

Memb.2
Core Lecture

Recent Reviews for Membrane Traffick include: (Antonny and Schekman, 2001; Gorelick and Shugrue, 2001; Mayer, 2002; Nehls et al., 2000; Paiement and Bergeron, 2001; Pelham, 2001; Pilon and Schekman, 1999; Tekirian, 2002; Zaal et al., 1999)

Organelle Definition and Mechanism of Production

Typically, membrane organelles are defined by marker proteins, often enzymes, that are chiefly resident in the compartment. In a cell at steady state, the resident marker proteins are often found 80-90% of the time in the designated compartment. However, the methods of definition are not sufficiently quantitative to enable us to know that number with a higher precision. What constitutes the boundary between various compartments is often blurry and there are regional and temporal

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, some proteins from the cytoplasm are transported across two membranes to the interior.

Protein Synthesis and Degradation

At steady state $d[\text{Protein}]/dt = 0$ then $(r_s - r_d)dt/N_o = dV/V_o$

where $r_s =$ translation rate (1 – error frequency)

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

and V_o is the cell volume

Flow and counterflow of membrane from one compartment to another

Ab initio or seeded

Basic synthesis and processing in ER (carbohydrate addition)

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

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)

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 30 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.

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 (assume that synthesis is stopped but the membrane area stays constant)?
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:

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Proteolysis and Degradation

Proteins have widely different half-lives in cells from minutes to days. What determines if a protein is degraded rapidly or slowly is related to 1) the proper folding of the protein (abnormal, unfolded proteins are degraded rapidly), 2) N-terminal amino acid, and 3) physical aggregation of the protein.

Ubiquitylation
Lysosomes (Man 6P system)
Autophagocytosis

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