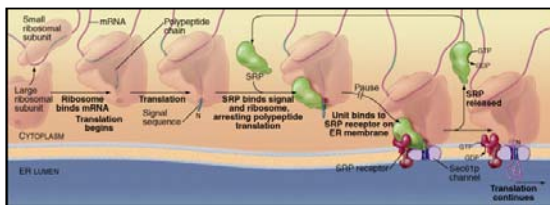


Initial steps in synthesis of secretory proteins

7. SRP dissociates
 - GTP hydrolysis on both SRP and SRP receptor
 - Translational arrest is lifted
8. Translation continues while peptide is translocated into ER
9. Once in ER signal peptide is cleaved by signal peptidase

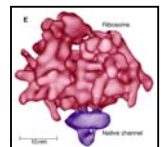
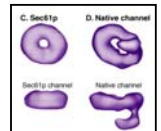


Steps in the synthesis of secretory proteins



Translocon

- Heterotrimer of Sec61p and 2 other proteins
 - Homolog of SecY and Sec E in bacteria
- Signal peptidase is associated with translocon



Translocation into ER lumen

- Signal sequence binds to translocon
- After the entire protein is finished and translocated signal peptidase cleaves signal sequence and releases polypeptide into ER



Insertion of integral membrane proteins into the ER membrane

- To be inserted into the cell membrane proteins have to be first inserted into ER membrane
- The insertion to the membrane is guided by special topogenic sequences



Insertion of integral membrane proteins into the ER membrane

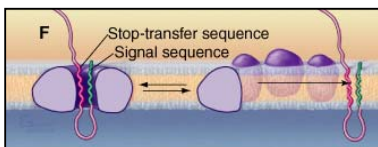
- Protein must be inserted into the membrane with proper orientation
 - The same it will assume in the plasma membrane
 - Inside of the ER becomes outside of the cell
- The “sidedness” of membranes is preserved during transport to the cell surface

Topogenic sequences

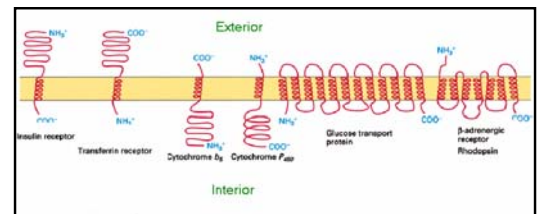
- Direct appropriate insertion of membrane proteins into the ER membrane
- Combination of N-terminal signal sequence and stop-transfer, start transfer internal sequences

Topogenic sequences

- N-terminal signal sequence is usually cleaved the internal sequences are not
- Internal transfer sequences become transmembrane domains



Orientation of membrane proteins



Synthesis of insulin receptor

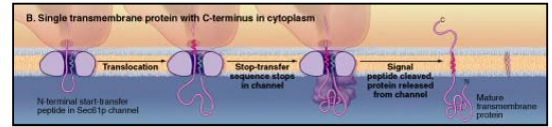


- Single transmembrane domain, C-terminus in cytoplasm
- Requires
 - Signal sequence to guide it to ER (cleaved later)
 - Stop-transfer sequence to stop translocation and insert into the membrane

Synthesis of insulin receptor



- Stop-transfer sequence stays as the transmembrane part of the protein



Synthesis of asialoglycoprotein receptor

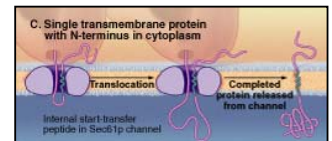


- Single transmembrane domain, N-terminus inside the cell (in the cytosol)
 - Different orientation of termini that insulin receptor !
- Needs internal start transfer sequence (this is also signal sequence that directs the polypeptide to ER) for anchoring to the membrane

Synthesis of asialoglycoprotein receptor



- Uncleaved internal signal sequence stays in the membrane (becomes transmembrane segment)
- The remaining part of the peptide is elongated into the ER

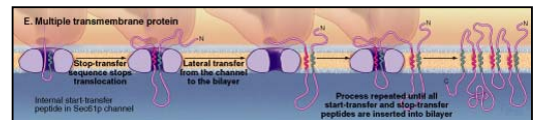


Multiple transmembrane domains require multiple signal sequences



- Proteins such as ion channels, receptors or transporters have multiple start and stop sequences that allow multiple passes through the membrane
- Each internal start-transfer sequence or stop-transfer sequence becomes transmembrane domain

Synthesis of multi-transmembrane protein



- For the N-terminus to be on the outside signal sequence must be localized at N-terminus and cleaved later
- For the N-terminus to remain in the cytosol signal sequence must be internal

Topogenic sequences



- Start-transfer sequence is like internal signal sequence - begins translocation toward ER
- Stop-transfer sequence stops the translocation (anchors the protein to the membrane) – next few residues become transmembrane domain and the rest of the chain elongates into cytosol

Posttranslational modifications



- Following translation (and translocation and insertion into ER membrane) all proteins are modified to assume their final structure and function
- Only properly modified proteins are transported from ER to the final destination