Assignment Questions

1. Dr. Theriot compares two types of eukaryotic cells, the amoeba and the neutrophil, which show some surprising similarities. From the list below, select all the statements that are correct:
   a. They evolved very recently from a common ancestor
   b. They use similar cellular and molecular processes to move
   c. They have very different shapes
   d. None of the above

2. A good example of a situation that involves cell motility is wound healing. From the list below, select all the statements that are correct:
   a. Neutrophils target bacteria and try to engulf them
   b. Epithelial cells move into the wound to close it up
   c. White blood cells target bacteria and try to engulf them
   d. Keratocytes move into the wound to close it up
   e. None of the above

3. Based on Dr. Theriot’s presentation of essential concepts in cell motility, choose all the statements that are correct:
   a. Actin depolymerization pushes the cells forward
   b. Some cells in our body can re-orient their movement within a few milliseconds
   c. Cell motility requires the establishment of cell polarity
   d. Cell motility requires inhibition of adhesion
   e. None of the above

4. Regarding the structure of actin filaments (choose all that apply):
   a. Actin filaments are connected to other actin filaments in a helical structure
   b. Actin monomers are connected to other actin monomers in a helical structure
   c. Branched structures amplify the individual properties of actin polymers
   d. None of the above
5. Regarding actin polymerization and depolymerization (choose all that apply):
   a. Individual actin filaments are constantly assembling and disassembling
   b. The lifetime of an individual actin filament is usually as long as the lifetime of the cell
   c. Actin polymerization and depolymerization are facilitated by actin-binding proteins
   d. Depolymerization of actin filaments enables protrusion to happen
   e. None of the above

6. To crawl across a substrate, the cell must push the leading edge forward. This is possible because (choose all that apply):
   a. The depolymerization reaction is always energetically favorable
   b. Polymerization releases energy used for an energetically unfavorable reaction
   c. A new monomer can sneak in between the load and the polymer to generate force
   d. None of the above

7. About actin monomers (choose all that apply):
   a. ADP-actin is more likely to polymerize
   b. ATP-actin is more likely to polymerize
   c. ATP is hydrolyzed when the actin monomer binds to a filament
   d. None of the above

8. Using RNAi, scientists found a gene which, when mutated, resulted in intracellular changes causing loss of motility. Based on these results, select possible hypotheses that could explain these changes from the list below:
   a. Fmax increased
   b. Ccrit was decreased and to a value lower than C
   c. The size of the individual subunits increased
   d. None of the above

9. Regarding the physical models proposed to describe how actin filaments can create space for a new actin monomer (choose all that apply):
   a. One model suggests that the actin filament is fixed in space, while the load undergoes thermal fluctuations, creating space for the monomer
b. One model suggests that the actin filament fluctuates through thermal motion, and that it is the bending of the filament that creates space for the monomer

c. None of the above

10. Compare the two major physical models proposed to describe how actin filaments can create space for a new actin monomer (choose all that apply):

a. They both help us predict how fast the actin polymer motors can go when pushing a load
b. The speed of movement predicted by these models is less than the speed of a cell
c. None of the above

11. Why is Listeria monocytogenes such a good model for studying cellular motility (choose all that apply)?

a. Because the mechanism by which actin polymerizes and depolymerizes inside Listeria is highly conserved
b. Because Listeria harnesses the depolymerization of actin in epithelial cells to move
c. Because Listeria can be used in vitro to study comet tail formation
d. None of the above

12. Regarding the comet tail (choose all that apply):

a. It has a similar structure to the actin network that causes protrusion of the leading edge
b. The tail end of the comet tail is the polymerization side
c. It is made up of actin filaments organized in branched networks
d. None of the above

