BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 3)

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EXERCISE 1 "BIOMECHANICS OF THE BACTERIAL CYTOSKELETON" :

Bacterial actin-like proteins (like MreB) self-assemble into dynamic filaments that can grow at both ends, shrink at both ends, or treadmill.

1. At low concentrations of ATP-MreB:
☐ MreB filaments grow at the plus end and grow at the minus end.
☐ MreB filaments shrink at the plus end and grow at the minus end.
☐ MreB filaments grow at the plus end and shrink at the minus end.
☒ MreB filaments shrink at the plus end and shrink at the minus end.
Explain your answer:

MreB (and other bacterial actin-like proteins) possesses an intrinsic ATPase activity that stochastically converts ATP-MreB to ADP-MreB. Only ATP-MreB can add to the ends of an MreB filament. When ADP-MreB subunits are exposed at the end of a filament they detach and the filament shrinks at that end. The rate of ATP-MreB addition is fast at the plus end and slow at the minus end. At low [ATP-MreB] the rate of ATP-MreB addition at both ends (plus and minus) is slower than the rate of conversion from ATP-MreB to ADP-MreB. Thus, the filament shrinks at both ends.

2. At high concentrations of ATP-MreB:
☒ MreB filaments grow at the plus end and grow at the minus end.
☐ MreB filaments shrink at the plus end and grow at the minus end.
☐ MreB filaments grow at the plus end and shrink at the minus end.
☐ MreB filaments shrink at the plus end and shrink at the minus end.
Explain your answer:

See answer to question 1, above. At high [ATP-MreB] the rate of addition at both ends (plus and minus) is faster than the rate of conversion from ATP-MreB to ADP-MreB. Therefore, the MreB filament grows at both ends.

3. At intermediate concentrations of ATP-MreB:
☐ MreB filaments grow at the plus end and grow at the minus end.
☐ MreB filaments shrink at the plus end and grow at the minus end.
☒ MreB filaments grow at the plus end and shrink at the minus end.
☐ MreB filaments shrink at the plus end and shrink at the minus end.
Explain your answer:

See answer to question 1, above. At intermediate [ATP-MreB] the rate of addition of ATP-MreB is faster at the plus end than the rate of conversion from ATP-MreB to ADP-MreB. Thus, the filament grows at the plus end. However, the rate of addition of ATP-MreB is slower at the minus end than the rate of conversion from ATP-MreB to ADP-MreB. Thus, the filament shrinks at the minus end. This behavior is called "treadmilling".

4. Bacterial actin-like proteins (like MreB) have intrinsic ATPase activity. Imagine an MreB "hypomorph" mutation that *decreases* its ATPase activity. At intermediate concentrations:
☐ A hypomorph MreB would form shorter filaments than wild-type MreB.
☒ A hypomorph MreB would form longer filaments than wild-type MreB.
☐ A hypomorph MreB would form filaments equally long as wild-type MreB.
Explain your answer:

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Conversion from ATP-MreB to ADP-MreB causes the ADP-MreB subunits to detach from the ends of a filament. If the rate of conversion from ATP-MreB to ADP-MreB is decreased due to a lower rate of ATP hydrolysis caused by a hypomorph mutation, this would shift the balance from shrinkage to growth. Thus, filaments formed by a hypomorph mutant form of MreB with decreased ATPase activity would tend to be longer than filaments formed by wild-type MreB.

5. Bacterial actin-like proteins (like MreB) have intrinsic ATPase activity. Imagine an MreB "hypermorph" mutation that *increases* its ATPase activity. At intermediate concentrations:
☒ A hypermorph MreB would form shorter filaments than wild-type MreB.
☐ A hypermorph MreB would form longer filaments than wild-type MreB.
☐ A hypermorph MreB would form filaments equally long as wild-type MreB.
Explain your answer:

Conversion from ATP-MreB to ADP-MreB causes the ADP-MreB subunits to detach from the ends of a filament. If the rate of conversion from ATP-MreB to ADP-MreB is increased due to a higher rate of ATP hydrolysis caused by a hypermorph mutation, this would shift the balance from growth to shrinkage. Thus, filaments formed by a hypermorph mutant form of MreB with increased ATPase activity would tend to be shorter than filaments formed by wild-type MreB.

