Highlighted Research

Monomer-sized Stepping of *Listeria monocytogenes*

Citation: Kuo & McGrath 2000, *Nature 407*, 1026-9

Summary:

We report the unexpected discovery of monomer-sized steps during actin-based motility of *Listeria monocytogenes*. Because *Listeria* is a model system for understanding many types of actin-based cell motilities, the existence of step-like motion severely constrains models of many cellular motilities. Apparently, only actin polymerization is needed to propel *Listeria* and many models have been proposed to explain how actin polymerization alone generates forces. In light of our discoveries, the most popular models, the Brownain ratchet models, are incorrect as published and must be recalculated if not discarded. The geometric constraints implied by monomer-sized stepping are very restrictive, and hence, very revealing.

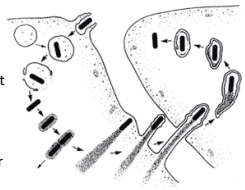
News Items: Highlighted by the Whiting School of Engineering, there is a video interview (A 'tail' of two cells) and news release (Tracking a microscopic 'rocket' by its tail) discussing this work. This research has been highlighted in JHU Gazette (Oct 30 2000, p8), Biophotonics International (Dec 2000, **7**:13), Photonics Spectra (Jan 2001, **35**:18), and ASEE Prism (Feb 2001, **10**:9). This research was presented in an invited minisymposium talk at the Dec 2000 Meeting of the American Society for Cell Biology and at various invited seminars.

Outline:

- Life cycle of *Listeria monocytogenes*
- Relevance to other pathogens and cell motility
- How can mere polymerization push? -- introduction to Brownian Ratchet models
- **Discovery:** *Listeria* moves with monomer-sized steps (~5.4 nm)
- **hy surprising:** Stepping shouldn't be observable!
- Hypothesis to explain the appearance of steps
- Implications of monomer-sized steps

The animations below require either Macromedia's Flash Player or Apple's QuickTime Player to view. Certain versions of Internet Explorer 5.5 interfere with playing the QuickTime format of the animations; download a newer version.

Life Cycle of *Listeria monocytogenes* The life cycle of the bacterial pathogen, *Listeria monocytogenes* is summarized in the image at the right (from Tilney & Portnoy 1989, *J Cell Biol* **109**:1597-1608). As part of normal defenses against infection, white blood cells (macrophages) "eat" *Listeria* (phagocytosis), intending to destroy the bacterium. However, *Listeria* makes specific enzymes that help it escape the cell's "stomach" (lysosome) into the cytoplasm. Somewhat "hidden" by the "cloak" of the host cell's outer membrane, *Listeria* evades the immune system by growing *within* the cytoplasm of host cells.



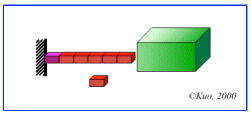
Once within the cytoplasm of the host cell, *Listeria* quickly assembles proteins from the host cell to form rocket-like tails containing F-actin. These F-actin tails propel bacteria through the cytoplasm (see QuickTime movie (3.8Mb) of F-actin tail propelling bacterium inside a host cell). When bacteria encounter the cell's outer membrane, they deform the membrane and attempt to infect neighboring cells. During the protrusion of *Listeria*, bacteria maintain the protection of original host cell's outer membrane. Long before the original host cell fills with bacteria and bursts, adjacent cells have already been infected.

The generation of a rocket-like F-actin tail and subsequent propulsion of bacteria is essential to *Listeria*'s pathogenicity. Only one bacterial protein (ActA) is required to generate rocket tails. Genetic deletion of this protein nearly blocks the virulence of this bacterium.

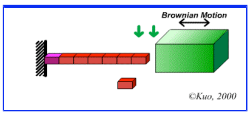
Relevance to Other Pathogens and Cell Motility Other pathogens also generate propulsive F-actin tails. These include *Shigella* (bacterial dysentery), *Rickettsia* (Rocky Mountain spotted fevers), cowpox (vaccinia) virus,and, presumably, smallpox virus. By analogy to *Listeria*, all these pathogens probably use their F-actin rocket-like tails for motility and to infect neighboring cells. Hence, the rocket-tail propulsion would be critical for the virulence of these pathogens.

