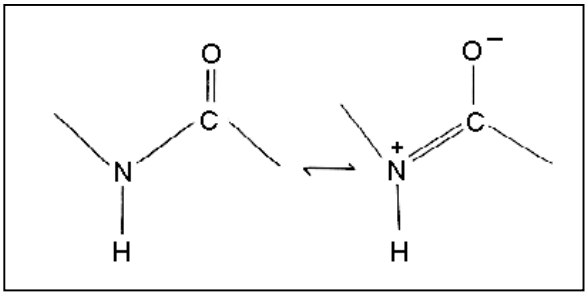
7.013 Recitation 10 – Spring 2018

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

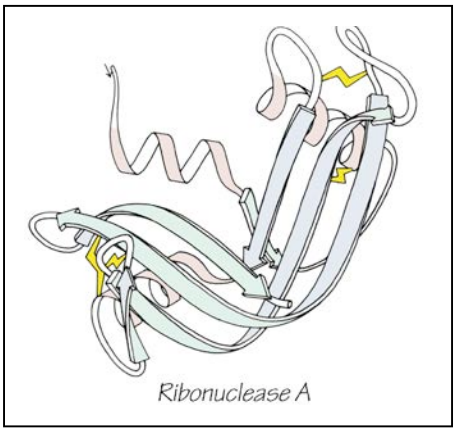
Summary of Lecture 17 (3/19)

Peptide bonds: This is a covalent bond formed between the α-NH2 and α-COOH group of two amino acids. It is important to note that the C-N bond length is 10% shorter than the usual C-N amine bonds. This is due to their double bond characteristics due to the resonance that occurs between amides. The two canonical structures are represented below.



As a consequence of this resonance, all peptide bonds are found to be almost planer. This rigidity of the peptide bond reduces the degree of polypeptide chain during its folding. Therefore the folding of peptide chain is mostly based on the side-chains of amino acids.

Anfinsen's Dogma (thermodynamics hypothesis): This dogma was championed by Anfinsen based on his studies on Ribonuclease A. This protein denatures if heated in test tube. However, if it is brought back to the normal temperature condition, it spontaneously refolds and regains its activity. So per this dogma, for the small globular proteins the native structure is determined by the protein's amino acid sequence.



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In the cells, chaperone proteins guide the folding of the protein in their active 3D conformation. The misfolded proteins can aggregate and lead to neurodegenerative diseases such as Alzheimer's, Parkinson's disease and Prion diseases.

Prions an example of neurodegenerative disease: Prions: are the infective proteinaceous particles. The diseases caused by prions are caused by a change in the conformation of specific proteins. The defects arise from errors in the folding of these proteins into the proper 3D- dimensional conformation. The protein with the altered conformation then seems to induce a change in the conformation of the normal protein counterpart so that it also becomes abnormal. The altered proteins have profound effects on its function in the cell. There is a long period of several years between the onset of the disease and the manifestations of the disease symptoms. Prions unlike the bacteria, viruses or nucleic acids cannot be altered or killed through UV irradiation. The transmissible spongiform encephalopathies (TSE), scrapie, kuru, mad cow disease and chronic wasting disease are some examples of prion related diseases.

- Nobel Prize awarded to Stanley Pruisner: http://www.nobelprize.org/nobel_prizes/medicine/laureates/1997/
- MIT tech news article: http://tech.mit.edu/V117/N48/nobel.48w.html

Protein Trafficking and Localization: The site of a polypeptide function may be far away from its point of synthesis in the cytoplasm, specifically in the eukaryotes. The polypeptide may stay in the cytosol once it is made, may translocate to a specific organelle, may become a part of the plasma membrane or may even be secreted by the cell. Furthermore, the proteins undergo different types of

post-translational modification i.e. cleavage of signal peptide, 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. The newly formed polypeptide chain also contains a signal sequence- an "address label" that indicates where in the cell does the polypeptide belongs. Proteins synthesis always begins on the free ribosomes in the cytosol. But as a polypeptide chain is made, its amino acid sequence gives it one of the two sets of instructions:

1. Complete translation and either stay and function in the cytosol or go to a specific organelle based on its 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, mitochondrial localization sequence for the mitochondrial protein etc. However these 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), the SRP aids in the docking of the nascent polypeptide to the ER receptor. The signal sequence then enters the ER lumen 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 the vesicles with the lipid bilayer. The N terminus of the membrane proteins faces the extracellular side of the cell whereas the C-terminus either faces the cytosol or the extracellular side of the cell depending on the number of transmembrane domains within the membrane protein.

Please visit the following site to visualize protein trafficking:

http://www.youtube.com/watch?v=ScAu0MZK3CU&feature=related

Protein localization can be determined by techniques such as fluorescence imaging or by creating a fluorescent protein.

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.

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