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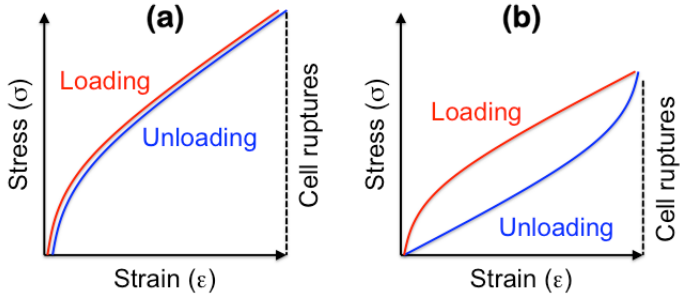
EXERCISE 2 "BIOMECHANICS OF THE BACTERIAL CYTOSKELETON" :

Cytoskeletal proteins contribute to the biomechanical properties of bacterial cells. The graphs (below) depict the stress-strain curves for two different cells: cell (a) and cell (b). Assume that the scales of the x-axis and y-axis are the same for both graphs.



1. Write the formula for Stress (σ). Write the formula for Strain (ε). Define the terms.

Stress (σ) = force per unit area = $N*m^{-2}$ = $kg*m^{-1}*s^{-2}$. Note that Stress and Pressure are expressed in the same terms.

Strain (ε) = [(stretched length) – (unstretched length)] * $(\text{unstretched length})^{-1}$. Note that strain is dimensionless.

2. Which cell is stiffer?
☒ Cell (a) is stiffer than cell (b).
☐ Cell (b) is stiffer than cell (a).
☐ Cell (a) and cell (b) are equally stiff.
☐ Impossible to tell from these graphs.
Explain your answer:

"Stiffness" is the amount of stress that must be imposed on an object in order to strain the object. A stiff object requires more stress than a soft object to achieve the same strain. In the graph, cell (a) requires more stress than cell (b) to achieve the same strain. Expressing it another way: cell (a) exhibits less strain than cell (b) under the same stress. Expressing it yet another way: the slope of the stress-strain curve is steeper for cell (a) than cell (b). All three interpretations of the graphs yield the same conclusion: cell (a) is stiffer than cell (b).

3. Which cell is more extensible?
☐ Cell (a) is more extensible than cell (b).
☐ Cell (b) is more extensible than cell (a).
☒ Cell (a) and cell (b) are equally extensible.
☐ Impossible to tell from these graphs.
Explain your answer:

"Extensibility" is the amount of strain that can be imposed on an object before it ruptures. In the graph, cell (a) and cell (b) rupture at the same amount of strain. Thus, cell (a) and cell (b) are equally extensible.

4. Which cell is stronger?
☒ Cell (a) is stronger than cell (b).
☐ Cell (b) is stronger than cell (a).

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☐ Cell (a) and cell (b) are equally strong.
☐ Impossible to tell from these graphs.
Explain your answer:

"Strength" is the amount of stress that can be imposed on an object before it ruptures. In the graph, cell (a) ruptures at a higher level of stress than cell (b). Thus, cell (a) is stronger than cell (b).

Note. People sometimes confuse "stiffness" and "strength". Although cell (a) is both stiffer and stronger than cell (b) in the examples shown here, these two properties are distinct. Stiff materials can be mechanically weak, and soft materials can be mechanically strong.

5. Which cell is more resilient?
☒ Cell (a) is more resilient than cell (b).
☐ Cell (b) is more resilient than cell (a).
☐ Cell (a) and cell (b) are equally resilient.
☐ Impossible to tell from these graphs.
Explain your answer:

"Resilience" is the ability of an object to absorb energy under loading (elastic deformation) and to release that energy during unloading (relaxation to the original shape). If the object is perfectly resilient, like cell (a), then the loading (stretching) and unloading (relaxing) curves will be perfectly superimposable. In other words, all of the work put into stretching the object will be recovered when the object is allowed to return to its original unstretched length. In an object that is not perfectly resilient, like cell (b), some of the work put into stretching the object is lost; consequently, the loading (stretching) and unloading (relaxing) curves will not be perfectly superimposable. In the stress/strain curve for cell (b), note that the amount of stress required to produce a specific strain is higher for the loading curve and lower for the unloading curve due to the work lost during loading (stretching), which is not recovered during unloading (relaxing).

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EXERCISE 3 "BIOMECHANICS OF THE BACTERIAL CYTOSKELETON" :

The "Minicell" (Min) system functions to ensure that the "FtsZ ring" assembles at the middle of the cell (along the longitudinal axis) with the ring oriented along the cell's short axis.

1. The Min system comprises three proteins: MinC, MinD, and MinE. Briefly describe the function of each of these proteins.

MinC stimulates the dissociation of FtsZ filaments. MinC binds to MinD.

