BMT-72106 CELLULAR BIOPHYSICS

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ABSTRACT

This week's exercise is about the general concept of the mechanotransduction,including the protein quanternary, ECM and the tool AFM. In calculation part, the Molecular dissociation constant is of our interest. The binding between advidin biotin pair and antibodies antigen pair are compared by calculation.

1. EXERCISE 1 CELLULAR CYTOSKELETON

Compare the three components of the cell cytoskeleton. E.g. How are they structured? What are their roles? The three main structural components of the cytoskeleton are microtubules (formed by tubulins) , microfilaments (formed by actins) and intermediate filaments. All three components interact with each other non-covalently. Microtubules are polymers of tubulin. They help with cell transport. They also help with the cell shape because it resists compression. It also helps facilitate cell motility. There are two motor proteins that assist organelles to move along the microtubules: kinesin and dynein. Microtubules also help movement of substances within the cell and are also involved in powering whole-cell movement by cilia and flagella. The microtubules provide tracks that can move vesicles from one organelle to the next in an efficient, directed fashion. Microtubules also segregate the duplicated chromosomes during mitosis.

Microfilaments are polymers of actin. They help with cell shape also because it bears tension in the cell. It is also involved in cell motility. There are three types of cell shape, which are microvilli, lamellipodia, and filopodia. Microfilaments are formed when individual actin monomers polymerize, in a process fueled by ATP hydrolysis, to form chains of filamentous actin. Microfilaments are dynamic structures, growing and shrinking in a controlled manner. Some microfilaments play a structrual role in the cell to maintain cell shape. These structural microfilaments have protein caps at both ends to prevent changes in microfilament length. Other microfilaments have functions that require dynamic changes in length. The microfilaments also mediate cytoplasmic streaming, a mixing of the cytoplasm that aids diffusion. Intermediate filaments are polymers of keratin. They help with cell shape also because it bears tension in the cell. It is primarily involved in organelle anchorage. The intermediate filaments also consist of various fibrous proteins that have a diameter of about 10 nm. Intermediate filaments often form a meshwork under the cell membrane and, in cells that lack a cell wall, help impart and maintain cell shape.

Microtubles have a larger diameter than microfilaments and intermediate filaments.Intermediate filaments are fairly stable and are not thought to undergo acute changes in length the way microfilaments.

2. EXERCISE 2 CELL ELASTICITY

Describe different methods to measure cell elasticity experimentally. Experimentally, main methods used to measure the cell elastics are basic mechanical methods, magnetic methods optical methods. Other methods, for instances the acoustic microscopy methods, laser tracking microrhelogy and hydrodynamic flow are also quite commonly used. 1) mechanical methods: Micropipette aspiration track the leading edge of aspirated cell (with accuracy in 25nm), suction pressure in micropipette (in range 0.1 to 1000Pa and force (range from 10pN to 10mN). Usually, the pressure ti edge position yields E. Cell poker can be used to tip mounted on flexible wire;position of both tip and wire arm are measured to determine deflection; Another type is the indentation, immedidate retraction, monitoring cell relaxation by reflaction interference contrast microscoppy(RICM). Silicon micromachines use poker fixed to beam of known elasticity, monitoring poker and sensor position,and stretching experiments by glueing tip to cell. Atomic Force Microscopy records player in cantilever scans sample with cantilever deflection recorded by laser reflection. FOrce-distance-maps can be produxed for each x-y-position. 2)Magnetic methods: Magnetic twisting magnetizes the surface-bound ferromagnetic beads y strong filed pulse and twist it by small perpendicular field. TH twisting angle measured by magnetometer reflects shear response. Attached magnetic beads apply force pulses to paramagnetic beads, recording and relaxing creeep response. Optical methods: Beads glued to cell are held in optical traps, measuring the second one by acousto-optical modulator which responds to the first moved one with quad-

rant detector. 3)Acoustic microscopy scans samples with acoustic lens, the phase and amplitude of the reflected wave which is used in elasticity calculation later are recorded to calculate the velocity and attenuation.

