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MOVING PROTEINS INTO MEMBRANES AND ORGANELLES

REVIEW THE CONCEPTS

1. a. In the absence of ER membranes, the entire protein is translated and the ER signal sequence remains on the protein.
   b. When translation occurs in the presence of ER-containing microsomes, the protein is translated into the lumen of the microsomes. Following this process, the signal sequence is cleaved producing a smaller protein.
   c. Translation and translocation across the ER membrane are simultaneous processes. If they do not occur at the same, the protein is not properly imported into the ER where the signal sequence can be cleaved (although there are some examples of post-translational translocation).

2. a. The energy source for cotranslational translocation comes from the translation process itself—in other words, the nascent chain is pushed through the translocon channel. Please note, however, that as translation is completed a portion of the newly synthesized protein still resides within the translocon. This portion is drawn into the ER lumen rather than being pushed.
   b. In post-translational translocation, the newly synthesized polypeptide chain is drawn through the translocon by an energy input from ATP hydrolysis by BiP. BiP is luminal protein of the ER and is a member of the ~~Hsc~~70 family of molecular chaperones. BiP-ATP activates by binding to the Sec63 complex that in turn binds to the Sec61 translocon complex. Activated BiP is enzymatically active and cleaves ATP to ADP plus P_i. It is BiP-ADP that binds to the entering, unfolded nascent chain. Sequential binding of BiP-ADP to the nascent chain serves to block any sliding of

the chain back and forth in the translocon and to ratchet the nascent chain through the translocon.

c. Translocation into the mitochondrial matrix occurs through a bipartite Tom/Tim complex in which Tom is the outer membrane translocon and Tim is the inner membrane translocon. Three energy inputs are required. First, ATP hydrolysis by a cytosolic Hsp70 chaperone keeps the newly synthesized mitochondrial precursor protein unfolded in the cytosol. Second, ATP hydrolysis by multiple ATP-driven matrix Hsp70 chaperones may serve to pull the translocating protein into the matrix. Matrix Hsp70s interact with Tim44 and hence may be analogous to the BiP/Sec63 interaction at the ER membrane. Third, energy input from the H^+ electrochemical gradient or proton-motive force is required. The inside-negative membrane electric potential may serve to electrophorese the amphipathic matrix-targeting sequence toward the matrix.

3. SRP (signal recognition particle) acts as a cycling cytosolic factor for the translocation of ER targeted proteins. It binds to both the signal sequence and SRP receptor, a heterodimer associated with the ER membrane. In doing this, SRP initiates ribosome binding to ER membranes and positions the nascent chain proximal to the translocon. Both SRP and the SRP receptor are GTPases. The unfolded nascent chain then translocates. Cytosolic Hsp70 functions as a cytosolic factor required for protein translocation into mitochondria. It acts as a molecular chaperone to keep the post-translationally targeted mitochondrial precursor protein in an open, extended conformation. At least two different cytosolic proteins are required for translocation of peroxisomal matrix proteins. These are Pex5, the soluble receptor protein for matrix proteins containing a C-terminal PTS1 targeting sequence, and Pex7, the soluble receptor protein for matrix proteins containing an N-terminal PTS2 targeting sequence. A different receptor, Pex19, is required for peroxisomal membrane proteins.

4. Many membrane proteins are embedded in the membrane by virtue of transmembrane α-helical segment(s). Such segments can be referred to as topogenic sequences. These segments share general principles or properties. They tend to be about 20 amino acids long, a length suffi cient to span the membrane, and hydrophobic, an appropriate property for a sequence embedded in the hydrophobic lipid bilayer. Application of these principles through computer algorithms is predictive. In brief, amino acid sequences of polypeptides may be scanned for hydrophobic segments of about 20 amino acids long. Each amino acid may be assigned a hydrophobic index value based on relative solubility in hydropbobic media versus water, and these values then can be summed by a computer for all 20 amino acid segments of a protein. Segments exceeding a threshold value are expected to be topogenic transmembrane segments. Internal signal anchor and stop-transfer anchor segments similarly can be identifi ed. Such sequences alternate within a multipass membrane protein. Because of this, the overall arrangement of the protein can be predicted as described in detail in the text.

5. The UPR pathway up-regulates transcription of protein chaperones. It is thought that the timing of glycosylation modifi cations is one manner in which misfolded ER proteins are identified in the ERAD pathway. Dislocation into the cytoplasm is necessary because the proteolytic machinery for these ER proteins is located in the cytoplasm.

6. The seven-sugar intermediate is synthesized by sugar addition to cytosolic-facing dolichol phosphate. The intermediate is fl ipped from the cytosolic face of the ER membrane to the luminal face. Further sugar additions then occur within the lumen of the ER. Short forms of the intermediate are on the wrong side of the membrane to add to nascent polypeptides within the ER lumen. Incomplete adductants within the ER lumen are located appropriately to *N*-glycosylate nascent polypeptide.