MinD is a bacterial cytoskeletal protein that has no known homologs in eukaryotes. MinD is an ATP-binding protein with intrinsic ATPase activity. ATP-MinD self-associates to form filaments; ADP-MinD dissociates from filaments. This dynamic behavior is similar to the dynamic behavior of bacterial actin-like proteins (like MreB), although MinD is not an actin-like protein.

MinE binds to the leading edge of the "cup" of MinD-MinC filaments that grow from the cell pole towards midcell. MinE activates the intrinsic ATPase activity of MinD, which results in conversion of ATP-MinD-ATP to ADP-MinD.

2. Explain how the components of the Min system work together to localize the FtsZ ring at midcell.

ATP-MinD nucleates filaments at sites of high negative curvature, especially at the cell poles, which are the curviest part of the cell. As a "cup" of ATP-MinD filaments grows outwards from a cell pole, the leading edge of the cup attracts molecules of MinE, which form a circumferential ring at the leading edge of the ATP-MinD cup. MinE promotes conversion of ATP-MinD to ADP-MinD and detachment of ADP-MinD subunits from the leading edge of the MinD cup. In this manner, the MinE ring "dissolves" the leading edge of the ATP-MinD cup back towards the cell pole until all of the ATP-MinD subunits have been converted to ADP-MinD and detached. Meanwhile, in the cytoplasm the free ADP-MinD subunits are converted to ATP-MinD subunits, which migrate to the opposite cell pole (where there is no MinE ring) and nucleate a new ATP-MinD cup, which grows outwards from the cell pole until a new MinE ring forms at the leading edge of the ATP-MinD cup…and the entire process starts all over again. Thus, over time, ATP-MinD filaments oscillate between the two cell poles and the region of the cell with the lowest time-averaged concentration of MinD subunits is midcell. MinC "hitchhikes" by binding to MinD and travels with it, back and forth, between the two cell poles. Thus, the region of the cell with the lowest time-averaged concentration of MinC is midcell. MinC promotes the dissociation of FtsZ filaments. Since the region of the cell with the lowest time-averaged concentration of MinC is the midcell region, this is the only region where FtsZ can polymerize into the "FtsZ ring" that determines where the cell will divide.

3. Imagine a bacterial cell treated with a drug that depletes the MinCDE proteins. What phenotype would you predict for the MinCDE-depleted cell?

In **untreated wild-type cells**, the "Minicell" (Min) system ensures that the FtsZ ring forms only at the correct place for cell division, i.e., at midcell. In **Min-depleted cells**, there is no mechanism to concentrate FtsZ filaments at midcell. Consequently, the "FtsZ ring" that drives cell division can form at locations other than midcell, which may result in asymmetric division and formation of very small cells ("Minicells") that lack chromosomal DNA. Because they lack chromosomal DNA, these "Minicells" are not viable.

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4. Imagine a bacterial cell treated with a drug that depletes the FtsZ protein. What phenotype would you predict for the FtsZ-depleted cell?

The FtsZ protein forms filaments that coalesce into an "FtsZ ring" at midcell prior to cell division. The FtsZ ring functions as a scaffold for assembly of the system of proteins involved in cell division (the "divisome"). Thus, localization of the FtsZ ring at midcell is the primary determinant of where cell division will occur in space. In the absence of FtsZ (in FtsZ-depleted cells), the FtsZ ring cannot form and, therefore, the divisome cannot form either. Consequently, FtsZ-depleted cells will form elongated filaments by growing longer and longer without dividing.

Note. Experimental compounds that target FtsZ are in fact under development as potential new antibiotics. These compounds work by interfering with FtsZ polymerization, rather than by depleting FtsZ, but the result is the same. You don't need to know this for the exam but I think it's a "fun fact".

5. The FtsZ protein forms dynamic filaments that "treadmill" around the short axis of the bacterial cell. Explain: what is "treadmilling" and how does it work?

FtsZ subunits bind to GTP and have an intrinsic GTPase activity that converts GTP to GDP. GTP-FtsZ subunits form filaments that grow faster at the "plus" end than at the "minus" end. At physiological concentrations of GTP-FtsZ, the FtsZ filament grows at the plus end by addition of GTP-FtsZ subunits, which form stable filaments. However, the rate of addition of GTP-FtsZ is slower at the minus end than the rate of conversion from GTP-FtsZ to GDP-FtsZ, and GDP-FtsZ subunits dissociate from the filament. Thus, the filament shrinks at the minus end. This behavior (growth of the FtsZ filament at the plus end with simultaneous shrinkage of the FtsZ filament at the minus end) is called "treadmilling". Note that this dynamic behavior is very similar to the dynamic behavior of bacterial actin-like protein filaments such as MreB (see Exercise 1, above). The differences are: FtsZ is a tubulin homolog whereas MreB is an actin homolog; FtsZ binds to and hydrolyzes GTP whereas MreB binds to and hydrolyzes ATP.

