Lecture #20
The Cell as a Machine

Fission, Fusion, and Flow

In the area of membrane biology, the traffic of membrane from one area of the cell to another is a critical parameter. We have talked about the translation, translocation, and processing events last time and how they need to be coordinated for the cell to export plasma membrane or secreted proteins. To reach the outside of the cell from the ER, the proteins must move to the Golgi and then on to the plasma membrane. Lysosomal proteins also pass through the Golgi and are sorted to a different transport compartment. Further, there is another area of membrane traffic that involves the endocytosis of membrane and material, from micropinocytosis to phagocytosis. Endocytosed material is processed to return most of the membrane to the plasma membrane. Contents of endocytic vesicles are concentrated in the late endosome which is acidified (pH 5 or so) and located near the Golgi. Finally, a small portion of the material is passed on to the lysosome that is packed with degradative enzymes to break down proteins, carbohydrates and lipids.

Studies of the overall quantity of traffic between the membrane compartments have been difficult but new GFP technologies make it possible to quantify the number of molecules moving between the different compartments (Lippincott-Schwartz et al., 2000).

ER to Golgi to Plasma Membrane

The major synthetic pathway for the cell membrane lipids, proteins, carbohydrates and secreted proteins flows from the ER to the Golgi to the plasma membrane. Recent GFP protein analyses have defined the rates of movement and quantitative modeling is being done to define the relative rates of the components in those pathways. They suggest that the rate of movement of a GFP-tagged viral glycoprotein, GFP-VSVG, from ER to Golgi to the plasma membrane can be described by simple rate equations with the rate constants of 2.8%/min for ER to Golgi and 3%/min for Golgi to plasma membrane.

Biophysical Problem

Considerable energy is required for the fission of membrane to form vesicles and for the fusion of vesicles to form larger structures.

GFP-VSVG

Brefeldin A

Bodipy-ceramide

Micropinocytosis and Endocytosis

Volume of Membrane (equal to the cell surface area in about an hour)

Site and Mechanism (clathrin and membrane bending)

Early processing (recycling and movement on to late Endosome)

Transport to TGN

Macropinocytosis and Phagocytosis

Questions:

1. We will use a 14° temperature block to hold a GFP-tagged membrane glycoprotein in the ER until a significant amount is synthesized. When the temperature is raised to 37°, the protein will be released to transit to the Golgi and on to the plasma membrane. If further synthesis of the protein is blocked and we use the constants defined in the Hirschberg et al. 1998 study, then about how long will it take before 10% of the protein reaches the plasma membrane.
2. If endocytosis is randomly sampling the surface and 4% of the surface is endocytosed every minute, then how long will it take to endocytose 80% of a membrane protein?
3. (Extra Credit, 10 pts) Many receptors are recycled after endocytosis but a fraction often moves on to the lysosome where it is degraded. If the endocytosis rate is the same as in problem 2 but 75% of the endocytosed protein is recycled, then what is the half-time for the degradation of the protein?

Reference:

Lippincott-Schwartz, J., T.H. Roberts, K. Hirschberg. (2000) Secretory protein trafficking and organelle dynamics in living cells. Ann. Rev. Cell Devel. Biol. 16:557-589.

Hirschberg, K., et al. (1998) Kinetic Analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. J. Cell Biol. 143:1485-1503.