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Julie Theriot's Lecture Part 2:

Force Generation by Actin Assembly: Theories and Experiments

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1. Keywords and Terms

optical trap, atomic force microscopy, acrosomal bundle, cantilever, ActA, keratocytes, force generation, actin bundle, branched network, stall force

2. Review Questions

1. Why was a bundle of filaments, and not a single filament, used to measure the force generated by actin polymerization?
2. In the optical trap experiment, how do individual filaments contribute to the total force generated by an actin bundle? For example, at a given time, is the measured force the sum of all the individual filaments within the bundle, a subset of the

filaments, or a single filament? What conclusions can be drawn about force generation of branched networks?

3. Why can't optical tweezers be used to measure the force generated by the comet tail of *Listeria*?
4. Name three mechanisms used by biological systems to generate force.
5. Explain the microscopic and mesoscopic physical models of actin-based motility.
6. What are the differences between the microscopic and mesoscopic models of actin-based motility?
7. What would the mesoscopic model of actin-based motility predict when a non-rigid object, such as a liposome, is coated with ActA? What is experimentally observed?
8. In the AFM experiment, how is the movement of the cantilever detected? What intrinsic property of the cantilever is used to calculate force?
9. Give three examples of keratocyte behavior where history dependent effects may be occurring.
10. Give one example of experimental evidence that supports the mesoscopic elastic gel model and two examples that refute the model.

3. Answers to Review Questions

1. Single actin filaments would present mechanical problems. For example, a single filament is less rigid and smaller in size. Thus, a single filament is more likely to bend, buckle or break and it is more difficult to grab and manipulate a single filament. In addition, the acrosomal bundle could be used to nucleate new filament growth at a predictable location in space.
2. The experimentally measured stall force of the actin bundle was approximately 1.5 pN, which is close to the predicted force from the polymerization of a single actin filament. This suggests that the force generated from the acrosomal bundle, which typically consists of six filaments, is dependent on the growth of a single filament. If the acrosomal bundle is an accurate model of actin bundles, then this experiment indicates that force generation from a bundle of filaments is not additive but instead contingent on only the longest filament in the bundle. This experiment is only

suggestive of small actin bundles and no conclusions can be made on branched networks.

3. The stall force needed to measure the force generated during actin-based motility is outside the range of the optical trap. The laser trap is able to measure forces in the picoNewton range, which is significantly less than the force generated by *Listeria*.
4. Molecular motors, rotary motors, Brownian ratchet, preloaded springs (e.g. the horseshoe crab sperm acrosomal bundle), and extrusion nozzles (e.g. Cyanobacterium can move by extruding a glycoprotein slime from their posterior end).
5. Microscopic model or the tethered Brownian ratchet model: In this model, the bacterium surface is modeled as a rigid surface that polymerizing filaments generate force against. In this model, a pushing force is generated by polymerizing filaments and a drag force is generated by filaments bound to the surface of the bacterium. The relationship between these forces can be modeled mathematically to predict the force generated and the speed of the bacterium.

Mesoscopic model or the elastic gel model: This model ignores individual filaments and instead treats the comet tail as an elastic gel. As the gel grows along the surface, the stress from the growing gel will increase moving towards the back of bacterium, which will create a strain that is greater at the back of the bacterium. The strain generates a squeezing force that pushes the bacterium forward.

6. The microscopic model focuses on individual filaments, while ignoring interactions between filaments and the geometry of the cell. In the mesoscopic model, the interactions between filaments and the shape of the cell are taken into consideration, but the contribution of individual filaments are neglected.
7. In the mesoscopic model, there is squeezing force that is generated at the posterior end of the object. Therefore, if a non-rigid object is used, the force of the elastic gel should squeeze and deform the back of the object. In addition, if the properties of the object are known, then the change in object shape can be used to estimate the amount of force generated at the surface of the object. Experimentally, it is observed

that liposomes do deform as if they were being squeezed, in qualitative and quantitative agreement with the predictions of this model.

8. The movement of the cantilever is detected by measuring the deflection of a laser beam that is reflected from the cantilever surface. Cantilever stiffness is an intrinsic property that must be known in order to calculate the amount of force corresponding to a particular deflection of the laser beam. When subjected to the same amount of force, a stiffer cantilever will bend less than a more compliant one. The relationship between the extent of laser deflection and the stiffness of the cantilever can be used to calculate the force.
9. In the lecture, three keratocyte observations were described. The importance of the past can be seen in the behavior keratocytes after they collide with a barrier, oscillations during persistent motion, and symmetry-breaking of non-motile symmetrical cells.
10. Deformation of the lipid vesicles suggest a squeezing force is generated during actin-based motility and supports the elastic gel model. Evidence against the mesoscopic model include the observation that changing the geometry at the end of the cantilever (round ball vs. flat surface) does not alter the force-velocity curve and the data showing a concave-down curve force-velocity curve while the model predicts a concave-up force-velocity curve.

4. Discussion Questions

1. In the optical trap experiment, laser light is used to hold the bead and the bundle next to the nanofabricated wall. Is work being done against the laser light focused at the bead and/or the light focused along the bundle?
2. What is the Reynolds number and why is it relevant when studying *Listeria* motility?
3. How would you expect the rate of actin growth to change when pulling up or pushing down on the back of the cantilever?
4. What might account for the load-independent behavior of ActA growth, as demonstrated using AFM?

