

(23) great!

(10 pts) (10)

Based on our studies of *Listeria* migration in class and discussion, we can establish a working model in which the bacterial protein ActA is synthesized and secreted asymmetrically by the bacteria. ActA presumably binds to and localizes the eukaryotic protein, profilin, to its "hind" end and the profilin, in turn, promotes polymerization of actin filaments needed for migration of the bacteria. This is supported by our observation that when a mutant strain of *Listeria* was generated lacking the proline-rich region (PRR) of ActA, profilin localization to the hind end of the *Listeria* was eliminated. We would expect, then, that localized actin polymerization would also be eliminated. Instead, we observed a small amount of actin still polymerizing at the tail end of the mutant strain.

Question #1: Assuming that binding of ActA to profilin has been eliminated truly and completely, give the simplest explanation possible for the observation that actin still polymerizes (albeit in very small amounts) at one end of the mutant *Listeria* cells.

4 ActA's actin binding region was still intact on the mutant strain. Actin polymerization can occur without profilin but not very effectively. The binding of three G-actin is most likely quite slow in this case ^(to initiate polymerization) and only little polymerization occurs.

great
Question #2: Design an experiment to test the hypothesis that you described in #1. Describe your methods and both of the expected outcomes should your hypothesis be a) correct or b) incorrect.

6 Other mutant forms of ActA would be constructed, that would also be lacking the PRR rich region, but also other domains of the protein. These new constructs would be compared to the wild type and construct only lacking PRR in ability to polymerize actin. If one of the constructs can not polymerize at all, the above hypothesis would seem to be supported, loss of actin binding domain and profilin domains, total loss of actin polymerization ability. If some polymerization does occur with all constructs, hypothesis not supported, another hypothesis needed.

Name _____

(15 pts)

(13)

Please indicate if the following statements are true or false. If they are false, correct them so that they are true.

- 1 T ✓ ES cells are capable of giving rise to every single type of cell in the adult mouse.
- 1 T ✓ In an S1 myosin-decorated actin filament, the pointy end of the arrowhead points to the (-) end of the actin filament.
- 1 F ✓ Integrins link the ECM with the actin cytoskeleton at ~~hemidesmosomes~~ focal contacts.
- 1 T ✓ The ability of actin to carry out so many diverse roles in cells is due in part to actin filament cross-linking proteins.
- 2 F ✓ F. Brown Multiple forms of ~~collagen~~ are produced by alternate RNA splicing.
- 1 T ✓ Matrix proteases help modulate the ~~actin~~ ECM cytoskeleton so that it is a dynamic, responsive structure during growth and development.
- 2 F ✓ One remedy for poisoning by phalloidin-containing mushrooms would be to eat a lot of raw meat because the high concentration of ~~myosin~~ actin in the ingested muscle tissue would bind to and inactivate the phalloidin.
- 1 T ✓ Skin blistering diseases can occur in any number of situations when dermal-epidermal adhesion is compromised.
- 1 T ✓ The hydrolysis of ATP to ADP within the actin monomer contributes to the dynamic instability of the actin filament.
- 2 F ✓ In gene targeting, the neo gene, in conjunction with the drug neomycin, ~~kills~~ live transfected ES cells which have randomly integrated the DNA construct (the targeting vector).
- 1 T ✓ In a dividing cell, the contractile ring contains both actin and myosin.