Not only an interesting bacterial pathogen, *Listeria* is a model system for understanding cell motility. The same host proteins recruited by *Listeria* are also used by the host cell for it's own motile and protrusive processes. When cells crawl (e.g. activated white blood cells), they must protrude their leading edge prior to gripping and pulling. Similarly, when cells "eat" (phagocytose), cells must locally protrude to engulf particles (see image above; see QuickTime movie of white blood cell "hunting down" a bacterium, 9Mb). Finally, when neuronal cells are extending axons, the leading edge of the axon protrudes finger-like filopodia to "taste" for guidance cues that direct neurite extension (see QuickTime movie of neuronal filopodia, 2Mb). Activated by the similar molecular processes, all of these processes require actin polymerization by the host cell to protrude. Because *Listeria* generates "stripped-down", unregulated motility, it helps focus investigators on the key host proteins required for all these motilities. So far, only proteins required to regulate actin polymerization into F-actin are believed necessary for *Listeria* motility (Loisel *et al.* 1999, *Nature* **401**:613-615).

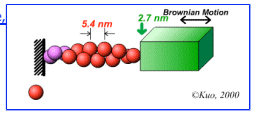
How can mere polymerization push? Despite its widespread acceptance, it seems paradoxical that actin polymerization alone can generate forces. To illustrate this paradox, consider the cartoon at the right and its companion animation (either Flash, 5Kb or QuickTime, 7Kb). Think of actin monomers as books being stacked onto a bookshelf. When reaching the bookend, there's not enough room for the next book. If we were to manually slide the bookend to make room for the next book, our action of moving the bookend generated the force; stacking books only stabilized the new position of the bookend. From this macroscopic analogy, polymerization alone could never generate forces.

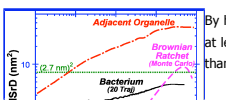


This apparent paradox can be resolved because nothing is truly stationary at the microscopic size scale. As shown in the cartoon at right with its companion animation (either Flash, 8Kb or QuickTime, 9Kb), heat (even at room temperature) causes all objects to "wiggle" with Brownian motion. The magnitude of Brownian motions are generally inversely related to size; hence, microscopic objects can "wiggle" a lot. In the macroscopic analogy, the bookend intrinsically wiggles more than the size of each book, and when it wiggles far enough, polymerization would "jam" the next book into the gap on the bookshelf. Each inserted book prevents any backsliding and "ratchets" the bookend forward. Called the "Brownian ratchet" by George Oster and colleagues, this type of model is very elegant and amenable to analytical exploration with a minimum of parameters (Peskin, Odell & Oster 1993, *Biophys J* **65**:316-324). To distinguish it from later Brownian ratchet models, it is often called the "**classic Brownian ratchet**" model.

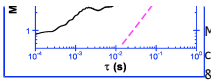


To give the **classic Brownian ratchet** some molecular details, consider the cartoon at right and its companion animation (either Flash, 7Kb or QuickTime, 9Kb). Actin monomers are 5.4 nm in diameter and F-actin filaments are composed of two intertwined protofilaments that are staggered by half a monomer (2.7 nm). As suggested by the cartoon and its animation, *Listeria* must "wiggle" at least 2.7 nm to allow intercalation of actin monomers. The magnitude of the "wiggles" are inversely proportional to the size of the bacterium and to the viscosity of its environment. Because diffusion is time-dependent, the longer you wait, the larger the magnitude of Brownian motions and intercalation eventually occurs. However, the high speed of *Listeria* motility implies that bacteria diffuse very readily. Rapid diffusion means that its Brownian motions are sufficiently large at the right time scales so that the rates of actin monomer intercalation can explain *Listeria*'s high speed.