13. How can comet tail formation be studied in vitro (choose all that apply)?

a. Adding bacteria to the right cytoplasmic extract can induce comet tail formation
b. Adding the right proteins to bacteria can induce bacterial motility in vitro
c. Adding the right proteins to polystyrene beads in a cytoplasmic extract can induce bacterial motility
d. Adding the right proteins to polystyrene beads in a cytoplasmic extract can induce bacteria-like motility.
14. About the biochemical events that are involved in comet tail formation and bacterial motility (choose all that apply):

   a. The epithelial cell expresses ActA, which binds and activates the Arp2/3 complex on the membrane of the bacterial cell
   b. Capping Protein disassembles old actin filaments so the monomer pool can be regenerated
   c. ADF keeps the free filament ends from continuing to polymerize
   d. Arp2/3 nucleates the growth of a new filament from pre-existing branched filaments
   e. None of the above

15. Using Dr. Theriot’s talk, synthesize the important molecular steps involved in comet tail formation and disassembly with:

   a. A drawing/sketch of the processes
   b. On the sketch, label the following molecules mentioned in the talk:
      Capping Protein, ATP-bound actin, ADP-bound actin, ADP/cofilin, Arp2/3 complex, actin filament
   c. Provide a short (one paragraph) description of the most important steps

16. Discussion Question:

   In her seminar, Dr Theriot describes a thermodynamic model that proposes a mechanism by which the energy associated with a polymerizing cytoskeletal filament can be converted to force for cell movements.

   Write a paragraph-long answer to the following question:

   What predictions of the model would you measure in order to test the idea that the proposed mechanism is used to propel a Listeria bacterium through cytoplasm in infected cells and how might this illuminate the mechanism of neutrophil motility in the blood?
Assignment Answers

1. b
2. a, b, c, and d
3. b and c
4. b and c
5. a and c
6. b and c
7. b and c
8. c
9. a and b
10. a
11. c
12. a and c
13. a, b, and c
14. d
   a. A drawing/sketch including at least 3 of the processes described in 15 c.
   b. Sketch should include labels for the following: Capping Protein, ATP-bound actin, ADP-bound actin, ADP/cofilin, Arp2/3 complex, actin filament
   c. Provide a short (one paragraph) description of the most important steps. The paragraph should include at least 3 of following processes:

15.
   a. Capping Protein binds to the end of older actin branches to prevent further polymerization
   b. Polymerization of actin filaments by addition of ATP-actin (at leading edge or branches)
   c. Arp2/3 complex binds to the side of a pre-existing actin filament and nucleates the growth of a new filament into a branched structure
   d. ADP/cofilin disassemble old actin filaments by severing and depolymerizing actin
   e. ADP-bound actin, released and phosphorylated from the depolymerizing end of the actin filaments is recycled to the polymerizing end
Rubric

<table>
<thead>
<tr>
<th>Assignment (10pts)</th>
<th>Pass (points)</th>
<th>No Pass (no point)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length and clarity (5 pts total)</td>
<td>The student provided a paragraph-long answer and used full sentences.</td>
<td>The student’s answer was too short (less than a paragraph long) or used short fragments of sentences.</td>
</tr>
<tr>
<td>Content of answer (5 pts total)</td>
<td>The answer relates directly to the discussion question and is based on valid scientific data, models or principles.</td>
<td>The answer is off topic and it is not based on any scientific data, models or principles.</td>
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In-Class Quiz Questions

1. Why is Listeria a good experimental model for studying amoeboid locomotion (5 pts)?

2. “ATP hydrolysis is required for actin to push on a Listeria or a neutrophil cell membrane.” Do you agree or disagree? Explain why.

3. How do random thermal fluctuations due to thermal energy in the system allow a cytoskeletal filament to polymerize and push on a Listeria bacterium or a membrane at the leading edge of a locomoting keratocyte?

In-Class Quiz Answers
1. Because the mechanism involved in comet tail formation is the same as the mechanism involved in cell motility, a process that is conserved across evolution.

2. Disagree – force is produced by polymerization of an ATP-monomer to a filament. ATP hydrolysis causes depolymerization of actin (and therefore of the comet tail).

3. Students describe one or both of the models described in Theriot Part 1 – 23:11