Lecture #3
The Cell as a Machine

Diffusive Transport vs. Active Transport

Readings: Berg, Random Walks in Biology: Howard, Mechanics of Motor Proteins and the Cytoskeleton

Summary: Transport of material in cells from one site to another can be purely diffusive. However, the time penalty is significant (diffusive transport time scales as the square of the distance or $<X^2> = 4Dt$) and there is a concentration penalty as well because diffusive transport requires a concentration gradient.

From the smallest to the largest cells there is a need to concentrate components in subcellular regions and to transport proteins and/or membranous organelles from one place to another in cytoplasm. There is an active debate about whether such transport is best accomplished by diffusion or by a motor-dependent system. Both diffusive transport and active transport have been observed and we will consider the mechanism of both today.

Diffusive Transport:
    We will consider a simple case of synthesis and assembly in cytoplasm. Site A is where a protein is being translated and folded properly. Site B is where the protein is assembled into a working complex. Proteins need to get from A to B for assembly. If they diffuse from A to B and are trapped at B, then the cell doesn't need to expend energy to move them. The penalty that the cell pays for diffusive transport is a long time constant that relies on a concentration gradient. The penalty for active transport is the need for ATP

    If we consider the case of one-dimensional diffusion, we can model the process of diffusive transport by considering what happens after one step to the concentrations molecules that were originally in a narrow slices at points, X and X + δ, where δ is a single step. If there are more molecules at X than at X + δ, then more molecules will step to X + δ than vice versa. The concentration will increase at X + δ, which is diffusive transport. Thus, diffusion can only produce a net transport down a concentration gradient and the rate of transport is directly proportional to the magnitude of the concentration gradient. The mathematical expression that describes the relationship between the concentration gradient, the diffusion coefficient and the transport rate is Fick's Law.

$$J_x = -D \, dC/dx \quad\quad\quad (12)$$

where Jx is the flux in the x direction, D is the diffusion coefficient, dC/dx is the concentration gradient in the x direction. The simplest case is one where there is a tube

connecting a large reservoir of concentration C_1 with a second reservoir of concentration C_2. The concentration gradient is then linear and the flux is constant through the tube.

Diffusive transport has a number of important implications for cellular processes because of its undirected nature. The most important is that the level of transport is proportional to the gradient of concentration. For any steady-state situation, a stable gradient will be found between the site of synthesis and utilization.

For example, actin mRNA is transported toward the front portion of migrating fibroblasts, which means that the actin protein will be preferentially synthesized at that site. Actin filament assembly occurs preferentially at the leading edge, which would then decrease the g-actin concentration. Thus, diffusive transport would occur between the site of synthesis and the leading edge. Since diffusion toward the back of the cell would occur with equal probability as toward the front, the concentration at the back of the cell, which has much less actin polymerization than at the front, would be the same as that at the site of synthesis. Thus, there is almost no advantage to the cell for transport purposes to place the site of synthesis near the site of utilization, if diffusion to the rest of the cell is open. On the other hand, if diffusion is restricted to other regions such as when there is a barrier, then efficient transport from source to sink occurs. The restriction of diffusion is very hard to accomplish for single proteins but is common for large aggregates in cytoplasm. Kate Luby-Phelps observations of diffusion of dextrans in cytoplasm show nonuniformity with larger particles (Luby-Phelps, 2000). The cortical actin region of cytoplasm is restrictive of large particle diffusion whereas the perinuclear region is not.

Active Transport

For directed transport of material within cells, it is much faster to tie transport to an active transport mechanism. The critical elements of such a system are the motor, the transport complex and the link between the two. Motor proteins in mammalian systems can move at rates up to about 5 microns/sec. (plants have myosin motors that move 80 microns/sec). Most movements are saltatory, which means that vesicles do not move continuously but rather move intermittently. Consequently, the amount of displacement per unit time is often less than the product of the observed vesicle velocity and elapsed time.

$$d < vt \tag{12}$$

Problems:

1. If we assume that a protein ($D = 2 \times 10^{-7}$ cm^2/s) is synthesized at a steady state rate of 100 molecules per second and assembled into a larger complex at the same rate, then you can calculate the gradient of protein concentration in the cell from a site of synthesis separated by 20 microns from the site of assembly (assume that all the molecules are moving down a 20 micron diameter tube). If the concentration at the site of assembly is 10^{-9} M and the assembly site is at the pole of a spherical cell of 40 microns in diameter,

what is the concentration at the synthesis site at the center of the cell and what is the concentration in the other half of the cell?

2. Many believe that the reduction in the number of dimensions of diffusion (e.g. from 3-D solution phase to a 2-D membrane phase or to a 1-D filament phase) will result in an increase in the rate of transport. How will problem 1 change if a microtubule extends from the site of synthesis to the site of assembly and all of the protein binds to the microtubule surface and diffuses with the same D as in Problem 1? Often the penalty for the binding of a protein to a filament or a membrane is a decrease in the diffusion coefficient. What would happen if the D is decreased by two orders of magnitude?

3. Axonal transport has been studied extensively by injecting the cell bodies with radioactive amino acids and then following the distribution of radioactivity in specific proteins along the axon ((Jung et al., 2000; Nixon et al., 1994; Dillman et al., 1996)). Originally the slow axonal transport of cytoskeletal proteins (1-2 mm/day) was interpreted as the bulk movement of the assembled cytoskeleton down the axon. In the case of studies of the rat optic nerve (radioactive amino acids were injected into the eye and the optic nerves, 1.6 cm in length, were cut up at various times after injection), we can compare three different models of the transport process. 1) active transport; in this case assume that the cytoskeletal proteins are carried in a complex that moves at the rate of 2 mm/day and all of the radioactive amino acids (10,000 counts per minute (cpm) total) were incorporated into protein within one hour. 2) diffusive transport; assume that the protein has a diffusion coefficient of 10^{-7} cm^2/sec. 3) Rapid transport and diffusion model; assume that the protein is part of a complex which moves at a fast axonal transport rate (200 mm/day) but for only 1% of the time. For the rest of the time the particles diffuse at a rate of 10^{-10} cm^2/sec. Please calculate the number of cpms that would be expected in 2 mm slices of the axon for each of these cases after 1.5 days and one week.

References:
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Jung, C., J. T. Yabe, and T. B. Shea. 2000. C-terminal phosphorylation of the high molecular weight neurofilament subunit correlates with decreased neurofilament axonal transport velocity. *Brain Res*. 856:12-19.
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Nixon, R. A., S. E. Lewis, M. Mercken, and R. K. Sihag. 1994. [32P]orthophosphate and [35S]methionine label separate pools of neurofilaments with markedly different axonal transport kinetics in mouse retinal ganglion cells in vivo. *Neurochem Res*. 19:1445-1453.