3. EXERCISE 3 CYTOSKELETAL DYNAMICS

Actin and microtubule polymers are extremely dynamic structures. These polymers are always assem- bling and dis-assembling. Where does this dynamic nature come from? What is the benefit of having a dynamic cytoskeleton compared to more static one?

There are two motor proteins that assist organelles to move along the microtubules: kinesin and dynein. Microtubules also help movement of substances within the cell and are also involved in powering whole-cell movement by cilia and flagella. filaments also help with the cell shape because it resists compression.These structural microfilaments have protein caps at both ends to prevent changes in microfilament length, supporting its function. It also helps facilitate cell motility.

Because cells are living entities that are highly dynamic themselves. They are constantly interacting with their environment and responding accordingly. In order to meet with the requirements of the cell, the cytoskeleton needs to be equally dynamic. If the skeleton is static, the tranportation cannot be made efficiently which might be constructed occupying large space and costing huge energy in order to connect essential parts. And if the shape itself can not be changed, the mobility of the cells transferred in the cytoskeleton is requires itself to be buit in different shape for transferring certain cells which is inconvenien and energy cost again.

4. EXERCISE 4 ACTIN POLYMERIZATION

Actin filaments can push the cell membrane while they are growing by polymerization and thus form extrusions as shown in the Figure 1 below. The force required for this feat can be calculated with equation.

a) When the actin filament is the force calculate with equation 1 is negative, the multipication of change in actin filament length and the log of the devision of monomer and Kc is negative because the boltzman's constant and absolute temperature is positive. Furthermore, the change in actin filament length is increasing during actin polymoerization according to figure one, thus it actually means the actin monomer concentration is larger than the critical concentration which requires the actin monomer concentration larger than 0.27 uM at least.

b) Assuming the globular actin monomer size is $4 * 10^{-9}$,T 1)plus end:$-1.43*10^{-12}$, minus end:$-5.74*10^{-12}$,

$$F = \frac{k_B T}{\Delta Z} ln \frac{[monomer]}{K_c}, \quad (1)$$

where F is the force (N), k_B is Boltzmann's constant, T is absolute temperature (K), ΔZ is the change in actin filament length (m) as shown in Figure 1, $[monomer]$ is the actin monomer concentration (M) and K_c is the critical concentration (M). Actin filaments can grow from both ends (called plus- and

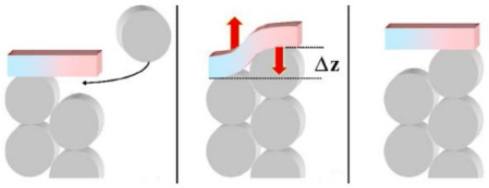

Figure 1: Actin polymerization while pushing the cell membrane.

minus-ends), but with different rates. This is reflected on their critical concentrations: plus-end has $K_c^+ = 0.1$ μM and minus-end $K_c^- = 0.8$ μM.

a) What is happening to the actin filament if the force calculated with Equation 1 is negative?

b) Given the actin monomer concentrations (i) 0.05 μM, (ii) 0.5 μM and (iii) 1 μM, calculate the forces for both the minus and plus ends. You can approximate ΔZ as a half of the globural actin monomer size, which you can find from the literature.

c) If you have a single actin fiber and actin monomer concentration of 0.5 μM, what do you think is happening to the fiber based on equation 1?

Fig. 1. actin polymerization.

2);2)$3.33*10^{-12}$,minus end:$-9.73*10^{-13}$;3)plus-end$4.77*10^{-12}$,minus$4.62 * 10^{-13}$

c)Because 0.5 is between 0.27 and 2.17 thus actin fiber might go either go through polymerization or not.And according to the phenomenon of microtubule treadmilling, the concentration near microtubule plus, the end will shrink while near the plus end will grow.