7.016 Recitation 13 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lecture 19 (10/26):

Protein Trafficking and Localization: The site of a polypeptide function may be far away from its point of synthesis in the eukaryotes. The newly synthesized protein may stay in the cytosol once it is made, translocate to a specific organelle, become a part of the plasma membrane or even be secreted by the cell. Furthermore, the newly synthesized proteins may undergo different types of post-translational modifications i.e. glycosylation (addition of carbohydrate moiety that occurs in golgi bodies), phosphorylation (addition of phosphate group) and lipid addition (in smooth ER).

As a polypeptide chain emerges from the ribosome, it folds into a specific three- dimensional structure based on its primary amino acid sequence. The newly formed polypeptide chain can also contain a signal sequence- an "address label" that indicates where in the cell does the polypeptide belongs.

It is important to note that for all proteins (irrespective of their final destination), translation always begins on the free ribosomes in the cytoplasm/ cytosol. But as a polypeptide chain is made, its amino acid sequence gives it one of two sets of instructions:

1. Complete translation and either stay and function in the cytosol or go to a specific organelle based on the inherent organelle specific amino acid sequence or…

2. Stop translation, go to the endoplasmic reticulum and finish translation.

Cytosolic proteins do not need to go anywhere in the cell. So they are made and stay in the cytosol and lack any signal sequence.

Each organelle specific protein contains an inherent signal sequence that serves as a zip code for the translocation of the protein to its specific destination. Proteins going to the specific organelles have a short stretch of amino acids that allows them to bind to docking protein receptors present on the membranes of appropriate organelles i.e. nuclear localization signal for nuclear proteins (a sequence of amino acids with positively charged side-chains, ---Lys_Lys-Lys-Lys---), mitochondrial localization sequence for the mitochondrial protein (a sequence of alternative amino acids with positively and negatively charged side-chains such as ----Lys-Glu-Lys-Glu-Lys-Glu----) or peroxisome localization sequence (tripeptide sequence with various amino acid combinations such as ---Ser-Lys-Leu---). However these organelle specific proteins are translated within the cytosol.

The transmembrane and secretory proteins have a stretch of 15- 20 hydrophobic amino acids as their signal sequence. This signal sequence is bound by a signal recognition particle (SRP), which temporarily stops protein translation and aids in the docking of the nascent polypeptide to the ER receptor. The signal sequence then enters the ER lumen (the inside of the ER) by passing through a channel in the receptor. The signal sequence is then removed and the protein synthesis once again resumes. The N- terminus of the membrane protein faces the inside of the ER i.e. ER lumen. It is to be noted that the membrane proteins have one or multiple hydrophobic stop transfer/ transmembrane domain sequences as a result of which they are NEVER released into the ER lumen unlike the secretory proteins. Once translated the membrane and secretory proteins are transported via vesicles to ER, golgi body for further modifications. The secretory proteins move out of the cell by the process of exocytosis. In comparison, the membrane proteins become a part of the plasma membrane due to the fusion of vesicles with the lipid bilayer.

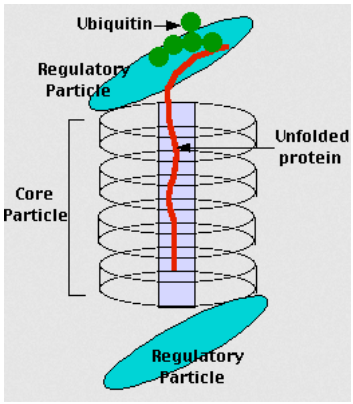
The N terminus of the membrane proteins faces the extracellular side of the cell whereas the C-terminus can either face the cytosol or the extracellular side of the cell depending on the number of transmembrane domains within the membrane protein.

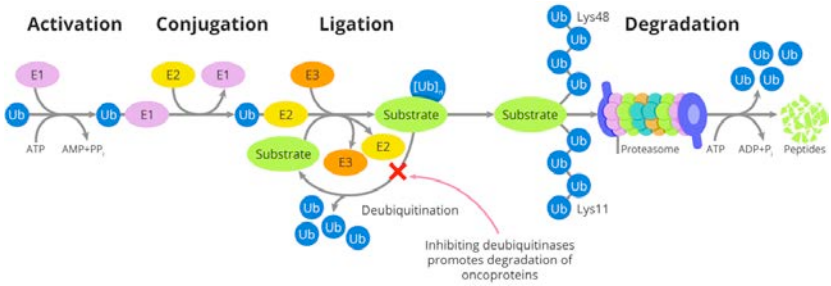
Protein trafficking: https://www.youtube.com/watch?v=4qf1BSXn_tk
Role of SRP: http://www.youtube.com/watch?v=ScAu0MZK3CU&feature=related

Proteasomes: Protein can either be degraded by the lysosomal enzymes or through the proteasome complex. **Lysosomes** deal primarily with **extracellular** proteins, e.g., plasma proteins, that are taken into the cell (endocytosis), cell-surface membrane proteins that are used in receptor-mediated endocytosis, autophagy (that engulfs the proteins and other molecules).