5. Suggest a role for ActA in addition to Arp2/3 complex mediated actin polymerization. Extend the watermelon seed analogy used in lecture. Compare wild-type *Listeria* with the ActA mutant. If the watermelon seed is *Listeria* and your fingers are a comet tail, what would the outcome be when squeezing your fingers when using wild-type *Listeria*, what would you expect when using the mutant?

5. Answers to Discussion Questions

1. In theory, work should only be done against the laser light focused at the bead. For the bead to move away from the wall, the force from actin polymerization must do work against the force applied by the laser light. The bead is positioned in an energy well where force is needed to displace the bead from its position in the trap. The laser light focused along the length of the bundle merely holds the bundle perpendicular to the wall.
2. The Reynolds number of an object moving at a velocity (v) in a fluid is defined by the equation

$$R = \frac{2vL\rho}{\eta}$$

Where L is the size of the object, ρ is the specific gravity of the fluid and η is the viscosity of the fluid. The Reynolds number can be thought of as a measure of the relative importance of inertial forces to viscous forces. At small Reynolds numbers, R less than 1, viscous forces dominate the motion of the particle and the inertia of the particle is negligible. Bacteria live at a Reynolds number well below one. This means that bacterial movement is characterized by viscous drag and inertia is essentially nonexistent. In these conditions, when a directional force is removed the bacterium will instantaneously lose any directional motility and its movement will be defined by thermal forces.

3. As described in the lecture the relationship between force and velocity is history dependent. When pulling up on the cantilever you reduce the force applied to the actin network and an increase in the rate of actin growth is seen. When pushing down on the cantilever, the force on the growing actin network increases and the growth rate will likely be dependent on the change in force. If there is a significant

increase in force, then there will likely be a significant reduction in the rate of growth, after which the linear relationship between force and velocity should be recovered. A slight increase in force should have little impact on growth rate, as actin growth occurs in a load-independent manner.

4. There are many possible answers to this question. The growth of the actin network could be limited by force-independent mechanisms. For example, the rate limiting step could be the time required for actin polymerization or nucleation of new filaments. Alternatively, the velocity may remain constant with increasing force if the density of the actin network increases with time. The density of the network should naturally increase with time because the branching rate should increase with time.

Lasa et al. describe a *Listeria* ActA mutant that exhibits a hopping behavior (Lasa et al. (1997). EMBO. 16: 1531-40). The mutant *Listeria* moves in a discontinuous manner, where periodic spots of dense actin (~2microns in length which is about the size of the bacterium) are spaced by distances of 1 to 4 microns. The speed of the bacterium occurs in bursts that coincide with the bursts of actin polymerization. The average speed of the wild-type *Listeria* is twice as fast as the mutant, but the burst speed of the mutant, which peaks as the bacterium emerges from the actin sheath, is around four times faster than wild-type *Listeria*.

5. The behavior of the ActA mutant suggests that the sheath of polymerized actin produces a squeezing force that drives bacterial motility. The bursts of actin density could result from the inability of the bacterium to remain in tethered to the growing actin network, which suggests that ActA is needed to tether the bacterium to the comet tail. The burst speed of the mutant would increase if there is no drag force produced by tethering between the tail and bacterium.

In the watermelon seed analogy, in wild-type conditions, squeezing the watermelon seed would result in forward movement of the seed and the elongation of your fingers. In mutant conditions, squeezing the watermelon seed would produce a squeezing force that shoots the seed forward while your fingers would remain stationary and not elongate.

6. Explain or Teach These Concepts to a Friend

1. Explain why cells need to move. Include examples of actin-based motility of eukaryotic and prokaryotic cells.
2. Why is drift a problem in AFM experiments? How does the second cantilever correct for this problem?
3. How could you directly observe the dynamic behavior of the proteins involved in actin-based motility of *Listeria*?

7. Research the Literature on Your Own

1. What experimental techniques are used to measure forces in the picoNewton to nanoNewton range? Describe the theory behind the technique and list at least two biological applications for each technique.
2. Describe in detail the events of actin-based motility in a eukaryotic cell or bacterium of your choosing. Next, use metaphors to describe each event in the process.
3. Actin polymerization drives the motility of *Listeria* and the protrusion of the leading edge of many motile cells. The speed of cell movement can vary over a wide range, from about 1 micron per second (for chemotaxing neutrophils) to about one micron per hour (for some fibroblasts). *Listeria* moves at a speed of about 0.2 microns per second in epithelial cells but up to five times faster in macrophages. What might cause the speed of actin-based motility to vary so much? How could you test your ideas?

8. Papers for Journal Club

1. Theriot, J.A., T.J. Mitchison, L.G. Tilney, D.A. Portnoy (1992). The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization. *Nature* 357: 257-60. *This is the first paper to closely examine the dynamics of actin polymerization during actin-based motility of Listeria.*

2. Loisel, P.L., R. Boujemaa, D. Pantaloni, M. Carlier (1999). Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* 401: 613-6. *This is the first reconstitution of actin-based motility. This paper helped to elucidate the mechanism of actin-based motility.*
3. Parekh, S.H., O. Chaudhuri, J.A. Theriot, D.A. Fletcher (2005). Loading history determines the velocity of actin-network growth. *Nature Cell Biology* 7: 1219-23. *This paper shows how a cantilever can be used to measure actin polymerization forces.*
4. Pollard, T.D. and G.G. Borisy (2003). Cell motility driven by assembly and disassembly of actin filaments. *Cell* 112: 453-65. *A good review on the importance of actin dynamics in cell motility and the proteins that modulate this process.*