7. Several proteins facilitate the modfi cation or folding of secretory proteins within the ER. These include signal peptidase, BiP, oligosaccharyl transferase, various glycosidases, calnexin and calreticulun, protein disulfi de isomerase, peptidyl-prolyl isomerase, and others. Of these, BiP and peptidyl-prolyl isomerase act to facilitate conformation changes. Protein disulfi de isomerase facilitates the making/breaking of disulfi de bonds to ensure correct protein folding. Calreticulin and calnexin are lectins that bind to glycoproteins during folding. The others all directly support the covalent modifi cation of proteins within the ER lumen.

8. Each mutation has a different effect.
   a. Tom22 together with Tom20 act as outer mitochondrial membrane receptor proteins for N-terminal matrix targeting sequences. A defective Tom22 receptor protein would result in accumulation of mitochondrial matrix targeted proteins in the cytosol, possibly followed by their turnover within the cytosol.
   b. Tom70 signal receptor is an outer mitochondrial membrane protein recognizing multipass mitochondrial membrane proteins that have internal signal sequences. Mutation in Tom70 will have no immediate effect on mitochondrial matrix protein import, as Tom70 does not recognize this class of protein.
   c. Matrix Hsp70 has a role in the folding of matrix proteins. Also, it is one source of energy for powering translocation. Defective matrix Hsp70 should result in clogging the Tom/Tim translocon complex with incompletely translocated proteins.
   d. Retention of the matrix targeting N-terminal signal sequence because of a defective matrix signal peptidase might well result in defective folding of the imported protein. The sequence normally is removed.

9. On the whole, protein import into the mitochondrial matrix and the chloroplast stroma, topologically equivalent locations, is by functionally equivalent mechanisms. Functionally analogous proteins mediate each process. However, the proteins are not homologous, indicating a separate evolutionary origin of mitochondria and chloroplasts. Energetically, unlike the situation for mitochondria, there is no need for a membrane electrochemical gradient for import into chloroplasts. Presumably, stromal Hsp70 pulls proteins into the stroma.

10. To answer this question, we must determine how many amino acids must span the Tom/Tim complex to expose the matrix-targeting sequence to the matrix-processing protease. DHFR in the presence of the drug methotrexate is locked into a folded state. A chimeric mitochondrial protein with folded DHFR fails to translocate fully into the mitochondria matrix. Instead, it is stuck in the Tom/Tim complex. The number of amino acids between the matrix targeting sequence

and the folded DHFR sequence could be varied to provide a 'molecular ruler'. Any unfolded N-terminal DHFR sequence must be included within the ruler. With respect to channel length, an overestimate will result from this approach, as the matrix targeting sequence must be spaced out from Tom/Tim to be accessible for cleavage.

11. Catalase is responsible for breaking down H_2O_2 to H_2O. Catalase, like most other peroxisome-localized enzymes, contains a peroxisome-targeting sequence (PTS1) consisting of three amino acids, serine-lysine-leucine, at its C-terminus. This PTS1 is recognized and binds in the cytosol to the Pex5 receptor. The cata-lase-Pex5 heterodimer moves to the peroxisome membrane, where it interacts with the Pex14 receptor located on the membrane. In this position, the complex interacts with three membrane proteins—Pex2, Pex10, and Pex12—that facilitate the translocation of catalase into the peroxisome.

12. Separate mechanisms are used to import peroxisomal matrix and membrane proteins. Hence, mutations can selectively affect one or the other. Either can result in the loss of functional peroxisomes. One approach to determining whether the mutant is primarily defective in insertion/assembly of peroxisomal membrane proteins or matrix proteins is to use antibodies to assess by microscopy if either class of proteins localize to “peroxisomal” structures (e.g., peroxisome ghosts). An alternate approach is cell fractionation, in which the assay determines whether the appropriate proteins are present in a membrane organelle fraction.

13. The NLS. The nuclear import receptor binds to the NLS on the cargo molecule and brings it into the nucleus. Here, the receptor binds to Ran-GTP, causing release of the cargo. The receptor is thought to interact with the FG repeats that are commonly found on nuclear pore complex proteins, moving from one to the next as it passes through the nuclear pore.

14. Ran-guanine nucleotide–exchange factor (Ran-GEF) must be present in the nucleus and Ran-GAP must be in the cytoplasm for unidirectional transport of cargo proteins across the nuclear pore complex. When the Ran is bound to GTP, it has high affinityforcargoproteins.Duringnuclearexport,Ran-GTPpicksup cargo proteins in the nucleus and carries them to the cytoplasm. To be able to release the cargo on the cytoplasmic side, GTP must be hydrolyzed to GDP, and this process is stimulated by Ran-GAP. Once translocated back into the nucleus, Ran needs to be in the GTP-bound state to pick up more cargo. Ran-GEF in the nucleus stimulates the exchange of GDP for GTP, and this process of export can start again.