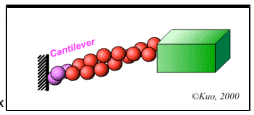




By high resolution laser-tracking of bacteria in living host cells, we have shown that the classic Brownian ratchet is not correct for explaining *Listeria* motility (Fig at left is colorized version of Fig.4 from Kuo & McGrath 2000, *Nature* **407**:1026-9). Not only do *Listeria* "wiggle" less than 2.7 nm, it wiggles at least 20-fold less than adjacent particles in the cell. Hence, *Listeria* are not limited by the viscosity of the subcellular environment. They must be binding their F-actin tails.

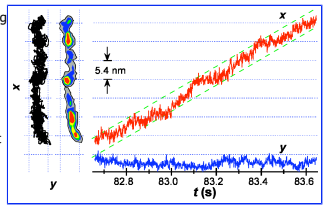


Microscopically, filaments readily bend so that bacteria do not have to "wiggle" to allow actin intercalation; the filaments can do almost all the "wiggling". Often called the "**elastic Brownian ratchet**", George Oster and colleagues (Mogilner & Oster 1996, *Biophys J* **71**: 3030-3045) developed an alternative to their original Brownian ratchet model.



Exaggerated in the cartoon at right and its companion animation (either Flash, 12Kb or QuickTime, 14Kb), the elastic Brownian ratchet considers the bending flexibility of filamentous actin. With little diffusion of the bacteria, most of the Brownian motion occurs in the flexing of F-actin. If filament tips flex sufficiently far from the bacterial surface, actin monomers can intercalate. Despite its statistical flexing, the longer filament applies increased pressure to the bacterial surface. Increased pressure will eventually cause bacteria to move.

***Listeria* moves with monomer-sized steps (~5.4 nm)** By high-resolution laser-tracking of bacteria in living host cells, we made a startling discovery. Bacteria often paused during actin-based motility, and pauses were spaced the size of actin monomers (5.4 nm). The figure at right (colorized version of Fig. 2 from Kuo & McGrath 2000, *Nature* **407**:1026-9) shows the "hotspots" in pausing. The left side of the figure shows both the trajectory of the moving bacterium and the "hotspots" of bacterial position next to the trajectory. When plotted against time, pauses are apparent, but some of the pauses that were visible in the *xy* trajectory become lost in the noise.

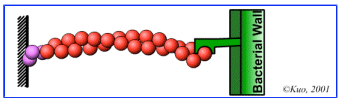


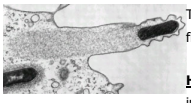
Unlike molecular motors that step (e.g. kinesin, Svoboda *et al.* 1993, *Nature* **365**:721-7), *Listeria* do not move with steps of rigorously constant sizes. Limited by the noise in our equipment, we can see half-monomer-sized steps (~3 nm) and episodes with no obvious pauses during motility (see Supplemental Information of Kuo & McGrath 2000, *Nature* **407**:1026-9). On average, steps are monomer-sized (~5.4 nm) despite the variability of individual intervals.

We believe that monomer-sized steps will prove to be general. In preliminary studies, we've seen monomer-sized steps in the actin-based motility of *Shigella flexneri* when infecting living host cells. We've also seen monomer-sized steps when reconstituting *Listeria* motility in a 'test tube' using tissue extracts to provide necessary host factors.

How might monomer-sized steps arise? Although actin monomers are staggered at the end of filaments (see cartoon), monomers are spaced ~5.4 nm along the sides of F-actin filaments. Progress of a side-binding protein would generate the appropriate spacing. However, deeper thought reveals that we shouldn't have seen *any* steps.

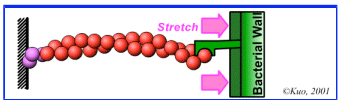
Why stepping is surprising Although binding to the sides of filaments could generate appropriate sized steps, steps should not be visible for two reasons. The first reason is that an actin filament is too floppy. This floppiness is the fundamental mechanism that allows the **elastic Brownian ratchet** to generate a gap large enough for monomers to insert between the ends of the filaments and the bacterium. If a filament were floppy enough to allow a monomer to insert, then the filament is not rigid enough to reveal monomer-sized steps -- wiggles would be as big as steps. Despite monomer-spaced distribution of actin along the sides of filaments, a single filament makes a very poor template for "stepping". Using exaggerated flexibility, the animation (either Flash, 27Kb or QuickTime, 29Kb) show how the flexibility would largely obscure steps.



