Lecture #8
Cell Physiology of Disease
2/16/04
Readings: Chapters 12 and 13 of MBOC 4 (Alberts et al.)

Protein Targeting and Translocation

During and sometimes following protein synthesis, there is a targeting of the proteins to intracellular organelles. The common targets are endoplasmic reticulum, mitochondria, chloroplasts, and peroxisomes; and of these, the major one is the endoplasmic reticulum (ER). ER constitutes 50% of cellular membrane and is the site of secreted and membrane protein synthesis as well as lipid and carbohydrate synthesis. Proteins, immature carbohydrate chains and membrane physically move from the ER to the Golgi apparatus and from the Golgi to either lysosomes, plasma membrane or secretory vesicles. In the case of mitochondria, most of the proteins are encoded in the cell genome and only a few proteins are produced from the Mitochondrial DNA. Since mitochondria have a double membrane, proteins from the cytoplasm must be transported across one or two membrane bilayers. Critical elements of these functions include the targeting mechanisms that direct proteins to one compartment or another, the control of synthesis and degradation rates, membrane flow from one compartment to another and the bases of the various membrane compartments.

Targeting of Membrane and Secreted Proteins

Viral glycoproteins have been used extensively to study the process of targeting because they are exogenous to the cell (not already at steady state) and can be expressed at high levels readily (viral infection will often take over the cell's machinery to produce a few proteins). Since viral particle assembly often occurs on specific sites on the cell surface, the viral membrane capsid glycoproteins must be targeted to those sites. It is, therefore, easy to assay for proper targeting. Conceptually, a lot of effort was devoted early on to finding a linear sequence of amino acids that would act as the address tag for a site in the cell. Such sequences do exist for proteins targeted for the peroxisome but more complex signals are needed to direct proteins to other sites. There is a consensus that the physical properties of the proteins and not the linear sequences are more important for the targeting. Thus, it is important to understand which physical properties are important in the organization of membranes, membrane dynamics and protein dynamics.

Protein Dynamics at Steady State

We will start by first considering the problem of protein dynamics in a cell at steady state and later add the complexities introduced by growth. Many proteins turn over at rates considerably faster than 24 hours (the typical time for cell division under optimal growth conditions). Thus, it is useful to model the levels of the proteins as the result of the balance between synthesis and degradation. Often it is assumed that degradation is a random first order decay process and that the rate of synthesis simply matches the rate of degradation under steady state conditions.

Protein Synthesis and Degradation

At steady state $d[Protein]/dt = 0$ then $(r_s - r_d)dt/N_o = dV/V$

where r_s = translation rate (1 – error frequency)

r_d = degradation rate, N_o = number of molecules/cell

and V is the cell volume

Flow and counterflow of membrane from one compartment to another

There is a large flux of membrane between different compartments in the cell that can best be described as the flow of water between different pools in a self-contained fountain. Membrane exchange rates are much faster than net membrane synthesis rates, which then means that flow rates between the pools are the primary determinants of pool size. There is also evidence for complicated return flow patterns between compartments that will affect compartment size. One good illustration of how alterations in this flow can change the morphology and perhaps size of a compartment is a paper on the movement of membrane as a result of the loss of microtubules [Cole et al., 1996]. We will discuss this paper on Wednesday and you can access the paper from any Columbia University site as described below.

References:

There are several programs for searching the literature and retrieving full articles on the internet. For making reports and papers, I suggest using EndNote that has links to PubMed for searching but will enable you to add references to Word documents as you are working on them. You can get more information on the cell biology by looking at the PubMed web site (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) and then find the paper with the authors last names (separated by commas). Once you find the article open the abstract and you will find "links" in the upper right area near the title. Click on links and you will find "books" as one of the 3 choices. Select "books" and then many of the words in the abstract will be highlighted in blue. Double clicking on those words will open a menu of Cell Biology textbooks which have descriptions of those terms and related materials.

Presley, J.F., N.B. Cole, T.A. Schroer, K. Hirschberg, K.J. Zaal, and J. Lippincott-Schwartz. 1997. ER-to-Golgi transport visualized in living cells. *Nature*. 389:81-5. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9288971

Basic synthesis and processing in ER (carbohydrate addition)

The steps in the synthesis of proteins while attached to the ER membrane are described in the Cell Biology texts and further information can be obtained through either more advanced texts or by searching PubMed for some of the important terms and select for recent reviews.

Concentration in regions of transport (budding) (VSV-g protein)

There has been considerable work on the transition from ER to Golgi. It is a critical step in the processing of glycoproteins and in their quality control. There is considerable evidence that the contents of the ER are not randomly sampled but rather are

concentrated at exit sites. Budding occurs at these sites and the membrane is then transported to the Golgi.

Movement to Golgi
Snap-Snare specificity of fusion
CopI and II plus clathrin coats for fission
Movement forward and backward in Golgi
Golgi to plasma membrane (apical vs. basolateral)
Ab initio or seeded

Read the following paper for Wednesday

Cole, N.B., N. Sciaky, A. Marotta, J. Song, and J. Lippincott-Schwartz. 1996. Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol Biol Cell*. 7:631-50. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8730104

Questions:

1. Polyribosomes represent an assembly line of protein synthesis on one mRNA, and clearly many copies are made in parallel. For this problem, we have induced synthesis of a protein by a stimulus. We want to determine how many proteins per cell are present at steady state if the half-time of the protein after synthesis is 10 hours and the rate of synthesis is 20 molecules per minute. (Assume that the degradation rate represents a first order decay process)
2. In the case of plasma membrane proteins, they need to be processed through the ER and Golgi before reaching the plasma membrane, which often takes 15-30 minutes. If a protein has a half-life of 10 minutes in the ER and 15 min in the Golgi, then what is the number of molecules of the protein in the ER and Golgi at steady state assuming a synthesis rate of 100 molecules per minute.