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EXERCISE 4 "BIOMECHANICS OF THE BACTERIAL CYTOSKELETON" :

1. In solid objects under stress, formation and propagation of cracks is the primary cause of material failure. Explain why.

In a solid object experiencing stress (e.g., an object that is being stretched or compressed), the stress concentrates at the tips of cracks that are oriented perpendicular to the direction of the stress. This is because the material "behind" the crack, being separated into two physically unconnected halves, cannot resist any of the stress exerted by stretching or compression. Consequently, the stress in transferred to the crack tip, which may (if the force is sufficient) result in progressive propagation of the crack through the material.

2. Intermediate filament proteins form "coiled coils". Explain: what is the overall structure of a "coiled coil"?

Each intermediate filament protein contains long stretches of alpha-helical structures, forming a first-order "coil". When two molecules of an intermediate filament protein interact, these alpha-helixes wrap around each other along their long axes, forming a second-order "coil". The overall structure (comprising first-order and second-order coils) is described as a "coiled coil".

3. Intermediate filament proteins form "coiled coils". Explain: what drives the formation of "coiled coils"?

Each intermediate filament protein contains long stretches of alpha-helical structures with a helical "stripe" of hydrophobic amino acids running along the length of the alpha-helix. As the term "hydrophobic" implies, these hydrophobic stripes do not "like" to be exposed to water. Consequently, when two molecules of an intermediate filament protein come together, their alpha helices wrap around each other lengthwise (forming a "coiled coil") in order to "bury" their hydrophobic stripes at the interface between the two proteins. This arrangement shields the hydrophobic amino acids from the surrounding water, thereby minimizing the free energy of the system.

4. "Coiled coils" of intermediate filament proteins are highly extensible. Explain how this works.

Intermediate filament proteins contain long stretches of alpha-helix structures that look and act like springs. Under tensile stress (stretching), intermediate filament proteins display strongly non-linear stress-strain curves with three distinct "stretching regimes". The first regime (called the "alpha-regime" in the slides) represents the overall stretching of the alpha-helix structures along the long axis, very similar to a "classical" Hookean spring. In this domain, the slope of the stress-strain curve is steep. The second regime (called the "beta-regime" in the slides) represents the breaking of hydrogen bonds between adjacent turns of the alpha helix. An equal amount of force is required to break each successive hydrogen bond. Consequently, in the "beta-regime" the slope of the stress-strain curve is very shallow (almost flat). The "beta-regime" of the stress-strain curve explains the remarkable extensibility of intermediate filament proteins, because the protein can undergo a lot of stretching without requiring a big increase in the force applied to it. The third regime (called the "gamma-regime" in the slides) represents the stretching of the actual polypeptide backbone, which occurs only after all of the hydrogen bonds between adjacent turns of the alpha-helix have been broken. A lot of force is required to stretch the polypeptide backbone

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of a protein. Correspondingly, the slope of the stress-strain curve is quite steep in this region of the curve.

5. Intermediate filaments form mesh-like networks that are resistant to crack propagation. Explain how this works.

Intermediate filament networks often have a mesh-like organization with individual filaments oriented along the x or y axis, like burlap cloth or "toile de jute". Individual intermediate filaments are "stretchy" and can undergo a lot of strain before breaking. Thus, networks of intermediate filaments can stretch quite a bit in every direction without breaking. If there is a crack in the intermediate filament network and tensile stress is applied perpendicular to the crack, normally this would" lead to stress concentration at the crack tips, crack propagation, and material failure. However, when the mesh-like structure of the intermediate filament network stretches along the same axis as the tensile stress, this leads to blunting ("rounding off") of the crack tip, thus reducing stress concentration and suppressing crack propagation. In fact, the more the mesh-like material is stretched the more the crack tip will be blunted in the direction perpendicular to the direction of tensile stress. Of course, this cannot continue indefinitely, as intermediate filaments are not infinitely extensible, and eventually the stress will exceed the material strength of the filaments, leading to breakage. Still, this mesh-like arrangement of filaments does make the material more resistant to crack propagation, which is the usual cause of material failure.