In comparison, **Proteasomes** deal primarily with endogenous proteins that are synthesized within the cell such as: transcription factors, cyclins, proteins encoded by viruses or other intracellular pathogens, proteins that have been damaged by other molecules within the cell and proteins that are folded incorrectly due to errors in translation or mutations within the gene.

The proteasome is made of core particle (CP) and regulatory particle (RP) and is a multimeric protein complex. The proteasome complex within the cell may chew of Proteins that have the incorrect amino acid sequence.





Proteins destined for destruction are conjugated to a molecule of ubiquitin. The ubiquitin-protein complex binds to the ubiquitin-recognition site(s) on RP. The protein is unfolded by ATPases using the energy from ATP and then translated into the central cavity of CP. As it passes through the CP it is degraded by hydrolysis of the peptide bonds. This produces a set of peptides that leave the CP and are broken down into individual amino acids by peptidases in the cytosol. The regulatory particle releases the ubiquitins for reuse.

A loss of these processes can result in neurodegenerative diseases and prions related diseases.

Proteasome mediated degradation is energy requiring, it is irreversible, requires ubiquitinylation of target proteins and this is regulated by E2 and E3 enzymes.

Question

You are interested in four different proteins in a yeast cell: protein 1 is a cytosolic protein, protein 2 is a secreted protein, protein 3 is a nuclear protein and protein 4 is a transmembrane protein. You plan to study how the proteins are localized to their specific destination by creating the following mutations in the genes encoding proteins 1-4.

a) Mutation A inactivates the SRP (signal recognition particle). Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

b) Mutation B removes the signal sequence from protein 2. Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

c) Mutation C removes the signal sequence from protein 4. Indicate how the localization of each protein (1-4) will be affected by this mutation. In additional state whether each protein will function as it does in a wild-type cell.

d) Mutation D prevents the fusion of vesicles to the golgi body membrane. Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

The key

You are interested in four different proteins in a yeast cell: protein 1 is a cytosolic protein, protein 2 is a secreted protein, protein 3 is a nuclear protein and protein 4 is a transmembrane protein. You plan to study how the proteins are localized to their specific destination by creating the following mutations in the genes encoding proteins 1-4.

1. Mutation A inactivates the SRP (signal recognition particle). Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

Protein 1: The localization and function will be unchanged.
Protein 3: The localization and function will be unchanged.
Proteins 2 and 4: With no SRP, translation for this protein may not be completed and if it is, the protein will be localized to the cytosol. As a transmembrane protein the natural function would NOT be intracellular, so protein 4 will NOT function as it would in a wild-type cell. Neither will Protein 2 whose function is outside the cell.

2. Mutation B removes the signal sequence from protein 2. Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

Protein 1: The localization and function will be unchanged.
Protein 2: With no signal sequence, translation for this protein will be completed in the cytosol and the protein will be localized to the cytosol. As a secreted protein the natural function would NOT be intracellular, so protein 2 will NOT function as it would in a wild-type cell.
Protein 3: The localization and function will be unchanged.
Protein 4: The localization and function will be unchanged.

3. Mutation C removes the signal sequence from protein 4. Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

Proteins 1, 2 & 3: The localization and function will be unchanged.
Protein 4: With no signal sequence, translation for this protein will be completed in the cytosol and the protein will be localized to the cytosol. As a transmembrane protein the natural function would NOT be intracellular, so protein 4 will NOT function as it would in a wild-type cell.

4. Mutation D prevents the fusion of vesicles to the golgi body membrane. Indicate how the localization of each protein (1-4) will be affected by this mutation. In addition, state whether each protein will function as it does in a wild-type cell.

Protein 1: The localization and function will likely be unchanged.
Protein 2: In a cell that cannot fuse vesicles to the golgi, secreted protein will become trapped in the vesicles in cytoplasm. So protein 2 will NOT function as it would in a wild-type cell.
Protein 3: The localization and function may be unchanged.
Protein 4: In a cell that cannot fuse vesicles to the golgi, protein will become trapped in the vesicles in cytoplasm. So protein 4 will NOT function as it would in a wild-type cell.

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