ML-13
The Cell as a Machine

Cytoskeleton and Cell Mechanics

During the next several lectures we will discuss the mechanical aspects of mammalian cells in culture. Since all mammals are shaped by cells, it is obvious that a lot of the effort and control information within the organism goes into the cell-cell and cell-matrix interactions that shape the organism. From the earliest involution of the embryo to the maturation of digits, there are many highly orchestrated motile events that rely upon the cell cytoskeleton, its structure and dynamics. The precision and reproducibility of these morphological changes indicates that there are complex control mechanisms involved under a genetically defined plan. Mutations of proteins, diseases, cancers and parasites can all alter the morphology of the organism in defined ways that belie targeted alterations in cytoskeletal functions. These are higher level functions and may not be involved in cell viability per se (clearly the aberrations that lead to many cancers result in cell immortality, i.e. circumvent normal growth controls). However, they are extremely important to our understanding of what defines a mouse versus a rat or a cancerous growth versus normal wound repair.

In general, the mechanical properties are defined by the cytoskeleton and the volume of the cell. The rigidity of the cytoskeleton is reinforced by the membrane which maintains constant cell volume on the short time scale (obviously cell growth does increase cell volume but slowly). From the point of view of materials, cells respond elastically to short periods of stress but respond plastically to long periods of stress. A rapid poke of a cell by an AFM tip will result in an elastic response and the cell will return to its previous morphology immediately. In contrast, a prolonged stretch of a cell will cause cell remodeling and the cell will not return to its previous morphology immediately upon relaxation. Thus, we need to consider not only the static organization of the cytoskeleton but also its dynamics to understand how it will function.

Many cells are designed to be part of tissues and they differentiate when they lose neighbors on all sides (this is presumably part of the response to wounding of a tissue). Thus, the protein composition of cells will change in response to alterations in physical environment as well as hormonal or chemical environment. The recent advent of gene chip technology has provided a rapid growth in our understanding of the changes in cell composition that occur but it is a long way from defining those changes in composition to understanding how they cause changes in complex cell functions. At present, we can talk about specific cell lines grown under defined conditions which have a reproducible behavior.

Organization of the Cell Cytoskeleton

A Professor of Biomechanics has described the mechanical properties of cells in suspension culture as similar to chocolate-covered cherries, i.e., they have a relatively rigid outer shell covering a relatively fluid cytoplasm with a relatively rigid nucleus (cherry) at the center. The outer shell is composed primarily of crosslinked actin filaments and is tightly linked to the membrane. The fluid cytoplasm has microtubules and intermediate filaments coursing through it but they do not support significant compressive stresses (the fluid volume of the cell supports most compressive stresses). The nucleus constitutes a small fraction of the cell volume in most non-immune cells and is therefore a minor factor in cell deformations.

Filament Self-assembly

(References: Alberts et al., Molecular Biology of the Cell, 3rd Ed., Chapter 16, and Howard, Mechanics of Motor Proteins and the Cytoskeleton, Chapters 9-11)

We will consider the self-assembly of two of the three major filaments in cytoplasm, their dynamics, polarity and distribution. The major filaments of animal cells in order of size are actin filaments (4-6 nm helical filaments), intermediate filaments (10 nm filaments), and microtubules (25 nm tubes). These filaments are all multi-stranded filaments and unlike a single stranded polymer shown below in which the dissociation constant for the dimer is the same as the dissociation constant for the monomers at the ends of the polymer.

$$A + A = A2 \quad + A = A3 \quad + A = A4 \quad + A = A5$$

The dissociation constant is commonly used to quantitatively evaluate the strength of binding because it has units of M (moles/liter) (remember in an equilibrium reaction the constant is described as the ratio of the products over the reactants or for the dissociation of the dimer, $K_d = [A][A]/[A2]$).

$$K_d = \exp(\Delta G/RT) \text{ moles/liter}$$

For multi-stranded filaments, the binding of the later subunits is stronger than the dimer formation because additional bonds are formed between the strands of the polymer. In the case of actin, which is a double-stranded polymer, the third subunit added will have two binding interactions (one with each of the subunits in the dimer) and will have a stronger bond (lower value for the K_d). Similarly, all subsequent monomers that bind have two interaction sites. The case of microtubules with 13-16 strands in the tubule is similar, except that the long axis of the tubulin dimer is oriented along the strand axis. This means that the dimer binds to the tubule initially through one interaction along a strand plus two inter-strand interactions. The multi-stranded polymer can be much more stable than a single stranded polymer because of the lateral interactions and is consequently a much better structural building block for the cell. An additional feature is that the internal subunits in a filament are unlikely to leave the filament because they have typically twice as many interactions as the subunits at the ends.

Seeding Polymerization

Another important aspect of the multi-stranded filaments is that they will preferentially polymerize on seeds. This comes directly from the fact that the rate of dissociation of the dimer is much more rapid than the rate of dissociation of a monomer from the end of a filament. Ramifications of seed formation are several fold: first, the rate of polymerization often has a lag phase if you start with pure monomer

(corresponding to the time needed to form seeds); second, the addition of polymeric seeds to monomers removes the lag phase; third, at the cellular level, the site of polymerization can be controlled by the site of seed formation; and fourth, a solution of pure actin monomer or tubulin dimer at the critical concentration (the concentration needed to give polymer from pure monomer) will polymerize onto permanent or crosslinked seeds. The critical concentration (Kc) is defined by rate of polymerization and depolymerization at the ends of the filaments.

$$Kc = k_{off}/k_{on} = K_D$$

A further implication is that the release of subunits from the side of a filament is unlikely. This can be understood by considering the number of bonds between subunits and the entropy of binding. As we have discussed before, the entropy of binding is negative, which results in an increase in the free energy of binding ($\Delta G = \Delta H - T\Delta S$); however, the DS term is the same for the binding of proteins to the ends of filaments as to the side of the filaments. In the case of actin, a monomer binding to the end of the filament forms bonds with 2 neighbors (one in the same protofilament and one between protofilaments) whereas a monomer in the filament has bonds with 4 neighbors (2 in the same protofilament and 2 in the adjacent protofilament).

Problem:

1. In the theoretical filaments that are linear assemblies of subunits where the binding constant for the dimer is the same as the binding constant for the n + 1 mer, the filament length is limited because of what? For an extra credit of 10 points, describe how you would compute the average filament length from the dissociation constant and the concentration of the protein (all forms).