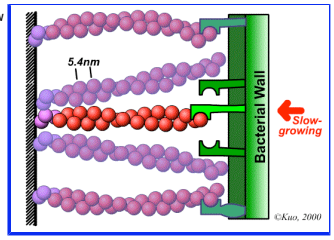


The second reason is that hundreds of actin filaments form the "rocket-like" tail that pushes bacteria. Stepping motion implies a molecular coordination, either filaments are molecularly aligned or filaments are growing in a molecularly coordinated fashion. Without some mechanism to impose molecular coordination,

Hypothesis to explain appearance of steps To generate monomer-sized steps, we need to suppress the intrinsic floppiness of actin filaments illustrated above. Like stretching a piece of string, stretching an actin filament will make it less floppy. With tension, filaments become "stiffer" and the fluctuations of a single filament drops considerably. A taut filament becomes an appropriate 'stepping template' to reveal monomer-sized steps. The animation (either Flash, 10Kb or QuickTime, 12Kb) shows how an individual stretched filament serves as an excellent template.



To generate a 'template' filament within the "rocket-like" tail, our hypothesis is that the multiple actin filaments of the tail are unlikely to grow at the same rates. Indeed, the slowest growing filaments must be stretched taut by the other, faster growing filaments. The animation (either Flash, 213Kb or QuickTime, 214Kb) shows the template filament (red) being stretched by other filaments (purple). Consistent with our thoughts about a binding protein, some of the "compressed" filaments (purple) are bound to the surface of bacteria, but some filaments may have released from the binding proteins and might elongate according to the elastic Brownian ratchet model. The motions of binding proteins along the sides of this slowest-growing (red) template filament will generate monomer-spaced steps of the bacterium. Statistically, a filament is 'template' for only a limited number of steps. Another filament becomes slowest-growing and takes over the role as template filament.



The cumulative action of many filaments pushing and a few filaments stretched taut makes the attachment of bacteria to their tails extremely strong. The strength of this cumulative attachment is revealed by the magnitude of the fluctuations of the bacterium. Although the 'wiggles' are smaller than directly detectable by our laser-tracking device, we can make an estimate that the 'wiggles' are <0.1nm (most chemical bonds are ~0.14nm) and correspond to >220pN to generate a 5.4nm step. The value of >220pN is particularly noteworthy because actin filaments *break* when forces exceed ~400pN (Tsuda *et al.* 1996, *Proc Nat'l Acad Sci, USA* **93**:12937-42). If our hypothesis is correct, forces near breaking levels are applied to the template filament!

Implications of monomer-sized steps There are two major implications of monomer-sized stepping. **First**, there must be an extremely tight binding complex that binds to the sides of actin filaments, and this complex can release and re-bind to filament at monomer-sized distances. Without a binding complex and taut filaments (see hypothesis above), bacteria would 'wiggle' >25-fold more and obscure any hint of monomer-sized steps. **Second**, biophysical models must be reconsidered. The 'classic' Brownian ratchet model must be discarded for describing *Listeria* motility. However, the physics behind the elastic Brownian ratchet model must occur, but the published calculations assume that the ends of filaments are free to 'wiggle' to allow intercalation (Mogilner & Oster 1996, *Biophys J* **71**: 3030-3045). If the ends of filaments are bound by a binding complex, the filaments must be longer and floppier for the model to work.

These implications are only two of many more implications. For example, a tight binding complex suggests additional mechanisms for regulating cellular protrusions generated by actin polymerization (see examples). The more points of regulation that we understand, the more likely we can control protrusive processes, both in clinical and engineering applications. Only additional research can determine if these more speculative ideas are correct.
